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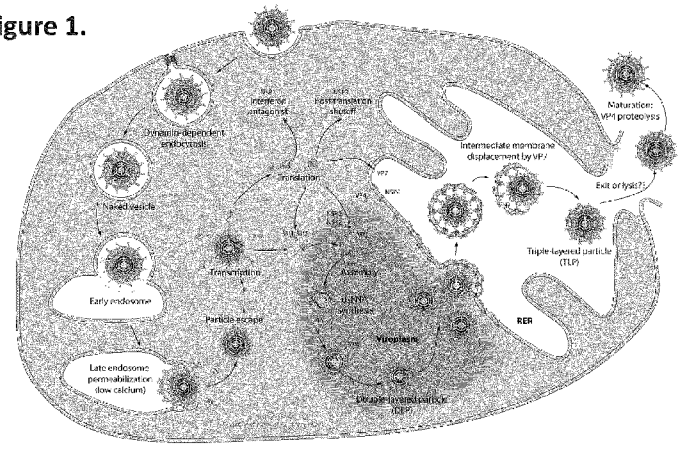
(54) Title: ROTAVIRUS-LIKE PARTICLE PRODUCTION IN PLANTS

Figure 1.



(57) Abstract: A method of producing a rotavirus-like particle (RLP) in a plant is provided. The method comprises expressing within a host or host cell for example a plant, portion of a plant or plant cell one or more nucleic acid comprising one or more regulatory region operatively linked to a first, second and third nucleotide sequence, the regulatory region active in the host or host cell. The first nucleotide sequence encoding a first rotavirus protein, the second nucleotide sequence encoding a second rotavirus protein and the third nucleotide sequence encoding a third rotavirus protein. The first, second and third encode rotavirus protein NSP4 and VP2 or VP6 and VP4 or VP7. The host or host cell is incubated under conditions that permit the expression of the nucleic acids, so that NSP4 and either VP2 of VP6 and VP4 or VP7 are expressed, thereby producing the RLP. Hosts comprising the RLP, compositions comprising the RLP and method for using the composition are also provided.

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Rotavirus-like particle production in plants

FIELD OF INVENTION

[0001] This invention relates to producing rotavirus–like particles in plants.

BACKGROUND OF THE INVENTION

[0002] Rotavirus infection is a global problem mainly affecting children under the age of five. It results in severe gastroenteritis and in worst cases death.

[0003] Rotaviruses are members of the Reoviridae family of viruses (genus Rotavirus) that affect the gastrointestinal system and respiratory tract. The name is derived from the wheel like appearance of virions when viewed by negative contrast electron microscopy. The rotavirus is usually globular shape and is named after the outer and inner shells or double-shelled capsid structure of the same. The outer capsid is about 70 nm, and inner capsid is about 55 nm in diameter, respectively. The double-shelled capsid of the rotavirus surrounds the core including the inner protein shell and genome. The genome of the rotavirus consists of double stranded RNA segments encoding at least 11 rotavirus proteins - either structural viral proteins (VP) or nonstructural proteins (NSP; Desselberger, Virus Res 190: 75-96 (2014)).

[0004] The dsRNA codes for six structural proteins (VP) and six non-structural proteins (NSP). The structural proteins comprise VP1, VP2, VP3, VP4, VP6 and VP7. Three concentric layers are formed by the assembly of VP2, VP6 and VP7 respectively, with VP4 forming “spikes” on the surface of the virus structure. VP4 is cleaved by trypsin to VP8* and VP5*. VP8* and VP5* are proteolytic products of VP4.

[0005] VP2 is a 102 kDa protein and is the most abundant protein of the viral core. It forms the inner-most structural protein layer and provides a scaffold for the correct assembly of the components and transcription enzymes of the viral core (Lawton, 2000). VP1, the largest viral protein at 125 kDa, acts as an RNA-dependent polymerase for rotavirus, creating a core replication intermediate, and associates with VP2 at its icosahedral vertices (Varani and Allain, 2002; Vende et al., 2002). VP3, a 98 kDa protein, is also directly associated with the viral genome, acting as an mRNA

capping enzyme that adds a 5' cap structure to viral mRNAs. Together, VP1 and VP3 form a complex that is attached to the outer 5-fold vertices of the VP2 capsid layer (Angel, 2007). VP6 is a 42 kDa protein which forms the middle shell of the viral core, is the major capsid protein and accounts for more than 50% of the total protein mass of the virion (González et al., 2004; Estes, 1996). It is required for gene transcription and may have a role in encapsulation of the rotavirus RNA by anchoring VP1 to VP2 in the core, as seen in bluetongue virus, another member of the Reoviridae family. It also determines the classification of rotaviruses into five groups (A to E) with group A most commonly affecting humans (Palombo, 1999). VP6 in rotavirus group A has at least four subgroups (SG), which depend on the presence or absence of SG specific epitopes: SG I, SG II, SG (I+II) and SG non-(I+II). Groups B and C lack a common group A antigen but are also known to infect humans, while group D only affects animals e.g. chickens and cows (Thongprachum, 2010).

[0006] The two outer capsid proteins VP7, a 37 kDa glycoprotein (G) and the 87 kDa protease sensitive VP4 (P), define the virus' serotypes. These two proteins induce neutralizing antibody responses and are thus used to classify rotavirus serotypes into a dual nomenclature system, depending on the G–P antigen combination (e.g. G1 P[8] or G2 P[4]) (Sanchez-Padilla et al., 2009, Rahman et al., J Clin Microbiol 41: 2088-2095 (2003)). The VP4 protein dimerizes to form 60 spikes on the outer shell of the virus, which are directly involved in the initial stages of host cell entry. The spike protein contains a cleavage site at amino acid (aa) position 248. Upon infection, it is cleaved by the protease trypsin to produce VP5 (529 aa, 60 kDa) and VP8 (246 aa, 28 kDa) (Denisova et al., 1999). This process enhances virus infectivity (cell attachment and invasion of host cell) and stabilizes the spike structure (Glass, 2006). The VP7 glycoprotein forms the third or outside layer of the virus. At present, 27 G and 35 P genotypes are known (Greenberg and Estes, 2009). VP4 and VP7 are the major antigens involved in virus neutralization and are important targets for vaccine development (Dennehy, 2007).

[0007] The non-structural proteins (NSPs) are synthesized in infected cells and function in various parts of the replication cycle or interact with some of the host proteins to influence pathogenesis or the immune response to infection (Greenberg and Estes, 2009). The rotavirus nonstructural protein, NSP4, has been shown to have

multiple functions including the release of calcium from the endoplasmic reticulum (ER; Tian et al, 1995); the disruption of the ER membranes and may play an important role in the removal of the transient envelope from budding particles during viral morphogenesis (see Figure 1); affecting membrane trafficking from the ER to the Golgi complex with its ability to bind to micro tubules (Xu et al 2000); and function as an intracellular receptor to aid in the budding of subviral particles into the ER (Tian et al 1996).

[0008] In infected mammalian cells, rotaviruses undergo a unique mode of morphogenesis to form the complete triple-layered VP2/6/4/7 viral particles (Lopez et al., 2005). The triple-layer capsid is a very stable complex which enables faecal–oral transmission and delivery of the virus into the small intestine where it infects non-dividing differentiated enterocytes near the tips of the villi (Greenberg and Estes, 2009). Firstly, the intact virus attaches to sialic acid-independent receptors via 60 VP4 dimer spikes on the surface of the virus (Lundgren and Svensson, 2001). The 60 VP4 dimer spikes on the surface of the virus allow the virus to attach to these cell receptors. VP4 is susceptible to proteolytic cleavage by trypsin which results in a conformational change that exposes additional attachment sites on the surface of the glycoprotein for interaction with a series of co-receptors.

[0009] The multi-step attachment and entry process is, however, not clearly understood but the virus is delivered across the host's plasma membrane. The VP7 outer capsid shell which is also involved in the entry process, is removed in the process and double-layered particles (DLP) are delivered into the cell cytoplasm in vesicles (Figure 1; prior art). The DLP escapes from the vesicle and goes into non-membrane bound cytoplasmic inclusions. Early transcription of the genome by VP1 begins in particles so that dsRNA is never exposed to the cytoplasm. RNA replication and core formation takes place in these non-membrane-bound cytoplasmic inclusions. The nascent (+) RNAs are then transported into the cytoplasm and serve as templates for viral protein synthesis. VP4 is produced in the cytosol and transported to the rough endoplasmic reticulum (RER), and VP7 is secreted into the RER. VP2 and VP6 are produced and assemble in the cytosol in virosomes and subsequently bud into the RER compartments, receiving a transient membrane envelope in the process (Lopez et al., 2005; Tian et al., 1996). In the RER, the transient envelopes of the viral

particles are removed and replaced by VP4 and VP7 protein monomers, with critical involvement of rotaviral glycoprotein NSP4 (Tian et al., 1996; Lopez et al., 2005; Gonzalez et al., 2000). NSP4 functions as an intracellular receptor in the ER membrane and binds newly made subviral particles and probably also the spike protein VP4 (Tian et al., 1996). NSP4 is also toxic to humans and is the causative agent of the diarrhea. The complete, mature particles are subsequently transferred from the RER through the Golgi apparatus to the plasma membrane for secretion (Lopez et al., 2005).

[0010] A variety of different approaches have been taken to generate a rotavirus vaccine suitable to protect human populations from the various serotypes of rotavirus. These approaches include various Jennerian approaches, use of live attenuated viruses, use of virus-like particles, nucleic acid vaccines and viral sub-units as immunogens. At present there are two oral vaccines available on the market, however, these have low efficacy in due to strain variation.

[0011] U.S. Pat. Nos. 4,624,850, 4,636,385, 4,704,275, 4,751,080, 4,927,628, 5,474,773, and 5,695,767, each describe a variety of rotavirus vaccines and/or methods of preparing these vaccines, where the whole viral particles is used to create each of the rotavirus vaccines.

[0012] Production of rotavirus-like particles is a challenging task, as both the synthesis and assembly of one or more recombinant proteins are required. Rotavirus comprises a capsid formed by 1860 monomers of four different proteins. For RLP production the simultaneous expression and assembly of two to three recombinant proteins may be required. For example, an inner layer comprising 120 molecules of VP2, 780 molecules of VP6 (middle layer) and an outer layer of 780 molecules of the glycoprotein VP7 and 60 VP4 dimers, to form a double or triple-layered particle (Libersou et al. J. of Virology, Mar. 2008).

[0013] Crawford et al. (J Virol. 1994 September; 68(9): 5945–5952) describe the expression of VP2, VP4, VP6, and VP7 in a baculovirus expression system. Co-expression of different combinations of the rotavirus major structural proteins resulted in the formation of stable virus-like particles (VLPs). The co-expression of VP2 and

VP6 alone or with VP4 resulted in the production of VP2/6 or VP2/4/6 VLPs, which were similar to double-layered rotavirus particles. Co-expression of VP2, VP6, and VP7, with or without VP4, produced triple-layered VP2/6/7 or VP2/4/6/7 VLPs, which were similar to native infectious rotavirus particles. The VLPs maintained the structural and functional characteristics of native particles, as determined by electron microscopic examination of the particles, the presence of non-neutralizing and neutralizing epitopes on VP4 and VP7, and hemagglutination activity of the VP2/4/6/7 VLPs.

[0014] Vaccine candidates generated from rotavirus-like particles of different protein compositions have shown potential as subunit vaccines. O'Neal et al. (J. Virology, 1997, 71(11):8707-8717) show that VLPs containing VP 2 and VP6, or VP2, VP6, and VP7, and administered to mice with and without the addition of cholera toxin induced protective immunity in immunized mice. Core-like particles (CLP) and VLPs have also been used to immunize cows with VLPs more effective than CLPs in inducing passive immunity Fernandez, et al., (Vaccine, 1998, 16(5):507-516).

[0015] Plants are increasingly being used for large-scale production of recombinant proteins. For example US 2003/0175303 discloses the expression of recombinant rotavirus structural protein VP6, VP2, VP4 or VP7 in stably transformed tomato plants.

[0016] Saldana et al. (Viral Immunol. 19: 42-53 (2006)) expressed VP2 and VP6 in the cytoplasm of tomato plants. Electron microscopy studies showed that a small proportion of the proteins had assembled into 2/6 VLPs. A protective immune response was detected in mice and this may have to some extent been contributed by the non-assembled VPs. Individual proteins have been shown to elicit immune responses in mice, as in the case of VP8 and VP6 (Rodriguez-Diaz et al. Biotechnol Lett. 2011, 33(6):1169-75, Zhou et al., Vaccine 28: 6021-6027 (2010)).

[0017] Matsumura et al., (Archives of Virology 147: 1263-1270 (2002)) report bovine rotavirus A VP6 expression in transgenic potato plants. The VP6 was expressed, purified and immunogenic studies performed. Immune-response in adult mice showed presence of VP6 antibodies in the sera. However, no evidence of assembled VP6 proteins was provided. It may have been that monomers or trimers of

VP6 were responsible for eliciting the immune response. O'Brien et al. (2000, Virol. 270: 10444–10453) show VP6 assembly in *Nicotiana benthamiana* using a potato virus X (PVX) vector. Assembly of VP6 protein into icosahedral VLPs was only observed when the VP6 was fused to the PVX protein rods. Following cleavage the VP6 assembled into the icosahedral VLPs.

[0018] Codon-optimized human rotavirus VP6 has been successfully expressed in *Chenopodium amaranticolor* using a Beet black scorch virus (BBSV) mediated expression system. ,The protein was engineered as a replacement to the coat protein of BBSV. Oral immunization of female BALB/c mice with the plant based VP6 protein induced high titers of anti-VP6 mucosal IgA and serum IgG (Zhou et al., Vaccine 28: 6021-6027 (2010)). However, there was no teaching that the VP6 proteins assembled into VLPs or particles.

[0019] Rotavirus VP7 has been expressed in potato plants and was shown to produce a neutralizing immune response in mice (Yu and Langridge, 2001 Nature Biotechnol 19: 548-552). In transgenic potato plants, the VP7 gene was stable over 50 generations, with the VP7 protein from the 50th generation induced both protective and neutralizing antibodies in adult mice (Li et al., 2006, Virol 356:171-178).

[0020] Yang et al. (Yang Y M, Li X, Yang H, et al. Science China Life Science 54: 82-89 (2011)) co-expressed three rotavirus capsid proteins VP2, VP6 and VP7 of group A RV (P[8]G1) in tobacco plants and expression levels of these proteins, as well as formation of rotavirus-like particles and immunogenicity were studied. VLPs were purified from transgenic tobacco plants and analyzed by electron microscopy and Western blot. These results indicate that the plant derived VP2, VP6 and VP7 protein self-assembled into 2/6 or 2/6/7 rotavirus like particle with a diameter of 60-80 nm.

[0021] WO 2013/166609 described the production of rotavirus-like particle (RLPs) in plants, by co-expressing rotavirus structural proteins VP2, VP4, VP6 and VP7 in plants and purifying the resulting RLPs in the presence of calcium.

[0022] Rotavirus NSP4 has been expressed and purified from insect cells (Tian et al. 1996, Arch Virol. 1996; Rodriguez-Diaz et al. Protein Expr. Purif. 2003) and in *E.*

coli (Sharif et al. Medical Journal of the Islamic Republic of Iran 2003). NSP4 has also been expressed as a fusion protein with the cholera toxin B (CTB) subunit in potato (Arakawa et al., Plant Cell Report 20 : 343-348 (2001)).

SUMMARY OF THE INVENTION

[0023] The present invention relates to producing rotavirus–like particles in plants.

[0024] It is an object of the invention to produce rotavirus–like particles in plants.

[0025] Several methods to produce a rotavirus like particle (RLP) in a plant, portion of a plant or plant cell are described.

[0026] For example, a method (A) for producing a rotavirus like particle (RLP) in a host or host cell may comprise:

a) providing a host or host cell comprising one or more nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP.

[0027] In the method (A) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third and fourth nucleotide

sequence encoding rotavirus protein VP2, VP4 or VP7 and wherein each of NSP4, VP6 and two of VP2, VP4 and VP7 are expressed from the one or more nucleic acid.

[0028] A method (B) to produce a rotavirus like particle (RLP) in a host or host cell is further described, the method may comprise:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second and third nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP.

[0029] In the method (B) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third and fourth nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and two of rotavirus protein VP2, VP7 or VP4 and wherein each of NSP4, VP6 and two of VP2, VP7 or VP4 are expressed from the one or more nucleic acid.

[0030] In the method (A) and (B) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein and a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

[0031] In the method (A) or (B) as described above, if a host or host cell is provided where the one or more nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein, a third nucleotide sequence encoding a third rotavirus protein, fourth nucleotide sequence encoding a fourth rotavirus protein and a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell, then the one or more nucleic acid may comprise the first, second, third, fourth and fifth nucleotide sequence encoding the first, second, third, fourth and fifth rotavirus protein, or the one or more nucleic acid may comprise for example two nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, and a second nucleic acid comprising the second, third, fourth and fifth nucleotide sequence encoding the second, third, fourth and fifth rotavirus protein, or the one or more nucleic acid may comprise for example two nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, and a second nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein or the one or more nucleic acid may comprise for example three nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, a second nucleic acid comprising the third and fourth nucleotide sequence encoding the third and fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein or the one or more nucleic acid may comprise for example three nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, and a third nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein or the one or more nucleic acid may comprise for example four nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid

comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth and fifth nucleotide sequence encoding the fourth and fifth rotavirus protein or the one or more nucleic acid may comprise for example five nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein.

[0032] The methods (A) or (B) as described above may further comprise the steps of:

c) harvesting the host or host cell, and

d) purifying the RLPs from the host or host cell, wherein the RLPs range in size from 70-100 nm.

[0033] The one or more nucleotide sequence of the method (A) or (B) as described above may be operatively linked to one or more expression enhancer. Furthermore, the expression enhancer may be selected from the group consisting of CPMV HT, CPMV 160, CPMV 160+ and CPMV HT+.

[0034] Also described herein is a method (C) of producing a rotavirus like particle (RLP) in host or host cell comprising:

a) introducing into the host or host cell one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP.

[0035] In the method (C) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third and fourth nucleotide sequence encoding rotavirus protein VP2, VP4 or VP7 and wherein each of NSP4, VP6 and two of VP2, VP4 and VP7 are expressed from the one or more nucleic acid.

[0036] Also described herein is a method (D) of producing a rotavirus like particle (RLP) in host or host cell comprising:

a) introducing into the host or host cell one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second and third nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP.

[0037] In the method (D) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third and fourth nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and two of rotavirus protein VP2, VP7 or VP4 and wherein each of NSP4, VP6 and two of VP2, VP7 or VP4 are expressed from the one or more nucleic acid.

[0038] In the method (C) and (D) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein and a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

[0039] The methods (C) or (D) as described above may further comprise the steps of:

c) harvesting the host or host cell, and

d) purifying the RLPs from the host or host cell, wherein the RLPs range in size from 70-100 nm.

[0040] In the method (C) or (D) as described above, if a host or host cell is provided where the one or more nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein, a third nucleotide sequence encoding a third rotavirus protein, fourth nucleotide sequence encoding a fourth rotavirus protein and a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell, then in the step of introducing (step a), the one or more nucleic acid may comprise two nucleic acids, with a first nucleic acid comprising encoding the NSP4, and a second nucleic acid encoding VP2, VP4, VP6 and VP7, the ratio of

an amount of the first nucleic acid relative to the second nucleic acid that is introduced into the plant, portion of a plant or plant cell is between 1:0.8 and 1:2. The ratio may also be 1:1. Alternatively, the one or more nucleic acid may comprise two nucleic acids, with a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein and the second nucleotide sequence encoding the second rotavirus protein, and a second nucleic acid comprising the third to fifth nucleotide sequence encoding the third to fifth rotavirus protein.

[0041] In the method (C) or (D) as described above, in the step of introducing (step a), the one or more nucleic acid may comprise three nucleic acids, with a first nucleic acid comprising the first and the second nucleotide sequence encoding the first and the second rotavirus protein, a second nucleic acid comprising the third and fourth nucleotide sequence encoding the third and the fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, and wherein the ratio of an amount of the first nucleic acid relative to the second nucleic acid and to the third nucleic that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1.

[0042] Alternatively, in the method (C) or (D) as described above, in the step of introducing (step a), the one or more nucleic acid may comprise three nucleic acids, with the first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, the second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, and the third nucleic acid comprising the third to fifth nucleotide sequence encoding the third to fifth rotavirus protein, and wherein the ratio of an amount of the first nucleic acid relative to the second nucleic acid and to the third nucleic that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1.

[0043] Furthermore, in method (C) or (D) described above, in the step of introducing (step a), the one or more nucleic acid may comprise four nucleic acids, a first nucleic acid comprising the first nucleic acid encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth

and the fifth nucleotide sequence encoding the fourth and fifth rotavirus protein, wherein the ratio of an amount of the first nucleic acid relative to the second nucleic acid, to the third nucleic and to the fourth nucleic acid that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1:1.

[0044] In the method (C) or (D) as described above, in the step of introducing (step a), the one or more nucleic acid may comprise five nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, and the ratio of an amount of the first nucleic acid relative to the second nucleic acid, to the third nucleic acid, to the fourth nucleic acid and to the fifth nucleic acid that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1:1:1.

[0045] The one or more nucleotide sequence of the method (C) or (D) as described above may be operatively linked to one or more expression enhancer. Furthermore, the expression enhancer may be selected from the group consisting of CPMV HT, CPMV 160, CPMV 160+ and CPMV HT+.

[0046] A method (E) of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) is also described, the method comprising:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[0047] In the method (E) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third and fourth nucleotide sequence encoding rotavirus protein VP2, VP4 or VP7 and wherein each of NSP4, VP6 and two of VP2, VP4 and VP7 are expressed from the one or more nucleic acid.

[0048] A method (F) of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) is also described, the method comprising:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second and third nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[0049] In the method (F) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third and fourth nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and two of rotavirus protein VP2, VP7 or VP4 and wherein each of NSP4, VP6 and two of VP2, VP7 or VP4 are expressed from the one or more nucleic acid.

[0050] In the method (E) and (F) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein and a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

[0051] The one or more nucleotide sequence of the method (E) or (F) as described above may be operatively linked to one or more expression enhancer. Furthermore, the expression enhancer may be selected from the group consisting of CPMV HT, CPMV 160, CPMV 160+ and CPMV HT+.

[0052] A method (G) of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) is also described, the method comprising:

a) introducing into a host or host cell one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[0053] In the method (G) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third and fourth nucleotide sequence encoding rotavirus protein VP2, VP4 or VP7 and wherein each of NSP4, VP6 and two of VP2, VP4 and VP7 are expressed from the one or more nucleic acid.

[0054] A method (H) of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) is also described, the method comprising:

a) introducing in a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence

being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second and third nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[0055] In the method (H) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third and fourth nucleotide sequence encoding one of rotavirus protein NSP4, VP6 and two of rotavirus protein VP2, VP7 or VP4 and wherein each of NSP4, VP6 and two of VP2, VP7 or VP4 are expressed from the one or more nucleic acid.

[0056] In the method (G) and (H) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein and a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

[0057] In the method (G) and (H) as described above the one or more nucleic acid may further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein and a fifth nucleotide sequence encoding a fifth rotavirus protein, the first,

second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

[0058] In the method (G) or (H) as described above, in the step of introducing (step a), the one or more nucleic acid may comprise two nucleic acids, with a first nucleic acid comprising encoding the NSP4, and a second nucleic acid encoding VP2, VP4, VP6 and VP7, the ratio of an amount of the first nucleic acid relative to the second nucleic acid that is introduced into the plant, portion of a plant or plant cell is between 1:0.8 and 1:2. The ratio may also be 1:1. Alternatively, the one or more nucleic acid may comprise two nucleic acids, with a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein and the second nucleotide sequence encoding the second rotavirus protein, and a second nucleic acid comprising the third to fifth nucleotide sequence encoding the third to fifth rotavirus protein.

[0059] In the method (G) or (H) as described above, in the step of introducing (step a), the one or more nucleic acid may comprise three nucleic acids, with a first nucleic acid comprising the first and the second nucleotide sequence encoding the first and the second rotavirus protein, a second nucleic acid comprising the third and fourth nucleotide sequence encoding the third and the fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, and wherein the ratio of an amount of the first nucleic acid relative to the second nucleic acid and to the third nucleic that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1.

[0060] Alternatively, in the method (G) or (H) as described above, in the step of introducing (step a), the one or more nucleic acid may comprise three nucleic acids, with the first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, the second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, and the third nucleic acid comprising the third to fifth nucleotide sequence encoding the third to fifth rotavirus protein, and wherein the ratio of an amount of the first nucleic acid relative to the second nucleic acid and

to the third nucleic that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1.

[0061] Furthermore, in the method (G) or (H) described above, in the step of introducing (step a), the one or more nucleic acid may comprise four nucleic acids, a first nucleic acid comprising the first nucleic acid encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth and the fifth nucleotide sequence encoding the fourth and fifth rotavirus protein, wherein the ratio of an amount of the first nucleic acid relative to the second nucleic acid, to the third nucleic and to the fourth nucleic acid that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1:1.

[0062] In the method in the method (G) or (H) as described above, in the step of introducing (step a), the one or more nucleic acid may comprise five nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, and the ratio of an amount of the first nucleic acid relative to the second nucleic acid, to the third nucleic acid, to the fourth nucleic acid and to the fifth nucleic acid that is introduced into the plant, the portion of the plant, or the plant cell, is 1:1:1:1:1.

[0063] The one or more nucleotide sequence of the method (G) or (H) as described above may be operatively linked to one or more expression enhancer. Furthermore, the expression enhancer may be selected from the group consisting of CPMV HT, CPM 160, CPMV 160+ and CPMV HT+.

[0064] The methods (G) or (H) as described above may further comprise the steps of:

c) harvesting the host or host cell, and

d) purifying the RLPs from the host or host cell, wherein the RLPs range in size from 70-100 nm.

[0065] In the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above, the one or more nucleic acid may comprise one nucleic acid comprising the first, second, third, fourth and fifth nucleotide sequence encoding the first, second, third, fourth, and fifth rotavirus protein.

[0066] In the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above, the one or more nucleic acid may comprise two nucleic acids, for example, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, and a second nucleic acid comprising the second to fifth nucleotide sequence encoding the second to fifth rotavirus protein. Alternatively, the one or more nucleic acid may comprise two nucleic acids, with a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein and the second nucleotide sequence encoding the second rotavirus protein, and a second nucleic acid comprising the third to fifth nucleotide sequence encoding the third to fifth rotavirus protein.

[0067] In the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above, the one or more nucleic acid may also comprise three nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, the second nucleotide sequence encoding the second rotavirus protein, a second nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein and fourth nucleotide sequence encoding the fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein. Alternatively, the one or more nucleic acid may comprise three nucleic acids, with a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, and a third nucleic acid comprising the third to fifth nucleotide sequence encoding the third to fifth rotavirus protein.

[0068] In the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic acid comprises four nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth and fifth nucleotide sequence encoding the fourth and fifth rotavirus protein.

[0069] Furthermore, in the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic acid may comprise five nucleic acids, with a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein.

[0070] The one or more nucleotide sequence of the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above may be operatively linked to one or more expression enhancer. Furthermore, the expression enhancer may be selected from the group consisting of CPMV HT, CPM 160, CPMV 160+ and CPMV HT+.

[0071] In the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the host or host cell may comprise insect cells, mammalian cells, plant, portion of a plant or plant cells. The plant may be *Nicotiana benthamiana.*

[0072] Also described herein is an RLP produced by the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) described above, wherein the RLP is a triple layered RLP comprising rotavirus protein, the rotavirus protein consists of VP2, VP4, VP6 and VP7. The RLP may not comprise NSP4.

[0073] A composition comprising an effective dose of the RLP for inducing an immune response in a subject, and a pharmaceutically acceptable carrier, and a method of inducing immunity to a rotavirus infection in a subject, that comprises administering the composition, are also described. In the method of inducing immunity, the composition may be administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[0074] Also described herein is plant matter comprising an RLP produced by the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above.

[0075] In the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above, the one or more nucleic acid may comprise one nucleic acid comprising the first, second and third nucleotide sequence encoding the first, second and third, rotavirus protein. Furthermore, in the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic acid may comprise two nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, and a second nucleic acid comprising the second and third nucleotide sequence encoding the second and third rotavirus protein. Furthermore, in the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic acid may comprise two nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, and a second nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein. Alternatively, in the method (A), the method (B), the method (C), the method (D), the method (E), the method (F), the method (G) or the method (H) as described above the one or more nucleic acid may comprise three nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein and a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein.

[0076] As described herein, by co-expressing NSP4 along with VP6 and VP4 or VP7 , in a host or host cell, for example a plant, portion of the plant, or a plant cell, RLPs comprising increased levels of VP4, VP7, or both VP4 and VP7 are observed, when compared to the level of VP4 and VP7 in RLPs produced by a second host or host cell for example a plant, portion of a second plant, or second plant cell, that expresses the one or more nucleic acid that encodes VP6 and VP4 or VP7, and does not encode NSP4, the second host or second host cell for example a second plant, the second portion of plant, or the second plant cell, incubated or grown, under the same conditions as the host or host cell for example a plant, portion of the plant, or plant cell.

[0077] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0078] 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:

[0079] **Figure 1** shows rotavirus cell entry and replication. When rotavirus enters a cell, VP4 and VP7 are lost, forming a double layered particle (DLP). Transcription of the dsRNA commences resulting in translation of VP2, VP4, VP6 and VP7. Progeny cores with replicase activity are produced in virus factories (also called viroplasms). Late transcription occurs in these progeny cores. At the periphery of virus factories, these core are coated with VP6, forming immature DLPs that bud across the membrane of the endoplasmic reticulum, acquiring a transient lipid membrane which is modified with the ER resident viral glycoproteins NSP4 and VP7; these enveloped particles also contain VP4. As the particles move towards the interior of the ER cisternae, the transient lipid membrane and the nonstructural protein NSP4 are lost, while the virus surface proteins VP4 and VP7 rearrange to form the outermost virus protein layer, yielding mature infectious triple-layered particles (see Swiss Institute of Bioinformatics (ViralZone): viralzone.expasy.org/viralzone/all_by_species/107.html)

[0080] **Figure 2** shows rotavirus-like particle purification by ultracentrifugation on iodixanol density gradient. **Figure 2A** presents the percentage of iodixanol and

volume for each layer of the gradient used for the purification of rotavirus-like particles. After centrifugation, the gradient was fractionated into 1 ml fractions starting from the bottom of the tube. The approximate localization of fractions 1 to 13 are indicated by arrows. **Figure 2B** shown a Coomassie-stained SDS-PAGE analysis of the protein content of fractions 1 to 10 from an iodixanol density gradient separation applied to a crude protein extract from leaves expressing rotavirus VP2, VP4, VP6 and VP7 antigens.

[0081] **Figure 3** shows rotavirus protein expression. **Figure 3A** shows a Coomassie-stained SDS-PAGE analysis of fractions 2 and 3 from an iodixanol density gradient separation applied to crude protein extracts from leaves expressing VP2, VP4, VP6, VP7 in the presence or absence of NSP4. Rotavirus structural proteins VP2, VP6, VP7, VP4 were expressed, left panel, using individual constructs for each structural antigen ("single gene constructs"), with NSP4 on a separate construct ; middle panel, two constructs, each having the genes of two structural antigens ("dual gene constructs"), with NSP4 on a separate construct ; or right panel, a single construct for the co-expression of the four structural antigens (Quadruple gene constructs) , with a separate construct for the expression of NSP4. Position of the rotavirus VP2 and VP6 antigen are shown by arrows. **Figure 3B** shows a Western blot analysis of fraction F2 from the same treatments as in figure 3A using an anti-rotavirus VP4 or VP7 antibody as specified.

[0082] **Figure 4** shows rotavirus protein expression in the presence of an expression enhancer. **Figure 4A** shows a Coomassie-stained SDS-PAGE analysis of fractions F2 and F3 from an iodixanol density gradient applied to crude protein extracts from leaves co-expressing VP2, VP4, VP6, VP7 and NSP4 . Rotavirus structural proteins VP2, VP6, VP7, VP4 were expressed from single gene constructs, and one construct expressing NSP4 (left panel), or from two dual gene constructs and one construct expressing NSP4 (middle and right panel)). Each construct comprised an expression enhancer, either CPMV HT (left and middle panels) or CMPV 160 (right panel), except for NSP4 which always comprised the CPMV-HT enhancer. The ratios indicate the proportion of the *Agrobacterium* strains in the bacterial suspension used for transformation: left panel - five single gene constructs (VP2, VP6, VP4, VP7 and NSP4; ratio of 1:1:1:1:1); middle and right panels – dual gene constructs encoding

structural proteins (VP6/2 and VP7/4) and the construct encoding non-structural protein (NSP4; ratio of 1:1:1). **Figure 4B** shows a Western blot analysis of F2 from the same treatments as in Figure 4A using an anti-rotavirus VP4 or VP7 antibodyas specified. The ratios indicate the proportion of the *Agrobacterium* strains in the bacterial suspension used for transformation: left panel - five single gene constructs (VP2, VP6, VP4, VP7 and NSP4; ratio of 1:1:1:1:1); middle and right panels – dual gene constructs encoding structural proteins (VP6/2 and VP7/4) and the construct encoding non-structural protein (NSP4; ratio of 1:1:1).

[0083] **Figure 5** shows rotavirus protein expression. Upper panel shows a Coomassie-stained SDS-PAGE analysis of fractions F2 and F3 an iodixanol density gradient applied to crude protein extracts from leaves co-expressing VP2, VP4, VP6, VP7 and NSP4, and the lower panel shows a Western blot analysis of the corresponding F2 fraction from the upper panel. Rotavirus structural proteins VP2, VP6, VP7 and VP4 were expressed within a quadruple gene construct, and the non-structural protein NSP4 was co-expressed from a distinct single gene construct (lanes 1 and 2), or structural proteins VP2, VP6, VP7, VP4 and the non-structural protein NSP4 were expressed within a quintuple gene construct (lane 3). The ratio of agroinfiltration of the constructs is indicated. An OD of 0.4 of *Agrobacterium* strains in the bacterial suspension is indicated as 1. An OD of 0.6 of *Agrobacterium* strains in the bacterial suspension is indicated as 1.5.

[0084] **Figure 6** shows a general schematic of an example of several enhancer sequences that may be used in the constructs of the present invention**. Figure 6A** and **Figure 6B** show a general schematic of the CPMV HT and CPMV HT+ enhancer sequences fused to a nucleotide sequence of interest (for example encoding a rotavirus structural protein VP2, VP4, VP6, VP7, or a non-structural protein NSP4). Not all of the elements shown in Figures 5A or 5B may be required within the enhancer sequence. Additional elements may be included at the 3'end of the nucleotide sequence of interest including a sequence encoding a comovirus 3' untranslated region (CPMV 3' UTR), or a plastocyanin 3' UTR (3'UTR). **Figure 6C and 6D** show a general schematic of the enhancer sequence of CPMVX, and CPMVX+ (comprising CPMVX, and a stuffer fragment, which in this non-limiting example, comprises a multiple cloning site and a plant kozak sequence), as described

herein. CPMVX and CPMVX+ are each shown as operatively linked to plant regulatory region at their 5'ends, and at their 3' ends, in series, a nucleotide sequence of interest (including an ATG initiation site and STOP site), a 3'UTR, and a terminator sequence. An example of construct CPMVX as described herein, is CPMV160. An example of construct CPMVX+ as described herein, is CPMV160+.

[0085] **Figure 7** shows sequence components used to prepare construct number 1710 (2X35S/CPMV-HT/ RVA(WA) VP2(opt)/ NOS. **Figure 7A** shows the nucleotide sequence of IF-WA_VP2(opt).s1+3c (SEQ ID NO: 19). **Figure 7B** shows the nucleotide sequence of IF-WA_VP2(opt).s1-4r (SEQ ID NO: 20). **Figure 7C** shows the optimized coding sequence of Rotavirus A VP2 from strain WA (SEQ ID NO: 21). **Figure 7D** shows the schematic representation of construct 1191. **Figure 7E** shows the nucleotide sequence of construct 1191 (SEQ ID NO: 22). **Figure 7F** shows the nucleotide sequence of expression cassette number 1710 (SEQ ID NO: 23). **Figure 7G** shows the amino acid sequence of VP2 from Rotavirus A WA strain (SEQ ID NO: 24). **Figure 7H** shows the schematic representation of construct number 1710.

[0086] **Figure 8** shows sequence components used to prepare construct number 1713 (2X35S/CPMV-HT/RVA(WA) VP6(opt)/NOS). **Figure 8A** shows the nucleotide sequence of IF-WA_VP6(opt).s1+3c (SEQ ID NO: 25). **Figure 8B** shows the nucleotide sequence of IF-WA_VP6(opt).s1-4r (SEQ ID NO: 26). **Figure 8C** shows the optimized coding sequence of Rotavirus A VP6 from strain WA (SEQ ID NO: 217. **Figure 8D** shows the nucleotide sequence of expression cassette number 1713 (SEQ ID NO: 28). **Figure 8E** shows the amino acid sequence of VP6 from Rotavirus A WA strain (SEQ ID NO: 29). **Figure 8F** shows the schematic representation of construct number 1713.

[0087] **Figure 9** shows sequence components used to prepare construct number 1730 (2X35S/CPMV-HT/ RVA(Rtx) VP4(opt)/ NOS). **Figure 9A** shows the nucleotide sequence of IF-Rtx_VP4(opt).s1+3c (SEQ ID NO: 30). **Figure 9B** shows the nucleotide sequence of IF-Rtx_VP4(opt).s1-4r (SEQ ID NO: 31). **Figure 9C** shows the optimized coding sequence of Rotavirus A VP4 from strain RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8] (SEQ ID NO:32). **Figure**

9D shows the nucleotide sequence of expression cassette number 1730 (SEQ ID NO: 33). **Figure 9E** shows the amino acid sequence of VP4 from Rotavirus A Rotarix strain (SEQ ID NO: 34). **Figure 9F** shows the schematic representation of construct number 1730.

[0088] **Figure 10** shows sequence components used to prepare construct number 1734 (2X35S/CPMV-HT/RVA(Rtx) VP7(Opt)/NOS). **Figure 10A** shows the nucleotide sequence of IF-TrSP+Rtx_VP7(opt).s1+3c (SEQ ID NO: 35). **Figure 10B** shows the nucleotide sequence of IF-Rtx_VP7(opt).s1-4r (SEQ ID NO: 36). **Figure 10C** shows the optimized coding sequence of Rotavirus A VP7 from strain RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8] (SEQ ID NO: 37). **Figure 10D** shows the nucleotide sequence of expression cassette number 1734 (SEQ ID NO: 38). **Figure 10E** shows the amino acid sequence of TrSp-VP7 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain (SEQ ID NO: 39). **Figure 10F** shows the schematic representation of construct number 1734.

[0089] **Figure 11** shows sequence components used to prepare construct number 1706 (2X35S/CPMV-HT/RVA(WA) NSP4/NOS). **Figure 11A** shows the nucleotide sequence of IF-WA_NSP4.s1+3c (SEQ ID NO: 40). **Figure 11B** shows the nucleotide sequence of IF-WA_NSP4.s1-4r (SEQ ID NO: 41). **Figure 11C** shows the coding sequence of Rotavirus A VP6 from strain WA (SEQ ID NO: 42). **Figure 11D** shows the nucleotide sequence of expression cassette number 1706 (SEQ ID NO: 43). **Figure 11E** shows the amino acid sequence of NSP4 from Rotavirus A WA strain (SEQ ID NO: 44). **Figure 11F** shows the schematic representation of construct number 1706.

[0090] **Figure 12** shows sequence components used to prepare construct number 1108 (2X35S/CPMV-160/ RVA(WA) VP2(opt)/ NOS). **Figure 12A** shows the nucleotide sequence of IF(C160)-WA_VP2(opt).c (SEQ ID NO: 45). **Figure 12B** shows a schematic representation of construct 1190. **Figure 12C** shows the nucleotide sequence of construct 1190 (SEQ ID NO: 46). **Figure 12D** shows the nucleotide sequence of expression cassette number 1108 (SEQ ID NO: 47). **Figure 12E** shows a schematic representation of construct number 1108.

[0091] **Figure 13** shows sequence components used to prepare construct number 1128 (2X35S/CPMV-160/RVA(WA) VP6(opt)/NOS). **Figure 13A** shows the nucleotide sequence of IF(C160)-WA_VP6(opt).c (SEQ ID NO: 48). **Figure 13B** shows the nucleotide sequence of expression cassette number 1128 (SEQ ID NO: 49). **Figure 13C** shows a schematic representation of construct number 1128.

[0092] **Figure 14** shows sequence components used to prepare construct number 1178 (2X35S/CPMV-160/ RVA(Rtx) VP4(opt)/ NOS). **Figure 14A** shows the nucleotide sequence of IF(C160)-Rtx_VP4(opt).c (SEQ ID NO: 50). Figure 14B shows the nucleotide sequence of expression cassette number 1178 (SEQ ID NO: 51). **Figure 14C** shows the schematic representation of construct number 1178.

[0093] **Figure 15** shows sequence components used to prepare construct number 1199 (2X35S/CPMV-160/TrSp-RVA(Rtx) VP7(Opt)/NOS). **Figure 15A** shows the nucleotide sequence of IF(C160)-TrSP+Rtx_VP7(opt).c (SEQ ID NO: 52). **Figure 15B** shows the nucleotide sequence of Expression cassette number 1199 (SEQ ID NO: 53). **Figure 15C** shows the schematic representation of construct number 1199.

[0094] **Figure 16** shows the schematic representation of construct number 1708 (double gene construct for the expression of VP6 and VP2 under CPMV-HT expression cassette).

[0095] **Figure 17** shows the schematic representation of construct number 1719 (double gene construct for the expression of VP7 and VP4 under CPMV-HT expression cassette).

[0096] **Figure 18** shows the schematic representation of construct number 2400 (double gene construct for the expression of VP6 and VP2 under CPMV-160 expression cassette).

[0097] **Figure 19** shows the schematic representation of construct number 2408 (double gene construct for the expression of VP7 and VP4 under CPMV-160 expression cassette).

[0098] **Figure 20** shows the schematic representation of construct number 1769 (quadruple gene construct for the expression of VP7, VP4, VP6 and VP2 under CPMV-HT expression cassette).

[0099] **Figure 21** shows the schematic representation of construct number 2441 (quintuple gene construct for the expression of VP4, VP7, NSP4, VP6 and VP2 under CPMV-HT expression cassette).

DETAILED DESCRIPTION

[00100] The following description is of a preferred embodiment.

[00101] The present invention relates to virus-like particles (VLPs) comprising one or more rotavirus structural protein (i.e. a rotavirus like particle, rotavirus VLP or RLP), and methods of producing rotavirus-like particle (RLPs) in any host, particularly in plants, a portion of a plant, or a plant cell. Other hosts might comprise, for example, insect cells and mammalian cells. The rotavirus like particle (RLP) may comprise one or more rotavirus structural protein. The RLP may triple layered. The RLP may be produced by co-expressing rotavirus structural and nonstructural proteins in plant, however, the RLP does not comprise any rotavirus nonstructural proteins.

[00102] The host or host cell may be from any source including plants, fungi, bacteria, insect and animals. In a preferred embodiment the host or host cell is a plant or plant cell.

[00103] The present invention in part provides further a method of producing a rotavirus-like particle (RLP) in a host, such as a plant, a portion of a plant, or a plant cell. The method may comprise introducing one or more nucleic acid comprising a regulatory region active in the host, such as a plant, a portion of a plant, or a plant cell, the regulatory region operatively linked to a nucleotide sequence encoding one or more rotavirus structural protein and one or more rotavirus nonstructural protein into the host, such as into a plant, portion of the plant, or plant cell. Followed by incubating the host, such as a plant, portion of the plant, or plant cell under conditions that permit the expression of the nucleic acids, thereby producing the RLP comprising one or more rotavirus structural protein. The one or more rotavirus structural protein

may be rotavirus protein VP2, VP4, VP6 or VP7. The rotavirus nonstructural protein may be NSP4. The RLP may be triple layered. The RLP may comprise rotavirus structural protein VP2, VP4, VP6 and VP7, and does not comprise the nonstructural protein NSP4.

[00104] The present invention in part provides further a method of producing a rotavirus like particle (RLP) in a host or host cell, the method may comprise:

providing a host or host cell comprising one or more nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second and third nucleotide sequence encoding NSP4 and one or two of rotavirus protein VP2 or VP6 and one or two of rotavirus protein VP7 or VP4;

incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that NSP4 and either VP2, VP4 and VP7, or VP2, VP6 and VP7, or VP2, VP6 and VP4, or VP6, VP4 and VP7, are expressed, thereby producing the RLP.

[00105] Furthermore, the one or more nucleic acid may comprise a fourth nucleotide sequence encoding a fourth rotavirus protein. The first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and the first nucleotide sequence, the second nucleotide sequence, the third and fourth nucleotide sequence encoding rotavirus protein VP2, or VP6, VP4 or VP7 and NSP4, wherein NSP4 and wherein either VP2 or VP6 and VP4 or VP7 are expressed from the one or more nucleic acid.

[00106] Furthermore, the present invention in part provides a method of producing a rotavirus-like particle (RLP) vaccine candidate in a host, such as a plant, a portion of the plant, or a plant cell. The method may comprise expressing in a host, such as in a plant or portion of a plant, one or more nucleic acid (R_1-R_5) comprising one or more regulatory region active in the host, such as in the plant, portion of a plant, or plant cell, the regulatory region operatively linked to nucleotide sequences R_1-R_5, wherein

nucleotide sequence R_1 encodes rotavirus protein X_1, nucleotide sequence R_2 encodes rotavirus protein X_2, nucleotide sequence R_3 encodes rotavirus protein X_3, nucleotide sequence R_4 encodes rotavirus protein X_4 and nucleotide sequence R_5 encodes rotavirus protein X_5 and each of X_1-X_5 are selected from the group of rotavirus protein VP2, VP4, VP6, VP7 and NSP4, so that each of VP2, VP4, VP6, VP7 and NSP4 are expressed in the host, such as in the plant, portion of the plant, or plant cell (see Table 1). The RLP may comprise rotavirus structural protein VP2, VP4, VP6 and VP7. The RLP does not comprise nonstructural protein NSP4.

[00107] It has been found that by introducing and co-expressing rotavirus structural protein and a rotavirus non-structural protein in the host, such as a plant or portion of the plant that the yield of the RLP produced may be modulated. In particular, it has been found that by co-expressing rotavirus structural proteins along with a rotavirus non-structural protein NSP4 in the host, such as a plant, portion of the plant, or plant cell, that the incorporation of structural protein VP4 ,VP7 or both VP4 and VP7 into the RLP may be increased, when compared to the level of VP4 and VP7 produced by a second host, such as a second plant, portion of a second plant, or second plant cell that expresses the same rotavirus structural proteins but that does not express the rotavirus non-structural protein, under the same conditions.

[00108] For example a method of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) is provided. The method comprises:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein, a third nucleotide sequence encoding a third rotavirus protein and a fourth nucleotide sequence encoding a fourth rotavirus protein; the first, second, third and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third and fourth nucleotide sequence encoding one of rotavirus protein VP7, VP4, NSP4 and VP2 or VP6;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of the VP7, VP4, NSP4 and VP2 or VP6 and are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[00109] Furthermore, an alternative method of increasing production of VP4 ,VP7 or both VP4 and VP7 in a rotavirus like particle (RLP) may comprises:

a) introducing into a plant, portion of a plant or plant cell one or more nucleic acid comprising one or more regulatory operatively linked to a first, second, third, fourth and fifth nucleotide sequence, the one or more regulatory region active in the plant, portion of the plant or the plant cell, the first nucleotide sequence encoding a first rotavirus protein, the second nucleotide sequence encoding a second rotavirus protein, the third nucleotide sequence encoding a third rotavirus protein, the fourth nucleotide sequence encoding a fourth rotavirus protein and the fifth nucleotide sequence encoding a fifth rotavirus protein, each of the first, second, third, fourth or fifth nucleotide sequence encoding one of VP2, VP4, VP6, VP7 or NSP4, and

b) incubating the plant, portion of a plant or plant cell under conditions that permit the transient expression of the one or more nucleic acid so that each of VP2, VP4, VP6, VP7 and NSP4 are transiently expressed, thereby producing the RLP with enhanced levels of VP4 and VP7 when compared to the level of VP4 and VP7 produced by a second plant, portion of a second plant, or second plant cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[00110] If desired, the method may further comprises the steps of:

c) harvesting the plant, portion of a plant or plant cell, and

d) purifying the RLPs from the plant, portion of a plant or plant cell, wherein the RLPs range in size from 70-100 nm.

[00111] An alternate method of increasing production of VP4 ,VP7 or both VP4 and VP7 in a rotavirus like particle (RLP) is also provided, the method comprising:

a) providing a plant, portion of a plant or plant cell comprising one or more nucleic acid comprising one or more regulatory region operatively linked to a first, second, third, fourth and fifth nucleotide sequence, the one or more regulatory region active in the plant, portion of the plant or the plant cell, the first nucleotide sequence encoding a first rotavirus protein, the second nucleotide sequence encoding a second rotavirus protein, the third nucleotide sequence encoding a third rotavirus protein, the fourth nucleotide sequence encoding a fourth rotavirus protein and the fifth nucleotide sequence encoding a fifth rotavirus protein, each of the first, second, third, fourth and fifth nucleotide sequence encoding one of VP2, VP4, VP6, VP7 or NSP4, and

b) incubating the plant, portion of a plant or plant cell under conditions that permit the expression of the one or more nucleic acid, so that each of the VP2, VP4, VP6, VP7 and NSP4 are expressed, thereby producing the RLP with enhanced levels of VP4 and VP7 when compared to the level of VP4 and VP7 produced by a second plant, portion of a second plant, or second plant cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

[00112] It has been further found that by modulating the ratio between constructs comprising nucleic acids encoding rotavirus structural proteins and the non-structural protein NSP4 during transient expression in the host, such as a plant, portion of the plant, or plant cell, the yield of RLP production and the incorporation of structural proteins VP4 and VP7 into the RLP may be improved.

[00113] Without wishing to be bound by theory, the co-expression of a rotavirus non-structural protein for example NSP4 together with one or more rotavirus structural protein for example VP2, VP4, VP6 and/or VP7 may lead to an increase in incorporation of VP4 and/or VP7 into RLPs. This increase of incorporation of VP4 and/or VP7 into RLPs may occur via an increase of expression or production of the rotavirus proteins and/or because of an increase of the efficacy of the assembly of RLPs and/or an increase of recruiting of the rotavirus proteins at the RLP assembly site.

[00114] As shown in Figure 3A and 3B, the co-expression of rotavirus non-structural protein NSP4 together with rotavirus structural protein VP2, VP6, VP4 and VP7 in plants lead to an increase in incorporation of VP4 and VP7 into RLPs, when compared to the expression of structural protein VP2, VP6, VP4 and VP7 without the presence of NSP4 (see Figure 3B).

[00115] The ratios of the constructs encoding the various structural and non-structural proteins that are transiently expressed in the host, such as a plant, portion of the plant, or plant cell, may be altered by providing constructs comprising nucleic acid sequences encoding rotavirus structural proteins and the non-structural protein NSP4 on two, three, four or five constructs and varying the amount of each construct during the step of introducing the construct in the host (using Agrobacterium to the plant, plant portion or plant cell). For example, five separate constructs, each encoding one structural protein and the non-structural protein, may be co-introduced at various ratios resulting co-expression of the nucleic acids at various ratios within a plant, plant portion, or plant cell. Alternatively, the nucleic acid sequences may be provided in various combinations on two, three or four constructs and the constructs co-introduced in the plant, portion of the plant, or plant cell, at various ratios, as described below.

[00116] Additionally, the nucleic acid sequences may be provided on the same construct.

[00117] "Rotavirus protein" may refer to rotavirus structural protein or rotavirus nonstructural proteins. A "rotavirus structural protein" may refer to all or a portion of a rotavirus structural protein isolated from rotavirus, present in any naturally occurring, or a variant of any naturally occurring, rotavirus strain or isolate. Thus, the term rotavirus structural protein includes a naturally occurring rotavirus structural protein, or a variant of a rotavirus structural protein that may be produced by mutation during the virus life-cycle or produced in response to selective pressure (e.g., drug therapy, expansion of host cell tropism, or infectivity, etc.). As one of skill in the art appreciates, such rotavirus structural proteins and variants thereof may be also produced using recombinant techniques. Rotavirus structural proteins may include capsid proteins such for example VP2 and VP6, surface proteins, for example VP4, or

a combination of capsid and surface proteins. The structural protein may further include for example VP7.

[00118] By rotavirus “non structural protein”, “nonstructural protein”, “non-structural protein”, “NSP” or “nonstructural rotavirus protein” it is meant a protein that is encoded by the rotavirus genome, but not packaged into the viral particle. Non-limiting examples of rotavirus nonstructural proteins are rotavirus NSP4.

[00119] By “co-expressed” it is meant that two, or more than two, nucleotide sequences are expressed at about the same time within the plant, within the same tissue of the plant and within the same cells in the plant. The nucleotide sequences need not be expressed at exactly the same time. Rather, the two or more nucleotide sequences are expressed in a manner such that the encoded products have a chance to interact within a desired cellular compartment. For example, the non-structural protein may be preferably expressed either before or during the period when the structural proteins are expressed. The two or more than two nucleotide sequences can be co-expressed using a transient expression system, where the two or more sequences are introduced within the plant at about the same time, under conditions that the two or more sequences are expressed. The two or more than two sequences may be present on different constructs, and co-expression requires introduction of each of the constructs into the plant, portion of plant or plant cell, or the two or more than two sequences may be present on one construct and the construct introduced into the plant, portion of plant or plant cell.

[00120] The term "virus-like particle" (VLP), or "virus-like particles" or "VLPs" refers to structures that self-assemble and comprise one or more structural proteins such as for example rotavirus structural protein, for example but not limited to VP2, VP4, VP6, VP7, or a combination of VP2, VP4, VP6, VP7, structural protein. VLPs comprising rotavirus structural protein maybe also be referred to “rotavirus VLP”, "rotavirus -like particle (RVLP)", "rotavirus -like particle (RLP)" , "rotavirus -like particle”, “RVLP” or “RLP” . VLPs or RLPs are generally morphologically and antigenically similar to virions produced in an infection, but lack genetic information sufficient to replicate and thus are non-infectious. VLPs may be produced in suitable eukaryotic host cells including plant host cells. Following extraction from the host

cell and upon isolation and further purification under suitable conditions, VLPs may be recovered as intact structures. The RLP may be a single, double, or triple-layered RLP. Triple-layered RLPs may be obtained by the simultaneous expression of three or more rotavirus structural proteins, and as described herein, co-expression with one or more non-structural protein. For example, the co-expression of structural proteins VP2, VP6, VP7, VP4 and nonstructural protein NSP4 results in producing triple-layered RLPs.

[00121] Co-expression of VP4, along with VP2, VP6, VP7, and one or more non-structural protein as required, results in a particle with spikes that resembles native rotavirus. VP4 may be processed or cleaved to produce VP5 and VP8. This processing may take place within the host using endogenous proteases, or by co-expressing a suitable protease, for example, trypsin, a trypsin-like protease, a serine protease, a chymotrypsin-like protease, subtilisin. Alternatively, VP4 may be processed to produce VP5 and VP8 by adding a suitable protease, for example, trypsin, a trypsin-like protease, a serine protease, a chymotrypsin-like protease, subtilisin during any step of the RLP extraction procedure, or after RLP purification.

[00122] Each of the rotavirus structural proteins has different characteristics and size, and is required in different amounts for assembly into RLP. The term “rotavirus VLP”, "rotavirus virus-like particle (RVLP)", "rotavirus virus-like particle (RLP)" , "rotavirus virus-like particle”, “RVLP” or “RLP” refers to a virus-like particle (VLP) comprising one or more rotavirus structural proteins. Example of rotavirus structural proteins may include, but are not limited to VP2, VP4 (or VP5 and VP8) VP6 and VP7 structural protein. The RLP may not comprise rotavirus nonstructural proteins.

[00123] The present invention provides for a method of producing RLPs in a plant, wherein one or more nucleic acid (N_1-N_5) comprising one or more regulatory region active in the plant are operatively linked to nucleotide sequences R_1-R_5, wherein nucleotide sequence R_1 encodes rotavirus protein X_1, nucleotide sequence R_2 encodes rotavirus protein X_2, nucleotide sequence R_3 encodes rotavirus protein X_3, nucleotide sequence R_4 encodes rotavirus protein X_4 and nucleotide sequence R_5 encodes rotavirus protein X_5 and wherein X_1-X_5 are selected from the group of rotavirus

protein VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are each selected once (see Table 1). The final set, or combination, of nucleic acids used to transform the host results in the expression of each rotavirus protein within the host resulting in expression of VP2, VP4, VP6, VP7 and NSP4 and formation of an RLP..

[00124] For example, with reference to Table 1, 2 nucleic acids (N_1 and N_2), may be used to transform a host, (see example # 2.1). In this case N_1 comprises the R_1 nucleotide sequence and R_1 may encode one of VP2, VP4, VP6, VP7, or NSP4. The nucleic acid N_2 comprises four sequences R_2 to R_5, each of R_2 to R_5 encoding one of VP2, VP4, VP6, VP7, or NSP4, but not the protein encoded by R_1, so that each of the VP2, VP4, VP6, VP7 and NSP4 are expressed within the host, thereby producing the RLP. As a non-limiting example, N_1 may comprise R_1 which may encode VP2, and N_2 may comprise R_2 to R_5 which may encode VP4, VP6, VP7 and NSP4 respectively.

[00125] Table 1, provides an overview of combinations, which is not to be considered limiting, of nucleic acids (N), and nucleotide sequences (R) that may be expressed or co-expressed within a host to produce an RLP comprising VP2, VP4, VP6, and VP7.

[00126] Table 1

Combination #							Total # Nucleic Acids
1.1	Nucleic Acids	N_1					1
1.2		N_1					1
1.3		N_1					1
2.1		N_1	N_2				2
2.2		N_1	N_2				2
2.3		N_1	N_2				2
2.4		N_1	N_2				2
2.5		N_1	N_2				2
3.1		N_1	N_2	N_3			3
3.2		N_1	N_2	N_3			3
3.3		N_1	N_2	N_3			3
3.4		N_1	N_2	N_3			3
4.1		N_1	N_2	N_3	N_4		4
4.2		N_1	N_2	N_3	N_4		4
5		N_1	N_2	N_3	N_4	N_5	5
	Nucleotide Sequence	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	
	Rotavirus Protein	**X_1**	**X_2**	**X_3**	**X_4**	**X_5**	**Protein type**
	$X_{(1-5)}$ may be*	VP2	VP2	VP2	VP2	VP2	Structural
		VP4	VP4	VP4	VP4	VP4	Structural
		VP6	VP6	VP6	VP6	VP6	Structural
		VP7	VP7	VP7	VP7	VP7	Structural
		NSP4	NSP4	NSP4	NSP4	NSP4	Non-structural

**For combinations 1.1, 2.1, 2.2, 3.1, 3.2, 4.1 and 5: X_1, X_2, X_3, X_4 and X_5 each have to be a different rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4.*

For combination 1.3, 2.5 and 3.4: X_1, X_2 and X_3 each have to *be a different rotavirus protein selected from either VP4, VP6 and NSP4 or VP7, VP6 and NSP4.*

For combination 1.2, 2.3, 2.4, 3.3 and 4.2: X_1, X_2, X_3 and X_4 each *have to be a different rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, VP2, VP7, VP6 and NSP4 or VP4, VP7, VP6 and NSP4.*

1. One Construct

1.1 Quintuple gene construct

[00127] As described herein, a method of producing RLPs in a plant is provided, wherein a nucleic acid (N_1; a first nucleic acid) comprising a first, second, third, fourth and fifth nucleotide sequences (R_1, R_2, R_3, R_4, R_5) encoding a first, second, third, fourth and fifth rotavirus protein (X_1, X_2, X_3, X_4, X_5) is expressed in a plant or portion of a plant (See Table 1, Combination #1.1).

[00128] Accordingly, nucleic acid N_1 comprises nucleotide sequences R_1, R_2, R_3, R_4 and R_5, wherein R_1 encodes rotavirus protein X_1, where X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_2-R_5 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 and R_3-R_5 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 R_2, R_4, and R_5 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 – R_3 and R_5 encode a rotavirus protein that is not X_4, where X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 – R_4 encode a rotavirus protein that is not X_5, with the result that a nucleotide sequence encoding for each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is comprised on nucleic acid N_1, thereby allowing for the expression of each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 in the transformed host.

[00129] The nucleic acid may comprise a nucleotide sequence R_1, wherein R_1 may encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and nucleotide sequences R_2-R_5, wherein R_2-R_5 encode a rotavirus protein selected from VP2, VP4, VP6, VP7 or NSP4, and wherein the rotavirus protein is not encoded by R_1. For example which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP2 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP4, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP2. In another example which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP4 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP2, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP4. In yet another non-limiting example, nucleotide sequence R_1 may encode rotavirus protein VP6 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP7 and NSP4, but R_2-R_5 may not encode VP6. In yet another example which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP7 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and NSP4, but R_2-R_5 may not encode VP7. In yet another example which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein NSP4 and nucleotide sequence

R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and VP7. but R_2-R_5 may not encode NSP4.

[00130] For example, which is not to be considered limiting, the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP7, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP4, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus protein NSP4, a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein, for example rotavirus protein VP6 and a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein, for example rotavirus protein VP2.

[00131] In a further non-limiting example the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP4, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP7, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus protein NSP4, a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein, for example rotavirus protein VP6 and a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein, for example rotavirus protein VP2.

[00132] In a further non-limiting example the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP4, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP7, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus protein VP6, a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein, for example rotavirus protein VP2 and a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein, for example rotavirus protein NSP4.

[00133] In another non-limiting example the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP7, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP4, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus protein VP6, a fourth

nucleotide sequences (R_4) encoding a fourth rotavirus protein, for example rotavirus protein VP2 and a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein, for example rotavirus protein NSP4. (see Figure 5) A plant may be transformed with a single nucleic acid (N_1) comprising a first, second, third, fourth and fifth nucleotide sequences (R_1, R_2, R_3, R_4, R_5) encoding a first, second, third, fourth and fifth rotavirus protein, so that each of the first, second, third, fourth and fifth protein are expressed in the plant. The rotavirus proteins are selected from the group of rotavirus protein VP2, VP4, VP6, VP7 and NSP4, so that each of VP2, VP4, VP6, VP7 and NSP4 are expressed in the plant. The single nucleic acid may be introduced in the plant in a transient manner, or in a stable manner.

[00134] The VP4 may be processed or cleaved to produce VP5 and VP8 within the host by co-expressing a nucleic acid encoding a suitable protease, for example, trypsin, a trypsin-like protease, a serine protease, a chymotrypsin-like protease, subtilisin. Alternatively, VP4 may be processed during any step of RLP extraction, or after RLP purification by adding a sutible protease, for example, trypsin, a trypsin-like protease, a serine protease, a chymotrypsin-like protease, subtilisin.

1.2. Quadruple gene construct

[00135] As described herein, a method of producing RLPs in a plant is provided, wherein a nucleic acid (N_1; a first nucleic acid) comprising a first, second, third and fourth nucleotide sequences (R_1, R_2, R_3, R_4) encoding a first, second, third and fourth rotavirus protein (X_1, X_2, X_3, X_4) is expressed in a plant or portion of a plant (See Table 1, Combination #1.2).

[00136] Accordingly, nucleic acid N_1 may comprises nucleotide sequences (R_1, R_2, R_3, R_4), wherein R_1 encodes rotavirus protein X_1, where X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 –R_3 encode a

rotavirus protein that is not X_4, with the result that a nucleotide sequence encoding for each rotavirus protein VP2, VP4, VP6 and NSP4 is comprised on nucleic acid N_1, thereby allowing for the expression of each rotavirus protein VP2, VP4, VP6 and NSP4 in the transformed host.

[00137] For example, which is not to be considered limiting, the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP6, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP4, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus proteinVP2, and a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein, for example rotavirus protein NSP4.

[00138] Further accordingly, nucleic acid N_1 may comprises nucleotide sequences (R_1, R_2, R_3, R_4), wherein R_1 encodes rotavirus protein X_1, where X_1 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that a nucleotide sequence encoding for each rotavirus protein VP2, VP7, VP6 and NSP4 is comprised on nucleic acid N_1, thereby allowing for the expression of each rotavirus protein VP2, VP7, VP6 and NSP4 in the transformed host.

[00139] For example, which is not to be considered limiting, the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP6, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP7, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus proteinVP2, and a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein, for example rotavirus protein NSP4.

[00140] Further accordingly, nucleic acid N_1 may comprises nucleotide sequences (R_1, R_2, R_3, R_4), wherein R_1 encodes rotavirus protein X_1, where X_1 may be any rotavirus protein selected from the group of VP7, VP4, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP7, VP4, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP7, VP4, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP7, VP4, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that a nucleotide sequence encoding for each rotavirus protein VP7, VP4, VP6 and NSP4 is comprised on nucleic acid N_1, thereby allowing for the expression of each rotavirus protein VP7, VP4, VP6 and NSP4 in the transformed host.

[00141] For example, which is not to be considered limiting, the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP6, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP4, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus proteinVP7, and a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein, for example rotavirus protein NSP4.

1. 3 Triple gene construct

[00142] As described herein, a method of producing RLPs in a plant is provided, wherein a nucleic acid (N_1; a first nucleic acid) comprising a first, second and third nucleotide sequences (R_1, R_2, R_3) encoding a first, second and third rotavirus protein (X_1, X_2, X_3) is expressed in a plant or portion of a plant (See Table 1, Combination #1.3).

[00143] Accordingly, nucleic acid N_1 may comprises nucleotide sequences (R_1, R_2, R_3), wherein R_1 encodes rotavirus protein X_1, where X_1 may be any rotavirus protein selected from the group of VP4, VP6 and NSP4, and wherein R_2-R_3 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP4, VP6 and NSP4, and wherein R_1 and R_3 encode a rotavirus protein

that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP4, VP6 and NSP4, and wherein R_1 R_2 encode a rotavirus protein that is not X_3, with the result that a nucleotide sequence encoding for each rotavirus protein VP4, VP6 and NSP4 is comprised on nucleic acid N_1, thereby allowing for the expression of each rotavirus protein VP4, VP6 and NSP4 in the transformed host.

[00144] For example, which is not to be considered limiting, the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP6, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP4, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus protein.

[00145] Further accordingly, nucleic acid N_1 may comprises nucleotide sequences (R_1, R_2, R_3), wherein R_1 encodes rotavirus protein X_1, where X_1 may be any rotavirus protein selected from the group of VP7, VP6 and NSP4, and wherein R_2-R_3 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP7, VP6 and NSP4, and wherein R_1 and R_3 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP7, VP6 and NSP4, and wherein R_1 R_2 encode a rotavirus protein that is not X_3, with the result that a nucleotide sequence encoding for each rotavirus protein VP7, VP6 and NSP4 is comprised on nucleic acid N_1, thereby allowing for the expression of each rotavirus protein VP7, VP6 and NSP4 in the transformed host.

[00146] For example, which is not to be considered limiting, the nucleotide sequence (N_1) comprises a first nucleotide sequences (R_1) encoding a first rotavirus protein, for example rotavirus protein VP6, a second nucleotide sequences (R_2) encoding a second rotavirus protein, for example rotavirus protein VP7, a third nucleotide sequences (R_3) encoding a third rotavirus protein, for example rotavirus protein.

2. Two Constructs

2.1. Quadruple gene construct + single gene construct

[00147] The present invention also provides for a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), is co-expressed with a second nucleic acid (N_2)

comprising four nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein (X_2-X_5)(see Table 1, Combination #2.1), so that the first, second, third, fourth and fifth nucleotide sequence (R_1-R_5) are co-expressed in the plant.

[00148] In this non-limiting example, N_1 comprises nucleotide sequence (R_1) and N_2 comprises nucleotide sequences (R_2, R_3, R_4, R_5), wherein each rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4 is encoded in the combination of both constructs N_1 and N_2, and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_2-R_5 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 and R_3-R_5 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 R_2, R_4, and R_5 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_3 and R_5 encode a rotavirus protein that is not X_4, where X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_4 encode a rotavirus protein that is not X_5, with the result that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the host.

[00149] For example, which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP2 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP4, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP2. In another non-limiting example nucleotide sequence R_1 may encode rotavirus protein VP4 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP2, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP4. In yet another non-limiting example nucleotide sequence R_1 may encode rotavirus protein VP6 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP7 and NSP4, but R_2-R_5 may not encode VP6. In yet another example which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP7 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and NSP4, but R_2-R_5 may not encode VP7. In yet another non-limiting example nucleotide

sequence R_1 may encode rotavirus protein NSP4 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and VP7, but R_2-R_5 may not encode NSP4.

[00150] The first nucleic acid (N_1) and second nucleic acid (N_2) may be introduced into the plant in the same step, or may be introduced to the plant sequentially.

[00151] For example, which is not to be considered limiting, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP7, VP4, VP6 and VP2, respectively (see Figure 5).

[00152] In another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP4, VP7, VP6 and VP2, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP7, VP4, VP2 and VP6, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP4, VP7, VP2 and VP6, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP6, VP2, VP4 and VP7, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus

protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP6, VP2, VP7 and VP4, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP2, VP4, VP6 and VP7, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example NSP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example VP7, VP6, VP4 and VP2, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example VP7, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example NSP4, VP2, VP6 and VP4, respectively. In yet another non-limiting example, a first nucleotide sequence (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein, for example VP4, is co-expressed with a second nucleic acid (N_2) comprising four sequential nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example NSP4, VP2, VP6 and VP7, respectively.

[00153] A plant that expresses a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), may be transformed with a second nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein (X_2-X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. Furthermore, a plant that expresses a first nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a rotavirus protein X_2-X_5 may be transformed with a second nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a rotavirus protein (X_1), so that the first and second nucleotide sequences R_1-R_5 are co-expressed in the plant. The rotavirus protein X_1 may be any rotavirus protein selected from the group of VP2,

VP4, VP6, VP7 and NSP4, and rotavirus proteins X_2-X_5 may be any rotavirus protein selected from the group of VP2, VP_4, VP6, VP7 and NSP4, but not X_1, so that each rotavirus protein VP2, VP4, VP_6, VP7 and NSP4 is expressed. Furthermore, a plant may be simultaneously co-transformed with a first nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a rotavirus protein X_2-X_5, and with a second nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a rotavirus protein (X_1), so that the first and second nucleotide sequences R_1-R_5 are co-expressed in the plant. The rotavirus protein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and rotavirus proteins X_2-X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, but not the protein selected for X_1, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed. The first nucleic acid (N_1) and second nucleic acid (N_2) may be introduced in the plant in a transient manner, or in a stable manner.

[00154] For example, which is not to be considered limiting, a plant that expresses a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) for example NSP4, may be transformed with a second nucleic acid encoding (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein (X_2-X_5), for example VP7, VP4, VP6 and VP2, so that NSP4, VP7, VP4, VP6 and VP2 are co-expressed in the plant.

[00155] A first plant expressing a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), may be crossed with a second plant expressing the second nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein (X_2-X_5) to produce a progeny plant (third plant) that co-expresses the first, second, third, fourth and fifth rotavirus protein (X_1-X_5). Furthermore, a first plant expressing a first nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a rotavirus protein X_2-X_5 may be crossed with a second plant expressing a second nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a rotavirus protein (X_1), so that nucleotide sequences R_1-R_5 are co-expressed in the progeny plant. The rotavirus protein may be any rotavirus protein selected from the group of rotavirus protein VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that

each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the progeny plant.

[00156] The VP4 may be processed or cleaved to produce VP5 and VP8 within the host by co-expressing a nucleic acid encoding a suitable protease, for example, trypsin, a trypsin-like protease, a serine protease, a chymotrypsin-like protease, subtilisin. Alternatively, VP4 may be processed during any step of RLP extraction, or after RLP purification by adding a suitable protease, for example, trypsin, a trypsin-like protease, a serine protease, a chymotrypsin-like protease, subtilisin.

2.2 Triple gene construct + Dual gene construct

[00157] The present invention also provides for a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising two nucleotide sequences (R_1 and R_2) encoding a first rotavirus protein (X_1) and second rotavirus protein (X_2) respectively, is co-expressed with a second nucleic acid (N_2) comprising three nucleotide sequences (R_3-R_5) encoding a third, fourth and fifth rotavirus proteins (X_3-X_5) (see Table 1, Combination #2.2), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant.

[00158] In a non-limiting example, N_1 comprises nucleotide sequences (R_1, R_2) and N_2 comprises nucleotide sequences (R_3, R_4, R_5), wherein each rotavirus protein selected from the group of VP2, VP4, VP6, V_{P7} and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_2-R_5 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 and R_3-R_5 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 R_2, R_4, and R_5 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_3 and R_5 encode a rotavirus protein that is not X_4, where X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_4 encode a rotavirus protein that is not X_5, with the result that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the host.

[00159] Therefore, the first nucleic acid (N_1) may comprise a nucleotide sequence R_1, wherein R_1 may encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and a second nucleotide sequence R_2 which may encode any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, that is not encoded by R_1. The second nucleic acid (N_2) may comprise nucleotide sequences R_3-R_5, wherein R_3-R_5 encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4, but not rotavirus protein that are encoded by R_1 or R_2. For example, which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP2 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP4, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP2. In another non-limiting example nucleotide sequence R_1 may encode rotavirus protein VP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP6, VP7 and NSP4. In yet another example nucleotide sequence R_1 may encode rotavirus protein VP6 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP7 and NSP4. In yet another non-limiting example nucleotide sequence R_1 may encode rotavirus protein VP7 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and NSP4. In yet another non-limiting example nucleotide sequence R_1 may encode rotavirus protein NSP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and VP7.

[00160] A plant that expresses a first nucleic acid (N_1) comprising a first and second nucleotide sequence (R_1 + R_2) encoding a first and second rotavirus protein (X_1 + X_2), may be transformed with a second nucleic acid (N_2) comprising three nucleotide sequences (R_3-R_5) encoding a third, fourth and fifth rotavirus protein (X_3-X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. The first nucleic acid (N_1) and second nucleic acid (N_2) may be introduced in the plant in a transient manner, or in a stable manner.

[00161] Furthermore, a plant that expresses a first nucleic acid (N_2) comprising three nucleotide sequences (R_3-R_5) encoding a first, second and third rotavirus protein (X_3-X_5) may be transformed with a second nucleic acid (N_1) comprising a fourth nucleotide sequence (R_1) encoding a fourth rotavirus protein (X_1) and a fifth rotavirus protein (X_2) so that the first and second nucleic acids R_1-R_5 are co-expressed in the plant. The rotavirus protein X_1 may be any rotavirus protein selected from the group

of VP2, VP4, VP6, VP7 and NSP4, and rotavirus proteins X_2-X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, but not X_1, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed. For example, which is not to be considered limiting, a plant that expresses a first nucleic acid (N_2) comprising three nucleotide sequences (R_3-R_5) encoding a first, second and third rotavirus proteins (X_3-X_5), for example VP7, VP4 and VP6 may be transformed with a second nucleic acid encoding (N_1) comprising a fourth and a fifth nucleotide sequences (R_1 –R_2) encoding a fourth and a fifth rotavirus protein (X_1-X_2) for example VP2 and NSP4, so that NSP4, VP7, VP4, VP6 and VP2 are co-expressed in the plant. Furthermore, a plant may be simultaneously co-transformed with a first nucleic acid (N_1) comprising a first and second nucleotide sequence (R_1 + R_2) encoding a first and second rotavirus protein (X_1 + X_2), and a second nucleic acid (N_2) comprising three nucleotide sequences (R_3-R_5) encoding a third, fourth and fifth rotavirus protein (X_3-X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. The first nucleic acid (N_1) and second nucleic acid (N_2) may be introduced in the plant in a transient manner, or in a stable manner.

[00162] A first plant expressing a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) and a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), may be crossed with a second plant expressing the second nucleic acid (N_2) comprising three nucleotide sequences (R_3-R_5) encoding a third, fourth and fifth rotavirus protein (X_3-X_5) to produce a progeny plant (third plant) that co-expresses the first, second, third, fourth and fifth rotavirus protein (X_1-X_5). Furthermore, a first plant expressing a first nucleic acid (N_2) comprising three nucleotide sequences (R_3-R_5) encoding a rotavirus protein X_3-X_5 may be crossed with a second plant expressing a second nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a rotavirus protein (X_1) and a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), so that nucleotide sequences R_1-R_5 are co-expressed in the progeny plant. The rotavirus protein may be any rotavirus protein selected from the group of rotavirus protein VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the progeny plant.

2.3. Triple gene construct + Single gene construct

[00163] The present invention also provides for a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising one nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), is co-expressed with a second nucleic acid (N_2) comprising three nucleotide sequences (R_2-R_4) encoding a second, third and fourth rotavirus proteins (X_2-X_4), so that the first, second, third and fourth nucleotide sequence are co-expressed in the plant (See Table 1, Combination #2.3).

[00164] In a non-limiting example, N_1 comprises nucleotide sequence (R_1) and N_2 comprises nucleotide sequences (R_2, R_3, R_4), wherein each rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP4, VP6 and NSP4 is expressed in the host.

[00165] In a further non-limiting example, N_1 comprises nucleotide sequence (R_1) and N_2 comprises nucleotide sequences (R_2, R_3, R_4), wherein each rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP7, VP6 and NSP4 is expressed in the host.

[00166] In yet a further non-limiting example, N_1 comprises nucleotide sequence (R_1) and N_2 comprises nucleotide sequences (R_2, R_3, R_4), wherein each rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP4, VP7, VP6 and NSP4 is expressed in the host.

2. 4 Two Double gene constructs

[00167] The present invention also provides for a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising nucleotide sequences (R_1-R_2) encoding a first and a second rotavirus proteins (X_1-X_2), is co-expressed with a second nucleic acid (N_2) comprising two nucleotide sequences (R_3-R_4) encoding a third and fourth rotavirus proteins (X_3-X_4), so that the first, second, third and fourth nucleotide sequences are co-expressed in the plant (See Table 1, Combination #2.4).

[00168] In a non-limiting example, N_1 comprises nucleotide sequences (R_1, R_2) and N_2 comprises nucleotide sequences (R_3, R_4), wherein each rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP4, VP6 and NSP4 is expressed in the host.

[00169] In a further non-limiting example, N_1 comprises nucleotide sequences (R_1, R_2) and N_2 comprises nucleotide sequences (R_3, R_4), wherein each rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP7, VP6 and NSP4 is expressed in the host.

[00170] In yet a further non-limiting example, N_1 comprises nucleotide sequences (R_1, R_2) and N_2 comprises nucleotide sequences (R_3, R_4), wherein each rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP4, VP7, VP6 and NSP4 is expressed in the host.

2.5 Double gene construct + Single gene construct

[00171] The present invention also provides for a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising nucleotide sequences (R_1-R_2) encoding a first and a second rotavirus proteins (X_1-X_2), is co-expressed with a second nucleic acid (N_2) comprising a nucleotide sequence (R_3) encoding a third rotavirus proteins (X_3), so that the first, second and third nucleotide sequences are co-expressed in the plant (See Table 1, Combination #2.5).

[00172] In a non-limiting example, N_1 comprises nucleotide sequences (R_1, R_2) and N_2 comprises nucleotide sequence (R_3), wherein each rotavirus protein selected from the group of VP4, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP4, VP6 and NSP4, and wherein R_2-R_3 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP4, VP6 and NSP4, and wherein R_1 and R_3 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP4, VP6 and NSP4, and wherein R_1 and R_2 encode a rotavirus protein that is not X_3, with the result that each rotavirus protein VP4, VP6 and NSP4 is expressed in the host.

[00173] In a further non-limiting example, N_1 comprises nucleotide sequences (R_1, R_2) and N_2 comprises nucleotide sequence (R_3), wherein each rotavirus protein selected from the group of VP7, VP6 and NSP4 is encoded and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP7, VP6 and NSP4, and wherein R_2-R_3 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP7, VP6 and NSP4, and wherein R_1 and R_3 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP7, VP6 and NSP4, and wherein R_1 and R_2 encode a rotavirus protein that is not X_3, with the result that each rotavirus protein VP7, VP6 and NSP4 is expressed in the host.

3. Three Constructs

3.1 Two Dual gene constructs + one Single gene construct

[00174] The present invention also provides for a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) and a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), is co-expressed with a second nucleic acid (N_2) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3) and a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) and a third nucleic acid (N_3) comprising a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5) (see Table 1, Combination #3.1) so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant.

[00175] In this non-limiting example, N_1 comprises (R_1, R_2), N_2 comprises (R_3, R_4) and N_3 comprises (R_5), wherein each rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4 is encoded once and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_2-R_5 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 and R_3-R_5 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 R_2, R_4, and R_5 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_3 and R_5 encode a rotavirus protein that is not X_4, where X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_4 encode a rotavirus protein that is not X_5, with the result that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the host.

[00176] For example, the first nucleic acid (N_1) may comprise a nucleotide sequence R_1, wherein R_1 may encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and a second nucleotide sequence R_2 which may encode any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4 that is not encoded by R_1. The second nucleotide sequence may comprise nucleotide sequences R_3 and R_4, wherein R_3 encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4, but not a rotavirus protein that are encoded by R_1 or R_2, and R_4 encodes rotavirus protein VP2, VP4, VP6, VP7 or NSP4, but not a rotavirus protein that are encoded by R_1, R_2 or R_3. The third nucleotide sequence may comprise nucleotide sequences R_5, wherein R_5 encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4, but not a rotavirus protein that are encoded by R_1, R_2, R_2 or R_4, so that each of VP2, VP4, VP6, VP7 or NSP4 are expressed in a host.

[00177] For example which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP2 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP4, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP2. In another example nucleotide sequence R_1 may encode rotavirus protein VP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP6, VP7

and NSP4. In yet another example nucleotide sequence R_1 may encode rotavirus protein VP6 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP7 and NSP4. In yet another non-limiting example nucleotide sequence R_1 may encode rotavirus protein VP7 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and NSP4. In yet another non-limiting example nucleotide sequence R_1 may encode rotavirus protein NSP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and VP7.

[00178] For example, which is not to be considered limiting, a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein, for example VP6 and a second nucleotide sequence (R_2) encoding a second rotavirus protein, for example VP2, is co-expressed with a second nucleic acid (N_2) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein for example VP7 and a fourth nucleotide sequence (R_4) encoding a fourth rotavirus protein, for example VP4 and a third nucleic acid (N_3) comprising a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein for example NSP4. (see Figures 2 and 3).

[00179] A plant that expresses a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) and a second rotavirus protein (X_2), may be transformed with a second nucleic acid (N_2) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3) and a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4). The plant may be further transformed with a third nucleic acid (R_3) comprising a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. Rotavirus protein X_1-X_5 may be selected from the group of VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the plant. Furthermore, a plant may be simultaneously co-transformed with a first nucleic acid (N_1) comprising a first and second nucleotide sequence ($R_1 + R_2$) encoding a first and second rotavirus protein ($X_1 + X_2$), a second nucleic acid (N_2) comprising a third and a fourth nucleotide sequences ($R_3 + R_4$) encoding a third and a fourth rotavirus protein ($X_3 + X_4$), and a third nucleic acid (N_3) comprising a fifth nucleotide sequences (R_5) encoding a fifth

rotavirus protein (X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. The first nucleic acid (N_1), second nucleic acid (N_2) and third nucleic acid (N_3) may be introduced in the plant in a transient manner, or in a stable manner.

3.2 Two Single gene constructs + one Triple gene construct

[00180] An alternated method of producing RLPs in a plant is also provided, wherein a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) is co-expressed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) and a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) and a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5) (see Table 1, Combination #3.2) so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant.

[00181] In an alternate example, N_1 comprises (R_1), N_2 comprises (R_2) and N_3 comprises (R_3, R_4, R_5), wherein each rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4 is encoded once and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_2-R_5 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 and R_3-R_5 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 R_2, R_4, and R_5 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_3 and R_5 encode a rotavirus protein that is not X_4, where X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_4 encode a rotavirus protein that is not X_5, with the result that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the host.

[00182] For example which is not to be considered limiting, the first nucleic acid (N_1) may comprise a nucleotide sequence R_1, wherein R_1 may encode rotavirus protein

VP2, VP4, VP6, VP7 or NSP4 and a second nucleotide sequence R_2 which may encode any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4 that is not encoded by R_1. The second nucleotide sequence may comprise nucleotide sequences R_3 and R_4, wherein R_3 encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4, but not a rotavirus protein that are encoded by R_1 or R_2 and R_4 encodes rotavirus protein VP2, VP4, VP6, VP7 or NSP4, but not a rotavirus protein that are encoded by R_1, R_2 or R_3. The third nucleotide sequence may comprise nucleotide sequences R_5, wherein R_5 encode rotavirus protein VP2, VP4, VP6, VP7 or NSP4, but not a rotavirus protein that are encoded by R_1, R_2, R_3 or R_4, and wherein VP2, VP4, VP6, VP7 or NSP4 are encoded once. For example nucleotide sequence R_1 may encode rotavirus protein VP2 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP4, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP2. In another example nucleotide sequence R_1 may encode rotavirus protein VP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP6, VP7 and NSP4. In yet another example nucleotide sequence R_1 may encode rotavirus protein VP6 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP7 and NSP4. In yet another example nucleotide sequence R_1 may encode rotavirus protein VP7 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and NSP4. In yet another example nucleotide sequence R_1 may encode rotavirus protein NSP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and VP7.

[00183] A plant that expresses a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) may be transformed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) . The plant may be further transformed with a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) and a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. Rotavirus protein X_1-X_5 may be selected from the group of VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is

expressed in the plant. Furthermore, a plant may be simultaneously co-transformed with a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), and a third nucleic acid (N_3) comprising a third, a fourth and a five nucleotide sequences (R_3-R_5) encoding a third, a fourth and a fifth rotavirus protein (X_3-X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. The first nucleic acid (N_1), second nucleic acid (N_2) and third nucleic acid (N_3) may be introduced in the plant in a transient manner, or in a stable manner.

[00184] For example, a first plant expressing a first nucleic acid (N_1) comprising a first, second and third nucleotide sequence (R_1, R_2, and R_3) encoding a first, second and third rotavirus protein (X_1, X_2, and X_3) may be crossed with a second plant expressing a second nucleic acid (N_2) comprising a fourth nucleotide sequence (R_4) encoding a fourth rotavirus protein (X_4) to produce a progeny plant (third plant). The third plant may be crossed with a fourth plant expressing a third nucleic acid (N_3) comprising a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5) to produces a progeny plant that co-expresses the first, second, third, fourth and fifth rotavirus protein (X_1-X_5). Furthermore, a first plant expressing a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) may be crossed with a second plant expressing a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a rotavirus protein (X_2) to produce a progeny plan (third plant). The third plant may be crossed with a fourth plant expressing a third nucleic acid (N_3) comprising a third, fourth and fifth nucleotide sequence (R_3, R_4 and R_5) encoding a third, fourth and fifth rotavirus protein (X_3, X_4, and X_5) to produce a progeny plant that co-expresses the first, second, third, fourth and fifth rotavirus protein (X_1-X_5). Rotavirus protein X_1-X_5 may be selected from the group of VP2, VP4, VP6, VP7 and NSP4, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the plant.

3.3 Two Single gene constructs + one Dual gene construct

[00185] An alternated method of producing RLPs in a plant is also provided, wherein a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a first

rotavirus protein (X_1) is co-expressed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) and a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), and a fourth nucleotide sequence (R_4) encoding a fourth rotavirus protein (X_4), so that the first, second, third and fourth nucleotide sequence are co-expressed in the plant (See Table 1, Combination #3.3).

[00186] As a non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2) and N_3 comprises (R_3, R_4), wherein each rotavirus protein selected from the group of VP2, VP4, VP6, and NSP4 is encoded once and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP4, VP6, and NSP4 is expressed in the host.

[00187] As another non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2) and N_3 comprises (R_3, R_4), wherein each rotavirus protein selected from the group of VP2, VP7, VP6, and NSP4 is encoded once and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP7, VP6, and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP7, VP6, and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP7, VP6, and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP7, VP6, and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP6, VP7 and NSP4 is expressed in the host.

[00188] As a further non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2) and N_3 comprises (R_3, R_4), wherein each rotavirus protein selected from the group of VP4, VP7, VP6, and NSP4 is encoded once and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP4, VP7, VP6, and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP4, VP7, VP6, and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP4, VP7, VP6, and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP4, VP7, VP6, and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP4, VP7, VP6, and NSP4 is expressed in the host.

3.4 Three Single gene constructs

[00189] An alternated method of producing RLPs in a plant is also provided, wherein a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) is co-expressed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) and a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), so that the first, second and third nucleotide sequence are co-expressed in the plant (See Table 1, Combination #3.4).

[00190] As a non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2) and N_3 comprises (R_3), wherein each rotavirus protein selected from the group of VP4, VP6, and NSP4 is encoded once and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP4, VP6, and NSP4, and wherein R_2-R_3 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP4, VP6, and NSP4, and wherein R_1 and R_3 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP4, VP6, and NSP4, and wherein R_1 and R_2 encode a rotavirus protein that is not X_3, with the result that each rotavirus protein VP4, VP6, and NSP4 is expressed in the host.

[00191] As a further non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2) and N_3 comprises (R_3), wherein each rotavirus protein selected from the group of VP7, VP6, and NSP4 is encoded once and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP7, VP6, and NSP4, and wherein R_2-R_3 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP7, VP6, and NSP4, and wherein R_1 and R_3 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP7, VP6, and NSP4, and wherein R_1 and R_2 encode a rotavirus protein that is not X_3, with the result that each rotavirus protein VP7, VP6, and NSP4 is expressed in the host.

4. *Four Constructs*

4.1. Three single gene constructs + one dual gene construct

[00192] Also provided herein is a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) is co-expressed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), and fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) and a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5) (see Table 1, Combination #4.1) so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant.

[00193] In this example, N_1 comprises (R_1), N_2 comprises (R_2), N_3 comprises (R_3), and N_4 comprises (R_4, and R_5), wherein each rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4 is encoded, and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_2-R_5 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 and R_3-R_5 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 R_2, R_4, and R_5 encode a rotavirus protein that is

not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_3 and R_5 encode a rotavirus protein that is not X_4, where X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_4 encode a rotavirus protein that is not X_5, with the result that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the host.

[00194] The four nucleic acids may be introduced into a plant in any order. For example, which is not be considered limiting, a plant that expresses a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) may be transformed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) . The plant may be further transformed with a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3). The plant then may be further be transformed with a fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) and a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. Furthermore, a plant may be simultaneously co-transformed with a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), and a fourth nucleic acid (N_4) comprising a fourth and a fifth nucleotide sequences (R_4-R_5) encoding a fourth and a fifth rotavirus protein (X_4-X_5) so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. The first nucleic acid (N_1), second nucleic acid (N_2), third nucleic acid (N_3) and fourth nucleic acid (N_4) may be introduced in the plant in a transient manner, or in a stable manner.

[00195] Furthermore, a plant that expresses a first nucleic acid (N_4) comprising a first nucleotide sequence (R_4) encoding a first rotavirus protein (X_4) and a second nucleotide sequence (R_5) encoding a second rotavirus protein (X_5) may be transformed with a second nucleic acid (N_1) comprising a third nucleotide sequence (R_1) encoding a third rotavirus protein (X_1) . The plant may be further transformed

with a third nucleic acid (N_2) comprising a third nucleotide sequence (R_2) encoding a fourth rotavirus protein (X_3). The plant then may be further be transformed with a fourth nucleic acid (N_3) comprising a fifth nucleotide sequences (R_3) encoding a fifth rotavirus protein and a fifth nucleotide sequence (R_3) encoding a fifth rotavirus protein (X_3), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. Rotavirus protein X_1-X_5 may be selected from the group of VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the plant. The first nucleic acid (N_4), second nucleic acid (N_1), third nucleic acid (N_2) and fourth nucleic acid (N_3) may be introduced in the plant in a transient manner, or in a stable manner.

[00196] A first plant expressing a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), may be crossed with a second plant expressing a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) to produce a progeny plant (third plant). The third plant may be crossed with a fourth plant expressing a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3) to produces a progeny plant (fifth plant). The fifth plant may be crossed with a sixth plant expressing a fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) and a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein (X_5) to produce a progeny plant that co-expresses the first, second, third, fourth and fifth rotavirus protein (X_1-X_5).

[00197] Furthermore, a first plant expressing a first nucleic acid (N_4) comprising a first nucleotide sequence (R_4) encoding a first rotavirus protein (X_4) and a second nucleotide sequence (R_5) encoding a second rotavirus protein (X_5) may be crossed with a second plant expressing a second nucleic acid (N_2) comprising a third nucleotide sequence (R_1) encoding a third rotavirus protein (X_1) to produce a progeny plan (third plant). The third plant may be crossed with a fourth plant expressing a third nucleic acid (N_2) comprising a fourth nucleotide sequence (R_2) encoding a fourth rotavirus protein (X_2) to produce a progeny plant (fifth plant). The fifth plant may be crossed with a sixth plant expressing a fifth nucleotide sequence (R_3) encoding a fifth rotavirus protein (X_3) to produce a progeny plant that co-

expresses the first, second, third, fourth and fifth rotavirus protein (X_1-X_5). Rotavirus protein X_1-X_5 may be selected from the group of VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the plant.

4.2 Four single gene constructs

[00198] Also provided herein is a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) is co-expressed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), and a fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant (See Table 1, Combination #4.2).

[00199] In a non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2), N_3 comprises (R_3), and N_4 comprises (R_4), wherein each rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4 is encoded, and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP4, VP6 and NSP4 is expressed in the host.

[00200] In another non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2), N_3 comprises (R_3), and N_4 comprises (R_4), wherein each rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4 is encoded, and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1,

where X_2 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP7, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP2, VP7, VP6 and NSP4 is expressed in the host.

[00201] In a further non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2), N_3 comprises (R_3), and N_4 comprises (R_4), wherein each rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4 is encoded, and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_2-R_4 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 and R_3-R_4 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 R_2, and R_4 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP4, VP7, VP6 and NSP4, and wherein R_1 –R_3 encode a rotavirus protein that is not X_4, with the result that each rotavirus protein VP4, VP7, VP6 and NSP4 is expressed in the host.

5. *Five Constructs*

5. Five single gene constructs

[00202] The present invention also provides for a method of producing RLPs in a plant, wherein a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) is co-expressed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) and a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3) and fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) and a fifth nucleic acid (R_5) comprising a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein (X_5) (see Table 1, Combination #5) so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant.

[00203] In this non-limiting example, N_1 comprises (R_1), N_2 comprises (R_2), N_3 comprises (R_3), N_4 comprises (R_4) and N_5 comprises (R_5), wherein each rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 encodes rotavirus protein X_1, wherein X_1 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_2-R_5 encode a rotavirus protein that is not X_1, where X_2 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 and R_3-R_5 encode a rotavirus protein that is not X_2, where X_3 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 R_2, R_4, and R_5 encode a rotavirus protein that is not X_3, where X_4 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_3 and R_5 encode a rotavirus protein that is not X_4, where X_5 may be any rotavirus protein selected from the group of VP2, VP4, VP6, VP7 and NSP4, and wherein R_1 –R_4 encode a rotavirus protein that is not X_5, with the result that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the host.

[00204] For example, which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP2 and nucleotide sequence R_2-R_5 may encode in any order rotavirus protein VP4, VP6, VP7 and NSP4, but R_2-R_5 may not encode VP2. In another non-limiting example nucleotide sequence R_1 may encode rotavirus protein VP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP6, VP7 and NSP4. In yet another non-limiting example nucleotide sequence R_1 may encode rotavirus protein VP6 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP7 and NSP4. In yet another example which is not to be considered limiting, nucleotide sequence R_1 may encode rotavirus protein VP7 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and NSP4. In yet another non-limiting example nucleotide sequence R_1 may encode rotavirus protein NSP4 and nucleotide sequences R_2-R_5 may encode in any order rotavirus protein VP2, VP4, VP6 and VP7.

[00205] For example, which is not to be considered limiting, a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein, for example VP2 is co-expressed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein, for example VP6, a

third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein for example VP4, a fourth nucleic acid (N_4) comprising a fourth nucleotide sequence (R_4) encoding a fourth rotavirus protein, for example VP7 and a fifth nucleic acid (N5) comprising a fifth nucleotide sequence (R_5) encoding a fifth rotavirus protein for example NSP4 (see Figure 3).

[00206] The five nucleic acids may be introduced into a plant in any order. For example, which is not to be considered limiting, a plant that expresses a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1) may be transformed with a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) . The plant may be further transformed with a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3). The plant then may be further transformed with a fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4). The plant then may be further transformed with a fifth nucleic acid (N_5) comprising a fourth nucleotide sequences (R_5) encoding a fourth rotavirus protein (X_5), so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. Rotavirus protein X_1-X_5 may be selected from the group of VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the plant. Furthermore, a plant may be co-transformed simultaneously with a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2), a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3), a fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth and a fifth rotavirus protein (X_4) , and a fifth nucleic acid (N_5) comprising a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein (X_5) so that the first, second, third, fourth and fifth nucleotide sequence are co-expressed in the plant. The first nucleic acid (N_1), second nucleic acid (N_2), third nucleic acid (N_3), fourth nucleic acid (N_4) and fifth nucleic acid (N_5) may be introduced in the plant in a transient manner, or in a stable manner.

[00207] A first plant expressing a first nucleic acid (N_1) comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein (X_1), may be crossed with a second plant expressing a second nucleic acid (N_2) comprising a second nucleotide sequence (R_2) encoding a second rotavirus protein (X_2) to produce a progeny plant (third plant). The third plant may be crossed with a fourth plant expressing a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein (X_3) to produces a progeny plant (fifth plant). The fifth plant may be crossed with a sixth plant expressing a fourth nucleic acid (N_4) comprising a fourth nucleotide sequences (R_4) encoding a fourth rotavirus protein (X_4) to produce a progeny plant (seventh plant). The seventh plant may be crossed with an eight plant expressing a fifth nucleic acid (N_5) comprising a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein (X_5) to produce a progeny plant that co-expresses the first, second, third, fourth and fifth rotavirus protein (X_1-X_5). Rotavirus protein X_1-X_5 may be selected from the group of VP2, VP4, VP6, VP7 and NSP4, wherein VP2, VP4, VP6, VP7 and NSP4 are selected once, so that each rotavirus protein VP2, VP4, VP6, VP7 and NSP4 is expressed in the plant.

Ratio of nucleic acids (N) used to transform a host

[00208] As may be seen in Figure 5, the level of RLP accumulation in the plant, portion of the plant or plant cell, may be influenced by the ratio of the nucleic acids encoding rotavirus structural and nonstructural proteins that are expressed in a plant. For example, which is not to be considered limiting, the ratio of nucleic acids (N) that are introduced into a plant may be modified by providing different amounts of *Agrobacterium*, that are used to infiltrate the plant, portion of the plant or plant cell, where each *Agrobacterium* comprises a construct comprising a nucleic acid (N) as set out in Table 1 (and accompanying text) above. For example which is not to be considered limiting, the ratio of the structural protein-containing to nonstructural protein-containing *Agrobacterium* may range for example from about 0.8:1 to about 2.5:1.5 (structural protein : nonstructural protein), or any amount therebetween, for example from about 0.8:1, 0.9:1, 1:1, 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:0.5, 2:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.5:1.1, 2.5:1.2, 2.5:1.3, 2.5:1.4, 2.5:1.5 (structural protein : nonstructural protein), or any amount therebetween.

For example, as described below, the ratio of structural rotavirus protein to nonstructural protein may be varied for example by introducing different ratios of *Agrobacterium* containing the one or more nucleic acid (N) comprising one or more nucleotide sequence (R) encoding the rotavirus structural and non-structural proteins (X) into a host, for example a plant, portion of the plant or plant cell. For example the absorbance or optical density (OD) of *Agrobacterium* strains in the bacterial suspension may be used as measure to establish a ratio between *Agrobacterium* strains that containing the structural protein to *Agrobacterium* strains that contain the nonstructural protein. For example, which is not considered limiting, the OD may range for example from about 0.2:0.4 to about 1:0.6 (*Agrobacterium* strains containing structural protein : *Agrobacterium* strains containing nonstructural protein in the bacterial suspension) or any amount therebetween, for example from about 0.2:0.4, 0.3:0.4, 0.4:0.4, 0.5:0.4, 0.6:0.4, 0.7:0.4, 0.8:0.4, 0.9:0.4, 1:0.4, 0.2:0.5, 0.3:0.5, 0.4:0.5, 0.5:0.5, 0.6:0.5, 0.7:0.5, 0.8:0.5, 0.9:0.5, 1:05, 0.2:0.6, 0.3:0.6, 0.4:0.6, 0.5:0.6, 0.6:0.6, 0.7:0.6, 0.8:0.6, 0.9:0.6, 1:06, 0.3:0.4, 0.3:0.5, 0.3:0.6, 0.4:0.4, 0.4:0.5, 0.4:0.6, 0.5:0.4, 0.5:0.5, 0.5:0.6, 0.6:0.4, 0.6:0.5, 0.6:0.6, 0.7:0.4, 0.7:0.5, 0.7:0.6, 0.8:0.4, 0.8:0.5, 0.8:0.6, 0.9:0.4, 0.9:0.5, 0.9:0.6, 1:0.4, 1:0.5, 1:0.6 (*Agrobacterium* strains containing structural protein : *Agrobacterium* strains containing nonstructural protein in the bacterial suspension) or any amount therebetween. For example, which is not considered limiting, an OD of 0.4 of *Agrobacterium* strains in bacterial suspension may be designated as a reference of 1. Therefore a ratio of 1.5:1 of structural to non structural protein may be achieved by using an OD of 0.6 of *Agrobacterium* strain containing structural protein to an OD of 0.4 of *Agrobacterium* strain containing nonstructural protein in bacterial suspension.

[00209] The ratio of rotavirus structural protein to nonstructural protein may be varied for example by introducing different ratios of *Agrobacterium* containing the one or more nucleic acid (N) comprising one or more nucleotide sequence (R) encoding the rotavirus structural and non-structural proteins (X) into the plant, portion of the plant or plant cell. Alternatively, if the rotavirus structural proteins and nonstructural proteins are present on the same construct, and therefore are introduced into the plant, plant portion or plant cell, using one *Agrobacterium*, they may be differentially expressed within the plant, portion of the plant or plant cell using suitable promoters

so that the desired ratio of rotavirus structural proteins and nonstructural proteins is obtained.

[00210] Therefore as described herein, a method is provided for increasing RLP production yield, increasing VP4 and VP7 yield, or increasing both RLPS and VP4 and VP7 yield, by modulating the ratio between the one or more nucleic acid (N) comprising one or more nucleotide sequence (R) encoding the rotavirus structural proteins (X) and the one or more nucleic acid (N) comprising one or more nucleotide sequence (R) encoding the rotavirus nonstructural proteins (X).

[00211] For example, the percentage of the *Agrobacterium* containing rotavirus nonstructural protein may be between 20% to 60% or any amount therebetween, of total amount of *Agrobacterium* use to infiltrate the plant, plant portion or plant cell. For example the percent ratio of *Agrobacterium* containing rotavirus nonstructural protein may be 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% 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, or any amount therebetween of the total *Agrobacterium* use to infiltrate the plant, plant portion or plant cell. Similarly, the percentage of *Agrobacterium* containing structural protein within the total amount of *Agrobacterium* infiltrated may be 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41% or 40% or any amount therebetween, of the total *Agrobacterium* use to infiltrate the plant, plant portion or plant cell.

[00212] For example, the percentage ratio of *Agrobacterium* containing rotavirus structural protein to *Agrobacterium* containing nonstructural protein may be 70%:30%, 60%:40%, 50%:50%, 40%:60% or any percentage ratio amount therebetween. For example, the percentage ratio between *Agrobacterium* containing structural protein and *Agrobacterium* containing nonstructural protein may be 50%:50%, 51%:49%, 52%:48%, 53%:47%, 54%:46%, 55%:45%, 56%:44%, 57%:43%, 58%:42%, 59%: 41%, 60%:40%, or any percentage ratio in between.

[00213] As described below, the ratio of rotavirus structural protein to rotavirus nonstructural protein may further be varied for example by differentially expressing the rotavirus structural protein and the rotavirus nonstructural protein. Expression may be varied by modulating for example replication, transcription, translation, or a combination thereof, of the rotavirus structural protein , the rotavirus nonstructural protein , or both the rotavirus structural protein and the rotavirus nonstructural protein . For example different regulatory elements, including promoters, amplification elements, enhancers or a combination thereof, may be used in addition to varying the ratio of the rotavirus structural protein -containing *Agrobacterium* to rotavirus nonstructural protein -containing *Agrobacterium* infiltrated as described above. A first set or combination of regulatory elements may be used to regulate the replication, transcription or a combination thereof, of the one or more nucleic acid comprising one or more nucleotide sequence encoding rotavirus structural protein and a second set or combination of regulatory elements may be used to regulate the replication, transcription or a combination thereof, of the one or more nucleotide sequence encoding rotavirus nonstructural protein. The first set or combination of regulatory elements is different from the second set or combination of regulatory elements and permits differential expression of the one or more nucleic acid comprising one or more nucleotide sequence encoding rotavirus structural protein and the one or more nucleic acid comprising one or more nucleotide sequence encoding rotavirus nonstructural protein to permit modulating the ratio of rotavirus structural protein:rotavirus nonstructural protein *in vivo*.

[00214] For example, which is not to be considered limiting, one set or combination of regulatory elements, for example the first set, may include an enhancer element for example elements obtained from CPMV, such as CPMV HT, or CPMV 160 (see Figure 6). CMPV HT is described in US 61/971,274 (which is incorporated herein by reference) and CPMV 160 is described in US 61/925,852 (which is incorporated herein by reference). The enhancer element, for example those obtained from CPMV, for example CPMV HT or CPMV 160 (see Figure 6; US 61/971,274, and US 61/925,852, respectively) may be absent in the other set or combination of regulatory elements, for example the second set. Alternatively, the second set may include an enhancer element (for example elements obtained from CPMV, (for example CPMV

HT or CPMV 160), while the amplification element (for example elements obtained from CPMV, (for example CPMV HT or CPMV 160) may be absent in the first set or combination of regulatory elements. In a similar manner, the strength of a promoters may differ between the first and second set or combination of regulatory elements, or one of the promoters may be inducible, and the other constitutive, so that differential expression between the rotavirus structural protein relative to the rotavirus nonstructural protein is achieved *in vivo*.

[00215] For example, the ratio of rotavirus structural protein to nonstructural protein may be varied for example by introducing different ratios of *Agrobacterium* containing a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding first rotavirus protein for example rotavirus nonstructural protein NSP4 to *Agrobacterium* containing a second nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein, for example in any order rotavirus structural proteins VP2, VP4, VP6 and VP7. For example the ratio of the *Agrobacterium* containing a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding rotavirus nonstructural protein NSP4 to the *Agrobacterium* containing a second nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding rotavirus structural proteins VP2, VP4, VP6 and VP7 may be 0.8:1 and 1:2 (*Agrobacterium* containing N_1 to N_2) or any amount there between for example 1:1.5 (*Agrobacterium* containing N_1 to N_2).

[00216] Furthermore, the ratio of rotavirus structural protein to nonstructural protein may be varied by differentially expressing within the plant, portion of the plant or plant cell the rotavirus structural protein to nonstructural protein using enhancer elements. For example, the ratio of rotavirus structural protein to nonstructural protein may be varied for example by co-expressing within the plant, portion of the plant or plant cell a first nucleic acid (N_1) comprising a nucleotide sequence (R_1) encoding a first rotavirus protein for example a nonstructural protein NSP4 with a second nucleic acid (N_2) comprising four nucleotide sequences (R_2-R_5) encoding a second, third, fourth and fifth rotavirus protein for example in any order rotavirus structural proteins VP2, VP4, VP6 and VP7, wherein the second, third, fourth and fifth nucleotide sequence are operatively linked to an enhancer sequence for example CPMV HT, CPM 160, CPMV 160+ and CPMV HT+ (described in US 61/971,274,

and US 61/925,852, respectively which are incorporated herein by reference), as described below. In another example, the ratio of rotavirus structural protein to nonstructural protein may be varied for example by co-expressing within the plant, portion of the plant or plant cell a first nucleic acid (N_1) comprising first nucleotide sequence (R_1) encoding a first rotavirus protein for example structural protein VP6 or VP7 and second nucleotide sequence (R_2) encoding a second rotavirus protein for example structural protein VP2 or VP4, second nucleic acid (N_2) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein for example structural protein VP7 or VP6 and a fourth nucleotide sequence (R_4) encoding a fourth rotavirus protein for example structural protein VP4 or VP2 and a third nucleic acid (N_3) comprising fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein for example nonstructural protein NSP4, wherein the first, second, third and fourth nucleotide sequence are operatively linked to an enhancer sequence for example CPMV HT, CPMV 160, CPMV 160+ and CPMV HT+, as described below.

[00217] In another example, the ratio of rotavirus structural protein to nonstructural protein may be varied for example by co-expressing within the plant, portion of the plant or plant cell one or more nucleic acids comprising a first nucleotide sequence (R_1) encoding a first rotavirus protein for example structural protein VP6 or VP7 and second nucleotide sequence (R_2) encoding a second rotavirus protein for example structural protein VP2 or VP4, a third nucleotide sequence (R_3) encoding a third rotavirus protein for example structural protein VP7 or VP6 and a fourth nucleotide sequence (R_4) encoding fourth rotavirus protein for example structural protein VP4 or VP2, and a fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein for example nonstructural protein NSP4, wherein the first, second, third and fourth nucleotide sequence are operatively linked to an enhancer sequence for example CPM 160, CPMV 160+ and CPMV HT+, as described below and the fifth nucleotide sequence is operatively linked to CPMV HT as described below.

[00218] In another example, the ratio of rotavirus structural protein to nonstructural protein may be varied for example by co-expressing within the plant, portion of the plant or plant cell a first nucleic acid (N_1) comprising first nucleotide sequence (R_1) encoding a first rotavirus protein for example structural protein VP6 or VP7, a second nucleic acid (N_2) comprising second nucleotide sequence (R_2) encoding a second

rotavirus protein for example structural protein VP2 or VP4, a third nucleic acid (N_3) comprising a third nucleotide sequence (R_3) encoding a third rotavirus protein for example structural protein VP7 or VP6, a fourth nucleic acid (N_4) comprising a fourth nucleotide sequence (R_4) encoding a fourth rotavirus protein for example structural protein VP4 or VP2 and a fifth nucleic acid (N_5) comprising fifth nucleotide sequences (R_5) encoding a fifth rotavirus protein for example nonstructural protein NSP4, wherein the first, second, third and fourth nucleotide sequence are operatively linked to an enhancer sequence for example CPMV HT, CPMV 160, CPMV 160+ and CPMV HT+, as described below.

Amplification Elements and Enhancer Elements/ Regulatory Elements

[00219] The rotavirus protein or polypeptide may be expressed in an expression system comprising a viral based, DNA or RNA, expression system, for example but not limited to, a comovirus-based expression cassette and geminivirus-based amplification element.

[00220] Enhancer elements may be used to achieve high level of transient expression of rotavirus structural and nonstructural proteins. Enhancer elements may be based on RNA plant viruses, including comoviruses, such as *Cowpea mosaic virus* (CPMV; see, for example, WO2007/135480; WO2009/087391; US 2010/0287670, Sainsbury F. et al., 2008, *Plant Physiology;* 148: 121-1218; Sainsbury F. et al., 2008, *Plant Biotechnology Journal;* 6: 82-92; Sainsbury F. et al., 2009, *Plant Biotechnology Journal;* 7: 682-693; Sainsbury F. et al. 2009, *Methods in Molecular Biology, Recombinant Proteins From Plants*, vol. 483: 25-39).

***CPMV 160 (*CPMVX) *and CPMV 160+* (CPMVX+)**

[00221] In an embodiment the enhancer Elements are "CPMVX" (also referred as "CPMV 160") and/ or "CPMVX+" (also referred to as "CPMV 160+") and are described in US 61/925,852 (which is incorporated herein by reference).

[00222] Expression enhancer "CPMVX" comprises a comovirus cowpea mosaic virus (CPMV) 5' untranslated region (UTR). The 5'UTR from nucleotides 1-160 of the CPMV RNA -2 sequence (SEQ ID NO: 1), starts at the transcription start site to the first in frame initiation start codon (at position 161), which serve as the initiation

site for the production of the longer of two carboxy coterminal proteins encoded by a wild-type comovirus genome segment. Furthermore a 'third' initiation site at (or corresponding to) position 115 in the CPMV RNA-2 genomic sequence may also be mutated, deleted or otherwise altered. It has been shown that removal of AUG 115 in addition to the removal of AUG 161 enhances expression when combined with an incomplete M protein (Sainsbury and Lomonossoff, 2008, *Plant Physiology;* 148: 1212-1218; WO 2009/087391; which are incorporated herein by reference).

[00223] CPMVX comprises X nucleotides of SEQ ID NO:1, where X=160, 155, 150, or 114 of SEQ ID NO:1, or a sequence that comprises between 80% to 100% sequence similarity with CPMVX, where X=160, 155, 150, or 114 of SEQ ID NO:93. This expression enhancer is generally referred to as CPMVX (see Figure 6c).

[00224] The expression enhancer CPMVX, where X=160, consists of nucleotides 1-160 of SEQ ID NO: 1:

```
1   tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc
61  ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc
121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca (SEQ ID NO:1)
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[00225] The CPMVX enhancer sequence may further be fused to a stuffer sequence, wherein the CMPVX comprises X nucleotides of SEQ ID NO:1, where X=160, 155, 150, or 114 of SEQ ID NO:1, or a sequence that comprises between 80 to 100 % sequence similarity with CPMVX, where X=160, 155, 150, or 114 of SEQ ID NO:1, and the stuffer sequence comprises from 1-100 nucleotides fused to the 3' end of the CMPVX sequence. For example, the stuffer sequence may comprise from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides, or any number of nucleotides therebetween.

[00226] If the CMPVX sequence comprises a stuffer fragment, then this expression enhancer may be referred to as CPMVX+ (see Figure 6d), where X=160, 155, 150, 114 of SEQ ID NO:1, it may also be referred to as CMPVX comprising a stuffer sequence, or it may be referred to as CPMV160+; CPMV155+; CPMV150+; CPMV114+, when X-160, 155, 150, or 114, respectively. Constructs comprising CPMVX that do not comprise a stuffer sequence may be termed CPMVX+, where

X=160, 155, 150, 114 of SEQ ID NO:1, and where the stuffer sequence is of 0 nucleotides in length.

[00227] The stuffer sequence may be modified by truncation, deletion, or replacement of the native CMPV5'UTR sequence that is located 3'to nucleotide 160. The modified stuffer sequence may be removed, replaced, truncated or shortened when compared to the initial or unmodified (i.e. native) stuffer sequence associated with the 5'UTR (as described in Sainsbury F., and Lomonossoff G.P., 2008, Plant Physiol. 148: pp. 1212-1218). The stuffer sequence may comprise a one or more restriction sites (polylinker, multiple cloning site, one or more cloning sites), one or more plant kozak sequences, one or more linker sequences, one or more recombination sites, or a combination thereof. For example, which is not to be considered limiting, a stuffer sequence may comprise in series, a multiple cloning site of a desired length fused to a plant kozak sequence. The stuffer sequence does not comprise a nucleotide sequence from the native 5'UTR sequence that is positioned 3' to nucleotide 160 of the native CPMV 5'UTR, for example nucleotides 161 to 512 as shown in Figure 1 of Sainsbury F., and Lomonossoff G.P. (2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference), or nucleotides 161-509 of prior art CPMV HT sequence. That is, the incomplete M protein present in the prior art CPMV HT sequence (Figure 1; of Sainsbury F., and Lomonossoff G.P., 2008) is removed from the 5'UTR in the present invention.

[00228] Plant Kozak consensus sequences are known in the art (see for example Rangan et al. Mol. Biotechnol., 2008, July 39(3), pp. 207-213). Both naturally occurring and synthetic Kozak sequences may be used in the expression enhancer or may be fused to the nucleotide sequence of interest as described herein.

[00229] The plant kozak sequence may be any known plant kozak sequences (see for example L. Rangan et. al. Mol. Biotechnol.2008), including, but not limited to the following plant consensus sequences:

caA(A/C)a (SEQ ID NO:2; plant kingdom)

aaA(A/C)a (SEQ ID NO:3; dicots)

aa(A/G)(A/C)a (SEQ ID NO:4; arabidopsis)

The plant kozak sequence may also be selected from the group of:

AGAAA (SEQ ID NO: 5)
AGACA (SEQ ID NO: 6)
AGGAA (SEQ ID NO: 7)
AAAAA (SEQ ID NO: 8)
AAACA (SEQ ID NO: 9)
AAGCA (SEQ ID NO: 10)
AAGAA (SEQ ID NO: 11)
AAAGAA (SEQ ID NO: 12)
AAAGAA (SEQ ID NO: 13)

(A/-)A(A/G)(A/G)(A/C)A. (SEQ ID NO: 14; Consensus sequence)

[00230] The expression enhancer CPMVX, or CPMVX+, may be operatively linked at the 5'end of the enhancer sequence with a regulatory region that is active in a plant, and operatively linked to a nucleotide sequence of interest at the 3'end of the expression enhancer (Figure 6c), in order to drive expression of the nucleotide sequence of interest within a plant host.

CPMV HT+ , CPMV HT+[WT115], CPMV HT+ [511]

[00231] In another embodiment the enhancer elements is "CPMV HT+" which is described in US 61/971,274 (which is incorporated herein by reference). Expression enhancer "CPMV HT+" (see Figure 6b) comprises a comovirus 5' untranslated region (UTR) and a modified, lengthened, or truncated stuffer sequence.

[00232] A plant expression system comprising a first nucleic acid sequence comprising a regulatory region, operatively linked with one or more than one expression enhancer as described herein (e.g. CPMV HT+, CPMV HT+[WT115], CPMV HT+ [511]), and a nucleotide sequence encoding a rotavirus structural or nonstructural proteins is also provided. Furthermore, a nucleic acid comprising a promoter (regulatory region) sequence, an expression enhancer (e.g. CPMV HT+ or CPMV HT+[WT115]) comprising a comovirus 5'UTR and a stuffer sequence with a plant kozak sequence fused to one or more nucleic acid sequences encoding a rotavirus structural or nonstructural proteins are described. The nucleic acid may further comprise a sequence comprising a comovirus 3' untranslated region (UTR), for example, a plastocyanin 3' UTR, or other 3'UTR active in a plant, and a terminator sequence, for example a NOS terminator, operatively linked to the 3'end of the nucleotide sequence encoding a rotavirus structural or nonstructural proteins (referred to as nucleotide of interest in Figure 6a), so that the nucleotide sequence

encoding the rotavirus structural or nonstructural proteins is inserted upstream from the comovirus 3' untranslated region (UTR), plastocyanin 3' UTR, or other 3'UTR sequence.

[00233] SEQ ID NO:15 comprises a "CPMV HT" expression enhancer as known in the prior art (e.g. Figure 1 of Sainsbury and Lomonossoff 2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference). CPMV HT includes the 5'UTR sequence from nucleotides 1-160 of SEQ ID NO:15 with modified nucleotides at position 115 (cgt), and an incomplete M protein with a modified nucleotide at position 162 (acg), and lacks a plant kozak sequence (5'UTR: nucleotides 1-160; incomplete M protein underlined, nucleotides 161 – 509). SEQ ID NO:15 also includes a multiple cloning site (italics, nucleotides 510-528) which is not present in the prior art CPMV HT sequence:

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

481 taagcttctg tatattctgc ccaaatttgt *cgggccc* SEQ ID NO: 15

[00234] CPMV HT+ with a plant kozak consensus sequence is provided in SEQ ID NO:16 (nucleotide 1-160, 5'UTR, including modified ATG at positions 115 (GTG) lower case bold and italics; stuffer fragment comprising: an incomplete M protein underlined, nucleotides 161 – 509, with modified nucleotide at 162 (ACG); a multiple cloning site, italics, nucleotides 510-528; and a consensus plant kozak sequence, caps and bold, nucleotides 529-534).

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc***gtg***agc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

481 taagcttctg tatattctgc ccaaatttg*t tcgggcccaa taccgcgg****(A/-)A(A/G)***

(A/G) (A/C)A (SEQ ID NO:16)

[00235] SEQ ID NO:17 ("CPMV HT+ 511") comprises a segment of the native sequence of the CPMV RNA 2 genome from nucleotides 1-154. The 5'UTR sequence from nucleotides 1-511 of SEQ ID NO:17 comprises modified "atg" sequences at positions 115 ("g" in place of "a"; italics bold) and 162 ("c" in place of "t"; italics bold), and an incomplete M protein (underlined) from nucleotides 161 – 511. CPMV HT+ 511 comprises a native M protein kozak consensus sequence (nucleotides 508-511; bold):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc***gtg***agc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

481 taagcttctg tatattctgc ccaaatt**tga a**... SEQ ID NO: 17

[00236] Another non-limiting example of a CPMV HT+ enhancer sequence is provided by the sequence of SEQ ID NO:18 (CPMV HT+[WT115]). Expression cassettes or vectors comprising CPMV HT+ and including a plant regulatory region in operative association with the expression enhancer sequence of SEQ ID NO: 18, and

the transcriptional start site (ATG) at the 3' end fused to a nucleotide sequence encoding rotavirus structural or nonstructural protein are also part o the present invention.

[00237] SEQ ID NO: 18 (CPMV HT+[WT115]) nucleotide 1-160, 5'UTR, with an ATG at position 115-117, lower case bold; stuffer fragment comprising: an incomplete M protein underlined, nucleotides 161 - 509; with a modified ATG at position 161-153 lower case bold, and underlined, a multiple cloning site, italics, nucleotides 510-528; and a plant kozak sequence, caps and bold, nucleotides 529-534).

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc***atg***agc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca ***acg***ttttctt tcactgaagc

181 gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc

241 ctattcttgt cggtgtggtc ttgggaaaag aaagcttgct ggaggctgct gttcagcccc

301 atacattact tgttacgatt ctgctgactt tcggcgggtg caatatctct acttctgctt

361 gacgaggtat tgttgcctgt acttctttct tcttcttctt gctgattggt tctataagaa

421 atctagtatt ttctttgaaa cagagttttc ccgtggtttt cgaacttgga gaaagattgt

481 taagcttctg tatattctgc ccaaatttgt *tcgggcccaa taccgcgg****AG AAAA***

(SEQ ID NO:18)

[00238] The plant kozak sequence of SEQ ID NO:18 may be any plant kozak sequence, including but not limited, to one of the sequences of SEQ ID NO's: 2-14.

[00239] A plant expression system comprising a first nucleic acid sequence comprising a regulatory region, operatively linked with one or more than one expression enhancer as described herein (e.g. CPMV HT+, CPMV HT+[WT115], CPMV HT+ [511]), and a nucleotide sequence encoding a rotavirus structural or nonstructural protein is also provided. Furthermore, a nucleic acid comprising a promoter (regulatory region) sequence, an expression enhancer (e.g. CPMV HT+ or CPMV HT+[WT115]) comprising a comovirus 5'UTR and a stuffer sequence with a plant kozak sequence fused to one or more nucleic acid sequences encoding rotavirus

structural or nonstructural protein are described. The nucleic acid may further comprise a sequence comprising a comovirus 3' untranslated region (UTR), for example, a plastocyanin 3' UTR, or other 3'UTR active in a plant, and a terminator sequence, for example a NOS terminator, operatively linked to the 3'end of the nucleotide sequence encoding rotavirus structural or nonstructural protein (referred to as nucleotide of interest in Figure 6a), so that the nucleotide sequence encoding rotavirus structural or nonstructural protein is inserted upstream from the comovirus 3' untranslated region (UTR), plastocyanin 3' UTR, or other 3'UTR sequence.

[00240] The occurrence of RLPs produced using the methods described herein may be detected using any suitable method for example density gradient centrifugation or size exclusion chromatography. RLPs may be assessed for structure and size, for example by electron microscopy, size exclusion chromatography, or other techniques that would be evident to one of skill in the art.

[00241] For size exclusion chromatography, total soluble proteins may be extracted from plant tissue by homogenizing (Polytron) sample of frozen-crushed plant material in extraction buffer, and insoluble material removed by centrifugation. Precipitation with ice cold acetone or PEG may also be of benefit. The soluble protein is quantified, and the extract passed through a SephacrylTM column, for example a SephacrylTM S500 column. Blue Dextran 2000 may be used as a calibration standard. Following chromatography, fractions may be further analyzed by immunoblot to determine the protein complement of the fraction.

[00242] The separated fraction may be for example a supernatant (if centrifuged, sedimented, or precipitated), or a filtrate (if filtered), and is enriched for proteins, or suprastructure proteins, and include higher molecular weight, particles such as single-layered (sl), double-layered (dl) or triple-layered (tl) RLPs.

[00243] The separated fraction may be further processed to isolate, purify, concentrate or a combination thereof, the proteins, suprastructure proteins or higher-order particles by, for example, additional centrifugation steps, precipitation, chromatographic steps (e.g. size exclusion, ion exchange, affinity chromatography), tangential flow filtration, or a combination thereof. The presence of purified proteins, suprastructure proteins or higher-order particles such as RLPs, may be confirmed by,

for example, native or SDS-PAGE, Western analysis using an appropriate detection antibody, capillary electrophoresis, electron microscopy, or any other method as would be evident to one of skill in the art.

[00244] The RLP's produced according to the present invention may be purified, partially purified from a plant, portion of a plant or plant matter, or may be administered as an oral vaccine, using methods as know to one of skill in the art.

[00245] RLP purification may involve gradient centrifugation, for example sucrose, iodixanol, OptiPrep™ or cesium chloride (CsCl) density gradients may be used to purify or partially purify the RLPs from transformed plant biomass. As shown for example in Figure 4, an iodixanol step gradient or iodixanol continuous gradient might be used to purify the RLP and/or expressed rotavirus structural proteins.

[00246] Calcium (Ca2+) concentration has been shown to be important for the triple-layer particle (TLP) to double layer particle (DLP) transformation and is strain dependent (see for example Martin et al. Journal of Virology, Jan 2002, which is incorporated herein by reference). Complete loss of the outer-capsid proteins from TLPs (TLP decapsidation) takes place in the nanomolar range of [Ca2+]. Therefore the extraction and/or purification of RLP may be performed in the presence of calcium, and the step of gradient centrifugation may be performed in the presence of calcium, for example in the present of CaCl2. The concentration of CaCl2 maybe between for example, 1 mM and 1000 mM, or any amount there between, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 50, 600, 650, 700, 750, 800, 850, 900, 950 mM or any amount therebetween.

[00247] The plants, or plant fragments may be minimally processed. By the term "minimal processing" it is meant plant matter, for example, a plant or portion thereof comprising a protein of interest and /or the RLP which is partially purified to yield a plant extract, homogenate, fraction of plant homogenate or the like (i.e. minimally processed). Partial purification may comprise, but is not limited to disrupting plant cellular structures thereby creating a composition comprising soluble plant components, and insoluble plant components which may be separated for example, but not limited to, by centrifugation, filtration or a combination thereof. In this regard,

proteins secreted within the extracellular space of leaf or other tissues could be readily obtained using vacuum or centrifugal extraction, or tissues could be extracted under pressure by passage through rollers or grinding or the like to squeeze or liberate the protein free from within the extracellular space. Minimal processing could also involve preparation of crude extracts of soluble proteins, since these preparations would have negligible contamination from secondary plant products. Further, minimal processing may involve aqueous extraction of soluble protein from leaves, followed by precipitation with any suitable salt. Other methods may include large scale maceration and juice extraction in order to permit the direct use of the extract. The RLPs may be purified or extracted using any suitable method for example mechanical or biochemical extraction.

[00248] The one or more rotavirus structural protein may be synthesized at an amount up to 2 g per kilogram of plant fresh weight. For example, the amount of synthesized structural protein maybe between 1 and 2 g per kilogram of fresh weight, or any amount there between, such as 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2 g per kilogram of fresh weight or any amount therebetween. For example, the structural protein may be synthesized at an amount up to 1.54 g per kilogram of plant fresh weight.

[00249] The size (i.e. the diameter) of the above-defined RLPs, maybe measures for example by dynamic light scattering (DLS) or electron microscope (EM) techniques, is usually between 50 to 110 nm, or any size therebetween. For example, the size of the intact RLP structure may range from about 70 nm to about 110 nm, or any size therebetween, such as 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm or any size therebetween.

Nucleotide Sequences

[00250] The present invention further provides a nucleic acid comprising a nucleotide sequence encoding one or more rotavirus structural protein operatively linked to a regulatory region active in a plant. The nucleotide sequence may be optimized for example for human codon usage or plant codon usage. Furthermore one or more rotavirus structural protein may be operatively linked to one or more than one amplification elements. In addition one or more rotavirus structural protein may be

operatively linked to one or more than one compartment targeting sequence. The one or more rotavirus structural protein encoded by the nucleotide sequence may be for example VP2, VP4, VP6 or VP7. Furthermore the one or more rotavirus structural protein encoded by the nucleotide sequence may be for example from any rotavirus group A to G, but more preferably from rotavirus group A. Furthermore, the one or more rotavirus structural protein encoded by the nucleotide sequence maybe from any rotavirus strain having a genotype of any combinations of G- and P- types from G1 to G27 and from P1 to P34, and more preferably from G1 to G19 and from P1 to P27, including, but not limited to G1P[8], G2P[4], G2P[8], G3P[8], G4P[8], G9P[6], G9P[8], rotavirus A WA strain, rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain or rotavirus SA11 strain.

[00251] A nucleic acid sequence referred to in the present invention, may be "substantially homologous" , "substantially similar" or "substantially identical" to a sequence, or a compliment of the sequence if the nucleic acid sequence hybridize to one or more than one nucleotide sequence or a compliment of the nucleic acid sequence as defined herein under stringent hybridization conditions. Sequences are "substantially homologous" "substantially similar" "substantially identical" when at least about 70%, or between 70 to 100%, or any amount therebetween, for example 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100%, or any amount therebetween, of the nucleotides match over a defined length of the nucleotide sequence providing that such homologous sequences exhibit one or more than one of the properties of the sequence, or the encoded product as described herein.

[00252] For example the present invention provides an isolated polynucleotide comprising a nucleotide sequence which encodes one or more rotavirus protein, for example a structural or nonstructural rotavirus protein, that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% 100% or any amount therebetween identical to sequences as defines for example in SEQ ID NOs: 21, 27, 32, 37 or 42. The polynucleotide may be human codon optimized by any of the methods known in the art. The nucleotide sequence may enclode for example a rotavirus protein that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99% 100% or any amount therebetween identical the amino acid sequence of SEQ ID NOs: 24, 29, 34, 39 or 44.

[00253] Furthermore, the present invention provides RLPS that comprise rotavirus structural proteins that are for example encoded by nucleic acids that are at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% 100% or any amount therebetween identical to sequences as defines for example in SEQ ID NOs: 21, 27, 32, 37 or 42.

[00254] Such a sequence similarity or identity may be determined using a nucleotide sequence comparison program, such as that provided within DNASIS (using, for example but not limited to, the following parameters: GAP penalty 5, #of top diagonals 5, fixed GAP penalty 10, k tuple 2, floating gap 10, and window size 5). However, other methods of alignment of sequences for comparison are well-known in the art for example the algorithms of Smith & Waterman (1981, Adv. Appl. Math. 2:482), Needleman & Wunsch (J. Mol. Biol. 48:443, 1970), Pearson & Lipman (1988, Proc. Nat'l. Acad. Sci. USA 85:2444), and by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and BLAST, available through the NIH.), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology, Ausubel et al., eds. 1995 supplement), or using Southern or Northern hybridization under stringent conditions (see Maniatis et al., in Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory, 1982). Preferably, sequences that are substantially homologous exhibit at least about 80% and most preferably at least about 90% sequence similarity over a defined length of the molecule.

[00255] An example of one such stringent hybridization conditions may be overnight (from about 16-20 hours) hybridization in 4 X SSC at 65ºC, followed by washing in 0.1 X SSC at 65ºC for an hour, or 2 washes in 0.1 X SSC at 65°C each for 20 or 30 minutes. Alternatively an exemplary stringent hybridization condition could be overnight (16-20 hours) in 50% formamide, 4 X SSC at 42ºC, followed by washing in 0.1 X SSC at 65ºC for an hour, or 2 washes in 0.1 X SSC at 65°C each for 20 or 30 minutes, or overnight (16-20 hours), or hybridization in Church aqueous phosphate buffer (7% SDS; 0.5M NaPO4 buffer pH 7.2; 10 mM EDTA) at 65ºC, with 2 washes

either at 50°C in 0.1 X SSC, 0.1% SDS for 20 or 30 minutes each, or 2 washes at 65°C in 2 X SSC, 0.1% SDS for 20 or 30 minutes each for unique sequence regions.

[00256] A nucleic acid encoding a rotavirus structural polypeptide may be described as a "rotavirus nucleic acid", a "rotavirus nucleotide sequence", a "rotavirus nucleic acid", or a "rotavirus nucleotide sequence". For example, which is not to be considered limiting, a virus-like particle comprising one or more rotavirus structural protein or rotavirus structural polypeptide, may be described as a “rotavirus VLP”, “RVLP” or “RLP”.

[00257] Many organisms display a bias for use of particular codons to code for insertion of a particular amino acid in a growing peptide chain. Codon preference or codon bias, differences in codon usage between organisms, is afforded by degeneracy of the genetic code, and is well documented among many organisms. Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, inter alia, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization. The process of optimizing the nucleotide sequence coding for a heterologously expressed protein can be an important step for improving expression yields. The optimization requirements may include steps to improve the ability of the host to produce the foreign protein.

[00258] “Codon optimization” is defined as modifying a nucleic acid sequence for enhanced expression in cells of interest by replacing at least one, more than one, or a significant number, of codons of the native sequence with codons that may be more frequently or most frequently used in the genes of another organism or species. Various species exhibit particular bias for certain codons of a particular amino acid.

[00259] The present invention includes synthetic polynucleotide sequences that have been codon optimized for example the sequences have been optimized for human codon usage or plant codon usage. The codon optimized polynucleotide sequences may then be expressed in plants. More specifically the sequences optimized for human codon usage or plant codon usage may be expressed in plants. Without

wishing to be bound by theory, it is believed that the sequences optimized for human codon increases the guanine-cytosine content (GC content) of the sequence and improves expression yields in plants.

[00260] There are different codon-optimisation techniques known in the art for improving, the translational kinetics of translationally inefficient protein coding regions. These techniques mainly rely on identifying the codon usage for a certain host organism. If a certain gene or sequence should be expressed in this organism, the coding sequence of such genes and sequences will then be modified such that one will replace codons of the sequence of interest by more frequently used codons of the host organism.

Amino acid Sequences

[00261] Non-limiting examples of rotavirus structural protein are rotavirus protein VP2, VP4, VP6 and VP7, and a fragment of VP2, VP4, VP6 and VP7. Non-limiting examples of VP2, VP4, VP6 and VP7, or fragments of VP2, VP4, VP6 and VP7 protein that may be used according to the present invention include those VP2, VP4 VP6 and VP7 protein from rotavirus strain G9 P[6], rotavirus A WA strain, rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain and rotavirus SA11 strain. For example, but not limited to Rotarix-A41CB052A: VP4 (accession # JN849113), VP7: (accession # JN849114), rotavirus A WA strain: VP2 (accession #X14942), VP4: (accession #L34161), VP6 (accession # K02086), VP7: (accession # GU723327), NSP4 (accession # K02032), rotavirus SA11 strain: VP2 (accession # NC_011506), VP4 (accession # NC_011510), VP6 (accession # NC_011509), VP7 (accession # NC_011503) and NSP4 (accession # NC_011504).

[00262] An example of a VP2 structural protein, which is not to be considered limiting, is set forth in the amino acid sequence of SEQ ID NO: 24. Furthermore, the VP2 structural protein may comprise the sequence set forth in SEQ ID NO: 24, or a sequence having at least about 90-100% sequence similarity thereto, including any percent similarity within these ranges, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto. In addition, a VP2 structural protein may be encoded by a nucleotide sequence as set forth in SEQ ID NO:21 or a sequence having at least about 80-100% sequence similarity thereto, including any percent similarity within these

ranges, such as 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto.

[00263] An example of a VP4 structural protein, which is not to be considered limiting, is set forth in the amino acid sequence of SEQ ID NO: 34. Furthermore, the VP4 structural protein may comprise the sequence set forth in SEQ ID NO: 34, or a sequence having at least about 90-100% sequence similarity thereto, including any percent similarity within these ranges, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto. In addition, a VP4 structural protein may be encoded by a nucleotide sequence as set forth in SEQ ID NO: 32 or a sequence having at least about 80-100% sequence similarity thereto, including any percent similarity within these ranges, such as 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto.

[00264] An example of a VP6 structural protein, which is not to be considered limiting, is set forth in the amino acid sequence of SEQ ID NO: 29. Furthermore, the VP6 structural protein may comprise the sequence set forth in SEQ ID NO: 29, or a sequence having at least about 90-100% sequence similarity thereto, including any percent similarity within these ranges, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto. In addition, a VP6 structural protein may be encoded by a nucleotide sequence as set forth in SEQ ID NO:27 or a sequence having at least about 80-100% sequence similarity thereto, including any percent similarity within these ranges, such as 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto.

[00265] An example of a VP7 structural protein, which is not to be considered limiting, is set forth in the amino acid sequence of SEQ ID NO: 39. Furthermore, the VP7 structural protein may comprise the sequence set forth in SEQ ID NO: 39, or a sequence having at least about 90-100% similarity thereto, including any percent similarity within these ranges, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto. In addition, a VP7 structural protein may be encoded by a nucleotide sequence as set forth in SEQ ID NO:37 or a sequence having at least about 80-100% sequence similarity thereto, including any percent similarity within these

ranges, such as 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto.

[00266] An example of a NSP4 structural protein, which is not to be considered limiting, is set forth in the amino acid sequence of SEQ ID NO: 44. Furthermore, the NSP4 nonstructural protein may comprise the sequence set forth in SEQ ID NO: 44, or a sequence having at least about 90-100% similarity thereto, including any percent similarity within these ranges, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto. In addition, a NSP4 nonstructural protein may be encoded by a nucleotide sequence as set forth in SEQ ID NO: 42 or a sequence having at least about 80-100% sequence similarity thereto, including any percent similarity within these ranges, such as 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity thereto.

[00267] Amino acid sequence similarity or identity may be computed by using the BLASTP and TBLASTN programs which employ the BLAST (basic local alignment search tool) 2.0 algorithm. Techniques for computing amino acid sequence similarity or identity are well known to those skilled in the art, and the use of the BLAST algorithm is described in ALTSCHUL et al. (1990, J Mol. Biol. 215: 403- 410) and ALTSCHUL et al. (1997, Nucleic Acids Res. 25: 3389-3402).

[00268] Without wishing to be bound by theory, the protein concentration and ratio of the different rotavirus structural proteins may be important for the assembly efficiency of RLPs. Therefore multiplicity and time of infection, may be important to manipulate protein concentration and the overall assembly efficiency of RLPs in plants.

[00269] The construct of the present invention may be transiently expressed in a plants or portion of a plant. A transient expression system relying on the epichromosomal expression of recombinant Agrobacterium tumefaciens in a plant, portion of a plant or plant cell may be used to express the rotavirus structural protein, targeted to various cell compartments or sub-compartments. A transient expression system allows for a high production speed. Furthermore, large amounts of protein can be attained within a few days after infiltration of recombinant Agrobacterium in plants (Rybicki, 2010; Fischer et al., 1999). It is also possible to express long gene

sequences and have more than one gene simultaneously expressed in the same cell, allowing for efficient assembly of multimeric proteins (Lombardi et al., 2009).

[00270] The nucleotide sequences encoding for the rotavirus structural proteins and nonstructural proteins may be transferred into the plant host using 1, 2, 3, 4 or 5 transformed *Agrobacterium tumefaciens* strains (as described in Table 1 and accompanying text

[00271] During transient expression post-transcriptional gene silencing may limit the expression of the heterologous proteins in plants. The co-expression of a suppressor of silencing, for example, but not limited to Nss from Tomato spotted wilt virus may be used to counteract the specific degradation of transgene mRNAs (Brigneti et al., 1998). Alternate suppressors of silencing are well known in the art and may be used as described herein (Chiba et al., 2006, Virology 346:7-14; which is incorporated herein by reference), for example but not limited to HcPro, TEV -p1/HC-Pro (Tobacco etch virus-p1/HC-Pro), BYV -p21, p19 of Tomato bushy stunt virus (TBSV p19), capsid protein of Tomato crinkle virus (TCV -CP), 2b of Cucumber mosaic virus; CMV-2b), p25 of Potato virus X (PVX-p25), p11 of Potato virus M (PVM-p11), p11 of Potato virus S (PVS-p11), p16 of Blueberry scorch virus, (BScV –p16), p23 of Citrus tristexa virus (CTV-p23), p24 of Grapevine leafroll-associated virus-2, (GLRaV-2 p24), p10 of Grapevine virus A, (GVA-p10), p14 of Grapevine virus B (GVB-p14), p10 of Heracleum latent virus (HLV-p10), or p16 of Garlic common latent virus (GCLV-p16). Therefore, a suppressor of silencing, for example HcPro, TEV -p1/HC-Pro, BYV-p21, TBSV p19, TCV-CP, CMV-2b, PVX-p25, PVM-p11, PVS-p11, BScV-p16, CTV-p23, GLRaV-2 p24, GBV-p14, HLV-p10, GCLV-p16or GVA-p10, may be co-expressed along with one or more rotavirus structural or non structural protein for example VP2, VP4, VP6, VP7 and NSP4 or a combination thereof, to further ensure high levels of protein production within a plant or portion of a plant.

[00272] The present invention also provides a methods as described above, wherein an additional (second, third, fourth, fifth or sixth) nucleotide sequence is expressed within the plant, the additional (second, third, fourth, fifth or sixth) nucleotide sequence encoding a suppressor of silencing is operatively linked with an additional

(second, third, fourth, fifth or sixth) regulatory region that is active in the plant. The nucleotide sequence encoding a suppressor of silencing may be, for example Nss, HcPro, TEV -p1/HC-Pro, BYV-p21, TBSV p19, TCV-CP, CMV-2b, PVX-p25, PVM-p11, PVS-p11, BScV-p16, CTV-p23, GLRaV-2 p24, GBV-p14, HLV-p10, GCLV-p16 or GVA-p10.

[00273] As described below, transient expression methods may be used to express the constructs of the present invention (see Liu and Lomonossoff, 2002, Journal of Virological Methods, 105:343-348; which is incorporated herein by reference). Alternatively, a vacuum-based transient expression method, as described by Kapila et al., 1997, which is incorporated herein by reference) 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 the production of the protein of interest in infected cells, however, the passage of t-DNA inside the nucleus is transient.

[00274] To aid in identification of transformed plant cells, the constructs of this invention may be further manipulated to include plant selectable markers. Useful selectable markers include enzymes that provide for resistance to chemicals such as an antibiotic for example, gentamycin, hygromycin, kanamycin, or herbicides such as phosphinothrycin, glyphosate, chlorosulfuron, and the like. Similarly, enzymes providing for production of a compound identifiable by colour change such as GUS (beta-glucuronidase), or luminescence, such as luciferase or GFP, may be used.

[00275] Also considered part of this invention are transgenic plants, plant cells or seeds containing the constructs as described herein. Methods of regenerating whole plants from plant cells are also known in the art. 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 cultures.

[00276] The use of the terms "regulatory region", "regulatory element" or "promoter" in the present application is meant to reflect 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 gene of interest, this may result in expression of the gene of interest. A regulatory element may be capable of mediating organ specificity, or controlling developmental or temporal gene activation. A "regulatory region" may 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, may 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.

[00277] 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 usually situated approximately 25 base pairs upstream of a transcriptional start site. A promoter element comprises a basal promoter element, responsible for the initiation of transcription, as well as other regulatory elements (as listed above) that modify gene expression.

[00278] 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, which is incorporated herein by reference).

[00279] 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, LR.P., 1998, Trends Plant Sci. 3, 352-358; which is incorporated by reference). Examples, of potential inducible promoters include, but not limited to, tetracycline-inducible promoter (Gatz, C.,1997, Ann. Rev. Plant Physiol. Plant Mol. BioI. 48,89-108; which is incorporated by reference), steroid inducible promoter (Aoyama. T. and Chua, N.H.,1997, Plant 1. 2, 397-404; which is incorporated by reference) and ethanol-inducible promoter (Salter, M.G., et aI, 1998, Plant 10urnal 16, 127-132; Caddick, M.X., et al,1998, Nature Biotech. 16, 177-180, which are incorporated by reference) cytokinin inducible IB6 and CKI 1 genes (Brandstatter, I. and K.ieber, 1.1.,1998, Plant Cell 10, 1009-1019; Kakimoto, T., 1996, Science 274,982-985; which are incorporated by reference) and the auxin inducible element, DR5 (Ulmasov, T., et aI., 1997, Plant Cell 9, 1963-1971; which is incorporated by reference).

[00280] 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 (Odell et aI., 1985, Nature, 313: 810-812), the rice actin 1 (Zhang et aI, 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, which is incorporated herein by reference), and triosephosphate isomerase 1 (Xu et. aI., 1994, Plant Physiol. 106: 459-467) genes, the maize ubiquitin 1 gene (Cornejo et ai, 1993, Plant Mol. BioI. 29: 637-646), the Arabidopsis ubiquitin 1 and 6 genes (Holtorf et aI, 1995, Plant Mol. BioI. 29: 637-646), and the tobacco translational initiation factor 4A gene (Mandel et aI, 1995, Plant Mol. BioI. 29: 995-1004).

[00281] The term "constitutive" as used herein does not necessarily indicate that a gene under control of the constitutive regulatory region is expressed at the same level in all cell types, but that the gene is expressed in a wide range of cell types even though variation in abundance is often observed. Constitutive regulatory elements may be coupled with other sequences to further enhance the transcription and/or translation of the nucleotide sequence to which they are operatively linked. For example, the CPMV-HT system is derived from the untranslated regions of the Cowpea mosaic virus (CPMV) and demonstrates enhanced translation of the associated coding sequence. By "native" it is meant that the nucleic acid or amino

acid sequence is naturally occurring, or "wild type". By "operatively linked" it is meant that the particular sequences, for example a regulatory element and a coding region of interest, interact either directly or indirectly to carry out an intended function, such as mediation or modulation of gene expression. The interaction of operatively linked sequences may, for example, be mediated by proteins that interact with the operatively linked sequences.

[00282] The RLP produced within a plant may produce a rotavirus VP7 structural protein comprising plant-specific N-glycans. Therefore, this invention also provides for a RLP comprising VP7 having plant specific N-glycans.

[00283] Furthermore, modification of N-glycan in plants is known (see for example U.S. 60/944,344; which is incorporated herein by reference) and VP7 having modified N-glycans may be produced. VP7 comprising a modified glycosylation pattern, for example with reduced fucosylated, xylosylated, or both, fucosylated and xylosylated, N-glycans may be obtained, or VP7 having a modified glycosylation pattern may be obtained, wherein the protein lacks fucosylation, xylosylation, or both, and comprises increased galactosylation. Furthermore, modulation of post-translational modifications, for example, the addition of terminal galactose may result in a reduction of fucosylation and xylosylation of the expressed VP7 when compared to a wild-type plant expressing VP7.

[00284] For example, which is not to be considered limiting, the synthesis of VP7 having a modified glycosylation pattern may be achieved by co-expressing VP7 along with a nucleotide sequence encoding beta-1.4 galactosyltransferase (GalT), for example, but not limited to mammalian GalT, or human GalT however GalT from another sources may also be used. The catalytic domain of GalT may also be fused to a CTS domain (i.e. the cytoplasmic tail, transmembrane domain, stem region) of N-acetylglucosaminyl transferase (GNT1), to produce a GNT1-GalT hybrid enzyme, and the hybrid enzyme may be co-expressed with VP7. The VP7 may also be co-expressed along with a nucleotide sequence encoding N-acetylglucosaminyl transferase III (GnT-III), for example but not limited to mammalian GnT-III or human GnT-III, GnT-III from other sources may also be used. Additionally, a GNT1-GnT-III hybrid enzyme, comprising the CTS of GNT1 fused to GnT-III may also be used.

[00285] Therefore the present invention also provides RLPs comprising VP7 having modified N-glycans.

[00286] Without wishing to be bound by theory, the presence of plant N-glycans on VP7 may stimulate the immune response by promoting the binding of VP7 by antigen presenting cells. Stimulation of the immune response using plant N glycan has been proposed by Saint-Jore-Dupas et al. (2007).

[00287] Table 2 lists sequences provided in various embodiments of the invention.

Table 2:

SEQ ID NO	Description	Page/Figure
1	expression enhancer CPMVX	
2	plant kingdom kozak consensus sequence	
3	Dicots kozak consensus sequence	
4	Arabidopsis kozak consensus sequence	
5-13	plant kozak sequences	
14	Kozak consensus sequence	
15	CPMV HT	
16	CPMV HT+	
17	CPMV HT+ 511	
18	CPMV HT+[WT115]	
19	IF-WA_VP2(opt).s1+3c	Fig. 7A
20	IF-WA_VP2(opt).s1-4r	Fig. 7B
21	Optimized coding sequence of Rotavirus A VP2 from strain WA	Fig. 7C
22	Construct 1191	Fig. 7E
23	Expression cassette number 1710	Fig. 7F
24	Amino acid sequence of VP2 from Rotavirus A WA strain	Fig. 7G
25	IF-WA_VP6(opt).s1+3c	Fig. 8A
26	IF-WA_VP6(opt).s1-4r	Fig. 8B
27	Optimized coding sequence of Rotavirus A VP6 from strain WA	Fig. 8C
28	Expression cassette number 1713	Fig. 8D
29	Amino acid sequence of VP6 from Rotavirus A WA strain	Fig. 8E
30	IF-Rtx_VP4(opt).s1+3c	Fig. 9A
31	IF-Rtx_VP4(opt).s1-4r	Fig. 9B
32	Optimized coding sequence of Rotavirus A VP4 from strain RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]	Fig. 9C
33	Expression cassette number 1730	Fig. 9D
34	Amino acid sequence of VP4 from Rotavirus A Rotarix strain	Fig. 9E
35	IF-TrSP+Rtx_VP7(opt).s1+3c	Fig. 10A

36	IF-Rtx_VP7(opt).s1-4r	Fig. 10B
37	Optimized coding sequence of Rotavirus A VP7 from strain RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]	Fig. 10C
38	Expression cassette number 1734	Fig. 10D
39	Amino acid sequence of TrSp-VP7 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain	Fig. 10E
40	IF-WA_NSP4.s1+3c	Fig. 11A
41	IF-WA_NSP4.s1-4r	Fig. 11B
42	Coding sequence of Rotavirus A NSV4 from strain WA	Fig. 11C
43	Expression cassette number 1706	Fig. 11D
44	Amino acid sequence of NSP4 from Rotavirus A WA strain	Fig. 11E
45	IF(C160)-WA_VP2(opt).c	Fig. 12A
46	Construct 1190	Fig. 12C
47	Expression cassette number 1108	Fig.12D
48	IF(C160)-WA_VP6(opt).c	Fig. 13A
49	Expression cassette number 1128	Fig. 13B
50	IF(C160)-Rtx_VP4(opt).c	Fig. 14A
51	Expression cassette number 1178	Fig. 14B
52	IF(C160)-TrSP+Rtx_VP7(opt).c	Fig. 15A
53	Expression cassette number 1199	Fig. 15B

[00288] The present invention will be further illustrated in the following examples.

Examples

Example 1 Materials and Methods

Table 3: Constructs

Constr. #	Description	Fig.	Constr.#	Description	Fig.
1108	160-VP2	3A/3B	1706	CPMV-HT NSP4	3A/3B 4A/4B
1128	160-VP6	3A/3B	1708	CPMV-HT VP6/2	3A/3B 4A/4B
1178	160-VP4	3A/3B	2408	160-VP7/4	3A/3B 4A/4B
1199	160-VP7	3A/3B	1769	CPMV-HT VP7/4/6/2	3A/3B/5
1710	CPMV-HT VP2	4A/4B	2441	CPMV-HT VP4/7/NSP4/6/2	5
1713	CPMV-HT VP6	4A/4B	2400	160-VP6/2	4A/4B
1730	CPMV-HT VP4	4A/4B	1719	CPMV-HT VP7/4	4A/4B
1734	CPMV-HT VP7	4A/4B			

1. 2X35S/CPMV-HT/ RVA(WA) VP2(opt)/ NOS (Construct number 1710)

[00289] An optimized sequence encoding VP2 from Rotavirus A WA strain was cloned into 2X35S-CPMV-HT-NOS expression system in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP2 coding sequence was amplified using primers IF-WA_VP2(opt).s1+3c (Figure 7a, SEQ ID NO: 19) and IF-WA_VP2(opt).s1-4r (Figure 7B, SEQ ID NO: 20), using optimized VP2 gene sequence (Figure 7C, SEQ ID NO :21) as template. For sequence optimization, VP2 protein sequence (Genbank accession number CAA33074) was backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR product was cloned in 2X35S/CPMV-HT/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1191 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1191 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a CPMV-HT-based expression cassette. 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 Figure 7E (SEQ ID NO: 22). The resulting construct was given number 1710 (Figure 7F, SEQ ID NO: 23). The amino acid sequence of VP2 from Rotavirus A strain WA is presented in Figure 7G (SEQ ID NO: 24). A representation of plasmid 1710 is presented in Figure 7H.

2. 2X35S/CPMV-HT/RVA(WA) VP6(opt)/NOS (Construct number 1713)

[00290] An optimized sequence encoding VP6 from Rotavirus A WA strain was cloned into 2X35S-CPMV-HT-NOS expression system in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP6 coding sequence was amplified using primers IF-WA_VP6(opt).s1+3c (Figure 8A, SEQ ID NO: 25) and IF-WA_VP6(opt).s1-4r (Figure 8B, SEQ ID NO: 26), using optimized VP6 gene sequence (Figure 8C, SEQ ID NO :27) as template. For sequence optimization, VP6 protein sequence (Genbank accession number AAA47311) was backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR product was cloned in 2X35S/CPMV-HT/NOS expression system using In-Fusion cloning system

(Clontech, Mountain View, CA). Construct number 1191 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1191 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a CPMV-HT-based expression cassette. 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 Figure 7E (SEQ ID NO: 22). The resulting construct was given number 1713 (Figure 8D, SEQ ID NO: 28). The amino acid sequence of VP6 from Rotavirus A strain WA is presented in Figure 8E (SEQ ID NO: 29). A representation of plasmid 1713 is presented in Figure 8F.

3. 2X35S/CPMV-HT/RVA(Rtx) VP4(opt)/NOS (Construct number 1730)

[00291] An optimized sequence encoding VP4 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain was cloned into 2X35S/CPMV-HT/NOS in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP4 coding sequence was amplified using primers IF-Rtx_VP4(opt).s1+3c (Figure 9A, SEQ ID NO: 30) and IF-Rtx_VP4(opt).s1-4r (Figure 9B, SEQ ID NO: 31), using optimized VP4 gene sequence (Figure 9C, SEQ ID NO: 32) as template. For sequence optimization, VP4 protein sequence (Genbank accession number AEX30660) was backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR product was cloned in 2X35S/CPMV-HT/NOS expression cassette using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1191 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1191 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a CPMV-HT-based expression cassette. 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 Figure 7E (SEQ ID NO: 22). The resulting construct was given number 1730 (Figure 9D, SEQ ID NO: 33). The amino acid sequence of VP4 from Rotavirus A vaccine USA/Rotarix-

A41CB052A/1988/G1P1A[8] is presented in Figure 9E (SEQ ID NO: 34). A representation of plasmid 1730 is presented in Figure 9F.

4. 2X35S/CPMV-HT/TrSp-RVA(Rtx) VP7(opt)/NOS (Construct number 1734)

[00292] An optimized sequence encoding VP7 with a truncated version of the native signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain was cloned into 2X35S-CPMV-HT-NOS expression system in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP7 coding sequence was amplified using primers IF-TrSP+Rtx_VP7(opt).s1+3c (Figure 10A, SEQ ID NO: 35) and IF-Rtx_VP7(opt).s1-4r (Figure 10B, SEQ ID NO: 36), using optimized VP7 gene sequence (corresponding to nt 88-891 from Figure 10C, SEQ ID NO: 37) as template. For sequence optimization, VP7 protein sequence (Genbank accession number AEX30682) was backtranslated and optimized for human codon usage, GC content and mRNA structure. The PCR product was cloned in 2X35S/CPMV-HT/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1191 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1191 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a CPMV-HT-based expression cassette. 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 Figure 7E (SEQ ID NO: 22). The resulting construct was given number 1734 (Figure 10D, SEQ ID NO: 38). The amino acid sequence of VP7 with truncated signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain is presented in Figure 10E (SEQ ID NO: 39). A representation of plasmid 1734 is presented in Figure 10F.

5. 2X35S/CPMV-HT/RVA(WA) NSP4/NOS (Construct number 1706)

[00293] A sequence encoding NSP4 from Rotavirus A WA strain was cloned into 2X35S-CPMV-HT-NOS expression system in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method.

A fragment containing the NSP4 coding sequence was amplified using primers IF-WA_NSP4.s1+3c (Figure 11A, SEQ ID NO: 40) and IF-WA_NSP4.s1-4r (Figure 11B, SEQ ID NO: 41), using synthesized NSP4 gene (corresponding to nt 42-569 from GenBank accession number K02032) (Figure 11C, SEQ ID NO : 42) as template. The PCR product was cloned in 2X35S/CPMV-HT/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1191 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1191 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a CPMV-HT-based expression cassette. 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 Figure 7E (SEQ ID NO: 22). The resulting construct was given number 1706 (Figure11D, SEQ ID NO: 43). The amino acid sequence of NSP4 from Rotavirus A strain WA is presented in Figure 11E (SEQ ID NO: 44). A representation of plasmid 1706 is presented in Figure 11F.

6. 2X35S/CPMV-160/ RVA(WA) VP2(opt)/ NOS (Construct number 1108)

[00294] An optimized sequence encoding VP2 from Rotavirus A WA strain was cloned into 2X35S/CPMV-160/NOS expression system in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP2 coding sequence was amplified using primers IF(C160)-WA_VP2(opt).c (Figure 12A, SEQ ID NO: 45) and IF-WA_VP2(opt).s1-4r (Figure 7B, SEQ ID NO: 20), using optimized VP2 gene sequence (Figure 7C, SEQ ID NO : 21) as template. For sequence optimization, VP2 protein sequence (Genbank accession number CAA33074) 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 (Figure 12B) 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 CPMV-160-based expression cassette. 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 Figure 12C (SEQ ID NO: 46). The resulting construct was given number 1108 (Figure 12D, SEQ ID NO: 47). The amino acid sequence of VP2 from Rotavirus A strain WA is presented in Figure 7G (SEQ ID NO: 24). A representation of plasmid 1108 is presented in Figure 12E.

7. -2X35S/CPMV-160/RVA(WA) VP6(opt)/NOS (Construct number 1128) –

An optimized sequence encoding VP6 from Rotavirus A WA strain was cloned into 2X35S/CPMV-160/NOS expression system in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP6 coding sequence was amplified using primers IF(C160)-WA_VP6(opt).c (Figure 13A, SEQ ID NO: 48) and IF-WA_VP6(opt).s1-4r (Figure 8B, SEQ ID NO: 26), using optimized VP6 gene sequence (Figure 8C, SEQ ID NO : 27) as template. For sequence optimization, VP6 protein sequence (Genbank accession number AAA47311) 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 (Figure 11B, SEQ ID NO:40) 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 CPMV-160-based expression cassette. 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 Figure 11C (SEQ ID NO: 41). The resulting construct was given number 1128 (Figure 13B, SEQ ID NO: 49). The amino acid sequence of VP6 from Rotavirus A strain WA is presented in Figure 8E (SEQ ID NO: 28). A representation of plasmid 1128 is presented in Figure 13C.

8. X35S/CPMV-160/RVA(Rtx) VP4(opt)/NOS (Construct number 1178)

An optimized sequence encoding VP4 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain was cloned into 2X35S/CPMV-160/NOS in a

plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP4 coding sequence was amplified using primers IF(C160)-Rtx_VP4(opt).c (Figure 14A, SEQ ID NO: 50) and IF-Rtx_VP4(opt).s1-4r (Figure 9B, SEQ ID NO: 30), using optimized VP4 gene sequence (Figure 9C, SEQ ID NO: 31) as template. For sequence optimization, VP4 protein sequence (Genbank accession number AEX30660) 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 (Figure 11B, SEQ ID NO: 40) 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 CPMV-160-based expression cassette. 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 Figure 11C (SEQ ID NO: 41). The resulting construct was given number 1178 (Figure H2, SEQ ID NO: H2). The amino acid sequence of VP4 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] is presented in Figure 9E (SEQ ID NO: 33). A representation of plasmid 1178 is presented in Figure 14C.

9. 2X35S/CPMV-160/TrSp-RVA(Rtx) VP7(opt)/NOS (Construct number 1199)

An optimized sequence encoding VP7 with a truncated version of the native signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain was cloned into 2X35S/CPMV-160/NOS expression system in a plasmid containing Plasto_pro/P19/Plasto_ter expression cassette using the following PCR-based method. A fragment containing the VP7 coding sequence was amplified using primers IF(C160)-TrSP+Rtx_VP7(opt).c (Figure 15A, SEQ ID NO: 52) and IF-Rtx_VP7(opt).s1-4r (Figure 10B, SEQ ID NO: 35), using optimized VP7 gene sequence (corresponding to nt 88-891 from Figure 10C, SEQ ID NO: 36) as template. For sequence optimization, VP7 protein sequence (Genbank accession number AEX30682) 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 (Figure 11B, SEQ ID NO: 40) 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 CPMV-160-based expression cassette. 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 Figure 11C (SEQ ID NO: 41). The resulting construct was given number 1199 (Figure 15B, SEQ ID NO: 53). The amino acid sequence of VP7 with truncated signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain is presented in Figure 10E (SEQ ID NO: 38). A representation of plasmid 1199 is presented in Figure 15C.

10. Double gene construct for the expression of VP6 and VP2 under CPMV-HT expression cassette (construct number 1708)

[00295] A single vector for the co-expression of VP6 from Rotavirus A WA strain and VP2 from Rotavirus A WA strain under the control of CPMV-HT expression system was assembled using the following restriction enzyme/ligase-based method. Donor plasmid DNA (construct number 1710; 2X35S/CPMV-HT/ RVA(WA) VP2(opt)/ NOS)(Figure 7F, SEQ ID NO: 23) was digested with AvrII (located before the 2X35S promoter) and AscI (located after the NOS terminator) restriction enzymes and the fragment corresponding to 2X35S/CPMV-HT/ RVA(WA) VP2(opt)/ NOS expression cassette was gel-purified. This fragment was then inserted into the acceptor construct number 1713 (2X35S/CPMV-HT/RVA(WA) VP6(opt)/NOS)(Figure 8D, SEQ ID NO: 28) linearized using XbaI and AscI restriction enzymes (both sites are located after the NOS terminator of VP6 expression cassette). The resulting construct was given number 1708. A representation of plasmid 1708 is presented in Figure 16.

11. Double gene construct for the expression of VP7 and VP4 under CPMV-HT expression cassette (construct number 1719)

[00296] A single vector for the co-expression of VP7 with a truncated version of the native signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain and VP4 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain under the control of CPMV-HT expression system was assembled using the following restriction enzyme/ligase-based method. Donor plasmid DNA (construct number 1730; 2X35S/CPMV-HT/ RVA(Rtx) VP4(opt)/ NOS)(Figure 9D, SEQ ID NO: 32) was digested with AvrII (located before the 2X35S promoter) and AscI (located after the NOS terminator) restriction enzymes and the fragment corresponding to 2X35S/CPMV-HT/ RVA(Rtx) VP4(opt)/ NOS expression cassette was gel-purified. This fragment was then inserted into the acceptor construct number 1734 (2X35S/CPMV-HT/TrSp-RVA(Rtx) VP7(opt)/NOS)(Figure 10D, SEQ ID NO: 37) linearized using XbaI and AscI restriction enzymes (both sites are located after the NOS terminator of VP7 expression cassette). The resulting construct was given number 1719. A representation of plasmid 1719 is presented in Figure 17.

12. Double gene construct for the expression of VP6 and VP2 under CPMV-160 expression cassette (construct number 2400)

[00297] A single vector for the co-expression of VP6 from Rotavirus A WA strain and VP2 from Rotavirus A WA strain under the control of CPMV-160 expression system was assembled using the following restriction enzyme/ligase-based method. Donor plasmid DNA (construct number 1108; 2X35S/CPMV-160/ RVA(WA) VP2(opt)/ NOS)(Figure 12D, SEQ ID NO: 47) was digested with AvrII (located before the 2X35S promoter) and AscI (located after the NOS terminator) restriction enzymes and the fragment corresponding to 2X35S/CPMV-160/ RVA(WA) VP2(opt)/ NOS expression cassette was gel-purified. This fragment was then inserted into the acceptor construct number 1128 (2X35S/CPMV-160/RVA(WA) VP6(opt)/NOS)(Figure 13B, SEQ ID NO: 49) linearized using XbaI and AscI restriction enzymes (both sites are located after the NOS terminator of VP6 expression cassette). The resulting construct was given number 2400. A representation of plasmid 2400 is presented in Figure 18.

13. Double gene construct for the expression of VP7 and VP4 under CPMV-160 expression cassette (construct number 2408)

A single vector for the co-expression of VP7 with a truncated version of the native signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain and VP4 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain under the control of CPMV-160 expression system was assembled using the following restriction enzyme/ligase-based method. Donor plasmid DNA (construct number 1178; 2X35S/CPMV-160/ RVA(Rtx) VP4(opt)/ NOS)(Figure 14B, SEQ ID NO: 51) was digested with AvrII (located before the 2X35S promoter) and AscI (located after the NOS terminator) restriction enzymes and the fragment corresponding to 2X35S/CPMV-160/ RVA(Rtx) VP4(opt)/ NOS expression cassette was gel-purified. This fragment was then inserted into the acceptor construct number 1199 (2X35S/CPMV-160/TrSp-RVA(Rtx) VP7(opt)/NOS)(Figure 15B, SEQ ID NO: 53) linearized using XbaI and AscI restriction enzymes (both sites are located after the NOS terminator of VP7 expression cassette). The resulting construct was given number 2408. A representation of plasmid 2408 is presented in Figure 19.

14. Quadruple gene construct for the expression of VP7, VP4, VP6 and VP2 under CPMV-HT expression cassette (construct number 1769)

A single vector for the co-expression of VP7 with a truncated version of the native signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain, VP4 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain, VP6 from Rotavirus A WA strain and VP2 from Rotavirus A WA strain under the control of CPMV-HT expression system was assembled using the following restriction enzyme/ligase-based method. Donor plasmid DNA (construct number 1730; 2X35S/CPMV-HT/ RVA(Rtx) VP4(opt)/ NOS)(Figure 9D, SEQ ID NO: 32) was digested with AvrII (located before the 2X35S promoter) and AscI (located after the NOS terminator) restriction enzymes and the fragment corresponding to 2X35S/CPMV-HT/ RVA(Rtx) VP4(opt)/ NOS expression cassette was gel-purified. This fragment was then inserted into the acceptor construct number 1734 (2X35S/CPMV-HT/TrSp-RVA(Rtx) VP7(opt)/NOS)(Figure 10D, SEQ ID NO: 37) linearized using XbaI and AscI restriction enzymes (both sites are located after the

NOS terminator of VP7 expression cassette). Ligation of cohesive ends produced by AvrII and XbaI destroyed the original restriction sites producing a temporary acceptor vector with the same unique XbaI and AscI restriction enzyme sites at the end of the NOS terminator of the second expression cassettes (from left to right T-DNA). VP6 (construct number 1713; Figure 8D, SEQ ID NO: 28) and VP2 (construct number 1710; Figure 7F, SEQ ID NO: 23) expressed under CPMV-HT expression system were then inserted sequentially in the resulting temporary acceptor vector using the same digestion strategy to give the final VP7/VP4/VP6/VP2 construct. The resulting construct was given number 1769. A representation of plasmid 1769 is presented in Figure 20.

15. Quintuple gene construct for the expression of VP4, VP7, NSP4, VP6 and VP2 under CPMV-HT expression cassette (construct number 2441)

A single vector for the co-expression of VP4 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain, VP7 with a truncated version of the native signal peptide from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain, NSP4 from Rotavirus A WA strain, VP6 from Rotavirus A WA strain and VP2 from Rotavirus A WA strain under the control of CPMV-HT expression system was assembled using the following restriction enzyme/ligase-based method. Donor plasmid DNA (construct number 1734; 2X35S/CPMV-HT/ TrSp-RVA(Rtx) VP7(opt)/ NOS)(Figure 10D, SEQ ID NO: 37) was digested with AvrII (located before the 2X35S promoter) and AscI (located after the NOS terminator) restriction enzymes and the fragment corresponding to 2X35S/CPMV-HT/ TrSp-RVA(Rtx) VP7(opt)/ NOS expression cassette was gel-purified. This fragment was then inserted into the acceptor construct number 1730 (2X35S/CPMV-HT/ RVA(Rtx) VP4(opt)/NOS)(Figure 9D, SEQ ID NO: 32) linearized using XbaI and AscI restriction enzymes (both sites are located after the NOS terminator of VP4 expression cassette). Ligation of cohesive ends produced by AvrII and XbaI destroyed the original restriction sites producing a temporary acceptor vector with the same unique XbaI and AscI restriction enzyme sites at the end of the NOS terminator of the second expression cassettes (from left to right T-DNA). NSP4 (construct number 1706; Figure 11D, SEQ ID NO: 42), VP6 (construct number 1713; Figure 8D, SEQ ID NO: 28) and VP2 (construct number 1710; Figure 7F, SEQ ID NO: 23) expressed

under CPMV-HT expression system were then inserted sequentially in the resulting temporary acceptor vector using the same digestion strategy to give the final VP4/VP7/NSP4/VP6/VP2 construct. The resulting construct was given number 2441. A representation of plasmid 2441 is presented in Figure 21.

Example 2 Co-expression of NSP4 increases VP4 and VP4 incorporation into RLPs

[00298] The rotavirus VP2, VP4, VP6 and VP7 structural antigens were transiently co-expressed in *Nicotiana benthamiana* plants in the presence or absence of a NSP4 expression construct using agroinfiltration as described in example 1. Crude protein extracts from RLP producing plants contain large amounts of host protein as shown by the banding pattern in Coomassie-stained SDS-PAGE (Figure 2B, load). Rotavirus-like particles can be separated from plant proteins by ultracentrifugation on a iodixanol density gradient. After centrifugation, analysis of the fractions from iodixanol density gradient showed that the RLPs migrated to the 35% iodixanol fraction (F2 and F3 in Figure 2B) while the majority of the host proteins remained in the 25-30% iodixanol fractions (F4-F10 in Figure 2B). RLPs from plants co-expressing rotavirus structural antigens were purified on iodixanol density gradients and the analysis of the RLP containing fractions (F2 and F3) showed that RLPs can be produced efficiently, irrespectively of the number of gene per construct as shown in figure 3A with single, dual and quadruple gene constructs. The results obtained also showed that the co-expression of NSP4 reduced RLP expression (compare fractions under –NSP4 and +NSP4 in figure 3A). Note that equal volumes of each fraction were loaded on the gel to compare RLP content per volume.

[00299] RLP-containing fraction 2 from the same experiments were analyzed by western blot to evaluate the impact of NSP4 co-expression on VP4 and VP7 incorporation. For that comparison, equal amounts of RLPS were loaded on the gel. The western blot results obtained showed stronger signals for VP4 and VP7 on the RLPs produced in the presence of NSP4 (Figure 3B, compare lanes under –NSP4 and +NSP4). These results clearly indicate that the co-expression of NSP4 increased VP4 and VP7 incorporation on the surface of the RLPs.

[00300] The genes encoding the four rotavirus antigens and the non-structural protein NSP4 were cloned into CPMV-HT and CPMV160 for comparison of expression. Co-expression studies followed by extraction and purification by ultracentrifugation in iodixanol density gradient showed that both expression efficiently produced RLPs, as demonstrated by the amount of VP6 in fractions 2 and 3 of the gradient (Figure 4A), and the amount of VP4 and VP7 in fraction 2 from the same treatments (Figure 4B). This study also showed that, when using the CPMV-HT system for expression of the rotavirus proteins, single gene constructs produced as much RLPs as dual gene constructs (Figure 4A, left panel vs middle panel) and resulted in similar coverage with the surface antigens, VP4 and VP7 (Figure 4B, left panel vs middle panel).

[00301] A quintuple gene construct (comprising 5 genes on the same plasmid) has been evaluated for the co-expression of the four structural antigens with NSP4. As shown in figure 5, the use of quintuple gene construct resulted in similar RLP production level as with the use of a quadruple gene construct with the NSP4 gene on a separate plasmid (Figure 5, top panel), as well as comparable levels of VP4 and VP7 incorporation (Figure 5, lower panel).

Agrobacterium transformation

[00302] All plasmids were used to transform *Agrobacterium tumefaciens* (AGL1; ATCC, Manassas, VA 20108, USA) by electroporation (Mattanovich et al., 1989, Nucleic Acid Res. 17:6747) alternatively, heat shock using CaCl2-prepared competent cells (XU et al., 2008, Plant Methods 4) may be used. The integrity of the plasmids in the *A. tumefaciens* strains created was confirmed by restriction mapping.

Preparation of plant biomass, inoculum, agroinfiltration, and harvesting

[00303] *Nicotiana 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.

[00304] Agrobacteria transfected with each construct were grown in a LB medium from vegetal origin and supplemented with 10 mM 2-(N-morpholino)ethanesulfonic acid (MES) and 50 µg/ml kanamycin pH5.6 until they reached an OD600 between 0.6 and 2.5. Agrobacterium suspensions were mixed to reach appropriate ratio for each construct and brought to 2.5X OD600 with infiltration medium (10 mM MgCl2 and 10 mM MES pH 5.6). *A. tumefaciens* suspensions were stored overnight at 4°C. On the day of infiltration, culture batches were diluted with infiltration medium 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. Following infiltration, plants were returned to the greenhouse for a 9 day incubation period until harvest. Harvested biomass was kept frozen (-80°C) until use for purification of particles.

Extraction and screening by ultracentrifugation of rotavirus-like particles

[00305] Proteins were extracted from frozen biomass by mechanical extraction in a blender with 2 volumes of extraction buffer (TNC: 10 mM Tris pH 7.4, 140 mM NaCl, 10 mM CaCl2). 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.

[00306] Analytical density gradient centrifugation was performed as follows. 38 ml tubes containing discontinuous iodixanol density gradient in TNC buffer (1.2 ml at 45%, 2 ml at 35%, 5 ml at 30% 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 120 000 g for 4 hours (4°C). After centrifugation, 1 ml fractions were collected from the bottom to the top and fractions 2 and 3 (corresponding to 35% iodixanol) were analysed by SDS-PAGE combined to protein staining or Western blot.

SDS-PAGE and immunoblotting

[00307] Protein concentrations were determined by the BCA protein assay (Pierce Biochemicals, Rockport, IL). 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).

[00308] For immunoblotting, electrophoresed proteins were electrotransferred onto polyvinylene difluoride (PVDF) membranes (Roche Diagnostics Corporation, Indianapolis, IN). Prior to immunoblotting, the membranes were blocked with 5% skim milk and 0.1% Tween-20 in Tris-buffered saline (TBS-T) for 16-18h at 4ºC.

[00309] Immunoblotting was performed by incubation with a suitable antibody (Table 4) in 2% skim milk in TBS-Tween 20 0.1%. Secondary antibodies used 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, Indianapolis, IN).

[00310] Table 4: Electrophoresis conditions, antibodies, and dilutions for immunoblotting of rotavirus antigens.

Rotavirus antigen	Electrophoresis condition	Primary antibody	Dilution	Secondary antibody	Dilution
VP4	Reducing	Rabbit serum from immunized Rabbit with recombinant VP4 (in house)	1:30 000	Goat anti-rabbit (JIR 111-035-144)	1:10 000
VP7	Reducing	Rabbit serum from immunized Rabbit with recombinant VP7 (in house)	1:50 000	Goat anti-rabbit (JIR 111-035-144)	1:10 000

[00311] All citations are hereby incorporated by reference.

[00312] 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 without departing from the scope of the invention as defined in the claims.

WHAT IS CLAIMED IS:

1. A method of producing a rotavirus like particle (RLP) in a host or host cell comprising:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP.

2. The method of claim 1, wherein the one or more nucleic acid further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third and fourth nucleotide sequence encoding rotavirus protein VP2, VP4 or VP7 and wherein each of NSP4, VP6 and two of VP2, VP4 and VP7 are expressed from the one or more nucleic acid.

3. The method of claim 2, wherein the one or more nucleic acid further comprises a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second,

third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

4. The method of claim 3, wherein the one or more nucleic acid comprises one nucleic acid comprising the first, second, third, fourth and fifth nucleotide sequence encoding the first, second, third, fourth and fifth rotavirus protein.

5. The method of claim 3, wherein the one or more nucleic acid comprises two nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, and a second nucleic acid comprising the second, third, fourth and fifth nucleotide sequence encoding the second, third, fourth and fifth rotavirus protein.

6. The method of claim 3, wherein the one or more nucleic acid comprises two nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, and a second nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein.

7. The method of claim 3, wherein the one or more nucleic acid comprises three nucleic acids, a first nucleic acid comprising the first and second nucleotide sequence encoding the first and second rotavirus protein, a second nucleic acid comprising the third and fourth nucleotide sequence encoding the third and fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein.

8. The method of claim 3, wherein the one or more nucleic acid comprises three nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, and a third nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein.

9. The method of claim 3, wherein the one or more nucleic acid comprises four nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth and fifth nucleotide sequence encoding the fourth and fifth rotavirus protein.

10. The method of claim 3, wherein the one or more nucleic acid comprises five nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein.

11. The method of claim 1, wherein the host or host cell comprises insect cells, mammalian cells, plant, portion of a plant or plant cells.

12. The method of claim 11, wherein the host or host cell consist of a plant, portion of a plant or plant cells.

13. The method of claim 12, wherein the plant is *Nicotiana benthamiana*.

14. The method of claim 1, wherein the one or more nucleotide sequence is operatively linked to one or more expression enhancer.

15. The method of claim 14, wherein the expression enhancer is selected from the group consisting of CPMV HT, CPMV 160, CPMV 160+ and CPMV HT+.

16. The method of any one of claims 1, 2 or 3, further comprising the steps of:

c) harvesting the host or host cell, and

d) purifying the RLPs from the host or host cell, wherein the RLPs range in size from 70-100 nm.

17. A method of producing a rotavirus like particle (RLP) in a host or host cell comprising:

a) introducing into the host or host cell one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP.

18. The method of claim 17, wherein the one or more nucleic acid further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third and fourth nucleotide sequence encoding of rotavirus protein VP2, VP4 or VP7 and wherein each of NSP4, VP6 and two of VP2, VP4 and VP7 are expressed from the one or more nucleic acid.

19. The method of claim 18, wherein the one or more nucleic acid further comprises a fifth nucleotide sequence encoding a fifth rotavirus protein, the first, second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

20. The method of claim 19, wherein in the step of introducing (step a), the one or more nucleic acid comprises two nucleic acids:

i) a first nucleic acid comprising a first nucleotide sequence encoding a first rotavirus protein, and a second nucleic acid comprising a second, third, fourth and fifth nucleotide sequence encoding a second, third, fourth and fifth rotavirus protein; or

ii) a first nucleic acid comprising a first and second nucleotide sequence encoding a first and second rotavirus protein, and a second nucleic acid comprising a third, fourth and fifth nucleotide sequence encoding a third, fourth and fifth rotavirus protein, and

wherein the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid that is introduced into the host or host cell being between 1:0.8 and 1:2.

21. The method of claim 20, wherein the first rotavirus protein is NSP4, and the second, third, fourth and fifth rotavirus proteins are VP2, VP4, VP6 and VP7.

22. The method of claim 20, wherein the ratio is 1:1.

23. The method of claim 19, wherein in the step of introducing (step a), the one or more nucleic acid comprises three nucleic acids:

i) a first nucleic acid comprising the first and the second nucleotide sequence encoding the first and the second rotavirus protein, a second nucleic acid comprising the third and fourth nucleotide sequence encoding the third and the fourth rotavirus protein, and a third nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, or

ii) the first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, the second nucleic acid comprising the second nucleotide

sequence encoding the second rotavirus protein, and the third nucleic acid comprising the third, fourth and fifth nucleotide sequence encoding the third, fourth and fifth rotavirus protein,

wherein the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid and to the amount of the third nucleic that is introduced into the host or host cell is 1:1:1.

24. The method of claim 19, wherein in the step of introducing (step a), the one or more nucleic acid comprises four nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, and a fourth nucleic acid comprising the fourth and the fifth nucleotide sequence encoding the fourth and fifth rotavirus protein, wherein the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid, to the amount of the third nucleic and to the amount of the fourth nucleic acid that is introduced into the host or host cell is 1:1:1:1.

25. The method of claim 19, wherein in the step of introducing (step a), the one or more nucleic acid comprises five nucleic acids, a first nucleic acid comprising the first nucleotide sequence encoding the first rotavirus protein, a second nucleic acid comprising the second nucleotide sequence encoding the second rotavirus protein, a third nucleic acid comprising the third nucleotide sequence encoding the third rotavirus protein, a fourth nucleic acid comprising the fourth nucleotide sequence encoding the fourth rotavirus protein, and a fifth nucleic acid comprising the fifth nucleotide sequence encoding the fifth rotavirus protein, and the ratio of an amount of the first nucleic acid relative to the amount of the second nucleic acid, to the amount of the third nucleic acid, to the amount of the fourth nucleic acid and to the amount of the fifth nucleic acid that is introduced into the host or host cell is 1:1:1:1:1.

26. An RLP produced by the method of claim 3 or 19, wherein the RLP is a triple layered RLP comprising rotavirus protein, the rotavirus protein consisting of VP2, VP4, VP6 and VP7.

27. A composition comprising an effective dose of the RLP of claim 26 for inducing an immune response in a subject, and a pharmaceutically acceptable carrier.

28. A method of inducing immunity to a rotavirus infection in a subject, comprising administering the composition of claim 27 to the subject.

29. The method of claim 28, wherein the composition is administered to a subject orally, intradermally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

30. A plant matter comprising an RLP produced by the method of claim 1, 2 or 3.

31. A plant matter comprising an RLP produced by the method of claim 17, 18 or 19.

32. A method of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) comprising:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

33. A method of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) comprising:

a) introducing into a host or host cell one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein and a third nucleotide sequence encoding a third rotavirus protein, the first, second and third nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third nucleotide sequence encoding one of rotavirus protein VP7 or VP4;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of NSP4, VP6 and VP7 or VP4 are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

34. The method of claim 32 or 33, wherein the one or more nucleic acid further comprises a fourth nucleotide sequence encoding a fourth rotavirus protein, the first, second, third, and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first nucleotide sequence encoding rotavirus protein NSP4, the second nucleotide sequence encoding rotavirus protein VP6, and the third and fourth nucleotide sequence encoding rotavirus protein VP2, VP4 or VP7 and wherein each of NSP4, VP6 and two of VP2, VP4 and VP7 are expressed from the one or more nucleic acid.

35. The method of claim 32 or 33, wherein the one or more nucleic acid further comprises a fifth nucleotide sequence encoding a fifth rotavirus protein, the first,

second, third, fourth and fifth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third, fourth and fifth nucleotide sequence encoding one of rotavirus protein VP2, VP4, VP6, VP7 or NSP4 and wherein each of VP2, VP4, VP6, VP7 and NSP4 are expressed from the one or more nucleic acid.

36. The method of claim 32, 33, 34 or 35, further comprising the steps of:

c) harvesting host or host cell, and

d) purifying the RLPs from the host or host cell, wherein the RLPs range in size from 70-100 nm.

37. Use of NSP4 for increasing incorporation of VP4, VP7, or both VP4 and VP7 on the surface of a rotavirus like particle (RLP) produced in insect cells, mammalian cells, plant, portion of a plant or plant cells.

38. A method of increasing incorporation of VP4, VP7, or both VP4 and VP7 in a rotavirus like particle (RLP) comprising:

a) providing a host or host cell comprising one or more nucleic acid comprising

a first nucleotide sequence encoding a first rotavirus protein, a second nucleotide sequence encoding a second rotavirus protein, a third nucleotide sequence encoding a third rotavirus protein and a fourth nucleotide sequence encoding a fourth rotavirus protein; the first, second, third and fourth nucleotide sequence being operatively linked to one or more regulatory region active in the host or host cell; and

the first, second, third and fourth nucleotide sequence encoding one of rotavirus protein VP7, VP4, NSP4 and VP2 or VP6;

b) incubating the host or host cell under conditions that permit the expression of the one or more nucleic acid, so that each of the VP7, VP4, NSP4 and VP2 or VP6 and are expressed, thereby producing the RLP with enhanced levels of VP4, VP7, or both VP4 and VP7 when compared to the level of VP4 or VP7 produced

by a second host or host cell that expresses the one or more nucleic acid that does not comprise NSP4, under the same conditions.

Figure 1.

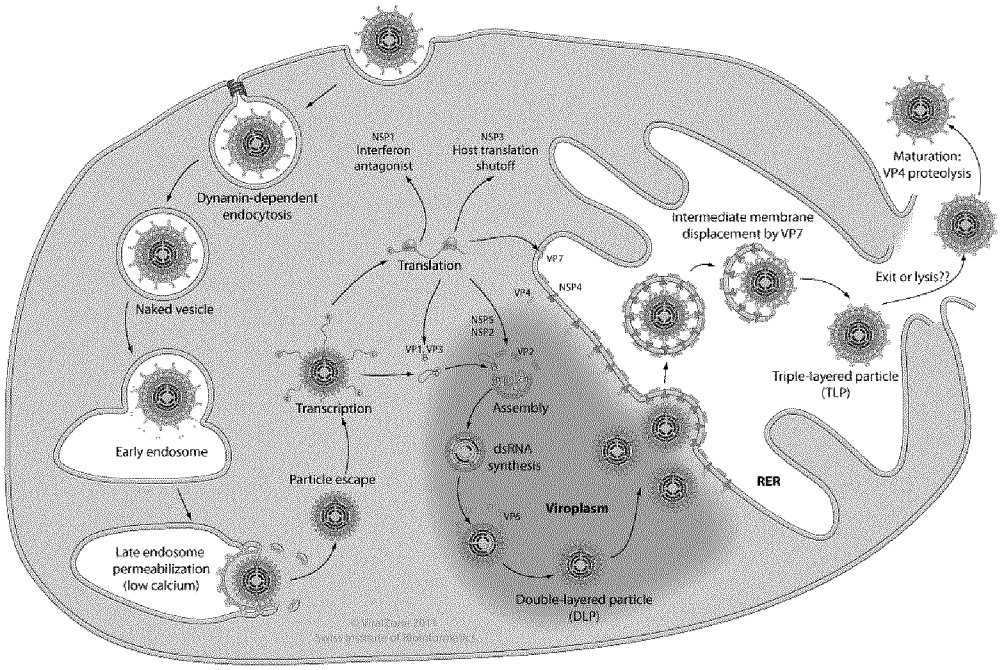

Dynamin-dependent endocytosis
Naked vesicle
Early endosome
Late endosome permeabilization (low calcium)
Particle escape
Transcription
Translation
NSP1 Interferon antagonist
NSP3 Host translation shutoff
VP7
NSP4
VP4
NSP5 NSP2
VP1 VP3
VP2
Assembly
dsRNA synthesis
VP6
Viroplasm
RER
Double-layered particle (DLP)
Intermediate membrane displacement by VP7
Maturation: VP4 proteolysis
Exit or lysis??
Triple-layered particle (TLP)

Figure 2A

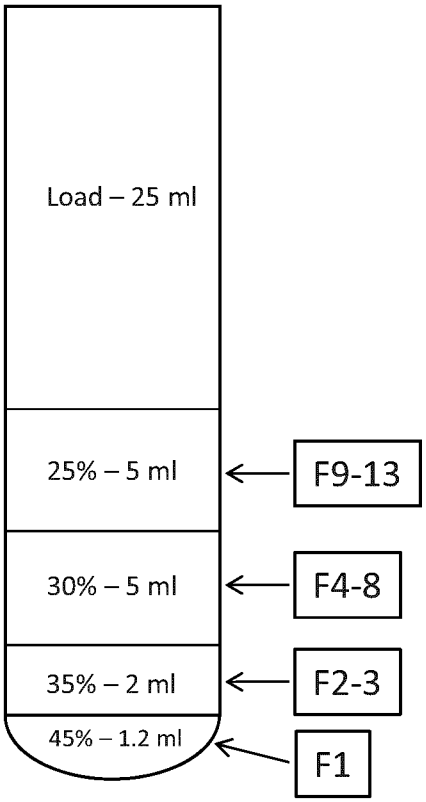


Figure 2B

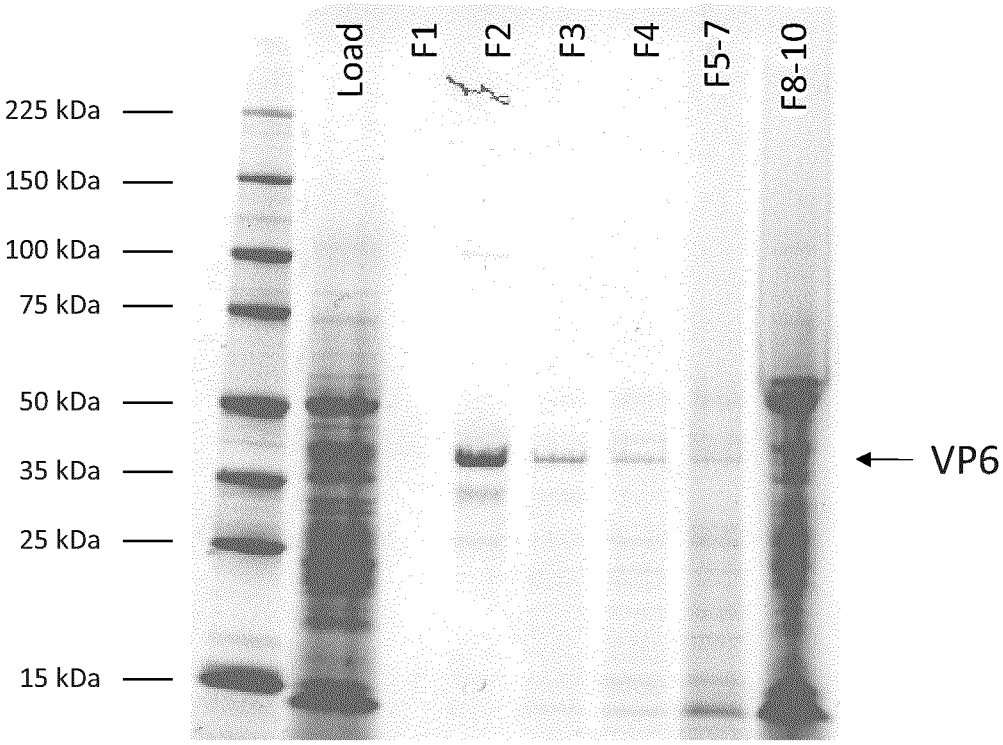


Figure 3A

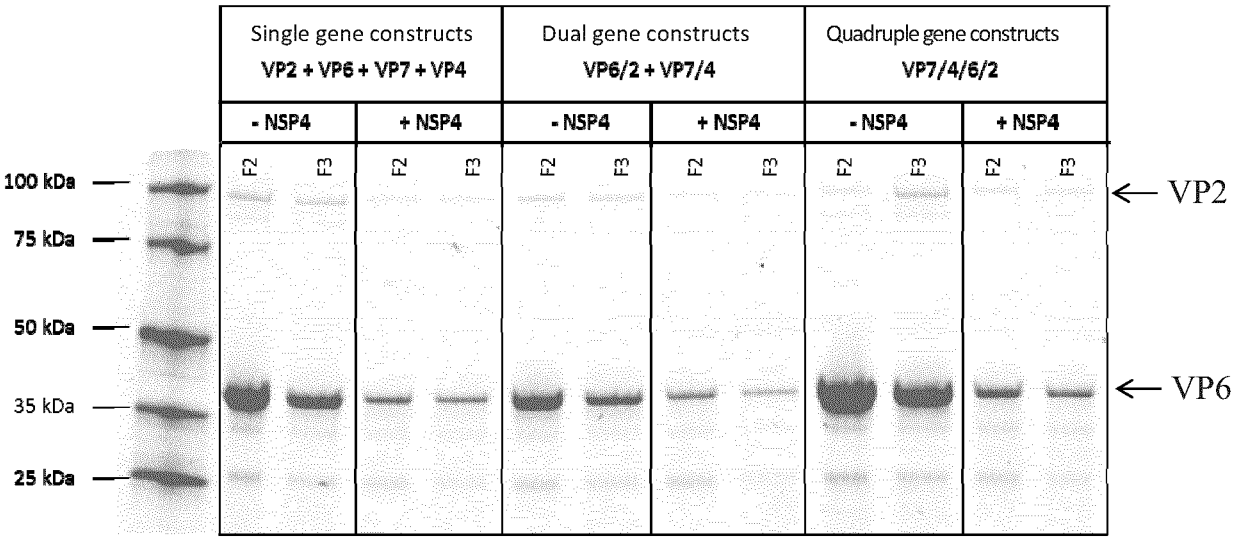


Figure 3B

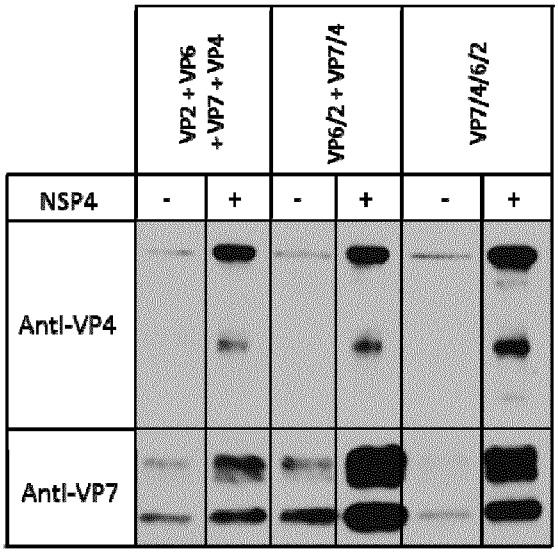


Figure 4A

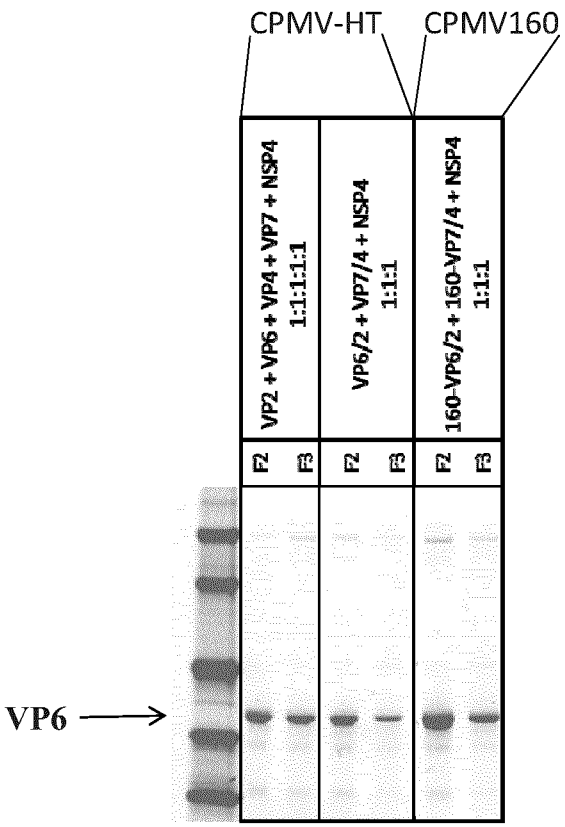


Figure 4B

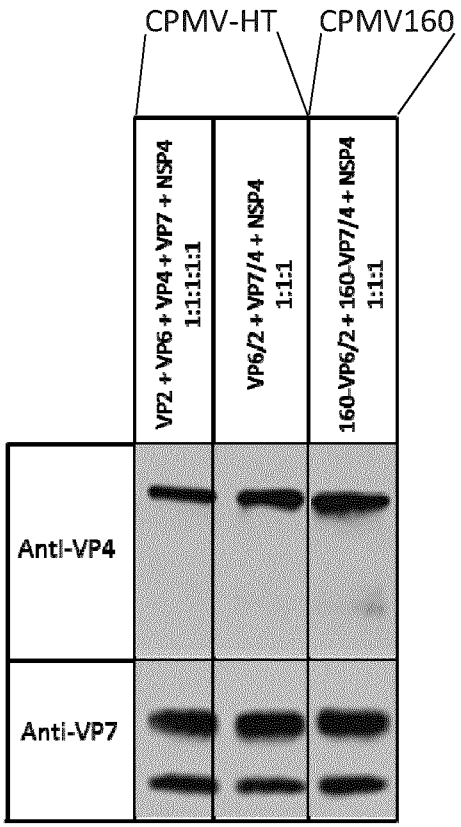


Figure 5

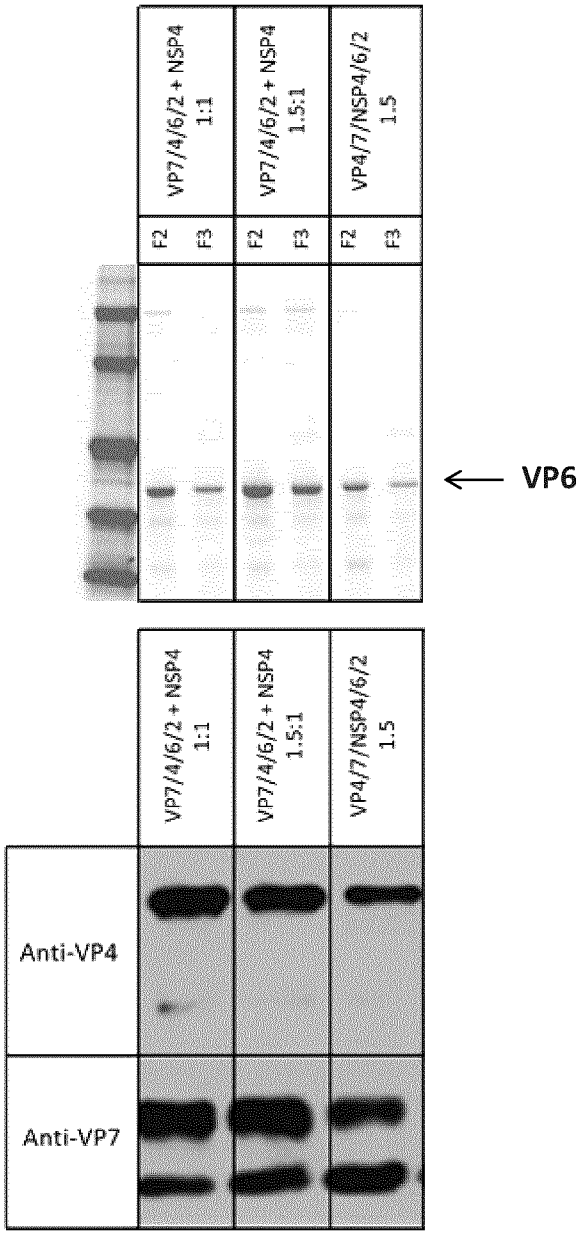
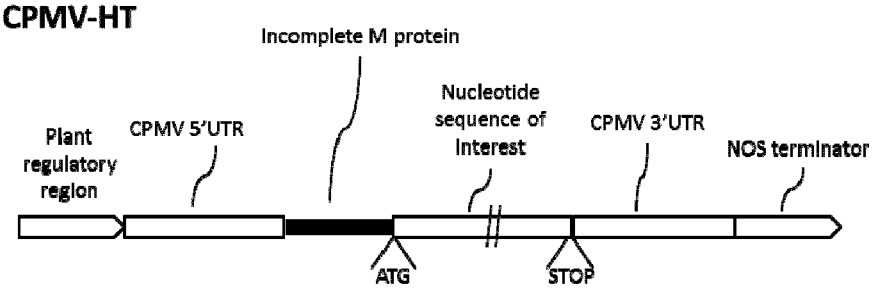
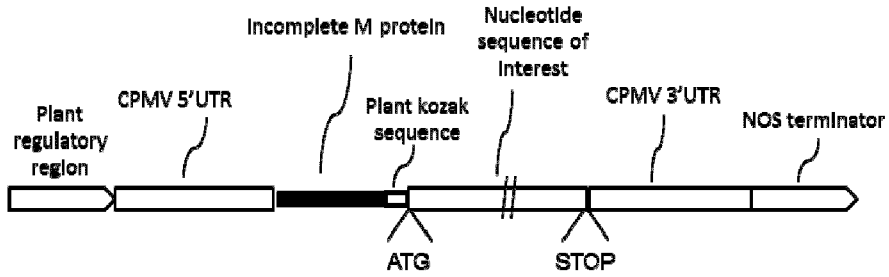

VP7/4/6/2 + NSP4 1:1
VP7/4/6/2 + NSP4 1.5:1
VP4/7/NSP4/6/2 1.5
F2
F3
VP6
Anti-VP4
Anti-VP7

Figure 6

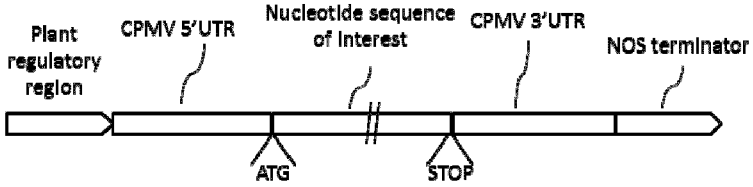
A) **CPMV-HT**



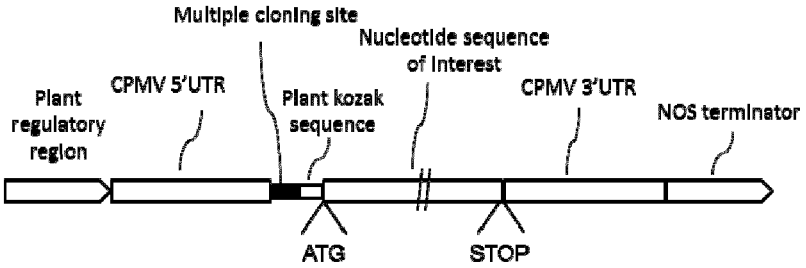
B) **CPMV-HT+**



C) **Construct comprising CPMV160**



D) **Construct comprising CPMV160+**



1. 2X35S/CPMV-HT/ RVA(WA) VP2(opt)/ NOS (Construct number 1710)

Figure 7A, SEQ ID NO: 19

IF-WA_VP2(opt).s1+3c

AAATTTGTCGGGCCCATGGCATACCGGAAGAGAGGAGCAAAGCGCGAA

Figure 7B, SEQ ID NO: 20

IF-WA_VP2(opt).s1-4r

ACTAAAGAAAATAGGCCTTTAAAGCTCGTTCATTATTCGCATATTGTCGA

Figure 7C, SEQ ID NO: 21

Optimized coding sequence of Rotavirus A VP2 from strain WA

ATGGCATACCGGAAGAGAGGAGCAAAGCGCGAAAACCTGCCGCAACAGAACGAGAGACTGCAAGAAAAAGAGAT
AGAGAAAGATGTCGACGTAACAATGGAAAACAAGAATAACAATAGGAAACAACAGCTGTCCGACAAAGTTCTGTCC
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TAGAGCCAGCATGCACATGCTGACTTCTAACCTTACGTTTACCGTCTACTCTGACCTACTGTCATTTGTTTCAGCGGAC
ACGGTAGAGCCCATTAACGCAGTCGCATTCGACAATATGCGAATAATGAACGAGCTTTAA

Figure 7D

Schematic representation of construct 1191. SacII and StuI restriction enzyme sites used for plasmid linearization are annotated on the representation.

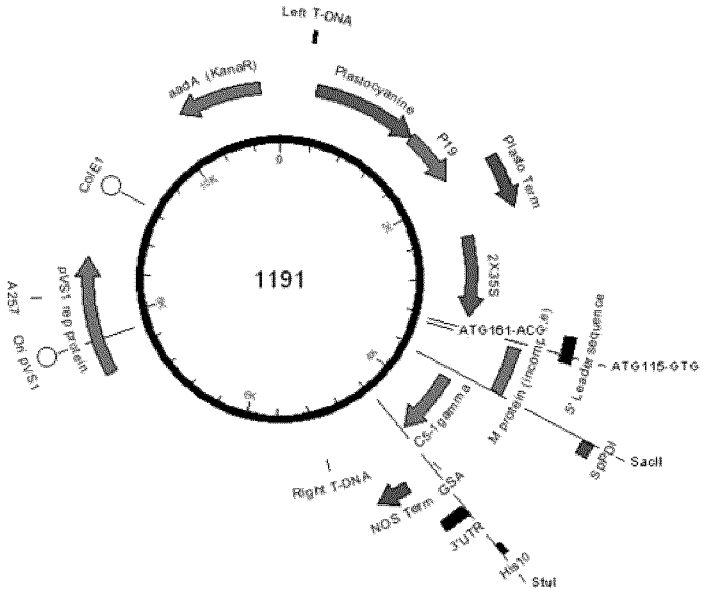

Figure 7E, SEQ ID NO: 22

Construct 1191 from left to right t-DNA borders (underlined). 2X35S/CPMV-HT/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette

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
CGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGA
GCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACA
CGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCT
GTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCT
GTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTTCGAACTT
GGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCGCGGATGGCGAAAAACGTTGCGATTTTCGGCTTATT
GTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCTGCAGGCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGC
CCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCT
GGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACT
GTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTG
TGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGAT
GTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTT
TGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTT
CCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGCGATCGCTCACCATCACCATCACCATCACCATCACCATTAAAGGCCTATT
TTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTA
ATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAA
AAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTG
TTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTAT
GAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAA
TTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCTAGAGTCTCAAGCTTGGCGCGCCCACGTGACTAGTGGCACTGGCCGTCGT
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GCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGAT
TGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAACCTAAGAGAAAAGAGCGTTTA

Figure 7F, SEQ ID NO: 23

Expression cassette number 1710 from 2X35S promoter to NOS terminator. VP2(opt) from Rotavirus A WA strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC
GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGG
AGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTA
TTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTT
TTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGCATACCGGAAGAGAGGAGCAAA
GCGCGAAAACCTGCCGCAACAGAACGAGAGACTGCAAGAAAAAGAGATAGAGAAAGATGTCGACGTAACAATGGAAAACAAGA
ATAACAATAGGAAACAACAGCTGTCCGACAAAGTTCTGTCCCAGAAGGAGGAAATTATCACTGACGCCCAGGACGATATTAAAAT
TGCCGGAGAAATAAAGAAGAGCTCGAAAGAAGAATCTAAACAGCTGCTCGAAATTCTGAAAACAAAAGAAGACCATCAGAAAGA
GATTCAATATGAAATTTTGCAAAAAACAATACCTACATTTGAGTCCAAAGAAAGTATCCTCAAGAAGCTTGAAGACATAAGACCGG
AGCAGGCAAAAAAACAGATGAAACTCTTTCGCATTTTCGAGCCAAAACAGCTCCCTATATATCGCGCCAATGGCGAGAAGGAGCT
ACGCAACCGGTGGTACTGGAAGTTGAAAAAAGACACCCTGCCAGATGGAGATTATGACGTCCGGGAGTATTTCCTCAATCTCTAT
GATCAGATCCTCATCGAAATGCCGGACTATCTGCTCCTCAAGGACATGGCCGTGGAGAACAAAAATAGCAGAGACGCCGGCAAA
GTTGTCGACTCTGAGACTGCCAATATTTGTGATGCCATCTTCCAGGATGAGGAGACCGAGGGAGTCGTCCGTAGATTCATCGCTG
ATATGCGGCAACAGGTCCAGGCTGATCGTAACATTGTCAATTACCCTTCCATCCTTCACCCTATTGATCATGCATTCAATGAGTATT
TTCTTAACCACCAGTTGGTGGAGCCGCTGAACAATGAGATAATCTTCAATTACATACCAGAGAGGATAAGGAATGACGTGAATTA
CATCCTGAACATGGATATGAATCTGCCATCTACAGCCAGGTATATCAGGCCAAACTTGTTGCAGGATAGACTGAATCTTCACGATA
ATTTTGAGTCCCTGTGGGATACCATCACAACATCCAACTACATTCTGGCCAGGTCCGTCGTTCCCGATTTGAAGGAGAAGGAGCTG
GTCTCCACCGAAGCACAGATCCAGAAAATGAGCCAGGACCTGCAGCTGGAGGCCCTCACTATTCAGAGCGAGACACAGTTTTTAG
CCGGGATTAACAGTCAGGCTGCCAATGATTGTTTCAAGACCCTCATAGCCGCCATGCTGTCTCAAAGAACCATGTCTTTGGACTTT
GTGACCACGAACTATATGAGCCTAATCTCCGGAATGTGGCTACTTACAGTGATTCCCAACGATATGTTCCTCCGGGAGTCACTAGT
GGCCTGTGAGCTGGCGATCATCAACACCATCGTGTATCCAGCATTCGGAATGCAGAGAATGCATTACCGGAATGGCGACCCTCAG
ACACCCTTCCAGATCGCAGAACAGCAGATCCAGAATTTCCAGGTGGCGAACTGGCTCCATTTTATTAACAATAACAGATTCAGGCA
AGTTGTGATTGATGGAGTTCTGAATCAGACTCTGAACGACAATATACGGAATGGACAGGTCATCAACCAGCTGATGGAAGCATTG
ATGCAACTCAGCAGACAGCAGTTCCCCACGATGCCTGTGGATTACAAACGGAGCATCCAACGGGGCATTCTGCTTCTCTCCAATAG
GCTGGGGCAGCTTGTCGACTTAACCCGACTGGTCTCCTATAACTACGAGACGCTAATGGCTTGTGTGACCATGAACATGCAGCAC
GTGCAAACCCTGACAACTGAGAAGTTGCAGCTCACTTCTGTGACTTCGCTTTGTATGTTAATTGGTAACACAACCGTGATTCCGTCC
CCACAGACACTGTTCCACTACTACAACATCAACGTGAATTTCCACTCCAATTATAATGAGCGGATCAACGACGCCGTCGCCATAATT
ACCGCAGCAAATAGGCTGAATCTTTATCAGAAAAAAATGAAGTCCATAGTGGAAGACTTTCTGAAACGGCTCCAGATTTTCGACG
TACCACGAGTGCCTGACGACCAAATGTACAGGCTGAGGGATCGCCTTCGGCTCTTACCCGTTGAACGGAGACGGCTTGACATATT
CAACTTGATCCTGATGAATATGGAGCAGATCGAACGCGCTTCTGATAAGATTGCTCAGGGGGTTATCATCGCATACCGAGATATG
CAGCTGGAACGCGACGAGATGTACGGATATGTTAATATTGCACGGAATCTTGATGGCTACCAGCAAATTAACTTGGAGGAACTCA
TGCGCACCGGTGATTACGGACAAATTACGAACATGCTTCTCAACAATCAACCCGTTGCCCTTGTGGGTGCATTGCCCTTCGTTACG
GACTCATCCGTGATCAGTCTAATCGCCAAGCTCGACGCAACCGTCTTCGCTCAGATAGTGAAGCTCAGGAAAGTTGACACACTGA
AGCCCATACTGTACAAAATAAACTCGGATTCCAATGACTTTTACCTTGTGGCCAACTACGACTGGATCCCCACAAGTACAACTAAG

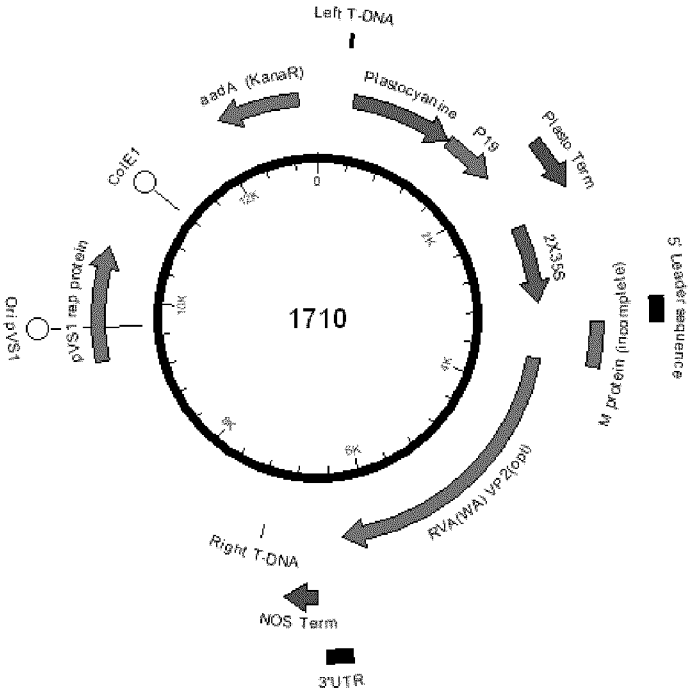
GTCTACAAACAGGTGCCACAACCATTCGACTTTAGAGCCAGCATGCACATGCTGACTTCTAACCTTACGTTTACCGTCTACTCTGAC
CTACTGTCATTTGTTTCAGCGGACACGGTAGAGCCCATTAACGCAGTCGCATTCGACAATATGCGAATAATGAACGAGCTTTAAAG
GCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTT
TATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAA
AAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGA
ATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGT
TATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAG
GATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

Figure 7G, SEQ ID NO: 24

Amino acid sequence of VP2 from Rotavirus A WA strain

MAYRKRGAKRENLPQQNERLQEKEIEKDVDVTMENKNNNRKQQLSDKVLSQKEEIITDAQDDIKIAGEIKKSSKEESKQLLEILKTKEDH
QKEIQYEILQKTIPTFESKESILKKLEDIRPEQAKKQMKLFRIFEPKQLPIYRANGEKELRNRWYWKLKKDTLPDGDYDVREYFLNLYDQILIE
MPDYLLLKDMAVENKNSRDAGKVVDSETANICDAIFQDEETEGVVRRFIADMRQQVQADRNIVNYPSILHPIDHAFNEYFLNHQLVEP
LNNEIIFNYIPERIRNDVNYILNMDMNLPSTARYIRPNLLQDRLNLHDNFESLWDTITTSNYILARSVVPDLKEKELVSTEAQIQKMSQDL
QLEALTIQSETQFLAGINSQAANDCFKTLIAAMLSQRTMSLDFVTTNYMSLISGMWLLTVIPNDMFLRESLVACELAIINTIVYPAFGMQ
RMHYRNGDPQTPFQIAEQQIQNFQVANWLHFINNNRFRQVVIDGVLNQTLNDNIRNGQVINQLMEALMQLSRQQFPTMPVDYKRS
IQRGILLLSNRLGQLVDLTRLVSYNYETLMACVTMNMQHVQTLTTEKLQLTSVTSLCMLIGNTTVIPSPQTLFHYYNINVNFHSNYNERIN
DAVAIITAANRLNLYQKKMKSIVEDFLKRLQIFDVPRVPDDQMYRLRDRLRLLPVERRRLDIFNLILMNMEQIERASDKIAQGVIIAYRDM
QLERDEMYGYVNIARNLDGYQQINLEELMRTGDYGQITNMLLNNQPVALVGALPFVTDSSVISLIAKLDATVFAQIVKLRKVDTLKPILY
KINSDSNDFYLVANYDWIPTSTTKVYKQVPQPFDFRASMHMLTSNLTFTVYSDLLSFVSADTVEPINAVAFDNMRIMNEL

Figure 7H Schematic representation of construct number 1710



2. 2X35S/CPMV-HT/RVA(WA) VP6(opt)/NOS (Construct number 1713)

Figure 8A, SEQ ID NO: 25

IF-WA_VP6(opt).s1+3c

AAATTTGTCGGGCCCATGGAGGTCCTTTATAGTCTCTCCAAAACGCTGA

Figure 8B, SEQ ID NO: 26

IF-WA_VP6(opt).s1-4r

ACTAAAGAAAATAGGCCTCTACTTGATCAACATACTCCGGATAGAGGCCACA

Figure 8C, SEQ ID NO :27

Optimized coding sequence of Rotavirus A VP6 from strain WA

ATGGAGGTCCTTTATAGTCTCTCCAAAACGCTGAAGGACGCTAGGGACAAGATCGTGGAGGGTACACTTTATAGCAATGTCAGCG
ACCTAATACAGCAGTTTAATCAAATGATCGTTACAATGAATGGGAATGATTTCCAAACTGGCGGTATTGGTAATCTGCCCGTGAGG
AACTGGACATTCGATTTCGGCCTGCTGGGCACGACTCTCCTTAATCTCGATGCAAATTATGTAGAAAACGCCAGAACGATTATCGA
GTACTTTATCGATTTCATTGATAACGTTTGTATGGATGAGATGGCCCGCGAGTCACAACGGAACGGAGTTGCTCCACAGTCCGAG
GCCCTTCGGAAACTCGCCGGCATTAAGTTCAAGCGTATTAATTTCGACAACTCCTCCGAATATATAGAGAACTGGAACTTGCAGAA
TCGTCGACAGAGAACCGGCTTCGTGTTCCATAAACCTAATATCTTTCCGTATAGCGCCTCATTCACCCTGAATAGGAGTCAGCCCAT
GCACGACAACCTCATGGGTACAATGTGGCTGAATGCGGGGAGTGAAATACAGGTCGCCGGGTTCGATTACTCCTGTGCCATTAAT
GCACCCGCAAACATCCAGCAGTTCGAACATATCGTGCAACTAAGACGGGCTCTCACGACCGCGACAATTACACTCCTGCCCGACG
CCGAGCGCTTCTCCTTTCCCCGCGTAATCAACTCAGCTGATGGCGCCACCACTTGGTTCTTCAACCCTGTTATATTGCGCCCTAACA
ACGTAGAGGTGGAGTTTCTCTTAAACGGACAGATCATCAATACCTACCAAGCCAGGTTCGGCACGATTATTGCAAGAAATTTCGAC
GCTATCAGGCTGCTCTTCCAACTGATGAGGCCCCCCAATATGACTCCCGCTGTGAACGCTTTGTTTCCGCAGGCTCAGCCTTTCCAG
CACCACGCCACCGTCGGCTTGACTCTTCGAATAGAGAGCGCGGTCTGCGAATCAGTGCTGGCAGACGCCAACGAGACGCTGCTG
GCAAACGTTACCGCCGTGCGGCAAGAGTATGCCATCCCAGTAGGGCCTGTGTTTCCACCCGGCATGAACTGGACTGAACTAATTA
CTAACTATAGCCCATCCAGAGAAGACAACTTGCAGCGGGTCTTCACTGTGGCCTCTATCCGGAGTATGTTGATCAAGTAG

Figure 8D, SEQ ID NO: 28

Expression cassette number 1713 from 2X35S promoter to NOS terminator. VP6(opt) from Rotavirus A WA strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC
GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGG
AGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTA

TTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTT
TTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGAGGTCCTTTATAGTCTCTCCAAAA
CGCTGAAGGACGCTAGGGACAAGATCGTGGAGGGTACACTTTATAGCAATGTCAGCGACCTAATACAGCAGTTTAATCAAATGAT
CGTTACAATGAATGGGAATGATTTCCAAACTGGCGGTATTGGTAATCTGCCCGTGAGGAACTGGACATTCGATTTCGGCCTGCTG
GGCACGACTCTCCTTAATCTCGATGCAAATTATGTAGAAAACGCCAGAACGATTATCGAGTACTTTATCGATTTCATTGATAACGTT
TGTATGGATGAGATGGCCCGCGAGTCACAACGGAACGGAGTTGCTCCACAGTCCGAGGCCCTTCGGAAACTCGCCGGCATTAAG
TTCAAGCGTATTAATTTCGACAACTCCTCCGAATATATAGAGAACTGGAACTTGCAGAATCGTCGACAGAGAACCGGCTTCGTGTT
CCATAAACCTAATATCTTTCCGTATAGCGCCTCATTCACCCTGAATAGGAGTCAGCCCATGCACGACAACCTCATGGGTACAATGT
GGCTGAATGCGGGGAGTGAAATACAGGTCGCCGGGTTCGATTACTCCTGTGCCATTAATGCACCCGCAAACATCCAGCAGTTCGA
ACATATCGTGCAACTAAGACGGGCTCTCACGACCGCGACAATTACACTCCTGCCCGACGCCGAGCGCTTCTCCTTTCCCCGCGTAA
TCAACTCAGCTGATGGCGCCACCACTTGGTTCTTCAACCCTGTTATATTGCGCCCTAACAACGTAGAGGTGGAGTTTCTCTTAAACG
GACAGATCATCAATACCTACCAAGCCAGGTTCGGCACGATTATTGCAAGAAATTTCGACGCTATCAGGCTGCTCTTCCAACTGATG
AGGCCCCCCAATATGACTCCCGCTGTGAACGCTTTGTTTCCGCAGGCTCAGCCTTTCCAGCACCACGCCACCGTCGGCTTGACTCTT
CGAATAGAGAGCGCGGTCTGCGAATCAGTGCTGGCAGACGCCAACGAGACGCTGCTGGCAAACGTTACCGCCGTGCGGCAAGA
GTATGCCATCCCAGTAGGGCCTGTGTTTCCACCCGGCATGAACTGGACTGAACTAATTACTAACTATAGCCCATCCAGAGAAGACA
ACTTGCAGCGGGTCTTCACTGTGGCCTCTATCCGGAGTATGTTGATCAAGTAGAGGCCTATTTTCTTTAGTTTGAATTTACTGTTAT
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TATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAG
TCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATG
TTACTAGAT

Figure 8E, SEQ ID NO: 29

Amino acid sequence of VP6 from Rotavirus A WA strain

MEVLYSLSKTLKDARDKIVEGTLYSNVSDLIQQFNQMIVTMNGNDFQTGGIGNLPVRNWTFDFGLLGTTLLNLDANYVENARTIIEYFID
FIDNVCMDEMARESQRNGVAPQSEALRKLAGIKFKRINFDNSSEYIENWNLQNRRQRTGFVFHKPNIFPYSASFTLNRSQPMHDNLM
GTMWLNAGSEIQVAGFDYSCAINAPANIQQFEHIVQLRRALTTATITLLPDAERFSFPRVINSADGATTWFFNPVILRPNNVEVEFLLNG
QIINTYQARFGTIIARNFDAIRLLFQLMRPPNMTPAVNALFPQAQPFQHHATVGLTLRIESAVCESVLADANETLLANVTAVRQEYAIPV
GPVFPPGMNWTELITNYSPSREDNLQRVFTVASIRSMLIK

Figure 8F

Schematic representation of construct number 1713

3. 2X35S/CPMV-HT/ RVA(Rtx) VP4(opt)/ NOS (Construct number 1730)

Figure 9A, SEQ ID NO: 30

IF-Rtx_VP4(opt).s1+3c

AAATTTGTCGGGCCCATGGCTAGCCTGATCTACAGACAACTCTTGACCAATTC

Figure 9B, SEQ ID NO: 31

IF-Rtx_VP4(opt).s1-4r

ACTAAAGAAAATAGGCCTTCAGAGTTTACATTGCAGGATTAATTGCTCAATCCTA

Figure 9C, SEQ ID NO: 32

Optimized coding sequence of Rotavirus A VP4 from strain RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]

ATGGCTAGCCTGATCTACAGACAACTCTTGACCAATTCATATTCTGTGGATCTTCATGACGAAATCGAGCAGATTGGGTCCGAGAA
GACCCAGAACGTGACCATCAACCCTGGACCTTTTGCTCAGACCCGCTATGCCCCTGTGAATTGGGATCACGGAGAAATCAACGAC

AGTACGACCGTCGAACCCATTCTGGACGGGCCATACCAACCCACCACCTTCACCCCACCTAATGATTATTGGATTTTAATCAACTCC
AACACAAACGGAGTGGTCTACGAGTCCACTAATAACTCCGATTTTTGGACCGCCGTTGTAGCCATCGAGCCACACGTCAATCCTGT
CGATCGCCAGTATATGATATTCGGCGAGTCCAAACAGTTTAACGTTTCCAATGACAGCAACAAATGGAAGTTTCTGGAGATGTTTC
GCAGCTCCTCTCAGAACGAATTCTATAATAGACGGACCCTTACCTCCGATACACGACTCGTGGGTATTTTTAAGTACGGCGGCAGG
GTGTGGACATTTCACGGTGAAACCCCTCGAGCAACCACTGACTCCAGTAGCACTGCAAACCTGAACAATATATCTATTACCATCCA
CAGCGAATTCTACATAATCCCAAGATCTCAGGAAAGTAAGTGTAACGAATATATCAACAACGGACTCCCCCCAATTCAGAATACAC
GGAACGTGGTGCCTCTCCCACTCAGTTCTCGGTCTATCCAGTATAAGAGAGCACAAGTGAATGAGGACATTATTGTGAGCAAGAC
TAGCCTTTGGAAAGAAATGCAGTACAACAGAGACATTATCATCCGGTTTAAGTTTGGGAACTCTATCGTGAAGATGGGCGGCCTG
GGGTACAAATGGTCAGAAATCTCATATAAAGCCGCCAACTATCAGTATAACTACTTGAGAGACGGCGAGCAGGTAACCGCCCACA
CAACATGCTCTGTCAACGGCGTTAATAACTTTAGCTACAACGGAGGCTTCCTTCCCACCGACTTCGGTATCAGCCGGTATGAAGTC
ATCAAGGAAAATTCTTATGTGTACGTAGATTACTGGGATGATAGCAAAGCGTTCCGCAACATGGTGTATGTTAGGAGCCTGGCTG
CTAATCTCAATTCTGTGAAGTGTACTGGTGGATCATATTATTTCTCAATTCCCGTGGGGGCTTGGCCAGTCATGAATGGCGGGGCA
GTCTCCCTCCATTTTGCTGGCGTGACGTTGAGCACTCAGTTTACCGATTTCGTGTCTCTGAACTCCCTGAGGTTCCGGTTTTCCCTTA
CTGTCGACGAGCCCCCATTCAGCATTCTGCGTACAAGAACTGTCAACCTCTACGGGTTACCTGCCGCGAATCCAAACAACGGCAAT
GAATACTATGAAATTTCGGGCCGCTTCTCTTTGATAAGTCTGGTACCAACTAATGACGACTATCAGACACCCATCATGAACAGCGT
GACTGTCAGACAGGACCTGGAAAGACAACTTACAGATCTGCGGGAAGAATTCAATTCTCTCAGTCAGGAGATTGCAATGGCCCAA
TTGATAGATCTTGCCCTACTGCCTCTCGATATGTTTAGTATGTTCTCCGGCATCAAATCAACTATAGATCTGACAAAGAGCATGGCT
ACTTCTGTGATGAAGAAGTTCAGGAAATCAAAACTTGCCACGAGCATATCAGAAATGACGAACTCTCTGAGTGATGCAGCATCAT
CAGCGTCACGCAACGTTTCCATTCGGTCGAATCTCAGCGCCATCAGCAACTGGACAAACGTGTCCAACGACGTCAGCAACGTGAC
CAACTCCTTGAACGATATTTCTACCCAGACGTCAACGATCAGTAAGAAACTCCGCTTGAAAGAAATGATCACCCAGACTGAGGGA
ATGTCTTTCGACGACATTTCCGCCGCCGTGCTAAAAACCAAAATCGATATGTCTACTCAGATCGGCAAGAACACTCTGCCGGATAT
CGTAACCGAAGCCTCCGAAAAGTTTATCCCTAAGCGCAGCTACAGAATATTGAAAGATGACGAGGTCATGGAGATCAACACAGAA
GGGAAGTTCTTCGCTTATAAGATCAACACCTTTGACGAGGTTCCGTTTGACGTCAATAAGTTTGCAGAGCTCGTGACAGATAGTCC
AGTGATTTCTGCCATCATTGACTTTAAGACTTTGAAGAACCTGAACGACAACTATGGAATAACACGGACCGAAGCGTTGAACCTCA
TTAAGTCCAATCCCAATATGTTGCGCAATTTCATTAACCAGAACAATCCAATCATAAGAAATAGGATTGAGCAATTAATCCTGCAAT
GTAAACTCTGA

Figure 9D, SEQ ID NO: 33

Expression cassette number 1730 from 2X35S promoter to NOS terminator. VP4(opt) from Rotavirus A Rotarix strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC
GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGG
AGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTA
TTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTT
TTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGCTAGCCTGATCTACAGACAACTCT
TGACCAATTCATATTCTGTGGATCTTCATGACGAAATCGAGCAGATTGGGTCCGAGAAGACCCAGAACGTGACCATCAACCCTGG
ACCTTTTGCTCAGACCCGCTATGCCCCTGTGAATTGGGATCACGGAGAAATCAACGACAGTACGACCGTCGAACCCATTCTGGACG
GGCCATACCAACCCACCACCTTCACCCCACCTAATGATTATTGGATTTTAATCAACTCCAACACAAACGGAGTGGTCTACGAGTCCA
CTAATAACTCCGATTTTTGGACCGCCGTTGTAGCCATCGAGCCACACGTCAATCCTGTCGATCGCCAGTATATGATATTCGGCGAG

TCCAAACAGTTTAACGTTTCCAATGACAGCAACAAATGGAAGTTTCTGGAGATGTTTCGCAGCTCCTCTCAGAACGAATTCTATAAT
AGACGGACCCTTACCTCCGATACACGACTCGTGGGTATTTTTAAGTACGGCGGCAGGGTGTGGACATTTCACGGTGAAACCCCTC
GAGCAACCACTGACTCCAGTAGCACTGCAAACCTGAACAATATATCTATTACCATCCACAGCGAATTCTACATAATCCCAAGATCTC
AGGAAAGTAAGTGTAACGAATATATCAACAACGGACTCCCCCCAATTCAGAATACACGGAACGTGGTGCCTCTCCCACTCAGTTCT
CGGTCTATCCAGTATAAGAGAGCACAAGTGAATGAGGACATTATTGTGAGCAAGACTAGCCTTTGGAAAGAAATGCAGTACAACA
GAGACATTATCATCCGGTTTAAGTTTGGGAACTCTATCGTGAAGATGGGCGGCCTGGGGTACAAATGGTCAGAAATCTCATATAA
AGCCGCCAACTATCAGTATAACTACTTGAGAGACGGCGAGCAGGTAACCGCCCACACAACATGCTCTGTCAACGGCGTTAATAAC
TTTAGCTACAACGGAGGCTTCCTTCCCACCGACTTCGGTATCAGCCGGTATGAAGTCATCAAGGAAAATTCTTATGTGTACGTAGA
TTACTGGGATGATAGCAAAGCGTTCCGCAACATGGTGTATGTTAGGAGCCTGGCTGCTAATCTCAATTCTGTGAAGTGTACTGGT
GGATCATATTATTTCTCAATTCCCGTGGGGGCTTGGCCAGTCATGAATGGCGGGGCAGTCTCCCTCCATTTTGCTGGCGTGACGTT
GAGCACTCAGTTTACCGATTTCGTGTCTCTGAACTCCCTGAGGTTCCGGTTTTCCCTTACTGTCGACGAGCCCCCATTCAGCATTCT
GCGTACAAGAACTGTCAACCTCTACGGGTTACCTGCCGCGAATCCAAACAACGGCAATGAATACTATGAAATTTCGGGCCGCTTCT
CTTTGATAAGTCTGGTACCAACTAATGACGACTATCAGACACCCATCATGAACAGCGTGACTGTCAGACAGGACCTGGAAAGACA
ACTTACAGATCTGCGGGAAGAATTCAATTCTCTCAGTCAGGAGATTGCAATGGCCCAATTGATAGATCTTGCCCTACTGCCTCTCG
ATATGTTTAGTATGTTCTCCGGCATCAAATCAACTATAGATCTGACAAAGAGCATGGCTACTTCTGTGATGAAGAAGTTCAGGAAA
TCAAAACTTGCCACGAGCATATCAGAAATGACGAACTCTCTGAGTGATGCAGCATCATCAGCGTCACGCAACGTTTCCATTCGGTC
GAATCTCAGCGCCATCAGCAACTGGACAAACGTGTCCAACGACGTCAGCAACGTGACCAACTCCTTGAACGATATTTCTACCCAGA
CGTCAACGATCAGTAAGAAACTCCGCTTGAAAGAAATGATCACCCAGACTGAGGGAATGTCTTTCGACGACATTTCCGCCGCCGT
GCTAAAAACCAAAATCGATATGTCTACTCAGATCGGCAAGAACACTCTGCCGGATATCGTAACCGAAGCCTCCGAAAAGTTTATCC
CTAAGCGCAGCTACAGAATATTGAAAGATGACGAGGTCATGGAGATCAACACAGAAGGGAAGTTCTTCGCTTATAAGATCAACAC
CTTTGACGAGGTTCCGTTTGACGTCAATAAGTTTGCAGAGCTCGTGACAGATAGTCCAGTGATTTCTGCCATCATTGACTTTAAGA
CTTTGAAGAACCTGAACGACAACTATGGAATAACACGGACCGAAGCGTTGAACCTCATTAAGTCCAATCCCAATATGTTGCGCAAT
TTCATTAACCAGAACAATCCAATCATAAGAAATAGGATTGAGCAATTAATCCTGCAATGTAAACTCTGAAGGCCTATTTTCTTTAGT
TTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTT
CTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGAC
CGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTC
TTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGG
TTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGC
GCGGTGTCATCTATGTTACTAGAT

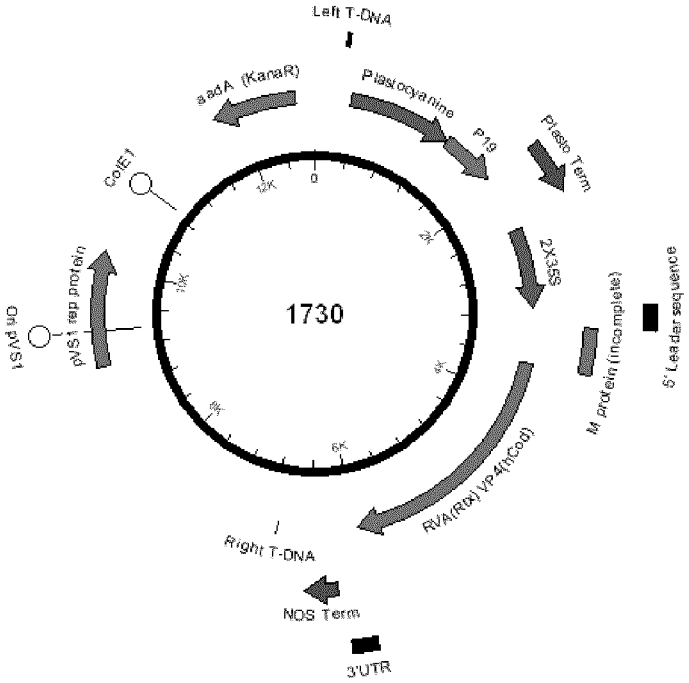
Figure 9E, SEQ ID NO: 34

Amino acid sequence of VP4 from Rotavirus A Rotarix strain

MASLIYRQLLTNSYSVDLHDEIEQIGSEKTQNVTINPGPFAQTRYAPVNWDHGEINDSTTVEPILDGPYQPTTFTPPNDYWILINSNTNG
VVYESTNNSDFWTAVVAIEPHVNPVDRQYMIFGESKQFNVSNDSNKWKFLEMFRSSSQNEFYNRRTLTSDTRLVGIFKYGGRVWTFH
GETPRATTDSSSTANLNNISITIHSEFYIIPRSQESKCNEYINNGLPPIQNTRNVVPLPLSSRSIQYKRAQVNEDIIVSKTSLWKEMQYNRDIII
RFKFGNSIVKMGGLGYKWSEISYKAANYQYNYLRDGEQVTAHTTCSVNGVNNFSYNGGFLPTDFGISRYEVIKENSYVYVDYWDDSKA
FRNMVYVRSLAANLNSVKCTGGSYYFSIPVGAWPVMNGGAVSLHFAGVTLSTQFTDFVSLNSLRFRFSLTVDEPPFSILRTRTVNLYGLP
AANPNNGNEYYEISGRFSLISLVPTNDDYQTPIMNSVTVRQDLERQLTDLREEFNSLSQEIAMAQLIDLALLPLDMFSMFSGIKSTIDLTKS
MATSVMKKFRKSKLATSISEMTNSLSDAASSASRNVSIRSNLSAISNWTNVSNDVSNVTNSLNDISTQTSTISKKLRLKEMITQTEGMSF
DDISAAVLKTKIDMSTQIGKNTLPDIVTEASEKFIPKRSYRILKDDEVMEINTEGKFFAYKINTFDEVPFDVNKFAELVTDSPVISAIIDFKTLK
NLNDNYGITRTEALNLIKSNPNMLRNFINQNNPIIRNRIEQLILQCKL

Figure 9F

Schematic representation of construct number 1730



4. 2X35S/CPMV-HT/RVA(Rtx) VP7(Opt)/NOS (Construct number 1734)

Figure 10A, SEQ ID NO: 35

IF-TrSP+Rtx_VP7(opt).s1+3c

AAATTTGTCGGGCCCATGGATTATATTATCTATCGTAGCCTCCTCATCTA

Figure 10B, SEQ ID NO: 36

IF-Rtx_VP7(opt).s1-4r

ACTAAAGAAAATAGGCCTCTAAACGCGATAATAGAAGGCTGCTGAGTTCAGGGA

Figure 10C, SEQ ID NO: 37

Optimized coding sequence of Rotavirus A VP7 from strain RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]

ATGTACGGCATCGAGTATACAACAATTTTAATTTTCCTGATTTCCATCATTCTGTTAAACTACATCCTTAAGTCCGTGACCAGAATTA
TGGATTATATTATCTATCGTAGCCTCCTCATCTACGTGGCCCTTTTTGCCCTGACCAGGGCCCAGAACTATGGCCTGAACTTACCAA
TCACCGGTTCAATGGATACCGTTTACGCTAATTCCACTCAAGAGGGGATATTTCTGACAAGTACCCTGTGCCTGTATTATCCAACAG
AAGCCTCTACCCAGATCAATGATGGGGAGTGGAAGGATAGTCTCTCACAGATGTTCCTAACCAAGGGCTGGCCCACCGGTTCCGT
CTACTTCAAGGAATACTCTAGTATTGTCGACTTCTCAGTTGACCCCCAGCTTTATTGCGACTACAACCTGGTACTTATGAAATACGA
CCAGAACCTGGAGCTGGATATGTCCGAGCTGGCTGACCTGATCCTCAATGAGTGGCTGTGCAACCCCATGGACATCACATTATATT
ACTACCAGCAGTCTGGAGAATCCAACAAGTGGATCAGTATGGGCTCAAGTTGCACCGTGAAGGTGTGTCCCTTGAACACCCAAAT
GCTGGGCATTGGTTGTCAGACAACTAATGTGGATTCGTTTGAAATGGTAGCCGAAAACGAGAAGCTGGCTATAGTGGACGTAGTC
GATGGGATTAACCACAAGATCAATCTGACTACCACCACTTGTACCATCAGAAACTGTAAAAAGCTCGGCCCCCGGGAGAACGTCG
CCGTGATCCAGGTGGGGGGGAGCAATGTGCTCGACATTACTGCCGACCCTACCACCAATCCACAGACGGAACGGATGATGAGAG
TCAACTGGAAGAAATGGTGGCAGGTCTTTTATACCATTGTGGACTACATTAACCAGATTGTGCAAGTCATGAGTAAACGGTCCAG
ATCCCTGAACTCAGCAGCCTTCTATTATCGCGTTTAG

Figure 10D, SEQ ID NO: 38

Expression cassette number 1734 from 2X35S promoter to NOS terminator. VP7 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC
GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGG
AGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTA
TTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTT
TTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGATTATATTATCTATCGTAGCCTCC
TCATCTACGTGGCCCTTTTTGCCCTGACCAGGGCCCAGAACTATGGCCTGAACTTACCAATCACCGGTTCAATGGATACCGTTTACG
CTAATTCCACTCAAGAGGGGATATTTCTGACAAGTACCCTGTGCCTGTATTATCCAACAGAAGCCTCTACCCAGATCAATGATGGG
GAGTGGAAGGATAGTCTCTCACAGATGTTCCTAACCAAGGGCTGGCCCACCGGTTCCGTCTACTTCAAGGAATACTCTAGTATTGT
CGACTTCTCAGTTGACCCCCAGCTTTATTGCGACTACAACCTGGTACTTATGAAATACGACCAGAACCTGGAGCTGGATATGTCCG
AGCTGGCTGACCTGATCCTCAATGAGTGGCTGTGCAACCCCATGGACATCACATTATATTACTACCAGCAGTCTGGAGAATCCAAC
AAGTGGATCAGTATGGGCTCAAGTTGCACCGTGAAGGTGTGTCCCTTGAACACCCAAATGCTGGGCATTGGTTGTCAGACAACTA
ATGTGGATTCGTTTGAAATGGTAGCCGAAAACGAGAAGCTGGCTATAGTGGACGTAGTCGATGGGATTAACCACAAGATCAATCT
GACTACCACCACTTGTACCATCAGAAACTGTAAAAAGCTCGGCCCCCGGGAGAACGTCGCCGTGATCCAGGTGGGGGGGAGCAA
TGTGCTCGACATTACTGCCGACCCTACCACCAATCCACAGACGGAACGGATGATGAGAGTCAACTGGAAGAAATGGTGGCAGGT
CTTTTATACCATTGTGGACTACATTAACCAGATTGTGCAAGTCATGAGTAAACGGTCCAGATCCCTGAACTCAGCAGCCTTCTATTA
TCGCGTTTAGAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAG
AGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATT
TTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTT
TCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTA
ATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGC
GCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

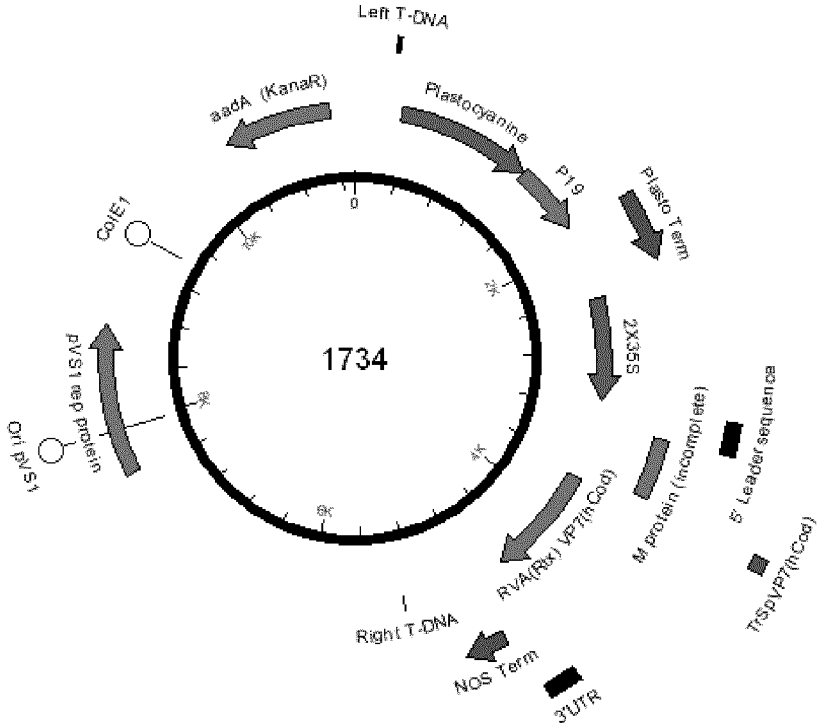
Figure 10E, SEQ ID NO: 39

Amino acid sequence of TrSp-VP7 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain

MDYIIYRSLLIYVALFALTRAQNYGLNLPITGSMDTVYANSTQEGIFLTSTLCLYYPTEASTQINDGEWKDSLSQMFLTKGWPTGSVYFKE
YSSIVDFSVDPQLYCDYNLVLMKYDQNLELDMSELADLILNEWLCNPMDITLYYYQQSGESNKWISMGSSCTVKVCPLNTQMLGIGCQ
TTNVDSFEMVAENEKLAIVDVVDGINHKINLTTTTCTIRNCKKLGPRENVAVIQVGGSNVLDITADPTTNPQTERMMRVNWKKWWQ
VFYTIVDYINQIVQVMSKRSRSLNSAAFYYRV

Figure 10F

Schematic representation of construct number 1734



5. 2X35S/CPMV-HT/RVA(WA) NSP4/NOS (Construct number 1706)

Figure 11A, SEQ ID NO: 40

IF-WA_NSP4.s1+3c

AAATTTGTCGGGCCCATGGATAAGCTTGCCGACCTCAACTACACATTGAGTG

Figure 11B, SEQ ID NO: 41

IF-WA_NSP4.s1-4r

ACTAAAGAAAATAGGCCTTCACATGGATGCAGTCACTTCTGACGGTTCATATGGA

Figure 11C, SEQ ID NO : 42

Coding sequence of Rotavirus A NSP4 from strain WA

ATGGATAAGCTTGCCGACCTCAACTACACATTGAGTGTAATCACTTCAATGAATGACACATTGCATTCTATAATTCAAGATCCTGGA
ATGGCGTATTTTCTATATATTGCATCTGTTCTAACAGTTTTGTTCACATTACATAAAGCTTCAATTCCAACCATGAAAATAGCATTGA
AAACATCAAAATGTTCATATAAAGTGATTAAATATTGTATAGTCACGATCATTAATACTCTTTTAAAATTGGCTGGATATAAAGAGC
AGGTTACTACAAAAGACGAAATTGAGCAACAGATGGACAGAATTGTGAAAGAGATGAGACGTCAGCTGGAGATGATTGATAAAC
TAACTACTCGTGAAATTGAACAGGTTGAATTGCTTAAACGTATACATGACAACCTGATAACTAGACCAGTTGACGTTATAGATATG
TCGAAGGAATTCAATCAGAAAAACATCAAAACGCTAGATGAATGGGAGAGTGGAAAAAATCCATATGAACCGTCAGAAGTGACT
GCATCCATGTGA

Figure 11D, SEQ ID NO: 43

Expression cassette number 1706 from 2X35S promoter to NOS terminator. NSP4 from Rotavirus A WA strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC
GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACGTTTTCTTTCACTGAAGCGAAATCAAAGATCTCTT
TGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGG
AGGCTGCTGTTCAGCCCCATACATTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTA
TTGTTGCCTGTACTTCTTTCTTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTT
TTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCATGGATAAGCTTGCCGACCTCAACTACA
CATTGAGTGTAATCACTTCAATGAATGACACATTGCATTCTATAATTCAAGATCCTGGAATGGCGTATTTTCTATATATTGCATCTGT
TCTAACAGTTTTGTTCACATTACATAAAGCTTCAATTCCAACCATGAAAATAGCATTGAAAACATCAAAATGTTCATATAAAGTGAT
TAAATATTGTATAGTCACGATCATTAATACTCTTTTAAAATTGGCTGGATATAAAGAGCAGGTTACTACAAAAGACGAAATTGAGC
AACAGATGGACAGAATTGTGAAAGAGATGAGACGTCAGCTGGAGATGATTGATAAACTAACTACTCGTGAAATTGAACAGGTTG
AATTGCTTAAACGTATACATGACAACCTGATAACTAGACCAGTTGACGTTATAGATATGTCGAAGGAATTCAATCAGAAAAACATC
AAAACGCTAGATGAATGGGAGAGTGGAAAAAATCCATATGAACCGTCAGAAGTGACTGCATCCATGTGAAGGCCTATTTTCTTTA
GTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAAT
TTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAG
ACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCG
GTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGAT
GGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCG
CGCGCGGTGTCATCTATGTTACTAGAT

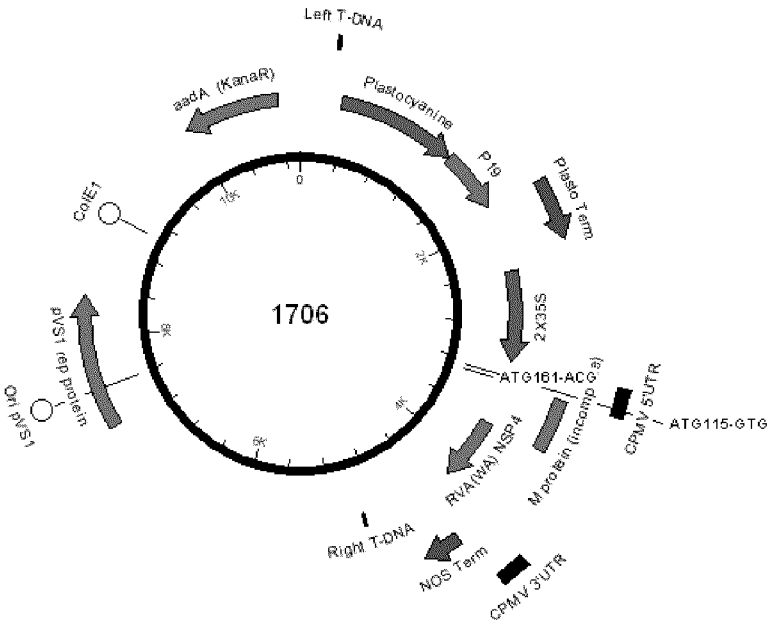
Figure11E, SEQ ID NO: 44

Amino acid sequence of NSP4 from Rotavirus A WA strain

MDKLADLNYTLSVITSMNDTLHSIIQDPGMAYFLYIASVLTVLFTLHKASIPTMKIALKTSKCSYKVIKYCIVTIINTLLKLAGYKEQVTTKDEI
EQQMDRIVKEMRRQLEMIDKLTTREIEQVELLKRIHDNLITRPVDVIDMSKEFNQKNIKTLDEWESGKNPYEPSEVTASM

Figure 11F

Schematic representation of construct number 1706



6. 2X35S/CPMV-160/ RVA(WA) VP2(opt)/ NOS (Construct number 1108)

Figure 12A, SEQ ID NO: 45

IF(C160)-WA_VP2(opt).c

TCGTGCTTCGGCACCAGTACAATGGCATACCGGAAGAGAGGAGCAAAGCGCGAA

Figure 12B

Schematic representation of construct 1190. SacII and StuI restriction enzyme sites used for plasmid linearization are annotated on the representation.

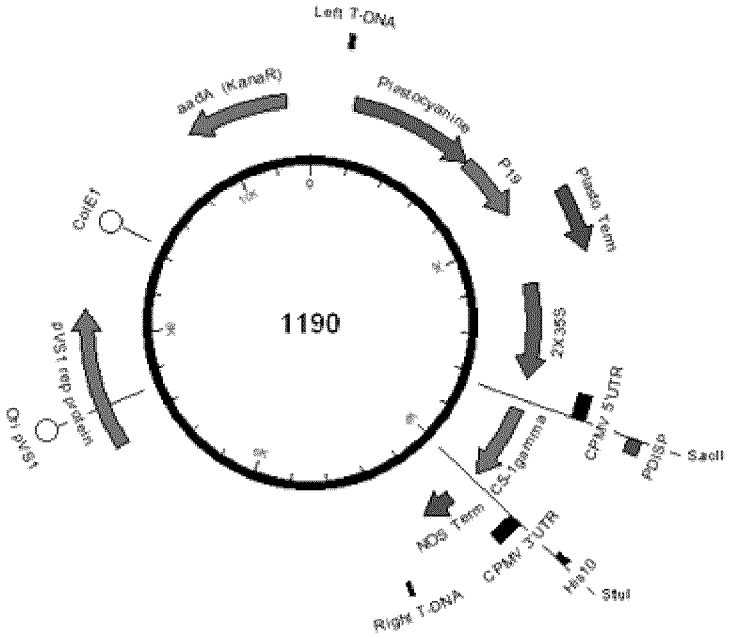


Figure 12C, SEQ ID NO: 46

Construct 1190 from left to right t-DNA borders (underlined). 2X35S/CPMV-160/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette

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
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TTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGT
GGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTTGATAAAAG
CGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGA
GCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCGCGGATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGT
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CCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC
CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACC
TGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGT
GGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATTACT
CTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGG
AGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCA
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TTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTG
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TTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGA
TGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTAT
GATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTG
TCATCTATGTTACTAGATCTCTAGAGTCTCAAGCTTGGCGCGCCCACGTGACTAGTGGCACTGGCCGTCGTTTTACAACGTCGTGA
CTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCA
CCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGATTGTCGTTTCCCGCCTT
CAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAACCTAAGAGAAAAGAGCGTTTA

Figure 12D, SEQ ID NO: 47

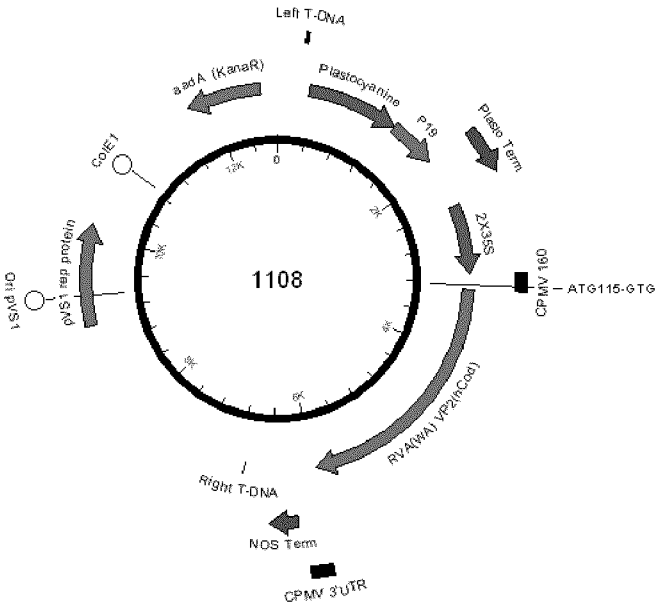
Expression cassette number 1108 from 2X35S promoter to NOS terminator. VP2(opt) from Rotavirus A WA strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC

GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGGCATACCGGAAGAGAGGAGCAAAGCGCGAAA
ACCTGCCGCAACAGAACGAGAGACTGCAAGAAAAAGAGATAGAGAAAGATGTCGACGTAACAATGGAAAACAAGAATAACAAT
AGGAAACAACAGCTGTCCGACAAAGTTCTGTCCCAGAAGGAGGAAATTATCACTGACGCCCAGGACGATATTAAAATTGCCGGA
GAAATAAAGAAGAGCTCGAAAGAAGAATCTAAACAGCTGCTCGAAATTCTGAAAACAAAAGAAGACCATCAGAAAGAGATTCAA
TATGAAATTTTGCAAAAAACAATACCTACATTTGAGTCCAAAGAAAGTATCCTCAAGAAGCTTGAAGACATAAGACCGGAGCAGG
CAAAAAAACAGATGAAACTCTTTCGCATTTTCGAGCCAAAACAGCTCCCTATATATCGCGCCAATGGCGAGAAGGAGCTACGCAA
CCGGTGGTACTGGAAGTTGAAAAAAGACACCCTGCCAGATGGAGATTATGACGTCCGGGAGTATTTCCTCAATCTCTATGATCAG
ATCCTCATCGAAATGCCGGACTATCTGCTCCTCAAGGACATGGCCGTGGAGAACAAAAATAGCAGAGACGCCGGCAAAGTTGTCG
ACTCTGAGACTGCCAATATTTGTGATGCCATCTTCCAGGATGAGGAGACCGAGGGAGTCGTCCGTAGATTCATCGCTGATATGCG
GCAACAGGTCCAGGCTGATCGTAACATTGTCAATTACCCTTCCATCCTTCACCCTATTGATCATGCATTCAATGAGTATTTTCTTAAC
CACCAGTTGGTGGAGCCGCTGAACAATGAGATAATCTTCAATTACATACCAGAGAGGATAAGGAATGACGTGAATTACATCCTGA
ACATGGATATGAATCTGCCATCTACAGCCAGGTATATCAGGCCAAACTTGTTGCAGGATAGACTGAATCTTCACGATAATTTTGAG
TCCCTGTGGGATACCATCACAACATCCAACTACATTCTGGCCAGGTCCGTCGTTCCCGATTTGAAGGAGAAGGAGCTGGTCTCCAC
CGAAGCACAGATCCAGAAAATGAGCCAGGACCTGCAGCTGGAGGCCCTCACTATTCAGAGCGAGACACAGTTTTTAGCCGGGAT
TAACAGTCAGGCTGCCAATGATTGTTTCAAGACCCTCATAGCCGCCATGCTGTCTCAAAGAACCATGTCTTTGGACTTTGTGACCAC
GAACTATATGAGCCTAATCTCCGGAATGTGGCTACTTACAGTGATTCCCAACGATATGTTCCTCCGGGAGTCACTAGTGGCCTGTG
AGCTGGCGATCATCAACACCATCGTGTATCCAGCATTCGGAATGCAGAGAATGCATTACCGGAATGGCGACCCTCAGACACCCTT
CCAGATCGCAGAACAGCAGATCCAGAATTTCCAGGTGGCGAACTGGCTCCATTTTATTAACAATAACAGATTCAGGCAAGTTGTG
ATTGATGGAGTTCTGAATCAGACTCTGAACGACAATATACGGAATGGACAGGTCATCAACCAGCTGATGGAAGCATTGATGCAAC
TCAGCAGACAGCAGTTCCCCACGATGCCTGTGGATTACAAACGGAGCATCCAACGGGGCATTCTGCTTCTCTCCAATAGGCTGGG
GCAGCTTGTCGACTTAACCCGACTGGTCTCCTATAACTACGAGACGCTAATGGCTTGTGTGACCATGAACATGCAGCACGTGCAAA
CCCTGACAACTGAGAAGTTGCAGCTCACTTCTGTGACTTCGCTTTGTATGTTAATTGGTAACACAACCGTGATTCCGTCCCCACAGA
CACTGTTCCACTACTACAACATCAACGTGAATTTCCACTCCAATTATAATGAGCGGATCAACGACGCCGTCGCCATAATTACCGCAG
CAAATAGGCTGAATCTTTATCAGAAAAAAATGAAGTCCATAGTGGAAGACTTTCTGAAACGGCTCCAGATTTTCGACGTACCACGA
GTGCCTGACGACCAAATGTACAGGCTGAGGGATCGCCTTCGGCTCTTACCCGTTGAACGGAGACGGCTTGACATATTCAACTTGA
TCCTGATGAATATGGAGCAGATCGAACGCGCTTCTGATAAGATTGCTCAGGGGGTTATCATCGCATACCGAGATATGCAGCTGGA
ACGCGACGAGATGTACGGATATGTTAATATTGCACGGAATCTTGATGGCTACCAGCAAATTAACTTGGAGGAACTCATGCGCACC
GGTGATTACGGACAAATTACGAACATGCTTCTCAACAATCAACCCGTTGCCCTTGTGGGTGCATTGCCCTTCGTTACGGACTCATCC
GTGATCAGTCTAATCGCCAAGCTCGACGCAACCGTCTTCGCTCAGATAGTGAAGCTCAGGAAAGTTGACACACTGAAGCCCATAC
TGTACAAAATAAACTCGGATTCCAATGACTTTTACCTTGTGGCCAACTACGACTGGATCCCCACAAGTACAACTAAGGTCTACAAA
CAGGTGCCACAACCATTCGACTTTAGAGCCAGCATGCACATGCTGACTTCTAACCTTACGTTTACCGTCTACTCTGACCTACTGTCA
TTTGTTTCAGCGGACACGGTAGAGCCCATTAACGCAGTCGCATTCGACAATATGCGAATAATGAACGAGCTTTAAAGGCCTATTTT
CTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAAT
TTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAA
AAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTT
GCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATG
AGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATT
ATCGCGCGCGGTGTCATCTATGTTACTAGAT

Figure 12E

Schematic representation of construct number 1108



7. 2X35S/CPMV-160/RVA(WA) VP6(opt)/NOS (Construct number 1128)

Figure 13A, SEQ ID NO: 48

IF(C160)-WA_VP6(opt).c

TCGTGCTTCGGCACCAGTACAATGGAGGTCCTTTATAGTCTCTCCAAAACGCTGA

Figure 13B, SEQ ID NO: 49

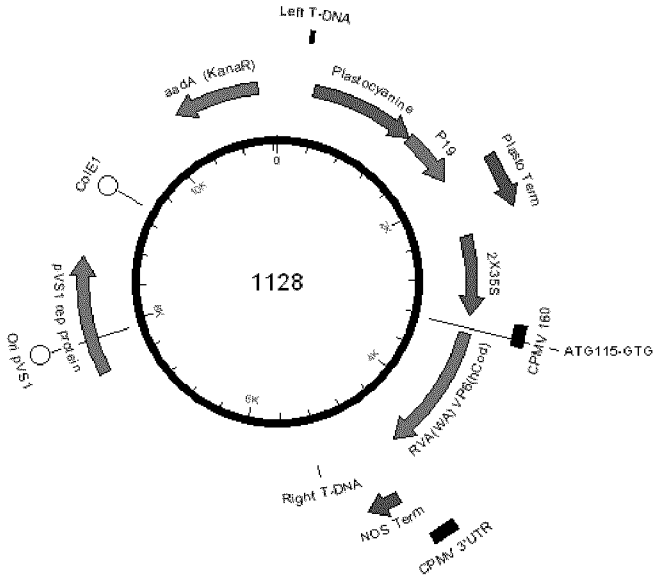
Expression cassette number 1128 from 2X35S promoter to NOS terminator. VP6(opt) from Rotavirus A WA strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC
GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC

TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGGAGGTCCTTTATAGTCTCTCCAAAACGCTGAAG
GACGCTAGGGACAAGATCGTGGAGGGTACACTTTATAGCAATGTCAGCGACCTAATACAGCAGTTTAATCAAATGATCGTTACAA
TGAATGGGAATGATTTCCAAACTGGCGGTATTGGTAATCTGCCCGTGAGGAACTGGACATTCGATTTCGGCCTGCTGGGCACGAC
TCTCCTTAATCTCGATGCAAATTATGTAGAAAACGCCAGAACGATTATCGAGTACTTTATCGATTTCATTGATAACGTTTGTATGGA
TGAGATGGCCCGCGAGTCACAACGGAACGGAGTTGCTCCACAGTCCGAGGCCCTTCGGAAACTCGCCGGCATTAAGTTCAAGCGT
ATTAATTTCGACAACTCCTCCGAATATATAGAGAACTGGAACTTGCAGAATCGTCGACAGAGAACCGGCTTCGTGTTCCATAAACC
TAATATCTTTCCGTATAGCGCCTCATTCACCCTGAATAGGAGTCAGCCCATGCACGACAACCTCATGGGTACAATGTGGCTGAATG
CGGGGAGTGAAATACAGGTCGCCGGGTTCGATTACTCCTGTGCCATTAATGCACCCGCAAACATCCAGCAGTTCGAACATATCGT
GCAACTAAGACGGGCTCTCACGACCGCGACAATTACACTCCTGCCCGACGCCGAGCGCTTCTCCTTTCCCCGCGTAATCAACTCAG
CTGATGGCGCCACCACTTGGTTCTTCAACCCTGTTATATTGCGCCCTAACAACGTAGAGGTGGAGTTTCTCTTAAACGGACAGATC
ATCAATACCTACCAAGCCAGGTTCGGCACGATTATTGCAAGAAATTTCGACGCTATCAGGCTGCTCTTCCAACTGATGAGGCCCCC
CAATATGACTCCCGCTGTGAACGCTTTGTTTCCGCAGGCTCAGCCTTTCCAGCACCACGCCACCGTCGGCTTGACTCTTCGAATAGA
GAGCGCGGTCTGCGAATCAGTGCTGGCAGACGCCAACGAGACGCTGCTGGCAAACGTTACCGCCGTGCGGCAAGAGTATGCCAT
CCCAGTAGGGCCTGTGTTTCCACCCGGCATGAACTGGACTGAACTAATTACTAACTATAGCCCATCCAGAGAAGACAACTTGCAGC
GGGTCTTCACTGTGGCCTCTATCCGGAGTATGTTGATCAAGTAGAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGC
ATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGT
CGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAGCTTATCG
ACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCT
GTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATT
ATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

Figure 13C

Schematic representation of construct number 1128



8. 2X35S/CPMV-160/ RVA(Rtx) VP4(opt)/ NOS (Construct number 1178)

Figure 14A, SEQ ID NO: 50

IF(C160)-Rtx_VP4(opt).c

TCGTGCTTCGGCACCAGTACAATGGCTAGCCTGATCTACAGACAACTCTTGACCAATTC

Figure 14B, SEQ ID NO: 51

Expression cassette number 1178 from 2X35S promoter to NOS terminator. VP4(opt) from Rotavirus A Rotarix strain is underlined.

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTT
TTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAA
GGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGAC
CCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGG
AGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGT
AATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACA
AATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAG
GAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC
GCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTTT
GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
TTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAATGGCTAGCCTGATCTACAGACAACTCTTGACCAAT
TCATATTCTGTGGATCTTCATGACGAAATCGAGCAGATTGGGTCCGAGAAGACCCAGAACGTGACCATCAACCCTGGACCTTTTGC
TCAGACCCGCTATGCCCCTGTGAATTGGGATCACGGAGAAATCAACGACAGTACGACCGTCGAACCCATTCTGGACGGGCCATAC
CAACCCACCACCTTCACCCCACCTAATGATTATTGGATTTTAATCAACTCCAACACAAACGGAGTGGTCTACGAGTCCACTAATAAC
TCCGATTTTTGGACCGCCGTTGTAGCCATCGAGCCACACGTCAATCCTGTCGATCGCCAGTATATGATATTCGGCGAGTCCAAACA
GTTTAACGTTTCCAATGACAGCAACAAATGGAAGTTTCTGGAGATGTTTCGCAGCTCCTCTCAGAACGAATTCTATAATAGACGGA
CCCTTACCTCCGATACACGACTCGTGGGTATTTTTAAGTACGGCGGCAGGGTGTGGACATTTCACGGTGAAACCCCTCGAGCAACC
ACTGACTCCAGTAGCACTGCAAACCTGAACAATATATCTATTACCATCCACAGCGAATTCTACATAATCCCAAGATCTCAGGAAAGT
AAGTGTAACGAATATATCAACAACGGACTCCCCCCAATTCAGAATACACGGAACGTGGTGCCTCTCCCACTCAGTTCTCGGTCTAT
CCAGTATAAGAGAGCACAAGTGAATGAGGACATTATTGTGAGCAAGACTAGCCTTTGGAAAGAAATGCAGTACAACAGAGACAT
TATCATCCGGTTTAAGTTTGGGAACTCTATCGTGAAGATGGGCGGCCTGGGGTACAAATGGTCAGAAATCTCATATAAAGCCGCC
AACTATCAGTATAACTACTTGAGAGACGGCGAGCAGGTAACCGCCCACACAACATGCTCTGTCAACGGCGTTAATAACTTTAGCTA
CAACGGAGGCTTCCTTCCCACCGACTTCGGTATCAGCCGGTATGAAGTCATCAAGGAAAATTCTTATGTGTACGTAGATTACTGGG
ATGATAGCAAAGCGTTCCGCAACATGGTGTATGTTAGGAGCCTGGCTGCTAATCTCAATTCTGTGAAGTGTACTGGTGGATCATAT
TATTTCTCAATTCCCGTGGGGGCTTGGCCAGTCATGAATGGCGGGGCAGTCTCCCTCCATTTTGCTGGCGTGACGTTGAGCACTCA
GTTTACCGATTTCGTGTCTCTGAACTCCCTGAGGTTCCGGTTTTCCCTTACTGTCGACGAGCCCCCATTCAGCATTCTGCGTACAAG
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GTCTGGTACCAACTAATGACGACTATCAGACACCCATCATGAACAGCGTGACTGTCAGACAGGACCTGGAAAGACAACTTACAGA
TCTGCGGGAAGAATTCAATTCTCTCAGTCAGGAGATTGCAATGGCCCAATTGATAGATCTTGCCCTACTGCCTCTCGATATGTTTAG
TATGTTCTCCGGCATCAAATCAACTATAGATCTGACAAAGAGCATGGCTACTTCTGTGATGAAGAAGTTCAGGAAATCAAAACTTG
CCACGAGCATATCAGAAATGACGAACTCTCTGAGTGATGCAGCATCATCAGCGTCACGCAACGTTTCCATTCGGTCGAATCTCAGC
GCCATCAGCAACTGGACAAACGTGTCCAACGACGTCAGCAACGTGACCAACTCCTTGAACGATATTTCTACCCAGACGTCAACGAT
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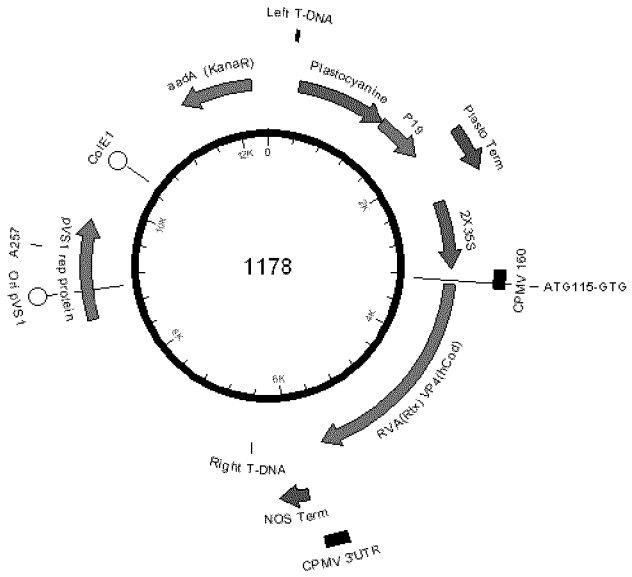
AGAACAATCCAATCATAAGAAATAGGATTGAGCAATTAATCCTGCAATGTAAACTCTGAAGGCCTATTTTCTTTAGTTTGAATTTAC

TGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGC
TCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCG
ATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGAT
TATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATT
AGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCAT
CTATGTTACTAGAT

Figure 14C

Schematic representation of construct number 1178



9. 2X35S/CPMV-160/TrSp-RVA(Rtx) VP7(Opt)/NOS (Construct number 1199)

Figure 15A, SEQ ID NO: 52

IF(C160)-TrSP+Rtx_VP7(opt).c

TCGTGCTTCGGCACCAGTACAATGGATTATATTATCTATCGTAGCCTCCTCATCTA

Figure 15B, SEQ ID NO: 53

Expression cassette number 1199 from 2X35S promoter to NOS terminator. VP7 from Rotavirus A vaccine USA/Rotarix-A41CB052A/1988/G1P1A[8] strain is underlined.

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GATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTC
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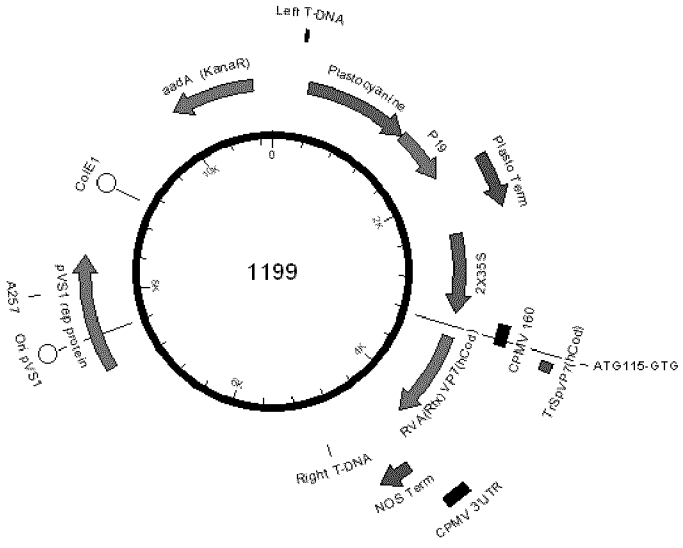
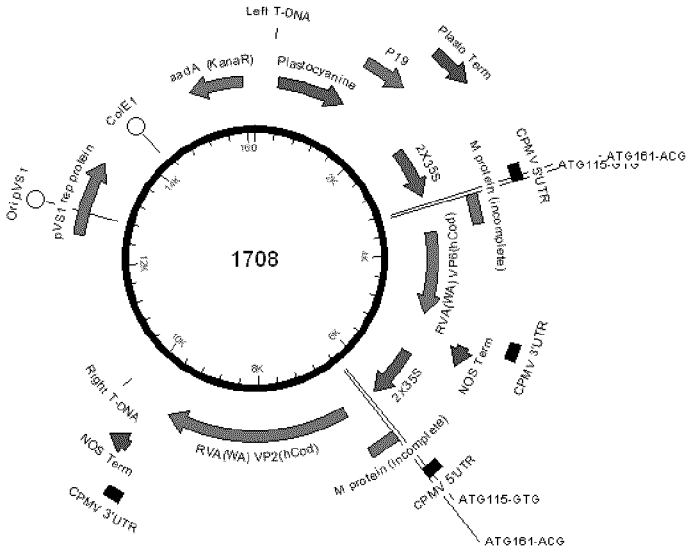
Figure 15C

Schematic representation of construct number 1199



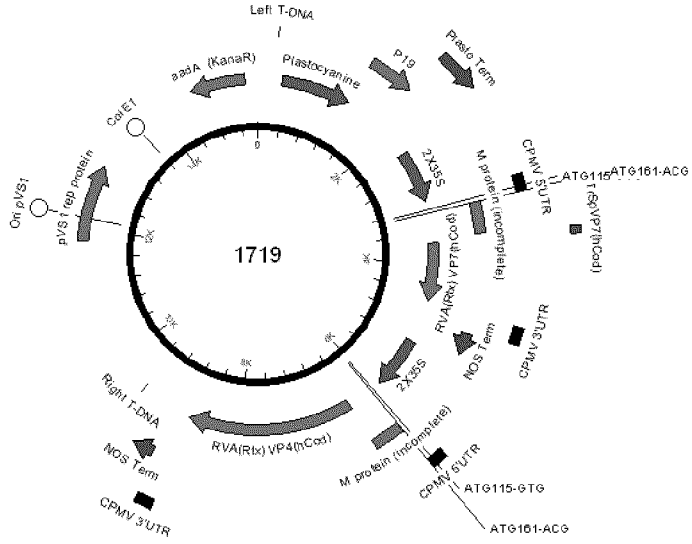
10. Double gene construct for the expression of VP6 and VP2 under CPMV-HT expression cassette (construct number 1708)

Figure 16 Schematic representation of construct number 1708



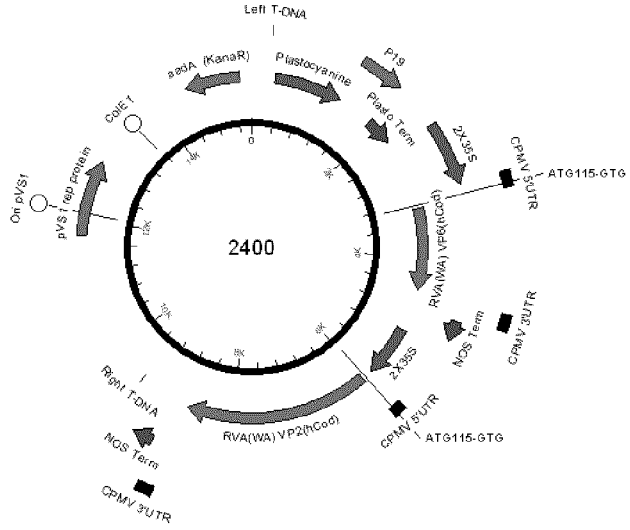
11. Double gene construct for the expression of VP7 and VP4 under CPMV-HT expression cassette (construct number 1719)

Figure 17 Schematic representation of construct number 1719



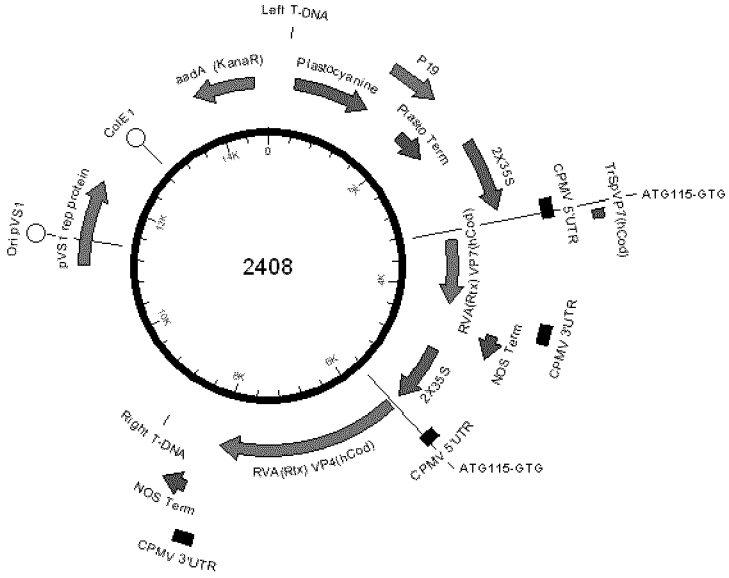
12. Double gene construct for the expression of VP6 and VP2 under CPMV-160 expression cassette (construct number 2400)

Figure 18 Schematic representation of construct number 2400



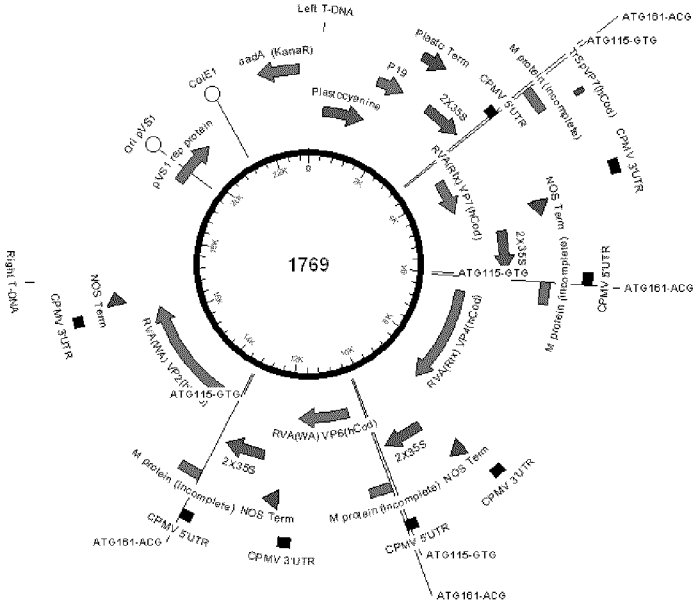
13. Double gene construct for the expression of VP7 and VP4 under CPMV-160 expression cassette (construct number 2408)

Figure 19 Schematic representation of construct number 2408



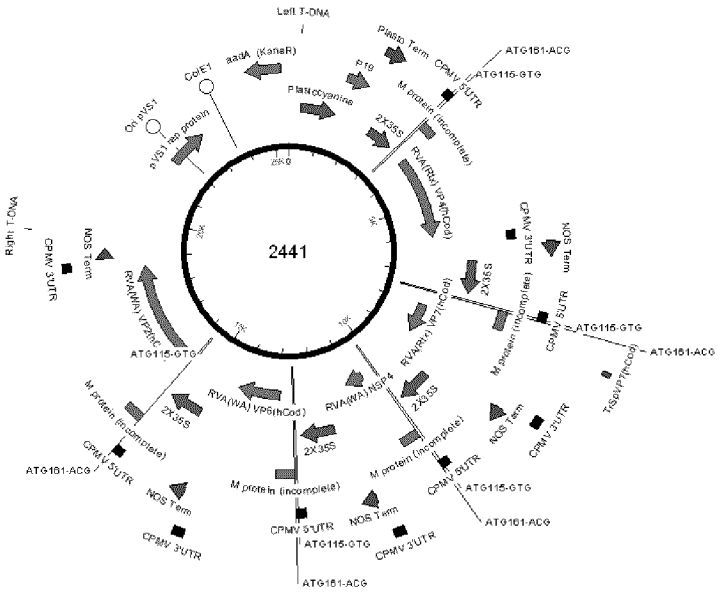
14. Quadruple gene construct for the expression of VP7, VP4, VP6 and VP2 under CPMV-HT expression cassette (construct number 1769)

Figure 20 Schematic representation of construct number 1769



15. Quintuple gene construct for the expression of VP4, VP7, NSP4, VP6 and VP2 under CPMV-HT expression cassette (construct number 2441)

Figure 21 Schematic representation of construct number 2441



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/050043

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 7/04*** (2006.01), ***A01H 5/00*** (2006.01), ***C12N 15/46*** (2006.01), ***C12N 15/82*** (2006.01), ***C12N 15/86*** (2006.01), ***C12N 7/01*** (2006.01), ***C12N 7/02*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 7/04 (2006.01), ***A01H 5/00*** (2006.01), ***C12N 15/46*** (2006.01), ***C12N 15/82*** (2006.01), ***C12N 15/86*** (2006.01), ***C12N 7/01*** (2006.01), ***C12N 7/02*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: PubMed, Canadian Patent Database, Questel Orbit
Keywords: RLP, NSP4, VP2, VP6, VP7, VP4, rotavirus, *Nicotiana benthamiana*, plant, vaccine, non-structural

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO2013166609 A1 (D'AOUST ET AL.) 14 November 2013 (14-11-2013) the whole document	1-38
A	US20050186219 A1 (LANGRIDGE ET AL.) 25 August 2005 (25-08-2005) the whole document	1-38

☐ Further documents are listed in the continuation of Box C.

☑ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent but published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
	"&" document member of the same patent family

Date of the actual completion of the international search 03 March 2016 (03-03-2016)	Date of mailing of the international search report 14 April 2016 (14-04-2016)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Keely Ingrey (819) 639-7697

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☑ forming part of the international application as filed:

☑ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 28 and 29
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 28 and 29 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to examine under **Rule 67.1 (iv) of the PCT**. Moreover, this Authority has established a written opinion based on the alleged use of a composition comprising an effective dose of the RLP as defined in instant claim 26 for inducing immunity to a rotavirus infection in a subject.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/050043

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2013166609A	14 November 2013 (14-11-2013)	WO2013166609A1	14 November 2013 (14-11-2013)
		AU2013258849A1	13 November 2014 (13-11-2014)
		CA2872803A1	14 November 2013 (14-11-2013)
		CN104284978A	14 January 2015 (14-01-2015)
		EA201492014A1	27 February 2015 (27-02-2015)
		EP2847324A1	18 March 2015 (18-03-2015)
		EP2847324A4	23 March 2016 (23-03-2016)
		HK1206059A1	31 December 2015 (31-12-2015)
		JP2015517304A	22 June 2015 (22-06-2015)
		KR20150013713A	05 February 2015 (05-02-2015)
		MX2014013671A	07 May 2015 (07-05-2015)
		PH12014502373A1	12 January 2015 (12-01-2015)
		SG11201406996VA	27 November 2014 (27-11-2014)
		TW201346028A	16 November 2013 (16-11-2013)
		US2015216961A1	06 August 2015 (06-08-2015)
US2005186219A1	25 August 2005 (25-08-2005)	US2005186219A1	25 August 2005 (25-08-2005)
		AT319822T	15 March 2006 (15-03-2006)
		AU750623B2	25 July 2002 (25-07-2002)
		AU1072499A	27 April 1999 (27-04-1999)
		AU3459201A	07 August 2001 (07-08-2001)
		AU2001234592B2	29 January 2004 (29-01-2004)
		AU3757499A	08 November 1999 (08-11-1999)
		CA2326373A1	28 October 1999 (28-10-1999)
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		CA2398462A1	02 August 2001 (02-08-2001)
		DE69930249D1	04 May 2006 (04-05-2006)
		DE69930249T2	07 December 2006 (07-12-2006)
		EP1076694A1	21 February 2001 (21-02-2001)
		EP1076694A4	10 April 2002 (10-04-2002)
		EP1076694B1	08 March 2006 (08-03-2006)
		EP1254156A1	06 November 2002 (06-11-2002)
		EP1254156A4	21 May 2003 (21-05-2003)
		US2002055618A1	09 May 2002 (09-05-2002)
		US6777546B2	17 August 2004 (17-08-2004)
		US2006277635A1	07 December 2006 (07-12-2006)
		US7422747B2	09 September 2008 (09-09-2008)
		US2003021803A1	30 January 2003 (30-01-2003)
		US2003165543A1	04 September 2003 (04-09-2003)
		US2005044588A1	24 February 2005 (24-02-2005)
		US2005241024A1	27 October 2005 (27-10-2005)
		US2009081256A1	26 March 2009 (26-03-2009)
		WO0155169A1	02 August 2001 (02-08-2001)
		WO9918225A1	15 April 1999 (15-04-1999)
		WO9954452A1	28 October 1999 (28-10-1999)

SEQUENCE LISTING

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gaaatcaaag atctctttgt ggacacgtag tgcggcgcca ttaaataacg tgtacttgtc 240

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565 570 575

His Val Gln Thr Leu Thr Thr Glu Lys Leu Gln Leu Thr Ser Val Thr
580 585 590

Ser Leu Cys Met Leu Ile Gly Asn Thr Thr Val Ile Pro Ser Pro Gln
595 600 605

Thr Leu Phe His Tyr Tyr Asn Ile Asn Val Asn Phe His Ser Asn Tyr
610 615 620

Asn Glu Arg Ile Asn Asp Ala Val Ala Ile Ile Thr Ala Ala Asn Arg
625 630 635 640

Leu Asn Leu Tyr Gln Lys Lys Met Lys Ser Ile Val Glu Asp Phe Leu
645 650 655

Lys Arg Leu Gln Ile Phe Asp Val Pro Arg Val Pro Asp Asp Gln Met
660 665 670

Tyr Arg Leu Arg Asp Arg Leu Arg Leu Leu Pro Val Glu Arg Arg Arg
675 680 685

Leu Asp Ile Phe Asn Leu Ile Leu Met Asn Met Glu Gln Ile Glu Arg
690 695 700

Ala Ser Asp Lys Ile Ala Gln Gly Val Ile Ile Ala Tyr Arg Asp Met
705 710 715 720

Gln Leu Glu Arg Asp Glu Met Tyr Gly Tyr Val Asn Ile Ala Arg Asn

725 730 735

Leu Asp Gly Tyr Gln Gln Ile Asn Leu Glu Glu Leu Met Arg Thr Gly
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Asp Tyr Gly Gln Ile Thr Asn Met Leu Leu Asn Asn Gln Pro Val Ala
755 760 765

Leu Val Gly Ala Leu Pro Phe Val Thr Asp Ser Ser Val Ile Ser Leu
770 775 780

Ile Ala Lys Leu Asp Ala Thr Val Phe Ala Gln Ile Val Lys Leu Arg
785 790 795 800

Lys Val Asp Thr Leu Lys Pro Ile Leu Tyr Lys Ile Asn Ser Asp Ser
805 810 815

Asn Asp Phe Tyr Leu Val Ala Asn Tyr Asp Trp Ile Pro Thr Ser Thr
820 825 830

Thr Lys Val Tyr Lys Gln Val Pro Gln Pro Phe Asp Phe Arg Ala Ser
835 840 845

Met His Met Leu Thr Ser Asn Leu Thr Phe Thr Val Tyr Ser Asp Leu
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Phe Asp Asn Met Arg Ile Met Asn Glu Leu
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<220>
<223> IF WA VP6 opt s1 plus 3c

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<400> 29

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35 40 45

Gly Gly Ile Gly Asn Leu Pro Val Arg Asn Trp Thr Phe Asp Phe Gly
50 55 60

Leu Leu Gly Thr Thr Leu Leu Asn Leu Asp Ala Asn Tyr Val Glu Asn
65 70 75 80

Ala Arg Thr Ile Ile Glu Tyr Phe Ile Asp Phe Ile Asp Asn Val Cys
85 90 95

Met Asp Glu Met Ala Arg Glu Ser Gln Arg Asn Gly Val Ala Pro Gln
100 105 110

Ser Glu Ala Leu Arg Lys Leu Ala Gly Ile Lys Phe Lys Arg Ile Asn
115 120 125

Phe Asp Asn Ser Ser Glu Tyr Ile Glu Asn Trp Asn Leu Gln Asn Arg
130 135 140

Arg Gln Arg Thr Gly Phe Val Phe His Lys Pro Asn Ile Phe Pro Tyr
145 150 155 160

Ser Ala Ser Phe Thr Leu Asn Arg Ser Gln Pro Met His Asp Asn Leu
165 170 175

Met Gly Thr Met Trp Leu Asn Ala Gly Ser Glu Ile Gln Val Ala Gly
180 185 190

Phe Asp Tyr Ser Cys Ala Ile Asn Ala Pro Ala Asn Ile Gln Gln Phe
195 200 205

Glu His Ile Val Gln Leu Arg Arg Ala Leu Thr Thr Ala Thr Ile Thr
210 215 220

Leu Leu Pro Asp Ala Glu Arg Phe Ser Phe Pro Arg Val Ile Asn Ser
225 230 235 240

Ala Asp Gly Ala Thr Thr Trp Phe Phe Asn Pro Val Ile Leu Arg Pro
245 250 255

Asn Asn Val Glu Val Glu Phe Leu Leu Asn Gly Gln Ile Ile Asn Thr
260 265 270

Tyr Gln Ala Arg Phe Gly Thr Ile Ile Ala Arg Asn Phe Asp Ala Ile
275 280 285

Arg Leu Leu Phe Gln Leu Met Arg Pro Pro Asn Met Thr Pro Ala Val
290 295 300

Asn Ala Leu Phe Pro Gln Ala Gln Pro Phe Gln His His Ala Thr Val
305 310 315 320

Gly Leu Thr Leu Arg Ile Glu Ser Ala Val Cys Glu Ser Val Leu Ala
325 330 335

Asp Ala Asn Glu Thr Leu Leu Ala Asn Val Thr Ala Val Arg Gln Glu
340 345 350

Tyr Ala Ile Pro Val Gly Pro Val Phe Pro Pro Gly Met Asn Trp Thr
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370 375 380

Val Phe Thr Val Ala Ser Ile Arg Ser Met Leu Ile Lys
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<212> DNA
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<223> IF Rtx VP4 opt s1 to 4r

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<223> Amino acid sequence of VP4 from Rotavirus A Rotarix strain

<400> 34

Met Ala Ser Leu Ile Tyr Arg Gln Leu Leu Thr Asn Ser Tyr Ser Val
1 5 10 15

Asp Leu His Asp Glu Ile Glu Gln Ile Gly Ser Glu Lys Thr Gln Asn
20 25 30

Val Thr Ile Asn Pro Gly Pro Phe Ala Gln Thr Arg Tyr Ala Pro Val
35 40 45

Asn Trp Asp His Gly Glu Ile Asn Asp Ser Thr Thr Val Glu Pro Ile
50 55 60

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Leu Asp Gly Pro Tyr Gln Pro Thr Thr Phe Thr Pro Pro Asn Asp Tyr
65                  70                  75                  80

Trp Ile Leu Ile Asn Ser Asn Thr Asn Gly Val Val Tyr Glu Ser Thr
                85                  90                  95

Asn Asn Ser Asp Phe Trp Thr Ala Val Val Ala Ile Glu Pro His Val
            100                 105                 110

Asn Pro Val Asp Arg Gln Tyr Met Ile Phe Gly Glu Ser Lys Gln Phe
        115                 120                 125

Asn Val Ser Asn Asp Ser Asn Lys Trp Lys Phe Leu Glu Met Phe Arg
    130                 135                 140

Ser Ser Ser Gln Asn Glu Phe Tyr Asn Arg Arg Thr Leu Thr Ser Asp
145                 150                 155                 160

Thr Arg Leu Val Gly Ile Phe Lys Tyr Gly Gly Arg Val Trp Thr Phe
                165                 170                 175

His Gly Glu Thr Pro Arg Ala Thr Thr Asp Ser Ser Ser Thr Ala Asn
            180                 185                 190

Leu Asn Asn Ile Ser Ile Thr Ile His Ser Glu Phe Tyr Ile Ile Pro
        195                 200                 205

Arg Ser Gln Glu Ser Lys Cys Asn Glu Tyr Ile Asn Asn Gly Leu Pro
    210                 215                 220

Pro Ile Gln Asn Thr Arg Asn Val Val Pro Leu Pro Leu Ser Ser Arg
225                 230                 235                 240

Ser Ile Gln Tyr Lys Arg Ala Gln Val Asn Glu Asp Ile Ile Val Ser
                245                 250                 255

Lys Thr Ser Leu Trp Lys Glu Met Gln Tyr Asn Arg Asp Ile Ile Ile
            260                 265                 270

Arg Phe Lys Phe Gly Asn Ser Ile Val Lys Met Gly Gly Leu Gly Tyr
        275                 280                 285
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Lys Trp Ser Glu Ile Ser Tyr Lys Ala Ala Asn Tyr Gln Tyr Asn Tyr
    290                 295                 300

Leu Arg Asp Gly Glu Gln Val Thr Ala His Thr Thr Cys Ser Val Asn
305                 310                 315                 320

Gly Val Asn Asn Phe Ser Tyr Asn Gly Gly Phe Leu Pro Thr Asp Phe
                325                 330                 335

Gly Ile Ser Arg Tyr Glu Val Ile Lys Glu Asn Ser Tyr Val Tyr Val
            340                 345                 350

Asp Tyr Trp Asp Asp Ser Lys Ala Phe Arg Asn Met Val Tyr Val Arg
        355                 360                 365

Ser Leu Ala Ala Asn Leu Asn Ser Val Lys Cys Thr Gly Gly Ser Tyr
    370                 375                 380

Tyr Phe Ser Ile Pro Val Gly Ala Trp Pro Val Met Asn Gly Gly Ala
385                 390                 395                 400

Val Ser Leu His Phe Ala Gly Val Thr Leu Ser Thr Gln Phe Thr Asp
                405                 410                 415

Phe Val Ser Leu Asn Ser Leu Arg Phe Arg Phe Ser Leu Thr Val Asp
            420                 425                 430

Glu Pro Pro Phe Ser Ile Leu Arg Thr Arg Thr Val Asn Leu Tyr Gly
        435                 440                 445

Leu Pro Ala Ala Asn Pro Asn Asn Gly Asn Glu Tyr Tyr Glu Ile Ser
    450                 455                 460

Gly Arg Phe Ser Leu Ile Ser Leu Val Pro Thr Asn Asp Asp Tyr Gln
465                 470                 475                 480

Thr Pro Ile Met Asn Ser Val Thr Val Arg Gln Asp Leu Glu Arg Gln
                485                 490                 495

Leu Thr Asp Leu Arg Glu Glu Phe Asn Ser Leu Ser Gln Glu Ile Ala
            500                 505                 510
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Met Ala Gln Leu Ile Asp Leu Ala Leu Leu Pro Leu Asp Met Phe Ser
        515                 520                 525

Met Phe Ser Gly Ile Lys Ser Thr Ile Asp Leu Thr Lys Ser Met Ala
    530                 535                 540

Thr Ser Val Met Lys Lys Phe Arg Lys Ser Lys Leu Ala Thr Ser Ile
545                 550                 555                 560

Ser Glu Met Thr Asn Ser Leu Ser Asp Ala Ala Ser Ser Ala Ser Arg
                565                 570                 575

Asn Val Ser Ile Arg Ser Asn Leu Ser Ala Ile Ser Asn Trp Thr Asn
            580                 585                 590

Val Ser Asn Asp Val Ser Asn Val Thr Asn Ser Leu Asn Asp Ile Ser
        595                 600                 605

Thr Gln Thr Ser Thr Ile Ser Lys Lys Leu Arg Leu Lys Glu Met Ile
    610                 615                 620

Thr Gln Thr Glu Gly Met Ser Phe Asp Asp Ile Ser Ala Ala Val Leu
625                 630                 635                 640

Lys Thr Lys Ile Asp Met Ser Thr Gln Ile Gly Lys Asn Thr Leu Pro
                645                 650                 655

Asp Ile Val Thr Glu Ala Ser Glu Lys Phe Ile Pro Lys Arg Ser Tyr
            660                 665                 670

Arg Ile Leu Lys Asp Asp Glu Val Met Glu Ile Asn Thr Glu Gly Lys
        675                 680                 685

Phe Phe Ala Tyr Lys Ile Asn Thr Phe Asp Glu Val Pro Phe Asp Val
    690                 695                 700

Asn Lys Phe Ala Glu Leu Val Thr Asp Ser Pro Val Ile Ser Ala Ile
705                 710                 715                 720

Ile Asp Phe Lys Thr Leu Lys Asn Leu Asn Asp Asn Tyr Gly Ile Thr
                725                 730                 735
```

Arg Thr Glu Ala Leu Asn Leu Ile Lys Ser Asn Pro Asn Met Leu Arg
740 745 750

Asn Phe Ile Asn Gln Asn Asn Pro Ile Ile Arg Asn Arg Ile Glu Gln
755 760 765

Leu Ile Leu Gln Cys Lys Leu
770 775

<210> 35
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> IF TrSP plus Rtx VP7j opt s1 plus 3c

<400> 35
aaatttgtcg ggcccatgga ttatattatc tatcgtagcc tcctcatcta 50

<210> 36
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> IF Rtx VP7 opt s1 to 4r

<400> 36
actaaagaaa ataggcctct aaacgcgata atagaaggct gctgagttca ggga 54

<210> 37
<211> 981
<212> DNA
<213> Artificial Sequence

<220>
<223> Optimized coding sequence of Rotavirus A VP7

<400> 37
atgtacggca tcgagtatac aacaatttta attttcctga tttccatcat tctgttaaac 60

tacatcctta agtccgtgac cagaattatg gattatatta tctatcgtag cctcctcatc 120

tacgtggccc tttttgccct gaccagggcc cagaactatg gcctgaactt accaatcacc 180

ggttcaatgg ataccgttta cgctaattcc actcaagagg ggatatttct gacaagtacc 240

ctgtgcctgt attatccaac agaagcctct acccagatca atgatgggga gtggaaggat 300

agtctctcac agatgttcct aaccaagggc tggcccaccg gttccgtcta cttcaaggaa 360

tactctagta ttgtcgactt ctcagttgac ccccagcttt attgcgacta caacctggta 420

cttatgaaat acgaccagaa cctggagctg gatatgtccg agctggctga cctgatcctc 480

aatgagtggc tgtgcaaccc catggacatc acattatatt actaccagca gtctggagaa 540

tccaacaagt ggatcagtat gggctcaagt tgcaccgtga aggtgtgtcc cttgaacacc 600

caaatgctgg gcattggttg tcagacaact aatgtggatt cgtttgaaat ggtagccgaa 660

aacgagaagc tggctatagt ggacgtagtc gatgggatta accacaagat caatctgact 720

accaccactt gtaccatcag aaactgtaaa aagctcggcc cccgggagaa cgtcgccgtg 780

atccaggtgg gggggagcaa tgtgctcgac attactgccg accctaccac caatccacag 840

acggaacgga tgatgagagt caactggaag aaatggtggc aggtctttta taccattgtg 900

gactacatta accagattgt gcaagtcatg agtaaacggt ccagatccct gaactcagca 960

gccttctatt atcgcgttta g 981

<210> 38
<211> 2634
<212> DNA
<213> Artificial Sequence

<220>
<223> Expression cassette number 1734

<400> 38
gtcaacatgg tggagcacga cacacttgtc tactccaaaa atatcaaaga tacagtctca 60

gaagaccaaa gggcaattga gacttttcaa caaagggtaa tatccggaaa cctcctcgga 120

ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180

tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240

ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300

acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360

tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420

agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480

aagatagtgg aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc 540

atcgttgaag atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc 600

atcgtggaaa aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatatc 660

tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720

taaggaagtt catttcattt ggagaggtat taaaatctta ataggttttg ataaaagcga 780

acgtggggaa acccgaacca aaccttcttc taaactctct ctcatctctc ttaaagcaaa 840

cttctctctt gtctttcttg cgtgagcgat cttcaacgtt gtcagatcgt gcttcggcac 900

cagtacaacg ttttctttca ctgaagcgaa atcaaagatc tctttgtgga cacgtagtgc 960

ggcgccatta aataacgtgt acttgtccta ttcttgtcgg tgtggtcttg ggaaaagaaa 1020

gcttgctgga ggctgctgtt cagccccata cattacttgt tacgattctg ctgactttcg 1080

gcgggtgcaa tatctctact tctgcttgac gaggtattgt tgcctgtact tctttcttct 1140

tcttcttgct gattggttct ataagaaatc tagtattttc tttgaaacag agttttcccg 1200

tggttttcga acttggagaa agattgttaa gcttctgtat attctgccca aatttgtcgg 1260

gcccatggat tatattatct atcgtagcct cctcatctac gtggcccttt ttgccctgac 1320

cagggcccag aactatggcc tgaacttacc aatcaccggt tcaatggata ccgtttacgc 1380

taattccact caagagggga tatttctgac aagtaccctg tgcctgtatt atccaacaga 1440

agcctctacc cagatcaatg atggggagtg gaaggatagt ctctcacaga tgttcctaac 1500

caagggctgg cccaccggtt ccgtctactt caaggaatac tctagtattg tcgacttctc 1560

agttgacccc cagctttatt gcgactacaa cctggtactt atgaaatacg accagaacct 1620

ggagctggat atgtccgagc tggctgacct gatcctcaat gagtggctgt gcaaccccat 1680

ggacatcaca ttatattact accagcagtc tggagaatcc aacaagtgga tcagtatggg 1740

ctcaagttgc accgtgaagg tgtgtccctt gaacacccaa atgctgggca ttggttgtca 1800

gacaactaat gtggattcgt ttgaaatggt agccgaaaac gagaagctgg ctatagtgga 1860

cgtagtcgat gggattaacc acaagatcaa tctgactacc accacttgta ccatcagaaa 1920

ctgtaaaaag ctcggccccc gggagaacgt cgccgtgatc caggtggggg ggagcaatgt 1980

gctcgacatt actgccgacc ctaccaccaa tccacagacg gaacggatga tgagagtcaa 2040

ctggaagaaa tggtggcagg tcttttatac cattgtggac tacattaacc agattgtgca 2100

agtcatgagt aaacggtcca gatccctgaa ctcagcagcc ttctattatc gcgtttagag 2160

gcctattttc tttagtttga atttactgtt attcggtgtg catttctatg tttggtgagc 2220

ggttttctgt gctcagagtg tgtttatttt atgtaattta atttctttgt gagctcctgt 2280

ttagcaggtc gtcccttcag caaggacaca aaaagatttt aattttatta aaaaaaaaaa 2340

aaaaaaagac cgggaattcg atatcaagct tatcgacctg cagatcgttc aaacatttgg 2400

caataaagtt tcttaagatt gaatcctgtt gccggtcttg cgatgattat catataattt 2460

ctgttgaatt acgttaagca tgtaataatt aacatgtaat gcatgacgtt atttatgaga 2520

tgggttttta tgattagagt cccgcaatta tacatttaat acgcgataga aaacaaaata 2580

tagcgcgcaa actaggataa attatcgcgc gcggtgtcat ctatgttact agat 2634

<210> 39
<211> 297
<212> PRT
<213> Artificial sequence

<220>
<223> Amino acid sequence of TrSp-VP7

<400> 39

Met Asp Tyr Ile Ile Tyr Arg Ser Leu Leu Ile Tyr Val Ala Leu Phe
1 5 10 15

Ala Leu Thr Arg Ala Gln Asn Tyr Gly Leu Asn Leu Pro Ile Thr Gly
20 25 30

Ser Met Asp Thr Val Tyr Ala Asn Ser Thr Gln Glu Gly Ile Phe Leu
35 40 45

Thr Ser Thr Leu Cys Leu Tyr Tyr Pro Thr Glu Ala Ser Thr Gln Ile
50 55 60

Asn Asp Gly Glu Trp Lys Asp Ser Leu Ser Gln Met Phe Leu Thr Lys
65 70 75 80

Gly Trp Pro Thr Gly Ser Val Tyr Phe Lys Glu Tyr Ser Ser Ile Val
85 90 95

Asp Phe Ser Val Asp Pro Gln Leu Tyr Cys Asp Tyr Asn Leu Val Leu
100 105 110

Met Lys Tyr Asp Gln Asn Leu Glu Leu Asp Met Ser Glu Leu Ala Asp
115 120 125

Leu Ile Leu Asn Glu Trp Leu Cys Asn Pro Met Asp Ile Thr Leu Tyr
130 135 140

Tyr Tyr Gln Gln Ser Gly Glu Ser Asn Lys Trp Ile Ser Met Gly Ser
145 150 155 160

Ser Cys Thr Val Lys Val Cys Pro Leu Asn Thr Gln Met Leu Gly Ile
165 170 175

Gly Cys Gln Thr Thr Asn Val Asp Ser Phe Glu Met Val Ala Glu Asn
180 185 190

Glu Lys Leu Ala Ile Val Asp Val Val Asp Gly Ile Asn His Lys Ile
195 200 205

Asn Leu Thr Thr Thr Thr Cys Thr Ile Arg Asn Cys Lys Lys Leu Gly
210 215 220

Pro Arg Glu Asn Val Ala Val Ile Gln Val Gly Gly Ser Asn Val Leu
225 230 235 240

Asp Ile Thr Ala Asp Pro Thr Thr Asn Pro Gln Thr Glu Arg Met Met
245 250 255

Arg Val Asn Trp Lys Lys Trp Trp Gln Val Phe Tyr Thr Ile Val Asp
260 265 270

Tyr Ile Asn Gln Ile Val Gln Val Met Ser Lys Arg Ser Arg Ser Leu
275 280 285

Asn Ser Ala Ala Phe Tyr Tyr Arg Val
290 295

<210> 40
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> IF WA NSP4 S1 plus 3C

<400> 40
aaatttgtcg ggcccatgga taagcttgcc gacctcaact acacattgag tg 52

<210> 41
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> IF WA NSP4 S1 to 4r

<400> 41
actaaagaaa ataggccttc acatggatgc agtcacttct gacggttcat atgga 55

<210> 42
<211> 528
<212> DNA
<213> Artificial Sequence

<220>
<223> Coding sequence of Rotavirus A NSP4 from strain WA

<400> 42
atggataagc ttgccgacct caactacaca ttgagtgtaa tcacttcaat gaatgacaca 60

ttgcattcta taattcaaga tcctggaatg gcgtattttc tatatattgc atctgttcta 120

acagttttgt tcacattaca taaagcttca attccaacca tgaaaatagc attgaaaaca 180

tcaaaatgtt catataaagt gattaaatat tgtatagtca cgatcattaa tactctttta 240

aaattggctg gatataaaga gcaggttact acaaaagacg aaattgagca acagatggac 300

agaattgtga aagagatgag acgtcagctg gagatgattg ataaactaac tactcgtgaa 360

attgaacagg ttgaattgct taaacgtata catgacaacc tgataactag accagttgac 420

gttatagata tgtcgaagga attcaatcag aaaaacatca aaacgctaga tgaatgggag 480

agtggaaaaa atccatatga accgtcagaa gtgactgcat ccatgtga 528

<210> 43
<211> 2268
<212> DNA
<213> Artificial Sequence

<220>
<223> Expression cassette number 1706

<400> 43
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gaagaccaaa gggcaattga gacttttcaa caaagggtaa tatccggaaa cctcctcgga 120

ttccattgcc cagctatctg tcactttatt gtgaagatag tggaaaagga aggtggctcc 180

tacaaatgcc atcattgcga taaaggaaag gccatcgttg aagatgcctc tgccgacagt 240

ggtcccaaag atggaccccc acccacgagg agcatcgtgg aaaaagaaga cgttccaacc 300

acgtcttcaa agcaagtgga ttgatgtgat aacatggtgg agcacgacac acttgtctac 360

tccaaaaata tcaaagatac agtctcagaa gaccaaaggg caattgagac ttttcaacaa 420

agggtaatat ccggaaacct cctcggattc cattgcccag ctatctgtca ctttattgtg 480

aagatagtgg aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggcc 540

atcgttgaag atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc 600

atcgtggaaa aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgatatc 660

tccactgacg taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata 720

taaggaagtt catttcattt ggagaggtat taaaatctta ataggttttg ataaaagcga 780

acgtggggaa acccgaacca aaccttcttc taaactctct ctcatctctc ttaaagcaaa 840

cttctctctt gtctttcttg cgtgagcgat cttcaacgtt gtcagatcgt gcttcggcac 900

cagtacaacg ttttctttca ctgaagcgaa atcaaagatc tctttgtgga cacgtagtgc 960

ggcgccatta aataacgtgt acttgtccta ttcttgtcgg tgtggtcttg ggaaaagaaa 1020

gcttgctgga ggctgctgtt cagccccata cattacttgt tacgattctg ctgactttcg 1080

gcgggtgcaa tatctctact tctgcttgac gaggtattgt tgcctgtact tctttcttct 1140

tcttcttgct gattggttct ataagaaatc tagtattttc tttgaaacag agttttcccg 1200

tggttttcga acttggagaa agattgttaa gcttctgtat attctgccca aatttgtcgg 1260

gcccatggat aagcttgccg acctcaacta cacattgagt gtaatcactt caatgaatga 1320

cacattgcat tctataattc aagatcctgg aatggcgtat tttctatata ttgcatctgt 1380

tctaacagtt ttgttcacat tacataaagc ttcaattcca accatgaaaa tagcattgaa 1440

aacatcaaaa tgttcatata aagtgattaa atattgtata gtcacgatca ttaatactct 1500

tttaaaattg gctggatata aagagcaggt tactacaaaa gacgaaattg agcaacagat 1560

ggacagaatt gtgaaagaga tgagacgtca gctggagatg attgataaac taactactcg 1620

tgaaattgaa caggttgaat tgcttaaacg tatacatgac aacctgataa ctagaccagt 1680

tgacgttata gatatgtcga aggaattcaa tcagaaaaac atcaaaacgc tagatgaatg 1740

ggagagtgga aaaaatccat atgaaccgtc agaagtgact gcatccatgt gaaggcctat 1800

tttctttagt ttgaatttac tgttattcgg tgtgcatttc tatgtttggt gagcggtttt 1860

ctgtgctcag agtgtgttta ttttatgtaa tttaatttct ttgtgagctc ctgtttagca 1920

ggtcgtccct tcagcaagga cacaaaaaga ttttaatttt attaaaaaaa aaaaaaaaaa 1980

agaccgggaa ttcgatatca agcttatcga cctgcagatc gttcaaacat ttggcaataa 2040

agtttcttaa gattgaatcc tgttgccggt cttgcgatga ttatcatata atttctgttg 2100

aattacgtta agcatgtaat aattaacatg taatgcatga cgttatttat gagatgggtt 2160

tttatgatta gagtcccgca attatacatt taatacgcga tagaaaacaa aatatagcgc 2220

gcaaactagg ataaattatc gcgcgcggtg tcatctatgt tactagat 2268

<210> 44
<211> 175
<212> PRT
<213> Rotavirus

<400> 44

Met Asp Lys Leu Ala Asp Leu Asn Tyr Thr Leu Ser Val Ile Thr Ser
1 5 10 15

Met Asn Asp Thr Leu His Ser Ile Ile Gln Asp Pro Gly Met Ala Tyr
20 25 30

Phe Leu Tyr Ile Ala Ser Val Leu Thr Val Leu Phe Thr Leu His Lys
35 40 45

Ala Ser Ile Pro Thr Met Lys Ile Ala Leu Lys Thr Ser Lys Cys Ser
50 55 60

Tyr Lys Val Ile Lys Tyr Cys Ile Val Thr Ile Ile Asn Thr Leu Leu
65 70 75 80

Lys Leu Ala Gly Tyr Lys Glu Gln Val Thr Thr Lys Asp Glu Ile Glu
85 90 95

Gln Gln Met Asp Arg Ile Val Lys Glu Met Arg Arg Gln Leu Glu Met
100 105 110

Ile Asp Lys Leu Thr Thr Arg Glu Ile Glu Gln Val Glu Leu Leu Lys
115 120 125

Arg Ile His Asp Asn Leu Ile Thr Arg Pro Val Asp Val Ile Asp Met
130 135 140

Ser Lys Glu Phe Asn Gln Lys Asn Ile Lys Thr Leu Asp Glu Trp Glu
145 150 155 160

Ser Gly Lys Asn Pro Tyr Glu Pro Ser Glu Val Thr Ala Ser Met
165 170 175

<210> 45
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> IF C160 WA VP2 opt c

<400> 45
tcgtgcttcg gcaccagtac aatggcatac cggaagagag gagcaaagcg cgaa 54

<210> 46
<211> 4540
<212> DNA
<213> Artificial sequence

<220>
<223> Construct 1190

<400> 46
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aataactcaa aaaccataaa agtttaagtt agcaagtgtg tacatttta cttgaacaaa 180

aatattcacc tactactgtt ataaatcatt attaaacatt agagtaaaga aatatggatg 240

ataagaacaa gagtagtgat attttgacaa caattttgtt gcaacatttg agaaaatttt 300

gttgttctct cttttcattg gtcaaaaaca atagagagag aaaaaggaag agggagaata 360

aaaacataat gtgagtatga gagagaaagt tgtacaaaag ttgtaccaaa atagttgtac 420

aaatatcatt gaggaatttg acaaaagcta cacaaataag ggttaattgc tgtaaataaa 480

taaggatgac gcattagaga gatgtaccat tagagaattt ttggcaagtc attaaaaaga 540

aagaataaat tatttttaaa attaaaagtt gagtcatttg attaaacatg tgattattta 600

atgaattgat gaaagagttg gattaaagtt gtattagtaa ttagaatttg gtgtcaaatt 660

taatttgaca tttgatcttt tcctatatat tgccccatag agtcagttaa ctcatttta 720

tatttcatag atcaaataag agaaataacg gtatattaat ccctccaaaa aaaaaaaacg 780

gtatatttac taaaaaatct aagccacgta ggaggataac aggatccccg taggaggata 840

acatccaatc caaccaatca caacaatcct gatgagataa cccactttaa gcccacgcat 900

ctgtggcaca tctacattat ctaaatcaca cattcttcca cacatctgag ccacacaaaa 960

accaatccac atctttatca cccattctat aaaaaatcac actttgtgag tctacacttt 1020

gattcccttc aaacacatac aaagagaaga gactaattaa ttaattaatc atcttgagag 1080

aaaatggaac gagctataca aggaaacgac gctagggaac aagctaacag tgaacgttgg 1140

gatggaggat caggaggtac cacttctccc ttcaaacttc ctgacgaaag tccgagttgg 1200

actgagtggc ggctacataa cgatgagacg aattcgaatc aagataatcc ccttggtttc 1260

aaggaaagct ggggtttcgg gaaagttgta tttaagagat atctcagata cgacaggacg 1320

gaagcttcac tgcacagagt ccttggatct tggacgggag attcggttaa ctatgcagca 1380

tctcgatttt tcggtttcga ccagatcgga tgtacctata gtattcggtt tcgaggagtt 1440

agtatcaccg tttctggagg gtcgcgaact cttcagcatc tctgtgagat ggcaattcgg 1500

tctaagcaag aactgctaca gcttgcccca atcgaagtgg aaagtaatgt atcaagagga 1560

tgccctgaag gtactcaaac cttcgaaaaa gaaagcgagt aagttaaaat gcttcttcgt 1620

ctcctattta taatatggtt tgttattgtt aattttgttc ttgtagaaga gcttaattaa 1680

tcgttgttgt tatgaaatac tatttgtatg agatgaactg gtgtaatgta attcatttac 1740

ataagtggag tcagaatcag aatgtttcct ccataactaa ctagacatga agacctgccg 1800

cgtacaattg tcttatattt gaacaactaa aattgaacat cttttgccac aactttataa 1860

gtggttaata tagctcaaat atatggtcaa gttcaataga ttaataatgg aaatatcagt 1920

tatcgaaatt cattaacaat caacttaacg ttattaacta ctaattttat atcatcccct 1980

ttgataaatg atagtacacc aattaggaag gagcatgctc gcctaggaga ttgtcgtttc 2040

ccgccttcag tttgcaagct gctctagccg tgtagccaat acgcaaaccg cctctccccg 2100

cgcgttggga attactagcg cgtgtcgaca agcttgcatg ccggtcaaca tggtggagca 2160

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摘要

提供在植物中产生轮状病毒样颗粒(RLP)的方法。该方法包括在宿主或宿主细胞(例如植物、植物的一部分或植物细胞)内表达一种或多种核酸，所述一种或多种核酸包含与第一核苷酸序列、第二核苷酸序列和第三核苷酸序列可操作地(operatively)连接的一种或多种调控区，所述调控区在宿主或宿主细胞中是有活性的(active)。第一核苷酸序列编码第一轮状病毒蛋白，第二核苷酸序列编码第二轮状病毒蛋白，以及第三核苷酸序列编码第三轮状病毒蛋白。第一核苷酸序列、第二核苷酸序列和第三核苷酸序列编码轮状病毒蛋白 NSP4、VP2 或 VP6，以及 VP4 或 VP7。在允许核酸表达的条件下孵育宿主或宿主细胞，使得 NSP4、VP2 或 VP6，以及 VP4 或 VP7 都被表达，从而产生 RLP。还提供了包含 RLP 的宿主，包含 RLP 的组合物和使用该组合物的方法。