Local implementation and optimization of rotavirus reverse genetics systems

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“ Sometimes the prize is not worth the costs. The means by which we achieve victory are as important as the victory itself. ”

- Brandon Sanderson, The Way of Kings

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Summary

Reverse genetics (RG) is one of the most powerful tools for the study of viral replication, pathogenesis and for the generation of rationally designed vaccine candidates. The main bottleneck in rotavirus (RV) research has been the lack of a robust, traceable, helper-virus independent RV RG system (Desselberger, 2014). The first viral RG system was developed in 1976 to rescue the dsDNA virus, λ-phage, from cultured monkey kidney cells . In 1981 the first RNA virus, poliovirus, was rescued from cell culture through the transfection of viral genome transcripts generated *in vitro* from cDNA plasmids. In 2006, 25 years later, the first RV RG system was developed . It was a helper-virus based system that relied on the segmented genome of the RV to undergo reassortment during co-infection and depended on a selection system for the isolation of recombinant viral progeny. In 2017, 41 years since the development of the first viral RG system, a Japanese group (Kanai *et al.,* 2017) published the first, plasmid only, helper-virus independent, pT7_SA11-L2 RV RG system. The pT7_SA11-L2 RG system, and its subsequent adaptations and optimizations (Komoto *et al.,* 2018; Komoto *et al.,* 2019), has opened up a new era of targeted, rationally guided RV research opportunities.

The main goal of this project was to establish a plasmid-based, helper-virus independent RV RG system at the NWU. To accomplish this, the project had three main objectives, namely: 1) To obtain and implement the Japanese pT7_SA11-L2 RV RG system, and to optimize it through the incorporation of insights gained from the bluetongue virus (BTV) and African horsesickness virus (AHSV) RG systems. 2) To finalize and implement the locally developed, consensus sequence-based pSmart_SA11-N5 RV RG system with all the optimizations used during the pT7_SA11-L2 RV RG systems implementation, and 3) to perform a comparative analysis of the pSmart_SA11-N5 and pT7_SA11-L2 RV RG systems and their various optimizations throughout the project via $TCID_{50}$ assay. The original pT7_SA11-L2 RV RG system was obtained from AddGene and implemented at the NWU with initial difficulty. Several aspects of the BTV and AHSV RG systems were incorporated into the original pT7_SA11-L2 RG system.

These optimizations included: 1) The design and implementation of alternative capping and fusion enzyme expression plasmids, phCMVdream_VV_D1R, phCMVdream_VV_D12L and phCMVdream_p10_FAST. 2) Exchanging the transfection and co-seeding cell-lines from BHK-T7 and MA104 cells to BSR-T5/7 and ST cells respectively. 3) Using 3x more capping enzyme expression plasmids and the adaptation of an equi-molar transfection mixture approach. These optimizations significantly increased the pT7_SA11-L2 RV RG systems' repeatability and increased viral yield 10-fold from $2.15x10^2$ $TCID_{50}$/ml to $2.15x10^3$ $TCID_{50}$/ml. Rescue of the recombinant SA11-L2 strain from the pT7_SA11-L2 RG system was verified through dsRNA extraction, cDNA synthesis and whole viral genome sequencing.

I finalized the locally developed pSmart_SA11-N5 RV RG system, as only 8 of the 11 SA11-N5 cDNA transcription plasmids were successfully sub-cloned by previous students. The three remaining transcription plasmids for GS8, GS9 and GS11 were sub-cloned using In-Fusion HD seamless cloning and were sequence-verified through next-generation sequencing (NGS). The full pSmart_SA11-N5 RV RG system was then successfully implemented with all the optimizations used in the pT7_SA11-L2 RV RG system. Recombinant SA11-N5 was rescued from the pSmart_SA11-N5 RG system, as verified by genome segment-specific, one-step RT-PCR and sequencing of GS4 (VP4).

Both the pSmart_SA11-N5 and pT7_SA11-L2 RV RG systems were then further optimized by replacing the vaccinia virus (VV) capping enzyme expression plasmids with the phCMVdream_C3P3 construct that expressed an African swine fever virus (ASFV) capping enzyme fused to a viral T7-RNA polymerase through a serine-glycine linker . This increased the viral titers of both systems 100-fold with the pT7_SA11-L2 RG system going from $2.15x10^3$ $TCID_{50}$/ml to $1.0x10^5$ $TCID_{50}$/ml, and the pSmart_SA11-N5 RG system going from $2.15x10^4$ $TCID_{50}$/ml to $3.16x10^6$ $TCID_{50}$/ml. The final optimization in this project entailed a 3x increase of the (GS8) NSP2 and (GS11) NSP5 transcription plasmids of both the pT7_SA11-L2 and pSmart_SA11-N5 RV RG system to increase viroplasm formation. This increased viral yield another 100-fold in both systems with pT7_SA11-L2 reaching $3.16x10^7$ $TCID_{50}$/ml, and the pSmart_SA11-N5 RG system reaching $1.7x10^8$ $TCID_{50}$/ml.

To conclude: The initial rescue efficiency of both RV RG systems was very low and not suitable for research purposes. The titer of the rescued virus from the pT7_SA11-L2 RG system was only $2.15x10^2$ $TCID_{50}$/ml and from the pSmart_SA11-N5 RG system only $2.15x10^4$ $TCID_{50}$/ml. After optimization, both systems are now ready for robust experimentation. The pT7_SA11-L2 RG system now reaches viral titers of $3.16x10^7$ $TCID_{50}$/ml, with the pSmart_SA11-N5 RG system reaching $1.7x10^8$ $TCID_{50}$/ml. The pSmart_SA11-N5 RG system consistently yielded higher viral titers, thus was more efficient, than the pT7_SA11-L2 RG system in every comparable experiment. This was most likely due to the reduction in plasmid backbone size, with pSmart (~2010bp) being roughly 1070bp smaller than the pCAG (~3080bp) backbone used in the pT7_SA11-L2 RG system. This decreased overall plasmid load of the pSmart_SA11-N5 RG system by up to 11770bp when compared to the pT7_SA11-L2 RG system, and significantly increased transfection efficiency. Both the pT7_SA11-L2 and pSmart_SA11-N5 RV RG systems can now be used to further RV research and development goals, such as the elucidation of the many unclear aspects of RV replication, pathogenesis and correlates of protection, as well as providing a platform for the generation of rationally designed, next-generation, regionally specific, safe RV vaccine candidates.

Keywords

Rotavirus (RV); reverse genetics (RG); rotavirus SA11 strain; consensus sequence; In-Fusion HD cloning; seamless cloning; transfection; viral rescue; Immuno-fluorescent staining; Immuno-fluorescent monolayer assay (IFMA); $TCID_{50}$; $TCID_{50}$/ml; viral titer; plasmid only reverse genetics; pSmart; phCMVdream; BHK-T7; BSR-T5/7; MA104; ST; cell culture monolayers.

Opsomming

Tru-genetika (TG) is een van die kragtigste instrumente vir die studie van virale replikasie, patogenese en vir die generering van rasioneel ontwerpte entstofkandidate. Tot onlangs was die grootste knelpunt in rotavirus (RV) navorsing tot onlangs was die gebrek aan 'n herhaalbare, naspeurbare, helper-virus-onafhanklike RV TG stelsel (Desselberger, 2014). Die eerste virale TG stelsel is in 1976 ontwikkel om die ddDNS-virus, faag-λ, van gekweekte aapnierselle te herwin (Goff & Berg, 1976). In 1981 is die eerste RNS-virus, poliovirus, uit selkultuur herwin deur die transfeksie van virusgenoomtranskripte wat *in vitro* gegenereer is vanaf kDNS-plasmiede. In 2006, 25 jaar later, is die eerste rotavirus (RV) TG stelsel ontwikkel (Komoto & Taniguchi, 2006). Dit was 'n helper-virus-gebaseerde stelsel wat berus het op die gesegmenteerde genoom van RV om tydens ko-infeksie te herrangskik, en afhanklik was van 'n seleksiestelsel vir die isolasie van rekombinante virus nageslagte. In 2017, 41 jaar sedert die ontwikkeling van die eerste virale TG stelsel, het 'n Japanese groep (Kanai et al., 2017) die eerste, uitsluitlike plasmied, helper-virus-onafhanklike, pT7_SA11-L2 RV TG stelsel, gepubliseer. Die pT7_SA11-L2 TG stelsel, en die daaropvolgende aanpassings en optimaliserings (Komoto et al., 2018; Komoto et al., 2019), het 'n nuwe era geopen vir geteikende, rasioneel geleide RV-navorsingsgeleenthede.

Die hoofdoel van hierdie projek was om 'n plasmied-gebaseerde, helper-virus-onafhanklike, RV TG stelsel aan die NWU te vestig. Om dit te bereik, het die projek drie hoofdoelwitte gehad, naamlik: 1) Om die Japannese pT7_SA11-L2 RV TG stelsel te bekom, te implementeer, en om dit te optimaliseer deur die inkorporering van insigte wat verkry is uit die TG stelsels van bloutong virus (BTV) en die Afrika perdesiekte virus (APS). 2) Om die plaaslik ontwikkelde, konsensus-volgorde-gebaseerde pSmart_SA11-N5 RV RG-stelsel te finaliseer en te implementeer met al die optimaliserings wat tydens die implementering van pT7_SA11-L2 TV RG-stelsels gebruik is, en 3) om ‘n vergelykende analise van die pSmart_SA11-N5 en pT7_SA11-L2 RV TG stelsels uit te voer met hul verskillende optimaliserings gedurende die projek deur die 50% selkultuur infektiewe doses ($SKID_{50}$) meeting. Die oorspronklike pT7_SA11-L2 RV TG stelsel is van AddGene verkry en met aanvanklike probleme by die NWU geïmplementeer. Verskeie aspekte van die BTV en APS TG stelsels is in die oorspronklike pT7_SA11-L2 TG stelsel geïnkorporeer. Hierdie optimaliserings het die

volgende ingesluit: 1) Die ontwerp en implementering van alternatiewe uitdrukkingsplasmiede, phCMVdream_VV_D1R, phCMVdream_VV_D12L en phCMVdream_p10_FAST. 2) Die uitruiling van die verskeie transfeksie en medesaai sel-lyne van BHK-T7 en MA104 na BSR-T5 / 7 en ST selle. 3) Die gebruik van 3x meer 5`-afrondings ensiem uitdrukkingsplasmiede en die aanpassing van 'n gelyk-molêre transfeksiemengsel benadering. Hierdie optimaliserings het die herhaalbaarheid van die pT7_SA11-L2 RV TG stelsels aansienlik verhoog en die virale opbrengs tienvoudig verhoog van $2{,}15x10^{2}$ $SKID_{50}$/ml tot $2{,}15x10^{3}$ $SKID_{50}$/ml. Die herwinning van die rekombinante SA11-L2 stam uit die pT7_SA11-L2 TG stelsel is bevestig deur ddRNS-ekstraksie, kDNS-sintese en die volledige virale genoomvolgorde bepaling.

Ek het die plaaslik ontwikkelde pSmart_SA11-N5 RV TG stelsel gefinaliseer, aangesien slegs 8 van die 11 SA11-N5 kDNS-transkripsieplasmiede suksesvol deur vorige studente gesubkloneer was. Die drie oorblywende transkripsieplasmiede vir GS8, GS9 en GS11 is gesubkloneer deur gebruik te maak van In-Fusion HD naatlose klonering, en is deur die volgende generasie volgordebepaling (NGS) geverifieer. Die volledige pSmart_SA11-N5 RV TG stelsel is toe suksesvol geïmplementeer met al die optimaliserings wat in die pT7_SA11-L2 RV TG stelsel gebruik is. Rekombinante SA11-N5 is herwin uit die pSmart_SA11-N5 TG stelsel, soos bevestig deur genome segment-spesifieke, een-stap TT-PKR en volgordebepaling van GS4 (VP4). Beide die pSmart_SA11-N5- en pT7_SA11-L2 RV TG stelsels is daarna verder geoptimaliseer deur die 5`-afrondings ensiem plasmiede van die vaccinia virus (VV) te vervang met die phCMVdream_C3P3-konstruk wat 'n Afrika-varkkoorsvirus (ASFV) afrondings ensiem uitdruk wat saamgesmelt is met ’n virale T7-RNA-polimerase deur 'n serien-glisien-skakelaar (Eaton et al., 2017). Dit verhoog die virale titers van albei stelsels 100-voudig met die pT7_SA11-L2 TG stelsel wat van $2.15x10^{3}$ $SKID_{50}$/ml na $1.0x10^{5}$ $SKID_{50}$/ml gegaan het, en die pSmart_SA11-N5 TG stelsel wat van $2.15x10^{4}$ $SKID_{50}$/ml na $3.16x10^{6}$ $SKID_{50}$/ml gegaan het. Die finale optimalisering in hierdie projek het 'n 3x toename in die (GS8) NSP2 en (GS11) NSP5 transkripsie plasmiede van beide die pT7_SA11-L2 en pSmart_SA11-N5 RV RG-stelsel behels om die vorming van die viroplasma te verhoog. Hierdie verhoogde virale opbrengs is 100-keer meer in albei stelsels, met die pT7_SA11-L2 wat $3{,}16x10^{7}$ $SKID_{50}$/ml bereik het, en die pSmart_SA11-N5 TG stelsel wat $1{,}7x10^{8}$ $SKID_{50}$/ml bereik het.

Om af te sluit: Die aanvanklike herwinning doeltreffendheid van beide RV TG stelsels was baie laag en was nie geskik vir navorsingsdoeleindes nie. Die titer van die herwinde virus vanaf die pT7_SA11-L2 RG-stelsel was slegs $2,15x10^2$ $SKID_{50}$/ml en van die pSmart_SA11-N5 TG stelsel slegs $2,15x10^4$ $SKID_{50}$/ml. Na optimalisering is albei stelsels nou gereed vir eksperimentering. Die pT7_SA11-L2 TG stelsel bereik nou virale titers van $3.16x10^7$ $SKID_{50}$/ml, met die pSmart_SA11-N5 TG stelsel wat $1.7x10^8$ $SKID_{50}$/ml bereik. Die pSmart_SA11-N5 TG stelsel het deurgaans hoër virale titers gelewer, wat dus meer doeltreffend was as die pT7_SA11-L2 TG stelsel in elke vergelykbare eksperiment. Dit was waarskynlik te danke aan die vermindering in die grootte van die plasmied-ruggraat, met pSmart (~2010bp) wat ongeveer 1070bp kleiner was as die pCAG (~3080bp) ruggraat wat in die pT7_SA11-L2 TG stelsel gebruik is. Dit het die totale plasmied-lading van die pSmart_SA11-N5 TG stelsel met tot 11770bp verminder het, in vergelyking met die pT7_SA11-L2 TG stelsel, wat die transfeksie-doeltreffendheid aansienlik verhoog het. Beide die pT7_SA11-L2 en pSmart_SA11-N5 RV TG stelsels kan nou gebruik word om RV-navorsing en ontwikkelingsdoelwitte te bevorder, soos die opklaring van die vele onduidelike aspekte van RV-replikasie, patogenese en merkers van beskerming, sowel as om 'n platform te bied vir die ontwikkeling van rasioneel ontwerpte, volgende generasie, streekspesifieke, veilige RV-entstofkandidate.

Sleutelwoorde

Rotavirus (RV); tru-genetika (TG); rotavirus SA11 stam; consensusvolgorde; In-Fusion HD klonering; naatlose cloning; transfeksie; virale herwinning; Immuno-fluoressensie kleuring; Immuno-fluoressensie monolaag prosedure (IFMP); selkultuur infektiewe doses ($SKID_{50}$); $SKID_{50}$/ml; virale titer; uitsluitlik plasmied gebaseerde tru-genetika; pSmart; phCMVdream; BHK-T7; BSR-T5/7; MA104; ST; selkultuur monolaag.

List of abbreviations

[DNA]	: Concentration of DNA
aa	: Amino acid
AGE	: Agarose gel electrophoresis
AHSV	: African horsesickness virus
ASFV	: African swine fever virus
BHK	: Baby hamster kidney cells
bp	: Base pair (nucleic acids)
BTV	: Bluetongue virus
C3P3	: Chimeric cytoplasmic capping-prone phage polymerase
CPE	: Cytopathogenic effect
CAF	: Stellenbosch University's Central Analytical Facilities
DLP	: Double layered particle
DMEM	: Dulbecco's Modified Eagle Medium
DNA	: Deoxyribonucleic acid
dNTP	: Deoxyribonucleotide triphosphate
EDTA	: Ethylenediamine tetra-acetic acid
Em	: Emission range
EtBr	: Ethidium bromide
EtOH	: Ethanol or Ethel alcohol
Ex	: Excitation range
FAST	: Fusion-associated small transmembrane
FBS	: Fetal bovine serum
GS	: Genome segment
HDV	: Hepatitis Delta virus
IFMA	: Immuno-fluorescent monolayer assay
IgG	: Immunoglobulin G
IVIS	: In Vivo Imaging System
kDa	: Kilodaltons
LB	: Lysogeny broth, or Luria broth or Luria-Bertani broth
LPS	: Lipopolysaccharides
MA104	: African green monkey kidney cells
MCS	: Multiple cloning site
MEM	: Minimal essential medium
MMOH	: Methyl mercury hydroxide
MW	: Molecular weight
NBV	: Nelson-bay virus
NEAA	: Non-essential amino acid
NGS	: Next generation sequencing
NICD	: National Institute for Communal Diseases
NSP	: Non-structural protein (virology)
NWU	: North-West University
OD	: Optical density
ORF	: Open reading frame
PAGE	: Polyacrylamide gel electrophoresis
PBS	: Phosphate buffered saline
PCR	: Polymerase chain reaction
RG	: Reverse genetic or reverse genetics

RNA : Ribonucleic acid
RNP : Ribonucleoprotein complex
RSA : South Africa
RV : Rotavirus
SA11 : Simian agent 11 (RV)
SDS : Sodium dodecyl sulphate
SG : Sub-group (taxonomy)
SLP : Single layered particle
ST : Swine testes cells
SNP : Single nucleotide polymorphism
SUV : Sub-unit vaccine
TAE : Tris-base, acetic acid and EDTA (buffer)
TBE : Tris-base, boric acid and EDTA (buffer)
$TCID_{50}$: 50% of the Tissue culture infective dose
TLP : Triple layered particle
U.K. : United Kingdom
USA : United States of America
UTR : untranslated region
VLP : Virus like particle
VP : Viral protein (structural protein) (virology)
VV : Vaccinia virus
w/v : weight to volume (buffer preparation)
WT : Wild type (virology)

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Chapter 1

Literature review

1.1 Background

Genetic research is at its very core the study of the correlation between an organism's genotype and its phenotype. It reveals the genes, or gene sequences, that produce specific enzymes or regulate various pathways, which in turn influence everything from cell differentiation during embryonic development to the metabolism and the immune response of the final organism. From beginning to end the genetic code inscribes an organism's life story, and we as geneticists aim to not only understand this story but also to understand how it is written, how it is read and how we can guide it to our advantage. This project utilized one of the most powerful tools in genetic research, namely reverse genetics (RG), as we implemented several RG systems for the generation and study of recombinant rotaviruses (RV).

One of the most definitive ways to study the role of specific genes or genetic elements in a viral genome is to modify its sequence and then generate infectious viruses through the process of RG. This can also be defined as the rescue of recombinant viruses from cell-culture based on modified transcripts or cDNA sequences . RG enables the targeted study of genetic changes in various viral genomes and can provide critical insights into viral replication, host range and the mechanisms that determine pathogenesis and virulence (Conradie *et al.*, 2016; Kanai *et al.*, 2017). This information is essential in the rationally guided combat of viral diseases, especially in terms of the highly adaptive and divergent RNA viruses, of which many are dangerous human pathogens. The impact of RNA virus RG systems on efforts to design effective and safe viral therapeutics and rationally guided vaccines is already enormous and will be even more significant in future.

For RVs, the lack of a robust and helper-virus independent RG system has until recently been the main bottleneck in progressive research and has left many elements of this virus's lifecycle and pathogenesis subject to speculation.

The recently developed, plasmid-only, pT7_SA11-L2 RV RG system (Sen *et al.*, 2009) has opened up new avenues in research and promises to answer some of the most burning questions pertaining to RV replication, genome packaging, correlates of protection and virus-host interactions. However, to date mixed results have been obtained in regards to the recreation of the originally published RV RG results at various institutions (Potgieter, A.C. Deltamune. Personal communication). This clearly illustrated the need for further development and optimization of the initial Japanese pT7_SA11-L2 RV RG system.

The plasmid-only pT7_SA11-L2 RG system does not require the use of a helper-virus and is thus independent of any recombinant virus selection system, which has historically been one of the main limitations of RV RG systems. Kanai and associates recovered RV SA11-L2 after transfection of baby hamster kidney cells (BHK) constitutively expressing a viral T7-RNA polymerase (BHK-T7) with 11 RV cDNA transcription plasmids and expression plasmids encoding the fusion-associated small transmembrane (FAST) protein from the Nelson-bay virus (NBV), and the two subunits of the vaccinia virus (VV) capping enzyme. The system was made available to the scientific community through the AddGene service and was purchased and implemented at various institutions, including the NWU.

Although the initial pT7_SA11-L2 RV RG system was quite difficult to repeat, its impact was clearly evident as publications on the various applications and optimizations of the system became available shortly after its release. Komoto and associates investigated RV replication using the pT7_SA11-L2 RV RG system and found that NSP6 was not necessary for propagation in cell culture (Dormitzer *et al.*, 2004; Kobayashi *et al.*, 2007; McClain *et al.*, 2010; Patton, 2012; Pretorius *et al.*, 2015), and then further improved on the initial pT7_SA11-L2 RV RG system by reducing the plasmid backbone size and increasing the ratios of viroplasm encoding gene sequences GS8 (NSP2) and GS11 (NSP5), threefold.

This increased the system's efficiency considerably and facilitated viral rescued without the use of the NBV FAST protein or the VV capping enzyme originally included in the pT7_SA11-L2 RV RG system.

At the NWU we have been working on the development of a helper-virus independent RV RG system for several years. My project entailed the implementation of the initial Japanese pT7_SA11-L2 RV RG system and the finalization and implementation of our own, locally developed, consensus sequence-based, plasmid only pSmart_SA11-N5 RV RG system. This will be followed by a comparative analysis between the Japanese and locally developed RV RG systems, as well as the rationally guided optimization of both. The optimizations considered in this project were based on the already established dsRNA virus RG systems of African horsesickness virus (AHSV) and bluetongue virus (BTV) .

1.2 Introduction to reverse genetics (RG)

Conventional genetic research (forward genetics), describes the process by which a variation in the phenotype of an organism, be it naturally occurring or induced through mutagenesis, is correlated to a specific gene sequence. The process moves from the identification of a divergent, hereditary, phenotypic characteristic to the identification and isolation of the corresponding allele (through genome mapping) and finishes with DNA extraction, sequencing and mapping of the divergent gene against a normal or wild-type (WT) gene sequence. In essence, the researcher already knows the nature of the mutation, as it is observed, and therefore seeks the origin thereof. Although this system can be used for gene function assays and has been used therefor in the past, it is often difficult to precisely define a correlation between a specific gene and the observed mutant phenotype due to the massive interplay of various genes and regulatory elements in the presentation of a specific phenotypic characteristic . This approach is also limited to non-lethal mutations that are hereditary and phenotypically visible, requiring the generation of bi-allelic mutants which is a lengthy and expensive procedure. This concept is however turned around with RG as it moves from the genetic sequence through to the phenotypic trait, a seemingly semantic shift, but a fundamentally different approach. The main purpose of RG is therefore not to identify a gene responsible for a variant trait, but to elucidate the function of a specific gene, or genetic sequence, through guided mutagenesis followed by observation of the corresponding mutant phenotype.

The use of RG more narrowly defines the specific function of the modified gene and directly correlates gene sequence to gene function through phenotypic evaluation. Currently, the majority of gene function assays are based on the rationally guided knock-out of specific genomic sequences through various means. These approaches have been used in various models, ranging from mouse to zebrafish , fruit flies and even various single-celled organisms (Argmann *et al.*, 2006; Bridgen, 2013; Goff & Berg, 1976; Racaniello & Baltimore, 1981). In terms of virology, RG is defined as the recovery, or rescue, of infectious viruses from cloned cDNA or mRNA that has been engineered to carry specific mutations or extra-genomic sequences (Baric & Sims, 2007; Sambrook & Russell, 2001; Wienholds *et al.*, 2002).

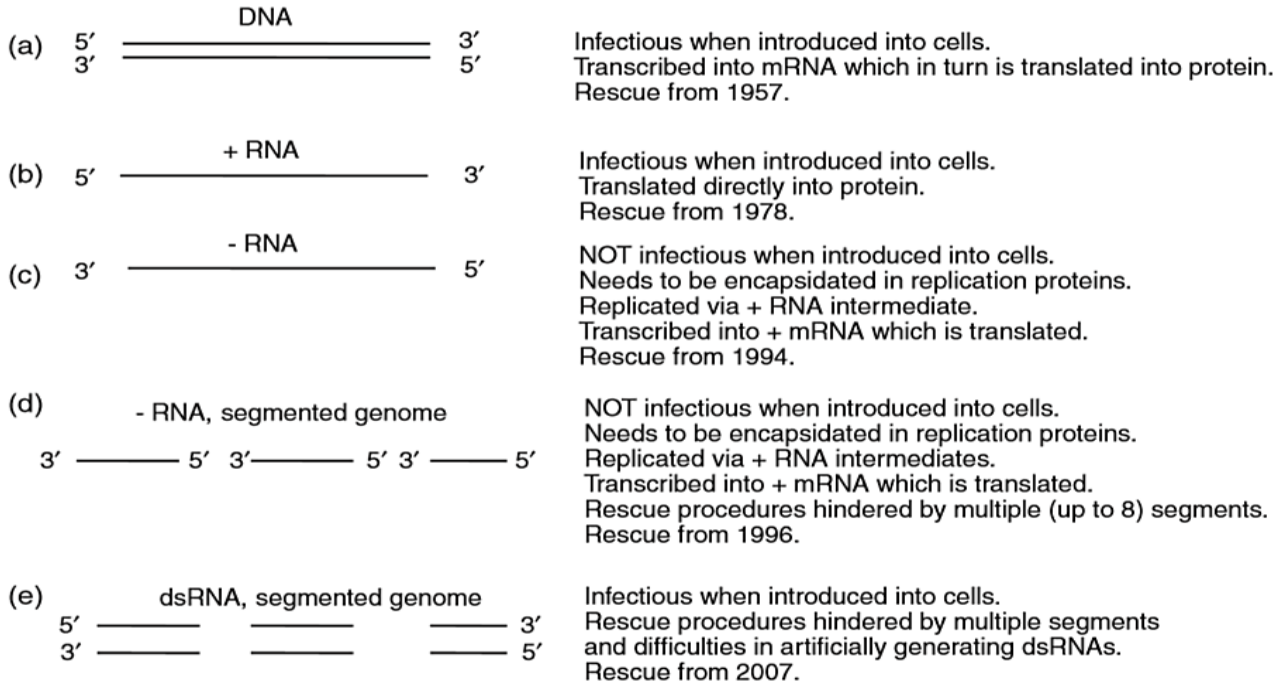

Figure 1: Illustration of the most common viral genome configurations in chronological rescue order, including methods and challenges associated with each. Image taken from Bridgen (2013) with permission.

Due to the diverse nature of viruses in terms of the replication cycle, genomic structure and host vectors, RG systems have to be adapted to the specific nature of the viral genome and correspondingly have very divergent techniques and strategies. Figure 1 illustrates and very briefly summarizes the basic variations of viral RG systems based on the most common viral genome configurations. The recovery of a dsDNA virus, for example, is more straightforward than that of a segmented dsRNA virus. The first published viral RG system was used to recover the dsDNA viruses, λ-phage, from

monkey kidney cells (Kao & Lee, 2013; van Gennip *et al.*, 2012). In this instance, the dsDNA used for transfection was deemed infectious as it was capable of producing infection viable viruses directly after transfection with no further adaptation or modification necessary. RNA viruses, however, require a bit more engineering to successfully rescue (Figure 2).

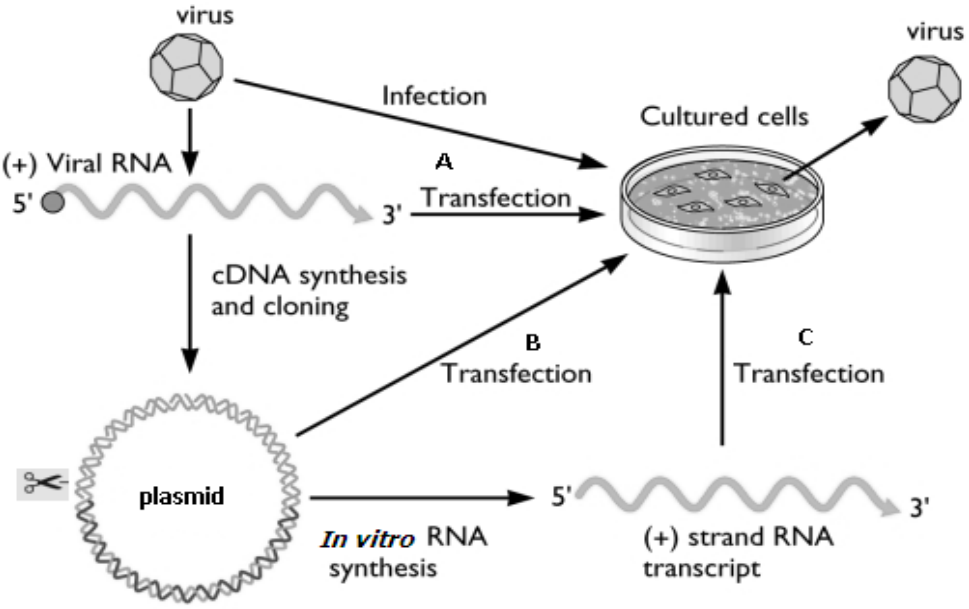

Figure 2: Illustration of RG strategies for +sense RNA viruses. A: Transfection of extracted wild-type viral transcripts into cells. **B**: Transfection of plasmids carrying cDNA copies of viral transcripts. **C**: in vitro transcription of cDNA followed by transfection

The first RNA virus to be rescued from a RG system was the poliomyelitis virus (poliovirus) in 1981. The positive-sense (+)RNA poliovirus was rescued by cloning a cDNA copy of the viral genome into the pBR322 plasmid, which was then used to generate transcripts that were transfected into cultured mammalian cells . The system was further developed to incorporate the SP6 polymerase promoter into the cDNA plasmid as a transcription regulatory factor. These transcription plasmids were then used to generate *in vitro* transcripts of the viral genome, which were then transfected into HeLa 3 cells to recover infectious recombinant poliovirus (Bhadauria *et al.*, 2009; Dickson, 2000). These strategies relied on the fact that the poliovirus has a (+)RNA genome which is deemed infectious once introduced into cells. Figure 2 illustrates the three main methods of rescuing (+)RNA viruses from cell-culture. **1**) Transfection of

viral mRNA derived from transcriptionally active viral particles. **2**) Transfection of cDNA carrying plasmids under the regulation of specific RNA-polymerase promoters into cells that express said RNA-polymerases. **3**) Transfection of *in vitro* generated transcripts from cDNA carrying plasmids. Each of these strategies generates positive-sense transcripts that mimic viral mRNAs and thus initiate viral replication.

In terms of negative-sense (-)RNA viruses, a lot of genetic engineering is required before a viable RG system can be implemented. The best example of this is most certainly the RG systems developed for influenza virus, as these systems had to cope with the fragility of ssRNA, the (-)sense nature of the viral genome and the need for ribonucleoprotein complexes (RNPs) formation before any virus could be rescued. The RNPs could be formed *in vitro* by adding viral genomic RNA to purified nucleoproteins and polymerase . Only through the formation of these complexes could the viral genome be stably carried into receptive cells and readily have available the various transcription and regulatory factors required for expression and replication. The influenza virus RG system is one of the most well-known and widespread viral RG systems to date and is used annually across the globe for the generation of rationally designed seasonal vaccines. The development of this system also closely mirrors the basic steps in the development and optimization of most viral RG systems, moving from a helper-virus based system to a solely plasmid-based system and finally to the incorporation of various genomic elements into a single plasmid.

Although not all viral RG systems follow this trajectory, the themes that guide its development and optimization are shared throughout viral RG. As illustrated by Figure 3, the first stage in the influenza RG system was based on the transfection of RNPs along with a cDNA transcription plasmid (carrying a cDNA copy of a single genome segment) into cultured eukaryotic cells. These cells were then later infected with a helper-virus (influenza A). This system relies on the propensity of the influenza virus to undergo reassortment, a process by which viral genome segments can be exchanged between related strains if co-infection occurs. Taking advantage of this naturally occurring phenomenon the RG system introduces numerous copies of a specific recombinant viral genome segment which will be incorporated into the genome of some of the viral progeny. This yields a viral population that is a mixture of unaltered helper-virus and recombinant influenza virus carrying the gene of interest.

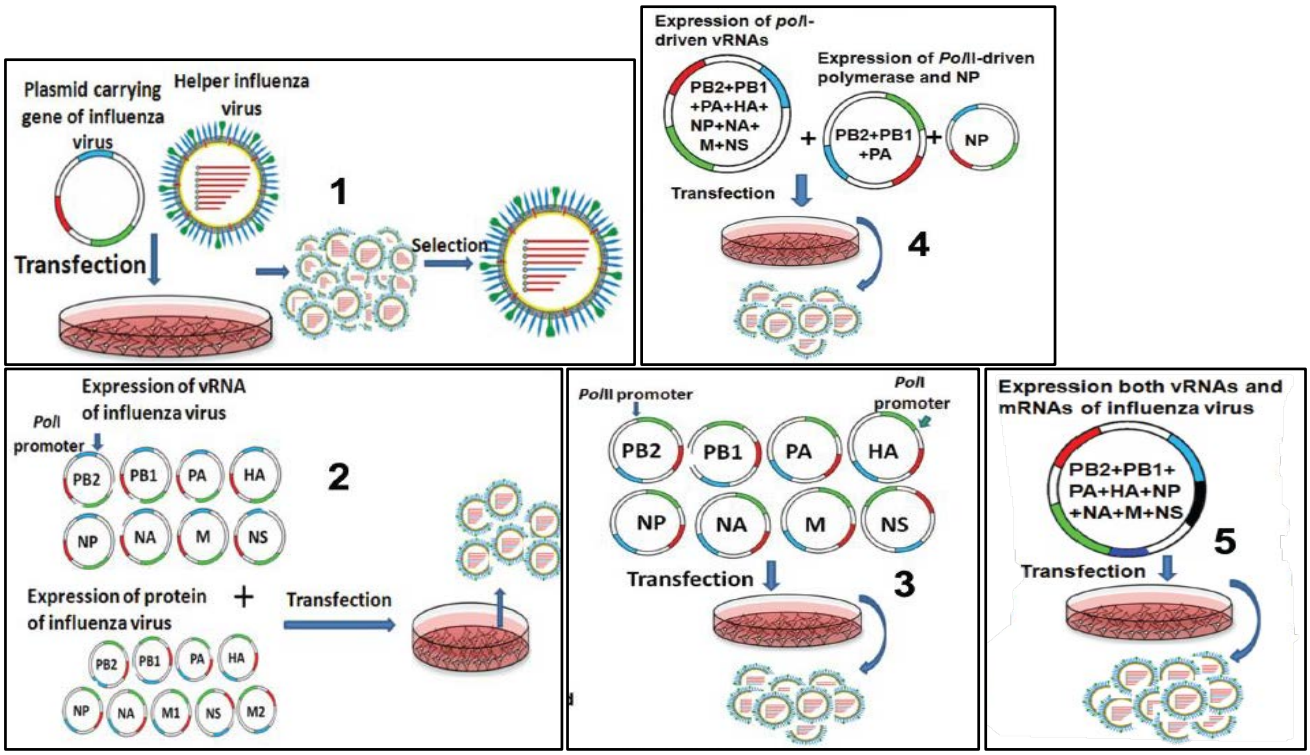

Figure 3: Illustration of the development and optimization of the influenza RG systems. 1: Helper-virus based system where a single genome segment is exchanged. Most commonly an outer capsid protein associated with a cellular or humoral immune response. **2**: Plasmid-based system that entails several expression plasmids encoding the various viral replication elements and transcription plasmids that produces precise viral mRNAs for viral genome packaging. **3**: Plasmid-based system that consists solely of transcription plasmids each with various regulatory elements associated with viral replication. **4**: The incorporation of various viral genome segments into single plasmids along with their various regulatory elements. **5**: Single plasmid system that encodes the entire viral genome as well as all the regulatory factors required for its expression, translation and packaging. Image adapted from Bio-engineered 4:1; 9-14; January/February (2013) with permission.

The unaltered helper virus is then removed from the population through some or other selection system, yielding mostly recombinant virus. This is the first stage of the influenza RG system as the selection of unaltered helper virus is never 100% efficient and only single gene segment reassortment can be achieved. Over many years this system was redesigned, further developed and optimized (Matthijnssens *et al.*, 2008) as illustrated in Figure 3. A solely plasmid-based system comprising of 12 rescue plasmids was successfully implemented and quickly replaced by one comprising of only 8 (Braks *et al.*, 2006; Chinnery *et al.*, 2004). This trend continued until a 5 plasmid system was developed . Currently, the majority of the commercial influenza A RG systems comprise of a highly optimized single vector system that is annually used around the globe to generate rationally designed, seasonal influenza vaccines (Luytjes *et al.*, 1989).

Although not precisely, most RG systems follow a similar development approach as the influenza virus RG system, moving from a single substitution helper-virus based system to more advanced transcript-based and plasmid-based systems which are then optimized and fine-tuned over time. The ability to engineer recombinant viruses makes it possible to study the biology of the virus and also to generate rationally designed vaccine candidates.

1.3 Rotavirus

1.3.1 Rotavirus classification

When viewed under an electron microscope the rotavirus particle has a distinct wheel-and-spoke like structure, hence the use of the name “rota”, Latin for “wheel” . Taxonomically, rotaviruses belong to the genus *Rotavirus* which in turn belongs to the *Reoviridae* family which comprises of two subfamilies (*Sedoreovirinae* and *Spinareovirinae*) and a total of 15 genera (Table 1). This family is characterised by viruses with genomes consisting of 9 to 12 linear segments of dsRNA (Jansen *et al.*, 1997; Maclean *et al.*, 2017).

Historically RV classification was based on the use of monoclonal antibodies to identify the presence or lack of specific VP6 epitopes. More recently however rotaviruses were divided into eight serogroups (A through H) based on the amino-acid (aa) sequence and immunogenic properties of their structural protein VP6 . Group A rotaviruses are the major cause of diarrhoea in humans, with groups B and C contributing to a lesser extent.

Table 1: Classification of dsRNA viruses within the Reoviridae family

Subfamily	Genus	Number of genome-segments	Particle diameter and genome size	Selected examples of members of the virus species
Spinareovirinae	*Aquareovirus*	11	75 nm (30,500 bp)	Aquareovirus A (Chum Salmon reovirus CS)
	Coltivirus	12	60 – 80 nm (29, 000 bp)	Colorado tick fever virus (strain Florio)
	Cypovirus	10	65 nm (25, 000 bp)	Lymantria dispar cypovirus 1
	Dinovernavirus	9	49 – 50 nm (unknown)	Aedes pseudoscutellaris reovirus
	Fijivirus	10	65 – 70 nm (27, 000 – 30, 000 bp)	Fiji disease virus
	Idnoreovirus	10 – 11	70 nm (27, 000 – 30, 000 bp)	Idnoreovirus 1: Diadromus pulchellus
	Mycoreovirus	11 or 12	80 nm (~ 23, 000 bp)	mycoreovirus 1: Cryphonectria parasitica mycoreovirus-1
	Orthoreovirus	10	80 nm (~23, 500 bp)	Mammalian orthoreovirus
	Oryzavirus	10	70 nm (26, 000 bp)	Rice ragged stunt virus
Sedoreovirinae	*Cardoreovirus*	11	55 nm (Not known)	Eriocheir sinensis reovirus
	Orbivirus	10	80 nm (19, 200 bp)	African horsesickness virus; bluetongue virus
	Mimoreovirus	11	90 nm – 95 nm (25, 400 bp)	Micromonas pusilla reovirus
	Phytoreovirus	12	70 nm (26, 000 bp)	Wound tumour virus
	Rotavirus	11	80 nm (~18, 500 bp)	Rotavirus A
	Seadornavirus	11	60 nm – 70nm (~21, 000 bp)	Banna virus

Data in table extracted from (Goff & Berg, 1976). Genus used in this project, i.e. rotavirus, highlighted in blue.

RVs were also classified through RNA electrophoretic separation profiles (based on GS11). This technique divided RV into sub-groups (SG) namely SGI, SGII, SGI and II or non-SGI and II (Bridgen & Elliott, 1996). Additionally, RNA-RNA hybridization studies group RVs into various genogroups represented by the Wa, DS-1 and Au-1 prototype strains (Table 2). The Wa strain is the prototype of the Wa-like genogroup which contains SGII rotaviruses with a long electropherotype whilst the DS-1-like genogroup is represented by the prototype RV DS-1 strain which is classified in SGI, with a characteristic short electropherotype. The AU-1 strain is the prototype of AU-1-like strains which have a long electropherotype and are also placed into SGI (Ebihara *et al.*, 2005).

Table 2: Whole-genome genotype constellation of selected prototype RV strains

Strain	Species	Genotype*									
		VP[7]	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Wa	Hu	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
DS-1	Hu	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
Au-1	Hu	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
OSU	Po	G5	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1
PO-13	Av	G18	P[17]	I4	R4	C4	M4	A4	N4	T4	E4	H4
SA11-H96	Si	G3	P[2]	I2	R2	C5	M5	A5	N5	T5	E2	H5

Reassortants are visualised by a non-homogenous constellation colour. *The colour scheme is used to enhance the visualisation of certain patterns or genome segment constellations. Green, red, and orange depict the human strains (Hu) Wa-like, DS-1-like, and AU-like genome segments, respectively. Yellow, blue, and purple respectively indicate the avian (Av) PO-13-like rotavirus genome segments; some typical porcine (Po) VP4, VP7, and VP6 genotypes; and the SA-11-like genome segments. Table taken from (Nakagomi *et al.*, 1985)(Kaplan *et al.*, 1985; Racaniello & Baltimore, 1981) with permission.

The great diversity observed among the RVs is thought to arise from the segmented nature of the viruses' genome and its propensity to undergo reassortment (Yu *et al.*, 2019). This makes classification difficult as zoonosis and reassortment frequently lead to novel strains.

To not only classify each specific strain fully, but also illustrate its genetic relationship to other strains, a full genome-based classification system was developed (Fodor *et al.*, 1999; Kaplan *et al.*, 1985). Sequence identity cut-off limits are used when comparing the sequences of each individual genome segment of a specific strain according to the following annotation: Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx which respectively represents the genotype for genome segments encoding VP7-VP4-VP6-

VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6. The capital letters in the genotype were derived from the function associated with the protein i.e., **g**lycoprotein, **p**rotease-sensitive, **i**nner capsid, **R**NA-dependent RNA polymerase, **c**ore, **m**ethyltransferase, interferon **a**ntagonist, **N**TPase, **t**ranslation enhancer, **e**nterotoxin and p**h**osphoprotein (Table 3). As of April 2011 27 G, 35 P, 16 I, 9 R, 9 C, 8 M, 16 A, 9 N, 12 T, 14 E and 11 H genotypes varieties are known and this number is expected to rise as more whole-genome sequencing projects are undertaken globally (Neumann *et al.*, 2005).

The outer most layer of the RV TLP comprises of the **p**rotease-sensitive VP4 and the **g**lycoprotein VP7 which are also commonly used to classify various RV into G- and P-serotypes. Historically this was done using neutralizing antibodies against various VP4 and VP7s, however, this approach is limited due to the lack of a wide range of antibodies and various other technical difficulties . More recently, RT-PCR and sequencing have been used to determine the P and G genotypes based on genome sequence correlations (Attoui *et al.*, 2011).

Current nomenclature dictates that as much information about the strain as possible is included in its identification. The Rotavirus Classification Working Group recommends that strains be named using a notation that states the RV group, species of origin, country of identification, common name, year of identification and G- and P-types (Mertens, 2004). For instance, the strain used in this project (simian agent 11, SA11) originally isolated in 1958 is named: RVA/Simian-tc/ZAF/SA11-H96/1958-N5/G3P5B[2].

Table 3: The rotavirus genome segments, encoded proteins and their known functions

GS	Size (bp)	Protein	Size (kDa)	Location	Genotype*	Protein function
1	3302	VP1	125	Core	R	RNA-dependent RNA polymerase; 3'-mRNA binding; forms transcription complex with VP3
2	2690	VP2	102	Inner capsid	C	Inner capsid structural protein; non-specific ssRNA and dsRNA binding; myristoylated; required for replicase activity of VP1.
3	2591	VP3	98	Core	M	Guanylyltransferase and methyltransferase; non-specific ssRNA binding; part of transcription complex with VP1
4	2362	VP4	VP4: 88 VP5*: 60 VP8*: 28	Outer capsid	P	Outer capsid spike protein; P-type neutralization antigen; virulence determinant; cell-attachment protein; trypsin cleavage into VP5* and VP8* enhances infectivity; VP5* permeabilises membranes; VP8* contains the haemagglutinin domain (in some strains)
5	1611	NSP1	59	Cytoplasm	A	Associates with cytoskeleton; high degree of sequence variation; role in suppression of host interferon response
6	1356	VP6	48	Middle capsid	I	Major virion protein; middle capsid structural protein; homotrimeric structure; group- and subgroup-specific antigen
7	1105	NSP3	35	Cytoplasm	T	Homodimer; virus-specific 3'-mRNA binding; binds elongation factor eIF4G1 and circularises mRNA on translation initiation complex; involved in translational regulation and host shut-off
8	1059	NSP2	37	Cytoplasm	N	NTPase and helicase activity; non-specific ssRNA binding; a major component of the viroplasm; binds NSP5 and VP1; essential for dsRNA synthesis and formation of infectious viral progeny
9	1062	VP7	37	Outer capsid	G	Outer capsid structural glycoprotein; G-type neutralization antigen; N-linked high mannose glycosylation and trimming; RER transmembrane calcium-binding
10	751	NSP4	20	Cytoplasm	E	Viral enterotoxin; receptor for double-layer particle budding through ER membrane; N-linked high mannose glycosylation; modulates intracellular calcium levels essential for viral RNA replication and formation of infectious viral progeny
11	667	NSP5/6	NSP5: 22 NSP6:11	Cytoplasm	H	NSP5: viroplasm formation; multimerizes; O-linked glycosylation; hyper-phosphorylated; autokinase activity; enhanced by NSP2 interaction; binds ssRNA; component of viroplasm NSP6: interacts with NSP5 and localises to viroplasms

*Function-dependent genotype annotation adapted from (Mertens, 2004). The size data in the table is based on the rotavirus SA11 strain (Kohli *et al.*, 1992). GS: genome segment; ds: double-stranded; ss: single-stranded; ER: endoplasmic reticulum; RsER: rough ER

1.3.2 Rotavirus genome structure and protein-coding assignment

The RV genome consists of 11 segments of dsRNA that range from 667bp to 3302bp in size, based of RV SA11 (Estes & Cohen, 1989; Ramig, 1997), which encode 6 structural and 6 nonstructural proteins (Table 3). Each genome segment (GS) is monocistronic (encodes a single protein) except for GS11 which is polycistronic and encodes NSP5 and NSP6 in two overlapping out of frame ORFs (Mitchell & Both, 1988). The viral dsRNA genomes can be extracted and fully visualized through polyacrylamide gel electrophoresis (PAGE) to produce a separation profile that is unique to specific RV strains (Figure 4).

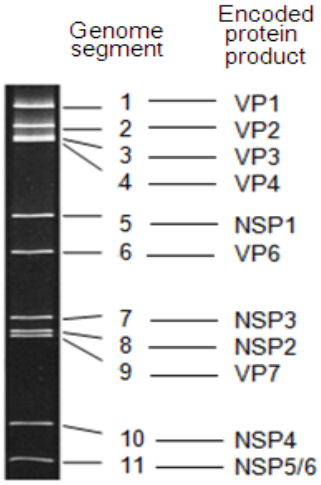

Figure 4: The rotavirus dsRNA genome segments resolved by SDS-PAGE along with encoded protein products. Image taken from Patton (2012) with permission.

The structure of each of the 11 dsRNA genome segments has a similar design starting with a 5`-$m^7GpppG^{(m)}$ cap (Imai *et al.*, 1983; Pizarro *et al.*, 1991) followed by the 5`-UTR (untranslated region) leading into the ORF and finally culminating in the UTR-3` of that segment (Patton, 1995). These UTRs vary in length and consensus-sequence and range from 9 to 49bp for the 5`- regions and 17 to 182bp for the -3` regions. Highly conserved, group-specific terminal sequences are present among the various serogroups, A through H, (Matthijnssens *et al.*, 2012), Table 4.

Table 4: Variations between the 5'- and 3'-terminal end sequence of selected rotavirus strains of the different serogroups

Group	Strain	5'-terminal sequence	3'-terminal sequence
A	SA11	5'-GGC(A/U)$_7$-	-AUGUGACC-3' *
B	IDIR	5'-GGCC/U-	-ACCC-3'
C	Bristol	5'-GGCC(A/U)$_7$-	-GGCU-3'
D	HS-58	5'-GG(U)$_5$(A)$_3$-	-GACC-3
H	ADRV-N	5'-GGCACU-	-ACCCC-3'

(Matthijnssens *et al.*, 2008b), *(Trojnar *et al.*, 2010)

With reference to RV_SA11, each of the genome segments contain a 5`-GGC(A/U)$_7$- and a 3`-AUGUGACC conserved terminal region (Chizhikov & Patton, 2000; Imai *et al.*, 1983; Patton, 1995; Tortorici *et al.*, 2006; Trojnar *et al.*, 2010; Wentz *et al.*, 1996). This characteristic of conserved 5` and 3` UTR sequences are observed among all RV strains although slight variations occur following the second G of the 5`-GGN sequence. These conserved regions are thought to play a critical role in genome packaging. For this reason, any RV RG system should be designed to generate very precise 5`- and -3` transcript termini to facilitate viral rescue.

1.3.3 Rotavirus particle structure

The mature RV particle, or virion, comprises of a triple-layered, non-enveloped, icosahedral capsid which encases the 11 dsRNA segments of the viral genome (Figure 5, A). Due to its structural properties, the mature virion is also referred to as the triple-layered particle or TLP and has a diameter of about 80 nm (Ludert *et al.*, 1986; McClain *et al.*, 2010). The interior of the capsid is comprised of mainly 60 VP2 dimers in a T = 2 icosahedral symmetry, where five dimers arrange in a fivefold axis to form a decamer which in turn is arranged into a uniform viral core particle of 12 decamers (thus 120 VP2 molecules in total). Small pores along the fivefold axis are left after core particle assembly to allow transcription of the viral genome by VP1 (viral RNA-dependent RNA polymerase) and VP3 (viral methyltransferase) (Desselberger, 2014; Matthijnssens *et al.*, 2008a).

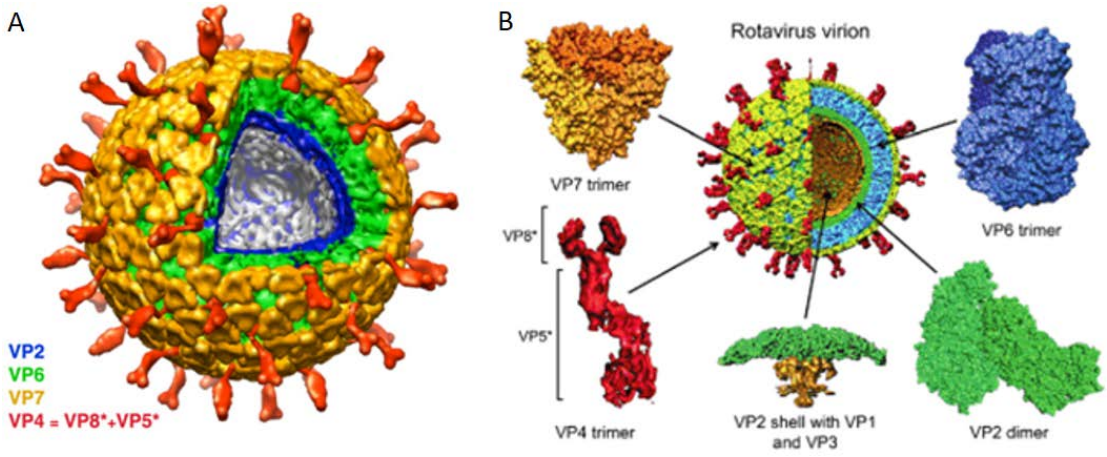

Figure 5: 3D graphic representation of the rotavirus virion and particle architecture. **A:** Cut-away model of a rotavirus triple-layered particle. The inner layer is composed of the structural protein VP2 (blue), the middle layer is composed of VP6 (green) and the outer layer is made up of VP4 (red) and VP7 (yellow-orange). **B:** Schematic illustration of rotavirus structure from cryoEM reconstruction, and the location of the structural protein components. Figure adapted from (Greenberg *et al.*, 1983), with permission.

VP1 and VP3, which together form the viral genome replication complex, are the remainder of the internal structure of the core particle and are arranged around the pores of the fivefold axis (Desselberger, 2014; Jere *et al.*, 2014; Kushnir *et al.*, 2012; Liu *et al.*, 2013; Pesavento *et al.*, 2006). The viral core is encased by VP6 in the form of 260 trimers arranged in a T= 13 icosahedral symmetry. VP6 forms the middle layer of the tVLP and interacts with the VP2 dimers as well as the outer layer VP7 and VP4 trimers. The outermost layer of the mature viral capsid is comprised of 260 VP7 trimers associated with 60 spikes of VP4 trimers in icosahedral symmetry. The virion also contains 132 channels along the fivefold axis used during viral genome transcription (capped mRNA exits through these channels and enters the cytoplasm without exposing the dsRNA genome to the intracellular environment).

1.3.4 Rotavirus replication and life-cycle

Rotavirus is transmitted mainly through the faecal-oral pathway with villus cells of the small intestine being the primary target for infection. The replication cycle of RV (Figure 6) is a complex set of steps that can be summarised as attachment, entry and uncoating, viral genome transcription and translation, viroplasm formation, DLP

formation, TLP formation and maturation and finally release of fully matured viral particles (Desselberger *et al.*, 2009; Estes *et al.*, 1979a; Flewett & Woode, 1978a).

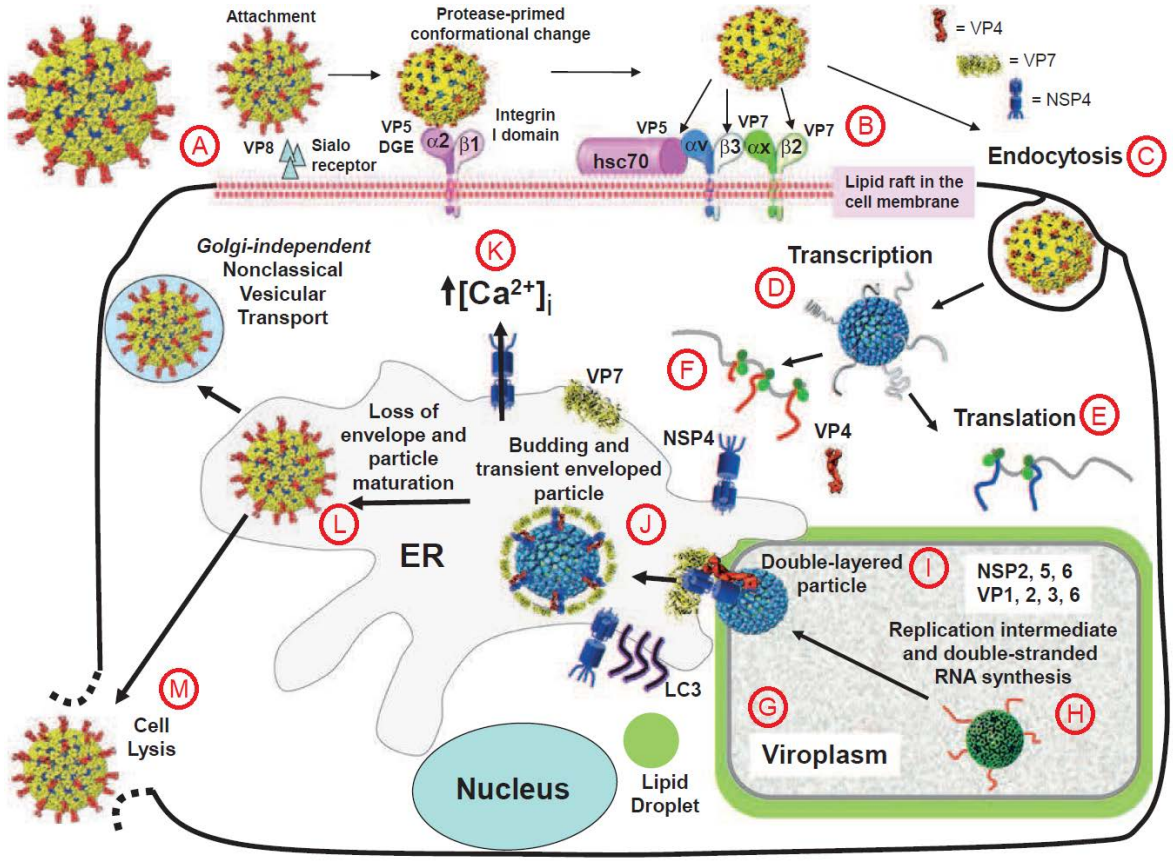

Figure 6: Graphic representation of the rotavirus life-cycle. **A**: Attachment of rotavirus to cell surface through sialo acid receptor mediated binding of VP8 (proteolytically cleaved subunit of VP4). **B**: Interaction of various integrin ligand motifs in VP5 and VP7 with integrins α2β1, αvβ3 and αxβ2 facilitating viral attachment and initiation of viral entry through endocytosis. Interactions with heat shock protein 70 also illustrated. **C**: Viral entry via endocytosis and formation of endosome. During this step the viral particle also undergoes uncoating and sheds the outer VP4 and VP7 layer exposing the transcriptionally active DLP to the cytoplasm. **D**: Transcription of the viral genome. **F**: Translation of the viral mRNA resulting in viral protein synthesis leading to the formation of the viroplasm. The precise mechanisms regulating viroplasm formation are still unclear, however it is well known that GS8 (NSP2) and GS11 (NSP5) are key components in its formation. **E**: Transcription of viral mRNA for incorporation into newly formed replication intermediary. **G**: Recruitment of lipid droplets for the formation of the viroplasm. NSP2, 5 and 6 are strongly associated with the formation of the viroplasm along with the structural units of the newly formed DLP namely VP1, 2, 3 and 6. **H**: Formation of the replication intermediary during which one of each of the 11 genome segments are encased in the core particle in association with VP1, VP3 and VP2. **I**: Attachment of VP6 to the core particle and the synthesis of dsRNA within the core particle through the activity of VP1. **J**: Transferral of newly formed DLP to ER for attachment of outer layer of VP7 and VP4. **K**: During initial uncoating and later maturation of the TLP, Ca^{2+} ion concentrations play a critical role in the stabilization of VP7 trimers. Low concentrations of Ca^{2+} (such as those found in the endosome) destabilize VP7 trimers and initiate uncoating. Increased concentrations of Ca^{2+} (facilitated through NSP4 activity) are required for proper coting of the DLP and maturation of the TLP. **L**: Maturation of TLP following the disassociation of the envelope and non-vesicle transport of virions to cell surface for viral budding or shedding. This process is independent of the Golgi apparatus and is not fully understood as of yet. **M**: final release of matured viral particles through cell lysis or viral budding. Matured TLPs are now deemed infectious and spread to adjacent cells. Image taken from (Gouvea *et al.*, 1990; Komoto *et al.*, 2017; Matthijnssens *et al.*, 2011; Matthijnssens *et al.*, 2008b; Pesavento *et al.*, 2006) with permission.

As illustrated by Figure 6, many of the key mechanisms regulating RV replication are still unknown. The elucidation of these mechanisms and their interactions is one of the most anticipated outcomes of a robust, traceable, helper-virus independent RV RG system. For a complete list of possible RV RG outputs see Section 1.4.3.4.

1.3.4.1 Viral attachment and cell entry

Viral attachment is mainly mediated through VP4 (Ludert *et al.*, 1996a), although integrin ligand motifs in VP7 also contribute to stable binding and have been correlated to the initiation of endocytosis (Coulson *et al.*, 1997; Gutierrez *et al.*, 2010). The VP4 (GS4) spike is proteolytically cleaved into the two sub-units, VP5 and VP8 (Arias *et al.*, 1996; Espejo *et al.*, 1981; Estes *et al.*, 1981) which increases infectivity and might be essential for the propagation of some RV strains (Figure 7).

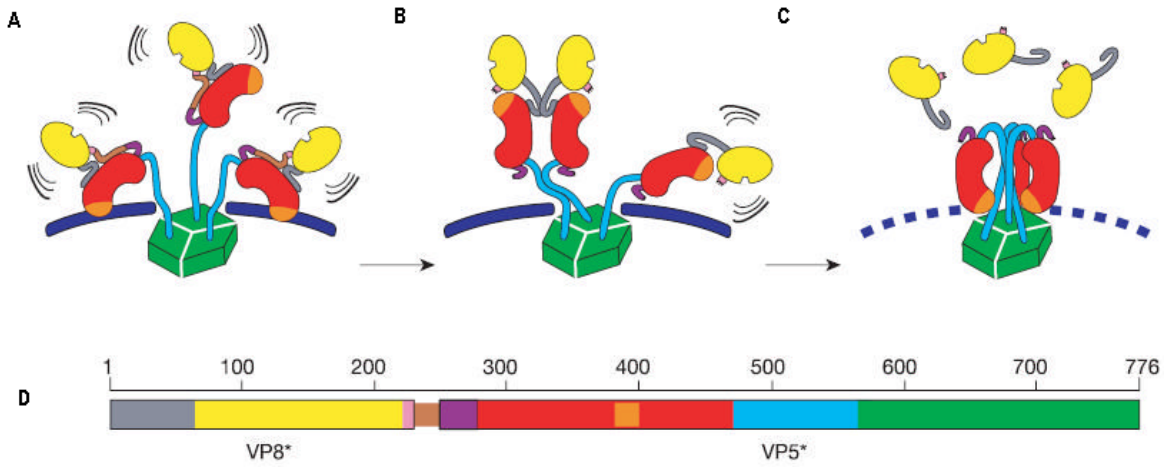

Figure 7: Model showing the rearrangement of VP4 during trypsin cleavage and cell entry. A: Non-cleaved (brown line) state with possible, flexible, free play indicated by wavy lines. **B**: Trypsin cleaved state. **C**: Folded-back state which exposes the hydrophobic regions during membrane penetration. **D**: Colour coded, linear VP4 indicating the location of amino acid regions in VP5* and VP8*. The head region is coloured in yellow (the VP8* core), notched at the sialoside-binding cleft. The body includes the red domain (VP5* antigenic region) and the orange cap (the potential membrane interaction loop), the purple appendage, and part of the grey and cyan tubes. The stalk is the lower part of the cyan tubes and the foot is shown in green. Image taken from (Matthijnssens et al., 2008a)(Neumann et al., 2005) with permission

The VP5 and VP8 subunits remain in a non-covalent association with the viral particle (Ludert *et al.*, 1996b; Trask *et al.*, 2012), with VP8 interacting with sialic acids on various cellular glycans and VP5 (alongside VP7) acting as co-receptors following initial viral attachment (Ciarlet & Estes, 1999; Haselhorst *et al.*, 2011; Haselhorst *et al.*, 2009). As illustrated in Figure 7, the dissociation of VP8 following trypsin cleavage allows the trimeric coiled chains to zip together, resulting in the body folding back to expose hydrophobic regions within VP5 (Dormitzer *et al.*, 2004; Trask *et al.*, 2012; Yoder *et al.*, 2009).

These regions, especially aa139, are responsible for permeabilisation of the cell membrane (Kim *et al.*, 2010) and facilitate viral entry. Mutations in this region (V139D) reduced infectivity 10 000-fold (Kim *et al.*, 2010) and truncations (385–404) abolishes membrane permeability altogether (Dowling *et al.*, 2000).

Integrins α2β1 and α4β1 are associated with viral attachment and integrin αvβ3 with viral entry (Ciarlet *et al.*, 2002; Guerrero *et al.*, 2000; Hewish *et al.*, 2000; Londrigan *et al.*, 2000). When blocking integrin αvβ3 with monoclonal antibodies in MA104 cell-cultures RV infection was inhibited (Guerrero *et al.*, 2000), indicating that integrin αvβ3 plays a crucial role in viral entry. Integrin α2β1, however, was later found to be non-essential for attachment, although it does promote cell entry. RV has also been shown to interact with heat shock protein 70 (Lopez & Arias, 2004). Following viral entry, low Ca^{2+} levels in the endosome and cytoplasm cause destabilization of the VP7 trimers coating the TLP, resulting in the solubilisation and uncoating of the VP7 and VP4 outer layer, and exposure of the transcriptionally active DLP to the cytoplasm (Charpilienne *et al.*, 2002; Estes *et al.*, 1979b; Ruiz *et al.*, 2007; Ruiz *et al.*, 1996; Ruiz *et al.*, 2000; Ruiz *et al.*, 2005).

1.3.4.2 Viral genome transcription and translation

During viral entry, the outer most layer of the TLP (VP 4 & VP7) is shed due to low Ca^{2+} concentrations within the endosome. This results in the exposure of the transcriptionally active DLP to the cytoplasm (Bican *et al.*, 1982). During this process it is thought that conformational changes within the DLP activate VP1 and VP3, initiating viral genome transcription (Dowling *et al.*, 2000; Gilbert *et al.*, 2001; Ruiz *et*

al., 2000). The activation is thought to be mediated by VP6 through its interactions with the inner core (VP2) and outer capsid protein VP4 as it detaches. This is supported by the fact that VP2 core particles (lacking VP6) are not transcriptionally active (Sandino *et al.*, 1986). VP1 uses the negative strand of the dsRNA genome as template to synthesise, non-polyadenylated plus-sense (+)ssRNA which is then capped by VP3 before being transferred to the cytoplasm (Guglielmi *et al.*, 2010; Patton, 1986).

This is a rapid process as transcripts are detectable 1hour post-infection (hpi) (Patton *et al.*, 2004). These (+)ssRNAs serve as both mRNA for viral protein synthesis and as template for dsRNA synthesis within the viroplasm (Chen *et al.*, 1994). Transcripts are not synthesized in equi-molar amounts as experiments with RNA interference have shown that smaller genome segments are synthesised more rapidly and at higher amounts than the larger ones (Ayala-Breton *et al.*, 2009). At 12 hpi the relative number of GS10 (NSP4, 751bp) transcripts is roughly 4.5 and 2 times greater than those of GS1 (VP1, 3302bp) and GS6 (VP6, 1356bp) respectively (Stacy-Phipps & Patton, 1987).

NSP3 has been shown to bind to the 3'-terminal end of RV transcripts and interact with eukaryotic initiation factor 2-α (eIF2-α) (Piron *et al.*, 1999; Piron *et al.*, 1998) in a similar way to how poly-A binding protein (PABP) binds to native poly-A mRNA tails. This is thought to result in circularisation of the RV transcripts, leading to enhanced expression of these transcripts over native mRNAs (Vende *et al.*, 2000). NSP3 has also been shown to suppress or completely silence the expression of various host cell proteins (Montero *et al.*, 2006) further aiding in the selective expression of RV transcripts.

1.3.4.3 Viroplasm formation and DLP assembly

The viroplasm is an electron-dense, membrane-free, cytoplasmic inclusion that is formed by NSP2 and NSP5 (Contin *et al.*, 2010; Fabbretti *et al.*, 1999), within which (-)ssRNA synthesis, viral genome replication and packaging occurs. Viral intermediaries (DLPs) are formed within the viroplasm. Multiple viroplasms can form during early infection, which usually merges together to form larger structures during later stages

of infection. Besides NSP2 and NSP5, viroplasms are also strongly associated with VP1, VP3, VP2 and VP6 (structural components of DLPs). Viral (+)ssRNA segments are also present in high concentrations (Eichwald *et al.*, 2004). Cellular tubulin is sequestered to the viroplasm during its formation, through unknown means, where it is depolymerised by NSP2. This provides structure and also aids in the evasion of the host-cells antiviral mechanisms (Martin *et al.*, 2010).

The viroplasm co-localizes with cytoplasmic lipids and lipid droplets, as well as the lipid droplet-associated proteins perilipin A and ADRP, which are thought to recruit droplets to the viroplasm during its formation. Currently, it is thought that these lipid-droplets are essential for the formation of the viroplasm and the production of infectious viral particles (Cheung *et al.*, 2010). Genomic dsRNA can be detected 2 to 4 hpi (Patton *et al.*, 2004) within the viroplasm in equi-molar amounts (Ayala-Breton *et al.*, 2009), despite the various genome segments size variations and cytoplasmic expression levels. It is postulated that (+)ssRNA is used as template for (-)ssRNA synthesis and dsRNA genome packaging. It is not clear if these (+)ssRNAs are incorporated into the viroplasm during its formation, or recruited and shuttled into it after its completion (Carreno-Torres *et al.*, 2010; Silvestri *et al.*, 2004). The -UGUG-3` terminal sequence is essential for viral genome replication (Patton *et al.*, 1996; Trojnar *et al.*, 2010) and is selectively recognised by VP1 (Lu *et al.*, 2008) for packaging and dsRNA synthesis. The exact mechanism regulating the recruitment and packaging of (+)ssRNA into the DLP is not currently understood.

One packaging model, based on the observed equi-molar ratios of genome segments in mature virions, suggests a process during which each individual (+)ssRNA GS forms a hybridization complex with another in a sequential manner until all 11 GSs are associated with one another through RNA-RNA interactions. This complex is then bound to free VP1, which is known to have a high affinity for (+)ssRNA, to form a ssRNA-polymerase complex (McDonald & Patton, 2011). Following this, VP2 then binds to the ssRNA-polymerase complex, simultaneously forming the structural units for the SLP (core particle) and inducing a conformational change in VP1 which induces (-)ssRNA synthesis and genome replication (Patton *et al.*, 1997). This model is based on that of the packaging model observed in influenza (Hutchinson *et al.*, 2010). Many alternative packaging models are also proposed, however to date no concrete evidence has been found definitively identifying one as correct.

Whether or not genome packaging takes place before or after DLP completion, the stages of the DLP auto-assembly are as follows, the VP1_VP3 polymerase complex binds to VP2 pentamers before the VP2 pentamers coalesce into a SLP (or core particle). The SLP is then coated by VP6 trimers to form the completed DLP viral intermediary. This process culminates in the transport of the DLP to the ER where NSP4 mediated coating of VP4 and VP7 takes place. The mechanism of transport between the viroplasm and ER is not fully understood, but it is clear that NSP4 plays a decisive role in its execution.

1.3.4.4 TLP formation and virion maturation

Viral maturation occurs in the ER and entails the coating of the DLP with VP4 (spikes) and VP7 to form a complete TLP. This process appears to be directly regulated by NSP4 (Hu *et al.*, 2012), as experiments wherein NSP4 was silenced through siRNAs in MA014 cells, the viral yield decreased with roughly 75% (Lopez *et al.*, 2005). NSP4 is a multi-functional, regulatory protein and exists in various forms in and around the infected cell. The majority of intracellular NSP4 is accumulated within the ER, with extracellular and cytoplasmic NSP4 also being present, but in lower concentrations. Trans-membrane intracellular NSP4 (iNSP4) is known to bind both DLPs budded from the viroplasm and VP4, in a chaperone-like manner (Trask *et al.*, 2012).

While the precise mechanism of DLP release from the viroplasm is unknown, it is evident that NSP4 recruits DLPs into the outer-capsid-assembly pathway (Au *et al.*, 1989; Berkova *et al.*, 2006; Trask *et al.*, 2012) whereby it mediates the binding of VP4 trimers onto the DLP (Figure 8). During this stage, the trans-membrane NSP4 is bound to both the DLP and VP4 and causes a deformation in the ER that results in encapsulation of the DLP and the formation of a VP7 containing envelope. The VP7 trimers comprising the envelope bind to the DLP and lock VP4 in place (Trask *et al.*, 2012). NSP4 also regulates the high Ca^{2+} concentration within the ER required for the formation of stable VP7 trimers and the proper coating of the DLP (Kuum *et al.*, 2012; Ruiz *et al.*, 2000; Waldron *et al.*, 1994).

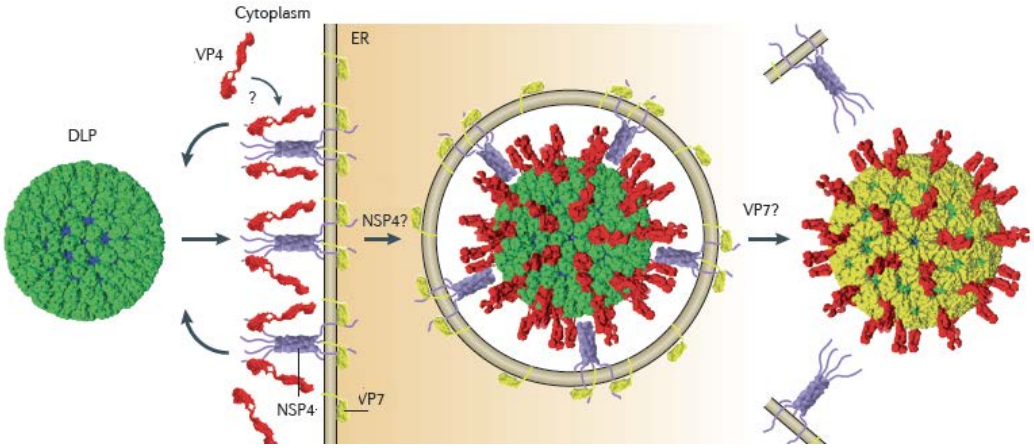

Figure 8: Illustration of NSP4 mediated DLP binding and ER penetration during outer capsid coating pathway. NSP4 binding to both VP4 and the DLP causes ER membrane deformation and entry into the ER. During this stage VP4 associates with the DLP and a temporary envelope containing VP7 is formed. VP7 binds to the DLP, locking VP4 in place and the envelope is lost through unknown means. Figure taken from Trask et al., 2012b with permission.

After the enveloped TLP has bound both VP4 and VP7, the envelope is lost through unknown means and the mature TLP is released at the apical surface of the now polarised cells before cell lysis (Cuadras *et al.*, 2006).

1.3.5 Rotavirus pathogenesis and vaccines

1.3.5.1 Burden of disease

Rotavirus was first described in the early 1940s as an “agent” causing “epidemic diarrhoea” in calves and infantile mice (Kraft, 1957; Light & Hodes, 1943). Its importance as a human pathogen was first identified in 1973 when RV particles were found in the duodenal mucosa (following electron microscopic examination) of nine infants presenting with severe diarrhoea (Bishop *et al.*, 1973; Davidson *et al.*, 1975). Viral particles similar to those described by Bishop and Davidson were subsequently identified and associated with individuals suffering from gastroenteritis (Flewett *et al.*, 1973; Flewett & Woode, 1978b).

Today RV is considered one of the major causes of severe gastroenteritis in both humans and animals and is responsible for roughly 125,000 deaths annually of children under the age of 5 (Clark *et al.*, 2017; WHO, 2016).

Although RV infections are ubiquitously spread across the globe, life-threatening dehydration due to diarrhoea mainly occurs in developing countries such as those in Africa and Asia, which contribute to upwards of 50% of the global mortality rate. RV also threatens food-stock security as it is a zoonotic virus capable of infecting rodents, several bird species and livestock.

As mentioned in Section 1.3.4, the target of rotavirus infection is the villus cells of the small intestine and is spread through the faecal-oral pathway. On a systemic level, following RV infection, necrosis of the epithelial cells of the small intestine is induced leading to villous atrophy (Desselberger *et al.*, 2009; Johnson *et al.*, 1986; Ramig, 2004). This is followed by enterocyte atrophy which in turn causes a loss of intestinal enzymes, low absorption capacity and increased osmotic pressure (Desselberger *et al.*, 2009). In addition to this damage to the endothelial cells result in crypt cells becoming hyperplasic which is also commonly accompanied by increased fluid secretion (Ramig, 2004), resulting in gastroenteritis and dehydration due to diarrhoea.

On a cellular level NSP4 (RV GS10) acts as an enterotoxin that disrupts the cellular electrolyte balance by directly simulating the enteric nervous system, leading to an intracellular increase of Ca^{2+} levels (Ball *et al.*, 1996; Dong *et al.*, 1997; Lundgren *et al.*, 2000; Ramig, 2004). Other major contributors to cellular toxicity and viral pathogenesis are VP4, VP3, NSP1 and NSP2 (Desselberger *et al.*, 2009).

1.3.5.2 Immune response

Rotavirus infections can elicit both an innate immune response and a cellular immune response depending on the specific strain and host cell type (Frias *et al.*, 2012; Sen *et al.*, 2011). In most cases, the innate immune response is the first line of defence against initial infection (García-Sastre & Biron, 2006) and comprises of various pattern recognition receptors (PRRs) (Levy *et al.*, 2011) and the production and secretion of cytokines such as type I (IFN-β) and type III interferon (IFN-λ) (Deal *et al.*, 2010; Frias *et al.*, 2012; Pott *et al.*, 2011; Sen *et al.*, 2011). The activation of the innate immune response initiates a cascade of cellular signals that ultimately establishes an anti-viral state within the cell and induces the activation of the cellular immune response (Angel *et al.*, 2012; Pott *et al.*, 2011; Pott *et al.*, 2012). The primary function of PRRs is the

detection of viral pathogen-associated molecular patterns (PAMPs) (Levy *et al.*, 2011), and include membrane-bound Toll-like receptors (TLRs), cytoplasmic retinoic acid-inducible gene I-like receptors (RLRs) and the cytoplasmic nucleotide oligomerisation domain (NOD)-like receptors (Akira *et al.*, 2006; Mogensen, 2009). One of the most common and strongly immunogenic PAMPs is dsRNA (Thompson & Locarnini, 2007), and various other viral replication intermediates. For this reason, RV has evolved various strategies to evade the innate immune response, some of which include the formation of the viroplasm, the transcriptionally active DLPs (no dsRNA exposed to cytoplasm) and the NSP1 mediated anti-interferon response, Figure 9, (Arnold & Patton, 2011; Barro & Patton, 2007; Sen *et al.*, 2009).

The cellular immune response induced by RV infections is well documented in rat, rabbit, pig and mouse models (Conner *et al.*, 1993; Franco & Greenberg, 1997; Knipping *et al.*, 2011; Yuan *et al.*, 1998) with the focus being on B-cell and T-cell activation as well as the production of various rotavirus-specific antibodies. When

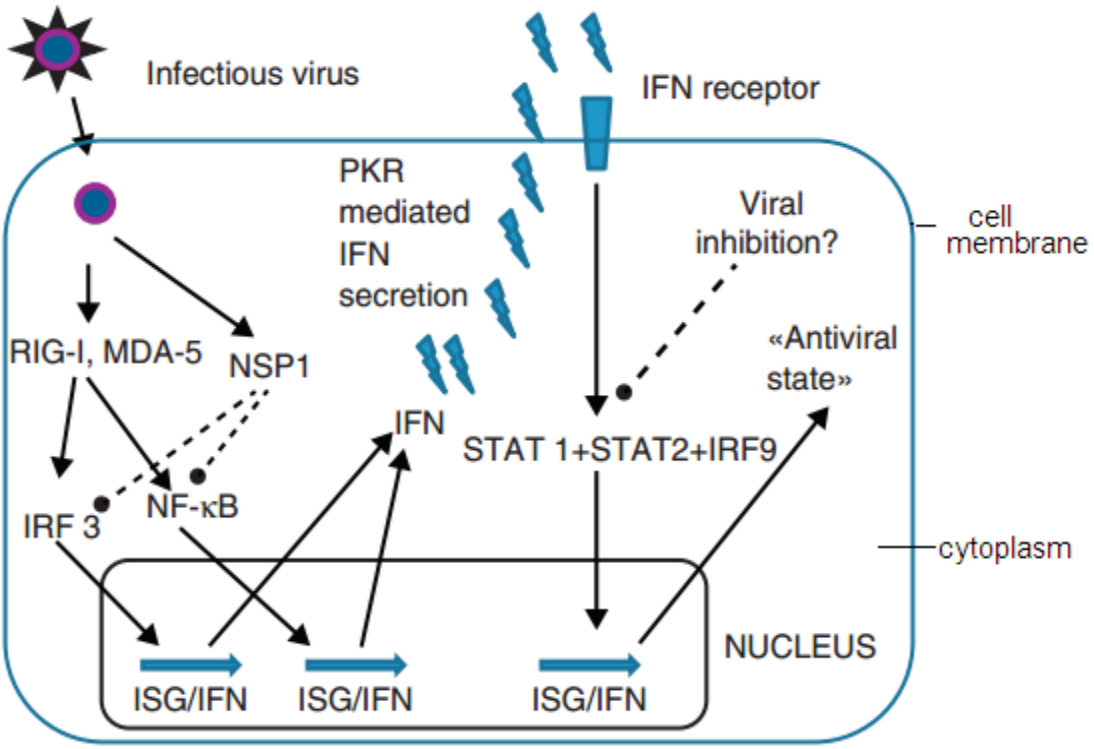

Figure 9: Schematic depiction of the innate immune response and the NSP1 mediated anti-interferon response. During initial infection IFN regulatory factor 3 (IRF3) or NF-κB is activated by the stimulation of various PRRs, MDA5 or RIG-I and PKRs by virus-specific ligands. The IFN regulatory factors then translocate to the nucleus and activate interferon stimulated genes (ISG) through the production of IFN. NSP1 inhibits IRF3 (Bridgen & Elliott, 1996; Falcone et al., 1999; Gentsch et al., 1992)(Komoto & Taniguchi, 2006)(Luytjes et al., 1989; Neumann & Kawaoka, 2001; Neumann et al., 1999), and in so doing circumvents the establishment of an anti-viral state. Figure from (Angel et al., 2012) with permission.

infecting adult mice with the virus, no diarrheal symptoms develop, however viral shedding is observed. This viral shedding required IgA and IgG-producing B lymphocytes to clear (Franco & Greenberg, 1999). Viral clearance is observed in T-cell deficient mice (Franco & Greenberg, 1995; Franco & Greenberg, 1997), however, the process is very slow, indicating that the initial response to RV infection is based on B-cell activation and is T-cell independent (Blutt *et al.*, 2002). In gnotobiotic piglets, the use of milk supplemented with anti-RV (human Wa strain) IgY antibodies (raised in chickens) passively increased rates of protection against Wa strains in a dose-dependent manner (Vega *et al.*, 2012). Similarly, anti-VP6 antibodies (raised in llamas) provided broad neutralising activity *in vitro* and provided protection against diarrhoea in mice models (Garaicoechea *et al.*, 2008).

Although extensive animal model studies have been performed, their findings do not necessarily translate well to humans where initial RV infection elicits the production of intestinal and systemic antibodies which protect against severe diarrhoea upon re-infection (Davidson *et al.*, 1983; Velázquez *et al.*, 1996; Ward & Bernstein, 1994). The majority of human infections result in protection levels against re-infection of roughly 40%, 75% against any diarrhoea and 88% against severe diarrhoea (Gladstone *et al.*, 2011; Velázquez *et al.*, 1996). The precise correlates of protection are not currently known. However intestinal IgA antibodies against the outer capsid proteins VP4 and VP7 seem to provide neutralising protection (Desselberger & Huppertz, 2011; Desselberger *et al.*, 2009; Franco *et al.*, 2006; Franco & Greenberg, 1999). This theory is supported by the findings of Blutt *et al.* (2012) where mice lacking mucosal IgA did not develop protective immunity against repeat infections (Blutt *et al.*, 2012), and refuted by Angel *et al.* (2012), as it has been shown that rotavirus-specific IgA antibodies are not optimal correlates of protection following vaccination of children in developing countries (Angel *et al.*, 2012).

1.3.5.3 Live-attenuated vaccines

The first two widely used commercial RV vaccines are, RotaTeq® (Merck) a pentavalent (G1, G2, G3, G4, G6: P[5], P[8]) live attenuated human-bovine reassortant vaccine, and Rotarix™ (GlaxoSmithKline), a live attenuated vaccine derived from a

human G1P[8] RV isolate (Vesikari *et al.*, 2006). The efficacy of an immune response to these vaccines are suboptimal in children in Africa and other developing countries (Clarke & Desselberger, 2015; Desselberger, 2014; Madhi *et al.*, 2010; Ruiz-Palacios *et al.*, 2006; WHO, 2016; Zaman *et al.*, 2010). Various reasons have been postulated for the reduced vaccine efficacy in these regions including interference of RV-specific antibodies acquired via breastfeeding, co-administration of the oral polio vaccine, malnutrition and co-infections such as HIV. It has also been speculated that the vaccines have a lower efficacy against the specific RV strains circulating in Africa and Asia, which are considerably more diverse than that of the other regions and countries. To devise suitable approaches towards local strain-based vaccine development it is important to gain a better understanding of the replication cycle of RVs and the extent of structural compatibility of proteins of various strains. Reverse genetics (RG) has been very useful to study this in many other viruses.

1.3.5.4 Sub-unit and VLP vaccines

Virus-like particles (VLPs) are comprised of several of the structural components of a virus without any of the viral genome, thus making the VLP immunogenic but eliminating the risk of reassortment and viral replication. This allows the VLP to elicit a humoral and cellular immune response without the risk of infection. However, VLPs are non-replicating meaning that repeated dosages of the antigen are required to elicit long term immunity. As a result of this, many studies have indicated that VLPs can be used as an immune booster that can provide extended immunity to that of the existing vaccines, in essence making VLPs complementary to attenuated vaccines instead of an alternative thereof (Jere et al. 2014; Shoja et al. 2013; Li et al. 2014; Petitpas et al. 1998; Azevedo et al., 2010).

1.3.6 Rotavirus SA11

Simian agent 11 (SA11), was first isolated by Dr Hubert Malherbe in 1958 from a rectal swab of an overtly healthy vervet monkey (*Cercopithecus aethiops pygerythrus*) at the National Institute of Virology (Johannesburg, South Africa). SA11 is the first rotavirus ever described (Malherbe & Harwin, 1963; Malherbe & Strickland-Cholmley, 1967).

SA11 was selected for this project as it propagates very well in cell culture, has never caused any form of diarrhoea (or any other RV associated pathology) and is universally viewed as a prototype strain for RV research pertaining to viral replication and genome segment function (Estes *et al.*, 1979b; Matthijnssens *et al.*, 2010; Small *et al.*, 2007). The specific strain selected for this project is RVA/Simian-tc/ZAF/SA11-H96/1958-N5/G3P5B[2]. Its genotype is G3-P[2]-I2-R2-C5-M5-A5-N5-T5-E2-H5 (Mlera *et al.*, 2013)

1.4 Overview of RG systems for the *Reoviridae* family

1.4.1 Reovirus RG

The first member of the *Reoviridae* family to be rescued from a RG system was reovirus (Roner *et al.*, 1990). The reovirus RG system entailed the transfection of either ssRNA, dsRNA or a mixture of the two in association with a reticulocyte lysate in which ssRNA or dsRNA had been translated. Reovirus strain ST3 was selected for this experiment and transfections were done in murine fibroblast cells (L929). After transfection, a helper-virus (reovirus ST2 which propagated much slower than the ST3 strain) was used to infect the L929 cells and viruses were harvested after 24 to 48 hours. The recovered viruses were then passaged and as expected the ST3 strain formed plaques after roughly 5 days whereas the helper-virus ST2 strain only formed plaques after 12 days. This early system was helper-virus based and required a selection system and had very low efficiency, roughly 4% of transfected cells yielded recombinant virus (Roner *et al.*, 1990). This system was however used to incorporate on cDNA level a CAT reporter gene into the viral genome (Roner & Joklik, 2001).

As with most RG systems various attempts were made to either increase the sensitivity of the selection system or to entirely remove it through the development of a plasmid-based system. This was achieved in 2007 when plasmids carrying viral cDNA were constructed for each of the viral genome segments flanked by a T7 polymerase promoter at the 5` end and a hepatitis D virus (HDV) ribozyme (Rib) at the 3'-end (Kobayashi *et al.*, 2007). The use of the T7 promoter and the HDV Rib ensured that the mRNA transcripts of the plasmids carrying viral cDNA produced exact 5`- and 3` termini mimicking that of the actual viral transcripts. The viral rescue was achieved through transfection of plasmids into L929 cells followed by infection with a recombinant vaccinia virus (rDIs-T7pol) which provided the T7 polymerase for transcription and capping of reovirus transcripts. Recombinant reovirus was recovered after 5 days. The system was later optimized by incorporating multiple genome segments into single plasmids (Figure 10).

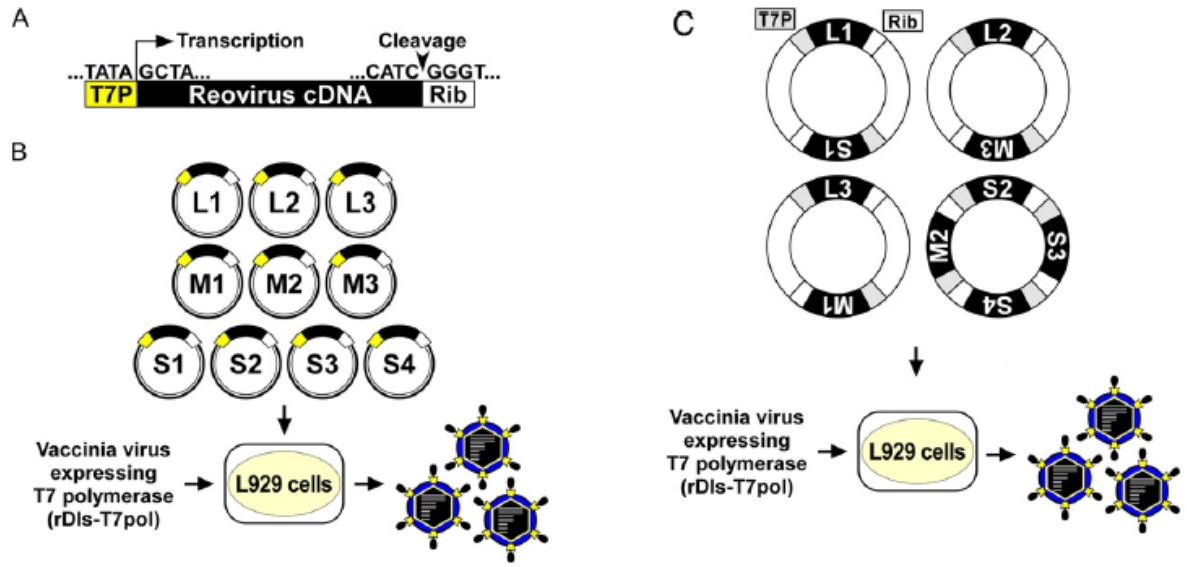

Figure 10: Illustration of reovirus RG setup and rational. A: Illustration of the layout of the cDNA carried in the transfection plasmid. Reovirus cDNA (in black) is flanked by the T7 polymerase promoter (in yellow) on the 5` end and by the HDV rib (in white) at the 3` end. **B**: Illustration of the 10 cDNA carrying plasmids representing the total viral genome being transfected into L929 cells expressing the T7 polymerase (derived from infection with recombinant VV-helper-virus), yielding recombinant reovirus. **C**: illustration of the improved reovirus RG system where multiple genome segments are carried in single plasmids. Image taken from (Matthijnssens et al., 2008c) with permission.

Although reovirus rescue has been achieved, the RG system has of yet not been widely applied for research or commercial purposes, indicating that it is technically difficult and requires optimization or further development. Boehme and associates

attempted to implement and recreate the system in 2011 and failed to rescue. According to the author, technical problems such as plasmid purity and concentration, transfection efficiency and T7 polymerase activity are most likely the cause of their failure, however, the occurrence of lethal mutations within the system cannot be ruled out (Boehme *et al.*, 2011). This illustrates the difficulty in the rescue of members of the *Reoviridae* family and underlies most of the technical challenges that RG systems for this family present.

1.4.2 Orbivirus RG

Another very important genus in the *Reoviridae* family that has been rescued with various RG system is the Orbiviruses, of which bluetongue virus (BTV) and African horsesickness virus (AHSV) will be discussed here. These RG systems also played a significant role in this project as the strategies used during their implementation and optimization were also used during the implementation and optimization of both the Japanese pT7_SA11-L2 RV RG system and the locally developed pSmart_SA11-N5 RV RG system. Both the BTV and AHSV RG systems followed a similar development and optimization approach, starting with transfections of viral-core-derived transcripts, then moving on to run-off transcripts synthesised from cDNA plasmids, to expression plasmids along with run-off transcripts and culminating in the development of plasmid-only RG systems. The research findings and applications of both BTV and AHSV RG systems have been very important and have led to the elucidation of several key viral replication and virulence factors and will have an economic impact in terms of the development of various, rationally designed, recombinant vaccine candidates.

1.4.2.1 BTV RG development and overview

BTV was first rescued in 2007 through the transfection of (+)ssRNA derived from transcriptionally active viral core particles (Boyce & Roy, 2007). This initial rescue proved that BTV can be produced from transfections of viral (+)ssRNA transcripts alone, and laid the foundations for rapid development and optimization of various BTV RG systems. In 2008 Boyce and associates rescued recombinant BTV containing both genome segments from serotype 1 and serotype 9 through transfection of core derived (+)ssRNA of both serotypes into BSR cells. This not only illustrated the capacity for recombinant viral engineering but also suggested that a similar approach could be used for the development of multi-serotype vaccine candidates. In the same year, transcription plasmids were constructed with cDNA copies of the entire viral genome under the control of a T7 viral RNA polymerase promoter (Boyce *et al.*, 2008a).

These plasmids were then used to generate *in vitro* transcripts, using a T7 viral RNA polymerase, which was used to successfully rescue recombinant BTV. This laid the foundations for further work in the field and resulted in the rescue of both virulent and non-virulent field strains of BTV (van Gennip *et al.*, 2012b). It was also reported that BTV can be rescued from *in vitro* reconstituted BTV sub-cores in a cell-free reverse genetics system (Lourenco & Roy, 2011). Further optimizations to the BTV RG system included the use of a two-step transfection protocol which entailed the transfection of expression plasmids, which express the viral replication complex (VP1 and NS2, and VP3 if the transcripts were not capped prior to transfection), followed by transfection of the 10 capped genome transcripts 18 hours later.

The initial transfection allows protein expression and initiates viral production and the second transfection is thought to provide transcripts for viral packaging and maturation (Boyce *et al.*, 2008b; Matsuo *et al.*, 2010; van Gennip *et al.*, 2012a). Following the success of the BTV transcript-based RG systems, a plasmid only RG approach was developed. This was achieved through the construction of transcription plasmids that flanked cDNA copies of the BTV genome segments with a 5`- T7 viral polymerase promoter and a 3`- HDV ribozyme (Figure 11).

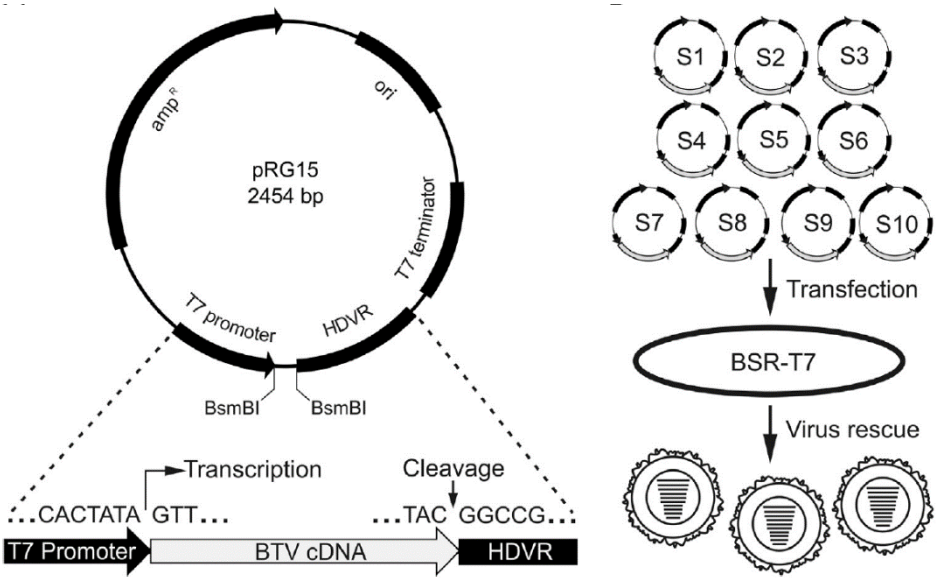

Figure 11: Illustration of the construction and execution of the BTV plasmid only RG system. The 10 transcription plasmids (left) were designed by incorporating a cDNA copy of each viral genome segment into the pRG15 plasmid (right) flanked by a 5`- T7 promoter and a 3`- HDV ribozyme. The plasmids are pooled and transfected into BSR-T7 cells. Recombinant virus is rescued after 3-5 days incubation. Image taken from with permission.

The plasmid only BTV RG system comprised of 10 transcription cassettes (similar to those used in the *in vitro* transcription model), each representing a single viral genome segment, and were transfected into BSR cells expressing the viral T7 polymerase. Recombinant BTV virus was rescued after 3-5 days from this system.

1.4.2.2 AHSV RG development and overview

AHSV was first rescued in 2010 through the transfection of (+)ssRNA derived from transcriptionally active viral core particles (Matsuo *et al.*, 2010) similar to that of the first BTV RG system. This was also quickly followed by the construction of transcription plasmids carrying cDNA copies of the 10 AHSV genome segments under the control of a T7-RNA polymerase promoter. Similar to the progression of the BTV RG systems, these transcription cassettes were used for the *in vitro* synthesis of run-off transcripts that were capped and transfected into BSR cells. Within the AHSV RG system, the two-step transfection approach significantly increased viral yield.

It was also shown that although AHSV could be rescued from non-capped transcripts, the viral yield was significantly increased once the transcripts were capped (Conradie *et al.*, 2016; van de Water *et al.*, 2015). This was one of the optimizations used during the implementation of the Japanese pT7_SA11-L2 RV RG and locally developed pSmart_SA11-N5 RV RG systems. Another key element of the BTV and AHSV RG systems that were incorporated into this project was the use of equi-molar amounts of transfection plasmids. AHSV RG systems also moved to a more plasmid-based approach over time. This started with the transfection of expression plasmids encoding the viral replication complex (VP1, VP3 and NS2, as well as VP4, VP6 and VP7 if transcripts were not capped prior to transfection), followed by the transfection of the 10 capped genome transcripts 18 hours later. Over time this model developed into a plasmid only version where expression plasmids were transfected first followed by transcription plasmids 18 hours later (Conradie *et al.*, 2016; van de Water *et al.*, 2015; van Rijn *et al.*, 2016). These transcription plasmids were designed similarly to those used in the BTV plasmid only RG system and entailed the cDNA copy of the AHSV genome segment being flanked by a 5`- viral T7 RNA polymerase promoter and a -3` HDV ribozyme, ensuring precise 5`- and -3` transcript termini (Figure 12).

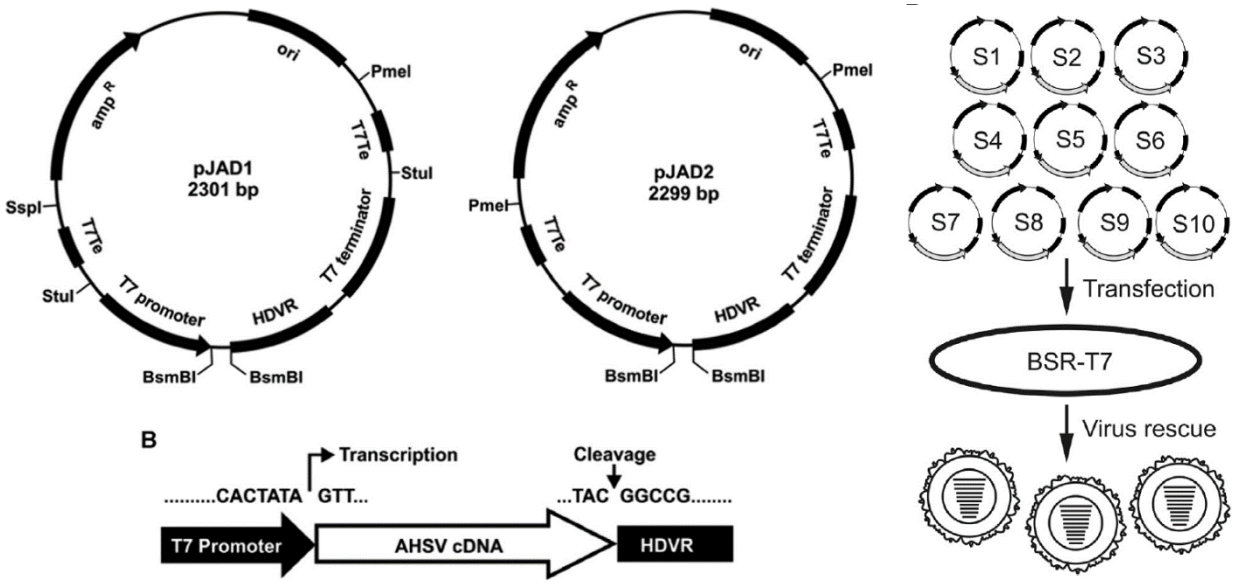

Figure 12: Illustration of the construction and execution of the AHSV plasmid only RG system. The 10 transcription plasmids (left) were designed by incorporating a cDNA copy of each viral genome segment into the pJAD1 and pJAD2 plasmids (right) flanked by a 5`- T7 promoter and a 3`- HDV ribozyme. The plasmids are pooled and transfected into BSR-T7 cells. Recombinant virus is rescued after 3-5 days incubation. Image taken from with permission

1.4.2.3 Orbivirus RG findings and applications

Both the AHSV and BTV RG systems have significant commercial applications. The most important research findings thus far are the elucidation of the function of NS3 and NS4 in both BTV and AHSV, the generation of Disabled Infectious Single Cycle/Cell (DISC) and Disabled Infectious Single Animal (DISA) vaccine candidates for both BTV and AHSV, the generation of multi-serotype (transcapsidation) vaccine candidates and the development of diagnostic strategies for the detection of variations between WT BTV and AHSV infection and the immune response to their respective recombinant vaccines.

For both AHSV and BTV the non-structural proteins NS3 and NS4 have long been suspected to be associated with virulence, however, their precise functions remained unknown until the development of the AHSV and BTV RG systems. It has now been shown that NS3 is required for viral egress from insect cells (during the midge vector part of the viral life cycle) and is cytotoxic in mammalian cells. NS3 also suppresses the interferon response in mammalian cells and is a key target in the development of DISA vaccines. DISA vaccines are rationally attenuated, recombinant vaccines that lack the NS3 genome segment and as such cannot be spread from mammalian hosts via insect vectors, as viral egress is inhibited (Feenstra *et al.*, 2014; Feenstra *et al.*, 2015; Ruiz *et al.*, 2007; van de Water *et al.*, 2015; van Rijn *et al.*, 2017; van Rijn *et al.*, 2018a; van Rijn *et al.*, 2018b).

NS4 has also been shown to interfere with the mammalian immune response and is a very important virulence factor. Recombinant BTV and AHSV lacking NS4 have been shown to elicit a strong immune response without the development of severe disease symptoms. Additionally, DISC vaccines are recombinant BTV and AHSV strains that lack the essential VP6 genome segment and can thus not replicate within the host. DISC vaccines are viewed as save vaccines that can elicit an immune response but cannot replicate within the host. DISC vaccines are produced by using a RG system that lacks the VP6 genome segment (that also contains the NS4 ORF), transfected into specialized cell-lines that express the required VP6 protein. This produces a recombinant infectious virus that cannot replicate (van Gennip *et al.*, 2012a; van Rijn *et al.*, 2016).

BTV and AHSV RG systems have also been used to generate multi-serotype vaccines by using a universal core particle and exchanging the VP2 from different serotypes. For AHSV all 9 serotypes can be rescued with a single universal core, whereas BTV requires the use of two core particles to facilitate the expression of every serotype (Conradie *et al.*, 2016; Potgieter *et al.*, 2003; van de Water *et al.*, 2015). Another very important research output and practical application of the BTV and AHSV RG systems was the development of diagnostic tests to distinguish between the presence of WT virus and vaccine virus in the blood and bodily fluids. In summary, the development of RG systems for BTV and AHSV have already provided much insight into viral replication, virus-host cell interactions and the correlation between protein structure and serotype-specific immunogenicity (Bishop, 2016). It can be safely assumed that the optimization of a robust helper-virus independent RV RG system will do the same.

1.4.3 Rotavirus RG

1.4.3.1 Helper-virus based RV RGs

The first RV RG systems (Figure 13) was mainly based on the ability of the viral genome segments to undergo reassortment (Komoto & Taniguchi, 2006). These early RG systems for RV primarily focused on single-segment replacement methods relying on infection of an appropriate cell-line with a helper virus that carries a specific selection property and the transfection of a plasmid carrying a cDNA copy of the recombinant genome segment. These systems mainly utilized RV SA11 as backbone due to its non-pathogenic nature, or RRV due to the wide range of temperature-sensitive mutants that are available (Johne *et al.*, 2015; Mansell *et al.*, 1994; Ramig & Gombold, 1991; Trask *et al.*, 2010). The majority of the early studies also focused on the exchange of the outer capsid protein VP4 as it elicits neutralizing antibodies. For RV RGs the efficiency of recovery is almost entirely dependent on the strength of the selection system used to isolate the single-segment recombinant RV from the unmodified helper virus population (Trask *et al.*, 2013).

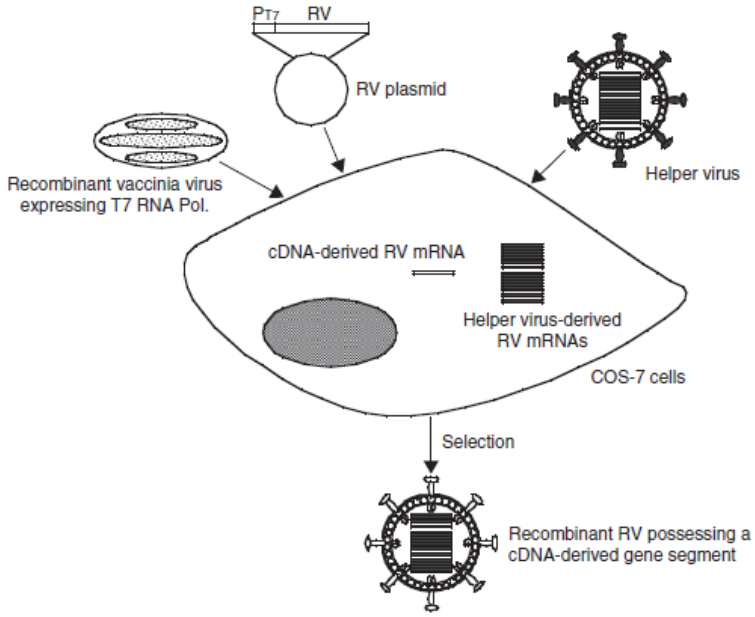

Figure 13: Illustration of the simplified helper-virus based RV RG system. The system entails the transfection of COS-7 cells with a transcription plasmid containing the selected recombinant RV cDNA genome segment under the regulation of a 5`-T7 promoter. The cells are then infected with a recombinant VV expressing the T7 RNA polymerase. This is then followed by infection of the cells with the helper virus. Following incubation and reassortment, recombinant viruses are screened and propagated further. Image taken from (Ramig, 1997) with permission.

As previously mentioned, the RV RG system relies on the T7-mediated transcription of cDNA containing plasmids followed by either antibody-, short interfering RNA (siRNA)- or temperature-sensitive mutation-based selection systems (Figure 14) for the selection of reassortants (Komoto & Taniguchi, 2006; Trask *et al.*, 2010). A recent adaptation of the temperature-sensitive selection technique was published by Johne *et al*. (2015) and entails the plasmid-based replacement of RNA genome segment 4 of the SA11 strain with that of an avian RV strain. The technique requires the transfection of a plasmid carrying the cDNA RV genome segment 4 (of the avian strain) into BSR-T5/7 cells. The BSR-T5/7 cells were selected due to their relative ease of transfection and their expression of the T7 polymerase (required for the expression of the previously mentioned recombinant plasmid under the regulation of the T7 promoter). The transfected cells were then infected with a temperature-sensitive RV SA11 strain (tsSA11) and allowed to proliferate. Rescued viruses (a mixture of reassortants and the original tsSA11) were then transferred to MA104 cells for temperature selection and recovery since reassorted viruses would be more temperature tolerant than the original unmodified tsSA11 helper virus (Johne *et al.*, 2015).

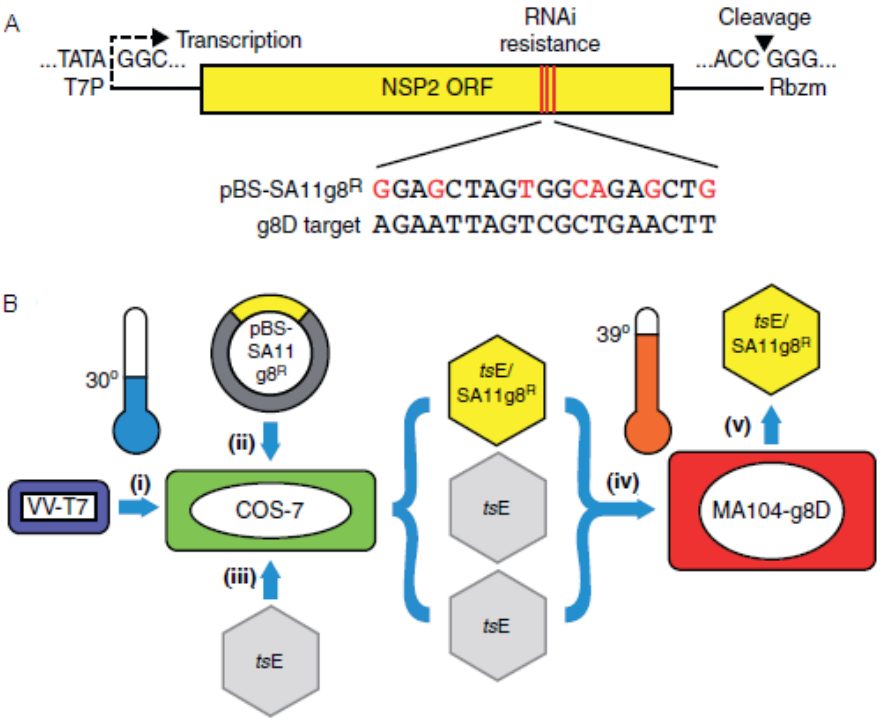

Figure 14: Graphic representation of various helper-virus based RV RG selection systems. A: RNAi selection system. During this type of selection, the helper virus contains an antagonist sequence in the gene of interest that is not present in the recombinant version. Following reassortment, the viral population is passaged and treated with interfering RNA specific to the unaltered helper-virus, thus selecting for recombinant strains. **B**: Temperature selection system. During this type of selection, the helper-virus selected contains mutations that inhibit its growth at 39°C. Following reassortment the viral population is passaged and grown at the higher temperature, thus selecting recombinant viruses. Image taken from with permission.

1.4.3.2 Transcript-based RV RGs

Before the advent of any helper-virus based RV RG systems, an attempt was made at rescuing recombinant RV based on the initial approaches of the BTV and AHSV RG systems, namely the generation of transcripts from viral cores (Chen *et al.*, 1999; Patton, 1986; Patton & Chen, 1999; Stacy-Phipps & Patton, 1987; van Rijn *et al.*, 2016). It was shown that RV transcripts can be synthesised *in vitro* if isolated RV particles are un-coated (outer capsid proteins VP4 and VP7 are removed) to expose the DLP. Early work was aimed at isolation of these transcripts followed by transfection into appropriate cells along with a transcript of a recombinant RV genome segment. This recombinant GS contained specific restriction sites not present in the core-derived transcripts, which allowed for screening of recombinant RV after reassortment.

This approach did yield successful reassortment, however, the technique was not fully implemented due to limitations in the screening process and the lack of an adequate selection system. The premise of the transcript based model remained a viable RV RG model, however, and was later attempted at various institutions (Potgieter, A.C. Deltamune. Personal communication), including the NWU. The development and implementation of a robust RV RG system have long been a goal of our research group at the NWU. In the past several attempts have been made to generate both a transcript based and plasmid-based RG system, and although significant insights have been gained through this process, no fully functional RV RG was obtained.

These systems were based on the work of Dr L. Mlera (Mlera, 2012), and Dr JF. Wentzel (Wentzel, 2014). In his PhD, Dr Mlera performed whole genome amplification and 454 pyrosequencing of the prototype DS1-like RV strain and derived the consensus sequence thereof. Dr Mlera did the same for the African SA11 strain, which is used throughout this project, and performed a molecular clock phylogenetic analysis thereon. Dr Mlera not only determined the SA11 consensus sequence but also characterized the sample obtained from the Diarrhoeal Pathogens Research Unit (DPRU), University of Limpopo (Pretoria, South Africa) and determined that the SA11 was the product of a mixed infection of the SA11-N5 and a bovine “O-agent” RV strains (Mlera *et al.*, 2013). Over time, and consecutive passages, the O-agent reassorted with the SA11-N5 strain to produce a small population of SA11-N2 reassortants. The SA11-N2 strain outcompeted the predominant SA11-N5 strain over time as evaluated by various whole genome sequencing experiments on the viral culture. Dr Mlera also designed a transcript based RV RG system that relied on the transfection of *in vitro* generated transcripts. Although the system could not rescue recombinant virus, it did illustrate the innate immune response elicited by RV transcripts, which heavily impacted later work on RV RG at the NWU.

For his PhD, Dr Wentzel developed a more plasmid-based approach to the RV RG system, designing four multi-genome segment encoding plasmids: **1**) pAlpha held GS1 (VP1), GS8 (NSP2) and GS11 (NSP5/6), **2**) pBeta held GS2 (VP2) and GS3 (VP3), **3**) pDelta (held GS7 (NSP3), GS10 (NSP4) and GS9 (VP7), and finally, **4**) pGamma held GS4 (VP4), GS6 (VP6) and GS5 (NSP1), Figure 51. These transcription plasmids (Appendix C) carried the consensus sequence cDNA of the SA11-N5 genome segments each flanked by a 5`- viral T7-RNA polymerase promoter and a -3` HDV

ribozyme. He used these plasmids to generate viral mRNA with exact 5`- and -3` termini which would be used in conjunction with several expression plasmids to attempt RV rescue. The four transcription plasmids were used for the construction of the pSmart_SA11-N5 RV RG rescue plasmid set by L. Geldenhuys and myself.

The expression plasmids encoded the ORFs of the viral replication complex (VP1, VP2, VP3, VP6, NSP2 and NSP5), codon-optimized for expression in MA104 cells. Similar to the AHSV RG system, these expression plasmids would be transfected first to initiate the formation of the viroplasm and viral replication complexes, followed by a later transfection of the viral mRNA transcripts for viral genome packaging and replication. Although many valuable insights were gain from Dr Wentzel's project, a coding error in his initial plasmid constructs resulted in the formation of incorrect non-coding regions in the viral mRNAs 5`- termini, which made viral genome packaging impossible. The 5`-GGGN sequence, although very beneficial for transcription, could not be packaged, and resulted in failed viral rescue and propagation. Later attempts at the NWU were aimed at correcting the 5`- sequence to the consensus 5`-GGN version and reattempting the transcription model approach (Section 3.1). He did, however, show that viral mRNA can be transfected into cells and could be expressed to generate viral proteins and that precise 5`- and 3`- termini are essential for viral genome packaging and viral replication (Wentzel, 2014).

1.4.3.3 Plasmid-based RV RGs

The long-awaited goal of a true selection-free, RV RG system which we and others globally worked towards has recently been achieved. The lack thereof was the main bottleneck of major progress in important basic and applied research on rotaviruses the past two decades. During early 2017, a Japanese group published their results that they succeeded in developing a plasmid-based reverse genetics system that is free from helper viruses and independent of any selection for the rotavirus simian agent 11, strain L2 (Kanai *et al.*, 2017). Their success was based on a very similar approach that our research group at the NWU were following for SA11-N5, but they incorporated two additional enzymes which significantly increased the efficiency of rescue. This is undoubtedly the most significant breakthrough in the field in the past

few decades. Although the introduction of the commercial vaccines, RotaTeq and Rotarix, in 2006 has reduced RV infections and mortality rates globally, their efficiency in developing countries such as South Africa has been shown to be much lower than countries such as Europe and USA (Madhi *et al.*, 2010). It is postulated that reassortment and interspecies transmission (zoonosis) along with the differences in the circulating field strains of Africa in comparison to that in Europe and America might explain the reduced efficiency of the vaccine. It is for this reason that the advent of the plasmid only RG system is so important. This could allow the generation of rationally designed, regionally specific RV vaccine candidates and answer some of the most burning questions pertaining to RV replication and pathogenesis.

The first fully plasmid-based RV RG system (pT7_SA11-L2) is illustrated in Figure 15 below. The system comprises of 14 plasmids, 11 of which carry cDNA copies of the RV genome segments, 2 of which encode the VV capping enzyme sub-units and the last encoding the NBV FAST protein (Section 2.1). The 11 cDNA RV genome segments are carried in transcription plasmids designed to flank each GS with a 5`-viral T7-promoter, and a 3`-HDV ribozyme. These additions to the GS ensured precise transcription and the generation of exact 5`- and 3`- ends, something that is known to be essential for viral genome packaging and replication. For rescue, the plasmids are extracted and mixed together in equi-ug amounts and transfected into BHK-T7 cells. The transfected cells are then incubated and co-seeded with MA104 cells to facilitate an increase in titer for any rescued virus. The co-seeded culture is incubated until CPE is visible, after which the culture is lysed and passaged for visualization. The initial pT7_SA11-L2 RV RG system was made available to the scientific community through the AddGene service and was acquired and implemented at various institutions across the globe. This system was then further developed through the incorporation of an eGFP flag system for quick visualization of viral rescue (Kanai *et al.*, 2017).

Shortly after the publication of the Kanai RV RG system another Japanese (Komoto *et al.*, 2017) group adapted the model and published their findings pertaining to the replication of RV in cell culture. This was one of the first practical applications of the plasmid only RV RG system and illustrated its potential for furthering RV research. Their findings suggested that NSP6 is non-essential for RV replication in cell culture.

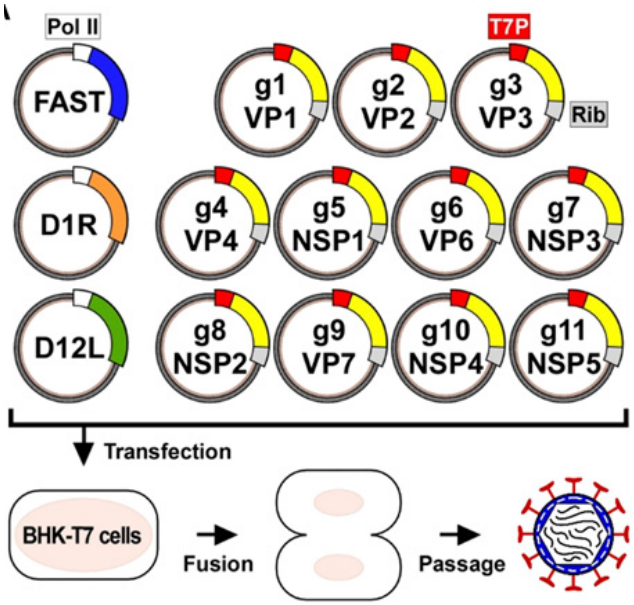

Figure 15: Illustration of the basic rescue procedure of the pT7_SA11-L2 RV RG system. Equi-ug amounts of the 14 rescue plasmids are mixed together and transfected into BHK cells expressing the viral T7 RNA polymerase. NSB FAST protein expression results in cellular fusion. Cell lysates are passaged after which rescued virus is visualized. Image adapted from (Matthijnssens et al., 2011) with permission.

Later the same group further developed the initial pT7_SA11-L2 RV RG system by reducing the size of the plasmid backbone and increasing the relative amounts of viroplasm forming genome segments NSP2 and NSP5. The primary co-seeding and propagation cell lines were also changed from MA104 to CV1 cells (Figure 16). These adaptations significantly improved viral recovery and also showed that the VV capping enzyme and NBV FAST protein was non-essential for viral rescue (Komoto *et al.*, 2018). Later the same group used the optimized system to incorporate a full-length GFP reporter into the ORF of the NSP1 gene segment (Komoto *et al.*, 2018). The NSP1 protein was supplied in these experiments through the incorporation of a NSP1 expression plasmid containing only the NSP1 ORF (no UTRs).

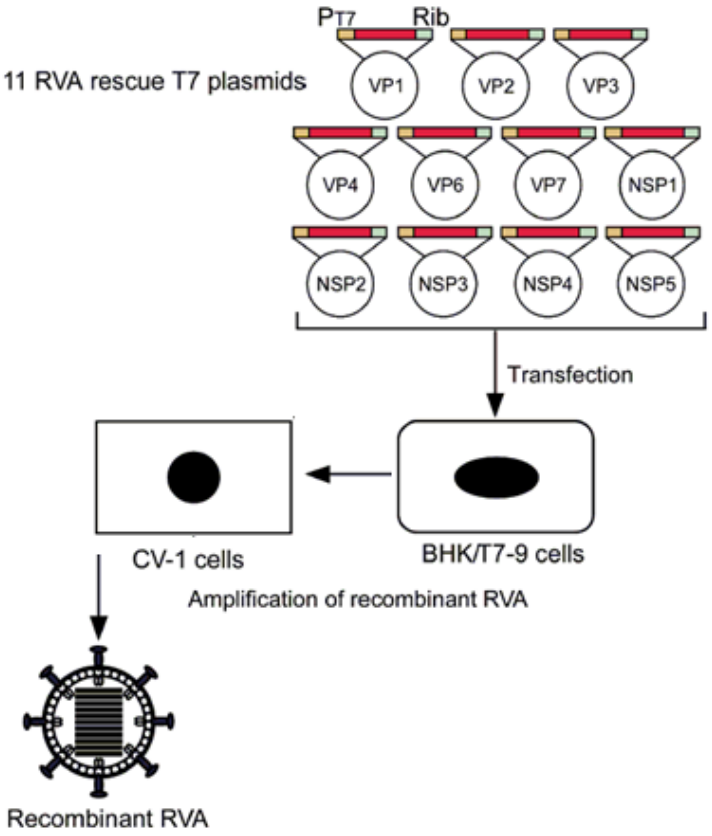

Figure 16: Illustration of the Komoto-optimized pT7_SA11-L2 RV RG system rescue procedure. Equi-ug amounts of the 11 rescue plasmids (with 3x more NSP2 and NSP5) are mixed together and transfected into BHK cells expressing the viral T7 RNA polymerase. Cell lysates are passaged onto CV-1 cells, after which rescued virus is visualized. Image adapted from (Matthijnssens et al., 2012) with permission.

The main focuses of the major research entities using RV RG currently are the elucidation of viral packaging and pathogenesis mechanisms as well as the development of human chimeric strains. To date no published human chimaera system has been developed that utilizes the SA11 DLP, however, there are several successful recombinations with the SA11 TLP that carries a single VP4 or VP7 reassortment. The Komoto research group also developed a human-SA11 hybrid (Komoto *et al.,* 2019) that had a KU TLP with a SA11 VP4 (Figure 17).

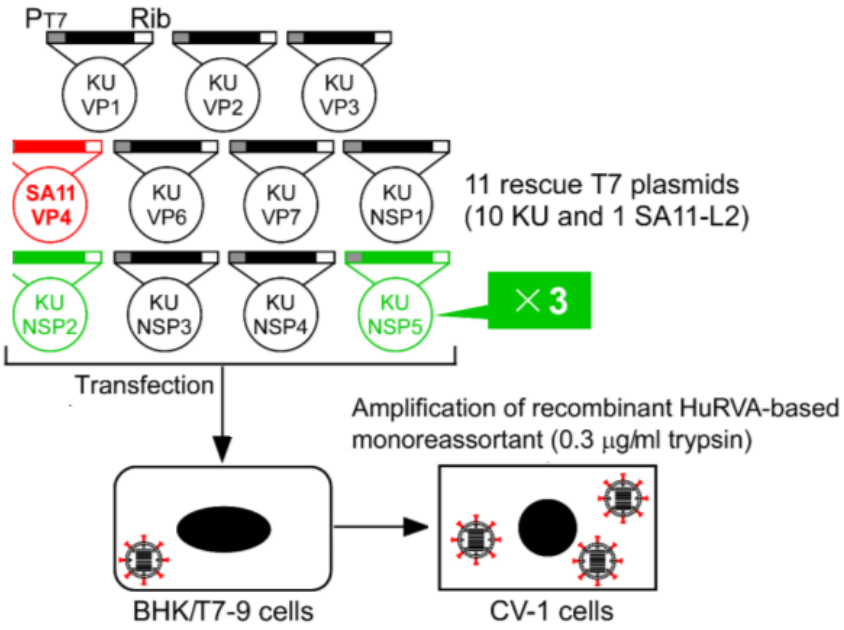

Figure 17: Illustration of the simplified rescue protocol for the KU-SA11 chimera RV RG system. Equi-ug amounts of the 10 KU rescue plasmids (with 3x more NSP2 and NSP5) are mixed together with an equi-ug amount of the SA11-VP4 rescue plasmid, and transfected into BHK cells expressing the viral T7 RNA polymerase. Cell lysates are passaged onto CV-1 cells, after which rescued virus is visualized. Image adapted from (Komoto et al., 2019) with permission.

1.4.3.4 Anticipated outcomes of a fully established, traceable, helper-virus independent RV RG system

The major bottleneck in terms of RV genetic research has untill recently been the lack of a robust, traceable, helper-virus independent RG system. Although RV has been extensively studied in vitro, many elements pertaining to its replication cycle are still unclear. It is the general consensus that a robust, helper-virus independent RG system for RV will aid in the elucidation of several key mechanisms that initiate, regulate and guide interactions between the virus and the host cell (Desselberger, 2014). The following is a list of subjects proposed by Desselberger in 2014 that are of particular note in terms of RV research, and questions that can potentially be answered through the guided application of the newly developed plasmid-only RV RG systems:

- The precise structure, orientation and interaction of dsRNA within the RV core particle, with a specific focus on RNA-RNA interactions and the interactions between dsRNA and VP2.

- Identification and characterization of the different receptors for various RV strains, as well as the mechanisms used by various strains during infection that add to strain specificity.
- The precise mechanism used during viroplasm formation for lipid droplet recruitment as well as the regulatory elements thereof.
- The regulatory mechanisms controlling assortment and reassortment, as well as the elements that limit reassortment of the 11 dsRNA segments during viral replication.
- Details pertaining to RNA-RNA interactions during viral genome packaging as well as RNA-protein interactions.
- Details pertaining to the formation and loss of the temporary enveloped RV particles in the ER during viral maturation, with a specific focus on the mechanisms involved in signalling and transportation.
- Development of intestinal organoid cell cultures to study and evaluate RV replication in a more native/natural environment.
- The elements that determine RV pathogenicity and virulence, with a specific focus on acquired mutations that increase or decrease host range and disease progression.
- Elucidation of the molecular basis that determines host range and more specifically organ- or cell-specific viral replication.
- The exact role that the various immune responses (innate, cellular and humoral) play in establishing protection against RV infection and disease.
- Elucidation of the precise correlates of protection against RV infection and disease.
- The factors determining and regulating the spread of RV through various communities and populations.
- The reasons for the apparent decreased efficiency of commercial RV vaccines in developing countries and areas of low socioeconomic status.
- The potential impact of universal mass vaccination against RV disease on the evolution and diversification of RV strains.
- The potential for the development of rationally attenuated or non-living RV vaccine candidates.

- The identification of various stages during viral replication that can be targeted by viral therapeutics, as well as the identification of various small molecules that can act as antiviral agents.

1.5 Problem identification

Rotavirus reverse genetics offers a powerful new way to research RV replication and pathogenesis, the applications of which can be significant and far-reaching. The first workable RV RG is now available, but its effectiveness and robustness still needs to be improved.

1.6 Aims and Objectives

This project entails the finalization, implementation and optimization of a plasmid-only pSmart_SA11-N5 RV RG system based on the Kanai (2017) plasmid-only pT7_SA11-L2 RV RG system but with several key adaptations.

Elements unique to this system include but are not limited to, the use of rotavirus-SA11 consensus sequence genome segments, codon optimization for increased expression in cell culture and the incorporation of various elements of both the BTV and AHSV RG systems (van Gennip *et al.*, 2012a; van Rijn *et al.*, 2016) such as increased amounts of capping enzymes and the use of equi-molar transfection mixtures

This pSmart_SA11-N5 RV RG platform will be established alongside that of the initial pT7_SA11-L2 RV RG system and compared throughout the development and implementation process (allowing a comparative analysis of both techniques).

The primary objectives of the project were:

1) To acquire and implement the Japanese, plasmid-only, pT7_SA11-L2 RV RG system at the NWU, and to increase the efficiency thereof through the incorporation of insights from the AHSV and BTV RG systems.

2) To finalize plasmid construction and implement the locally designed, plasmid-only, consensus sequence-based, pSmart_SA11-N5 RV RG system with all optimizations used throughout the implementation and optimization of the Japanese SA11-L2 RV RG system.

3) To compare the various optimizations of the Japanese pT7_SA11-L2 and locally developed pSmart_SA11-N5 RV RG systems throughout the project via $TCID_{50}$ assay.

Chapter 2

Local implementation and optimization of plasmid only pT7_SA11-L2 RV RG system

2.1 Introduction

The pT7_SA11-L2 RV RG system was developed in 2017 by a Japanese group (Kanai *et al.*, 2017), and made available to the scientific community through the AddGene service. The system, comprising of 14 plasmids, was acquired by various institutions across the globe, including the NWU. In this chapter I will be presenting my findings in terms of the implementation and rationally guided optimization of the pT7_SA11-L2 RV RG system, outlining the approaches utilized and the rationale behind each.

Initial attempts to implement the Japanese pT7_SA11-L2 RV RG system to obtain the results originally published by the group proved to be more difficult than anticipated. Although rescue was possible, repeatability was a major concern. Through communication with other institutions that were also in the process of implementing the system, it became apparent that we were not the only group that was struggling to implement the original pT7_SA11-L2 RV RG system (Prof. A.C. Potgieter, Deltamune, personal communication). This illustrated the need for further development and optimization of the Japanese, plasmid-based RV RG approach.

My approach was to improve the system through the incorporation of techniques and strategies relating to two other dsRNA RG systems, namely the AHSV and BTV RG systems (Section 1.4). I also designed and incorporated several alternative expression plasmids encoding different capping and fusion proteins into the system, and incorporated newly published findings pertaining to this RG system throughout its implementation (Komoto *et al.*, 2018). This accumulative approach was successful and allowed me to significantly optimize both the repeatability and overall efficiency of the system in terms of the rescued virus yield.

I used the implementation and optimization of the pT7_SA11-L2 RG system for testing and to familiarize myself fully with the techniques involved in the RG system, for the later implementation of our own locally developed, consensus sequence-based, pSmart_SA11-N5 RV RG system and preformed comparative analyses throughout the entire process (Chapter 3).

2.2 Materials and Methods

2.2.1 Transformation of chemically competent cells

The protocol used for the transformation of chemically competent cells during this project is based on the original calcium-based DNA transformation protocol published in 1970 (Mandel & Higa, 1970). The underlying biochemical principles of the technique rely on the treatment of bacterial cells with calcium ions (Ca^{2+}) to neutralise charges on the cell surface, eliminating electrostatic repulsion and allowing binding of foreign DNA to the cell membrane. The cell-DNA mixture is then exposed to an increase in temperature that facilitates the formation of pores in the cell membrane and creates a pressure differential that results in the cell taking up foreign DNA in its immediate surroundings. The temperature is then lowered so that the cell membranes can stabilize and the cells are incubated in media that is selective for transformed cells (Sambrook & Russell, 2001).

For this project, chemically competent DH5α (Novagen, Merck Biosciences, Darmstadt, Germany) and Stellar cells (Takara Bio, Separations, Johannesburg, RSA) were purchased and transformed using the heat-shock protocol. Cells were thawed on wet ice for roughly 15min after which 50ul was transferred to a pre-chilled 15ml round bottom Falcon tube. Between 1ng and 100ng of plasmid DNA was added to the cells keeping the volume of DNA less than 5% of the volume of the cells. The cells and DNA were gently mixed through swirling and incubated on ice for 30min. This was followed by heat shock for 45sec in a pre-heated water bath at 42°C. Directly after heat shock, the cells were incubated on ice for 2min after which 950ul of pre-warmed recovery medium was added.

The transformation mixture was then incubated for 60min at 37°C in a shaking incubator at 250rpm. Finally, 50ul of the transformation mixture was spread out onto pre-warmed nutrient agar plates containing the appropriate antibiotic selection and incubated at 37°C overnight.

2.2.2 Preparation of bacterial glycerol stocks

The preparation of glycerol stocks allows the long-term storage of bacterial samples at extremely low temperatures (-80°C). The biochemical principles that underlie this practice is called cryo-protection and is based on glycerol's membrane permeability and its ability to prevent cell shrinkage, prevent ice crystal formation and its ability to regulate isotonic pressure (Sambrook & Russell, 2001). For this project glycerol stocks were prepared from overnight bacterial cultures to which glycerol was added to a final concentration of 15%. The samples were then labelled, frozen and stored at -80°C.

2.2.3 Colony selection and master-plate preparation

Following transformation and plating, bacterial colonies were evaluated, isolated and transferred to a master-plate for further use. The basic premise of plating is that the bacterial culture is diluted and spread over a large surface area to isolate individual bacterial cells (Sambrook & Russell, 2001). During overnight incubation, these individual cells will replicate and form mono-clonal colonies that can be propagated for further use. During colony selection, it is important to identify and avoid non-desirable colonies such as satellite colonies, colonies arising from clumps of cells or cells that were not properly separate during plating. Once a properly isolated monoclonal colony was selected it was aseptically transferred to a pre-labelled agar master-plate containing the appropriate antibiotic selection for the desired experiment. The plate was then incubated overnight and then stored at 4°C until needed.

2.2.4 Miniprep plasmid extraction

In this project, the QIAprep Spin Miniprep kit (Qiagen, Whitehead Scientific, Johannesburg, RSA) was used for all plasmid extractions except for when endotoxin-free plasmid extraction was specifically required. The kit is designed for the small-scale isolation of plasmid DNA from bacterial cultures using a simple bind-wash-elute method. The biochemical principles underlying this technique are the alkaline denaturing of high molecular weight chromosomal DNA (Birnboim & Doly, 1979) and the binding of DNA to a silica membrane in the presence of a high salt concentration (Sambrook & Russell, 2001). Very briefly, the technique takes advantage of the very narrow range of pH (between 12.0 and 12.5) where chromosomal DNA is denatured but closed-circular plasmid DNA remains double-stranded (ds). This high pH is achieved through the addition of NaOH and is neutralised through the addition of sodium acetate. During neutralization the chromosomal DNA partially re-natures and forms an insoluble clot along with various proteins while the plasmid DNA remains in suspension. The insoluble genomic DNA and proteins are then separated from the plasmid DNA through centrifugation and the plasmid DNA (present in the supernatant) is then bound to a silica membrane in the presence of a high salinity solution. The membrane is then washed with 70% ethanol through centrifugation and the plasmid DNA is eluted with a low salinity solution such as molecular grade water or elution buffer. Directly prior to the use of this kit the LyseBlue reagent was added to Buffer P1 along with an RNase-A solution (1:1000 ratio). Buffer P1 was correspondingly labelled and stored at 4°C as per the manufacturers' protocol. LyseBlue is a colour indicator that will visualize optimal mixing and pH during the procedure. Additionally, 220µl of 100% ethanol was added to Buffer PE and correspondingly labelled. Extraction was performed as per the manufacturers' guidelines.

Overnight bacterial cultures of 5ml LB in a 15ml Falcon tube, were pelleted at room temperature through centrifugation at 6000g for 5 minutes. The supernatant was carefully decanted, and the pellet was dried for roughly 10min by leaving the tubes upside down on a paper towel. After drying, the pellet was re-suspended in 250ul buffer P1 (containing EDTA, Tris, RNase-A and glucose) and homogenised through pipetting.

The EDTA serves to chelate divalent cations such as magnesium (Mg^{2+}) and calcium (Ca^{2+}) that are required by DNases to function. In doing so the EDTA not only prevents damage to plasmids but also helps to destabilise the cell walls of the bacteria aiding in lysis. RNase-A degrades any RNAs released after cell lysis, a necessary step as RNA will also bind to the silica membrane and contaminate the extracted plasmids after elution. Glucose functions as an osmotic pressure buffer and increases the overall plasmid yield.

The homogenate was then moved to a sterile 2ml microcentrifuge tube and 250ul of chilled Buffer P2 was added and thoroughly mixed by inverting the tube six times. The reaction should turn blue due to the LyseBlue reagent indicating a pH of between 12.0 and 12.5. If no colour change was observed additional NaOH was added until a colour change was clearly visible. Buffer P2 is the lysis buffer and contains sodium hydroxide (NaOH) and sodium dodecyl sulphate (SDS).

The SDS will solubilise the cell membrane while the NaOH breaks down the cell wall and causes denaturing of proteins and genomic DNA. The lysis reaction should not exceed 5 minutes. Following lysis, 350ul of neutralizing buffer N3 was added to the solution and mixed through inverting the tube six times. Buffer N3 contains potassium acetate (CH_3COOK) which decreases the alkalinity of the solution and turns it from blue to clear (colour-less or a milky opaque). The solution was then centrifuged at 16 000g for 10 minutes to pellet the insoluble genomic DNA-protein complex along with cellular debris and other insoluble components. The supernatant was then carefully transferred to a QIAprep 2.0 spin column through pipetting and centrifuged at 13 000g for 60sec, after which the flow-through was discarded.

The membrane was then washed by adding 750ul PE buffer and centrifuged again for 60 seconds. If it was suspected that the sample might have a very high salt content, this washing buffer was incubated on the membrane at room temperature for 5min before centrifugation. Following the wash step, the flow-through was discarded and the membrane was again centrifuged without the addition of any buffers for 60sec to remove any residual buffer from the membrane and prepare it for elution. The QIAprep spin column was then transferred to a sterile 1.5ml Low-Bind micro-centrifuge tube and the plasmid DNA was eluted from the membrane by adding 50ul of preheated (75°C) nuclease-free H_2O (Ion torrent nuclease-free H_2O, ThermoFisher), allowed to

incubate for 60 seconds and then centrifuged at 13 000g for 60sec. The flow-through (containing the DNA) was collected, evaluated through AGE (Section 2.2.7) and quantified through spectrophotometry (Section 2.2.6). All centrifugation steps were carried out in an Eppendorf 2J20 bench centrifuge.

2.2.5 Endotoxin-free maxiprep plasmid extraction

The most common and commercially available bacterial cells used for transformations are gram-negative bacilli such as *E. coli.* This is due to the nature and structure of their thin, double-layered, cell walls that lack the thick peptidoglycan layer more commonly associated with gram-positive bacteria. The thick peptidoglycan layer makes transformation much more difficult. However, gram-negative bacteria have an abundance of highly variable cell membrane components known as lipopolysaccharides (LPS). LPSs are molecules that contain various hydrophobic, hydrophilic, and charged regions and are biologically active as cell membrane receptors, structural components and even facilitate intracellular communication. Due to the unique composition of LPSs, they can have various unanticipated and unwanted interactions with other molecules. It is for this reason that LPSs are also known as endotoxins and are known to dramatically reduce transfection efficiency and increase cellular toxicity (Butash *et al.*, 2000). Especially when transfecting DNA into mammalian cells the levels of endotoxins must be reduced as far as possible. Therefore, all plasmids and DNA constructs that would be transfected in this project were first purified with the EndoFree® Plasmid Maxi kit (Qiagen, Whitehead Scientific).

The kit is based on the same biochemical principles as that of the miniprep kits (Section 2.2.4) but with the addition of a wash step designed to specifically remove LPSs from the sample. Endotoxin-free plasmid extraction was performed as per the manufacturer's guidelines from an overnight bacterial culture of 100ml which was separated into two 50ml Falcon tubes and pelleted through centrifugation at 6000g for 15-30 minutes at 4ºC. The supernatant was decanted and both pellets were each resuspended in 2ml of buffer P1. The re-suspended pellets were homogenized through pipetting and added together. Additional buffer P1 was added to the suspension to a final volume of 10ml.

After re-suspension, 10ml of lysis buffer P2 was added and mixed by inverting the tube 6 times during which the suspension should turn blue due to the LyseBlue reagent. The lysis reaction was incubated at room temperature for 5min after which 10ml of chilled Buffer P3 (neutralizing buffer) was added and mixed through inverting the tube six times. During this step, the suspension should change colour from blue to clear (transparent with a milky/opaque cloud suspended within). The suspension was then transferred to a provided QAI-filter cartridge and incubated for 10 minutes at room temperature.

The cell lysate was filtered into the 50ml Falcon collection tube after which 2.5ml ER Buffer was added and incubated on ice for 30min. The ER buffer is designed to remove endotoxins (LPSs) from the sample through the formation of various binding complexes and as a result, will change the filtrate from clear to slightly opaque over time. During this incubation, a QAI-tip column was equilibrated with 10ml of QBT buffer. The buffer was added to the column and allowed to empty by means of gravity flow. Following the 30min filtrate incubation and the column equilibration, the filtrate was added to the column and allowed to enter the resin by means of gravity flow. The flow-through was discarded and the column was washed with 60ml of QC buffer (done in two sessions as the column can only hold 30ml at a time).

The QAI-tip column was then moved to a 50ml endotoxin-free tube for elution. DNA was eluted by adding 15ml of QN buffer to the column and allowed to drain by means of gravity flow. The DNA was precipitated by the addition of 10.5ml of isopropanol and incubated for 10min. The DNA was pelleted through centrifugation at 15 000g for 30min, the supernatant was carefully removed and the pellet was washed with 5ml of a 70% endotoxin-free ethanol solution.

The sample was again centrifuged for 30min at 15 000g after which the ethanol was carefully removed and the pellet was air-dried for 5-10min. Finally, the dried pellet was dissolved in 100ul endotoxin-free TE buffer or nuclease-free H_2O (Ion Torrent nuclease-free H_2O, ThermoFisher). The plasmid DNA was evaluated through AGE (Section 2.2.7) and quantified through spectrophotometry (Section 2.2.6).

2.2.6 Spectrophotometric evaluation of nucleic acids

All spectrophotometric evaluations of nucleic acids were performed using a NanoDrop One (Thermo Scientific, Waltham, MA, USA) in accordance with the manufacturer's guidelines. The NanoDrop was cleaned with molecular grade water before and after each sample read and was calibrated and blanked using the same buffer the sample was suspended in. The concentration of DNA samples was measured at 260nm and RNA samples at 280nm. The purity of DNA samples were determined by the $OD_{260/280}$ nm values, with pure DNA expected to have a ratio of 1.8. Any $OD_{260/280}$ ratios lower than 1.8 were taken as indicative of protein contamination and ratios higher than 2.0 of RNA contamination. Sample volumes of 1ul were used for each evaluation.

2.2.7 Agarose gel electrophoresis (AGE)

The primary analysis of all nucleic acids was performed through separation and visualization via agarose gel electrophoresis (AGE). Standard 1% (w/v) agarose gels were used with 0.5ug/ml ethidium bromide (EtBr) as intercalating and visualization agent. Gels were prepared by adding agar powder to either a 1x TAE buffer (Tris-acetate and EDTA) or a 0.5x TBE buffer (Tris base, boric acid and EDTA). An agarose gel concentration of 1% was used as it is the optimal concentration to study DNA fragments varying in size from 250bp to 12000bp (Sambrook & Russell, 2001). TAE based gels were used for higher resolution of larger nucleic acids, whereas TBE based gels provided better ionic strength and buffering capacity in addition to the increased resolution of smaller nucleic acids. The agarose was dissolved in the selected buffer through heating and cooled to about 45°C before adding the EtBr and casting the gel into a 10cm x 7cm x 1cm casting tray, yielding a gel of roughly 10cm x 7cm x 0.7cm. The gels were then left to cool to room temperature for about an hour before being cured at 4°C for 30min. All gels were freshly prepared directly prior to each AGE step. The gels were electrophoresed at between 10 and 15 volts per cm of gel (70V - 100V) for roughly an hour or until samples were sufficiently separated.

All AGE steps were performed in a BioRad system and voltage was kept constant throughout the electrophoresis process. The gels were then visualised under fluorescent light in a ChemiDoc™ MP Imaging System from Bio-Rad laboratories. All gels and accompanying buffers were made in accordance with standard molecular procedures (Sambrook & Russell, 2001). All nucleic acid samples were mixed in a 5:1 (nucleic acid: loading dye) ratio with a 6x mass ruler DNA loading dye prior to being loaded into the wells. Each AGE step also included a gene ruler sample (GeneRuler™ 1kb DNA Ladder) that acted as an internal standard and DNA fragment size reference.

2.2.8 Cell-cultures

In this project baby hamster kidney cells constitutively expressing the T7-RNA polymerase (BHK-T7 and BSR-T5/7) were selected as transfection cell-lines and African green monkey kidney (MA104) and swine testes (ST) cells were selected for co-seeding and propagation cell-lines. Although RV does not propagate well in either BHK-T7 or BSR-T5/7 cell-lines, these cells transfect much more efficiently than MA104 and ST cells. RV can, however, propagate well in both MA104 (Desselberger, 2017; Estes *et al.*, 1979a; Gutierrez *et al.*, 2010; Londrigan *et al.*, 2000; Teimoori *et al.*, 2014) and ST (Welter *et al.*, 1991) cells with MA104 cells being the industry standard (Estes *et al.*, 1979a) for RV propagation.

Complete media, for the growth of each cell-line except ST cells (Table 5), consists of Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with the following to a final concentration of 5% fetal bovine serum (FBS, Gibco), 100 units/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B (Anti-Anti, Gibco) and 1% (v/v) non-essential amino acids (1mM of each amino acid) (NEAA, Gibco). Incomplete media, or splitting media, is the same as complete media but without any FBS. Table 6 and Table 7 show the composition of the NEAA and Anti-Anti respectively.

BHK-T7 cells were grown in complete media supplemented with hygromycin (Gibco, Thermo scientific, MA, USA) to a final concentration of 600µg/ml, every third passage to ensure T7-RNA polymerase expression. BSR-T5/7 cells were grown in complete media supplemented with geneticin (Gibco) to a final concentration of 1mg/ml, with each passage to ensure T7-RNA polymerase expression.

MA104 cells were grown in complete media. ST cells were grown in minimal essential medium (MEM) supplemented with 10% FBS, 100 units/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B, 1% (v/v) sodium pyruvate (1mM) (Gibco) and 1% (v/v) non-essential amino acids (NEAA). All cell cultures were incubated at 37°C in a humidified incubator with 5% CO_2.

Table 5: Composition of complete media for each selected cell-line

Cell-line	DMEM	MEM	FBS	Anti-Anti	S. Pyruvate	NEAA
BHK-T7	✓	N/A	5%	1%	N/A	1% (optional)
BSR-T5/7	✓	N/A	5%	1%	N/A	1% (optional)
MA104	✓	N/A	5%	1%	N/A	1%
ST	N/A	✓	10%	1%	1%	1%

Table 6: Composition of 100x NEAA

Amino-acid	mg/L	mM
Glycine	750.0	10.0
L-Alanine	890.0	10.0
L-Asparagine	1320.0	10.0
L-Aspartic acid	1330.0	10.0
L-Glutamic Acid	1470.0	10.0
L-Proline	1150.0	10.0
L-Serine	1050.0	10.0
Each diluted to 0.1mM in complete media		

Table 7: Composition of 1% Anti-Anti

Penicillin	100 units/mL
Streptomycin	100 µg/mL
Amphotericin B	0.25 µg/mL
Final concentration in complete media	

The BHK-T7 and BSR-T5/7 cells were grown in 25cm^2 tissue culture flasks (Nunc, Thermo Scientific, MA, USA) to roughly 95% confluence before being split 1:5 for further propagation. MA104 and ST cell-lines were split 1:3 for propagation. However MA104 cells reached confluence faster (2-3 days) than ST cells (3-5 days). MA104 cells were split once monolayers reached roughly 95% confluency, whereas ST cells were split upon reaching 80% confluency. If ST cells were grown past 80% confluency, their trypsinization efficiency dramatically reduced.

All cell lines were split according to the following procedure. Upon reaching the desired confluence the media was removed and the monolayers were washed twice with 1% sterile phosphate-buffered saline (PBS, Gibco) solution at room temperature. The monolayers were then treated with room temperature 0.05% trypsin EDTA (Gibco) and incubated in a humidified incubator at 37°C until the cells started to detach, at which

point any residual trypsin was removed and the cells were manually re-suspended in 100% fetal bovine serum (FBS) through pipetting. The re-suspended cells were then aliquoted into new flasks containing the appropriate volume of media (without FBS). Trypsin incubation times for BHK-T7 cells ranged from 2-3min and BSR-T5/7 cells ranging from 1.5-2min. MA104 cells required a consistent 4 min incubation and ST cells ranged from 4-12min. The ST cells seemed to become more resilient to the trypsin treatment with increasing confluence, with a roughly 75% confluent culture requiring 4 min of trypsin incubation to start to detach and a 95% confluent culture requiring up to 12min. 100% confluent ST monolayers could not be fully detached without damaging the cells even with extended incubation times.

2.2.9 Plasmid design

The following five expression plasmids were designed *in silico* using the SnapGene gene editing and visualization software: 1) phCMVdream_VV_D1R, 2) phCMVdream_VV_D12L, 3) phCMVdream_p10_FAST, 4) phCMVdream_ASFV and 5) phCMVdream_C3P3. Refer to Appendix A for plasmid maps (Figure 42 and Figure 43) and sequences. The vector selected for each of these constructs is the phCMVdream plasmid (2558 bp) and is based on the pSmart plasmid. This backbone was selected due to its successful use in the development and optimization of bluetongue virus (BTV) (van Gennip *et al.*, 2012a; van Rijn *et al.*, 2016) and African horsesickness virus (AHSV) (van de Water *et al.*, 2015; van Rijn *et al.*, 2016) RG systems as well as its relatively small size. Each of the constructs were designed by using the ORF of the gene of interest and placing it in the multiple cloning site (MCS) of the pdCMVdream plasmid under the CMV promoter.

The construct designs were then sent through to GenScript for *Cricetinae* (hamster embryonic cell) codon optimization and synthesis. The vaccinia virus (VV) capping enzyme has two subunits encoded by ORFs, D1R and D12L. The coding regions of these two subunits were obtained from GenBank (NC006998.1) and used to construct phCMVdream_VV_D1R and phCMVdream_VV_D12L (Figure 42) respectively. The Nelson bay orthoreovirus (NBV) p10 fusion-associated small transmembrane (FAST) protein-coding region (GenBank no. AB908284) was used to design the

phCMVdream_p10_FAST plasmid (Figure 43). The phCMVdream_ASFV capping plasmid (Figure 42) was designed to carry the coding region of the African swine fever virus (ASFV) capping enzyme (GenBank no. NP868R). This sequence was also used to design the phCMVdream_C3P3 construct (Eaton *et al.*, 2017) which contained both the ORFs of the ASFV capping enzyme and a synthetic viral T7-RNA polymerase (GenBank no. KY446063.1) fused together through a serine-glycine linker (Figure 42).

2.2.10 Preparation of transfection mixtures

For the various plasmid-only RV RG systems in this project multiple transcription plasmids along with expression plasmids encoding the various capping and FAST proteins needed to be transfected into cells in very specific ratios. These plasmids were prepared through Endotoxin-free MaxiPreps (Section 2.2.5) and mixed together to form either a 1) equi-ug or 2) equi-molar transfection mixture. In both scenarios, the concentration of the final transfection mixture was determined through spectrophotometry (Section 2.2.6) to calculate what volume of the transfection mixture will be used during each transfection (Equation 1 and Equation 2). Optimal transfection and rescue efficiency required 30ug of total DNA per 25cm^2 transfection reaction. An equi-ug transfection mixture means that an equal amount (in terms of ug weight) of each plasmid is mixed together. This does not, however, result in an equal number of each plasmid being present in the mixture. Due to the large distribution of RV genome segment sizes (from GS1: 3302bp to GS11: 667bp), the plasmids vary largely in size. This type of transfection mixture contains more copies of the smaller plasmids than that of the larger ones, roughly 5 times more GS11 (NSP5/6) than GS1 (VP1).

For calculation purposes, the specific amount of plasmid selected in this instance is quite arbitrary as long as it remains the same for each plasmid (p = 10ug was selected for this project) (see equation 1 and Table 8 for details). It is also important to mention that this equi-ug ratio was not applied to the p10 FAST plasmids. Adding too much of the FAST coding plasmid will result in massive cellular trans-membrane fusion after transfection and will negatively impact viral rescue.

An equi-molar transfection mixture is prepared by calculating the molecular weight (MW) of each plasmid and correlating it to its concentration. An equal molar amount of each plasmid is then mixed together to ensure that the mix contains the DNA equivalent of one copy of each genome segment, similar to that of a natural virion. This results in larger plasmids contributing more than smaller plasmids to the overall weight (in ug) of the DNA in the mixture. This was however not applied to the FAST plasmid as a mere 40ng per 25cm^2 reaction was necessary and too much of it will cause the reverse genetic system to fail. The MW of each plasmid was calculated based on its sequence *in silico* using DNAMAN gene editing and visualization software and shown in Table 8. Table 8 illustrates the steps involved in the construction of either an equi-ug or equi-molar transfection mixture based on the implementation of the unaltered pT7_SA11-L2 RV RG system. The equations that were used are annotated as equation 1 and equation 2. The Table was constructed in Excel and used to determine the amounts (ul) of each plasmid required based on their concentration. In the table the labels p=10ug and p=1x10^{-9} mol refer to the amounts selected for this first experiment with p=10ug referring to 10ug of each plasmid involved in the mix and p=1x10^{-9} mol referring to 1x10^{-9} mol of each plasmid in the mix. These were arbitrary amounts selected solely to ensure equal amounts of each plasmid.

Table 8: Calculations for the preparation of equi-ug and equi-molar transfection mixtures

				Equi-ug	Equi-molar
Plasmid name	plasmid size (bp)	Plasmid weight (kDa)	Working stock (ng/ul)	Plasmid required (ul)	Plasmid required (ul)
pT7-VP1SA11	6387	3930,31	2064	4,8	1,9
pT7-VP2SA11	5777	3554,32	1950	5,1	1,8
pT7-VP3SA11	5675	3491,35	1274	7,8	2,7
pT7-VP4SA11	5447	3350,98	2103	4,8	1,6
pT7-VP6SA11	4441	2730,98	2603	3,8	1,0
pT7-VP7SA11	4147	2549,74	740	13,5	3,4
pT7-NSP1SA11	4695	2887,43	1075	9,3	2,7
pT7-NSP2SA11	4144	2576,21	2241	4,5	1,1
pT7-NSP3SA11	4190	2547,87	2089	4,8	1,2
pT7-NSP4SA11	3836	2358,08	987	10,1	2,4
pT7-NSP5-6SA11	3751	2305,68	2040	4,9	1,1
pCAG-FAST	6012	3907,3	720	13,9	5,4
pCAG-D1R	8258	5084,84	2574	3,9	2,0
pCAG-D12L	6586	4054,97	2301	4,3	1,8
Total				95,6	30,3
				p=10ug stock	p=1x10^{-9} mol stock

Total DNA per transfection (ug)	Concentration of Equi-ug plasmid mix (ng/ul)	Concentration of Equi-molar plasmid mix (ng/ul)	Volume of equi-ug plasmid mix per transfection (ul)	Volume of equi-molar plasmid mix per transfection (ul)
30	1125	1409	26,67	21,29

Equation 1: Calculation of the volume of plasmid (ul) required to yield a specific amount of DNA (ug)

For the construction of the p = 10ug plasmid stock the following equation was used:

p is defined as: The standard amount of DNA (in ug) selected for each plasmid.

[DNA] is defined as: The concentration of plasmid DNA (in ng/ul).

V is defined as: The volume (in ul) of plasmid required to obtain a specific amount (in ug) of DNA (p).

Thus $[DNA] = \frac{ng}{ul}$ and $V = \frac{p}{[DNA]}$ where p = 10ug of DNA.

Equation 2: Calculation of the volume of plasmid (ul) required to yield a specific amount of DNA (mol)

For the construction of the p = $1x10^{-9}$ mol stock the following equations were used:

$mol_{(DNA)}$ is defined as: The amount of DNA (in g) divided by the molecular weight (MW) of the DNA (in Dalton).

$[DNA]_{ng}$ is defined as: The concentration of plasmid DNA (in ng/ul).

$[DNA]_g$ is defined as: The concentration of plasmid DNA (in g/ul). Conversion is $ng_{(DNA)} \times 10^{-6} = g_{(DNA)}$

Meaning $mol_{(DNA)} = \frac{g_{(DNA)}}{MW_{(DNA\ in\ Da)}}$ and $[DNA]_g = \frac{ng_{(DNA)} \times 10^{-6}}{ul}$ thus

$g(DNA) = [DNA]g \times ul$ and $mol_{(DNA)} = \frac{[DNA]g \times ul}{MW_{(DNA\ in\ Da)}}$ meaning

$mol_{(DNA)} \times MW_{(DNA\ in\ Da)} = [DNA]g \times ul$ and $ul = \frac{mol_{(DNA)} \times MW_{(DNA\ in\ Da)}}{[DNA]g}$

2.2.11 Rescue protocol

All transfections were performed in 25cm^2 tissue culture flasks (Nunc, Thermo Scientific, MA, USA) on either BHK-T7 or BSR-T5/7 cell monolayers of roughly 80% confluence. This was done due to the fact that optimal transfection requires actively growing monolayers that are between 75% and 85% confluent. Monolayers that have reached 100% confluence reduce metabolic activity and have a lower overall transfection efficiency and viral yield. Once the desired cell-line reached 80% confluence the growth media was removed and the monolayer was washed with 5ml of sterile, room temperature, 1x PBS. This was done to remove excess FBS and residual antibiotics that might interact with the transfection reagent and adversely influence transfection efficiency. The monolayer was then covered in 1.5ml OptiMEM (Gibco) containing no antibiotics or FBS. The monolayer was then transfected with the desired transfection plasmid mix (Figure 18) using TransIT-LT transfection reagent.

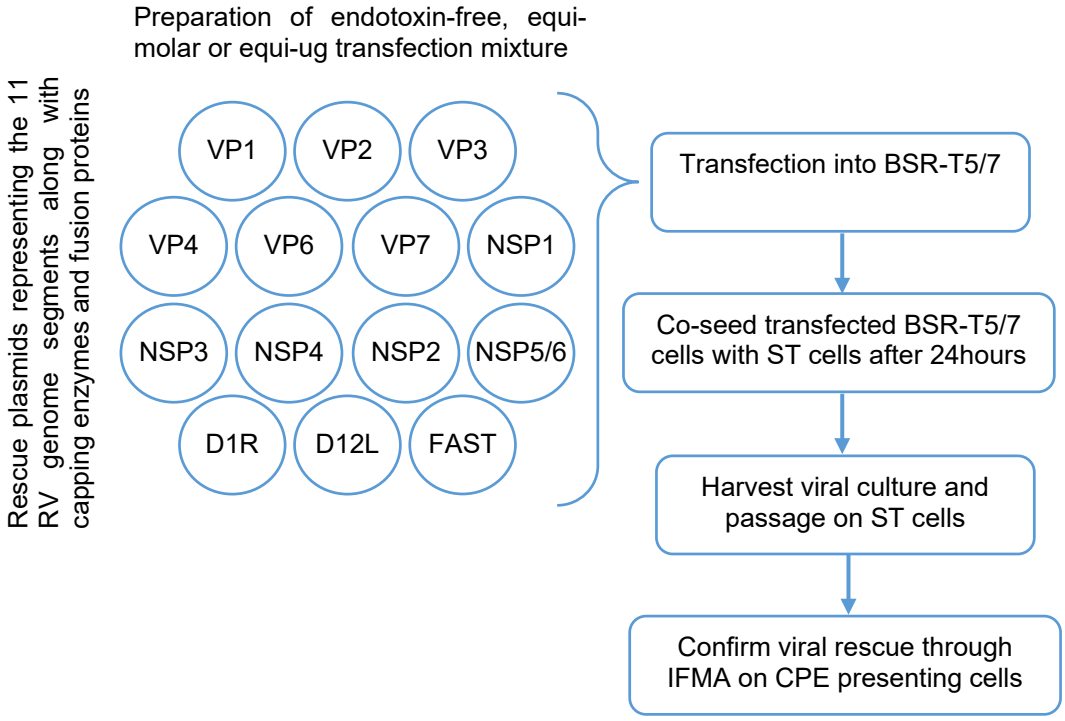

Figure 18: Diagram depicting the simplified rescue protocol used throughout this project for both the pT7_SA11-L2 and pSmart_SA11-N5 RV RG systems

transfection of DNA and proteins into a variety of commercial and primary cell-lines. The reagent is well known for its low toxicity and high transfection efficiency and was

used for all transfections in this project. Although the transfection reagent is tolerant to FBS, it is inhibited by various antibiotics and is thus used in conjunction with un-supplemented Opti-MEM throughout the transfection process. All transfections were carried out in accordance with the manufacturers' guidelines and followed the same basic protocol.

The selected transfection plasmids were freshly mixed as described in Table 8, and its concentration measured through spectrophotometry (Section 2.2.6). Dilute 30ug of the plasmid mixture in 500ul of un-supplemented Opti-MEM in a 5ml round bottom polystyrene tube and mix gently through pipetting. Next, gently mix the TransIT-LT reagent through flicking the tube and then transfer 90ul of the reagent to the diluted DNA tube being careful not to let the reagent touch the sides of the tube. Optimal results were obtained by using 3ul of transfection reagent per ug of DNA. Introduce the reagent directly into the 500ul of diluted DNA and mix through pipetting. Let the transfection mix incubate at room temperature for 15-30min so that the DNA-lipid complex can form.

During this incubation, the growth media was removed from the BHK-T7 or BRS5-T7 monolayers and replaced with Opti-MEM. The transfection complex was not incubated for more than 30 minutes. The total volume of the transfection complex was added drop-wise to the prepared monolayers making sure that the complex directly interacts with the cells. The cells were then incubated with the transfection complex for 12-16 hours at 37°C in a humidified CO_2 (5%) incubator, where-after the transfection complex was removed and replaced with complete media for a further 12-16 hour incubation. It was found that even though the TransIT-LT transfection reagent has low toxicity, the 16-hour incubation did damage a significant portion of the cells and better results were obtained when this damage was counteracted with the second 12-16 hour incubation in complete media.

Following this recovery incubation, the co-seeding cells (MA104 or ST cells were grown to confluence beforehand) were prepared and introduced to the transfected monolayer. The co-seeding cells were washed twice with 1% PBS and treated with 0.05% trypsin EDTA (as per splitting procedure). The cells were then re-suspended in 500ul of FBS and added directly to the transfected monolayer in complete media. The co-seeded culture was then incubated for 1-3 hours to facilitate attachment of the co-

seeding cells. After attachment the complete media was removed and labelled as co-seeding supernatant, it was replaced with maintenance media, growth media lacking FBS and supplemented with either 0.5 ug/ml (for MA104 cells), or 0.3 µg/ml (for ST cells) porcine trypsin (catalogue no. T0303-1G, Merck) and incubated for 2-3 days or until CPE is clearly visible. The addition of MA104 or ST cells was designed to significantly increase the viral titer as both these cell-lines are known to propagate rotavirus very well.

The rescue protocol was concluded by viral harvest, passaged on either MA104 or ST monolayers and viral visualization through an immuno-fluorescent monolayer assay (IFMA), as depicted in Appendix D.

2.2.12 Immuno-fluorescent monolayer assay (IFMA)

Initial verification and visualization of viral recovery were done through an immuno-fluorescent monolayer assay, IFMA (Bishop, 2016; van Gennip *et al.*, 2012). The primary antibody used was either a polyclonal rabbit anti-RV VP2/6 DLP IgG (serum generously provided by Prof. A.C. Potgieter, Deltamune, RSA), a polyclonal rabbit anti-RV NSP4 IgG or a polyclonal rabbit anti-RV VP7 peptide IgG (provided by Prof. A.C. Potgieter, Deltamune, RSA, in association with Prof. H.G. O'Neill, University of the Free State, RSA). The secondary antibody used was a fluorescently labelled (Alexa Fluor 488) goat anti-rabbit IgG conjugate (ThermoFisher).

The basic premise of this technique is that during the organic solvent fixing step the cell monolayer is fixed to the bottom of the flask and all proteins including any viral particles are precipitated. This step also enhances membrane permeability and allows the primary antibodies to interact with the precipitated proteins. The primary antibody is then introduced to the monolayer and binds to specific antigenic epitopes on any viral particles present. All excessive antibody is washed off and the secondary antibody is introduced. The second antibody binds specifically to the constant region (long-chain end) of the rabbit antibodies and is conjugated with an Alexa Fluor® 488 fluorescent complex.

This fluorescent complex has an excitation range of Ex: 495nm and emission range of Em: 519nm. Finally, the excess conjugate was washed off and the monolayer viewed under a fluorescent microscope (conjugate will emit green light). IFMA was performed on cell-culture monolayers that had presented with cytopathogenic effects (CPE) indicating viral growth. The cells were fixed by removal of any media and addition of 5mL of ice-cold fixing solution (1:1 methanol: acetone) followed by incubation at -20°C for 30min. The media removed in this step was labelled and stored at 4°C. If the IFMA results indicated viral rescue, this media was used as primary viral stock (P1 stock) or used for dsRNA (viral genome) extraction. The fixed cells were washed with washing buffer (1x PBS with 0.05% Tween-20), after which the blocking buffer (1x PBS, 0.05% Tween-20 and 1% (w/v) tryptone) was added and incubated at 37°C for 60 min. Organic solvent fixation, such as methanol/acetone, does not require a permeabilisation step as it is capable of removing lipids from the cell membranes, dehydrating the cells and causing precipitation of proteins in the cellular structure (Jamur & Oliver, 2010a; Jamur & Oliver, 2010b).

The blocking buffer prevents unspecific binding of the various antibodies through the coating of the exposed areas of the tissue flask with tryptone. The blocking buffer was then replaced with the primary antibody solution (serum-containing above-mentioned rabbit anti-RV IgG diluted 1:2000 in blocking buffer) and allowed to incubate for 60min at 37°C. After this incubation, the primary antibody solution was removed and the fixed cells were washed three times with washing buffer. The secondary antibody solution (anti-rabbit goat IgG conjugated diluted 1:2000 in blocking buffer) was then added and incubated for 60min at 37°C followed by two washes with 1x PBS. The cells were then immediately viewed under a fluorescent microscope (Nikon eclipse TE2000-S, Nikon Instruments Inc. Melville, NY, USA) and photos were taken.

2.2.13 Viral propagation

After obtaining the primary viral stock (P1 stock), propagation of the virus is required for dsRNA extraction, $TCID_{(50)}$ assays or serial passaging. For each of these, the viral stock must first be activated and then used to infect new cells. As described in Section 1.3.4, rotavirus outer capsid protein VP4 must first be proteolytically cleaved into VP5

and VP8 before it can interact with cell receptors and initiate infection. The cleavage of the VP4 spike is referred to as activating the virus and is achieved through the addition of porcine trypsin (Merck) to a final concentration of 10ug/ml, and incubation of the P1 stock at 37°C for 30min. Viral propagation was carried out in either MA104 or ST cells grown in 75cm^2 tissue culture flasks at 90% confluence. The growth media was removed and the monolayer washed with 1x sterile PBS to remove FBS.

To 1ml inoculum 1ml of growth media without any antibiotics or FBS was added and poured over the monolayer and incubated for one hour at 37°C with slight agitation. The inoculum was removed after 60min and fresh propagation media is added to the cells. Propagation media simply referrers to the cells' standard growth media without any FBS and with the addition of porcine trypsin to a final concentration of 0.3ug/ml.

Monolayers were then incubated at 37°C in a humidified, 5% CO_2 incubator for 3-5 days or until CPE developed to more than 75%. At this stage, the cells were either harvested or the supernatant was removed and stored as a viral stock (Pn+1) whereafter the CPE presenting cells were fixed for IFMA (Section 2.2.12). When harvesting viruses from CPE presenting cultures, the cells must first be detached from the tissue culture flask through scraping or pipetting. The entire content of the flask is then transferred to a centrifugation tube and homogenised through pipetting. From here on two distinct options were considered, namely the preparation of a supernatant stock or a whole lysate stock. In general supernatant, stocks were preferred as cellular debris can contain various cytotoxic components which could interfere with infection. However, in this project, the initial passage (from P0 stock to P1 for IFMA) was done using a whole-cell lysate. For a supernatant stock, the homogenised cell suspension was centrifuged to pellet out all intact cells and cellular debris after which the stock was activated.

Whole lysate stocks were simply activated directly after homogenization. After activation, the standard viral propagation protocol was followed.

2.2.14 Sequencing

All transcription plasmids used throughout this project were sent for sequence verification via Sanger sequencing at Stellenbosch University's Central Analytical Facility (CAF), DNA Sequencing Unit, using the standard T7 promoter forward- and T7 terminator reverse-sequencing primers (supplied by CAF sequencing unit). The standard miniprep plasmid extraction protocol was followed for samples sent for Sanger sequencing.

Plasmids used for transfection and cDNA derived from rescued viral genomes were sent for next-generation sequencing (NGS). Plasmids sent for NGS were extracted and purified using the endotoxin-free maxiprep protocol. All NGS was done by Dr Arshad Ismail at the National Institute for Communal Diseases (NICD) with the Illumina MySeq instrument. Sequence analyses were performed with the use of the CLC Genomic workbench software.

2.3 Results and Discussion

2.3.1 Plasmid extraction and sequence verification of the pT7_SA11-L2 RV RG system

This step of the project entailed the extraction of the Japanese pT7_SA11-L2 RV RG plasmid set from bacterial cultures and validation of their sequences through NGS. The plasmids arrived in the form of 14 bacterial stab cultures each harbouring a single plasmid. The cultures were received from AddGene and immediately used to inoculate 14 overnight cultures for endotoxin-free plasmid extraction to be transfection ready (Section 2.2.5), glycerol stock preparation (Section 2.2.2) and sequencing (Section 2.2.14). The overnight cultures comprised of 100ml standard LB medium (Melford Laboratories Ltd. Bildeston Road, Suffolk, U.K) supplemented with carbenicillin (Melford, U.K) to a final concentration of 100µg/ml, and were inoculated with one of the 14 bacterial stab cultures. Following the 16hour incubation at 37°C with shaking at 250rpm, plasmids were extracted and a 2ul sample of each was visualised through AGE (Section 2.2.7), Figure 19.

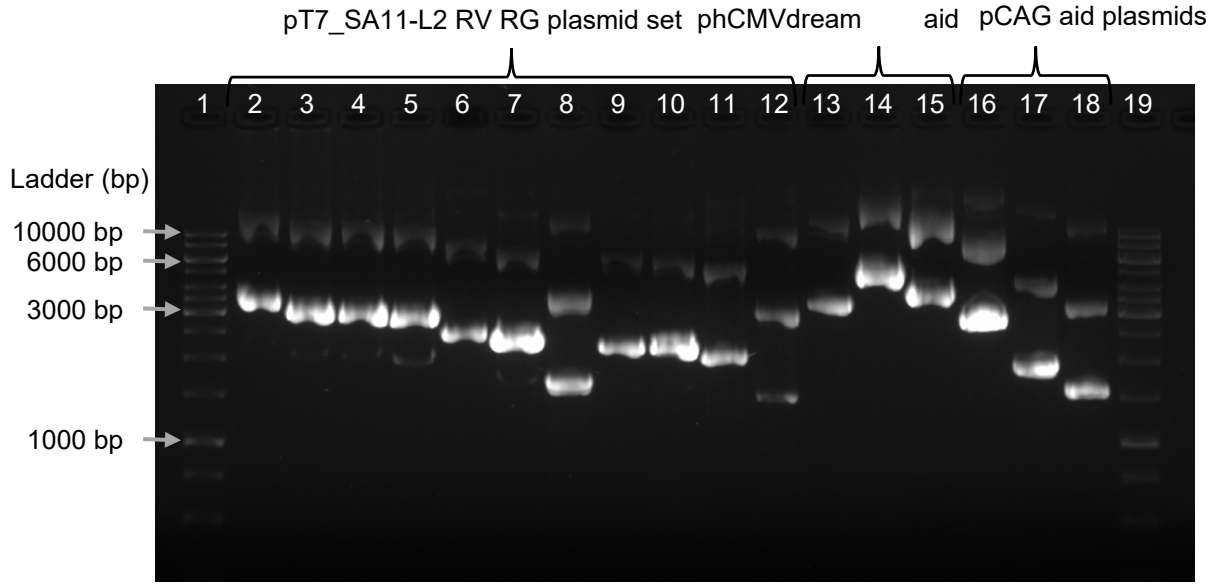

Figure 19: Agarose gel of pT7_SA11-L2 RV RG plasmid set. Lane 1 & 19: dsDNA ladder (O'GeneRuler 1 kb DNA Ladder, ThermoFisher). Lanes 2 to 12: pT7 plasmids carrying the RV_SA11-L2 genome segments cDNA in order (from GS1 in lane 2 through to GS11 in lane 12). Lane 16: pCAG_FAST plasmid. Lanes 17 & 18: the pCAG expression plasmids encoding the two subunits of the VV capping enzyme. Lanes 13 & 15: the two phCMVdream_VV-capping expression plasmids. Lane 14: phCMVdream_FAST expression plasmid.

Plasmid extractions yielded concentrations between 200ng/ul and 700ng/ul (Table 9), and as a result slightly distorted the gel, as evident by the bowing and slight smudging of the plasmids in the gel. This AGE was however purely to confirm and visualize plasmid extraction. Final confirmation would be done through sequence verification.

Aliquots of the 11 pT7_SA11-L2 cDNA transcription plasmids were pooled and sent for NGS, which was much more affordable than sequencing each plasmid alone. Pooling the plasmids allowed single reaction sequencing of the entire plasmid set but had the disadvantage of not correlating the backbone sequence to each individual RV genome segment sequence (see Appendix B for sequence alignments and NGS reads mapped to reference genomes). The sequences of the 11 RV genome segments could, however, be fully verified, as well as the consensus sequence of the pT7 plasmid backbone. Only the 5`- and -3` flanking regions between the plasmid backbone and the individual genome segments could not be directly verified. The NGS reads were mapped against the SA11-L2 reference genome (LC333802 - LC333812) and the *in silico* constructs for each plasmid.

The consensus sequence (cs) obtained from the NGS as compared to the SA11-L2 reference genome showed no mutations or sequence errors. The backbone cs also aligned perfectly. Based on these results the plasmid sequences were deemed correct and ready for use in the implementation of the pT7_SA11-L2 RV RG system.

2.3.2 Implementation of the original, equi-ug pT7_SA11-L2 RV RG system and comparison to an equi-molar approach

This experiment entailed the implementation of the exact pT7_SA11-L2 RV RG system as described by Kanai (2017) alongside an equi-molar variation thereof. This was done in an attempt to recreate the original RV rescue results (Kanai *et al.*, 2017) and to test if the system could be improved using the equi-molar approach as used in the AHSV and BTV RG systems (Section 1.4.2). The exact experimental approach as used by Kanai (2017) was followed with the only modification being the use of 25cm^2 tissue culture flasks for transfections instead of the 96-well plates used during the original experiment.

In short, the 14 rescue plasmids were extracted, purified and mixed together to form both an equi-ug and equi-molar, endotoxin-free, transfection mixture (Section 2.2.10) as shown in Table 9. For each of the systems a negative control was also constructed following the same procedure as the original but without the inclusion of an essential component, the plasmid encoding the viral RNA-dependent RNA-polymerase (GS1, VP1). Following this, the transfection protocol (Section 2.2.11) was followed for each system and its corresponding negative control, and rescue was verified through IFMA (Section 2.2.12). In addition to this SA11-N2 (our WT) was passaged on MA104 cells and visualized through IFMA as a positive control for the IFMA.

Table 9: Calculations for the preparation of equi-ug and equi-molar pT7_SA11-L2 RV RG transfection mixtures

				Equi-ug	Equi-molar
Plasmid name	plasmid size (bp)	Plasmid weight (kDa)	Working stock (ng/ul)	Plasmid required (ul)	Plasmid required (ul)
pT7-VP1SA11	6387	3930,31	2064	4,8	1,9
pT7-VP2SA11	5777	3554,32	1950	5,1	1,8
pT7-VP3SA11	5675	3491,35	1274	7,8	2,7
pT7-VP4SA11	5447	3350,98	2103	4,8	1,6
pT7-VP6SA11	4441	2730,98	2603	3,8	1,0
pT7-VP7SA11	4147	2549,74	740	13,5	3,4
pT7-NSP1SA11	4695	2887,43	1075	9,3	2,7
pT7-NSP2SA11	4144	2576,21	2241	4,5	1,1
pT7-NSP3SA11	4190	2547,87	2089	4,8	1,2
pT7-NSP4SA11	3836	2358,08	987	10,1	2,4
pT7-NSP5-6SA11	3751	2305,68	2040	4,9	1,1
pCAG-FAST	6012	3907,3	720	13,9	5,4
pCAG-D1R	8258	5084,84	2574	3,9	2,0
pCAG-D12L	6586	4054,97	2301	4,3	1,8
Total				95,6	30,3
				p=10ug stock	p=1x10^{-9} mol stock

Total DNA per transfection (ug)	Equi-ug mixture (ng/ul)	Equi-molar mixture (ng/ul)	Volume equi-ug mixture / transfection (ul)	Volume equi-molar mixture / transfection (ul)
30	1125	1409	26,67	21,29

The experiment was carried out several times with every attempt yielding very few, and very faint fluorescent foci, indicative of low viral titer, or weak viral growth. Viral rescue, as evaluated by IFMA of the P1 passage at 5 to 7 days post-infection, could only be visually confirmed once with the original equi-ug pT7_SA11-L2 system (Figure 20, A) and twice with the equi-molar approach (Figure 20, B). IFMA results of the WT SA11 infection (Figure 20, C) serves as an example of high levels of infection in MA104 cells compared to the viral rescue. For each reiteration of the experiment the negative controls never showed fluorescence following IFMA, the results of which are not shown. The system was also tried at various other institutions globally, and similar difficulties were experienced (personal communication). Since no significant differences could be seen between the equi-ug and equi-molar approaches it was arbitrarily decided to use the equi-molar approach for follow-up experiments as it was considered the standard approach in both the AHSV and BTV RG systems.

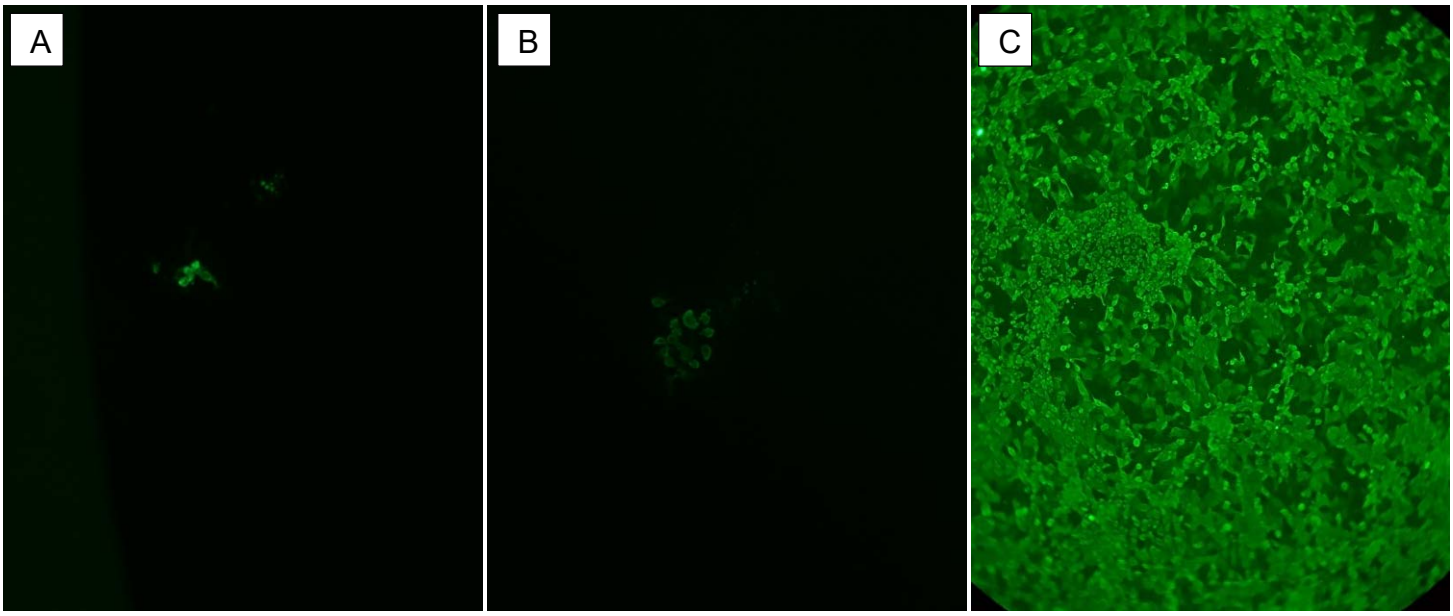

Figure 20: IFMA of equi-ug (A), equi-molar (B) transfections of the original pT7_SA11-L2 RV RG system and infection with SA11-N2 (C). IFMAs were done on MA104 monolayers following the final 7 day incubation step (no CPE was visible for A and B). 400x magnification

When compared to the RG systems for AHSV and BTV RG, the reasons for the low efficacy of the pT7_SA11-L2 RV RG system could include a large number of plasmid (14 plasmids ranging from 6387bp to 3751bp) that needed to be transfected into a single cell and the total plasmid load. To address this it was decided to **1**) Reduce the

plasmid load with roughly 3000bp by replacing the pCAG backbone (~3080bp) with the phCMVdream backbone (~2010bp) for the two capping enzyme and fusion protein expression plasmids. **2**) Replace the VV capping enzyme with a single subunit capping enzyme, such as the ASFV capping enzyme (which is a key element of the BTV RG system), and **3**) Codon optimize each of the aid plasmids for expression in BSR-T5/7 cells. These optimizations were investigated in follow-up experiments.

2.3.3 Replacement of pCAG capping and fusion expression plasmids with *Cricetinae* codon-optimized phCMVdream versions

This experiment entailed the exchange of the original pT7 capping and FAST plasmids with plasmids of our own design, phCMVdream_VV_D1R, phCMVdream_D12L and phCMVdream_p10-FAST (Figure 42 and Figure 43, Appendix A). The reasoning behind this was that although the pT7_SA11-L2 rescue plasmids were producing exact mRNA copies of the viral genome, these RNAs might not have been properly capped and thus were not being translated efficiently. The VV capping enzyme subunits (D1R and D12L) used in our plasmids were codon-optimized for expression in BSR-T5/7 cells (Chinese hamster ovary, *Cricetinae*, codon optimization) and were carried in the much smaller phCMVdream plasmids backbone. In theory, the smaller backbone would result in more copies of the plasmids being transfected into cells and the codon optimizations would lead to increased expression of the capping and fusion enzymes which in turn would increase capping efficiency and trans-membrane fusion. Similar to the previous experiment both an equi-ug and equi-molar transfection mixture was prepared, but this time with the phCMVdream based capping and FAST plasmids. For each version of the system a negative control was included (not adding VP1 coding plasmid). The standard rescue protocol (Section 2.2.11) was followed and viral recovery was confirmed through IFMA (Figure 21).

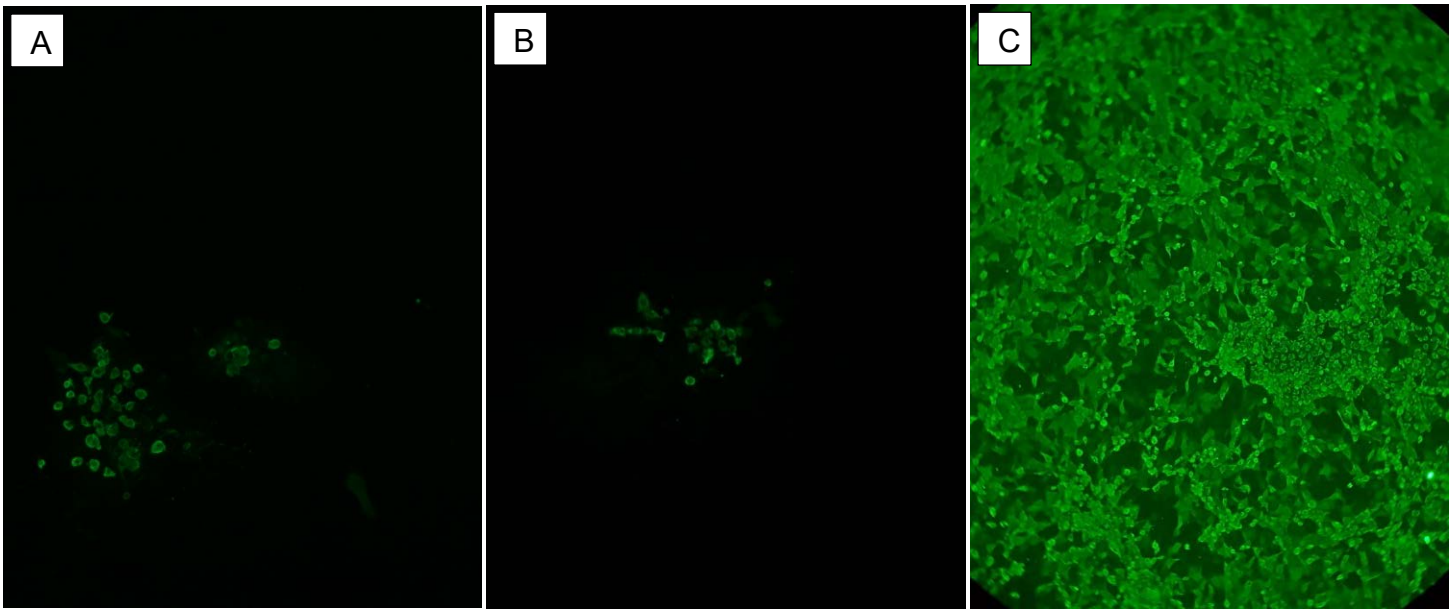

Figure 21: IFMA of equi-molar (A), equi-ug (B) transfection of the pT7_SA11-L2 system using phCMVdream based capping and FAST plasmids and infection with SA11-N2 (C). IFMAs were done on MA104 monolayers following a 7 day incubation with the P1 stock recovered from the co-seeded culture. No CPE was visible for A or B. 400x magnification.

In both the equi-molar and equi-ug systems cellular fusion was visibly increased, however, only a slight increase in efficiency was visible as indicated by fluorescent response following IFMA (Figure 21). Repeatability remained a major concern with multiple rescue attempts confirmed through IFMA, but no consecutive rescues obtained from P1 passages.

Following the transfection and co-seeding steps of the rescue protocol (Section 2.2.11), both systems' monolayers were monitored and various signs of viral propagation were observed during each reiteration of the experiment. These possible viral indicators however rarely translated into positive IFMA results, implying that: **1**) The signs of viral propagation were the result of plasmid carry-over, meaning viral proteins were being expressed, but no actual rescued virus was present to propagate. **2**) The recombinant virus is being rescued but not propagating efficiently enough in MA104 cells, thus making visualization through IFMA difficult. **3**) The signs of viral propagation were the result of trypsin toxicity and/or the carry-over of cytotoxic elements from the P1 stock.

Due to the nature of the rescue protocol, it was difficult to eliminate carry-over of plasmids and cytotoxic elements from the transfected culture to the propagation monolayer. For this reason, it was decided that the focus of the next experiments would be to ensure viral replication before P1 passage instead of trying to reduce carry-over and cellular toxicity. It was decided to change the co-seeding and propagation cell-line from MA104 to ST cells, which are known to be capable of propagating RV at lower (non-toxic) trypsin levels and to be highly susceptible to RV infection (Estes *et al.*, 1979; Welter *et al.*, 1991).

As with the previous experiment, no significant difference in visual viral yield was observed between the equi-molar and equi-ug approaches. It was thus decided that all follow-up experiments would only be based on an equi-molar approach as it more closely represents natural viral infection (Section 2.2.10).

2.3.4 Use of ST cells for co-seeding and as propagation cell line instead of MA104s

This experiment entailed the use of ST cells as co-seeding and propagation cell-line along with the increase of the relative amounts of plasmids encoding the sub-units of the VV capping enzyme. The reasoning behind the use of ST cells was based on the ability of ST cells to propagate RV at lower trypsin concentrations (possibly reducing toxicity) and the high susceptibility of ST cells to RV infection (Welter *et al.*, 1991). Additionally, concerns were raised about the high passage number of our MA104 cells and their continued susceptibility to RV infection. MA104 cells are the industry standard for RV propagation (Estes *et al.*, 1979; Gutierrez *et al.*, 2010; Londrigan *et al.*, 2000; Teimoori *et al.*, 2014) as they are a simian cell line and SA11 is also a simian virus. However, at Deltamune ST cells are also used for propagating non-porcine viruses (including SA11) and seem to be more susceptible to RV infection than MA104 cells (Potgieter, A.C. Deltamune, Personal communication). It should be noted that SA11 reaches much higher viral titers when passaged on MA104 cells than in ST cells. For this experimental approach it was decided that during the initial infection, following co-seeding, susceptibility to infection was more important than generating very high viral titers.

The suggested increase of the capping enzymes was based on experience with the AHSV RG system where increased capping of the viral transcripts directly resulted in increased viral yield. Based on these factors two equi-molar transfection mixtures were prepared, one containing the standard 1:1 ratio of phCMVdream based capping plasmids to pT7_SA11-L2 rescue plasmids, and the other containing three times as much (1:3 ratio), see Table 10 below for precise composition. Negative controls for each of the systems were constructed (lacking the VP1 transcription plasmid) followed by standard rescue protocol (Section 2.2.11), and verification of viral recovery through IFMA (Section 2.2.12).

Table 10: Construction of equi-molar transfection mixtures with increased ratios of capping enzyme encoding plasmids

	1:1 Ratio	1:3 Ratio	Total DNA
Rescue plasmids	25.8ug	20.2ug	30ug
Capping plasmids	4.2ug	9.8ug	30ug

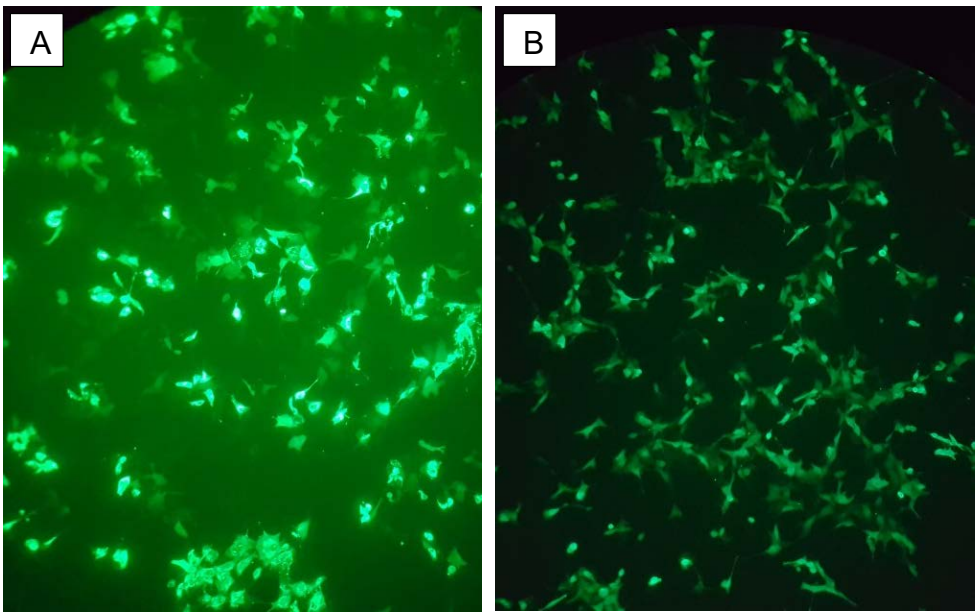

Figure 22: IFMA of equi-molar transfections of the pT7_SA11-L2 system using ST cells for co-seeding and propagation and varying the ratio of capping plasmids to rescue plasmids (A) 1:1 and (B) 1:3. IFMAs were done on CPE presenting ST cells 3-5 days after P1 passage. 400x magnification.

The results of the IFMA (Figure 22) clearly show high levels of viral infection and indicate definitive viral rescue. There was a clear distinction between infected cells and back-ground fluorescent interference. Although the majority of ST cells were infected, some cells remain un-infected and non-fluorescent. Repeatability dramatically increased with viral rescue visually confirmed 4 out of 5 times via IFMA.

It is noteworthy that the ST cells did not survive as well as MA104 in the presence of the propagation media. This is most likely due to the presence of porcine trypsin in the propagation media, a protease required for RV propagation that is toxic to ST cells even at levels that are considered non-toxic to MA104 cells (0.3ug/ml). Additionally, the use of ST cells instead of MA104 cells reduced incubation times to see CPE, from 7 days to between 3-5 days. CPE was also clearly visible in ST monolayers whereas MA104 monolayers did not present with clear CPE even after 7 days. This apparent discrepancy in CPE is thought to be due to the increased susceptibility of ST cells to infection as compared to MA104 cells. It was thus decided that ST cells would form the bases for further experiments with MA104 cells only being used for serial passages and $TCID_{50}$ assays.

Visual IFMA evaluation of the results also suggested a slight increase in efficiency for the system that provided more capping enzyme, further supporting the notion that the capping of the RV mRNA transcripts is essential for viral rescue. Another hypothesis for improving the efficiency of translation, and by extension viral rescue, was to use a more stringent selection system for the regulation of viral T7-RNA polymerase expression. This would not only increase the amount of T7-RNA polymerase available in each cell but also increase the number of cells actively expressing the enzyme.

2.3.5 Replacing BHK-T7 cells with BSR-T5/7 cells as transfection cell-line

In BHK-T7 cells hygromycin is used for the selection of the plasmid carrying the T7-RNA polymerase (Ito *et al.*, 2003). Due to its toxicity, hygromycin pressure is only applied to the cells every third passage. In contrast, BSR-T5/7 cells have a different T7-RNA polymerase expression plasmid that requires geneticin selection, which can be applied continuously (Liu *et al.*, 2005).

This experiment entailed the exchange of the initial transfection cell-line (BHK-T7) with BSR-T5/7 cells that more stringently selects for the expression of the viral T7-RNA polymerase. The hypothesis was that more stringent T7-RNA polymerase selection would increase the number of cells expressing the viral T7-RNA polymerase, which in turn would increase transcription of the rescue plasmids, providing more mRNA for translation and increasing overall rescue efficiency. The exact same experimental approach as the previous experiment (Section 2.3.4) was followed with the exception of BSR-T5/7 cells being used as primary transfection cell-line.

The combination of increased T7-RNA polymerase expression and the increased amounts of capping and fusion plasmids yielded a repeatable and consistent viral rescue system (Figure 23). Not only did this configuration of the RG system dramatically increase repeatability (rescue confirmed 5 out of 5 times), but it also increased viral titer as evidenced by the advanced CPE visible after only three to four days (whereas the unmodified system showed no CPE after 7 days). From this point forward the final incubation step, visual CPE formation was reduced to between 3 to 5 days.

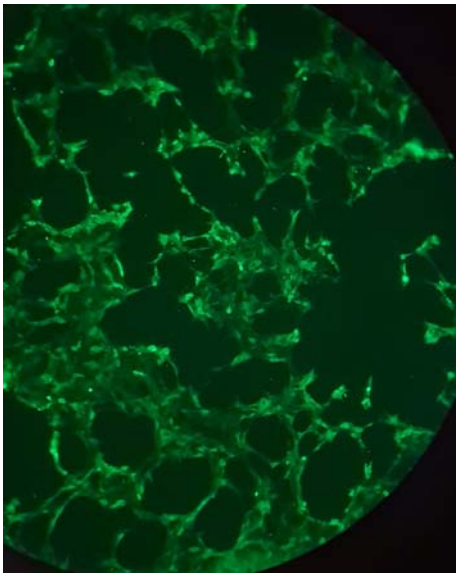

Figure 23: IFMA of equi-molar transfections of the pT7_SA11-L2 system into BSR-T5/7 cells, using ST cells for co-seeding and propagation. IFMAs were done on CPE presenting ST cells 3-5 days after P1 passage. 400x magnification.

2.4 Summary

The pT7_SA11-L2 RV RG system was made available to the scientific community through the AddGene service in 2017. The system was acquired by the NWU and was implemented with initial difficulty. Communication with various other groups also using the original system proved that the initial system required further development and optimization. The system proved to be very hard to consistently repeat and viral yields were very low (roughly $2x10^2$ $TCID_{50}$/ml, see Chapter 3, Section 3.3.5). Based on the strategies used during the implementation and optimization of the BTV and AHSV RG systems, it was decided to incorporate the following modifications to the initial pT7_SA11-L2 RV RG system.

1) Using equi-molar transfection mixtures instead of equi-ug mixtures. This was the standard approach in both the AHSV and BTV RG systems and more closely replicated the actual conditions during viral infection and replication.

2) Reducing the plasmid load with roughly 3000bp by exchanging the two pCAG capping and pCAG FAST expression plasmids (3010bp backbone) with phCMVdream versions (2080bp backbone) I designed.

3) The phCMVdream capping and FAST plasmids were also codon-optimized for expression in hamster cells (primary transfection cell-lines) which in theory would increase capping and fusion efficiency.

4) Using ST cells instead of MA104 cells for co-seeding and propagation cell-line. Not only did this significantly increase repeatability of the pT7_SA11-L2 RV RG system, but also reduced reporting time with 2-3 days.

5) Increasing the ratio of capping to rescue plasmids from 1:1 to 3:1. In the AHSV RG system, the increase in capping efficiency directly resulted in increased viral yield.

6) Using BSR-T5/7 cells instead of BHK-T7 cells as primary transfection cell-line. This increased the availability of the viral T7-RNA polymerase and increased the number of cells actively expressing the enzyme.

Through the incorporation of these modifications, I was able to greatly improve both the overall repeatability of the pT7_SA11-L2 system and its efficiency as evaluated

through relative viral yield. The optimized pT7_SA11-L2 system served to fully familiarize me with the basic methods involved in viral rescue and as a useful reference for the implementation of our own consensus sequence-based, plasmid only pSmart_SA11-N5 RV RG system (Chapter 3). Following the implementation of the NWU developed pSmart_SA11-N5 RV RG system, side-by-side comparisons were done with the pT7_SA11-L2 system also incorporating all the optimizations implemented in order to perform a comparative analysis between the different RG systems.

Evaluation of viral titers, sequencing results and comparative analysis of the pT7_SA11-L2 RV RG system is presented and discussed in Chapter 3 as it pertains to the implementation and evaluation of the locally developed, pSmart_SA11-N5 RV RG system.

Chapter 3

Implementation, optimization and comparative analysis of our pSmart_SA11-N5 RV RG system against the Japanese pT7_SA11-L2 RV RG system

3.1 Introduction

As mentioned in Section 1.4.3.2, the development and implementation of a robust RV RG system have long been a goal of our research group at the NWU. Before the 2017 publication of the Japanese pT7_SA11-L2 RG system, the NWU research group was in the process of developing our own RV RG systems based on the work of Dr L. Mlera (Mlera, 2012), and Dr JF. Wentzel (Wentzel, 2014). This chapter entails the finalization and implementation of the locally developed, consensus sequence-based, plasmid only pSmart_SA11-N5 RV RG system as well as the comparative analysis of it to the Japanese pT7_SA11-L2 RV RG system. Further improvements and optimizations were introduced to both systems and again compared via $TCID_{50}$ assay.

Following the work of Dr Wentzel (2014), another member of the NWU research group, L. Geldenhuys, focused on the construction of a fully plasmid-based RV RG system that would not rely on *in vitro* transcription, but rather on the transfection of transcription plasmids alone. Geldenhuys amplified the 11, consensus sequence, genome segments of RV SA11-N5 from the plasmids designed by Dr Wentzel (Figure 51, Appendix C) using the primers listed in Table 12. Each of these cDNA amplicons was then sub-cloned into the pSmart backbone, through the use of In-Fusion and fast cloning techniques, to generate the basis of the pSmart_SA11-N5 RV RG system. The primers used by Geldenhuys were also designed to correct the 5`-GGGN sequence that inhibited viral genome packaging in the Dr Wentzel transcription-based RV RG system. The plasmid only approach followed by Geldenhuys was also heavily influenced by the techniques and strategies used during the development of the AHSV and BTV RG systems.

The pSmart backbone used throughout this project was obtained from Prof. A.C. Potgieter (Deltamune, RSA) where it was being used as the backbone for the plasmid only AHSV RG system (van Rijn, *et al.,* 2016). Each of the pSmart_SA11-N5 constructs was designed to carry a single genome segment flanked by a 5`- T7-RNA polymerase promoter and a 3`- HDV ribozyme for the generation of precise viral transcript termini following transfection. Geldenhuys correctly generated and sequence-verified 8 of the 11 pSmart_SA11-N5 RV RG constructs during her MSc, with only GS3 (VP3), GS9 (VP7) and GS11 (NSP5/6) not being finalized. The final part of Geldenhuys's MSc and the initial part of my MSc overlapped, with the main goal of both being the implementation of a plasmid only RV RG system. As a result of this, many of the primers and constructs used during Geldenhuys's MSc are also used in mine.

Upon the publication of the pT7_SA11-L2 RV RG system in 2017 the focus of our research group at the NWU temporarily shifted from further fine-tuning the pSmart_SA11-N5 RV RG system to the implementation of the Japanese pT7_SA11-L2 RV RG system (Chapter 2). Upon successfully rescuing recombinant RV from the optimized pT7_SA11-L2 RV RG system, the focus again shifted to the finalization of the pSmart_SA11-N5 RV RG system and its implementation with the newly discovered optimizations (Section 2.4) in mind. This was initiated through the correction of the last three pSmart_SA11-N5 plasmids (GS3, GS9 and GS11) through In-Fusion HD cloning. Additionally, the phCMVdream capping and fusion encoding plasmids designed for the optimization of the Japanese pT7_SA11-L2 RV RG system (Figure 42 and Figure 43, Appendix A) were also incorporated into the pSmart_SA11-N5 RV RG system.

3.2 Materials and methods

3.2.1 dsRNA extraction

The protocol for dsRNA extraction and sequence-independent primer ligation was basically the same as that described by Potgieter and associates (Potgieter *et al.*, 2009). Following IFMA confirmation of viral rescue, the corresponding P1 stock was used to infect confluent monolayers of MA104 or ST cells in a 75cm^2 tissue culture flask for viral propagation (Section 2.2.13). After infection the monolayer was incubated at 37°C in a humidified, 5% CO_2 incubator for 3-5 days or until CPE progressed to more than 75%, at which stage the cells were harvested. All remaining cells were detached from the bottom of the tissue culture flask through scraping or pipetting and the total content of the flask was emptied into a 15ml Falcon tube. The cells were pelleted through centrifugation at room temperature for 10min at 2500 x g. The supernatant was decanted into a new 15ml Flacon tube, labelled as the P2 viral stock and stored at 4°C. The pellet was then used for Tri-reagent mediated viral dsRNA genome extraction.

TRIzol, also known as Tri-reagent, is a monophasic solution of phenol and guanidinium isothiocyanate that is commercially available for the solubilisation, extraction and deproteinizing of RNA (Rio *et al.*, 2010). The reagent maintains RNA integrity during tissue homogenizing whilst simultaneously solubilizing biological material and denaturing proteins. It is especially useful in protecting RNAs from endogenous RNases during cell breakdown and homogenizing. It is used in conjunction with chloroform to preform organic-phase:aqueous-phase extraction during which proteins are extracted to the organic phase, DNA resolves at the interface, and RNA remains in the aqueous phase.

For RNA extraction the cell-pellet was re-suspended in 750µL TRIzol® (Invitrogen, Carlsbad, California, USA), followed by the addition of 200µL chloroform. The sample was vortexed and incubated at room temperature for 5min, after which the sample was centrifuged for 15 - 30min at 16 000g at 4°C. The aqueous phase containing the RNA was carefully removed, after which an equivalent volume of isopropanol was added to it, thoroughly mixed and allowed to stand for 5-10min to facilitate RNA precipitation.

RNA is pelleted through centrifugation at 16 000g for 15-30min followed by removal of supernatant and drying of the pellet through another 10min centrifugation at 16 000g. Any remaining supernatant was carefully removed via pipetting and the pellet was air-dried for 5min. The RNA pellet was then dissolved in 100µl elution buffer (EB; Qiagen) for 15-30min at room temperature with intermittent mixing.

ssRNA was precipitated by adding lithium chloride (LiCl) to a final concentration of 2M to the sample, followed by overnight incubation at 4°C. ssRNA was pelleted through centrifugation at 16 000g at 4°C for 15 - 30min, after which the supernatant containing the dsRNA was moved to a new sterile PCR tube. The dsRNA could not be visualised through AGE at this stage as the LiCl in the sample distorts the gel and results in smears during separation. Before being visualized and used for sequence-independent primer ligation and cDNA synthesis the dsRNA was first purified using the MEGAclear™ kit (Invitrogen).

Before first use of the kit 20ml of 100% ethanol was added to the washing buffer and correspondingly labelled. The LiCl containing dsRNA sample was brought up to 100ul with elution buffer and transferred to a sterile, low-bind 1,5ml Eppendorf micro-centrifuge tube. Following this, 350µl of the provided binding solution concentrate was added and mixed through pipetting after which 250µl of 100% ethanol was added and again thoroughly mixed through pipetting. The solution was then transferred to the provided binding column which in turn was then placed into a 2ml collection tube. The sample was centrifuged at 13 000rpm for 60 seconds during which the RNA was bound to the membrane. The flow-through was discarded and the membrane was washed twice with 500ul of washing solution through centrifugation for 60 seconds, after which the flow-through was discarded. The sample was centrifuged again without the addition of any buffers for 60 seconds to remove any residual buffers from the membrane and to prepare it for elution.

RNA was eluted from the membrane by adding 30ul of 95°C nuclease-free H_2O to the column and incubating it for 5min after which it was centrifuged for 60 seconds at 13 000rmp. This elution step was repeated twice yielding 60ul of purified dsRNA of which 3ul was used for visualization through AGE (Section 2.2.7).

The purity and concentration of the dsRNA were determined by spectrophotometry (Section 2.2.6). All centrifugation steps were performed in an Eppendorf 2J20 bench centrifuge.

3.2.2 Polyacrylamide gel electrophoresis (PAGE)

For higher resolution evaluation of dsRNA, either from viral genome extraction or from various dsRNA ligation reactions, RNA-PAGE was used instead of the standard AGE approach. Although reducing PAGE techniques (such as sodium dodecyl sulphate polyacrylamide gel electrophoresis, SDS-PAGE) is traditionally used for the separation of proteins, the technique can be applied to the separation of nucleic acids, especially RNA. The basic principles of AGE and PAGE are the same with charged molecules moving through a matrix with various pores. The larger the molecule, the more resistance is provided by the matrix and the less it moves, correspondingly the smaller the molecule the less resistance is experienced and the further along with the gel it moves, thus causing separation. In RNA-PAGE the pores are formed by cross-linking various polyacrylamide chains together using bifunctional agents such as N, N`-methylene-bis-acrylamide. The concentrations and ratios of these base reagents determine the density of the gel and the pore size.

For this experiment, the separation of dsRNA was done with a 5% stacking, and 12% resolving gel at 130V. The standard Laemmli (1970) method, as described in Sambrook and Russell (2001), was used to construct and run the PAGE gel. The resolving gel was prepared first (Table 11) and cast into an assembled 0.75mm Bio-Rad Mini Protean gel casting apparatus (BIORAD). The gel was then covered with H_2O-saturated isobutanol to prevent the diffusion of O_2 into the gel (which would inhibit polymerization) and allowed to fully polymerize at room temperature for 30-60 min. Following this, the H_2O-saturated isobutanol is removed and the top of the gel is washed with deionized H_2O to remove any unpolymerized acrylamide. Any remaining H_2O is removed with Whattman filter paper, and the stacking gel is prepared (Table 11) and cast onto the resolving gel.

Table 11: Preparation of resolving and stacking gel for RNA-PAGE

	12% Resolving gel		5% Stacking Gel	
Stock reagent	Volume (ml)	Final concentration	Volume (ml)	Final concentration
40% Acrylamide mix	4.5	12%	0.75	5%
1.5 M Tris (pH 8.8)	3.8	0.38 M	N/A	N/A
1.0 M Tris (pH 6.8)	N/A	N/A	0.75	0.125 M
10% SDS	0.15	0.1%	0.06	0.1%
10% APS	0.15	0.1%	0.06	0.1%
TEMED	0.006	0.04 %	0.006	0.1%
H_2O	6.4 ml	-	4.35	-
Total	15 ml	-	6 ml	-

The acrylamide mixture and Ammonium persulfate (APS) reagents were obtained from Sigma, whilst the tetramethylethylenediamine (TEMED) was obtained from Merck.

The comb is inserted into the electrophoresis apparatus directly after the stacking gel has been cast, making sure not to trap any air bubbles, and left to fully polymerize at room temperature for 30-60min. After polymerization has occurred, the comb was gently removed and the wells are washed with deionized H_2O. The gel was then mounted into a Bio-Rad electrophoresis apparatus and place into the running tank filled with TGS buffer (25 mM Tris, 250 mM Glycine, 0.1% (m/v) SDS, pH 8.3). Samples were electrophoresed at 130V for 60min after which gels were fixed and stained.

3.2.3 Silver staining

Silver staining of RNA-PAGE gels was selected for this project as the technique is more sensitive to low concentration nucleic acids than conventional ethidium bromide (EtBr) staining methods, and could more clearly visualize experiments with low RNA yields (such as ligation reactions and RNA purification steps). The Silver Stain Plus (Bio-Rad) staining kit was used throughout the project. This kit is based on the original Gottlieb and Chavko method (Gottlieb & Chavko, 1987), and entails the fixation of the RNA, followed by staining through the use of carrier-complex chemistry, and finally development of the stain and stopping of the resolving (visualization) reaction.

Following electrophoresis, gels were fixed by submersion in the fixing solution (50% v/v reaction grade methanol, 10% v/v glacial acetic acid, 10% v/v Silver Stain Plus

fixative enhancer concentrate, and 30% v/v deionized distilled water). For two standard RNA-PAGE mini gels, 400ml of this solution was sufficient. Gels were submerged and incubated at room temperature with slight agitation for 20min, after which the fixing solution was removed and the gels were rinsed twice with 400ml of deionized water for a further 20min.

Preparation of the staining and developing reagents took place no more than 5min before staining and all staining steps took place in a pre-prepared glass staining vessel. The staining reaction mixture was prepared by adding 5ml of each of the following Silver Stain Plus reagents to 35ml of deionized water directly prior to each reaction; silver complex solution, reduction moderator solution and image developing reagent. To this mixture directly before use 50ml of Silver Stain Plus, development accelerator solution was added, mixed well through swirling and added to the fixed gels. Gels were incubated at room temperature for 20min with slight agitation.

The staining and development reactions took place simultaneously and were stopped after 20min with a 5% acetic acid incubation step. The staining and development reaction solution was removed from the gel and 400ml of the stopping solution (5% glacial acetic acid v/v in deionized distilled water) was added. This reaction was incubated at room temperature for 15min with slight agitation. Finally, the stopping solution was rinsed off with 400ml of high purity water for 5min and the gels were photographed.

3.2.4 Sequence-independent cDNA synthesis and genome amplification

For sequence-independent cDNA synthesis and viral genome amplification and sequencing, 12µl of the MEGAclear purified dsRNA (Section 2.2.14) was used in the PC3-T7 oligonucleotide ligation reaction (Potgieter *et al.*, 2009). The ligation reaction contained the following: 300ng PC3-T7 loop oligonucleotide (5'-p–GGATCCCGGGAATTCGGTAATACGACTCACTATATTTTTATAGTGAGTCGTATTA–OH-3', TIB MOLBIOL, Berlin, Germany), 12µl purified dsRNA, 50 mM HEPES/NaOH (pH 8.8; Sigma), 18mM $MgCl_2$, (Sigma, St. Louis, Missouri, United States), 0.01% bovine serum albumin (BSA; TaKaRa, Kusatsu, Shiga Prefecture, Japan), 1 mM ATP (Roche, Basel, Switzerland), 3 mM DTT (Roche), 10% DMSO (Sigma), 20% PEG_{6000}

(Calbiochem, Sigma), and 30 U T4 RNA ligase (TaKaRa). The reaction was thoroughly mixed and incubated at 37°C overnight. The ligated RNA was then purified using the MEGAclear kit and yielded 30µl purified PC3-T7 loop-ligated dsRNA which was used for cDNA synthesis.

Directly prior to cDNA synthesis 15ul of the PC3-T7 loop-ligated dsRNA was denatured through the addition of 1ul methyl mercury hydroxide (MMOH; Alfa Aesar, Haverhill, Massachusetts, USA) to a final concentration of 30mM, and incubation at room temperature for 30min in a fume hood. This denaturing step was directly followed by a reverse transcription reaction that contained: 1mM dNTPs, 1X Transcriptor High Fidelity buffer (Roche), 30mM 2-mercaptoethanol, 0.5U RNase inhibitor (Roche) and 10U Transcriptor High Fidelity Reverse Transcriptase (Roche). The reaction was mixed well and incubated for 45min at 42°C, immediately followed by a 15min incubation at 55°C. During the initial 42°C incubation, the mercaptoethanol reduced the methyl mercury hydroxide and allowed the reverse transcriptase to synthesise cDNA from ssRNA. The final 55°C incubation step de-activated the reverse transcriptase and prepared the sample for the next step.

The excess dsRNA was removed through the addition of 1M NaOH to the sample to a final concentration of 0.1 M, followed by incubation at 65°C for 30min. This was followed by addition of 1M HCl to a final concentration of 0.1 M and 1 M Tris/HCl (pH 7.5) to a final concentration of 0.1 M, and incubation at 65°C for 60min. The viral genomic cDNA was then amplified through polymerase chain reaction (PCR) using the PC2 primer (5'-p–CCGAATTCCCGGGATCC-3' from TIB MOLBIOL). The PCR reaction mixture contained: 5ul TaKaRa Ex Taq 10x reaction buffer, 4ul 25mM TaKaRa Ultrapure dNTPs, 1ul PC2 primer (50pmol/ul), 0.6ul TaKaRa Ex-Taq High Fidelity DNA polymerase (5 units/µl) and 10ul of the cDNA synthesised in the previous step. The reaction was brought to a final volume of 50ul with nuclease-free H_2O and placed in a thermocycler (T100 Thermal Cycler, Biorad, California, USA). All TaKaRa products were purchased from Clontech (Clontech, TaKaRa, Kusatsu, Shiga Prefecture, Japan).

PCR conditions were as follows: initial incubation at 72^{o}C for 1 minute to fill in cDNA ends to ensure amplification of the entire cDNA segment. This was followed by the initial denaturation at 94^{o}C for 2 minutes followed by 25 - 30 cycles of denaturation at

94ºC for 30 seconds, annealing at 65ºC for 30 seconds and extension at 72ºC for 4 minutes. Lastly, the final elongation step was 72°C for 5min and then storage at 4°C until retrieved. The PCR product was then analysed through AGE (Section 2.2.7) before being sent through for NGS (Section 2.2.14).

3.2.5 Genome segment-specific cDNA synthesis using SuperScript One-step RT-PCR kit

Genome segment-specific (gs-specific) cDNA synthesis and PCR amplification were done in one step with the SuperScript III One-step RT-PCR Platinum kit (Invitrogen, Carlsbad, California, USA) using gs-specific primers. The kit was optimized for the amplification of RNA fragments between 200 and 4500bp from a starting concentration of as little as 0.1pg. The One-step reaction mixture was set up on ice in a PCR tube and contained: 25ul of the 2x reaction buffer, 1ul of both the sense and anti-sense primers (0.2 uM), 10ul of purified template RNA (between 0.2ug and 1ug) and 2ul of the SuperScript RT: Platinum Tac enzyme mix. The reaction volume was adjusted to 50ul using nuclease-free H_2O and placed into a thermocycler.

The cycling conditions were as follows: 1 cycle of cDNA synthesis and pre-denaturation for 30min at 50°C followed by 2min at 94°C. Next, 40 cycles of denaturing at 94°C for 30 seconds followed by annealing at 60°C for 30 seconds and extension at 68°C for 4min. The last step was 1 cycle at 68°C for 10min for the final extension. The PCR product was then analysed through AGE (Section 2.2.7) and sent for NGS (Section 2.2.14).

3.2.6 Gel extraction and PCR clean-up

All gel extraction steps in this project were performed using the MiniElute® Gel extraction kit (Qiagen, Whitehead Scientific). Gel extraction was only required for procedures involving In-Fusion®HD cloning reactions. Before the first use of the kit 24ml of 100% pure ethanol was added to the EP buffer and correspondingly labelled. Following AGE (Section 2.2.7) the gel is viewed on a Dark reader and the desired

band is excised from the gel using a sterile scalpel blade and placed in a pre-weighed 2ml RNase, DNase free micro-centrifuge tube. It is important that the gel not be exposed to the high-intensity light that visualizers such as the ChemiDoc use as these wavelengths will damage the DNA during visualization. During excision of the desired band, it is also important to remove as much as possible of the agarose gel from the sample as the reaction volume for the following steps is based on the weight of the gel fragment.

QC buffer is added to the fragment to a volume three times more than its weight (3ul of buffer per ug of gel). The sample is then incubated in a heating block at 50°C for an hour (extended incubations have been correlated with increased yield) with vortexing every 10-15min to ensure complete homogenization. The QC buffer is a chaotropic salt buffer that aids in the breakdown of the agarose gel and provides the high salinity environment that is required for binding of the DNA to the silica membrane. The QC buffer also contains a pH indicator that will change from orange to violet if the pH is too high. Optimal pH for binding of the DNA to the membrane is 7.5.

After the gel fragment is completely dissolved a volume of isopropanol is added to the mixture equal to the weight of the gel and mixed thoroughly. The entire sample volume is then transferred to a MiniElute column which in turn is placed in a collection tube. The sample is then centrifuged for 60 seconds to bind the DNA to the membrane. The flow-through is discarded and an additional 500ul of QC buffer is added to the column and again centrifuged for 30 seconds to remove any traces of the agarose gel. After this, the sample is washed with 750ul of PE buffer and gain centrifuged for 30 seconds. The flow-through is discarded and the column is again centrifuged without the addition of any buffers to remove any residual buffers from the membrane and prepare it for elution.

Finally, the column is moved to a new sterile low-bind micro-centrifuge tube and the DNA is eluted with 30ul of pre-heated nuclease-free water. The 75°C water is placed on the membrane and incubated for 60 seconds after which it is centrifuged at 11 000g for one minute. The extracted DNA was evaluated through AGE (Section 2.2.7) and quantified through spectrophotometry (Section 2.2.6). All centrifugation steps were carried out in an Eppendorf 2J20 bench centrifuge.

All PCR clean-up steps in this project were performed using the MiniElute® PCR clean-up kit (Qiagen, Whitehead Scientific). This kit is designed for the purification of PCR products ranging from 70bp to 4000bp. The kit removes extraneous nucleotides, primers and polymerases from the sample and is based on the same principles than that of gel extraction. The procedure is however only recommended for PCR products that show a clear/well-defined band with very little to no background or non-specific amplification. If the sample meets these requirements its volume is adjusted to 100ul with nuclease-free water and 300ul of QC buffer is added to it and mixed through light vortexing. The rest of the procedure is the same as that for gel extraction.

3.2.7 In-Fusion® HD cloning

In-Fusion® HD cloning is a commercial cloning system based on the FastCloning protocol (Li *et al.*, 2011). In-Fusion can be described as a directional, ligation-independent cloning method that allows fast, single-tube cloning with high cloning accuracy. The system requires the linearization of both the insert and backbone through either restriction enzymes digest or through PCR amplification using primers designed to have overlapping complementary regions. In this project, PCR was selected for the linearization of both the inserts and plasmid backbone.

All In-Fusion cloning reactions were set-up in accordance with the manufacturer's (Clontech, Takara) specifications and only PCR linearized and gel-extracted amplicons were used to reduce the likelihood of off-target results. The standard In-Fusion reaction contains 200ng of insert DNA, 100ng of backbone DNA and 2ul of the 5x In-Fusion HD enzyme premix. The reaction volume is then adjusted to 10ul using nuclease-free water and mixed through pipetting.

The reaction mixture is incubated in a thermocycler (T100 Thermal Cycler, Biorad, California, USA) at 50°C for 15min and then placed on ice until the transformation step. Extended incubation times will not increase efficiency and could, in fact, lead to a reduction in overall yield. After the 15min incubation the inserts and backbones should be hybridised and in the form of closed, circular plasmids with nicks in the phosphate backbone. The In-Fusion HD enzyme premix contains 3`-exonucleases that digest the PCR products yielding long single-stranded regions that can hybridise

with their complementary sequences. Upon transformation, the nicks in the plasmid backbone are repaired by the bacterial cells in the presence of selective pressure. Provided with the In-Fusion HD cloning kit are chemically competent Stellar cells which were meant to be used after In-Fusion. Optimal transformation efficiency requires the use of 5ul of the In-Fusion reaction along with 50ul of the Stellar cells. Standard transformation protocol is followed with the Stellar cells instead of DH5α (Section 2.2.1).

3.2.8 $TCID_{50}$ assay

The purpose of the $TCID_{50}$ assay is to determine the infective dose at which 50% of the cell-culture would die. This is achieved through serial dilution of the selected viral stock and passage onto prepared cell monolayers in a 96-well plate. The viral stock is incubated to facilitate viral attachment and then replaced with propagation media (Sections 2.2.8 and 2.2.13). The 96-well plate is then incubated for a set time after which viral propagation is visualized through IFMA.

For further detail and illustrations of the equations used see Appendix D. For each $TCID_{(50)}$ MA104 cells were grown in a 96-well plate (LabTec, Nunc, Thermo scientific) to roughly 85% confluence. The viral stock in question was then diluted in serial 10-fold dilutions from 10^0 through to 10^{-7}. The growth media of the MA104 cells was removed and the serial dilution of the viral stock was used to infect the cells with each dilution being represented by 6 adjacent wells.

After an hour incubation, the viral dilution was removed and 100ul of propagation media was added to each well.

The 96-well plate was incubated for 5 days and viral propagation was investigated through IFMA (Section 2.2.12). The relative viral titers were expressed as 50% of the tissue culture infective dose ($TCID_{50}$) as per methods described by Reed and Muench (REED & MUENCH, 1938).

Equation 3: Calculation of relative $TCID_{50}$/ml from 96-well IFMA results

$$\text{Proportionate Distance (PD)} = \frac{(\text{Next \% higher than 50}) - 50\%}{(\text{Next \% higher than 50}) - (\text{Next \% lower than 50})}$$

$$\text{End-point dilution (EP)} = \text{Log}_{10}(\text{lowest positive dilution})$$

$$\text{Log}_{10}(TCID_{50}) = (PD + EP) \text{ thus } TCID_{50} = \frac{1}{10^{(PD + EP)}}$$

3.3 Results and Discussion

3.3.1 PCR amplification of SA11-N5 genome segments for pSmart In-Fusion

As mentioned in the introduction, my MSc overlapped with the last part of that of L. Geldenhuys resulting in us working in parallel to generate a complete set of pSmart_SA11-N5 RV RG rescue plasmids. To familiarize myself with the techniques involved in In-Fusion sub-cloning, and to serve as a control for the work done by Geldenhuys, I decided to generate my own complete set of pSmart_SA11-N5 RV RG rescue plasmids. The first step of this process was the amplification of the 11 SA11-N5 genome segments from the consensus sequences, cDNA transcription plasmids designed by Dr Wentzel (Figure 51, Appendix C). The primers designed by Geldenhuys for this purpose had a wide range of proposed annealing temperatures and several of the primer pairs had very different annealing temperatures. For this reason, I decided to first perform a temperature gradient PCR amplification (Figure 24) of each of the 11 SA11-N5 genome segments using the primers listed in Table 12. The temperatures selected were 53.4, 58.3, 63.7 and 68.0°C, based on the average annealing temperatures proposed for the primers, and 50°C selected for amplification of the pSmart backbone.

Table 12: Optimal annealing temperature and primers for pSmart, SA11-N5 In-Fusion reaction

SA11-N5 GS	Amplicon size (bp)	Forword primer	Reverse primer	optimal annealing temp. (°C)
GS1	3334	5'-TAATACGACTCACTATAGGCTATTAAAGCTG-3'	5'-GGGACCATGCCGGCCGGTCACATCTAAGCG-3'	63,7
GS2	2687	5'-TAATACGACTCACTATAGGCTATTAAAGGCTC-3'	5'-GGGACCATGCCGGCCGGTCATATCTCCAC-3'	58,3
GS3	2585	5'-TAATACGACTCACTATAGGCTATTAAAGCAG-3'	5'-GGGACCATGCCGGCCGGTCACATCATGACTAG-3'	53,4
GS4	2356	5'-TAATACGACTCACTATAGGCTATAAAATGGC-3'	5'-GGGACCATGCCGGCCGGTCACATCCTCTAG-3'	68,0
GS5	1608	5'-TAATACGACTCACTATAGGCTTTTTTTTTGAAAAG-3'	5'-GGGACCATGCCGGCCGGTCACATCCTCTAG-3'	58,3
GS6	1350	5'-TAATACGACTCACTATAGGCTTTTAAACGAAG-3'	5'-GGGACCATGCCGGCCGGTCACATCCTCTCAC-3'	63,7
GS7	1099	5'-TAATACGACTCACTATAGGCATTTAATGC-3'	5'-GGGACCATGCCGGCCGGCCACATAACGCCC-3'	58,3
GS8	1053	5'-TAATACGACTCACTATAGGCTTTTAAAGCG-3'	5'-GGGACCATGCCGGCCGGTCACATAAGCGC-3'	58,3
GS9	1057	5'-TAATACGACTCACTATAGGCTTTAAAAAGAG-3'	5'-GGGACCATGCCGGCCGGTCACATCATACAATTC-3'	58,3
GS10	745	5'-TAATACGACTCACTATAGGCTTTTAAAAGTTC-3'	5'-GGGACCATGCCGGCCGGTCACATTAAGACC-3'	58,3
GS11	660	5'-TAATACGACTCACTATAGCTTTTAAAGCGC-3'	5'-GGGACCATGCCGGCCGGTCACAAAACGGGAG-3'	63,7
pSmart	2018	5'-GGCCGGCATGGTCCCAGCCTCCTCGC-3'	5'-TATAGTGAGTCGTATTAGATATC-3'	50,0

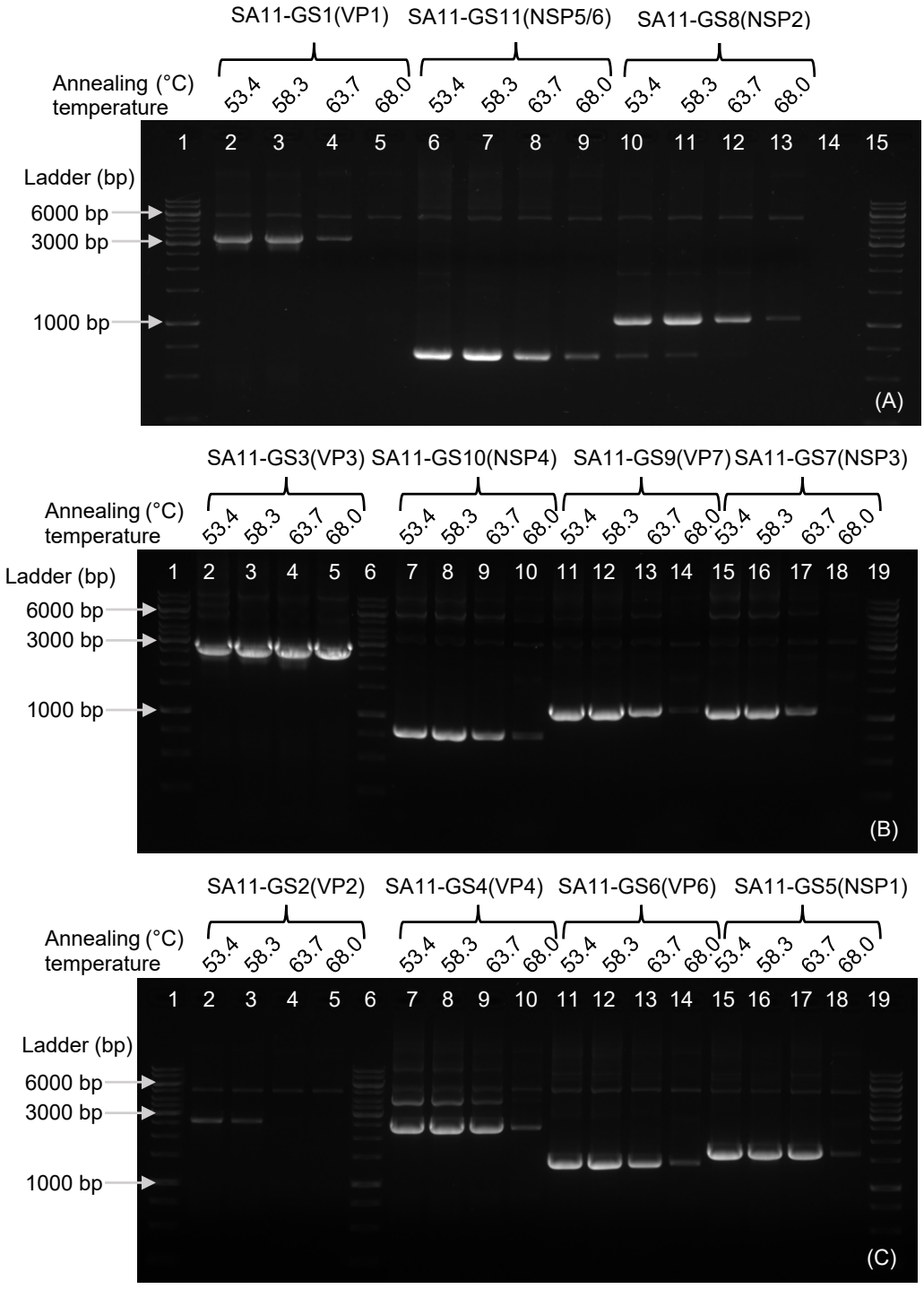

Figure 24: Agarose gels of temperature gradient PCR amplicons from pAlpha (A), pBeta (lanes 2-5, B and C), pDelta (lanes 7-18, B) and pGamma (lanes 7-18, C). A) Lanes 1 & 15: Ladder, 2-5: GS1 (VP1), 6-9: GS11 (NSP5/6), 10-14: GS8 (NSP2). **B)** Lanes 1, 6 & 19: Ladder, 2-5: GS3 (VP3), 7-10: GS10 (NSP4), 11-14: GS9 (VP7), 15-18: GS7 (NSP3). **C)** Lanes 1, 6 & 19: Ladder, 2-5: GS2 (VP2), 7-10: GS4 (VP4), 11-14: GS6 (VP6), 15-18: GS5 (NSP1).

Figure 24 illustrates the agarose gels of the temperature gradient PCR amplicons from the SA11-N5 cDNA genome segment carrying pAlpha (A), pBeta (lanes 2-5 in B and C), pDelta (lanes 7-18, B) and pGamma (lanes 7-18, C) plasmids. Optimal annealing temperatures (Table 12) were determined as the temperature at which both the highest amplicon yield was generated and the lowest amount of non-specific amplification was detected. Faint non-specific amplification can be seen in every reaction (Figure 24, A-C C) irrelevant of annealing temperature, indicating consistent and conserved interactions between the genome segment-specific primers and the pAlpha, pBeta, pDelta and pGamma plasmid backbones. This hypothesis is further supported by the fact that the majority of the non-specific amplification is consistent across each reaction and of a similar size (roughly 6000bp). Considering the variations in the pAlpha, pBeta, pDelta and pGamma plasmids, as well as that of the genome segment-specific primers, the non-specific binding must have occurred in conserved regions shared by each of the cDNA carrying plasmids (ergo the backbone) and in conserved regions shared by each of the primers (In-Fusion specific tails required for pSmart hybridization during sub-cloning). This is a necessary design flaw that is required for later In-Fusion sub-cloning and cannot be removed. This also accounts for the wide range in annealing temperatures amongst the In-Fusion primers and also necessitates agarose gel extraction and purification of the specific SA11-N5 genome segment amplicons prior to In-Fusion.

As expected, the lower the annealing temperature, the higher the yield of the genome segment amplicons, and the higher the non-specific amplification. Correspondingly less amplification was observed as the annealing temperature increases. This was true for each genome segment amplification, except for GS2, GS3, GS4 and GS8, which each show slight irregularities. For GS2 (VP2), Figure 24, C (lanes 2-5): Very low amplification was observed even at the lowest annealing temperatures. This is most likely due to the significant differences in melting point between the forward (59.2 °C) and reverse (64.1 °C) primers. Additionally, the low yield could be contributed to primer decay, however, each of the In-Fusion primer sets was ordered at the same time and kept under the same conditions, and no other primer set shows this kind of discrepancy. For GS3 (VP3), Figure 24, B (lanes 2-5): No distinctive difference was observed between the various annealing temperatures, indicating a high level of specificity and low efficient annealing temperature.

For GS4 (VP4), Figure 24, C (lanes 7-10): Unexpectedly high levels of non-specific amplification present at lower temperatures, with only the highest (68.0 °C) indicating a decrease. The non-specific amplicon at roughly 4000bp is most likely due to off-target binding of the GS4 In-Fusion primer in addition to the expected background amplification at roughly 6000bp. This off-target binding was eliminated at 68.0°C. For GS8 (NSP2), Figure 24, A (lanes 10-13): Unintentional amplification of GS11 (NSP5/6) present at lower temperatures (53.4 and 58.3°C). This is most likely due to off-target binding of the GS8 In-Fusion primers to the NSP5/6 genome segment at low temperatures. This off-target binding was eliminated at 63.7°C. Overall the amplifications were successful and the optimal annealing temperatures were recorded in Table 12.

3.3.2 Completion of the GS8 (NSP2), GS9 (VP7) and GS11 (NSP5/6) pSmart_SA11-N5 RV RG plasmids through In-Fusion HD cloning

As mentioned in the introduction this part of my MSc overlapped with that of L. Geldenhuys's MSc as we were working in parallel to complete the pSmart_SA11-N5 RV RG plasmid set. At this stage I have already amplified, gel extracted and purified each of the SA11-N5 consensus sequence genome segments in preparation for In-Fusion HD cloning. During this stage of my MSc however, the sequencing results of the pSmart_SA11-N5 RV RG rescue plasmids constructed by Geldenhuys were returned. The sequencing results indicated that Geldenhuys successfully sub-cloned SA11-N5 GS1 through GS7, and GS10 into the pSmart backbone with only GS8, GS9 and GS11 containing incorrect sequences. As a result of this, it was decided that my efforts should be focused on the finalization of these three constructs instead of the construction (and sequence verification) of my own entire pSmart_SA11-N5 RV RG plasmid set. This experiment, therefore, entails the correction of the 5`-GGG-, and -CC-3` ends of the pSmart_SA11_GS11 (NSP5/6) construct, as well as the reconstruction of the pSmart_SA11_GS8 (NSP2) and pSmart_SA11_GS9 (VP7), constructs using the In-Fusion HD cloning technique (Section 3.2.5).

For GS11 (NSP5/6) new In-Fusion seamless cloning primers were designed to amplify the GS from the pAlpha (Figure 51) plasmid whilst also correcting the 5`- and -3` termini (Table 12). For GS8 (NSP2) and GS9 (VP7), the standard In-Fusion primers designed by L. Geldenhuys was used for amplification. In all three instances, the pSmart backbone was amplified using the corresponding In-Fusion primers and each amplicon was visualized through AGE (using a Dark reader) and gel extracted before In-Fusion. Following the standard In-Fusion protocol (Section 3.2.5) overnight plates were evaluated for colony formation and five random colonies were selected (Section 2.2.3) for propagation and endotoxin-free plasmid extraction (Section 2.2.4).

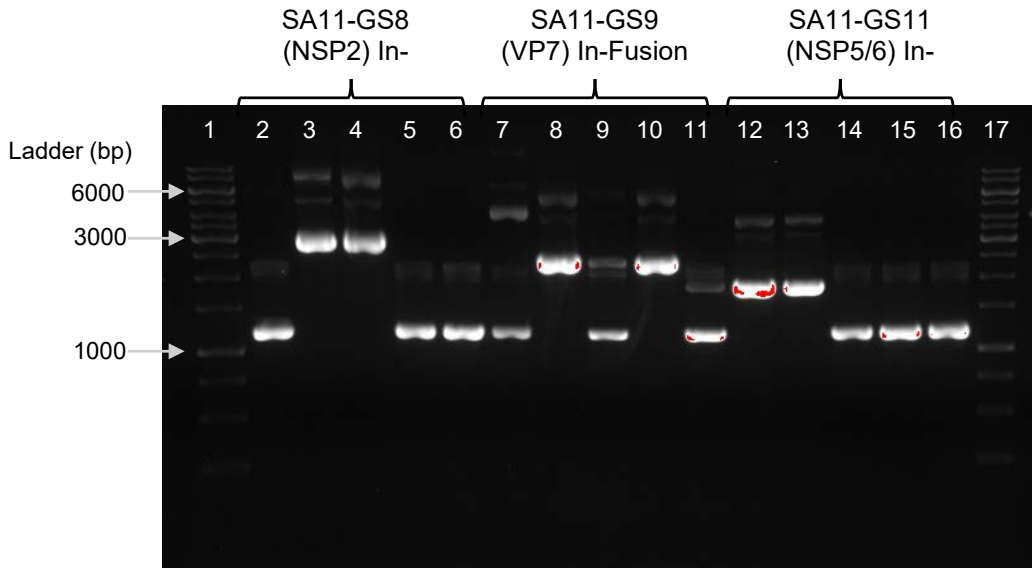

Figure 25: Agarose gel of plasmids extracted following In-Fusion HD cloning colony selection. Lanes 1 & 17: Ladder. Colonies selected from In-Fusion reactions for GS8: lanes 2-6, GS9: lanes 7-11 and GS11: lanes 12-16.

Figure 25 shows the agarose gel of the extracted plasmids from the selected In-Fusion reaction colonies. Based purely on the electrophoretic patterns of the supercoiled plasmid bands 4 distinct groups can be identified. Group I contains the bands present in lanes 2, 5-7, 9, 11 and 14-16, each appearing to be of the same size and having a similar electrophoretic pattern, suggesting a similar composition. As lanes 2-6, 7-11 and 12-16, represent three distinct In-Fusion reactions, the only explanation of group I plasmids being similar is that group I represents the empty pSmart backbone resulting from the linearized backbone closing onto itself without incorporating any of the amplified SA11-N5 genome segments during In-Fusion.

The bands present in lanes 3 and 4 are similar and form group II. Lanes 8 and 10 together from group III and group IV is represented by the plasmids present in lanes 12 and 13. Again based purely on the electrophoretic patterns of these plasmids it was determined that group II represented plasmids that potentially incorporated GS8 (NSP2) into the pSmart backbone, group III represented the incorporation of GS9 (VP7) and group IV represented the incorporation of GS11 (NSP5/6). Comparison of the relative positions of groups II, III and IV to one another and to group I further supported this hypothesis as group II is the largest and contains the largest of the inserts, GS8 (NSP2). Correspondingly group III is slightly lower as it contains GS9 (VP7), and group IV is the lowest (except for group I that is presumed to be the empty backbone) and contains the smallest insert, GS11 (NSP5/6). The colonies that represented groups II, III and IV were cultured overnight for endotoxin-free plasmid extraction (to be transfection ready), glycerol stock preparation and plasmid sequence verification.

The apparent low efficiency of this experiment (9 out of 15 reactions returning an empty plasmid backbone) can be attributed to parental plasmid carry-over, non-optimal In-Fusion ratios or non-representative colony selection. Parental plasmid carry-over refers to the presence of template plasmid (used during PCR linearization in preparation for In-Fusion) in the In-Fusion reaction. The template plasmid would then be transfected along with the In-Fusion products and could outcompete the In-Fusion product over the incubation period. This can be addressed through the incorporation of a DpnI restriction enzyme digestion step before the In-Fusion reaction to eliminate the methylated parental plasmids from the reaction. Non-optimal In-Fusion ratios refers to the ratio of the vector (backbone) to insert during the In-Fusion reaction. This ratio is based on the relative molar amounts of both the vector and inserts and is influenced by GC-content, bp length and the hybridization regions in both the vector and insert (Section 3.2.6). These ratios can be experimentally optimized for each reaction, however, this would have been an unnecessary expenditure during this experiment and was thus not attempted.

Finally, the apparent low efficiency of the experiment could have been due to the random selection of colonies that did not fully represent the total experimental results. Due to time and financial restraints, not all of the resulting In-Fusion colonies were tested and only 5 colonies were selected for evaluation.

3.3.3 NGS sequence verification of our pSmart_SA11-N5 RV RG plasmid set

This step of the project entailed the endotoxin-free extraction of the locally developed, pSmart_SA11-N5 RV RG plasmid set followed by sequence verification through NGS (Section 2.2.14). Eight of the 11 pSmart_SA11-N5 rescue plasmids (GS1 through GS7, and GS10) were designed by L. Geldenhuys and stored as bacterial glycerol stocks. Three separate glycerol stock samples were selected for each of these eight constructs and used to inoculate 24 overnight cultures for endotoxin-free plasmid extraction (for NGS and to be transfection ready). I was in the process of constructing the remaining three plasmids (GS8, GS9 and GS11), and used the overnight cultures described in Section 3.3.2 for endotoxin-free plasmid extractions of the selected (group II, III and IV) In-Fusion colonies.

The overnight cultures comprised of 100ml standard LB medium (Melford Laboratories Ltd. Bildeston Road, Suffolk, U.K) supplemented with carbenicillin (Melford, U.K) to a final concentration of 100µg/ml, and incubated at 37°C with shaking at 250rpm for 16hours. Plasmids were extracted (Section 2.2.5), concentration determined (Table 13) through NanoDrop spectrophotometry (Section 2.2.6) and a 2ul sample of each was visualised through AGE (Section 2.2.7). The plasmid extracts yielded between 630.3ng/ul and 2314.4ng/ul and had acceptable $OD_{260/280}$ readings (between 1.7 and 1.9).

Figure 26 illustrates the agarose gels of the endotoxin-free plasmid extractions from the glycerol stocks of the eight pSmart_SA11-N5 RV RG constructs designed by Geldenhuys. The gels showed very clear bands representing the various configurations that plasmids can take (relaxed, supercoiled, etc.) and had homologues electrophoretic separation profiles for each of the selected glycerol stocks, except for lane 2 in gel B, which showed an unexpectedly high electrophorotype. Even though this divergent electrophoretic separation profile does not definitively confirm a plasmid miss-match, the sample represented in lane 2, gel B was excluded from further testing. Endotoxin-free plasmid extractions were also made from the overnight cultures obtained from the GS8 (NSP2), GS9 (VP7) and GS11 (NSP5/6) In-Fusion HD cloning colonies discussed in Section 3.3.2 and illustrated in Figure 26.

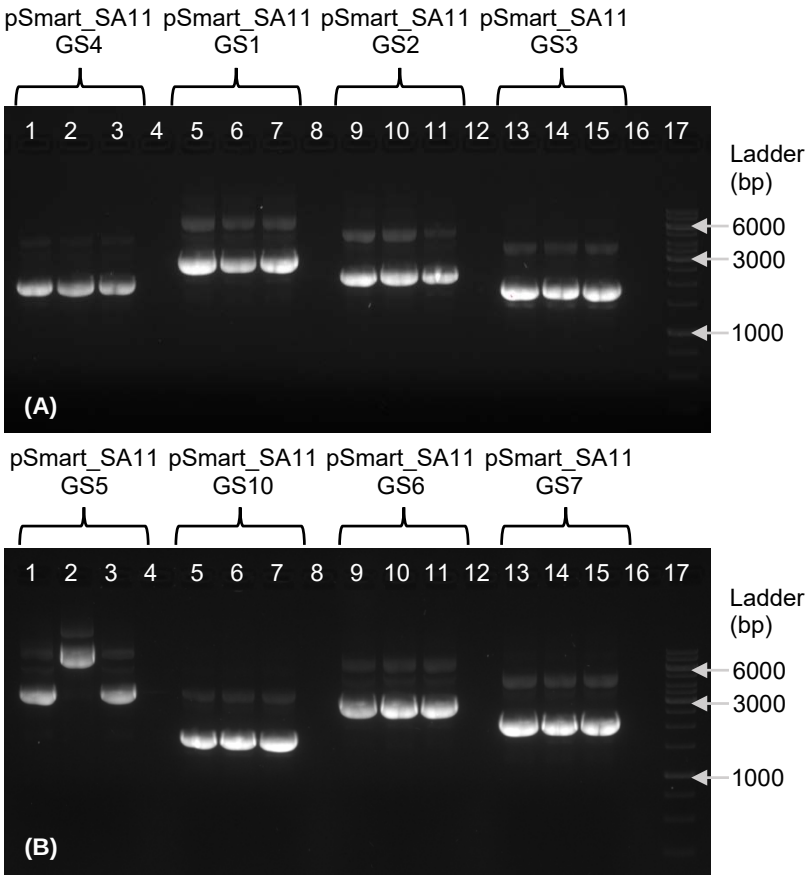

Figure 26: Agarose gels of endotoxin-free plasmid extractions of pSmart_SA11-N5 RV RG plasmids. (A & B) Lane 17: Ladder. Lanes 4,8,12 & 16 : Empty. **(A)** Lanes 1-3: pSmart_SA11_GS4 (VP4). Lanes 5-7: pSmart_SA11_GS1 (VP1). Lanes 9-11: pSmart_SA11_GS2 (VP2). Lanes 13-15: pSmart_SA11_GS3 (VP3). **(B)** Lanes 1-3: pSmart_SA11_GS5 (NSP1). Lanes 5-7: pSmart_SA11_ GS10 (NSP4). Lanes 9-10: pSmart_SA11_GS6 (VP6). Lanes 13-15: pSmart_SA11_GS7 (NSP3).

Following plasmid extraction and visualization, a 30ul sample of 100ng/ul was prepared from one of each of the 11 endotoxin-free plasmid extractions (eight from the Geldenhuys glycerol stocks, and three from the In-Fusion HD cloning experiment) and sent for individual NGS (Section 2.2.14). Although this approach was much more expensive than the mixed sample approach used during the NGS of the Japanese pT7_SA11-L2 RV RG plasmid set, it would allow a much more comprehensive analysis of the pSmart_SA11-N5 RG transcription plasmids. This individual plasmid sequencing approach allowed the precise evaluation of the 5`- and -3` termini of each individual plasmid (Section 1.4.3.3). The sequencing results were analysed on CLC-Bio workbench and fully annotated (Appendix C). Sample reads were mapped against *in silico* constructs and the consensus sequence was extracted and again aligned with the *in silico* plasmid references.

No statistically relevant deviations were obtained from the mapping and the consensus sequence of each of the plasmids aligned perfectly against the *in silico* plasmids constructs (Appendix C).

3.3.4 Implementation of pSmart_SA11-N5 RV RG system

This experiment entailed the implementation of our full, locally developed, consensus sequence-based, plasmid only pSmart_SA11-N5 RV RG system. This experiment followed the initial optimization of the pT7_SA11-L2 system (Section 2.3.5) and the finalization and sequence verification of the corrected pSmart_SA11-N5 RG constructs (Sections 3.3.2 and 3.3.3). The exact same plasmid preparation (Section 2.2.10) and transfection (Section 2.2.11) protocols used in the pT7_SA11-L2 optimized system were followed.

This system comprised of the 11 pSmart_SA11-N5 RG transcription plasmids, harbouring the 11 RV cDNA genome segments, as well as the two phCMVdream_VV capping enzyme expression plasmids and the phCMVdream_p10_FAST expression plasmid, for a total of 14 plasmids. The plasmids were each extracted and purified (Section 2.2.5) and then mixed together in equi-molar ratios with 3x higher relative amounts of the capping enzyme expression plasmids, the precise composition is annotated in Table 13 below. The negative control used in this experiment was an exact copy of the pSmart_SA11-N5 system but without the incorporation of the viral RNA-dependent-RNA-polymerase, GS1 (VP1), transcription plasmid. The rescue was confirmed through IFMA (Section 2.2.12). As illustrated in Figure 27, recombinant RV was indeed recovered from the system. Rescue from this system proved to be much easier and more reliable due to the implementation of the various optimizations tested and verified with the pT7_SA11-L2 system. The system seemed to be quite efficient as evaluated through visual indications of high viral titer. Upon confirmation of viral rescue, a comparative analysis between this system and the optimized pT7_SA11-L2 RG system was preformed through $TCID_{50}$ assay (Section 3.3.5), and the supernatant saved from the IFMA culture was propagated on MA104 cells for viral genome extraction (Section 3.2.1).

Table 13: Composition of equi-ug and equi-molar transfection mixtures

				Equi-ug	Equi-molar
Plasmid name	Plasmid size (bp)	Plasmid weight (kDa)	Working stock (ng/ul)	Plasmid required (ul)	Plasmid required (ul)
pSmart_SA11_GS1 (VP1)	5320	3930,31	1146,9	8,7	3,4
pSmart_SA11_GS2 (VP2)	4712	3554,32	1329,7	7,5	2,7
pSmart_SA11_GS3 (VP3)	4609	3491,35	1198,8	8,3	2,9
pSmart_SA11_GS4 (VP4)	4380	3350,98	1050,9	9,5	3,2
pSmart_SA11_GS5 (NSP1)	3632	2887,43	630,3	15,9	4,6
pSmart_SA11_GS6 (VP6)	3372	2730,98	2314,4	4,3	1,2
pSmart_SA11_GS7 (NSP3)	3123	2547,87	852	11,7	3,0
pSmart_SA11_GS8 (NSP2)	3125	2576,21	713,8	14,0	3,6
pSmart_SA11_GS9 (VP7)	3096	2549,74	1286,9	7,8	2,0
pSmart_SA11_GS10 (NSP4)	2769	2358,08	750	13,3	3,1
pSmart_SA11_GS11 (NSP5/6)	2684	2305,68	658,9	15,2	3,5
phCMVdream_p10_FATS	2843	3700,00	519,9	19,2	7,1
phCMVdream_VV_D1R	5090	3138,19	1352	7,4	2,3
phCMVdream_VV_D12L	3419	2107,88	1054	9,5	2,0
Total				152,4	44,6

			p=10ug stock	p=1x10^{-9} mol stock
Total DNA per transformation (ug)	Equi-ug mixture (ng/ul)	Equi-molar mixture (ng/ul)	Volume equi-ug mixture / transformation (ul)	Volume equi-molar mixture / transformation (ul)
30	1125	1409	26,67	21,29

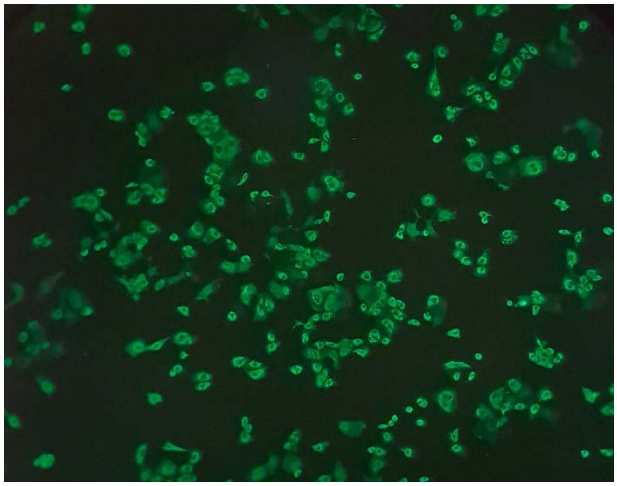

Figure 27: IFMA of equi-molar transfection of pSmart_SA11-N5 RG system in BSR-T5/7 cells with the pDream_VV_capping plasmids, co-seeded and propagated with ST cells. IFMAs done on CPE presenting cells 3 days after P1 passage. 400x magnification.

The incubation time between the initial passage and IFMA was shortened from roughly 5 days to 3, due to the excessive CPE formed in the pT7_SA11-L2 experiment where BSR-T5/7 cells were used as primary transfection cell line (Section 2.3.5). In that experiment, the advanced CPE made IFMA visualization of initial infection and propagation difficult.

3.3.5 $TCID_{50}$ comparison of basic pSmart_SA11-N5, original pT7_SA11-L2 and optimized pT7_SA11-L2 RV RG systems

In this experiment, the relative viral titer of each of the selected RV RG systems was evaluated through $TCID_{50}$ assay directly after confirmation of viral rescue via IFMA. As stated in Section 2.2.12, the supernatant of a CPE presenting culture is stored as a P1 viral stock prior to IFMA. These P1 stocks were then used for the construction of a dilution series as described in Section 3.2.6. MA104 cells were seeded into a 96-well plate for the $TCID_{50}$, infected with the viral dilution series (see Appendix D for a more detailed description of the preparation process) and incubated for 3 to 5 days. Following this incubation viral propagation was evaluated via IFMA and annotated in a calculation table such as Table 14, Table 15, and Table 16 below. Any fluorescent signal obtained from a well was viewed as viral propagation and annotated as “+”. Correspondingly if no fluorescent signal was found in a well it was deemed virus free and annotated as “-“. Calculations of $TCID_{50}$ and $TCID_{50}$/ml were done in Excel using the equations annotated in Equation 1. The relative $TCID_{50}$/ml obtained were used to construct Figure 28, a bar chart used for visual analysis and comparison of the various RG systems.

Table 14: $TCID_{50}$ calculation table for optimized pT7_SA11-L2 RV RG system

Jap_NWU-opti	pT7_SA11-L2 with NWU capping & FAST equi-molar set						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	Dilution factor
	IFMA results											
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	11	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	7	0	100	1,E-01
10^{-2}	+	-	+	+			3	1	3	1	75	1,E-02
10^{-3}	-	-	-	-			0	4	0	5	0	1,E-03
10^{-4}	-	-	-	-			0	4	0	9	0	1,E-04
10^{-5}	-	-	-	-			0	4	0	13	0	1,E-05
10^{-6}	-	-	-	-			0	4	0	17	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	21	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	25	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	29	0	1,E-09
$TCID_{50}$			2,15E+02									
$TCID_{50}$/ml			2,15E+03									

Table 15: $TCID_{50}$ calculation table for original pSmart_SA11-N5 RV RG system

NWU_Ori	Full pSmart_SA11-N5 equi-molar set						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	Dilution factor
	IFMA results											
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	15	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	11	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	7	0	100	1,E-02
10^{-3}	+	-	+	+			3	1	3	1	75	1,E-03
10^{-4}	-	-	-	-			0	4	0	5	0	1,E-04
10^{-5}	-	-	-	-			0	4	0	9	0	1,E-05
10^{-6}	-	-	-	-			0	4	0	13	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	17	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	21	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	25	0	1,E-09
$TCID_{50}$			2,15E+03									
$TCID_{50}$/ml			2,15E+04									

Table 16: $TCID_{50}$ calculation table for original pT7_SA11-L2 RV RG system

Jap_Ori	Origional pT7_SA11-L2 equi-ug set						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	Dilution factor
	IFMA results											
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	7	0	100	1,E+00
10^{-1}	+	+	+	-			3	1	3	1	75	1,E-01
10^{-2}	-	-	-	-			0	4	0	5	0	1,E-02
10^{-3}	-	-	-	-			0	4	0	9	0	1,E-03
10^{-4}	-	-	-	-			0	4	0	13	0	1,E-04
10^{-5}	-	-	-	-			0	4	0	17	0	1,E-05
10^{-6}	-	-	-	-			0	4	0	21	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	25	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	29	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	33	0	1,E-09
$TCID_{50}$			2,15E+01									
$TCID_{50}$/ml			2,15E+02									

The results annotated in Table 14 were obtained from the optimized pT7_SA11-L2 RV RG system (Section 2.3.5). This was compared to the original (unmodified) pT7_SA11-L2 RG system (Section 2.3.2, Table 16) and to the NWU developed pSmart_SA11-N5 RV RG system (Section 3.3.4, Table 14) under the same conditions (using the same 96well plate). For each RG system, the P1 stock (saved prior to IFMA confirmation of rescue) was used for the construction of the dilution series (Section 3.2.7). Standard IFMA was used for visualization of viral propagation.

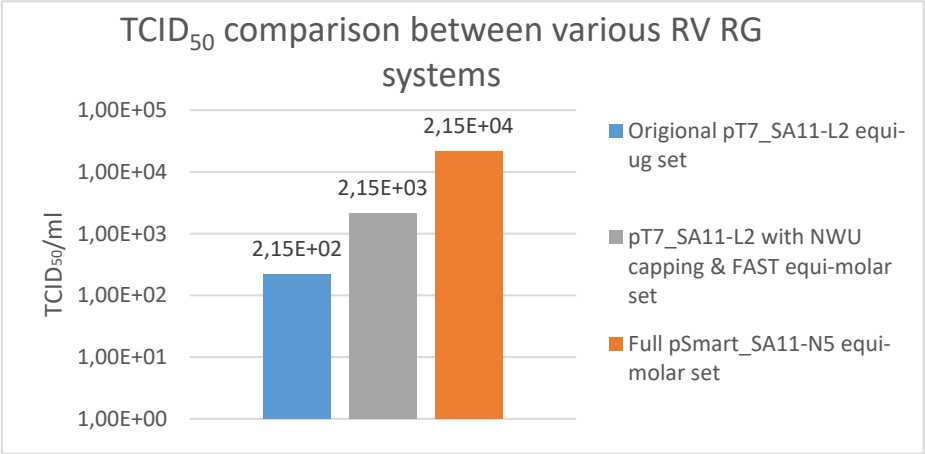

Figure 28: $TCID_{50}$ comparison of original pT7_SA11-L2, optimized pT7_SA11-L2 and original pSmart_SA11-N5 RV RG systems

As illustrated by Figure 28, the original pT7_SA11-L2 RG system (blue) yielded a viral titer of $2.15x10^2$ $TCID_{50}$/ml. This is very low yield and might not be representative of a fully functional RG system, however, these results were obtained from the use of the P1 stock of the only rescue attempt that yielded a visible fluorescent response (Section 2.3.2). During the implementation of the original pT7_SA11-L2 system, several attempts at rescue were made with only one yielding a positive result. It was concluded that the system required further optimization and thus no further rescue attempts were made. The $TCID_{50}$ results of this experiment were included to evaluate the relative increase in rescue efficiency resulting from the implementation of the various optimizations discussed in Chapter 2. As expected the optimized pT7_SA11-L2 RV RG system (grey) performed better than the original, reaching a viral titer of $2.15x10^3$ $TCID_{50}$/ml, a 10-fold increase. Although this is still a relatively low yield, it is indicative that the optimizations implemented, based on experience with the BTV and AHSV RG systems did, in fact, increase repeatability and overall viral yield.

Finally, the two pT7_SA11-L2 systems were compared with the locally developed, pSmart_SA11-N5 system (orange). Viral titers of approximately $2.15x10^4$ $TCID_{50}$/ml were obtained from the pSmart system, again increasing the yield 10-fold as compared with the optimized pT7_SA11-L2 system and a 100-fold (two log rise) increase when compared to the unmodified pT7_SA11-L2 system. This increase might be due to the reduced backbone size and increased transfection efficiency of the pSmart_SA11-N5 RG system as compared to the optimized pT7_SA11-L2 RG system (Section 3.3.9).

3.3.6 Incorporation of ASFV capping constructs and $TCID_{50}$ comparison of pSmart_SA11-N5 and pT7_SA11-L2 RG systems

This experiment entails the exchange of the VV capping subunits (D1R & D12L) carried in the phCMVdream plasmid with the single subunit African swine-fever virus (ASFV) capping enzyme alone (phCMVdream_ASFV, Figure 42, Appendix A), and fused to a viral T7-polymerase through a serine-glycine linker (phCMVdream_C3P3, Figure 42, Appendix A). This was done to reduce the plasmid load being transfected and also to increase the transcription and capping efficiency of the system. Standard rescue protocol (Section 2.2.11) was followed with the addition of negative controls (plasmid mixtures lacking VP1 representation) and rescue was confirmed through IFMA (Section 2.2.12). Initial experiments with the exchange of the VV capping plasmids with the pDream_ASFV capping plasmid (not fused with the viral T7-polymerase) did not yield any significant improvements, with IFMA results of the VV based and ASFV based RG systems being visually identical (Figure 29, A & B). The incorporation of the C3P3 construct, however, yielded much higher viral titers as evaluated in terms of the progression of CPE over a set time of 3 days. As illustrated by Figure 29 (C), not only was rescue consistent but CPE progressed to more than 60% within only 3 days indicating an increased viral load and therefore increased viral titer.

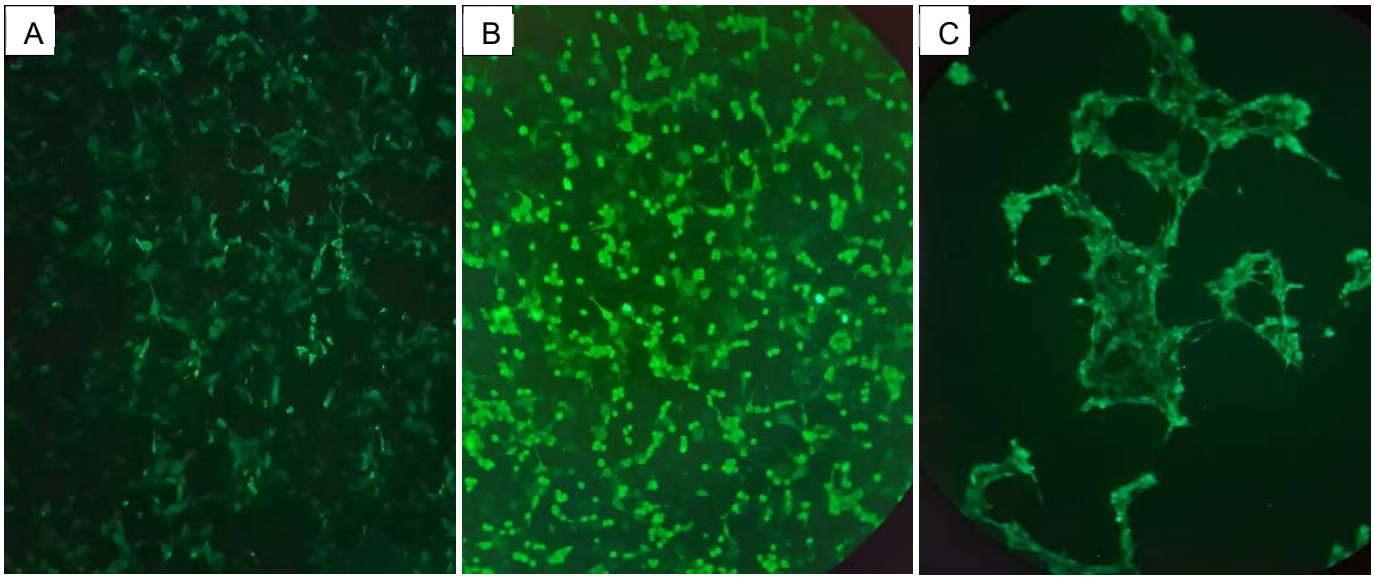

Figure 29: IFMA results of pSmart_SA11-N5 RV RG systems using (A) VV capping plasmids, (B) the ASFV capping plasmid and (C) the C3P3 capping-polymerase construct. Transfected into BSR-T5/7 cells and co-seeded and propagated in ST cells. IFMAs done on CPE presenting cells 3 days after P1 passage. 400x magnification

Confirmation of viral rescue was also done through dsRNA extraction and visualization, the results of which are discussed in Section 3.3.7. The dsRNA was then converted to cDNA and sent for sequence verification at the NICD through NGS, results of which are discussed in Section 3.3.7. Following the rescue of the pSmart_SA11-N5 RG system with the C3P3 construct, the same was done with the pT7_SA11-L2 RG system, yielding similar results. To compare these systems a $TCID_{50}$ assay was constructed for each under the same conditions and annotated in the tables below.

Table 17: $TCID_{50}$ calculation table for original pSmart_SA11-N5 RV RG system

NWU_Ori	Full pSmart_SA11-N5 equi-molar set						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1
	IFMA results											Dilution factor
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	15	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	11	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	7	0	100	1,E-02
10^{-3}	+	-	+	+			3	1	3	1	75	1,E-03
10^{-4}	-	-	-	-			0	4	0	5	0	1,E-04
10^{-5}	-	-	-	-			0	4	0	9	0	1,E-05
10^{-6}	-	-	-	-			0	4	0	13	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	17	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	21	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	25	0	1,E-09
$TCID_{50}$			2,15E+03									
$TCID_{50}$/ml			2,15E+04									

Table 18: TCID50 calculation table for pSmart_SA11-N5 RV RG system with ASFV capping plasmid

NWU_ASFV	pSmart_SA11-N5 with 3x ASFV-capping and NWU-FAST, equi-molar IFMA results						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1 Dilution factor
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	16	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	12	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	8	0	100	1,E-02
10^{-3}	+	+	+	-			3	1	4	1	80	1,E-03
10^{-4}	-	+	-	-			1	3	1	4	20	1,E-04
10^{-5}	-	-	-	-			0	4	0	8	0	1,E-05
10^{-6}	-	-	-	-			0	4	0	12	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	16	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	20	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	24	0	1,E-09
$TCID_{50}$			3,16E+04									
$TCID_{50}$/ml			3,16E+05									

Table 19: $TCID_{50}$ calculation table for pSmart_SA11-N5 RV RG system with C3P3

NWU_C3P3	pSmart_SA11-N5 with 3x C3P3-capping and NWU-FAST, equi-molar IFMA results						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1 Dilution factor
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	20	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	16	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	12	0	100	1,E-02
10^{-3}	+	+	+	+			4	0	8	0	100	1,E-03
10^{-4}	+	+	+	-			3	1	4	1	80	1,E-04
10^{-5}	+	-	-	-			1	3	1	4	20	1,E-05
10^{-6}	-	-	-	-			0	4	0	8	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	12	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	16	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	20	0	1,E-09
$TCID_{50}$			3,16E+05									
$TCID_{50}$/ml			3,16E+06									

Table 20: $TCID_{50}$ calculation table for pT7_SA11-L2 RV RG system with C3P3

Jap_C3P3	pT7_SA11-L2 with 3x C3P3-capping and NWU-FAST, equi-molar set IFMA results						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1 Dilution factor
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	18	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	14	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	10	0	100	1,E-02
10^{-3}	+	+	+	+			4	0	6	0	100	1,E-03
10^{-4}	+	+	-	-			2	2	2	2	50	1,E-04
10^{-5}	-	-	-	-			0	4	0	6	0	1,E-05
10^{-6}	-	-	-	-			0	4	0	10	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	14	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	18	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	22	0	1,E-09
$TCID_{50}$			1,00E+04									
$TCID_{50}$/ml			1,00E+05									

The results annotated in Table 17were obtained from the original pSmart_SA11-N5 RV RG system (Section 3.3.4) and was included to evaluate the efficacy of the ASFV and C3P3 constructs on base-line pSmart_SA11-N5 RG rescue. This was compared to the pSmart_SA11-N5 RG system utilizing the ASFV capping plasmid (Table 18), the pSmart_SA11-N5 RG system utilizing the C3P3 construct (Table 19) and the optimized pT7_SA11-L2 RG system utilizing the C3P3 construct (Table 20), under the same conditions (using the same 96 well plate). For each RG system, the P1 stock (saved directly prior to IFMA confirmation of rescue) was used for the construction of the dilution series (Section 3.2.7). Standard IFMA was used for visualization of viral propagation.

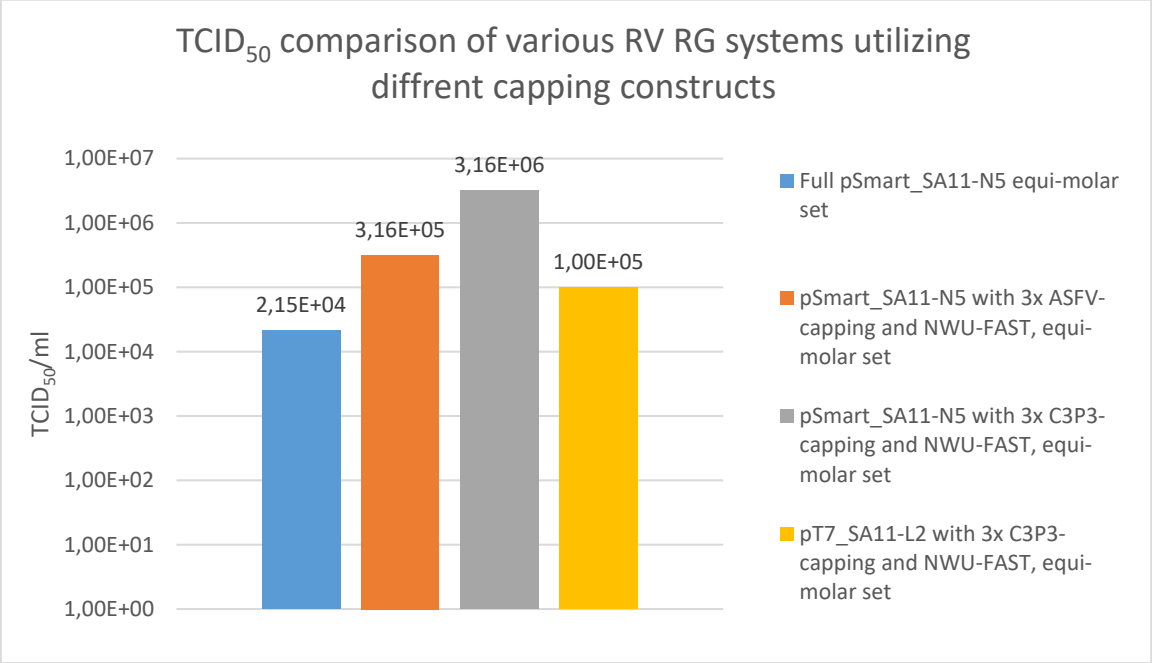

Figure 30: $TCID_{50}$ comparison of pT7_SA11-L2 and pSmart_SA11-N5 RV RG systems utilizing various capping constructs.

As illustrated by Figure 30, the original pSmart_SA11-N5 RG system (blue) yielded a viral titer of $2.15x10^4$ $TCID_{50}$/ml, and served as the benchmark against which the performance of the different RG systems including the various capping constructs would be measured. Contrary to the visual evaluation (where no significant difference was evident), the pSmart_SA11-N5 RG system utilizing the ASFV capping plasmid (orange) did perform better than the benchmark, reaching a viral titer of $3.16x10^5$ $TCID_{50}$/ml, a roughly 10-fold increase.

The pSmart_SA11-N5 RG system utilizing the C3P3 construct (grey) performed even better with a viral titer of $3.16x10^6$ $TCID_{50}$/ml. And finally, the optimized pT7_SA11-L2 RG system unitizing the C3P3 construct (yellow) reached a viral titer of $1.0x10^5$ $TCID_{50}$/ml, not as high as the pSmart_SA11-N5 RG system using the phCMVdream_C3P3 construct, but significantly higher than the pT7_SA11-L2 RG system that utilized the VV capping plasmids (~$2.15x10^3$ $TCID_{50}$/ml, Section 3.3.5). These results indicate that the capping of viral transcripts and the abundance of the viral T7 polymerase play a decisive role in the efficacy of a RV RG system.

3.3.7 dsRNA extraction, cDNA synthesis and viral genome sequencing for both optimized pSmart_SA11-N5 and pT7_SA11-L2 RV RG systems

Although IFMA confirmed viral recovery and propagation, viral genome extraction and sequencing was required for definitive confirmation of rescue of recombinant RV strains. The dsRNA extraction protocol (Section 3.2.1) was followed after successful viral rescue and dsRNA was visualised through AGE (Section 2.2.7) and RNA-PAGE (Section 3.2.2). Both rescued SA11-L2 and SA11-N5, as well as a SA11-N2 WT control, were propagated (Section 2.2.13) for dsRNA extraction followed by cDNA synthesis.

Because the optimized pT7_SA11-L2 RG system (Section 2.3.5) was fully implemented and SA11-L2 was rescued first (before the finalization of the pSmart_SA11-N5 RG system, Section 3.3.4), the dsRNA of rescued SA11-L2 was isolated and compared to that of our WT SA11-N5 first. This was done to familiarize myself with the techniques involved in dsRNA extraction and cDNA synthesis and to definitively prove that recombinant SA11-L2 was rescued from the optimized pT7_SA11-L2 RG system. Figure 31 illustrates dsRNA isolated from both our SA11-N2 WT RV and the recovered SA11-L2 RV from the optimized pT7_SA11-L2 RG system. The electrophoresis pattern of both samples correlates to the expected pattern for RV. The size of the dsRNA genome segments ranged from 3302bp (GS1, VP1) to 667bp (GS11, NSP5/6).

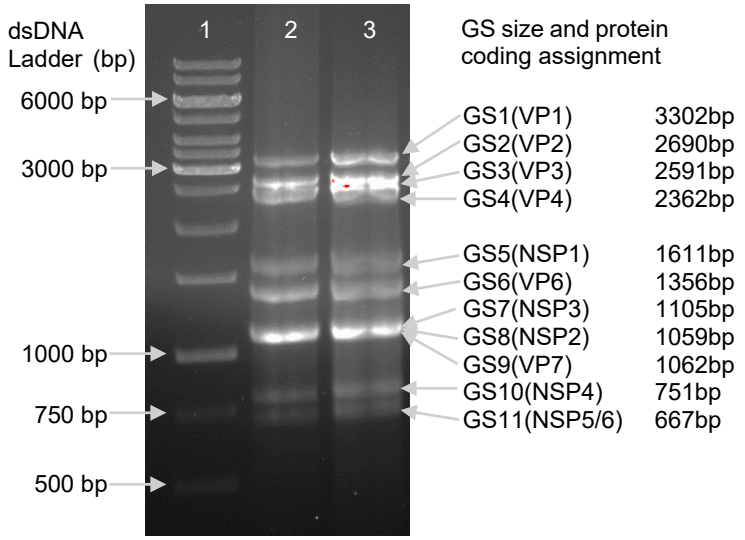

Figure 31: Agarose gel of RV dsRNA. Lane 1: dsDNA Ladder. Lane 2: dsRNA extracted from MA104 cells infected with RV SA11-N2. Lane 3: dsRNA extracted from ST cells infected with P1 stock of optimized pT7_SA11-L2 RV RG system.

Following dsRNA extraction and visualization, sequence-independent primer ligation (Section 3.2.4) was performed and visualised through AGE (Figure 32). The ligation of the sequencing primers was visible as a very slight increase in genome segment size, which corresponds to the size of the ligated primers (2x 55bp). The increase is a bit more apparent for the smaller genome segments than the largest ones.

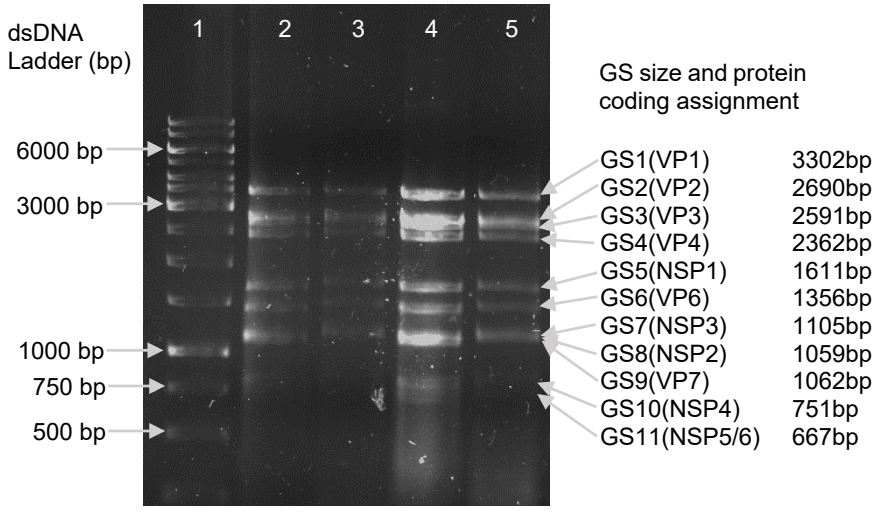

Figure 32: Agarose gel of primer ligated and non-ligated RV dsRNA. Lane 1: dsDNA Ladder. Lane 2: dsRNA extracted from MA104 cells infected with RV SA11-N2. Lane 3: Sequence independent primer ligated dsRNA of SA11-N2. Lane 4: dsRNA extracted from ST cells infected with P1 stock of optimized pT7_SA11-L2 RV RG system. Lane 5: Sequence independent primer ligated dsRNA of SA11-L2.

The oligo-ligated dsRNA was used for cDNA synthesis (Section 3.2.4). The cDNA was then purified (Section 3.2.6) and PCR amplified for sequencing. The resulting NGS reads were mapped against both the RV SA11-L2 reference genome (LC333802-LC333812) and the RV SA11-N2 reference genome (JN827244-JN827250 and JN827252- JN827255). The same was done with dsRNA extracted from the RV SA11-N2 and compared to that of the pT7_SA11-L2 RG system. For all mapped reads and sequence alignments of each of the 11 GS, see Appendix B. The results for GS8 (NSP2) are shown in Figure 33, as it most clearly illustrates the differences between the SA11-L2 and SA11-N2 genotypes. The sequences of GS8 for the SA11-L2 and SA11-N2 references only share a 79.79% identity and therefor best visually illustrate the corresponding strains without showing the actual sequences (available in Appendix B).

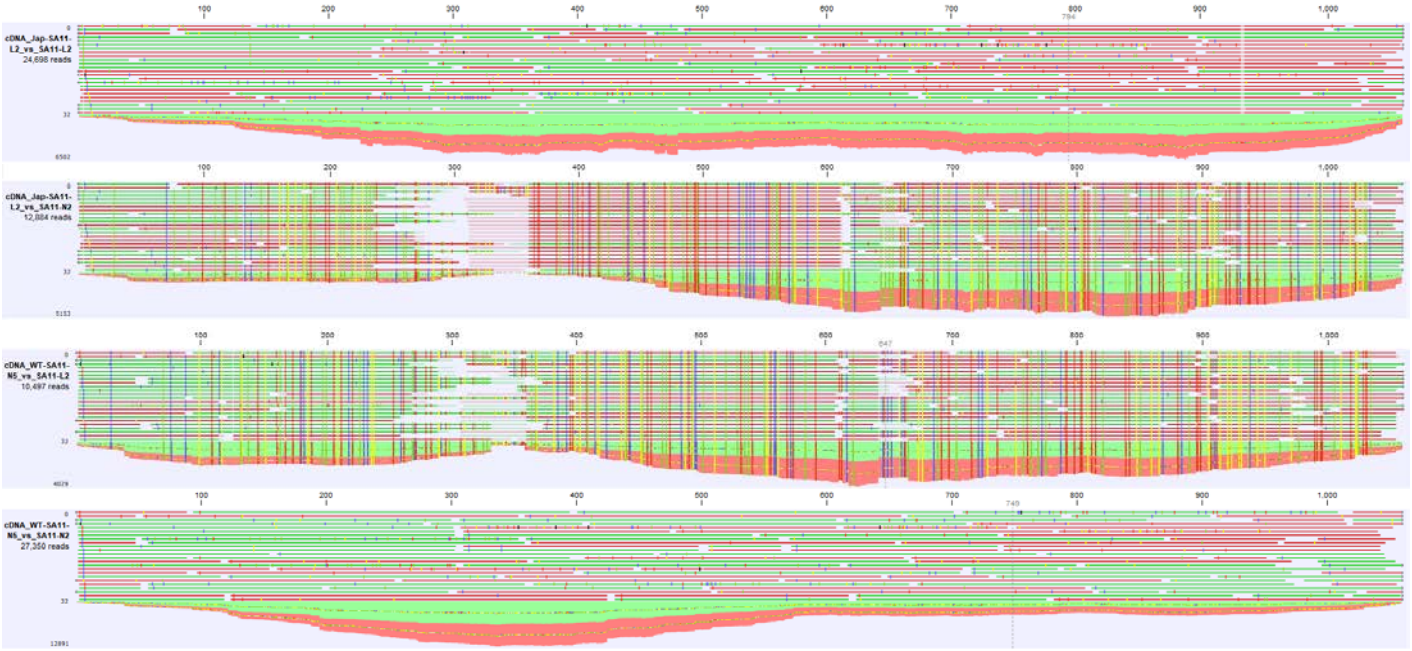

Figure 33: NGS reads of GS8 (NSP2) from rescued SA11-L2 and SA11-N2 cDNA mapped against GS8 (NSP2) of SA11-L2 (LC333809) and SA11-N2 (JN827252) reference genomes. cDNA of SA11-L2 (from optimized pT7_SA11-L2 RG system) mapped against, GS8 of the SA11-L2 reference genome (LC333809), and B: GS8 of the SA11-N2 reference genome (JN827252). C: cDNA of SA11-N5 (WT control) mapped against, GS8 of the SA11-L2 reference genome, and D: GS8 of the SA11-N2 reference genome. Mismatches illustrated by blue, red, yellow and green verticle lines (in B & C). A & B mapped without incongruities.

As illustrated by Figure 33 (A & B), the virus recovered from the optimized pT7_SA11-L2 RG system is clearly RV SA11-L2 and not the WT RV SA11-N2, as (A) mapped perfectly to the SA11-L2 reference sequence, whilst (B) could not.

Correspondingly the WT reads did not map against the SA11-L2 reference genome Figure 33 (C) but did map perfectly against the SA11-N2 reference genome Figure 33 (D). This along with the sequence alignments in Appendix B definitively confirm the rescue of the intended RV SA11-L2 strain from the optimized pT7_SA11-L2 RG system and eliminates the possibility that results could arise from SA11-N2 WT cross-contamination. This same process was followed for the dsRNA extraction, cDNA synthesis and NGS whole-genome sequencing for the RV SA11-N5 rescued from the optimized pSmart_SA11-N5 RG system (Section 3.3.6). Figure 34 (Lane 2) depicts the agarose gel of the dsRNA extracted from ST cells infected with the P1 stock of the optimized pSmart_SA11-N5 RG system. The electrophoretic separation pattern is that expected of RV SA11. Figure 34 (Lane 3) shows the corresponding sequence-independent primer ligated dsRNA of the rescued SA11-N5 genome.

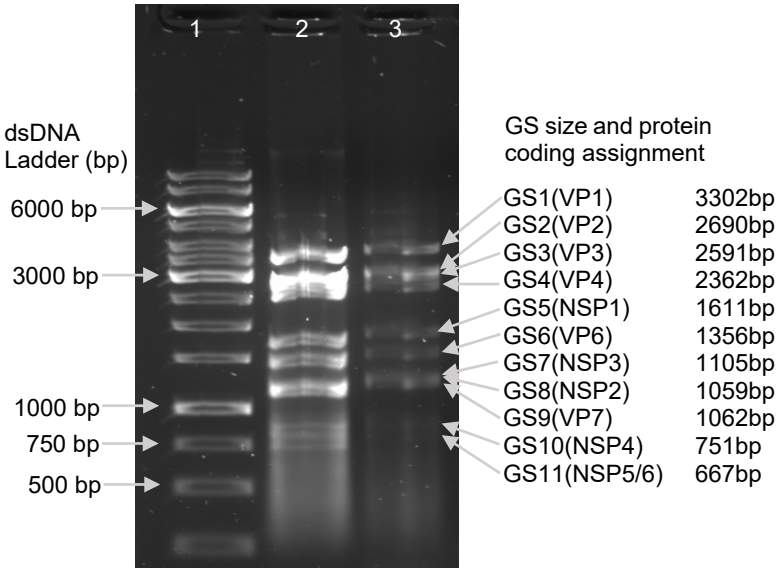

Figure 34: Agarose gel visualizing the 11 segments of the RV SA11-N5 genome adjacent the primer ligated dsRNA of SA11-N5. Lane 1: dsDNA Ladder. Lane 2: dsRNA extracted from ST cells infected with P1 stock of optimized pSmart_SA11-N5 RV RG system. Lane 3: sequence independent primer ligated dsRNA from SA11-N5.

dsRNA was successfully isolated from ST cells infected with the P1 stock of the optimized pSmart_SA11-N5 RG system (Figure 34), confirming viral prop agation. Due to the very slight increase in the corresponding primer ligated dsRNA (Figure 34, Lane 3), especially visible for the smallest genome segments, it was assumed that the sequence-independent primer ligation step (Section 3.2.4) was successful. The primer ligated dsRNA from this experiment could however not be converted to cDNA (Section

3.2.4) as was done for the pT7_SA11-L2 RG system. I then repeated the experiment with both the dsRNA from the pSmart_SA11-N5 RG system and that of the pT7_SA11-L2 RG system as control. I then separated the extracted and primer ligated dsRNA from both the recovered SA11-L2 (Figure 35, lanes 2 and 3) and SA11-N5 (Figure 35, lanes 4 and 5) on a RNA-PAGE gel which was visualized through silver staining which is more sensitive than EtBr staining, to verify if primer ligation had occurred.

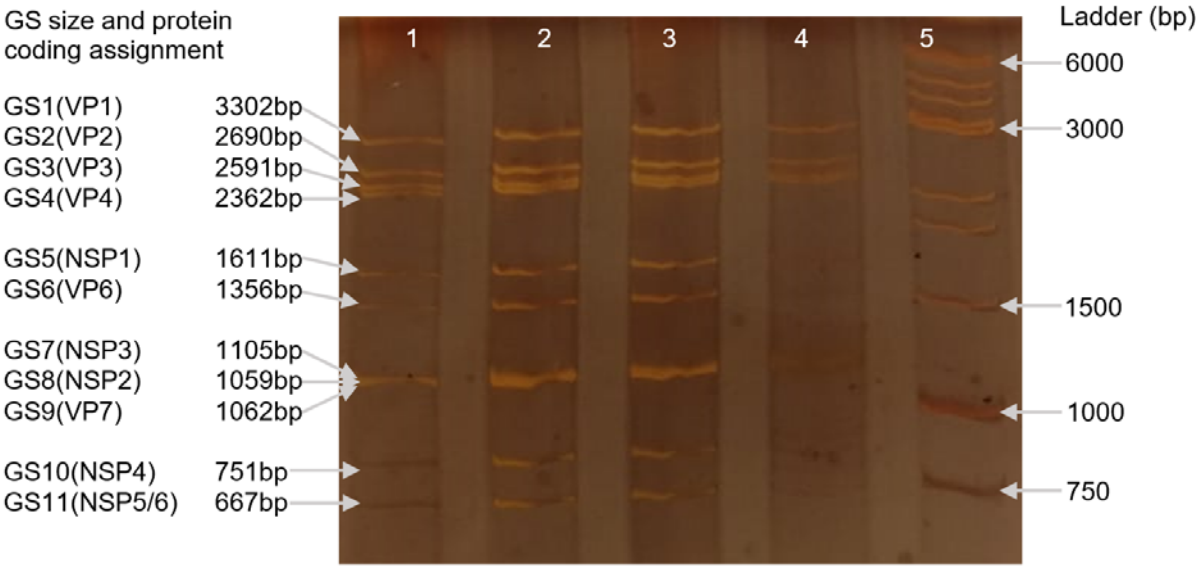

Figure 35: RNA-PAGE gel of dsRNA extracted from MA104 cells infected with SA11-N2 WT (2) and ST cells infected with P1 stock from the optimized pSmart_SA11-N5 RG system, next to their sequence independent primer ligated dsRNA genome segments. Lane 5: dsDNA Ladder. Lane 1: dsRNA extracted from MA104 cells infected with SA11-N2 WT. Lane 2: sequence independent primer ligated dsRNA from SA11-N2 WT. Lane 3: dsRNA extracted from ST cells infected with P1 stock of optimized pSmart_SA11-N5 RV RG system. Lane 4: sequence independent primer ligated dsRNA from SA11-N5.

Based on the results obtained from the RNA-PAGE it was concluded that the primer ligation step prior to the reverse transcriptase reaction was not successful. The primary goal of the whole genome amplification and NGS steps in this project was to verify the identity of the recovered virus and definitively indicate that the intended recombinant virus was rescued from the specific RG system, and that recovered virus was not the result of cross-contamination from another strain. This was achieved for the SA11-L2 strain through sequence-independent primer ligation, whole genome amplification and NGS.

This entire process was however not strictly required for definitive verification of rescue and viral identification. Only a single genome segment that is distinct enough to distinguish between the SA11-L2 and SA11-N5 strains was required. During the implementation and optimization of the pT7_SA11-L2 RG system, our in-house SA11-N2 WT was used as a control, and as such the virus recovered from the pT7_SA11-L2 RG system needed to be distinguished from that of the SA11-N2 WT. During the implementation and optimization of the pSmart_SA11-N5 RG system, the pT7_SA11-L2 RG system served as control and no SA11-N2 WT was ever used. For this reason, the recovered virus from the pSmart_SA11-N5 RG system needed to be distinguished from that of the SA11-L2 strain.

Due to time constraints at this stage of the project, it was decided that instead of troubleshooting and re-optimizing the entire sequence-independent primer ligation and cDNA synthesis workflow for the pSmart_SA11-N5 RG system, a genome segment-specific approach would be followed to isolate, reverse transcribe and PCR amplify a single GS which would then be sent for sequencing. GS4 (VP4) was selected for this purpose and GS4 specific primers were designed that would amplify both the SA11-L2 GS4 and the SA11-N5 GS4. The primer set: Forward, 5`-GGCTATAAAATGGCTTCGC-3`, and Reverse, 5`-GCCGCTGAAATATCATCAAA ATTCATACC-3` was designed to amplify a variable region that could be used to distinguish between the SA11-L2 and SA11-N5 RV strains.

Standard SuperScript III One-step RT-PCR Platinum kit (Invitrogen) protocol (Section 3.2.5) was followed for the GS4 (VP4) cDNA and PCR amplification steps, results of which are illustrated inFigure 36. Both the recovered SA11-L2 (Figure 36, lane 2) and SA11-N5 (Figure 36, lane 3) dsRNA (GS4, VP4) was successfully reverse transcribed and PCR amplified. The resulting amplicons were of the expected size (1926bp), were purified (Section 3.2.6) and were sent for Sanger sequencing (Section 2.2.14).

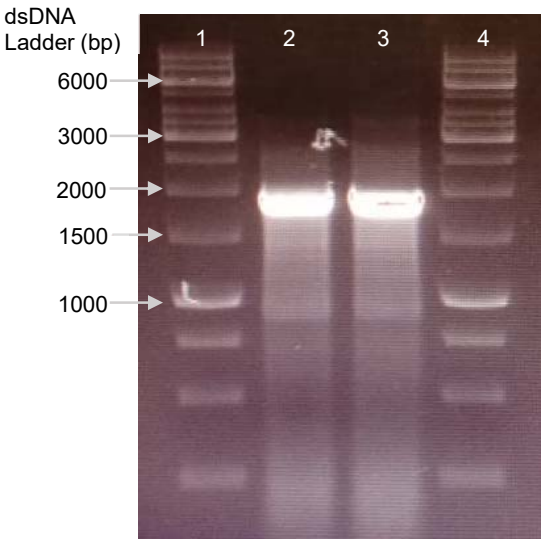

Figure 36: Agarose gel of GS4 specific cDNA synthesis for (2) SA11-L2 and (3) SA11-N5 rescued virus. Lanes 1 & 4: dsDNA Ladder. Lanes 2 & 3: SuperScript One-step RT-PCR product from dsRNA extracted from ST cells infected with (2) P1 stock from the optimized pT7_SA11-L2 RG system, and (3) P1 stock from the optimized pSmart_SA11-N5 RG system, using GS4 specific primers.

Figure 37 illustrates the GS4 (VP4) cDNA sequences of SA11-N5 aligned to both the SA11-N5 (1st row) and SA11-L2 (2nd row) GS4 (VP4) reference sequences. The highlighted nucleotides represent the 6 nucleic acid sequence differences used for differentiation of the SA11-N5 and SA11-L2 GS4 sequences. These are 194 (A to G), 224 (T to C), 318 (G to A), 478 (T to C), 569 (G to C) and 1004 (C to A) with the first being the SA11-N5 consensus sequence and the second being the SA11-L2 variation. Of the 6 selected sequence differences, 5 correlated to the SA11-N5 reference (highlighted in green) and one correlated to the SA11-L2 reference (highlighted in red).

Based on these findings it was confirmed that the rescued virus from the pSmart_SA11-N5 RG system was indeed the intended SA11-N5, even though a single SNP (224: T to C) resembled the SA11-L2 strain. This can be further validated with additional genome segment-specific amplifications, however, for the purposes of this MSc, this confirmation was deemed sufficient and additional genome segment-specific amplifications were not attempted. Additionally, the 224 T in the SA11-N5 reference is based on the consensus sequence and might not be retained in the actual viral genome during rescue and passage, and might be a naturally occurring mutation. This will have to be confirmed through NGS.

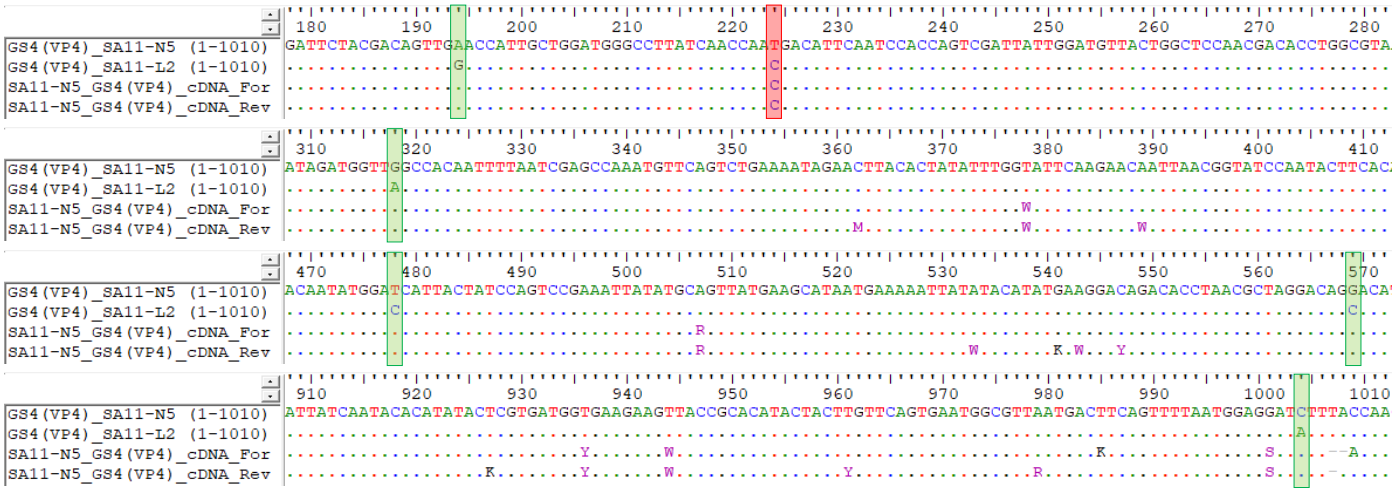

Figure 37: Sanger sequencing alignments of GS4(VP4) cDNA from recovered SA11-N5 aligned against both SA11-N5 and SA11-L2 GS4(VP4) reference sequences. Figure depicts 100pb segments of the full alignment that contain the conserved sequence variations used to distinguish between SA11-N5 and SA11-L2. These are: 194 (A to G), 224 (T to C), 318 (G to A), 478 (T to C), 569 (G to C) and 1004 (C to A). Of the 6 selected nucleic acid sequence differences, 5 correlated to the SA11-N5 reference (highlighted in green) and one correlated to the SA11-L2 reference (highlighted in red).

3.3.8 Effect of a 3x increase of NSP2 and NSP5 transcription plasmids on both optimized pSmart_SA11-N5 and pT7_SA11-L2 RV RG systems

This experiment was based on the 2018 Komoto publication that indicated that recovery of recombinant RV from the pT7_SA11-L2 system could be greatly improved by increasing the number of transcription plasmids encoding the genome segments responsible for viroplasm formation, GS8 (NSP2) and GS11 (NSP5). In addition to this, I wanted to compare the relative efficiency of the equi-molar based system that we were using against the equi-ug based system that Komoto and associates were using. The optimised pT7_SA11-L2 system comprised of the standard 11 pT7_SA11-L2 rescue plasmids in equi-molar amounts along with our pDream_p10_FAST expression plasmid, and 3x molar amounts of the phCMVdream_C3P3 expression plasmid. In parallel, an equi-ug mixture was prepared that simply mixed equivalent amounts of each of the plasmids of the system with the addition of 3x the relative amounts of NSP2 and NSP5 transcription plasmids and 3x the relative amount of the phCMVdream_C3P3 expression plasmid. Standard rescue protocol was followed for both systems with the inclusion of negative controls for each (lacking VP1 representation). BSR-T5/7 cells were used as primary transfection cell-line and ST cells for co-seeding and propagation cell-lines.

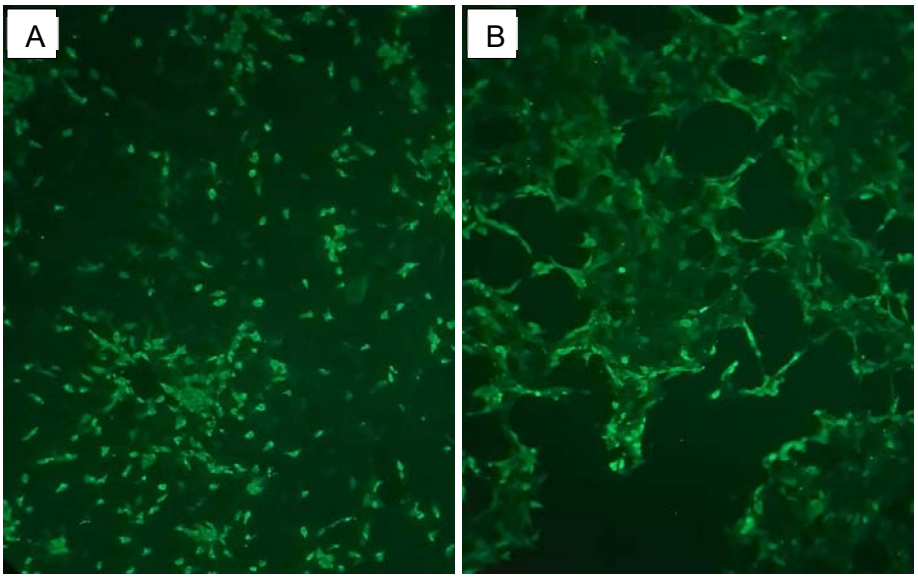

Figure 38: IFMA of the optimized pT7_SA11-L2 RV RG system using (A), an equi-molar approach with 3x phCMVdream_C3P3 expression plasmid, compared to (B), an equi-ug approach using 3x NSP2 and NSP5 transcription plasmids and 3x phCMVdream_C3P3 expression plasmid. Transfected into BSR-T5/7 cells and co-seeded and propagated in ST cells. IFMAs were done on CPE presenting cells 3 days after P1 passage. 400x magnification.

Confirmation of rescue and visual comparison was done through IFMA (Figure 38). With both approaches, it was possible to consistently rescue recombinant RV. However, the equi-ug approach consistently formed more CPE than the equi-molar approach. A visual comparison was done following each IFMA with the assumption that greater CPE represents higher viral titer and thus represents the more efficient system. This was a qualitative evaluation and not a quantitative one. The exact relative efficiencies of the two different approaches were determined through $TCID_{50}$ comparison (Section 3.3.9). These results were unexpected because the experiments described in Section 2.3.2 and Section 2.3.3 indicated either a negligible difference between the equi-molar and equi-ug approaches or a slight increase of efficiency for the equi-molar approach.

This difference could be due to the very nature of the equi-ug approach, which has more copies of the relatively small NSP2 and NSP5 transcription plasmids, than the equi-molar approach. This suggests that it might be possible to overcome the difference in efficiency between the equi-ug and equi-molar systems by increasing the NSP2 and NSP5 transcription plasmids in the equi-molar system even more.

The reason my initial experiments did not indicate a significant difference between the two approaches might be due to the overall low efficiency of both the equi-molar and equi-ug systems at that stage. To accurately monitor the effects of our optimizations to the initial system would have required each experiment to have both the equi-molar and equi-ug versions with their respective negative controls and each of the specific optimizations. This duel approach was not followed due to financial and time constraints and that is why, based on early experiments, the equi-molar approach was selected for further investigation. It is however noteworthy that both the equi-molar and equi-ug systems containing the various optimizations incorporated from the AHSV and BTV RG systems, resulted in the reliable rescue of the virus, illustrating that the optimizations were indeed effective and improved the recovery capacity of the initial pT7_SA11-l2 RG system significantly. Table 20 summarises the various adaptations and optimizations made to the optimized pT7_SA11-L2 RV RG system as compared to the original unmodified system and our locally developed pSmart_SA11-N5 RV RG system.

Table 20: Summary of optimizations made to the original pT7_SA11-L2 RV RG system as compared to our pSmart_SA11-N5 RV RG system

RV RG system	**pT7_SA11-L2 (Original)**	**pT7_SA11-L2 (NWU adapted)**	**pT7_SA11-L2 (NWU optimized)**	**pSmart_SA11-N5 (NWU optimised)**
Transfected cells	BHK-T7	BSR-T5/7	BSR-T5/7	BSR-T5/7
Co-seeding cells	MA104	ST	ST	ST
Capping enzyme	VV (D1R & D12L)	VV (D1R & D12L)	C3P3	C3P3
Additional T7pol	No	No	Yes	Yes
SA11 strain	L2	L2	L2	N5
Detection system	IVIS	IFMA	IFMA	IFMA
Ribozyme	Genomic HDV	Genomic HDV	Anti-genomic HDV	Anti-genomic HDV
Backbone	pT7 (~3080bp)	pT7 (~3080bp)	pT7 (~3080bp)	pSmart (~2010bp)
Plasmid mix	Equi-µg	Equi-molar & -µg	Equi- µg	Equi-µg
Capping : cDNA	1:1	2:1	3:1	3:1
Fusion protein	NBV p10 FAST	NBV p10 FAST	NBV p10 FAST	NBV p10 FAST
Additional NSP2/5	No	No	Yes	Yes

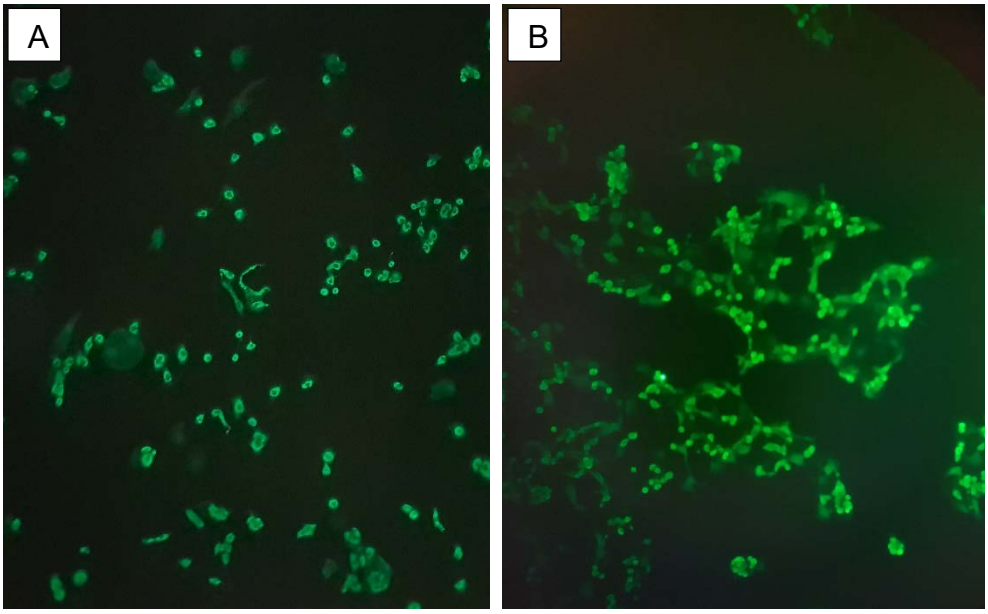

Figure 39: IFMA of the optimized pSmart_SA11-N5 RV RG system using (A), an equi-molar approach with 3x phCMVdream_C3P3 expression plasmid, compared to (B), an equi-ug approach using 3x NSP2 and NSP5 transcription plasmids and 3x phCMVdream_C3P3 expression plasmid. Transfected into BSR-T5/7 cells and co-seeded and propagated in ST cells. IFMA was done on CPE presenting cells 3 days after P1 passage. 400x magnification

The exact same approach used for the evaluation of the pT7_SA11-L2 RV RG systems was also applied to the NWU developed pSmart_SA11-N5 RV RG system, with one being the optimized equi-molar (Figure 39, A) and the other being an equi-ug (Figure 39, B) version with 3x more NSP2, NSP5 transcription plasmids and 3x more phCMVdream_C3P3 expression plasmid. Following IFMA confirmation of viral rescue, a $TCID_{50}$ comparison was performed on each of the RG versions as described in Sections 3.3.5 and 3.2.8. As with the results of the pT7_SA11-L2 experiment, both the equi-molar and equi-ug systems repeatedly and consistently rescued, with the equi-ug version consistently outperforming the equi-molar one in terms of relative viral titer, evaluated visually following IFMA. Precise comparison of relative viral titers was done through $TCID_{50}$ assay and annotated in Section 3.3.9, Tables 22 - 25.

3.3.9 $TCID_{50}$ evaluation of optimized pSmart_SA11-N5 and pT7_SA11-L2 RG systems using an equi-molar approach as compared to an equi-ug approach with 3x more NSP2 and NSP5 transcription plasmids

In this experiment, the impact of increasing the relative amounts of viroplasm forming GS8 (NSP2) and GS11 (NSP5) transcription plasmids on viral titer was evaluated through $TCID_{50}$ assays. As stated in Section 2.2.12, the supernatant of a CPE presenting culture is stored as a P1 viral stock prior to IFMA. The P1 stocks of the various RG systems described in Section 3.3.8, were used for the construction of several dilution series as described in Section 3.2.8. MA104 cells were seeded into a 96-well plate for the $TCID_{50}$, infected with the viral dilution series and incubated for 3 to 5 days. Following this incubation viral propagation was evaluated via IFMA. Any fluorescent signal obtained from a well was viewed as viral propagation and annotated as “+”. Correspondingly if no fluorescent signal was found in a well it was deemed virus free and annotated as “-“. Calculations of $TCID_{50}$ and $TCID_{50}$/ml were done in Excel using the equations annotated in Equation 2.

Results shown in Table 21 were obtained from the optimized, equi-molar pSmart_SA11-N5 RV RG system (Section 3.3.6), and were included to be compared to the optimized, equi-molar pT7_SA11-L2 RG system that utilized the phCMVdream_C3P3 construct (Table 22). These two equi-molar RG systems were used as a benchmark to evaluate the efficiency of the equi-ug RG systems that increased the amount of NSP2 and NSP3 transcription plasmids (Table 23). This was compared to the equi-ug pSmart_SA11-N5 RG system with 3x NSP2 and NSP5 transcription plasmids (Table 24) and the equi-ug pT7_SA11-L2 RG system with 3x NSP2 and NSP5 transcription plasmids (Table 24), under the same conditions (using the same 96 well plate).

Table 21: $TCID_{50}$ calculation table for optimized, equi-molar pSmart_SA11-N5 RV RG system with 3x phCMVdream_C3P3 expression plasmid.

NWU_C3P3	pSmart_SA11-N5 with 3x C3P3-capping and NWU-FAST, equi-molar IFMA results						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1 Dilution factor
Dilution	1	2	3	4	5	6						
10^0	+	+	+	+			4	0	20	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	16	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	12	0	100	1,E-02
10^{-3}	+	+	+	+			4	0	8	0	100	1,E-03
10^{-4}	+	+	+	-			3	1	4	1	80	1,E-04
10^{-5}	+	-	-	-			1	3	1	4	20	1,E-05
10^{-6}	-	-	-	-			0	4	0	8	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	12	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	16	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	20	0	1,E-09

$TCID_{50}$ 3,16E+05

$TCID_{50}$/ml 3,16E+06

Table 22: $TCID_{50}$ calculation table for equi-ug pT7_SA11-L2 RV RG system with 3x NSP2 and NSP5 transcription plasmids and 3x phCMVdream_C3P3 expression plasmid.

Jap_3x NSP2/5	pT7_SA11-L2 origional with 3x NSP2/5, equi-ug set IFMA results						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1 Dilution factor
Dilution	1	2	3	4	5	6						
10^0	+	+	+	+			4	0	24	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	20	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	16	0	100	1,E-02
10^{-3}	+	+	+	+			4	0	12	0	100	1,E-03
10^{-4}	+	+	+	+			4	0	8	0	100	1,E-04
10^{-5}	+	+	+	-			3	1	4	1	80	1,E-05
10^{-6}	-	-	+	-			1	3	1	4	20	1,E-06
10^{-7}	-	-	-	-			0	4	0	8	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	12	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	16	0	1,E-09

$TCID_{50}$ 3,16E+06 $TCID_{50}$/ml 3,16E+07

Table 23: $TCID_{50}$ calculation table for optimized equi-molar pT7_SA11-L2 RV RG system with 3x phCMVdream_C3P3 expression plasmid.

Jap_C3P3	pT7_SA11-L2 with 3x C3P3-capping and NWU-FAST, equi-molar set IFMA results						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1 Dilution factor
Dilution	1	2	3	4	5	6						
10^0	+	+	+	+			4	0	18	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	14	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	10	0	100	1,E-02
10^{-3}	+	+	+	+			4	0	6	0	100	1,E-03
10^{-4}	+	+	-	-			2	2	2	2	50	1,E-04
10^{-5}	-	-	-	-			0	4	0	6	0	1,E-05
10^{-6}	-	-	-	-			0	4	0	10	0	1,E-06
10^{-7}	-	-	-	-			0	4	0	14	0	1,E-07
10^{-8}	-	-	-	-			0	4	0	18	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	22	0	1,E-09

$TCID_{50}$ 1,00E+04 $TCID_{50}$/ml 1,00E+05

Table 24: $TCID_{50}$ calculation table for equi-ug pSmart_SA11-N5 RV RG system with 3x NSP2 and NSP5 transcription plasmids and 3x phCMVdream_C3P3 expression plasmid.

NWU_3x NSP2/5	pSmart_SA11-N5 with 3x C3P3-capping and 3x NSP2/5, equi-ug						No. infected wells	No. non-infected wells	Σno. Infected wells (lowest to highest dilution)	ΣNo. Non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣNo. Non-infected wells	0,1
	IFMA results											Dilution factor
Dilution	1	2	3	4	5	6						
10^{0}	+	+	+	+			4	0	27	0	100	1,E+00
10^{-1}	+	+	+	+			4	0	23	0	100	1,E-01
10^{-2}	+	+	+	+			4	0	19	0	100	1,E-02
10^{-3}	+	+	+	+			4	0	15	0	100	1,E-03
10^{-4}	+	+	+	+			4	0	11	0	100	1,E-04
10^{-5}	+	+	+	+			4	0	7	0	100	1,E-05
10^{-6}	-	+	+	-			2	2	3	2	60	1,E-06
10^{-7}	-	+	-	-			1	3	1	5	17	1,E-07
10^{-8}	-	-	-	-			0	4	0	9	0	1,E-08
10^{-9}	-	-	-	-			0	4	0	13	0	1,E-09
$TCID_{50}$			1,70E+07				$TCID_{50}$/ml		1,70E+08			

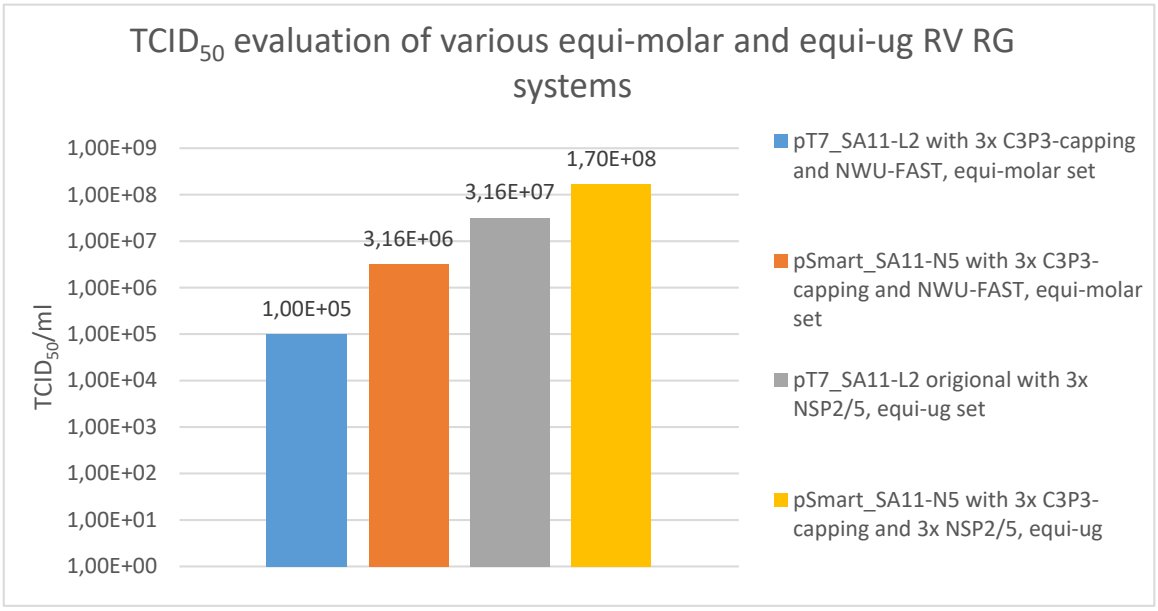

Figure 40: $TCID_{50}$ comparison of both equi-molar and equi-ug approaches of the pT7_SA11-L2 and pSmart_SA11-N5 RV RG systems, with the equi-ug approach using 3x NSP2 and NSP5 transcription plasmids.

As illustrated by Figure 40, the optimized, equi-molar pT7_SA11-L2 RG system (blue) yielded a titer of $1.0x10^{5}$ $TCID_{50}$/ml, and the optimized, equi-molar pSmart_SA11-N5 RG system (orange) yielded a titer of $3.16x10^{6}$ $TCID_{50}$/ml. This comparison not only provided a benchmark against which the performance of the equi-ug RG systems with increased NSP2 and NSP5 constructs would be measured but also confirms that the pSmart_SA11-N5 RG system is more efficient than the pT7_SA11-L2 RG system in every comparable experiment thus far.

The equi-ug pT7_SA11-L2 RG system with 3x NSP2 and NSP5 transcription plasmids (grey) reached a viral titer of $3.17x10^7$ $TCID_{50}$/ml, a 10-fold increase of that of the optimized, equi-molar pSmart_SA11-N5 RG system, and a 100-fold (two logs higher) increase of that of the optimized, equi-molar pT7_SA11-L2 RG system. This substantial increase in efficiency clearly highlights the importance of the viroplasm forming GS8 (NSP2) and GS11 (NSP5) during viral replication (Desselberger, 2014; Desselberger, 2017; Desselberger, 2019; Estes & Cohen, 1989).

As mentioned previously, initial experiments where the equi-molar and equi-ug approaches were compared through IFMA, did not indicate a significant difference between the two systems and suggested (based purely on visual evaluation) that the equi-molar approach might be slightly more efficient. This is in sharp contrast to the findings of this experiment. If we, however, take into consideration the substantial effect of the increased viroplasm forming GSs had on rescue, we can infer that the equi-ug approach only outperformed the equi-molar approach because it would provide even more copies of the relatively small GS8 (NSP2) and GS11 (NSP5) transcription plasmids than the equi-molar approach. This notion is further supported by the equi-ug pSmart_SA11-N5 RG system reaching the highest viral titer of $1.7x10^8$ $TCID_{50}$/ml. In this experiment, the reduced size of the pSmart plasmid backbone would further increase the number of small plasmids represented in an equi-ug transfection mixture and therefor would further support the formation of the viroplasm.

This comparison also illustrates that the pSmart_SA11-N5 RG system consistently outperforms the pT7_SA11-L2 RG system in every comparable experiment. This again (as stated in Section 3.3.5) can most likely be attributed to the smaller plasmid backbone size of the pSmart_SA11-N5 RG system (~2010bp) as compared to that of the pT7_SA11-L2 RG system (~3080bp). The smaller backbone allowed higher transfection efficiency and decreases plasmid load by roughly 11700bp, resulting in higher transcription levels and an increase in over-all rescue efficiency.

3.4 Summary

The NWU research team has long been working towards a robust, helper-virus independent RV RG system. Work towards this goal was primarily based on the work done by Dr L. Mlera and Dr J.F. Wentzel who determined the SA11-N5 consensus sequence and generated several transcription plasmids carrying the SA11-N5 cDNA genome segments respectively. Prior to the start of this project, the bases of the pSmart_SA11-N5 RV RG system was already being worked on by various members of the NWU research team, with L. Geldenhuys generating 8 of the 11 SA11-N5 cDNA rescue plasmids by the end of 2017. I took over the project and constructed the remaining 3 SA11-N5 cDNA rescue plasmids (GS8: NSP2, GS9: VP7 and GS11: NSP5/6) in addition to the various capping and fusion expression plasmids used with both the pSmart_SA11-N5 and pT7_SA11-L2 RV RG systems.

The various optimizations used during the implementation of the Japanese pT7_SA11-L2 RV RG system (Section 2.4) were also incorporated into the pSmart_SA11-N5 RV RG system, which was successfully implemented and used for the rescue of recombinant RV SA11-N5 (Section 3.3.7). The pSmart_SA11-N5 RG system was compared to the pT7_SA11-L2 RG system at various stages during the optimization process and consistently outperformed the pT7_SA11-L2 RG system in comparable experiments (Figure 41). The original pSmart_SA11-N5 RG system was further optimized through the incorporation of the single-subunit capping enzyme expression plasmid, phCMVdream_ASFV and phCMVdream_C3P3 (Section 3.3.6) and the 3x increase of the viroplasm forming NSP2 and NSP5 transcription plasmids (Section 3.3.8). Each of these optimizations increased the relative viral titer obtained from the system roughly 10-fold (on average) and were also incorporated into the pT7_SA11-L2 system and again compared to the pSmart_SA11-N5 system via $TCID_{50}$ assay (Figure 41).

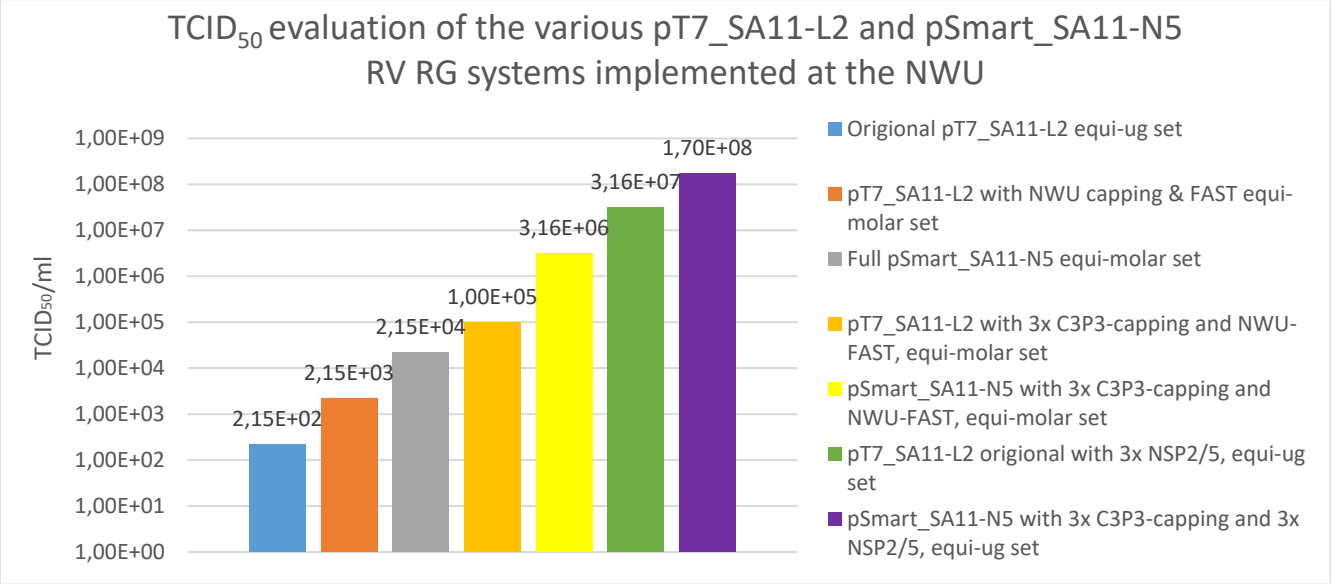

Figure 41: $TCID_{50}$ evaluation of each of the various pT7_SA11-L2 and pSmart_SA11-N5 RV RG systems throughout their implementation and optimization processes.

As illustrated in Figure 41, the initial (unmodified) Japanese pT7_SA11-L2 RV RG system was successfully implemented at the NWU and was used to rescue recombinant RV SA11-L2. Consistently rescuing from the initial system proved very difficult and the only IFMA confirmed rescue only yielded a viral titer of $2.15x10^2$ $TCID_{50}$/ml (blue). The initial pT7_SA11-L2 RG system was then optimized through the incorporation of various insights gained from the BTV and AHSV RG systems to form the optimized pT7_SA11-L2 RG system, which yielded a viral titer of $2.15x10^3$ $TCID_{50}$/ml (orange). This 10-fold increase indicated successful optimization and concluded the first objective of this project.

The locally developed pSmart_SA11-N5 RV RG system was then finalized and implemented with all the optimizations used during the pT7_SA11-L2 optimization process, including the use of the phCMVdream capping and fusion expression plasmids I designed as well as the use of BSR-T5/7 cells for transfections and ST cells for co-seeding and propagation cells. The full pSmart_SA11-N5 RG system yielded a viral titer of $2.15x10^4$ $TCID_{50}$/ml (grey), a 10-fold increase from the optimized pT7_SA11-L2 RG system and a 100-fold increase from the initial pT7_SA11-L2 RG system. This completed the second objective of this project and established two working, plasmid-based RV RG systems at the NWU.

Further optimizations to both the pT7_SA11-L2 and pSmart_SA11-N5 RG systems included the use of the phCMVdream_C3P3 construct that expressed the ASFV capping enzyme linked to a viral T7-RNA polymerase. This construct replaced the phCMVdream_VV_D1R and phCMVdream_VV_D12L VV capping enzyme expression plasmids in both systems. This yielded a viral titer of $1.00x10^5$ $TCID_{50}$/ml (gold) for the pT7_SA11-$L2_{C3P3}$ RG system and a viral titer of $3.16x10^6$ $TCID_{50}$/ml (yellow) for the pSmart_SA11-$N5_{C3P3}$ RG system. The pT7_SA11-$L2_{C3P3}$ RG system showed a 100-fold increase in viral yield as compared to the optimized pT7_SA11-L2 RG system, as did the pSmart_SA11-$N5_{C3P3}$ RG system when compared to the initial pSmart_SA11-N5 RG system. This substantial increase illustrates the importance of adequate viral transcript capping and the availability of viral T7-polymerase during the viral rescue.

Finally, the importance of the viroplasm forming GS8 (NSP2) and GS11 (NSP5) transcription plasmids was evaluated through the 3x increase of said plasmids in both the pT7_SA11-$L2_{C3P3}$ and pSmart_SA11-$N5_{C3P3}$ RV RG systems. The equi-ug pT7_SA11-$L2_{3xNSP2/5}$ RG system reached a viral titer of $3.16x10^7$ $TCID_{50}$/ml (green), another 100-fold increase over the equi-molar pT7_SA11-$L2_{C3P3}$ RG system, and the pSmart_SA11-$N5_{3xNSP2/5}$ RG system reached a viral titer of $1.7x10^8$ $TCID_{50}$/ml (purple), also a 100-fold increase over the equi-molar pSmart_SA11-$N5_{C3P3}$ RG system. This illustrated the profound impact the early formation of the viroplasm can have on viral rescue, and also re-affirmed that the pSmart_SA11-N5 RG system outperforms the pT7_SA11-L2 RG system in every comparable experiment. This increase in efficiency is most likely due to the smaller pSmart backbone (~2010bp) used in the pSmart_SA11-N5 RG system than that used by the pT7_SA11-L2 RG system (~3080bp). Considering the 11 RV cDNA transcription plasmids and the various capping and fusion expression plasmids, the pSmart_SA11-N5 RG system reduces plasmid load by up to 14000bp. The final comparative analysis between the pT7_SA11-L2 and pSmart_SA11-N5 RG systems also completed the final objective of the project and thus finalized the experimental components of my MSc.

Chapter 4

Concluding remarks and future prospects

The long-awaited goal of a true selection free RV RG system was first reported in 2017 and was made available to the scientific community through the AddGene service. This Japanese developed pT7_SA11-L2 RV RG system was acquired by various institutions, including our research team at the NWU, but could not immediately be implemented robustly. This notwithstanding still represented a major breakthrough in RV research and is sure to significantly impact our understanding of RV replication, the biological mechanisms used by the virus, its pathogenesis and correlates of protection, and could provide systems for the identification of targets for viral therapeutics and act as a platform for the generation of novel, rationally designed RV vaccine candidates.

During this project, several plasmid-based RV RG systems were implemented and rationally optimized to form a platform from which future RV research can be initiated. The pT7_SA11-L2 RV RG system was implemented at the NWU with initial difficulty and was optimized through the incorporation of insights gained for the BTV and AHSV RG systems. These optimizations included 1) The design and implementation of alternative capping and fusion enzyme expression plasmids, phCMVdream_VV_D1R, phCMVdream_VV_D12L and phCMVdream_p10_FAST. 2) Exchanging the transfection and co-seeding cell-lines from BHK-T7 and MA104 cells to BSR-T5/7 and ST cells respectively. 3) Using 3x more capping enzyme expression plasmids and the adaptation of an equi-molar transfection mixture approach. These optimizations significantly increased the pT7_SA11-L2 RV RG systems' repeatability and increased viral yield 10-fold from $2.15x10^2$ $TCID_{50}$/ml, to $2.15x10^3$ $TCID_{50}$/ml. Rescue of the recombinant SA11-L2 strain from the pT7_SA11-L2 RG system was verified through dsRNA extraction, cDNA synthesis and whole viral genome sequencing.

The pSmart_SA11-N5 RV RG plasmid set was already partially completed by another member of the NWU RV research team (L. Geldenhuys) with only three constructs not being completed, GS8(NSP2), GS9(VP7) and GS11(NSP5/6). These three constructs were finalized by me and sub-cloned into the pSmart plasmid backbone using In-Fusion HD seamless cloning.

The strategies used during the implementation and optimization of the Japanese pT7_SA11-L2 RV RG system were then applied to the implementation of the full, locally developed, consensus sequence-based pSmart_SA11-N5 RV RG system. Rescue of recombinant SA11-N5 was verified by genome segment-specific, one-step RT-PCR and sequencing of GS4 (VP4). Comparative $TCID_{50}$ analysis was performed on both systems and their respective optimizations throughout the project and the pSmart_SA11-N5 RV RG system outperformed the pT7_SA11-L2 RV RG system in terms of repeatability and overall viral yield in each comparable experiment.

Further optimizations were made to both the pT7_SA11-L2 and pSmart_SA11-N5 RV RG systems through the incorporation of the phCMVdream_C3P3 construct that expressed the ASFV capping enzyme fused to a viral T7-RNA polymerase through a serine-glycine linker. Additionally, the relative amounts of the GS8(NSP2) and GS11(NSP5) transcription plasmids were increased three-fold to produce more viroplasm. Both these two final optimizations significantly increased viral recovery, each yielding a roughly 100-fold increase. All the various optimizations and adaptations performed in this project thus culminated in an increase of efficiency of viral rescue from $2.15x10^2$ $TCID_{50}$/ml to viral titers of $1.7x10^8$ $TCID_{50}$/ml. The project initially set out to establish a robust and helper-virus independent, plasmid-based RV RG system at the NWU, and although this process was arduous, it has been achieved and will form an integral part of many future RV research projects at the NWU and its collaborators in South Africa and elsewhere.

The implementation and optimization of the two RV RG systems described in this project are needed for a wide range of RV research projects at the NWU and elsewhere. The transcription plasmids that make up each of the pT7_SA11-L2 RG and pSmart_SA11-N5 RG systems, were transformed into DH5α cells (New England Biolabs, NEB, Massachusetts, USA) and stored as glycerol stocks at -80°C, as is standard protocol. Samples of these glycerol stocks (in DH5α cells) were shared with the University of the Free State (UFS) and another MSc student in our research group. As per standard protocol, these glycerol stocks were cultured for plasmid extraction and sequence verification. However, the sequencing results of the pSmart_SA11-N5 RG plasmid set indicated several mutations, rearrangements and large scale scrambling in the GS2(VP2), GS9(VP7), GS8(NSP2) and GS10(NSP4) transcription plasmids.

These findings were also confirmed by UFS, but the sequence variants obtained from their samples, differed from ours, indicating instability of the pSmart_SA11-N5 RV RG plasmids in DH5α cells. This hypothesis was further supported by the culturing of various pSmart_SA11-N5 RG plasmid glycerol stocks which were plasmid extracted and sent for sequencing, the results of which returned different mutations than those initially observed, indicating mutagenesis during glycerol stock culturing. The instability of the pSmart_SA11-N5 RV RG plasmids is currently being addressed through the testing of different bacterial hosts, such as JM109 (Zymo, Inqaba Biotec), Epi400 (Lucigen, Wisconsin,.USA) and NEB10β (NEB). Preliminary results indicate that the pSmart_SA11-N5 RV RG transcription plasmids that were not stable in DH5α cells are indeed stable in JM109, NEB10β or Epi400 cells. No investigation into the stability of the pT7_SA11-L2 RV RG plasmids has been done as of yet.

The plasmid-based RV RG systems implementation and optimization during this project are not stand-alone projects and will form part of many other RV research projects at the NWU in the future, once the plasmid stability issue has been resolved. Some of the future development proposals include the incorporation of fluorescent markers into both the established RV RG systems to facilitate the real-time evaluation of rescue progression and to eliminate IFMA confirmation of viral rescue (reducing time between transfection and confirmation of viral rescue). Another common use of a well-established RG system is the generation of rationally designed chimeric viruses. At the NWU, this work will be based on the RG strategies used for the generation of multi-strain BTV and AHSV vaccine candidates (transcapsidation). Furthermore, RV strains can undergo reassortment during co-infection, however, the genetic and biochemical determinants permitting or restricting this is not understood. As such the RV RG systems optimized in this project will be used to investigate the determinants of reassortment in SA11.

Results obtained during this MSc were presented at various national and international symposiums and meetings pertaining to dsRNA viruses and reverse genetics. The following is a list of symposiums, conferences and seminars where elements of this project where presented, as well as the format in which it was presented:

1. **MGJ Huyzers**, AC Potgieter, AA van Dijk. Local implementation and optimization of rotavirus reverse genetics systems. SASBMB-FASBMB conference, Potchefstroom, South Africa, 8 - 11 July 2018. (Oral and Poster presentation)
2. **MGJ Huyzers**, AC Potgieter, AA van Dijk. Local implementation and optimization of rotavirus reverse genetics systems. 13th international dsRNA virus symposium, Houffalize, Belgium, 24 - 28 September 2018. (Shotgun oral presentation and Poster)
3. **MGJ Huyzers**, AC Potgieter, AA van Dijk. Rotavirus reverse genetics, optimizations and future development potential. 12th African Rotavirus Symposium, Johannesburg, South Africa, 30 July - 1 August 2019. (Oral and Poster presentation)
4. **MGJ Huyzers**, AC Potgieter, AA van Dijk. Rotavirus reverse genetics, local implementation and optimizations. 1st Annual AfRota meeting, BFR, Berlin, 20 - 22 September 2018. (Oral presentation and progress report).
5. **MGJ Huyzers**, AC Potgieter, AA van Dijk. Rotavirus reverse genetics, local implementation and optimizations. 2nd Annual AfRota meeting, UFS, South Africa, 09 - 11 September 2019. (Oral presentation and progress report).

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Appendix A

Plasmid maps of the phCMVdream constructs designed for, and implemented in both the pT7_SA11-L2 and pSmart_SA11-N5 RV RG systems.

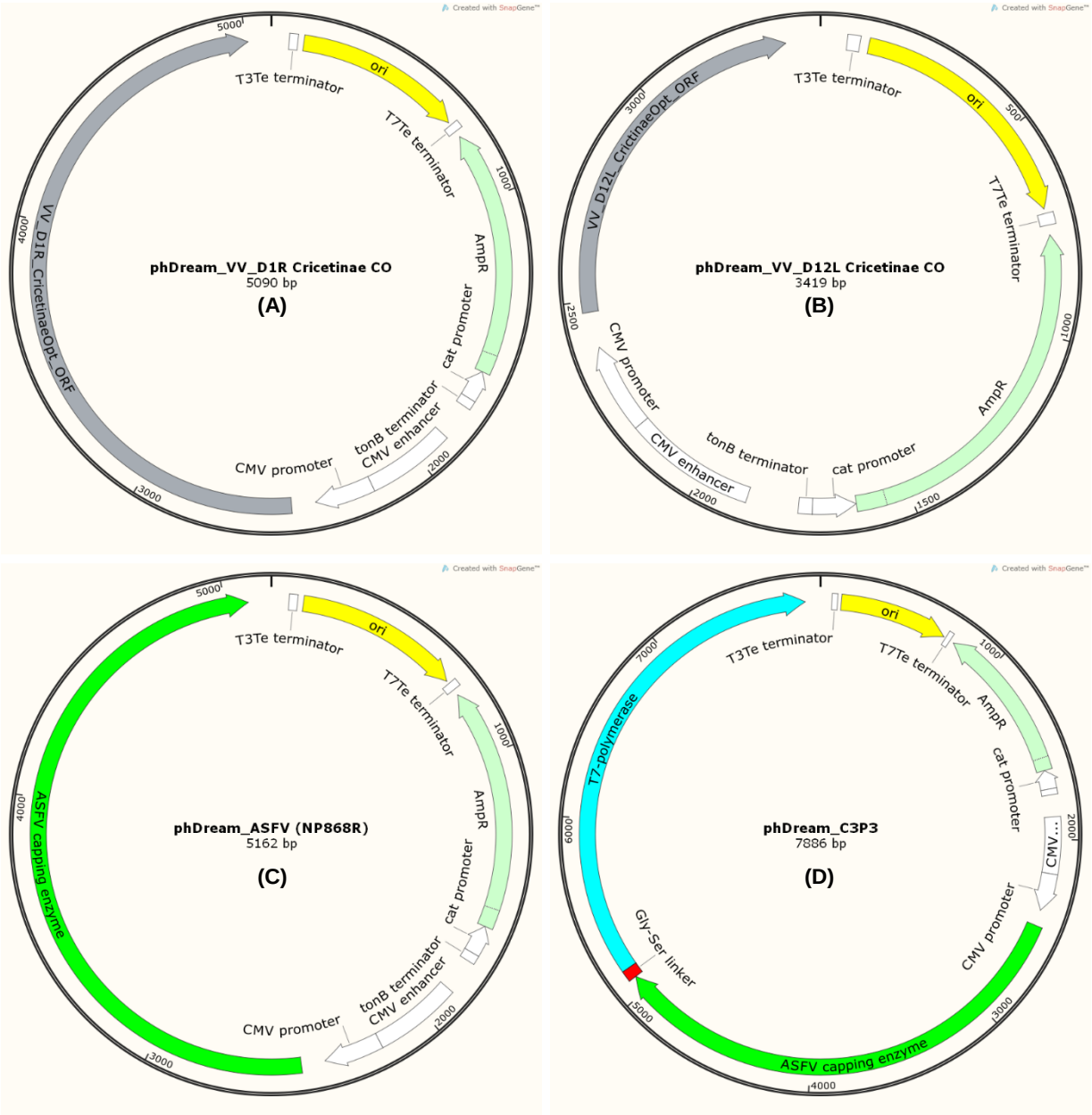

Figure 42: Plasmid maps of the phCMVdream expression plasmids for A: the VV D1R capping enzyme subunit, B: the VV D12L capping enzyme subunit, C: the ASFV capping enzyme and D: the ASFV capping enzyme fused to a viral T7-RNA polymerase through a serine-glycine linker (C3P3 construct). Images displayed show the short-hand annotations used during the early development and implantation of the project, ergo the phCMVdream plasmids are annotated as phDream. Plasmid maps were constructed using SnapGene software.

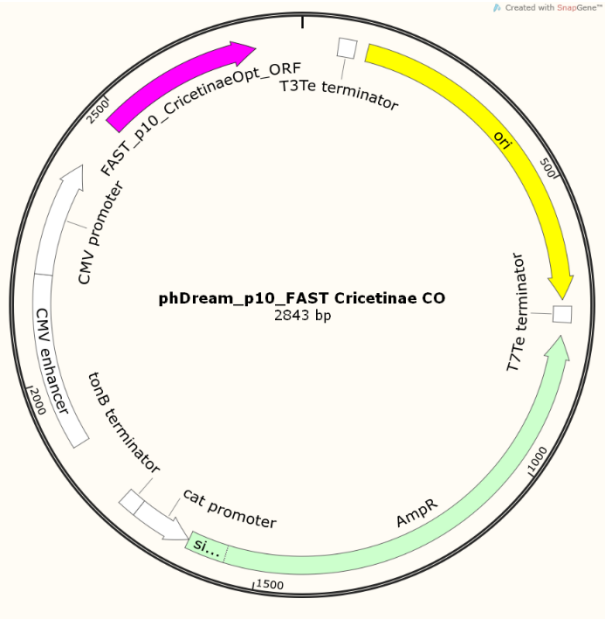

Figure 43: Map of phCMVdream_p10_FAST NBV fusion protein expression plasmid. Image shows the short-hand annotations used during the early development and implantation of the project, ergo the phCMVdream plasmid is annotated as phDream. Plasmid map was constructed using SnapGene software.

Appendix B

Plasmid maps of the Japanese pT7_SA11-L2 RV RG transcription plasmids along with NGS reads of the dsRNA extracted from cell cultures infected with the P1 viral stocks obtained from the pT7_SA11-L2 RG system, mapped against the RV SA11-L2 reference genome (LC333802 - LC3338012).

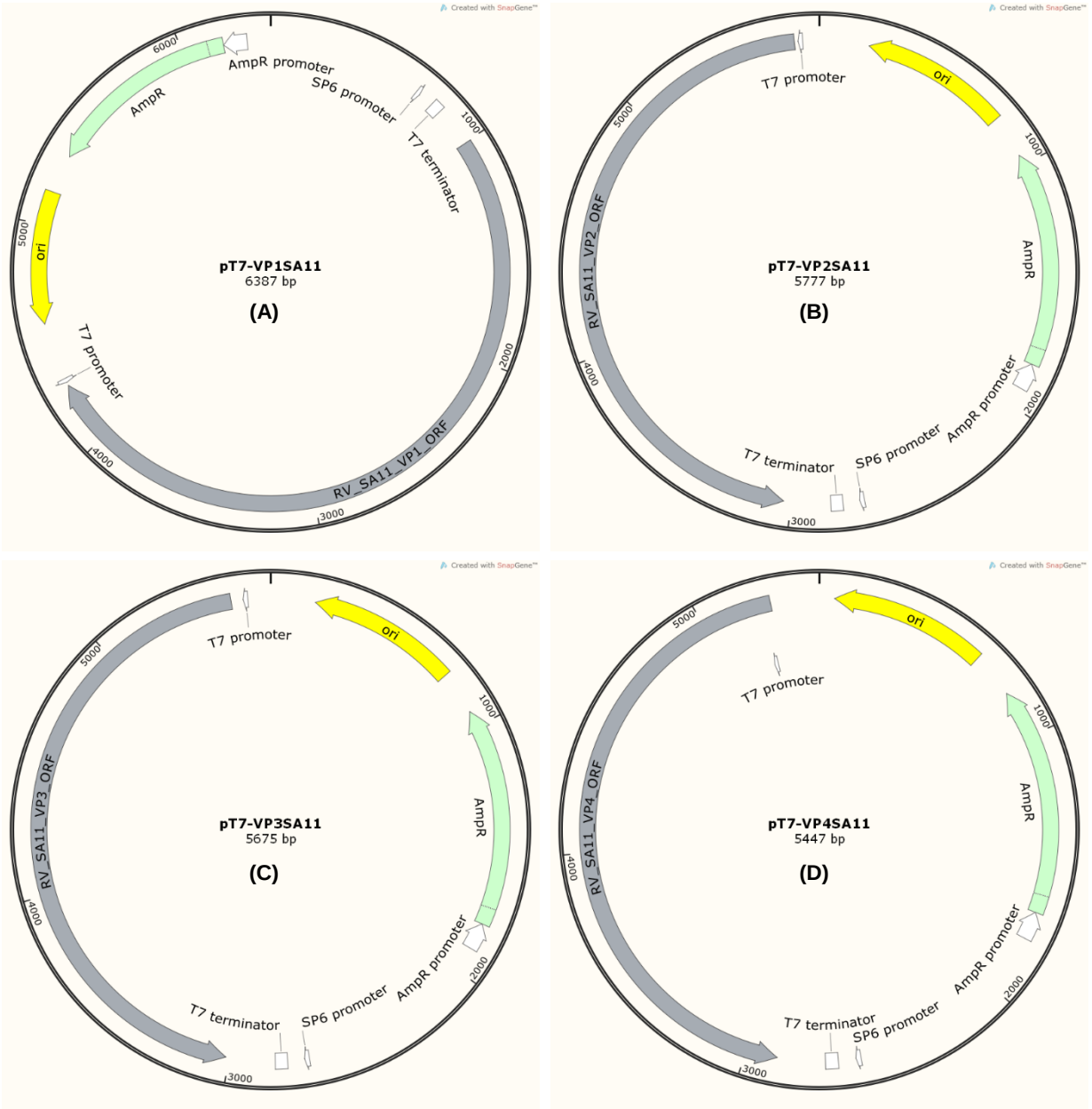

Figure 44: Plasmid maps of the pT7_SA11-L2 transcription plasmids for A: GS1(VP1), B: GS2(VP2), C: GS3(VP3) and D: GS4(VP4). Plasmid maps were constructed using SnapGene software.

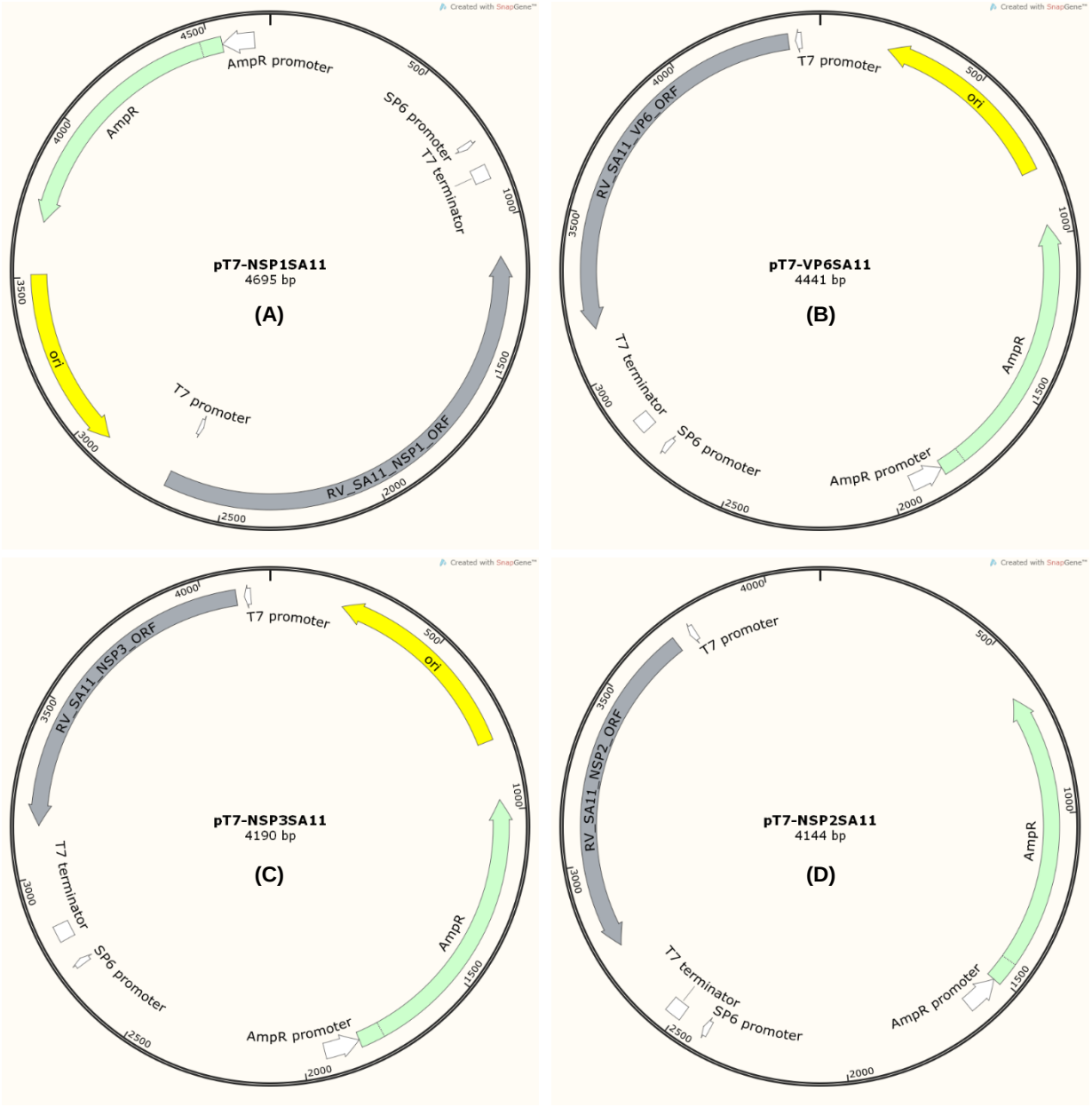

Figure 45: Plasmid maps of the pT7_SA11-L2 transcription plasmids for A: GS5(NSP1), B: GS6(VP6), C: GS7(NSP3) and D: GS8(NSP2). Plasmid maps were constructed using SnapGene software.

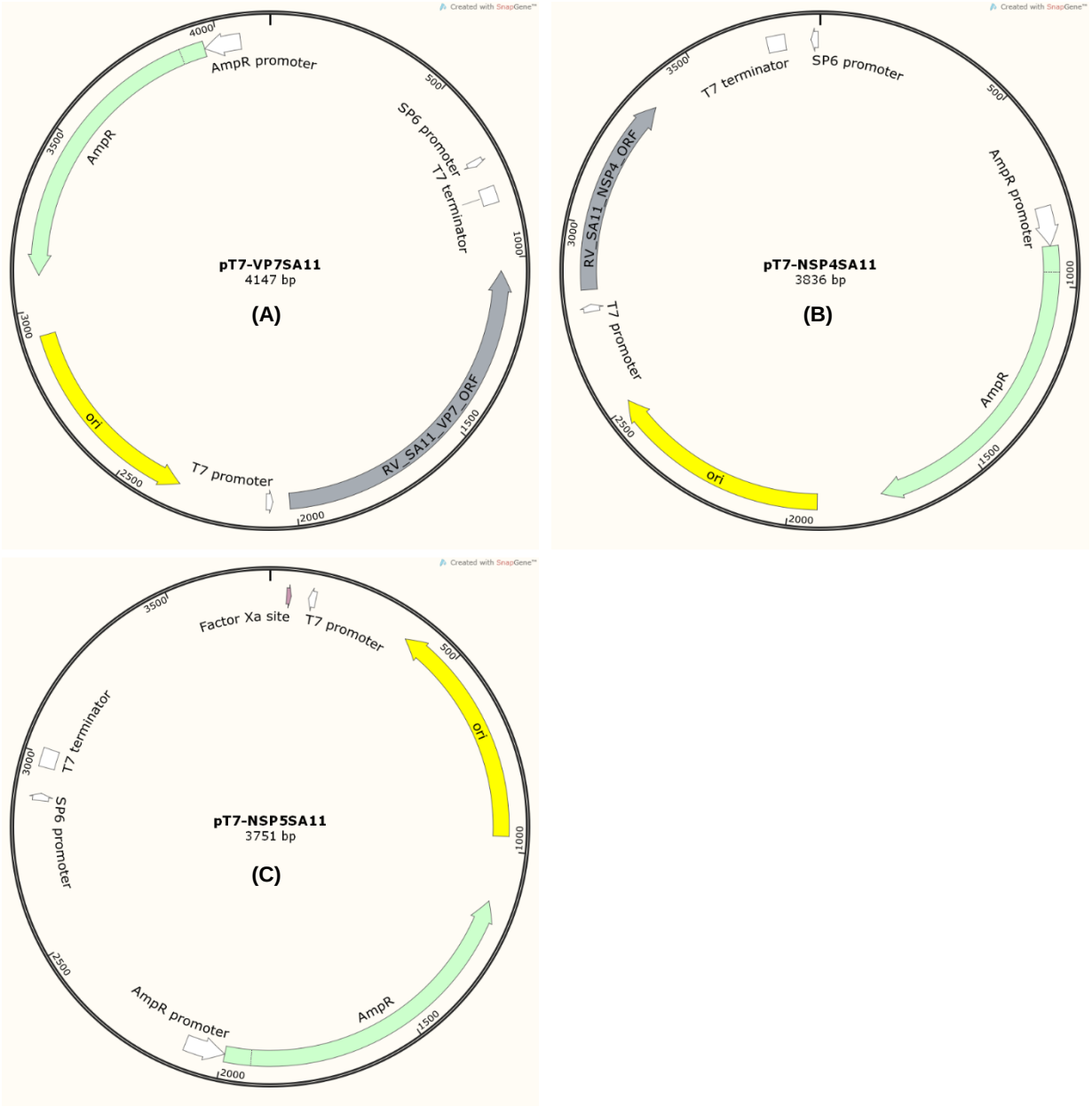

Figure 46: Plasmid maps of the pT7_SA11-L2 transcription plasmids for A: GS9(VP7), B: GS10 (NSP4) and C: GS11(NSP5/6). Plasmid maps were constructed using SnapGene software.

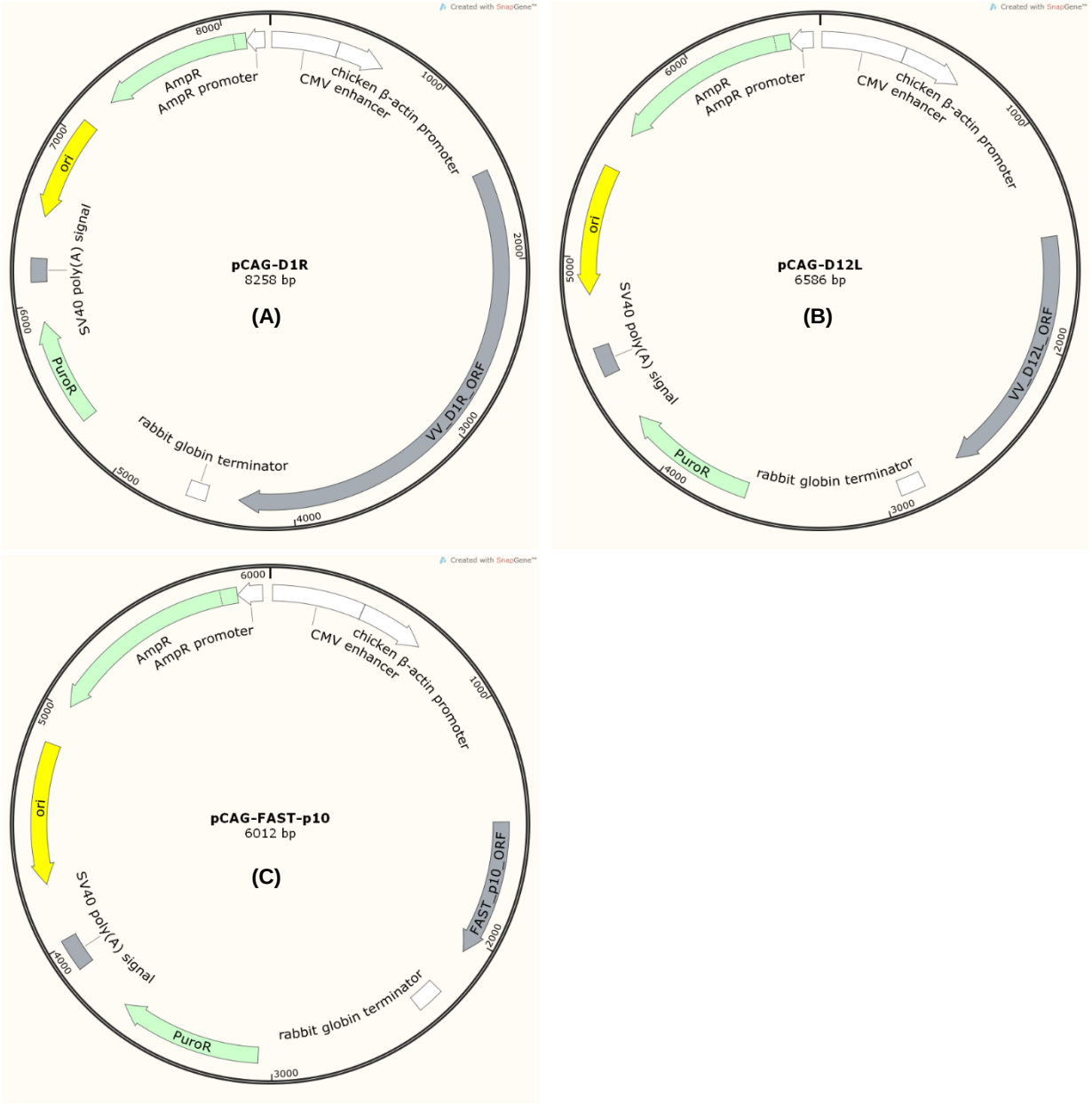

Figure 47: Plasmid maps of the pCAG expression plasmids for A: the VV D1R capping enzyme subunit, B: the VV D12L capping enzyme subunit and C: the NBV fusion protein. Plasmid maps were constructed using SnapGene software.

As stated in Section 3.3.7, dsRNA was extracted from cultured cells that were infected with P1 stocks of the optimized pT7_SA11-L2 RV RG system. The dsRNA was purified (Section 3.2.1) and used for sequence-independent primer ligation (Section 3.2.4). The oligo-ligated RNA was then converted to cDNA and PCR amplified for NGS. Figure 48, Figure 49 and Figure 50 are the visual illustrations of the NGS reads of the rescued SA11-L2 as mapped against the SA11-L2 reference genome (GenBank no: LC333802-LC333812).

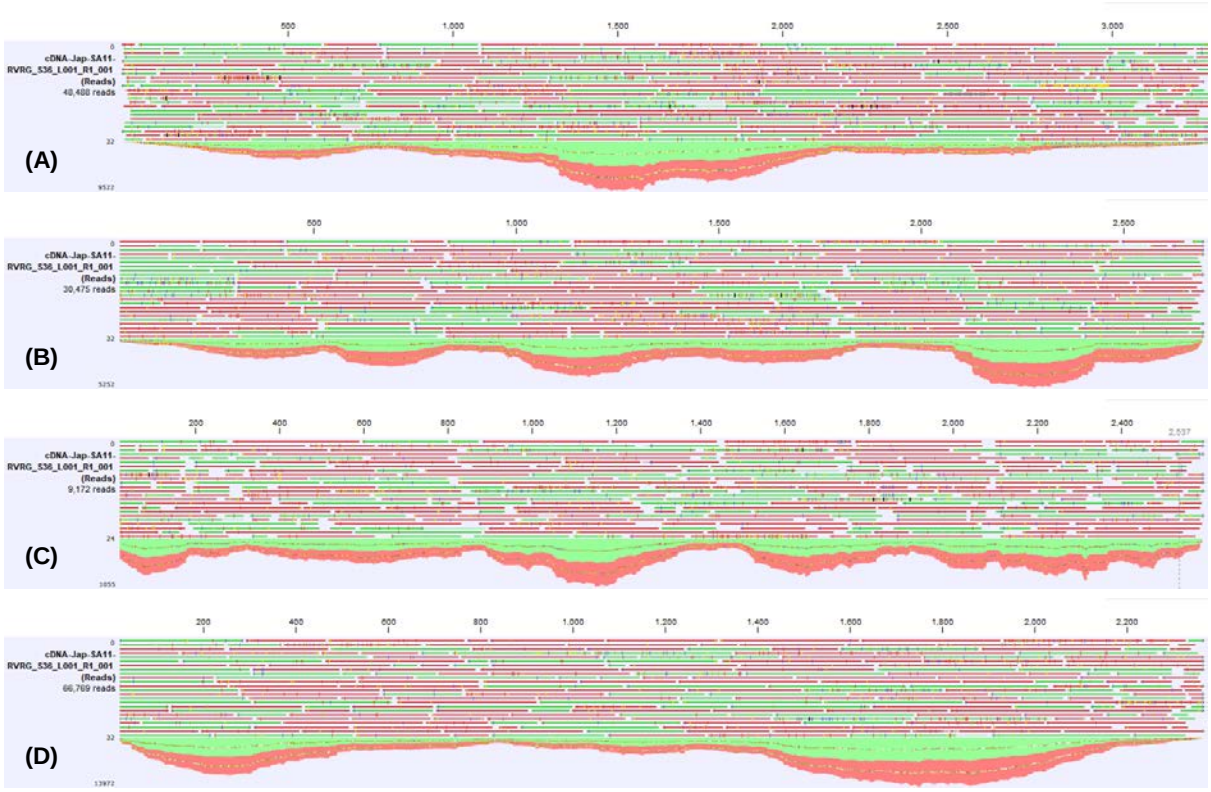

Figure 49: NGS reads of dsRNA extracted from cell cultures infected with P1 stocks obtained from the optimized pT7_SA11-L2 RV RG system mapped against the RV SA11-L2 reference genome (LC333802-LC333812) for A: GS1(VP1), B: GS2(VP2), C: GS3(VP3) and D: GS4(VP4). NGS reads compiled and mapped against SA11-L2 reference genome on CLC-Bio software and aligned on BioEdit software.

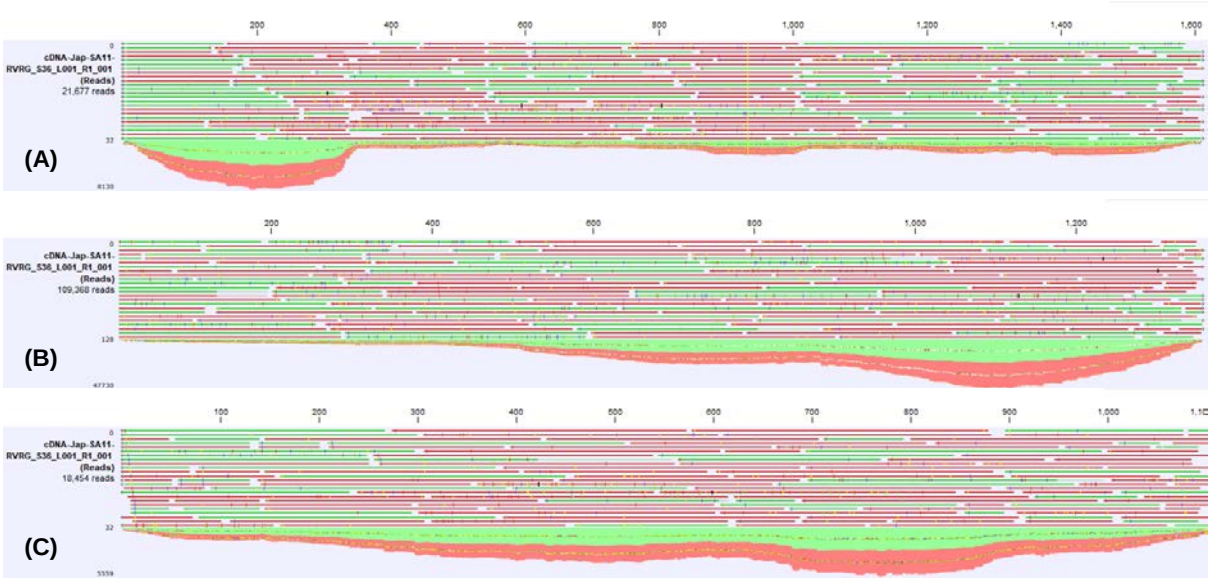

Figure 48: NGS reads of dsRNA extracted from cell cultures infected with P1 stocks obtained from the optimized pT7_SA11-L2 RV RG system mapped against the RV SA11-L2 reference genome (LC333802-LC333812) for A: GS5(NSP1), B: GS6(VP6) and C: GS7(NSP3). NGS reads compiled and mapped against SA11-L2 reference genome on CLC-Bio software and aligned on BioEdit software.

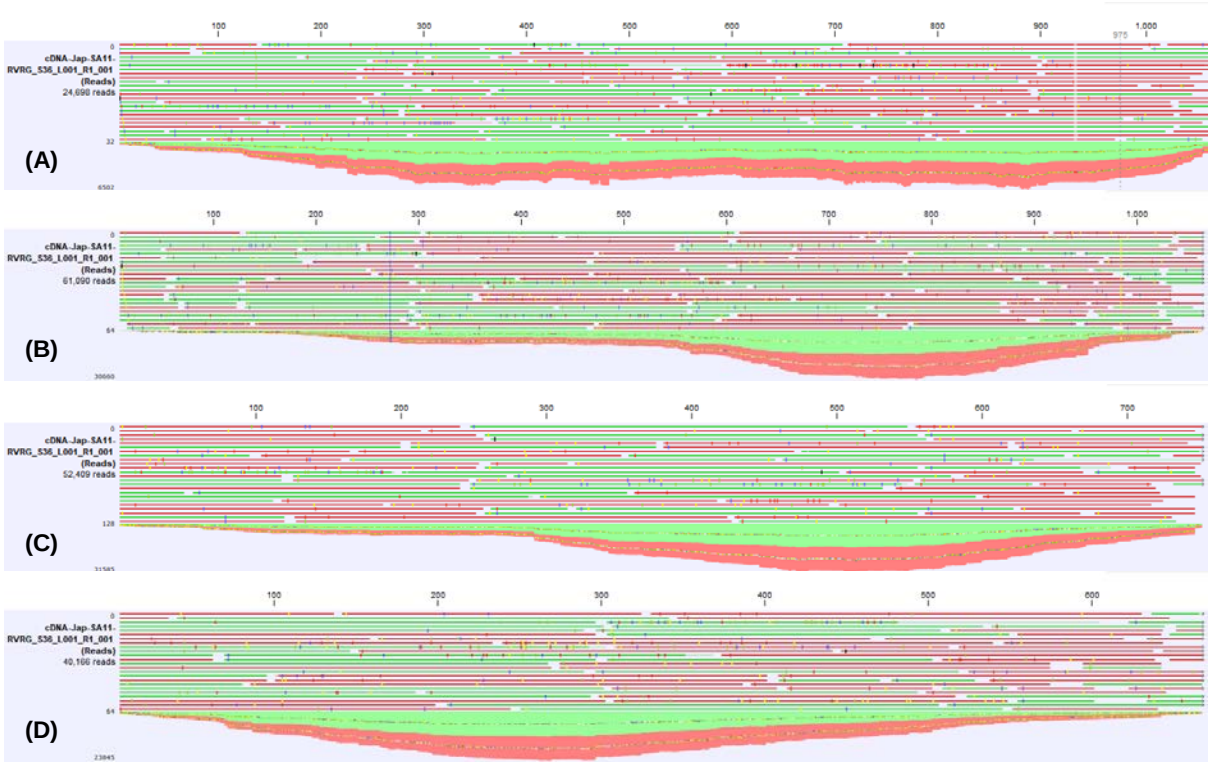

Figure 50: NGS reads of dsRNA extracted from cell cultures infected with P1 stocks obtained from the optimized pT7_SA11-L2 RV RG system mapped against the RV SA11-L2 reference genome (LC333802-LC333812) for A: GS8(NSP2), B: GS9(VP7), C: GS10(NSP4) and D: GS11(NSP5/6). NGS reads compiled and mapped against SA11-L2 reference genome on CLC-Bio software and aligned on BioEdit software.

See Supplementary Documentation for full consensus sequence alignments

Appendix C

Plasmid maps of the four SA11 consensus sequence-based transcription plasmids designed by Dr Wentzel.

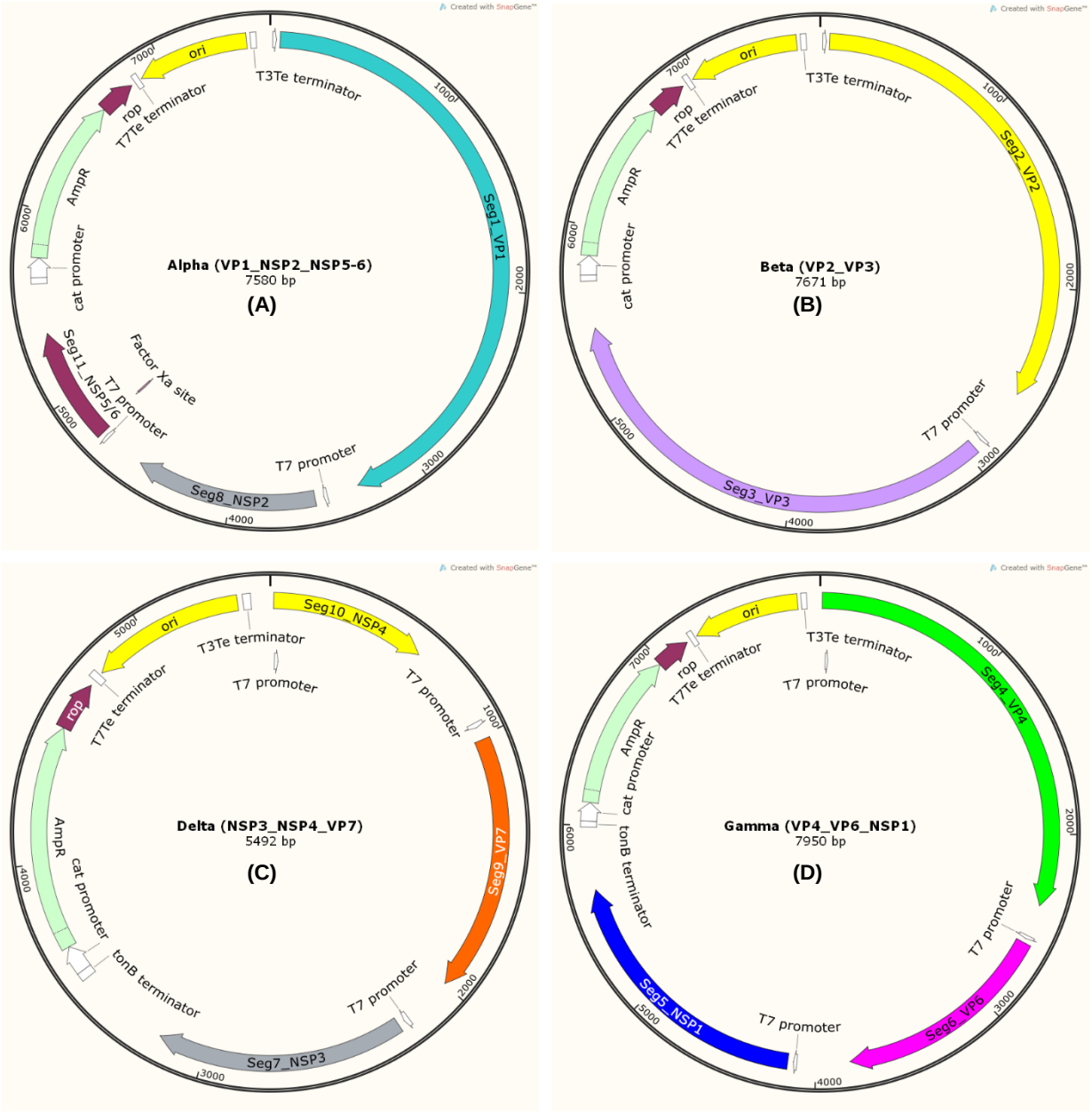

Figure 51: Plasmid maps of the four, consensus sequence based SA11-N5 transcription plasmids designed by Dr. Wentzel. A: pAlpha, harbouring GS1(VP1), GS8(NSP2) and GS11(NSP5/6). B: pBeta, harbouring GS2(VP2 and GS3(VP3). C: pDelta, harbouring GS7(NSP3), GS10(NSP4) and GS9(VP7). D: pGamma, harbouring GS4(VP4), GS6(VP6) and GS5(NSP1). Plasmid maps were constructed using SnapGene software.

Maps of the pSmart transcription plasmids that make up the pSmart_SA11-N5 RV RG system.

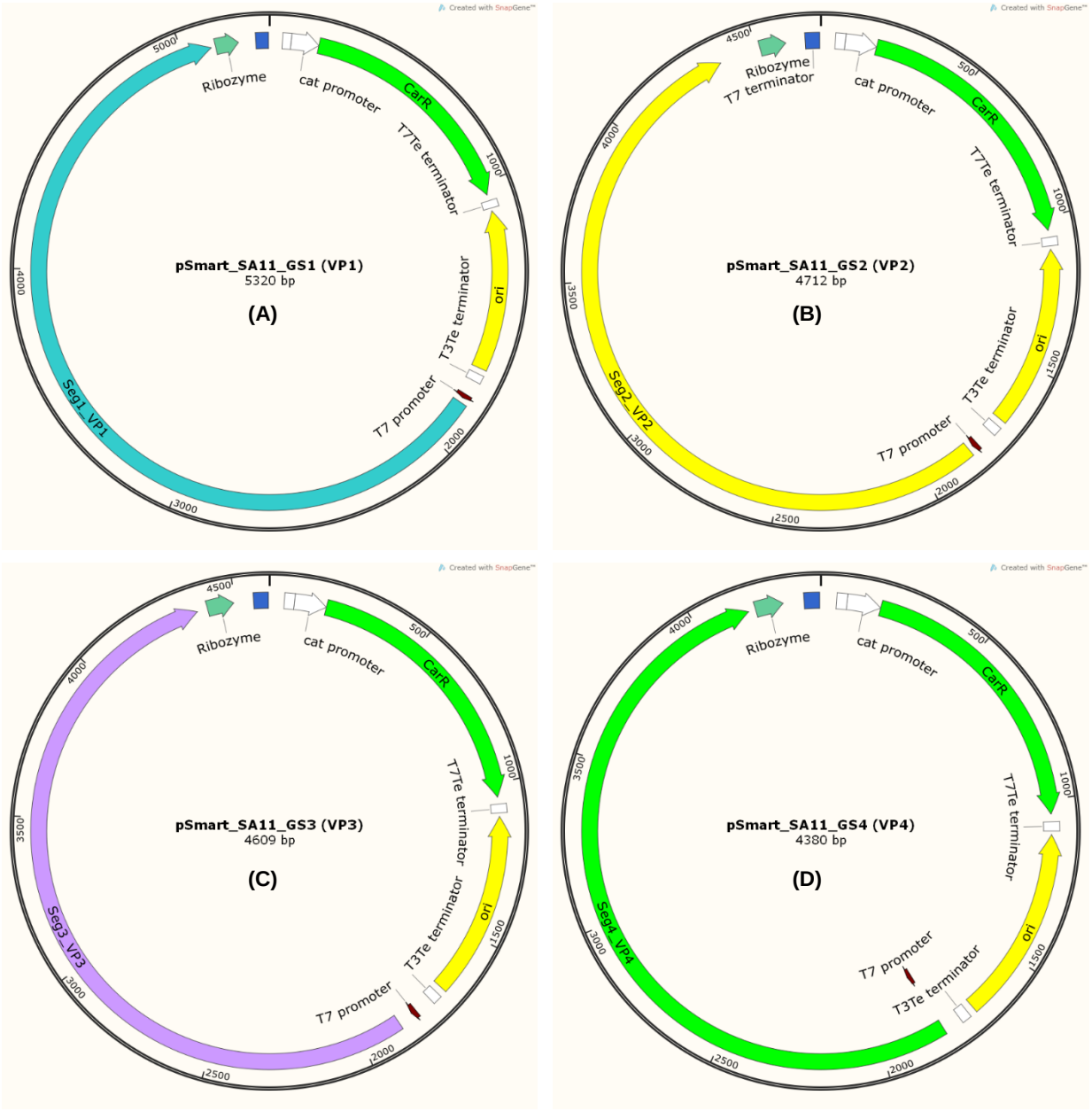

Figure 52: Maps of the SA11-N5 consensus sequence based pSmart transcription plasmids for A: GS1(VP1), B: GS2(VP2), C: GS3(VP3) and D: GS4(VP4). Plasmid maps were constructed using SnapGene software.

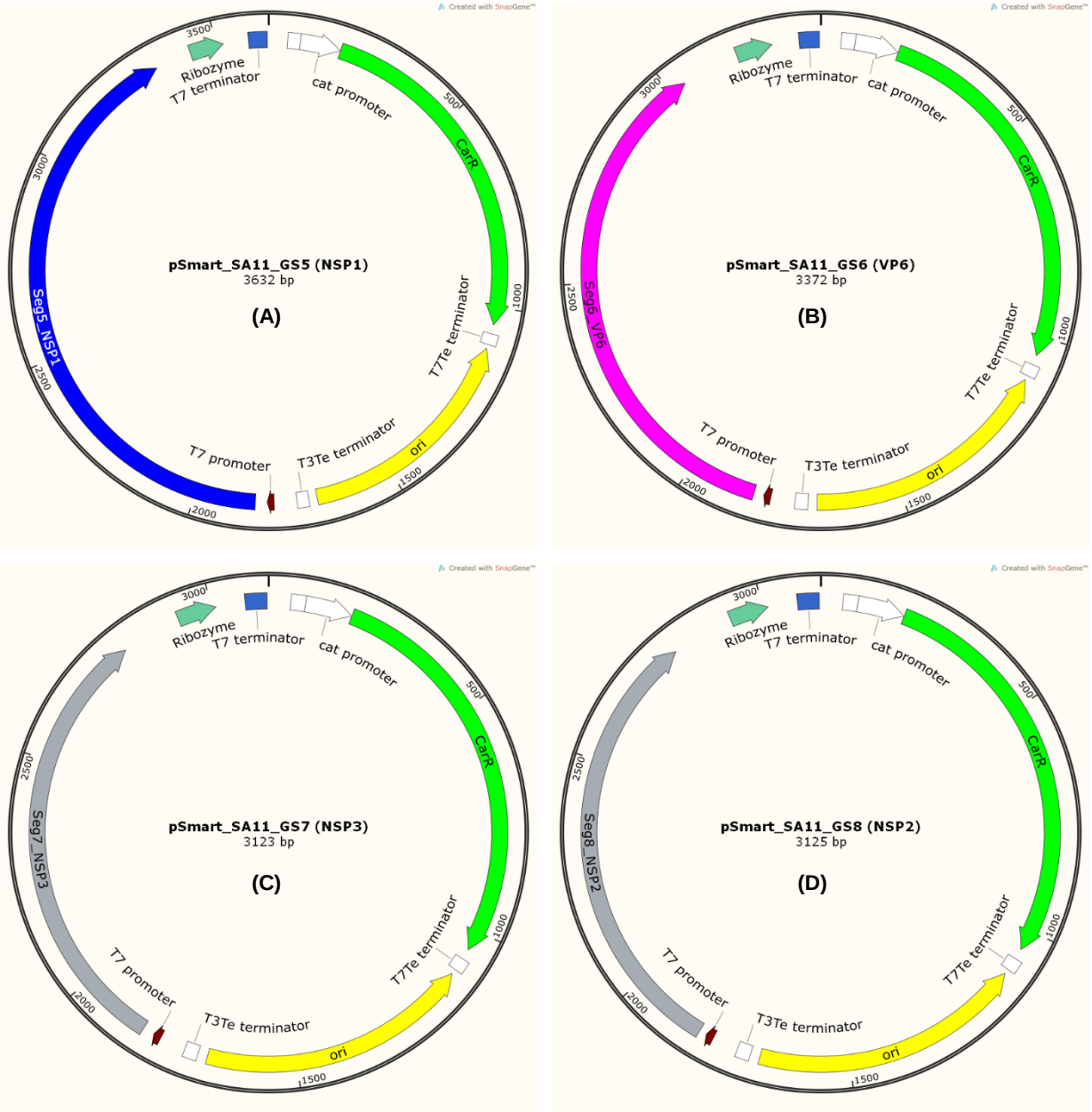

Figure 53: Maps of the SA11-N5 consensus sequence based pSmart transcription plasmids for A: GS5(NSP1), B: GS6(VP6), C: GS7(NSP3) and D: GS8(NSP2). Plasmid maps were constructed using SnapGene software.

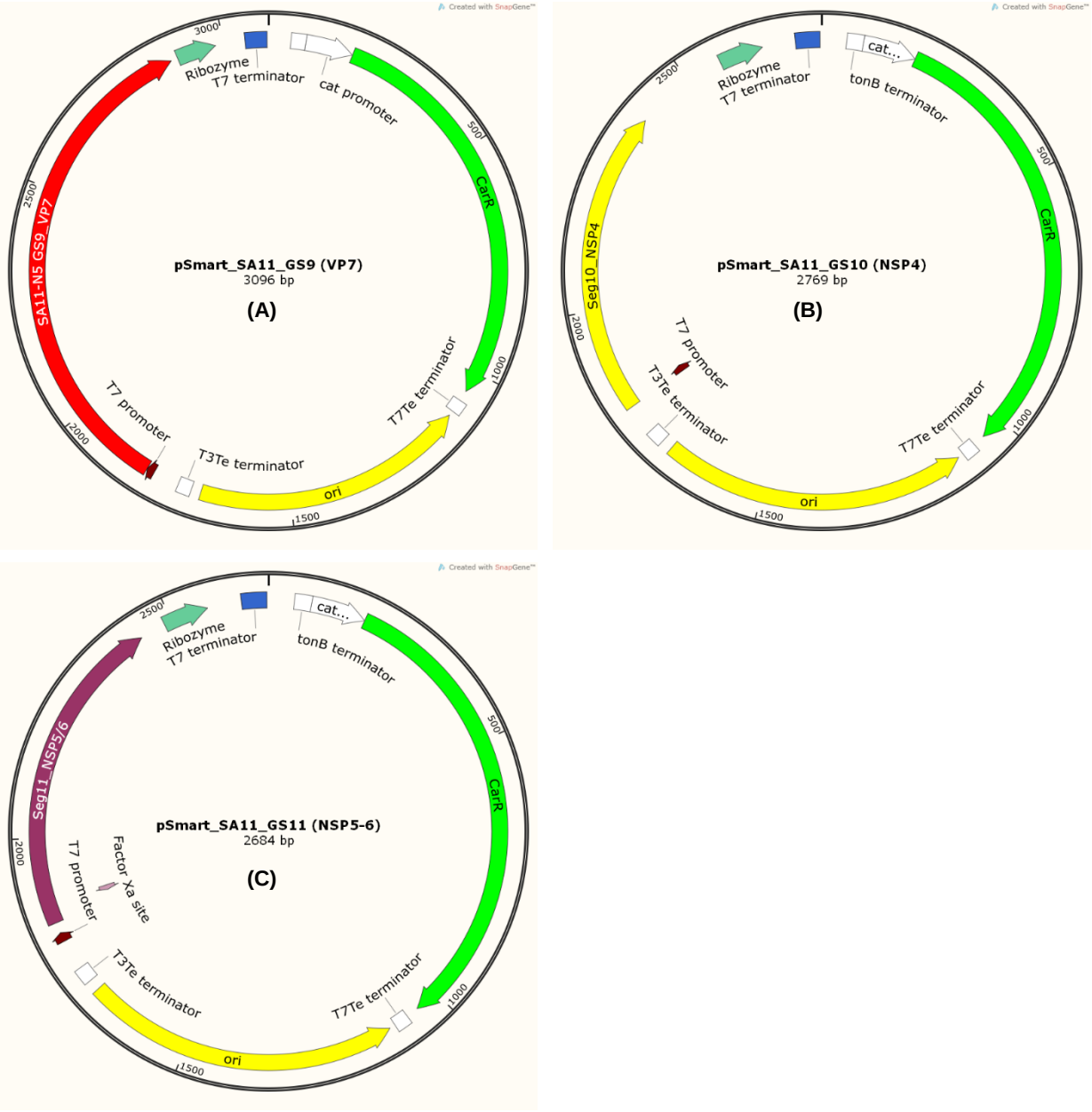

Figure 54: Maps of the SA11-N5 consensus sequence based pSmart transcription plasmids for A: GS9(VP7), B: GS10(NSP4) and C: GS11(NSP5/6). Plasmid maps were constructed using SnapGene software.

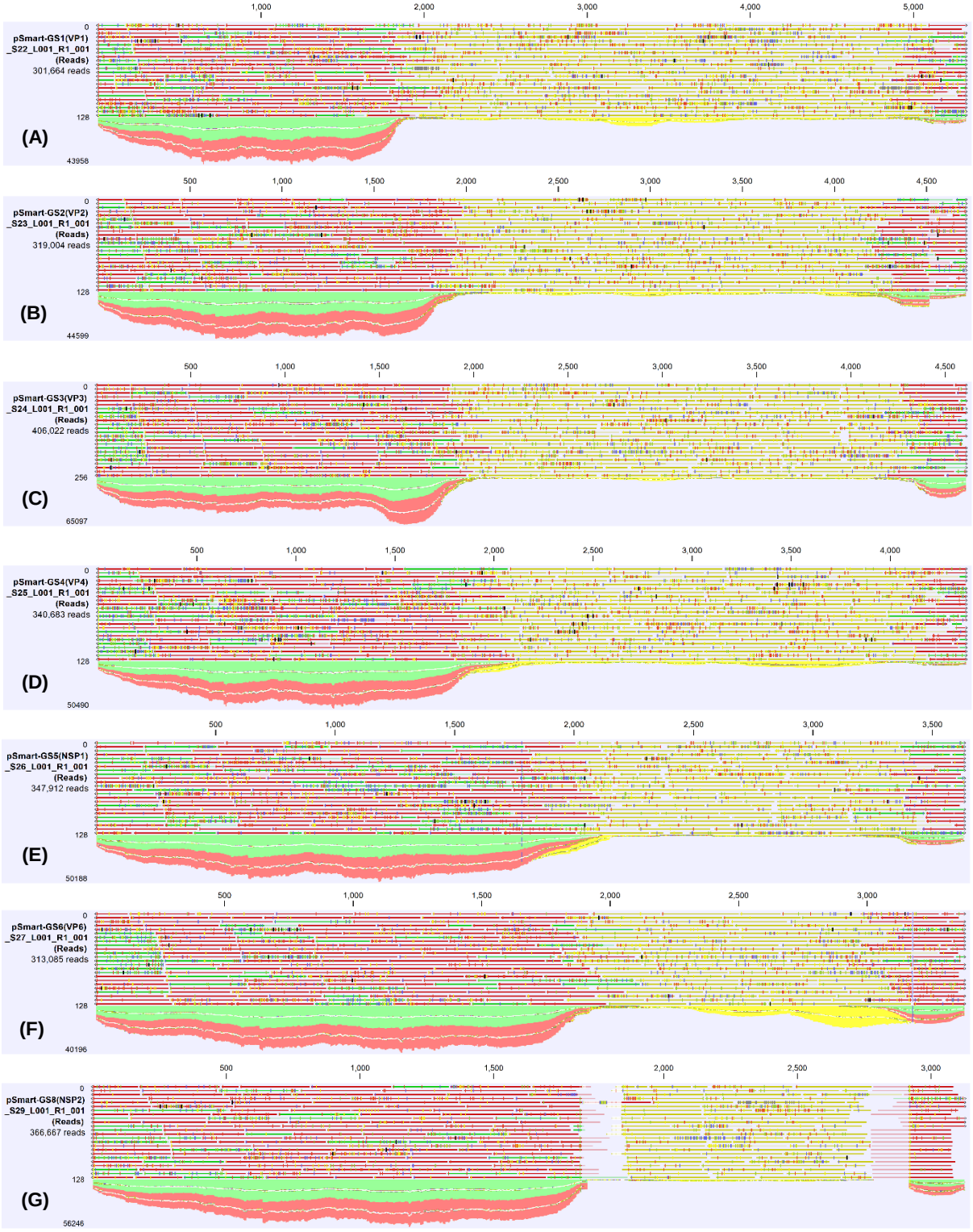

Figure 55: NGS reads of pSmart_SA11-N5 RV RG transcription plasmids mapped against the RV SA11-N5 reference genome and in silico pSmart_SA11-N5 RG constructs for A: GS1(VP1), B: GS2(VP2), C: GS3(VP3), D: GS4(VP4), E: GS5(NSP1), F: GS6(VP6) and G: GS7(NSP3). NGS reads compiled and mapped against SA11-N5 reference genome (JN827244-JN827250 and JN827252-JN827255) on CLC-Bio software and aligned on BioEdit software.

Figure 56: NGS reads of pSmart_SA11-N5 RV RG transcription plasmids mapped against the RV SA11-N5 reference genome and in silico pSmart_SA11-N5 RG constructs for A: GS8(NSP2), B: GS9(VP7), C: GS10(NSP4) and D: GS11(NSP5/6). NGS reads compiled and mapped against SA11-N5 reference genome (JN827244-JN827250 and JN827252- JN827255) on CLC-Bio software and aligned on BioEdit software.

See Supplementary Documentation for full consensus sequence alignments

Appendix D

$TCID_{50}$ extended description and calculation illustration.

The $TCID_{50}$ begins with the preparation of the dilution media. 1x MEM is supplemented with 1% (v/v) NEAA, 1mg/ml porcine trypsin and 100 units/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B (Anti-anti Gibco). This dilution media is then used to construct the serial dilution series of the isolated viral culture as per Table 25 below. The initial dilution (10^0) is merely 500µl of activated viral inoculum. From this 50µl is transferred to 450µl of dilution media to create the second dilution (10^{-1}). The second dilution is mixed very well and then a new tip is used to transfer 50µl of it to a new 450µl of dilution media, thus forming the third dilution (10^{-2}). This process is repeated, using a new tip each time, until the dilution factor is 10^{-7}.

Table 25: Construction of dilution series for $TCID_{50}$ assay

Dilution	Cell control	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Volume MEM (µl)	500	0	450	450	450	450	450	450	450
Volume virus (µl)	0	500	50	50	50	50	50	50	50

When transferring the viral serial dilution to a 96-well plate, ensure that each dilution is represented by four consecutive wells. Thus wells A1 through to A4 will each receive 100µl of the 10^0 dilutions while wells B1 through to B4 each receive 100µl of the 10^{-1} dilution. This quadruplicate layout is required for the calculation of the 50% extinction coefficient and the $TCID_{50}$. Once each dilution has been transferred to the 96-well plate the plate is incubated for 60min while MA104 or ST cells are prepared. A roughly 90% confluent 25cm^2 tissue culture flask of either cell-line will provide enough cells to seed the entire 96-well plate. The monolayer of cells has its growth media removed and is washed twice with 1x PBS. The cells are treated with 0.05% trypsin EDTA and incubated at 37°C until the cells start to detach. The trypsin is removed and the cells are then re-suspended in 500µl of 100% FBS and added to 9.5ml room-temperature growth media (lacking FBS). This yields 10ml of re-suspended MA104 or ST cells that

are of appropriate density to seed the entire 96-well plate. 100µl of this cell suspension is then added to each of the wells of the 96-well viral dilution. This results in each well-containing 200µl of media in which there is activated virus, 0.5µg/ml porcine trypsin and roughly $0.1x10^6$ cells.

The seeded 96-well plate is then incubated at 37°C for 3-5 days or until CPE is clearly visible, after which an IFMA is performed on the plate to visualise any viral activity. Any fluorescence detectable in a well is deemed sufficient indication of viral infection and propagation and that well is then annotated as positive. If no fluorescence is visible the well is annotated as negative. This is a slight deviation of the standard $TCID_{(50)}$ protocol which relied on plaque formation and cell death to visualise viral activity. In this case, we visualise the virus not by evaluating cell viability but by directly illuminating the virus. It is assumed that if a viral focus is formed that is visually fluorescent the cells in that well, given enough time, would all be infected and would perish. From this annotation, the basic calculations of $TCID_{50}$ can be done (Barrett *et al.*, 1996; LaBarre & Lowy, 2001; Smith *et al.*, 1996; Wang *et al.*, 2018). Table 26 below illustrates the annotation style and will be used to explain the calculation of the $TCID_{(50)}$ and viral titer.

Table 26: Depiction of $TCID_{50}$ result annotation

Dilution factor	Viral culture A				Viral culture B			
0	++	++	++	++	+++	+++	+++	+++
10^{-1}	+	+	+	+	+++	++	++	+++
10^{-2}	+	+	-	-	++	+	+	++
10^{-3}	-	-	-	-	+	-	+	+
10^{-4}	-	-	-	-	-	-	-	+
10^{-5}	-	-	-	-	-	-	-	-
10^{-6}	-	-	-	-	-	-	-	-
10^{-7}	-	-	-	-	-	-	-	-

As illustrated in Table 26 above each dilution is represented by 4 wells and fluorescent activity is annotated as “+” whereas the lack of fluorescence is annotated as “-“. To calculate the $TCID_{50}$ a table is constructed that summarises the dilution factor and correlates it with the number of wells that are either infected or non-infected. The sum total of infected wells is then calculated from the lowest dilution to highest. This is then

followed by the sum total of non-infected wells calculated from highest to lowest dilution. Finally, the percentage of infected wells as measured against the total number of wells is calculated. Table 27 below illustrates this calculation for the example used in Table 26 above.

Table 27: Calculation of $TCID_{50}$

Viral culture	Dilution	No. infected wells	No. non-infected wells	ΣNo. infected wells (lowest to highest dilution)	ΣNo. non-infected wells (highest to lowest dilution)	% of ΣNo. infected wells / ΣTotal no. wells
A	10^{-1}	4	0	6	0	100
	10^{-2}	2	2	2	2	50
	10^{-3}	0	4	0	4	0
B	10^{-1}	4	0	12	0	100
	10^{-2}	4	0	8	0	100
	10^{-3}	3	1	4	1	80
	10^{-4}	1	3	1	4	20
	10^{-5}	0	4	0	8	0

From Table 27 above we can see that the $TCID_{50}$ (or the dilution at which half of the wells died as a result of viral activity) for viral culture A is precisely 10^{-2}, however, the value for viral culture B lies somewhere between 10^{-3} and 10^{-4}. Using the calculations described in Reed and Muench (1938) the $TCID_{50}$/ml for viral culture A is $5x10^2$ and viral culture B is $1.58x10^4$ (REED & MUENCH, 1938).

Supplementary Documentation

Sequence alignments for the dsRNA extracted from cell cultures infected with P1 stocks of the pT7_SA11-L2 RV RG system, and the pSmart_SA11-N5 RV RG transcription plasmids.

Alignment: SA11-L2_GS1(VP1)

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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS1(VP1) reference   GGCTATTAAA GCTGTACAAT GGGGAAGTAC AATCTAATCT TGTCAGAATA TCTATCATTT ATATATAATT CACAATCTGC AGTTCAAATT CCAATATATT
SA11-L2_GS1(VP1) NGS cs      GGCTATTAAA GCTGTACAAT GGGGAAGTAC AATCTAATCT TGTCAGAATA TCTATCATTT ATATATAATT CACAATCTGC AGTTCAAATT CCAATATATT


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 110        120        130        140        150        160        170        180        190        200
SA11-L2_GS1(VP1) reference   ACTCTTCCAA CAGTGAATTA GAAAATAGAT GTATTGAATT TCATTCCAAG TGTTTAGAGA ACTCAAAGAA TGGGTTATCG TTAAGAAAGT TGTTTGTTGA
SA11-L2_GS1(VP1) NGS cs      ACTCTTCCAA CAGTGAATTA GAAAATAGAT GTATTGAATT TCATTCCAAG TGTTTAGAGA ACTCAAAGAA TGGGTTATCG TTAAGAAAGT TGTTTGTTGA


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                                 210        220        230        240        250        260        270        280        290        300
SA11-L2_GS1(VP1) reference   ATATAATGAT GTCATAGAAA ATGCCACATT ACTGTCAATA CTATCATATT CTTACGACAA GTATAACGCT GTTGAAAGAA AATTGGTGAA GTATGCGAAA
SA11-L2_GS1(VP1) NGS cs      ATATAATGAT GTCATAGAAA ATGCCACATT ACTGTCAATA CTATCATATT CTTACGACAA GTATAACGCT GTTGAAAGAA AATTGGTGAA GTATGCGAAA


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                                 310        320        330        340        350        360        370        380        390        400
SA11-L2_GS1(VP1) reference   GGCAAACCAT TGGAGGCAGA CTTAACAGTG AATGAATTGG ATTATGAGAA CAATAAAATA ACATCTGAAT TATTTCCAAC AGCGGAGGAA TATACGGACT
SA11-L2_GS1(VP1) NGS cs      GGCAAACCAT TGGAGGCAGA CTTAACAGTG AATGAATTGG ATTATGAGAA CAATAAAATA ACATCTGAAT TATTTCCAAC AGCGGAGGAA TATACGGACT


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                                 410        420        430        440        450        460        470        480        490        500
SA11-L2_GS1(VP1) reference   CACTAATGGA TCCAGCAATT TTAACTTCGC TATCATCAAA TTTAAATGCA GTCATGTTCT GGTTGGAAAA ACATGAAAAT GATGTCGCTG AAAAACTTAA
SA11-L2_GS1(VP1) NGS cs      CACTAATGGA TCCAGCAATT TTAACTTCGC TATCATCAAA TTTAAATGCA GTCATGTTCT GGTTGGAAAA ACATGAAAAT GATGTCGCTG AAAAACTTAA


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                                 510        520        530        540        550        560        570        580        590        600
SA11-L2_GS1(VP1) reference   AGTTTATAAA AGGAGATTAG ACCTATTCAC CATAGTAGCC TCAACGATAA ATAAATATGG CGTACCAAGG CATAACGCAA AGTACAGATA TGAATACGAC
SA11-L2_GS1(VP1) NGS cs      AGTTTATAAA AGGAGATTAG ACCTATTCAC CATAGTAGCC TCAACGATAA ATAAATATGG CGTACCAAGG CATAACGCAA AGTACAGATA TGAATACGAC


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                                 610        620        630        640        650        660        670        680        690        700
SA11-L2_GS1(VP1) reference   GTAATGAAAG ATAAACCGTA CTACTTAGTG ACATGGGCAA ATTCTTCAAT TGAAATGTTA ATGTCAGTTT TCTCTCATGA CGACTATTTG ATAGCAAAAG
SA11-L2_GS1(VP1) NGS cs      GTAATGAAAG ATAAACCGTA CTACTTAGTG ACATGGGCAA ATTCTTCAAT TGAAATGTTA ATGTCAGTTT TCTCTCATGA CGACTATTTG ATAGCAAAAG


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                                 710        720        730        740        750        760        770        780        790        800
SA11-L2_GS1(VP1) reference   AGTTAATAGT GTTATCATAT TCTAATAGAT CTACTCTAGC AAAGTTAGTG TCATCACCAA TGTCGATTTT GGTAGCCTTG GTGGATATTA ATGGAACATT
SA11-L2_GS1(VP1) NGS cs      AGTTAATAGT GTTATCATAT TCTAATAGAT CTACTCTAGC AAAGTTAGTG TCATCACCAA TGTCGATTTT GGTAGCCTTG GTGGATATTA ATGGAACATT


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                                 810        820        830        840        850        860        870        880        890        900
SA11-L2_GS1(VP1) reference   TATTACAAAT GAAGAATTAG AATTGGAATT TTCAAATAAA TATGTACGAG CAATAGTTCC GGATCAAACA TTTGACGAAT TAAATCAAAT GCTTGACAAT
SA11-L2_GS1(VP1) NGS cs      TATTACAAAT GAAGAATTAG AATTGGAATT TTCAAATAAA TATGTACGAG CAATAGTTCC GGATCAAACA TTTGACGAAT TAAATCAAAT GCTTGACAAT
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                                910        920        930        940        950        960        970        980        990       1000
SA11-L2_GS1(VP1) reference   ATGAGGAAAG CTGGATTAGT TGACATACCT AAGATGATAC AGGACTGGTT AGTTGATCGT TCTATCGAAA AATTTCCATT AATGGCTAAG ATATATTCAT
SA11-L2_GS1(VP1) NGS cs      ATGAGGAAAG CTGGATTAGT TGACATACCT AAGATGATAC AGGACTGGTT AGTTGATCGT TCTATCGAAA AATTTCCATT AATGGCTAAG ATATATTCAT


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                               1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-L2_GS1(VP1) reference   GGTCGTTTCA TGTTGGATTT AGAAAGCAAA AAATGCTAGA TGCTGCGCTG GATCAATTGA AAACTGAGTA TACAGAAAAT GTGGACGATG AAATGTATCG
SA11-L2_GS1(VP1) NGS cs      GGTCGTTTCA TGTTGGATTT AGAAAGCAAA AAATGCTAGA TGCTGCGCTG GATCAATTGA AAACTGAGTA TACAGAAAAT GTGGACGATG AAATGTATCG


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                               1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-L2_GS1(VP1) reference   GGAATATACA ATGTTAATAA GAGATGAAGT AGTTAAAATG CTTGAAGAAC CAGTTAAACA TGATGATCAC TTGCTACGAG ATTCTGAGTT AGCTGGTTTA
SA11-L2_GS1(VP1) NGS cs      GGAATATACA ATGTTAATAA GAGATGAAGT AGTTAAAATG CTTGAAGAAC CAGTTAAACA TGATGATCAC TTGCTACGAG ATTCTGAGTT AGCTGGTTTA


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                               1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-L2_GS1(VP1) reference   CTATCAATGT CGTCAGCATC GAATGGTGAG TCAAGGCAGC TAAAGTTTGG TAGGAAAACA ATTTTTTCAA CTAAAAAGAA TATGCATGTC ATGGATGATA
SA11-L2_GS1(VP1) NGS cs      CTATCAATGT CGTCAGCATC GAATGGTGAG TCAAGGCAGC TAAAGTTTGG TAGGAAAACA ATTTTTTCAA CTAAAAAGAA TATGCATGTC ATGGATGATA


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                               1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-L2_GS1(VP1) reference   TGGCTAACGA AAGATACACG CCTGGTATAA TACCACCAGT GAATGTTGAT AAACCAATAC CATTAGGAAG AAGAGATGTT CCAGGAAGAA GGACTAGAAT
SA11-L2_GS1(VP1) NGS cs      TGGCTAACGA AAGATACACG CCTGGTATAA TACCACCAGT GAATGTTGAT AAACCAATAC CATTAGGAAG AAGAGATGTT CCAGGAAGAA GGACTAGAAT


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                               1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-L2_GS1(VP1) reference   AATATTCATT CTGCCATACG AATATTTCAT AGCACAGCAC GCTGTAGTTG AAAAAATGTT GATTTACGCA AAACATACGA GAGAATACGC TGAATTTTAT
SA11-L2_GS1(VP1) NGS cs      AATATTCATT CTGCCATACG AATATTTCAT AGCACAGCAC GCTGTAGTTG AAAAAATGTT GATTTACGCA AAACATACGA GAGAATACGC TGAATTTTAT


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                               1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
SA11-L2_GS1(VP1) reference   TCACAATCAA ACCAATTATT GTCATACGGC GATGTAACGC GTTTTTTGTC TAATAACACA ATGGTCTTGT ATACGGATGT ATCTCAGTGG GATTCGTCTC
SA11-L2_GS1(VP1) NGS cs      TCACAATCAA ACCAATTATT GTCATACGGC GATGTAACGC GTTTTTTGTC TAATAACACA ATGGTCTTGT ATACGGATGT ATCTCAGTGG GATTCGTCTC


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                               1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-L2_GS1(VP1) reference   AGCATAATAC ACAGCCATTT AGGAAAGGAA TAATAATGGG ACTGGACATA TTAGCTAACA TGACTAATGA TGCTAAAGTT CTTCAGACAT TAAACTTATA
SA11-L2_GS1(VP1) NGS cs      AGCATAATAC ACAGCCATTT AGGAAAGGAA TAATAATGGG ACTGGACATA TTAGCTAACA TGACTAATGA TGCTAAAGTT CTTCAGACAT TAAACTTATA


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                               1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-L2_GS1(VP1) reference   CAAACAAACA CAAATCAATC TCATGGATTC ATACGTTCAA ATACCAGATG GCAACGTCAT TAAGAAAATA CAATACGGGG CAGTAGCATC AGGAGAGAAA
SA11-L2_GS1(VP1) NGS cs      CAAACAAACA CAAATCAATC TCATGGATTC ATACGTTCAA ATACCAGATG GCAACGTCAT TAAGAAAATA CAATACGGGG CAGTAGCATC AGGAGAGAAA
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                                  1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-L2_GS1(VP1) reference    CAAACGAAAG CAGCAAATTC AATAGCAAAT TTGGCACTGA TTAAAACGGT TTTGTCACGT ATTTCTAACA AACATTCATT CGCAACAAAA ATAATAAGAG
SA11-L2_GS1(VP1) NGS cs       CAAACGAAAG CAGCAAATTC AATAGCAAAT TTGGCACTGA TTAAAACGGT TTTGTCACGT ATTTCTAACA AACATTCATT CGCAACAAAA ATAATAAGAG

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                                  1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-L2_GS1(VP1) reference    TTGATGGAGA TGATAACTAT GCGGTGCTAC AATTTAATAC AGAGGTGACT AAGCAGATGA TCCAAGACGT ATCGAACGAT GTAAGAGAAA CTTATGCACG
SA11-L2_GS1(VP1) NGS cs       TTGATGGAGA TGATAACTAT GCGGTGCTAC AATTTAATAC AGAGGTGACT AAGCAGATGA TCCAAGACGT ATCGAACGAT GTAAGAGAAA CTTATGCACG

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                                  2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-L2_GS1(VP1) reference    CATGAATGCT AAAGTTAAAG CTCTGGTATC CACAGTAGGA ATAGAAATTG CTAAAAGGTA CATTGCAGGT GGAAAAATAT TTTTTCGAGC TGGAATAAAT
SA11-L2_GS1(VP1) NGS cs       CATGAATGCT AAAGTTAAAG CTCTGGTATC CACAGTAGGA ATAGAAATTG CTAAAAGGTA CATTGCAGGT GGAAAAATAT TTTTTCGAGC TGGAATAAAT

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                                  2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-L2_GS1(VP1) reference    CTACTTAATA ATGAAAAGAG AGGGCAGAGT ACGCAGTGGG ATCAAGCAGC AATTTTATAT TCAAATTATA TTGTAAATAG ACTTAGAGGA TTTGAAACTG
SA11-L2_GS1(VP1) NGS cs       CTACTTAATA ATGAAAAGAG AGGGCAGAGT ACGCAGTGGG ATCAAGCAGC AATTTTATAT TCAAATTATA TTGTAAATAG ACTTAGAGGA TTTGAAACTG

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                                  2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-L2_GS1(VP1) reference    ATAGGGAGTT TATTTTAACT AAGATAATGC AGATGACGTC AGTCGCAATT ACTGGATCAT TAAGACTATT TCCTTCTGAA CGCGTATTAA CTACGAATTC
SA11-L2_GS1(VP1) NGS cs       ATAGGGAGTT TATTTTAACT AAGATAATGC AGATGACGTC AGTCGCAATT ACTGGATCAT TAAGACTATT TCCTTCTGAA CGCGTATTAA CTACGAATTC

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                                  2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-L2_GS1(VP1) reference    AACATTTAAA GTATTTGACT CGGAGGATTT TATTATAGAG TACGGAACGA CTGATGACGA AGTATATATA CAAAGAGCGT TCATGTCTTT ATCAAGTCAG
SA11-L2_GS1(VP1) NGS cs       AACATTTAAA GTATTTGACT CGGAGGATTT TATTATAGAG TACGGAACGA CTGATGACGA AGTATATATA CAAAGAGCGT TCATGTCTTT ATCAAGTCAG

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                                  2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-L2_GS1(VP1) reference    AAATCAGGAA TAGCCGATGA GATAGCGGCA TCATCAACAT TTAAAAATTA CGTCACGAGA CTATCTGAAC AGTTATTATT TTCAAAGAAT AATATAGTGT
SA11-L2_GS1(VP1) NGS cs       AAATCAGGAA TAGCCGATGA GATAGCGGCA TCATCAACAT TTAAAAATTA CGTCACGAGA CTATCTGAAC AGTTATTATT TTCAAAGAAT AATATAGTGT

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                                  2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-L2_GS1(VP1) reference    CCAGAGGAAT AGCTTTGACT GAAAAAGCGA AATTGAATTC ATACGCTCCA ATATCGCTTG AGAAAAGACG TGCACAGATA TCAGCTTTAT TGACTATGTT
SA11-L2_GS1(VP1) NGS cs       CCAGAGGAAT AGCTTTGACT GAAAAAGCGA AATTGAATTC ATACGCTCCA ATATCGCTTG AGAAAAGACG TGCACAGATA TCAGCTTTAT TGACTATGTT

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                                  2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-L2_GS1(VP1) reference    GCAGAAACCG GTCACCTTCA AATCAAGTAA AATAACAATA AATGACATAC TCAGAGATAT AAAACCATTT TTTACAGTAA GTGATGCACA CTTACCTATA
SA11-L2_GS1(VP1) NGS cs       GCAGAAACCG GTCACCTTCA AATCAAGTAA AATAACAATA AATGACATAC TCAGAGATAT AAAACCATTT TTTACAGTAA GTGATGCACA CTTACCTATA
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                                  2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-L2_GS1(VP1) reference   CAATACCAAA AATTTATGCC AACTTTGCCA GATAACGTAC AGTATATAAT TCAATGTATA GGATCCAGAA CTTATCAAAT TGAAGATGAC GGTTCGAAGT
SA11-L2_GS1(VP1) NGS cs      CAATACCAAA AATTTATGCC AACTTTGCCA GATAACGTAC AGTATATAAT TCAATGTATA GGATCCAGAA CTTATCAAAT TGAAGATGAC GGTTCGAAGT


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                                  2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-L2_GS1(VP1) reference   CAGCCATATC TAGACTAATA TCAAAGTATT CAGTTTATAA GCCATCAATT GAAGAATTGT ATAAAGTGAT TTCATTGCAT GAAAACGAAA TACAATTATA
SA11-L2_GS1(VP1) NGS cs      CAGCCATATC TAGACTAATA TCAAAGTATT CAGTTTATAA GCCATCAATT GAAGAATTGT ATAAAGTGAT TTCATTGCAT GAAAACGAAA TACAATTATA


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                                  2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-L2_GS1(VP1) reference   TCTGATTTCA TTAGGAATAC CGAAAATAGA CGCTGACACG TATGTTGGAT CAAAGATTTA TTCTCAAGAT AAGTATAGAA TACTAGAATC ATACGTGTAC
SA11-L2_GS1(VP1) NGS cs      TCTGATTTCA TTAGGAATAC CGAAAATAGA CGCTGACACG TATGTTGGAT CAAAGATTTA TTCTCAAGAT AAGTATAGAA TACTAGAATC ATACGTGTAC


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                                  3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-L2_GS1(VP1) reference   AATTTATTGT CCATTAATTA TGGATGCTAT CAATTATTTG ATTTCAATTC ACCGGACTTG GAGAAGCTGA TAAGAATACC ATTTAAGGGA AAAATACCAG
SA11-L2_GS1(VP1) NGS cs      AATTTATTGT CCATTAATTA TGGATGCTAT CAATTATTTG ATTTCAATTC ACCGGACTTG GAGAAGCTGA TAAGAATACC ATTTAAGGGA AAAATACCAG


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                                  3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
SA11-L2_GS1(VP1) reference   CTGTTACATT CATATTACAC TTATATGCAA AGCTAGAAGT TATAAACTAC GCTATAAAAA ATGGTTCATG GATAAGCCTA TTTTGCAATT ACCCTAAATC
SA11-L2_GS1(VP1) NGS cs      CTGTTACATT CATATTACAC TTATATGCAA AGCTAGAAGT TATAAACTAC GCTATAAAAA ATGGTTCATG GATAAGCCTA TTTTGCAATT ACCCTAAATC


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                                  3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
SA11-L2_GS1(VP1) reference   AGAAATGATA AAATTATGGA AGAAGATGTG GAACATCACG TCATTACGTT CGCCGTACAC TAACGCGAAC TTCTTTCAAG ATTAGAACGC TTAGATGTGA
SA11-L2_GS1(VP1) NGS cs      AGAAATGATA AAATTATGGA AGAAGATGTG GAACATCACG TCATTACGTT CGCCGTACAC TAACGCGAAC TTCTTTCAAG ATTAGAACGC TTAGATGTGA


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SA11-L2_GS1(VP1) reference   CC
SA11-L2_GS1(VP1) NGS cs      CC
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Alignment: SA11-L2_GS2(VP2)

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                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS2(VP2) reference   GGCTATTAAA GGCTCAATGG CGTATCGAAA ACGTGGAGCG CGTCGTGAGA CGAATCTAAA ACAAGATGAA CGAATGCAAG AAAAAGAAGA TAGCAAGAAC
SA11-L2_GS2(VP2) NGS cs      GGCTATTAAA GGCTCAATGG CGTATCGAAA ACGTGGAGCG CGTCGTGAGA CGAATCTAAA ACAAGATGAA CGAATGCAAG AAAAAGAAGA TAGCAAGAAC

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                                 110        120        130        140        150        160        170        180        190        200
SA11-L2_GS2(VP2) reference   ATTAATAATG ACAGTCCTAA ATCACAATTA TCAGAAAAAG TATTATCTAA GAAAGAAGAG ATAATTACAG ATAATCAAGA AGAAGTTAAG ATATCTGATG
SA11-L2_GS2(VP2) NGS cs      ATTAATAATG ACAGTCCTAA ATCACAATTA TCAGAAAAAG TATTATCTAA GAAAGAAGAG ATAATTACAG ATAATCAAGA AGAAGTTAAG ATATCTGATG

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                                 210        220        230        240        250        260        270        280        290        300
SA11-L2_GS2(VP2) reference   AGGTAAAAAA ATCTAATAAA GAAGAATCGA AACAGTTGTT AGAAGTACTT AAAACAAAAG AGGAACATCA AAAAGAAGTT CAGTATGAAA TATTACAAAA
SA11-L2_GS2(VP2) NGS cs      AGGTAAAAAA ATCTAATAAA GAAGAATCGA AACAGTTGTT AGAAGTACTT AAAACAAAAG AGGAACATCA AAAAGAAGTT CAGTATGAAA TATTACAAAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 310        320        330        340        350        360        370        380        390        400
SA11-L2_GS2(VP2) reference   AACTATCCCT ACATTTGAAC CAAAAGAGTC AATACTCAAA AAATTAGAAG ACATAAAACC AGAACAAGCA AAGAAACAAA CTAAACTGTT TCGAATATTT
SA11-L2_GS2(VP2) NGS cs      AACTATCCCT ACATTTGAAC CAAAAGAGTC AATACTCAAA AAATTAGAAG ACATAAAACC AGAACAAGCA AAGAAACAAA CTAAACTGTT TCGAATATTT

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                                 410        420        430        440        450        460        470        480        490        500
SA11-L2_GS2(VP2) reference   GAACCGAAAC AATTGCCTAT TTATAGAGCT AATGGAGAAA GAGAGCTTCG TAATAGATGG TATTGGAAAT TGAAACGAGA TACTCTTCCT GATGGAGATT
SA11-L2_GS2(VP2) NGS cs      GAACCGAAAC AATTGCCTAT TTATAGAGCT AATGGAGAAA GAGAGCTTCG TAATAGATGG TATTGGAAAT TGAAACGAGA TACTCTTCCT GATGGAGATT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 510        520        530        540        550        560        570        580        590        600
SA11-L2_GS2(VP2) reference   ATGATGTTAG AGAGTATTTT TTAAATTTAT ATGATCAAGT ATTAATGGAA ATGCCGGATT ATCTATTACT TAAAGATATG GCTGTAGAGA ATAAAAATTC
SA11-L2_GS2(VP2) NGS cs      ATGATGTTAG AGAGTATTTT TTAAATTTAT ATGATCAAGT ATTAATGGAA ATGCCGGATT ATCTATTACT TAAAGATATG GCTGTAGAGA ATAAAAATTC

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                                 610        620        630        640        650        660        670        680        690        700
SA11-L2_GS2(VP2) reference   AAGGGATGCT GGCAAAGTAG TTGATTCTGA AACAGCCGCA ATATGCGATG CTATTTTTCA AGATGAAGAA ACCGAAGGTG CAGTAAGAAG ATTCATAGCT
SA11-L2_GS2(VP2) NGS cs      AAGGGATGCT GGCAAAGTAG TTGATTCTGA AACAGCCGCA ATATGCGATG CTATTTTTCA AGATGAAGAA ACCGAAGGTG CAGTAAGAAG ATTCATAGCT

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                                 710        720        730        740        750        760        770        780        790        800
SA11-L2_GS2(VP2) reference   GAGATGAGAC AACGAGTTCA AGCTGATCGA AATGTAGTCA ATTATCCATC TATATTGCAT CCAATTGACC ATGCGTTTAA CGAATACTTC TTACAACATC
SA11-L2_GS2(VP2) NGS cs      GAGATGAGAC AACGAGTTCA AGCTGATCGA AATGTAGTCA ATTATCCATC TATATTGCAT CCAATTGACC ATGCGTTTAA CGAATACTTC TTACAACATC

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                                 810        820        830        840        850        860        870        880        890        900
SA11-L2_GS2(VP2) reference   AGTTGGTAGA ACCATTAAAT AATGATATCA TTTTCAATTA CATACCAGAG AGAATAAGAA ATGATGTCAA CTATATATTA AATATGGACA GGAATTTACC
SA11-L2_GS2(VP2) NGS cs      AGTTGGTAGA ACCATTAAAT AATGATATCA TTTTCAATTA CATACCAGAG AGAATAAGAA ATGATGTCAA CTATATATTA AATATGGACA GGAATTTACC
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                                  910        920        930        940        950        960        970        980        990       1000
SA11-L2_GS2(VP2) reference    GTCTACTGCT AGATATATCA GACCAAACTT GCTACAAGAT AGGTTAAATT TACATGATAA TTTTGAGTCA CTCTGGGATA CTATAACTAC ATCTAATTAT
SA11-L2_GS2(VP2) NGS cs       GTCTACTGCT AGATATATCA GACCAAACTT GCTACAAGAT AGGTTAAATT TACATGATAA TTTTGAGTCA CTCTGGGATA CTATAACTAC ATCTAATTAT


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                                 1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-L2_GS2(VP2) reference    ATTTTAGCAA GATCTGTGGT GCCAGACCTA AAAGAATTAG TATCTACTGA GGCACAAATC CAGAAAATGT CACAAGATTT GCAATTGGAA GCTTTGACAA
SA11-L2_GS2(VP2) NGS cs       ATTTTAGCAA GATCTGTGGT GCCAGACCTA AAAGAATTAG TATCTACTGA GGCACAAATC CAGAAAATGT CACAAGATTT GCAATTGGAA GCTTTGACAA


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                                 1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-L2_GS2(VP2) reference    TACAATCAGA GACTCAGTTT TTAACAGGTA TAAACTCACA AGCCGCTAAT GATTGTTTTA AAACTTTGAT TGCTGCTATG TTGAGTCAGA GAACCATGTC
SA11-L2_GS2(VP2) NGS cs       TACAATCAGA GACTCAGTTT TTAACAGGTA TAAACTCACA AGCCGCTAAT GATTGTTTTA AAACTTTGAT TGCTGCTATG TTGAGTCAGA GAACCATGTC


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                                 1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-L2_GS2(VP2) reference    ATTAGATTTC GTAACGACAA ATTACATGTC ACTTATTTCA GGCATGTGGT TACTCACTGT GATTCCAAAT GATATGTTTA TAAGAGAATC ATTAGTAGCA
SA11-L2_GS2(VP2) NGS cs       ATTAGATTTC GTAACGACAA ATTACATGTC ACTTATTTCA GGCATGTGGT TACTCACTGT GATTCCAAAT GATATGTTTA TAAGAGAATC ATTAGTAGCA


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                                 1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-L2_GS2(VP2) reference    TGTCAACTAG CCATAATAAA TACCATTGTT TATCCGGCAT TCGGAATGCA AAGAATGCAT TATAGGAATG GTGATCCACA GACTCCCTTT CAAATTGCAG
SA11-L2_GS2(VP2) NGS cs       TGTCAACTAG CCATAATAAA TACCATTGTT TATCCGGCAT TCGGAATGCA AAGAATGCAT TATAGGAATG GTGATCCACA GACTCCCTTT CAAATTGCAG


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                                 1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-L2_GS2(VP2) reference    AGCAACAGAT TCAAAATTTT CAGGTAGCTA ATTGGTTACA TTTTGTTAAT TATAATCAGT TTAGACAAGT AGTGATTGAT GGAGTGTTAA ATCAAGTCTT
SA11-L2_GS2(VP2) NGS cs       AGCAACAGAT TCAAAATTTT CAGGTAGCTA ATTGGTTACA TTTTGTTAAT TATAATCAGT TTAGACAAGT AGTGATTGAT GGAGTGTTAA ATCAAGTCTT


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                                 1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
SA11-L2_GS2(VP2) reference    GAATGATAAT ATAAGAAATG GTCATGTAGT CAACCAATTA ATGGAAGCTC TGATGCAATT ATCTAGACAA CAGTTTCCCA CAATGCCAGT TGATTATAAA
SA11-L2_GS2(VP2) NGS cs       GAATGATAAT ATAAGAAATG GTCATGTAGT CAACCAATTA ATGGAAGCTC TGATGCAATT ATCTAGACAA CAGTTTCCCA CAATGCCAGT TGATTATAAA


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                                 1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-L2_GS2(VP2) reference    AGATCTATAC AGAGAGGAAT TTTGCTGCTT TCTAACAGAC TTGGTCAGCT TGTCGATTTA ACAAGATTGT TATCATACAA TTATGAGACA TTAATGGCAT
SA11-L2_GS2(VP2) NGS cs       AGATCTATAC AGAGAGGAAT TTTGCTGCTT TCTAACAGAC TTGGTCAGCT TGTCGATTTA ACAAGATTGT TATCATACAA TTATGAGACA TTAATGGCAT


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                                 1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-L2_GS2(VP2) reference    GCATAACAAT GAATATGCAG CATGTTCAAA CATTAACAAC TGAAAAATTG CAATTAACAT CAGTAACATC ATTATGTATG CTAATTGGAA ATGCTACGGT
SA11-L2_GS2(VP2) NGS cs       GCATAACAAT GAATATGCAG CATGTTCAAA CATTAACAAC TGAAAAATTG CAATTAACAT CAGTAACATC ATTATGTATG CTAATTGGAA ATGCTACGGT
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                                  1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-L2_GS2(VP2) reference    TATACCGAGT CCGCAAACAT TGTTCCATTA CTATAATGTG AATGTCAATT TTCATTCAAA TTATAATGAA AGAATTAATG ACGCAGTTGC AATTATAACT
SA11-L2_GS2(VP2) NGS cs       TATACCGAGT CCGCAAACAT TGTTCCATTA CTATAATGTG AATGTCAATT TTCATTCAAA TTATAATGAA AGAATTAATG ACGCAGTTGC AATTATAACT

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                                  1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-L2_GS2(VP2) reference    GCGGCAAATA GATTAAATTT ATATCAAAAG AAAATGAAAT CAATAGTTGA GGACTTTCTG AAAAGATTAC AGATATTTGA TGTTGCGAGA GTACCAGATG
SA11-L2_GS2(VP2) NGS cs       GCGGCAAATA GATTAAATTT ATATCAAAAG AAAATGAAAT CAATAGTTGA GGACTTTCTG AAAAGATTAC AGATATTTGA TGTTGCGAGA GTACCAGATG

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                                  2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-L2_GS2(VP2) reference    ACCAAATGTA TAGATTGAGA GATAGATTAA GACTATTACC AGTTGAAATA AGAAGATTAG ATATTTTTAA TTTGATAGCA ATGAATATGG AACAGATTGA
SA11-L2_GS2(VP2) NGS cs       ACCAAATGTA TAGATTGAGA GATAGATTAA GACTATTACC AGTTGAAATA AGAAGATTAG ATATTTTTAA TTTGATAGCA ATGAATATGG AACAGATTGA

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                                  2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-L2_GS2(VP2) reference    ACGTGCATCA GATAAAATTG CACAAGGAGT TATAATAGCA TACCGAGATA TGCAGTTAGA ACGAGATGAG ATGTATGGTT ACGTCAATAT TGCCAGAAAC
SA11-L2_GS2(VP2) NGS cs       ACGTGCATCA GATAAAATTG CACAAGGAGT TATAATAGCA TACCGAGATA TGCAGTTAGA ACGAGATGAG ATGTATGGTT ACGTCAATAT TGCCAGAAAC

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                                  2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-L2_GS2(VP2) reference    TTGGACGGAT TTCAACAAAT AAATCTTGAA GAATTGATGA GATCAGGAGA TTATGCTCAA ATTACTAACA TGCTACTTAA TAATCAACCA GTAGCTTTAG
SA11-L2_GS2(VP2) NGS cs       TTGGACGGAT TTCAACAAAT AAATCTTGAA GAATTGATGA GATCAGGAGA TTATGCTCAA ATTACTAACA TGCTACTTAA TAATCAACCA GTAGCTTTAG

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                                  2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-L2_GS2(VP2) reference    TTGGAGCGCT ACCATTTATA ACGGATTCAT CAGTGATTTC GTTAATAGCT AAACTAGATG CAACCGTTTT TGCACAGATT GTCAAACTTA GAAAGGTCGA
SA11-L2_GS2(VP2) NGS cs       TTGGAGCGCT ACCATTTATA ACGGATTCAT CAGTGATTTC GTTAATAGCT AAACTAGATG CAACCGTTTT TGCACAGATT GTCAAACTTA GAAAGGTCGA

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                                  2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-L2_GS2(VP2) reference    CACGTTAAAA CCCATCCTAT ATAAGATAAA TTCAGATTCT AATGACTTTT ATTTGGTGGC TAATTATGAT TGGATTCCTA CATCTACTAC AAAAGTGTAT
SA11-L2_GS2(VP2) NGS cs       CACGTTAAAA CCCATCCTAT ATAAGATAAA TTCAGATTCT AATGACTTTT ATTTGGTGGC TAATTATGAT TGGATTCCTA CATCTACTAC AAAAGTGTAT

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                                  2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-L2_GS2(VP2) reference    AAACAAGTTC CACAACAATT TGATTTTAGA GCGTCAATGC ATATGTTAAC GTCTAACCTA ACATTTACCG TATATTCAGA TTTGCTTGCG TTCGTTTCAG
SA11-L2_GS2(VP2) NGS cs       AAACAAGTTC CACAACAATT TGATTTTAGA GCGTCAATGC ATATGTTAAC GTCTAACCTA ACATTTACCG TATATTCAGA TTTGCTTGCG TTCGTTTCAG

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                                  2610       2620       2630       2640       2650       2660       2670       2680       2690
SA11-L2_GS2(VP2) reference    CTGATACTGT TGAACCAATT AATGCTGTTG CTTTTGATAA TATGCGCATC ATGAACGAAC TGTAAACGCC AACCCCATTG TGGAGATATG ACC
SA11-L2_GS2(VP2) NGS cs       CTGATACTGT TGAACCAATT AATGCTGTTG CTTTTGATAA TATGCGCATC ATGAACGAAC TGTAAACGCC AACCCCATTG TGGAGATATG ACC
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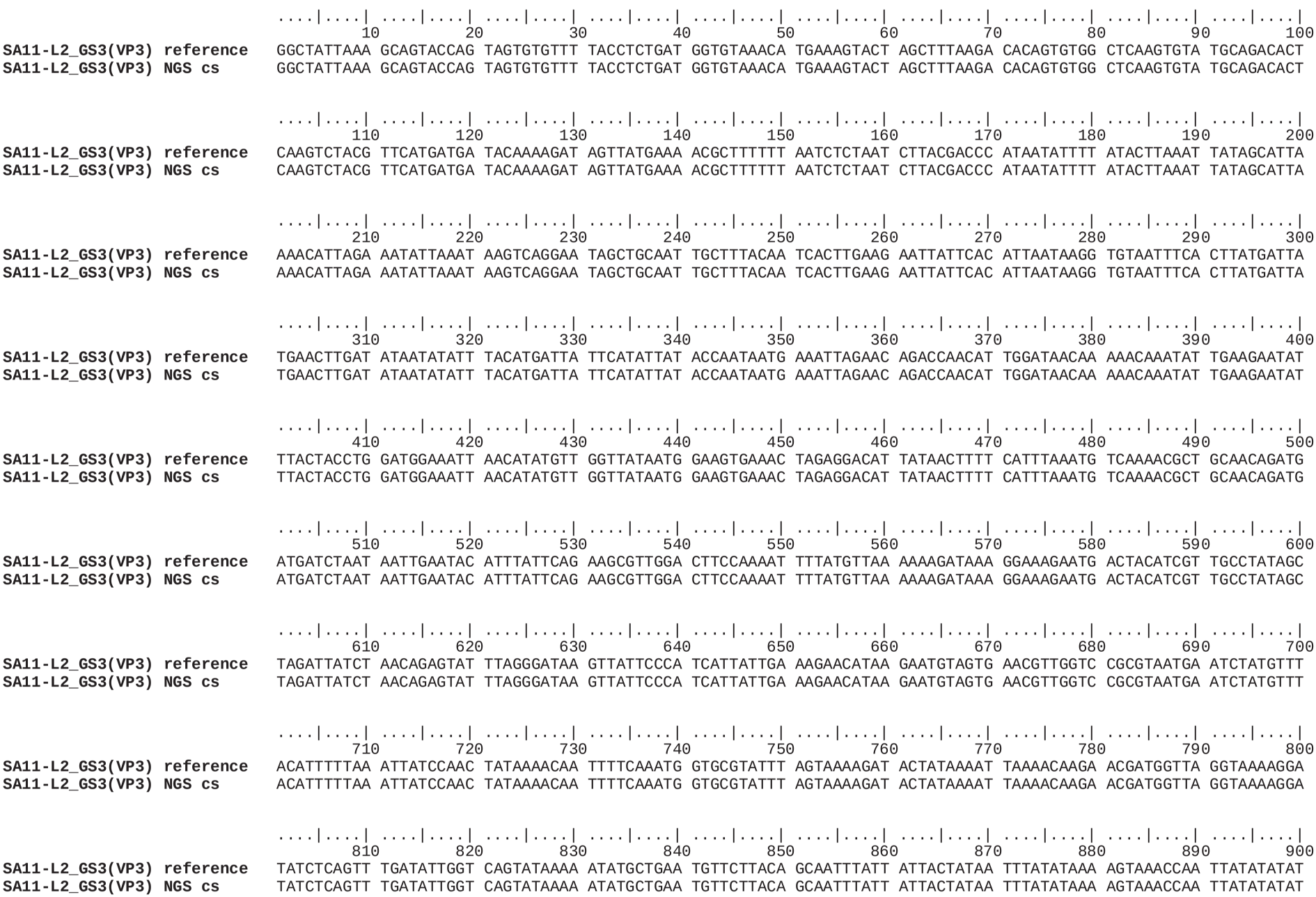
Alignment: SA11-L2_GS3(VP3)



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                                910        920        930        940        950        960        970        980        990       1000
SA11-L2_GS3(VP3) reference   GATCGGATCT GCTCCATCTT ATTGGATATA TGACGTTAGG CATTATTCCG ATTTTTTCTT TGAAACTTGG GATCCATTGG ACACACCATA TTCATCAATC
SA11-L2_GS3(VP3) NGS cs      GATCGGATCT GCTCCATCTT ATTGGATATA TGACGTTAGG CATTATTCCG ATTTTTTCTT TGAAACTTGG GATCCATTGG ACACACCATA TTCATCAATC


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                               1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-L2_GS3(VP3) reference   CATCACAAAG AATTATTTTT TATAAATGAT GTGAAGAAAC TGAAGGATAA CTCAATATTG TATATTGATA TAAGAACCGA TAGGGGCAAT GCTGATTGGA
SA11-L2_GS3(VP3) NGS cs      CATCACAAAG AATTATTTTT TATAAATGAT GTGAAGAAAC TGAAGGATAA CTCAATATTG TATATTGATA TAAGAACCGA TAGGGGCAAT GCTGATTGGA


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                               1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-L2_GS3(VP3) reference   AAAAATGGAG AAAGACAGTA GAAGAACAAA CTATTAATAA TTTGGACATA GCTTATGAAT ATTTACGAAC GGGTAAAGCG AAGGTGTGTT GTGTTAAGAT
SA11-L2_GS3(VP3) NGS cs      AAAAATGGAG AAAGACAGTA GAAGAACAAA CTATTAATAA TTTGGACATA GCTTATGAAT ATTTACGAAC GGGTAAAGCG AAGGTGTGTT GTGTTAAGAT


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                               1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-L2_GS3(VP3) reference   GACAGCTATG GATTTGGAAC TGCCAATTTC AGCTAAATTA CTGCACCACC CAACTACGGA AATAAGATCA GAATTTTATT TATTACTAGA TACTTGGGAT
SA11-L2_GS3(VP3) NGS cs      GACAGCTATG GATTTGGAAC TGCCAATTTC AGCTAAATTA CTGCACCACC CAACTACGGA AATAAGATCA GAATTTTATT TATTACTAGA TACTTGGGAT


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                               1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-L2_GS3(VP3) reference   TTAACTAACA TTAGGAGGTT CATTCCTAAA GGCGTGTTAT ATTCATTTAT AAACAATATA ATAACTGAAA ATGTGTTTAT TCAACAACCA TTTAAAGTAA
SA11-L2_GS3(VP3) NGS cs      TTAACTAACA TTAGGAGGTT CATTCCTAAA GGCGTGTTAT ATTCATTTAT AAACAATATA ATAACTGAAA ATGTGTTTAT TCAACAACCA TTTAAAGTAA


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                               1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-L2_GS3(VP3) reference   AAGTACTGAA TGATAGTTAT ATTGTAGCGT TATATGCATT ATCAAATGAT TTTAATAATA GATCAGAAGT AATTAAATTA ATTAATAATC AGAAACAATC
SA11-L2_GS3(VP3) NGS cs      AAGTACTGAA TGATAGTTAT ATTGTAGCGT TATATGCATT ATCAAATGAT TTTAATAATA GATCAGAAGT AATTAAATTA ATTAATAATC AGAAACAATC


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
SA11-L2_GS3(VP3) reference   TCTAATAACT GTTAGAATAA ATAATACGTT TAAGGATGAA CCAAAAGTTG GGTTCAAAAA TATCTATGAT TGGACCTTTC TTCCAACCGA CTTTGATACC
SA11-L2_GS3(VP3) NGS cs      TCTAATAACT GTTAGAATAA ATAATACGTT TAAGGATGAA CCAAAAGTTG GGTTCAAAAA TATCTATGAT TGGACCTTTC TTCCAACCGA CTTTGATACC


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-L2_GS3(VP3) reference   AAAGAAGCTA TAATTACTTC ATACGACGGT TGTTTAGGAC TCTTTGGTTT GTCTATATCG TTAGCATCAA AACCAACAGG GAATAATCAT TTATTCATTT
SA11-L2_GS3(VP3) NGS cs      AAAGAAGCTA TAATTACTTC ATACGACGGT TGTTTAGGAC TCTTTGGTTT GTCTATATCG TTAGCATCAA AACCAACAGG GAATAATCAT TTATTCATTT


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                               1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-L2_GS3(VP3) reference   TAAGTGGTAC AGATAAGTAT TATAAATTGG ATCAATTTGC TAATCACACC AGTATATCGA GAAGATCACA CCAAATTAGG TTTTCGGAAT CTGCTACTTC
SA11-L2_GS3(VP3) NGS cs      TAAGTGGTAC AGATAAGTAT TATAAATTGG ATCAATTTGC TAATCACACC AGTATATCGA GAAGATCACA CCAAATTAGG TTTTCGGAAT CTGCTACTTC
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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-L2_GS3(VP3) reference   ATATTCAGGT TATATATTTA GAGATTTGTC CAATAATAAT TTTAATCTAA TTGGTACTAA TATAGAGAAT TCAGTATCAG GTCATGTATA TAATGCTTTA
SA11-L2_GS3(VP3) NGS cs      ATATTCAGGT TATATATTTA GAGATTTGTC CAATAATAAT TTTAATCTAA TTGGTACTAA TATAGAGAAT TCAGTATCAG GTCATGTATA TAATGCTTTA


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                                  1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-L2_GS3(VP3) reference   ATTTATTATA GATATAATTA TTCATTTGAT CTTAAACGCT GGATTTATTT ACATTCTATA GATAAAGTTG ATATAGAAGG AGGAAAGTAT TATGAACACG
SA11-L2_GS3(VP3) NGS cs      ATTTATTATA GATATAATTA TTCATTTGAT CTTAAACGCT GGATTTATTT ACATTCTATA GATAAAGTTG ATATAGAAGG AGGAAAGTAT TATGAACACG


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                                  2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-L2_GS3(VP3) reference   CACCAATAGA ATTAATTTAT GCATGTAGAT CAGCAAAAGA ATTTGCTACA TTGCAGGATG ACTTAACTGT ATTGAGATAT TCAAACGAAA TAGAGAATTA
SA11-L2_GS3(VP3) NGS cs      CACCAATAGA ATTAATTTAT GCATGTAGAT CAGCAAAAGA ATTTGCTACA TTGCAGGATG ACTTAACTGT ATTGAGATAT TCAAACGAAA TAGAGAATTA


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-L2_GS3(VP3) reference   TATTAATACA GTATATAGTA TAACATACGC TGATGATCCG AATTACTTTA TCGGAATACA ATTTAGAAAT ATACCATATA AATATGATGT TAAAATACCG
SA11-L2_GS3(VP3) NGS cs      TATTAATACA GTATATAGTA TAACATACGC TGATGATCCG AATTACTTTA TCGGAATACA ATTTAGAAAT ATACCATATA AATATGATGT TAAAATACCG


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-L2_GS3(VP3) reference   CATTTAACCT TCGGAGTATT ACATATTTCT GATAACATGG TGCCAGACGT GATTGACATA CTAAAGATAA TGAAGAATGA ATTATTTAAA ATGGATATTA
SA11-L2_GS3(VP3) NGS cs      CATTTAACCT TCGGAGTATT ACATATTTCT GATAACATGG TGCCAGACGT GATTGACATA CTAAAGATAA TGAAGAATGA ATTATTTAAA ATGGATATTA


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                                  2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-L2_GS3(VP3) reference   CGACCAGTTA TACATATATG TTATCAGATG GAATCTACGT AGCAAATGTT AGTGGAGTAT TATCTACATA CTTTAAAATC TATAACGTAT TTTATAAAAA
SA11-L2_GS3(VP3) NGS cs      CGACCAGTTA TACATATATG TTATCAGATG GAATCTACGT AGCAAATGTT AGTGGAGTAT TATCTACATA CTTTAAAATC TATAACGTAT TTTATAAAAA


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                                  2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-L2_GS3(VP3) reference   TCAAATAACT TTTGGCCAAT CCAGAATGTT TATTCCGCAC ATAACATTAA GCTTCAATAA CATGAGAACA GTAAGGATAG AGACTACTAA ATTACAAATT
SA11-L2_GS3(VP3) NGS cs      TCAAATAACT TTTGGCCAAT CCAGAATGTT TATTCCGCAC ATAACATTAA GCTTCAATAA CATGAGAACA GTAAGGATAG AGACTACTAA ATTACAAATT


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                                  2510       2520       2530       2540       2550       2560       2570       2580       2590
SA11-L2_GS3(VP3) reference   AAATCCATTT ATTTAAGAAA GATTAAGGGT GATACAGTGT TTGATATGGT TGAGTGAGCT AAAAACTTAA CACACTAGTC ATGATGTGAC C
SA11-L2_GS3(VP3) NGS cs      AAATCCATTT ATTTAAGAAA GATTAAGGGT GATACAGTGT TTGATATGGT TGAGTGAGCT AAAAACTTAA CACACTAGTC ATGATGTGAC C
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Alignment: SA11-L2_GS4(VP4)

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                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS3(VP4) reference   GGCTATAAAA TGGCTTCGCT CATTTATAGA CAATTGCTCA CGAATTCTTA TACAGTAGAT TTATCCGATG AGATACAAGA GATTGGATCA ACTAAATCAC
SA11-L2_GS4(VP4) NGS cs      GGCTATAAAA TGGCTTCGCT CATTTATAGA CAATTGCTCA CGAATTCTTA TACAGTAGAT TTATCCGATG AGATACAAGA GATTGGATCA ACTAAATCAC

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                                 110        120        130        140        150        160        170        180        190        200
SA11-L2_GS3(VP4) reference   AAAATGTCAC AATTAATCCT GGACCATTTG CGCAAACAGG TTATGCTCCA GTTAACTGGG GACCTGGAGA AATTAATGAT TCTACGACAG TTGGACCATT
SA11-L2_GS4(VP4) NGS cs      AAAATGTCAC AATTAATCCT GGACCATTTG CGCAAACAGG TTATGCTCCA GTTAACTGGG GACCTGGAGA AATTAATGAT TCTACGACAG TTGGACCATT

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                                 210        220        230        240        250        260        270        280        290        300
SA11-L2_GS3(VP4) reference   GCTGGATGGG CCTTATCAAC CAACGACATT CAATCCACCA GTCGATTATT GGATGTTACT GGCTCCAACG ACACCTGGCG TAATTGTTGA AGGTACAAAT
SA11-L2_GS4(VP4) NGS cs      GCTGGATGGG CCTTATCAAC CAACGACATT CAATCCACCA GTCGATTATT GGATGTTACT GGCTCCAACG ACACCTGGCG TAATTGTTGA AGGTACAAAT

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                                 310        320        330        340        350        360        370        380        390        400
SA11-L2_GS3(VP4) reference   AATACAGATA GATGGTTAGC CACAATTTTA ATCGAGCCAA ATGTTCAGTC TGAAAATAGA ACTTACACTA TATTTGGTAT TCAAGAACAA TTAACGGTAT
SA11-L2_GS4(VP4) NGS cs      AATACAGATA GATGGTTAGC CACAATTTTA ATCGAGCCAA ATGTTCAGTC TGAAAATAGA ACTTACACTA TATTTGGTAT TCAAGAACAA TTAACGGTAT

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                                 410        420        430        440        450        460        470        480        490        500
SA11-L2_GS3(VP4) reference   CCAATACTTC ACAAGACCAG TGGAAATTTA TTGATGTCGT AAAAACAACT GCAAATGGAA GTATAGGACA ATATGGACCA TTACTATCCA GTCCGAAATT
SA11-L2_GS4(VP4) NGS cs      CCAATACTTC ACAAGACCAG TGGAAATTTA TTGATGTCGT AAAAACAACT GCAAATGGAA GTATAGGACA ATATGGACCA TTACTATCCA GTCCGAAATT

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                                 510        520        530        540        550        560        570        580        590        600
SA11-L2_GS3(VP4) reference   ATATGCAGTT ATGAAGCATA ATGAAAAATT ATATACATAT GAAGGACAGA CACCTAACGC TAGGACAGCA CATTATTCAA CAACGAATTA TGATTCTGTT
SA11-L2_GS4(VP4) NGS cs      ATATGCAGTT ATGAAGCATA ATGAAAAATT ATATACATAT GAAGGACAGA CACCTAACGC TAGGACAGCA CATTATTCAA CAACGAATTA TGATTCTGTT

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                                 610        620        630        640        650        660        670        680        690        700
SA11-L2_GS3(VP4) reference   AACATGACTG CTTTTTGTGA CTTTTATATA ATTCCTAGAT CTGAAGAGTC TAAATGTACG GAATACATTA ATAATGGATT ACCACCAATA CAAAATACTA
SA11-L2_GS4(VP4) NGS cs      AACATGACTG CTTTTTGTGA CTTTTATATA ATTCCTAGAT CTGAAGAGTC TAAATGTACG GAATACATTA ATAATGGATT ACCACCAATA CAAAATACTA

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                                 710        720        730        740        750        760        770        780        790        800
SA11-L2_GS3(VP4) reference   GAAATGTTGT ACCATTATCG TTGACTGCTA GAGATGTAAT ACACTATAGA GCTCAAGCTA ATGAAGATAT TGTGATATCC AAGACATCAT TATGGAAAGA
SA11-L2_GS4(VP4) NGS cs      GAAATGTTGT ACCATTATCG TTGACTGCTA GAGATGTAAT ACACTATAGA GCTCAAGCTA ATGAAGATAT TGTGATATCC AAGACATCAT TATGGAAAGA

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                                 810        820        830        840        850        860        870        880        890        900
SA11-L2_GS3(VP4) reference   AATGCAATAT AATAGAGATA TAACTATTAG ATTTAAATTT GCAAATACAA TTATAAAATC AGGAGGGCTG GGATATAAGT GGTCAGAAAT ATCATTTAAG
SA11-L2_GS4(VP4) NGS cs      AATGCAATAT AATAGAGATA TAACTATTAG ATTTAAATTT GCAAATACAA TTATAAAATC AGGAGGGCTG GGATATAAGT GGTCAGAAAT ATCATTTAAG
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                                910        920        930        940        950        960        970        980        990       1000
SA11-L2_GS3(VP4) reference  CCAGCGAATT ATCAATACAC ATATACTCGT GATGGTGAAG AAGTTACCGC ACATACTACT TGTTCAGTGA ATGGCGTTAA TGACTTCAGT TTTAATGGAG
SA11-L2_GS4(VP4) NGS cs     CCAGCGAATT ATCAATACAC ATATACTCGT GATGGTGAAG AAGTTACCGC ACATACTACT TGTTCAGTGA ATGGCGTTAA TGACTTCAGT TTTAATGGAG

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                               1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-L2_GS3(VP4) reference  GATATTTACC AACTGATTTT GTTGTATCTA AATTTGAAGT AATTAAAGAG AATTCATACG TCTATATCGA TTACTGGGAT GATTCACAAG CATTTCGTAA
SA11-L2_GS4(VP4) NGS cs     GATATTTACC AACTGATTTT GTTGTATCTA AATTTGAAGT AATTAAAGAG AATTCATACG TCTATATCGA TTACTGGGAT GATTCACAAG CATTTCGTAA

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                               1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-L2_GS3(VP4) reference  CGTGGTGTAT GTCCGATCGT TAGCAGCAAA CTTGAATTCA GTTATGTGTA CTGGAGGCAG CTATAATTTT AGTCTACCAG TTGGACAATG GCCTGTTTTA
SA11-L2_GS4(VP4) NGS cs     CGTGGTGTAT GTCCGATCGT TAGCAGCAAA CTTGAATTCA GTTATGTGTA CTGGAGGCAG CTATAATTTT AGTCTACCAG TTGGACAATG GCCTGTTTTA

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                               1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-L2_GS3(VP4) reference  ACTGGGGGAG CAGTTTCTTT ACATTCAGCT GGTGTAACAC TATCTACTCA ATTTACAGAT TTCGTATCAT TAAATTCATT AAGATTTAGA TTTAGACTAG
SA11-L2_GS4(VP4) NGS cs     ACTGGGGGAG CAGTTTCTTT ACATTCAGCT GGTGTAACAC TATCTACTCA ATTTACAGAT TTCGTATCAT TAAATTCATT AAGATTTAGA TTTAGACTAG

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                               1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-L2_GS3(VP4) reference  CTGTCGAAGA ACCACACTTT AAACTGACTA GAACTAGATT AGATAGATTG TATGGTCTGC CTGCTGCAGA TCCAAATAAT GGTAAAGAAT ATTATGAAAT
SA11-L2_GS4(VP4) NGS cs     CTGTCGAAGA ACCACACTTT AAACTGACTA GAACTAGATT AGATAGATTG TATGGTCTGC CTGCTGCAGA TCCAAATAAT GGTAAAGAAT ATTATGAAAT

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                               1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-L2_GS3(VP4) reference  TGCTGGACGA TTTTCACTTA TATCATTAGT GCCATCAAAT GATGACTATC AGACTCCTAT AGCAAACTCA GTTACTGTAC GACAAGATTT AGAAAGGCAG
SA11-L2_GS4(VP4) NGS cs     TGCTGGACGA TTTTCACTTA TATCATTAGT GCCATCAAAT GATGACTATC AGACTCCTAT AGCAAACTCA GTTACTGTAC GACAAGATTT AGAAAGGCAG

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                               1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
SA11-L2_GS3(VP4) reference  TTAGGAGAAC TAAGAGAAGA GTTTAACGCT TTGTCTCAAG AAATTGCAAT GTCGCAGTTA ATCGATTTAG CGCTTCTACC ATTAGATATG TTCTCAATGT
SA11-L2_GS4(VP4) NGS cs     TTAGGAGAAC TAAGAGAAGA GTTTAACGCT TTGTCTCAAG AAATTGCAAT GTCGCAGTTA ATCGATTTAG CGCTTCTACC ATTAGATATG TTCTCAATGT

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                               1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-L2_GS3(VP4) reference  TTTCTGGCAT TAAAAGTACT ATTGATGCTG CAAAATCAAT GGCTACTAAT GTTATGAAAA AATTCAAAAA GTCAGGATTA GCGAATTCAG TTTCAACACT
SA11-L2_GS4(VP4) NGS cs     TTTCTGGCAT TAAAAGTACT ATTGATGCTG CAAAATCAAT GGCTACTAAT GTTATGAAAA AATTCAAAAA GTCAGGATTA GCGAATTCAG TTTCAACACT

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                               1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-L2_GS3(VP4) reference  GACAGATTCT TTATCAGACG CAGCATCATC AATATCAAGA GGTTCATCTA TACGTTCGAT TGGATCTTCA GCATCAGCAT GGACGGATGT ATCAACACAA
SA11-L2_GS4(VP4) NGS cs     GACAGATTCT TTATCAGACG CAGCATCATC AATATCAAGA GGTTCATCTA TACGTTCGAT TGGATCTTCA GCATCAGCAT GGACGGATGT ATCAACACAA
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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-L2_GS3(VP4) reference   ATAACTGATA TATCGTCATC AGTAAGTTCA GTTTCGACAC AAACGTCAAC TATCAGTAGA AGATTGAGAC TAAAGGAAAT GGCAACACAA ACTGAGGGTA
SA11-L2_GS4(VP4) NGS cs      ATAACTGATA TATCGTCATC AGTAAGTTCA GTTTCGACAC AAACGTCAAC TATCAGTAGA AGATTGAGAC TAAAGGAAAT GGCAACACAA ACTGAGGGTA


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                                  1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-L2_GS3(VP4) reference   TGAATTTTGA TGATATATCA GCGGCTGTTT TGAAGACTAA GATAGATAAA TCGACTCAAA TATCACCAAA CACAATACCT GACATTGTTA CTGAAGCATC
SA11-L2_GS4(VP4) NGS cs      TGAATTTTGA TGATATATCA GCGGCTGTTT TGAAGACTAA GATAGATAAA TCGACTCAAA TATCACCAAA CACAATACCT GACATTGTTA CTGAAGCATC


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                                  2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-L2_GS3(VP4) reference   GGAAAAATTC ATACCAAATA GGGCTTACCG CGTTATAAAC AACGATGATG TGTTTGAAGC TGGAATTGAT GGGAAATTTT TTGCTTATAA AGTGGATACA
SA11-L2_GS4(VP4) NGS cs      GGAAAAATTC ATACCAAATA GGGCTTACCG CGTTATAAAC AACGATGATG TGTTTGAAGC TGGAATTGAT GGGAAATTTT TTGCTTATAA AGTGGATACA


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                                  2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-L2_GS3(VP4) reference   TTTGAGGAAA TACCATTTGA TGTACAAAAA TTCGCTGACT TAGTTACAGA TTCTCCAGTA ATATCCGCTA TAATTGATTT TAAAACACTT AAAAATTTGA
SA11-L2_GS4(VP4) NGS cs      TTTGAGGAAA TACCATTTGA TGTACAAAAA TTCGCTGACT TAGTTACAGA TTCTCCAGTA ATATCCGCTA TAATTGATTT TAAAACACTT AAAAATTTGA


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                                  2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-L2_GS3(VP4) reference   ACGATAATTA CGGCATTACT AAGCAACAAG CATTTAATCT TTTAAGATCT GACCCAAGAG TTTTACGTGA ATTCATTAAT CAGGACAATC CTATAATTAG
SA11-L2_GS4(VP4) NGS cs      ACGATAATTA CGGCATTACT AAGCAACAAG CATTTAATCT TTTAAGATCT GACCCAAGAG TTTTACGTGA ATTCATTAAT CAGGACAATC CTATAATTAG


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                                  2310       2320       2330       2340       2350       2360
SA11-L2_GS3(VP4) reference   AAATAGAATT GAACAACTGA TTATGCAATG CAGGTTGTGA GTAATTTCTA GAGGATGTGA CC
SA11-L2_GS4(VP4) NGS cs      AAATAGAATT GAACAACTGA TTATGCAATG CAGGTTGTGA GTAATTTCTA GAGGATGTGA CC
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Alignment: SA11-L2_GS5(NSP1)

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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS5(NSP1) reference   GGCTTTTTTT TGAAAAGTCT TGTGTTAGCC ATGGCTACTT TTAAAGATGC ATGCTTTCAT TATCGTAGAT TAACTGCTTT AAATCGGAGA TTATGCAACA
SA11-L2_GS5(NSP1) NGS cs      GGCTTTTTTT TGAAAAGTCT TGTGTTAGCC ATGGCTACTT TTAAAGATGC ATGCTTTCAT TATCGTAGAT TAACTGCTTT AAATCGGAGA TTATGCAACA

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                                 110        120        130        140        150        160        170        180        190        200
SA11-L2_GS5(NSP1) reference   TTGGTGCAAA TTCTATTTGG ATGCCAGTTC CTGATGCGAA AATTAAGGGG TGGTGTTTAG AATGTTGTCA AATAGCTGAT TTAACCCATT GTTATGGTTG
SA11-L2_GS5(NSP1) NGS cs      TTGGTGCAAA TTCTATTTGG ATGCCAGTTC CTGATGCGAA AATTAAGGGG TGGTGTTTAG AATGTTGTCA AATAGCTGAT TTAACCCATT GTTATGGTTG

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                                 210        220        230        240        250        260        270        280        290        300
SA11-L2_GS5(NSP1) reference   CTCATTGCCG CATGTTTGCA AATGGTGTGT TCAGAACAGA AGATGCTTCC TTGACAATGA ACCTCATTTG CTTAAGCTTA GAACTGTGAA ACATCCAATT
SA11-L2_GS5(NSP1) NGS cs      CTCATTGCCG CATGTTTGCA AATGGTGTGT TCAGAACAGA AGATGCTTCC TTGACAATGA ACCTCATTTG CTTAAGCTTA GAACTGTGAA ACATCCAATT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 310        320        330        340        350        360        370        380        390        400
SA11-L2_GS5(NSP1) reference   ACCAAAGACA AATTACAGTG TATCATAGAC TTGTACAATA TAATATTTCC AATTAATGAT AAAGTAATTA GAAAATTTGA AAGAATGATA AAGCAAAGAG
SA11-L2_GS5(NSP1) NGS cs      ACCAAAGACA AATTACAGTG TATCATAGAC TTGTACAATA TAATATTTCC AATTAATGAT AAAGTAATTA GAAAATTTGA AAGAATGATA AAGCAAAGAG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 410        420        430        440        450        460        470        480        490        500
SA11-L2_GS5(NSP1) reference   AATGTAGGAA TCAATATAAA ATTGAATGGT ATAATCATTT GCTGCTCCCA ATTACATTAA ATGCTGCTGC ATTTAAGTTT GATGAAAATA ATCTTTATTA
SA11-L2_GS5(NSP1) NGS cs      AATGTAGGAA TCAATATAAA ATTGAATGGT ATAATCATTT GCTGCTCCCA ATTACATTAA ATGCTGCTGC ATTTAAGTTT GATGAAAATA ATCTTTATTA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 510        520        530        540        550        560        570        580        590        600
SA11-L2_GS5(NSP1) reference   TGTTTTTGGG TTATATGAGA AATCAGTCAG TGATATATAT GCTCCATATA GAATTGTTAA CTTTATAAAT GAATTTGATA AATTATTGCT TGATCATATT
SA11-L2_GS5(NSP1) NGS cs      TGTTTTTGGG TTATATGAGA AATCAGTCAG TGATATATAT GCTCCATATA GAATTGTTAA CTTTATAAAT GAATTTGATA AATTATTGCT TGATCATATT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 610        620        630        640        650        660        670        680        690        700
SA11-L2_GS5(NSP1) reference   AACTTTACAA GAATGTCCAA TCTACCAATA GAGTTGAGAA ACCATTACGC AAAGAAATAC TTCCAATTAT CAAGACTGCC ATCATCAAAA CTAAAGCAAA
SA11-L2_GS5(NSP1) NGS cs      AACTTTACAA GAATGTCCAA TCTACCAATA GAGTTGAGAA ACCATTACGC AAAGAAATAC TTCCAATTAT CAAGACTGCC ATCATCAAAA CTAAAGCAAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 710        720        730        740        750        760        770        780        790        800
SA11-L2_GS5(NSP1) reference   TTTACTTTTC AGATTTTACT AAAGAAACTG TGATTTTTAA TACTTATACA AAAACGCCAG GAAGATCAAT ATACAGAAAT GTAACTGAAT TTAATTGGAG
SA11-L2_GS5(NSP1) NGS cs      TTTACTTTTC AGATTTTACT AAAGAAACTG TGATTTTTAA TACTTATACA AAAACGCCAG GAAGATCAAT ATACAGAAAT GTAACTGAAT TTAATTGGAG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 810        820        830        840        850        860        870        880        890        900
SA11-L2_GS5(NSP1) reference   AGATGAATTG GAGCTTTATT CTGATTTAAA AAATGATAAG AATAAATTAA TTGCTGCAAT GATGACGAGT AAGTATACTC GGTTCTATGC TCATGATAAT
SA11-L2_GS5(NSP1) NGS cs      AGATGAATTG GAGCTTTATT CTGATTTAAA AAATGATAAG AATAAATTAA TTGCTGCAAT GATGACGAGT AAGTATACTC GGTTCTATGC TCATGATAAT
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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                910        920        930        940        950        960        970        980        990       1000
SA11-L2_GS5(NSP1) reference   AATTTTGGAA GGTTGAAAAT GACAATATTT GAGTTGGGAC ATCATTGTCA GCCTAACTAC GTGGCATCTA ATCACCCAGG CAATGCTTCC GATATCCAGT
SA11-L2_GS5(NSP1) NGS cs      AATTTTGGAA GGTTGAAAAT GACAATATTT GGGTTGGGAC ATCATTGTCA GCCTAACTAC GTGGCATCTA ATCACCCAGG CAATGCTTCC GATATCCAGT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-L2_GS5(NSP1) reference   ACTGTAAATG GTGTAATATA AAATATTTTC TTAGTAAAAT TGATTGGCGG ATTCGTGATA TGTATAATTT ATTGATGGAA TTTATTAAGG ATTGTTATAA
SA11-L2_GS5(NSP1) NGS cs      ACTGTAAATG GTGTAATATA AAATATTTTC TTAGTAAAAT TGATTGGCGG ATTCGTGATA TGTATAATTT ATTGATGGAA TTTATTAAGG ATTGTTATAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-L2_GS5(NSP1) reference   AAGTAATGTT AACGTTGGAC ATTGTAGTTC TGTTGAAAAC ATATATCCTT TAATTAAAAG ATTAATTTGG AGTTTGTTTA CTAATCACAT GGATCAAACA
SA11-L2_GS5(NSP1) NGS cs      AAGTAATGTT AACGTTGGAC ATTGTAGTTC TGTTGAAAAC ATATATCCTT TAATTAAAAG ATTAATTTGG AGTTTGTTTA CTAATCACAT GGATCAAACA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-L2_GS5(NSP1) reference   ATTGAAGAAG TGTTTAATCA CATGTCGCCA GTGTCAGTTG AAGGTACGAA TGTCATCATG TTGATTCTTG GATTGAATAT TAGTTTGTAT AATGAAATTA
SA11-L2_GS5(NSP1) NGS cs      ATTGAAGAAG TGTTTAATCA CATGTCGCCA GTGTCAGTTG AAGGTACGAA TGTCATCATG TTGATTCTTG GATTGAATAT TAGTTTGTAT AATGAAATTA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-L2_GS5(NSP1) reference   AGCGCACTTT GAATGTAGAT AGCATACCAA TGGTACTTAA TTTAAATGAA TTCAGTAGTA TAGTTAAATC AATTAGCAGT AAATGGTATA ATGTTGATGA
SA11-L2_GS5(NSP1) NGS cs      AGCGCACTTT GAATGTAGAT AGCATACCAA TGGTACTTAA TTTAAATGAA TTCAGTAGTA TAGTTAAATC AATTAGCAGT AAATGGTATA ATGTTGATGA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-L2_GS5(NSP1) reference   ATTGGATAAA TTGCCAATGT CAATAAAATC AACGGAGGAA CTGATTGAAA TGAAGAATTC TGGAACTTTA ACTGAAGAAT TTGAGCTACT GATCTCCAAC
SA11-L2_GS5(NSP1) NGS cs      ATTGGATAAA TTGCCAATGT CAATAAAATC AACGGAGGAA CTGATTGAAA TGAAGAATTC TGGAACTTTA ACTGAAGAAT TTGAGCTACT GATCTCCAAC

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
SA11-L2_GS5(NSP1) reference   TCAGAAGATG ACAATGAGTG AAATTATGTC ACTATCTAAT TATACAGTAT TTAGCCATCA CAAGACCGTC CAGACTAGAG TAGCGCCTAG CTGGCAAAAT
SA11-L2_GS5(NSP1) NGS cs      TCAGAAGATG ACAATGAGTG AAATTATGTC ACTATCTAAT TATACAGTAT TTAGCCATCA CAAGACCGTC CAGACTAGAG TAGCGCCTAG CTGGCAAAAT

                          ....|....|
                               1610
SA11-L2_GS5(NSP1) reference   ACTGTGAACC
SA11-L2_GS5(NSP1) NGS cs      ACTGTGAACC
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Alignment: SA11-L2_GS6(VP6)

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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS6(VP6) reference   GGCTTTTAAA CGAAGTCTTC AACATGGATG TCCTATACTC TTTGTCAAAG ACTCTTAAAG ACGCTAGAGA CAAAATTGTC GAAGGCACAT TGTATTCTAA
SA11-L2_GS6(VP6) NGS cs      GGCTTTTAAA CGAAGTCTTC AACATGGATG TCCTATACTC TTTGTCAAAG ACTCTTAAAG ACGCTAGAGA CAAAATTGTC GAAGGCACAT TGTATTCTAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  110        120        130        140        150        160        170        180        190        200
SA11-L2_GS6(VP6) reference   CGTGAGTGAT CTAATTCAAC AATTTAATCA AATGATAATT ACTATGAATG GAAATGAATT TCAAACTGGA GGAATCGGTA ATTTGCCAAT TAGAAACTGG
SA11-L2_GS6(VP6) NGS cs      CGTGAGTGAT CTAATTCAAC AATTTAATCA AATGATAATT ACTATGAATG GAAATGAATT TCAAACTGGA GGAATCGGTA ATTTGCCAAT TAGAAACTGG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  210        220        230        240        250        260        270        280        290        300
SA11-L2_GS6(VP6) reference   AATTTTAATT TCGGGTTACT TGGAACAACT TTGCTGAACT TAGACGCTAA TTATGTTGAA ACGGCAAGAA ATACAATTGA TTATTTCGTG GATTTTGTAG
SA11-L2_GS6(VP6) NGS cs      AATTTTAATT TCGGGTTACT TGGAACAACT TTGCTGAACT TAGACGCTAA TTATGTTGAA ACGGCAAGAA ATACAATTGA TTATTTCGTG GATTTTGTAG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  310        320        330        340        350        360        370        380        390        400
SA11-L2_GS6(VP6) reference   ACAATGTATG CATGGATGAG ATGGTTAGAG AATCACAAAG GAACGGAATT GCACCTCAAT CAGACTCGCT AAGAAAGCTG TCAGCCATTA AATTCAAAAG
SA11-L2_GS6(VP6) NGS cs      ACAATGTATG CATGGATGAG ATGGTTAGAG AATCACAAAG GAACGGAATT GCACCTCAAT CAGACTCGCT AAGAAAGCTG TCAGCCATTA AATTCAAAAG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  410        420        430        440        450        460        470        480        490        500
SA11-L2_GS6(VP6) reference   AATAAATTTT GATAATTCGT CGGAATACAT AGAAAACTGG AATTTGCAAA ATAGAAGACA GAGGACAGGT TTCACTTTTC ATAAACCAAA CATTTTTCCT
SA11-L2_GS6(VP6) NGS cs      AATAAATTTT GATAATTCGT CGGAATACAT AGAAAACTGG AATTTGCAAA ATAGAAGACA GAGGACAGGT TTCACTTTTC ATAAACCAAA CATTTTTCCT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  510        520        530        540        550        560        570        580        590        600
SA11-L2_GS6(VP6) reference   TATTCAGCAT CATTTACACT AAATAGATCA CAACCCGCTC ATGATAATTT GATGGGCACA ATGTGGTTAA ACGCAGGATC GGAAATTCAA GTCGCTGGAT
SA11-L2_GS6(VP6) NGS cs      TATTCAGCAT CATTTACACT AAATAGATCA CAACCCGCTC ATGATAATTT GATGGGCACA ATGTGGTTAA ACGCAGGATC GGAAATTCAA GTCGCTGGAT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  610        620        630        640        650        660        670        680        690        700
SA11-L2_GS6(VP6) reference   TTGACTACTC ATGTGCTATT AACGCACCAG CCAATATACA ACAATTTGAG CATATTGTGC CACTCCGAAG AGTGTTAACT ACAGCTACGA TAACTCTTCT
SA11-L2_GS6(VP6) NGS cs      TTGACTACTC ATGTGCTATT AACGCACCAG CCAATATACA ACAATTTGAG CATATTGTGC CACTCCGAAG AGTGTTAACT ACAGCTACGA TAACTCTTCT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  710        720        730        740        750        760        770        780        790        800
SA11-L2_GS6(VP6) reference   ACCAGACGCG GAAAGGTTTA GTTTTCCAAG AGTGATCAAT TCAGCTGACG GGGCAACTAC ATGGTTTTTC AACCCAGTGA TTCTCAGGCC GAATAACGTT
SA11-L2_GS6(VP6) NGS cs      ACCAGACGCG GAAAGGTTTA GTTTTCCAAG AGTGATCAAT TCAGCTGACG GGGCAACTAC ATGGTTTTTC AACCCAGTGA TTCTCAGGCC GAATAACGTT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  810        820        830        840        850        860        870        880        890        900
SA11-L2_GS6(VP6) reference   GAAGTGGAGT TTCTATTGAA TGGACAGATA ATAAACACTT ATCAAGCAAG ATTTGGAACT ATCGTAGCTA GAAATTTTGA TACTATTAGA CTATCATTCC
SA11-L2_GS6(VP6) NGS cs      GAAGTGGAGT TTCTATTGAA TGGACAGATA ATAAACACTT ATCAAGCAAG ATTTGGAACT ATCGTAGCTA GAAATTTTGA TACTATTAGA CTATCATTCC
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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  910        920        930        940        950        960        970        980        990       1000
SA11-L2_GS6(VP6) reference    AGTTAATGAG ACCACCAAAC ATGACACCAG CAGTAGCAGT ACTATTCCCG AATGCACAGC CATTCGAACA TCATGCAACA GTGGGATTGA CACTTAGAAT
SA11-L2_GS6(VP6) NGS cs       AGTTAATGAG ACCACCAAAC ATGACACCAG CAGTAGCAGT ACTATTCCCG AATGCACAGC CATTCGAACA TCATGCAACA GTGGGATTGA CACTTAGAAT


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-L2_GS6(VP6) reference    TGAGTCTGCA GTTTGTGAGT CTGTACTCGC CGATGCAAGT GAAACTCTAT TAGCAAATGT AACATCCGTT AGGCAAGAGT ACGCAATACC AGTTGGACCA
SA11-L2_GS6(VP6) NGS cs       TGAGTCTGCA GTTTGTGAGT CTGTACTCGC CGATGCAAGT GAAACTCTAT TAGCAAATGT AACATCCGTT AGGCAAGAGT ACGCAATACC AGTTGGACCA


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-L2_GS6(VP6) reference    GTCTTTCCAC CAGGTATGAA CTGGACTGAT TTAATCACCA ATTATTCACC GTCTAGGGAG GACAATTTGC AACGCGTATT TACAGTGGCT TCCATTAGAA
SA11-L2_GS6(VP6) NGS cs       GTCTTTCCAC CAGGTATGAA CTGGACTGAT TTAATCACCA ATTATTCACC GTCTAGGGAG GACAATTTGC AACGCGTATT TACAGTGGCT TCCATTAGAA


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-L2_GS6(VP6) reference    GCATGCTCAT TAAATGAGGA CCAAGCTAAC AACTTGGTAT CCAACTTTGG TGAGTATGTA GCTATATCAA GCTGTTTGAA CTCTGTAAGT AAGGATGCGT
SA11-L2_GS6(VP6) NGS cs       GCATGCTCAT TAAATGAGGA CCAAGCTAAC AACTTGGTAT CCAACTTTGG TGAGTATGTA GCTATATCAA GCTGTTTGAA CTCTGTAAGT AAGGATGCGT


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|.
                                 1310       1320       1330       1340       1350
SA11-L2_GS6(VP6) reference    ATACGCATTC GCTACACAGA GTAATCACTC AGATGGTATA GTGAGAGGAT GTGACC
SA11-L2_GS6(VP6) NGS cs       ATACGCATTC GCTACACAGA GTAATCACTC AGATGGTATA GTGAGAGGAT GTGACC
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Alignment: SA11-L2_GS7(NSP3)

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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS7(NSP3) reference   GGCATTTAAT GCTTTTCAGT GGTTGATGCT CAAGATGGAG TCTACGCAAC AGATGGCCGT CTCAATTATT AACTCTTCTT TTGAAGCTGC AGTTGTAGCT
SA11-L2_GS7(NSP3) NGS cs      GGCATTTAAT GCTTTTCAGT GGTTGATGCT CAAGATGGAG TCTACGCAAC AGATGGCCGT CTCAATTATT AACTCTTCTT TTGAAGCTGC AGTTGTAGCT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 110        120        130        140        150        160        170        180        190        200
SA11-L2_GS7(NSP3) reference   GCAACCTCAG CTCTTGAGAA TATGGGAATA GAATATGATT ATCAGGATAT ATATTCTAGA GTAAAGAATA AATTTGATTT TGTGATGGAC GATTCTGGTG
SA11-L2_GS7(NSP3) NGS cs      GCAACCTCAG CTCTTGAGAA TATGGGAATA GAATATGATT ATCAGGATAT ATATTCTAGA GTAAAGAATA AATTTGATTT TGTGATGGAC GATTCTGGTG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 210        220        230        240        250        260        270        280        290        300
SA11-L2_GS7(NSP3) reference   TTAAAAATAA TCTGATTGGT AAAGCAATAA CTATTGATCA AGCTTTGAAT AATAAATTTG GATCTGCTAT AAGAAATAGA AACTGGCTTG CTGATACTTC
SA11-L2_GS7(NSP3) NGS cs      TTAAAAATAA TCTGATTGGT AAAGCAATAA CTATTGATCA AGCTTTGAAT AATAAATTTG GATCTGCTAT AAGAAATAGA AACTGGCTTG CTGATACTTC

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 310        320        330        340        350        360        370        380        390        400
SA11-L2_GS7(NSP3) reference   TAGAGCAGCT AAATTAGATG AGGATGTAAA CAAACTAAGA ATGATGTTAT CATCAAAAGG AATTGATCAA AAAATGAGAG TTTTAAACGC ATGCTTCAGT
SA11-L2_GS7(NSP3) NGS cs      TAGAGCAGCT AAATTAGATG AGGATGTAAA CAAACTAAGA ATGATGTTAT CATCAAAAGG AATTGATCAA AAAATGAGAG TTTTAAACGC ATGCTTCAGT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 410        420        430        440        450        460        470        480        490        500
SA11-L2_GS7(NSP3) reference   GTAAAAAGAA TACCTGGAAA ATCATCATCT ATTATTAAAT GCACAAAATT GATGCGTGAT AAATTGGAAC GTGGTGAAGT TGAAGTGGAT GATTCATTTG
SA11-L2_GS7(NSP3) NGS cs      GTAAAAAGAA TACCTGGAAA ATCATCATCT ATTATTAAAT GCACAAAATT GATGCGTGAT AAATTGGAAC GTGGTGAAGT TGAAGTGGAT GATTCATTTG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 510        520        530        540        550        560        570        580        590        600
SA11-L2_GS7(NSP3) reference   TGGATGAAAA AATGGAAGTG GATACCATTG ACTGGAAATC GCGCTATGAG CAATTGGAGC AAAGGTTTGA ATCATTGAAA TCCAGGGTAA ATGAAAAATA
SA11-L2_GS7(NSP3) NGS cs      TGGATGAAAA AATGGAAGTG GATACCATTG ACTGGAAATC GCGCTATGAG CAATTGGAGC AAAGGTTTGA ATCATTGAAA TCCAGGGTAA ATGAAAAATA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 610        620        630        640        650        660        670        680        690        700
SA11-L2_GS7(NSP3) reference   TAATAATTGG GTGTTGAAAG CAAGAAAAAT GAATGAAAAT ATGCATTCTC TTCAAAATGT CATCTCTCAA CAGCAAGCAC ATATAGCTGA GCTTCAAGTG
SA11-L2_GS7(NSP3) NGS cs      TAATAATTGG GTGTTGAAAG CAAGAAAAAT GAATGAAAAT ATGCATTCTC TTCAAAATGT CATCTCTCAA CAGCAAGCAC ATATAGCTGA GCTTCAAGTG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 710        720        730        740        750        760        770        780        790        800
SA11-L2_GS7(NSP3) reference   TACAATAATA AACTAGAACG TGATTTGCAA AATAAAATTG GATCCCTTAC TTCTTCGATT GAATGGTATT TAAGATCAAT GGAATTAGAC CCTGAAATAA
SA11-L2_GS7(NSP3) NGS cs      TACAATAATA AACTAGAACG TGATTTGCAA AATAAAATTG GATCCCTTAC TTCTTCGATT GAATGGTATT TAAGATCAAT GGAATTAGAC CCTGAAATAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 810        820        830        840        850        860        870        880        890        900
SA11-L2_GS7(NSP3) reference   AGGCAGACAT TGAACAGCAA ATTAACTCAA TTGATGCGAT AAATCCATTG CACGCTTTTG ATGACTTAGA ATCAGTAATA CGTAATTTGA TATCTGATTA
SA11-L2_GS7(NSP3) NGS cs      AGGCAGACAT TGAACAGCAA ATTAACTCAA TTGATGCGAT AAATCCATTG CACGCTTTTG ATGACTTAGA ATCAGTAATA CGTAATTTGA TATCTGATTA
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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        910        920        930        940        950        960        970        980        990        1000
SA11-L2_GS7(NSP3) reference     TGACAAATTA TTCCTTATGT TCAAAGGATT AATACAGAGA TGTAATTATC AATATTCATT TGGTTGCGAA TAACCATTTT GATACATGTT GAACAATCAA
SA11-L2_GS7(NSP3) NGS cs        TGACAAATTA TTCCTTATGT TCAAAGGATT AATACAGAGA TGTAATTATC AATATTCATT TGGTTGCGAA TAACCATTTT GATACATGTT GAACAATCAA


                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-L2_GS7(NSP3) reference     ATACAGTGTT AGTATGTTGT CATCTATGCA TAACCCTCTA TGAGCACAAT AGTTAAAAGC TAACACTGTC AAAAACCTAA ATGGCTATAG GGGCGTTATG
SA11-L2_GS7(NSP3) NGS cs        ATACAGTGTT AGTATGTTGT CATCTATGCA TAACCCTCTA TGAGCACAAT AGTTAAAAGC TAACACTGTC AAAAACCTAA ATGGCTATAG GGGCGTTATG


                                ....|
SA11-L2_GS7(NSP3) reference     TGGCC
SA11-L2_GS7(NSP3) NGS cs        TGGCC
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Alignment: SA11-L2_GS8(NSP2)

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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS8(NSP2) reference   GGCTTTTAAA GCGTCTCAGT CGCCGTTTGA GCCTTGCGGT GTAGCCATGG CTGAGCTAGC TTGCTTTTGC TATCCTCATT TGGAGAATGA TAGCTATAAA
SA11-L2_GS8(NSP2) NGS cs      GGCTTTTAAA GCGTCTCAGT CGCCGTTTGA GCCTTGCGGT GTAGCCATGG CTGAGCTAGC TTGCTTTTGC TATCCTCATT TGGAGAATGA TAGCTATAAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 110        120        130        140        150        160        170        180        190        200
SA11-L2_GS8(NSP2) reference   TTTATTCCTT TTAATAATTT AGCTATTAAA GCTATGCTGA CAGCTAAAGT AGACAAAAAG GACATGGATA AGTTTTATGA TTCAATTATT TATGGAATAG
SA11-L2_GS8(NSP2) NGS cs      TTTATTCCTT TTAATAATTT AGCTATTAAA GCTATGCTGA CAGCTAAAGT AGACAAAAAG GACATGGATA AGTTTTATGA TTCAATTATT TATGGAATAG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 210        220        230        240        250        260        270        280        290        300
SA11-L2_GS8(NSP2) reference   CACCGCCTCC TCAATTTAAG AAACGGTATA ATACTAATGA TAATTCAAGA GGCATGAATT TTGAAACAAT TATGTTTACT AAGGTGGCTA TGTTGATATG
SA11-L2_GS8(NSP2) NGS cs      CACCGCCTCC TCAATTTAAG AAACGGTATA ATACTAATGA TAATTCAAGA GGCATGAATT TTGAAACAAT TATGTTTACT AAGGTGGCTA TGTTGATATG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 310        320        330        340        350        360        370        380        390        400
SA11-L2_GS8(NSP2) reference   TGAAGCTCTA AATTCATTGA AAGTGACGCA AGCAAACGTC TCTAATGTAT TATCACGAGT AGTATCAATA AGGCATTTAG AAAATTTGGT GATACGTAAA
SA11-L2_GS8(NSP2) NGS cs      TGAAGCTCTA AATTCATTGA AAGTGACGCA AGCAAACGTC TCTAATGTAT TATCACGAGT AGTATCAATA AGGCATTTAG AAAATTTGGT GATACGTAAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 410        420        430        440        450        460        470        480        490        500
SA11-L2_GS8(NSP2) reference   GAAAATCCAC AGGATATTCT ATTTCATTCA AAAGATTTAC TTTTGAAATC AACACTGATT GCTATTGGAC AGTCTAAAGA AATTGAAACT ACAATAACTG
SA11-L2_GS8(NSP2) NGS cs      GAAAATCCAC AGGATATTCT ATTTCATTCA AAAGATTTAC TTTTGAAATC AACACTGATT GCTATTGGAC AGTCTAAAGA AATTGAAACT ACAATAACTG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 510        520        530        540        550        560        570        580        590        600
SA11-L2_GS8(NSP2) reference   CAGAAGGAGG AGAAATTGTA TTTCAAAACG CTGCCTTCAC CATGTGGAAA CTAACTTATT TAGAACATCA ATTGATGCCA ATTCTGGATC AGAATTTTAT
SA11-L2_GS8(NSP2) NGS cs      CAGAAGGAGG AGAAATTGTA TTTCAAAACG CTGCCTTCAC CATGTGGAAA CTAACTTATT TAGAACATCA ATTGATGCCA ATTCTGGATC AGAATTTTAT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 610        620        630        640        650        660        670        680        690        700
SA11-L2_GS8(NSP2) reference   TGAATATAAA GTTACATTGA ACGAAGATAA ACCAATTTCA GATGTTCATG TTAAAGAATT AGTCGCTGAA CTTCGATGGC AATATAACAA GTTTGCTGTA
SA11-L2_GS8(NSP2) NGS cs      TGAATATAAA GTTACATTGA ACGAAGATAA ACCAATTTCA GATGTTCATG TTAAAGAATT AGTCGCTGAA CTTCGATGGC AATATAACAA GTTTGCTGTA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 710        720        730        740        750        760        770        780        790        800
SA11-L2_GS8(NSP2) reference   ATCACACATG GTAAGGGTCA TTATAGAATT GTAAAGTATT CATCAGTTGC TAATCACGCT GACAGAGTAT ATGCAACTTT CAAGAGTAAT GTTAAAACTG
SA11-L2_GS8(NSP2) NGS cs      ATCACACATG GTAAGGGTCA TTATAGAATT GTAAAGTATT CATCAGTTGC TAATCACGCT GACAGAGTAT ATGCAACTTT CAAGAGTAAT GTTAAAACTG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                 810        820        830        840        850        860        870        880        890        900
SA11-L2_GS8(NSP2) reference   GAGTTAATAA TGATTTTAAC CTACTTGATC AAAGAATTAT TTGGCAAAAC TGGTATGCAT TTACATCATC AATGAAACAG GGTAATACAC TTGACGTGTG
SA11-L2_GS8(NSP2) NGS cs      GAGTTAATAA TGATTTTAAC CTACTTGATC AAAGAATTAT TTGGCAAAAC TGGTATGCAT TTACATCATC AATGAAACAG GGTAATACAC TTGACGTGTG
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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        910        920        930        940        950        960        970        980        990        1000
SA11-L2_GS8(NSP2) reference     TAAAAGGTTG CTTTTCCAAA AAATGAAACC AGAAAAAAAT CCATTTAAAG GGCTGTCAAC GGATAGAAAA ATGGACGAAG TTTCTCAAGT TGGCGTTTAA
SA11-L2_GS8(NSP2) NGS cs        TAAAAGGTTG CTTTTCCAAA AAATGAAACC AGAAAAAAAT CCATTTAAAG GGCTGTCAAC GGATAGAAAA ATGGACGAAG TTTCTCAAGT TGGCGTTTAA


                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....
                                        1010       1020       1030       1040       1050
SA11-L2_GS8(NSP2) reference     TTCGCTATCA ATTTGAGGAT GATGATGGCT TAGCAAGAAT AGAAAGCGCT TATGTGACC
SA11-L2_GS8(NSP2) NGS cs        TTCGCTATCA ATTTGAGGAT GATGATGGCT TAGCAAGAAT AGAAAGCGCT TATGTGACC
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Alignment: SA11-L2_GS9(VP7)

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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100
SA11-L2_GS9(VP7) reference   GGCTTTAAAA AGAGAGAATT TCCGTTTGGC TAGCGGTTAG CTCCTTTTAA TGTATGGTAT TGAATATACC ACAGTTCTAA CCTTTCTGAT ATCGATTATT
SA11-L2_GS9(VP7) NGS cs      GGCTTTAAAA AGAGAGAATT TCCGTTTGGC TAGCGGTTAG CTCCTTTTAA TGTATGGTAT TGAATATACC ACAGTTCTAA CCTTTCTGAT ATCGATTATT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  110        120        130        140        150        160        170        180        190        200
SA11-L2_GS9(VP7) reference   CTACTAAATT ACATACTTAA ATCATTAACT AGAATAATGG ACTTTATAAT TTATAGATTT CTTTTTATAA TTGTGATATT GTCACCATTT CTCAGAGCAC
SA11-L2_GS9(VP7) NGS cs      CTACTAAATT ACATACTTAA ATCATTAACT AGAATAATGG ACTTTATAAT TTATAGATTT CTTTTTATAA TTGTGATATT GTCACCATTT CTCAGAGCAC

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  210        220        230        240        250        260        270        280        290        300
SA11-L2_GS9(VP7) reference   AAAATTATGG TATTAATCTT CCAATCACAG GCTCCATGGA CATTGCATAC GCTAATTCAA CGCAAGAAGA AACATTCCTC ACTTCTACAC TTTGCCTATA
SA11-L2_GS9(VP7) NGS cs      AAAATTATGG TATTAATCTT CCAATCACAG GCTCCATGGA CATTGCATAC GCTAATTCAA CGCAAGAAGA ACCATTCCTC ACTTCTACAC TTTGCCTATA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  310        320        330        340        350        360        370        380        390        400
SA11-L2_GS9(VP7) reference   TTATCCGACT GAGGCTGCGA CTGAAATAAA CGATAATTCA TGGAAAGACA CACTGTCACA ACTATTTCTT ACGAAAGGGT GGCCAACTGG ATCCGTATAT
SA11-L2_GS9(VP7) NGS cs      TTATCCGACT GAGGCTGCGA CTGAAATAAA CGATAATTCA TGGAAAGACA CACTGTCACA ACTATTTCTT ACGAAAGGGT GGCCAACTGG ATCCGTATAT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  410        420        430        440        450        460        470        480        490        500
SA11-L2_GS9(VP7) reference   TTTAAAGAAT ATACTAACAT TGCATCGTTT TCTGTTGATC CGCAGTTGTA TTGTGATTAT AACGTAGTAC TAATGAAATA TGACGCGACG TTGCAATTGG
SA11-L2_GS9(VP7) NGS cs      TTTAAAGAAT ATACTAACAT TGCATCGTTT TCTGTTGATC CGCAGTTGTA TTGTGATTAT AACGTAGTAC TAATGAAATA TGACGCGACG TTGCAATTGG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  510        520        530        540        550        560        570        580        590        600
SA11-L2_GS9(VP7) reference   ATATGTCAGA ACTTGCGGAT CTAATATTAA ACGAATGGTT GTGTAATCCA ATGGATATTA CTCTGTATTA TTATCAGCAA ACTGACGAAG CGAATAAATG
SA11-L2_GS9(VP7) NGS cs      ATATGTCAGA ACTTGCGGAT CTAATATTAA ACGAATGGTT GTGTAATCCA ATGGATATTA CTCTGTATTA TTATCAGCAA ACTGACGAAG CGAATAAATG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  610        620        630        640        650        660        670        680        690        700
SA11-L2_GS9(VP7) reference   GATATCAATG GGCTCATCAT GTACAATTAA AGTATGTCCA CTTAATACAC AAACTCTTGG AATTGGATGC TTGACAACTG ATGCTACAAC TTTTGAAGAA
SA11-L2_GS9(VP7) NGS cs      GATATCAATG GGCTCATCAT GTACAATTAA AGTATGTCCA CTTAATACAC AAACTCTTGG AATTGGATGC TTGACAACTG ATGCTACAAC TTTTGAAGAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  710        720        730        740        750        760        770        780        790        800
SA11-L2_GS9(VP7) reference   GTTGCGACAG CTGAAAAGTT GGTAATTACT GACGTGGTTG ATGGCGTTAA TCATAAGCTG GATGTCACAA CAGCAACGTG TACTATTAGA AACTGTAAGA
SA11-L2_GS9(VP7) NGS cs      GTTGCGACAG CTGAAAAGTT GGTAATTACT GACGTGGTTG ATGGCGTTAA TCATAAGCTG GATGTCACAA CAGCAACGTG TACTATTAGA AACTGTAAGA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  810        820        830        840        850        860        870        880        890        900
SA11-L2_GS9(VP7) reference   AATTGGGACC AAGAGAAAAC GTAGCCGTTA TACAAGTTGG TGGTTCTGAC ATCCTCGATA TAACTGCTGA TCCAACTACT GCACCACAGA CAGAACGGAT
SA11-L2_GS9(VP7) NGS cs      AATTGGGACC AAGAGAAAAC GTAGCCGTTA TACAAGTTGG TGGTTCTGAC ATCCTCGATA TAACTGCTGA TCCAACTACT GCACCACAGA CAGAACGGAT
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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  910        920        930        940        950        960        970        980        990        1000
SA11-L2_GS9(VP7) reference    GATGCGAATT AACTGGAAAA AATGGTGGCA AGTTTTTTAT ACTGTAGTAG ACTATGTAGA TCAGATAATA CAAGTTATGT CCAAAAGATC AAGATCACTA
SA11-L2_GS9(VP7) NGS cs       GATGCGAATT AACTGGAAAA AATGGTGGCA AGTTTTTTAT ACTGTAGTAG ACTATGTAGA TCAGATAATA CAAGTTATGT CCAGAAGATC AAGATCACTA


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ...
                                  1010       1020       1030       1040       1050       1060
SA11-L2_GS9(VP7) reference    AATTCAGCAG CATTTTATTA CAGAGTGTAG GTATAACTTA GGTTAGAATT GTATGATGTG ACC
SA11-L2_GS9(VP7) NGS cs       AATTCAGCAG CATTTTATTA CAGAGTGTAG GTATAACTTA GGTTAGAATT GTATGATGTG ACC
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Alignment: SA11-L2_GS10(NSP4)

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        10         20         30         40         50         60         70         80         90        100
SA11-L2_GS10(NSP4) reference    GGCTTTTAAA AGTTCTGTTC CGAGAGAGCG CGTGCGGAAA GATGGAAAAG CTTACCGACC TCAATTATAC ATTGAGTGTA ATCACTCTAA TGAACAATAC
SA11-L2_GS10(NSP4) NGS cs       GGCTTTTAAA AGTTCTGTTC CGAGAGAGCG CGTGCGGAAA GATGGAAAAG CTTACCGACC TCAATTATAC ATTGAGTGTA ATCACTCTAA TGAACAATAC

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       110        120        130        140        150        160        170        180        190        200
SA11-L2_GS10(NSP4) reference    ATTGCACACA ATACTTGAGG ATCCAGGAAT GGCGTATTTT CCTTATATAG CATCTGTCTT AACAGTTTTG TTTGCGCTAC ATAAAGCATC CATTCCAACA
SA11-L2_GS10(NSP4) NGS cs       ATTGCACACA ATACTTGAGG ATCCAGGAAT GGCGTATTTT CCTTATATAG CATCTGTCTT AACAGTTTTG TTTGCGCTAC ATAAAGCATC CATTCCAACA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       210        220        230        240        250        260        270        280        290        300
SA11-L2_GS10(NSP4) reference    ATGAAAATTG CATTGAAAAC GTCAAAATGT TCATATAAAG TGGTGAAATA TTGTATTGTA ACAATTTTTA ATACGTTGTT AAAATTGGCA GGTTATAAAG
SA11-L2_GS10(NSP4) NGS cs       ATGAAAATTG CATTGAAAAC GTCAAAATGT TCATATAAAG TGGTGAAATA TTGTATTGTA ACAATTTTTA ATACGTTGTT AAAATTGGCA GGTTATAAAG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       310        320        330        340        350        360        370        380        390        400
SA11-L2_GS10(NSP4) reference    AGCAGATAAC TACTAAAGAT GAGATAGAAA AGCAAATGGA CAGAGTAGTC AAAGAAATGA GACGCCAGCT AGAAATGATT GACAAATTGA CTACACGTGA
SA11-L2_GS10(NSP4) NGS cs       AGCAGATAAC TACTAAAGAT GAGATAGAAA AGCAAATGGA CAGAGTAGTC AAAGAAATGA GACGCCAGCT AGAAATGATT GACAAATTGA CTACACGTGA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       410        420        430        440        450        460        470        480        490        500
SA11-L2_GS10(NSP4) reference    AATTGAACAA GTAGAGTTGC TTAAACGCAT TTACGATAAA TTGACGGTGC AAACGACAGG TGAAATAGAT ATGACAAAAG AGATCAATCA AAAAAACGTG
SA11-L2_GS10(NSP4) NGS cs       AATTGAACAA GTAGAGTTGC TTAAACGCAT TTACGATAAA TTGACGGTGC AAACGACAGG TGAAATAGAT ATGACAAAAG AGATCAATCA AAAAAACGTG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       510        520        530        540        550        560        570        580        590        600
SA11-L2_GS10(NSP4) reference    AGAACGCTAG AAGAATGGGA AAGTGGAAAA AATCCTTATG AACCAAGAGA AGTGACTGCA GCAATGTAAG AGGTTGAGCT GCCGTCGACT GTCCTCGGAA
SA11-L2_GS10(NSP4) NGS cs       AGAACGCTAG AAGAATGGGA AAGTGGAAAA AATCCTTATG AACCAAGAGA AGTGACTGCA GCAATGTAAG AGGTTGAGCT GCCGTCGACT GTCCTCGGAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       610        620        630        640        650        660        670        680        690        700
SA11-L2_GS10(NSP4) reference    GCGGCGGAGT TCTTTACAGT AAGCACCATC GGACCTGATG GCTGACTGAG AAGCCACAGT CAGCCATATC GCGTGTGGCT CAAGCCTTAA TCCCGTTTAA
SA11-L2_GS10(NSP4) NGS cs       GCGGCGGAGT TCTTTACAGT AAGCACCATC GGACCTGATG GCTGACTGAG AAGCCACAGT CAGCCATATC GCGTGTGGCT CAAGCCTTAA TCCCGTTTAA

                                ....|....| ....|....| ....|....| ....|....| ....|...| .
                                       710        720        730        740        750
SA11-L2_GS10(NSP4) reference    CCAATCCGGT CAGCACCGGA CGTTAATGGA AGGAACGGTC TTAATGTGAC C
SA11-L2_GS10(NSP4) NGS cs       CCAATCCGGT CAGCACCGGA CGTTAATGGA AGGAACGGTC TTAATGTGAC C
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Alignment: SA11-N5_GS1(VP1)

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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen  ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS1 NGS      ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  110        120        130        140        150        160        170        180        190        200
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen  CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS1 NGS      CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  210        220        230        240        250        260        270        280        290        300
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen  CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS1 NGS      CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  310        320        330        340        350        360        370        380        390        400
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen  GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS1 NGS      GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  410        420        430        440        450        460        470        480        490        500
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen  TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS1 NGS      TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  510        520        530        540        550        560        570        580        590        600
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen  CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS1 NGS      CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  610        620        630        640        650        660        670        680        690        700
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen  ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS1 NGS      ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  710        720        730        740        750        760        770        780        790        800
SA11-N5_GS1(VP1) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS1 referen   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS1 NGS       ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

                             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                    810        820        830        840        850        860        870        880        890        900
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS1 NGS       AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

                             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                    910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS1 NGS       TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

                             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                   1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS1 NGS       GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

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                                   1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS1 NGS       GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

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                                   1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS1 NGS       TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

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                                   1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS1 NGS       ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

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                                   1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS1 NGS       CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

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                                   1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS1 NGS       GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

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                                     1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS1 NGS       TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

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                                     1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS1(VP1) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen   CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS1 NGS       CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                     1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS1(VP1) reference   ---------- ---------G GCTATTAAAG CTGTACAATG GGGAAGTACA ATCTAATCTT GTCAGAATAT CTATCATTTA TATATAATTC ACAATCTGCA
pSmart_SA11-N5_GS1 referen   TCTAATACGA CTCACTATAG GCTATTAAAG CTGTACAATG GGGAAGTACA ATCTAATCTT GTCAGAATAT CTATCATTTA TATATAATTC ACAATCTGCA
pSmart_SA11-N5_GS1 NGS       TCTAATACGA CTCACTATAG GCTATTAAAG CTGTACAATG GGGAAGTACA ATCTAATCTT GTCAGAATAT CTATCATTTA TATATAATTC ACAATCTGCA

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                                     1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS1(VP1) reference   GTTCAAATTC CAATATATTA CTCTTCCAAC AGTGAATTAG AAAATAGATG TATTGAATTT CATTCCAAGT GTTTAGAGAA CTCAAAGAAT GGGTTATCGT
pSmart_SA11-N5_GS1 referen   GTTCAAATTC CAATATATTA CTCTTCCAAC AGTGAATTAG AAAATAGATG TATTGAATTT CATTCCAAGT GTTTAGAGAA CTCAAAGAAT GGGTTATCGT
pSmart_SA11-N5_GS1 NGS       GTTCAAATTC CAATATATTA CTCTTCCAAC AGTGAATTAG AAAATAGATG TATTGAATTT CATTCCAAGT GTTTAGAGAA CTCAAAGAAT GGGTTATCGT

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                                     2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS1(VP1) reference   TAAGAAAGTT GTTTGTTGAA TATAATGATG TCATAGAAAA TGCCACATTA CTGTCAATAC TATCATATTC TTACGACAAG TATAACGCTG TTGAAAGAAA
pSmart_SA11-N5_GS1 referen   TAAGAAAGTT GTTTGTTGAA TATAATGATG TCATAGAAAA TGCCACATTA CTGTCAATAC TATCATATTC TTACGACAAG TATAACGCTG TTGAAAGAAA
pSmart_SA11-N5_GS1 NGS       TAAGAAAGTT GTTTGTTGAA TATAATGATG TCATAGAAAA TGCCACATTA CTGTCAATAC TATCATATTC TTACGACAAG TATAACGCTG TTGAAAGAAA

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                                     2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS1(VP1) reference   ATTGGTGAAG TATGCGAAAG GCAAACCATT GGAGGCAGAC TTAACAGTGA ATGAATTGGA TTATGAGAAC AATAAAATAA CATCTGAATT ATTTCCAACA
pSmart_SA11-N5_GS1 referen   ATTGGTGAAG TATGCGAAAG GCAAACCATT GGAGGCAGAC TTAACAGTGA ATGAATTGGA TTATGAGAAC AATAAAATAA CATCTGAATT ATTTCCAACA
pSmart_SA11-N5_GS1 NGS       ATTGGTGAAG TATGCGAAAG GCAAACCATT GGAGGCAGAC TTAACAGTGA ATGAATTGGA TTATGAGAAC AATAAAATAA CATCTGAATT ATTTCCAACA

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                                     2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS1(VP1) reference   GCGGAGGAAT ATACGGACTC ACTAATGGAT CCAGCAATTT TAACTTCGCT ATCATCAAAT TTAAATGCAG TCATGTTCTG GTTGGAAAAA CATGAAAATG
pSmart_SA11-N5_GS1 referen   GCGGAGGAAT ATACGGACTC ACTAATGGAT CCAGCAATTT TAACTTCGCT ATCATCAAAT TTAAATGCAG TCATGTTCTG GTTGGAAAAA CATGAAAATG
pSmart_SA11-N5_GS1 NGS       GCGGAGGAAT ATACGGACTC ACTAATGGAT CCAGCAATTT TAACTTCGCT ATCATCAAAT TTAAATGCAG TCATGTTCTG GTTGGAAAAA CATGAAAATG

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS1(VP1) reference      ATGTCGCTGA AAAACTTAAA GTTTATAAAA GGAGATTAGA CCTATTCACC ATAGTAGCCT CAACGATAAA TAAATATGGC GTACCAAGGC ATAACGCAAA
pSmart_SA11-N5_GS1 referen      ATGTCGCTGA AAAACTTAAA GTTTATAAAA GGAGATTAGA CCTATTCACC ATAGTAGCCT CAACGATAAA TAAATATGGC GTACCAAGGC ATAACGCAAA
pSmart_SA11-N5_GS1 NGS          ATGTCGCTGA AAAACTTAAA GTTTATAAAA GGAGATTAGA CCTATTCACC ATAGTAGCCT CAACGATAAA TAAATATGGC GTACCAAGGC ATAACGCAAA

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS1(VP1) reference      GTACAGATAT GAATACGACG TAATGAAAGA TAAACCATAC TACTTAGTGA CATGGGCAAA TTCTTCAATT GAAATGTTAA TGTCAGTTTT CTCTCATGAC
pSmart_SA11-N5_GS1 referen      GTACAGATAT GAATACGACG TAATGAAAGA TAAACCATAC TACTTAGTGA CATGGGCAAA TTCTTCAATT GAAATGTTAA TGTCAGTTTT CTCTCATGAC
pSmart_SA11-N5_GS1 NGS          GTACAGATAT GAATACGACG TAATGAAAGA TAAACCATAC TACTTAGTGA CATGGGCAAA TTCTTCAATT GAAATGTTAA TGTCAGTTTT CTCTCATGAC

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS1(VP1) reference      GACTATTTGA TAGCAAAAGA GTTAATAGTG TTATCATATT CTAATAGATC TACTCTAGCA AAGTTAGTGT CATCACCAAT GTCGATTTTG GTAGCCTTGG
pSmart_SA11-N5_GS1 referen      GACTATTTGA TAGCAAAAGA GTTAATAGTG TTATCATATT CTAATAGATC TACTCTAGCA AAGTTAGTGT CATCACCAAT GTCGATTTTG GTAGCCTTGG
pSmart_SA11-N5_GS1 NGS          GACTATTTGA TAGCAAAAGA GTTAATAGTG TTATCATATT CTAATAGATC TACTCTAGCA AAGTTAGTGT CATCACCAAT GTCGATTTTG GTAGCCTTGG

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS1(VP1) reference      TGGATATTAA TGGAACATTT ATTACAAATG AAGAATTAGA ATTGGAATTT TCAAATAAAT ATGTACGAGC AATAGTTCCG GATCAAACAT TTGACGAATT
pSmart_SA11-N5_GS1 referen      TGGATATTAA TGGAACATTT ATTACAAATG AAGAATTAGA ATTGGAATTT TCAAATAAAT ATGTACGAGC AATAGTTCCG GATCAAACAT TTGACGAATT
pSmart_SA11-N5_GS1 NGS          TGGATATTAA TGGAACATTT ATTACAAATG AAGAATTAGA ATTGGAATTT TCAAATAAAT ATGTACGAGC AATAGTTCCG GATCAAACAT TTGACGAATT

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                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS1(VP1) reference      AAATCAAATG CTTGACAATA TGAGGAAAGC TGGATTAGTT GACATACCTA AGATGATACA GGACTGGTTA GTTGATCGTT CTATCGAAAA ATTTCCATTA
pSmart_SA11-N5_GS1 referen      AAATCAAATG CTTGACAATA TGAGGAAAGC TGGATTAGTT GACATACCTA AGATGATACA GGACTGGTTA GTTGATCGTT CTATCGAAAA ATTTCCATTA
pSmart_SA11-N5_GS1 NGS          AAATCAAATG CTTGACAATA TGAGGAAAGC TGGATTAGTT GACATACCTA AGATGATACA GGACTGGTTA GTTGATCGTT CTATCGAAAA ATTTCCATTA

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                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS1(VP1) reference      ATGGCTAAGA TATATTCATG GTCGTTTCAT GTTGGATTTA GAAAGCAAAA AATGCTAGAT GCTGCGCTGG ATCAATTGAA AACTGAGTAT ACAGAAAATG
pSmart_SA11-N5_GS1 referen      ATGGCTAAGA TATATTCATG GTCGTTTCAT GTTGGATTTA GAAAGCAAAA AATGCTAGAT GCTGCGCTGG ATCAATTGAA AACTGAGTAT ACAGAAAATG
pSmart_SA11-N5_GS1 NGS          ATGGCTAAGA TATATTCATG GTCGTTTCAT GTTGGATTTA GAAAGCAAAA AATGCTAGAT GCTGCGCTGG ATCAATTGAA AACTGAGTAT ACAGAAAATG

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                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS1(VP1) reference      TGGACGATGA AATGTATCGG GAATATACAA TGTTAATAAG AGATGAAGTA GTTAAAATGC TTGAAGAACC AGTTAAACAT GATGATCACT TGCTACGAGA
pSmart_SA11-N5_GS1 referen      TGGACGATGA AATGTATCGG GAATATACAA TGTTAATAAG AGATGAAGTA GTTAAAATGC TTGAAGAACC AGTTAAACAT GATGATCACT TGCTACGAGA
pSmart_SA11-N5_GS1 NGS          TGGACGATGA AATGTATCGG GAATATACAA TGTTAATAAG AGATGAAGTA GTTAAAATGC TTGAAGAACC AGTTAAACAT GATGATCACT TGCTACGAGA

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                                3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-N5_GS1(VP1) reference      TTCTGAGTTA GCTGGTTTAC TATCAATGTC GTCAGCATCG AATGGTGAGT CAAGGCAGCT AAAGTTTGGT AGGAAAACAA TTTTTTCAAC TAAAAAGAAT
pSmart_SA11-N5_GS1 referen      TTCTGAGTTA GCTGGTTTAC TATCAATGTC GTCAGCATCG AATGGTGAGT CAAGGCAGCT AAAGTTTGGT AGGAAAACAA TTTTTTCAAC TAAAAAGAAT
pSmart_SA11-N5_GS1 NGS          TTCTGAGTTA GCTGGTTTAC TATCAATGTC GTCAGCATCG AATGGTGAGT CAAGGCAGCT AAAGTTTGGT AGGAAAACAA TTTTTTCAAC TAAAAAGAAT
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                                  3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
SA11-N5_GS1(VP1) reference    ATGCATGTCA TGGATGATAT GGCTAACGAA AGATACACGC CTGGTATAAT ACCACCAGTG AATGTTGATA AACCAATACC ATTAGGAAGA AGAGATGTTC
pSmart_SA11-N5_GS1 referen    ATGCATGTCA TGGATGATAT GGCTAACGAA AGATACACGC CTGGTATAAT ACCACCAGTG AATGTTGATA AACCAATACC ATTAGGAAGA AGAGATGTTC
pSmart_SA11-N5_GS1 NGS        ATGCATGTCA TGGATGATAT GGCTAACGAA AGATACACGC CTGGTATAAT ACCACCAGTG AATGTTGATA AACCAATACC ATTAGGAAGA AGAGATGTTC

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                                  3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
SA11-N5_GS1(VP1) reference    CAGGAAGAAG GACTAGAATA ATATTCATTC TGCCATACGA ATATTTCATA GCACAGCACG CTGTAGTTGA AAAAATGTTG ATTTACGCAA AACATACGAG
pSmart_SA11-N5_GS1 referen    CAGGAAGAAG GACTAGAATA ATATTCATTC TGCCATACGA ATATTTCATA GCACAGCACG CTGTAGTTGA AAAAATGTTG ATTTACGCAA AACATACGAG
pSmart_SA11-N5_GS1 NGS        CAGGAAGAAG GACTAGAATA ATATTCATTC TGCCATACGA ATATTTCATA GCACAGCACG CTGTAGTTGA AAAAATGTTG ATTTACGCAA AACATACGAG

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                                  3310       3320       3330       3340       3350       3360       3370       3380       3390       3400
SA11-N5_GS1(VP1) reference    AGAATACGCT GAATTTTATT CACAATCAAA CCAATTATTG TCATACGGCG ATGTAACGCG TTTTTTGTCT AATAACACAA TGGTCTTGTA TACGGATGTA
pSmart_SA11-N5_GS1 referen    AGAATACGCT GAATTTTATT CACAATCAAA CCAATTATTG TCATACGGCG ATGTAACGCG TTTTTTGTCT AATAACACAA TGGTCTTGTA TACGGATGTA
pSmart_SA11-N5_GS1 NGS        AGAATACGCT GAATTTTATT CACAATCAAA CCAATTATTG TCATACGGCG ATGTAACGCG TTTTTTGTCT AATAACACAA TGGTCTTGTA TACGGATGTA

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                                  3410       3420       3430       3440       3450       3460       3470       3480       3490       3500
SA11-N5_GS1(VP1) reference    TCTCAGTGGG ATTCGTCTCA GCATAATACA CAGCCATTTA GGAAAGGAAT AATAATGGGA CTGGACATAT TAGCTAACAT GACTAATGAT GCTAAAGTTC
pSmart_SA11-N5_GS1 referen    TCTCAGTGGG ATTCGTCTCA GCATAATACA CAGCCATTTA GGAAAGGAAT AATAATGGGA CTGGACATAT TAGCTAACAT GACTAATGAT GCTAAAGTTC
pSmart_SA11-N5_GS1 NGS        TCTCAGTGGG ATTCGTCTCA GCATAATACA CAGCCATTTA GGAAAGGAAT AATAATGGGA CTGGACATAT TAGCTAACAT GACTAATGAT GCTAAAGTTC

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                                  3510       3520       3530       3540       3550       3560       3570       3580       3590       3600
SA11-N5_GS1(VP1) reference    TTCAGACATT AAATTTATAC AAACAAACAC AAATCAATCT CATGGATTCA TACGTTCAAA TACCAGATGG CAACGTCATT AAGAAAATAC AATACGGGGC
pSmart_SA11-N5_GS1 referen    TTCAGACATT AAATTTATAC AAACAAACAC AAATCAATCT CATGGATTCA TACGTTCAAA TACCAGATGG CAACGTCATT AAGAAAATAC AATACGGGGC
pSmart_SA11-N5_GS1 NGS        TTCAGACATT AAATTTATAC AAACAAACAC AAATCAATCT CATGGATTCA TACGTTCAAA TACCAGATGG CAACGTCATT AAGAAAATAC AATACGGGGC

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                                  3610       3620       3630       3640       3650       3660       3670       3680       3690       3700
SA11-N5_GS1(VP1) reference    AGTAGCATCA GGAGAGAAAC AAACGAAAGC AGCAAATTCA ATAGCAAATT TGGCACTGAT TAAAACGGTT TTGTCACGTA TTTCTAACAA ACATTCATTC
pSmart_SA11-N5_GS1 referen    AGTAGCATCA GGAGAGAAAC AAACGAAAGC AGCAAATTCA ATAGCAAATT TGGCACTGAT TAAAACGGTT TTGTCACGTA TTTCTAACAA ACATTCATTC
pSmart_SA11-N5_GS1 NGS        AGTAGCATCA GGAGAGAAAC AAACGAAAGC AGCAAATTCA ATAGCAAATT TGGCACTGAT TAAAACGGTT TTGTCACGTA TTTCTAACAA ACATTCATTC

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                                  3710       3720       3730       3740       3750       3760       3770       3780       3790       3800
SA11-N5_GS1(VP1) reference    GCAACAAAAA TAATAAGAGT TGATGGAGAT GATAACTATG CGGTGCTACA ATTTAATACA GAGGTGACTA AGCAGATGAT CCAAGACGTA TCGAACGATG
pSmart_SA11-N5_GS1 referen    GCAACAAAAA TAATAAGAGT TGATGGAGAT GATAACTATG CGGTGCTACA ATTTAATACA GAGGTGACTA AGCAGATGAT CCAAGACGTA TCGAACGATG
pSmart_SA11-N5_GS1 NGS        GCAACAAAAA TAATAAGAGT TGATGGAGAT GATAACTATG CGGTGCTACA ATTTAATACA GAGGTGACTA AGCAGATGAT CCAAGACGTA TCGAACGATG

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                                  3810       3820       3830       3840       3850       3860       3870       3880       3890       3900
SA11-N5_GS1(VP1) reference    TAAGAGAAAC TTATGCACGC ATGAATGCTA AAGTTAAAGC TCTGGTATCC ACAGTAGGAA TAGAAATTGC TAAAAGGTAC ATTGCAGGTG GAAAAATATT
pSmart_SA11-N5_GS1 referen    TAAGAGAAAC TTATGCACGC ATGAATGCTA AAGTTAAAGC TCTGGTATCC ACAGTAGGAA TAGAAATTGC TAAAAGGTAC ATTGCAGGTG GAAAAATATT
pSmart_SA11-N5_GS1 NGS        TAAGAGAAAC TTATGCACGC ATGAATGCTA AAGTTAAAGC TCTGGTATCC ACAGTAGGAA TAGAAATTGC TAAAAGGTAC ATTGCAGGTG GAAAAATATT
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                            3910       3920       3930       3940       3950       3960       3970       3980       3990       4000
SA11-N5_GS1(VP1) reference  TTTTCGAGCT GGAATAAATC TACTTAATAA TGAAAAGAGA GGGCAGAGTA CGCAGTGGGA TCAAGCAGCA ATTTTATATT CAAATTATAT TGTAAATAGA
pSmart_SA11-N5_GS1 referen  TTTTCGAGCT GGAATAAATC TACTTAATAA TGAAAAGAGA GGGCAGAGTA CGCAGTGGGA TCAAGCAGCA ATTTTATATT CAAATTATAT TGTAAATAGA
pSmart_SA11-N5_GS1 NGS      TTTTCGAGCT GGAATAAATC TACTTAATAA TGAAAAGAGA GGGCAGAGTA CGCAGTGGGA TCAAGCAGCA ATTTTATATT CAAATTATAT TGTAAATAGA


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                            4010       4020       4030       4040       4050       4060       4070       4080       4090       4100
SA11-N5_GS1(VP1) reference  CTTAGAGGAT TTGAAACTGA TAGGGAGTTT ATTTTAACTA AGATAATGCA GATGACGTCA GTCGCAATTA CTGGATCATT AAGACTATTT CCTTCTGAAC
pSmart_SA11-N5_GS1 referen  CTTAGAGGAT TTGAAACTGA TAGGGAGTTT ATTTTAACTA AGATAATGCA GATGACGTCA GTCGCAATTA CTGGATCATT AAGACTATTT CCTTCTGAAC
pSmart_SA11-N5_GS1 NGS      CTTAGAGGAT TTGAAACTGA TAGGGAGTTT ATTTTAACTA AGATAATGCA GATGACGTCA GTCGCAATTA CTGGATCATT AAGACTATTT CCTTCTGAAC


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                            4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
SA11-N5_GS1(VP1) reference  GCGTATTAAC TACGAATTCA ACATTTAAAG TATTTGACTC GGAGGATTTT ATTATAGAGT ACGGAACGAC TGATGACGAA GTATATATAC AAAGAGCGTT
pSmart_SA11-N5_GS1 referen  GCGTATTAAC TACGAATTCA ACATTTAAAG TATTTGACTC GGAGGATTTT ATTATAGAGT ACGGAACGAC TGATGACGAA GTATATATAC AAAGAGCGTT
pSmart_SA11-N5_GS1 NGS      GCGTATTAAC TACGAATTCA ACATTTAAAG TATTTGACTC GGAGGATTTT ATTATAGAGT ACGGAACGAC TGATGACGAA GTATATATAC AAAGAGCGTT


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                            4210       4220       4230       4240       4250       4260       4270       4280       4290       4300
SA11-N5_GS1(VP1) reference  CATGTCTTTA TCAAGTCAGA AATCAGGAAT AGCCGATGAG ATAGCGGCAT CATCAACATT TAAAAATTAC GTCACGAGAC TATCTGAACA GTTATTATTT
pSmart_SA11-N5_GS1 referen  CATGTCTTTA TCAAGTCAGA AATCAGGAAT AGCCGATGAG ATAGCGGCAT CATCAACATT TAAAAATTAC GTCACGAGAC TATCTGAACA GTTATTATTT
pSmart_SA11-N5_GS1 NGS      CATGTCTTTA TCAAGTCAGA AATCAGGAAT AGCCGATGAG ATAGCGGCAT CATCAACATT TAAAAATTAC GTCACGAGAC TATCTGAACA GTTATTATTT


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                            4310       4320       4330       4340       4350       4360       4370       4380       4390       4400
SA11-N5_GS1(VP1) reference  TCAAAGAATA ATATAGTGTC CAGAGGAATA GCTTTGACTG AAAAAGCGAA ATTGAATTCA TACGCTCCAA TATCGCTTGA GAAAAGACGT GCACAGATAT
pSmart_SA11-N5_GS1 referen  TCAAAGAATA ATATAGTGTC CAGAGGAATA GCTTTGACTG AAAAAGCGAA ATTGAATTCA TACGCTCCAA TATCGCTTGA GAAAAGACGT GCACAGATAT
pSmart_SA11-N5_GS1 NGS      TCAAAGAATA ATATAGTGTC CAGAGGAATA GCTTTGACTG AAAAAGCGAA ATTGAATTCA TACGCTCCAA TATCGCTTGA GAAAAGACGT GCACAGATAT


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                            4410       4420       4430       4440       4450       4460       4470       4480       4490       4500
SA11-N5_GS1(VP1) reference  CAGCTTTATT GACTATGTTG CAGAAACCGG TCACTTTCAA ATCAAGTAAA ATAACAATAA ATGACATACT CAGAGATATA AAACCATTTT TTACAGTAAG
pSmart_SA11-N5_GS1 referen  CAGCTTTATT GACTATGTTG CAGAAACCGG TCACTTTCAA ATCAAGTAAA ATAACAATAA ATGACATACT CAGAGATATA AAACCATTTT TTACAGTAAG
pSmart_SA11-N5_GS1 NGS      CAGCTTTATT GACTATGTTG CAGAAACCGG TCACTTTCAA ATCAAGTAAA ATAACAATAA ATGACATACT CAGAGATATA AAACCATTTT TTACAGTAAG


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                            4510       4520       4530       4540       4550       4560       4570       4580       4590       4600
SA11-N5_GS1(VP1) reference  TGATGCACAC TTACCTATAC AATACCAAAA ATTTATGCCA ACTTTGCCAG ATAACGTACA GTATATAATT CAATGTATAG GATCCAGAAC TTATCAAATT
pSmart_SA11-N5_GS1 referen  TGATGCACAC TTACCTATAC AATACCAAAA ATTTATGCCA ACTTTGCCAG ATAACGTACA GTATATAATT CAATGTATAG GATCCAGAAC TTATCAAATT
pSmart_SA11-N5_GS1 NGS      TGATGCACAC TTACCTATAC AATACCAAAA ATTTATGCCA ACTTTGCCAG ATAACGTACA GTATATAATT CAATGTATAG GATCCAGAAC TTATCAAATT


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                            4610       4620       4630       4640       4650       4660       4670       4680       4690       4700
SA11-N5_GS1(VP1) reference  GAAGATGACG GTTCGAAGTC AGCCATATCT AGACTAATAT CAAAGTATTC AGTTTATAAG CCATCAATTG AAGAATTGTA TAAAGTGATT TCATTGCATG
pSmart_SA11-N5_GS1 referen  GAAGATGACG GTTCGAAGTC AGCCATATCT AGACTAATAT CAAAGTATTC AGTTTATAAG CCATCAATTG AAGAATTGTA TAAAGTGATT TCATTGCATG
pSmart_SA11-N5_GS1 NGS      GAAGATGACG GTTCGAAGTC AGCCATATCT AGACTAATAT CAAAGTATTC AGTTTATAAG CCATCAATTG AAGAATTGTA TAAAGTGATT TCATTGCATG
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                                  4710       4720       4730       4740       4750       4760       4770       4780       4790       4800
SA11-N5_GS1(VP1) reference    AAAACGAAAT ACAATTATAT CTGATTTCAT TAGGAATACC GAAAATAGAC GCTGACACGT ATGTTGGATC AAAGATTTAT TCTCAAGATA AGTATAGAAT
pSmart_SA11-N5_GS1 referen    AAAACGAAAT ACAATTATAT CTGATTTCAT TAGGAATACC GAAAATAGAC GCTGACACGT ATGTTGGATC AAAGATTTAT TCTCAAGATA AGTATAGAAT
pSmart_SA11-N5_GS1 NGS        AAAACGAAAT ACAATTATAT CTGATTTCAT TAGGAATACC GAAAATAGAC GCTGACACGT ATGTTGGATC AAAGATTTAT TCTCAAGATA AGTATAGAAT

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                                  4810       4820       4830       4840       4850       4860       4870       4880       4890       4900
SA11-N5_GS1(VP1) reference    ACTAGAATCA TACGTGTACA ATTTATTGTC CATTAATTAT GGATGCTATC AATTATTTGA TTTCAATTCA CCGGACTTGG AGAAGCTGAT AAGAATACCA
pSmart_SA11-N5_GS1 referen    ACTAGAATCA TACGTGTACA ATTTATTGTC CATTAATTAT GGATGCTATC AATTATTTGA TTTCAATTCA CCGGACTTGG AGAAGCTGAT AAGAATACCA
pSmart_SA11-N5_GS1 NGS        ACTAGAATCA TACGTGTACA ATTTATTGTC CATTAATTAT GGATGCTATC AATTATTTGA TTTCAATTCA CCGGACTTGG AGAAGCTGAT AAGAATACCA

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                                  4910       4920       4930       4940       4950       4960       4970       4980       4990       5000
SA11-N5_GS1(VP1) reference    TTTAAGGGAA AAATACCAGC TGTTACATTC ATATTACACT TATATGCAAA GCTAGAAGTT ATAAACTACG CTATAAAAAA TGGTTCATGG ATAAGCCTAT
pSmart_SA11-N5_GS1 referen    TTTAAGGGAA AAATACCAGC TGTTACATTC ATATTACACT TATATGCAAA GCTAGAAGTT ATAAACTACG CTATAAAAAA TGGTTCATGG ATAAGCCTAT
pSmart_SA11-N5_GS1 NGS        TTTAAGGGAA AAATACCAGC TGTTACATTC ATATTACACT TATATGCAAA GCTAGAAGTT ATAAACTACG CTATAAAAAA TGGTTCATGG ATAAGCCTAT

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                                  5010       5020       5030       5040       5050       5060       5070       5080       5090       5100
SA11-N5_GS1(VP1) reference    TTTGCAATTA CCCTAAATCA GAAATGATAA AATTATGGAA GAAGATGTGG AACATCACGT CATTACGTTC GCCGTACACT AACGCGAACT TCTTTCAAGA
pSmart_SA11-N5_GS1 referen    TTTGCAATTA CCCTAAATCA GAAATGATAA AATTATGGAA GAAGATGTGG AACATCACGT CATTACGTTC GCCGTACACT AACGCGAACT TCTTTCAAGA
pSmart_SA11-N5_GS1 NGS        TTTGCAATTA CCCTAAATCA GAAATGATAA AATTATGGAA GAAGATGTGG AACATCACGT CATTACGTTC GCCGTACACT AACGCGAACT TCTTTCAAGA

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                                  5110       5120       5130       5140       5150       5160       5170       5180       5190       5200
SA11-N5_GS1(VP1) reference    TTAGAACGCT TAGATGTGAC C--------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen    TTAGAACGCT TAGATGTGAC CGGCCGGCAT GGTCCCAGCC TCCTCGCTGG CGCCGGCTGG GCAACATTCC GAGGGGACCG TCCCCTCGGT AATGGCGAAT
pSmart_SA11-N5_GS1 NGS        TTAGAACGCT TAGATGTGAC CGGCCGGCAT GGTCCCAGCC TCCTCGCTGG CGCCGGCTGG GCAACATTCC GAGGGGACCG TCCCCTCGGT AATGGCGAAT

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                                  5210       5220       5230       5240       5250       5260       5270       5280       5290       5300
SA11-N5_GS1(VP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS1 referen    GGGACGGATC CGGCTGCTAA CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC TGCCACCGCT GAGCAATAAC TAGCATAACC CCTTGGGGCC TCTAAACGGG
pSmart_SA11-N5_GS1 NGS        GGGACGGATC CGGCTGCTAA CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC TGCCACCGCT GAGCAATAAC TAGCATAACC CCTTGGGGCC TCTAAACGGG

                          ....|....| ....|....|
                                  5310       5320
SA11-N5_GS1(VP1) reference    ---------- ----------
pSmart_SA11-N5_GS1 referen    TCTTGAGGGG TTTTTTGGAT
pSmart_SA11-N5_GS1 NGS        TCTTGAGGGG TTTTTTGGAT
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Alignment: SA11-N5_GS2(VP2)

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                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS2 NGS          ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

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                                        110        120        130        140        150        160        170        180        190        200
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS2 NGS          CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

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                                        210        220        230        240        250        260        270        280        290        300
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS2 NGS          CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

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                                        310        320        330        340        350        360        370        380        390        400
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS2 NGS          GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

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                                        410        420        430        440        450        460        470        480        490        500
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS2 NGS          TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

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                                        510        520        530        540        550        560        570        580        590        600
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS2 NGS          CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

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                                        610        620        630        640        650        660        670        680        690        700
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS2 NGS          ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        710        720        730        740        750        760        770        780        790        800
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS2 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS2 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

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                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS2(VP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS2 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

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                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS2(VP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS2 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

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                                       1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS2(VP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS2 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

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                                       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS2(VP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS2 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

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                                       1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS2(VP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS2 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

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                                       1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS2(VP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS2 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

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                                       1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS2(VP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS2 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

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                                       1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS2 NGS          GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

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                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS2 NGS          TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

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                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS2 NGS          CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS2(VP2) reference      ---------- ---------G GCTATTAAAG GCTCAATGGC GTATCGAAAA CGTGGAGCGC GTCGTGAGAC GAATCTAAAA CAAGATGAAC GAATGCAAGA
pSmart_SA11-N5_GS2 reference    TCTAATACGA CTCACTATAG GCTATTAAAG GCTCAATGGC GTATCGAAAA CGTGGAGCGC GTCGTGAGAC GAATCTAAAA CAAGATGAAC GAATGCAAGA
pSmart_SA11-N5_GS2 NGS          TCTAATACGA CTCACTATAG GCTATTAAAG GCTCAATGGC GTATCGAAAA CGTGGAGCGC GTCGTGAGAC GAATCTAAAA CAAGATGAAC GAATGCAAGA

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                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS2(VP2) reference      AAAAGAAGAT AGCAAGAACA TTAATAATGA CAGTCCTAAA TCACAATTAT CAGAAAAAGT ATTATCTAAG AAAGAAGAGA TAATTACAGA TAATCAAGAA
pSmart_SA11-N5_GS2 reference    AAAAGAAGAT AGCAAGAACA TTAATAATGA CAGTCCTAAA TCACAATTAT CAGAAAAAGT ATTATCTAAG AAAGAAGAGA TAATTACAGA TAATCAAGAA
pSmart_SA11-N5_GS2 NGS          AAAAGAAGAT AGCAAGAACA TTAATAATGA CAGTCCTAAA TCACAATTAT CAGAAAAAGT ATTATCTAAG AAAGAAGAGA TAATTACAGA TAATCAAGAA

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                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS2(VP2) reference      GAAGTTAAGA TATCTGATGA GGTAAAAAAA TCTAATAAAG AAGAATCGAA ACAGTTGTTA GAAGTACTTA AAACAAAAGA GGAACATCAA AAAGAAGTTC
pSmart_SA11-N5_GS2 reference    GAAGTTAAGA TATCTGATGA GGTAAAAAAA TCTAATAAAG AAGAATCGAA ACAGTTGTTA GAAGTACTTA AAACAAAAGA GGAACATCAA AAAGAAGTTC
pSmart_SA11-N5_GS2 NGS          GAAGTTAAGA TATCTGATGA GGTAAAAAAA TCTAATAAAG AAGAATCGAA ACAGTTGTTA GAAGTACTTA AAACAAAAGA GGAACATCAA AAAGAAGTTC

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                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS2(VP2) reference      AGTATGAAAT ATTACAAAAA ACTATCCCTA CATTTGAACC AAAAGAGTCA ATACTCAAAA AATTAGAAGA CATAAAACCA GAACAAGCAA AGAAACAAAC
pSmart_SA11-N5_GS2 reference    AGTATGAAAT ATTACAAAAA ACTATCCCTA CATTTGAACC AAAAGAGTCA ATACTCAAAA AATTAGAAGA CATAAAACCA GAACAAGCAA AGAAACAAAC
pSmart_SA11-N5_GS2 NGS          AGTATGAAAT ATTACAAAAA ACTATCCCTA CATTTGAACC AAAAGAGTCA ATACTCAAAA AATTAGAAGA CATAAAACCA GAACAAGCAA AGAAACAAAC

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                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS2(VP2) reference      TAAACTGTTT CGAATATTTG AACCGAAACA ATTGCCTATT TATAGAGCTA ATGGAGAAAG AGAGCTTCGT AATAGATGGT ATTGGAAATT GAAACGAGAT
pSmart_SA11-N5_GS2 reference    TAAACTGTTT CGAATATTTG AACCGAAACA ATTGCCTATT TATAGAGCTA ATGGAGAAAG AGAGCTTCGT AATAGATGGT ATTGGAAATT GAAACGAGAT
pSmart_SA11-N5_GS2 NGS          TAAACTGTTT CGAATATTTG AACCGAAACA ATTGCCTATT TATAGAGCTA ATGGAGAAAG AGAGCTTCGT AATAGATGGT ATTGGAAATT GAAACGAGAT

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS2(VP2) reference      ACTCTTCCTG ATGGAGATTA TGATGTTAGA GAGTATTTTT TAAATTTATA TGATCAAGTA TTAATGGAAA TGCCGGATTA TCTATTACTT AAAGATATGG
pSmart_SA11-N5_GS2 reference    ACTCTTCCTG ATGGAGATTA TGATGTTAGA GAGTATTTTT TAAATTTATA TGATCAAGTA TTAATGGAAA TGCCGGATTA TCTATTACTT AAAGATATGG
pSmart_SA11-N5_GS2 NGS          ACTCTTCCTG ATGGAGATTA TGATGTTAGA GAGTATTTTT TAAATTTATA TGATCAAGTA TTAATGGAAA TGCCGGATTA TCTATTACTT AAAGATATGG

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS2(VP2) reference      CTGTAGAGAA TAAAAATTCA AGGGATGCTG GCAAAGTAGT TGATTCTGAA ACAGCCGCAA TATGCGATGC TATTTTTCAA GATGAAGAAA CCGAAGGTGC
pSmart_SA11-N5_GS2 reference    CTGTAGAGAA TAAAAATTCA AGGGATGCTG GCAAAGTAGT TGATTCTGAA ACAGCCGCAA TATGCGATGC TATTTTTCAA GATGAAGAAA CCGAAGGTGC
pSmart_SA11-N5_GS2 NGS          CTGTAGAGAA TAAAAATTCA AGGGATGCTG GCAAAGTAGT TGATTCTGAA ACAGCCGCAA TATGCGATGC TATTTTTCAA GATGAAGAAA CCGAAGGTGC

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS2(VP2) reference      AGTAAGAAGA TTCATAGCTG AGATGAGACA ACGAGTTCAA GCTGATCGAA ATGTAGTCAA TTATCCATCT ATATTGCATC CAATTGACCA TGCGTTTAAC
pSmart_SA11-N5_GS2 reference    AGTAAGAAGA TTCATAGCTG AGATGAGACA ACGAGTTCAA GCTGATCGAA ATGTAGTCAA TTATCCATCT ATATTGCATC CAATTGACCA TGCGTTTAAC
pSmart_SA11-N5_GS2 NGS          AGTAAGAAGA TTCATAGCTG AGATGAGACA ACGAGTTCAA GCTGATCGAA ATGTAGTCAA TTATCCATCT ATATTGCATC CAATTGACCA TGCGTTTAAC

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS2(VP2) reference      GAATACTTCT TACAACATCA GTTGGTAGAA CCATTAAATA ATGATATCAT TTTCAATTAC ATACCAGAGA GAATAAGAAA TGATGTCAAC TATATATTAA
pSmart_SA11-N5_GS2 reference    GAATACTTCT TACAACATCA GTTGGTAGAA CCATTAAATA ATGATATCAT TTTCAATTAC ATACCAGAGA GAATAAGAAA TGATGTCAAC TATATATTAA
pSmart_SA11-N5_GS2 NGS          GAATACTTCT TACAACATCA GTTGGTAGAA CCATTAAATA ATGATATCAT TTTCAATTAC ATACCAGAGA GAATAAGAAA TGATGTCAAC TATATATTAA

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                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS2(VP2) reference      ATATGGACAG GAATTTACCG TCTACTGCTA GATATATCAG ACCAAACTTG CTACAAGATA GGTTAAATTT ACATGATAAT TTTGAGTCAC TCTGGGATAC
pSmart_SA11-N5_GS2 reference    ATATGGACAG GAATTTACCG TCTACTGCTA GATATATCAG ACCAAACTTG CTACAAGATA GGTTAAATTT ACATGATAAT TTTGAGTCAC TCTGGGATAC
pSmart_SA11-N5_GS2 NGS          ATATGGACAG GAATTTACCG TCTACTGCTA GATATATCAG ACCAAACTTG CTACAAGATA GGTTAAATTT ACATGATAAT TTTGAGTCAC TCTGGGATAC

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                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS2(VP2) reference      TATAACTACA TCTAATTATA TTTTAGCAAG ATCTGTGGTG CCAGACCTAA AAGAATTAGT ATCTACTGAG GCACAAATCC AGAAAATGTC ACAAGATTTG
pSmart_SA11-N5_GS2 reference    TATAACTACA TCTAATTATA TTTTAGCAAG ATCTGTGGTG CCAGACCTAA AAGAATTAGT ATCTACTGAG GCACAAATCC AGAAAATGTC ACAAGATTTG
pSmart_SA11-N5_GS2 NGS          TATAACTACA TCTAATTATA TTTTAGCAAG ATCTGTGGTG CCAGACCTAA AAGAATTAGT ATCTACTGAG GCACAAATCC AGAAAATGTC ACAAGATTTG

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                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS2(VP2) reference      CAATTGGAAG CTTTGACAAT ACAATCAGAG ACTCAGTTTT TAACAGGTAT AAACTCACAA GCCGCTAATG ATTGTTTTAA AACTTTGATT GCTGCTATGT
pSmart_SA11-N5_GS2 reference    CAATTGGAAG CTTTGACAAT ACAATCAGAG ACTCAGTTTT TAACAGGTAT AAACTCACAA GCCGCTAATG ATTGTTTTAA AACTTTGATT GCTGCTATGT
pSmart_SA11-N5_GS2 NGS          CAATTGGAAG CTTTGACAAT ACAATCAGAG ACTCAGTTTT TAACAGGTAT AAACTCACAA GCCGCTAATG ATTGTTTTAA AACTTTGATT GCTGCTATGT

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                                3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-N5_GS2(VP2) reference      TGAGTCAGAG AACCATGTCA TTAGATTTCG TAACGACAAA TTACATGTCA CTTATTTCAG GCATGTGGTT ACTCACTGTG ATTCCAAATG ATATGTTTAT
pSmart_SA11-N5_GS2 reference    TGAGTCAGAG AACCATGTCA TTAGATTTCG TAACGACAAA TTACATGTCA CTTATTTCAG GCATGTGGTT ACTCACTGTG ATTCCAAATG ATATGTTTAT
pSmart_SA11-N5_GS2 NGS          TGAGTCAGAG AACCATGTCA TTAGATTTCG TAACGACAAA TTACATGTCA CTTATTTCAG GCATGTGGTT ACTCACTGTG ATTCCAAATG ATATGTTTAT
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                                  3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
SA11-N5_GS2(VP2) reference      AAGAGAATCA TTAGTAGCAT GTCAACTAGC CATAATAAAT ACCATTGTTT ATCCGGCATT CGGAATGCAA AGAATGCATT ATAGGAATGG TGATCCACAG
pSmart_SA11-N5_GS2 reference    AAGAGAATCA TTAGTAGCAT GTCAACTAGC CATAATAAAT ACCATTGTTT ATCCGGCATT CGGAATGCAA AGAATGCATT ATAGGAATGG TGATCCACAG
pSmart_SA11-N5_GS2 NGS          AAGAGAATCA TTAGTAGCAT GTCAACTAGC CATAATAAAT ACCATTGTTT ATCCGGCATT CGGAATGCAA AGAATGCATT ATAGGAATGG TGATCCACAG

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                                  3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
SA11-N5_GS2(VP2) reference      ACTCCCTTTC AAATTGCAGA GCAACAGATT CAAAATTTTC AGGTAGCTAA TTGGTTACAT TTTGTTAATT ATAATCAGTT TAGACAAGTA GTGATTGATG
pSmart_SA11-N5_GS2 reference    ACTCCCTTTC AAATTGCAGA GCAACAGATT CAAAATTTTC AGGTAGCTAA TTGGTTACAT TTTGTTAATT ATAATCAGTT TAGACAAGTA GTGATTGATG
pSmart_SA11-N5_GS2 NGS          ACTCCCTTTC AAATTGCAGA GCAACAGATT CAAAATTTTC AGGTAGCTAA TTGGTTACAT TTTGTTAATT ATAATCAGTT TAGACAAGTA GTGATTGATG

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                                  3310       3320       3330       3340       3350       3360       3370       3380       3390       3400
SA11-N5_GS2(VP2) reference      GAGTGTTAAA TCAAGTCTTG AATGATAATA TAAGAAATGG TCATGTAGTC AACCAATTAA TGGAAGCTCT GATGCAATTA TCTAGACAAC AGTTTCCCAC
pSmart_SA11-N5_GS2 reference    GAGTGTTAAA TCAAGTCTTG AATGATAATA TAAGAAATGG TCATGTAGTC AACCAATTAA TGGAAGCTCT GATGCAATTA TCTAGACAAC AGTTTCCCAC
pSmart_SA11-N5_GS2 NGS          GAGTGTTAAA TCAAGTCTTG AATGATAATA TAAGAAATGG TCATGTAGTC AACCAATTAA TGGAAGCTCT GATGCAATTA TCTAGACAAC AGTTTCCCAC

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                                  3410       3420       3430       3440       3450       3460       3470       3480       3490       3500
SA11-N5_GS2(VP2) reference      AATGCCAGTT GATTATAAAA GATCTATACA GAGAGGAATT TTGCTGCTTT CTAACAGACT TGGTCAGCTT GTCGATTTAA CAAGATTGTT ATCATACAAT
pSmart_SA11-N5_GS2 reference    AATGCCAGTT GATTATAAAA GATCTATACA GAGAGGAATT TTGCTGCTTT CTAACAGACT TGGTCAGCTT GTCGATTTAA CAAGATTGTT ATCATACAAT
pSmart_SA11-N5_GS2 NGS          AATGCCAGTT GATTATAAAA GATCTATACA GAGAGGAATT TTGCTGCTTT CTAACAGACT TGGTCAGCTT GTCGATTTAA CAAGATTGTT ATCATACAAT

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                                  3510       3520       3530       3540       3550       3560       3570       3580       3590       3600
SA11-N5_GS2(VP2) reference      TATGAGACAT TAATGGCATG CATAACAATG AATATGCAGC ATGTTCAAAC ATTAACAACT GAAAAATTGC AATTAACATC AGTAACATCA TTATGTATGC
pSmart_SA11-N5_GS2 reference    TATGAGACAT TAATGGCATG CATAACAATG AATATGCAGC ATGTTCAAAC ATTAACAACT GAAAAATTGC AATTAACATC AGTAACATCA TTATGTATGC
pSmart_SA11-N5_GS2 NGS          TATGAGACAT TAATGGCATG CATAACAATG AATATGCAGC ATGTTCAAAC ATTAACAACT GAAAAATTGC AATTAACATC AGTAACATCA TTATGTATGC

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                                  3610       3620       3630       3640       3650       3660       3670       3680       3690       3700
SA11-N5_GS2(VP2) reference      TAATTGGAAA TGCTACGGTT ATACCGAGTC CGCAAACATT GTTCCATTAC TATAATGTGA ATGTCAATTT TCATTCAAAT TATAATGAAA GAATTAATGA
pSmart_SA11-N5_GS2 reference    TAATTGGAAA TGCTACGGTT ATACCGAGTC CGCAAACATT GTTCCATTAC TATAATGTGA ATGTCAATTT TCATTCAAAT TATAATGAAA GAATTAATGA
pSmart_SA11-N5_GS2 NGS          TAATTGGAAA TGCTACGGTT ATACCGAGTC CGCAAACATT GTTCCATTAC TATAATGTGA ATGTCAATTT TCATTCAAAT TATAATGAAA GAATTAATGA

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                                  3710       3720       3730       3740       3750       3760       3770       3780       3790       3800
SA11-N5_GS2(VP2) reference      CGCAGTTGCA ATTATAACTG CGGCAAATAG ATTAAATTTA TATCAAAAGA AAATGAAATC AATAGTTGAG GACTTTCTGA AAAGATTACA GATATTTGAT
pSmart_SA11-N5_GS2 reference    CGCAGTTGCA ATTATAACTG CGGCAAATAG ATTAAATTTA TATCAAAAGA AAATGAAATC AATAGTTGAG GACTTTCTGA AAAGATTACA GATATTTGAT
pSmart_SA11-N5_GS2 NGS          CGCAGTTGCA ATTATAACTG CGGCAAATAG ATTAAATTTA TATCAAAAGA AAATGAAATC AATAGTTGAG GACTTTCTGA AAAGATTACA GATATTTGAT

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                                  3810       3820       3830       3840       3850       3860       3870       3880       3890       3900
SA11-N5_GS2(VP2) reference      GTTGCGAGAG TACCAGATGA CCAAATGTAT AGATTGAGAG ATAGATTAAG ACTATTACCA GTTGAAATAA GAAGATTAGA TATTTTTAAT TTGATAGCAA
pSmart_SA11-N5_GS2 reference    GTTGCGAGAG TACCAGATGA CCAAATGTAT AGATTGAGAG ATAGATTAAG ACTATTACCA GTTGAAATAA GAAGATTAGA TATTTTTAAT TTGATAGCAA
pSmart_SA11-N5_GS2 NGS          GTTGCGAGAG TACCAGATGA CCAAATGTAT AGATTGAGAG ATAGATTAAG ACTATTACCA GTTGAAATAA GAAGATTAGA TATTTTTAAT TTGATAGCAA
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                                  3910       3920       3930       3940       3950       3960       3970       3980       3990       4000
SA11-N5_GS2(VP2) reference      TGAATATGGA ACAGATTGAA CGTGCATCAG ATAAAATTGC ACAAGGAGTT ATAATAGCAT ACCGAGATAT GCAGTTAGAA CGAGATGAGA TGTATGGTTA
pSmart_SA11-N5_GS2 reference    TGAATATGGA ACAGATTGAA CGTGCATCAG ATAAAATTGC ACAAGGAGTT ATAATAGCAT ACCGAGATAT GCAGTTAGAA CGAGATGAGA TGTATGGTTA
pSmart_SA11-N5_GS2 NGS          TGAATATGGA ACAGATTGAA CGTGCATCAG ATAAAATTGC ACAAGGAGTT ATAATAGCAT ACCGAGATAT GCAGTTAGAA CGAGATGAGA TGTATGGTTA

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                                  4010       4020       4030       4040       4050       4060       4070       4080       4090       4100
SA11-N5_GS2(VP2) reference      CGTCAATATT GCCAGAAACT TGGACGGATT TCAACAAATA AATCTTGAAG AATTGATGAG ATCAGGAGAT TATGCTCAAA TTACTAACAT GCTACTTAAT
pSmart_SA11-N5_GS2 reference    CGTCAATATT GCCAGAAACT TGGACGGATT TCAACAAATA AATCTTGAAG AATTGATGAG ATCAGGAGAT TATGCTCAAA TTACTAACAT GCTACTTAAT
pSmart_SA11-N5_GS2 NGS          CGTCAATATT GCCAGAAACT TGGACGGATT TCAACAAATA AATCTTGAAG AATTGATGAG ATCAGGAGAT TATGCTCAAA TTACTAACAT GCTACTTAAT

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                                  4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
SA11-N5_GS2(VP2) reference      AATCAACCAG TAGCTTTAGT TGGAGCGCTA CCATTTATAA CGGATTCATC AGTGATTTCG TTAATAGCTA AACTAGATGC AACCGTTTTT GCACAGATTG
pSmart_SA11-N5_GS2 reference    AATCAACCAG TAGCTTTAGT TGGAGCGCTA CCATTTATAA CGGATTCATC AGTGATTTCG TTAATAGCTA AACTAGATGC AACCGTTTTT GCACAGATTG
pSmart_SA11-N5_GS2 NGS          AATCAACCAG TAGCTTTAGT TGGAGCGCTA CCATTTATAA CGGATTCATC AGTGATTTCG TTAATAGCTA AACTAGATGC AACCGTTTTT GCACAGATTG

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                                  4210       4220       4230       4240       4250       4260       4270       4280       4290       4300
SA11-N5_GS2(VP2) reference      TCAAACTTAG AAAGGTCGAC ACGTTAAAAC CCATCCTATA TAAGATAAAT TCAGATTCTA ATGACTTTTA TTTGGTGGCT AATTATGATT GGATTCCTAC
pSmart_SA11-N5_GS2 reference    TCAAACTTAG AAAGGTCGAC ACGTTAAAAC CCATCCTATA TAAGATAAAT TCAGATTCTA ATGACTTTTA TTTGGTGGCT AATTATGATT GGATTCCTAC
pSmart_SA11-N5_GS2 NGS          TCAAACTTAG AAAGGTCGAC ACGTTAAAAC CCATCCTATA TAAGATAAAT TCAGATTCTA ATGACTTTTA TTTGGTGGCT AATTATGATT GGATTCCTAC

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                                  4310       4320       4330       4340       4350       4360       4370       4380       4390       4400
SA11-N5_GS2(VP2) reference      ATCTACTACA AAAGTGTATA AACAAGTTCC ACAACAATTT GATTTTAGAG CGTCAATGCA TATG-TTAAC GTCTAACCTA ACATTTACCG TATATTCAGA
pSmart_SA11-N5_GS2 reference    ATCTACTACA AAAGTGTATA AACAAGTTCC ACAACAATTT GATTTTAGAG CGTCAATGCA TATGGTTAAC GTCTAACCTA ACATTTACCG TATATTCAGA
pSmart_SA11-N5_GS2 NGS          ATCTACTACA AAAGTGTATA AACAAGTTCC ACAACAATTT GATTTTAGAG CGTCAATGCA TATGGTTAAC GTCTAACCTA ACATTTACCG TATATTCAGA

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                                  4410       4420       4430       4440       4450       4460       4470       4480       4490       4500
SA11-N5_GS2(VP2) reference      TTTGCTTGCG TTCGTTTCAG CTGATACTGT TGAACCAATT AATGCTGTTG CTTTTGATAA TATGCGCATC ATGAACGAAC TGTAAACGCC AACCCCATTG
pSmart_SA11-N5_GS2 reference    TTTGCTTGCG TTCGTTTCAG CTGATACTGT TGAACCAATT AATGCTGTTG CTTTTGATAA TATGCGCATC ATGAACGAAC TGTAAACGCC AACCCCATTG
pSmart_SA11-N5_GS2 NGS          TTTGCTTGCG TTCGTTTCAG CTGATACTGT TGAACCAATT AATGCTGTTG CTTTTGATAA TATGCGCATC ATGAACGAAC TGTAAACGCC AACCCCATTG

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                                  4510       4520       4530       4540       4550       4560       4570       4580       4590       4600
SA11-N5_GS2(VP2) reference      TGGAGATATG ACC------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    TGGAGATATG ACCGGCCGGC ATGGTCCCAG CCTCCTCGCT GGCGCCGGCT GGGCAACATT CCGAGGGGAC CGTCCCCTCG GTAATGGCGA ATGGGACGGA
pSmart_SA11-N5_GS2 NGS          TGGAGATATG ACCGGCCGGC ATGGTCCCAG CCTCCTCGCT GGCGCCGGCT GGGCAACATT CCGAGGGGAC CGTCCCCTCG GTAATGGCGA ATGGGACGGA

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                                  4610       4620       4630       4640       4650       4660       4670       4680       4690       4700
SA11-N5_GS2(VP2) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS2 reference    TCCGGCTGCT AACAAAGCCC GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA ACTAGCATAA CCCCTTGGGG CCTCTAAACG GGTCTTGAGG
pSmart_SA11-N5_GS2 NGS          TCCGGCTGCT AACAAAGCCC GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA ACTAGCATAA CCCCTTGGGG CCTCTAAACG GGTCTTGAGG
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                                      4710
SA11-N5_GS2(VP2) reference    ---------- --
pSmart_SA11-N5_GS2 reference  GGTTTTTTGG AT
pSmart_SA11-N5_GS2 NGS        GGTTTTTTGG AT
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Alignment: SA11-N5_GS3(VP3)

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                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       110        120        130        140        150        160        170        180        190        200
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       210        220        230        240        250        260        270        280        290        300
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       310        320        330        340        350        360        370        380        390        400
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       410        420        430        440        450        460        470        480        490        500
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       510        520        530        540        550        560        570        580        590        600
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       610        620        630        640        650        660        670        680        690        700
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       710        720        730        740        750        760        770        780        790        800
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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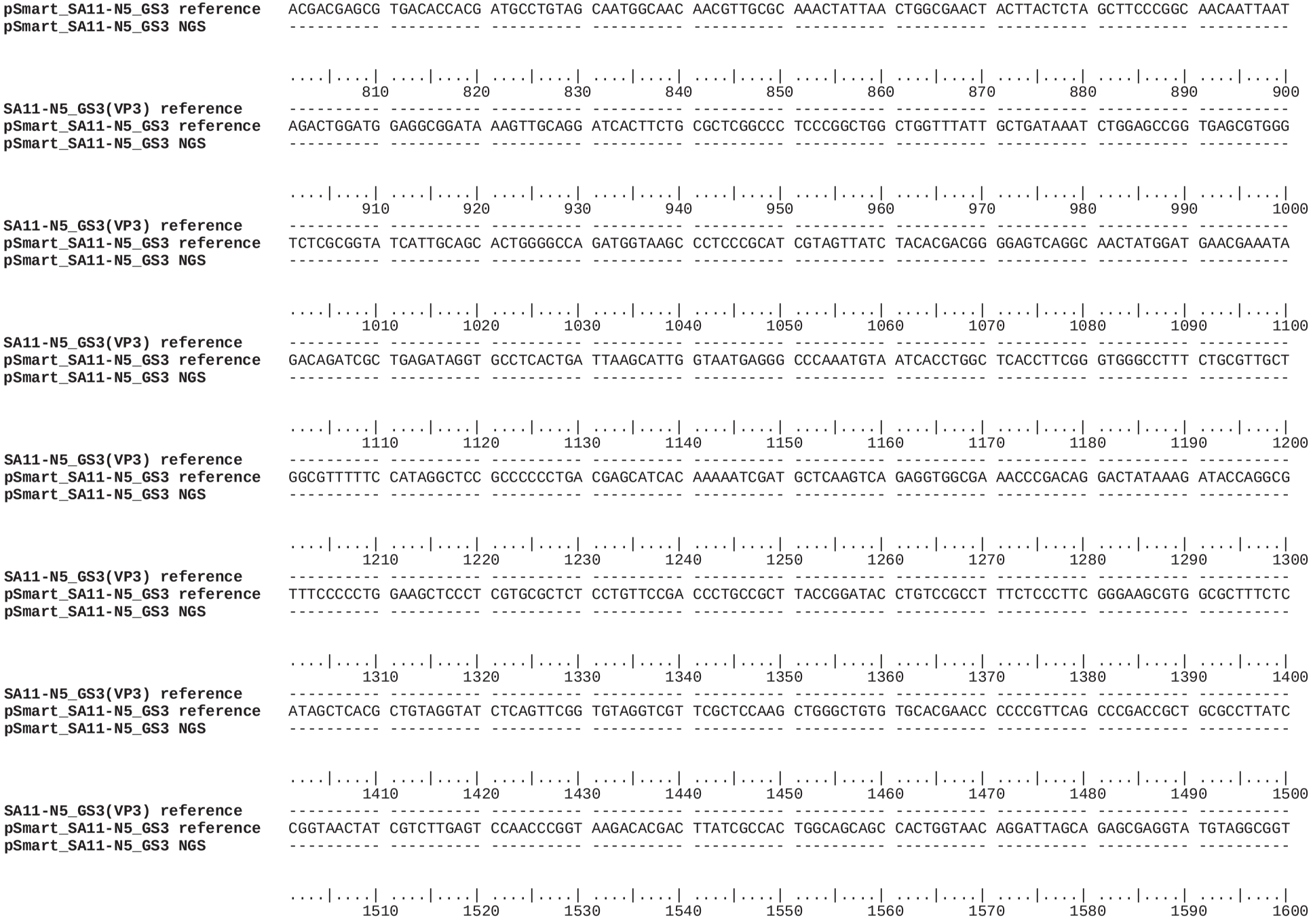
pSmart_SA11-N5_GS3 reference ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS3 NGS
810 820 830 840 850 860 870 880 890 900
SA11-N5_GS3(VP3) reference
pSmart_SA11-N5_GS3 reference AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS3 NGS
910 920 930 940 950 960 970 980 990 1000
SA11-N5_GS3(VP3) reference
pSmart_SA11-N5_GS3 reference TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS3 NGS
1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
SA11-N5_GS3(VP3) reference
pSmart_SA11-N5_GS3 reference GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS3 NGS
1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
SA11-N5_GS3(VP3) reference
pSmart_SA11-N5_GS3 reference GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS3 NGS
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
SA11-N5_GS3(VP3) reference
pSmart_SA11-N5_GS3 reference TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS3 NGS
1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
SA11-N5_GS3(VP3) reference
pSmart_SA11-N5_GS3 reference ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS3 NGS
1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
SA11-N5_GS3(VP3) reference
pSmart_SA11-N5_GS3 reference CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS3 NGS
1510 1520 1530 1540 1550 1560 1570 1580 1590 1600

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SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS3(VP3) reference      ---------- ---------G GCTATTAAAG CAGTACCAGT AGTGTGTTTT ACCTCTGATG GTGTAAACAT GAAAGTACTA GCTTTAAGAC ACAGTGTGGC
pSmart_SA11-N5_GS3 reference    TCTAATACGA CTCACTATAG GCTATTAAAG CAGTACCAGT AGTGTGTTTT ACCTCTGATG GTGTAAACAT GAAAGTACTA GCTTTAAGAC ACAGTGTGGC
pSmart_SA11-N5_GS3 NGS          ---------- ---------G GCTATTAAAG CAGTACCAGT AGTGTGTTTT ACCTCTGATG GTGTAAACAT GAAAGTACTA GCTTTAAGAC ACAGTGTGGC

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                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS3(VP3) reference      TCAAGTGTAT GCAGACACTC AAGTCTACGT TCATGATGAT ACAAAAGATA GTTATGAAAA CGCTTTTTTA ATCTCTAATC TTACGACCCA TAATATTTTA
pSmart_SA11-N5_GS3 reference    TCAAGTGTAT GCAGACACTC AAGTCTACGT TCATGATGAT ACAAAAGATA GTTATGAAAA CGCTTTTTTA ATCTCTAATC TTACGACCCA TAATATTTTA
pSmart_SA11-N5_GS3 NGS          TCAAGTGTAT GCAGACACTC AAGTCTACGT TCATGATGAT ACAAAAGATA GTTATGAAAA CGCTTTTTTA ATCTCTAATC TTACGACCCA TAATATTTTA

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                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS3(VP3) reference      TACTTAAATT ATAGCATTAA AACATTAGAA ATATTAAATA AGTCAGGAAT AGCTGCAATT GCTTTACAAT CACTTGAAGA ATTATTCACA TTAATAAGGT
pSmart_SA11-N5_GS3 reference    TACTTAAATT ATAGCATTAA AACATTAGAA ATATTAAATA AGTCAGGAAT AGCTGCAATT GCTTTACAAT CACTTGAAGA ATTATTCACA TTAATAAGGT
pSmart_SA11-N5_GS3 NGS          TACTTAAATT ATAGCATTAA AACATTAGAA ATATTAAATA AGTCAGGAAT AGCTGCAATT GCTTTACAAT CACTTGAAGA ATTATTCACA TTAATAAGGT

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                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS3(VP3) reference      GTAATTTCAC TTATGATTAT GAACTTGATA TAATATATTT ACATGATTAT TCATATTATA CCAATAATGA AATTAGAACA GACCAACATT GGATAACAAA
pSmart_SA11-N5_GS3 reference    GTAATTTCAC TTATGATTAT GAACTTGATA TAATATATTT ACATGATTAT TCATATTATA CCAATAATGA AATTAGAACA GACCAACATT GGATAACAAA
pSmart_SA11-N5_GS3 NGS          GTAATTTCAC TTATGATTAT GAACTTGATA TAATATATTT ACATGATTAT TCATATTATA CCAATAATGA AATTAGAACA GACCAACATT GGATAACAAA

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                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS3(VP3) reference      AACAAATATT GAAGAATATT TACTACCTGG ATGGAAATTA ACATATGTTG GTTATAATGG AAGTGAAACT AGAGGACATT ATAACTTTTC ATTTAAATGT
pSmart_SA11-N5_GS3 reference    AACAAATATT GAAGAATATT TACTACCTGG ATGGAAATTA ACATATGTTG GTTATAATGG AAGTGAAACT AGAGGACATT ATAACTTTTC ATTTAAATGT
pSmart_SA11-N5_GS3 NGS          AACAAATATT GAAGAATATT TACTACCTGG ATGGAAATTA ACATATGTTG GTTATAATGG AAGTGAAACT AGAGGACATT ATAACTTTTC ATTTAAATGT

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS3(VP3) reference      CAAAACGCTG CAACAGATGA TGATCTAATA ATTGAATACA TTTATTCAGA AGCGTTGGAC TTCCAAAATT TTATGTTAAA AAAGATAAAG GAAAGAATGA
pSmart_SA11-N5_GS3 reference    CAAAACGCTG CAACAGATGA TGATCTAATA ATTGAATACA TTTATTCAGA AGCGTTGGAC TTCCAAAATT TTATGTTAAA AAAGATAAAG GAAAGAATGA
pSmart_SA11-N5_GS3 NGS          CAAAACGCTG CAACAGATGA TGATCTAATA ATTGAATACA TTTATTCAGA AGCGTTGGAC TTCCAAAATT TTATGTTAAA AAAGATAAAG GAAAGAATGA

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS3(VP3) reference      CTACATCGTT GCCTATAGCT AGATTATCTA ACAGAGTATT TAGGGATAAG TTATTCCCAT CATTATTGAA AGAACATAAG AATGTAGTGA ACGTTGGTCC
pSmart_SA11-N5_GS3 reference    CTACATCGTT GCCTATAGCT AGATTATCTA ACAGAGTATT TAGGGATAAG TTATTCCCAT CATTATTGAA AGAACATAAG AATGTAGTGA ACGTTGGTCC
pSmart_SA11-N5_GS3 NGS          CTACATCGTT GCCTATAGCT AGATTATCTA ACAGAGTATT TAGGGATAAG TTATTCCCAT CATTATTGAA AGAACATAAG AATGTAGTGA ACGTTGGTCC

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS3(VP3) reference      GCGTAATGAA TCTATGTTTA CATTTTTAAA TTATCCAACT ATAAAACAAT TTTCAAATGG TGCGTATTTA GTAAAAGATA CTATAAAATT AAAACAAGAA
pSmart_SA11-N5_GS3 reference    GCGTAATGAA TCTATGTTTA CATTTTTAAA TTATCCAACT ATAAAACAAT TTTCAAATGG TGCGTATTTA GTAAAAGATA CTATAAAATT AAAACAAGAA
pSmart_SA11-N5_GS3 NGS          GCGTAATGAA TCTATGTTTA CATTTTTAAA TTATCCAACT ATAAAACAAT TTTCAAATGG TGCGTATTTA GTAAAAGATA CTATAAAATT AAAACAAGAA

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS3(VP3) reference      CGATGGTTAG GTAAAAGGAT ATCTCAGTTT GATATTGGTC AGTATAAAAA TATGCTGAAT GTTCTTACAG CAATTTATTA TTACTATAAT TTATATAAAA
pSmart_SA11-N5_GS3 reference    CGATGGTTAG GTAAAAGGAT ATCTCAGTTT GATATTGGTC AGTATAAAAA TATGCTGAAT GTTCTTACAG CAATTTATTA TTACTATAAT TTATATAAAA
pSmart_SA11-N5_GS3 NGS          CGATGGTTAG GTAAAAGGAT ATCTCAGTTT GATATTGGTC AGTATAAAAA TATGCTGAAT GTTCTTACAG CAATTTATTA TTACTATAAT TTATATAAAA

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                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS3(VP3) reference      GTAAACCAAT TATATATATG ATCGGATCTG CTCCATCTTA TTGGATATAT GACGTTAGGC ATTATTCCGA TTTTTTCTTT GAAACTTGGG ATCCATTGGA
pSmart_SA11-N5_GS3 reference    GTAAACCAAT TATATATATG ATCGGATCTG CTCCATCTTA TTGGATATAT GACGTTAGGC ATTATTCCGA TTTTTTCTTT GAAACTTGGG ATCCATTGGA
pSmart_SA11-N5_GS3 NGS          GTAAACCAAT TATATATATG ATCGGATCTG CTCCATCTTA TTGGATATAT GACGTTAGGC ATTATTCCGA TTTTTTCTTT GAAACTTGGG ATCCATTGGA

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                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS3(VP3) reference      CACACCATAT TCATCAATCC ATCACAAAGA ATTATTTTTT ATAAATGATG TGAAGAAACT GAAGGATAAC TCAATATTGT ATATTGATAT AAGAACCGAT
pSmart_SA11-N5_GS3 reference    CACACCATAT TCATCAATCC ATCACAAAGA ATTATTTTTT ATAAATGATG TGAAGAAACT GAAGGATAAC TCAATATTGT ATATTGATAT AAGAACCGAT
pSmart_SA11-N5_GS3 NGS          CACACCATAT TCATCAATCC ATCACAAAGA ATTATTTTTT ATAAATGATG TGAAGAAACT GAAGGATAAC TCAATATTGT ATATTGATAT AAGAACCGAT

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                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS3(VP3) reference      AGGGGCAATG CTGATTGGAA AAAATGGAGA AAGACAGTAG AAGAACAAAC TATTAATAAT TTGGACATAG CTTATGAATA TTTACGAACG GGTAAAGCGA
pSmart_SA11-N5_GS3 reference    AGGGGCAATG CTGATTGGAA AAAATGGAGA AAGACAGTAG AAGAACAAAC TATTAATAAT TTGGACATAG CTTATGAATA TTTACGAACG GGTAAAGCGA
pSmart_SA11-N5_GS3 NGS          AGGGGCAATG CTGATTGGAA AAAATGGAGA AAGACAGTAG AAGAACAAAC TATTAATAAT TTGGACATAG CTTATGAATA TTTACGAACG GGTAAAGCGA

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                                3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-N5_GS3(VP3) reference      AGGTGTGTTG TGTTAAGATG ACAGCTATGG ATTTGGAACT GCCAATTTCA GCTAAATTAC TGCACCACCC AACTACGGAA ATAAGATCAG AATTTTATTT
pSmart_SA11-N5_GS3 reference    AGGTGTGTTG TGTTAAGATG ACAGCTATGG ATTTGGAACT GCCAATTTCA GCTAAATTAC TGCACCACCC AACTACGGAA ATAAGATCAG AATTTTATTT
pSmart_SA11-N5_GS3 NGS          AGGTGTGTTG TGTTAAGATG ACAGCTATGG ATTTGGAACT GCCAATTTCA GCTAAATTAC TGCACCACCC AACTACGGAA ATAAGATCAG AATTTTATTT
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                                  3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
SA11-N5_GS3(VP3) reference      ATTACTAGAT ACTTGGGATT TAACTAACAT TAGGAGGTTC ATTCCTAAAG GCGTGTTATA TTCATTTATA AACAATATAA TAACTGAAAA TGTGTTTATT
pSmart_SA11-N5_GS3 reference    ATTACTAGAT ACTTGGGATT TAACTAACAT TAGGAGGTTC ATTCCTAAAG GCGTGTTATA TTCATTTATA AACAATATAA TAACTGAAAA TGTGTTTATT
pSmart_SA11-N5_GS3 NGS          ATTACTAGAT ACTTGGGATT TAACTAACAT TAGGAGGTTC ATTCCTAAAG GCGTGTTATA TTCATTTATA AACAATATAA TAACTGAAAA TGTGTTTATT

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                                  3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
SA11-N5_GS3(VP3) reference      CAACAACCAT TTAAAGTAAA AGTACTGAAT GATAGTTATA TTGTAGCGTT ATATGCATTA TCAAATGATT TTAATAATAG ATCAGAAGTA ATTAAATTAA
pSmart_SA11-N5_GS3 reference    CAACAACCAT TTAAAGTAAA AGTACTGAAT GATAGTTATA TTGTAGCGTT ATATGCATTA TCAAATGATT TTAATAATAG ATCAGAAGTA ATTAAATTAA
pSmart_SA11-N5_GS3 NGS          CAACAACCAT TTAAAGTAAA AGTACTGAAT GATAGTTATA TTGTAGCGTT ATATGCATTA TCAAATGATT TTAATAATAG ATCAGAAGTA ATTAAATTAA

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                                  3310       3320       3330       3340       3350       3360       3370       3380       3390       3400
SA11-N5_GS3(VP3) reference      TTAATAATCA GAAACAATCT CTAATAACTG TTAGAATAAA TAATACGTTT AAGGATGAAC CAAAAGTTGG GTTCAAAAAT ATCTATGATT GGACCTTTCT
pSmart_SA11-N5_GS3 reference    TTAATAATCA GAAACAATCT CTAATAACTG TTAGAATAAA TAATACGTTT AAGGATGAAC CAAAAGTTGG GTTCAAAAAT ATCTATGATT GGACCTTTCT
pSmart_SA11-N5_GS3 NGS          TTAATAATCA GAAACAATCT CTAATAACTG TTAGAATAAA TAATACGTTT AAGGATGAAC CAAAAGTTGG GTTCAAAAAT ATCTATGATT GGACCTTTCT

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                                  3410       3420       3430       3440       3450       3460       3470       3480       3490       3500
SA11-N5_GS3(VP3) reference      TCCAACCGAC TTTGATACCA AAGAAGCTAT AATTACTTCA TACGACGGTT GTTTAGGACT CTTTGGTTTG TCTATATCGT TAGCATCAAA ACCAACAGGG
pSmart_SA11-N5_GS3 reference    TCCAACCGAC TTTGATACCA AAGAAGCTAT AATTACTTCA TACGACGGTT GTTTAGGACT CTTTGGTTTG TCTATATCGT TAGCATCAAA ACCAACAGGG
pSmart_SA11-N5_GS3 NGS          TCCAACCGAC TTTGATACCA AAGAAGCTAT AATTACTTCA TACGACGGTT GTTTAGGACT CTTTGGTTTG TCTATATCGT TAGCATCAAA ACCAACAGGG

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                                  3510       3520       3530       3540       3550       3560       3570       3580       3590       3600
SA11-N5_GS3(VP3) reference      AATAATCATT TATTCATTTT AAGTGGTACA GATAAGTATT ATAAATTGGA TCAATTTGCT AATCACACCA GTATATCGAG AAGATCACAC CAAATTAGGT
pSmart_SA11-N5_GS3 reference    AATAATCATT TATTCATTTT AAGTGGTACA GATAAGTATT ATAAATTGGA TCAATTTGCT AATCACACCA GTATATCGAG AAGATCACAC CAAATTAGGT
pSmart_SA11-N5_GS3 NGS          AATAATCATT TATTCATTTT AAGTGGTACA GATAAGTATT ATAAATTGGA TCAATTTGCT AATCACACCA GTATATCGAG AAGATCACAC CAAATTAGGT

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                                  3610       3620       3630       3640       3650       3660       3670       3680       3690       3700
SA11-N5_GS3(VP3) reference      TTTCGGAATC TGCTACTTCA TATTCAGGTT ATATATTTAG AGATTTGTCC AATAATAATT TTAATCTAAT TGGTACTAAT ATAGAGAATT CAGTATCAGG
pSmart_SA11-N5_GS3 reference    TTTCGGAATC TGCTACTTCA TATTCAGGTT ATATATTTAG AGATTTGTCC AATAATAATT TTAATCTAAT TGGTACTAAT ATAGAGAATT CAGTATCAGG
pSmart_SA11-N5_GS3 NGS          TTTCGGAATC TGCTACTTCA TATTCAGGTT ATATATTTAG AGATTTGTCC AATAATAATT TTAATCTAAT TGGTACTAAT ATAGAGAATT CAGTATCAGG

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                                  3710       3720       3730       3740       3750       3760       3770       3780       3790       3800
SA11-N5_GS3(VP3) reference      TCATGTATAT AATGCTTTAA TTTATTATAG ATATAATTAT TCATTTGATC TTAAACGCTG GATTTATTTA CATTCTATAG ATAAAGTTGA TATAGAAGGA
pSmart_SA11-N5_GS3 reference    TCATGTATAT AATGCTTTAA TTTATTATAG ATATAATTAT TCATTTGATC TTAAACGCTG GATTTATTTA CATTCTATAG ATAAAGTTGA TATAGAAGGA
pSmart_SA11-N5_GS3 NGS          TCATGTATAT AATGCTTTAA TTTATTATAG ATATAATTAT TCATTTGATC TTAAACGCTG GATTTATTTA CATTCTATAG ATAAAGTTGA TATAGAAGGA

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                                  3810       3820       3830       3840       3850       3860       3870       3880       3890       3900
SA11-N5_GS3(VP3) reference      GGAAAGTATT ATGAACACGC ACCAATAGAA TTAATTTATG CATGTAGATC AGCAAAAGAA TTTGCTACAT TGCAGGATGA CTTAACTGTA TTGAGATATT
pSmart_SA11-N5_GS3 reference    GGAAAGTATT ATGAACACGC ACCAATAGAA TTAATTTATG CATGTAGATC AGCAAAAGAA TTTGCTACAT TGCAGGATGA CTTAACTGTA TTGAGATATT
pSmart_SA11-N5_GS3 NGS          GGAAAGTATT ATGAACACGC ACCAATAGAA TTAATTTATG CATGTAGATC AGCAAAAGAA TTTGCTACAT TGCAGGATGA CTTAACTGTA TTGAGATATT
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                                  3910       3920       3930       3940       3950       3960       3970       3980       3990       4000
SA11-N5_GS3(VP3) reference      CAAACGAAAT AGAGAATTAT ATTAATACAG TATATAGTAT AACATACGCT GATGATCCGA ATTACTTTAT CGGAATACAA TTTAGAAATA TACCATATAA
pSmart_SA11-N5_GS3 reference    CAAACGAAAT AGAGAATTAT ATTAATACAG TATATAGTAT AACATACGCT GATGATCCGA ATTACTTTAT CGGAATACAA TTTAGAAATA TACCATATAA
pSmart_SA11-N5_GS3 NGS          CAAACGAAAT AGAGAATTAT ATTAATACAG TATATAGTAT AACATACGCT GATGATCCGA ATTACTTTAT CGGAATACAA TTTAGAAATA TACCATATAA


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                                  4010       4020       4030       4040       4050       4060       4070       4080       4090       4100
SA11-N5_GS3(VP3) reference      ATATGATGTT AAAATACCGC ATTTAACCTT CGGAGTATTA CATATTTCTG ATAACATGGT GCCAGACGTG ATTGACATAC TAAAGATAAT GAAGAATGAA
pSmart_SA11-N5_GS3 reference    ATATGATGTT AAAATACCGC ATTTAACCTT CGGAGTATTA CATATTTCTG ATAACATGGT GCCAGACGTG ATTGACATAC TAAAGATAAT GAAGAATGAA
pSmart_SA11-N5_GS3 NGS          ATATGATGTT AAAATACCGC ATTTAACCTT CGGAGTATTA CATATTTCTG ATAACATGGT GCCAGACGTG ATTGACATAC TAAAGATAAT GAAGAATGAA


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                                  4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
SA11-N5_GS3(VP3) reference      TTATTTAAAA TGGATATTAC GACCAGTTAT ACATATATGT TATCAGATGG AATCTACGTA GCAAATGTTA GTGGAGTATT ATCTACATAC TTTAAAATCT
pSmart_SA11-N5_GS3 reference    TTATTTAAAA TGGATATTAC GACCAGTTAT ACATATATGT TATCAGATGG AATCTACGTA GCAAATGTTA GTGGAGTATT ATCTACATAC TTTAAAATCT
pSmart_SA11-N5_GS3 NGS          TTATTTAAAA TGGATATTAC GACCAGTTAT ACATATATGT TATCAGATGG AATCTACGTA GCAAATGTTA GTGGAGTATT ATCTACATAC TTTAAAATCT


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                                  4210       4220       4230       4240       4250       4260       4270       4280       4290       4300
SA11-N5_GS3(VP3) reference      ATAACGTATT TTATAAAAAT CAAATAACTT TTGGCCAATC CAGAATGTTT ATTCCGCACA TAACATTAAG CTTCAATAAC ATGAGAACAG TAAGGATAGA
pSmart_SA11-N5_GS3 reference    ATAACGTATT TTATAAAAAT CAAATAACTT TTGGCCAATC CAGAATGTTT ATTCCGCACA TAACATTAAG CTTCAATAAC ATGAGAACAG TAAGGATAGA
pSmart_SA11-N5_GS3 NGS          ATAACGTATT TTATAAAAAT CAAATAACTT TTGGCCAATC CAGAATGTTT ATTCCGCACA TAACATTAAG CTTCAATAAC ATGAGAACAG TAAGGATAGA


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                                  4310       4320       4330       4340       4350       4360       4370       4380       4390       4400
SA11-N5_GS3(VP3) reference      GACTACTAAA TTACAAATTA AATCCATTTA TTTAAGAAAG ATTAAGGGTG ATACAGTGTT TGATATGGTT GAGTGAGCTA AAAACTTAAC ACACTAGTCA
pSmart_SA11-N5_GS3 reference    GACTACTAAA TTACAAATTA AATCCATTTA TTTAAGAAAG ATTAAGGGTG ATACAGTGTT TGATATGGTT GAGTGAGCTA AAAACTTAAC ACACTAGTCA
pSmart_SA11-N5_GS3 NGS          GACTACTAAA TTACAAATTA AATCCATTTA TTTAAGAAAG ATTAAGGGTG ATACAGTGTT TGATATGGTT GAGTGAGCTA AAAACTTAAC ACACTAGTCA


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                                  4410       4420       4430       4440       4450       4460       4470       4480       4490       4500
SA11-N5_GS3(VP3) reference      TGATGTGACC ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    TGATGTGACC GGCCGGCATG GTCCCAGCCT CCTCGCTGGC GCCGGCTGGG CAACATTCCG AGGGGACCGT CCCCTCGGTA ATGGCGAATG GGACGGATCC
pSmart_SA11-N5_GS3 NGS          TGATGTGACC ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------


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                                  4510       4520       4530       4540       4550       4560       4570       4580       4590       4600
SA11-N5_GS3(VP3) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS3 reference    GGCTGCTAAC AAAGCCCGAA AGGAAGCTGA GTTGGCTGCT GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT CTAAACGGGT CTTGAGGGGT
pSmart_SA11-N5_GS3 NGS          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------


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SA11-N5_GS3(VP3) reference      ---------
pSmart_SA11-N5_GS3 reference    TTTTTGGAT
pSmart_SA11-N5_GS3 NGS          ---------
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Alignment: SA11-N5_GS4(VP4)

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS4 NGS          ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

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                                       110        120        130        140        150        160        170        180        190        200
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS4 NGS          CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

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                                       210        220        230        240        250        260        270        280        290        300
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS4 NGS          CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

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                                       310        320        330        340        350        360        370        380        390        400
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS4 NGS          GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       410        420        430        440        450        460        470        480        490        500
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS4 NGS          TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

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                                       510        520        530        540        550        560        570        580        590        600
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS4 NGS          CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

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                                       610        620        630        640        650        660        670        680        690        700
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS4 NGS          ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       710        720        730        740        750        760        770        780        790        800
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS4 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS4 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

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                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS4(VP4) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS4 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

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                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS4(VP4) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS4 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

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                                       1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS4(VP4) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS4 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

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                                       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS4(VP4) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS4 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

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                                       1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS4(VP4) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS4 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

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                                       1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS4(VP4) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS4 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

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                                       1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS4(VP4) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS4 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

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                                       1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS4 NGS          GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

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                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS4 NGS          TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

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                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS4 NGS          CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS4(VP4) reference      ---------- ---------G GCTATAAAAT GGCTTCGCTC ATTTATAGAC AATTGCTCAC GAATTCTTAT ACAGTAGATT TATCCGATGA GATACAAGAG
pSmart_SA11-N5_GS4 reference    TCTAATACGA CTCACTATAG GCTATAAAAT GGCTTCGCTC ATTTATAGAC AATTGCTCAC GAATTCTTAT ACAGTAGATT TATCCGATGA GATACAAGAG
pSmart_SA11-N5_GS4 NGS          TCTAATACGA CTCACTATAG GCTATAAAAT GGCTTCGCTC ATTTATAGAC AATTGCTCAC GAATTCTTAT ACAGTAGATT TATCCGATGA GATACAAGAG

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                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS4(VP4) reference      ATTGGATCAA CTAAATCACA AAATGTCACA ATTAATCCTG GACCATTTGC GCAAACAGGT TATGCTCCAG TTAACTGGGG ACCTGGAGAA ATTAATGATT
pSmart_SA11-N5_GS4 reference    ATTGGATCAA CTAAATCACA AAATGTCACA ATTAATCCTG GACCATTTGC GCAAACAGGT TATGCTCCAG TTAACTGGGG ACCTGGAGAA ATTAATGATT
pSmart_SA11-N5_GS4 NGS          ATTGGATCAA CTAAATCACA AAATGTCACA ATTAATCCTG GACCATTTGC GCAAACAGGT TATGCTCCAG TTAACTGGGG ACCTGGAGAA ATTAATGATT

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                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS4(VP4) reference      CTACGACAGT TGAACCATTG CTGGATGGGC CTTATCAACC AATGACATTC AATCCACCAG TCGATTATTG GATGTTACTG GCTCCAACGA CACCTGGCGT
pSmart_SA11-N5_GS4 reference    CTACGACAGT TGAACCATTG CTGGATGGGC CTTATCAACC AATGACATTC AATCCACCAG TCGATTATTG GATGTTACTG GCTCCAACGA CACCTGGCGT
pSmart_SA11-N5_GS4 NGS          CTACGACAGT TGAACCATTG CTGGATGGGC CTTATCAACC AATGACATTC AATCCACCAG TCGATTATTG GATGTTACTG GCTCCAACGA CACCTGGCGT

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                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS4(VP4) reference      AATTGTTGAA GGTACAAATA ATACAGATAG ATGGTTGGCC ACAATTTTAA TCGAGCCAAA TGTTCAGTCT GAAAATAGAA CTTACACTAT ATTTGGTATT
pSmart_SA11-N5_GS4 reference    AATTGTTGAA GGTACAAATA ATACAGATAG ATGGTTGGCC ACAATTTTAA TCGAGCCAAA TGTTCAGTCT GAAAATAGAA CTTACACTAT ATTTGGTATT
pSmart_SA11-N5_GS4 NGS          AATTGTTGAA GGTACAAATA ATACAGATAG ATGGTTGGCC ACAATTTTAA TCGAGCCAAA TGTTCAGTCT GAAAATAGAA CTTACACTAT ATTTGGTATT

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                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS4(VP4) reference      CAAGAACAAT TAACGGTATC CAATACTTCA CAAGACCAGT GGAAATTTAT TGATGTCGTA AAAACAACTG CAAATGGAAG TATAGGACAA TATGGATCAT
pSmart_SA11-N5_GS4 reference    CAAGAACAAT TAACGGTATC CAATACTTCA CAAGACCAGT GGAAATTTAT TGATGTCGTA AAAACAACTG CAAATGGAAG TATAGGACAA TATGGATCAT
pSmart_SA11-N5_GS4 NGS          CAAGAACAAT TAACGGTATC CAATACTTCA CAAGACCAGT GGAAATTTAT TGATGTCGTA AAAACAACTG CAAATGGAAG TATAGGACAA TATGGATCAT

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS4(VP4) reference      TACTATCCAG TCCGAAATTA TATGCAGTTA TGAAGCATAA TGAAAAATTA TATACATATG AAGGACAGAC ACCTAACGCT AGGACAGGAC ATTATTCAAC
pSmart_SA11-N5_GS4 reference    TACTATCCAG TCCGAAATTA TATGCAGTTA TGAAGCATAA TGAAAAATTA TATACATATG AAGGACAGAC ACCTAACGCT AGGACAGGAC ATTATTCAAC
pSmart_SA11-N5_GS4 NGS          TACTATCCAG TCCGAAATTA TATGCAGTTA TGAAGCATAA TGAAAAATTA TATACATATG AAGGACAGAC ACCTAACGCT AGGACAGGAC ATTATTCAAC

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS4(VP4) reference      AACGAATTAT GATTCTGTTA ACATGACTGC TTTTTGTGAC TTTTATATAA TTCCTAGATC TGAAGAGTCT AAATGTACGG AATACATTAA TAATGGATTA
pSmart_SA11-N5_GS4 reference    AACGAATTAT GATTCTGTTA ACATGACTGC TTTTTGTGAC TTTTATATAA TTCCTAGATC TGAAGAGTCT AAATGTACGG AATACATTAA TAATGGATTA
pSmart_SA11-N5_GS4 NGS          AACGAATTAT GATTCTGTTA ACATGACTGC TTTTTGTGAC TTTTATATAA TTCCTAGATC TGAAGAGTCT AAATGTACGG AATACATTAA TAATGGATTA

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS4(VP4) reference      CCACCAATAC AAAATACTAG AAATGTTGTA CCATTATCGT TGACTGCTAG AGATGTAATA CACTATAGAG CTCAAGCTAA TGAAGATATT GTGATATCCA
pSmart_SA11-N5_GS4 reference    CCACCAATAC AAAATACTAG AAATGTTGTA CCATTATCGT TGACTGCTAG AGATGTAATA CACTATAGAG CTCAAGCTAA TGAAGATATT GTGATATCCA
pSmart_SA11-N5_GS4 NGS          CCACCAATAC AAAATACTAG AAATGTTGTA CCATTATCGT TGACTGCTAG AGATGTAATA CACTATAGAG CTCAAGCTAA TGAAGATATT GTGATATCCA

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS4(VP4) reference      AGACATCATT ATGGAAAGAA ATGCAATATA ATAGAGATAT AACTATTAGA TTTAAATTTG CAAATACAAT TATAAAATCA GGAGGGCTGG GATATAAGTG
pSmart_SA11-N5_GS4 reference    AGACATCATT ATGGAAAGAA ATGCAATATA ATAGAGATAT AACTATTAGA TTTAAATTTG CAAATACAAT TATAAAATCA GGAGGGCTGG GATATAAGTG
pSmart_SA11-N5_GS4 NGS          AGACATCATT ATGGAAAGAA ATGCAATATA ATAGAGATAT AACTATTAGA TTTAAATTTG CAAATACAAT TATAAAATCA GGAGGGCTGG GATATAAGTG

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                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS4(VP4) reference      GTCAGAAATA TCATTTAAGC CAGCGAATTA TCAATACACA TATACTCGTG ATGGTGAAGA AGTTACCGCA CATACTACTT GTTCAGTGAA TGGCGTTAAT
pSmart_SA11-N5_GS4 reference    GTCAGAAATA TCATTTAAGC CAGCGAATTA TCAATACACA TATACTCGTG ATGGTGAAGA AGTTACCGCA CATACTACTT GTTCAGTGAA TGGCGTTAAT
pSmart_SA11-N5_GS4 NGS          GTCAGAAATA TCATTTAAGC CAGCGAATTA TCAATACACA TATACTCGTG ATGGTGAAGA AGTTACCGCA CATACTACTT GTTCAGTGAA TGGCGTTAAT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS4(VP4) reference      GACTTCAGTT TTAATGGAGG ATCTTTACCA ACTGATTTTG TTGTATCTAA ATTTGAAGTA ATTAAAGAGA ATTCATACGT CTATATCGAT TACTGGGATG
pSmart_SA11-N5_GS4 reference    GACTTCAGTT TTAATGGAGG ATCTTTACCA ACTGATTTTG TTGTATCTAA ATTTGAAGTA ATTAAAGAGA ATTCATACGT CTATATCGAT TACTGGGATG
pSmart_SA11-N5_GS4 NGS          GACTTCAGTT TTAATGGAGG ATCTTTACCA ACTGATTTTG TTGTATCTAA ATTTGAAGTA ATTAAAGAGA ATTCATACGT CTATATCGAT TACTGGGATG

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                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS4(VP4) reference      ATTCACAAGC ATTTCGTAAC GTGATGTATG TCCGATCGTT AGCAGCAAAC TTGAATTCAG TTATGTGTAC TGGAGGCAGC TATAATTTTA GTCTACCAGT
pSmart_SA11-N5_GS4 reference    ATTCACAAGC ATTTCGTAAC GTGATGTATG TCCGATCGTT AGCAGCAAAC TTGAATTCAG TTATGTGTAC TGGAGGCAGC TATAATTTTA GTCTACCAGT
pSmart_SA11-N5_GS4 NGS          ATTCACAAGC ATTTCGTAAC GTGATGTATG TCCGATCGTT AGCAGCAAAC TTGAATTCAG TTATGTGTAC TGGAGGCAGC TATAATTTTA GTCTACCAGT

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                                3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-N5_GS4(VP4) reference      TGGACAATGG CCTGTTTTAA CTGGGGGAGC AGTTTCTTTA CATTCAGCTG GTGTAACACT ATCTACTCAA TTTACAGATT TCGTATCATT AAATTCATTA
pSmart_SA11-N5_GS4 reference    TGGACAATGG CCTGTTTTAA CTGGGGGAGC AGTTTCTTTA CATTCAGCTG GTGTAACACT ATCTACTCAA TTTACAGATT TCGTATCATT AAATTCATTA
pSmart_SA11-N5_GS4 NGS          TGGACAATGG CCTGTTTTAA CTGGGGGAGC AGTTTCTTTA CATTCAGCTG GTGTAACACT ATCTACTCAA TTTACAGATT TCGTATCATT AAATTCATTA
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                                  3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
SA11-N5_GS4(VP4) reference    AGATTTAGAT TTAGACTAGC TGTCGAAGAA CCACACTTTA AACTGACTAG AACTAGATTA GATAGATTGT ATGGTCTGCC TGCTGCAGAT CCAAATAATG
pSmart_SA11-N5_GS4 reference  AGATTTAGAT TTAGACTAGC TGTCGAAGAA CCACACTTTA AACTGACTAG AACTAGATTA GATAGATTGT ATGGTCTGCC TGCTGCAGAT CCAAATAATG
pSmart_SA11-N5_GS4 NGS        AGATTTAGAT TTAGACTAGC TGTCGAAGAA CCACACTTTA AACTGACTAG AACTAGATTA GATAGATTGT ATGGTCTGCC TGCTGCAGAT CCAAATAATG

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                                  3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
SA11-N5_GS4(VP4) reference    GTAAAGAATA TTATGAAATT GCTGGACGAT TTTCACTTAT ATCATTAGTG CCATCAAATG ATGACTATCA GACTCCTATA GCAAACTCAG TTACTGTACG
pSmart_SA11-N5_GS4 reference  GTAAAGAATA TTATGAAATT GCTGGACGAT TTTCACTTAT ATCATTAGTG CCATCAAATG ATGACTATCA GACTCCTATA GCAAACTCAG TTACTGTACG
pSmart_SA11-N5_GS4 NGS        GTAAAGAATA TTATGAAATT GCTGGACGAT TTTCACTTAT ATCATTAGTG CCATCAAATG ATGACTATCA GACTCCTATA GCAAACTCAG TTACTGTACG

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                                  3310       3320       3330       3340       3350       3360       3370       3380       3390       3400
SA11-N5_GS4(VP4) reference    ACAAGATTTA GAAAGGCAGT TAGGAGAACT AAGAGAAGAG TTTAACGCTT TGTCTCAAGA AATTGCAATG TCGCAGTTAA TCGATTTAGC GCTTCTACCA
pSmart_SA11-N5_GS4 reference  ACAAGATTTA GAAAGGCAGT TAGGAGAACT AAGAGAAGAG TTTAACGCTT TGTCTCAAGA AATTGCAATG TCGCAGTTAA TCGATTTAGC GCTTCTACCA
pSmart_SA11-N5_GS4 NGS        ACAAGATTTA GAAAGGCAGT TAGGAGAACT AAGAGAAGAG TTTAACGCTT TGTCTCAAGA AATTGCAATG TCGCAGTTAA TCGATTTAGC GCTTCTACCA

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  3410       3420       3430       3440       3450       3460       3470       3480       3490       3500
SA11-N5_GS4(VP4) reference    TTAGATATGT TCTCAATGTT TTCTGGCATT AAAAGTACTA TTGATGCTGC AAAATCAATG GCTACTAATG TTATGAAAAA ATTCAAAAAG TCAGGATTAG
pSmart_SA11-N5_GS4 reference  TTAGATATGT TCTCAATGTT TTCTGGCATT AAAAGTACTA TTGATGCTGC AAAATCAATG GCTACTAATG TTATGAAAAA ATTCAAAAAG TCAGGATTAG
pSmart_SA11-N5_GS4 NGS        TTAGATATGT TCTCAATGTT TTCTGGCATT AAAAGTACTA TTGATGCTGC AAAATCAATG GCTACTAATG TTATGAAAAA ATTCAAAAAG TCAGGATTAG

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                                  3510       3520       3530       3540       3550       3560       3570       3580       3590       3600
SA11-N5_GS4(VP4) reference    CGAATTCAGT TTCAACACTG ACAGATTCTT TATCAGACGC AGCATCATCA ATATCAAGAG GTTCATCTAT ACGTTCGATT GGATCTTCAG CATCAGCATG
pSmart_SA11-N5_GS4 reference  CGAATTCAGT TTCAACACTG ACAGATTCTT TATCAGACGC AGCATCATCA ATATCAAGAG GTTCATCTAT ACGTTCGATT GGATCTTCAG CATCAGCATG
pSmart_SA11-N5_GS4 NGS        CGAATTCAGT TTCAACACTG ACAGATTCTT TATCAGACGC AGCATCATCA ATATCAAGAG GTTCATCTAT ACGTTCGATT GGATCTTCAG CATCAGCATG

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                                  3610       3620       3630       3640       3650       3660       3670       3680       3690       3700
SA11-N5_GS4(VP4) reference    GACGGATGTA TCAACACAAA TAACTGATAT ATCGTCATCA GTAAGTTCAG TTTCGACACA AACGTCAACT ATCAGTAGAA GATTGAGACT AAAGGAAATG
pSmart_SA11-N5_GS4 reference  GACGGATGTA TCAACACAAA TAACTGATAT ATCGTCATCA GTAAGTTCAG TTTCGACACA AACGTCAACT ATCAGTAGAA GATTGAGACT AAAGGAAATG
pSmart_SA11-N5_GS4 NGS        GACGGATGTA TCAACACAAA TAACTGATAT ATCGTCATCA GTAAGTTCAG TTTCGACACA AACGTCAACT ATCAGTAGAA GATTGAGACT AAAGGAAATG

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                                  3710       3720       3730       3740       3750       3760       3770       3780       3790       3800
SA11-N5_GS4(VP4) reference    GCAACACAAA CTGAGGGTAT GAATTTTGAT GATATATCAG CGGCTGTTTT GAAGACTAAG ATAGATAAAT CGACTCAAAT ATCACCAAAC ACAATACCTG
pSmart_SA11-N5_GS4 reference  GCAACACAAA CTGAGGGTAT GAATTTTGAT GATATATCAG CGGCTGTTTT GAAGACTAAG ATAGATAAAT CGACTCAAAT ATCACCAAAC ACAATACCTG
pSmart_SA11-N5_GS4 NGS        GCAACACAAA CTGAGGGTAT GAATTTTGAT GATATATCAG CGGCTGTTTT GAAGACTAAG ATAGATAAAT CGACTCAAAT ATCACCAAAC ACAATACCTG

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                                  3810       3820       3830       3840       3850       3860       3870       3880       3890       3900
SA11-N5_GS4(VP4) reference    ACATTGTTAC TGAAGCATCG GAAAAATTCA TACCAAATAG GGCTTACCGC GTTATAAACA ACGATGATGT GTTTGAAGCT GGAATTGATG GAAAATTTTT
pSmart_SA11-N5_GS4 reference  ACATTGTTAC TGAAGCATCG GAAAAATTCA TACCAAATAG GGCTTACCGC GTTATAAACA ACGATGATGT GTTTGAAGCT GGAATTGATG GAAAATTTTT
pSmart_SA11-N5_GS4 NGS        ACATTGTTAC TGAAGCATCG GAAAAATTCA TACCAAATAG GGCTTACCGC GTTATAAACA ACGATGATGT GTTTGAAGCT GGAATTGATG GAAAATTTTT
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                                  3910       3920       3930       3940       3950       3960       3970       3980       3990       4000
SA11-N5_GS4(VP4) reference      TGCTTATAAA GTGGATACAT TTGAGGAAAT ACCATTTGAT GTACAAAAAT TCGCTGACTT AGTTACAGAT TCTCCAGTAA TATCCGCTAT AATTGATTTT
pSmart_SA11-N5_GS4 reference    TGCTTATAAA GTGGATACAT TTGAGGAAAT ACCATTTGAT GTACAAAAAT TCGCTGACTT AGTTACAGAT TCTCCAGTAA TATCCGCTAT AATTGATTTT
pSmart_SA11-N5_GS4 NGS          TGCTTATAAA GTGGATACAT TTGAGGAAAT ACCATTTGAT GTACAAAAAT TCGCTGACTT AGTTACAGAT TCTCCAGTAA TATCCGCTAT AATTGATTTT


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                                  4010       4020       4030       4040       4050       4060       4070       4080       4090       4100
SA11-N5_GS4(VP4) reference      AAAACACTTA AAAATTTGAA CGATAATTAC GGCATTACTA AGCAACAAGC ATTTAATCTT TTAAGATCTG ACCCAAGAGT TTTACGTGAA TTCATTAATC
pSmart_SA11-N5_GS4 reference    AAAACACTTA AAAATTTGAA CGATAATTAC GGCATTACTA AGCAACAAGC ATTTAATCTT TTAAGATCTG ACCCAAGAGT TTTACGTGAA TTCATTAATC
pSmart_SA11-N5_GS4 NGS          AAAACACTTA AAAATTTGAA CGATAATTAC GGCATTACTA AGCAACAAGC ATTTAATCTT TTAAGATCTG ACCCAAGAGT TTTACGTGAA TTCATTAATC


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                                  4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
SA11-N5_GS4(VP4) reference      AGGACAATCC TATAATTAGA AATAGAATTG AACAACTGAT TATGCAATGC AGGTTGTGAG TAATTTCTAG AGGATGTGAC C--------- ----------
pSmart_SA11-N5_GS4 reference    AGGACAATCC TATAATTAGA AATAGAATTG AACAACTGAT TATGCAATGC AGGTTGTGAG TAATTTCTAG AGGATGTGAC CGGCCGGCAT GGTCCCAGCC
pSmart_SA11-N5_GS4 NGS          AGGACAATCC TATAATTAGA AATAGAATTG AACAACTGAT TATGCAATGC AGGTTGTGAG TAATTTCTAG AGGATGTGAC CGGCCGGCAT GGTCCCAGCC


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                                  4210       4220       4230       4240       4250       4260       4270       4280       4290       4300
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    TCCTCGCTGG CGCCGGCTGG GCAACATTCC GAGGGGACCG TCCCCTCGGT AATGGCGAAT GGGACGGATC CGGCTGCTAA CAAAGCCCGA AAGGAAGCTG
pSmart_SA11-N5_GS4 NGS          TCCTCGCTGG CGCCGGCTGG GCAACATTCC GAGGGGACCG TCCCCTCGGT AATGGCGAAT GGGACGGATC CGGCTGCTAA CAAAGCCCGA AAGGAAGCTG


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                                  4310       4320       4330       4340       4350       4360       4370       4380
SA11-N5_GS4(VP4) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS4 reference    AGTTGGCTGC TGCCACCGCT GAGCAATAAC TAGCATAACC CCTTGGGGCC TCTAAACGGG TCTTGAGGGG TTTTTTGGAT
pSmart_SA11-N5_GS4 NGS          AGTTGGCTGC TGCCACCGCT GAGCAATAAC TAGCATAACC CCTTGGGGCC TCTAAACGGG TCTTGAGGGG TTTTTTGGAT
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Alignment: SA11-N5_GS5(NSP1)

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                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference    ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS5 NGS          ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

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                                        110        120        130        140        150        160        170        180        190        200
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference    CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS5 NGS          CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

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                                        210        220        230        240        250        260        270        280        290        300
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference    CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS5 NGS          CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

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                                        310        320        330        340        350        360        370        380        390        400
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference    GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS5 NGS          GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

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                                        410        420        430        440        450        460        470        480        490        500
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference    TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS5 NGS          TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

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                                        510        520        530        540        550        560        570        580        590        600
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference    CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS5 NGS          CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

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                                        610        620        630        640        650        660        670        680        690        700
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference    ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS5 NGS          ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

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                                        710        720        730        740        750        760        770        780        790        800
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS5 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS5 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

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                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS5 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

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                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS5 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

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                                      1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS5 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

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                                      1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS5 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

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                                      1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS5 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

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                                      1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS5 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

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                                      1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS5 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

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                                      1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS5 NGS         GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS5 NGS         TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS5(NSP1) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference   CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS5 NGS         CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCC GAGGCTCGTC CTGAATGATA

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS5(NSP1) reference    ---------- ---------G GCTTTTTTTT GAAAAGTCTT GTGTTAGCCA TGGCTACTTT TAAAGATGCA TGCTTTCATT ATCGTAGATT AACTGCTTTA
pSmart_SA11-N5_GS5 reference   TCTAATACGA CTCACTATAG GCTTTTTTTT GAAAAGTCTT GTGTTAGCCA TGGCTACTTT TAAAGATGCA TGCTTTCATT ATCGTAGATT AACTGCTTTA
pSmart_SA11-N5_GS5 NGS         TCTAATACGA CTCACTATAG GCTTTTTTTT GAAAAGTCTT GTGTTAGCCA TGGCTACTTT TAAAGATGCA TGCTTTCATT ATCGTAGATT AACTGCTTTA

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS5(NSP1) reference    AATCGGAGAT TATGCAACAT TGGTGCAAAT TCTATTTGGA TGCCAGTTCC TGATGCGAAA ATTAAGGGGT GGTGTTTAGA ATGTTGTCAA ATAGCTGATT
pSmart_SA11-N5_GS5 reference   AATCGGAGAT TATGCAACAT TGGTGCAAAT TCTATTTGGA TGCCAGTTCC TGATGCGAAA ATTAAGGGGT GGTGTTTAGA ATGTTGTCAA ATAGCTGATT
pSmart_SA11-N5_GS5 NGS         AATCGGAGAT TATGCAACAT TGGTGCAAAT TCTATTTGGA TGCCAGTTCC TGATGCGAAA ATTAAGGGGT GGTGTTTAGA ATGTTGTCAA ATAGCTGATT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS5(NSP1) reference    TAACCCATTG TTATGGTTGC TCATTGCCGC ATGTTTGCAA ATGGTGTG-- ---------T TCAG------ ------AACA GAAGATGC-- ----------
pSmart_SA11-N5_GS5 reference   TAACCCATTG TTATGGTTGC TCATTGCCGC ATGTTTGCAA AGGAAGCGGA GCTACTAACT TCAGCCTGCT GAAGCAGGCT GGAGACGTGG AGGAGAACCC
pSmart_SA11-N5_GS5 NGS         TAACCCATTG TTATGGTTGC TCATTGCCGC ATGTTTGCAA ATGGTGTG-- ---------T TCAG------ ------AACA GAAGATGC-- ----------

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS5(NSP1) reference    ---------- --TTCCTTGA CAATGAACCT CATTTGCTT- AAGCTT---- -----AGAAC TGTGAA---- ---------- -----ACATC CAATTACCAA
pSmart_SA11-N5_GS5 reference   TGGACCTATG AACTCCCTGA TTAAGGAAAA CATGCACATG AAGCTCTACA TGGAGGGAAC CGTCAATAAC CATCACTTCA AGTGTACGTC CGAGGGAGAA
pSmart_SA11-N5_GS5 NGS         ---------- --TTCCTTGA CAATGAACCT CATTTGCTT- AAGCTT---- -----AGAAC TGTGAA---- ---------- -----ACATC CAATTACCAA

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS5(NSP1) reference    AGACAAAT-T ACAGTGTATC ATAGACT--- ---------- ---------- ---------- ---------- ---------- ---------- ----TGTACA
pSmart_SA11-N5_GS5 reference   GGAAAACCGT ACGAGGGGAC TCAGACTATG CGGATCAAGG TGGTGGAAGG AGGTCCGCTG CCGTTCGCAT TTGACATTCT GGCCACTTCC TTCATGTACG
pSmart_SA11-N5_GS5 NGS         AGACAAAT-T ACAGTGTATC ATAGACT--- ---------- ---------- ---------- ---------- ---------- ---------- ----TGTACA

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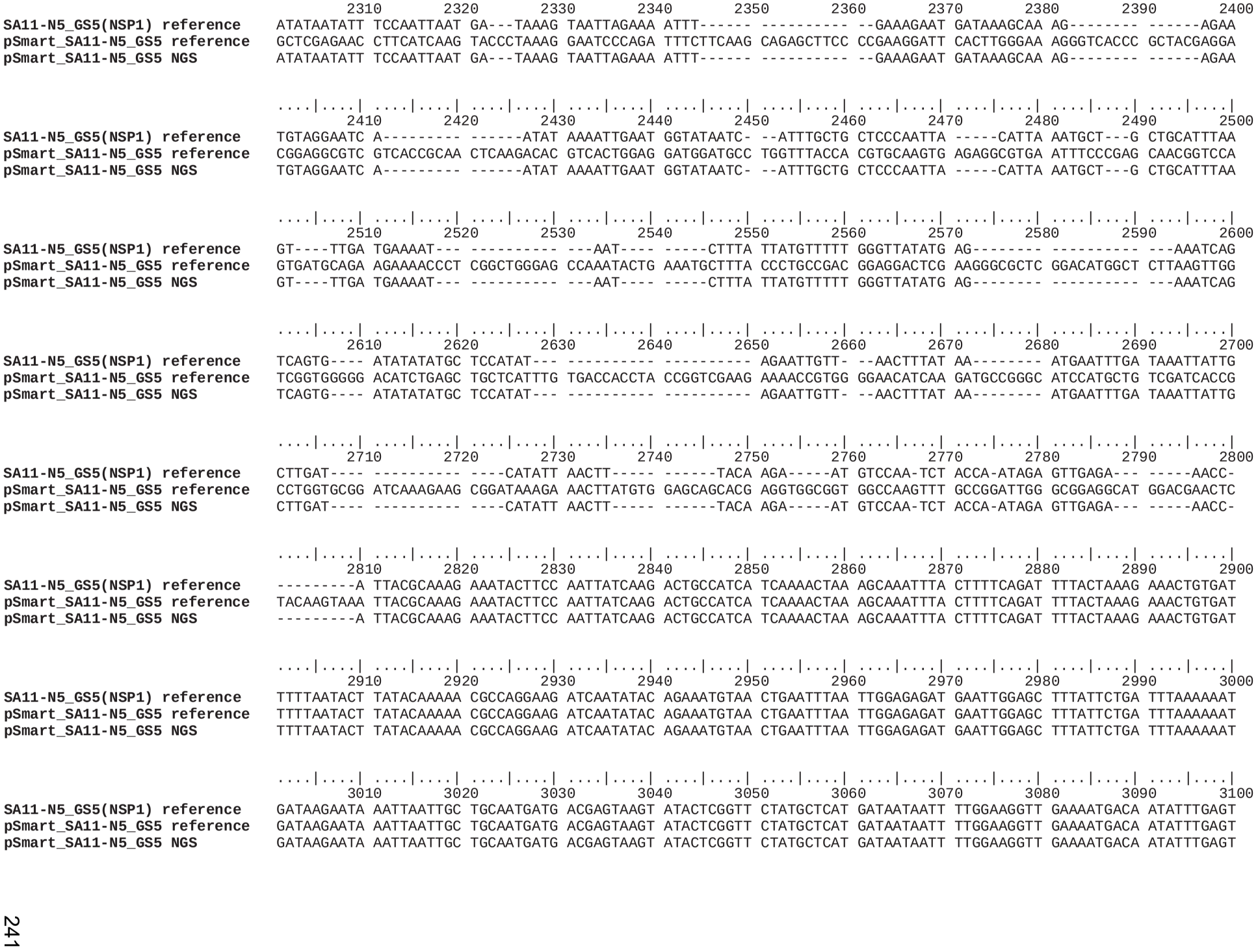
2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
SA11-N5_GS5(NSP1) reference ATATAATATT TCCAATTAAT GA---TAAAG TAATTAGAAA ATTT------ ---------- --GAAAGAAT GATAAAGCAA AG-------- ------AGAA
pSmart_SA11-N5_GS5 reference GCTCGAGAAC CTTCATCAAG TACCCTAAAG GAATCCCAGA TTTCTTCAAG CAGAGCTTCC CCGAAGGATT CACTTGGGAA AGGGTCACCC GCTACGAGGA
pSmart_SA11-N5_GS5 NGS ATATAATATT TCCAATTAAT GA---TAAAG TAATTAGAAA ATTT------ ---------- --GAAAGAAT GATAAAGCAA AG-------- ------AGAA
2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
SA11-N5_GS5(NSP1) reference TGTAGGAATC A--------- ------ATAT AAAATTGAAT GGTATAATC- --ATTTGCTG CTCCCAATTA -----CATTA AATGCT---G CTGCATTTAA
pSmart_SA11-N5_GS5 reference CGGAGGCGTC GTCACCGCAA CTCAAGACAC GTCACTGGAG GATGGATGCC TGGTTTACCA CGTGCAAGTG AGAGGCGTGA ATTTCCCGAG CAACGGTCCA
pSmart_SA11-N5_GS5 NGS TGTAGGAATC A--------- ------ATAT AAAATTGAAT GGTATAATC- --ATTTGCTG CTCCCAATTA -----CATTA AATGCT---G CTGCATTTAA
2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
SA11-N5_GS5(NSP1) reference GT----TTGA TGAAAAT--- ---------- ---AAT---- -----CTTTA TTATGTTTTT GGGTTATATG AG-------- ---------- ---AAATCAG
pSmart_SA11-N5_GS5 reference GTGATGCAGA AGAAAACCCT CGGCTGGGAG CCAAATACTG AAATGCTTTA CCCTGCCGAC GGAGGACTCG AAGGGCGCTC GGACATGGCT CTTAAGTTGG
pSmart_SA11-N5_GS5 NGS GT----TTGA TGAAAAT--- ---------- ---AAT---- -----CTTTA TTATGTTTTT GGGTTATATG AG-------- ---------- ---AAATCAG
2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
SA11-N5_GS5(NSP1) reference TCAGTG---- ATATATATGC TCCATAT--- ---------- ---------- AGAATTGTT- --AACTTTAT AA-------- ATGAATTTGA TAAATTATTG
pSmart_SA11-N5_GS5 reference TCGGTGGGGG ACATCTGAGC TGCTCATTTG TGACCACCTA CCGGTCGAAG AAAACCGTGG GGAACATCAA GATGCCGGGC ATCCATGCTG TCGATCACCG
pSmart_SA11-N5_GS5 NGS TCAGTG---- ATATATATGC TCCATAT--- ---------- ---------- AGAATTGTT- --AACTTTAT AA-------- ATGAATTTGA TAAATTATTG
2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
SA11-N5_GS5(NSP1) reference CTTGAT---- ---------- ----CATATT AACTT----- ------TACA AGA-----AT GTCCAA-TCT ACCA-ATAGA GTTGAGA--- -----AACC-
pSmart_SA11-N5_GS5 reference CCTGGTGCGG ATCAAAGAAG CGGATAAAGA AACTTATGTG GAGCAGCACG AGGTGGCGGT GGCCAAGTTT GCCGGATTGG GCGGAGGCAT GGACGAACTC
pSmart_SA11-N5_GS5 NGS CTTGAT---- ---------- ----CATATT AACTT----- ------TACA AGA-----AT GTCCAA-TCT ACCA-ATAGA GTTGAGA--- -----AACC-
2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
SA11-N5_GS5(NSP1) reference ---------A TTACGCAAAG AAATACTTCC AATTATCAAG ACTGCCATCA TCAAAACTAA AGCAAATTTA CTTTTCAGAT TTTACTAAAG AAACTGTGAT
pSmart_SA11-N5_GS5 reference TACAAGTAAA TTACGCAAAG AAATACTTCC AATTATCAAG ACTGCCATCA TCAAAACTAA AGCAAATTTA CTTTTCAGAT TTTACTAAAG AAACTGTGAT
pSmart_SA11-N5_GS5 NGS ---------A TTACGCAAAG AAATACTTCC AATTATCAAG ACTGCCATCA TCAAAACTAA AGCAAATTTA CTTTTCAGAT TTTACTAAAG AAACTGTGAT
2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
SA11-N5_GS5(NSP1) reference TTTTAATACT TATACAAAAA CGCCAGGAAG ATCAATATAC AGAAATGTAA CTGAATTTAA TTGGAGAGAT GAATTGGAGC TTTATTCTGA TTTAAAAAAT
pSmart_SA11-N5_GS5 reference TTTTAATACT TATACAAAAA CGCCAGGAAG ATCAATATAC AGAAATGTAA CTGAATTTAA TTGGAGAGAT GAATTGGAGC TTTATTCTGA TTTAAAAAAT
pSmart_SA11-N5_GS5 NGS TTTTAATACT TATACAAAAA CGCCAGGAAG ATCAATATAC AGAAATGTAA CTGAATTTAA TTGGAGAGAT GAATTGGAGC TTTATTCTGA TTTAAAAAAT
3010 3020 3030 3040 3050 3060 3070 3080 3090 3100
SA11-N5_GS5(NSP1) reference GATAAGAATA AATTAATTGC TGCAATGATG ACGAGTAAGT ATACTCGGTT CTATGCTCAT GATAATAATT TTGGAAGGTT GAAAATGACA ATATTTGAGT
pSmart_SA11-N5_GS5 reference GATAAGAATA AATTAATTGC TGCAATGATG ACGAGTAAGT ATACTCGGTT CTATGCTCAT GATAATAATT TTGGAAGGTT GAAAATGACA ATATTTGAGT
pSmart_SA11-N5_GS5 NGS GATAAGAATA AATTAATTGC TGCAATGATG ACGAGTAAGT ATACTCGGTT CTATGCTCAT GATAATAATT TTGGAAGGTT GAAAATGACA ATATTTGAGT

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                                  3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
SA11-N5_GS5(NSP1) reference       TGGGACATCA TTGTCAGCCT AACTACGTGG CATCTAATCA CCCAGGCAAT GCTTCCGATA TCCAGTACTG TAAATGGTGT AATATAAAAT ATTTTCTTAG
pSmart_SA11-N5_GS5 reference      TGGGACATCA TTGTCAGCCT AACTACGTGG CATCTAATCA CCCAGGCAAT GCTTCCGATA TCCAGTACTG TAAATGGTGT AATATAAAAT ATTTTCTTAG
pSmart_SA11-N5_GS5 NGS            TGGGACATCA TTGTCAGCCT AACTACGTGG CATCTAATCA CCCAGGCAAT GCTTCCGATA TCCAGTACTG TAAATGGTGT AATATAAAAT ATTTTCTTAG


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                                  3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
SA11-N5_GS5(NSP1) reference       TAAAATTGAT TGGCGGATTC GTGATATGTA TAATTTATTG ATGGAATTTA TTAAGGATTG TTATAAAAGT AATGTTAACG TTGGACATTG TAGTTCTGTT
pSmart_SA11-N5_GS5 reference      TAAAATTGAT TGGCGGATTC GTGATATGTA TAATTTATTG ATGGAATTTA TTAAGGATTG TTATAAAAGT AATGTTAACG TTGGACATTG TAGTTCTGTT
pSmart_SA11-N5_GS5 NGS            TAAAATTGAT TGGCGGATTC GTGATATGTA TAATTTATTG ATGGAATTTA TTAAGGATTG TTATAAAAGT AATGTTAACG TTGGACATTG TAGTTCTGTT


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                                  3310       3320       3330       3340       3350       3360       3370       3380       3390       3400
SA11-N5_GS5(NSP1) reference       GAAAACATAT ATCCTTTAAT TAAAAGATTA ATTTGGAGTT TGTTTACTAA TCACATGGAT CAAACAATTG AAGAAGTGTT TAATCACATG TCGCCAGTGT
pSmart_SA11-N5_GS5 reference      GAAAACATAT ATCCTTTAAT TAAAAGATTA ATTTGGAGTT TGTTTACTAA TCACATGGAT CAAACAATTG AAGAAGTGTT TAATCACATG TCGCCAGTGT
pSmart_SA11-N5_GS5 NGS            GAAAACATAT ATCCTTTAAT TAAAAGATTA ATTTGGAGTT TGTTTACTAA TCACATGGAT CAAACAATTG AAGAAGTGTT TAATCACATG TCGCCAGTGT


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                                  3410       3420       3430       3440       3450       3460       3470       3480       3490       3500
SA11-N5_GS5(NSP1) reference       CAGTTGAAGG TACGAATGTC ATCATGTTGA TTCTTGGATT GAATATTAGT TTGTATAATG AAATTAAGCG CACTTTGAAT GTAGATAGCA TACCAATGGT
pSmart_SA11-N5_GS5 reference      CAGTTGAAGG TACGAATGTC ATCATGTTGA TTCTTGGATT GAATATTAGT TTGTATAATG AAATTAAGCG CACTTTGAAT GTAGATAGCA TACCAATGGT
pSmart_SA11-N5_GS5 NGS            CAGTTGAAGG TACGAATGTC ATCATGTTGA TTCTTGGATT GAATATTAGT TTGTATAATG AAATTAAGCG CACTTTGAAT GTAGATAGCA TACCAATGGT


                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  3510       3520       3530       3540       3550       3560       3570       3580       3590       3600
SA11-N5_GS5(NSP1) reference       ACTTAATTTA AATGAATTCA GTAGTATAGT TAAATCAATT AGCAGTAAAT GGTATAATGT TGATGAATTG GATAAATTGC CAATGTCAAT AAAATCAACG
pSmart_SA11-N5_GS5 reference      ACTTAATTTA AATGAATTCA GTAGTATAGT TAAATCAATT AGCAGTAAAT GGTATAATGT TGATGAATTG GATAAATTGC CAATGTCAAT AAAATCAACG
pSmart_SA11-N5_GS5 NGS            ACTTAATTTA AATGAATTCA GTAGTATAGT TAAATCAATT AGCAGTAAAT GGTATAATGT TGATGAATTG GATAAATTGC CAATGTCAAT AAAATCAACG


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                                  3610       3620       3630       3640       3650       3660       3670       3680       3690       3700
SA11-N5_GS5(NSP1) reference       GAGGAACTGA TTGAAATGAA GAATTCTGGA ACTTTAACTG AAGAATTTGA GCTACTGATC TCCAACTCAG AAGATGACAA TGAGTGAAAT TACTAATGTC
pSmart_SA11-N5_GS5 reference      GAGGAACTGA TTGAAATGAA GAATTCTGGA ACTTTAACTG AAGAATTTGA GCTACTGATC TCCAACTCAG AAGATGACAA TGAGTGAAAT TACTAATGTC
pSmart_SA11-N5_GS5 NGS            GAGGAACTGA TTGAAATGAA GAATTCTGGA ACTTTAACTG AAGAATTTGA GCTACTGATC TCCAACTCAG AAGATGACAA TGAGTGAAAT TACTAATGTC


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                                  3710       3720       3730       3740       3750       3760       3770       3780       3790       3800
SA11-N5_GS5(NSP1) reference       ACTATCTAAT TATACAGTAT TTAGCCATCA CAAGACCGTC CAGACTAGAG TAGCGCCTAG CTGGCAAAAT ACTGTGAACC ---------- ----------
pSmart_SA11-N5_GS5 reference      ACTATCTAAT TATACAGTAT TTAGCCATCA CAAGACCGTC CAGACTAGAG TAGCGCCTAG CTGGCAAAAT ACTGTGAACC GGCCGGCATG GTCCCAGCCT
pSmart_SA11-N5_GS5 NGS            ACTATCTAAT TATACAGTAT TTAGCCATCA CAAGACCGTC CAGACTAGAG TAGCGCCTAG CTGGCAAAAT ACTGTGAACC GGCCGGCATG GTCCCAGCCT


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                                  3810       3820       3830       3840       3850       3860       3870       3880       3890       3900
SA11-N5_GS5(NSP1) reference       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS5 reference      CCTCGCTGGC GCCGGCTGGG CAACATTCCG AGGGGACCGT CCCCTCGGTA ATGGCGAATG GGACGGATCC GGCTGCTAAC AAAGCCCGAA AGGAAGCTGA
pSmart_SA11-N5_GS5 NGS            CCTCGCTGGC GCCGGCTGGG CAACATTCCG AGGGGACCGT CCCCTCGGTA ATGGCGAATG GGACGGATCC GGCTGCTAAC AAAGCCCGAA AGGAAGCTGA
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                                        3910       3920       3930       3940       3950       3960       3970
SA11-N5_GS5(NSP1) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------
pSmart_SA11-N5_GS5 reference    GTTGGCTGCT GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT CTAAACGGGT CTTGAGGGGT TTTTTGGAT
pSmart_SA11-N5_GS5 NGS          GTTGGCTGCT GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT CTAAACGGGT CTTGAGGGGT TTTTTGGAT
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Alignment: SA11-N5_GS6(VP6)

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                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS6 NGS          ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

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                                       110        120        130        140        150        160        170        180        190        200
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS6 NGS          CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       210        220        230        240        250        260        270        280        290        300
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS6 NGS          CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       310        320        330        340        350        360        370        380        390        400
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS6 NGS          GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

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                                       410        420        430        440        450        460        470        480        490        500
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS6 NGS          TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       510        520        530        540        550        560        570        580        590        600
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS6 NGS          CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

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                                       610        620        630        640        650        660        670        680        690        700
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS6 NGS          ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       710        720        730        740        750        760        770        780        790        800
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS6 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS6 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

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                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS6(VP6) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS6 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

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                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS6(VP6) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS6 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS6(VP6) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS6 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS6(VP6) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS6 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS6(VP6) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS6 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS6(VP6) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS6 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS6(VP6) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS6 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS6 NGS          GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS6 NGS          TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS6 NGS          CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS6(VP6) reference      ---------- ---------G GCTTTTAAAC GAAGTCTTCA ACATGGATGT CCTATACTCT TTGTCAAAGA CTCTTAAAGA CGCTAGAGAC AAAATTGTCG
pSmart_SA11-N5_GS6 reference    TCTAATACGA CTCACTATAG GCTTTTAAAC GAAGTCTTCA ACATGGATGT CCTATACTCT TTGTCAAAGA CTCTTAAAGA CGCTAGAGAC AAAATTGTCG
pSmart_SA11-N5_GS6 NGS          TCTAATACGA CTCACTATAG GCTTTTAAAC GAAGTCTTCA ACATGGATGT CCTATACTCT TTGTCAAAGA CTCTTAAAGA CGCTAGAGAC AAAATTGTCG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS6(VP6) reference      AAGGCACATT GTATTCTAAC GTGAGTGATC TAATTCAACA ATTTAATCAA ATGATAATTA CTATGAATGG AAATGAATTT CAAACTGGAG GAATCGGTAA
pSmart_SA11-N5_GS6 reference    AAGGCACATT GTATTCTAAC GTGAGTGATC TAATTCAACA ATTTAATCAA ATGATAATTA CTATGAATGG AAATGAATTT CAAACTGGAG GAATCGGTAA
pSmart_SA11-N5_GS6 NGS          AAGGCACATT GTATTCTAAC GTGAGTGATC TAATTCAACA ATTTAATCAA ATGATAATTA CTATGAATGG AAATGAATTT CAAACTGGAG GAATCGGTAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS6(VP6) reference      TTTGCCAATT AGAAACTGGA ATTTTAATTT CGGGTTACTT GGAACAACTT TGCTGAACTT AGACGCTAAT TATGTTGAAA CGGCAAGAAA TACAATTGAT
pSmart_SA11-N5_GS6 reference    TTTGCCAATT AGAAACTGGA ATTTTAATTT CGGGTTACTT GGAACAACTT TGCTGAACTT AGACGCTAAT TATGTTGAAA CGGCAAGAAA TACAATTGAT
pSmart_SA11-N5_GS6 NGS          TTTGCCAATT AGAAACTGGA ATTTTAATTT CGGGTTACTT GGAACAACTT TGCTGAACTT AGACGCTAAT TATGTTGAAA CGGCAAGAAA TACAATTGAT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS6(VP6) reference      TATTTCGTGG ATTTTGTAGA CAATGTATGC ATGGATGAGA TGGTTAGAGA ATCACAAAGG AACGGAATTG CACCTCAATC AGACTCGCTA AGAAAGCTGT
pSmart_SA11-N5_GS6 reference    TATTTCGTGG ATTTTGTAGA CAATGTATGC ATGGATGAGA TGGTTAGAGA ATCACAAAGG AACGGAATTG CACCTCAATC AGACTCGCTA AGAAAGCTGT
pSmart_SA11-N5_GS6 NGS          TATTTCGTGG ATTTTGTAGA CAATGTATGC ATGGATGAGA TGGTTAGAGA ATCACAAAGG AACGGAATTG CACCTCAATC AGACTCGCTA AGAAAGCTGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS6(VP6) reference      CAGCCATTAA ATTCAAAAGA ATAAATTTTG ATAATTCGTC GGAATACATA GAAAACTGGA ATTTGCAAAA TAGAAGACAG AGGACAGGTT TCACTTTTCA
pSmart_SA11-N5_GS6 reference    CAGCCATTAA ATTCAAAAGA ATAAATTTTG ATAATTCGTC GGAATACATA GAAAACTGGA ATTTGCAAAA TAGAAGACAG AGGACAGGTT TCACTTTTCA
pSmart_SA11-N5_GS6 NGS          CAGCCATTAA ATTCAAAAGA ATAAATTTTG ATAATTCGTC GGAATACATA GAAAACTGGA ATTTGCAAAA TAGAAGACAG AGGACAGGTT TCACTTTTCA

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS6(VP6) reference      TAAACCAAAC ATTTTTCCTT ATTCAGCATC ATTTACACTA AATAGATCAC AACCCGCTCA TGATAATTTG ATGGGCACAA TGTGGTTAAA CGCAGGATCG
pSmart_SA11-N5_GS6 reference    TAAACCAAAC ATTTTTCCTT ATTCAGCATC ATTTACACTA AATAGATCAC AACCCGCTCA TGATAATTTG ATGGGCACAA TGTGGTTAAA CGCAGGATCG
pSmart_SA11-N5_GS6 NGS          TAAACCAAAC ATTTTTCCTT ATTCAGCATC ATTTACACTA AATAGATCAC AACCCGCTCA TGATAATTTG ATGGGCACAA TGTGGTTAAA CGCAGGATCG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS6(VP6) reference      GAAATTCAAG TCGCTGGATT TGACTACTCA TGTGCTATTA ACGCACCAGC CAATATACAA CAATTTGAGC ATATTGTGCC ACTCCGAAGA GTGTTAACTA
pSmart_SA11-N5_GS6 reference    GAAATTCAAG TCGCTGGATT TGACTACTCA TGTGCTATTA ACGCACCAGC CAATATACAA CAATTTGAGC ATATTGTGCC ACTCCGAAGA GTGTTAACTA
pSmart_SA11-N5_GS6 NGS          GAAATTCAAG TCGCTGGATT TGACTACTCA TGTGCTATTA ACGCACCAGC CAATATACAA CAATTTGAGC ATATTGTGCC ACTCCGAAGA GTGTTAACTA

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS6(VP6) reference      CAGCTACGAT AACTCTTCTA CCAGACGCGG AAAGGTTTAG TTTTCCAAGA GTGATCAATT CAGCTGACGG GGCAACTACA TGGTTTTTCA ACCCAGTGAT
pSmart_SA11-N5_GS6 reference    CAGCTACGAT AACTCTTCTA CCAGACGCGG AAAGGTTTAG TTTTCCAAGA GTGATCAATT CAGCTGACGG GGCAACTACA TGGTTTTTCA ACCCAGTGAT
pSmart_SA11-N5_GS6 NGS          CAGCTACGAT AACTCTTCTA CCAGACGCGG AAAGGTTTAG TTTTCCAAGA GTGATCAATT CAGCTGACGG GGCAACTACA TGGTTTTTCA ACCCAGTGAT

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS6(VP6) reference      TCTCAGGCCG AATAACGTTG AAGTGGAGTT TCTATTGAAT GGACAGATAA TAAACACTTA TCAAGCAAGA TTTGGAACTA TCGTAGCTAG AAATTTTGAT
pSmart_SA11-N5_GS6 reference    TCTCAGGCCG AATAACGTTG AAGTGGAGTT TCTATTGAAT GGACAGATAA TAAACACTTA TCAAGCAAGA TTTGGAACTA TCGTAGCTAG AAATTTTGAT
pSmart_SA11-N5_GS6 NGS          TCTCAGGCCG AATAACGTTG AAGTGGAGTT TCTATTGAAT GGACAGATAA TAAACACTTA TCAAGCAAGA TTTGGAACTA TCGTAGCTAG AAATTTTGAT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS6(VP6) reference      ACTATTAGAC TATCATTCCA GTTAATGAGA CCACCAAACA TGACACCAGC AGTAGCAGTA CTATTCCCGA ATGCACAGCC ATTCGAACAT CATGCAACAG
pSmart_SA11-N5_GS6 reference    ACTATTAGAC TATCATTCCA GTTAATGAGA CCACCAAACA TGACACCAGC AGTAGCAGTA CTATTCCCGA ATGCACAGCC ATTCGAACAT CATGCAACAG
pSmart_SA11-N5_GS6 NGS          ACTATTAGAC TATCATTCCA GTTAATGAGA CCACCAAACA TGACACCAGC AGTAGCAGTA CTATTCCCGA ATGCACAGCC ATTCGAACAT CATGCAACAG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS6(VP6) reference      TGGGATTGAC ACTTAGAATT GAGTCTGCAG TTTGTGAGTC TGTACTCGCC GATGCAAGTG AAACTCTATT AGCAAATGTA ACATCCGTTA GGCAAGAGTA
pSmart_SA11-N5_GS6 reference    TGGGATTGAC ACTTAGAATT GAGTCTGCAG TTTGTGAGTC TGTACTCGCC GATGCAAGTG AAACTCTATT AGCAAATGTA ACATCCGTTA GGCAAGAGTA
pSmart_SA11-N5_GS6 NGS          TGGGATTGAC ACTTAGAATT GAGTCTGCAG TTTGTGAGTC TGTACTCGCC GATGCAAGTG AAACTCTATT AGCAAATGTA ACATCCGTTA GGCAAGAGTA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS6(VP6) reference      CGCAATACCA GTTGGACCAG TCTTTCCACC AGGTATGAAC TGGACTGATT TAATCACCAA TTATTCACCG TCTAGGGAGG ACAATTTGCA ACGCGTATTT
pSmart_SA11-N5_GS6 reference    CGCAATACCA GTTGGACCAG TCTTTCCACC AGGTATGAAC TGGACTGATT TAATCACCAA TTATTCACCG TCTAGGGAGG ACAATTTGCA ACGCGTATTT
pSmart_SA11-N5_GS6 NGS          CGCAATACCA GTTGGACCAG TCTTTCCACC AGGTATGAAC TGGACTGATT TAATCACCAA TTATTCACCG TCTAGGGAGG ACAATTTGCA ACGCGTATTT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-N5_GS6(VP6) reference      ACAGTGGCTT CCATTAGAAG CATGCTCATT AAATGAGGAC CAAGCTAACA ACTTGGTATC CAACTTTGGT GAGTATGTAG CTATATCAAG CTGTTTGAAC
pSmart_SA11-N5_GS6 reference    ACAGTGGCTT CCATTAGAAG CATGCTCATT AAATGAGGAC CAAGCTAACA ACTTGGTATC CAACTTTGGT GAGTATGTAG CTATATCAAG CTGTTTGAAC
pSmart_SA11-N5_GS6 NGS          ACAGTGGCTT CCATTAGAAG CATGCTCATT AAATGAGGAC CAAGCTAACA ACTTGGTATC CAACTTTGGT GAGTATGTAG CTATATCAAG CTGTTTGAAC
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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        3110       3120       3130       3140       3150       3160       3170       3180       3190       3200
SA11-N5_GS6(VP6) reference      TCTGTAAGTA AGGATGCGTA TACGCATTCG CTACACAGAG TAATCACTCA GATGGTATAG TGAGAGGATG TGACC----- ---------- ----------
pSmart_SA11-N5_GS6 reference    TCTGTAAGTA AGGATGCGTA TACGCATTCG CTACACAGAG TAATCACTCA GATGGTATAG TGAGAGGATG TGA--GGCCG GCATGGTCCC AGCCTCCTCG
pSmart_SA11-N5_GS6 NGS          TCTGTAAGTA AGGATGCGTA TACGCATTCG CTACACAGAG TAATCACTCA GATGGTATAG TGAGAGGATG TGACCGGCCG GCATGGTCCC AGCCTCCTCG


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                                        3210       3220       3230       3240       3250       3260       3270       3280       3290       3300
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS6 reference    CTGGCGCCGG CTGGGCAACA TTCCGAGGGG ACCGTCCCCT CGGTAATGGC GAATGGGACG GATCCGGCTG CTAACAAAGC CCGAAAGGAA GCTGAGTTGG
pSmart_SA11-N5_GS6 NGS          CTGGCGCCGG CTGGGCAACA TTCCGAGGGG ACCGTCCCCT CGGTAATGGC GAATGGGACG GATCCGGCTG CTAACAAAGC CCGAAAGGAA GCTGAGTTGG


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                                        3310       3320       3330       3340       3350       3360       3370
SA11-N5_GS6(VP6) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----
pSmart_SA11-N5_GS6 reference    CTGCTGCCAC CGCTGAGCAA TAACTAGCAT AACCCCTTGG GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GGAT
pSmart_SA11-N5_GS6 NGS          CTGCTGCCAC CGCTGAGCAA TAACTAGCAT AACCCCTTGG GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GGAT
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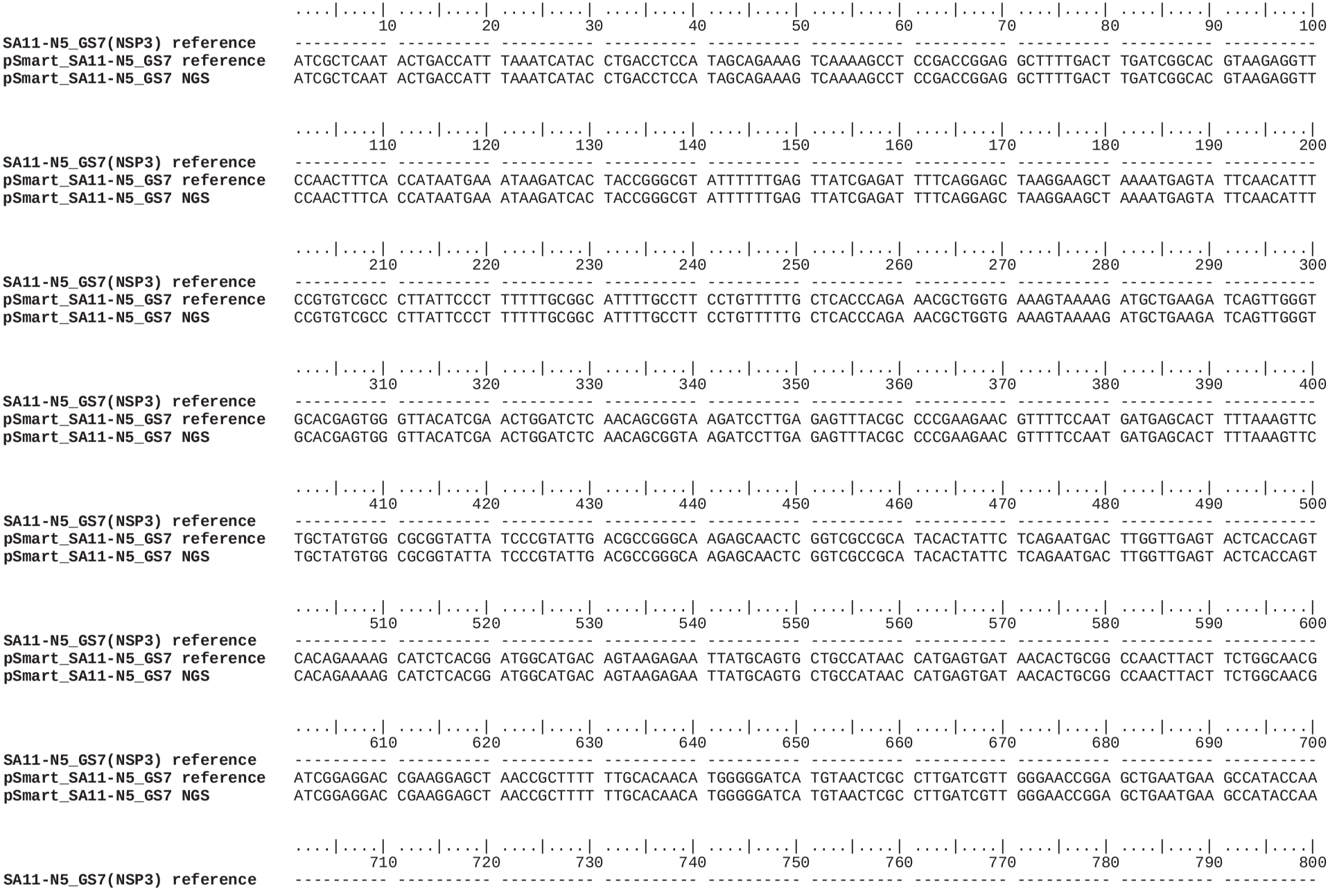
Alignment: SA11-N5_GS7(NSP3)



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pSmart_SA11-N5_GS7 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS7 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

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                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS7 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS7 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS7 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS7 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

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                                      1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS7 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS7 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS7 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS7 NGS         GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS7 NGS         TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

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                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS7(NSP3) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference   CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS7 NGS         CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS7(NSP3) reference    ---------- ---------G GCATTTAATG CTTTTCAGTG GTTGATGCTC AAGATGGAGT CTACGCAACA GATGGCCGTC TCAATTATTA ACTCTTCTTT
pSmart_SA11-N5_GS7 reference   TCTAATACGA CTCACTATAG GCATTTAATG CTTTTCAGTG GTTGATGCTC AAGATGGAGT CTACGCAACA GATGGCCGTC TCAATTATTA ACTCTTCTTT
pSmart_SA11-N5_GS7 NGS         TCTAATACGA CTCACTATAG GCATTTAATG CTTTTCAGTG GTTGATGCTC AAGATGGAGT CTACGCAACA GATGGCCGTC TCAATTATTA ACTCTTCTTT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS7(NSP3) reference    TGAAGCTGCA GTTGTAGCTG CAACCTCAGC TCTTGAGAAT ATGGGAATAG AATATGATTA TCAGGATATA TATTCTAGAG TAAAGAATAA ATTTGATTTT
pSmart_SA11-N5_GS7 reference   TGAAGCTGCA GTTGTAGCTG CAACCTCAGC TCTTGAGAAT ATGGGAATAG AATATGATTA TCAGGATATA TATTCTAGAG TAAAGAATAA ATTTGATTTT
pSmart_SA11-N5_GS7 NGS         TGAAGCTGCA GTTGTAGCTG CAACCTCAGC TCTTGAGAAT ATGGGAATAG AATATGATTA TCAGGATATA TATTCTAGAG TAAAGAATAA ATTTGATTTT

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                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS7(NSP3) reference    GTGATGGACG ATTCTGGTGT TAAAAATAAT CTGATTGGTA AAGCAATAAC TATTGATCAA GCTTTGAATA ATAAATTTGG ATCTGCTATA AGAAATAGAA
pSmart_SA11-N5_GS7 reference   GTGATGGACG ATTCTGGTGT TAAAAATAAT CTGATTGGTA AAGCAATAAC TATTGATCAA GCTTTGAATA ATAAATTTGG ATCTGCTATA AGAAATAGAA
pSmart_SA11-N5_GS7 NGS         GTGATGGACG ATTCTGGTGT TAAAAATAAT CTGATTGGTA AAGCAATAAC TATTGATCAA GCTTTGAATA ATAAATTTGG ATCTGCTATA AGAAATAGAA

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                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS7(NSP3) reference    ACTGGCTTGC TGATACTTCT AGAGCAGCTA AATTGGATGA GGATGTAAAC AAACTAAGAA TGATGTTATC ATCAAAAGGA ATTGATCAAA AAATGAGAGT
pSmart_SA11-N5_GS7 reference   ACTGGCTTGC TGATACTTCT AGAGCAGCTA AATTGGATGA GGATGTAAAC AAACTAAGAA TGATGTTATC ATCAAAAGGA ATTGATCAAA AAATGAGAGT
pSmart_SA11-N5_GS7 NGS         ACTGGCTTGC TGATACTTCT AGAGCAGCTA AATTGGATGA GGATGTAAAC AAACTAAGAA TGATGTTATC ATCAAAAGGA ATTGATCAAA AAATGAGAGT

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                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS7(NSP3) reference    TTTAAACGCA TGCTTCAGTG TAAAAAGAAT ACCTGGAAAA TCATCATCTA TTATTAAATG CACAAAATTG ATGCGTGATA AATTGGAACG TGGTGAAGTT
pSmart_SA11-N5_GS7 reference   TTTAAACGCA TGCTTCAGTG TAAAAAGAAT ACCTGGAAAA TCATCATCTA TTATTAAATG CACAAAATTG ATGCGTGATA AATTGGAACG TGGTGAAGTT
pSmart_SA11-N5_GS7 NGS         TTTAAACGCA TGCTTCAGTG TAAAAAGAAT ACCTGGAAAA TCATCATCTA TTATTAAATG CACAAAATTG ATGCGTGATA AATTGGAACG TGGTGAAGTT

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS7(NSP3) reference     GAAGTGGATG ATTCATTTGT GGATGAAAAA ATGGAAGTGG ATACCATTGA CTGGAAATCG CGCTATGAGC AATTGGAGCA AAGGTTTGAA TCATTGAAAT
pSmart_SA11-N5_GS7 reference    GAAGTGGATG ATTCATTTGT GGATGAAAAA ATGGAAGTGG ATACCATTGA CTGGAAATCG CGCTATGAGC AATTGGAGCA AAGGTTTGAA TCATTGAAAT
pSmart_SA11-N5_GS7 NGS          GAAGTGGATG ATTCATTTGT GGATGAAAAA ATGGAAGTGG ATACCATTGA CTGGAAATCG CGCTATGAGC AATTGGAGCA AAGGTTTGAA TCATTGAAAT

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS7(NSP3) reference     CCAGGGTAAA TGAAAAATAT AATAATTGGG TGTTGAAAGC AAGAAAAATG AATGAAAATA TGCATTCTCT TCAAAATGTC ATCTCTCAAC AGCAAGCACA
pSmart_SA11-N5_GS7 reference    CCAGGGTAAA TGAAAAATAT AATAATTGGG TGTTGAAAGC AAGAAAAATG AATGAAAATA TGCATTCTCT TCAAAATGTC ATCTCTCAAC AGCAAGCACA
pSmart_SA11-N5_GS7 NGS          CCAGGGTAAA TGAAAAATAT AATAATTGGG TGTTGAAAGC AAGAAAAATG AATGAAAATA TGCATTCTCT TCAAAATGTC ATCTCTCAAC AGCAAGCACA

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS7(NSP3) reference     TATAGCTGAG CTTCAAGTGT ACAATAATAA ACTAGAACGT GATTTGCAAA ATAAAATTGG ATCCCTTACT TCTTCGATTG AATGGTATTT AAGATCAATG
pSmart_SA11-N5_GS7 reference    TATAGCTGAG CTTCAAGTGT ACAATAATAA ACTAGAACGT GATTTGCAAA ATAAAATTGG ATCCCTTACT TCTTCGATTG AATGGTATTT AAGATCAATG
pSmart_SA11-N5_GS7 NGS          TATAGCTGAG CTTCAAGTGT ACAATAATAA ACTAGAACGT GATTTGCAAA ATAAAATTGG ATCCCTTACT TCTTCGATTG AATGGTATTT AAGATCAATG

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS7(NSP3) reference     GAATTAGACC CTGAAATAAA GGCAGACATT GAACAGCAAA TTAACTCAAT TGATGCGATA AATCCATTGC ACGCTTTTGA TGACTTAGAA TCAGTAATAC
pSmart_SA11-N5_GS7 reference    GAATTAGACC CTGAAATAAA GGCAGACATT GAACAGCAAA TTAACTCAAT TGATGCGATA AATCCATTGC ACGCTTTTGA TGACTTAGAA TCAGTAATAC
pSmart_SA11-N5_GS7 NGS          GAATTAGACC CTGAAATAAA GGCAGACATT GAACAGCAAA TTAACTCAAT TGATGCGATA AATCCATTGC ACGCTTTTGA TGACTTAGAA TCAGTAATAC

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                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS7(NSP3) reference     GTAATTTGAT ATCTGATTAT GACAAATTAT TCCTTATGTT CAAAGGATTA ATACAGAGAT GTAATTATCA ATATTCATTT GGTTGCGAAT AACCATTTTG
pSmart_SA11-N5_GS7 reference    GTAATTTGAT ATCTGATTAT GACAAATTAT TCCTTATGTT CAAAGGATTA ATACAGAGAT GTAATTATCA ATATTCATTT GGTTGCGAAT AACCATTTTG
pSmart_SA11-N5_GS7 NGS          GTAATTTGAT ATCTGATTAT GACAAATTAT TCCTTATGTT CAAAGGATTA ATACAGAGAT GTAATTATCA ATATTCATTT GGTTGCGAAT AACCATTTTG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS7(NSP3) reference     ATACATGTTG AACAATCAAA TACAGTGTTA GTATGTTGTC ATCTATGCAT AACCCTCTAT GAGCACAATA GTTAAAAGCT AACACTGTCA AAAACCTAAA
pSmart_SA11-N5_GS7 reference    ATACATGTTG AACAATCAAA TACAGTGTTA GTATGTTGTC ATCTATGCAT AACCCTCTAT GAGCACAATA GTTAAAAGCT AACACTGTCA AAAACCTAAA
pSmart_SA11-N5_GS7 NGS          ATACATGTTG AACAATCAAA TACAGTGTTA GTATGTTGTC ATCTATGCAT AACCCTCTAT GAGCACAATA GTTAAAAGCT AACACTGTCA AAAACCTAAA

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                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS7(NSP3) reference     TGGCTATAGG GGCGTTATGT GGCC------ ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference    TGGCTATAGG GGCGTTATGT GGCCGGCCGG CATGGTCCCA GCCTCCTCGC TGGCGCCGGC TGGGCAACAT TCCGAGGGGA CCGTCCCCTC GGTAATGGCG
pSmart_SA11-N5_GS7 NGS          TGGCTATAGG GGCGTTATGT GGCCTGCCGG CATGGTCCCA GCCTCCTCGC TGGCGCCGGC TGGGCAACAT TCCGAGGGGA CCGTCCCCTC GGTAATGGCG

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                                3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-N5_GS7(NSP3) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS7 reference    AATGGGACGG ATCCGGCTGC TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA ACCCCTTGGG GCCTCTAAAC
pSmart_SA11-N5_GS7 NGS          AATGGGACGG ATCCGGCTGC TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA ACCCCTTGGG ----------
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                                ....|....| ....|....| ...
                                        3110       3120
SA11-N5_GS7(NSP3) reference     ---------- ---------- ---
pSmart_SA11-N5_GS7 reference    GGGTCTTGAG GGGTTTTTTG GAT
pSmart_SA11-N5_GS7 NGS          ---------- ---------- ---
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Alignment: SA11-N5_GS8(NSP2)

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS8 NGS          ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

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                                        110        120        130        140        150        160        170        180        190        200
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS8 NGS          CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

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                                        210        220        230        240        250        260        270        280        290        300
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS8 NGS          CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        310        320        330        340        350        360        370        380        390        400
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS8 NGS          GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        410        420        430        440        450        460        470        480        490        500
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS8 NGS          TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        510        520        530        540        550        560        570        580        590        600
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS8 NGS          CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        610        620        630        640        650        660        670        680        690        700
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS8 NGS          ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        710        720        730        740        750        760        770        780        790        800
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS8 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS8 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS8 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS8 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS8 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS8 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS8 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS8 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS8 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                      1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS8 NGS         GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference   TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS8 NGS         TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS8(NSP2) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------GGCT
pSmart_SA11-N5_GS8 reference   CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS8 NGS         CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS8(NSP2) reference    TTTAAAGCGT CTCAGT---C GCCGTTTGAG CCTTGCGGT- ---------- ---------G TAGCCATGGC TGAGCTAGCT TGCTTTTGCT ATCCTCATTT
pSmart_SA11-N5_GS8 reference   TCTAATACGA CTCACTATAG GCTATTAAAG G--CTCA--- ---------- ---------- -----ATGGC TGAGCTAGCT TGCTTTTGCT ATCCTCATTT
pSmart_SA11-N5_GS8 NGS         TCTAATACGA CTCACTATAG GCTTTTAAAG CGTCTCAGTC GCCGTTTGAG CCTTGCGGTG TAGCCATGGC TGAGCTAGCT TGCTTTTGCT ATCCTCATTT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS8(NSP2) reference    GGAGAATGAT AGCTATAAAT TTATTCCTTT TAATAATTTA GCTATTAAAG CTATGCTGAC AGCTAAAGTA GACAAAAAGG ACATGGATAA GTTTTATGAT
pSmart_SA11-N5_GS8 reference   GGAGAATGAT AGCTATAAAT TTATTCCTTT TAATAATTTA GCTATTAAAG CTATGCTGAC AGCTAAAGTA GACAAAAAGG ACATGGATAA GTTTTATGAT
pSmart_SA11-N5_GS8 NGS         GGAGAATGAT AGCTATAAAT TTATTCCTTT TAATAATTTA GCTATTAAAG CTATGCTGAC AGCTAAAGTA GACAAAAAGG ACATGGATAA GTTTTATGAT

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS8(NSP2) reference    TCAATTATTT ATGGAATAGC ACCGCCTCCT CAATTTAAGA AACGGTATAA TACTAATGAT AATTCAAGAG GCATGAATTT TGAAACAATT ATGTTTACTA
pSmart_SA11-N5_GS8 reference   TCAATTATTT ATGGAATAGC ACCGCCTCCT CAATTTAAGA AACGGTATAA TACTAATGAT AATTCAAGAG GCATGAATTT TGAAACAATT ATGTTTACTA
pSmart_SA11-N5_GS8 NGS         TCAATTATTT ATGGAATAGC ACCGCCTCCT CAATTTAAGA AACGGTATAA TACTAATGAT AATTCAAGAG GCATGAATTT TGAAACAATT ATGTTTACTA

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                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS8(NSP2) reference    AGGTGGCTAT GTTGATATGT GAAGCTCTAA ATTCATTGAA AGTGACGCAA GCAAACGTCT CTAATGTATT ATCACGAGTA GTATCAATAA GGCATTTAGA
pSmart_SA11-N5_GS8 reference   AGGTGGCTAT GTTGATATGT GAAGCTCTAA ATTCATTGAA AGTGACGCAA GCAAACGTCT CTAATGTATT ATCACGAGTA GTATCAATAA GGCATTTAGA
pSmart_SA11-N5_GS8 NGS         AGGTGGCTAT GTTGATATGT GAAGCTCTAA ATTCATTGAA AGTGACGCAA GCAAACGTCT CTAATGTATT ATCACGAGTA GTATCAATAA GGCATTTAGA

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                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS8(NSP2) reference    AAATTTGGTG ATACGTAAAG AAAATCCACA GGATATTCTA TTTCATTCAA AAGATTTACT TTTGAAATCA ACACTGATTG CTATTGGACA GTCTAAAGAA
pSmart_SA11-N5_GS8 reference   AAATTTGGTG ATACGTAAAG AAAATCCACA GGATATTCTA TTTCATTCAA AAGATTTACT TTTGAAATCA ACACTGATTG CTATTGGACA GTCTAAAGAA
pSmart_SA11-N5_GS8 NGS         AAATTTGGTG ATACGTAAAG AAAATCCACA GGATATTCTA TTTCATTCAA AAGATTTACT TTTGAAATCA ACACTGATTG CTATTGGACA GTCTAAAGAA

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS8(NSP2) reference     ATTGAAACTA CAATAACTGC AGAAGGAGGA GAAATTGTAT TTCAAAACGC TGCCTTCACC ATGTGGAAAC TAACTTATTT AGAACATCAA TTGATGCCAA
pSmart_SA11-N5_GS8 reference    ATTGAAACTA CAATAACTGC AGAAGGAGGA GAAATTGTAT TTCAAAACGC TGCCTTCACC ATGTGGAAAC TAACTTATTT AGAACATCAA TTGATGCCAA
pSmart_SA11-N5_GS8 NGS          ATTGAAACTA CAATAACTGC AGAAGGAGGA GAAATTGTAT TTCAAAACGC TGCCTTCACC ATGTGGAAAC TAACTTATTT AGAACATCAA TTGATGCCAA

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS8(NSP2) reference     TTCTGGATCA GAATTTTATT GAATATAAAG TTACATTGAA CGAAGATAAA CCAATTTCAG ATGTTCATGT TAAAGAATTA GTCGCTGAAC TTCGATGGCA
pSmart_SA11-N5_GS8 reference    TTCTGGATCA GAATTTTATT GAATATAAAG TTACATTGAA CGAAGATAAA CCAATTTCAG ATGTTCATGT TAAAGAATTA GTCGCTGAAC TTCGATGGCA
pSmart_SA11-N5_GS8 NGS          TTCTGGATCA GAATTTTATT GAATATAAAG TTACATTGAA CGAAGATAAA CCAATTTCAG ATGTTCATGT TAAAGAATTA GTCGCTGAAC TTCGATGGCA

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS8(NSP2) reference     ATATAACAAG TTTGCTGTAA TCACACATGG TAAGGGTCAT TATAGAATTG TAAAGTATTC ATCAGTTGCT AATCACGCTG ACAGAGTATA TGCAACTTTC
pSmart_SA11-N5_GS8 reference    ATATAACAAG TTTGCTGTAA TCACACATGG TAAGGGTCAT TATAGAATTG TAAAGTATTC ATCAGTTGCT AATCACGCTG ACAGAGTATA TGCAACTTTC
pSmart_SA11-N5_GS8 NGS          ATATAACAAG TTTGCTGTAA TCACACATGG TAAGGGTCAT TATAGAATTG TAAAGTATTC ATCAGTTGCT AATCACGCTG ACAGAGTATA TGCAACTTTC

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS8(NSP2) reference     AAGAGTAATG TTAAAACTGG AGTTAATAAT GATTTTAACC TACTTGATCA AAGAATTATT TGGCAAAACT GGTATGCATT TACATCATCA ATGAAACAGG
pSmart_SA11-N5_GS8 reference    AAGAGTAATG TTAAAACTGG AGTTAATAAT GATTTTAACC TACTTGATCA AAGAATTATT TGGCAAAACT GGTATGCATT TACATCATCA ATGAAACAGG
pSmart_SA11-N5_GS8 NGS          AAGAGTAATG TTAAAACTGG AGTTAATAAT GATTTTAACC TACTTGATCA AAGAATTATT TGGCAAAACT GGTATGCATT TACATCATCA ATGAAACAGG

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                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS8(NSP2) reference     GTAATACACT TGACGTGTGT AAAAGGTTGC TTTTCCAAAA AATGAAACCA GAAAAAAATC CATTTAAAGG GCTGTCAACG GATAGAAAAA TGGACGAAGT
pSmart_SA11-N5_GS8 reference    GTAATACACT TGACGTGTGT AAAAGGTTGC TTTTCCAAAA AATGAAACCA GAAAAAAATC CATTTAAAGG GCTGTCAACG GATAGAAAAA TGGACGAAGT
pSmart_SA11-N5_GS8 NGS          GTAATACACT TGACGTGTGT AAAAGGTTGC TTTTCCAAAA AATGAAACCA GAAAAAAATC CATTTAAAGG GCTGTCAACG GATAGAAAAA TGGACGAAGT

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                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS8(NSP2) reference     TTCTCAAGTT GGCGTTTAAT TCGCTATCAA TTTGAGGATG ATGATGGCTT A-----GCAA GAATAGAAAG CGCTTATGTG ACC------- ----------
pSmart_SA11-N5_GS8 reference    TTCTCAAGTT GGCGTTTAAC CTAACATTTA CCGTATATTC AGATTTGCTT GCGTTCGTTT CAGCTGATAC TGTTGAACCA ATTAATGCTG TTGCTTTTGA
pSmart_SA11-N5_GS8 NGS          TTCTCAAGTT GGCGTTTAAC NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    TAATATGCGC ATCATGAACG AACTGTAAAC GCCAACCCCA TTGTGGAGAT ATGACCGGCC GGCATGGTCC CAGCCTCCTC GCTGGCGCCG GCTGGGCAAC
pSmart_SA11-N5_GS8 NGS          NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNGGAGAT GTGACCGGCC GGCATGGTCC CAGCCTCCTC GCTGGCGCCG GCTGGGCAAC

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                                3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS8 reference    ATTCCGAGGG GACCGTCCCC TCGGTAATGG CGAATGGGAC GGATCCGGCT GCTAACAAAG CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA
pSmart_SA11-N5_GS8 NGS          ATTCCGAGGG GACCGTCCCC TCGGTAATGG CGAATGGGAC GGATCCGGCT GCTAACAAAG CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA
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                                        3110       3120       3130       3140       3150
SA11-N5_GS8(NSP2) reference     ---------- ---------- ---------- ---------- ---------- -----
pSmart_SA11-N5_GS8 reference    ATAACTAGCA TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGGAT
pSmart_SA11-N5_GS8 NGS          ATAACTAGCA TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGGAT
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Alignment: SA11-N5_GS9(VP7)

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                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS9 NGS          ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

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                                       110        120        130        140        150        160        170        180        190        200
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS9 NGS          CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

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                                       210        220        230        240        250        260        270        280        290        300
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS9 NGS          CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

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                                       310        320        330        340        350        360        370        380        390        400
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS9 NGS          GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

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                                       410        420        430        440        450        460        470        480        490        500
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS9 NGS          TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       510        520        530        540        550        560        570        580        590        600
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS9 NGS          CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       610        620        630        640        650        660        670        680        690        700
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS9 NGS          ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       710        720        730        740        750        760        770        780        790        800
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS9 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS9 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

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                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS9(VP7) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS9 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS9(VP7) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS9 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

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                                       1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS9(VP7) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS9 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

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                                       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS9(VP7) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS9 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

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                                       1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS9(VP7) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS9 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS9(VP7) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS9 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

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                                       1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS9(VP7) reference     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS9 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

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                                       1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS9 NGS          GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

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                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS9 NGS          TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

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                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS9 NGS          CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS9(VP7) reference      ---------- ---------G GCTTTAAAAA GAGAGAATTT CCGTTTGGCT AGCGGTTAGC TCCTTTTAAT GTATGGTATT GAATATACCA CAGTTCTAAC
pSmart_SA11-N5_GS9 reference    TCTAATACGA CTCACTATAG GCTTTAAAAA GAGAGAATTT CCGTTTGGCT AGCGGTTAGC TCCTTTTAAT GTATGGTATT GAATATACCA CAGTTCTAAC
pSmart_SA11-N5_GS9 NGS          TCTAATACGA CTCACTATAG GCTTTAAAAA GAGAGAATTT CCGTTTGGCT AGCGGTTAGC TCCTTTTAAT GTATGGTATT GAATATACCA CAGTTCTAAC

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                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS9(VP7) reference      CTTTCTGATA TCGATTATTC TACTAAATTA CATACTTAAA TCATTAACTA GAATAATGGA CTTTATAATT TATAGATTTC TTTTTATAAT TGTGATATTG
pSmart_SA11-N5_GS9 reference    CTTTCTGATA TCGATTATTC TACTAAATTA CATACTTAAA TCATTAACTA GAATAATGGA CTTTATAATT TATAGATTTC TTTTTATAAT TGTGATATTG
pSmart_SA11-N5_GS9 NGS          CTTTCTGATA TCGATTATTC TACTAAATTA CATACTTAAA TCATTAACTA GAATAATGGA CTTTATAATT TATAGATTTC TTTTTATAAT TGTGATATTG

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                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS9(VP7) reference      TCACCATTTC TCAGAGCACA AAATTATGGT ATTAATCTTC CAATCACAGG CTCCATGGAC ATTGCATACG CTAATTCAAC GCAAGAAGAA ACATTCCTCA
pSmart_SA11-N5_GS9 reference    TCACCATTTC TCAGAGCACA AAATTATGGT ATTAATCTTC CAATCACAGG CTCCATGGAC ATTGCATACG CTAATTCAAC GCAAGAAGAA ACATTCCTCA
pSmart_SA11-N5_GS9 NGS          TCACCATTTC TCAGAGCACA AAATTATGGT ATTAATCTTC CAATCACAGG CTCCATGGAC ATTGCATACG CTAATTCAAC GCAAGAAGAA ACATTCCTCA

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                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS9(VP7) reference      CTTCTACACT TTGCCTATAT TATCCGACTG AGGCTGCGAC TGAAATAAAC GATAATTCAT GGAAAGACAC ACTGTCACAA CTATTTCTTA CGAAAGGGTG
pSmart_SA11-N5_GS9 reference    CTTCTACACT TTGCCTATAT TATCCGACTG AGGCTGCGAC TGAAATAAAC GATAATTCAT GGAAAGACAC ACTGTCACAA CTATTTCTTA CGAAAGGGTG
pSmart_SA11-N5_GS9 NGS          CTTCTACACT TTGCCTATAT TATCCGACTG AGGCTGCGAC TGAAATAAAC GATAATTCAT GGAAAGACAC ACTGTCACAA CTATTTCTTA CGAAAGGGTG

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                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS9(VP7) reference      GCCAACTGGA TCCGTATATT TTAAAGAATA TACTAACATT GCATCGTTTT CTGTTGATCC GCAGTTGTAT TGTGATTATA ACGTAGTACT AATGAAATAT
pSmart_SA11-N5_GS9 reference    GCCAACTGGA TCCGTATATT TTAAAGAATA TACTAACATT GCATCGTTTT CTGTTGATCC GCAGTTGTAT TGTGATTATA ACGTAGTACT AATGAAATAT
pSmart_SA11-N5_GS9 NGS          GCCAACTGGA TCCGTATATT TTAAAGAATA TACTAACATT GCATCGTTTT CTGTTGATCC GCAGTTGTAT TGTGATTATA ACGTAGTACT AATGAAATAT

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS9(VP7) reference      GACGCGACGT TGCAATTGGA TATGTCAGAA CTTGCGGATC TAATATTAAA CGAATGGTTG TGTAATCCAA TGGATATTAC TCTGTATTAT TATCAGCAAA
pSmart_SA11-N5_GS9 reference    GACGCGACGT TGCAATTGGA TATGTCAGAA CTTGCGGATC TAATATTAAA CGAATGGTTG TGTAATCCAA TGGATATTAC TCTGTATTAT TATCAGCAAA
pSmart_SA11-N5_GS9 NGS          GACGCGACGT TGCAATTGGA TATGTCAGAA CTTGCGGATC TAATATTAAA CGAATGGTTG TGTAATCCAA TGGATATTAC TCTGTATTAT TATCAGCAAA

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS9(VP7) reference      CTGACGAAGC GAATAAATGG ATATCAATGG GCTCATCATG TACAATTAAA GTATGTCCAC TTAATACACA AACTCTTGGA ATTGGATGCT TGACAACTGA
pSmart_SA11-N5_GS9 reference    CTGACGAAGC GAATAAATGG ATATCAATGG GCTCATCATG TACAATTAAA GTATGTCCAC TTAATACACA AACTCTTGGA ATTGGATGCT TGACAACTGA
pSmart_SA11-N5_GS9 NGS          CTGACGAAGC GAATAAATGG ATATCAATGG GCTCATCATG TACAATTAAA GTATGTCCAC TTAATACACA AACTCTTGGA ATTGGATGCT TGACAACTGA

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS9(VP7) reference      TGCTACAACT TTTGAAGAAG TTGCGACAGC TGAAAAGTTG GTAATTACTG ACGTGGTTGA TGGCGTTAAT CATAAGCTGG ATGTCACAAC AGCAACGTGT
pSmart_SA11-N5_GS9 reference    TGCTACAACT TTTGAAGAAG TTGCGACAGC TGAAAAGTTG GTAATTACTG ACGTGGTTGA TGGCGTTAAT CATAAGCTGG ATGTCACAAC AGCAACGTGT
pSmart_SA11-N5_GS9 NGS          TGCTACAACT TTTGAAGAAG TTGCGACAGC TGAAAAGTTG GTAATTACTG ACGTGGTTGA TGGCGTTAAT CATAAGCTGG ATGTCACAAC AGCAACGTGT

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS9(VP7) reference      ACTATTAGAA ACTGTAAGAA ATTGGGACCA AGAGAAAACG TAGCCGTTAT ACAAGTTGGT GGTTCTGACA TCCTCGATAT AACTGCTGAT CCAACTACTG
pSmart_SA11-N5_GS9 reference    ACTATTAGAA ACTGTAAGAA ATTGGGACCA AGAGAAAACG TAGCCGTTAT ACAAGTTGGT GGTTCTGACA TCCTCGATAT AACTGCTGAT CCAACTACTG
pSmart_SA11-N5_GS9 NGS          ACTATTAGAA ACTGTAAGAA ATTGGGACCA AGAGAAAACG TAGCCGTTAT ACAAGTTGGT GGTTCTGACA TCCTCGATAT AACTGCTGAT CCAACTACTG

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                                2710       2720       2730       2740       2750       2760       2770       2780       2790       2800
SA11-N5_GS9(VP7) reference      CACCACAGAC AGAACGGATG ATGCGAATTA ACTGGAAAAA ATGGTGGCAA GTTTTTTATA CTGTAGTAGA CTATGTAGAT CAGATAATAC AAGTTATGTC
pSmart_SA11-N5_GS9 reference    CACCACAGAC AGAACGGATG ATGCGAATTA ACTGGAAAAA ATGGTGGCAA GTTTTTTATA CTGTAGTAGA CTATGTAGAT CAGATAATAC AAGTTATGTC
pSmart_SA11-N5_GS9 NGS          CACCACAGAC AGAACGGATG ATGCGAATTA ACTGGAAAAA ATGGTGGCAA GTTTTTTATA CTGTAGTAGA CTATGTAGAT CAGATAATAC AAGTTATGTC

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                                2810       2820       2830       2840       2850       2860       2870       2880       2890       2900
SA11-N5_GS9(VP7) reference      CAAAAGATCA AGATCACTAA ATTCAGCAGC ATTTTATTAC AGAGTGTAGG TATAACTTAG GTTAGAATTG TATGATGTGA CC-------- ----------
pSmart_SA11-N5_GS9 reference    CAAAAGATCA AGATCACTAA ATTCAGCAGC ATTTTATTAC AGAGTGTAGG TATAACTTAG GTTAGAATTG TATGATGTGA CCGGGCGTTA TGTGGCCGGC
pSmart_SA11-N5_GS9 NGS          CAAAAGATCA AGATCACTAA ATTCAGCAGC ATTTTATTAC AGAGTGTAGG TATAACTTAG GTTAGAATTG TATGATGTGA CCGGGNNNNA TGTGACCGGC

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                                2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS9 reference    CGGCATGGTC CCAGCCTCCT CGCTGGCGCC GGCTGGGCAA CATTCCGAGG GGACCGTCCC CTCGGTAATG GCGAATGGGA CGGATCCGGC TGCTAACAAA
pSmart_SA11-N5_GS9 NGS          CGGCATGGTC CCAGCCTCCT CGCTGGCGCC GGCTGGGCAA CATTCCGAGG GGACCGTCCC CTCGGTAATG GCGAATGGGA CGGATCCGGC TGCTAACAAA

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                                3010       3020       3030       3040       3050       3060       3070       3080       3090
SA11-N5_GS9(VP7) reference      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------
pSmart_SA11-N5_GS9 reference    GCCCGAAAGG AAGCTGAGTT GGCTGCTGCC ACCGCTGAGC AATAACTAGC ATAACCCCTT GGGGCCTCTA AACGGGTCTT GAGGGGTTTT TTGGAT
pSmart_SA11-N5_GS9 NGS          GCCCGAAAGG AAGCTGAGTT GGCTGCTGCC ACCGCTGAGC AATAACTAGC ATAACCCCTT GGGGCCTCTA AACGGGTCTT GAGGGGTTTT TTGGAT
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Alignment: SA11-N5_GS10(NSP4)

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                                        10         20         30         40         50         60         70         80         90        100
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT
pSmart_SA11-N5_GS10 NGS         ATCGCTCAAT ACTGACCATT TAAATCATAC CTGACCTCCA TAGCAGAAAG TCAAAAGCCT CCGACCGGAG GCTTTTGACT TGATCGGCAC GTAAGAGGTT

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                                       110        120        130        140        150        160        170        180        190        200
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT
pSmart_SA11-N5_GS10 NGS         CCAACTTTCA CCATAATGAA ATAAGATCAC TACCGGGCGT ATTTTTTGAG TTATCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGAGTA TTCAACATTT

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                                       210        220        230        240        250        260        270        280        290        300
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT
pSmart_SA11-N5_GS10 NGS         CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT

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                                       310        320        330        340        350        360        370        380        390        400
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
pSmart_SA11-N5_GS10 NGS         GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTACGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC

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                                       410        420        430        440        450        460        470        480        490        500
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
pSmart_SA11-N5_GS10 NGS         TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       510        520        530        540        550        560        570        580        590        600
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG
pSmart_SA11-N5_GS10 NGS         CACAGAAAAG CATCTCACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGGCAACG

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                                       610        620        630        640        650        660        670        680        690        700
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
pSmart_SA11-N5_GS10 NGS         ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA

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                                       710        720        730        740        750        760        770        780        790        800
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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pSmart_SA11-N5_GS10 reference   ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS10 NGS         ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

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                                       810        820        830        840        850        860        870        880        890        900
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS10 NGS         AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

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                                       910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS10 NGS         TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

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                                      1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS10 NGS         GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

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                                      1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS10 NGS         GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

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                                      1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS10 NGS         TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

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                                      1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS10 NGS         ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

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                                      1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS10 NGS         CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

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                                      1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS10 NGS         GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

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                                       1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS10 NGS         TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

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                                       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS10 NGS         CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                       1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS10(NSP4) reference    ---------- ---------G GCTTTTAAAA GTTCTGTTCC GAGAGAGCGC GTGCGGAAAG ATGGAAAAGC TTACCGACCT CAATTATACA TTGAGTGTAA
pSmart_SA11-N5_GS10 reference   TCTAATACGA CTCACTATAG GCTTTTAAAA GTTCTGTTCC GAGAGAGCGC GTGCGGAAAG ATGGAAAAGC TTACCGACCT CAATTATACA TTGAGTGTAA
pSmart_SA11-N5_GS10 NGS         TCTAATACGA CTCACTATAG GCTTTTAAAA GTTCTGTTCC GAGAGAGCGC GTGCGGAAAG ATGGAAAAGC TTACCGACCT CAATTATACA TTGAGTGTAA

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                                       1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS10(NSP4) reference    TCACTCTAAT GAACAATACA TTGCACACAA TACTTGAGGA TCCAGGAATG GCGTATTTTC CTTATATAGC ATCTGTCTTA ACAGTTTTGT TTGCGCTACA
pSmart_SA11-N5_GS10 reference   TCACTCTAAT GAACAATACA TTGCACACAA TACTTGAGGA TCCAGGAATG GCGTATTTTC CTTATATAGC ATCTGTCTTA ACAGTTTTGT TTGCGCTACA
pSmart_SA11-N5_GS10 NGS         TCACTCTAAT GAACAATACA TTGCACACAA TACTTGAGGA TCCAGGAATG GCGTATTTTC CTTATATAGC ATCTGTCTTA ACAGTTTTGT TTGCGCTACA

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                                       2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS10(NSP4) reference    TAAAGCATCC ATTCCAACAA TGAAAATTGC ATTGAAAACG TCAAAATGTT CATATAAAGT GGTGAAATAT TGTATTGTAA CAATTTTTAA TACGTTGTTA
pSmart_SA11-N5_GS10 reference   TAAAGCATCC ATTCCAACAA TGAAAATTGC ATTGAAAACG TCAAAATGTT CATATAAAGT GGTGAAATAT TGTATTGTAA CAATTTTTAA TACGTTGTTA
pSmart_SA11-N5_GS10 NGS         TAAAGCATCC ATTCCAACAA TGAAAATTGC ATTGAAAACG TCAAAATGTT CATATAAAGT GGTGAAATAT TGTATTGTAA CAATTTTTAA TACGTTGTTA

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                                       2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS10(NSP4) reference    AAATTGGCAG GTTATAAAGA GCAGATAACT ACTAAAGATG AGATAGAAAA GCAAATGGAC AGAGTAGTCA AAGAAATGAG ACGCCAGCTA GAAATGATTG
pSmart_SA11-N5_GS10 reference   AAATTGGCAG GTTATAAAGA GCAGATAACT ACTAAAGATG AGATAGAAAA GCAAATGGAC AGAGTAGTCA AAGAAATGAG ACGCCAGCTA GAAATGATTG
pSmart_SA11-N5_GS10 NGS         AAATTGGCAG GTTATAAAGA GCAGATAACT ACTAAAGATG AGATAGAAAA GCAAATGGAC AGAGTAGTCA AAGAAATGAG ACGCCAGCTA GAAATGATTG

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                                       2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS10(NSP4) reference    ACAAATTGAC TACACGTGAA ATTGAACAAG TAGAGTTGCT TAAACGCATT TACGATAAAT TGACGGTGCA AACGACAGGC GAAATAGATA TGACAAAAGA
pSmart_SA11-N5_GS10 reference   ACAAATTGAC TACACGTGAA ATTGAACAAG TAGAGTTGCT TAAACGCATT TACGATAAAT TGACGGTGCA AACGACAGGC GAAATAGATA TGACAAAAGA
pSmart_SA11-N5_GS10 NGS         ACAAATTGAC TACACGTGAA ATTGAACAAG TAGAGTTGCT TAAACGCATT TACGATAAAT TGACGGTGCA AACGACAGGC GAAATAGATA TGACAAAAGA

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS10(NSP4) reference    GATCAATCAA AAAAACGTGA GAACGCTAGA AGAATGGGAA AGTGGAAAAA ATCCTTATGA ACCAAGAGAA GTGACTGCAG CAATGTAAGA GGTTGAGCTG
pSmart_SA11-N5_GS10 reference   GATCAATCAA AAAAACGTGA GAACGCTAGA AGAATGGGAA AGTGGAAAAA ATCCTTATGA ACCAAGAGAA GTGACTGCAG CAATGTAAGA GGTTGAGCTG
pSmart_SA11-N5_GS10 NGS         GATCAATCAA AAAAACGTGA GAACGCTAGA AGAATGGGAA AGTGGAAAAA ATCCTTATGA ACCAAGAGAA GTGACTGCAG CAATGTAAGA GGTTGAGCTG

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS10(NSP4) reference    CCGTCGACTG TCCTCGGAAG CGGCGGAGTT CTTTACAGTA AGCACCATCG GACCTGATGG CTGACTGAGA AGCCACAGTC AGCCATATCG CGTGTGGCTC
pSmart_SA11-N5_GS10 reference   CCGTCGACTG TCCTCGGAAG CGGCGGAGTT CTTTACAGTA AGCACCATCG GACCTGATGG CTGACTGAGA AGCCACAGTC AGCCATATCG CGTGTGGCTC
pSmart_SA11-N5_GS10 NGS         CCGTCGACTG TCCTCGGAAG CGGCGGAGTT CTTTACAGTA AGCACCATCG GACCTGATGG CTGACTGAGA AGCCACAGTC AGCCATATCG CGTGTGGCTC

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                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS10(NSP4) reference    AAGCCTTAAT CCCGTTTAAC CAATCCGGTC AGCACCGGAC GTTAATGGAA GGAACGGTCT TAATGTGACC ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   AAGCCTTAAT CCCGTTTAAC CAATCCGGTC AGCACCGGAC GTTAATGGAA GGAACGGTCT TAATGTGACC GGCCGGCATG GTCCCAGCCT CCTCGCTGGC
pSmart_SA11-N5_GS10 NGS         AAGCCTTAAT CCCGTTTAAC CAATCCGGTC AGCACCGGAC GTTAATGGAA GGAACGGTCT TAATGTGACC TGCCGGCATG GTCCCAGCCT CCTCGCTGGC

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                                2610       2620       2630       2640       2650       2660       2670       2680       2690       2700
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS10 reference   GCCGGCTGGG CAACATTCCG AGGGGACCGT CCCCTCGGTA ATGGCGAATG GGACGGATCC GGCTGCTAAC AAAGCCCGAA AGGAAGCTGA GTTGGCTGCT
pSmart_SA11-N5_GS10 NGS         GCCGGCTGGG CAACATTCCG AGGGGACCGT CCCCTCGGTA ATGGCGAATG GGACGGATCC GGCTGCTAAC AAAGCCCGAA AGGAAGCTGA GTTGGCTGCT

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                                2710       2720       2730       2740       2750       2760       260
SA11-N5_GS10(NSP4) reference    ---------- ---------- ---------- ---------- ---------- ---------- ---------
pSmart_SA11-N5_GS10 reference   GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT CTAAACGGGT CTTGAGGGGT TTTTTGGAT
pSmart_SA11-N5_GS10 NGS         GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT CTAAACGGGT CTTGAGGGGT TTTTTGGAT
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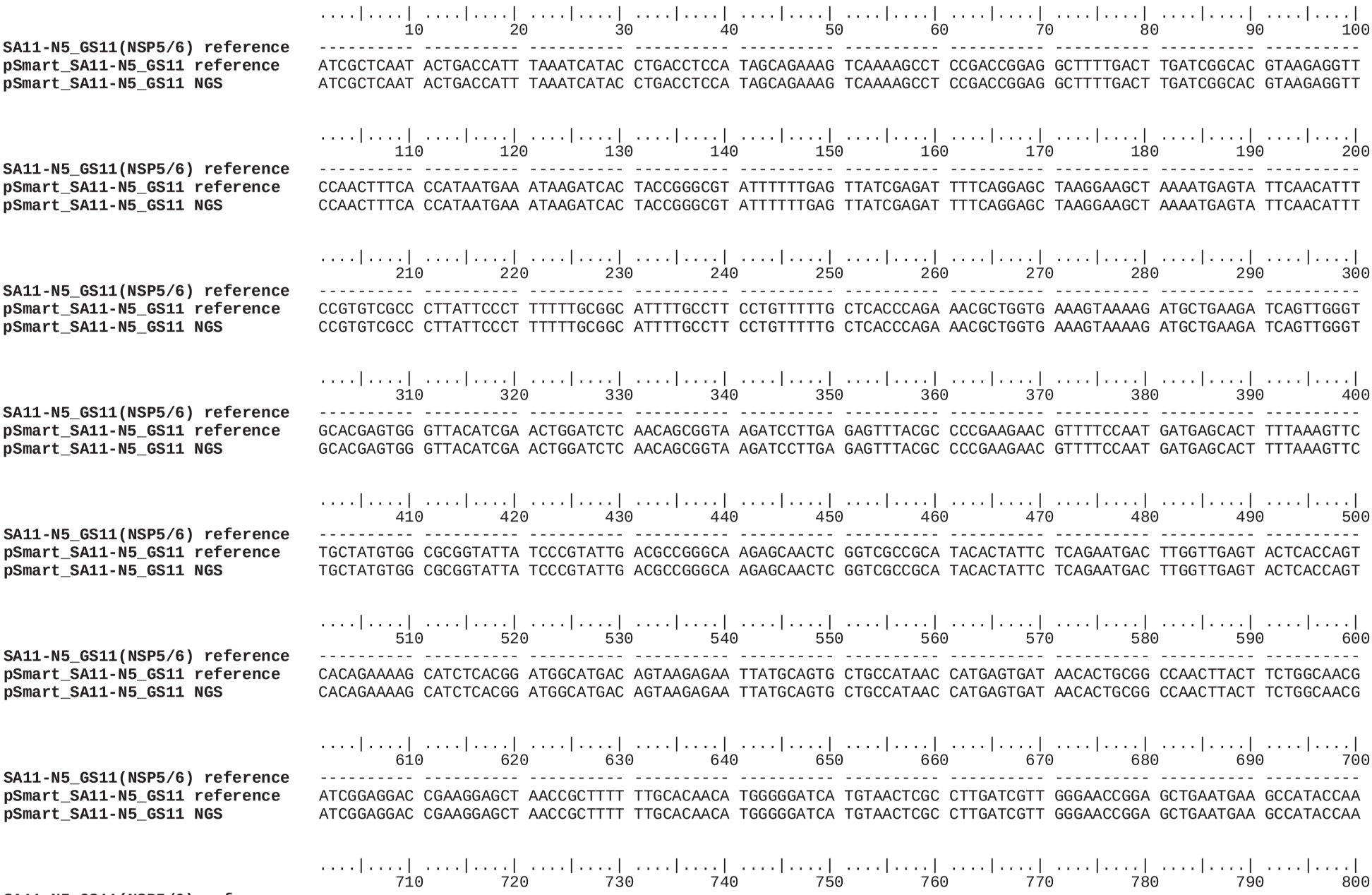
Alignment: SA11-N5_GS11(NSP5/6)



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pSmart_SA11-N5_GS11 reference    ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT
pSmart_SA11-N5_GS11 NGS          ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT

                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        810        820        830        840        850        860        870        880        890        900
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
pSmart_SA11-N5_GS11 NGS          AGACTGGATG GAGGCGGATA AAGTTGCAGG ATCACTTCTG CGCTCGGCCC TCCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG

                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        910        920        930        940        950        960        970        980        990       1000
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
pSmart_SA11-N5_GS11 NGS          TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGCAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA

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                                       1010       1020       1030       1040       1050       1060       1070       1080       1090       1100
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT
pSmart_SA11-N5_GS11 NGS          GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAATGAGGG CCCAAATGTA ATCACCTGGC TCACCTTCGG GTGGGCCTTT CTGCGTTGCT

                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG
pSmart_SA11-N5_GS11 NGS          GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAT GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1210       1220       1230       1240       1250       1260       1270       1280       1290       1300
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC
pSmart_SA11-N5_GS11 NGS          TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

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                                       1310       1320       1330       1340       1350       1360       1370       1380       1390       1400
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC
pSmart_SA11-N5_GS11 NGS          ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

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                                       1410       1420       1430       1440       1450       1460       1470       1480       1490       1500
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT
pSmart_SA11-N5_GS11 NGS          CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

                                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       1510       1520       1530       1540       1550       1560       1570       1580       1590       1600
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SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT
pSmart_SA11-N5_GS11 NGS          GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTCG GAAAAAGAGT

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                                         1610       1620       1630       1640       1650       1660       1670       1680       1690       1700
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
pSmart_SA11-N5_GS11 NGS          TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT

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                                         1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference    CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA
pSmart_SA11-N5_GS11 NGS          CCTTTGATTT TCTACCGAAG AAAGGCCCAC CCGTGAAGGT GAGCCAGTGA GTTGATTGCA GTCCAGTTAC GCTGGAGTCT GAGGCTCGTC CTGAATGATA

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                                         1810       1820       1830       1840       1850       1860       1870       1880       1890       1900
SA11-N5_GS11(NSP5/6) reference   ---------- ---------- GCTTTTAAAG CGCTACAGTG ATGTCTCTCA GTATTGACGT GACGAGTCTT CCTTCTATTC CTTCAACTAT ATATAAGAAT
pSmart_SA11-N5_GS11 reference    TCTAATACGA CTCACTATA- GCTTTTAAAG CGCTACAGTG ATGTCTCTCA GTATTGACGT GACGAGTCTT CCTTCTATTC CTTCAACTAT ATATAAGAAT
pSmart_SA11-N5_GS11 NGS          TCTAATACGA CTCACTATAG GCTTTTAAAG CGCTACAGTG ATGTCTCTCA GTATTGACGT GACGAGTCTT CCTTCTATTC CTTCAACTAT ATATAAGAAT

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                                         1910       1920       1930       1940       1950       1960       1970       1980       1990       2000
SA11-N5_GS11(NSP5/6) reference   GAATCGTCTT CAACAACGTC AACTCTTTCT GGAAAATCTA TTGGTAGGAG TGAACAGTAC ATTTCACCAG ATGCAGAAGC ATTCAATAAA TACATGCTGT
pSmart_SA11-N5_GS11 reference    GAATCGTCTT CAACAACGTC AACTCTTTCT GGAAAATCTA TTGGTAGGAG TGAACAGTAC ATTTCACCAG ATGCAGAAGC ATTCAATAAA TACATGCTGT
pSmart_SA11-N5_GS11 NGS          GAATCGTCTT CAACAACGTC AACTCTTTCT GGAAAATCTA TTGGTAGGAG TGAACAGTAC ATTTCACCAG ATGCAGAAGC ATTCAATAAA TACATGCTGT

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                                         2010       2020       2030       2040       2050       2060       2070       2080       2090       2100
SA11-N5_GS11(NSP5/6) reference   CGAAGTCTCC AGAGGATATT GGACCATCTG ATTCTGCTTC AAACGATCCA CTCACCAGTT TTTCGATTAG ATCGAATGCA GTTAAGACAA ATGCAGACGC
pSmart_SA11-N5_GS11 reference    CGAAGTCTCC AGAGGATATT GGACCATCTG ATTCTGCTTC AAACGATCCA CTCACCAGTT TTTCGATTAG ATCGAATGCA GTTAAGACAA ATGCAGACGC
pSmart_SA11-N5_GS11 NGS          CGAAGTCTCC AGAGGATATT GGACCATCTG ATTCTGCTTC AAACGATCCA CTCACCAGTT TTTCGATTAG ATCGAATGCA GTTAAGACAA ATGCAGACGC

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                                         2110       2120       2130       2140       2150       2160       2170       2180       2190       2200
SA11-N5_GS11(NSP5/6) reference   TGGCGTGTCT ATGGATTCAT CAGCACAATC ACGACCTTCA AGTAATGTCG GATGCGATCA AGTGGATTTC TCCTTAAATA AAGGCTTAAA AGTAAAAGCT
pSmart_SA11-N5_GS11 reference    TGGCGTGTCT ATGGATTCAT CAGCACAATC ACGACCTTCA AGTAATGTCG GATGCGATCA AGTGGATTTC TCCTTAAATA AAGGCTTAAA AGTAAAAGCT
pSmart_SA11-N5_GS11 NGS          TGGCGTGTCT ATGGATTCAT CAGCACAATC ACGACCTTCA AGTAATGTCG GATGCGATCA AGTGGATTTC TCCTTAAATA AAGGCTTAAA AGTAAAAGCT

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                                         2210       2220       2230       2240       2250       2260       2270       2280       2290       2300
SA11-N5_GS11(NSP5/6) reference   AATTTGGACT CATCAATATC AATATCTACG GATACTAAAA AGGAGAAATC AAAACAAAAC CATAAAAGTA GGAAGCACTA CCCAAGAATT GAAGCAGAGT
pSmart_SA11-N5_GS11 reference    AATTTGGACT CATCAATATC AATATCTACG GATACTAAAA AGGAGAAATC AAAACAAAAC CATAAAAGTA GGAAGCACTA CCCAAGAATT GAAGCAGAGT
pSmart_SA11-N5_GS11 NGS          AATTTGGACT CATCAATATC AATATCTACG GATACTAAAA AGGAGAAATC AAAACAAAAC CATAAAAGTA GGAAGCACTA CCCAAGAATT GAAGCAGAGT

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                                2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
SA11-N5_GS11(NSP5/6) reference  CTGATTCAGA TGATTATGTA CTGGATGATT CAGATAGTGA TGATGGTAAA TGTAAGAACT GTAAATATAA GAAGAAATAC TTCGCATTAA GAATGAGAAT
pSmart_SA11-N5_GS11 reference   CTGATTCAGA TGATTATGTA CTGGATGATT CAGATAGTGA TGATGGTAAA TGTAAGAACT GTAAATATAA GAAGAAATAC TTCGCATTAA GAATGAGAAT
pSmart_SA11-N5_GS11 NGS         CTGATTCAGA TGATTATGTA CTGGATGATT CAGATAGTGA TGATGGTAAA TGTAAGAACT GTAAATATAA GAAGAAATAC TTCGCATTAA GAATGAGAAT

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                                2410       2420       2430       2440       2450       2460       2470       2480       2490       2500
SA11-N5_GS11(NSP5/6) reference  GAAACAAGTC GCAATGCAAT TGATTGAAGA TTTGTAAGTC TGACCTGGGA ACACACTAGG GAGCTCCCCA CTCCCGTTTT GTGACC---- ----------
pSmart_SA11-N5_GS11 reference   GAAACAAGTC GCAATGCAAT TGATTGAAGA TTTGTAAGTC TGACCTGGGA ACACACTAGG GAGCTCCCCA CTCCCGTTTT GTGACCGGCC GGCATGGTCC
pSmart_SA11-N5_GS11 NGS         GAAACAAGTC GCAATGCAAT TGATTGAAGA TTTGTAAGTC TGACCTGGGA ACACACTAGG GAGCTCCCCA CTCCCGTTTT GTGACCGGCC GGCATGGTCC

                                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                2510       2520       2530       2540       2550       2560       2570       2580       2590       2600
SA11-N5_GS11(NSP5/6) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
pSmart_SA11-N5_GS11 reference   CAGCCTCCTC GCTGGCGCCG GCTGGGCAAC ATTCCGAGGG GACCGTCCCC TCGGTAATGG CGAATGGGAC GGATCCGGCT GCTAACAAAG CCCGAAAGGA
pSmart_SA11-N5_GS11 NGS         CAGCCTCCTC GCTGGCGCCG GCTGGGCAAC ATTCCGAGGG GACCGTCCCC TCGGTAATGG CGAATGGGAC GGATCCGGCT GCTAACAAAG CCCGAAAGGA

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                                2610       2620       2630       2640       2650       2660       2670       2680
SA11-N5_GS11(NSP5/6) reference  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----
pSmart_SA11-N5_GS11 reference   AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAACTAGCA TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGGAT
pSmart_SA11-N5_GS11 NGS         AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAACTAGCA TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGGAT
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