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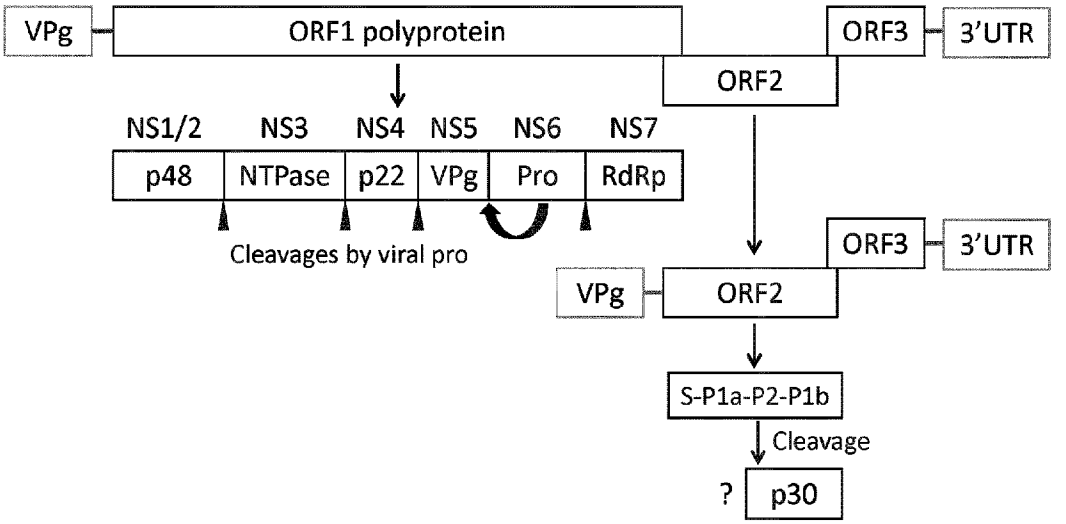
(72) **Inventeurs/Inventors:**
LAVOIE, PIERRE-OLIVIER, CA;
D'AOUST, MARC-ANDRE, CA

(73) **Propriétaire/Owner:**
MEDICAGO INC., CA

(74) **Agent:** GOWLING WLG (CANADA) LLP

(54) **Titre : PROTEINES DE FUSION DE NOROVIRUS ET PPV COMPRENANT DES PROTEINES DE FUSION DE NOROVIRUS**
(54) **Title: NOROVIRUS FUSION PROTEINS AND VLPS COMPRISING NOROVIRUS FUSION PROTEINS**



(57) **Abrégé/Abstract:**
Nucleic acids encoding norovirus VP1 fusion proteins and VLPs comprising the norovirus VP1 fusion proteins are provided. Methods for norovirus VP1 fusion protein and norovirus VLP production in plants are also described. The VP1 fusion protein comprises, a first sequence encoding an S domain derived from a first norovirus strain, and a second sequence encoding a P domain derived from a second norovirus strain.

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(71) Applicant: **MEDICAGO INC.** [CA/CA]; Suite 600 - 1020 Route de l'Eglise, Quebec, Québec G1V 3V9 (CA).

(72) Inventors: **LAVOIE, Pierre-Olivier**; 621 Franklin, Quebec, Québec G1N 2L7 (CA). **D'AOUST, Marc-Andre**; 3925 rue Louise-Fiset, Quebec, Québec G1X 4N4 (CA).

(74) Agent: **SECHLEY, Konrad** et al.; Gowling WLG (Canada) LLP., 2300 - 550 Burrard Street, Vancouver, British Columbia V6C 2B5 (CA).

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(54) Title: NOROVIRUS FUSION PROTEINS AND VLPS COMPRISING NOROVIRUS FUSION PROTEINS

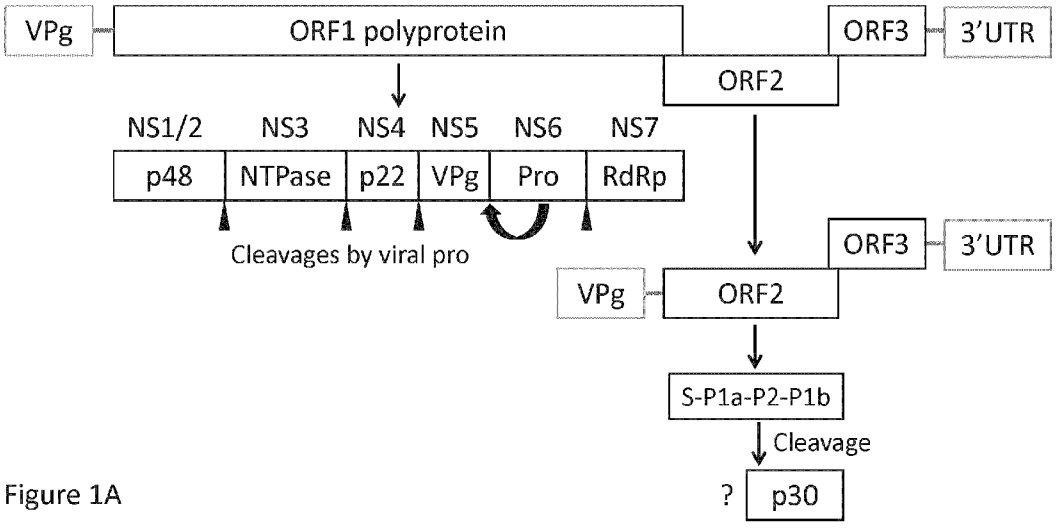

Figure 1A

(57) Abstract: Nucleic acids encoding norovirus VP1 fusion proteins and VLPs comprising the norovirus VP1 fusion proteins are provided. Methods for norovirus VP1 fusion protein and norovirus VLP production in plants are also described. The VP1 fusion protein comprises, a first sequence encoding an S domain derived from a first norovirus strain, and a second sequence encoding a P domain derived from a second norovirus strain.

[Continued on next page]

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Norovirus fusion proteins and VLPs comprising norovirus fusion proteins

FIELD OF INVENTION

[0001] The present invention relates to norovirus fusion proteins, VLPs comprising norovirus fusion proteins, and methods of producing the same.

BACKGROUND OF THE INVENTION

[0002] The global disease burden attributed to norovirus infection is high, being associated with an estimated 20% of all worldwide diarrheal cases and causing over 200,000 deaths annually. Noroviruses are the primary cause of foodborne disease outbreaks in North America and are the causative agent for the majority of healthcare-associated outbreaks amongst the elderly. Norovirus strains are also recognized as being the leading cause of pediatric gastrointestinal illness worldwide.

[0003] Noroviruses comprise one of a number of genera of the family *Caliciviridae*. The human norovirus genome is a single-stranded, positive-sense RNA molecule encoding three open reading frames (ORFs) and capped on its 5' end by a VPg protein. ORF1 encodes six non-structural viral proteins, including VPg, an RNA-dependent RNA polymerase, and a viral protease. ORF2 encodes the major structural capsid protein (VP1). ORF3 encodes a minor capsid protein (VP2).

[0004] VP1 is comprised of 2 domains: a shell (S) domain, and a protruding (P) domain. The P domain is further comprised of a P1 sub-domain and a P2 sub-domain. The P2 sub-domain is referred to as the hypervariable domain and is thought to play an important role in receptor binding and immune reactivity.

[0005] VP1 proteins form dimers via P domain-mediated protein interactions. Dimerization increases the stability of the virion capsid and results in formation of the protrusions extending from the base core of the norovirus particle formed by S domains. When expressed, norovirus VP1 proteins can automatically assemble to form 2 virion structures: a 180-mer capsid structure with T=3 icosahedral symmetry having a 38-40 nm diameter; and a 60-mer capsid structure with T=1 icosahedral symmetry having a 23 nm diameter.

[0006] VP2, the minor structural protein, has a molecular weight (MW) of approximately 21-24 kDa. Studies suggest that VP2 is highly basic and located inside the capsid. The function of VP2 has not yet been fully understood but it is generally believed to play a role in capsid stability by protecting the virions from disassembly and degradation (Bertolotti-Ciarlet A., Crawford S.E., Hutson A.M., Estes M.K. 2003, J. Virol. 77:11603-11615). VP2 may also have a function during RNA genome packaging. The amount of VP2 minor structural protein in virions is relatively low with 1.5 to 8 copies incorporated into the mature virion. Bertolotti-Ciarlet et. al. (2003) report that in insect and mammalian cells, VLPs composed of VP1/VP2 are more resistant to protease cleavage than those with only VP1, and that expression of VP2 in *cis*, results in an increase in VP1 protein production. In addition, the presence of the 3'UTR downstream of the ORF2 gene increases the steady-state levels of NV ORF2 mRNA. The greatest increase in VP1 expression was observed when ORF2 + ORF3 + 3'UTR, residing on the same construct and under regulation of one promoter, was expressed. Expression of VP2 in *trans* did not result in any increase in VP1 expression, indicating that the subgenomic organization of ORF2-ORF2-3'UTR was required for the observed increase in VP1 production.

[0007] Noroviruses are classified according to their phylogenetic clustering of the VP1 amino acid sequence. Seven genogroups have been classified to date (GI through GVII) with only genogroups GI, GII, and GIV known to infect humans. Of the 32 specific genotypes currently associated with human infections, GII.4 noroviruses have been responsible for the majority of recent norovirus outbreaks. New strains of GII.4 emerge every two to three years, evolving by a process driven by mutations in epitope determining regions of the hypervariable P2 domain of VP1. This process allows the norovirus to escape humoral immune responses acquired by previous exposure to earlier strains.

[0008] While faced with the difficulty of rapidly evolving and genetically diverse norovirus strains, the development of effective norovirus vaccines has been exacerbated by additional challenges. For instance, until recently, human norovirus could not be grown in cell culture and even now, robust cell culture systems for both VLPs and live attenuated noroviruses are lacking.

[0009] An additional challenge in vaccine development is that immunity to norovirus infection is strain and genotype specific with minimal cross-immunity conferred against other genogroups. Furthermore, immunity to a norovirus strain is not life-long and is estimated to persist from anywhere between six months and nine years.

[0010] Various approaches have been undertaken to develop a suitable vaccine against norovirus infection including the production of recombinant norovirus proteins in plants and recombinant generation of fusion/chimeric VP1 proteins.

[0011] Mason *et al.* (*Proc Natl Acad Sci U.S.A.*, 1996, 93(11):5335-40) teach the use of genetically engineered tobacco plants and potato tubers to express GI.1 norovirus VLPs from native VP1 protein. The plant produced norovirus VLPs are morphologically and physically similar to the 38 nm Norwalk VLPs produced in insect cells. Oral administration of purified tobacco-produced Norwalk VLPs from native capsid protein, or potato tubers expressing GI.1 capsid protein induced a humoral immune response in mice and humans (Tacket *et al.*, *J. Infect. Dis.*, 2000, 182(1):302-5).

[0012] Huang *et al.* (*Biotechnol. Bioeng.*, 2009, 103(4):706-14) describe a geminivirus-derived DNA replicon vector for production of GI.1 norovirus VLP in plants. Co-delivery of bean yellow dwarf virus-derived vector and Rep/RepA-supplying vector in *Nicotiana benthamiana* resulted in rapid and robust protein production.

[0013] Coit *et al.* (WO 2007/081447; US 7,527,801; US 8,119,145; US 8,124,104; US 8, 142, 793; US 9,428, 739) teach polynucleotides encoding capsid proteins and other immunogenic proteins from norovirus. The production of norovirus-derived multiple epitope fusion antigens comprising a norovirus NTPase-polymerase fusion protein is also described. The fusion protein may comprise a linker sequence. Methods to produce norovirus fusion proteins comprising VP1 are also disclosed.

[0014] Steadman *et al.* (U.S. 8,980,275) describe a chimeric protein comprising a Calicivirus capsid protein and at least one heterologous antigen, and the formation of VLPs when the chimeric protein is expressed in a host cell. A chimeric protein

comprising a heterologous antigen, or fragment thereof, inserted into a P2 domain of the Calicivirus protein is also disclosed.

[0015] Lin *et al.* (WO 2016/019890) teach a fusion protein in which an antigen is fused, with or without a linker sequence, on both its N-terminal and C-terminal ends, to viral structural proteins, or fragments thereof, and wherein fusion improves the folding and antigenicity of the antigen. The viral structural protein may be any protein that contributes to the structure of the capsid or protein core of the virus, and the norovirus S domain or P domain are mentioned as examples.

[0016] Settembre *et al.* (U.S. 14/946,324) disclose immunogenic compositions comprising chimeric norovirus VP1 proteins capable of forming VLPs produced in insect cells, mammalian cells, avian cells, bacterial cells, yeast cells, or *Tetrahymena* cells. The chimeric VP1 proteins have all, or a portion, of a VP1 P domain from one strain of norovirus replaced with all, or a portion, of a P domain from a non-homologous norovirus strain.

[0017] Noroviruses are known to bind specific histo-blood group antigens (HBGA). Huo *et al.* (*Virus Res.*, 2016, 224:1-5) teach the production of chimeric VP1 capsid proteins where the P2 domain of a GII.4 Sydney 2012-like variant norovirus is exchanged for the P2 domain of a GII.3 strain norovirus. Results from *in vitro* HBGA-binding blockade assays indicate that although GII.3 norovirus VLPs do not bind to any synthetic or salivary HBGAs tested, the chimeric VLPs are capable of binding synthetic blood type A (trimer) and Le(x) HBGAs and blood type A, B and O salivary HBGAs. Furthermore, Huo *et al.* demonstrate that this binding can be competitively inhibited by anti-GII.3 serum but not anti-GI.2 or anti GII.4 serum.

SUMMARY OF THE INVENTION

[0018] The present invention relates to norovirus fusion proteins, virus like particles (VLPs) comprising norovirus fusion proteins, and methods of producing the same.

[0019] It is an object of the invention to produce norovirus fusion proteins, VLPs comprising norovirus fusion proteins, and to producing VLPs comprising norovirus fusion proteins in plants.

[0020] As described herein there is provided a nucleic acid encoding a norovirus VP1 fusion protein comprising, a first sequence encoding an S domain derived from a first norovirus strain, and a second sequence encoding a P domain derived from a second norovirus strain, the first and second sequence are selected from norovirus genogroups GI, GII, and GIV.

[0021] Also provided is the nucleic acid encoding the norovirus VP1 fusion protein as described above, wherein the first and second norovirus strains are independently selected from norovirus genotypes GI.1, GI.2, GI.3, GI.4, GI.5, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21 is also provided. For example, which is not to be considered limiting, the first norovirus strain and the second norovirus strain may be independently selected from norovirus subtypes:

[0022] GI.1/US/1968,

[0023] GI.2/Leuven/2003/Bel,

[0024] GI.3/S29/2008/Lilla Edet/Sweden,

[0025] GI.5/AlbertaEI390/2013/CA,

[0026] GII.1/Ascension208/2010/USA,

[0027] GII.12/HS206/2010/USA,

[0028] GII.13/VA173/2010/USA,

[0029] GII.14/8610/Saga/2008/JPN,

[0030] GII.17/Kawazaki/2014/A0A077KVU6, and

[0031] GII.21/Salisbury150/2011/USA.

[0032] The nucleic acid as described above may also comprise a fifth sequence encoding a CPMV enhancer, the CPMV enhancer operatively linked with the first, second, third, and fourth sequences. The nucleic acid as described above may also be optimized for human codon usage, increased GC content, or a combination thereof.

[0033] A norovirus VP1 fusion protein encoded by the nucleic acid as described above is also described herein. Furthermore, a virus like particle (VLP) comprising the norovirus VP1 fusion protein encoded by the nucleic acid is also disclosed.

[0034] Methods to produce an antibody or antibody fragment using the norovirus fusion VP1 fusion protein or the VLP encoded by the nucleic acid, or the VLP comprising the norovirus VP1 fusion protein and norovirus VP2 protein encoded by the nucleic acid complex, are described herein. An antibody, an antibody fragment, or a combination thereof, produced using these methods is also provided.

[0035] The present disclosure also describes a method of producing a norovirus VP1 fusion protein in a plant host cell, for example the plant, the portion of a plant, or the plant cell. The method comprises introducing the nucleic acid, or nucleic acid complex, as described above into the plant host cell, and incubating the plant host cell under conditions that permit expression of the norovirus VP1 fusion protein. The method may further comprises a step of harvesting the plant host cell, for example the plant, the portion of a plant, or the plant cell, and purifying the norovirus VP1 fusion protein.

[0036] As described herein, there is a method of producing a VLP comprising a norovirus VP1 fusion protein in a plant, portion of the plant, or a plant cell. The method comprises introducing the nucleic acid as described herein into the plant, portion of the plant, or the plant cell, and incubating the plant, portion of the plant, or the plant cell under conditions that permit expression of the nucleic acid and the formation of the VLP. The method of producing the VLP may further comprise a step of harvesting the plant, portion of the plant, or the plant cell, producing a plant extract, and purifying the VLP, wherein the VLP has a diameter of about 15 nm to 50 nm, for example, about 23 nm (for T=1 icosahedral symmetry) or about 38 to about 40 nm (for T=3 icosahedral symmetry). Furthermore, in the step of introducing, a second nucleic acid sequence encoding a norovirus VP2 protein may be introduced in the plant, portion of the plant, or the plant cell, and in the step of incubating, the conditions permit co-expression and co-production, of both the VP1 fusion protein and the VP2 protein in the plant, the portion of a plant, or the plant cell

[0037] A plant, portion of a plant, or a plant cell comprising the VLP produced by the method described above is also provided herein. A plant extract comprising the VLP produced by this method is also described.

[0038] Also provided is a composition for inducing an immune response. The composition comprises, an effective dose of the norovirus VP1 fusion protein encoded by the nucleic acid as described herein; and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient. Alternatively, the composition may comprise, an effective dose of the VLP produced by the method described herein, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

[0039] A method of producing an antibody or an antibody fragment is also described. The method comprises, administering the norovirus fusion VP1 fusion protein as described above to a subject in need thereof, or a host animal, thereby producing the antibody or the antibody fragment.

[0040] Additionally there is provided a vaccine for inducing an immune response in a subject in need thereof, the vaccine comprising an effective dose of the norovirus VP1 fusion protein encoded by the nucleic acid described herein. Alternatively, the vaccine may comprise an effective dose of VLP produced by the method described herein.

[0041] The present disclosure also provides a method of inducing immunity to a norovirus infection in a subject comprising administering the norovirus VP1 fusion protein encoded by the nucleic acid described herein. The norovirus VP1 fusion protein may be administered orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously. Also provided is a method of inducing immunity to a norovirus infection in a subject comprising of administering the VLP produced by the method described herein. The VLP may be administered orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[0042] Also described herein is a nucleic acid complex comprising, a VP1 sequence encoding a norovirus VP1 fusion protein, and a VP2 sequence encoding a norovirus VP2 protein, the VP1 sequence comprising a first and a second nucleic acid sequence, the first nucleic acid sequence encoding an S domain derived from a first

norovirus strain, the second nucleic acid sequence encoding a P domain derived from a second norovirus strain, the VP2 sequence comprising a third nucleic acid sequence derived from the first norovirus strain and encoding the norovirus VP2 protein, wherein the VP1 sequence is operatively linked to a first regulatory region, and the VP2 sequence is operatively linked to a second regulatory region, and the VP1 sequence and the VP2 sequence are located on one nucleic acid segment, or the VP1 sequence and the VP2 sequence are located on separate nucleic acid segments. The first regulatory region, the second regulatory region, or the first and second regulatory region of the nucleic acid complex may comprise a CPMV enhancer element that is operatively linked with a promoter active in the plant. For example, the first and the second regulatory region may comprise the CPMV enhancer element, and the first and the second regulatory region may comprise the same promoter. Furthermore, the first and the second regulatory region may comprise a CPMV enhancer element, and the CPMV enhancer sequence of the first and the second regulatory region may be the same CPMV enhancer sequence. The first, the second, the third nucleic acid sequence, or all of the first, second and third nucleic acid sequence, may be optimized for human codon usage, increased GC content, or a combination thereof.

[0043] Also provided herein is a VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein encoded by the nucleic acid complex as defined above. The VLP may have a diameter of about 15 nm to 50 nm, for example, from about 23 nm or about 38nm.

[0044] A method of producing a virus like particle (VLP) in a plant, portion of a plant, or a plant cell is also described. The method comprises introducing the nucleic acid complex as defined above into the plant, the portion of a plant, or the plant cell, and incubating the plant, the portion of a plant, or the plant cell under conditions that permit the production of the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein. The method may further comprises a step of harvesting the plant, the portion of a plant, or the plant cell. Furthermore, the method may comprises a step of extracting, purifying, or both extracting and purifying, the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein, from the plant, the portion of a plant, or the plant cell.

[0045] Also included herein is a plant, portion of the plant, or the plant cell comprising the nucleic acid complex as described above. Furthermore, a plant extract comprising the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein, produced by the method described above is provided

[0046] Also described herein is the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein produced by the method described above. The VLP may have a diameter of about 15 nm to 50 nm, for example about 23 nm or about 38nm. Furthermore, a plant, portion of the plant, or the plant cell comprising the VLP comprising the VP1 fusion protein and the norovirus VP2 protein as described above is also provided.

[0047] A composition for inducing an immune response comprising, an effective dose of the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein described above, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient is also presented herein. Also provided, is a method for inducing immunity to a norovirus infection in a subject, comprising, administering the composition as just described to the subject. Furthermore, the composition may be administered to the subject orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[0048] Also described is a method for inducing immunity to a norovirus infection in a subject, the method comprising administering the VLP comprising, an effective dose of the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein as described above, to the subject. The VLP may be administered to the subject orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[0049] A vaccine is also described herein. The vaccine comprising an effective dose of the VLP of claim 66 for inducing an immune response. Also presented is a method for inducing immunity to a norovirus infection in a subject, comprising administering the vaccine as just described to the subject. The vaccine may be administered to the subject orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[0050] The present disclosure also describes a method of producing an antibody or an antibody fragment comprising, administering the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein, described above, to a subject in need thereof, or a host animal, thereby producing the antibody or the antibody fragment, is also provided. For example, the antibody or antibody fragment may recognizes an epitope of the P domain.

[0050a] There is provided a norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI, and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

[0051] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0053] **FIGURE 1A** shows a schematic representation of the linear structure of the norovirus genome and the polyprotein and proteins translated therefrom. **FIGURE 1B** shows a ribbon diagram representation of the 3-dimensional structure of the norovirus VP1 protein comprising a shell (S) domain, a P1 subdomain (P1), and a P2 subdomain (P2). **FIGURE 1C** shows a ribbon diagram representation of the 3-dimensional structure of a norovirus VP1 protein dimer comprising of two S domains (S), two P1 subdomains (P1), and two P2 subdomains (P2).

[0054] **FIGURE 2A** shows Uniprot and NCBI references for several norovirus VP1 (upper panel) and VP2 (lower panel) proteins. **FIGURE 2B** shows NCBI references for several norovirus VP1 and VP2 nucleic acid sequences. **FIGURE 2C** shows the amino acid sequence identity between norovirus VP1 (upper panel) and VP2 (lower

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panel) proteins. G1.1 (SEQ ID NO:1), G1.2 (SEQ ID NO:2), GI.3 (SEQ ID NO:3), GII.4 (SEQ ID NO:4), GII.6 (SEQ ID NO:5), GII.13 (SEQ ID NO:6), GII.17 (SEQ ID NO:7). **FIGURE 2D** shows the amino acid sequence identity between several strains of norovirus GII.4 VP1 proteins (upper panel), the amino acid sequence identity between several strains of norovirus GII.4 VP1 P domain (middle panel), and the amino acid sequence identity between several strains of norovirus GII.4 P2 domain (lower panel). US96: GII.4/Dresden174/1997/DE (GII.4 variant: US

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1995/96; SEQ ID NO:8); **FH02**: GII.4/FarmingtonHills/2002/US (SEQ ID NO:9); **Hnt04**:GII.4/Hunter-NSW504D/2004/AU (SEQ ID NO:10); **2006b:** GII.4/Shellharbour-NSW696T/2006/AU (SEQ ID NO:11); **NO09:** GII.4/Orange-NSW001P/2008/AU (GII.4 variant New Orleans 2009; SEQ ID NO:12); **Syd12:** Hu/GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:4).

[0055] **FIGURE 3A** shows norovirus protein production. Crude protein extracts prepared from *N. benthamiana* leaves, six and nine days post infiltration (DPI) of native (wildtype; wt) GI.1/United States/Norwalk/1968 ORF2 alone (VP1) (SEQ ID NO's: 1 (aa) and 13 (na); construct # 2720) and ORF 3 alone (VP2) (SEQ ID NO's: 14 (aa) and 15 (na); construct # 2721), ORF2/ORF3 (VP1/VP2; SEQ ID NO:16 (na); construct # 2722) and ORF2/ORF3/3'UTR (VP1/VP2/3'UTR; SEQ ID NO:17 (na); construct # 2723), and human codon-optimized (hCod) GI.1/United States/Norwalk/1968 ORF2 alone (VP1) (SEQ ID NO:18; construct # 2724), ORF3 alone (hCod VP2) (SEQ ID NO:19 (na); construct # 2725), ORF2/ORF3 (hCod VP1/VP2; in *cis*, on the same construct; SEQ ID NO:20 (na); construct # 2726) and ORF2/ORF3/3'UTR (hCod VP1/PV2/3'UTR; SEQ ID NO:21; construct # 2727), expression constructs. VP1+VP2: ORF2 (SEQ ID NO's: 1 (aa) and 13 (na); construct # 2720) and ORF 3 (SEQ ID NO's: 14 (aa) and 15 (na); construct # 2721) expressed on separated constructs, in *trans*. Proteins were analyzed by Coomassie-stained SDS-PAGE. **FIGURE 3B** upper panel shows norovirus protein expression and VLP assembly using Coomassie-stained SDS-PAGE analysis of fractions from an iodixanol density gradient separation of crude protein extracts prepared from *N. benthamiana* leaves expressing human codon-optimized GI.1/United States/Norwalk/1968 VP1 (construct #2724), or human codon-optimized VP1 (construct #2724) and co-expressed with human codon-optimized VP2 (construct #2725). Lower panel shows electron micrographs of norovirus VLPs purified from 33% iodixanol gradient fractions of VP1 or co-expression of VP1 and VP2 proteins. An electron micrograph of native norovirus VLP is shown for comparison. **FIGURE 3C** shows norovirus protein expression and VLP assembly using Coomassie-stained SDS-PAGE analysis of fractions from an iodixanol density gradient separation of crude protein extracts prepared from *N. benthamiana* leaves expressing: upper panel – human codon optimized GI.1/United States/Norwalk/1968 native VP1 (construct

#2724); lower panel (left hand side) - human codon optimized GI.2 Leu03 native VP1 (construct #3300); lower panel (right hand side) human codon optimized VP1 S(GI.1)+P (GI.2) fusion protein (construct 3360).

[0056] **FIGURE 4A** shows the alignment of several amino acid sequences of norovirus S domain-P domain boundary for GI.1 (VP1 Norwalk 1968 GI.1 Q83884 Rf; SEQ ID NO:88), GI.2 (VP1 Leuven 2003 Gi.1 D2DEL3; SEQ ID NO:89), GI.3 (VP1 LillaEdet 2008 Gi.3 H2DG70; SEQ ID NO:90), GII.4 (VP1 Sydney 2012 GII.3 K4LM89; SEQ ID NO:91), GII.6 (VP1 Ohio 2012 GII.6 M9T020; SEQ ID NO:92), GII.13 (VP1 VA173 2010 GII.13 H9AWU4; SEQ ID NO:93), GII.17 (VP1 awasaki 2014 GII.17 A0A077KVU6; SEQ ID NO:94), and the consensus sequence (SEQ ID NO:95). **FIGURE 4B** shows amino acid sequence identity between a GI.1 native norovirus VP1 protein (SEQ ID NO:1) and several VP1 fusion proteins as described herein (S(GI.1)+P(GI.2), SEQ ID NO:22; S(GI.1)+P(GI.3), SEQ ID NO:23; S(GI.1)+P(GII.4), SEQ ID NO:24; S(GI.1)+P(GII.6), SEQ ID NO:25; S(GI.1)+P(GII.12), SEQ ID NO:71; S(GI.1)+P(GII.13), SEQ ID NO:26; and S(GI.1)+P(GII.17), SEQ ID NO:27**). FIGURE 4C** shows amino acid sequence identity between a GII.12 norovirus native VP1 protein (SEQ ID NO:28) and several VP1 fusion proteins demonstrating that GII-S domain may be used for VP1 fusions as described herein (S(GII.12)+P(GI.1), SEQ ID NO: 29; S(GII.12)+P(GI.2), SEQ ID NO: 30; S(GII.12)+P(GI.3), SEQ ID NO: 31; S(GII.12)+P(GI.5), SEQ ID NO: 32; S(GII.12)+P(GII.1), SEQ ID NO: 33; S(GII.12)+P(GII.2), SEQ ID NO: 34; S(GII.12)+P(GII.3), SEQ ID NO: 35; S(GII.12)+P(GII.4), SEQ ID NO: 36; S(GII.12)+P(GII.5), SEQ ID NO: 37; S(GII.12)+P(GII.6), SEQ ID NO: 38; S(GII.12)+P(GII.7), SEQ ID NO: 39; S(GII.12)+P(GII.13), SEQ ID NO: 40; S(GII.12)+P(GII.14), SEQ ID NO: 41; S(GII.12)+P(GII.17), SEQ ID NO: 42; S(GII.12)+P(GII.21), SEQ ID NO: 43). **FIGURE 4D** shows amino acid sequence identity between a GI.5 native VP1 protein (SEQ ID NO: 44), a GII.1 norovirus native VP1 protein (SEQ ID NO: 45), a GII.14 norovirus native VP1 protein (SEQ ID NO: 46), a GII.21 norovirus native VP1 protein (SEQ ID NO: 47) and several VP1 fusion proteins as described herein (S(GI.5)+P(GII.4), SEQ ID NO: 48; S(GII.1)+P(GI.3), SEQ ID NO: 49; S(GII.1)+P(GII.4), SEQ ID NO: 50;

S(GII.1)+P(GII.17), SEQ ID NO: 51; S(GII.14)+P(GII.4), SEQ ID NO: 52; S(GII.21)+P(GII.4), SEQ ID NO: 53). S: shell domain; P: P domain.

[0057] **FIGURE 5A** shows Coomassie-stained SDS-PAGE analysis of crude protein extracts prepared from *N. benthamiana* leaves producing native norovirus VP1 and VP1 fusion proteins, six and nine days post infiltration (DPI) with expression vectors encoding human codon optimized (hCod) norovirus native VP1 (GI.1, construct #2724, SEQ ID NO's:1 (aa) and 18 (na); GI.2, construct #3300, SEQ ID NO's:2 (aa) and 54(na); GI.3, construct #3302, SEQ ID NO's:3(aa) and 55(na): GII.4, construct #3304, SEQ ID NO's:4(aa) and 56(na)) or norovirus VP1 fusion proteins (GI.1+GI.2, construct #3360, SEQ ID NO's:22(aa) and 57(na); GI.1+GI.3, construct #3361, SEQ ID NO's:23(aa) and 58(na); GI.1+GII.4, construct #3362, SEQ ID NO's:24(aa) and 59(na)). **FIGURE 5B** shows Coomassie-stained SDS-PAGE analysis of crude protein extracts prepared from *N. benthamiana* leaves, producing native norovirus VP1 and VP1 fusion proteins, six and nine days post infiltration (DPI) with expression vectors encoding native (wildtype) norovirus VP1 (GI.1, construct #2724, SEQ ID NO's:1(aa) and 18(na); GII.6, construct #3306, SEQ ID NO's:5(aa) and 60(na); GII.13, construct #3308, SEQ ID NO's:6(aa) and 61(na): GII.17, construct #3310, SEQ ID NO"s:7(aa) and 62(na)) or norovirus VP1 fusion proteins (GI.1+GII.6, construct #3363, SEQ ID NO' s:25(aa) and 63(na); GI.1+GII.13, construct #3364, SEQ ID NO's:26(aa) and 64(na); GI.1+GII.17, construct #3365, SEQ ID NO's:27(aa) and 65(na)). **FIGURE 5C** shows electron micrographs of human codon optimized native norovirus VLPs from iodixanol gradient fractions. GI.3 S29/2008/Lilla Edet/Sweeden (SEQ ID NO:3 (aa);55 (na); Figure 15B); GI.5 Siklos/HUN5407/2013/HUN (SEQ ID NO:44: Figure 16A); GII.1 Ascension208/2010/USA SEQ ID NO:45; Figures 16B); GII.7 Musashimurayama/2010/JP (SEQ I NO:69; Figure 16F). **FIGURE 5D** shows electron micrographs of human codon optimized native norovirus VLPs from iodixanol gradient fractions. GII.12 HS206/2010/USA (SEQ ID NO:28 , Figure 22A); GII.13 VA173/2010/USA (SEQ ID NO:61 , Figure 19B); GII.14 8610/Saga/2008/JPN (SEQ ID NO:46 , Figure 22B); GII.21 Salisbury150/2011/USA (SEQ ID NO:47 , Figure 22B).**FIGURE 5E** shows norovirus protein expression and VLP assembly using Coomassie-stained SDS-PAGE analysis of fractions from an

iodixanol density gradient separation of crude protein extracts prepared from *N. benthamiana* leaves expressing: Panel A (left hand side) - human codon optimized GII.4/Sydney/NSW0514/2012/AU native VP1 (construct #3304); Panel A (right hand side) - human codon optimized GII.4/Sydney/NSW0514/2012/AU (construct #3304; SEQ ID NO:56; Figure 17B) native VP1 co-expressed with GII.4/Sydney/NSW0514/2012/AU native VP2 (construct #3305; SEQ ID NO:120; Figure 23D); Panel B (left hand side) - human codon optimized VP1 S(GI.1)+P(GII.4) fusion protein (construct 3362; SEQ ID NO:59; Figure 27B); Panel B (right hand side) - human codon optimized VP1 S(GI.1)+P(GII.4) fusion protein (construct 3362; SEQ ID NO:59; Figure 27B) co-expressed with human codon optimized GI.1/Norwalk native VP2 (construct #2725; SEQ ID NO:19; Figure 23C); Panel C (left hand side) - human codon optimized VP1 S (GI.1)+P (GII.4) fusion protein (construct 3362; SEQ ID NO:59; Figure 27B) co-expressed with human codon optimized GI.1/Norwalk native VP2 (construct #2725; SEQ ID NO:19; Figure 23C); Panel C (right hand side) - human codon optimized VP1 S(GI.1)+P(GII.4) fusion protein (construct 3362; SEQ ID NO:59; Figure 27B) co-expressed with human codon optimized GII.4/Sydney native VP2 (construct #3305; SEQ ID NO:120; Figure 23D).

[0058] **FIGURE 6A** upper panel shows production of native norovirus VP1, native norovirus VLPs, VP1 fusion proteins and VLPs comprising VP1 fusion proteins using Coomassie-stained SDS-PAGE analysis of fractions from density gradients using crude protein extracts prepared from *N. benthamiana* leaves, nine days post infiltration (DPI) with expression vectors encoding human codon optimized norovirus VP1 (GI.2, construct #3300, SEQ ID NO's:2(aa) and 54(na)) or norovirus human codon optimized VP1 fusion (GI.1+GI.2, construct #3360, SEQ ID NO's:22(aa) and 57(na)). Lower panel shows electron micrographs of wildtype norovirus GI.2 VLPs and VLPs comprising norovirus GI.1+GI.2 VP1 fusions proteins from iodixanol gradient fractions. **FIGURE 6B** upper panel shows production of native norovirus VP1, native norovirus VLPs, VP1 fusion proteins and VLPs comprising VP1 fusion proteins using Coomassie-stained SDS-PAGE analysis of fractions from density gradients using crude protein extracts prepared from *N. benthamiana* leaves, nine days post infiltration (DPI) with expression vectors encoding norovirus human codon optimized VP1 (GI.3, construct #3302, SEQ ID

NO's:3(aa) and 55(na)) or norovirus VP1 fusion (GI.1+GI.3, construct #3361, SEQ ID NO's:23(aa) and 58(na)). Lower panel shows electron micrographs of wildtype norovirus GI.3 VLPs and VLPs comprising norovirus GI.1+GI.3 VP1 fusions proteins from iodixanol gradient fractions. **FIGURE 6C** upper panel shows production of VP1 fusion proteins and VLPs comprising VP1 fusion proteins using Coomassie-stained SDS-PAGE analysis of fractions from density gradients using crude protein extracts prepared from *N. benthamiana* leaves, nine days post infiltration (DPI) with expression vectors encoding norovirus human codon optimized VP1 fusion (GI.1+GII.13, construct #3364, SEQ ID NO's; 26(aa) and 64(na) GI.1+GII.17, construct #3365, SEQ ID NO's:27(aa) and 65(na)). Lower panel shows electron micrographs of VLPs comprising norovirus GI.1+GII.13 or GI.1+GII.17 VP1 fusions proteins from iodixanol gradient fractions. **FIGURE 6D** shows an electron micrographs of a human codon optimized norovirus VLP from iodixanol gradient fractions, the VLP comprising the VP1 fusion S(GI.1) +P(GII.4) (S(GI.1 Nor/68) +P (GII.4/Sydney/NSW0514/12; SEQ ID NO:59, Figure 27B). **FIGURE 6E** shows GI.1 VLP-specific total IgG titers measured in serum samples from animals after IM immunization with one dose (Day 21) and two doses (Day 42) of 1 μg or 10 μg of each formulation. Total IgG titers were measured by ELISA using GI.1 VLP-coated plates (LOQ − 100). Total IgG titers per treatment group (n−8 animals/group) are represented by geometric mean titer (GMT) with a 95 % confidence interval. Same letter (A, B, C, D): no significant difference detected between treatment groups ($p > 0.05$).

[0059] **FIGURE 7A** shows the nucleotide sequence of primer IF-NoV(US68)VP1(ORF2).c (SEQ ID NO: 72); **FIGURE 7B** shows the nucleotide sequence of primer IF-NoV(US68)VP1(ORF2).r (SEQ ID NO: 73); **FIGURE 7C** shows the nucleotide sequence of construct 1190 from left to right t-DNA borders (underlined). 2X35S/CPMV-160/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette (SEQ ID NO: 74); **FIGURE 7D** shows a schematic representation of construct 1190.

[0060] **FIGURE 8A** shows the nucleotide sequence of construct 2720 from 2X35S promoter to NOS terminator. Wild-type VP1 from Norovirus GI.1/Norwalk/1968/US

strain is underlined. (SEQ ID NO: 75); **FIGURE 8B** shows a schematic representation of construct 2720.

[0061] **FIGURE 9A** shows the nucleotide sequence of primer IF-NoV(US68)VP1(ORF2)(hCod).c (SEQ ID NO: 76); **FIGURE 9B** shows the nucleotide sequence of primer IF-NoV(US68)VP1(ORF2)(hCod).r (SEQ ID NO: 77); **FIGURE 9C** shows the nucleotide sequence of construct 2724 from 2X35S promoter to NOS terminator. Human codon-optimized VP1 from Norovirus GI.1/Norwalk/1968/US strain is underlined. (SEQ ID NO: 78); **FIGURE 9D** shows a schematic representation of construct 2724.

[0062] **FIGURE 10A** shows the nucleotide sequence of primer IF-NoV(US68)VP2(ORF3)(hCod).c (SEQ ID NO: 79);**FIGURE 10B** shows the nucleotide sequence of primer IF-NoV(US68)VP2(ORF3)(hCod).r (SEQ ID NO: 80); **FIGURE 10C** shows the nucleotide sequence of construct 2725 from 2X35S promoter to NOS terminator. Human codon-optimized VP2 from Norovirus GI.1/Norwalk/1968/US strain is underlined. (SEQ ID NO: 81); **FIGURE 10D** shows a schematic representation of construct 2725.

[0063] **FIGURE 11A** shows the nucleotide sequence of primer IF-GI2Leu03VP1.c (SEQ ID NO: 82); **FIGURE 11B** shows the nucleotide sequence of primer IF-GI2Leu03VP1.r (SEQ ID NO: 83); **FIGURE 11C** shows the nucleotide sequence of construct 3300 from 2X35S promoter to NOS terminator. Human codon-optimized VP1 from Norovirus GI.2/Leuven/2003/Bel strain is underlined. (SEQ ID NO: 84); **FIGURE 11D** shows a schematic representation of construct 3300.

[0064] **FIGURE 12A** shows the nucleotide sequence of primer GI2Leu+GI1VP1.r (SEQ ID NO: 85); **FIGURE 12B** shows the nucleotide sequence of primer GI1VP1+GI2Leu.c (SEQ ID NO: 86); **FIGURE 12C** shows the nucleotide sequence of construct 3360 from 2X35S promoter to NOS terminator. Human codon-optimized fusion VP1 S(GI.1)+P(GI.2) protein gene sequence is underlined. (SEQ ID NO: 87); **FIGURE 12D** shows a schematic representation of construct 3360.

[0065] **FIGURE 13A** shows the amino acid sequence of VP1 GI.1 United States Norwalk 1968 (SEQ ID NO:1); **FIGURE 13B** shows the nucleic acid sequence of

wild type VP1 GI.1 United States Norwalk 1968 (SEQ ID NO:13); **FIGURE 13C** shows the nucleic acid sequence of human codon optimized VP1 GI.1 United States Norwalk 1968 (SEQ ID NO:18).

[0066] **FIGURE 14A** shows the amino acid sequence of VP1 G1.2 Leuven 2003 D2DEL3 (SEQ ID NO:2); **FIGURE 14B** shows the nucleic acid sequence of human codon optimized VP1 G1.2 Leuven 2003 D2DEL3 (SEQ ID NO:54).

[0067] **FIGURE 15A** shows the amino acid sequence of VP1 GI.3 LillaEdet 2008 H2DG70 (SEQ ID NO:3); **FIGURE 15B** shows the nucleic acid sequence of human codon optimized GI.3 LillaEdet 2008 H2DG70 (SEQ ID NO:55).

[0068] **FIGURE 16A** shows the amino acid sequence of VP1 GI.5 Siklos HUN5407 2013 HUN AHW99832 (SEQ ID NO:44); **FIGURE 16B** shows the amino acids sequence of VP1 GII.1 Ascension208 2010 USA AFA55174 (SEQ ID NO: 45); **FIGURE 16C** shows the amino acid sequence of VP1 GII.2 CGMH47 2011 TW AGT39206 (SEQ ID NO: 66); **FIGURE 16D** shows the amino acid sequence of VP1 GII.3 Jingzhou 2013402 CHN AGX01095 (SEQ ID NO: 67); **FIGURE 16E** shows the amino acid sequence of VP1 GII.5 Alberta 2013 CA ALT54485 (SEQ ID NO: 68); **FIGURE 16F** shows the amino acid sequence of VP1 GII.7 Musa 2010 AII73774 (SEQ ID NO: 69); **FIGURE 16G** shows the amino acid sequence of VP1 consensus sequence from genotypes GI.1, GI.2, GI.3, GII.4, GII.6, GII.13 and GII.17 (SEQ ID NO: 70) the S-P domain boundary sequence is underlined, and the boundary indicated with a "‖".

[0069] **FIGURE 17A** shows the amino acid sequence of VP1 GII.4 Sydney 2012 K4LM89 (SEQ ID NO:4); **FIGURE 17B** shows the nucleic acid sequence of human codon optimized VP1 GII.4 Sydney 2012 K4LM89 (SEQ ID NO:56).

[0070] **FIGURE 18A** shows the amino acid sequence of VP1 GII.6 Ohio 2012 M9T020 (SEQ ID NO: 5); **FIGURE 18B** shows the nucleic acid sequence of human codon optimized VP1 GII.6 Ohio 2012 M9T020 (SEQ ID NO: 60).

[0071] **FIGURE 19A** shows the amino acid sequence of VP1 GII.13 VA173 2010 H9AWU4 (SEQ ID NO: 6); **FIGURE 19B** shows the nucleic acid sequence of human codon optimized VP1 GII.13 VA173 2010 H9AWU4 (SEQ ID NO: 61).

[0072] **FIGURE 20A** shows the amino acid sequence of VP1 GII.17 Kawa 2014 A0A077KVU6 (SEQ ID NO: 7); **FIGURE 20B** shows the nucleic acid sequence of human codon optimized VP1 GII.17 Kawa 2014 A0A077KVU6 (SEQ ID NO: 62).

[0073] **FIGURE 21A** shows the amino acid sequence of VP1 US96: GII.4/Dresden174/1997/DE AY741811 (SEQ ID NO: 8); **FIGURE 21B** shows the Amino acid sequence of VP1 FH02: GII.4/FarmingtonHills/2002/US AY502023 (SEQ ID NO: 9); **FIGURE 21C** shows the amino acid sequence of VP1 Hnt04:GII.4/Hunter-NSW504D/2004/AU DQ078814 (SEQ ID NO: 10); **FIGURE 21D** shows the amino acid sequence of VP1 2006b: GII.4/Shellharbour-NSW696T/2006/AU EF684915 (SEQ ID NO: 11); **FIGURE 21E** shows the amino acid sequence of VP1 NO09: GII.4/Orange-NSW001P/2008/AU GQ845367 (SEQ ID NO: 12);

[0074] **FIGURE 22A** shows the amino acid sequence of VP1 GII.12 HS206 2010 USA AEI29586 (SEQ ID NO: 28); **FIGURE 22B** shows the amino acid sequence of VP1GII.14 Saga 2008 JPN ADE28701 (SEQ ID NO: 46); **FIGURE 22C** shows the amino acid sequence of VP1 GII.21 Sali 2011 USA AFC89665 (SEQ ID NO: 47).

[0075] **FIGURE 23A** shows the amino acid sequence of native VP2 G1.1 (SEQ ID NO: 14); **FIGURE 23B** shows the nucleic acid sequence of wild-type VP2 G1.1 (SEQ ID NO: 15); **FIGURE 23C** shows the nucleic acid sequence of human codon-optimized VP2 G1.1 (SEQ ID NO: 19). **FIGURE 23D** shows the nucleic acid sequence of human codon-optimized VP2 GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:120). **FIGURE 23E** shows the amino acid sequence of VP2 GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:121).

[0076] **FIGURE 24A** shows the nucleic acid sequence of wild-type VP1/VP2 G1.1 (SEQ ID NO: 16); **FIGURE 24B** shows the nucleic acid sequence of wild-type VP1/VP2/3'UTR G1.1 (SEQ ID NO: 17); **FIGURE 24C** shows the nucleic acid sequence of human codon-optimized VP1/VP2 G1.1 (SEQ ID NO: 20); **FIGURE**

24D shows the nucleic acid sequence of human codon-optimized VP1/VP2/3'UTR G1.1 (SEQ ID NO: 21).

[0077] **FIGURE 25A** shows the amino acid sequence of S(GI.1)+P(GI.2) fusion VP1 (SEQ ID NO: 22); **FIGURE 25B** shows the nucleic acid sequence of human codon optimized S(GI.1)+P(GI.2) fusion VP1 (SEQ ID NO: 57).

[0078] **FIGURE 26A** shows the amino acid sequence of of S(GI.1)+P(GI.3) fusion VP1 (SEQ ID NO: 23); **FIGURE 26B** shows the nucleic acid sequence of human codon optimized S(GI.1)+P(GI.3) fusion VP1 (SEQ ID NO: 58).

[0079] **FIGURE 27A** shows the amino acid sequence of S(GI.1)+P(GII.4) fusion VP1 (SEQ ID NO: 24); **FIGURE 27B** shows the nucleic acid sequence of human codon optimized S(GI.1)+P(GII.4) fusion VP1 (SEQ ID NO: 59).

[0080] **FIGURE 28A** shows the amino acid sequence of S(GI.1)+P(GII.6) fusion VP1 (SEQ ID NO: 25); **FIGURE 28B** shows the nucleic acid sequence of human codon optimized S(GI.1)+P(GII.6) fusion VP1 (SEQ ID NO: 63).

[0081] **FIGURE 29A** shows the amino acid sequence of S(GI.1)+P(GII.13) fusion VP1 (SEQ ID NO: 26); **FIGURE 29B** shows the nucleic acid sequence of human codon optimized S(GI.1)+P(GII.13) fusion VP1 (SEQ ID NO: 64).

[0082] **FIGURE 30A** shows the amino acid sequence of S(GI.1)+P(GII.17) fusion VP1 (SEQ ID NO: 27); **FIGURE 30B** shows the nucleic acid sequence of human codon optimized S(GI.1)+P(GII.17) fusion VP1 (SEQ ID NO: 65).

[0083] **FIGURE 31A** shows the amino acid sequence of S(GI.1)+P(GII.12) fusion VP1 (SEQ ID NO: 71); **FIGURE 31B** shows the amino acid sequence of S(GI.5)+P(GII.4) fusion VP1 (SEQ ID NO: 48).

[0084] **FIGURE 32A** shows the amino acid sequence of S(GII.1)+P(GI.3) fusion VP1 (SEQ ID NO: 49); **FIGURE 32B** shows the amino acid sequence of S(GII.1)+P(GII.4) fusion VP1 (SEQ ID NO: 50); **FIGURE 32C** shows the amino acid sequence of S(GII.1)+P(GII.17) fusion VP1 (SEQ ID NO: 51).

[0085] **FIGURE 33A** shows the amino acid sequence of S(GII.12)+P(GI.1) fusion VP1 (SEQ ID NO: 29); **FIGURE 33B** shows the amino acid sequence of S(GII.12)+P(GI.2)fusion VP1 (SEQ ID NO: 30); **FIGURE 33C** shows the amino acid sequence of S(GII.12)+P(GI.3) fusion VP1 (SEQ ID NO: 31); **FIGURE 33D** shows the amino acid sequence of S(GII.12)+P(GI.5) fusion VP1 (SEQ ID NO: 32); **FIGURE 33E** shows the amino acid sequence of S(GII.12)+P(GII.1) fusion VP1 (SEQ ID NO: 33);**FIGURE 33F** shows the amino acid sequence of S(GII.12)+P(GII.2) fusion VP1 (SEQ ID NO: 34);**FIGURE 33G** shows the amino acid sequence of S(GII.12)+P(GII.3) fusion VP1 (SEQ ID NO: 35); **FIGURE 33H** shows the amino acid sequence of S(GII.12)+P(GII.4) fusion VP1 (SEQ ID NO: 36); **FIGURE 33I** shows the amino acid sequence of S(GII.12)+P(GII.5) fusion VP1 (SEQ ID NO: 37); **FIGURE 33J** shows the amino acid sequence of S(GII.12)+P(GII.6) fusion VP1 (SEQ ID NO: 38); **FIGURE 33K** shows the amino acid sequence of S(GII.12)+P(GII.7) fusion VP1 (SEQ ID NO: 39); **FIGURE 33L** shows the amino acid sequence of S(GII.12)+P(GII.13) fusion VP1 (SEQ ID NO: 40); **FIGURE 33M** shows the amino acid sequence of S(GII.12)+P(GII.14) fusion VP1 (SEQ ID NO: 41); **FIGURE 33N** shows the amino acid sequence of S(GII.12)+P(GII.17) fusion VP1 (SEQ ID NO: 42); **FIGURE 33O** shows the amino acid sequence of S(GII.12)+P(GII.21) fusion VP1 (SEQ ID NO: 43).

[0086] **FIGURE 34A** shows the amino acid sequence of S(GII.14)+P(GII.4) fusion VP1 (SEQ ID NO: 52); **FIGURE 34B** shows the amino acid sequence of S(GII.21)+P(GII.4) fusion VP1 (SEQ ID NO: 53).

DETAILED DESCRIPTION

[0087] The following description is of a preferred embodiment.

[0088] As used herein, the terms "comprising," "having," "including" and "containing," and grammatical variations thereof, are inclusive or open-ended and do not exclude additional, un-recited elements and/or method steps. The term "consisting essentially of" when used herein in connection with a use or method, denotes that additional elements and/or method steps may be present, but that these

additions do not materially affect the manner in which the recited method or use functions. The term "consisting of" when used herein in connection with a use or method, excludes the presence of additional elements and/or method steps. A use or method described herein as comprising certain elements and/or steps may also, in certain embodiments, consist essentially of those elements and/or steps, and in other embodiments consist of those elements and/or steps, whether or not these embodiments are specifically referred to. In addition, the use of the singular includes the plural, and "or" means "and/or" unless otherwise stated. The term "plurality" as used herein means more than one, for example, two or more, three or more, four or more, and the like. Unless otherwise defined herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. As used herein, the term "about" refers to an approximately +/-10% variation from a given value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to. The use of the word "a" or "an" when used herein in conjunction with the term "comprising" may mean "one," but it is also consistent with the meaning of "one or more," "at least one" and "one or more than one."

[0089] The term "plant", "portion of a plant", "plant portion', "plant matter", "plant biomass", "plant material", plant extract", or "plant leaves", as used herein, may comprise an entire plant, tissue, cells, or any fraction thereof, intracellular plant components, extracellular plant components, liquid or solid extracts of plants, or a combination thereof, that are capable of providing the transcriptional, translational, and post-translational machinery for expression of one or more than one nucleic acids described herein, and/or from which an expressed protein or VLP may be extracted and purified. Plants may include, but are not limited to, agricultural crops including for example canola, Brassica spp., maize, Nicotiana spp., (tobacco) for example, *Nicotiana benthamiana, Nicotiana rustica, Nicotiana, tabacum, Nicotiana alata, Arabidopsis thaliana*, alfalfa, potato, sweet potato (*Ipomoea batatus*), ginseng, pea, oat, rice, soybean, wheat, barley, sunflower, cotton, corn, rye (*Secale cereale*), sorghum (*Sorghum bicolor, Sorghum vulgare*), safflower (*Carthamus tinctorius*).

[0090] The term "plant portion", as used herein, refers to any part of the plant including but not limited to leaves, stem, root, flowers, fruits, a plant cell obtained

from leaves, stem, root, flowers, fruits, a plant extract obtained from leaves, stem, root, flowers, fruits, or a combination thereof. The term "plant extract", as used herein, refers to a plant-derived product that is obtained following treating a plant, a portion of a plant, a plant cell, or a combination thereof, physically (for example by freezing followed by extraction in a suitable buffer), mechanically (for example by grinding or homogenizing the plant or portion of the plant followed by extraction in a suitable buffer), enzymatically (for example using cell wall degrading enzymes), chemically (for example using one or more chelators or buffers), or a combination thereof. A plant extract may be further processed to remove undesired plant components for example cell wall debris. A plant extract may be obtained to assist in the recovery of one or more components from the plant, portion of the plant or plant cell, for example a protein (including protein complexes, protein surprastructures and/or VLPs), a nucleic acid, a lipid, a carbohydrate, or a combination thereof from the plant, portion of the plant, or plant cell. If the plant extract comprises proteins, then it may be referred to as a protein extract. A protein extract may be a crude plant extract, a partially purified plant or protein extract, or a purified product, that comprises one or more proteins, protein complexes, protein suprastructures, and/or VLPs, from the plant tissue. If desired a protein extract, or a plant extract, may be partially purified using techniques known to one of skill in the art, for example, the extract may be subjected to salt or pH precipitation, centrifugation, gradient density centrifugation, filtration, chromatography, for example, size exclusion chromatography, ion exchange chromatography, affinity chromatography, or a combination thereof. A protein extract may also be purified, using techniques that are known to one of skill in the art.

[0091] The term nucleic acid segment as used herein refers to a sequence of nucleic acids that encodes a protein of interest. In addition to the sequence of nucleic acids, the nucleic acid segment comprise a regulatory region and a terminator that are operatively linked to the sequence of nucleic acids. The regulatory region may for example comprise a promoter, and optionally, an enhancer element operatively linked to the promoter.

[0092] The term "nucleic acid complex" as used herein refers to a combination of two or more than two nucleic acid segments. The two or more than two nucleic acid

segments may be present in a single nucleic acid, so that the nucleic acid complex comprises two, or more than two nucleic acid segments, with each nucleic acid segment under the control of a regulatory region and a terminator. Alternatively, the nucleic acid complex may comprise two or more separate nucleic acids, each of the nucleic acids comprising one or more than one nucleic acid segment, where each nucleic acid segment is under the control of a regulatory region and a terminator. For example a nucleic acid complex may comprise one nucleic acid that comprises two nucleic acid segments, a nucleic acid complex may comprise two nucleic acids, each nucleic acid comprising one nucleic acid segment, or a nucleic acid complex may comprise two or more than two nucleic acids, with each nucleic acid comprising one or more than one nucleic acid segment.

[0093] The term "vector" or "expression vector", as used herein, refers to a recombinant nucleic acid for transferring exogenous nucleic acid sequences into host cells (e.g. plant cells) and directing expression of the exogenous nucleic acid sequences in the host cells. "Expression cassette" refers to a nucleotide sequence comprising a nucleic acid of interest under the control of, and operably (or operatively) linked to, an appropriate promoter or other regulatory elements for transcription of the nucleic acid of interest in a host cell. As one of skill in the art would appreciate, the expression cassette may comprise a termination (terminator) sequence that is any sequence that is active the plant host. For example the termination sequence may be derived from the RNA-2 genome segment of a bipartite RNA virus, e.g. a comovirus, the termination sequence may be a NOS terminator, or terminator sequence may be obtained from the 3'UTR of the alfalfa plastocyanin gene.

[0094] The constructs of the present disclosure may further comprise a 3' untranslated region (UTR). A 3' untranslated region contains a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by effecting the addition of polyadenylic acid tracks to the 3' end of the mRNA precursor. Polyadenylation signals are commonly recognized by the presence of homology to the canonical form 5' AATAAA-3' although variations are not uncommon. Non-limiting examples of suitable 3' regions are the 3' transcribed non-translated regions

containing a polyadenylation signal of *Agrobacterium* tumor inducing (Ti) plasmid genes, such as the nopaline synthase (Nos gene) and plant genes such as the soybean storage protein genes, the small subunit of the ribulose-1, 5-bisphosphate carboxylase gene (ssRUBISCO; US 4,962,028), the promoter used in regulating plastocyanin expression.

[0095] By "regulatory region" "regulatory element" or "promoter" it is meant a portion of nucleic acid typically, but not always, upstream of the protein coding region of a gene, which may be comprised of either DNA or RNA, or both DNA and RNA. When a regulatory region is active, and in operative association, or operatively linked, with a nucleotide sequence of interest, this may result in expression of the nucleotide sequence of interest. A regulatory element may be capable of mediating organ specificity, or controlling developmental or temporal gene activation. A "regulatory region" includes promoter elements, core promoter elements exhibiting a basal promoter activity, elements that are inducible in response to an external stimulus, elements that mediate promoter activity such as negative regulatory elements or transcriptional enhancers. "Regulatory region", as used herein, also includes elements that are active following transcription, for example, regulatory elements that modulate gene expression such as translational and transcriptional enhancers, translational and transcriptional repressors, upstream activating sequences, and mRNA instability determinants. Several of these latter elements may be located proximal to the coding region.

[0096] In the context of this disclosure, the term "regulatory element" or "regulatory region" typically refers to a sequence of DNA, usually, but not always, upstream (5') to the coding sequence of a structural gene, which controls the expression of the coding region by providing the recognition for RNA polymerase and/or other factors required for transcription to start at a particular site. However, it is to be understood that other nucleotide sequences, located within introns, or 3' of the sequence may also contribute to the regulation of expression of a coding region of interest. An example of a regulatory element that provides for the recognition for RNA polymerase or other transcriptional factors to ensure initiation at a particular site is a promoter element. Most, but not all, eukaryotic promoter elements contain a TATA box, a conserved nucleic acid sequence comprised of adenosine and thymidine nucleotide base pairs

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usually situated approximately 25 base pairs upstream of a transcriptional start site. A promoter element may comprise a basal promoter element, responsible for the initiation of transcription, as well as other regulatory elements that modify gene expression.

[0097] There are several types of regulatory regions, including those that are developmentally regulated, inducible or constitutive. A regulatory region that is developmentally regulated, or controls the differential expression of a gene under its control, is activated within certain organs or tissues of an organ at specific times during the development of that organ or tissue. However, some regulatory regions that are developmentally regulated may preferentially be active within certain organs or tissues at specific developmental stages, they may also be active in a developmentally regulated manner, or at a basal level in other organs or tissues within the plant as well. Examples of tissue-specific regulatory regions, for example see-specific a regulatory region, include the napin promoter, and the cruciferin promoter (Rask et al., 1998, J. Plant Physiol. 152: 595-599; Bilodeau et al., 1994, Plant Cell 14: 125-130). An example of a leaf-specific promoter includes the plastocyanin promoter (see US 7,125,978).

[0098] An inducible regulatory region is one that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor that binds specifically to an inducible regulatory region to activate transcription may be present in an inactive form, which is then directly or indirectly converted to the active form by the inducer. However, the protein factor may also be absent. The inducer can be a chemical agent such as a protein, metabolite, growth regulator, herbicide or phenolic compound or a physiological stress imposed directly by heat, cold, salt, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus. A plant cell containing an inducible regulatory region may be exposed to an inducer by externally applying the inducer to the cell or plant such as by spraying, watering, heating or similar methods. Inducible regulatory elements may be derived from either plant or non plant genes (e.g. Gatz, C. and Lenk, I.R.P., 1998, Trends Plant Sci. 3, 352-358). Examples, of potential inducible promoters include, but not limited to,

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tetracycline-inducible promoter (Gatz, C.,1997, Ann. Rev. Plant Physiol. Plant Mol. Biol. 48, 89-108), steroid inducible promoter (Aoyama, T. and Chua, N.H.,1997, Plant J. 2, 397-404) and ethanol-inducible promoter (Salter, M.G., et al, 1998, Plant Journal 16, 127-132; Caddick, M.X., et al,1998, Nature Biotech. 16, 177-180) cytokinin inducible IB6 and CKI1 genes (Brandstatter, I. and Kieber, J.J.,1998, Plant Cell 10, 1009-1019; Kakimoto, T., 1996, Science 274, 982-985) and the auxin inducible element, DR5 (Ulmasov, T., et al., 1997, Plant Cell 9, 1963-1971).

[0099] A constitutive regulatory region directs the expression of a gene throughout the various parts of a plant and continuously throughout plant development. Examples of known constitutive regulatory elements include promoters associated with the CaMV 35S transcript. (p35S; Odell et al., 1985, Nature, 313: 810-812), the rice actin 1 (Zhang et al, 1991, Plant Cell, 3: 1155-1165), actin 2 (An et al., 1996, Plant J., 10: 107-121), or tms 2 (U.S. 5,428,147), and triosephosphate isomerase 1 (Xu et. al., 1994, Plant Physiol. 106: 459-467) genes, the maize ubiquitin 1 gene (Cornejo et al, 1993, Plant Mol. Biol. 29: 637-646), the Arabidopsis ubiquitin 1 and 6 genes (Holtorf et al, 1995, Plant Mol. Biol. 29: 637-646), the tobacco translational initiation factor 4A gene (Mandel et al, 1995 Plant Mol. Biol. 29: 995-1004). the Cassava Vein Mosaic Virus promoter, pCAS, (Verdaguer et al., 1996); the promoter of the small subunit of ribulose biphosphate carboxylase, pRbcS: (Outchkourov et al., 2003), the pUbi (for monocots and dicots).

[00100] The term "constitutive" as used herein does not necessarily indicate that a nucleotide sequence under control of the constitutive regulatory region is expressed at the same level in all cell types, but that the sequence is expressed in a wide range of cell types even though variation in abundance is often observed.

[00101] The expression constructs as described above may be present in a vector. The vector may comprise border sequences which permit the transfer and integration of the expression cassette into the genome of the organism or host. The construct may be a plant binary vector, for example a binary transformation vector based on pPZP (Hajdukiewicz, et al. 1994). Other example constructs include pBin19

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(see Frisch, D. A., L. W. Harris-Haller, et al. 1995, *Plant Molecular Biology* 27: 405-409).

[00102] The term "native", "native protein" or "native domain", as used herein, refers to a protein or domain having a primary amino acid sequence identical to wildtype. Native proteins or domains may be encoded by nucleotide sequences having 100% sequence similarity to the wildtype sequence. A native amino acid sequence may also be encoded by a human codon (hCod) optimized nucleotide sequence or a nucleotide sequence comprising an increased GC content when compared to the wild type nucleotide sequence provided that the amino acid sequence encoded by the hCod-nucleotide sequence exhibits 100% sequence identity with the native amino acid sequence.

[00103] By a nucleotide sequence that is "human codon optimized" or a "hCod" nucleotide sequence, it is meant the selection of appropriate DNA nucleotides for the synthesis of an oligonucleotide sequence or fragment thereof that approaches the codon usage generally found within an oligonucleotide sequence of a human nucleotide sequence. By "increased GC content" it is meant the selection of appropriate DNA nucleotides for the synthesis of an oligonucleotide sequence or fragment thereof in order to approach codon usage that, when compared to the corresponding native oligonucleotide sequence, comprises an increase of GC content, for example, from about 1 to about 30%, or any amount therebetween, over the length of the coding portion of the oligonucleotide sequence. For example, from about 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30%, or any amount therebetween, over the length of the coding portion of the oligonucleotide sequence. As described below, a human codon optimized nucleotide sequence, or a nucleotide sequence comprising an increased GC content (when compared to the wild type nucleotide sequence) exhibits increased expression within a plant, portion of a plant, or a plant cell, when compared to expression of the non-human optimized (or lower GC content) nucleotide sequence.

[00104] Norovirus VP1 fusion proteins and methods of producing norovirus VP1 fusion proteins in plants are described herein. The norovirus VP1 fusion protein, comprises an S domain derived from a first norovirus strain fused to a P domain, or a

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portion of the P domain, derived from a second norovirus strain. It has been observed that expression of the VP1 fusion protein increases the yield of the P domain, or a portion of the P domain, derived from the second norovirus strain in plants, when compared to the yield of the P domain, or a portion of the P domain, of the second norovirus strain, when expressed in the same plant and under the same conditions, as a native VP1 protein comprising both an S domain and the P domain (or comprising the P domain that comprises a portion of the P domain), from the same second norovirus strain.

[00105] For example, the norovirus VP1 fusion protein, and methods of producing the norovirus VP1 fusion protein, may include a VP1 fusion protein comprising an S domain derived from a first norovirus strain fused to the P1 and P2 subdomains derived from a second norovirus strain:

$S_{1st\ strain}$-P1$a_{2nd\ strain}$-P2$2_{nd\ strain}$-P1$b_{2nd\ strain}$.

also referred to as: "S_1-P1a_2-P2$2_2$-P1b_2", or "S_1-P_2".

The VP1 fusion protein, S_1- P_2, was observed to maintain or increase the yield of the P1 and P2 subdomains (P domain) derived from the second norovirus strain, as compared to the yield of the P subdomain of the second strain, when expressed in the same plant and under the same conditions as a native VP1 protein comprising both an S domain and the P domain, that comprises the P1 and P2 subdomains, from the same second norovirus strain. The sequence encoding the VP1 fusion protein may be optimized for human codon usage, for having an increased GC content, or a combination thereof.

[00106] Also provided herein are methods of increasing production of VLPs comprising norovirus VP1 fusion proteins in plants, wherein a nucleic acid encoding a norovirus VP1 fusion protein as described herein, for example $S_{1st\ strain}$-P1$a_{2nd\ strain}$-P2$2_{nd\ strain}$-P1$b_{2nd\ strain}$ (S_1-P1a_2-P2$2_2$-P1b_2; S_1- P_2) is introduced into the plant or a portion of the plant. One or more than one type of norovirus fusion protein may be expressed in a plant or portion of the plant in order to produce a VLP comprising one or more than one type of norovirus fusion protein.

[00107] The methods of producing a VLP comprising a VP1 fusion protein may also comprise a step of co-expressing a nucleic acid sequence encoding a VP2 protein in the plant or portion of the plant.

[00108] The term "single construct" or "single constructs", as used herein, refers to nucleic acid vectors comprising a single nucleic acid sequence. The term "dual construct" or "dual constructs", as used herein, refers to a nucleic acid vector comprising two nucleic acid sequences.

[00109] By co-expression it is meant the introduction and expression of two or more nucleotide sequences, each of the two or more nucleotide sequences encoding a protein of interest, or a fragment of a protein of interest within a plant, portion of a plant or a plant cell. The two or more nucleotide sequences may be introduced into the plant, portion of the plant or the plant cell within one vector, so that each of the two or more nucleotide sequences is under the control of a separate regulatory region (e.g. comprising a dual construct). Alternatively, the two or more nucleotide sequences may be introduced into the plant, portion of the plant or the plant cell within separate vectors (e.g. comprising single constructs), and each vector comprising appropriate regulatory regions for the expression of the corresponding nucleic acid. For example, two nucleotide sequences, each on a separate vector and introduced into separate *A. tumefaciens* hosts, may be co-expressed by mixing suspensions of each *A. tumefaciens* host in a desired volume (for example, an equal volume, or the ratios of each *A. tumefaciens* host may be altered) before vacuum infiltration. In this manner, co-infiltration of multiple Agrobacterium suspensions permits co-expression of multiple transgenes.

[00110] The nucleic acid comprising encoding a norovirus VP1 fusion protein as described herein, for example, S_1- P_2, may further comprise sequences that enhance expression of the norovirus VP1 fusion protein in the plant, or in a portion of the plant. Sequences that enhance expression may include, a CPMV enhancer element in operative association with the nucleic acid encoding the norovirus VP1 fusion protein. The sequence encoding the VP1 fusion protein may also be optimized for human codon usage, for having an increased GC content, or a combination thereof. Furthermore, a nucleic acid encoding VP2 may be co-expressed along with the

sequence encoding the VP1 fusion protein. The co-expression of a nucleic acid encoding VP2 may lead to increased stability, an increased yield, or both an increase in stability and yield, of VLPs that comprise the one or more than one type of VP1 fusion protein.

[00111] The term "CPMV enhancer element", as used herein, refers to a nucleotide sequence encoding the 5'UTR regulating the Cowpea Mosaic Virus (CPMV) RNA2 polypeptide or a modified CPMV sequence as is known in the art. For example, a CPMV enhancer element or a CPMV expression enhancer, includes a nucleotide sequence as described in WO2015/14367; WO2015/103704; WO2007/135480; WO2009/087391; Sainsbury F., and Lomonossoff G.P., (2008, Plant Physiol. 148: pp. 1212-1218). A CPMV enhancer sequence can enhance expression of a downstream heterologous open reading frame (ORF) to which they are attached. The CPMV expression enhancer may include CPMV HT, CPMVX, CPMVX+, CPMV-HT+, CPMV HT+[WT115], or CPMV HT+ [511] (WO2015/14367; WO2015/103704). The CPMV expression enhancer may be used within a plant expression system comprising a regulatory region that is operatively linked with the CPMV expression enhancer sequence and a nucleotide sequence of interest. The term "5'UTR" or "5' untranslated region" or "5' leader sequence" refers to regions of an mRNA that are not translated. The 5'UTR typically begins at the transcription start site and ends just before the translation initiation site or start codon of the coding region. The 5" UTR may modulate the stability and/or translation of an mRNA transcript.

[00112] By "operatively linked" it is meant that the particular sequences interact either directly or indirectly to carry out an intended function, such as mediation or modulation of expression of a nucleic acid sequence. The interaction of operatively linked sequences may, for example, be mediated by proteins that interact with the operatively linked sequences.

[00113] When one or more than one type of the norovirus VP1 fusion protein is expressed in the plant, portion of the plant or the plant cell, the one or more than one type of VP1 fusion proteins auto-assemble into VLPs. The plant or portion of the plant

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plant may be harvested under suitable extraction and purification conditions to maintain the integrity of the VLP, and the VLP comprising the one or more than one type of VP1 fusion protein may be purified. The one or more than one VP1 fusion protein may also be co-expressed with nucleotide sequence encoding VP2, so that the VLP may comprise both VP1 fusion protein and VP2 protein. The present disclosure also provides for the production of one or more than one type of VP1 fusion protein as described herein within a plant, portion of a plant, or plant cell, and the extraction and purification of the one or more than one type of VP1 fusion protein from the plant, the portion of the plant, or the plant cell to produce plant matter, a plant extract, or a protein extract, comprising the VP1 fusion protein.

[00114] Plant matter, a plant extract, or a protein extract comprising the norovirus VP1 fusion protein, for example S_1- P_2, or VLPs comprising the norovirus a VP1 fusion protein as described herein, for example S_1- P_2 is also provided. The plant matter, plant extract, or protein extract may be used to induce immunity to norovirus infection in a subject. Alternatively, the VP1 fusion protein, or the VLP comprising the VP1 fusion protein (and optionally VP2), may be purified or partially purified, and the purified or partially purified preparation may be used in inducing immunity to norovirus infection in a subject.

[00115] The present disclosure also provides a composition comprising an effective dose of one or more than one type of norovirus VP1 fusion protein, for example, S_1-P_2, a combination thereof, or VLPs comprising one or more than one type of norovirus VP1 fusion protein, and optionally VP2, for example S_1- P_2, for inducing an immune response, and a pharmaceutically acceptable carrier, adjuvant, vehicle, or excipient.

[00116] Also provided herein are methods of inducing immunity to a norovirus infection in a subject comprising of administering one or more than one type of norovirus VP1 fusion protein or VLPs comprising one or more than one types of norovirus VP1 fusion proteins to a subject orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[00117] The term "norovirus", as used herein, refers to a non-enveloped viral strain of the genus *norovirus* of the family *Caliciviridae* that is characterized as having a

single-stranded, positive-sense RNA. The norovirus genome is 7,654 nucleotides in length. The ORF1 encodes a nonstructural polyprotein that is cleaved by viral 3C-like protease into 6 proteins, including an RNA-dependent RNA polymerase. ORF2 and ORF3 encode a major (VP1) and a minor (VP2) capsid proteins, respectively (see Figure 1A).

[00118] Norovirus strains as disclosed herein include, any known norovirus strain, but also modifications to known norovirus strains that are known to develop on a regular basis over time (See for example Parra G.I. et. al. PLoS Pathog 13(1): e1006136. doi:10.1371/journal. ppat.1006136). For example norovirus strains may include, but are not limited to GI.1/Norwalk/1968/US (GI.1; SEQ ID NO:1; Figure 13A), GI.2/Leuven/2003/Bel (GI.2; SEQ ID NO:2; Figure 14A), GI.3/S29/2008/Lilla Edet/Sweden (GI.3; SEQ ID NO:3; Figure 15A), GI.5/Siklos/Hun5407/2013/HUN (GI.5; SEQ ID NO:44; Figure 16A), GII.1/Ascension208/2010/USA (GII.1; SEQ ID NO:45; Figure 16B), GII.2/CGMH47/2011/TW (GII.2; SEQ ID NO:66; Figure 16C), GII.3/Jingzhou/2013402/CHN (GII.3; SEQ ID NO:67; Figure16D), GII.4/Sydney/NSW0514/2012/AU (GII.4; SEQ ID NO:4; Figure 17A), GII.5/AlbertaEI390/2013/CA (GII.5; SEQ ID NO:68; Figure 16E), GII.7/Musahimurayama/2010/JP (GII.7; SEQ ID NO:69; Figure 16F), GII.12/HS206/2010/USA (GII.12; SEQ ID NO:28; Figure 22A), GII.13/VA173/2010/USA (GII.13; SEQ ID NO:6; Figure 19A), GII.14/8610/Saga/2008/JP (GII.14; SEQ ID NO:46; Figure 46B), GII.17/Kawasaki323/2014/JP (GII.17; SEQ ID NO:7; Figure 20A), and GII.21/Salisbury150/2011/USA (GII.21; SEQ ID NO:47; Figure 22C). Norovirus strains also include strains having from about 30-100%, or any amount therebetween, amino acid sequence identity to the VP1 protein, the VP2 protein, or both the VP1 and the VP2 proteins, with any of the above norovirus strains of the strains listed in Figures 2A and 2B, provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject. For example, norovirus strains also include strains having 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100%, or any amount therebetween, amino acid sequence identity (sequence similarity; percent identity; percent similarity) to the VP1 protein, the VP2 protein, or both the

VP1 and the VP2 proteins, with any of the above norovirus strains of the strains listed in Figures 2A and 2B, provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject. An amino acid sequence identity comparison between the VP1 and the VP2 proteins of several norovirus strains, which are not to be considered limiting, is shown in Figure 2C (VP1, upper panel; VP2, lower panel).

[00119] The terms "percent similarity", "sequence similarity", "percent identity", or "sequence identity", when referring to a particular sequence, are used for example as set forth in the University of Wisconsin GCG software program, or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology, Ausubel et al., eds. 1995 supplement). Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, using for example the algorithm of Smith & Waterman, (1981, Adv. Appl. Math. 2:482), by the alignment algorithm of Needleman & Wunsch, (1970, J. Mol. Biol. 48:443), by the search for similarity method of Pearson & Lipman, (1988, Proc. Natl. Acad. Sci. USA 85:2444), by computerized implementations of these algorithms (for example: GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis.).

[00120] An example of an algorithm suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977, Nuc. Acids Res. 25:3389-3402) and Altschul et al., (1990, J. Mol. Biol. 215:403-410), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. For example the BLASTN program (for nucleotide sequences) may use as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program may use as defaults a word length of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, 1989, Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. Software for performing BLAST

analyses is publicly available through the National Center for Biotechnology Information (see URL: ncbi.nlm.nih.gov/).

[00121] The term "VP1", as used herein, refers to the norovirus major capsid protein or polypeptide comprising an amino acid sequence similar to the protein or polypeptide encoded by ORF2 of one or more strains of norovirus as described herein. The major capsid protein folds into two principal domains, a shell (S) domain and a protruding (P) domain, which contains two subdomains, P1 and P2 (see Figure 1B). The VP1 protein forms a dimer (Figure 1C) when incorporated into a virion particle, or a VLP. The nucleotide sequence encoding the VP1 protein is comprised, in series, of: a first sequence, encoding the S domain; a second sequence, encoding a first portion of the P1 domain; a third sequence, encoding the P2 domain; and a fourth sequence, encoding a second portion of the P1 domain. When translated, the resulting polypeptide folds into the VP1 protein as depicted in FIGURE 1B, comprising of a globular S domain (bottom of ribbon structure), a P1 subdomain (middle of ribbon structure), and a P2 subdomain (top of ribbon structure).

[00122] As shown in Figure 1C, the VP1 protein dimerizes via P-domain interactions. These interactions stabilize the spontaneous assembly of norovirus capsid molecules.

Norovirus VP1 Protein Production in Plants

[00123] The VP1 protein as disclosed herein includes any VP1 protein comprising an amino acid sequence having from about 40 to about 100%, from about 50 to about 100%, from about 60 to about 100%, from about 70 to about 100%, from about 80 to about 100%, from about 85 to about 100% from about 90 to about 100%, or from about 95 to about 100% or any amount therebetween, sequence identity (which may be also termed sequence similarity) with a VP1 amino acid sequenced from a norovirus GI.1 (SEQ ID NO:1; Figure 13A), GI.2 (SEQ ID NO:2; Figure 14A), GI.3 (SEQ ID NO:3; Figure 15A), GII.4 (SEQ ID NO:4; Figure 17A), GII.6 (SEQ ID NO:5; Figure 18A), GII.13 (SEQ ID NO:6; Figure 19A), GII.17(SEQ ID NO:7; Figure 20A), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject. For example, the VP1 protein may comprise an amino acid sequence exhibiting from about 40, 42, 44, 46,

48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween sequence identity with a VP1 amino acid sequence from a norovirus GI.1 (SEQ ID NO:1), GI.2 (SEQ ID NO:2), GI.3 (SEQ ID NO:3), GII.4 (SEQ ID NO:4), GII.6 (SEQ ID NO:5), GII.13 (SEQ ID NO:7; GII.17 (SEQ ID NO:7; see Figure 2C, upper panel), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00124] It is well known in the art that the sequence of the P domain of the norovirus VP1 protein is hypervariable and readily mutates. For example as shown in Figure 2D, the amino acid identity of six known GII.4 strains is compared. In this example, the full length amino acid sequence of the GII.4 VP1 protein exhibits from 93.1% to 97.4% sequence identity (upper panel Figure 2D). However, the P domain of these same six GII.4 strains exhibits an amino acid sequence identity from 88.7% to 96.9%, and the P2 subdomain of these same six GII.4 strains exhibits an amino acid sequence identity from 81.3% to 94.4%. The P domain of VP1 proteins obtained from other norovirus strains exhibits a similar range of amino acid sequence identity. An example of the consensus amino acid sequence for VP1 is shown in Figure16G (SEQ ID NO:70).

[00125] The present disclosure therefore includes nucleic acid sequences that exhibit from about 60% to about 100%, or any amount therebetween, sequence identity with any of the nucleic acid sequences encoding VP1, including the S, P or both the S and P domains, between the strains identified above, and as listed in Figures 2A and 2B. For example, nucleic acid sequences may exhibit from about 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100%, or any amount therebetween, sequence identity with any of the nucleic acid sequences encoding a norovirus VP1, including the S domain, P domain or both the S and P domains, from GI.1 (SEQ ID NO:1), GI.2 (SEQ ID NO:2), GI.3 (SEQ ID NO:3), GII.4 (SEQ ID NO:4), GII.6 (SEQ ID NO:5), GII.13 (SEQ ID NO:6), GII.17 (SEQ ID NO:7), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00126] Similarly, the present invention includes amino acid sequences that exhibit from about 40% to about 100% or any amount therebetween, sequence similarity with any of the VP1 sequences, including the S, P or both the S and P domains, from GI.1 (SEQ ID NO:1), GI.2 (SEQ ID NO:2), GI.3 (SEQ ID NO:3), GII.4 (SEQ ID NO:4), GII.6 (SEQ ID NO:5), GII.13 (SEQ ID NO:6), GII.17 (SEQ ID NO:7). For example, from about 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100% or any amount therebetween, sequence similarity with any of the VP1 amino acid sequences, including the S domain, the P domain, or both the S and P domains. For example, as shown in Figure 2C (upper panel), amino acid sequences of VP1 sequences between several norovirus strains, including but not limited to, GI.1, GI.2, GI.3, GII.4, GII.6, GII.13), exhibit a sequence identity from about 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100% or any amount therebetween, provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00127] Figure 2C (lower panel) also shows that these same norovirus strains (from GI.1 (SEQ ID NO:1), GI.2 (SEQ ID NO:2), GI.3 (SEQ ID NO:3), GII.4 (SEQ ID NO:4), GII.6 (SEQ ID NO:5), GII.13 (SEQ ID NO:6), GII.17 (SEQ ID NO:7), exhibit from about 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100% or any amount therebetween, amino acid sequence similarity of the VP2 protein.

[00128] Figure 16G shows the consensus amino acid sequence (SEQ ID NO:70) of GI.1 (SEQ ID NO:1), GI.2 (SEQ ID NO:2), GI.3 (SEQ ID NO:3), GII.4 (SEQ ID NO:4), GII.6 (SEQ ID NO:5), GII.13 (SEQ ID NO:6), GII.17 (SEQ ID NO:7). Any of these VP1 proteins, or the VP1 consensus amino acid sequence may be used to prepare the VP1 fusion proteins described herein.

[00129] By "VP1 fusion protein" or "chimeric VP1 protein" it is meant, a protein comprising an S domain derived from a first norovirus strain fused to the P1 and P2 subdomains derived from a second norovirus strain:

$S_{1st\ strain}$-$P1a_{2nd\ strain}$ -$P2_{2nd\ strain}$ -$P1b_{2nd\ strain}$ (S_1-$P1a_2$-$P2_2$-$P1b_2$; S_1-P_2).

The boundary between the S domain and the P domain of the norovirus VP1 amino acid sequence is well conserved (see Figure 4A) and comprise of the following consensus sequence:

...LVPPtvE||skTkpFsl... (SEQ ID NO:95),

where "||" indicates the boundary between the S and P domains.

[00130] Examples of VP1 fusion protein of the form: S_1-P1a_2-P2$_2$-P1b_2 include, but are not limited to:

- S(GI.1 Nor/68) +P (GI.2/Leuven/03) (SEQ ID NO:22, Figure 25A; S(GI.1)+P(GI.2)): comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GI.2/Leuven/03 (SEQ ID NO:2), or a sequence that exhibits from about 59-100% or any amount therebetween (see Figure 4B), sequence similarity with the amino acid sequence of the GI.1 VP1 protein, or the S_1-P1a_2-P2$_2$-P1b_2 fusion amino acid sequence shown in Figure 25A, for example from about 59, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence similarity with the amino acid sequence of the S_1-P1a_2-P2$_2$-P1b_2 fusions (see Figure 4B), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject;

- S(GI.1 Nor/68) +P (GI.3/S29/08/Lilla Edet) (SEQ ID NO:23, Figure 26A; S(GI.1)+P(GI.3)): comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GI.3/S29/08/Lilla Edet (SEQ ID NO:3), or a sequence that exhibits from about 59-100% or any amount therebetween (see Figure 4B), sequence similarity with the amino acid sequence of the GI.1 VP1 protein, or the S_1-P1a_2-P2$_2$-P1b_2 fusion amino acid sequence shown in Figure 26A, for example from about 59, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence similarity with the amino acid sequence of the S_1-P1a_2-P2$_2$-P1b_2 fusions (see Figure 4B), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject;

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- S(GI.1 Nor/68) +P (GII.4/Sydney/NSW0514/12) (SEQ ID NO:24, Figure 27A; S(GI.1)+P(GII.4)): comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GII.4/Sydney/NSW0514/12 (SEQ ID NO:4), or a sequence that exhibits from about 59-100% or any amount therebetween (see Figure 4B), sequence similarity with the amino acid sequence of the GI.1 VP1 protein, or the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusion amino acid sequence shown in Figure 27A, for example from about 59, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence similarity with the amino acid sequence of the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusions (see Figure 4B), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject;

- S(GI.1 Nor/68) +P (GII.6/Ohio/490/12) (SEQ ID NO:25, Figure 28A; S(GI.1)+P(GII.6)): comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GII.6/Ohio/490/12 (SEQ ID NO:5), or a sequence that exhibits from about 59-100% or any amount therebetween, sequence similarity with the amino acid sequence of the GI.1 VP1 protein, or the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusion shown in Figure 28A, for example from about 59, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence similarity with the amino acid sequence of the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusions, provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject;

- S(GI.1 Nor/68) +P (GII.12/HS206/2010/USA) (SEQ ID NO:71, Figure 31A; S(GI.1)+P(GII.12)): comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GII.12/HS206/2010/USA (SEQ ID NO:28), or a sequence that exhibits from about 59-100% or any amount therebetween (see Figure 4B), sequence similarity with the amino acid sequence of the GI.1 VP1 protein, or the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusion amino acid sequence shown in Figure 31A, for example from about 59, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence similarity with the amino acid sequence of the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusions (see Figure 4B), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject;

- (GI.1 Nor/68) +P (GII.13/VA173/10) (SEQ ID NO:26, Figure 29A; S(GI.1)+P(GII.13): comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GII.13/VA173/10 (SEQ ID NO:6), or a sequence that exhibits from about 59-100% or any amount therebetween (see Figure 4B), sequence similarity with the amino acid sequence of the GI.1 VP1 protein, or the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusion amino acid sequence shown in Figure 29A, for example from about 59, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence similarity with the amino acid sequence of the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusions (see Figure 4B), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject; or

- (GI.1 Nor/68) +P (GII.17/Kawasaki323/14) (SEQ ID NO:27, Figure 30A; S(GI.1)+P(GII.17): comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GII.17/Kawasaki323/14 (SEQ ID NO: 7), or a sequence that exhibits from about 59-100% or any amount therebetween (see Figure 4B), sequence similarity with the amino acid sequence of the GI.1 VP1 protein, or the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusion amino acid sequence shown in Figure 30A, for example from about 59, 60, 62, 64, 66, 68, 70, 72, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence similarity with the amino acid sequence of the S_1-$P1a_2$-$P2_2$-$P1b_2$ fusions (see Figure 4B), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00131] The VP1 fusion protein is heterologous (or chimeric) in that the fusion protein comprises an S domain from a first VP1 protein and a P domain from a second VP1 protein. The heterologous VP1 fusion protein may comprise an amino acid sequence that falls within, or the amino acid sequence is found within (or maps against) the consensus sequence of the VP1 sequence shown in Figure 16G (S-P boundary underlined and indicated by "‖"; SEQ ID NO:70), provided that the S and P domains of the VP1 fusion protein is heterologous, and that the VP1 fusion protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00132] Additional non-limiting examples of VP1 fusion proteins include those that comprise an S domain from: GI.1, for example but not limited to, the VP1 fusion shown in Figures 32A-32C (SEQ ID NO's:49-51); GI.5, for example but not limited to, the VP1 fusion shown in Figure 31B (SEQ ID NO:48); GII.12, for example but not limited to, the VP1 fusion as shown in Figures 33A – 33O (SEQ ID NO's:29-43), GII.14 for example, but not limited to, the VP1 fusion shown in Figure 34A (SEQ ID NO:52), GII.21 for example but not limited to, the VP1 fusion shown in Figure 34B (SEQ ID NO:53), or a sequence that exhibits from about 40-100% or any amount therebetween, sequence similarity with the amino acid sequence of the S domain provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject. Furthermore, the VP1 fusion protein may comprise an S domain that comprises an amino acid sequence that falls within (i.e. the amino acid sequence is found within, or maps against) the consensus sequence of the S domain as shown in Figure 16G (i.e. the N terminal portion of the consensus sequence; S-P boundary underlined and indicated by "‖"; SEQ ID NO:70), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00133] VP1 fusion proteins may also comprise a P domain obtained from GI.1/Norwalk/1968/US (SEQ ID NO:1); GI.5 Siklos/Hun5407/2013/HUN (SEQ ID NO:44); GII.1 Ascension 208/2010/USA (SEQ ID NO:45); GII.2 CGMH47/2011/TW (SEQ ID NO:66); GII.3 Jingzhou/2013402/CHN (SEQ ID NO:67); GII.4/Dresden174/1997/DE(variant:US1995/96); GII.4/FarmingtonHills/2002/US (SEQ ID NO:9); GII.4/Hunter-NSW504D/2004/AU (SEQ ID NO:10); GII.4/Shellharbour-NSW696T/2006/AU (11) ; GII.4/Orange-NSW001P/2008/AU (variant New Orleans 2009) (SEQ ID NO:12); GII.5 AlbertaEI390/2013/CA (SEQ ID NO:68); GII.7 Musashimurayama/2010/JP (SEQ ID NO:69; GII.14 8610/Saga/2008/JPN (SEQ ID NO:46); GII.21 Salisbury150/2011/USA (SEQ ID NO:47), or a sequence that exhibits from about 40-100% or any amount therebetween, sequence similarity with the amino acid sequence of the P domain from any one of GI.1/Norwalk/1968/US; GI.5 Siklos/Hun5407/2013/HUN; GII.1 Ascension 208/2010/USA; GII.2 CGMH47/2011/TW; GII.3 Jingzhou/2013402/CHN; GII.4/Dresden174/1997/DE(variant:US1995/96); GII.4/FarmingtonHills/2002/US;

GII.4/Hunter-NSW504D/2004/AU; GII.4/Shellharbour-NSW696T/2006/AU (11) ; GII.4/Orange-NSW001P/2008/AU (variant New Orleans 2009); GII.5 AlbertaEI390/2013/CA; GII.7 Musashimurayama/2010/JP; GII.14 8610/Saga/2008/JPN; GII.21 Salisbury150/2011/USA, for example from about 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween sequence similarity with the amino acid sequence of the P or the P2 domain from any one of GI.1/Norwalk/1968/US; GI.5 Siklos/Hun5407/2013/HUN; GII.1 Ascension 208/2010/USA; GII.2 CGMH47/2011/TW; GII.3 Jingzhou/2013402/CHN; GII.4/Dresden174/1997/DE(variant:US1995/96); GII.4/FarmingtonHills/2002/US; GII.4/Hunter-NSW504D/2004/AU; GII.4/Shellharbour-NSW696T/2006/AU (11) ; GII.4/Orange-NSW001P/2008/AU (variant New Orleans 2009); GII.5 AlbertaEI390/2013/CA; GII.7 Musashimurayama/2010/JP; GII.14 8610/Saga/2008/JPN; GII.21 Salisbury150/2011/USA, provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject. Furthermore, the VP1 fusion protein may comprise a P domain that comprises an amino acid sequence that falls within (i.e. the amino acid sequence maps against, or is found within) the consensus sequence of the P domain as shown in Figure 16G (i.e. the C terminal portion of the consensus sequence; S-P boundary underlined and indicated by "‖"; SEQ ID NO:70), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00134] In the VP1 fusion protein examples provided above, the S domain may comprise an amino acid sequence that exhibits from about 80-100%, or any amount therebetween, sequence similarity with the amino acid sequence of the S domain from any norovirus, for example but not limited to, the S domain from GI.1 Nor/68 (SEQ ID NO:1; see Figures13A, and 4B), GII.12/HS206/2010/USA (SEQ ID NO:28 ; see Figure 22A and 4C), or GI.5 Siklos/Hun5407/2013/HUN (SEQ ID NO:44 ; see Figure 16A and 4D). For example the S domain may comprise an amino acid sequence that exhibits from about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween sequence similarity with the amino acid sequence of the S domain from GI.1 Nor/68 (SEQ ID NO:1),

GII.12/HS206/2010/USA (SEQ ID NO:28), or GI.5 Siklos/Hun5407/2013/HUN (SEQ ID NO:44), provided that the VP1 protein induces immunity to norovirus in a subject, when the VP1 protein is administered to the subject.

[00135] As shown in Figures 5A, 5B, 6A, 6B and 6C, VP1 fusion proteins comprising S(GI.1)+P(GI.,2), S(GI.1)+P(GI.3), S(GI.1)+P(GII.4), S(GI.1)+P(GII.13), S(GI.1)+P(GII.17) resulted in VP1 fusion protein production in plants (as determined using a SDS-Comassie stained gel, or Western analysis) that was similar to or greater than the yield of the native VP1 protein that comprised the corresponding P domain (as determined using a SDS-Comassie stained gel, or Western analysis).

[00136] Expression of native GII.6/Ohio/490/12 VP1 protein has proven to be challenging (e.g. Figure 5B) and VP1 protein production varies from below detectable levels to detectable levels (see Example 4 below). Additionally expression of a VP1 fusion protein comprising an S domain from GI.1 Nor/68 (SEQ ID NO: 1) and a P domain from GII.6/Ohio/490/12 (SEQ ID NO: 5), was also bellow detection levels (Figure 5B).

[00137] The term "virus-like particle", VLP, "virus-like particles", or "VLPs", as used herein, refers to a norovirus virus like particles that comprise one or more than one type of norovirus VP1 protein, one or more than one type of VP1 fusion protein, or a combination thereof, and that self-assemble into non-replicating, non-enveloped, non-infectious viral capsid structures lacking all parts of the norovirus genome. For example, the VLP may comprise one type of VP1 fusion protein, or the VLP may comprise two or more different VP1 fusion proteins. Furthermore the VLP may comprise a VP2 protein. VLPs comprising VP1 protein, VP1+VP2 protein, VP1 fusion protein, or VP1 fusion protein +VP2 protein are of the size from about 15nm to 50nm or any amount therebetween, for example 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50nm, or any amount therebetween. For example, for T=1 icosahedral symmetry, VLPs may about 23 nm, or for T=3 icosahedral symmetry, VLPs may be from about 38 to about 40 nm.

[00138] As shown in Figures 6A, 6B, 6C, VLPS may be produced in plants from expressing human codon optimized nucleotide sequences encoding VP1 fusion

proteins described herein. For example, VLPs were produced comprising an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GI.2/Leuven/03 (SEQ ID NO:2; Figure 6A), an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GI.3/S29/08/Lilla Edet (SEQ ID NO:3 ; Figure 6B), an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GII.13/VA173/10 (SEQ ID NO:6; Figure 6C), and an S domain from GI.1 Nor/68 (SEQ ID NO:1) and a P domain from GII.17/Kawasaki323/14 (SEQ ID NO:7 ; Figure 6C).

[00139] An aspect of the present disclosure provides for the production of norovirus VP1 protein in plants. As shown in Figure 3A, leaves (from *N. benthamiana*) were vacuum infiltrated with *Argrobacterium tumifaciens* comprising expression vectors encoding GI.1 VP1 as a single nucleic acid construct, GI.1 VP2 as a single nucleic acid construct, both GI.1 VP1 and VP2, with VP1 and VP2 nucleic acid sequences introduced in separate vectors ("VP1+VP2"; dual constructs), or on the same vector ("VP1/VP2" or "VP1/VP2/3'UTR"; single nucleic acid constructs) to permit co-expression of the VP1 and/or VP2 sequences and the leaves examined for VP1 and VP2 production. After 6 or 9 days post infiltration (6 DPI and 9 DPI, respectively), total crude protein extracts were prepared from leaf homogenates, separated by SDS-PAGE, and stained with Coomassie Brilliant Blue dye. As seen in lanes 3-4, 7-10 and 19-20, leaves infiltrated with expression vectors comprising nucleotide sequences that correspond to wildtype GI.1 ORF2, encoding the VP1 protein, produced low or non-detectable levels of GI.1 VP1 as determined using Coomassie stained gels (VP1 expression was observed when expression was assayed by Western analysis, data not provided). In contrast, leaves infiltrated with expression vectors comprising GI.1 VP1 nucleotide sequences that were codon optimized for human expression (hCod), or enriched for GC content when compared to the GC content of the wildtype VP1 nucleic acid sequence, produced increased amounts of GI.1 VP1 protein (see lanes 11, 12, 21, 22; "VP1", 55-70 kDa band) in Coomassie stained gels. These results show that hCod VP1 may be produced in plants when VP1 is expressed on its own.

[00140] Furthermore, as seen in lanes 7-10 and 15-18 of Figure 3A, leaves infiltrated with vectors comprising either wildtype GI.1 VP1 and VP2 (lanes 7-10) or human codon optimized GI.1 VP1 and VP2 (lanes 15-18; dual nucleic acid

constructs) produced low or non-detectable levels of GI.1 VP1 protein in Coomassie stained gels, suggesting that expression of VP1 is not enhanced by the presence of VP2 when co-expressed in *cis* on the same vector, using the same organization as found in the viral genome (using one promoter to control expression). However, when VP1 or human codon optimized VP1 was co-expressed in *trans* (on a separate construct) along with VP2 or hCod VP2 (hCod VP1+VP2), respectively, an increase in VP1 protein (approx. 55-60kDa band) was observed (see lanes 15, 16, and 21, 22; Figure 3A). In this example, VP1 and VP2 nucleic acid segments, with each nucleic acid segment comprising a regulatory region and a terminator, were introduced into the plants as a nucleic acid complex, and this resulted in a corresponding increase in VP1 protein yield.

[00141] This observation is in contrast to that observed in insect and mammalian cells (Bertolotti-Ciarlet A., Crawford S.E., Hutson A.M., Estes M.K. 2003, J. Virol. 77:11603-11615), who reported that an increase in VP1 expression was only observed when VP1 and VP2 (or VP1+VP2+3'UTR) resided in *cis*, and were co-expressed using the same organization as that found in the viral genome, under the control of one promoter and terminator. No increase in VP1 expression was observed by Bertolotti-Ciarlet (2003) in insect or mammalian cells, when VP1 and VP2 were co-expressed in *trans*.

[00142] As described in more detail below (see "Norovirus VP1 Fusion Proteins"; reference to Figure 5E), when VP1 fusion proteins are expressed in plants, it is preferred that the ORF3 sequence encoding VP2 is obtained from the same norovirus genotype and strain as that used to obtain the S domain of fusion VP1 sequence.

[00143] The data presented in Figure 3A show that in plants, hCod VP1 may be expressed on its own, and that if hCod VP1 is co-expressed along with VP2, then both VP1 and VP2 should be expressed using separate expression systems, for example, on separate plasmids, or VP1 and VP2 may be expressed on the same vector but each of the sequences encoding VP1 and VP2 should be under the control of separate promoter and terminator sequences, so that they have a separate expression system.

Assembly of Plant-produced Norovirus VP1 into VLPs

[00144] FIGURE 3B (upper panel) shows a Coomassie-stained SDS-PAGE analysis of fractions from an iodixanol density gradient separation of crude protein extracts prepared from *N. benthamiana* leaves expressing GI.1 VP1 (single nucleic acid human codon optimized constructs), or VP1 and VP2 (dual nucleic acid human codon optimized constructs). Norovirus VP1 proteins (approx. 55-60kDa band) in high density fractions were analyzed by scanning electron microscopy. As seen in FIGURE 3B (lower panel), norovirus VP1 proteins and norovirus VP1+VP2 proteins self- assemble into VLPs in plants. The isolated VLPs exhibit a structural conformation similar to that of wildtype norovirus GI.1 virion particles (insert, Figure 3B).

[00145] Differential Expression of Norovirus VP1 in Plants

[00146] The expression levels of norovirus VP1 protein derived from six norovirus strains having the highest occurrence of outbreaks between September 1, 2013 and August 31, 2015 (as reported by the Centers for Disease Control and Prevention, see URL: www.cdc.gov/norovirus/reporting/calicinet/data.html) were compared in *N. benthamiana*.

[00147] VP1 protein production was determined using Coomassie-stained SDS-PAGE analysis (approx. 55-60kDa band) of extracts obtained from plant leaves vacuum infiltrated with expression vectors comprising human codon optimized sequences of VP1 derived from GI.1/Norwalk/1968/US (SEQ ID NO:18), GI.2/Leuven/2003/Bel (SEQ ID NO:54), GI.3/S29/2008/Lilla Edet/Sweden (SEQ ID NO:55), GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:56), GII.6/Ohio/490/12 (SEQ ID NO:60), GII.13/VA173/2010/USA (SEQ ID NO:61), GII.17/Kawasaki323/2014/JP (SEQ ID NO:62), strains. As shown in Figures 5A and 5B, VP1 protein production was observed in plant leaves expressing GI.1/Norwalk/1968/US (SEQ ID NO:1), GI.2/Leuven/2003/Bel (SEQ ID NO:2), GI.3/S29/2008/Lilla Edet/Sweden (SEQ ID NO:3), and GII.13/VA173/2010/USA (SEQ ID NO:6). A low or non-detectable amount of VP1 protein production was observed in plant leaves expressing GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:4), GII.6/Ohio/490/12 (SEQ ID NO:5), and GII.17/Kawasaki323/2014/JP (SEQ ID NO7).

[00148] VP1 protein expression was also observed when GI.3 (/S29/2008/Lilla Edet/Sweden; SEQ ID NO:3, Figure 15A), GI.5 (Siklos/Hun5407/2013/HUN; SEQ ID NO: 44, Figure 16A), GII.1 (Ascension208/2010/USA; SEQ ID NO: 45, Figure 16B), GII.2 (CGMH47/2011/TW; SEQ ID NO: 66, Figure 16C), GII.3 (Jingzhou/2013402/CHN; SEQ ID NO:67, Figure 16D), GII.5 (AlbertaEI390/2013/CA; SEQ ID NO: 68, Figure 16E), GII.6 (Ohio/490/12; SEQ ID NO: 60, Figure 18B), GII.7 (Musahimurayama/2010/JP; SEQ ID NO: 69, Figure 16F), GII.12 (HS206/2010/USA; SEQ ID NO:28, Figure 22A), GII.14 (8610/Saga/2008/JP; SEQ ID NO: 46, Figure 22B), and GII.21 (Salisbury150/2011/USA; SEQ ID NO: 47, Figure 22C), were expressed in plants (see Example 4 below).

[00149] As shown in the electron micrographs of Figures 3B, 5C, 5D, 6A and 6B, plant produced VP1 proteins derived from several norovirus strains self-assembled into VLPs. VLPs were observed in plant extracts following expression of strains GI.2/Leuven/2003/Bel (SEQ ID NO:54, Figure 14B), GI.3 S29/2008/Lilla Edet/Sweeden (SEQ ID NO's:3 (aa); 55 (na); Figure 15B), GI.5 Siklos/HUN5407/2013/HUN (SEQ ID NO:44: Figure 16A), GII.1 Ascension208/2010/USA SEQ ID NO:45; Figures 16B); GII.7 Musashimurayama/2010/JP (SEQ I NO:69; Figure 16F), GII.12 HS206/2010/USA (SEQ ID NO:28 , Figure 22A), GII.13 VA173/2010/USA (SEQ ID NO:61, Figure 19B), GII.14 8610/Saga/2008/JPN (SEQ ID NO:46 , Figure 22B), and GII.21 Salisbury150/2011/USA (SEQ ID NO:47 , Figure 22B). The VLPs have a structural conformation and diameter of about 15 nm to 50 nm, for example, of either about 23 nm, for T=1 icosahedral symmetry; or about 38 to 40 nm, for T=3 icosahedral symmetry, similar to that of wildtype norovirus.

[00150] Even though expression levels of VP1 protein in leaves infiltrated with vectors expressing GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:4), GII.6/Ohio/490/12 (SEQ ID NO:5), GII.17/Kawasaki323/2014/JP (SEQ ID NO:7), was either low or undetectable using Coomassie-stained SDS-PAGE analysis (see Figures 5A and 5B), expression of these VP1 proteins was observed at low levels (see Example 4, below).

[00151] Norovirus VP1 Fusion Proteins

[00152] Expression vectors were constructed which encoded norovirus VP1 fusion proteins wherein the S domain of GI.1 was fused to the following P domains:

GI.2 (S(GI.1)+P(GI.2); S(GI.1 Nor/68) +P (GI.2/Leuven/03); SEQ ID NO's:22(aa), 57(na), Figures 25A, 25B; construct 3360; SEQ ID NO:87; Figure 12C,

GI.3 (S(GI.1)+P(GI.3); GI.3 S(GI.1 Nor/68) +P (GI.3/S29/08/Lilla Edet) SEQ ID NO:23(aa), 58(na), Figures 26A, 26B; construct 3361),

GII.4 (S(GI.1)+P(GII.4); S(GI.1 Nor/68) +P (GII.4/Sydney/NSW0514/12) SEQ ID NO:24(aa), 59na), Figures 27A, 27B; construct 3362),

GII.6 (S(GI.1)+P(GII.6); S(GI.1 Nor/68) +P(GII.6/Ohio/490/12) SEQ ID NO:25(aa), 63(na), Figures 28A, 28B; construct 3363),

GII.13 (S(GI.1)+P(GII.13); S(GI.1 Nor/68) +P (GII.13/VA173/10) SEQ ID NO:26(aa); 64(na), Figures 29A, 29B; construct 3364), and

GII.17 (S(GI.1)+P(GII.17); S(GI.1 Nor/68) +P (GII.17/Kawasaki323/14) SEQ ID NO:27(aa), 65(na), Figures 30A, 30B; construct 3365)

[00153] VP1 fusion protein production was determined using Coomassie-stained SDS-PAGE analysis (approx. 55-60kDa band) of extracts obtained from plant leaves vacuum infiltrated with expression vectors comprising the above nucleotide sequences encoding the various VP1 fusion proteins, and VP2. As shown in Figures 5A and 5B, the fusion of the GI.1 Norwalk S domain to the P domain of GI.2 (GI.1S-GI.2P), GI.3 (GI.1S-GI.3P), or GII.13 (GI.1S-GII.13P), resulted in similar levels of expression of norovirus VP1 fusion proteins as compared to their native non-fusion counterparts. Demonstrating that VP1 fusion proteins may be expressed in a plant, portion of a plant, or a plant cell.

[00154] Even though protein product was not observed using SDS-PAGE analysis for the VP1 fusion S(GI.1) +P(GII.4) (S(GI.1 Nor/68)+P (GII.4/Sydney/NSW0514/12; SEQ ID NO:59, Figure 27B), and S(GI.1)+P(GII.17) (S(GI.1 Nor/68) +P (GII.17/Kawasaki323/14; SEQ ID NO:65, Figurer 30B), electron

micrograph analysis of S(GI.1) +P(GII.4) and GII.17 (GI.1S-GII.17P), revealed that VLPs were produced (see Figures 6C and 6D).

[00155] The fusion of the GI.1 Norwalk S domain to the P domains of low-expressing GII.6 (GI.1S-GII.6P) did not result in enhanced expression of norovirus VP1 fusion protein as compared to their native non-fusion counterparts. Without wishing to be bound by theory, these results suggest that the S domain may not be responsible for the low-level of expression in plants for these particular norovirus strains.

[00156] When VP1 fusion proteins are expressed in plants, it is preferred that the ORF3 sequence encoding VP2 is obtained from the same norovirus strain as used to obtain the S domain of fusion VP1 sequence. Support for this observation may be found with reference to Panels B and C of Figure 5E, which show that, in this example, the level of expression of a VP1 fusion protein, when co-expressed with VP2 obtained from the same genotype and strain as the S domain of the VP1 fusion, results in greater VLP yield (Panel B; Figure 5E, right hand side), then co-expression of the same VP1 fusion protein with a VP2 obtained from a different genotype and strain (Figure 5E; Panel C, right hand side). Rather, the VLP yield observed following co-expression of the VP1 fusion along with a heterologous VP2 (i.e. the S domain of the VP1 fusion and VP2 are from different genotypes and strains) decreased and approximated the VLP yield observed when VP1 was expressed alone (Figure 5E, Panel A, left hand side).

[00157] It is also of interest to note that the VLP yield obtained from co-expressing a VP1 fusion along with a VP2, where the S domain and the VP2 area obtained from the same genotype and strain (Panel B; Figure 5E, right hand side), is greater than the VLP yield observed following expression of the VP1 fusion when expressed alone (Panel B; Figure 5E, left hand side).

[00158] As shown in the electron micrographs of Figures 6A and 6B, VP1 fusion proteins derived from several of the strains including GI.1 Nor68+ GI.2/Leuven/2003/Bel (Figure 6A, right hand side); GI.1 Nor68+GI.3/S29/2008/Lilla Edet/Sweden (Figure 6B, right hand side), GI.1+GII.13Vas10 (Figure 6C left hand side), GI.1+GII.17Kaw14 (Figure 6C right hand side), self-assembled into VLPs

having a structural conformation and diameter of about 15 nm to 50 nm, for example, for T=1 icosahedral symmetry, about 23 nm, or for T=3 icosahedral symmetry, about 38 to about 40 nm, similar to that of wildtype norovirus. Of note is that the VP1 fusion product encoded by GI.1+GII.17Kaw14 resulted in a low yield (Figure 5B), however, VLPs comprising this VP1 protein could be purified from plant extracts.

[00159] However, no VLPs were obtained from plant extracts expressing the VP1 fusion protein GI.1+ GII.6/Ohio/490/12 (also see Example 5), consistent with the low or undetectable expression levels of this VP1 fusion protein as shown in Figure 5B.

[00160] Additional human codon optimized VP1 fusion proteins were prepared and co-expressed with VP2 in *N. benthamiana* leaves, as described in Example 5 below. The VP1 fusion proteins included:

S(GI.1)+P(X), where X= GI.2, GI.3, GII.4, GII.6, GII.12, GII.13, GII.17;

S(GI.5)+P(Y), where Y= GII.4;

S(GII.1)+P(Z), where Z= GI.3, GII.4, GII.17;

S(GII.12)+P(W), where W= GI.1, GI.2, GI.3, GI.5, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.13, GII.14, GII.17, GII.21;

S(GII.14)+P(T), where T= GII.4;

S(GII.21)+P(Q), where Q= GII.4

Expression of VP1 fusion proteins in a plant, a portion of a plant or a plant cell was observed (see Example 5) with the following VP1 fusion constructs:

S(GI.1)+P(GI.,2), S(GI.1)+P(GI.3), S(GI.1)+P(GII.4), S(GI.1)+P(GII.6), S(GI.1)+P(GII.12), S(GI.1)+P(GII.13), S(GI.1)+P(GII.17),

S(GI.5)+P(GII.4),

S(GII.1)+P(GI.3), S(GII.1)+P(GII.4),

S(GII.12)+P(GI.1), S(GII.12)+P(GI.2), S(GII.12)+P(GI.3), S(GII.12)+P(GI.5), S(GII.12)+P(GII.1), S(GII.12)+P(GII.2), S(GII.12)+P(GII.4), S(GII.12)+P(GII.7),

S(GII.12)+P(GII.13), S(GII.12)+P(GII.14), S(GII.12)+P(GII.17), S(GII.12)+P(GII.21).

[00161] Enhanced Stability of VLPs Comprising Norovirus VP1 Fusion Proteins

[00162] As shown in Figure 3C (upper panel), the levels of VLPs comprising of high-expressing native GI.1 VP1 protein (encoded by construct 2724) peaked in fractions four through eight following iodixanol gradient centrifugation. In contrast, VLPs comprising of native GI.2 VP1 protein (encoded by construct 3300) Figure 3C lower panel, left hand side) peaked in lower-density fractions six through nine. These results indicate that the assembly of native GI.2 VLPs may be less stable than GI.1 VLPs and may be more susceptible to malformed capsid particles and the generation of fragmentation products.

[00163] However, VLPs comprising S(GI.1 Nor68) +P(GI.2 Leu03) norovirus VP1 fusion proteins (encoded by construct 3360; Figure 3C, lower panel, right hand side), peaked in fractions four through eight, indicating that VLPs comprising norovirus VP1 fusion proteins having a GI.1 S domain core maybe more stable than their native VLP counterparts where the S domain is not derived from GI.1.

[00164] A similar shift in density was also observed in VLPs comprising of S(GI.1)+P(GI.3P) norovirus VP1 fusion proteins (Figure 6B; GI.1 Nor68+GI.3 Lil08, encoded by construct 3360).

[00165] Additionally, as shown with reference to Figure 5E, when a VP1 fusion protein is co-expressed with a VP2 minor structural protein that is obtained from the same genotype and strain as the S domain of the VP1 fusion, then the VP2 protein is incorporated on the VLPs (see high-molecular weight fractions of the density gradient that contain Norovirus VLPs, band of 21-24 kDa, indicated by a square; Figure 5E; Panel B right hand side, and Panel C left hand side). The VP2 band is absent when the VP2 protein is obtained from a different genotype and strain as the S domain of the VP1 fusion (Figure 5E, Panel C, right hand side). Without wishing to be bound by theory, these results are consistent with the proposal that VP2 is located on the inside of the viral particle and that VP2 may play a role in particle stability.

[00166] Induction of Immunity Against Norovirus Infection

[00167] An "immune response" generally refers to a response of the adaptive immune system of a subject. The adaptive immune system generally comprises a humoral response, and a cell-mediated response. The humoral response is the aspect of immunity that is mediated by secreted antibodies, produced in the cells of the B lymphocyte lineage (B cell). Secreted antibodies bind to antigens on the surfaces of invading microbes (such as viruses or bacteria), which flags them for destruction. Humoral immunity is used generally to refer to antibody production and the processes that accompany it, as well as the effector functions of antibodies, including Th2 cell activation and cytokine production, memory cell generation, opsonin promotion of phagocytosis, pathogen elimination and the like. The terms "modulate" or "modulation" or the like refer to an increase or decrease in a particular response or parameter, as determined by any of several assays generally known or used, some of which are exemplified herein.

[00168] A cell-mediated response is an immune response that does not involve antibodies but rather involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Cell-mediated immunity is used generally to refer to some Th cell activation, Tc cell activation and T-cell mediated responses. Cell mediated immunity may be of particular importance in responding to viral infections.

[00169] For example, the induction of antigen specific CD8 positive T lymphocytes may be measured using an ELISPOT assay; stimulation of CD4 positive T-lymphocytes may be measured using a proliferation assay. Anti-norovirus antibody titres may be quantified using an ELISA assay; isotypes of antigen-specific or cross reactive antibodies may also be measured using anti-isotype antibodies (e.g. anti -IgG, IgA, IgE or IgM). Methods and techniques for performing such assays are well-known in the art.

[00170] Cytokine presence or levels may also be quantified. For example a T-helper cell response (Th1/Th2) will be characterized by the measurement of IFN-γ and IL-4 secreting cells using by ELISA (e.g. BD Biosciences OptEIA kits). Peripheral blood mononuclear cells (PBMC) or splenocytes obtained from a subject may be cultured,

and the supernatant analyzed. T lymphocytes may also be quantified by fluorescence-activated cell sorting (FACS), using marker specific fluorescent labels and methods as are known in the art.

[00171] A microneutralization assay may also be conducted to characterize an immune response in a subject, see for example the methods of Rowe *et al.*, 1973. Virus neutralization titers may be quantified in a number of ways, including: enumeration of lysis plaques (plaque assay) following crystal violent fixation/coloration of cells; microscopic observation of cell lysis in *in vitro* culture; and 2) ELISA and spectrophotometric detection of norovirus.

[00172] The term "epitope" or "epitopes", as used herein, refers to a structural part of an antigen to which an antibody specifically binds.

[00173] With reference to Figure 6E, an immune response is observed following the administration of GI.1 VP1 VLPs, produced as described herein, to mice. Mice immunized with plant-made Norovirus native VP1 VLPs from GI.1 genotype exhibited GI.1 VLP-specific IgG antibody titers in sera on Days 21 and 42. IgG titer levels induced by each treatment on Days 21 and 42 were statistically higher than the titers quantified for the placebo group ($p < 0.05$). IgG titer level increased in a dose-dependent manner with a significant difference observed between the 1 μg and 10 μg treatments. The addition of Alhydrogel to the Norovirus VLP vaccine enhanced the immune response. These results demonstrate the ability of plant produced Norovirus native VP1 VLPs to elicit a robust immune response in mice. Similar results are observed with the administration of VP1 fusion proteins produced as described herein.

[00174] Plant expression

[00175] The constructs of the present invention can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, micro-injection, electroporation, etc. For reviews of such techniques see for example Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academy Press, New York VIII, pp. 421-463 (1988); Geierson and Corey, *Plant Molecular Biology*, 2d Ed. (1988); and Miki and Iyer, Fundamentals of Gene Transfer in Plants. In *Plant*

Metabolism, 2d Ed. DT. Dennis, DH Turpin, DD Lefebrvre, DB Layzell (eds), Addison Wesly, Langmans Ltd. London, pp. 561-579 (1997). Other methods include direct DNA uptake, the use of liposomes, electroporation, for example using protoplasts, micro-injection, microprojectiles or whiskers, and vacuum infiltration. See, for example, Bilang, et al. (1991, Gene 100: 247-250), Scheid et al. (1991, Mol. *Gen. Genet.* 228: 104-112), Guerche et al. (1987, Plant Science 52: 111-116), Neuhause et al. (1987, *Theor. Appl Genet.* 75: 30-36), Klein et al. (2987, Nature 327: 70-73); Freeman et al. (1984, *Plant Cell Physiol.* 29: 1353), Howell et al. (1980, Science 208: 1265), Horsch et al. (1985, Science 227: 1229-1231), DeBlock et al. (1989, *Plant Physiology* 91: 694-701), Methods for Plant Molecular Biology (Weissbach and Weissbach, eds., Academic Press Inc., 1988), Methods in Plant Molecular Biology (Schuler and Zielinski, eds., Academic Press Inc., 1989), WO 92/09696, WO 94/00583, EP 331083, EP 175966, Liu and Lomonossoff (2002, J *Virol Meth*, 105:343-348), EP 290395; WO 8706614; U.S. Pat. Nos. 4,945,050; 5,036,006; and 5,100,792, U.S. patent application Ser. Nos. 08/438,666, filed May 10, 1995, and 07/951,715, filed Sep. 25, 1992.

[00176] Transient expression methods may be used to express the constructs of the present invention (see D'Aoust et al., 2009, *Methods in molecular biology*, Vol 483, pages41-50; Liu and Lomonossoff, 2002, *Journal of Virological Methods*, 105:343-348). Alternatively, a vacuum-based transient expression method, as described by Kapila et al. (1997, *Plant Sci.* **122**, 101-108), or WO 00/063400, WO 00/037663 may be used. These methods may include, for example, but are not limited to, a method of Agro-inoculation or Agro-infiltration, syringe infiltration, however, other transient methods may also be used as noted above. With Agro-inoculation, Agro-infiltration, or syringe infiltration, a mixture of Agrobacteria comprising the desired nucleic acid enter the intercellular spaces of a tissue, for example the leaves, aerial portion of the plant (including stem, leaves and flower), other portion of the plant (stem, root, flower), or the whole plant. After crossing the epidermis the Agrobacteria infect and transfer t-DNA copies into the cells. The t-DNA is episomally transcribed and the mRNA translated, leading to

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the production of the protein of interest in infected cells, however, the passage of t-DNA inside the nucleus is transient.

[00177] Also considered part of this invention are transgenic plants, plant cells or seeds containing the gene construct of the present invention that may be used as a platform plant suitable for transient protein expression described herein. Methods of regenerating whole plants from plant cells are also known in the art (for example see Guerineau and Mullineaux (1993, Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148). In general, transformed plant cells are cultured in an appropriate medium, which may contain selective agents such as antibiotics, where selectable markers are used to facilitate identification of transformed plant cells. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be used to establish repetitive generations, either from seeds or using vegetative propagation techniques. Transgenic plants can also be generated without using tissue culture. Methods for stable transformation, and regeneration of these organisms are established in the art and known to one of skill in the art. Available techniques are reviewed in Vasil et al. (Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications, Academic Press, 1984), and Weissbach and Weissbach (Methods for Plant Molecular Biology, Academic Press, 1989). The method of obtaining transformed and regenerated plants is not critical to the present invention.

[00178] If plants, plant portions or plant cells are to be transformed or co-transformed by two or more nucleic acid constructs, the nucleic acid construct may be introduced into the *Agrobacterium* in a single transfection event so that the nucleic acids are pooled, and the bacterial cells transfected. Alternatively, the constructs may be introduced serially. In this case, a first construct is introduced into the *Agrobacterium* as described, the cells are grown under selective conditions (e.g. in the presence of an antibiotic) where only the singly transformed bacteria can grow. Following this first selection step, a second nucleic acid construct is introduced into the *Agrobacterium* as described, and the cells are grown under doubly-selective

conditions, where only the doubly-transformed bacteria can grow. The doubly-transformed bacteria may then be used to transform a plant, plant portion or plant cell as described herein, or may be subjected to a further transformation step to accommodate a third nucleic acid construct.

[00179] Alternatively, if plants, plant portions, or plant cells are to be transformed or co-transformed by two or more nucleic acid constructs, the nucleic acid construct may be introduced into the plant by co-infiltrating a mixture of *Agrobacterium* cells with the plant, plant portion, or plant cell, each *Agrobacterium* cell may comprise one or more constructs to be introduced within the plant. In order to vary the relative expression levels within the plant, plant portion or plant cell, of a nucleotide sequence of interest within a construct, during the step of infiltration, the concentration of the various *Agrobacteria* populations comprising the desired constructs may be varied.

[00180] Table 1: Norovirus strains and constructs.

Trivial name	strain	SEQ ID NO:	Construct #	Figure #
VP1				
GI.1 (VP1; aa)	GI.1/Norwalk/1968/US	1	--	13A
GI.1 (VP1; na)	Wild type GI.1/Norwalk/1968/US	13		13B
GI.1 hCod (VP1; na)	VP1-hCod GI.1/US/Norwalk/1968	18	2724	13C
GI.2 (VP1; aa)	GI.2/Leuven/2003/Bel	2	--	14A
GI.2 (VP1; (na)	hCod GI.2/Leuven/2003/Bel	54	3300	14B
GI.3 (VP1; aa)	GI.3/S29/2008/Lilla Edet/Sweden	3	--	15A
GI.3 (VP1; na)	hCod VP1 GI.3/S29/2008/Lilla Edet/Sweden	55	3302	15B
GI.5 (VP1; aa)	GI.5/Siklos/Hun5407/2013/HUN	44		16A
GII.1 (VP1; aa)	GII.1/Ascension208/2010/USA	45		16B
GII.2 (VP1; aa)	GII.2/CGMH47/2011/TW	66		16C
GII.3 (VP1; aa)	GII.3/Jingzhou/2013402/CHN	67		16D
GII.4 Syd12 (VP1; aa)	GII.4/Sydney/NSW0514/2012/AU	4	--	17A
GII.4 (VP1; na)	hCod VP1 GII.4/Sydney/NSW0514/2012/AU	56	3304	17B
GII.4 US96 (VP1; aa)	GII.4/Dresden174/1997/DE (variant: US 1995/96)	8	--	21A
GII.4 FH02 (VP1; aa)	GII.4/FarmingtonHills/2002/US	9	--	21B
GII.4 Hnt04 (VP1; aa)	GII.4/Hunter-NSW504D/2004/AU	10	--	21C
GII.4 2006b (VP1; aa)	GII.4/Shellharbour-NSW696T/2006/AU	11	--	21D
GII.4 NO09 (VP1; aa)	GII.4/Orange-NSW001P/2008/AU (variant New Orleans 2009)	12	--	21E
GII.5 (VP1; aa)	GII.5/AlbertaEI390/2013/CA	68		16E
GII.6 (VP1; aa)	GII.6/Ohio/490/12	5		18A
GII.6 (VP1; na)	hCod GII.6/Ohio/490/12	60	3306	18B
GII.7 (VP1; aa)	GII.7/Musahimurayama/2010/JP	69		16F

GII.12 (VP1; aa)	GII.12/HS206/2010/USA	28		22A
GII.13 (VP1; aa)	GII.13/VA173/2010/USA	6		19A
GII.13 (VP1; na)	hCod GII.13/VA173/2010/USA	61	3308	19B
GII.14 (VP1; aa)	GII.14/8610/Saga/2008/JP	46		22B
GII.17 (VP1; aa)	GII.17/Kawasaki323/2014/JP	7		20A
GII.17 (VP1; na)	hCod GII.17/Kawasaki323/2014/JP	62	3310	20B
GII.21 (VP1; aa)	GII.21/Salisbury150/2011/USA	47		22C
VP1 consensus (aa)	VP1 consensus sequence	70	--	16G
VP2				
GI.1 (VP2; aa)	VP2 GI.1/US/Norwalk/1968	14		23A
GI.1 (VP2; na)	VP2-GI.1/US/Norwalk/1968	15	2721	23B
GI.1 hCod (VP2; na)	VP2-hCod GI.1/US/Norwalk/1968	19	2725	23C
GII.4 hCod (VP2; na)	VP2 hCod GII.4/Sydney/NSW0514/2012/AU	120	3305	23D
GII.4 (VP2; aa)	VP2 GII.4/ Sydney/NSW0514/2012/AU	121		23E
VP1 fusions				
S(GI.1)+P(GI.2) (aa)	S(GI.1 Nor/68) +P (GI.2/Leuven/03)	22		25A
S(GI.1)+P(GI.2) (na)	hCod S(GI.1 Nor/68) +P (GI.2/Leuven/03)	57	3360	25B
S(GI.1)+P(GI.3) (aa)	S(GI.1 Nor/68) +P (GI.3/S29/08/Lilla Edet)	23	--	26A
S(GI.1)+P(GI.3) (na)	hCod S(GI.1 Nor/68) +P (GI.3/S29/08/Lilla Edet)	58	3361	26B
S(GI.1)+P(GII.4) (aa)	S(GI.1 Nor/68) +P (GII.4/Sydney/NSW0514/12	24	--	27A
S(GI.1)+P(GII.4) (na)	hCod S(GI.1 Nor/68) +P (GII.4/Sydney/NSW0514/12	59	3362	27B
S(GI.1)+P(GII.6) (aa)	S(GI.1 Nor/68) +P(GII.6/Ohio/490/12)	25	--	28A
S(GI.1)+P(GII.6) (na)	hCod S(GI.1 Nor/68) +P(GII.6/Ohio/490/12)	63	3363	28B
S(GI.1)+P(GII.12)(aa)	S(GI.1 Nor/68) +P (GII.12/HS206/2010/USA)	71	--	31A
S(GI.1)+P(GII.13) (aa)	S(GI.1 Nor/68) +P (GII.13/VA173/10)	26	--	29A
S(GI.1)+P(GII.13) (na)	hCod S(GI.1 Nor/68) +P (GII.13/VA173/10)	64	3364	29B
S(GI.1)+P(GII.17) (aa)	S(GI.1 Nor/68) +P (GII.17/Kawasaki323/14)	27	--	30A
S(GI.1)+P(GII.17) (na)	hCod S(GI.1 Nor/68) +P (GII.17/Kawasaki323/14)	65	3365	30B
S(GI.5)+P(GII.4) (aa)		48		31B
S(GII.1)+P(GI.3) (aa)		49		32A
S(GII.1)+P(GII.4) (aa)		50		32B
S(GII.1)+P(GII.17) (aa)		51		32C
S(GII.12)+P(GI.1) (aa)		29	--	33A
S(GII.12)+P(GI.2) (aa)		30	--	33B
S(GII.12)+P(GI.3) (aa)		31	--	33C
S(GII.12)+P(GI.5) (aa)		32	--	33D
S(GII.12)+P(GII.1) (aa)		33	--	33E
S(GII.12)+P(GII.2) (aa)		34	--	33F
S(GII.12)+P(GII.3) (aa)		35	--	33G
S(GII.12)+P(GII.4) (aa)		36	--	33H
S(GII.12)+P(GII.5) (aa)		37	--	33I

S(GII.12)+P(GII.6) (aa)		38	--	33J
S(GII.12)+P(GII.7) (aa)		39	--	33K
S(GII.12)+P(GII.13) (aa)		40	--	33L
S(GII.12)+P(GII.14) (aa)		41	--	33M
S(GII.12)+P(GII.17) (aa)		42	--	33N
S(GII.12)+P(GII.21) (aa)		43	--	33O
S(GII.14)+P(II.4) (aa)		52		34A
S9GII.21)+P(GII.4) (aa)		53		34B
S-P boundary				
S-P GI.1	S-P GI.1/Norwalk/1968/US	88	--	4A
S-P GI.2	S-P GI.2/Leuven/2003/Bel	89	--	4A
S-P GI.3	S-P GI.3/S29/2008/Lilla Edet/Sweden	90	--	4A
S-P GII,4	S-P GII.4/Sydney/NSW0514/2012/AU	91	--	4A
S-P GII.6	S-P GII.6/Ohio/490/12	92	--	4A
S-P GII.13	S-P GII.13/VA173/2010/USA	93	--	4A
S-P GII.17	S-P GII.17/Kawasaki323/2014/JP	94	--	4A
S-P boundary consensus		95		4A
ORF2/ORF3				
GI.1 VP1/VP2 (na)	GI.1/US/Norwalk/1968	16	2722	24A
GI.1 VP1/VP2/3'UTR (na)	GI.1/US/Norwalk/1968	17	2723	24B
GI.1 hCod VP1/VP2 (na)	hCod GI.1/US/Norwalk/1968	20	2726	24C
GI.1 hCod VP1/VP2/3'UTR (na)	hCod GI.1/US/Norwalk/1968	21	2727	24D
Primers				
lF-NoV(US68)VP1(ORF2).c		72		7A
lF-NoV(US68)VP1(ORF2).r		73		7B
lF-NoV(US68)VP1(ORF2)(hCod).c		76		9A
lF-NoV(US68)VP1(ORF2)(hCod).r		77		9B
lF-NoV(US68)VP2(ORF3)(hCod).c		79		10A
lF-NoV(US68)VP2(ORF3)(hCod).r		80		10B
lF-GI2Leu03VP1.c		82		11A
lF-GI2Leu03VP1.r		83		11B
GI2Leu+GI1VP1.r		85		12A
GI1VP1+GI2Leu.c		86		12B
lF-GI3Lil08VP1.c		111		35
lF-GI3Lil08VP1.r		98		35
lF-GII4Syd12VP1.c		112		35
lF-GII4Syd12VP1.r		101		35
lF-GII6Ohi12VP1.c		113		35
lF-GII6Ohi12VP1.r		104		35
lF-GII13VA10VP1.c		114		35
lF-GII13VA10VP1.r		107		35

IF-GII17Kaw14VP1.c		115		35
IF-GII17Kaw14VP1.r		110		35
GI3Lil+GI1VP1.r		96		35
GI1VP1+GI3Lil.c		97		35
GII4Syd+GI1VP1.r		99		35
GI1VP1+GII4Syd.c		100		35
GII6Ohi+GI1VP1.r		102		35
GI1VP1+GII6Ohi.c		103		35
GII13Va+GI1VP1.r		105		35
GI1VP1+GII13Va.c		106		35
GII17Kaw+GI1VP1.r		108		35
GI1VP1+GII17Kaw.c		109		35
IF-GII4Syd12VP2.c		117		35
IF-GII4Syd12VP2.r		119		35
IF-NoV(US68)VP2(ORF3).c		116		35
IF-NoV(US68)VP2(ORF3).r		122		
IF-NoV(US68)VP1/VP2(ORF3)NoV3'UTR.r		118		35
Constructs				
2X35S/CPMV-160/NOS (na)	2X35S/CPMV-160/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor	74	1190	7C
2X35S-WT VP1 GI.1/Norwalk/1968/US-NOS terminator (na)	2X35S promoter to NOS terminator. Wild-type VP1 from Norovirus GI.1/Norwalk/1968/US strain	75	2720	8A
2X35S-hCod-optimized VP1 GI.1/Norwalk/1968/US-NOS terminator	2X35S promoter to NOS terminator. Human codon-optimized VP1 from Norovirus GI.1/Norwalk/1968/US	78	2724	9C
2X35S-hCod-optimized VP2 GI.1/Norwalk/1968/US-NOS terminator	2X35S promoter to NOS terminator. Human codon-optimized VP2 from Norovirus GI.1/Norwalk/1968/US	81	2725	10C
2X35S-hCod optimized VP1 GI.2/Leuven/2003/Bel-NOS terminator	2X35S promoter to NOS terminator. Human codon-optimized VP1 from Norovirus GI.2/Leuven/2003/Bel	84	3300	11C
2X35S-hCod-optimized fusion VP1 S(GI.1)+P(GI.2)- NOS terminator	2X35S promoter to NOS terminator. Human codon-optimized fusion VP1 S(GI.1)+P(GI.2)	87	3360	12C

[00181] The present invention will be further illustrated in the following examples.

Example 1: Norovirus VP1 Constructs

[00182] The candidate sequences for VP1 and VP2 are available in Genbank (see Figures 2A and 2B). Non-limiting examples of these sequences are:

[00183] GI.1/Norwalk/1968/US (GI.1) NCBI M87661 (SEQ ID NO:1); VP1

[00184] GI.2/Leuven/2003/Bel (GI.2) NCBI FJ515294 (SEQ ID NO:2)

[00185] GI.3/S29/2008/Lilla Edet/Sweden NCBI JN603244 (SEQ ID NO:3)

[00186] GI.5/Siklos/Hun5407/2013/HUN (SEQ ID NO:44)

[00187] GII.1/Ascension208/2010/USA (SEQ ID NO:45)

[00188] GII.2/CGMH47/2011/TW (SEQ ID NO:66)

[00189] GII.3/Jingzhou/2013402/CHN (SEQ ID NO:67)

[00190] GII.4/Sydney/NSW0514/2012/AU NCBI JX459908 (SEQ ID NO:4)

[00191] GII.5/AlbertaEI390/2013/CA (SEQ ID NO:68)

[00192] GII.6/Ohio/490/2012/USA NCBI KC464321 (SEQ ID NO:5; VP1), NCBI J407072 (VP2)

[00193] GII.7/Musahimurayama/2010/JP (SEQ ID NO:69)

[00194] GII.12/HS206/2010/USA (SEQ ID NO:28)

[00195] GII.13/VA173/2010/USA NCBI JN899242 (SEQ ID NO:6)

[00196] GII.14/8610/Saga/2008/JP (SEQ ID NO:46)

[00197] GII.17/Kawasaki323/2014/JP NCBI AB983218 (SEQ ID NO:7)

[00198] GII.21/Salisbury150/2011/USA NCBI XX (SEQ ID NO:47)

A2X35S/CPMV 160/ wt VP1 GI.1/ NOS (Construct number 2720)

[00199] A wild-type sequence encoding VP1 from Norovirus strain GI.1/Norwalk/1968/US was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. A fragment containing the GI.1 VP1 coding sequence was amplified using primers IF-NoV(US68)VP1(ORF2).c (SEQ ID NO: 72) and IF-NoV(US68)VP1(ORF2).r (SEQ ID NO: 73), using native GI.1 VP1 gene sequence (SEQ ID NO: 13; Figure 13B) as template. The PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a 2X35S/CPMV 160/NOS-based

expression cassette (for a description of CMPV 160 see WO2015/103704 and WO2015/143567). It also incorporates a gene construct for the co-expression of the TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCAMBIA binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 74 (Figure 7C). The resulting construct was given number 2720 (SEQ ID NO: 75; Figure 8A). The amino acid sequence of native VP1 from Norovirus strain GI.1/Norwalk/1968/US is presented in SEQ ID NO: 1. A representation of plasmid 2720 is presented in Figure 8B.

2X35S/CPMV 160/ VP1 GI.1 (hCod)/ NOS (Construct number 2724)

[00200] A human codon-optimized sequence encoding VP1 from Norovirus strain GI.1/Norwalk/1968/US was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. A fragment containing the GI.1 VP1 coding sequence was amplified using primers IF-NoV(US68)VP1(ORF2)(hCod).c (SEQ ID NO: 76) and IF-NoV(US68)VP1(ORF2)(hCod).r (SEQ ID NO: 77), using human codon-optimized GI.1 VP1 gene sequence (SEQ ID NO: 18; Figure 13C) as template. For sequence optimization, GI.1/Norwalk/1968/US VP1 protein sequence (Genbank accession number NP_056821) was backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figures 7C and 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a 2X35S/CPMV 160/NOS-based expression cassette (For a description of CMPV 160 see WO2015/103704 and WO2015/143567). It also incorporates a gene construct for the co-expression of the TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCAMBIA binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 74. The resulting construct was given number 2724 (SEQ ID NO: 78; Figure 9C). The amino acid sequence of native VP1 from Norovirus strain

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GI.1/Norwalk/1968/US is presented in SEQ ID NO: 1. A representation of plasmid 2724 is presented in Figure 9D.

2X35S/CPMV 160/ VP2 GI.1 (hCod)/ NOS (Construct number 2725)

[00201] A human codon-optimized sequence encoding VP2 from Norovirus strain GI.1/Norwalk/1968/US was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. A fragment containing the GI.1 VP2 coding sequence was amplified using primers IF-NoV(US68)VP2(ORF3)(hCod).c (SEQ ID NO: 79) and IF-NoV(US68)VP2(ORF3)(hCod).r (SEQ ID NO: 80), using human codon-optimized GI.1 VP2 gene sequence (SEQ ID NO: 19; Figure 23C) as template. For sequence optimization, GI.1/Norwalk/1968/US VP2 protein sequence (Genbank accession number NP_056822) was backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figures 7C and 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a 2X35S/CPMV 160/NOS-based expression cassette (for a description of CMPV 160 see WO2015/103704 and WO2015/143567). It also incorporates a gene construct for the co-expression of the TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCAMBIA binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 74. The resulting construct was given number 2725 (SEQ ID NO: 81; Figure 10C). The amino acid sequence of native VP2 from Norovirus strain GI.1/Norwalk/1968/US is presented in SEQ ID NO: 14. A representation of plasmid 2725 is presented in Figure 10D.

2X35S/CPMV 160/ VP1 GI.2 (hCod)/ NOS (Construct number 3300)

[00202] A human codon-optimized sequence encoding VP1 from Norovirus strain GI.2/Leuven/2003/Bel was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. A fragment containing the GI.2 VP1 coding sequence was amplified using primers IF-GI2Leu03VP1.c (SEQ ID NO: 82)

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and IF-GI2Leu03VP1.r (SEQ ID NO: 83), using human codon-optimized GI.2 VP1 gene sequence (SEQ ID NO: 54; Figure 14B) as template. For sequence optimization, GI.2/Leuven/2003/Bel VP1 protein sequence (Genbank accession number ACU56258) was backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figures 7C and 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a 2X35S/CPMV 160/NOS-based expression cassette (for a description of CMPV 160 see WO2015/103704 and WO2015/143567). It also incorporates a gene construct for the co-expression of the TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCAMBIA binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 74. The resulting construct was given number 3300 (SEQ ID NO: 84; Figure 11C). The amino acid sequence of native VP1 from Norovirus strain GI.2/Leuven/2003/Bel is presented in SEQ ID NO: 2. A representation of plasmid 3300 is presented in Figure 11D.

[00203] A summary of the primers and templates used to preparer the above VP1 and VP2 constructs described above is provided in Table 2 below.

[00204] Norovirus VP1 Fusion Constructs

2X35S/CPMV 160/ fusion VP1 S(GI.1)+P(GI.2) (hCod)/ NOS (Construct number 3360)

[00205] A human codon-optimized sequence encoding fusion VP1 comprising of S domain from Norovirus strain GI.1/Norwalk/1968/US fused to P domain from Norovirus strain GI.2/Leuven/2003/Bel (VP1 S(GI.1)+P(GI.2)) was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. In a first round of PCR, a fragment containing S domain from Norovirus strain GI.1/Norwalk/1968/US was amplified using primers IF-NoV(US68)VP1(ORF2).c (SEQ ID NO: 72) and GI2Leu+GI1VP1.r (SEQ ID NO: 85), using human codon-

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optimized GI.1 VP1 gene sequence (SEQ ID NO: 18; Figure 13C) as template. A second fragment containing the P domain of GI.2/Leuven/2003/Bel was amplified using GI1VP1+GI2Leu.c (SEQ ID NO: 86) and IF-GI2Leu03VP1.r (SEQ ID NO: 83), using human codon-optimized GI.2 VP1 gene sequence (SEQ ID NO: 54; Figure 14B) as template. For sequence optimization, GI.1/Norwalk/1968/US VP1 protein sequence (Genbank accession number NP_056821) and GI.2/Leuven/2003/Bel VP1 protein sequence (Genbank accession number ACU56258) were backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR products from both amplifications were then mixed and used as template for a second round of amplification using IF-NoV(US68)VP1(ORF2).c (SEQ ID NO: 72) and IF-GI2Leu03VP1.r (SEQ ID NO: 83) as primers. The final PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a 2X35S/CPMV 160/NOS-based expression cassette (for a description of CMPV 160 see WO2015/103704 and WO2015/143567). It also incorporates a gene construct for the co-expression of the TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCAMBIA binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 74, Figure 7C. The resulting construct was given number 3360 (SEQ ID NO: 87; Figure 12C). The amino acid sequence of fusion VP1, VP1 S(GI.1)+P(GI.2) is presented in SEQ ID NO: 22 (Figure 25A). A representation of plasmid 3360 is presented in Figure 12D.

[00206] A summary of the VP1 fusion proteins, primers, templates and products is provided in Table 2 below. The VP1 fusion proteins were constructed using the same methods as described above, with reference to construct # 3360.

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Table 2: Summary of VP1 and VP2 proteins of interest, primers, templates, construct numbers, and final sequence identifiers of the resulting proteins. Rach construct also comprises a 2X 35S promoter, a CPMV-160 5'UTR*^, a CPMV 3" UTR, a Poly-A linker and a NOS terminator.

Protein of Interest	Construct No	Primer 1*	Primer 2**	Primer 3***	Primer 4^	Template ^^	Resulting protein (s)
NoV (GI.1/US/68) VP1 (hCod)	2724	76	N/Ap~	N/Ap	77	18	SEQ ID NO: 1
NoV (GI.2 Leu03) VP1 (Opt)	3300	82	N/Ap	N/Ap	83	54	SEQ ID NO: 2
NoV (GI.3 Lil08) VP1 (Opt)	3302	111	N/Ap	N/Ap	98	55	SEQ ID NO: 3
NoV (GII.4 Syd12) VP1 (Opt)	3304	112	N/Ap	N/Ap	101	56	SEQ ID NO: 4
NoV (GII.6 Ohi12) VP1 (Opt)	3306	113	N/Ap	N/Ap	104	60	SEQ ID NO: 5
NoV (GII.13 VA10) VP1 (Opt)	3308	114	N/Ap	N/Ap	107	61	SEQ ID NO: 6
NoV (GII.17 Kaw14) VP1 (Opt)	3310	115	N/Ap	N/Ap	110	62	SEQ ID NO: 7
NoV (GI.1/US/68) VP2	2721	116	N/Ap	N/Ap	122	13	SEQ ID NO: 14
NoV (GI.1/US/68) VP2 (hCod)	2725	79	N/Ap	N/Ap	80	19	SEQ ID NO: 14
VP1 S(GI.1)+P(GI.2)	3360	76	85	86	83	18 (S) and 54 (P)	SEQ ID NO: 22
VP1 S(GI.1)+P(GI.3)	3361	76	96	97	98	18 (S) and 55 (P)	SEQ ID NO: 23
VP1 S(GI.1)+P(GII.4)	3362	76	99	100	101	18 (S) and 56 (P)	SEQ ID NO: 24
VP1 S(GI.1)+P(GII.6)	3363	76	102	103	104	18 (S) and 60 (P)	SEQ ID NO: 25
VP1 S(GI.1)+P(GII.13)	3364	76	105	106	107	18 (S) and 61 (P)	SEQ ID NO: 26

VP1 S(GI.1)+P(GII.17)	3365	76	108	109	110	18 (S) and 62 (P)	SEQ ID NO: 27
NoV (GI.1/US/68) VP1/2	2722	72	N/Ap	N/Ap	122	17	SEQ ID NO: 1 and 14
NoV (GI.1/US/68) VP1/2/3'UTR	2723	72	N/Ap	N/Ap	118	17	SEQ ID NO: 1 and 14
NoV (GI.1/US/68) VP1/2 (hCod)	2726	76	N/Ap	N/Ap	80	21	SEQ ID NO: 1 and 14
NoV (GI.1/US/68) VP1/2 (hCod)/3'UTR	2727	76	N/Ap	N/Ap	118	21	SEQ ID NO:1 and 14
NoV (GI.1/US/68) VP1	2720	72	N/Ap	N/Ap	73	13	SEQ ID NO: 1
NoV (GII.4 Syd12) VP2 (Opt)	3305	117	N/Ap	N/Ap	119	120	SEQ ID NO: 121

*^ For a description of CMPV 160 see WO2015/103704 and WO2015/143567
* For In-fusion cloning; SEQ ID NO:
** To amplify S domain with primer 1; SEQ ID NO:
*** To amplify P domain with primer 4; SEQ ID NO:
^ For In-fusion cloning; SEQ ID NO:
^^ Complete VP1, S domain or P domain; SEQ ID NO:)
~ Not Applicable

Example 2: Methods

Agrobacterium tumefaciens Transfection

[00207] *Agrobacterium tumefaciens* strain AGL1 was transfected by electroporation with the native norovirus VP1, native norovirus VP2, or norovirus VP1 fusion protein expression vectors using the methods described by D'Aoust *et al.*, 2008 (*Plant Biotech. J.* 6:930-40). Transfected *Agrobacterium* were grown in YEB medium supplemented with 10 mM 2-(N-morpholino)ethanesulfonic acid (MES), 20 µM acetosyringone, 50 µg/ml kanamycin and 25 µg/ml of carbenicillin pH5.6 to an OD_{600} between 0.6 and 1.6. *Agrobacterium* suspensions were centrifuged before use and resuspended in infiltration medium (10 mM $MgCl_2$ and 10 mM MES pH 5.6).

[00208] Preparation of Plant Biomass, Inoculum and Agroinfiltration

[00209] *N. benthamiana* plants were grown from seeds in flats filled with a commercial peat moss substrate. The plants were allowed to grow in the greenhouse under a 16/8 photoperiod and a temperature regime of 25° C. day/20° C. night. Three weeks after seeding, individual plantlets were picked out, transplanted in pots and left to grow in the greenhouse for three additional weeks under the same environmental conditions

[00210] Agrobacteria transfected with each native norovirus VP1, native norovirus VP2, or norovirus VP1 fusion expression vector were grown in a YEB medium supplemented with 10 mM 2-(N-morpholino)ethanesulfonic acid (MES), 20 µM acetosyringone, 50 µg/ml kanamycin and 25 µg/ml of carbenicillin pH5.6 until they reached an OD_{600} between 0.6 and 1.6. *Agrobacterium* suspensions were centrifuged before use and resuspended in infiltration medium (10 mM $MgCl_2$ and 10 mM MES pH 5.6) and stored overnight at 4° C. On the day of infiltration, culture batches were diluted in 2.5 culture volumes and allowed to warm before use. Whole plants of *N. benthamiana* were placed upside down in the bacterial suspension in an air-tight stainless steel tank under a vacuum of 20-40 Torr for 2-min. Plants were returned to the greenhouse for a 6 or 9 day incubation period until harvest.

[00211] Leaf Harvest and Total Protein Extraction

[00212] Following incubation, the aerial part of plants was harvested, frozen at −80° C. and crushed into pieces. Total soluble proteins were extracted by homogenizing (Polytron) each sample of frozen-crushed plant material in 3 volumes of cold 100 mM NaOAc pH 5.2 + 150 mM NaCl, 0.4 µg/ml Metabisulfite and 1 mM phenylmethanesulfonyl fluoride. After homogenization, the slurries were centrifuged at 10,000 g for 10 min at 4° C. and these clarified crude extracts (supernatant) kept for analyses.

[00213] The total protein content of clarified crude extracts was determined by the Bradford assay (Bio-Rad, Hercules, California) using bovine serum albumin as the reference standard. Proteins were separated by SDS-PAGE under reducing conditions using Criterion™ TGX Stain-Free™ precast gels (Bio-Rad Laboratories, Hercules, CA) and proteins were visualized with Gel Doc™ EZ imaging system (Bio-Rad Laboratories, Hercules, CA) and electrotransferred onto polyvinylene difluoride (PVDF) membranes (Roche Diagnostics Corporation, Indianapolis, Indiana) for immunodetection. Prior to immunoblotting, the membranes were blocked with 5% skim milk and 0.1% Tween-20 in Tris-buffered saline (TBS-T) for 16-18 h at 4° C.

[00214] Protein Analysis and Immunoblotting

[00215] Immunoblotting was performed with a first incubation with a primary mAb 242P antibody specific to VP1 from GI and GII genotypes, diluted 1/500 in 2% skim milk in TBS-Tween 20 0.1%. Peroxydase-conjugated goat anti-mouse (Jackson Immunoresearch, cat #115-035-146) diluted 1/10000 was used as secondary antibody for chemiluminescence detection were as indicated in Table 4, diluted as indicated in 2% skim milk in TBS-Tween 20 0.1% Immunoreactive complexes were detected by chemiluminescence using luminol as the substrate (Roche Diagnostics Corporation). Horseradish peroxidase-enzyme conjugation of human IgG antibody was carried out by using the EZ-Link Plus® Activated Peroxidase conjugation kit (Pierce, Rockford, Ill.).

[00216] Analysis of VLP Formation/Iodixanol Gradients

[00217] Proteins were extracted from frozen biomass by mechanical extraction in a blender with 2 volumes of extraction buffer (100 mM NaOAc pH 5.2 + 150 mM NaCl). The slurry was filtered through a large pore nylon filter to remove large debris and centrifuged 5000 g for 5 min at 4°C. The supernatant was collected and centrifuged again at 5000 g for 30 min (4°C) to remove additional debris. The supernatant is then loaded on a discontinuous iodixanol density gradient. Analytical density gradient centrifugation was performed as follows: 38 ml tubes containing discontinuous iodixanol density gradient in acetate buffer (1 ml at 45%, 2 ml at 35%, 2 ml at 33%, 2 ml at 31%, 2 ml at 29% and 5 ml at 25% of iodixanol) were prepared and overlaid with 25 ml of the extracts containing the rotavirus-like particles. The gradients were centrifuged at 175 000 g for 4 hours (4°C). After centrifugation, 1 ml fractions were collected from the bottom to the top and fractions were analyzed by SDS-PAGE combined with protein staining or Western blot.

[00218] Electron microscopy

[00219] Following centrifugation of partially clarified plant extracts on discontinuous iodixanol density gradients, as described above, fractions (1 ml/fraction) containing the samples were pooled, mixed with 100 mM PBS pH 7.2 + 150 mM NaCl buffer to completely fill the tube and centrifuged 120 minutes at 100000g. The pellets were re-suspended in 300-1000 µl of buffer depending of the VP1 quantity.

[00220] Carbon-coated copper grids with a 200 nm mesh size were made hydrophilic by placing the carbon side face up on a Whatman paper in a petri dish and incubating overnight at 4 deg C. Pooled fractions (20 µl) from density gradient centrifugation to be observed by transmission electron microscopy (TEM) were deposited on a Parafilm and grids were floated with the carbon side facing down and incubated at room temperature for 5 minutes. Grids were washed 4 times on 20 µl water droplet and the excess water from the last wash drained by touching a Whatman paper with the side of the grid. Grids were incubated 1 minute on a 20 µl droplet of 2% uranyl acetate in water. Grids were allowed to dry 5 minutes on a Whatman paper. Observation was performed under transmission electron microscopy at magnifications ranging from 10,000X to 150,000X.

Example 3: VP1 protein production in plants

[00221] *N. benthamiana* leaves were, vacuum infiltrated, as described in Example 2, with *Agrobacterium tumifaciens* comprising expression vectors encoding wildtype GI.1 VP1 as a single nucleic acid construct, GI.1 VP2 (GI.1/Norwalk/1968/US; SEQ ID NO:15, Figure 23B) as a single nucleic acid construct, both GI.1 VP1 and VP2, with VP1 and VP2 nucleic acid sequences introduced in separate vectors ("VP1+/VP2"; dual constructs), or on the same vector ("VP1/VP2" or "VP1/VP2/3'UTR"; single nucleic acid constructs) to permit co-expression of the VP1 and/or VP2 sequences, and the leaves examined for VP1 and VP2 production. After 6 or 9 days post infiltration (6 DPI and 9 DPI, respectively), total crude protein extracts were prepared from leaf homogenates, separated by SDS-PAGE, and stained with Coomassie Brilliant Blue dye. The results are shown in Figure 3A,

[00222] Leaves infiltrated with expression vectors comprising nucleotide sequences that correspond to wildtype GI.1 VP1 (GI.1/Norwalk/1968/US; SEQ ID NO:13; Figure 13B), produced low or non-detectable levels of GI.1 VP1 as determined using Coomassie stained gels. See lanes 3-4, 7-10 and 19-20 of Figure 3A. VP1 expression was observed when expression was assayed by Western analysis (data not provided).

[00223] Leaves infiltrated with expression vectors comprising GI.1 VP1 nucleotide sequences that were codon optimized for human expression (hCod GI.1/Norwalk/1968/US; SEQ ID NO:18; Figure 13C), or enriched for GC content when compared to the GC content of the wildtype VP1 nucleic acid sequence (hCod), produced increased amounts of GI.1 VP1 protein (see lanes 11, 12, 21, 22; "VP1", 55-70 kDa band, Figure 3A).

[00224] Leaves infiltrated with vectors comprising either wildtype GI.1 VP1 (GI.1/Norwalk/1968/US; SEQ ID NO:13; Figure 13B) and VP2 (GI.1/Norwalk/1968/US; SEQ ID NO:15, Figure 23B; see lanes 7-10, Figure 3A) or human codon optimized GI.1 VP1 and VP2 (lanes 15-18, Figure 3A; dual nucleic acid

constructs) produced low or non-detectable levels of GI.1 VP1 protein in Coomassie stained gels.

[00225] Co-expression of human codon optimized GI.1 VP1 (GI.1/Norwalk/1968/US; SEQ ID NO:13; Figure 13B) and GI.1 VP2 (GI.1/Norwalk/1968/US; SEQ ID NO:15, Figure 23B) using separate vectors resulted in production of increased amounts of VP1 protein of approx. 55-60kDa (lanes 21-22, Figure 3A; single nucleic acid constructs). These data show that VP1 protein can be expressed in plants in the presence or absence of VP2 co-expression.

Norovirus VP1 VLPs

[00226] Components of crude plant extracts prepared from *N. benthamiana* leaves expressing GI.1 VP1 (single nucleic acid human codon optimized constructs; hCod GI.1/Norwalk/1968/US; SEQ ID NO:18; Figure 13C), or single nucleic acid human codon optimized constructs one vector comprising VP1 (hCod GI.1/Norwalk/1968/US; SEQ ID NO:18; Figure 13C) and the second vector comprising VP2 (GI.1/Norwalk/1968/US; SEQ ID NO:19, Figure 23C), were separated using discontinuous iodixanol density gradients as described in Example 2. Fractions following density gradient centrifugation were examined using Coomassie-stained SDS-PAGE analysis. The results are shown in Figure 3B (upper panel), with norovirus VP1 proteins, of approx. 55-60kDa band, observed in in high density (33% and 36%) fractions.

[00227] The protein components from the high density iodixanol gradient fractions were analyzed by scanning electron microscopy Figure 3B; lower panel). Norovirus VP1 proteins and norovirus VP1+VP2 proteins were found to self- assemble into VLPs in plants. The isolated VLPs exhibit a structural conformation similar to that of wildtype norovirus GI.1 virion particles (insert, Figure 3B).

[00228] Example 4: Differential Expression of Norovirus VP1 in Plants

[00229] The expression levels of norovirus human codon optimized sequences encoding VP1 protein from norovirus strains, GI.1/Norwalk/1968/US (SEQ ID NO:18; Figure 13C), GI.2/Leuven/2003/Bel (SEQ ID NO:54; Figurer 14B),

GI.3/S29/2008/Lilla Edet/Sweden (SEQ ID NO:55; Figure 15B), GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:56; Figure 17B), GII.6/Ohio/490/12 (SEQ ID NO:60; Figure 18B), GII.13/VA173/2010/USA (SEQ ID NO:61; Figure 19B), and GII.17/Kawasaki323/2014/JP (SEQ ID NO:62; Figure 20B), were compared in *N. benthamiana*. VP1 protein production was determined using Coomassie-stained SDS-PAGE analysis of extracts obtained from plant leaves vacuum infiltrated with expression vectors as described in Example 2. The results are presented in Figures 5A and 5B

[00230] Strong, or high, VP1 protein production was observed when human codon optimized GI.1/Norwalk/1968/US (SEQ ID NO:18), GI.3/S29/2008/Lilla Edet/Sweden (SEQ ID NO:55), GII.13/VA173/2010/USA (SEQ ID NO:61), and good, or medium, expression of VP1 was observed when GI.2/Leuven/2003/Bel (SEQ ID NO:54), were expressed in plant leaves (Figures 5A and 5B). Low or non-detectable amount of VP1 protein production was observed in plant leaves expressing human codon optimized GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:56), GII.6/Ohio/490/12 (SEQ ID NO:60), and GII.17/Kawasaki323/2014/JP (SEQ ID NO:62).

[00231] Strong, or high expression of GI.5/Siklos/HUN5407/2013/HUN; GII.1/Ascension208/2010/USA; GII.12/HS206/2010/USA; GII.12/HS206/2010/USA; and GII.21/Salisbury150/2011/USA, and good or medium expression of GII.2/CGMH47/2011/TW; GII.5/AlbertaE1390/2013/CA; GII.7/Musashimurayama/2010/JP, was also observed in plants.

[00232] Electron micrographs (prepared as described in Example 2), of high density iodixanol gradient fractions of several human codon optimized VP1 preparations were observed following expression of the following norovirus strains in plants (see Figures 5C, 5D, 6A (left hand panel) and Figure 6B (left hand panel):

- GI.2/Leuven/2003/Bel (SEQ ID NO's: 2 (aa) and 54 (na); Figure 14B);
- GI.3 S29/2008/Lilla Edet/Sweeden (SEQ ID NO:3 (aa);55 (na); Figure 15B);
- GI.5 Siklos/HUN5407/2013/HUN (SEQ ID NO:44: Figure 16A);

- GII.1 Ascension208/2010/USA SEQ ID NO:45; Figures 16B);

-GII.7 Musashimurayama/2010/JP (SEQ I NO:69; Figure 16F)

- GII.12 HS206/2010/USA (SEQ ID NO:28 , Figure 22A);

- GII.13 VA173/2010/USA (SEQ ID NO:61 , Figure 19B);

- GII.14 8610/Saga/2008/JPN (SEQ ID NO:46 , Figure 22B); and

- GII.21 Salisbury150/2011/USA (SEQ ID NO:47 , Figure 22B).

[00233] VP1 proteins derived from the above strains were observed to self-assembled into VLPs having a structural conformation and diameter of about 15 nm to 50 nm (for example, of either about 23 nm, for T=1 icosahedral symmetry; or about 38 to 40 nm, for T=3 icosahedral symmetry), similar to that of wildtype norovirus.

Example 5: VP1 fusion protein production in plants

[00234] *N. benthamiana* leaves were, vacuum infiltrated, as described in Example 2, with *Argrobacterium tumifaciens* comprising expression vectors encoding VP1 fusion proteins described below were co-expressed with VP2 (GI.1/Norwalk/1968/US; SEQ ID NO:15, Figure 23B). Nucleic acid segments encoding VP1 fusion proteins and VP2 were provided to the plants as a nucleic acid complex. After 6 or 9 days post infiltration (6 DPI and 9 DPI, respectively), total crude protein extracts were prepared from leaf homogenates, separated by SDS-PAGE, and stained with Coomassie Brilliant Blue dye to determine VP1 fusion protein production. Additionally electron micrographs of high density iodixanol gradient fractions of several VP1 fusion products were also prepared. The results are shown in Figures 5A, 5B, 5E, 6A (right hand panel), 6B (right hand panel), Figure 6C and 6D.

[00235] Leaves were infiltrated with expression vectors (nucleic acid complex) comprising human codon optimized nucleotide sequences encoding VP1 fusion of the GI.1 Norwalk S domain (GI.1/Norwalk/1968/US (SEQ ID NO:18; Figure 13C), to the P domain of:

- GI.2/Leuven/2003/Bel (SEQ ID NO:54; Figure 14B); to produce "GI.1-GI.2", comprising S(GI.1)+P(GI.,2); SEQ ID NO's 22(aa), 57(na);

- GI.3/S29/2008/Lilla Edet/Sweden (SEQ ID NO:55; Figure 15B); to produce "GI.1-GI.3", comprising S(GI.1)+P(GI.3); SEQ ID NO's: 23 (aa) and 58 (na);

- GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:56; Figure 17B); to produce "GI.1+GII.4", comprising S(GI.1)+P(GII.4); SEQ ID NO's: 24 (aa) and 59 (na);

- GII.6/Ohio/490/12 (SEQ ID NO:60; Figure 18B); to produce "GI.1+GII.6", comprising S(GI.1)+P(GII.6); SEQ ID NO's:25 (aa) and 63(na);

- GII.13/VA173/2010/USA (SEQ ID NO:61; Figure 19B); to produce "GI.1S+GII.13P", comprising S(GI.1)+P(GII.13); SEQ ID NO's:26 (aa) and 64 (na); and

- GII.17/Kawasaki323/2014/JP (SEQ ID NO:62; Figure 20B); to produce "GI.1+GII.17", comprising S(GI.1)+P(GII.17); SEQ ID NO's:27 (aa) and 65 (na).

[00236] In this example, VP1 and VP2 nucleic acid segments, with each nucleic acid segment comprising a regulatory region and a terminator, were introduced into the plants as a nucleic acid complex. As described below, with reference to Figure 5E, when VP1 fusion proteins are expressed in plants, it is preferred that the ORF3 sequence encoding VP2 is obtained from the same norovirus strain as used to obtain the S domain of fusion VP1 sequence.

[00237] Expression of VP1 fusion proteins comprising S(GI.1)+P(GI.,2), S(GI.1)+P(GI.3), S(GI.1)+P(GII.4), S(GI.1)+P(GII.13), S(GI.1)+P(GII.17), when co-expressed with VP2, resulted in similar or greater levels of expression of norovirus VP1 fusion proteins as compared to their native non-fusion counterparts (see Example 3; Figures 5A and 5B). These results demonstrate that VP1 fusion proteins may be expressed in a plant, portion of a plant, or a plant cell. Expression of the VP1 fusion proteins S(GI.1)+P(GII.6) was bellow detection levels (Figure 5B).

[00238] Electron micrographs (prepared as described in Example 2), of high density iodixanol gradient fractions of several human codon optimized VP1 fusion preparations were prepared as shown in Figure 6A (right hand panel), Figure 6B (right hand panel), Figure 6C (left and right hand panel) and 6D. VP1 fusion proteins

derived from S(GI.1)+P(GI.2), S(GI.1)+P(GII.3), S(GI.1)+P(GII.13), S(GI.1)+P(GII.17) and S(GI.1) +P(GII.4), and co-expressed with VP2, were observed to self-assembled into VLPs having a structural conformation and diameter of about 15 nm to 50 nm, for example, of either about 23 nm, for T=1 icosahedral symmetry; or about 38 to 40 nm, for T=3 icosahedral symmetry, similar to that of wildtype norovirus.

[00239] Even though protein product was below detectable levels using SDS-PAGE analysis for the VP1 fusion S(GI.1) +P(GII.4) (S(GI.1 Nor/68)+P (GII.4/Sydney/NSW0514/12; SEQ ID NO:59, Figure 27B) in Figure 5A, and for S(GI.1)+P(GII.17) (S(GI.1 Nor/68)+P (GII.17/Kawasaki323/14; SEQ ID NO:65, Figurer 30B) in Figure 5B, VLPs comprising S(GI.1) +P(GII.4) and S(GI.1)+P(GII.17) VP1 fusion protein could be purified from plant extracts (see Figures 6C and 6D). However, no VLPs were detected from plant extracts expressing S(GI.1)+P(GII.6) consistent with the low or undetectable expression levels of this VP1 fusion protein (Figure 5B).

[00240] Additional nucleic acid segments encoding human codon optimized VP1 fusion proteins were prepared and co-expressed with nucleic acid segments encoding VP2, in *N. benthamiana* leaves, as described above. These VP1 fusion proteins included:

S(GI.1)+P(X), where X= GI.2, GI.3, GII.4, GII.6, GII.12, GII.13, GII.17;

S(GI.5)+P(Y), where Y= GII.4;

S(GII.1)+P(Z), where Z= GI.3, GII.4, GII.17;

S(GII.12)+P(W), where W= GI.1, GI.2, GI.3, GI.5, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.13, GII.14, GII.17, GII.21;

S(GII.14)+P(T), where T= GII.4;

S(GII.21)+P(Q), where Q= GII.4

[00241] After 6 or 9 days post infiltration (6 DPI and 9 DPI, respectively) with the nucleic acid coonmplex, total crude protein extracts were prepared from leaf

homogenates, separated by SDS-PAGE, and stained with Coomassie Brilliant Blue dye to determine VP1 fusion protein production. Expression levels of the various VP1 fusion proteins was determined from the Coomassie stained gels. Additionally electron micrographs of high density iodixanol gradient fractions of several VP1 fusion products were also prepared.

[00242] Expression of various a nucleic acid segments encoding VP1 fusion proteins, comprising an S domain fused with heterologous P domain, with both domains obtained from VP1 proteins from a range of norovirus strains was observed, including, an S domain from GI.1, GI.5, GII.1, GII.12, GII.14 and GII.21, and a P domain obtained from GI.1, GI.2, GI.3, GI.5, GII.1, GII.2, GII.4, GII.6, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21. For example S(GI.1)+P(GI.2), S(GI.1)+P(GI.3), S(GI.1)+P(GII.4), S(GI.1)+P(GII.6), S(GI.1)+P(GII.12), S(GI.1)+P(GII.13), S(GI.1)+P(GII.17); S(GI.5)+P(GII.4); S(GII.1)+P(GI.3), S(GII.1)+P(GII.4), S(GII.12)+P(GI.1), S(GII.12)+P(GI.2), S(GII.12)+P(GI.3), S(GII.12)+P(GI.5), S(GII.12)+P(GII.1), S(GII.12)+P(GII.2), S(GII.12)+P(GII.4), S(GII.12)+P(GII.7), S(GII.12)+P(GII.13), S(GII.12)+P(GII.14), S(GII.12)+P(GII.17), S(GII.12)+P(GII.21), when co-expressed with a nucleic acid segment encoding VP2.

[00243] For example, strong, or high levels of expression of VP1 fusion protein in plants was observed using nucleic acid segments encoding: S(GI.1/US/68)+P(X); or S(GI.5/Siklos/HUN5407/2013/HUN)+P(Y), or S(GII.1/Ascension208/2010/USA)+P(Z); S(GII.12/HS206/2010/USA)+P(W); where:

X= P(GI.2/Leuven/2003/BEL); P(GII.4/Sydney/NSW0514/2012/AU); P(GII.12/HS206/2010/USA); P(GII.13/ VA173/2010/USA);

Y= P(GII.4/Sydney/NSW0514/2012/AU);

Z= P(GI.3/ S29/2008/Lilla Edet/Sweeden); or

W= P(GI.1/US/68): P(GI.3/S29/2008/Lilla Edet/Sweeden); P(GI.5/Siklos/HUN5407/2013/HUN); P(GII.1/Ascension208/2010/USA); P(GII.13/VA173/2010/USA); P(GII.14/8610/Saga/2008/JPN); P(GII.21/Salisbury150/2011/USA).

[00244] Good, or medium levels of expression levels in plants were observed using nucleic acid segments encoding VP1 fusion proteins comprising: S(GI.1/US/68)+P(X); S(GII.12/HS206/2010/USA)+P(W); or S(GII.14/8610/Saga/2008/JPN)+P(T), where:

X= P(GI.3/S29/2008/Lilla Edet/Sweeden); P(GII.6/Ohio/490/2012/USA); P(GII.17/Kawasaki323/2014/JP);

W=P(GI.2/Leuven/2003/BEL); P(GII.2/CGMH47/2011/TW); P(GII.7/Musashimurayama/2010/JP); P(GII.17/Kawasaki323/2014/JP); or

T= P(GII.4/Sydney/NSW0514/2012/AU).

[00245] Expression that was below detectable levels was observed with nucleic acid segments encoding VP1 fusion proteins comprising: S(GII.12/HS206/2010/USA)+P(GII.4/Sydney/NSW0514/2012/AU); S(GII.1/Ascension208/2010/USA+ P(GII.4/Sydney/NSW0514/2012/AU); or S(GII.21/Salisbury150/2011/USA)+ P(GII.4/Sydney/NSW0514/2012/AU).

[00246] These results demonstrate that VP1 fusion proteins comprising various combinations of S domains and P domains may be produced when expressed in plants.

Increased expression of VLPs comprising Norovirus VP1 fusion proteins and VP2 native proteins from the S domain genotype

[00247] The expression of a nucleic acid complex comprising norovirus nucleic acid segments encoding VP1 protein or VP1 fusion proteins co-expressed with a nucleic acid segment encoding VP2, were compared in *N. benthamiana* as described in Example 2. VP1 or VP1 fusion protein production was determined using Coomassie-stained SDS-PAGE analysis of extracts obtained from plant leaves vacuum infiltrated with expression vectors were loaded onto discontinuous iodixanol density gradients. Fractions collected from the bottom to the top and fractions were analyzed by SDS-PAGE. The following constructs were expressed and analyzed:

VP1 GII.4: human codon optimized native VP1 GII.4/Sydney/NSW0514/2012/AU (construct #3304; SEQ ID NO:56; Figure 17B)

VP1 GII.4 and VP2 GII.4: human codon optimized native VP1 co-expressed with human codon optimized native VP2 GII.4/Sydney/NSW0514/2012/AU (construct #3305; SEQ ID NO:120; Figure 23D);

VP1 fusion S(GI.1)+P(GII.4): human codon optimized VP1 S(GI.1)+P(GII.4) fusion protein (construct 3362; SEQ ID NO:59; Figure 27B);

VP1 fusion S(GI.1)+P(GII.4) and VP2 GI.1: human codon optimized native VP1 S(GI.1)+P(GII.4) fusion protein (construct 3362; SEQ ID NO:59; Figure 27B) co-expressed with human codon optimized native VP2 GI.1/Norwalk (construct #2725; SEQ ID NO:19; Figure 23C);

VP1 fusion S(GI.1)+P(GII.4) and VP2 GII.4: human codon optimized VP1 S(GI.1)+P(GII.4) fusion protein (construct 3362; SEQ ID NO:59; Figure 27B) co-expressed with human codon optimized native VP2 GII.4/Sydney (construct #3305; SEQ ID NO:133; Figure 23D).

The results are presented in Figure 5E.

[00248] The level of expression of the human codon-optimized GII.4/Sydney native VP1 protein (construct #3304) is low when the GII.4 VP1 construct is expressed alone (Figure 5E; Panel A, left hand side). The level of expression of the same GII.4 VP1 (construct #3304) remains low when co-expressed with human codon-optimized native minor structural VP2 protein from the GII.4 genotype and strain (construct #3305; Figure 5E Panel A, left hand side). T

[00249] The level of expression of the VP1 fusion, human codon optimized VP1 S (GI.1)+P (GII.4; construct #3362), is greatly increased when compared to the native VP1 of the GII.4 genotype (Panel B, Figure 5E, left hand side). Furthermore, the level of expression of the VP1 fusion S(GI.1)+P(GII.4; construct #3362), when co-expressed with human codon-optimized VP2 from GI.1/Norwalk (construct #2725), that is where the S domain of the VP1 fusion and the VP2 protein are obtained from the same genotype and strain, resulted in even higher VLP yield (Panel

B; Figure 5E, right hand side) when compared to the expression of the VP1 fusion S(GI.1)+P(GII.4). Additionally, the VP2 minor structural protein is incorporated on the VLPs as it is visible at the correct protein size (21-24 kDa; indicated by a square; Figure 5E; Panel B right hand side, and Panel C left hand side) in the high-molecular weight fractions of the density gradient that contain Norovirus VLPs.

[00250] This production of VLPs when co-expressing a VP1 fusion having an S domain and VP2 from same genotype and strain is to be contrast with the co-expression of a VP1 fusion, human codon optimized VP1 S (GI.1)+P (GII.4; construct #3362) with human codon-optimized VP2 from GII.4/Sydney (construct #3305), where the S domain of the VP1 fusion and the VP2 protein are obtained from different genotypes and strains. Co-expression of VP1 fusion comprising an S domain with a VP2 obtained from a different genotype and strain resulted in a dramatic decrease in VLP production (Panel C; Figure 5E, right hand side), when compared to the co-expression of the VP1 fusion S(GI.1)+P(GII.4) and VP2 from GI.1/Norwalk, construct #2725; Panel C; Figure 5E, left hand side) where the S domain and the VP2 are obtained from the same genotype and strain.

[00251] Without wishing to be bound by theory, these results are consistent with the proposal that VP2 is located on the inside of the viral particle and that VP2 may play a role in particle stability. When VP1 fusion proteins are expressed in plants, it is preferred that the ORF3 sequence encoding VP2 is obtained from the same norovirus strain as used to obtain the S domain of fusion VP1 sequence.

Enhanced Stability of VLPs Comprising Norovirus VP1 Fusion Proteins

[00252] Levels of VLPs comprising of high-expressing native VP1 GI.1/Norwalk/1968/US (encoded by construct 2724; SEQ ID NO:78; Figure 9C) peaked in fractions four through eight following iodixanol gradient centrifugation (see Figure 3C upper panel). In contrast, VLPs comprising of native VP1 GI.2/Leuven/2003/Bel (encoded by construct 3300; SEQ ID NO:84; Figure 11C) Figure 3C lower panel, left hand side) peaked in lower-density fractions six through nine, suggesting that native GI.2 VLPs may be less stable, more susceptible to

malformed capsid particles and the generation of fragmentation products, or a combination thereof, when compared to GI.1 VLPs.

[00253] In contrast, increased stability of VP1 fusion protein was observed with VLPs comprising S(GI.1 Nor68) +P(GI.2 Leu03) norovirus VP1 fusion proteins (encoded by construct 3360; SEQ ID NO:87; Figure 12C). As shown in Figure 3C, (lower panel, right hand side) VP1 fusion protein peaked in fractions four through eight, indicating that VLPs comprising norovirus VP1 fusion proteins having a GI.1 S domain core maybe more stable than their native VLP counterparts where the S domain is not derived from GI.1.

[00254] A similar shift in density was also observed in VLPs comprising S(GI.1)+P(GI.3) norovirus VP1 fusion proteins GI.1 Nor68+GI.3 Lil08 (see Figure 6B, right hand pannel), encoded by construct 33601; SEQ ID NO:58; Figure 26B) when compared to expression of VP1 GI.3 Lil08 (SEQ ID NO:55; Figure 15B) as shown in Figure 6B (left hand panel).

Example 6: Immune response using VP1

[00255] Studies on the immune response to Norovirus native GI.1 (SEQ ID NO:1) VLP administration were performed with 6-8 week old female BALB/c mice (Charles River Laboratories). Thirty seven mice were randomly divided into four groups of eight animals for Norovirus VLP vaccine and a group of five animals for placebo. All groups were injected using intramuscular immunization. All groups were immunized in a two-dose regimen, the boost immunization being administered 3 weeks following the first immunization.

[00256] For intramuscular administration in hind legs, two groups (eight animals) of unanaesthetized mice were immunized with the plant-made VLP native VP1 from Norovirus GI.1 genotype vaccine (1 or 10 µg). Placebo group (five animals) was immunized using the same route and regimen as the candidate vaccine using vaccine buffer (PBS at pH 6.0). In a similar manner plant-produced VP1 fusion proteins as described herein, for example VP1 fusion proteins produced using construct #3360, 3361, 3361, 3363, 3364, 3365, or SEQ ID NO's: 22 to 27, 29 to 43, 49 to 53, and 71,

may also also administered to mice following the same protocol as described in this example.

[00257] To measure the potential benefit of adjuvant, two groups of animals (8 animals) were immunized by intramuscular administration in hind legs on unanaesthetized mice with 1 or 10 µg plant-made VLP Norovirus vaccine plus one volume Alhydrogel 2% (alum, Cedarlane Laboratories Ltd., Burlington, Ontario, Canada). All groups were immunized according to a prime-boost regimen with the boost immunization performed 3 weeks following the first immunization.

[00258] Mice were evaluated through clinical observations during the in-life period as followed: daily monitoring for mortality and clinical signs, weekly detailed examinations, injection site observations and body weight measurements. All animals were under observation and sacrificed on Day 42 for gross examination. Blood was collected from all animals prior to dosing on Day 0, on Days 21 and 42 (21 days after each immunization). Samples were processed to isolate the serum for specific antibody response analyses.

[00259] Serum samples from blood collected on Days 21 and 42 from all animals were analyzed individually by ELISA for GI.1 VLP-specific total IgG and IgA antibodies using GI.1 VLP-coated plates. Pre-immune serum samples (Day 0 – prior dosing) collected from all animals were pooled by treatment group and each pool was analyzed to insure that they were negative (or below the cut-off value of the analytical test).

[00260] Descriptive statistics were performed using GraphPad Prism software (Version 6.05; GraphPad Software, La Jolla, CA, USA). Antibody titers measured for each group were reported as geometric mean titer (GMT) with 95 % confidence intervals (CI). Half of the value of the limit of detection was attributed to antibody titers below the limit of detection of the method specific to the tested antibodies. Therefore, in this study, an animal was considered to be a positive responder if its GMT value for a determined condition was equal or above the limit of detection of the method (LOQ = 100). Statistical comparisons between IgG titers of treatment groups were performed using one-way ANOVA followed by a Tukey's test on log10-transformed data. A comparison between the placebo group and each treatment group

was also performed using oneway ANOVA followed by a post hoc Dunnett's test on log10-transformed data.

[00261] The GI.1 VLP-specific total IgG titers that were measured in serum samples from all animals after IM immunization with one dose (Day 21) and two doses (Day 42) of 1 μg or 10 μg of each formulation. Total IgG titers were measured by ELISA using GI.1 VLP-coated plates (LOQ = 100). The results are present in Figure 6E. Total IgG titers per treatment group (n=8 animals/group) are represented by geometric mean titer (GMT) with a 95 % confidence interval. Statistical comparisons between IgG titers of treatment groups were performed using one-way ANOVA followed by a Tukey's test on log10-transformed data. A comparison between the placebo group and each treatment group was also performed using one-way ANOVA followed by a post-hoc Dunnett's test on log10-transformed data. Significant differences were annotated as letters in Figure 6E (the same letter indicates that no significant difference was detected between treatment groups; $p > 0.05$).

[00262] Mouse immune response to Norovirus native VP1 VLPs

[00263] As demonstrated in Figure 6E, mice immunized with plant-made Norovirus native VP1 VLPs from GI.1 genotype had shown GI.1 VLP-specific IgG antibody titers in sera and were detected for each treatment group on Days 21 and 42. IgG titer levels that were induced by each treatment on Days 21 and 42 were statistically higher than the titers quantified for the placebo group ($p < 0.05$). On each day, IgG titer level increased in a dose-dependent manner as demonstrated by the significant differences detected between the 1 μg and 10 μg treatments formulated with Alhydrogel or not ($p < 0.05$) and the addition of Alhydrogel to the NoV VLP vaccine enhanced significantly the induced immune response at doses of 1 μg and 10 μg ($p < 0.05$). A significant increase of IgG titer level was also detected for each treatment group between Days 21 and 42 ($p < 0.05$). These results collectively demonstrate the ability of plant produced Norovirus native VP1 VLPs to elicit a robust immune response in mice.

[00264] Similar results area observed with the administration of VP1 fusion proteins, VP1 fusion proteins produced for example, using construct #3360, 3361,

3361, 3363, 3364, 3365, or SEQ ID NO's: 22 to 27, 29 to 43, 49 to 53, and 71, as described herein.

[00265]

[00266] The present invention has been described with regard to one or more embodiments. However, it will be apparent to persons skilled in the art that a number of variations and modifications can be made to the described subject matter. The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole

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WHAT IS CLAIMED IS:

1. A norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI, and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

2. The norovirus VP1 fusion protein of claim 1 wherein the second norovirus strain is selected from norovirus genotypes GI.1, GI.2, GI.3, GI.4, GI.5, GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21.

3. The norovirus VP1 fusion protein of claim 1 or claim 2, wherein:

the first norovirus strain is GI.1 and the second norovirus strain is selected from the group consisting of GI.2, GI.3, GII.4, GII.12, GII.13 and GII.17;

the first norovirus strain is GI.5 and the second norovirus strain is GII.4;

the first norovirus strain is GII.1 and the second norovirus strain is selected from the group consisting of GI.3, GII.4 and GII.17; or

the first norovirus strain is GII.12 and the second norovirus strain is selected from the group consisting of GI.1, GI.2, GI.3, GI.5, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.13, GII.14, GII.17 and GII.21;

the first norovirus strain is from GII.14 and the second norovirus strain is GII.4; or

the first norovirus strain is GII.21 and the second norovirus strain is GII.4.

4. The norovirus VP1 fusion protein of claim 3 wherein the first norovirus strain and the second norovirus strain are selected from norovirus subtypes GI.1/US/1968; GI.2/Leuven/2003/Bel;

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GI.3/S29/2008/Lilla Edet/Sweden; I.5/AlbertaEI390/2013/CA; GII.1/Ascension208/2010/USA; GII.12/HS206/2010/USA; GII.13/VA173/2010/USA; GII.14/8610/Saga/2008/JPN; and GII.21/Salisbury150/2011/USA.

5. The norovirus VP1 fusion protein of any one of claims 1 to 4, the first norovirus strain selected from SEQ ID NO: 1, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 28, SEQ ID NO: 46 and SEQ ID NO: 47.

6. The norovirus VP1 fusion protein of any one of claims 1 to 5, the VP1 fusion protein comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 22-27, 29-43, 48-53 and 71.

7. A nucleic acid encoding the norovirus VP1 fusion protein of any one of claims 1 to 6, the nucleic acid comprising a first sequence encoding the S domain and a second sequence encoding the P domain, the first and second sequences under the control of a promoter that is active in plants.

8. The nucleic acid of claim 7 further comprising a third sequence encoding a CPMV enhancer, the CPMV enhancer operatively linked with the first and second sequences.

9. The nucleic acid of claim 7, where the first, the second, or both the first and second sequence is optimized for human codon usage, has increased GC content when compared to a corresponding wildtype nucleic acid sequence, or a combination thereof.

10. A virus like particle (VLP) comprising the norovirus VP1 fusion protein of any one of claims 1 to 6.

11. The VLP of claim 10, further comprising a norovirus VP2 protein.

12. A method of producing a norovirus VP1 fusion protein in a plant, a portion of the plant, or a plant cell comprising, introducing the nucleic acid of claim 7 into the plant, the portion of the plant, or the plant cell, and incubating the plant, the portion of the plant, or the plant cell under conditions that permit the expression and production of the norovirus VP1 fusion protein.

13. The method of claim 12, wherein the method further comprises a step of harvesting the plant, the portion of the plant, or the plant cell.

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14. The method of claim 13, wherein the method further comprises a step of extracting, purifying, or both extracting and purifying, the norovirus VP1 fusion protein from the plant, the portion of the plant, or the plant cell.

15. A norovirus VP1 fusion protein produced by the method of claim 14, the norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI, and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

16. The method of claim 13, wherein the method further comprises a step of purifying a virus like particle (VLP) from the plant, the portion of the plant, or the plant cell, wherein the VLP comprises the norovirus VP1 fusion protein and has a diameter of about 15 nm to 50 nm.

17. A virus like particle (VLP) comprising the norovirus VP1 fusion protein produced by the method of claim 16, the norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

18. The method of claim 16, wherein the VLP has a diameter of either about 23 nm or about 38 nm.

19. The method of claim 12, wherein in the step of introducing, a second nucleic acid sequence encoding a norovirus VP2 protein is introduced in the plant, the portion of the plant, or the plant cell, and in the step of incubating, the conditions permit co-expression and co-production of both the

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norovirus VP1 fusion protein and the norovirus VP2 protein in the plant, the portion of the plant, or the plant cell.

20. The method of claim 19 further comprising a step of harvesting the plant, the portion of the plant, or the plant cell.

21. The method of claim 20 further comprising a step of extracting, purifying, or both extracting and purifying the norovirus VP1 fusion protein and norovirus VP2 protein.

22. The method of claim 20 further comprising a step of extracting, purifying, or both extracting and purifying a virus like particle (VLP) from the plant, the portion of the plant, or the plant cell, wherein the VLP comprises the norovirus VP1 fusion protein and the norovirus VP2 protein, and has a diameter of about 15 nm to 50 nm.

23. A virus like particle (VLP) comprising the norovirus VP1 fusion protein and the norovirus VP2 protein produced by the method of claim 22, the norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI, and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

24. The method of claim 22, wherein the VLP has a diameter of either about 23 nm or about 38nm.

25. A plant cell comprising the norovirus VP1 fusion protein of any one of claims 1 to 6.

26. A plant cell comprising the norovirus VP1 fusion protein produced by the method of any one of claims 12 to 14, the norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI and GIV or selected from norovirus genotypes

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GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

27. A plant cell comprising the VLP of claim 10 or claim 11.

28. A plant cell comprising a VLP, the VLP comprising the norovirus VP1 fusion protein produced by the method of any one of claims 12 to 14, the norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

29. A plant cell comprising a VLP, the VLP comprising the norovirus VP1 fusion protein and VP2 protein produced by the method of claim 19, the norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

30. A plant cell comprising the nucleic acid of claim 7.

31. A plant extract comprising the norovirus VP1 fusion protein according to any one of claims 1 to 6.

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32. A plant extract comprising the norovirus VP1 fusion protein according to any one of claims 1 to 6 and a VP2 protein.

33. A plant extract comprising the virus like particle (VLP) according to claim 10 or claim 11.

34. A composition for inducing an immune response comprising, an effective dose of the norovirus VP1 fusion protein of any one of claims 1 to 6 or claim 15 and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

35. A composition for inducing an immune response comprising, an effective dose of the VLP of any one of claims 10, 11, 17 or 23, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

36. A vaccine comprising an effective dose of the norovirus VP1 fusion protein of any one of claims 1 to 6 or claim 15, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient, for inducing an immune response.

37. A vaccine comprising an effective dose of the VLP of any one of claims 10, 11, 17 or 23, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient, for inducing an immune response.

38. The VLP of any one of claims 10, 11, 17 or 23 for use in inducing immunity to a norovirus infection in a subject.

39. Use of the VLP of any one of claims 10, 11, 17 or 23 for inducing immunity to a norovirus infection in a subject.

40. Use of the VLP of any one of claims 10, 11, 17 or 23 in the manufacture of a medicament for inducing immunity to a norovirus infection in a subject.

41. The use of claim 40, wherein the VLP is for administration orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

42. A composition comprising, a VP1 sequence encoding the norovirus VP1 fusion protein of any one of claims 1 to 6, and a VP2 sequence encoding a norovirus VP2 protein, the VP1 sequence comprising a first and a second nucleic acid sequence, the first nucleic acid sequence encoding an S

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domain from a first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, the second nucleic acid sequence encoding a P domain from a second norovirus strain selected from norovirus genogroups GI, and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different, the VP2 sequence comprising a third nucleic acid sequence from the first norovirus strain and encoding the norovirus VP2 protein, wherein the VP1 sequence is operatively linked to a first regulatory region, and the VP2 sequence is operatively linked to a second regulatory region,

wherein the VP1 sequence and the VP2 sequence are located on one nucleic acid segment, or the VP1 sequence and the VP2 sequence are located on separate nucleic acid segments,

the composition further comprising a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

43. The composition of claim 42, wherein the first regulatory region, the second regulatory region, or the first and second regulatory region comprise a CPMV enhancer element operatively linked with a promoter active in the plant.

44. The composition of claim 43, wherein the first and the second regulatory region comprise the CPMV enhancer element, and the first and the second regulatory region comprise the same promoter.

45. The composition of claim 43, wherein the first and the second regulatory region comprise a CPMV enhancer element, and the CPMV enhancer sequence of the first and the second regulatory region is the same.

46. The composition of claim 42, wherein the first, the second, the third nucleic acid sequence, or all of the first, second and third nucleic acid sequence is optimized for human codon usage, has increased GC content when compared to a corresponding wildtype nucleic acid sequence, or a combination thereof.

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47. A method of producing a virus like particle (VLP) in a plant, a portion of the plant, or a plant cell comprising, introducing the composition of any one of claims 42 to 46 into the plant, the portion of the plant, or the plant cell, and incubating the plant, the portion of the plant, or the plant cell under conditions that permit the production of the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein.

48. The method of claim 47, wherein the method further comprises a step of harvesting the plant, the portion of the plant, or the plant cell.

49. The method of claim 48, wherein the method further comprises a step of extracting, purifying, or both extracting and purifying, the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein from the plant, the portion of the plant, or the plant cell.

50. The method of claim 49, wherein the VLP comprising the norovirus VP1 fusion protein and the norovirus VP2 protein has a diameter of about 15 nm to 50 nm.

51. A virus like particle (VLP) comprising the norovirus VP1 fusion protein and the norovirus VP2 protein produced by the method of any one of claims 47 to 50, the norovirus VP1 fusion protein comprising, a shell (S) domain from a first norovirus strain fused to a protruding (P) domain from a second norovirus strain, the first norovirus strain selected from norovirus genotypes GI.1, GI.5, GII.1, GII.12, GII.14, and GII.21, and the second norovirus strain selected from norovirus genogroups GI and GIV or selected from norovirus genotypes GII.1, GII.2, GII.3, GII.4, GII.5, GII.7, GII.12, GII.13, GII.14, GII.17 and GII.21, wherein the boundary between the S domain and the P domain of the norovirus VP1 fusion protein is defined by the consensus sequence LVPP--E||--T--F- wherein || indicates the boundary between the S domain and the P domain, wherein the first norovirus strain and second norovirus strain are different.

52. The method of claim any one of claims 47 to 50, wherein the VLP has a diameter of either about 23 nm or about 38 nm.

53. A plant cell comprising the VLP of claim 51.

54. A plant cell comprising the composition of any one of claims 42 to 46.

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55. A plant extract comprising the VLP according to claim 51.

56. A composition for inducing an immune response comprising, an effective dose of the VLP of claim 50, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

57. The composition of claim 56 for use in inducing immunity to a norovirus infection in a subject.

58. The composition for use of claim 57, wherein the composition is for administration orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

59. Use of the composition of claim 56 for inducing immunity to a norovirus infection in a subject.

60. Use of the composition of claim 56 in the manufacture of a medicament for inducing immunity to a norovirus infection in a subject.

61. A vaccine comprising an effective dose of the VLP of claim 51, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient, for inducing an immune response.

62. The vaccine of claim 61 for use in inducing immunity to a norovirus infection in a subject.

63. The vaccine of claim 62, wherein the vaccine is for administration orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

64. The VLP of claim 51 for use in inducing immunity to a norovirus infection in a subject.

65. The VLP of claim 64, wherein the VLP is for administration orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

66. Use of the VLP of claim 51 for inducing immunity to a norovirus infection in a subject.

67. Use of the VLP of claim 51 in the manufacture of a medicament for inducing immunity to a norovirus infection in a subject.

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Figure 1A

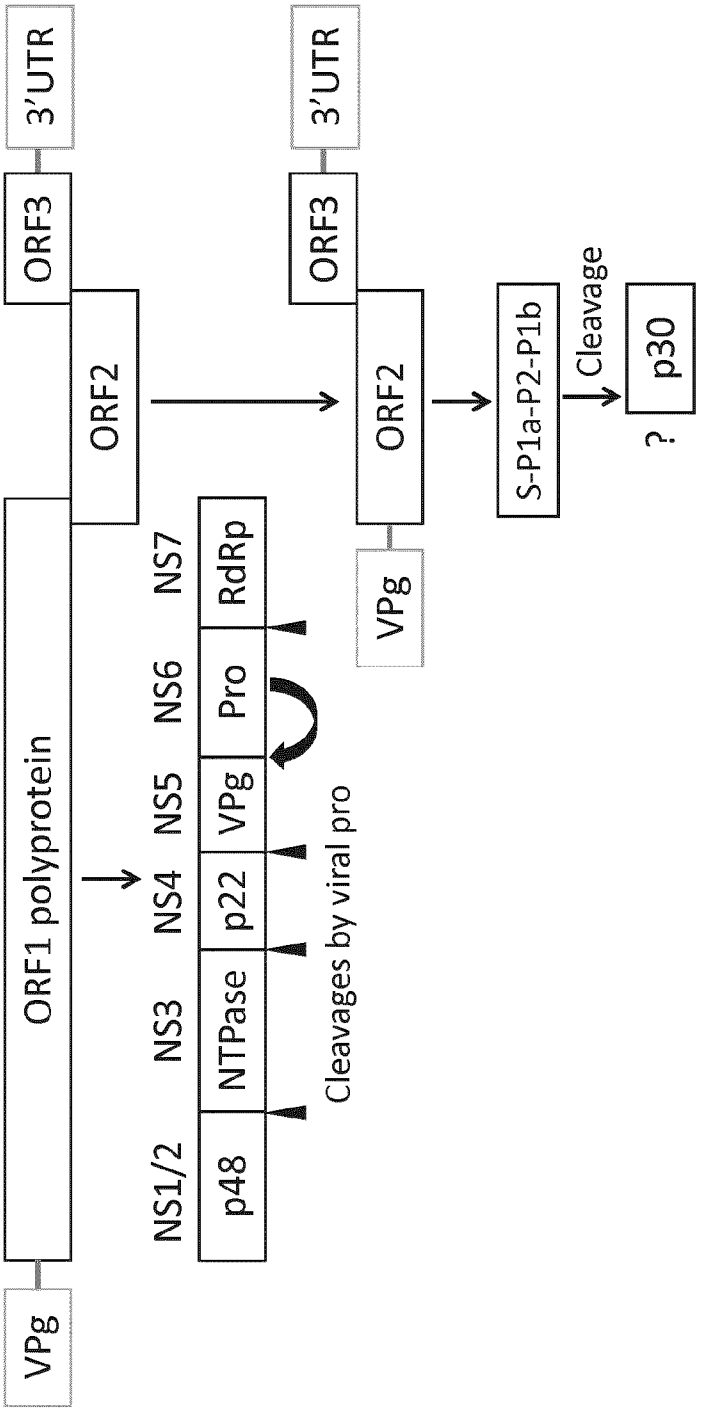
VPg
ORF1 polyprotein
ORF2
ORF3
3'UTR
NS1/2
NS3
NS4
NS5
NS6
NS7
p48
NTPase
p22
VPg
Pro
RdRp
Cleavages by viral pro
VPg
ORF2
ORF3
3'UTR
S-P1a-P2-P1b
Cleavage
?
p30

Figure 1C

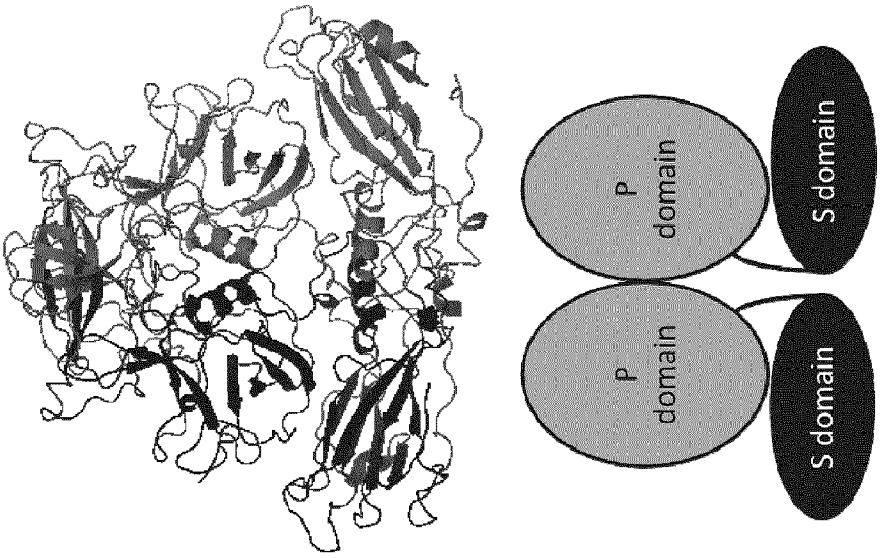

Figure 1B

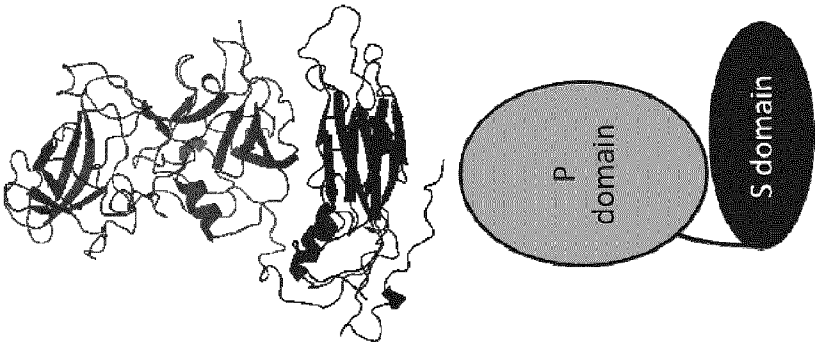

FIGURE 2A

Norovirus VP1 major capsid amino acid sequences		
Access (Uniprot)	Access (NCBI)	Strain name
Q83884	NP_056821	Hu/GI.1/United States/Norwalk/1968
D2DEL3	ACU56258	Hu/GI.2/Leuven/2003/BEL
H2DG70	AEY77318	Hu/GI.3/S29/2008/Lilla Edet/Sweden
K4LM89	AFV08795	Hu/GII.4/Sydney/NSW0514/2012/AU
M9T020	AGI96397	Hu/GII.6/Ohio/490/2012/USA
H9AWU4	AFC89656	Hu/GII.13/VA173/2010/USA
A0A077KVU6	AID51513	Hu/GII.17/Kawasaki323/2014/JP

Norovirus VP2 major capsid amino acid sequences		
Access (Uniprot)	Access (NCBI)	Strain name
Q83885	NP_056822	Hu/GI.1/United States/Norwalk/1968
D2DEL4	ACU56259	Hu/GI.2/Leuven/2003/BEL
H2DG71	AEY77319	Hu/GI.3/S29/2008/Lilla Edet/Sweden
K4LB50	AFV08796	Hu/GII.4/Sydney/NSW0514/2012/AU
W6APL0	AHI59155	Hu/GII.6/HS245/2010/USA
H9AWU5	AFC89657	Hu/GII.13/VA173/2010/USA
A0A077KP84	BAP33935	Hu/GII.17/Kawasaki323/2014/JP

FIGURE 2B

DNA accession numbers for VP1 and VP2 gene sequences		
Genotype	Genome access no (NCBI)	Strain name
GI.1	M87661	Hu/GI.1/United States/Norwalk/1968
GI.2	FJ515294	Hu/GI.2/Leuven/2003/BEL
GI.3	JN603244	Hu/GI.3/S29/2008/Lilla Edet/Sweden
GII.4	JX459908	Hu/GII.4/Sydney/NSW0514/2012/AU
GII.6	KC464321 (VP1), KJ407072 (VP2)	Hu/GII.6/Ohio/490/2012/USA (VP1), Hu/GII.6/HS245/2010/USA (VP2)
GII.13	JN899242	Hu/GII.13/VA173/2010/USA
GII.17	AB983218	Hu/GII.17/Kawasaki323/2014/JP

FIGURE 2C

Norovirus VP1 major capsid protein homology							
	GI.1	GI.2	GI.3	GII.4	GII.6	GII.13	GII.17
GI.1		69.5%	65.8%	41.8%	44.2%	44.4%	44.7%
GI.2			66.1%	41.7%	42.1%	43.5%	43.2%
GI.3				43.3%	44.9%	43.5%	45.4%
GII.4					60.8%	62.3%	65.6%
GII.6						66.6%	71.5%
GII.13							75.7%

Norovirus VP2 minor capsid protein homology							
	GI.1	GI.2	GI.3	GII.4	GII.6	GII.13	GII.17
GI.1		70.4%	62.5%	31.1%	35.0%	36.3%	34.1%
GI.2			63.0%	30.6%	34.1%	36.6%	34.9%
GI.3				30.5%	34.2%	38.3%	31.7%
GII.4					52.4%	53.9%	57.3%
GII.6						62.6%	60.3%
GII.13							67.2%

FIGURE 2D

VP1 protein homology (GII.4 strains)						
	US96	FH02	Hnt04	2006b	NO09	Syd12
US96		95.4%	94.1%	93.1%	93.3%	92.4%
FH02			95.7%	95.0%	94.1%	93.1%
Hnt04				94.1%	93.9%	93.1%
2006b					95.2%	94.8%
NO09						97.4%
Syd12						

VP1 (P domain only) protein homology (GII.4 strains)						
	US96	FH02	Hnt04	2006b	NO09	Syd12
US96		93.7%	92.2%	89.7%	90.3%	88.7%
FH02			94.7%	92.5%	91.2%	90.3%
Hnt04				92.2%	91.5%	90.9%
2006b					93.4%	92.8%
NO09						96.9%
Syd12						

VP1 (P2 sub-domain only) protein homology (GII.4 strains)						
	US96	FH02	Hnt04	2006b	NO09	Syd12
US96		89.6%	86.1%	81.3%	83.3%	81.3%
FH02			91.0%	84.0%	83.3%	82.6%
Hnt04				86.1%	84.0%	84.0%
2006b					87.5%	87.5%
NO09						94.4%
Syd12						

FIGURE 3A

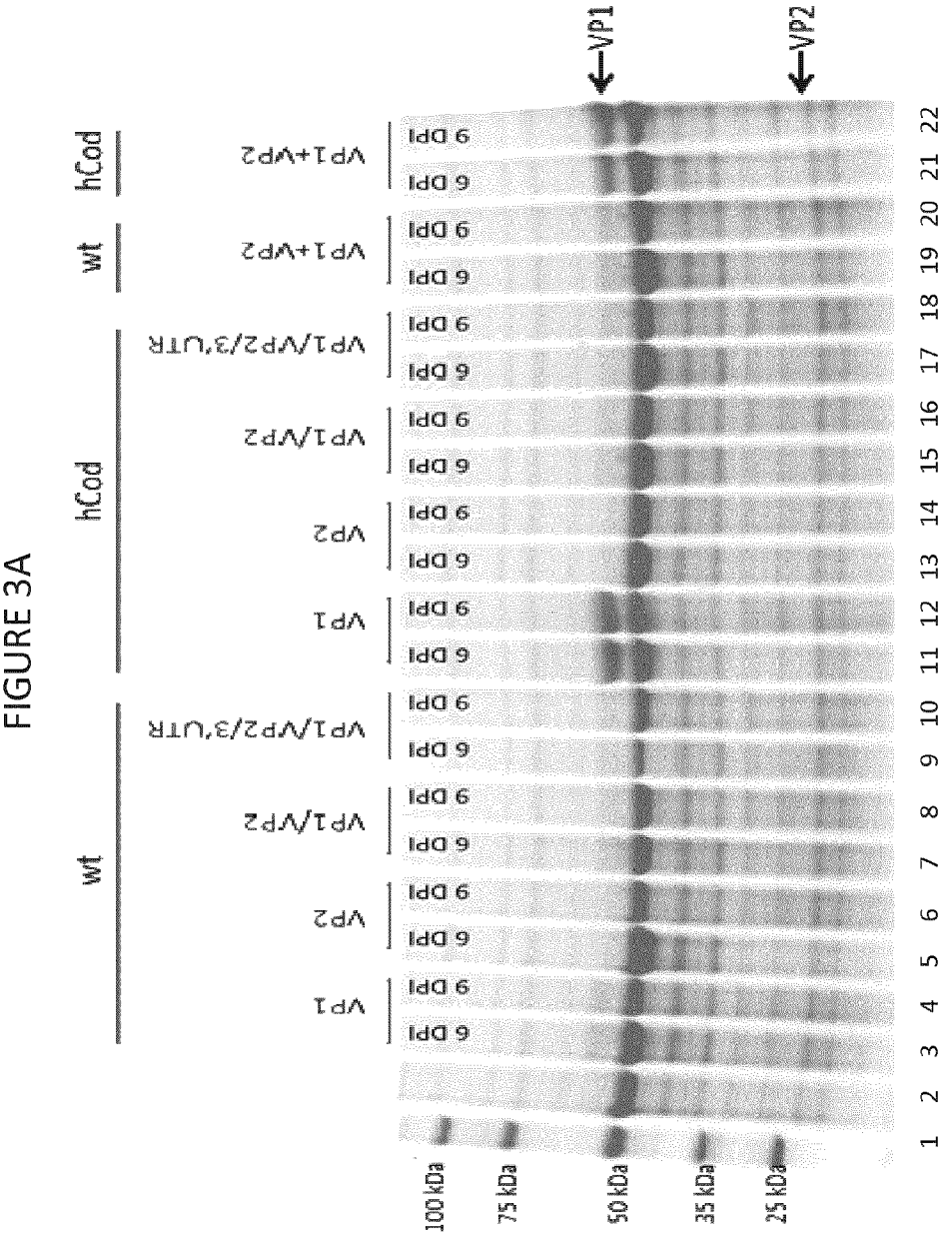
VP1
VP2
100 kDa
75 kDa
50 kDa
35 kDa
25 kDa
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
6 DPI
9 DPI
VP1
VP2
VP1/VP2
VP1/VP2/3'UTR
VP1+VP2
wt
hCod

FIGURE 3B

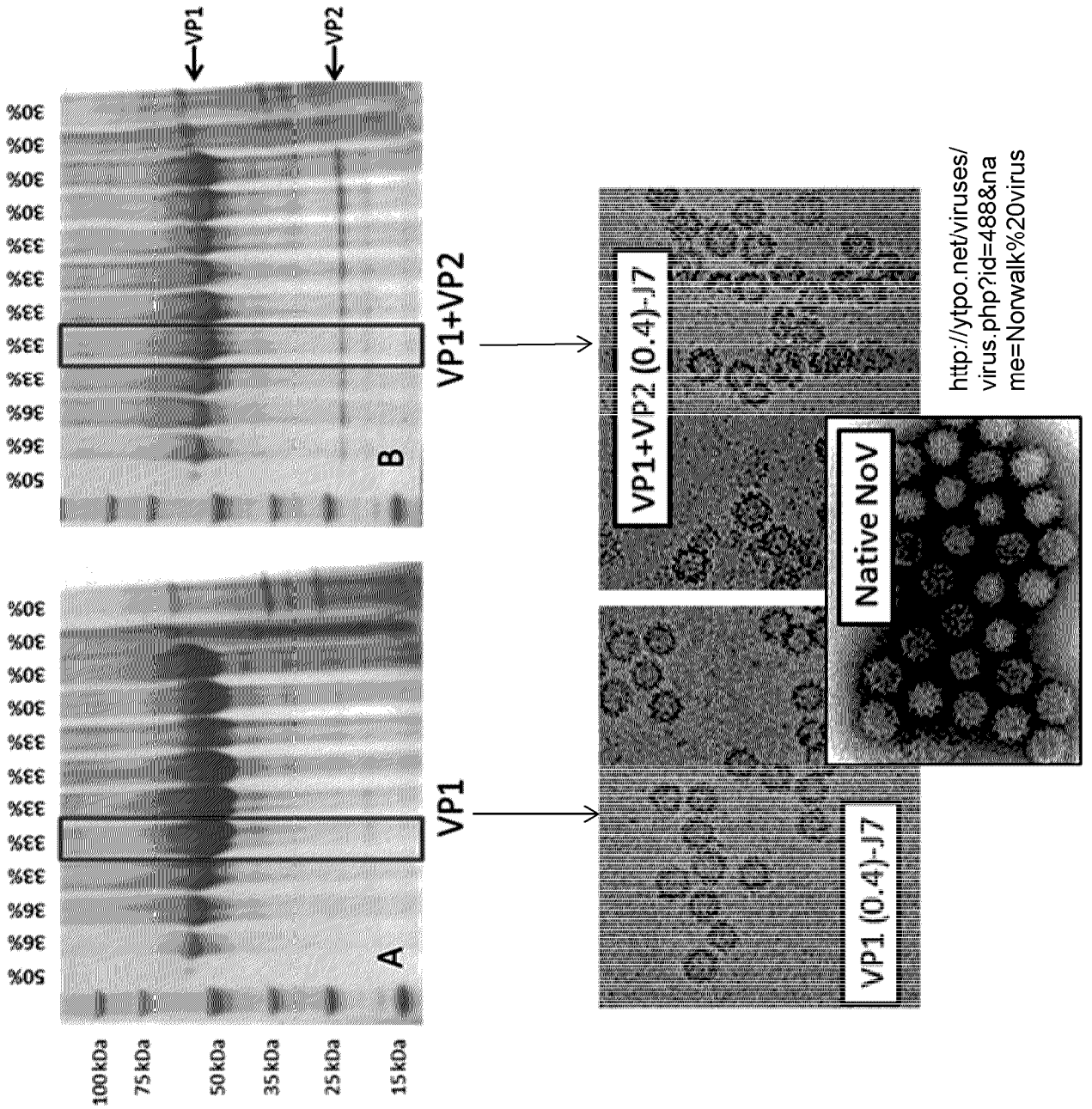

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FIGURE 3C

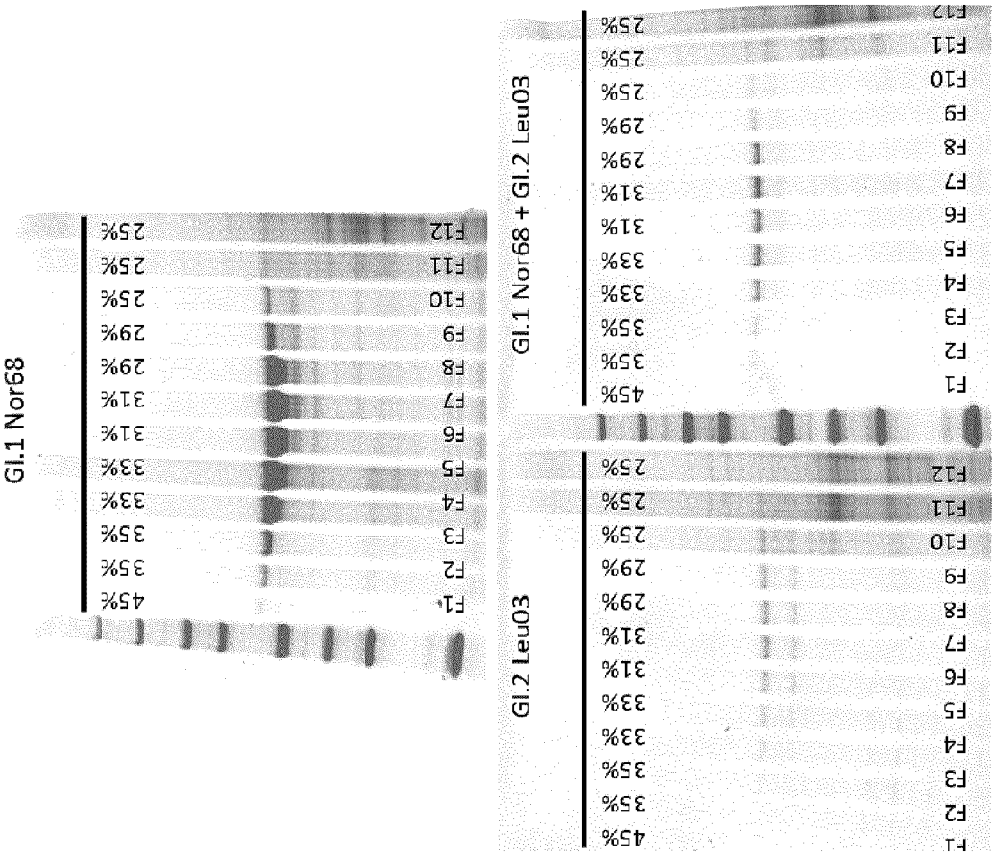

GI.1 Nor68
F12 25%
F11 25%
F10 25%
F9 29%
F8 29%
F7 31%
F6 31%
F5 33%
F4 33%
F3 35%
F2 35%
F1 45%
GI.1 Nor68 + GI.2 Leu03
F12 25%
F11 25%
F10 25%
F9 29%
F8 29%
F7 31%
F6 31%
F5 33%
F4 33%
F3 35%
F2 35%
F1 45%
GI.2 Leu03
F12 25%
F11 25%
F10 25%
F9 29%
F8 29%
F7 31%
F6 31%
F5 33%
F4 33%
F3 35%
F2 35%
F1 45%

FIGURE 4A

	← S DOMAIN	→ P DOMAIN 230	SEQ ID NO:
VP1_Norwalk_1968_GI_1_Q83884_Rf	LVPPTVE	QKTRPFTL	88
VP1_Leuven_2003_GI_2_D2DEL3	LVPPTIE	QKTRAFTV	89
VP1_LillaEdet_2008_GI_3_H2DG70	LVPPNVE	QKTKPFSV	90
VP1_Sydney_2012_GII_4_K4LM89	LVPPTVE	SRTKPFSV	91
VP1_Ohio_2012_GII_6_M9T020	VLPPTVE	SKTKPFSL	92
VP1_VA173_2010_GII_13_H9AWU4	LVPPSVE	SKTKPFTL	93
VP1_Kawasaki_2014_GII_17_A0A077KVU6	LVPPSVE	SKTKPFSL	94
CONSENSUS	LVPPtvE	skTkpFsl	95

Date Reçue/Date Received 2020-10-22

FIGURE 4B

	Norovirus VP1 major capsid protein homology							
	GI.1	S(GI.1) +P(GI.2)	S(GI.1) +P(GI.3)	S(GI.1) +P(GII.4)	S(GI.1) +P(GII.6)	S(GI.1) +P(GII.12)	S(GI.1) +P(GII.13)	S(GI.1) +P(GII.17)
GI.1		75.8%	75.0%	60.4%	60.8%	62.6%	61.8%	62.4%
S(GI.1) +P(GI.2)			74.6%	59.6%	59.1%	61.3%	60.5%	60.7%
S(GI.1) +P(GI.3)				60.2%	61.2%	60.9%	60.2%	62.2%
S(GI.1) +P(GII.4)					70.2%	75.0%	73.0%	76.1%
S(GI.1) +P(GII.6)						76.6%	74.7%	79.1%
S(GI.1) +P(GII.12)							78.3%	80.8%
S(GI.1) +P(GII.13)								79.0%

FIGURE 4C

	Norovirus VP1 major capsid protein homology									
	GII.12	S(GII.12) +P(GI.1)	S(GII.12) +P(GI.2)	S(GII.12) +P(GI.3)	S(GII.12) +P(GI.5)	S(GII.12) +P(GII.1)	S(GII.12) +P(GII.2)	S(GII.12) +P(GII.3)	S(GII.12) +P(GII.4)
GII.12		62.2%	61.0%	60.7%	60.1%	86.9%	80.3%	77.1%	74.6%
S(GII.12) +P(GI.1)									60.0%
S(GII.12) +P(GI.2)									59.3%
S(GII.12) +P(GI.3)									59.6%
S(GII.12) +P(GI.5)									60.1%
S(GII.12) +P(GII.1)									73.5%
S(GII.12) +P(GII.2)									70.7%
S(GII.12) +P(GII.3)									73.2%
	S(GII.12) +P(GII.5)	S(GII.12) +P(GII.6)	S(GII.12) +P(GII.7)	S(GII.12) +P(GII.13)	S(GII.12) +P(GII.14)	S(GII.12) +P(GII.17)	S(GII.12) +P(GII.21)		
GII.12		82.1%	76.2%	76.6%	77.9%	75.7%	80.4%	79.5%	
S(GII.12) +P(GII.3)		73.0%	70.0%	73.6%	72.8%	72.7%	76.0%	72.7%	

FIGURE 4D

	Norovirus VP1 major capsid protein homology									
	GI.5	S(GI.5) +P(GII.4)	GII.1	S(GII.1) +P(GI.3)	S(GII.1) +P(GII.4)	S(GII.1) +P(GII.17)	GII.14	S(GII.14) +P(GII.4)	GII.21	S(GII.21) +P(GII.4)
GI.5		60.6%	45.7%	60.1%	44.6%	45.9%	46.0%	44.0%	45.9%	44.9%
S(GI.5) +P(GII.4)			57.4%	44.3%	83.7%	60.1%	56.0%	83.2%	57.0%	84.1%
GII.1				60.8%	73.3%	80.4%	69.0%	65.7%	71.9%	66.7%
S(GII.1) +P(GI.3)					59.6%	61.9%	52.6%	52.2%	54.6%	53.1%
S(GII.1) +P(GII.4)						76.0%	64.9%	92.4%	65.9%	93.3%
S(GII.1) +P(GII.17)							68.5%	68.4%	71.3%	69.4%
GII.14								72.5%	65.1%	63.8%
S(GII.14) +P(GII.4)									63.9%	91.3%
GII.21										72.5%
S(GII.21) +P(GII.4)										

FIGURE 5A

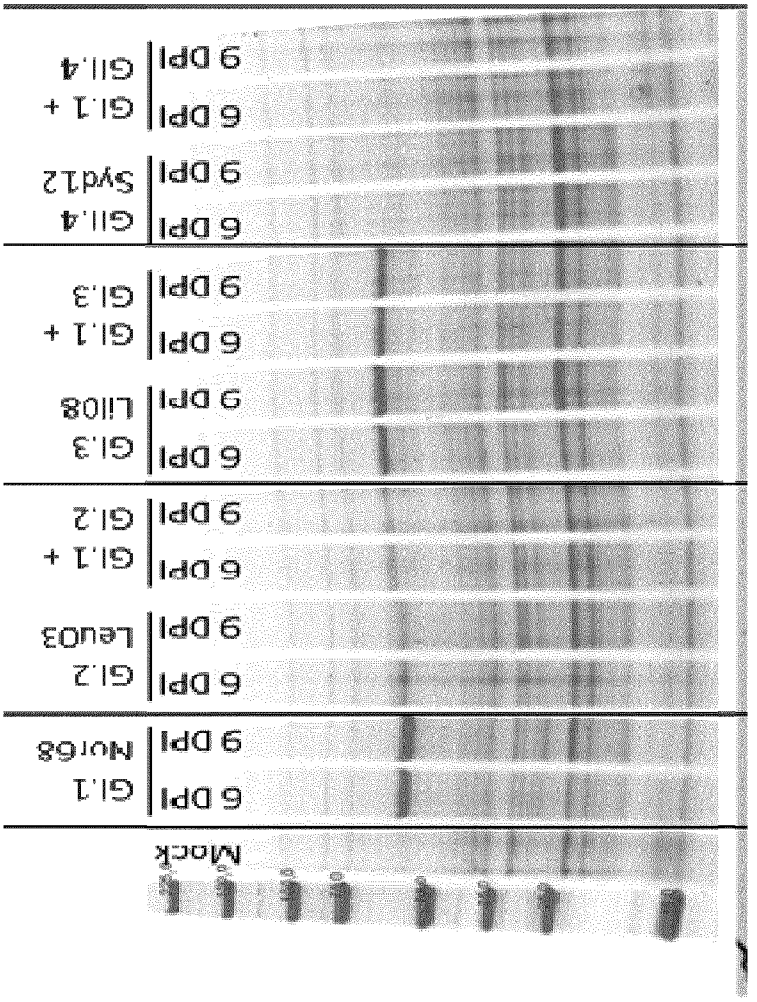

Mock
6 DPI
9 DPI
GI.1
Nor68
GI.2
Leu03
GI.1 +
GI.2
GI.3
Li08
GI.1 +
GI.3
GII.4
Syd12
GI.1 +
GII.4

FIGURE 5B

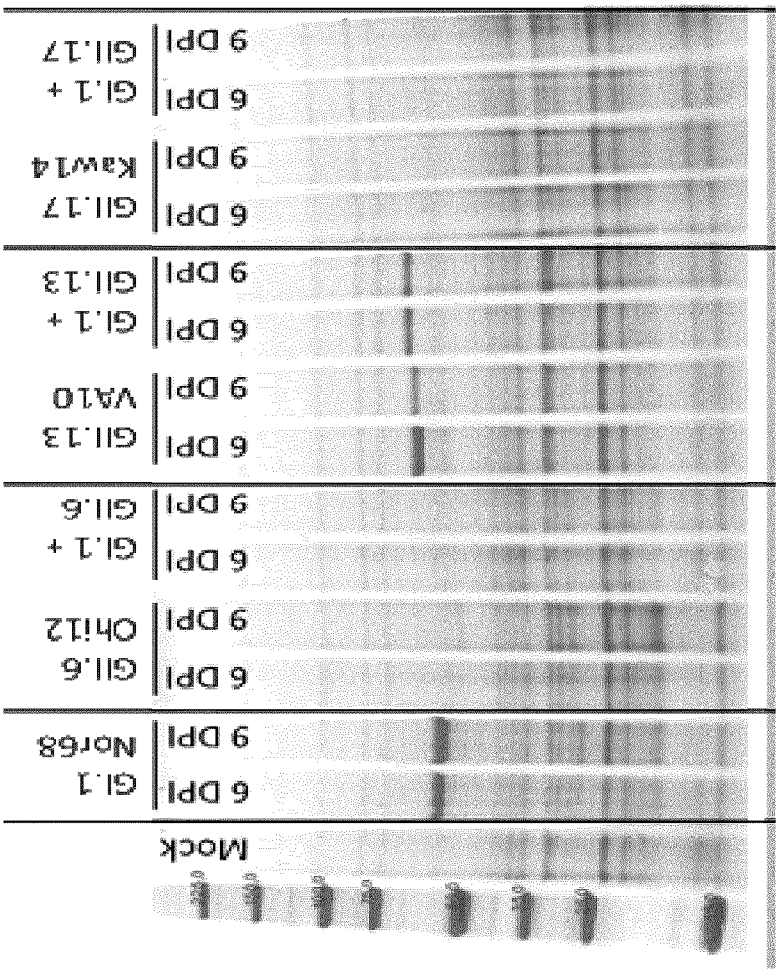

Mock
GI.1
Nor68
6 DPI
9 DPI
GII.6
Ohi12
6 DPI
9 DPI
GI.1 +
GII.6
6 DPI
9 DPI
GII.13
VA10
6 DPI
9 DPI
GI.1 +
GII.13
6 DPI
9 DPI
GII.17
Kaw14
6 DPI
9 DPI
GI.1 +
GII.17
6 DPI
9 DPI

FIGURE 5C

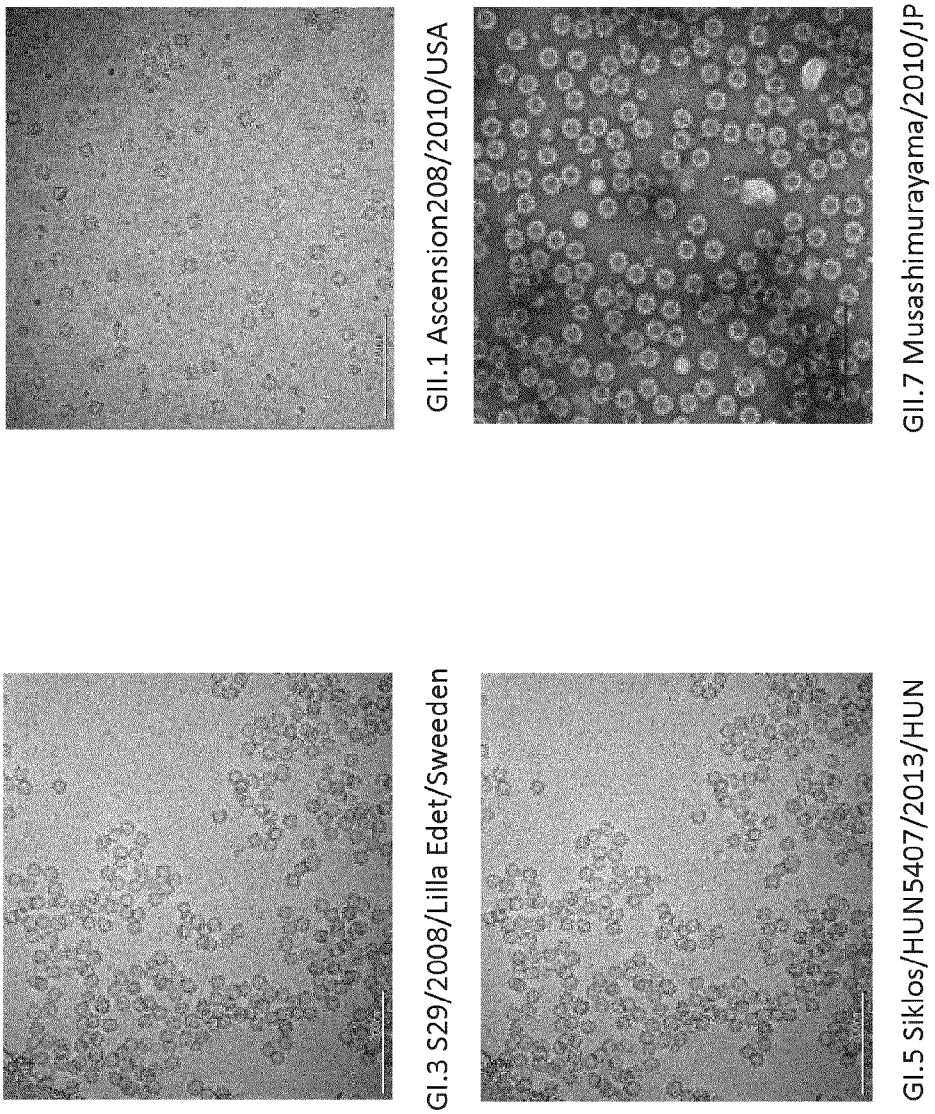
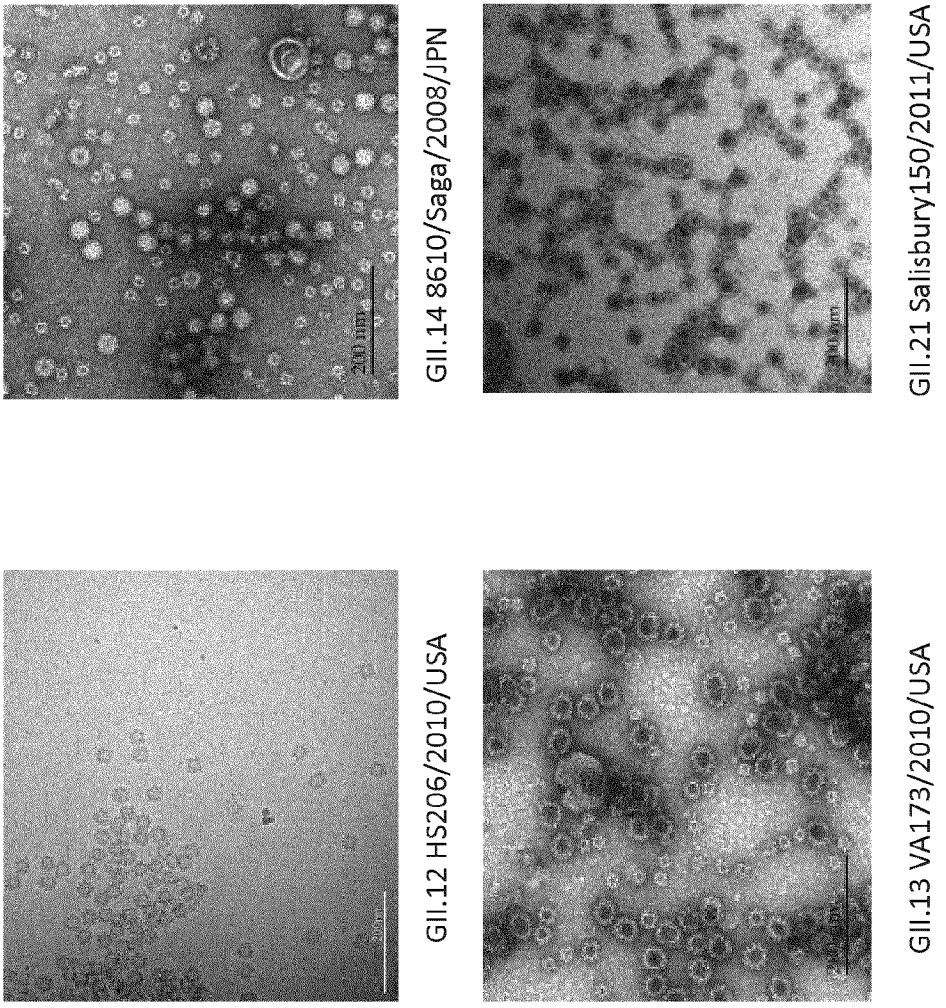

GII.1 Ascension208/2010/USA
GII.7 Musashimurayama/2010/JP
GI.3 S29/2008/Lilla Edet/Sweeden
GI.5 Siklos/HUN5407/2013/HUN

FIGURE 5D


GII.14 8610/Saga/2008/JPN
GII.21 Salisbury150/2011/USA
GII.12 HS206/2010/USA
GII.13 VA173/2010/USA

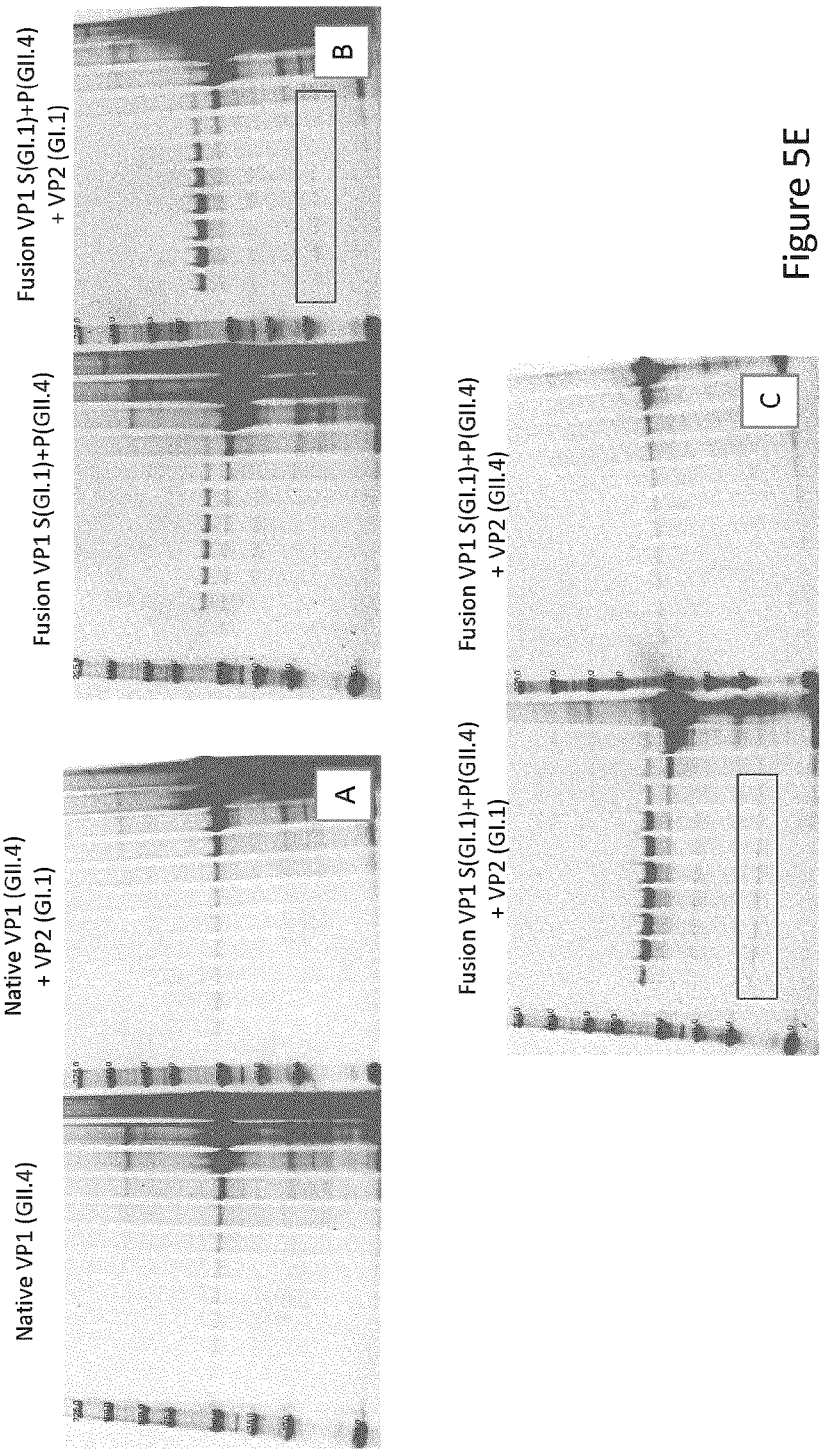

Native VP1 (GII.4)
Native VP1 (GII.4)
+ VP2 (GI.1)
A
Fusion VP1 S(GI.1)+P(GII.4)
Fusion VP1 S(GI.1)+P(GII.4)
+ VP2 (GI.1)
B
Fusion VP1 S(GI.1)+P(GII.4)
Fusion VP1 S(GI.1)+P(GII.4)
+ VP2 (GI.1)
C

Figure 5E

FIGURE 6A

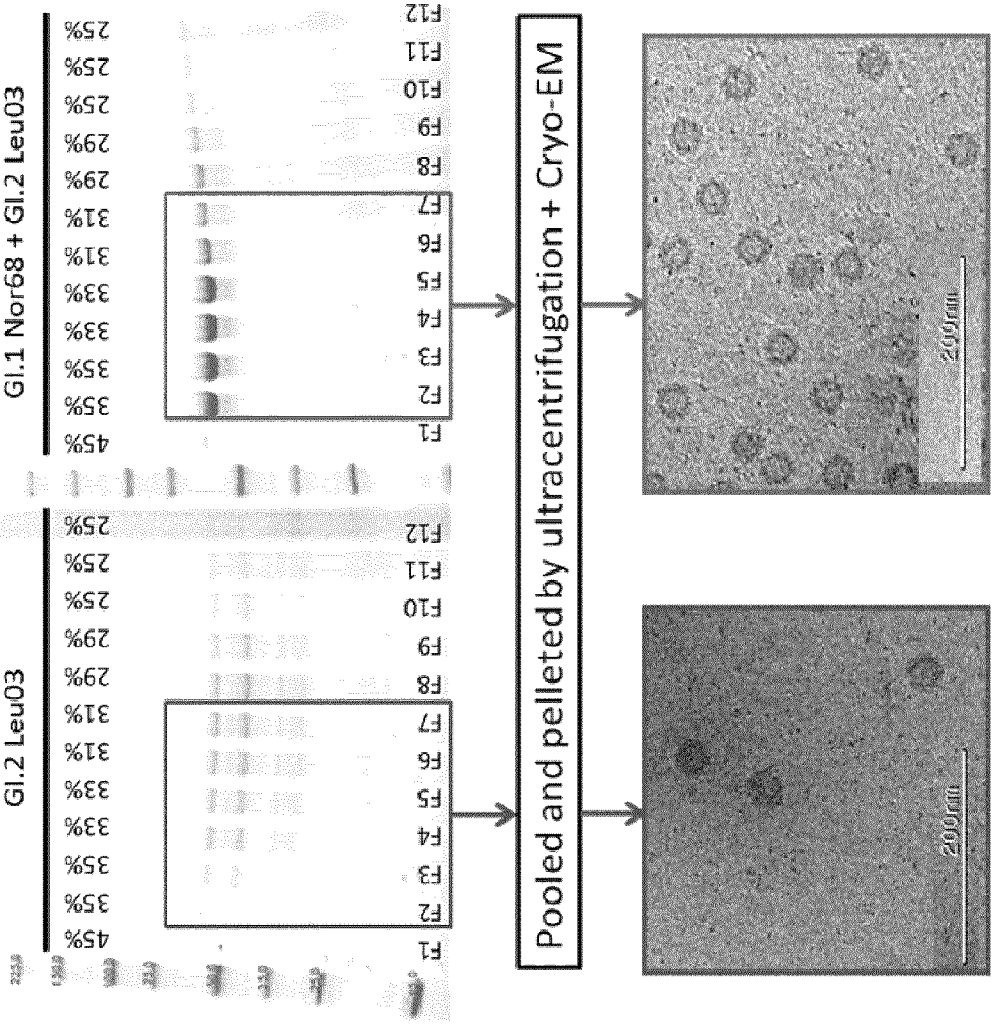

GI.1 Nor68 + GI.2 Leu03
GI.2 Leu03
45%
35%
35%
33%
33%
31%
31%
29%
29%
25%
25%
25%
25%
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11
F12
Pooled and pelleted by ultracentrifugation + Cryo-EM
200nm
200nm

FIGURE 6B

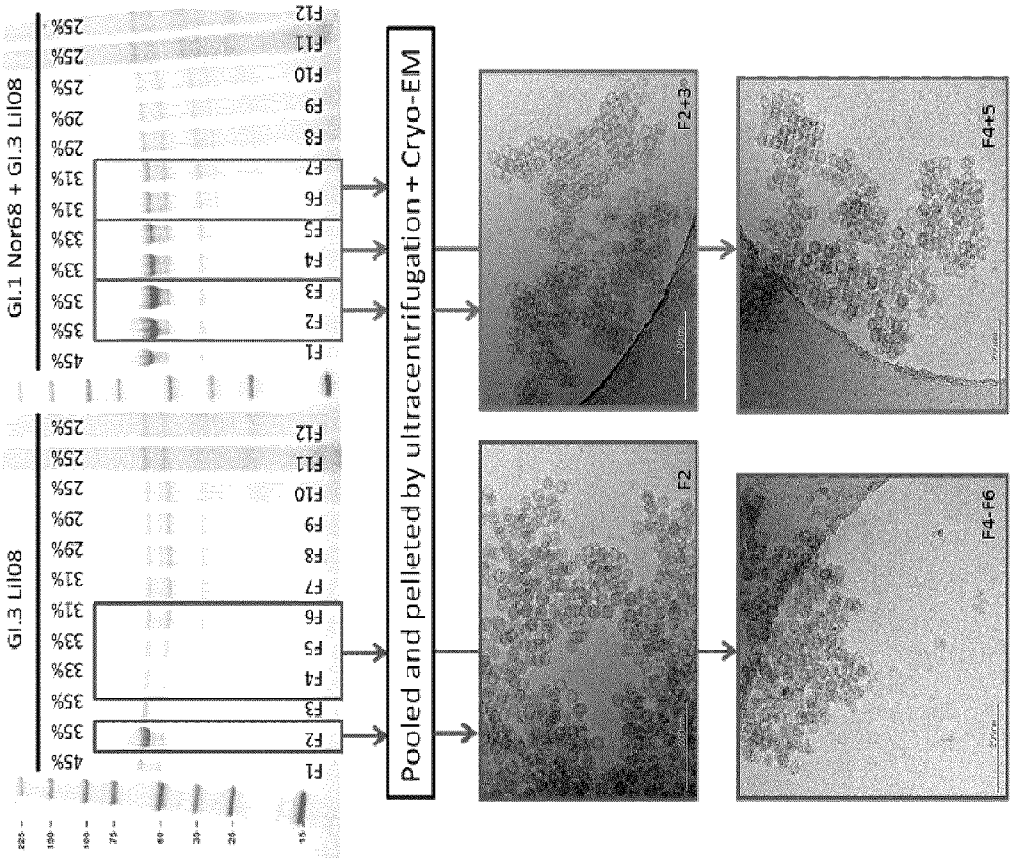
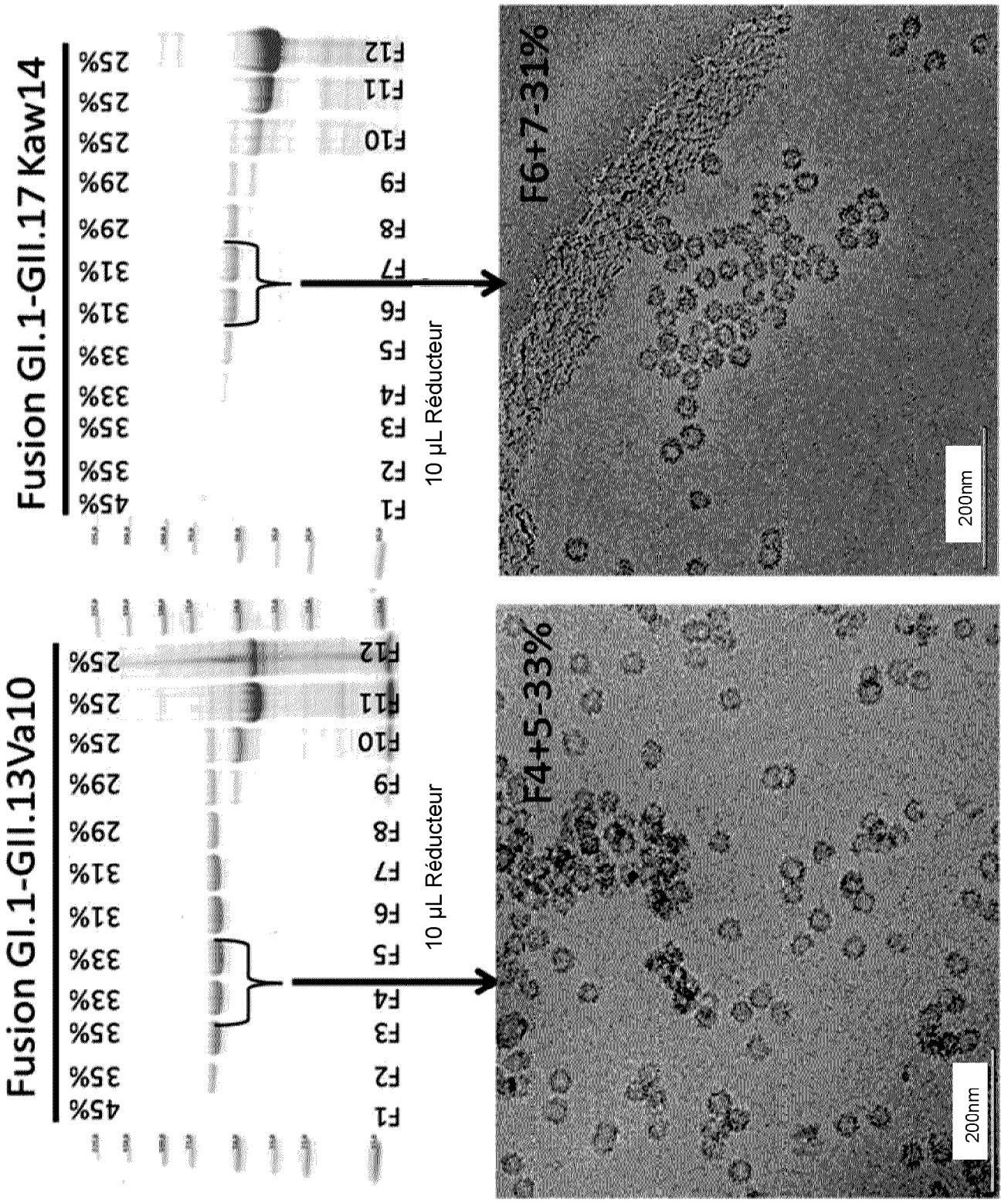
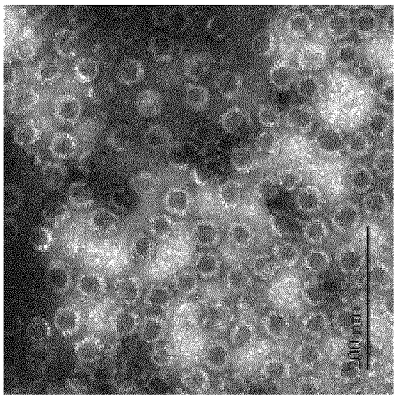

GI.1 Nor68 + GI.3 LilO8
GI.3 LilO8
Pooled and pelleted by ultracentrifugation + Cryo-EM
F2
F2+3
F4-F6
F4+5

FIGURE 6C



Date Reçue/Date Received 2020-05-14

FIGURE 6D



S(GI.1) +P(GII.4)
S(GI.1/Norwalk/68/US)+
P(GII.4 Sydney/NSW0514/2012/AU)

FIGURE 6E

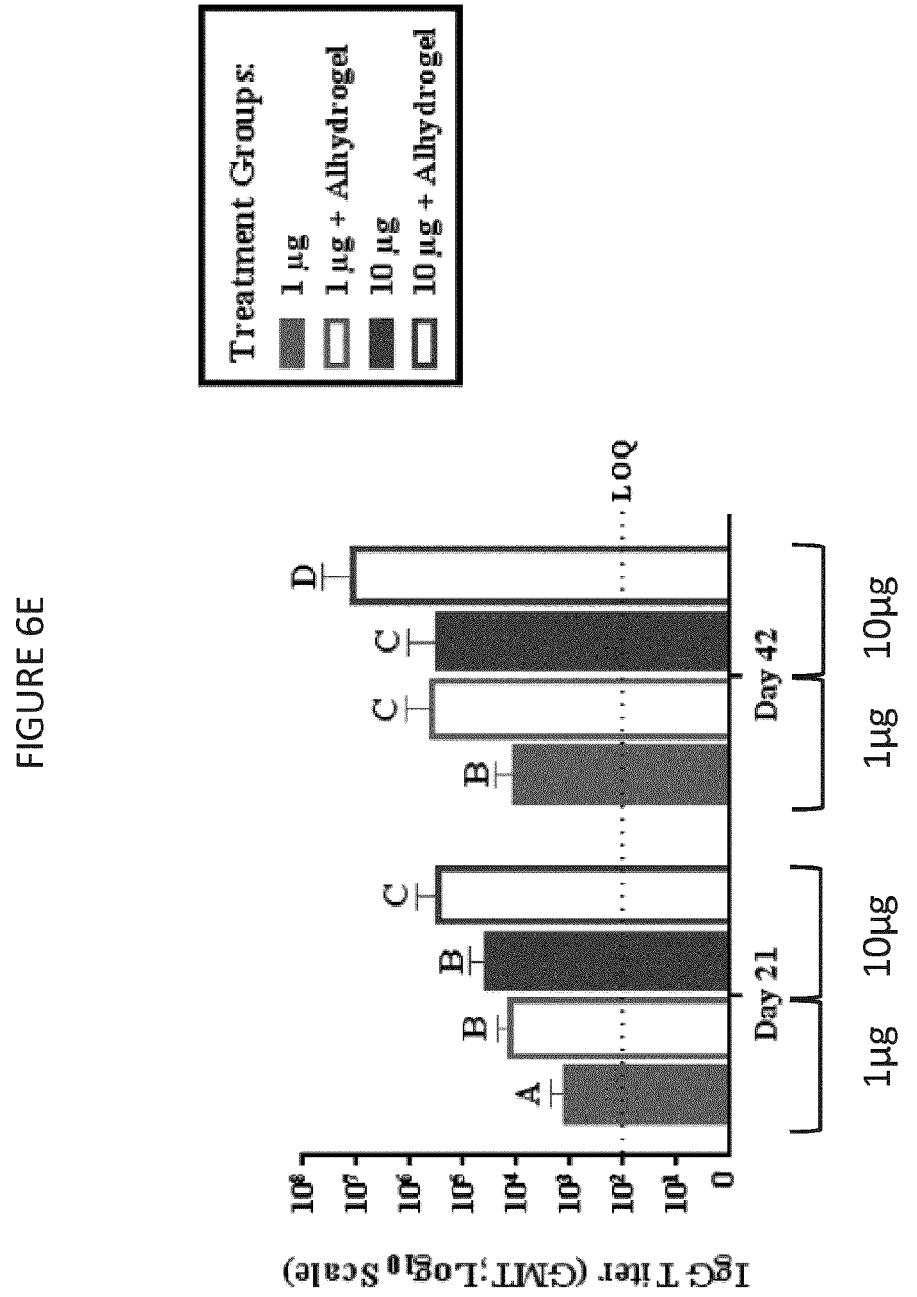

Treatment Groups:
1 µg
1 µg + Alhydrogel
10 µg
10 µg + Alhydrogel
IgG Titer (GMT; Log10 Scale)
10⁸
10⁷
10⁶
10⁵
10⁴
10³
10²
10¹
0
LOQ
A
B
B
B
C
C
C
D
Day 21
Day 42
1µg
10µg
1µg
10µg

Figure 7A

IF-NoV(US68)VP1(ORF2).c (SEQ ID NO: 72)

TCGTGCTTCGGCACCAGTACAATGATGATGGCGTCTAAGGACGCTACAT

Figure 7B

IF-NoV(US68)VP1(ORF2).r (SEQ ID NO: 73)

ACTAAAGAAAATAGGCCTTTATCGGCGCAGACCAAGCCTACCTCTTGCCGAGCTGGCAG

Figure 7C

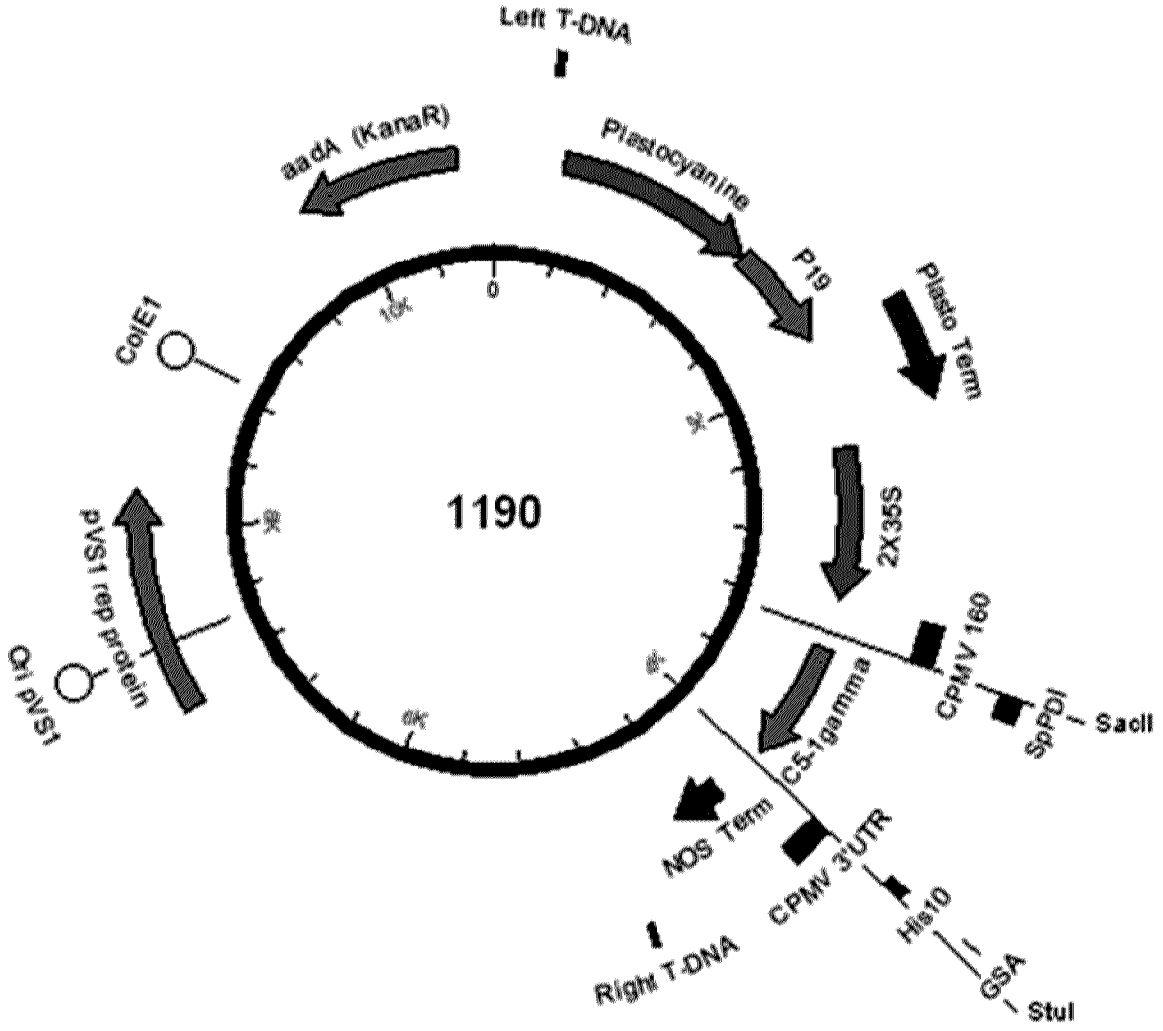
Construct 1190 from left to right t-DNA borders (underlined). 2X35S/CPMV-160/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette (SEQ ID NO: 74)

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAATTAACG
CCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTCAAAAACCATAAAAGTTTAAGTTAGCAAGTGTGTACATTTTTACTT
GAACAAAAATATTCACCTACTACTGTTATAAATCATTATTAAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAGTGA
TATTTTGACAACAATTTTGTTGCAACATTTGAGAAAATTTTGTTGTTCTCTCTTTTCATTGGTCAAAAACAATAGAGAGAGAAAAAG
GAAGAGGGAGAATAAAAACATAATGTGAGTATGAGAGAGAAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATATCATT
GAGGAATTTGACAAAAGCTACACAAATAAGGGTTAATTGCTGTAAATAAATAAGGATGACGCATTAGAGAGATGTACCATTAGAG
AATTTTTGGCAAGTCATTAAAAAGAAAGAATAAATTATTTTTAAAATTAAAAGTTGAGTCATTTGATTAAACATGTGATTATTTAAT
GAATTGATGAAAGAGTTGGATTAAAGTTGTATTAGTAATTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATATA
TTGCCCCATAGAGTCAGTTAACTCATTTTTATATTTCATAGATCAAATAAGAGAAATAACGGTATATTAATCCCTCCAAAAAAAAAA
AACGGTATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTAGGAGGATAACATCCAATCCAACCAATCAC
AACAATCCTGATGAGATAACCCACTTTAAGCCCACGCATCTGTGGCACATCTACATTATCTAAATCACACATTCTTCCACACATCTG
AGCCACACAAAAACCAATCCACATCTTTATCACCCATTCTATAAAAAATCACACTTTGTGAGTCTACACTTTGATTCCCTTCAAACAC
ATACAAAGAGAAGAGACTAATTAATTAATTAATCATCTTGAGAGAAAATGGAACGAGCTATACAAGGAAACGACGCTAGGGAAC
AAGCTAACAGTGAACGTTGGGATGGAGGATCAGGAGGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGA
GTGGCGGCTACATAACGATGAGACGAATTCGAATCAAGATAATCCCCTTGGTTTCAAGGAAAGCTGGGGTTTCGGGAAAGTTGTA
TTTAAGAGATATCTCAGATACGACAGGACGGAAGCTTCACTGCACAGAGTCCTTGGATCTTGGACGGGAGATTCGGTTAACTATG
CAGCATCTCGATTTTTCGGTTTCGACCAGATCGGATGTACCTATAGTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGG
TCGCGAACTCTTCAGCATCTCTGTGAGATGGCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAAGTGGAAAGTA
ATGTATCAAGAGGATGCCCTGAAGGTACTCAAACCTTCGAAAAAGAAAGCGAGTAAGTTAAAATGCTTCTTCGTCTCCTATTTATA
ATATGGTTTGTTATTGTTAATTTTGTTCTTGTAGAAGAGCTTAATTAATCGTTGTTGTTATGAAATACTATTTGTATGAGATGAACTG
GTGTAATGTAATTCATTTACATAAGTGGAGTCAGAATCAGAATGTTTCCTCCATAACTAACTAGACATGAAGACCTGCCGCGTACA
ATTGTCTTATATTTGAACAACTAAAATTGAACATCTTTTGCCACAACTTTATAAGTGGTTAATATAGCTCAAATATATGGTCAAGTTC
AATAGATTAATAATGGAAATATCAGTTATCGAAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCCCTTT
GATAAATGATAGTACACCAATTAGGAAGGAGCATGCTCGCCTAGGAGATTGTCGTTTCCCGCCTTCAGTTTGCAAGCTGCTCTAGC
CGTGTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGGAATTACTAGCGCGTGTCGACAAGCTTGCATGCCGGTCAACATGG
TGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAG
GGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCT
ACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCAC
GAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACAC
ACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGA
AACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCA
TTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGT
GGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAG

CGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCT
TCAACGTTGTCAGATCGTGCTTCGGCACCGCGGATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCA
GATCTTCGCCTGCAGGCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGAC
CCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGC
TGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCAC
CCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGT
CTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCC
GAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTC
AGTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGCGATCGCTCACCATCACCATCACCATCACCATCACCATTAAAGG
CCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAAT
TTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACC
GGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATG
ATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGT
CCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGA
TCTCTAGAGTCTCAAGCTTGGCGCGCCCACGTGACTAGTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCA
ACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCT
GAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCG
GGTAAACCTAAGAGAAAAGAGCGTTTA

Date Reçue/Date Received 2020-05-14

Figure 7D



Date Reçue/Date Received 2020-05-14

Figure 8A

Expression cassette number 2720 from 2X35S promoter to NOS terminator. Wild-type VP1 from Norovirus GI.1/Norwalk/1968/US strain is underlined. (SEQ ID NO: 75)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAA
TTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGA
AGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTC
TGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCT
TCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGT
CTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAG
CTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAG
GCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAG
AAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCT
CTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGATGATGGCGTCTAAG
GACGCTACATCAAGCGTGGATGGCGCTAGTGGCGCTGGTCAGTTGGTACCGGAGGTTAATGCTTCTGACCCTCTTGC
AATGGATCCTGTAGCAGGTTCTTCGACAGCAGTCGCGACTGCTGGACAAGTTAATCCTATTGATCCCTGGATAATTA
ATAATTTTGTGCAAGCCCCCCAAGGTGAATTTACTATTTCCCCAAATAATACCCCCGGTGATGTTTTGTTTGATTTGAG
TTTGGGTCCCCATCTTAATCCTTTCTTGCTCCATCTATCACAAATGTATAATGGTTGGGTTGGTAACATGAGAGTCAG
GATTATGCTAGCTGGTAATGCCTTTACTGCGGGGAAGATAATAGTTTCCTGCATACCCCCTGGTTTTGGTTCACATAA
TCTTACTATAGCACAAGCAACTCTCTTTCCACATGTGATTGCTGATGTTAGGACTCTAGACCCCATTGAGGTGCCTTT
GGAAGATGTTAGGAATGTTCTCTTTCATAATAATGATAGAAATCAACAAACCATGCGCCTTGTGTGCATGCTGTACA
CCCCCCTCCGCACTGGTGGTGGTACTGGTGATTCTTTTGTAGTTGCAGGGCGAGTTATGACTTGCCCCAGTCCTGATT
TTAATTTCTTGTTTTTAGTCCCTCCTACGGTGGAGCAGAAAACCAGGCCCTTCACACTCCCAAATCTGCCATTGAGTTC
TCTGTCTAACTCACGTGCCCCTCTCCCAATCAGTAGTATGGGCATTTCCCCAGACAATGTCCAGAGTGTGCAGTTCCA
AAATGGTCGGTGTACTCTGGATGGCCGCCTGGTTGGCACCACCCCAGTTTCATTGTCACATGTTGCCAAGATAAGAG
GGACCTCCAATGGCACTGTAATCAACCTTACTGAATTGGATGGCACACCCTTTCACCCTTTTGAGGGCCCTGCCCCCA
TTGGGTTTCCAGACCTCGGTGGTTGTGATTGGCATATCAATATGACACAGTTTGGCCATTCTAGCCAGACCCAGTAT
GATGTAGACACCACCCCTGACACTTTTGTCCCCCATCTTGGTTCAATTCAGGCAAATGGCATTGGCAGTGGTAATTAT
GTTGGTGTTCTTAGCTGGATTTCCCCCCCATCACACCCGTCTGGCTCCCAAGTTGACCTTTGGAAGATCCCCAATTAT
GGGTCAAGTATTACGGAGGCAACACATCTAGCCCCTTCTGTATACCCCCCTGGTTTCGGAGAGGTATTGGTCTTTTTC
ATGTCAAAAATGCCAGGTCCTGGTGCTTATAATTTGCCCTGTCTATTACCACAAGAGTACATTTCACATCTTGCTAGT
GAACAAGCCCCTACTGTAGGTGAGGCTGCCCTGCTCCACTATGTTGACCCTGATACCGGTCGGAATCTTGGGGAATT
CAAAGCATACCCTGATGGTTTCCTCACTTGTGTCCCCAATGGGGCTAGCTCGGGTCCACAACAGCTGCCGATCAATG
GGGTCTTTGTCTTTGTTTCATGGGTGTCCAGATTTTATCAATTAAAGCCTGTGGGAACTGCCAGCTCGGCAAGAGGT
AGGCTTGGTCTGCGCCGATAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGT
GAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCC
TTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTA
TCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTAT
CATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTT

ATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGC
GGTGTCATCTATGTTACTAGAT

Figure 8B

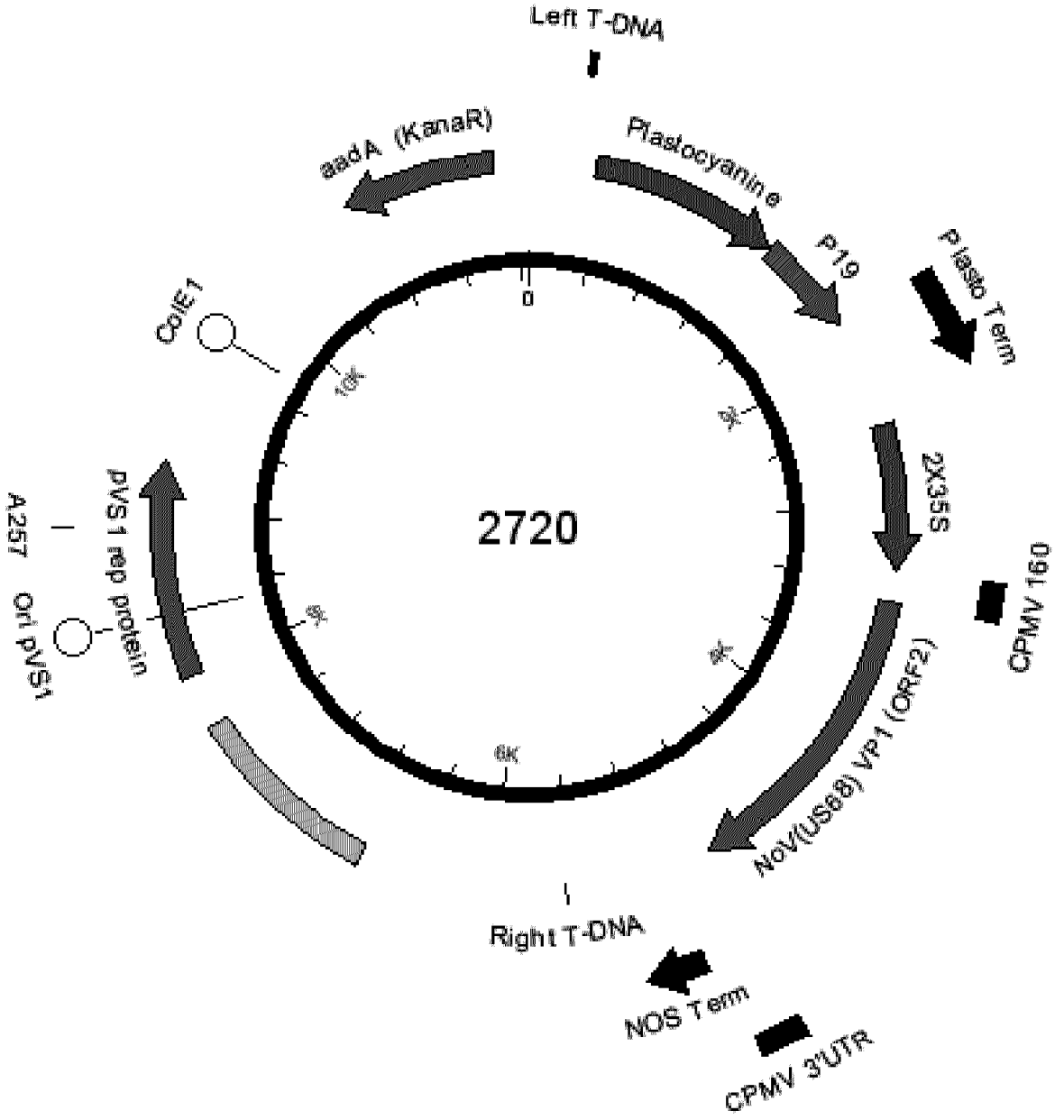


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Figure 9A

IF-NoV(US68)VP1(ORF2)(hCod).c (SEQ ID NO: 76)

TCGTGCTTCGGCACCAGTACAATGATGATGGCTAGTAAAGATGCGACCT

Figure 9B

IF-NoV(US68)VP1(ORF2)(hCod).r (SEQ ID NO: 77)

ACTAAAGAAAATAGGCCTTTATCTCCGCAGACCGAGGCGTCCGCGGGCAGAA

Figure 9C

Expression cassette number 2724 from 2X35S promoter to NOS terminator. Human codon-optimized VP1 from Norovirus GI.1/Norwalk/1968/US strain is underlined. (SEQ ID NO: 78)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAA
TTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGA
AGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTC
TGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCT
TCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGT
CTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAG
CTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAG
GCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAG
AAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCT
CTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGATGATGGCTAGTAAA
GATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAAACGCCAGCGACCCACTTG
CCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCCAATTGATCCGTGGATTATC
AACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGGGCGATGTGCTATTCGATCT
TTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTGGGTAGGAAACATGAGAGT
CCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTCCTCCCGGATTTGGATCTCA
TAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCTGGACCCCATCGAGGTGCC
CCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATGAGACTTGTCTGTATGCTCT
ATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAGTGATGACATGCCCCTCCCC
CGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGCAGAAGACGCGGCCCTTTACACTGCCCAATCTCCCGCTT
TCAAGTCTGAGTAATTCACGGGCCCCATTGCCGATCTCCTCAATGGGAATCTCCCCCGACAACGTCCAGTCTGTCCAA
TTCCAAAATGGGAGATGCACACTGGACGGTCGCCTGGTGGGAACAACTCCGGTGTCCCTCTCACATGTCGCCAAAAT
CCGCGGCACATCAAATGGTACCGTAATCAATCTGACAGAACTTGATGGCACGCCCTTCCATCCCTTTGAAGGACCAG
CCCCTATTGGATTTCCTGATCTGGGAGGTTGCGACTGGCACATAAACATGACACAGTTTGGCCACTCCAGCCAGACA
CAGTATGATGTCGATACAACCCCAGATACCTTCGTGCCACACCTGGGATCTATTCAAGCTAACGGTATTGGATCCGG
CAACTACGTGGGAGTCTTATCTTGGATCTCACCACCATCCCACCCCTCAGGATCCCAGGTTGACTTGTGGAAGATACC
GAATTATGGATCCTCGATCACTGAAGCCACGCACCTCGCACCTTCCGTCTACCCACCAGGTTTTGGAGAAGTCTTGGT

GTTTTTCATGAGCAAAATGCCCGGCCCTGGAGCCTACAATCTCCCTTGCCTACTCCCTCAAGAGTATATTAGTCACCTCGCATCT
GAGCAGGCCCCGACCGTTGGCGAGGCAGCCCTGCTGCATTATGTGGATCCGGACACCGGCAGGAACCTGGGTGAGTTCAAAG
CTTATCCTGACGGTTTTCTAACATGTGTACCAAATGGCGCTTCCAGCGGCCCTCAACAGCTCCCAATCAATGGCGTGTTCGTTTT
TGTCAGCTGGGTAAGCCGCTTCTACCAGCTGAAGCCCGTGGGGACAGCTTCTTCTGCCCGCGGACGCCTCGGTCTGCGGAGAT
AAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGT
GTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTA
TTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTT
TCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACAT
GTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAAT
ATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

Figure 9D

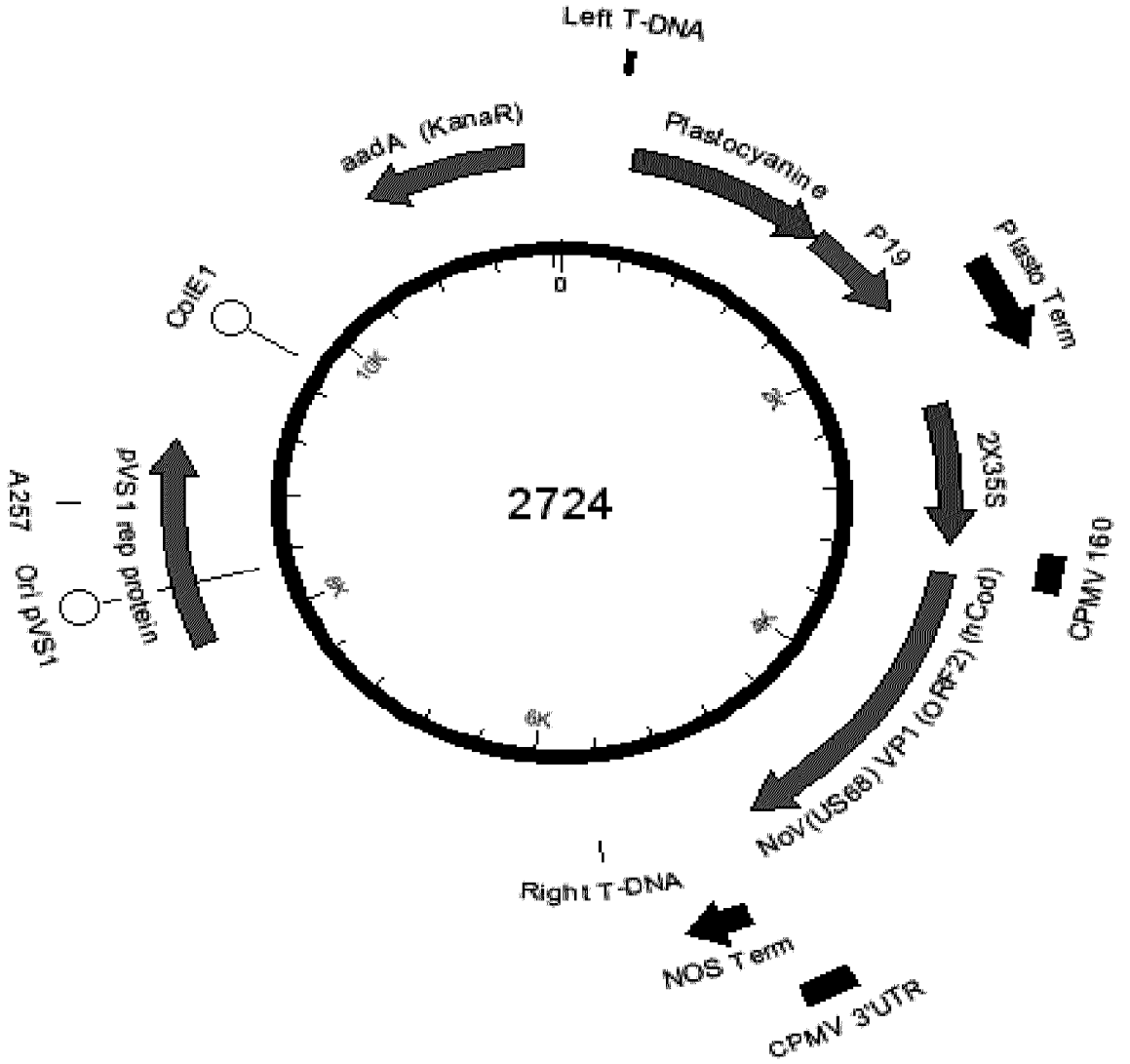

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Figure 10A

IF-NoV(US68)VP2(ORF3)(hCod).c (SEQ ID NO: 79)

TCGTGCTTCGGCACCAGTACAATGGCTCAGGCCATTATTGGCGCCAT

Figure 10B

IF-NoV(US68)VP2(ORF3)(hCod).r (SEQ ID NO: 80)

ACTAAAGAAAATAGGCCTTCAGCGGCGGTTGTTAGCGAACAGAGGAAGTC

Figure 10C

Expression cassette number 2725 from 2X35S promoter to NOS terminator. Human codon-optimized VP2 from Norovirus GI.1/Norwalk/1968/US strain is underlined. (SEQ ID NO: 81)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAA
TTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGA
AGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTC
TGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCT
TCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGT
CTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAG
CTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAG
GCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAG
AAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCT
CTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGGCTCAGGCCATTATT
GGCGCCATCGCTGCAAGTACAGCCGGGAGTGCATTGGGGGCCGGAATACAGGTGGGCGGGGAAGCTGCATTGCA
GAGCCAGCGGTACCAGCAAAACCTGCAGTTACAGGAGAATAGCTTTAAACACGACAGGGAGATGATTGGATATCA
GGTGGAGGCCAGCAATCAGCTGCTCGCCAAAAACTTGGCTACTCGATACTCATTACTGCGCGCCGGGGGGTTGACT
AGCGCCGACGCCGCACGATCTGTCGCAGGCGCCCCCGTGACTCGGATCGTAGACTGGAACGGGGTACGAGTCTCG
GCTCCCGAGTCGTCTGCAACCACCCTGAGGTCGGGAGGGTTTATGTCCGTGCCCATCCCATTCGCTAGCAAACAGAA
ACAGGTCCAGAGCTCCGGAATCTCCAATCCCAATTACTCCCCTAGCTCTATCTCTCGTACCACTTCCTGGGTCGAGAG
TCAGAACAGCAGTAGATTTGGCAACCTGAGCCCCTACCATGCTGAAGCCCTGAACACTGTGTGGTTGACTCCACCTG
GTAGCACGGCCTCCTCAACCCTGAGTTCCGTGCCTCGCGGGTACTTCAATACCGACAGACTTCCTCTGTTCGCTAACA
ACCGCCGCTGAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTT
CTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGG
ACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCA
GATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTC
TGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAG

TCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTA
TGTTACTAGAT

Figure 10D

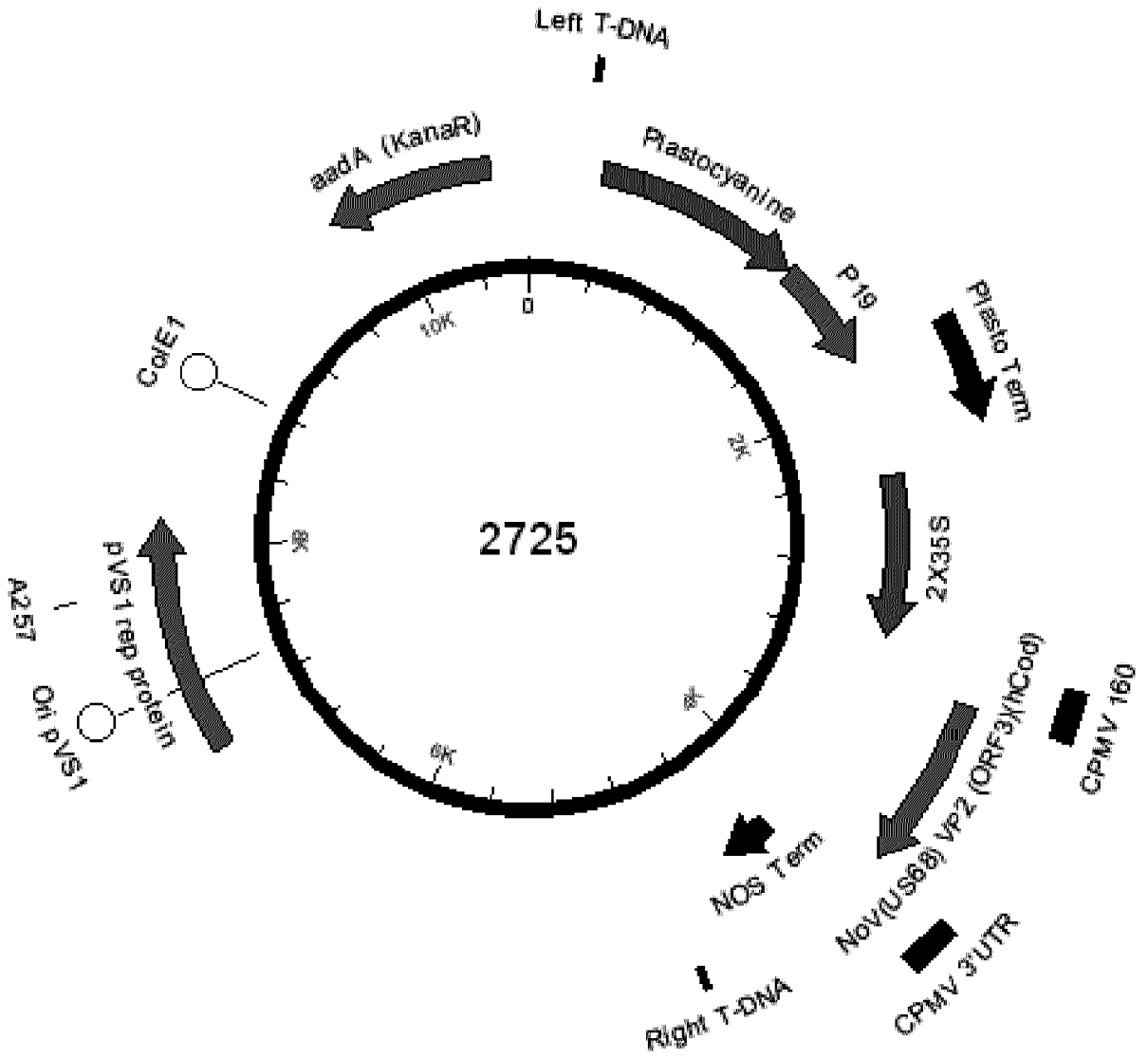

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Figure 11A

IF-GI2Leu03VP1.c (SEQ ID NO: 82)

TCGTGCTTCGGCACCAGTACAATGATGATGGCTTCAAAGGATGCTCCCCAAA

Figure 11B

IF-GI2Leu03VP1.r (SEQ ID NO: 83)

ACTAAAGAAAATAGGCCTTCAGATTCGGCGGACCCCTAGCCTGCCGCGTGCCGTAGA

Figure 11C

Expression cassette number 3300 from 2X35S promoter to NOS terminator. Human codon-optimized VP1 from Norovirus GI.2/Leuven/2003/Bel strain is underlined. (SEQ ID NO: 84)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAA
TTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGA
AGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTC
TGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCT
TCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGT
CTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAG
CTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAG
GCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAG
AAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCT
CTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGATGATGGCTTCAAAG
GATGCTCCCCAAAGCGCGGACGGAGCTAGCGGCGCCGGACAGTTGGTTCCGGAAGTCAACACTGCCGATCCACTGC
CCATGGAACCCGTAGCTGGTCCAACAACCGCTGTTGCCACCGCCGGCCAGGTTAACATGATCGATCCATGGATTGTT
AATAACTTTGTACAGAGCCCCCAGGGGGAGTTCACAATTTCTCCGAACAATACCCCTGGGGACATTCTGTTCGATCT
GCAACTGGGCCCACACTTGAATCCTTTCCTGAGCCATCTTTCACAGATGTACAACGGATGGGTTGGGAACATGCGTG
TTCGGATCCTCCTTGCTGGCAACGCCTTCAGTGCTGGCAAGATTATCGTGTGCTGCGTACCACCAGGGTTTACCTCGA
GTTCATTAACCATTGCTCAGGCCACCCTTTTCCCTCACGTGATCGCAGACGTGCGTACCTTAGAACCAATCGAAATGC
CCCTGGAAGATGTACGGAACGTGCTGTACCATACTAATGATAACCAGCCAACGATGAGATTAGTGTGCATGCTGTAC
ACCCCCCTGAGAACTGGAGGAGGTTCTGGAAATTCCGACAGTTTTGTGGTGGCTGGCAGGGTCCTGACCGCTCCCA
GTAGCGACTTCAGCTTTTTGTTCCTCGTTCCTCCTACAATCGAACAAAAAACAAGAGCATTCACAGTGCCCAACATTC
CACTGCAGACTTTAAGCAATTCCAGGTTTCCCAGCTTGATCCAGGGTATGATCCTTTCTCCCGACGCCTCCCAAGTTG
TGCAGTTCCAGAATGGGAGATGTCTTATCGACGGTCAGCTTCTGGGAACAACCCCTGCCACCTCCGGGCAACTCTTC
CGGGTGAGAGGCAAAATCAATCAGGGCGCCAGAACACTGAATCTGACAGAAGTGGACGGGAAACCCTTTATGGCG
TTCGATAGCCCCGCGCCCGTTGGATTCCCTGACTTCGGCAAGTGTGATTGGCACATGCGCATCAGTAAGACTCCCAA

CAACACTTCATCTGGAGACCCCATGAGGAGCGTGGATGTCCAGACCGACGTGCAGGGCTTCGTGCCGCACTTGGGATCTATCC
AGTTCGATGAGGTGTTCAATCACCCTACTGGCGACTACATAGGCACAATTGAGTGGATAAGTCAACCATCTACACCTCCAGGG
ACCGACATAAACCTGTGGGAAATTCCTGATTACGGGTCATCCCTGAGTCAAGCTGCCAATCTTGCACCCCCTGTCTTTCCCCCCG
GCTTTGGTGAGGCTCTTGTTTACTTCGTCTCTGCATTTCCTGGTCCTAACAACCGCTCCGCCCCTAACGATGTTCCGTGTTTGTTA
CCCCAGGAATATGTGACTCATTTCGTTTCCGAACAGGCACCCACCATGGGGGACGCTGCCCTGCTACACTATGTGGACCCCGAC
ACCAATAGAAACCTCGGCGAGTTCAAACTCTACCCCGGGGGATACCTGACCTGTGTTCCAAATGGAGTGGGAGCAGGCCCAC
AACAGCTGCCCCTGAATGGGGTCTTCCTGTTCGTTTCTTGGGTGTCACGCTTTTACCAGCTGAAGCCCGTTGGCACAGCTTCTAC
GGCACGCGGCAGGCTAGGGGTCCGCCGAATCTGAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTA
TGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCC
CTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGAC
CTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCT
GTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCA
ATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACT
AGAT

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Figure 11D

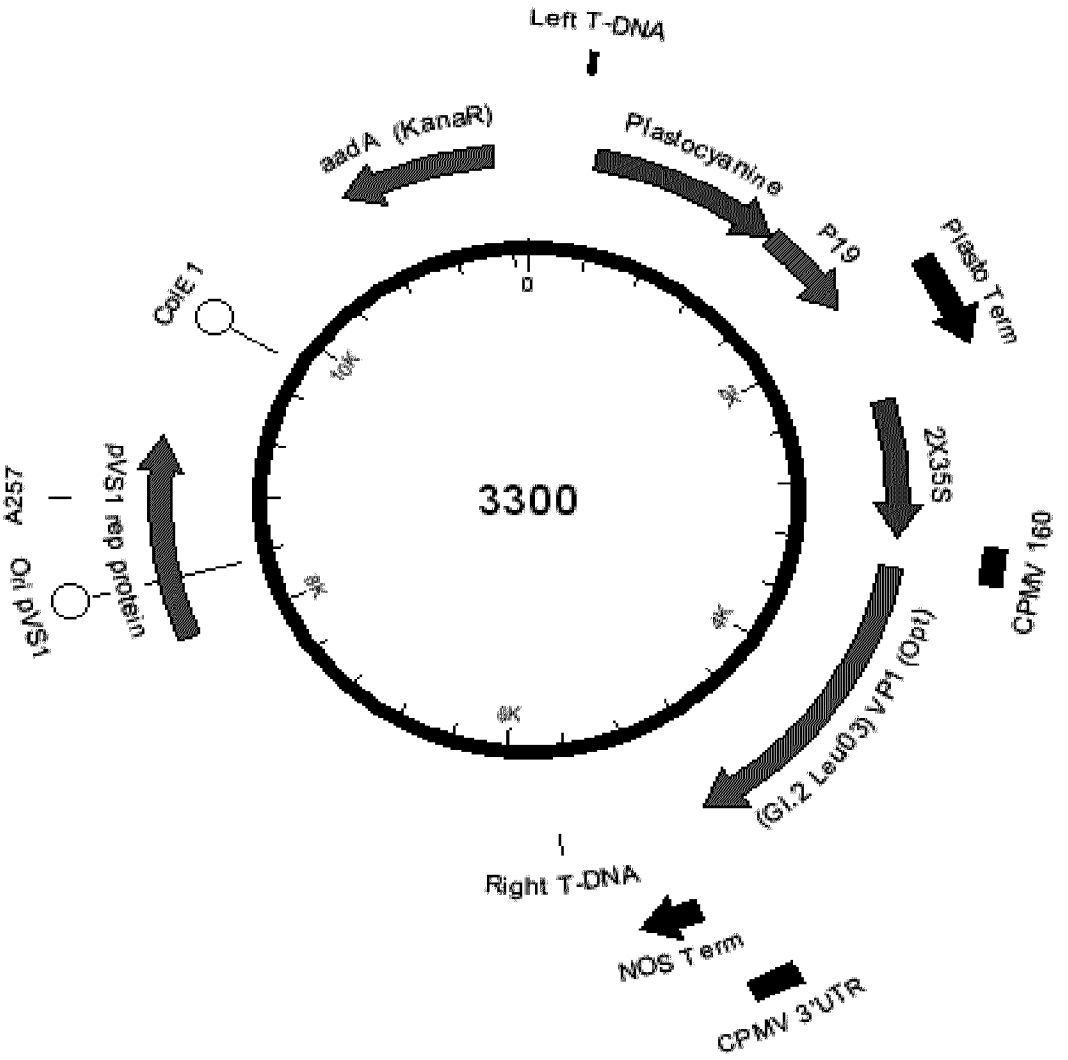

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Figure 12A

GI2Leu+GI1VP1.r (SEQ ID NO: 85)

ATGCTCTTGTTTTTTGCTCAACGGTTGGTGGGACCAGAAAAAGAAAGTTGAAGTCG

Figure 12B

GI1VP1+GI2Leu.c (SEQ ID NO: 86)

TCCCACCAACCGTTGAGCAAAAAACAAGAGCATTCACAGTGCCCAACAT

Figure 12C

Expression cassette number 3360 from 2X35S promoter to NOS terminator. Human codon-optimized fusion VP1 S(GI.1)+P(GI.2) protein gene sequence is underlined. (SEQ ID NO: 87)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAA
TTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGA
AGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTC
TGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCT
TCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGT
CTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAG
CTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAG
GCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAG
AAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCT
CTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGATGATGGCTAGTAAA
GATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAAACGCCAGCGACCCACTTG
CCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCCAATTGATCCGTGGATTATC
AACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGGGCGATGTGCTATTCGATCT
TTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTGGGTAGGAAACATGAGAGT
CCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTCCTCCCGGATTTGGATCTCA
TAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCTGGACCCCATCGAGGTGCC
CCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATGAGACTTGTCTGTATGCTCT
ATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAGTGATGACATGCCCCTCCCC
CGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGCAAAAAACAAGAGCATTCACAGTGCCCAACATTCCACT
GCAGACTTTAAGCAATTCCAGGTTTCCCAGCTTGATCCAGGGTATGATCCTTTCTCCCGACGCCTCCCAAGTTGTGCA
GTTCCAGAATGGGAGATGTCTTATCGACGGTCAGCTTCTGGGAACAACCCCTGCCACCTCCGGGCAACTCTTCCGGG
TGAGAGGCAAAATCAATCAGGGCGCCAGAACACTGAATCTGACAGAAGTGGACGGGAAACCCTTTATGGCGTTCG
ATAGCCCCGCGCCCGTTGGATTCCCTGACTTCGGCAAGTGTGATTGGCACATGCGCATCAGTAAGACTCCCAACAAC
ACTTCATCTGGAGACCCCATGAGGAGCGTGGATGTCCAGACCGACGTGCAGGGCTTCGTGCCGCACTTGGGATCTA
TCCAGTTCGATGAGGTGTTCAATCACCCTACTGGCGACTACATAGGCACAATTGAGTGGATAAGTCAACCATCTACA

CCTCCAGGGACCGACATAAACCTGTGGGAAATTCCTGATTACGGGTCATCCCTGAGTCAAGCTGCCAATCTTGCACCCCCTGTC
TTTCCCCCCGGCTTTGGTGAGGCTCTTGTTTACTTCGTCTCTGCATTTCCTGGTCCTAACAACCGCTCCGCCCCTAACGATGTTCC
GTGTTTGTTACCCCAGGAATATGTGACTCATTTCGTTTCCGAACAGGCACCCACCATGGGGGACGCTGCCCTGCTACACTATGT
GGACCCCGACACCAATAGAAACCTCGGCGAGTTCAAACTCTACCCCGGGGGATACCTGACCTGTGTTCCAAATGGAGTGGGA
GCAGGCCCACAACAGCTGCCCCTGAATGGGGTCTTCCTGTTCGTTTCTTGGGTGTCACGCTTTTACCAGCTGAAGCCCGTTGGC
ACAGCTTCTACGGCACGCGGCAGGCTAGGGGTCCGCCGAATCTGAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGT
GTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTA
GCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCA
AGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATC
ATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTA
GAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCA
TCTATGTTACTAGAT

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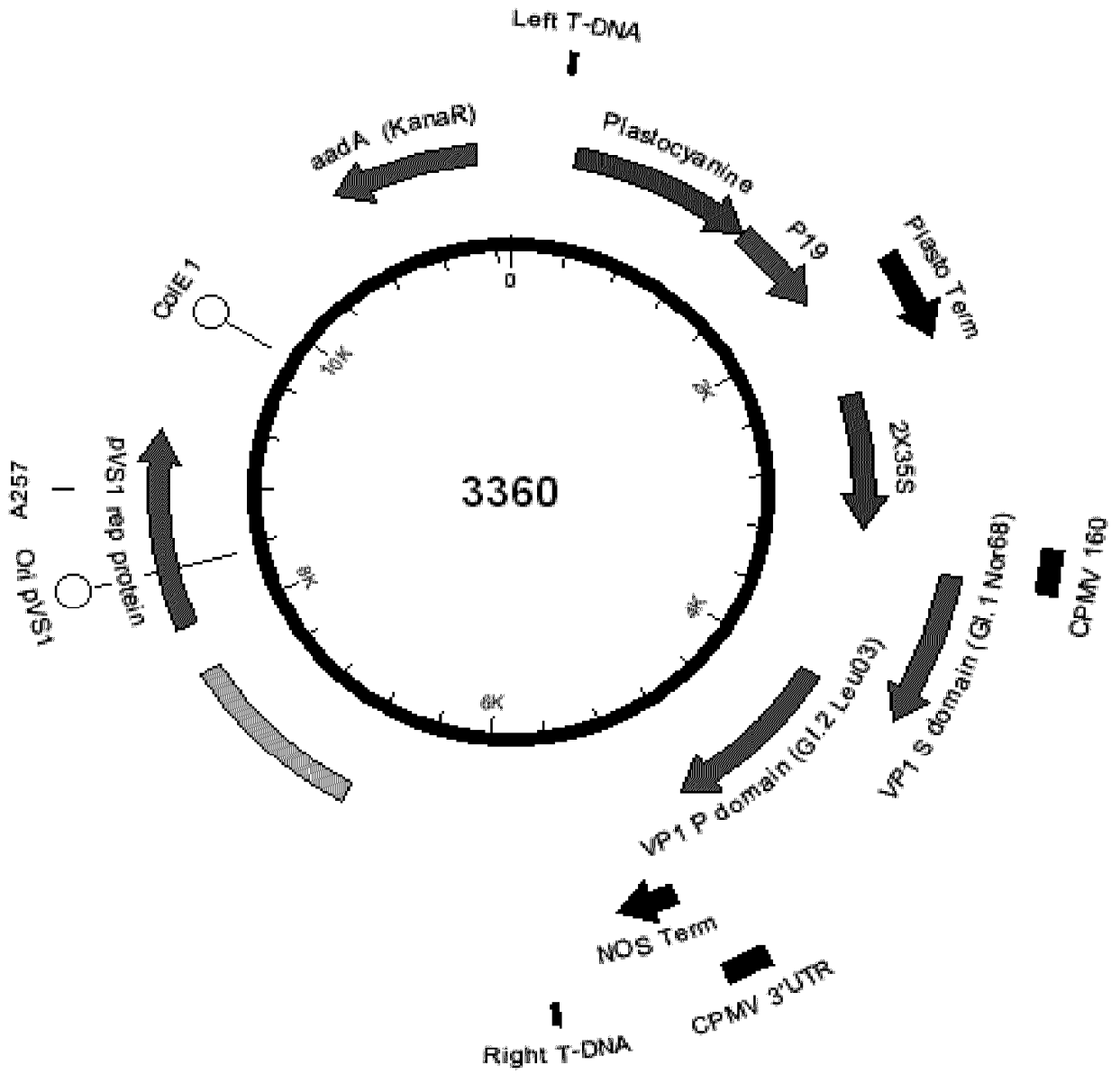
Figure 12D



Date Reçue/Date Received 2020-05-14

Figure 13A

Amino acid sequence of VP1 G1.1 (SEQ ID NO: 1)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVEQKTRPFTLPN
LPLSSLSNSRAPLPISSMGISPDNVQSVQFQNGRCTLDGRLVGTTPVSLSHVAKIRGTSNGTVINLTELDGTPFHPFEGPAPI
GFPDLGGCDWHINMTQFGHSSQTQYDVDTTPDTFVPHLGSIQANGIGSGNYVGVLSWISPPSHPSGSQVDLWKIPNYG
SSITEATHLAPSVYPPGFGEVLVFFMSKMPGPGAYNLPCLLPQEYISHLASEQAPTVGEAALLHYVDPDTGRNLGEFKAYP
DGFLTCVPNGASSGPQQLPINGVFVFVSWVSRFYQLKPVGTASSARGRLGLRR

Figure 13B

Nucleic acid sequence of wild-type VP1 G1.1 (SEQ ID NO: 13)

ATGATGATGGCGTCTAAGGACGCTACATCAAGCGTGGATGGCGCTAGTGGCGCTGGTCAGTTGGTACCGGAGGTTA
ATGCTTCTGACCCTCTTGCAATGGATCCTGTAGCAGGTTCTTCGACAGCAGTCGCGACTGCTGGACAAGTTAATCCTA
TTGATCCCTGGATAATTAATAATTTTGTGCAAGCCCCCCAAGGTGAATTTACTATTTCCCCAAATAATACCCCCGGTG
ATGTTTTGTTTGATTTGAGTTTGGGTCCCCATCTTAATCCTTTCTTGCTCCATCTATCACAAATGTATAATGGTTGGGTT
GGTAACATGAGAGTCAGGATTATGCTAGCTGGTAATGCCTTTACTGCGGGGAAGATAATAGTTTCCTGCATACCCCC
TGGTTTTGGTTCACATAATCTTACTATAGCACAAGCAACTCTCTTTCCACATGTGATTGCTGATGTTAGGACTCTAGAC
CCCATTGAGGTGCCTTTGGAAGATGTTAGGAATGTTCTCTTTCATAATAATGATAGAAATCAACAAACCATGCGCCTT
GTGTGCATGCTGTACACCCCCCTCCGCACTGGTGGTGGTACTGGTGATTCTTTTGTAGTTGCAGGGCGAGTTATGAC
TTGCCCCAGTCCTGATTTTAATTTCTTGTTTTTAGTCCCTCCTACGGTGGAGCAGAAAACCAGGCCCTTCACACTCCCA
AATCTGCCATTGAGTTCTCTGTCTAACTCACGTGCCCCTCTCCCAATCAGTAGTATGGGCATTTCCCCAGACAATGTCC
AGAGTGTGCAGTTCCAAAATGGTCGGTGTACTCTGGATGGCCGCCTGGTTGGCACCACCCCAGTTTCATTGTCACAT
GTTGCCAAGATAAGAGGGACCTCCAATGGCACTGTAATCAACCTTACTGAATTGGATGGCACACCCTTTCACCCTTTT
GAGGGCCCTGCCCCCATTGGGTTTCCAGACCTCGGTGGTTGTGATTGGCATATCAATATGACACAGTTTGGCCATTC
TAGCCAGACCCAGTATGATGTAGACACCACCCCTGACACTTTTGTCCCCCATCTTGGTTCAATTCAGGCAAATGGCAT
TGGCAGTGGTAATTATGTTGGTGTTCTTAGCTGGATTTCCCCCCCATCACACCCGTCTGGCTCCCAAGTTGACCTTTG
GAAGATCCCCAATTATGGGTCAAGTATTACGGAGGCAACACATCTAGCCCCTTCTGTATACCCCCCTGGTTTCGGAG
AGGTATTGGTCTTTTTCATGTCAAAAATGCCAGGTCCTGGTGCTTATAATTTGCCCTGTCTATTACCACAAGAGTACA
TTTCACATCTTGCTAGTGAACAAGCCCCTACTGTAGGTGAGGCTGCCCTGCTCCACTATGTTGACCCTGATACCGGTC
GGAATCTTGGGGAATTCAAAGCATACCCTGATGGTTTCCTCACTTGTGTCCCCAATGGGGCTAGCTCGGGTCCACAA
CAGCTGCCGATCAATGGGGTCTTTGTCTTTGTTTCATGGGTGTCCAGATTTTATCAATTAAAGCCTGTGGGAACTGCC
AGCTCGGCAAGAGGTAGGCTTGGTCTGCGCCGATAA

Figure 13C

Nucleic acid sequence of human codon-optimized VP1 G1.1 (SEQ ID NO: 18)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGCAGAAGACGCGGCCCTTTA
CACTGCCCAATCTCCCGCTTTCAAGTCTGAGTAATTCACGGGCCCCATTGCCGATCTCCTCAATGGGAATCTCCCCCG
ACAACGTCCAGTCTGTCCAATTCCAAAATGGGAGATGCACACTGGACGGTCGCCTGGTGGGAACAACTCCGGTGTC
CCTCTCACATGTCGCCAAAATCCGCGGCACATCAAATGGTACCGTAATCAATCTGACAGAACTTGATGGCACGCCCTT
CCATCCCTTTGAAGGACCAGCCCCTATTGGATTTCCTGATCTGGGAGGTTGCGACTGGCACATAAACATGACACAGT
TTGGCCACTCCAGCCAGACACAGTATGATGTCGATACAACCCCAGATACCTTCGTGCCACACCTGGGATCTATTCAA
GCTAACGGTATTGGATCCGGCAACTACGTGGGAGTCTTATCTTGGATCTCACCACCATCCCACCCCTCAGGATCCCAG
GTTGACTTGTGGAAGATACCGAATTATGGATCCTCGATCACTGAAGCCACGCACCTCGCACCTTCCGTCTACCCACCA
GGTTTTGGAGAAGTCTTGGTGTTTTTCATGAGCAAAATGCCCGGCCCTGGAGCCTACAATCTCCCTTGCCTACTCCCT
CAAGAGTATATTAGTCACCTCGCATCTGAGCAGGCCCCGACCGTTGGCGAGGCAGCCCTGCTGCATTATGTGGATCC
GGACACCGGCAGGAACCTGGGTGAGTTCAAAGCTTATCCTGACGGTTTTCTAACATGTGTACCAAATGGCGCTTCCA
GCGGCCCTCAACAGCTCCCAATCAATGGCGTGTTCGTTTTTGTCAGCTGGGTAAGCCGCTTCTACCAGCTGAAGCCC
GTGGGGACAGCTTCTTCTGCCCGCGGACGCCTCGGTCTGCGGAGATAA

Figure 14A

Amino acid sequence of VP1 G1.2_Leuven_2003_D2DEL3 (SEQ ID NO: 2)

MMMASKDAPQSADGASGAGQLVPEVNTADPLPMEPVAGPTTAVATAGQVNMIDPWIVNNFVQSPQGEFTISPNNT
PGDILFDLQLGPHLNPFLSHLSQMYNGWVGNMRVRILLAGNAFSAGKIIVCCVPPGFTSSSLTIAQATLFPHVIADVRTLEP
IEMPLEDVRNVLYHTNDNQPTMRLVCMLYTPLRTGGGSGNSDSFVVAGRVLTAPSSDFSFLFLVPPTIEQKTRAFTVPNIP
LQTLSNSRFPSLIQGMILSPDASQVVQFQNGRCLIDGQLLGTTPATSGQLFRVRGKINQGARTLNLTEVDGKPFMAFDSP
APVGFPDFGKCDWHMRISKTPNNTSSGDPMRSVDVQTDVQGFVPHLGSIQFDEVFNHPTGDYIGTIEWISQPSTPPGT
DINLWEIPDYGSSLSQAANLAPPVFPPGFGEALVYFVSAFPGPNNRSAPNDVPCLLPQEYVTHFVSEQAPTMGDAALLHY
VDPDTNRNLGEFKLYPGGYLTCVPNGVGAGPQQLPLNGVFLFVSWVSRFYQLKPVGTASTARGRLGVRRI

Figure 14B

Nucleic acid sequence of human codon-optimized VP1 G1.2_Leuven_2003_D2DEL3 (SEQ ID NO: 54)

ATGATGATGGCTTCAAAGGATGCTCCCCAAAGCGCGGACGGAGCTAGCGGCGCCGGACAGTTGGTTCCGGAAGTC
AACACTGCCGATCCACTGCCCATGGAACCCGTAGCTGGTCCAACAACCGCTGTTGCCACCGCCGGCCAGGTTAACAT
GATCGATCCATGGATTGTTAATAACTTTGTACAGAGCCCCCAGGGGGAGTTCACAATTTCTCCGAACAATACCCCTG
GGGACATTCTGTTCGATCTGCAACTGGGCCCACACTTGAATCCTTTCCTGAGCCATCTTTCACAGATGTACAACGGAT
GGGTTGGGAACATGCGTGTTCGGATCCTCCTTGCTGGCAACGCCTTCAGTGCTGGCAAGATTATCGTGTGCTGCGTA
CCACCAGGGTTTACCTCGAGTTCATTAACCATTGCTCAGGCCACCCTTTTCCCTCACGTGATCGCAGACGTGCGTACC
TTAGAACCAATCGAAATGCCCCTGGAAGATGTACGGAACGTGCTGTACCATACTAATGATAACCAGCCAACGATGA
GATTAGTGTGCATGCTGTACACCCCCCTGAGAACTGGAGGAGGTTCTGGAAATTCCGACAGTTTTGTGGTGGCTGG
CAGGGTCCTGACCGCTCCCAGTAGCGACTTCAGCTTTTTGTTCCTCGTTCCTCCTACAATCGAACAAAAAACAAGAGC
ATTCACAGTGCCCAACATTCCACTGCAGACTTTAAGCAATTCCAGGTTTCCCAGCTTGATCCAGGGTATGATCCTTTCT
CCCGACGCCTCCCAAGTTGTGCAGTTCCAGAATGGGAGATGTCTTATCGACGGTCAGCTTCTGGGAACAACCCCTGC
CACCTCCGGGCAACTCTTCCGGGTGAGAGGCAAAATCAATCAGGGCGCCAGAACACTGAATCTGACAGAAGTGGAC
GGGAAACCCTTTATGGCGTTCGATAGCCCCGCGCCCGTTGGATTCCCTGACTTCGGCAAGTGTGATTGGCACATGCG
CATCAGTAAGACTCCCAACAACACTTCATCTGGAGACCCCATGAGGAGCGTGGATGTCCAGACCGACGTGCAGGGC
TTCGTGCCGCACTTGGGATCTATCCAGTTCGATGAGGTGTTCAATCACCCTACTGGCGACTACATAGGCACAATTGA
GTGGATAAGTCAACCATCTACACCTCCAGGGACCGACATAAACCTGTGGGAAATTCCTGATTACGGGTCATCCCTGA
GTCAAGCTGCCAATCTTGCACCCCCTGTCTTTCCCCCCGGCTTTGGTGAGGCTCTTGTTTACTTCGTCTCTGCATTTCCT
GGTCCTAACAACCGCTCCGCCCCTAACGATGTTCCGTGTTTGTTACCCCAGGAATATGTGACTCATTTCGTTTCCGAA
CAGGCACCCACCATGGGGGGACGCTGCCCTGCTACACTATGTGGACCCCGACACCAATAGAAACCTCGGCGAGTTCA
AACTCTACCCCGGGGGATACCTGACCTGTGTTCCAAATGGAGTGGGAGCAGGCCCACAACAGCTGCCCCTGAATGG
GGTCTTCCTGTTCGTTTCTTGGGTGTCACGCTTTTACCAGCTGAAGCCCGTTGGCACAGCTTCTACGGCACGCGGCAG
GCTAGGGGTCCGCCGAATCTGA

Figure 15A

Amino acid sequence of VP1 GI.3_LillaEdet_2008_H2DG70 (SEQ ID NO: 3)

MMMASKDAPTNMDGTSGAGQLVPEVSTAEPISMEPVAGAATAAATAGQVNMIDPWIMSNYVQAPQGEFTISPNNT
PGDILFDLQLGPHLNPFLSHLAQMYNGWVGNMKVRVLLAGNAFTAGKIIISCVPPGFAAQNVSIAQATMFPHVIADVRV
LEPIEVPLEDVRNVLFHNNDSTPTMRLICMLYTPLRASGSSSGTDPFVIAGRVLTCPSPDFNFLFLVPPNVEQKTKPFSVPNL
PLNVLSNSRVPSLIKSMMVSQDHGQMVQFQNGRVTLDGQLQGTTPTSASQLCKIRGTVYHATGGQGLNLTEIDGTPYH
AFESPAPIGFPDLGECDWHINASPANAFTDGSIIHRIDVAQDSTFAPHLGTIHYTNADYNANVGLICSLEWLSPPSGGAPK
VNPWAIPRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSDFPIANGSDGLSVPCTIPQEFVTHFVNEQAPTRGEAALLHYVDP
DTHRNLGEFKLYPEGFMTCVPNSSGSGPQTLPINGVFTFISWVSRFYQLKPVGTTGPVRRLGIRRS

Figure 15B

Nucleic acid sequence of human codon-optimized VP1 GI.3_LillaEdet_2008_H2DG70 (SEQ ID NO: 55)

ATGATGATGGCTTCCAAGGATGCTCCCACAAACATGGATGGAACAAGCGGCGCGGGGCAACTTGTGCCGGAGGTG
TCCACGGCGGAACCCATTTCCATGGAACCTGTGGCCGGCGCAGCCACTGCTGCCGCCACCGCAGGACAGGTAAACA
TGATCGACCCCTGGATCATGTCAAATTACGTTCAGGCTCCACAGGGGGAGTTTACCATAAGCCCAAACAACACCCCG
GGTGACATCTTGTTTGACCTGCAGCTAGGACCACACTTGAATCCGTTTCTGAGTCACTTGGCTCAGATGTATAATGGA
TGGGTTGGAAACATGAAGGTGCGCGTGCTCCTGGCGGGCAATGCATTCACAGCCGGGAAGATTATTATCTCTTGCG
TGCCACCTGGATTTGCAGCCCAGAACGTGTCTATCGCACAGGCAACCATGTTTCCGCATGTCATCGCAGATGTGCGC
GTGCTAGAGCCCATCGAGGTGCCCCTTGAGGACGTGCGCAACGTCCTATTCCATAACAATGATAGCACCCCCACCAT
GCGCTTGATATGTATGTTATATACTCCCCTCCGCGCCAGTGGGTCCAGCTCCGGGACCGATCCTTTTGTGATTGCTGG
GCGGGTGTTGACTTGTCCTAGCCCTGACTTCAACTTCCTTTTTCTGGTGCCTCCAAATGTAGAACAGAAAACAAAGCC
ATTCAGCGTGCCAAACCTGCCCCTTAACGTGCTGTCGAATTCCCGAGTGCCTTCCCTTATTAAGTCCATGATGGTATC
TCAGGATCACGGTCAAATGGTGCAGTTTCAGAACGGCCGAGTGACGTTAGACGGGCAGCTGCAGGGCACAACCCC
AACCAGTGCCAGTCAGCTGTGTAAGATCAGAGGCACCGTCTACCACGCAACTGGCGGACAGGGGCTGAATCTTACT
GAGATCGATGGTACCCCCTACCATGCATTCGAGTCACCTGCACCTATTGGATTTCCCGATCTTGGGGAGTGTGATTG
GCATATCAATGCTTCACCTGCCAACGCTTTCACAGACGGGTCTATTATTCATCGCATTGACGTAGCACAGGATAGCAC
ATTTGCCCCGCACCTGGGTACCATCCACTATACGAACGCAGATTACAACGCAAACGTGGGTCTTATCTGTAGCCTAG
AGTGGCTATCTCCGCCAAGCGGTGGGGCCCCTAAAGTTAACCCATGGGCTATTCCTCGGTACGGGTCTACGCTGACT
GAGGCCGCTCAGCTGGCACCCCCCATATATCCACCAGGATTCGGGGAAGCCATTGTTTTCTTTATGTCCGATTTTCCG
ATAGCCAACGGTTCAGATGGCCTTAGTGTCCCTTGCACGATTCCACAGGAATTTGTGACACACTTCGTAAACGAGCA
GGCTCCTACTCGGGGCGAGGCTGCCTTGTTGCATTACGTAGACCCCGATACCCATAGAAACCTGGGCGAATTCAAAC
TCTACCCTGAAGGTTTCATGACCTGCGTACCTAACTCCTCCGGCAGTGGCCCTCAAACCTTGCCGATCAACGGCGTGT
TCACGTTTATCAGCTGGGTTTCACGGTTTTACCAACTCAAGCCCGTCGGAACAACTGGGCCAGTTCGGAGGCTCGGG
ATCAGACGGAGCTAG

Figure 16A

Amino acid sequence of VP1 GI.5_Siklos_HUN5407_2013_HUN_AHW99832 (SEQ ID NO: 44)

MMMASKDAPSSADGANGAGQLVPEVNNAEPLPLDPVAGASTALATAGQVNMIDPWIFNNFVQAPQGEFTISPNNTP
GDILFDLQLGPHLNPFLAHLSQMYNGWVGNMRVRVILAGNAFTAGKVIICCVPPGFQSRTLSIAQATLFPHIIADVRTLEPI
EIPLEDVRNTLYHTNDNQPTMRLLCMLYTPLRTGGGSGGTDAFVVAGRVLTCPSSDFNFLFLVPPTVEQKTRPFSVPNIPL
QLLSNSRVPNLIQSMVLSPDQAQNVQFQNGRCTTDGQLLGTTPVSVSQILKFRGKVSAGSKVINLTELDGSPFLAFEAPAP
TGFPDLGTSDWHVEMSLNSNSQSSGNPILLRDIHPNSSEFVPHLGSVCVTAAIEVAGDYTGTIQWTSQPSNVTPVPDVNF
WTIPHYGSNLAEASQLAPVVYPPGFGEAIVYFMSPIPGPNTAHKPNLVPCLLPQEFVTHFVSEQAPSMGEAALVHYVDPD
TNRNLGEFKLYPEGFITCVPNGTGPQQLPLNGVFVFASWVSRFYQLKPVGTASSARGRLGVRR

Figure 16B

Amino acid sequence of GII.1_Ascension208_2010_USA_AFA55174 native VP1 (SEQ ID NO: 45)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRMNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPFLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAIPPHFPLENLSPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQPEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPILTIGELSNS
RFPVPIDELYTSPNEGVVVQPQNGRSTLDGELLGTTQLVPSNICALRGRINAQVPDDHHQWNLQVTNANGTSFDPTEDV
PAPLGTPDFLANIYGVTSQRNPDNTCRAHDGVLATWSPKFTPKLGSVVLGTWEESDLDLNQPTRFTPVGLYDTGHFDQ
WVLPNYSGRLTLNMNLAPSVAPLFPGEQILFFRSHIPLKGGTSNGAIDCLLPQEWIQHFYQESAPSPTDVALIRYTNPDTG
RVLFEAKLHRQGFITVANSGSRPIVVPPNGYFRFDSWVNQFYSLAPMGTGNGRRRVQ

Figure 16C

Amino acid sequence of VP1 GII.2_CGMH47_2011_TW_AGT39206 (SEQ ID NO: 66)

MKMASNDAAPSTDGAAGLVPESNNEVMALEPVAGAALAAPVTGQTNIIDPWIRANFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLARMYNGYAGGMEVQVMLAGNAFTAGKLVFAAVPPHFPVENLSPQQITMFPHVIIDVRTLEPVLL
PLPDVRNNFFHYNQKDDPKMRIVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFTYLVPPTVESKTKPFTLPILTLGELS
NSRFPVSIDQMYTSPNEVISVQCQNGRCTLDGELQGTTQLQVSGICAFKGEVTAHLHDNDHLYNVTITNLNGSPFDPSED
IPAPLGVPDFQGRVFGVISQRDKHNTPGHNEPANRAHDAVVPTYTAQYTPKLGQIQIGTWQTDDLTVNQPVKFTPVGL
NDTDHFNQWVVPRYAGALNLNTNLAPSVAPVFPGERLLFFRSYIPLKGGYGTPAIDCLLPQEWVQHFYQEAAPSMSEVA
LVRYINPDTGRALFEAKLHRAGFMTVSSNTSAPVV

VPANGYFRFDSWVNQFYSLAPMGTGNGRRRIQ

Figure 16D

Amino acid sequence of VP1 GII.3_Jingzhou_2013402_CHN_AGX01095 (SEQ ID NO: 67)

MKMASNDAAPSNDGAAGLVPEISSEAMALEPVAGAAIAAPLTGQQNIIDPWIMNNFVQAPGGEFTVSPRNSPGEVLLN
LELGPEINPYLAHLARMYNGYAGGFEVQVVLAGNAFTAGKIIFAAIPPNFPIDNLSAAQITMCPHVIVDVRQLEPVNLPMP
DVRNNFFHYNQGSDSRLRLIAMLYTPLRANNSGDDVFTVSCRVLTRPSPDFSFNFLVPPTVESKTKPFSLPILTISEMSNSRF
PVPIDSLHTSPTENIVVQCQNGRVTLDGELMGTTQLLPSQICAFRGTLTRSTSRASDQADTATPRLFNYYWHIQLDNLNG
TPYDPAEDIPAPLGTPDFRGKVFGVASQRNPDATTRAHEAKIDTTSGRFTPKLGSLEISTESGDFDQNQPTRFTPVGIGVD
HEPDFQQWALPDYAGQFTHNMNLAPAVAPNFPGEQLLFFRSQLPSSGGRSNGILDCLVPQEWVQHFYQESAPSQTQV
ALVRYVNPDTGRVLFEAKLHKLRFMTIAKSGDSPITVPPNGYFRFESWVNPFYTLAPMGTGNGRRRIQ

Figure 16E

Amino acid sequence of VP1 GII.5_Alberta_2013_CA_ALT54485 (SEQ ID NO: 68)

MKMASNDATPSNDGAAGLVPESNNEAMALEPVVGASLAAPVTGQTNIIDPWIRTNFVQAPNGEFTVSPRNSPGEILVN
LELGPELNPYLAHLARMYNGYAGGMEVQVLLAGNAFTAGKIIFAAVPPYFPVENLSPSQITMFPHVIIDVRTLEPVLLPMP
DVRSTLFHFNQKDEPKMRLVAMLYTPLRSNGSGDDVFTVSCRILTRPSPEFDFTYLVPPTVESKTKPFTLPVLTLGELSNSRF
PLSIDEMVTSPNESIVVQPQNGRVTLDGELLGTTQLQACNICSIRGKVTGQVPNEQHMWNLEITNLNGTQFDPTDDVPA
PLGVPDFAGEVFGVLSQRNRGESNPANRAHDAVVATYSDKYTPKLGLVQIGTWNTNDVENQPTKFTPIGLNEVANGHR
FEQWTLPRYSGALTLNMNLAPAVAPLFPGERLLFFRSYVPLKGGFGNPAIDCLVPQEWVQHFYQESAPSLGDVALVRYV
NPDTGRVLFEAKLHKGGFLTVSSTSTGPVVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRFQ

Figure 16F

Amino acid sequence of VP1 GII.7_Musa_2010_AII73774 (SEQ ID NO: 69)

MKMASNDAAPSNDGAAGLVPEINNEVMPLEPVAGASLATPVVGQQNIIDPWIRNNFVQAPAGEFTVSPRNSPGEILLD
LELGPELNPYLAHLARMYNGHAGGMEVQIVLAGNAFTAGKIIFAAIPPGFPYENLSPSQITMCPHVIIDVRQLEPVLLPMP
DIRNNFFHYNQGNDPKLRLIAMLYTPLRANNSGDDVFTVSCRVLTKPSPDFEFTFLVPPTVESKTKQFTLPILKISEMTNSRF
PVPVEMMYTARNENQVVQPQNGRVTLDGELLGTTPLLAVNICKFKGEVIAKNGDVRSYRMDMEITNTDGTPIDPTEDT
PGPIGSPDFQGILFGVASQRNKNEQNPATRAHEANINTGGDQYAPKLAQVKFFSESQDFEVHQPTVFTPVGVAGDTSHP
FRQWVLPRYGGHLTNNTHLAPAVAPLFPGEQILFFRSQIPSSGGHELGYMDCLVPQEWVQHFYQEAATAQSEVALIRFIN
PDTGRVLFEAKLHKQGFITVAHTGDNPIVMPPNGYFRFEAWVNQFYSLAPVGTGNGRRRIQ

Figure 16G

Amino acid sequence of VP1 consensus sequence from genotypes GI.1, GI.2, GI.3, GII.4, GII.6, GII.13 and GII.17 (SEQ ID NO: 70)

```
GI.1 SEQ ID NO:1     MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNF
GI.2 SEQ ID NO:2     MMMASKDAPQSADGASGAGQLVPEVNTADPLPMEPVAGPTTAVATAGQVNMIDPWIVNNF
GI.3 SEQ ID NO:3     MMMASKDAPTNMDGTSGAGQLVPEVSTAEPISMEPVAGAATAAATAGQVNMIDPWIMSNY
GII.4 SEQ ID NO:4    MKMASSDANPSDGS---AANLVPEVNN-EVMALEPVVGAAIAAPVAGQQNVIDPWIRNNF
GII.6 SEQ ID NO:5    MKMASNDAAPSNDG---AANLVPEANN-EVMALEPVVGASIAAPVVGQQNIIDPWIRENF
GII.13 SEQ ID NO:6   MKMASNDAAPSNDG---AASLVPEAIN-ETMPLEPVAGASIAAPVAGQTNIIDPWIRTNF
GII.17 SEQ ID NO:7   MKMASNDAAPSNDG---AAGLVPEGNN-ETLPLEPVAGAAIAAPVTGQNNIIDPWIRTNF
VP1 Con SEQ ID NO:70 MkMASXDAXpsXdGaSgAaXLVPEvnXneXXXlePVaGaXiAapVaGQXNXIDPWIrXNf

GI.1 SEQ ID NO:1     VQAPQGEFTISPNNTPGDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTA
GI.2 SEQ ID NO:2     VQSPQGEFTISPNNTPGDILFDLQLGPHLNPFLSHLSQMYNGWVGNMRVRILLAGNAFSA
GI.3 SEQ ID NO:3     VQAPQGEFTISPNNTPGDILFDLQLGPHLNPFLSHLAQMYNGWVGNMKVRVLLAGNAFTA
GII.4 SEQ ID NO:4    VQAPGGEFTVSPRNAPGEILWSAPLGPDLNPYLSHLARMYNGYAGGFEVQVILAGNAFTA
GII.6 SEQ ID NO:5    VQAPQGEFTVSPRNSPGEMLLNLELGPELNPYLSHLSRMYNGYAGGMQVQVVLAGNAFTA
GII.13 SEQ ID NO:6   VQAPNGEFTVSPRNSPGEILLNLELGPDLNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTA
GII.17 SEQ ID NO:7   VQAPNGEFTVSPRNSPGEILLNLELGPDLNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTA
VP1 Con SEQ ID NO:70 VQaPqGEFTvSPrNXPGeiLXXlXLGPXLNPyLsHLsrMYNGYaGgmXVqvlLAGNAFtA

GI.1 SEQ ID NO:1     GKIIVSCIPPGFGSHNLTLAQATLFPHVIADVRTLDPIEVPLEDVRNVLFHNNDRNQQTM
GI.2 SEQ ID NO:2     GKIIVCCVPPGFTSSSLTLAQATLFPHVIADVRTLEPIEMPLEDVRNVLYHTND-NQFTM
GI.3 SEQ ID NO:3     GKIIISCVPPGFAAQNVSIAQATMFPHVIADVRVLEPIEVPLEDVRNVLFHNND-STFTM
GII.4 SEQ ID NO:4    GKVIFAAVPPNFPTEGLSPSQVTMFPHIVVDVRQLEPVLIPLPDVRNNFYHYNQSNDPTI
GII.6 SEQ ID NO:5    GKIIFAAVPPHFPVENINAAQTTMCPHVIVDVRQLEPVLLPLPDIRNRFFHYNQENTSRM
GII.13 SEQ ID NO:6   GKILFAAIPPNFPVDMISPAQTTMLPHLIVDVRTLEPIMIPLPDVRNVFYHFNNQPQPRM
GII.17 SEQ ID NO:7   GKILFAAVPPNFPVEFLSPAQTTMLPHLIVDVRTLEPIMIPLPDVRNTFFHYNNQPNSRM
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VP1 Con SEQ ID NO:70  GKiifaavPPXFpXXXlsXaQXTmfPHvivDVRtLePiXXPLpDvRNvffHXNXXnXptm

GI.1 SEQ ID NO:1      RLVCMLYTPLRTGCGTG--DSFVVAGRVMTCPSPDFNTLFLVPPTVE QKTRPFTLPNLPL
G1.2 SEQ ID NO:2      RLVCMLYTPLRTGGGSGNSDSFVVAGRVLTAPSSDFSTFLVPPTIE QKTRAFTVPNIPL
GI.3 SEQ ID NO:3      RLICMLYTPLRASGSSSGTDPTVIAGRVLTCPSPDFNTLFLVPPNVE QKTKPFSVPNLPL
GII.4 SEQ ID NO:4     KLIAMLYTPLRANNA--GDDVFTVSCRVLTRPSPDFDTFFLVPPTVE SRTKPFSVPVLTV
GII.6 SEQ ID NO:5     RLVAMLYTPLRAN-S--GEDVFTVSCRVLTRPAPDFETTLVPPTVE SKTKPFSLPILTL
GII.13 SEQ ID NO:6    RLVAMLYTPLRSNGS--GDDVFTVSCRVLTRPTPDTEFTYLVPPSVE SKTKPFTLPILTI
GII.17 SEQ ID NO:7    RLVAMLYTPLRSNGS--GDDVFTVSCRVLTRPTPDFEFTYLVPPSVE SKTKPFSLPILTL
VP1 Con SEQ ID NO:70  rLvaMLYTPLRXngsXXXXDvfTvscRVlTrPspDFXFLVPPtvE skTkpFslPXltl

GI.1 SEQ ID NO:1      SSLSNSRAPLPISSMGISPDNVQSVQFQNGRCTLDGRLVGTTPVSLSHVAKIRGTSN---
G1.2 SEQ ID NO:2      QTLSNSRFPSLIQGMLSPDASQVVQFQNGRCLIDGQLLGTTPATSGQLFRVRGKIN--Q
GI.3 SEQ ID NO:3      NVLSNSRVPSLIKSMMTVSQDHGQMVQFQNGRVTLDGQLQGTTPTSASQLCKIRGTVYHAT
GII.4 SEQ ID NO:4     EEMTNSRFPIPLEKLTGPSSAFVVQFQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHIT
GII.6 SEQ ID NO:5     GELSNSRFPAPIDMLYTDPNEGIVVQFQNGRCTLDGTLQGTTQLVPTQICAFRGTLIGQT
GII.13 SEQ ID NO:6    SELTNSRFPISIEQLYTAPNENNVVQCQNGRCTLDGSLQGTTQLISSAVCSYRGRTVANS
GII.17 SEQ ID NO:7    SELTNSRFPVPIDSLFTAQNNVLQVQCQNGRCTLDGSLQGTTQLLPSGICAFRGRVTAET
VP1 Con SEQ ID NO:70  XelsNSRfPXpiXXlXtXpXXXXvVQXQNGRctlDGXlqGTTqlXXsXXcXXRGXXXXXt

GI.1 SEQ ID NO:1      -GT----------VINLTELDGTPFHPF-EGPAPIGFPDLGGCDWHINMTQFGHS
G1.2 SEQ ID NO:2      GAR----------TLNLTEVDGKPFMAF-DSPAPVGFPDFGKCDWHMRISKTPNN
GI.3 SEQ ID NO:3      GGQ----------GLNLTEIDGTPYHAF-ESPAPIGFPDLGECDWHINASPANAF
GII.4 SEQ ID NO:4     GSR----------NYTMNLASQNWNDYDPTEETPAPLGTPDFVGKIQGVLTQTTR--
GII.6 SEQ ID NO:5     SRSPDSTDSAPRRRDHPLHVQLKNLDGTQYDPTDEVPAVLGAIDFKGTVFGVASQRDVSG
GII.13 SEQ ID NO:6    GDN----------WDQNVLQLTYPSGASYDPTDEVPAPLGTQDFSGILYGVLTQDNVRE
GII.17 SEQ ID NO:7    D-H----------RDKWHMQLQNLNGTTYDPTDDVPAPLGTPDFKGVVFGVASQRNVGN
VP1 Con SEQ ID NO:70  gRSPDSTDSAPRdXXXXXXXXnLtXXdgtXydptdXXPApIGXpDfXgXXXgvXXqXXXXX

GI.1 SEQ ID NO:1      S------QTQYDVDTTPDTFVPHLGSIQANGI---GSG----NYVGVLSWISPPSHPSGS
G1.2 SEQ ID NO:2      TS-SGDPMRSVDVQTDVQGFVPHLGSIQFDEVFNHPTG----DYIGTIEWISQPSTPPGT
GI.3 SEQ ID NO:3      -T-DGSIIHRIDVA-QDSTFAPHLGTIHYTNADYNANV----GLICSLEWLSPPSGG-AP
GII.4 SEQ ID NO:4     -TDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQDGGTTHRN

                                CONTINUATION OF FIGURE 16G
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GII.6 SEQ ID NO:5       QQVGATRAHEVHTNTDERYTPKLGSILMYSE-SDDFVTGQPVRFTPIGMGDN--------
GII.13 SEQ ID NO:6      NTGEAKNAKGVYSTTSGKFTPKIGSIGLHSI-TEDVRPNQQSRFTPVGVAQNENT----
GII.17 SEQ ID NO:7      DAPGSTRAHEAVTSTYSPQFVPKLGSVNFRSN-DNDFQ-LQPTKFTPVGINDDGDH----
VP1 Con SEQ ID NO:70    XXXgXXXXhXXXXXtXsXXXXpkXGsXXXXXXXXXdfXXXXXXXXftpXgXXXXXXXpXXX

GI.1 SEQ ID NO:1        QVDLWKIPNYGSSITEATHLAPSVYPPGFGEVLVFFMSKMPGPGA----YNLPCLLPQE
G1.2 SEQ ID NO:2        DINLWEIPDYGSSLSQAANLAPPVFPPGFGEALVYFVSAFPGPNNRS-APNDVPCLLPQE
GI.3 SEQ ID NO:3        KVNPWAIPRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSDFPIANG-S-DGLSVPCTIPQE
GII.4 SEQ ID NO:4       EPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYP--NMDLDCLLPQE
GII.6 SEQ ID NO:5       DWHQWELPDYPGHLTLNMNLAPAVAPAFPGRILFFRSIVPSAGGYG--SGQIDCLIPQE
GII.13 SEQ ID NO:6      PFQQWVLPRYAGAIAINTNLAPAVAPTFPGEQLLFFRSRVPCVQGLQGQDAFIDCLLPQE
GII.17 SEQ ID NO:7      PFRQWELPDYSGLLTLNMNLAPPVAPNFPGEQLLFFRSFVPCSGGYN--QGIVDCLIPQE
VP1 Con SEQ ID NO:70    XXXqWXlPXYXgXltXnXnLAPXvaPXfpGEXllfYrSXXPXXXgyXXQXXXXdCllPQE

GI.1 SEQ ID NO:1        YISHLASEQAPTVGEAALLHYVDPDTGRNLGEFKAYPDGFLTCVPNGASSGPQQLPINGV
G1.2 SEQ ID NO:2        YVTHFVSEQAPTMGDAALLHYVDPDTNRNLGEFKLYPGGYLTCVPNGVGAGPQQLPLNGV
GI.3 SEQ ID NO:3        FVTHFVNEQAPTRGEAALLHYVDPDTHRNLGEFKLYPEGFMTCVPNSSGSGPQTLPINGV
GII.4 SEQ ID NO:4       WVQYFYQEAAPAQSDVALLRFVNPDTGRVLETECKLHKSGYVTVAH--TGQHDLVTPPNGY
GII.6 SEQ ID NO:5       WVQHFYQEAAPSQSAVALIRYVNPDTGRNITEAKLHREGFITVAN--SGNNFIVVPPNGY
GII.13 SEQ ID NO:6      WVNHFYQEAAPSQADVALIRYVNPDTGRTLETEAKLHRSGFITVSH--TGAYPLVVPPNGH
GII.17 SEQ ID NO:7      WIQHFYQESAPSQSDVALIRYVNPDTGRTLETEAKLHRSGYITVAH--SGDYPLVVPANGY
VP1 Con SEQ ID NO:70    wvXhfyqEXAPXqXdvALlryVnPDTgRnlfEXKlhXXGfXTvXXXgXgXXpXvXPXNGX

GI.1 SEQ ID NO:1        FVFVSWVSRFYQLKPVGTASSARGRLGLRR-
G1.2 SEQ ID NO:2        FLFVSWVSRFYQLKPVGTASTARGRLGVRRI
GI.3 SEQ ID NO:3        FTF1SWVSRFYQLKPVGTTGPVR-RLGIRRS
GII.4 SEQ ID NO:4       FRFDSWVNQFYTLAPMGNGTGRRRAV-----
GII.6 SEQ ID NO:5       FRFEAWVNQFYTLTPMGTGQGRRRDQ-----
GII.13 SEQ ID NO:6      FRFDSWVNQFYSLAPMGTGNGRRRVQ-----
GII.17 SEQ ID NO:7      FRFDSWVNQFYSLAPMGTGNGRRRAQ-----
VP1 Con SEQ ID NO:70    FrFXsWVnqFYXlXPmGtgXgrRrXXGXRRX
```

CONTINUATION OF FIGURE 16G

Figure 17A

Amino acid sequence of VP1 GII.4_Sydney_2012_K4LM89 (SEQ ID NO: 4)

MKMASSDANPSDGSAANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIRNNFVQAPGGEFTVSPRNAPGEIL
WSAPLGPDLNPYLSHLARMYNGYAGGFEVQVILAGNAFTAGKVIFAAVPPNFPTEGLSPSQVTMFPHIVVDVRQLEPVLI
PLPDVRNNFYHYNQSNDPTIKLIAMLYTPLRANNAGDDVFTVSCRVLTRPSPDFDFIFLVPPTVESRTKPFSVPVLTVEEMT
NSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDYDPTEEIP
APLGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQDGGTTHR
NEPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQSDVAL
LRFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV

Figure 17B

Nucleic acid sequence of human codon-optimized VP1 GII.4_Sydney_2012_K4LM89 (SEQ ID NO: 56)

ATGAAAATGGCCTCGAGTGACGCTAACCCTAGTGACGGCAGCGCCGCCAATCTTGTGCCTGAGGTTAATAATGAGG
TGATGGCCCTGGAGCCTGTGGTGGGCGCAGCCATAGCAGCGCCCGTGGCCGGTCAGCAGAATGTGATTGACCCGT
GGATACGCAACAATTTTGTCCAAGCCCCTGGTGGGGAGTTCACCGTTAGCCCGAGAAATGCGCCAGGAGAAATCCT
GTGGTCGGCCCCATTGGGACCCGATCTGAACCCCTATTTGTCACATCTCGCTCGGATGTACAACGGGTATGCCGGCG
GATTTGAAGTGCAGGTGATTCTGGCTGGGAACGCGTTCACTGCTGGCAAAGTGATCTTTGCAGCGGTGCCTCCCAAC
TTCCCCACTGAAGGACTGTCTCCAAGCCAGGTCACAATGTTTCCACACATCGTGGTGGACGTACGGCAGCTAGAGCC
TGTCCTGATTCCCCTCCCTGATGTACGCAATAATTTCTACCACTACAATCAATCCAATGATCCGACCATTAAACTCATC
GCGATGTTGTACACCCCTCTGCGCGCTAACAATGCTGGAGACGACGTATTCACCGTGTCATGCAGAGTGCTCACCAG
ACCTTCACCAGACTTTGACTTTATCTTCTTAGTGCCCCCCACTGTTGAGAGCCGAACCAAGCCCTTTAGTGTCCCCGTA
CTCACAGTCGAGGAGATGACAAATAGCCGCTTTCCAATCCCCCTTGAGAAACTGTTCACAGGACCTTCCTCGGCATTC
GTGGTTCAGCCACAGAACGGACGCTGCACAACTGACGGCGTGCTGCTCGGAACCACCCAGCTTAGCCCTGTTAATAT
CTGTACGTTTAGAGGCGACGTAACTCACATAACTGGCTCACGGAACTATACCATGAATCTGGCATCACAGAATTGGA
ATGACTACGACCCAACCGAAGAGATTCCCGCACCTCTTGGAACCCCCGACTTTGTGGGAAAAATACAGGGCGTCCTG
ACACAAACCACCAGAACCGATGGCTCCACACGGGGACACAAGGCAACCGTCTACACTGGCTCTGCCGATTTTGCCCC
GAAACTGGGTAGAGTGCAGTTTGAGACCGACACTGACCGGGACTTTGAAGCCAATCAGAATACTAAGTTCACACCT
GTAGGAGTGATTCAGGACGGGGGCACCACTCACCGGAACGAGCCGCAACAATGGGTCCTGCCCTCTTATAGCGGG
AGGAATACTCATAATGTGCATTTGGCTCCTGCAGTGGCTCCCACGTTTCCCGGGGAACAACTGCTCTTTTTTCGTTCA
ACCATGCCTGGATGCTCCGGATATCCCAATATGGATCTCGATTGCCTGCTCCCACAGGAATGGGTGCAGTATTTTTAT
CAAGAGGCCGCACCAGCCCAATCCGACGTCGCACTTCTGCGGTTCGTGAATCCAGACACAGGCCGCGTGTTGTTTGA
GTGCAAATTGCACAAATCAGGATACGTTACAGTGGCTCATACTGGACAGCATGACCTGGTGATCCCACCCAACGGAT
ATTTTAGGTTCGACTCCTGGGTGAATCAGTTTTATACATTAGCCCCCATGGGGAATGGGACTGGCAGACGCAGGGCT
GTCTGA

Figure 18A

Amino acid sequence of VP1 GII.6_Ohio_2012_M9T020 (SEQ ID NO: 5)

MKMASNDAAPSNDGAANLVPEANNEVMALEPVVGASIAAPVVGQQNIIDPWIRENFVQAPQGEFTVSPRNSPGEMLL
NLELGPELNPYLSHLSRMYNGYAGGMQVQVVLAGNAFTAGKIIFAAVPPHFPVENINAAQITMCPHVIVDVRQLEPVLLP
LPDIRNRFFHYNQENTSRMRLVAMLYTPLRANSGEDVFTVSCRVLTRPAPDFEFTFLVPPTVESKTKPFSLPILTLGELSNSR
FPAPIDMLYTDPNEGIVVQPQNGRCTLDGTLQGTTQLVPTQICAFRGTLIGQTSRSPDSTDSAPRRRDHPLHVQLKNLDG
TQYDPTDEVPAVLGAIDFKGTVFGVASQRDVSGQQVGATRAHEVHINTTDPRYTPKLGSILMYSESDDFVTGQPVRFTPI
GMGDNDWHQWELPDYPGHLTLNMNLAPAVAPAFPGERILFFRSIVPSAGGYGSGQIDCLIPQEWVQHFYQEAAPSQS
AVALIRYVNPDTGRNIFEAKLHREGFITVANSGNNPIVVPPNGYFRFEAWVNQFYTLTPMGTGQGRRRDQ

Figure 18B

Nucleic acid sequence of human codon-optimized VP1 GII.6_Ohio_2012_M9T020 (SEQ ID NO: 60)

ATGAAGATGGCAAGCAACGACGCAGCTCCCTCCAATGATGGTGCCGCCAACCTGGTCCCCGAAGCTAATAATGAGG
TGATGGCGTTAGAGCCGGTGGTTGGCGCATCTATTGCAGCGCCTGTGGTCGGACAGCAGAACATCATTGATCCCTG
GATTCGCGAGAACTTCGTACAAGCTCCACAGGGGGAGTTCACAGTCTCCCCCCGGAACTCCCCGGGCGAGATGCTG
CTCAATCTGGAACTCGGCCCTGAACTAAACCCTTATCTGTCACACCTTTCACGGATGTACAATGGCTACGCAGGAGG
AATGCAAGTTCAGGTGGTCCTGGCCGGCAATGCTTTCACCGCGGGCAAAATCATCTTTGCGGCCGTTCCTCCACACT
TCCCTGTCGAAAATATCAACGCCGCCCAGATTACTATGTGCCCCCACGTGATTGTGGATGTGCGACAGTTAGAGCCA
GTTCTGCTGCCCCTGCCCGACATCAGAAACCGGTTCTTCCATTACAATCAAGAGAATACTTCACGGATGAGACTTGTT
GCGATGCTGTACACCCCTCTTCGTGCAAATTCCGGCGAAGACGTGTTCACTGTGTCTTGTCGAGTACTTACCCGACCC
GCCCCCGATTTCGAATTCACCTTCCTGGTTCCCCCTACTGTGGAGAGCAAGACAAAACCCTTCAGCCTCCCAATCTTA
ACACTCGGGGAGCTGTCTAATTCACGCTTCCCCGCACCTATTGATATGCTGTATACTGACCCCAACGAGGGGATAGT
GGTGCAGCCCCAAAATGGACGGTGTACTCTCGACGGCACGCTCCAGGGCACAACCCAACTGGTGCCAACCCAGATT
TGTGCATTCAGGGGCACTTTGATTGGGCAGACATCGAGATCTCCAGATTCTACTGATTCCGCGCCAAGGAGGAGGG
ACCACCCACTCCACGTTCAGTTAAAAAACCTGGACGGAACCCAGTACGACCCTACAGACGAGGTCCCCGCTGTCCTC
GGAGCCATCGACTTTAAAGGAACTGTATTTGGAGTGGCATCCCAAAGGGATGTCTCGGGGCAGCAGGTGGGAGCT
ACGAGAGCACATGAAGTCCACATTAACACCACAGACCCAAGATATACCCCAAAACTAGGGTCAATTTTAATGTATTC
GGAATCAGACGATTTTGTTACAGGTCAGCCCGTGCGGTTTACCCCGATCGGAATGGGGGACAACGATTGGCACCAG
TGGGAATTGCCCGATTACCCTGGACACCTCACCTTGAATATGAATCTGGCCCCAGCCGTCGCGCCCGCCTTCCCCGGT
GAGCGGATCCTCTTTTTTAGAAGCATAGTGCCCTCCGCAGGTGGGTATGGATCAGGGCAGATTGATTGCCTGATCCC
CCAAGAATGGGTACAGCATTTCTACCAGGAAGCAGCCCCTAGCCAGTCCGCAGTAGCACTGATCAGATATGTTAATC
CTGATACGGGAAGGAACATCTTCGAAGCAAAACTGCACCGTGAGGGCTTCATTACCGTCGCCAACAGTGGTAATAA
CCCTATTGTGGTGCCTCCTAATGGATACTTCAGGTTTGAGGCATGGGTGAATCAGTTTTATACTCTGACTCCCATGGG
GACAGGCCAGGGGCGACGCCGGGATCAGTGA

Figure 19A

Amino acid sequence of VP1 GII.13_VA173_2010_H9AWU4 (SEQ ID NO: 6)

MKMASNDAAPSNDGAASLVPEAINETMPLEPVAGASIAAPVAGQTNIIDPWIRTNFVQAPNGEFTVSPRNS
PGEILLNLELGPDLNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKILFAAIPPNFPVDMISPAQITMLPHLI
VDVRTLEPIMIPLPDVRNVFYHFNNQPQPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPTPDFEFIYLVPP
SVESKTKPFTLPILTISELTNSRFPISIEQLYTAPNENNVVQCQNGRCTLDGELQGTTQLLSSAVCSYRGRTVANS
GDNWDQNVLQLTYPSGASYDPTDEVPAPLGTQDFSGILYGVLTQDNVRENTGEAKNAKGVYISTTSGKFTPK
IGSIGLHSITEDVRPNQQSRFTPVGVAQNENTPFQQWVLPHYAGALALNTNLAPAVAPTFPGEQLLFFRSRVP
CVQGLQGQDAFIDCLLPQEWVNHFYQEAAPSQADVALIRYVNPDTGRTLFEAKLHRSGFITVSHTGAYPLVV
PPNGHFRFDSWVNQFYSLAPMGTGNGRRRVQ

Figure 19B

Nucleic acid sequence of human codon-optimized VP1 GII.13_VA173_2010_H9AWU4 (SEQ ID NO: 61)

ATGAAAATGGCTTCTAATGATGCCGCGCCCAGCAATGATGGTGCCGCCAGCCTTGTGCCCGAAGCAATTAACGAGA
CAATGCCCTTGGAGCCAGTCGCCGGGGCTTCTATTGCGGCCCCAGTTGCTGGACAGACGAATATCATCGATCCTTGG
ATACGGACTAATTTTGTTCAAGCTCCTAACGGAGAGTTCACTGTCTCCCCCCGTAATAGTCCTGGCGAGATCCTGTTG
AACCTCGAGTTGGGGCCAGATCTCAATCCTTACCTGGCTCATCTGTCGAGAATGTACAACGGGTACGCGGGGGGGG
TTGAGGTGCAGGTCTTACTGGCAGGTAACGCATTCACAGCAGGCAAGATTCTGTTTGCGGCCATCCCTCCTAATTTTC
CAGTGGATATGATATCTCCAGCACAGATTACAATGCTGCCCCATTTGATAGTGGATGTGCGGACACTTGAACCTATC
ATGATCCCTTTGCCCGATGTCCGAAATGTGTTTTATCATTTCAACAACCAGCCGCAGCCAAGAATGCGTCTCGTCGCG
ATGCTGTACACCCCGTTGCGGTCCAACGGCTCTGGCGATGATGTTTTCACAGTGTCGTGTCGAGTGTTAACCCGCCCT
ACCCCAGATTTTGAGTTTATATATCTAGTTCCCCCTTCTGTGGAAAGCAAGACTAAACCCTTTACTCTTCCCATTCTGA
CTATATCCGAGCTTACCAACTCCCGGTTCCCCATCTCAATCGAGCAACTGTACACTGCACCCAACGAGAACAACGTAG
TCCAGTGCCAGAACGGGAGATGTACCCTGGACGGGGAGCTCCAAGGGACCACGCAACTGTTAAGTTCAGCCGTTTG
CAGTTACAGAGGCAGGACTGTGGCGAACTCTGGTGATAACTGGGATCAAAATGTGTTGCAGCTGACTTACCCATCC
GGCGCAAGCTACGATCCAACAGATGAGGTGCCAGCGCCCCTTGGCACACAGGATTTCTCAGGAATTCTATACGGGG
TGCTTACTCAGGATAATGTGCGAGAAAATACTGGCGAGGCCAAGAATGCTAAAGGAGTGTATATAAGCACGACAAG
CGGTAAGTTTACCCCCAAAATTGGCAGTATTGGGCTCCACAGCATTACTGAGGACGTCCGCCCAAACCAGCAGTCTC
GTTTCACTCCCGTGGGGGTGGCACAGAACGAGAACACACCTTTCCAGCAGTGGGTCTTGCCCCATTATGCAGGTGCT
TTGGCGCTCAATACAAATCTGGCACCCGCCGTAGCGCCGACATTTCCTGGGGAGCAATTGCTGTTCTTTAGAAGCCG
CGTCCCGTGTGTTCAGGGCTTGCAGGGCCAGGACGCGTTCATTGATTGCCTCTTGCCCCAGGAATGGGTCAACCACT
TTTATCAGGAGGCAGCGCCCTCTCAAGCAGATGTGGCCCTGATAAGATATGTGAATCCCGACACAGGACGGACTTT
GTTTGAGGCAAAACTCCACCGGTCAGGATTCATTACTGTGAGTCACACAGGAGCCTATCCCCTTGTGGTTCCACCTAA
TGGCCACTTCAGGTTCGACTCTTGGGTCAATCAGTTTTATTCGCTGGCACCAATGGGTACCGGGAATGGTCGCCGTC
GGGTGCAATGA

Figure 20A

Amino acid sequence of VP1 GII.17_Kawa_2014_A0A077KVU6 (SEQ ID NO: 7)

MKMASNDAAPSNDGAAGLVPEGNNETLPLEPVAGAAIAAPVTGQNNIIDPWIRTNFVQAPNGEFTVSPRNS
PGEILLNLELGPDLNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKILFAAVPPNFPVEFLSPAQITMLPHLI
VDVRTLEPIMIPLPDVRNTFFHYNNQPNSRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPTPDFEFTYLVPP
SVESKTKPFSLPILTLSELTNSRFPVPIDSLFTAQNNVLQVQCQNGRCTLDGELQGTTQLLPSGICAFRGRVTAE
TDHRDKWHMQLQNLNGTTYDPTDDVPAPLGTPDFKGVVFGVASQRNVGNDAPGSTRAHEAVISTYSPQFV
PKLGSVNFRSNDNDFQLQPTKFTPVGINDDGDHPFRQWELPDYSGLLTLNMNLAPPVAPNFPGEQLLFFRSF
VPCSGGYNQGIVDCLIPQEWIQHFYQESAPSQSDVALIRYVNPDTGRTLFEAKLHRSGYITVAHSGDYPLVVPA
NGYFRFDSWVNQFYSLAPMGTGNGRRRAQ

Figure 20B

Nucleic acid sequence of human codon-optimized VP1 GII.17_Kawa_2014_A0A077KVU6 (SEQ ID NO: 62)

ATGAAAATGGCATCTAACGACGCAGCCCCCTCAAACGATGGCGCTGCTGGACTCGTGCCGGAGGGGAATAATGAG
ACACTTCCACTAGAGCCGGTTGCAGGCGCCGCTATAGCTGCCCCAGTGACAGGGCAGAATAATATTATAGACCCTTG
GATTCGGACAAACTTCGTGCAGGCACCCAACGGCGAGTTTACAGTATCCCCCCGGAACTCCCCAGGTGAGATACTCC
TGAATCTTGAGCTCGGCCCTGACCTCAATCCATATCTGGCTCATCTGAGCCGCATGTACAATGGTTACGCTGGGGGG
GTCGAAGTGCAGGTCCTCCTGGCCGGAAACGCCTTTACCGCTGGCAAAATTCTGTTTGCCGCCGTTCCACCAAACTTT
CCAGTCGAATTCCTCTCTCCCGCGCAAATAACCATGCTGCCACATTTGATCGTTGACGTGCGGACCCTGGAGCCAATA
ATGATTCCCCTGCCGGATGTGCGTAACACCTTTTTCCATTATAACAATCAGCCAAACTCTCGGATGAGACTTGTTGCT
ATGCTGTACACCCCCCTGCGGAGCAACGGCAGTGGCGATGATGTGTTTACCGTGAGTTGCAGAGTCCTGACGCGCC
CAACCCCGGACTTCGAGTTCACCTACCTGGTGCCCCCTTCTGTGGAATCTAAGACCAAACCGTTTTCACTGCCAATCT
TAACTCTCTCCGAACTGACTAACAGCCGGTTTCCAGTACCCATAGATTCTCTTTTTACCGCTCAAAACAACGTACTCCA
AGTCCAGTGCCAGAACGGCCGCTGTACGCTTGATGGTGAGTTGCAGGGGACAACACAGCTACTCCCCAGTGGCATC
TGTGCATTCCGGGGCCGCGTGACCGCTGAGACAGACCATCGTGACAAATGGCACATGCAACTCCAAAACTTAAACG
GGACCACCTACGACCCAACCGACGACGTCCCTGCTCCGCTAGGGACTCCTGACTTTAAGGGGGTGGTGTTCGGAGT
GGCCTCTCAGCGGAATGTTGGGAATGACGCCCCCGGCTCTACCCGAGCTCACGAGGCCGTTATCTCAACATATAGCC
CCCAATTTGTGCCCAAGCTCGGATCCGTTAATTTTCGTAGTAACGACAACGACTTCCAACTGCAACCAACGAAGTTTA
CGCCAGTGGGGATTAATGATGATGGAGACCATCCTTTCCGCCAATGGGAACTACCAGATTATTCTGGGCTGCTCACC
CTCAATATGAACCTCGCCCCACCCGTGGCCCCTAATTTCCCCGGTGAGCAGCTGCTGTTTTTTCGGAGCTTTGTGCCA
TGCAGTGGCGGATATAATCAAGGCATCGTAGACTGCTTGATTCCCCAAGAGTGGATACAACATTTTTACCAGGAAAG
TGCGCCCTCCCAGTCCGATGTGGCCCTGATACGGTACGTTAACCCCGATACCGGAAGAACATTATTCGAAGCGAAAT
TGCACAGATCAGGGTACATTACCGTTGCACATTCCGGCGATTATCCCCTGGTGGTTCCCGCCAACGGTTACTTTAGGT
TCGATAGTTGGGTCAACCAGTTCTATTCACTAGCCCCAATGGGCACCGGTAACGGCAGACGCCGGGCTCAGTAG

Figure 21A

Amino acid sequence of VP1 US96: GII.4/Dresden174/1997/DE_AY741811 (SEQ ID NO: 8)

MKMASNDANPSDGSTANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIRNNFVQAPGGEFTVSPRNAPGEIL
WSAPLGPDLNPYLSHLARMYNGYAGGFEVQVILAGNAFTAGKVIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLIP
LPDVRNNFYHYNQSNDSTIKLIAMLYTPLKANNAGDDVFTVSCRVLTRPSPDFDFIFLVPPTVESRTKPFTVPILTVEEMSN
SRFPIPLEKLYTGPSSAFVVQPQNGRCTTDGVLLGTTQLSAVNICTFRGDVTHIAGSHDYTMNLASQNWNNYDPTEEIPA
PLGTPDFVGKIQGMLTQTTREDGSTRAHKATVSTGSVHFTPKLGSVQYTTDTNNDFQTGQNTKFTPVGVIQDGNNHQN
EPQQWVLPNYSGRTGHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMNLDCLLPQEWVQHFYQEAAPAQSDVALL
RFVNPDTGRVLFECKLHKSGYVTVAHTGPHDLVIPPNGYFRFDSWVNQFYTLAPMGNGAGRRRAL

Figure 21B

Amino acid sequence of VP1 FH02: GII.4/FarmingtonHills/2002/US_AY502023 (SEQ ID NO: 9)

MKMASNDANPSDGSTANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIRNNFVQAPGGEFTVSPRNAPGEIL
WSAPLGPDLNPYLSHLARMYNGYAGGFEVQVILAGNAFTAGKIIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLIP
LPDVRNNFYHYNQLNDPTIKLIAMLYTPLRANNAGEDVFTVSCRVLTRPSPDFDFIFLVPPTVESRTKPFTVPILTVEEMTN
SRFPIPLEKLFTGPSGAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHIAGTHNYTMNLASQNWNNYDPTEEIPA
PLGTPDFVGRIQGMLTQTTRGDGSTRGHKATVSTGDVHFTPKLGSIQFNTDTNNDFETGQNTKFTPVGVVQDGNGTH
QNEPQQWVLPSYSGRTGHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMNLDCLLPQEWVQHFYQEAAPAQSDV
ALLRFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAL

Figure 21C

Amino acid sequence of VP1 Hnt04:GII.4/Hunter-NSW504D/2004/AU_DQ078814 (SEQ ID NO: 10)

MKMASNDATPSDGSTANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIRNNFVQAPGGEFTVSPRNAPGEIL
WSAPLGPDLNPYLSHLARMYNGYAGGFEVQVILAGNAFTAGKIIFAAVPPNFPTEVLSPSQVTMFPHIIVDVRQLEPVLIP
LPDVRNNLYHYNQSNDPTIRLIAMLYTPLRANNAGDDVFTVSCRVLTRPSPDFDFIFLVPPTVESRTKPFSVPILTVEEMTN
SRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHIAGTQNYTMNLASQNWNNYDPTEEIPA
PLGTPDFVGRIQGVLTQTTRRDGSTRGHKATVSTGSVHFTPKLGSVQFSTDTSNDFETGQNTRFTPVGVVQDGSTTHQN
EPQQWVLPDYSGRDSHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMNLDCLLPQEWVQHFYQESAPAQSDVALL
RFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGAGRRRAL

Figure 21D

Amino acid sequence of VP1 2006b: GII.4/Shellharbour-NSW696T/2006/AU_EF684915 (SEQ ID NO: 11)

MKMASNDANPSDGSAANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIRNNFVQAPGGEFTVSPRNAPGEIL
WSAPLGPDLNPYLSHLARMYNGYAGGFEVQVILAGNAFTAGKIIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLIP
LPDVRNNFYHYNQSNDSTIKLIAMLYTPLRANNAGEDVFTVSCRVLTRPSPDFDFIFLVPPTVESRTKPFTVPILTVEEMTNS
RFPIPLEKLFTGPSGAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHIAGSRNYTMNLASLKWNKYDPTEEIPAPL
GTPDFVGKIQGVLTQTTKGDGSTRGHKATIYTGSAPFTPKLGSVQFSTDTENDFETHQNTKFTPVGVTQDGSTTHRNEPQ
QWVLPSYSGRNVHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQHFYQEAAPAQSDVALLRFV
NPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAL

Figure 21E

Amino acid sequence of VP1 NO09: GII.4/Orange-NSW001P/2008/AU_GQ845367 (SEQ ID NO: 12)

MKMASSDANPSDGSTANLVPEVNNEVMALEPVVGAAIAAPVAGQQNVIDPWIRNNFVQAPGGEFTVSPRNAPGEIL
WSAPLGPDMNPYLSHLARMYNGYAGGFEVQVILAGNAFTAGKIIFAAVPPNFPTEGLSPSQVTMFPHIIVDVRQLEPVLI
PLPDVRNNFYHYNQSNDPTIKLIAMLYTPLRANNAGDDVFTVSCRVLTRPSPDFDFIFLVPPTVESRTKPFSVPILTVEEMT
NSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHIAGSRNYTMNLASQNWNSYDPTEEIPA
PLGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFSPKLGRVQFATDTDNDFDANQNTKFTPVGVIQDGNTAHR
NEPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQSDVAL
LRFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAL

Figure 22A

Amino acid sequence of VP1 GII.12_HS206_2010_USA_AEI29586 (SEQ ID NO: 28)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPILTIGELTNS
RFPVPIDELYTSPNESLVVQPQNGRCALDGELQGTTQLLPTAICSFRGRINQKVSGENHVWNMQVTNIDGTPFDPTEDV
PAPLGTPDFSGKLFGVLSQRDHDNACRSHDAVIATNSAKFTPKLGAIQIGTWEQDDVHINQPTKFTPVGLFESEGFNQW
TLPNYSGALTLNMGLAPPVAPTFPGEQILFFRSHIPLKGGVADPVIDCLLPQEWIQHLYQESAPSQTDVALIRFTNPDTGRV
LFEAKLHRSGYITVANTGSRPIVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRVQ

Figure 22B

Amino acid sequence of GII.14_Saga_2008_JPN_ADE28701 native VP1 (SEQ ID NO: 46)

MKMASNDATPSDDGAAGLVPEINNEVMALEPVAGASIAAPVVGQQNIIDPWIRNNFVQAPAGEFTVSPRNSPGELLLD
LELGPELNPYLAHLARMYNGHAGGMEVQIVLAGNAFTAGKILFAAIPPSFPYENLSPAQLTMCPHVIVDVRQLEPVLLPM
PDIRNVFYHYNQNNSPKLRLVAMLYTPLRANNSGDDVFTVSCRVLTRPSPDFQFTFLVPPTVESKTKNFTLPVLRVSEMTN
SRFPVVLDQMYTSRNENIIVQPQNGRCTTDGELLGTTILQSVSICNFKGTMQAKLNEEPRYQLQLTNLDGSPIDPTDDMP
APLGTPDFQAMLYGVASQRSSIDNATRAHDAQIDTAGDTFAPKIGQVRFKSSSNDFDLHDPTKFTPIGVNVDDQHPFRQ
WSLPNYGGHLALNNHLAPAVTPLFPGEQILFFRSYIPSAGGHTDGAMDCLLPQEWVEHFYQEAAPSQSDIALVRFINPDT
GRVLFEAKLHKQGFLTIAASGDHPIVMPTNGYFRFEAWVNPFYTLAPVGTGSGRRRIQ

Figure 22C

Amino acid sequence of VP1 GII.21_Sali_2011_USA_AFC89665 (SEQ ID NO: 47)

MKMASNDAAPSNDGATGLVPEINTETLPLEPVAGAAIAAPVTGQNNIIDPWIRNNFVQAPNGEFTVSPRNSPGEILMNL
ELGPDLNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKILFAAVPPNFPVDMLSPAQITMLPHLIVDVRTLEPIMIPLP
DVRNVFYHFNNQPAPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPTPDFEFTYLVPPSVESKTKPFTLPILTIGELTNSR
FPAPIDQLYTSPNADVVVQPQNGRCTLDGELQGTTQLLTTAICSYRGMTSNPTSDYWDDHLLHLVHPNGATYDPTEDVP
APFGTQDFRGILYGMLTQNPRTSGDEAANSHGIYISSTSEKFTPKLGTIGLHQVQGDIASNQQSKFTPVGIAVNGNTPFRQ
WELPNYSGALTLNTNLAPAVGPNFPGEQILFFRSNVPSVQGGQPIEIDCLIPQEWVSHFYQESAPSQSDVALVRYVNPDT
GRTIFEAKLHRQGFITIAATGSNPVVVPPNGYFRFDSWVNQFYALAPMGTGNGRRRVQ

Figure 23A

Amino acid sequence of native VP2 G1.1 (SEQ ID NO: 14)

MAQAIIGAIAASTAGSALGAGIQVGGEAALQSQRYQQNLQLQENSFKHDREMIGYQVEASNQLLAKNLATRYSLLRAGG
LTSADAARSVAGAPVTRIVDWNGVRVSAPESSATTLRSGGFMSVPIPFASKQKQVQSSGISNPNYSPSSISRTTSWVESQ
NSSRFGNLSPYHAEALNTVWLTPPGSTASSTLSSVPRGYFNTDRLPLFANNRR

Figure 23B

Nucleic acid sequence of wild-type VP2 G1.1 (SEQ ID NO: 15)

ATGGCCCAAGCCATAATTGGTGCAATTGCTGCTTCCACAGCAGGTAGTGCTCTGGGAGCGGGCATACAGGTTGGTG
GCGAAGCGGCCCTCCAAAGCCAAAGGTATCAACAAAATTTGCAACTGCAAGAAAATTCTTTTAAACATGACAGGGA
AATGATTGGGTATCAGGTTGAAGCTTCAAATCAATTATTGGCTAAAAATTTGGCAACTAGATATTCACTCCTCCGTGC
TGGGGGTTTGACCAGTGCTGATGCAGCAAGATCTGTGGCAGGAGCTCCAGTCACCCGCATTGTAGATTGGAATGGC
GTGAGAGTGTCTGCTCCCGAGTCCTCTGCTACCACATTGAGATCCGGTGGCTTCATGTCAGTTCCCATACCATTTGCC
TCTAAGCAAAAACAGGTTCAATCATCTGGTATTAGTAATCCAAATTATTCCCCTTCATCCATTTCTCGAACCACTAGTT
GGGTCGAGTCACAAAACTCATCGAGATTTGGAAATCTTTCTCCATACCACGCGGAGGCTCTCAATACAGTGTGGTTG
ACTCCACCCGGTTCAACAGCCTCTTCTACACTGTCTTCTGTGCCACGTGGTTATTTCAATACAGACAGGTTGCCATTAT
TCGCAAATAATAGGCGATGA

Figure 23C

Nucleic acid sequence of human codon-optimized VP2 G1.1 (SEQ ID NO: 19)

ATGGCTCAGGCCATTATTGGCGCCATCGCTGCAAGTACAGCCGGGAGTGCATTGGGGGCCGGAATACAGGTGGGC
GGGGAAGCTGCATTGCAGAGCCAGCGGTACCAGCAAAACCTGCAGTTACAGGAGAATAGCTTTAAACACGACAGG
GAGATGATTGGATATCAGGTGGAGGCCAGCAATCAGCTGCTCGCCAAAAACTTGGCTACTCGATACTCATTACTGCG
CGCCGGGGGGGTTGACTAGCGCCGACGCCGCACGATCTGTCGCAGGCGCCCCCGTGACTCGGATCGTAGACTGGAA
CGGGGTACGAGTCTCGGCTCCCGAGTCGTCTGCAACCACCCTGAGGTCGGGAGGGTTTATGTCCGTGCCCATCCCAT
TCGCTAGCAAACAGAAACAGGTCCAGAGCTCCGGAATCTCCAATCCCAATTACTCCCCTAGCTCTATCTCTCGTACCA
CTTCCTGGGTCGAGAGTCAGAACAGCAGTAGATTTGGCAACCTGAGCCCCTACCATGCTGAAGCCCTGAACACTGTG
TGGTTGACTCCACCTGGTAGCACGGCCTCCTCAACCCTGAGTTCCGTGCCTCGCGGGTACTTCAATACCGACAGACTT
CCTCTGTTCGCTAACAACCGCCGCTGA

FIGURE 23D

Nucleic acid sequence of human codon-optimized VP2 GII.4/ Sydney/NSW0514/2012/AU (SEQ ID NO:120)

ATGGCTGGGGCCTTTTTTGCAGGTTTGGCTAGTGACGTCCTCGGGTCGGGCCTCGGAAGTCTGATCAATGCAGGAG
CTGGAGCGATTAATCAAAAAGTCGAGTTCGAGAATAACCGGAAACTTCAACAGGCAAGTTTCCAATTCTCAAGCAAT
TTGCAGCAGGCCTCATTCCAACACGACAAGGAAATGCTCCAGGCCCAGATTGAGGCCACCAAGAAACTGCAACAAG
AGATGATGAAGGTCAAACAGGCCATGCTGCTGGAGGGCGGCTTTTTCGAGACCGATGCTGCGCGCGGAGCCATCA
ACGCCCCAATGACAAAGGCACTCGACTGGAGTGGCACACGGTATTGGGCACCCGATGCCAGAACGACTACCTATAA
TGCCGGAAGGTTCTCCACACCTCAACCTTCTGGTGCCCTCCCAGGGCGCGCAAATCTGAGGGACGCTGTGCCCGCTA
GGGGCTCCTCATCAAAGTCTTCCAATAGCTCCACTGCAACCTCGGTTTACTCAAACCAGACCACTTCAACAAGATTAG
GATCAACCGCCGTGTCGGGAACATCTGTCTCCTCTTTTCCTTCTACAGCACGAACCAGGTCTTGGGTCGAGGACCAG
AGCAGAAACCTGTCTCCGTCCATGCGCGGTGCTCACAACATTTCCTTCGTGACCCCTCCTTCTTCCCGATCCTCCAGCC
AAGGGACAGTGTCCACAGTGCCCAAAGAAGTGCTCGACTCTTGGACAGGTGCGTTCAATACCAGACGCCAGCCTCT
CTTTGCTCATATACGCAAAAGGGGTGAGTCACGAGCA

Figure 23E

Amino acid sequence of VP2 GII.4/Sydney/NSW0514/2012/AU (SEQ ID NO:121)

MAGAFFAGLASDVLGSGLGSLINAGAGAINQKVEFENNRKLQQASFQFSSNLQQASFQHDKEMLQAQIEATKKLQQEM
MKVKQAMLLEGGFFETDAARGAINAPMTKALDWSGTRYWAPDARTTTYNAGRFSTPQPSGALPGRANLRDAVPARGS
SSKSSNSSTATSVYSNQTTSTRLGSTAVSGTSVSSFPSTARTRSWVEDQSRNLSPSMRGAHNISFVTPPSSRSSSQGTVSTV
PKEVLDSWTGAFNTRRQPLFAHIRKRGESRA

Figure 24A

Nucleic acid sequence of wild-type VP1/VP2 G1.1 (SEQ ID NO: 16)

ATGATGATGGCGTCTAAGGACGCTACATCAAGCGTGGATGGCGCTAGTGGCGCTGGTCAGTTGGTACCGGAGGTTA
ATGCTTCTGACCCTCTTGCAATGGATCCTGTAGCAGGTTCTTCGACAGCAGTCGCGACTGCTGGACAAGTTAATCCTA
TTGATCCCTGGATAATTAATAATTTTGTGCAAGCCCCCCAAGGTGAATTTACTATTTCCCCAAATAATACCCCCGGTG
ATGTTTTGTTTGATTTGAGTTTGGGTCCCCATCTTAATCCTTTCTTGCTCCATCTATCACAAATGTATAATGGTTGGGTT
GGTAACATGAGAGTCAGGATTATGCTAGCTGGTAATGCCTTTACTGCGGGGAAGATAATAGTTTCCTGCATACCCCC
TGGTTTTGGTTCACATAATCTTACTATAGCACAAGCAACTCTCTTTCCACATGTGATTGCTGATGTTAGGACTCTAGAC
CCCATTGAGGTGCCTTTGGAAGATGTTAGGAATGTTCTCTTTCATAATAATGATAGAAATCAACAAACCATGCGCCTT
GTGTGCATGCTGTACACCCCCCTCCGCACTGGTGGTGGTACTGGTGATTCTTTTGTAGTTGCAGGGCGAGTTATGAC
TTGCCCCAGTCCTGATTTTAATTTCTTGTTTTTAGTCCCTCCTACGGTGGAGCAGAAAACCAGGCCCTTCACACTCCCA
AATCTGCCATTGAGTTCTCTGTCTAACTCACGTGCCCCTCTCCCAATCAGTAGTATGGGCATTTCCCCAGACAATGTCC
AGAGTGTGCAGTTCCAAAATGGTCGGTGTACTCTGGATGGCCGCCTGGTTGGCACCACCCCAGTTTCATTGTCACAT
GTTGCCAAGATAAGAGGGACCTCCAATGGCACTGTAATCAACCTTACTGAATTGGATGGCACACCCTTTCACCCTTTT
GAGGGCCCTGCCCCCATTGGGTTTCCAGACCTCGGTGGTTGTGATTGGCATATCAATATGACACAGTTTGGCCATTC
TAGCCAGACCCAGTATGATGTAGACACCACCCCTGACACTTTTGTCCCCCATCTTGGTTCAATTCAGGCAAATGGCAT
TGGCAGTGGTAATTATGTTGGTGTTCTTAGCTGGATTTCCCCCCCATCACACCCGTCTGGCTCCCAAGTTGACCTTTG
GAAGATCCCCAATTATGGGTCAAGTATTACGGAGGCAACACATCTAGCCCCTTCTGTATACCCCCCTGGTTTCGGAG
AGGTATTGGTCTTTTTCATGTCAAAAATGCCAGGTCCTGGTGCTTATAATTTGCCCTGTCTATTACCACAAGAGTACA
TTTCACATCTTGCTAGTGAACAAGCCCCTACTGTAGGTGAGGCTGCCCTGCTCCACTATGTTGACCCTGATACCGGTC
GGAATCTTGGGGAATTCAAAGCATACCCTGATGGTTTCCTCACTTGTGTCCCCAATGGGGCTAGCTCGGGTCCACAA
CAGCTGCCGATCAATGGGGTCTTTGTCTTTGTTTCATGGGTGTCCAGATTTTATCAATTAAAGCCTGTGGGAACTGCC
AGCTCGGCAAGAGGTAGGCTTGGTCTGCGCCGATAATGGCCCAAGCCATAATTGGTGCAATTGCTGCTTCCACAGC
AGGTAGTGCTCTGGGAGCGGGCATACAGGTTGGTGGCGAAGCGGCCCTCCAAAGCCAAAGGTATCAACAAAATTT
GCAACTGCAAGAAAATTCTTTTAAACATGACAGGGAAATGATTGGGTATCAGGTTGAAGCTTCAAATCAATTATTGG
CTAAAAATTTGGCAACTAGATATTCACTCCTCCGTGCTGGGGGTTTGACCAGTGCTGATGCAGCAAGATCTGTGGCA
GGAGCTCCAGTCACCCGCATTGTAGATTGGAATGGCGTGAGAGTGTCTGCTCCCGAGTCCTCTGCTACCACATTGAG
ATCCGGTGGCTTCATGTCAGTTCCCATACCATTTGCCTCTAAGCAAAAACAGGTTCAATCATCTGGTATTAGTAATCC
AAATTATTCCCCTTCATCCATTTCTCGAACCACTAGTTGGGTCGAGTCACAAAACTCATCGAGATTTGGAAATCTTTCT
CCATACCACGCGGAGGCTCTCAATACAGTGTGGTTGACTCCACCCGGTTCAACAGCCTCTTCTACACTGTCTTCTGTG
CCACGTGGTTATTTCAATACAGACAGGTTGCCATTATTCGCAAATAATAGGCGATGA

Figure 24B

Nucleic acid sequence of wild-type VP1/VP2/3'UTR G1.1 (SEQ ID NO: 17)

ATGATGATGGCGTCTAAGGACGCTACATCAAGCGTGGATGGCGCTAGTGGCGCTGGTCAGTTGGTACCGGAGGTTA
ATGCTTCTGACCCTCTTGCAATGGATCCTGTAGCAGGTTCTTCGACAGCAGTCGCGACTGCTGGACAAGTTAATCCTA
TTGATCCCTGGATAATTAATAATTTTGTGCAAGCCCCCCAAGGTGAATTTACTATTTCCCCAAATAATACCCCCGGTG
ATGTTTTGTTTGATTTGAGTTTGGGTCCCCATCTTAATCCTTTCTTGCTCCATCTATCACAAATGTATAATGGTTGGGTT
GGTAACATGAGAGTCAGGATTATGCTAGCTGGTAATGCCTTTACTGCGGGGAAGATAATAGTTTCCTGCATACCCCC
TGGTTTTGGTTCACATAATCTTACTATAGCACAAGCAACTCTCTTTCCACATGTGATTGCTGATGTTAGGACTCTAGAC
CCCATTGAGGTGCCTTTGGAAGATGTTAGGAATGTTCTCTTTCATAATAATGATAGAAATCAACAAACCATGCGCCTT
GTGTGCATGCTGTACACCCCCCTCCGCACTGGTGGTGGTACTGGTGATTCTTTTGTAGTTGCAGGGCGAGTTATGAC
TTGCCCCAGTCCTGATTTTAATTTCTTGTTTTTAGTCCCTCCTACGGTGGAGCAGAAAACCAGGCCCTTCACACTCCCA
AATCTGCCATTGAGTTCTCTGTCTAACTCACGTGCCCCTCTCCCAATCAGTAGTATGGGCATTTCCCCAGACAATGTCC
AGAGTGTGCAGTTCCAAAATGGTCGGTGTACTCTGGATGGCCGCCTGGTTGGCACCACCCCAGTTTCATTGTCACAT
GTTGCCAAGATAAGAGGGACCTCCAATGGCACTGTAATCAACCTTACTGAATTGGATGGCACACCCTTTCACCCTTTT
GAGGGCCCTGCCCCCATTGGGTTTCCAGACCTCGGTGGTTGTGATTGGCATATCAATATGACACAGTTTGGCCATTC
TAGCCAGACCCAGTATGATGTAGACACCACCCCTGACACTTTTGTCCCCCATCTTGGTTCAATTCAGGCAAATGGCAT
TGGCAGTGGTAATTATGTTGGTGTTCTTAGCTGGATTTCCCCCCCATCACACCCGTCTGGCTCCCAAGTTGACCTTTG
GAAGATCCCCAATTATGGGTCAAGTATTACGGAGGCAACACATCTAGCCCCTTCTGTATACCCCCCTGGTTTCGGAG
AGGTATTGGTCTTTTTCATGTCAAAAATGCCAGGTCCTGGTGCTTATAATTTGCCCTGTCTATTACCACAAGAGTACA
TTTCACATCTTGCTAGTGAACAAGCCCCTACTGTAGGTGAGGCTGCCCTGCTCCACTATGTTGACCCTGATACCGGTC
GGAATCTTGGGGAATTCAAAGCATACCCTGATGGTTTCCTCACTTGTGTCCCCAATGGGGCTAGCTCGGGTCCACAA
CAGCTGCCGATCAATGGGGTCTTTGTCTTTGTTTCATGGGTGTCCAGATTTTATCAATTAAAGCCTGTGGGAACTGCC
AGCTCGGCAAGAGGTAGGCTTGGTCTGCGCCGATAATGGCCCAAGCCATAATTGGTGCAATTGCTGCTTCCACAGC
AGGTAGTGCTCTGGGAGCGGGCATACAGGTTGGTGGCGAAGCGGCCCTCCAAAGCCAAAGGTATCAACAAAATTT
GCAACTGCAAGAAAATTCTTTTAAACATGACAGGGAAATGATTGGGTATCAGGTTGAAGCTTCAAATCAATTATTGG
CTAAAAATTTGGCAACTAGATATTCACTCCTCCGTGCTGGGGGTTTGACCAGTGCTGATGCAGCAAGATCTGTGGCA
GGAGCTCCAGTCACCCGCATTGTAGATTGGAATGGCGTGAGAGTGTCTGCTCCCGAGTCCTCTGCTACCACATTGAG
ATCCGGTGGCTTCATGTCAGTTCCCATACCATTTGCCTCTAAGCAAAAACAGGTTCAATCATCTGGTATTAGTAATCC
AAATTATTCCCCTTCATCCATTTCTCGAACCACTAGTTGGGTCGAGTCACAAAACTCATCGAGATTTGGAAATCTTTCT
CCATACCACGCGGAGGCTCTCAATACAGTGTGGTTGACTCCACCCGGTTCAACAGCCTCTTCTACACTGTCTTCTGTG
CCACGTGGTTATTTCAATACAGACAGGTTGCCATTATTCGCAAATAATAGGCGATGATGTTGTAATATGAAATGTGG
GCATCATATTCATTTAATTAGGTTTAATTAGGTTTAATTTGATGTT

Figure 24C

Nucleic acid sequence of human codon-optimized VP1/VP2 G1.1 (SEQ ID NO: 20)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGCAGAAGACGCGGCCCTTTA
CACTGCCCAATCTCCCGCTTTCAAGTCTGAGTAATTCACGGGCCCCATTGCCGATCTCCTCAATGGGAATCTCCCCCG
ACAACGTCCAGTCTGTCCAATTCCAAAATGGGAGATGCACACTGGACGGTCGCCTGGTGGGAACAACTCCGGTGTC
CCTCTCACATGTCGCCAAAATCCGCGGCACATCAAATGGTACCGTAATCAATCTGACAGAACTTGATGGCACGCCCTT
CCATCCCTTTGAAGGACCAGCCCCTATTGGATTTCCTGATCTGGGAGGTTGCGACTGGCACATAAACATGACACAGT
TTGGCCACTCCAGCCAGACACAGTATGATGTCGATACAACCCCAGATACCTTCGTGCCACACCTGGGATCTATTCAA
GCTAACGGTATTGGATCCGGCAACTACGTGGGAGTCTTATCTTGGATCTCACCACCATCCCACCCCTCAGGATCCCAG
GTTGACTTGTGGAAGATACCGAATTATGGATCCTCGATCACTGAAGCCACGCACCTCGCACCTTCCGTCTACCCACCA
GGTTTTGGAGAAGTCTTGGTGTTTTTCATGAGCAAAATGCCCGGCCCTGGAGCCTACAATCTCCCTTGCCTACTCCCT
CAAGAGTATATTAGTCACCTCGCATCTGAGCAGGCCCCGACCGTTGGCGAGGCAGCCCTGCTGCATTATGTGGATCC
GGACACCGGCAGGAACCTGGGTGAGTTCAAAGCTTATCCTGACGGTTTTCTAACATGTGTACCAAATGGCGCTTCCA
GCGGCCCTCAACAGCTCCCAATCAATGGCGTGTTCGTTTTTGTCAGCTGGGTAAGCCGCTTCTACCAGCTGAAGCCC
GTGGGGACAGCTTCTTCTGCCCGCGGACGCCTCGGTCTGCGGAGATAATGGCTCAGGCCATTATTGGCGCCATCGCT
GCAAGTACAGCCGGGAGTGCATTGGGGGCCGGAATACAGGTGGGCGGGGAAGCTGCATTGCAGAGCCAGCGGTA
CCAGCAAAACCTGCAGTTACAGGAGAATAGCTTTAAACACGACAGGGAGATGATTGGATATCAGGTGGAGGCCAG
CAATCAGCTGCTCGCCAAAAACTTGGCTACTCGATACTCATTACTGCGCGCCGGGGGGTTGACTAGCGCCGACGCCG
CACGATCTGTCGCAGGCGCCCCCGTGACTCGGATCGTAGACTGGAACGGGGTACGAGTCTCGGCTCCCGAGTCGTC
TGCAACCACCCTGAGGTCGGGAGGGTTTATGTCCGTGCCCATCCCATTCGCTAGCAAACAGAAACAGGTCCAGAGCT
CCGGAATCTCCAATCCCAATTACTCCCCTAGCTCTATCTCTCGTACCACTTCCTGGGTCGAGAGTCAGAACAGCAGTA
GATTTGGCAACCTGAGCCCCTACCATGCTGAAGCCCTGAACACTGTGTGGTTGACTCCACCTGGTAGCACGGCCTCC
TCAACCCTGAGTTCCGTGCCTCGCGGGTACTTCAATACCGACAGACTTCCTCTGTTCGCTAACAACCGCCGCTGA

Figure 24D

Nucleic acid sequence of human codon-optimized VP1/VP2/3'UTR G1.1 (SEQ ID NO: 21)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGCAGAAGACGCGGCCCTTTA
CACTGCCCAATCTCCCGCTTTCAAGTCTGAGTAATTCACGGGCCCCATTGCCGATCTCCTCAATGGGAATCTCCCCCG
ACAACGTCCAGTCTGTCCAATTCCAAAATGGGAGATGCACACTGGACGGTCGCCTGGTGGGAACAACTCCGGTGTC
CCTCTCACATGTCGCCAAAATCCGCGGCACATCAAATGGTACCGTAATCAATCTGACAGAACTTGATGGCACGCCCTT
CCATCCCTTTGAAGGACCAGCCCCTATTGGATTTCCTGATCTGGGAGGTTGCGACTGGCACATAAACATGACACAGT
TTGGCCACTCCAGCCAGACACAGTATGATGTCGATACAACCCCAGATACCTTCGTGCCACACCTGGGATCTATTCAA
GCTAACGGTATTGGATCCGGCAACTACGTGGGAGTCTTATCTTGGATCTCACCACCATCCCACCCCTCAGGATCCCAG
GTTGACTTGTGGAAGATACCGAATTATGGATCCTCGATCACTGAAGCCACGCACCTCGCACCTTCCGTCTACCCACCA
GGTTTTGGAGAAGTCTTGGTGTTTTTCATGAGCAAAATGCCCGGCCCTGGAGCCTACAATCTCCCTTGCCTACTCCCT
CAAGAGTATATTAGTCACCTCGCATCTGAGCAGGCCCCGACCGTTGGCGAGGCAGCCCTGCTGCATTATGTGGATCC
GGACACCGGCAGGAACCTGGGTGAGTTCAAAGCTTATCCTGACGGTTTTCTAACATGTGTACCAAATGGCGCTTCCA
GCGGCCCTCAACAGCTCCCAATCAATGGCGTGTTCGTTTTTGTCAGCTGGGTAAGCCGCTTCTACCAGCTGAAGCCC
GTGGGGACAGCTTCTTCTGCCCGCGGACGCCTCGGTCTGCGGAGATAATGGCTCAGGCCATTATTGGCGCCATCGCT
GCAAGTACAGCCGGGAGTGCATTGGGGGCCGGAATACAGGTGGGCGGGGAAGCTGCATTGCAGAGCCAGCGGTA
CCAGCAAAACCTGCAGTTACAGGAGAATAGCTTTAAACACGACAGGGAGATGATTGGATATCAGGTGGAGGCCAG
CAATCAGCTGCTCGCCAAAAACTTGGCTACTCGATACTCATTACTGCGCGCCGGGGGGTTGACTAGCGCCGACGCCG
CACGATCTGTCGCAGGCGCCCCCGTGACTCGGATCGTAGACTGGAACGGGGTACGAGTCTCGGCTCCCGAGTCGTC
TGCAACCACCCTGAGGTCGGGAGGGTTTATGTCCGTGCCCATCCCATTCGCTAGCAAACAGAAACAGGTCCAGAGCT
CCGGAATCTCCAATCCCAATTACTCCCCTAGCTCTATCTCTCGTACCACTTCCTGGGTCGAGAGTCAGAACAGCAGTA
GATTTGGCAACCTGAGCCCCTACCATGCTGAAGCCCTGAACACTGTGTGGTTGACTCCACCTGGTAGCACGGCCTCC
TCAACCCTGAGTTCCGTGCCTCGCGGGTACTTCAATACCGACAGACTTCCTCTGTTCGCTAACAACCGCCGCTGATGT
TGTAATATGAAATGTGGGCATCATATTCATTTAATTAGGTTTAATTAGGTTTAATTTGATGTT

Figure 25A

Amino acid sequence of S(GI.1)+P(GI.2) fusion VP1 (SEQ ID NO: 22)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVEQKTRAFTVP
NIPLQTLSNSRFPSLIQGMILSPDASQVVQFQNGRCLIDGQLLGTTPATSGQLFRVRGKINQGARTLNLTEVDGKPFMAF
DSPAPVGFPDFGKCDWHMRISKTPNNTSSGDPMRSVDVQTDVQGFVPHLGSIQFDEVFNHPTGDYIGTIEWISQPSTPP
GTDINLWEIPDYGSSLSQAANLAPPVFPPGFGEALVYFVSAFPGPNNRSAPNDVPCLLPQEYVTHFVSEQAPTMGDAALL
HYVDPDTNRNLGEFKLYPGGYLTCVPNGVGAGPQQLPLNGVFLFVSWVSRFYQLKPVGTASTARGRLGVRRI*

Figure 25B

Nucleic acid sequence of human codon optimized S(GI.1)+P(GI.2) fusion VP1 (SEQ ID NO: 57)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGCAAAAAACAAGAGCATTCA
CAGTGCCCAACATTCCACTGCAGACTTTAAGCAATTCCAGGTTTCCCAGCTTGATCCAGGGTATGATCCTTTCTCCCG
ACGCCTCCCAAGTTGTGCAGTTCCAGAATGGGAGATGTCTTATCGACGGTCAGCTTCTGGGAACAACCCCTGCCACC
TCCGGGCAACTCTTCCGGGTGAGAGGCAAAATCAATCAGGGCGCCAGAACACTGAATCTGACAGAAGTGGACGGG
AAACCCTTTATGGCGTTCGATAGCCCCGCGCCCGTTGGATTCCCTGACTTCGGCAAGTGTGATTGGCACATGCGCAT
CAGTAAGACTCCCAACAACACTTCATCTGGAGACCCCATGAGGAGCGTGGATGTCCAGACCGACGTGCAGGGCTTC
GTGCCGCACTTGGGATCTATCCAGTTCGATGAGGTGTTCAATCACCCTACTGGCGACTACATAGGCACAATTGAGTG
GATAAGTCAACCATCTACACCTCCAGGGACCGACATAAACCTGTGGGAAATTCCTGATTACGGGTCATCCCTGAGTC
AAGCTGCCAATCTTGCACCCCCTGTCTTTCCCCCCGGCTTTGGTGAGGCTCTTGTTTACTTCGTCTCTGCATTTCCTGG
TCCTAACAACCGCTCCGCCCCTAACGATGTTCCGTGTTTGTTACCCCAGGAATATGTGACTCATTTCGTTTCCGAACA
GGCACCCACCATGGGGGACGCTGCCCTGCTACACTATGTGGACCCCGACACCAATAGAAACCTCGGCGAGTTCAAA
CTCTACCCCGGGGGATACCTGACCTGTGTTCCAAATGGAGTGGGAGCAGGCCCACAACAGCTGCCCCTGAATGGGG
TCTTCCTGTTCGTTTCTTGGGTGTCACGCTTTTACCAGCTGAAGCCCGTTGGCACAGCTTCTACGGCACGCGGCAGGC
TAGGGGTCCGCCGAATCTGA

Figure 26A

Amino acid sequence of S(GI.1)+P(GI.3) fusion VP1 (SEQ ID NO: 23)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVEQKTKPFSVPN
LPLNVLSNSRVPSLIKSMMVSQDHGQMVQFQNGRVTLDGQLQGTTPTSASQLCKIRGTVYHATGGQGLNLTEIDGTPY
HAFESPAPIGFPDLGECDWHINASPANAFTDGSIIHRIDVAQDSTFAPHLGTIHYTNADYNANVGLICSLEWLSPPSGGAP
KVNPWAIPRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSDFPIANGSDGLSVPCTIPQEFVTHFVNEQAPTRGEAALLHYVD
PDTHRNLGEFKLYPEGFMTCVPNSSGSGPQTLPINGVFTFISWVSRFYQLKPVGTTGPVRRLGIRRS*

Figure 26B

Nucleic acid sequence of human codon optimized S(GI.1)+P(GI.3) fusion VP1 (SEQ ID NO: 58)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGCAGAAAACAAAGCCATTCA
GCGTGCCAAACCTGCCCCTTAACGTGCTGTCGAATTCCCGAGTGCCTTCCCTTATTAAGTCCATGATGGTATCTCAGG
ATCACGGTCAAATGGTGCAGTTTCAGAACGGCCGAGTGACGTTAGACGGGCAGCTGCAGGGCACAACCCCAACCA
GTGCCAGTCAGCTGTGTAAGATCAGAGGCACCGTCTACCACGCAACTGGCGGACAGGGGCTGAATCTTACTGAGAT
CGATGGTACCCCCTACCATGCATTCGAGTCACCTGCACCTATTGGATTTCCCGATCTTGGGGAGTGTGATTGGCATAT
CAATGCTTCACCTGCCAACGCTTTCACAGACGGGTCTATTATTCATCGCATTGACGTAGCACAGGATAGCACATTTGC
CCCGCACCTGGGTACCATCCACTATACGAACGCAGATTACAACGCAAACGTGGGTCTTATCTGTAGCCTAGAGTGGC
TATCTCCGCCAAGCGGTGGGGCCCCTAAAGTTAACCCATGGGCTATTCCTCGGTACGGGTCTACGCTGACTGAGGCC
GCTCAGCTGGCACCCCCCATATATCCACCAGGATTCGGGGAAGCCATTGTTTTCTTTATGTCCGATTTTCCGATAGCC
AACGGTTCAGATGGCCTTAGTGTCCCTTGCACGATTCCACAGGAATTTGTGACACACTTCGTAAACGAGCAGGCTCC
TACTCGGGGCGAGGCTGCCTTGTTGCATTACGTAGACCCCGATACCCATAGAAACCTGGGCGAATTCAAACTCTACC
CTGAAGGTTTCATGACCTGCGTACCTAACTCCTCCGGCAGTGGCCCTCAAACCTTGCCGATCAACGGCGTGTTCACGT
TTATCAGCTGGGTTTCACGGTTTTACCAACTCAAGCCCGTCGGAACAACTGGGCCAGTTCGGAGGCTCGGGATCAGA
CGGAGCTAG

Figure 27A

Amino acid sequence of S(GI.1)+P(GII.4) fusion VP1 (SEQ ID NO: 24)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVESRTKPFSVPV
LTVEEMTNSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDY
DPTEEIPAPLGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQD
GGTTHRNEPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPA
QSDVALLRFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV*

Figure 27B

Nucleic acid sequence of human codon optimized S(GI.1)+P(GII.4) fusion VP1 (SEQ ID NO: 59)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGAGCCGAACCAAGCCCTTTAG
TGTCCCCGTACTCACAGTCGAGGAGATGACAAATAGCCGCTTTCCAATCCCCCTTGAGAAACTGTTCACAGGACCTTC
CTCGGCATTCGTGGTTCAGCCACAGAACGGACGCTGCACAACTGACGGCGTGCTGCTCGGAACCACCCAGCTTAGC
CCTGTTAATATCTGTACGTTTAGAGGCGACGTAACTCACATAACTGGCTCACGGAACTATACCATGAATCTGGCATCA
CAGAATTGGAATGACTACGACCCAACCGAAGAGATTCCCGCACCTCTTGGAACCCCCGACTTTGTGGGAAAAATACA
GGGCGTCCTGACACAAACCACCAGAACCGATGGCTCCACACGGGGACACAAGGCAACCGTCTACACTGGCTCTGCC
GATTTTGCCCCGAAACTGGGTAGAGTGCAGTTTGAGACCGACACTGACCGGGACTTTGAAGCCAATCAGAATACTA
AGTTCACACCTGTAGGAGTGATTCAGGACGGGGGCACCACTCACCGGAACGAGCCGCAACAATGGGTCCTGCCCTC
TTATAGCGGGAGGAATACTCATAATGTGCATTTGGCTCCTGCAGTGGCTCCCACGTTTCCCGGGGAACAACTGCTCT
TTTTTCGTTCAACCATGCCTGGATGCTCCGGATATCCCAATATGGATCTCGATTGCCTGCTCCCACAGGAATGGGTGC
AGTATTTTTATCAAGAGGCCGCACCAGCCCAATCCGACGTCGCACTTCTGCGGTTCGTGAATCCAGACACAGGCCGC
GTGTTGTTTGAGTGCAAATTGCACAAATCAGGATACGTTACAGTGGCTCATACTGGACAGCATGACCTGGTGATCCC
ACCCAACGGATATTTTAGGTTCGACTCCTGGGTGAATCAGTTTTATACATTAGCCCCCATGGGGAATGGGACTGGCA
GACGCAGGGCTGTCTGA

Figure 28A

Amino acid sequence of S(GI.1)+P(GII.6) fusion VP1 (SEQ ID NO: 25)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVESKTKPFSLPIL
TLGELSNSRFPAPIDMLYTDPNEGIVVQPQNGRCTLDGTLQGTTQLVPTQICAFRGTLIGQTSRSPDSTDSAPRRRDHPLH
VQLKNLDGTQYDPTDEVPAVLGAIDFKGTVFGVASQRDVSGQQVGATRAHEVHINTTDPRYTPKLGSILMYSESDDFVT
GQPVRFTPIGMGDNDWHQWELPDYPGHLTLNMNLAPAVAPAFPGERILFFRSIVPSAGGYGSGQIDCLIPQEWVQHFY
QEAAPSQSAVALIRYVNPDTGRNIFEAKLHREGFITVANSGNNPIVVPPNGYFRFEAWVNQFYTLTPMGTGQGRRRDQ*

Figure 28B

Nucleic acid sequence of human codon optimized S(GI.1)+P(GII.6) fusion VP1 (SEQ ID NO: 63)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGAGCAAGACAAAACCCTTCA
GCCTCCCAATCTTAACACTCGGGGAGCTGTCTAATTCACGCTTCCCCGCACCTATTGATATGCTGTATACTGACCCCA
ACGAGGGGATAGTGGTGCAGCCCCAAAATGGACGGTGTACTCTCGACGGCACGCTCCAGGGCACAACCCAACTGG
TGCCAACCCAGATTTGTGCATTCAGGGGCACTTTGATTGGGCAGACATCGAGATCTCCAGATTCTACTGATTCCGCG
CCAAGGAGGAGGGACCACCCACTCCACGTTCAGTTAAAAAACCTGGACGGAACCCAGTACGACCCTACAGACGAGG
TCCCCGCTGTCCTCGGAGCCATCGACTTTAAAGGAACTGTATTTGGAGTGGCATCCCAAAGGGATGTCTCGGGGCA
GCAGGTGGGAGCTACGAGAGCACATGAAGTCCACATTAACACCACAGACCCAAGATATACCCCAAAACTAGGGTCA
ATTTTAATGTATTCGGAATCAGACGATTTTGTTACAGGTCAGCCCGTGCGGTTTACCCCGATCGGAATGGGGGACAA
CGATTGGCACCAGTGGGAATTGCCCGATTACCCTGGACACCTCACCTTGAATATGAATCTGGCCCCAGCCGTCGCGC
CCGCCTTCCCCGGTGAGCGGATCCTCTTTTTTAGAAGCATAGTGCCCTCCGCAGGTGGGTATGGATCAGGGCAGATT
GATTGCCTGATCCCCCAAGAATGGGTACAGCATTTCTACCAGGAAGCAGCCCCTAGCCAGTCCGCAGTAGCACTGAT
CAGATATGTTAATCCTGATACGGGAAGGAACATCTTCGAAGCAAAACTGCACCGTGAGGGCTTCATTACCGTCGCCA
ACAGTGGTAATAACCCTATTGTGGTGCCTCCTAATGGATACTTCAGGTTTGAGGCATGGGTGAATCAGTTTTATACTC
TGACTCCCATGGGGACAGGCCAGGGGCGACGCCGGGATCAGTGA

Figure 29A

Amino acid sequence of S(GI.1)+P(GII.13) fusion VP1 (SEQ ID NO: 26)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVESKTKPFTLPIL
TISELTNSRFPISIEQLYTAPNENNVVQCQNGRCTLDGELQGTTQLLSSAVCSYRGRTVANSGDNWDQNVLQLTYPSGAS
YDPTDEVPAPLGTQDFSGILYGVLTQDNVRENTGEAKNAKGVYISTTSGKFTPKIGSIGLHSITEDVRPNQQSRFTPVGVA
QNENTPFQQWVLPHYAGALALNTNLAPAVAPTFPGEQLLFFRSRVPCVQGLQGQDAFIDCLLPQEWVNHFYQEAAPSQ
ADVALIRYVNPDTGRTLFEAKLHRSGFITVSHTGAYPLVVPPNGHFRFDSWVNQFYSLAPMGTGNGRRRVQ*

Figure 29B

Nucleic acid sequence of human codon optimized S(GI.1)+P(GII.13) fusion VP1 (SEQ ID NO: 64)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGAGCAAGACTAAACCCTTTAC
TCTTCCCATTCTGACTATATCCGAGCTTACCAACTCCCGGTTCCCCATCTCAATCGAGCAACTGTACACTGCACCCAAC
GAGAACAACGTAGTCCAGTGCCAGAACGGGAGATGTACCCTGGACGGGGAGCTCCAAGGGACCACGCAACTGTTA
AGTTCAGCCGTTTGCAGTTACAGAGGCAGGACTGTGGCGAACTCTGGTGATAACTGGGATCAAAATGTGTTGCAGC
TGACTTACCCATCCGGCGCAAGCTACGATCCAACAGATGAGGTGCCAGCGCCCCTTGGCACACAGGATTTCTCAGGA
ATTCTATACGGGGTGCTTACTCAGGATAATGTGCGAGAAAATACTGGCGAGGCCAAGAATGCTAAAGGAGTGTATA
TAAGCACGACAAGCGGTAAGTTTACCCCCAAAATTGGCAGTATTGGGCTCCACAGCATTACTGAGGACGTCCGCCCA
AACCAGCAGTCTCGTTTCACTCCCGTGGGGGTGGCACAGAACGAGAACACACCTTTCCAGCAGTGGGTCTTGCCCCA
TTATGCAGGTGCTTTGGCGCTCAATACAAATCTGGCACCCGCCGTAGCGCCGACATTTCCTGGGGAGCAATTGCTGT
TCTTTAGAAGCCGCGTCCCGTGTGTTCAGGGCTTGCAGGGCCAGGACGCGTTCATTGATTGCCTCTTGCCCCAGGAA
TGGGTCAACCACTTTTATCAGGAGGCAGCGCCCTCTCAAGCAGATGTGGCCCTGATAAGATATGTGAATCCCGACAC
AGGACGGACTTTGTTTGAGGCAAAACTCCACCGGTCAGGATTCATTACTGTGAGTCACACAGGAGCCTATCCCCTTG
TGGTTCCACCTAATGGCCACTTCAGGTTCGACTCTTGGGTCAATCAGTTTTATTCGCTGGCACCAATGGGTACCGGGA
ATGGTCGCCGTCGGGTGCAATGA

Figure 30A

Amino acid sequence of S(GI.1)+P(GII.17) fusion VP1 (SEQ ID NO: 27)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVEQKTRPFTLPN
LPLSSLSNSRAPLPISSMGISPDNVQSVQFQNGRCTLDGRLVGTTQLLPSGICAFRGRVTAETDHRDKWHMQLQNLNGT
TYDPTDDVPAPLGTPDFKGVVFGVASQRNVGNDAPGSTRAHEAVISTYSPQFVPKLGSVNFRSNDNDFQLQPTKFTPVG
INDDGDHPFRQWELPDYSGLLTLNMNLAPSVYPPGFGEVLVFFMSKMPGPGAYNLPCLLPQEYISHLASEQAPTVGEAA
LLHYVDPDTGRNLGEFKAYPDGFLTCVPNGASSGPQQLPINGVFVFVS

Figure 30B

Nucleic acid sequence of human codon optimized S(GI.1)+P(GII.17) fusion VP1 (SEQ ID NO: 65)

ATGATGATGGCTAGTAAAGATGCGACCTCCTCTGTGGATGGTGCGTCAGGGGCAGGACAACTCGTACCCGAGGTAA
ACGCCAGCGACCCACTTGCCATGGACCCCGTTGCCGGAAGTTCCACAGCAGTGGCCACAGCCGGTCAAGTGAATCC
AATTGATCCGTGGATTATCAACAATTTCGTCCAGGCACCCCAGGGCGAGTTCACAATTTCACCAAACAATACACCGG
GCGATGTGCTATTCGATCTTTCCTTGGGTCCTCACCTTAACCCTTTTCTACTCCATCTCTCACAGATGTACAATGGTTG
GGTAGGAAACATGAGAGTCCGGATCATGCTGGCTGGCAATGCCTTTACCGCTGGCAAGATCATCGTCAGTTGTATTC
CTCCCGGATTTGGATCTCATAATCTGACCATTGCTCAAGCGACTCTCTTTCCCCATGTCATCGCCGACGTTAGGACCCT
GGACCCCATCGAGGTGCCCCTGGAGGACGTCCGGAATGTTTTGTTCCACAACAACGACAGAAACCAGCAGACGATG
AGACTTGTCTGTATGCTCTATACCCCACTGCGGACTGGAGGCGGGACTGGAGACTCCTTCGTTGTGGCAGGAAGAG
TGATGACATGCCCCTCCCCCGACTTCAACTTTCTTTTTCTGGTCCCACCAACCGTTGAGTCTAAGACCAAACCGTTTTC
ACTGCCAATCTTAACTCTCTCCGAACTGACTAACAGCCGGTTTCCAGTACCCATAGATTCTCTTTTTACCGCTCAAAAC
AACGTACTCCAAGTCCAGTGCCAGAACGGCCGCTGTACGCTTGATGGTGAGTTGCAGGGGACAACACAGCTACTCC
CCAGTGGCATCTGTGCATTCCGGGGCCGCGTGACCGCTGAGACAGACCATCGTGACAAATGGCACATGCAACTCCA
AAACTTAAACGGGACCACCTACGACCCAACCGACGACGTCCCTGCTCCGCTAGGGACTCCTGACTTTAAGGGGGTG
GTGTTCGGAGTGGCCTCTCAGCGGAATGTTGGGAATGACGCCCCCGGCTCTACCCGAGCTCACGAGGCCGTTATCTC
AACATATAGCCCCCAATTTGTGCCCAAGCTCGGATCCGTTAATTTTCGTAGTAACGACAACGACTTCCAACTGCAACC
AACGAAGTTTACGCCAGTGGGGATTAATGATGATGGAGACCATCCTTTCCGCCAATGGGAACTACCAGATTATTCTG
GGCTGCTCACCCTCAATATGAACCTCGCCCCACCCGTGGCCCCTAATTTCCCCGGTGAGCAGCTGCTGTTTTTTCGGA
GCTTTGTGCCATGCAGTGGCGGATATAATCAAGGCATCGTAGACTGCTTGATTCCCCAAGAGTGGATACAACATTTT
TACCAGGAAAGTGCGCCCTCCCAGTCCGATGTGGCCCTGATACGGTACGTTAACCCCGATACCGGAAGAACATTATT
CGAAGCGAAATTGCACAGATCAGGGTACATTACCGTTGCACATTCCGGCGATTATCCCCTGGTGGTTCCCGCCAACG
GTTACTTTAGGTTCGATAGTTGGGTCAACCAGTTCTATTCACTAGCCCCAATGGGCACCGGTAACGGCAGACGCCGG
GCTCAGTAG

Figure 31A

Amino acid sequence of S(GI.1)+P(GII.12) fusion VP1 (SEQ ID NO: 71)

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPIDPWIINNFVQAPQGEFTISPNNTP
GDVLFDLSLGPHLNPFLLHLSQMYNGWVGNMRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIADVRTLD
PIEVPLEDVRNVLFHNNDRNQQTMRLVCMLYTPLRTGGGTGDSFVVAGRVMTCPSPDFNFLFLVPPTVESKTKPFTLPIL
TIGELTNSRFPVPIDELYTSPNESLVVQPQNGRCALDGELQGTTQLLPTAICSFRGRINQKVSGENHVWNMQVTNIDGTP
FDPTEDVPAPLGTPDFSGKLFGVLSQRDHDNACRSHDAVIATNSAKFTPKLGAIQIGTWEQDDVHINQPTKFTPVGLFES
EGFNQWTLPNYSGALTLNMGLAPPVAPTFPGEQILFFRSHIPLKGGVADPVIDCLLPQEWIQHLYQESAPSQTDVALIRFT
NPDTGRVLFEAKLHRSGYITVANTGSRPIVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRVQ*

Figure 31B

Amino acid sequence of S(GI.5)+P(GII.4) fusion VP1 (SEQ ID NO: 48)

MMMASKDAPSSADGANGAGQLVPEVNNAEPLPLDPVAGASTALATAGQVNMIDPWIFNNFVQAPQGEFTISPNNTP
GDILFDLQLGPHLNPFLAHLSQMYNGWVGNMRVRVILAGNAFTAGKVIICCVPPGFQSRTLSIAQATLFPHIIADVRTLEPI
EIPLEDVRNTLYHTNDNQPTMRLLCMLYTPLRTGGGSGGTDAFVVAGRVLTCPSSDFNFLFLVPPTVESRTKPFSVPVLTV
EEMTNSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDYDP
TEEIPAPLGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQDGG
TTHRNEPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQS
DVALLRFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV*

Figure 32A

Amino acid sequence of S(GII.1)+P(GI.3) fusion VP1 (SEQ ID NO: 49)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRMNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPFLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAIPPHFPLENLSPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQPEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVEQKTKPFSVPNLPLNVLS
NSRVPSLIKSMMVSQDHGQMVQFQNGRVTLDGQLQGTTPTSASQLCKIRGTVYHATGGQGLNLTEIDGTPYHAFESPA
PIGFPDLGECDWHINASPANAFTDGSIIHRIDVAQDSTFAPHLGTIHYTNADYNANVGLICSLEWLSPPSGGAPKVNPWAI
PRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSDFPIANGSDGLSVPCTIPQEFVTHFVNEQAPTRGEAALLHYVDPDTHRNL
GEFKLYPEGFMTCVPNSSGSGPQTLPINGVFTFISWVSRFYQLKPVGTTGPVRRLGIRRS*

Figure 32B

Amino acid sequence of S(GII.1)+P(GII.4) fusion VP1 (SEQ ID NO: 50)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRMNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPFLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAIPPHFPLENLSPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQPEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESRTKPFSVPVLTVEEMT
NSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDYDPTEEIP
APLGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQDGGTTHR
NEPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQSDVAL
LRFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV*

Figure 32C

Amino acid sequence of S(GII.1)+P(GII.17) fusion VP1 (SEQ ID NO: 51)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRMNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPFLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAIPPHFPLENLSPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQPEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFSLPILTLSELTNS
RFPVPIDSLFTAQNNVLQVQCQNGRCTLDGELQGTTQLLPSGICAFRGRVTAETDHRDKWHMQLQNLNGTTYDPTDDV
PAPLGTPDFKGVVFGVASQRNVGNDAPGSTRAHEAVISTYSPQFVPKLGSVNFRSNDNDFQLQPTKFTPVGINDDGDHP
FRQWELPDYSGLLTLNMNLAPPVAPNFPGEQLLFFRSFVPCSGGYNQGIVDCLIPQEWIQHFYQESAPSQSDVALIRYVN
PDTGRTLFEAKLHRSGYITVAHSGDYPLVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRAQ*

Figure 33A

Amino acid sequence of S(GII.12)+P(GI.1) fusion VP1 (SEQ ID NO: 29)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVEQKTRPFTLPNLPLSSLSN
SRAPLPISSMGISPDNVQSVQFQNGRCTLDGRLVGTTPVSLSHVAKIRGTSNGTVINLTELDGTPFHPFEGPAPIGFPDLG
GCDWHINMTQFGHSSQTQYDVDTTPDTFVPHLGSIQANGIGSGNYVGVLSWISPPSHPSGSQVDLWKIPNYGSSITEAT
HLAPSVYPPGFGEVLVFFMSKMPGPGAYNLPCLLPQEYISHLASEQAPTVGEAALLHYVDPDTGRNLGEFKAYPDGFLTC
VPNGASSGPQQLPINGVFVFVSWVSRFYQLKPVGTASSARGRLGLRR*

Figure 33B

Amino acid sequence of S(GII.12)+P(GI.2)fusion VP1 (SEQ ID NO: 30)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVEQKTRAFTVPNIPLQTLS
NSRFPSLIQGMILSPDASQVVQFQNGRCLIDGQLLGTTPATSGQLFRVRGKINQGARTLNLTEVDGKPFMAFDSPAPVGF
PDFGKCDWHMRISKTPNNTSSGDPMRSVDVQTDVQGFVPHLGSIQFDEVFNHPTGDYIGTIEWISQPSTPPGTDINLW
EIPDYGSSLSQAANLAPPVFPPGFGEALVYFVSAFPGPNNRSAPNDVPCLLPQEYVTHFVSEQAPTMGDAALLHYVDPDT
NRNLGEFKLYPGGYLTCVPNGVGAGPQQLPLNGVFLFVSWVSRFYQLKPVGTASTARGRLGVRRI*

Figure 33C

Amino acid sequence of S(GII.12)+P(GI.3) fusion VP1 (SEQ ID NO: 31)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVEQKTKPFSVPNLPLNVLS
NSRVPSLIKSMMVSQDHGQMVQFQNGRVTLDGQLQGTTPTSASQLCKIRGTVYHATGGQGLNLTEIDGTPYHAFESPA
PIGFPDLGECDWHINASPANAFTDGSIIHRIDVAQDSTFAPHLGTIHYTNADYNANVGLICSLEWLSPPSGGAPKVNPWAI
PRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSDFPIANGSDGLSVPCTIPQEFVTHFVNEQAPTRGEAALLHYVDPDTHRNL
GEFKLYPEGFMTCVPNSSGSGPQTLPINGVFTFISWVSRFYQLKPVGTTGPVRRLGIRRS*

Figure 33D

Amino acid sequence of S(GII.12)+P(GI.5) fusion VP1 (SEQ ID NO: 32)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVEQKTRPFSVPNIPLQLLSN
SRVPNLIQSMVLSPDQAQNVQFQNGRCTTDGQLLGTTPVSVSQILKFRGKVSAGSKVINLTELDGSPFLAFEAPAPTGFP
DLGTSDWHVEMSLNSNSQSSGNPILLRDIHPNSSEFVPHLGSVCVTAAIEVAGDYTGTIQWTSQPSNVTPVPDVNFWTIP
HYGSNLAEASQLAPVVYPPGFGEAIVYFMSPIPGPNTAHKPNLVPCLLPQEFVTHFVSEQAPSMGEAALVHYVDPDTNR
NLGEFKLYPEGFITCVPNGTGPQQLPLNGVFVFASWVSRFYQLKPVGTASSARGRLGVRR*

Figure 33E

Amino acid sequence of S(GII.12)+P(GII.1) fusion VP1 (SEQ ID NO: 33)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPILTIGELSNS
RFPVPIDELYTSPNEGVVVQPQNGRSTLDGELLGTTQLVPSNICALRGRINAQVPDDHHQWNLQVTNANGTSFDPTEDV
PAPLGTPDFLANIYGVTSQRNPDNTCRAHDGVLATWSPKFTPKLGSVVLGTWEESDLDLNQPTRFTPVGLYDTGHFDQ
WVLPNYSGRLTLNMNLAPSVAPLFPGEQILFFRSHIPLKGGTSNGAIDCLLPQEWIQHFYQESAPSPTDVALIRYTNPDTG
RVLFEAKLHRQGFITVANSGSRPIVVPPNGYFRFDSWVNQFYSLAPMGTGNGRRRVQ*

Figure 33F

Amino acid sequence of S(GII.12)+P(GII.2) fusion VP1 (SEQ ID NO: 34)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPILTLGELSNS
RFPVSIDQMYTSPNEVISVQCQNGRCTLDGELQGTTQLQVSGICAFKGEVTAHLHDNDHLYNVTITNLNGSPFDPSEDIP
APLGVPDFQGRVFGVISQRDKHNTPGHNEPANRAHDAVVPTYTAQYTPKLGQIQIGTWQTDDLTVNQPVKFTPVGLND
TDHFNQWVVPRYAGALNLNTNLAPSVAPVFPGERLLFFRSYIPLKGGYGTPAIDCLLPQEWVQHFYQEAAPSMSEVALV
RYINPDTGRALFEAKLHRAGFMTVSSNTSAPVVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRIQ*

Figure 33G

Amino acid sequence of S(GII.12)+P(GII.3) fusion VP1 (SEQ ID NO: 35)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFSLPILTISEMSNS
RFPVPIDSLHTSPTENIVVQCQNGRVTLDGELMGTTQLLPSQICAFRGTLTRSTSRASDQADTATPRLFNYYWHIQLDNLN
GTPYDPAEDIPAPLGTPDFRGKVFGVASQRNPDATTRAHEAKIDTTSGRFTPKLGSLEISTESGDFDQNQPTRFTPVGIGV
DHEPDFQQWALPDYAGQFTHNMNLAPAVAPNFPGEQLLFFRSQLPSSGGRSNGILDCLVPQEWVQHFYQESAPSQTQ
VALVRYVNPDTGRVLFEAKLHKLRFMTIAKSGDSPITVPPNGYFRFESWVNPFYTLAPMGTGNGRRRIQ*

Figure 33H

Amino acid sequence of S(GII.12)+P(GII.4) fusion VP1 (SEQ ID NO: 36)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESRTKPFSVPVLTVEEMT
NSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDYDPTEEIP
APLGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQDGGTTHR
NEPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQSDVAL
LRFVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV*

Figure 33I

Amino acid sequence of S(GII.12)+P(GII.5) fusion VP1 (SEQ ID NO: 37)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPVLTLGELSN
SRFPLSIDEMVTSPNESIVVQPQNGRVTLDGELLGTTQLQACNICSIRGKVTGQVPNEQHMWNLEITNLNGTQFDPTDD
VPAPLGVPDFAGEVFGVLSQRNRGESNPANRAHDAVVATYSDKYTPKLGLVQIGTWNTNDVENQPTKFTPIGLNEVAN
GHRFEQWTLPRYSGALTLNMNLAPAVAPLFPGERLLFFRSYVPLKGGFGNPAIDCLVPQEWVQHFYQESAPSLGDVALV
RYVNPDTGRVLFEAKLHKGGFLTVSSTSTGPVVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRFQ*

Figure 33J

Amino acid sequence of S(GII.12)+P(GII.6) fusion VP1 (SEQ ID NO: 38)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFSLPILTLGELSNS
RFPAPIDMLYTDPNEGIVVQPQNGRCTLDGTLQGTTQLVPTQICAFRGTLIGQTSRSPDSTDSAPRRRDHPLHVQLKNLD
GTQYDPTDEVPAVLGAIDFKGTVFGVASQRDVSGQQVGATRAHEVHINTTDPRYTPKLGSILMYSESDDFVTGQPVRFT
PIGMGDNDWHQWELPDYPGHLTLNMNLAPAVAPAFPGERILFFRSIVPSAGGYGSGQIDCLIPQEWVQHFYQEAAPSQ
SAVALIRYVNPDTGRNIFEAKLHREGFITVANSGNNPIVVPPNGYFRFEAWVNQFYTLTPMGTGQGRRRDQ*

Figure 33K

Amino acid sequence of S(GII.12)+P(GII.7) fusion VP1 (SEQ ID NO: 39)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKQFTLPILKISEMTN
SRFPVPVEMMYTARNENQVVQPQNGRVTLDGELLGTTPLLAVNICKFKGEVIAKNGDVRSYRMDMEITNTDGTPIDPTE
DTPGPIGSPDFQGILFGVASQRNKNEQNPATRAHEANINTGGDQYAPKLAQVKFFSESQDFEVHQPTVFTPVGVAGDTS
HPFRQWVLPRYGGHLTNNTHLAPAVAPLFPGEQILFFRSQIPSSGGHELGYMDCLVPQEWVQHFYQEAATAQSEVALIR
FINPDTGRVLFEAKLHKQGFITVAHTGDNPIVMPPNGYFRFEAWVNQFYSLAPVGTGNGRRRIQ*

Figure 33L

Amino acid sequence of S(GII.12)+P(GII.13) fusion VP1 (SEQ ID NO: 40)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPILTISELTNS
RFPISIEQLYTAPNENNVVQCQNGRCTLDGELQGTTQLLSSAVCSYRGRTVANSGDNWDQNVLQLTYPSGASYDPTDEV
PAPLGTQDFSGILYGVLTQDNVRENTGEAKNAKGVYISTTSGKFTPKIGSIGLHSITEDVRPNQQSRFTPVGVAQNENTPF
QQWVLPHYAGALALNTNLAPAVAPTFPGEQLLFFRSRVPCVQGLQGQDAFIDCLLPQEWVNHFYQEAAPSQADVALIR
YVNPDTGRTLFEAKLHRSGFITVSHTGAYPLVVPPNGHFRFDSWVNQFYSLAPMGTGNGRRRVQ*

Figure 33M

Amino acid sequence of S(GII.12)+P(GII.14) fusion VP1 (SEQ ID NO: 41)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKNFTLPVLRVSEMT
NSRFPVVLDQMYTSRNENIIVQPQNGRCTTDGELLGTTILQSVSICNFKGTMQAKLNEEPRYQLQLTNLDGSPIDPTDDM
PAPLGTPDFQAMLYGVASQRSSIDNATRAHDAQIDTAGDTFAPKIGQVRFKSSSNDFDLHDPTKFTPIGVNVDDQHPFR
QWSLPNYGGHLALNNHLAPAVTPLFPGEQILFFRSYIPSAGGHTDGAMDCLLPQEWVEHFYQEAAPSQSDIALVRFINPD
TGRVLFEAKLHKQGFLTIAASGDHPIVMPTNGYFRFEAWVNPFYTLAPVGTGSGRRRIQ*

Figure 33N

Amino acid sequence of S(GII.12)+P(GII.17) fusion VP1 (SEQ ID NO: 42)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFSLPILTLSELTNS
RFPVPIDSLFTAQNNVLQVQCQNGRCTLDGELQGTTQLLPSGICAFRGRVTAETDHRDKWHMQLQNLNGTTYDPTDDV
PAPLGTPDFKGVVFGVASQRNVGNDAPGSTRAHEAVISTYSPQFVPKLGSVNFRSNDNDFQLQPTKFTPVGINDDGDHP
FRQWELPDYSGLLTLNMNLAPPVAPNFPGEQLLFFRSFVPCSGGYNQGIVDCLIPQEWIQHFYQESAPSQSDVALIRYVN
PDTGRTLFEAKLHRSGYITVAHSGDYPLVVPANGYFRFDSWVNQFYSLAPMGTGNGRRRAQ*

Figure 33O

Amino acid sequence of S(GII.12)+P(GII.21) fusion VP1 (SEQ ID NO: 43)

MKMASNDAAPSNDGAAGLVPEVNNETMALEPVAGASIAAPLTGQNNVIDPWIRLNFVQAPNGEFTVSPRNSPGEVLL
NLELGPELNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKLVFAAVPPHFPLENISPGQITMFPHVIIDVRTLEPVLLPLP
DVRNNFFHYNQQNEPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPSPDFDFNYLVPPTVESKTKPFTLPILTIGELTNS
RFPAPIDQLYTSPNADVVVQPQNGRCTLDGELQGTTQLLTTAICSYRGMTSNPTSDYWDDHLLHLVHPNGATYDPTEDV
PAPFGTQDFRGILYGMLTQNPRTSGDEAANSHGIYISSTSEKFTPKLGTIGLHQVQGDIASNQQSKFTPVGIAVNGNTPFR
QWELPNYSGALTLNTNLAPAVGPNFPGEQILFFRSNVPSVQGGQPIEIDCLIPQEWVSHFYQESAPSQSDVALVRYVNPD
TGRTIFEAKLHRQGFITIAATGSNPVVVPPNGYFRFDSWVNQFYALAPMGTGNGRRRVQ*

Figure 34A

Amino acid sequence of S(GII.14)+P(GII.4) fusion VP1 (SEQ ID NO: 52)

MKMASNDATPSDDGAAGLVPEINNEVMALEPVAGASIAAPVVGQQNIIDPWIRNNFVQAPAGEFTVSPRNSPGELLLD
LELGPELNPYLAHLARMYNGHAGGMEVQIVLAGNAFTAGKILFAAIPPSFPYENLSPAQLTMCPHVIVDVRQLEPVLLPM
PDIRNVFYHYNQNNSPKLRLVAMLYTPLRANNSGDDVFTVSCRVLTRPSPDFQFTFLVPPTVESRTKPFSVPVLTVEEMTN
SRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDYDPTEEIPAP
LGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQDGGTTHRNE
PQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQSDVALLR
FVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV*

Figure 34B

Amino acid sequence of S(GII.21)+P(GII.4) fusion VP1 (SEQ ID NO: 53)

MKMASNDAAPSNDGATGLVPEINTETLPLEPVAGAAIAAPVTGQNNIIDPWIRNNFVQAPNGEFTVSPRNSPGEILMNL
ELGPDLNPYLAHLSRMYNGYAGGVEVQVLLAGNAFTAGKILFAAVPPNFPVDMLSPAQITMLPHLIVDVRTLEPIMIPLP
DVRNVFYHFNNQPAPRMRLVAMLYTPLRSNGSGDDVFTVSCRVLTRPTPDFEFTYLVPPSVESRTKPFSVPVLTVEEMTN
SRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGTTQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDYDPTEEIPAP
LGTPDFVGKIQGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTDRDFEANQNTKFTPVGVIQDGGTTHRNE
PQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTMPGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQSDVALLR
FVNPDTGRVLFECKLHKSGYVTVAHTGQHDLVIPPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV*

Figure 35

Sequence identifier	Primer name	Nucleic acid sequence (5' to 3')
SEQ ID NO: 111	IF-GI3LilO8VP1.c	TCGTGCTTCGGCACCAGTACAATGATGGCTTCCAAGGATGCTCCCA
SEQ ID NO: 98	IF-GI3LilO8VP1.r	ACTAAAGAAAATAGGCCTCTAGCTCCGTCTGATCCCGAGCCTCCGAACT
SEQ ID NO: 112	IF-GII4Syd12VP1.c	TCGTGCTTCGGCACCAGTACAATGAAAATGGCCTCGAGTGACGCTAACC
SEQ ID NO: 101	IF-GII4Syd12VP1.r	ACTAAAGAAAATAGGCCTTCAGACAGCCCTGCGTCTGCCAGTCCCATT
SEQ ID NO: 113	IF-GII6Ohi12VP1.c	TCGTGCTTCGGCACCAGTACAATGAAGATGGCAAGCAACGACGCAGCTC
SEQ ID NO: 104	IF-GII6Ohi12VP1.r	ACTAAAGAAAATAGGCCTTCACTGATCCCGGCGTCGCCCCTGGCCTGTCCCCAT
SEQ ID NO: 114	IF-GII13VA10VP1.c	TCGTGCTTCGGCACCAGTACAATGAAAATGGCTTCTAATGATGCCGCGCCCAGCAATGA
SEQ ID NO: 107	IF-GII13VA10VP1.r	ACTAAAGAAAATAGGCCTTCATTGCACCCGACGGCGACCATTCCCGGTACCCA
SEQ ID NO: 115	IF-GII17Kaw14VP1.c	TCGTGCTTCGGCACCAGTACAATGAAAATGGCATCTAACGACGCAGCCCCCTC
SEQ ID NO: 110	IF-GII17Kaw14VP1.r	ACTAAAGAAAATAGGCCTCTACTGAGCCCGGCGTCTGCCGTTACCGGTGCCCATTG
SEQ ID NO: 96	GI3LiI+GI1VP1.r	GAATGGCTTTGTTTTCTGCTCAACGGTTGGTGGGACCAGAAAAAGAAAGTTGAAGT
SEQ ID NO: 97	GI1VP1+GI3LiI.c	TCCCACCAACCGTTGAGCAGAAAACAAAGCCATTCAGCGTGCCAAACC
SEQ ID NO: 99	GII4Syd+GI1VP1.r	AAGGGCTTGGTTCGGCTCTCAACGGTTGGTGGGACCAGAAAAAGAAAGT
SEQ ID NO: 100	GI1VP1+GII4Syd.c	TCCCACCAACCGTTGAGAGCCGAACCAAGCCCTTTAGTGTCCCGTACT

Figure 35 continued

SEQ ID NO: 102	GII6Ohi+GI1VP1.r
SEQ ID NO: 103	GI1VP1+GII6Ohi.c
SEQ ID NO: 105	GII13Va+GI1VP1.r
SEQ ID NO: 106	GI1VP1+GII13Va.c
SEQ ID NO: 108	GII17Kaw+GI1VP1.r
SEQ ID NO: 109	GI1VP1+GII17Kaw.c
SEQ ID NO: 117	IF-GII4Syd12VP2.c
SEQ ID NO: 119	IF-GII4Syd12VP2.r
SEQ ID NO:116	IF-NoV(US68)VP2(ORF3).c
SEQ ID NO:122	IF-NoV(US68)VP2(ORF3).r
SEQ ID NO:118	IF-NoV(US68)VP1/VP2(ORF3)NoV3'UTR.r

AAGGGTTTTGTCTTGCTCTCAACGGTTGGTGGGACCAGAAAAAGAAAGT
TCCCACCAACCGTTGAGAGCAAGACAAAACCCTTCAGCCTCCCAATCTTA
AAGGGTTTAGTCTTGCTCTCAACGGTTGGTGGGACCAGAAAAAGAAAGT
TCCCACCAACCGTTGAGAGCAAGACTAAACCCTTTACTCTTCCCATTCTG
AACGGTTTGGTCTTAGACTCAACGGTTGGTGGGACCAGAAAAAGAAAGTTGAAGT
TCCCACCAACCGTTGAGTCTAAGACCAAACCGTTTTCACTGCCAATCT
TCGTGCTTCGGCACCAGTACAATGGCTGGGGCCTTTTTTGCAGGTTTGGCTAGT
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TCGTGCTTCGGCACCAGTACAATGGCCCAAGCCATAATTGGTGCAATT
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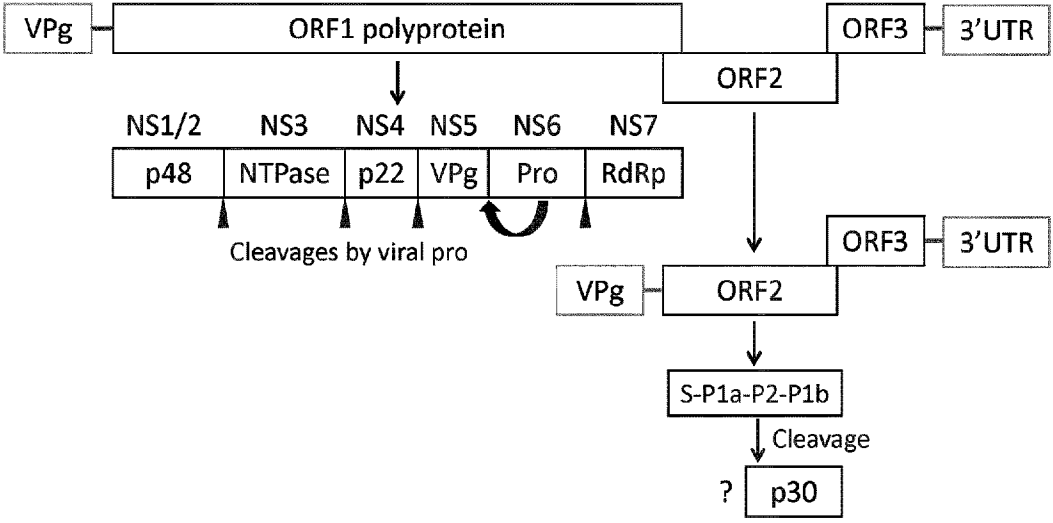
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ORF1 polyprotein
ORF3
3'UTR
ORF2
NS1/2
NS3
NS4
NS5
NS6
NS7
p48
NTPase
p22
VPg
Pro
RdRp
Cleavages by viral pro
ORF3
3'UTR
VPg
ORF2
S-P1a-P2-P1b
Cleavage
?
p30