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(54) Title: CPMV ENHANCER ELEMENTS

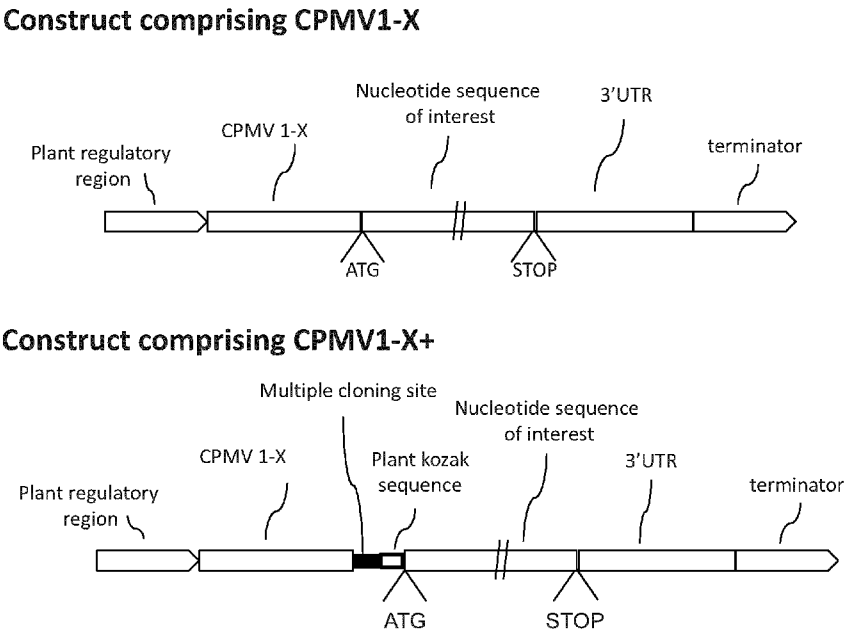


Figure 1a

(57) Abstract: An expression enhancer comprising a CPMV 5'UTR nucleotide sequence consisting of X nucleotides (CMPVX), where X=160, 155, 150, or 114 of SEQ ID NO:1, or consisting of a nucleotide sequence comprising from about 80% to 100% sequence similarity with CMPVX, where X=160, 155, 150, or 114 of SEQ ID NO:1SEQ ID NO:1 is provided. The expression enhancer may further comprise a stuffer sequence fused to the 3' end of the 5'UTR nucleotide sequence (CMPVX+, where X=160, 155, 150, or 114 of SEQ ID NO:1). The stuffer sequence may comprise one or more plant kozak sequences. Plants comprising the expression enhancer and methods using the expression enhancer are also described.

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# CPMV ENHANCER ELEMENTS

## FIELD OF INVENTION

[0001] The present invention relates to the expression of proteins of interest in plants. The present invention also provides methods and compositions for the production of proteins of interest in plants.

## BACKGROUND OF THE INVENTION

[0002] Plants offer great potential as production systems for recombinant proteins. One approach to producing foreign proteins in plants is to generate stable transgenic plant lines. However this is a time consuming and labor intensive process. An alternative to transgenic plants is the use of plant virus-based expression vectors. Plant virus-based vectors allow for the rapid, high level, transient expression of proteins in plants.

[0003] One method to achieve high level transient expression of foreign proteins in plants involves the use of vectors 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).

[0004] Comoviruses are RNA viruses with a bipartite genome. The segments of the comoviral RNA genome are referred to as RNA- 1 and RNA-2. RNA- 1 encodes the VPg, replicase and protease proteins. The replicase is required by the virus for replication of the viral genome. The RNA-2 of the comovirus cowpea mosaic virus (CPMV) produces a polyprotein of 105 kDa or 95 kDa processed into 4 functional peptides.

[0005] The 5' region of CPMV RNA-2 comprises start codons (AUGs) at positions 115, 161, 512 and 524. The start codons at positions 161 and 512 are in the same triplet reading frame. Initiation at the start codon at position 161 results in the

synthesis of the 105K polyprotein while initiation at the start codon at position 512 directs the synthesis of the 95K polyprotein. Initiation of translation at the start codon at position 512 in CPMV is more efficient than initiation at position 161, resulting in the production of more 95K polyprotein than 105K polyprotein. The start codon at position 115 is not essential for virus replication (Wellink et al., 1993 Biochimie. 75(8):741-7).

[0006] Maintenance of the frame between the initiation sites at positions 161 and 512 in CPMV RNA-2 is required for efficient replication of RNA-2 by the RNA-1-encoded replicase (Holness et al., 1989; Virology 172, 311- 320; van Bokhoven et al. 1993, Virology 195, 377-386; Rohll et al., 1993 Virology 193, 672-679; Wellink et al., 1993, Biochimie. 75(8):741-7). This requirement impacts the length of sequences which can be inserted upstream of the 512 start codon in replicative forms of CPMV RNA-2 expression vectors. Furthermore, the use of polylinkers must be used with caution as they may shift the codon reading frame (ORF) between these initiation sites.

[0007] CPMV has served as the basis for the development of vector systems suitable for the production of heterologous polypeptides in plants (Liu et al., 2005 Vaccine 23, 1788-1792; Sainsbury et al., 2007 Virus Expression Vectors (Hefferon, K. ed), pp. 339-555). These systems are based on the modification of RNA-2 but differ in whether full-length or deleted versions are used. Replication of the modified RNA-2 is achieved by co-inoculation with RNA-1. Foreign proteins are fused to the C-terminus of the RNA-2-derived polyproteins. Release of the N-terminal polypeptide is mediated by the action of the 2A catalytic peptide sequence from foot-and-mouth-disease virus (Gopinath et al., 2000, Virology 267: 159-173). The resulting RNA-2 molecules are capable of spreading both within and between plants. This strategy has been used to express a number of recombinant proteins, such as the Hepatitis B core antigen (HBcAg) and Small Immune Proteins (SIPs), in cowpea plants (Mechtcheriakova et al. J. Virol. Methods 131, 10-15; 2006; Monger et al., 2006, Plant Biotechnol. J. 4, 623-631; Alamillo et al., 2006, Biotechnol. J. 1, 1103-1111). Though successful, the use of a full-length viral vector limits the size of inserted sequences, and movement between plants raises concerns about biocontainment of the virus.

[0008] To address the issue of biocontainment and insert size, Canizares et al. (2006 Plant Biotechnol, J 4:183-193) replaced the majority of the coding region of RNA-2 with a sequence of interest to produce a disabled version of CPMV RNA-2 (deIRNA-2). The sequence to be expressed was fused to the AUG at position 512 of RNA-2, immediately upstream of the 3' untranslated region (UTR) to create a molecule that mimics RNA-2. Such constructs were capable of replication when introduced into plants in the presence of RNA-1 and a suppressor of silencing, and directed the synthesis of substantial levels of heterologous proteins (Sainsbury et al., 2008 Plant Biotechnol J 6:82-92).

[0009] Mutation of the start codon at position 161 in a CPMV RNA-2 vector (U162C; HT) increases the levels of expression of a protein encoded by a sequence inserted after the start codon at position 512. This permits the production of high levels of foreign proteins without the need for viral replication and was termed the CPMV-HT system (WO2009/087391; Sainsbury and Lomonossoff, 2008, Plant Physiol. 148, 1212-1218). In pEAQ expression plasmids (Sainsbury et al., 2009, Plant Biotechnology Journal, 7, pp 682-693; US 2010/0287670), the sequence to be expressed is positioned between the 5'UTR and the 3' UTR. The 5'UTR in the pEAQ series carries the U162C (HT) mutation.

## SUMMARY OF THE INVENTION

[0010] The present invention relates to the expression of proteins of interest in plants. The present invention also provides methods and compositions for the production of proteins of interest in plants.

[0011] As described herein, there is provided an expression enhancer comprising a CPMV 5'UTR nucleotide sequence consisting of X nucleotides (CMPVX), where X=160, 155, 150, or 114 of SEQ ID NO:1, or consisting of a nucleotide sequence comprising from about 80% to 100% sequence similarity with CMPVX, where X=160, 155, 150, or 114 of SEQ ID NO:1. The expression enhancer may comprise a nucleotide sequence selected from the group of SEQ ID NO: 24, 27, 68, 69, 70 and 71.

[0012] The present invention also provides the expression enhancer as defined above, where the expression enhancer furthers comprises a stuffer sequence (CPMVX+, where X=160, 155, 150, 114 of SEQ ID NO:1). The stuffer sequence may comprise comprises a length from 0 to about 100 nucleotides, or any length therein between, one or more plant kozak sequences, a multiple cloning site, one or more linker sequences, one or more recombination sites, or a combination thereof. The present invention also provides the expression enhancer as defined above, where the kozak sequence is selected from the group of sequences as shown in SEQ ID NO's: 5 - 17. The expression enhancer as just defined (CPMVX+, where X=160, 155, 150, or 114 of SEQ ID NO:1) may comprise a nucleotide sequence selected from the group of SEQ ID NO: 2, 72, 73, 74, 75, 76 and 77.

[0013] Also provided is a plant expression system comprising a nucleic acid sequence comprising a regulatory region, operatively linked with the expression enhancer CPMVX, CPMVX+, as defined above, the expression enhancer operatively linked with a nucleotide sequence of interest. The plant expression system may further comprising a comovirus 3' UTR. The plant expression system may further comprise a second nucleic acid sequence encoding a suppressor of silencing, for example HcPro or p19.

[0014] The nucleotide sequence of interest of the plant expression system as defined above may encodes a viral protein or an antibody. For example, the viral protein may be an influenza hemagglutinin and may be s selected from the group of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, and an influenza type B hemagglutinin. The nucleotide sequence encoding the viral protein or the antibody may comprise a native signal peptide sequence, or a non-native signal peptide, for example the non-native signal peptide may be obtained from Protein disulfide isomerase (PDI).

[0015] As described herein there is provided a method of producing a protein of interest in a plant or in a portion of a plant comprising, introducing into the plant or in the portion of a plant the plant expression system comprising CPMVX or CPMVX+, as defined above, and incubating the plant or the portion of a plant under conditions that permit expression of the nucleotide sequence encoding the protein of interest.

[0016] The present invention also provides a plant or portion of a plant transiently transfected or stably transformed with the plant expression system as described above.

[0017] Plant-based expression systems as described herein result in increasing or enhancing expression of a nucleotide sequence encoding a heterologous open reading frame that is operatively linked to the expression enhancer, either CPMVX, or CPMVX+ as defined herein. The increase in expression may be determined by comparing the level of expression obtained using the CPMVX based, or CPMVX+ based expression enhancers with the level of expression of the same nucleotide sequence encoding the heterologous open reading frame operatively linked to the prior art enhancer sequence (CPMV HT) comprising an incomplete M protein (as described in Sainsbury F., and Lomonossoff G.P., 2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference). An example of a prior art CPMV HT sequence is provided in SEQ ID NO:4.

[0018] The plant based expression systems as described herein may also have a number of properties such as, for example, containing convenient cloning sites for genes or nucleotide sequences of interest, may easily infect plants in a cost-effective manner, may cause efficient local or systemic infection of inoculated plants. In addition, the infection should provide a good yield of useful protein material.

[0019] This summary of the invention does not necessarily describe all features of the invention.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0020] 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:

[0021] **FIGURE 1A** shows a general schematic of an example of several enhancer sequences, CPMVX, and CPMVX+ (comprising CPMVX, and a stuffer fragment, which in this non-limiting example, comprises a multiple cloning site and plant kozak sequence), as described herein. CPMCX 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+. **FIGURE 1B** shows examples of several variants of constructs comprising enhancer sequences, as described herein (CPMV160, complete sequence provided as SEQ ID NO:1; CPMV155, complete sequence provided as SEQ ID NO:24; CPMV150, complete sequence provided as SEQ ID NO:27; and CPMV114, complete sequence provided as SEQ ID NO:68), operatively linked to plant regulatory region (in these non-limiting examples 2X35S) at their 5'ends, and at their 3' ends, a nucleotide sequence of interest, or "GOI", which includes a plant kozak sequence adjacent to the ATG initiation site (elements shown within the square brackets are include for context, and they are not part of the CPMVX or CPMVX+ enhancer sequences). **FIGURE 1C** shows examples of several variants of constructs comprising enhancer sequences, as described herein (CPMV160+, complete sequence provided as SEQ ID NO:2; CPMV155+, complete sequence provided as SEQ ID NO:72; CPMV150+, complete sequence provided as SEQ ID NO:73; and CPMV114+, complete sequence provided as SEQ ID NO:74), operatively linked to plant regulatory region (in these non-limiting examples 2X35S) at their 5'ends, and at their 3' ends, a stuffer fragment (in these non-limiting examples, comprising a multiple cloning site and plant kozak sequence), a nucleotide sequence of interest, "GOI" comprising an ATG initiation site (elements shown within the square brackets are include for context, and they are not part of the CPMVX or CPMVX+ enhancer sequences).

[0022] **FIGURE 2** shows the relative hemagglutination titre (HMG) in crude protein extracts of proteins produced in plants comprising CPMV-HT (prior art) expression constructs, and CPMV160+ based expression constructs, operatively linked with a nucleotide sequence of interest. Data for the expression of HA from H1 A/California/07/2009 with a PDI signal peptide (PDI-H1 Cal; construct number 484 5' UTR: CPMV HT; and construct number 1897, 5'UTR: CPMV160+; see Example 5), H3 A/Victoria/361/2011 with a PDI signal peptide (PDI-H3 Vic; construct number 1391, 5'UTR: CPMV HT; and construct number 1800, 5'UTR: CMPV160+; see Examples 1 and 2, respectively), H5 from Influenza A/Indonesia/5/2005 with a native signal peptide (WtSp-H5 Indo; construct number 489, 5'UTR: CMPV HT; and construct number 1880, 5'UTR: CMPV160+; see Example 6), and

B/Wisconsin/1/2010 with deleted proteolytic loop and with a native signal peptide (WtSp-B Wis-PrL; construct number 1445, 5'UTR: CMPV HT; and construct number 1975, 5'UTR: CMPV160+, see Example 13) are shown. PDI: Protein disulfide isomerase signal peptide; PrL-: deleted proteolytic loop.

[0023] **FIGURE 3** shows the relative hemagglutination titres (HMG) in crude protein extracts of proteins produced in plants comprising CPMV-HT (prior art) expression constructs, and CPMV160+ based expression constructs. Data for the expression of HA from H1 A/California/07/2009 with a PDI signal peptide (construct number 484, 5'UTR: CMPV HT; and construct number 1897 5'UTR: CMPV160+, see Example 5), H3 A/Victoria/361/2011 with a PDI signal peptide (construct number 1391, 5'UTR: CMPV HT; and construct number 1800 5'UTR: CMPV160+, see Examples 1 and 2, respectively), B Brisbane/60/08 with deleted proteolytic loop and with a PDI signal peptide (construct number 1039, 5'UTR: CMPV HT; and construct number 1937, 5'UTR: CMPV160+; see Example 9), B Brisbane/60/08+H1Tm, with deleted proteolytic loop, with transmembrane domain and cytoplasmic tail replaced by those of H1 A/California/07/2009, and with a PDI signal peptide (construct number 1067, 5'UTR: CMPV HT; and construct number 1977, 5'UTR: CMPV160+; see Example 10), B Massachusetts/2/2012 2012 with deleted proteolytic loop and with a PDI signal peptide (construct number 2072, 5'UTR: CMPV HT; and construct number 2050, 5'UTR: CMPV160+; see Example 11), B Massachusetts/2/2012+H1Tm with deleted proteolytic loop, with transmembrane domain and cytoplasmic tail replaced by those of H1 A/California/07/2009 and with a PDI signal peptide (construct number 2074, 5'UTR: CMPV HT; and construct number 2060, 5'UTR: CMPV160+; see Example 12), B Wisconsin/1/2010 with deleted proteolytic loop and with the native signal peptide (construct number 1445, 5'UTR: CMPV HT; and construct number 1975, 5'UTR: CMPV160+; see Example 13), and B Wisconsin/1/2010+H1Tm with deleted proteolytic loop, with transmembrane domain and cytoplasmic tail replaced by those of H1 A/California/07/2009 and with the native signal peptide (construct number 1454, 5'UTR: CMPV HT; and construct number 1893, 5'UTR: CMPV160+; see Example 14), are shown.

[0024] **FIGURE 4A** shows examples of variants of plant Kozak sequences tested. Constructs showing a partial sequence of the CPMVX+, a plant regulatory region, a

stuffer fragment, and a nucleotide sequence of interest (GOI). In this non-limiting example, the construct comprises a 2X35S regulatory region, CPMV160+, a stuffer fragment comprising a multiple cloning site and a plant kozak sequence (the 5'end of a nucleotide sequence of interest is also indicated: "ATG...GOI"; where the GOI is H3 A/Victoria/361). Variants of plant kozak sequences are also shown below the sequence (also see Figure 9). Each variant plant Kozak sequence was fused to the 3' end of the stuffer fragment, and to the 5'-ATG site of the nucleotide sequence of interest (in these non-limiting examples, H3 A/Victoria/361). The other elements of the constructs remained the same). **FIGURE 4B** shows HA titers of a nucleotide sequence of interest produced in plants comprising CMPV160+ expression construct and a variant plant Kozak sequence as indicated.

[0025] **FIGURE 5** shows the expression of the antibody rituximab (Rituxan) under the control of CPMV-HT (construct numbers 5001 and 5002, see examples 15 and 16) or CPMV160 (construct numbers 2100 and 2109, see example 15 and 16) and with either its native signal peptide or the native signal peptide replaced with the signal peptide of Protein disulfide isomerase (PDI).

[0026] **FIGURE 6** shows the sequence components used to prepare construct number 1391(A-2X35S CPMV-HT PDISP H3Victoria NOS; see example 1). Construct number 1391 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/H3 Victoria)). PDISP: protein disulfide isomerase signal peptide. NOS: nopaline synthase terminator. **FIGURE 6A** shows primer sequence IF-PDI.S1=3c (SEQ ID NO:67). **FIGURE 6B** shows primer sequence IF-H3V36111.s1-4r (SEQ ID NO:17). **FIGURE 6C** shows the sequence of PDISP/H3 Victoria (SEQ ID NO:18). **FIGURE 6D** shows a schematic representation of construct 1191. **FIGURE 6E** shows construct 1191; from left to right t-DNA borders (underlined), 2X35S CPMV-HT NOS, with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette (SEQ ID NO:19). **FIGURE 6F** shows expression cassette number 1391 from 2X35S promoter to NOS terminator. The PDISP/H3 Victoria nucleotide sequence is underlined; CPMV 5'UTR in bold; incomplete M-protein in italics (SEQ ID NO:20). **FIGURE 6G** shows the amino acid

sequence of PDISP/H3 Victoria (SEQ ID NO:21). **FIGURE 6H** shows a schematic representation of construct number 1391 (a reference construct).

[0027] **FIGURE 7** shows the sequence components used to prepare construct number 1800 (A-2X35S CPMV160+ PDISP H3Victoria NOS; see example 2). Construct number 1800 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide. NOS: nopaline synthase terminator. **FIGURE 7A** shows primer sequence IF**(SacII)-PDI.s1+4c (SEQ ID NO:22). **FIGURE 7B** shows primer sequence IF-H3V36111.s1-4r (SEQ ID NO:23). The sequence of PDISP/H3 Victoria is shown in Figure 6C (SEQ ID NO:18). **FIGURE 7C** shows a schematic representation of construct 2171 (SacII and StuI restriction enzyme sites used for plasmid linearization are indicated). **FIGURE 7D** shows construct 2171 from left to right t-DNA borders (underlined), 2X35S/CPMV160+/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette, an H1 California transmembrane cytoplasmic tail, and the CPMV3'UTR (SEQ ID NO:25). **FIGURE 7E** shows expression cassette number 1800 from 2X35S promoter to NOS terminator. PDISP/H3 Victoria nucleotide sequence is underlined; 5'UTR is shown in bold; plant kozak sequence double underline; a stuffer fragment (multiple cloning site) of 16 base pairs is positioned between the 5'UTR and plant kozak sequence (SEQ ID NO:26). The amino acid sequence of PDISP/H3 Victoria is shown in Figure 6G (SEQ ID NO:27). **FIGURE 7F** shows a schematic representation of construct number 1800 (a CPMVX+ based construct, where X=160).

[0028] **FIGURE 8** shows the sequence components used to prepare construct number 1935 (2X35S/CPMV160/ PDISP/H3 Victoria/ NOS; see example 3). Construct number 1935 includes a CPMV 5'UTR comprising 160 nucleotides, and does not include a stuffer fragment (multiple cloning site), or a plant kozak sequence (this construct also does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160 (CPMVX, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide. NOS: nopaline synthase terminator. **FIGURE 8A** shows primer sequence IF-CPMV(fl5'UTR)_SpPDI.c (SEQ ID NO:28).

**FIGURE 8B** shows a schematic representation of construct 1190. **FIGURE 8C** shows the nucleic acid sequence of construct 1190 from left to right t-DNA borders (underlined), 2X35S/CPMV160/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette, and a CPMV3'UTR (SEQ ID NO:29). **FIGURE 8D** shows expression cassette number 1935 from 2X35S promoter to NOS terminator. PDISP/H3 Victoria nucleotide sequence is underlined, 5'UTR is shown in bold (SEQ ID NO:30). This cassette does not include a plant kozak sequence or a stuffer fragment (multiple cloning site). **FIGURE 8E** shows a schematic representation of construct number 1935 (a CPMVX based construct, where X=160).

[0029] **FIGURE 9** shows sequences comprising variations in a plant kozak sequence used to prepare a selection of "CPMV160+" based constructs (constructs number 1992 to 1999). Variation of sequence between SacII restriction site and ATG of PDISP/H3 Victoria in 2X35S/CPMV160+/NOS expression system, comprising variations in a plant kozak sequence are shown (the sequences are shown as variations from the corresponding sequence from construct 1800; see Example 4). The variant plant kozak sequence are underlined. PDISP: protein disulfide isomerase signal peptide. **FIGURE 9A** shows the nucleotide sequence of IF-HT1*(-Mprot)-PDI.c (SEQ ID NO: 31; used to prepare construct number 1992). **FIGURE 9B** shows the nucleotide sequence of IF-HT2*(-Mprot)-PDI.c (SEQ ID NO:32; used to prepare construct number 1993). **FIGURE 9C** shows the nucleotide sequence of IF-HT3*(-Mprot)-PDI.c (SEQ ID NO:33; used to prepare construct number 1994). **FIGURE 9D** shows the nucleotide sequence of IF-HT4*(-Mprot)-PDI.c (SEQ ID NO:34; used to prepare construct number 1995). **FIGURE 9E** shows the nucleotide sequence of IF-HT5*(-Mprot)-PDI.c (SEQ ID NO:35; used to prepare construct number 1996). **FIGURE 9F** shows the nucleotide sequence of IF-HT6*(-Mprot)-PDI.c (SEQ ID NO:36 used to prepare construct number 1997). **FIGURE 9G** shows the nucleotide sequence of IF-HT7*(-Mprot)-PDI.c (SEQ ID NO:37; used to prepare construct number 1998). **FIGURE 9H** shows the nucleotide sequence of IF-HT8*(-Mprot)-PDI.c (SEQ ID NO:38; used to prepare construct number 1999). **FIGURE 9I** shows a schematic representation of construct number 1992 comprising a plant kozak sequence (Kozak1) using SEQ ID NO:31 (FIGURE 9A). Constructs 1993 -1999 comprise the same features as construct 1992, except that each construct (1993-1999)

comprises a modified plant Kozak sequence (Kozak1) as shown in Figures 9B to 9H (SEQ ID NOs: 32 to 38), respectively.

[0030] **FIGURE 10** shows sequence components used to prepare construct numbers 484 and 1897 (2X35S/CPMV HT PDISP/H1 California NOS and 2X35S/CPMV160+ PDISP/H1 California NOS, respectively; see Example 5). Construct number 484 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/H1 California). Construct number 1897 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide. NOS: nopaline synthase terminator. **FIGURE 10A** shows the nucleotide sequence of PDISP/H1 California (SEQ ID NO: 39). **FIGURE 10B** shows the amino acid sequence of PDISP/H1 California (SEQ ID NO: 40). **FIGURE 10C** shows a schematic representation of construct number 484 (2X35S/CPMV HT; reference construct). **FIGURE 10D** shows a schematic representation of construct number 1897 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0031] **FIGURE 11** shows sequence components used to prepare construct numbers 489, 1880 and 1885 (2X35S/CPMV HT H5 Indonesia NOS; CPMV160+ H5 Indonesia NOS, and CPMV160 H5 Indonesia NOS, respectively; see Example 6). Construct number 489 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/H1 California). Construct number 1880 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. Construct number 1885 includes a CPMV 5'UTR comprising 160 nucleotides, and does not include a stuffer fragment (multiple cloning site), or a plant kozak sequence (this construct also does not

comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160 (CPMVX, where X=160) based construct. NOS: nopaline synthase terminator. **FIGURE 11A** shows the nucleotide sequence of native H5 Indonesia (SEQ ID NO: 41). **FIGURE 11B** shows the amino acid sequence of native H5 Indonesia (SEQ ID NO: 42). **FIGURE 11C** shows a schematic representation of construct number 489 (2X35S/CPMV HT; reference constrcut). **FIGURE 11D** shows a schematic representation of construct number 1880 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160). **FIGURE 11E** shows a schematic representation of construct number 1885 (2X35S/CPMV160, a CPMVX based construct, where X=160).

[0032] **FIGURE 12** shows sequence components used to prepare construct numbers 1240 and 2168 (2X35S/CPMV HT PDISP/H7 Hangzhou NOS and 2X35S/CPMV160+ PDISP/H7 Hangzhou NOS, respectively; see Example 7). Construct number 1240 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/H7 Hangzhou). Construct number 1897 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide. NOS: nopaline synthase terminator. **FIGURE 12A** shows the nucleotide sequence of PDISP/H7 Hangzhou (SEQ ID NO: 43). **FIGURE 12B** shows the amino acid sequence of PDISP/H7 Hangzhou (SEQ ID NO: 44). **FIGURE 12C** shows a schematic representation of construct number 2140 (2X35S/CPMV HT; reference construct). **FIGURE 12D** shows a schematic representation of construct number 2168 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0033] **FIGURE 13** shows sequence components used to prepare construct numbers 2130 and 2188 (2X35S/CPMV HT PDISP/H7 Hangzhou+H5 Indonesia TMCT NOS and 2X35S/CPMV160+ PDISP/H7 Hangzhou+H5 Indonesia TMCT NOS, respectively; see Example 8). Construct number 2130 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a

sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/H7 Hangzhou+H5 Indonesia TMCT). Construct number 1897 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide; NOS: nopaline synthase terminator; TMCT: transmembrane domain cytoplasmic tail. **FIGURE 13A** shows the nucleotide sequence of PDISP/H7 Hangzhou+H5 Indonesia TMCT (SEQ ID NO: 45). **FIGURE 13B** shows the amino acid sequence of PDISP/H7 Hangzhou+H5 Indonesia TMCT (SEQ ID NO: 46). **FIGURE 13C** shows a schematic representation of construct number 2130 (2X35S/CPMV HT; reference construct). **FIGURE 13D** shows a schematic representation of construct number 2188 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0034] **FIGURE 14** shows sequence components used to prepare construct numbers 1039 and 1937 (2X35S/CPMV HT PDISP/HA B Brisbane (PrL-) NOS and 2X35S/CPMV160+ PDISP/HA B Brisbane (PrL-) NOS, respectively; see Example 9). Construct number 1039 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/HA B Brisbane (PrL-)). Construct number 1937 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide; NOS: nopaline synthase terminator; PrL-: deleted proteolytic loop. **FIGURE 14A** shows the nucleotide sequence of PDISP/HA B Brisbane (PrL-) (SEQ ID NO: 47). **FIGURE 14B** shows the amino acid sequence of PDISP/HA B Brisbane (PrL-) (SEQ ID NO: 48). **FIGURE 14C** shows a schematic representation of construct number 1039 (2X35S/CPMV HT; reference construct). **FIGURE 14D** shows a schematic representation of construct number 1937 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0035] **FIGURE 15** shows sequence components used to prepare construct numbers 1067 and 1977 (2X35S/CPMV HT PDISP/HA B Brisbane (Prl-)+H1 California TMCT NOS and 2X35S/CPMV160+ PDISP/HA B Brisbane (PrL-)+H1 California TMCT NOS, respectively; see Example 10). Construct number 1067 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/HA B Brisbane (PrL-)+H1 California TMCT). Construct number 1977 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide; NOS: nopaline synthase terminator; PrL-: deleted proteolytic loop; TMCT: transmembrane domain cytoplasmic tail. **FIGURE 15A** shows the nucleotide sequence of PDISP/HA B Brisbane (PrL-)+H1 California TMCT (SEQ ID NO: 49). **FIGURE 15B** shows the amino acid sequence of PDISP/HA B Brisbane (PrL-)+H1 California TMCT (SEQ ID NO: 50). **FIGURE 15C** shows a schematic representation of construct number 1067 (2X35S/CPMV HT; reference construct). **FIGURE 15D** shows a schematic representation of construct number 1977 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0036] **FIGURE 16** shows sequence components used to prepare construct numbers 2072 and 2050 (2X35S/CPMV HT PDISP/HA B Massachusetts (PrL-) NOS and 2X35S/CPMV160+ PDISP/HA B Massachusetts (PrL-) NOS, respectively; see Example 11). Construct number 2072 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/HA B Massachusetts (PrL-)). Construct number 2050 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide; NOS: nopaline synthase terminator; PrL-:

deleted proteolytic loop. **FIGURE 16A** shows the nucleotide sequence of PDISP/HA B Massachusetts (PrL-) (SEQ ID NO: 51). **FIGURE 16B** shows the amino acid sequence of PDISP/HA B Massachusetts (PrL-) (SEQ ID NO: 52). **FIGURE 16C** shows a schematic representation of construct number 2072 (2X35S/CPMV HT; reference construct). **FIGURE 16D** shows a schematic representation of construct number 2050 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0037] **FIGURE 17** shows sequence components used to prepare construct numbers 2074 and 2060 (2X35S/CPMV HT PDISP/HA B Massachusetts (PrL-)+H1 California TMCT NOS and 2X35S/CPMV160+ PDISP/HA B Massachusetts (PrL-)+H1 California TMCT NOS, respectively; see Example 12). Construct number 2074 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/HA B Massachusetts (PrL-)+H1 California TMCT). Construct number 2060 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide; NOS: nopaline synthase terminator; PrL-: deleted proteolytic loop; TMCT: transmembrane domain cytoplasmic tail. **FIGURE 17A** shows the nucleotide sequence of PDISP/HA B Massachusetts (PrL-)+H1 California TMCT (SEQ ID NO: 53). **FIGURE 17B** shows the amino acid sequence of PDISP/HA B Massachusetts (PrL-)+H1 California TMCT (SEQ ID NO: 54). **FIGURE 17C** shows a schematic representation of construct number 2074 (2X35S/CPMV HT; reference construct). **FIGURE 17D** shows a schematic representation of construct number 2060 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0038] **FIGURE 18** shows sequence components used to prepare construct numbers 1445, 1820 and 1975 (2X35S/CPMV HT HA B Wisconsin (PrL-) NOS, 2X35S/CPMV160+ HA B Wisconsin (PrL-) NOS and 2X35S/CPMV160 HA B Wisconsin (PrL-) NOS, respectively; see Example 13). Construct number 1445

incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (HA B Wisconsin (PrL-)). Construct number 1820 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. Construct number 1975 includes a CPMV 5'UTR comprising 160 nucleotides, and does not include a stuffer fragment (multiple cloning site), or a plant kozak sequence (this construct also does not comprise a sequence encoding an incomplete M protein) and is an example of a "CPMV160" (CPMVX) based construct. PrL-: deleted proteolytic loop; NOS: nopaline synthase terminator. **FIGURE 18A** shows the nucleotide sequence of HA B Wisconsin (PrL-) (SEQ ID NO: 55). **FIGURE 18B** shows the amino acid sequence of HA B Wisconsin (PrL-) (SEQ ID NO: 56). **FIGURE 18C** shows a schematic representation of construct number 1445 (2X35S/CPMV HT; reference construct). **FIGURE 18D** shows a schematic representation of construct number 1820 (2X35S/CPMV160+; a CPMVX+ based construct). **FIGURE 18E** shows a schematic representation of construct number 1975 (2X35S/CPMV160; a CPMVX based construct, where X=160).

[0039] **FIGURE 19** shows sequence components used to prepare construct numbers 1454 and 1893 (2X35S/CPMV HT HA B Wisconsin (PrL-)+H1 California TMCT NOS and 2X35S/CPMV160+ HA B Wisconsin (PrL-)+H1 California TMCT NOS, respectively; see Example 14). Construct number 1454 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (HA B Wisconsin (PrL-)+H1 California TMCT). Construct number 1893 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. NOS: nopaline synthase terminator; PrL-: deleted proteolytic loop; TMCT: transmembrane domain cytoplasmic tail. **FIGURE 19A**

shows the nucleotide sequence of HA B Wisconsin (PrL-)+H1 California TMCT (SEQ ID NO: 57). **FIGURE 19B** shows the amino acid sequence of PDISP/HA B Wisconsin (PrL-)+H1 California TMCT (SEQ ID NO: 58). **FIGURE 19C** shows a schematic representation of construct number 1454 (2X35S/CPMV HT; reference construct). **FIGURE 19D** shows a schematic representation of construct number 1893 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0040] **FIGURE 20** shows sequence components used to prepare construct numbers 5001 and 2100 (2X35S/CPMV HT HC rituximab (Rituxan) NOS and 2X35S/CPMV160+ HC rituximab (Rituxan) NOS, respectively; see Example 15). Construct number 5001 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (HC rituximab (Rituxan)). Construct number 2100 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. HC: heavy chain; NOS: nopaline synthase terminator. **FIGURE 20A** shows the nucleotide sequence of HC rituximab (Rituxan; SEQ ID NO: 59). **FIGURE 20B** shows the amino acid sequence of HC rituximab (Rituxan; SEQ ID NO: 60). **FIGURE 20C** shows a schematic representation of construct number 5001 (2X35S/CPMV HT; reference construct). **FIGURE 20D** shows a schematic representation of construct number 2100 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0041] **FIGURE 21** shows sequence components used to prepare construct numbers 5002 and 2109 (2X35S/CPMV HT PDISP/HC rituximab (Rituxan) NOS and 2X35S/CPMV160+ PDISP/HC rituximab (Rituxan) NOS, respectively; see Example 16). Construct number 5001 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (PDISP/HC Rituzan). Construct number 2100 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not

comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide; HC: heavy chain; NOS: nopaline synthase terminator. **FIGURE 21A** shows the nucleotide sequence of PDISP/HC rituximab (Rituxan; SEQ ID NO: 61). **FIGURE 21B** shows the amino acid sequence of PSISP/HC rituximab (Rituxan; SEQ ID NO: 62). **FIGURE 21C** shows a schematic representation of construct number 5002 (2X35S/CPMV HT; reference construct). **FIGURE 21D** shows a schematic representation of construct number 2109 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0042] **FIGURE 22** shows sequence components used to prepare construct numbers 5021 and 2120 (2X35S/CPMV HT LC rituximab (Rituxan) NOS and 2X35S/CPMV160+ LC rituximab (Rituxan) NOS, respectively; see Example 17). Construct number 5021 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between the 5'UTR and the nucleotide sequence of interest (LC rituximab (Rituxan)). Construct number 2120 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. LC: light chain; NOS: nopaline synthase terminator. **FIGURE 22A** shows the nucleotide sequence of LC rituximab (Rituxan; SEQ ID NO: 63). **FIGURE 22B** shows the amino acid sequence of LC rituximab (Rituxan; SEQ ID NO: 64). **FIGURE 22C** shows a schematic representation of construct number 5021 (2X35S/CPMV HT; reference construct). **FIGURE 22D** shows a schematic representation of construct number 2120 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

[0043] **FIGURE 23** shows sequence components used to prepare construct numbers 5022 and 2129 (2X35S/CPMV HT PDISP/LC rituximab (Rituxan) NOS and 2X35S/CPMV160+ PDISP/LC rituximab (Rituxan) NOS, respectively; see Example 18). Construct number 5001 incorporates a prior art CPMV-HT sequence (CMPV 5'UTR with mutated start codon at position 161 fused to a sequence encoding an incomplete M protein) and does not comprise a heterologous kozak sequence between

the 5'UTR and the nucleotide sequence of interest (PDISP/LC rituximab (Rituxan)). Construct number 2100 includes a CPMV 5'UTR comprising 160 nucleotides, a stuffer fragment (multiple cloning site), and a plant kozak sequence (this construct does not comprise a sequence encoding an incomplete M protein) and is an example of a CPMV160+ (CPMVX+, where X=160) based construct. PDISP: protein disulfide isomerase signal peptide; HC: heavy chain; NOS: nopaline synthase terminator. **FIGURE 23A** shows the nucleotide sequence of PDISP/LC rituximab (Rituxan; SEQ ID NO: 65). **FIGURE 23B** shows the amino acid sequence of PSISP/LC rituximab (Rituxan; SEQ ID NO: 66). **FIGURE 23C** shows a schematic representation of construct number 5022 (2X35S/CPMV HT; reference construct). **FIGURE 23D** shows a schematic representation of construct number 2129 (2X35S/CPMV160+; a CPMVX+ based construct, where X=160).

**DETAILED DESCRIPTION**

[0044] The present invention relates to the expression of proteins of interest in plants. The present invention also provides methods and compositions for the production of proteins of interest in plants.

[0045] In the description that follows, a number of terms are used extensively, the following definitions are provided to facilitate understanding of various aspects of the invention. Use of examples in the specification, including examples of terms, is for illustrative purposes only and is not intended to limit the scope and meaning of the embodiments of the invention herein.

[0046] As used herein, the use of the word "a" or "an" when used herein in conjunction with the term "comprising" may mean "one," but it is also consistent with the meaning of "one or more," "at least one" and "one or more than one". The term "about" refers to an approximately +/-10% variation from a given value. The term "plurality", means more than one, for example, two or more, three or more, four or more, and the like.

[0047] The present invention provides an expression enhancer comprising a CPMV 5' untranslated region (UTR), "CPMVX", comprising 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. This expression enhancer is generally referred to as CPMVX (see Figure 1A).

[0048] 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.

[0049] If the CMPVX sequence comprises a stuffer fragment, then this expression enhancer may be referred to as CPMVX+ (see Figure 1A), 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.

[0050] 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 SEQ ID NO:4. 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.

[0051] 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 1A), in order to drive expression of the nucleotide sequence of interest within a plant host.

[0052] Expression systems to produce one or more proteins of interest in a plant using either CMPVX or CPMVX+ are also provided. The expression systems described herein comprise an expression cassette comprising CPMVX, or a sequence that comprises 80% sequence similarity with CPMVX, and optionally, a stuffer sequence fused to CMPVX (CPMVX+). The expression cassette comprising CMPVX or CMPVX+, may further comprise a regulatory region that is active in a plant that is operatively linked to the 5'end of the expression enhancer. A nucleotide sequence of interest may be operatively linked to the 3'end of the expression cassette so that when introduced within a plant, expression of the nucleotide sequence of interest within a plant host is achieved.

[0053] Plant cells, plant tissues, whole plants, inoculum, nucleic acids, constructs comprising nucleotide sequences of interest encoding proteins of interest, expression cassettes or expression systems comprising CPMVX or CPMVX+ as described herein, and methods of expressing a protein of interest in plants are also provided.

[0054] With reference to Figures 1A, 1B and 1C, non-limiting examples of an expression enhancer comprising a CPMV 5' UTR (CPMVX) sequence comprising nucleotides from X of SEQ ID NO:1, where X=160, 155, 150, or 114 of SEQ ID NO:1 are provided. The expression enhancer CMPVX may also be referred to as

CPMV160; CPMV155; CPMV150; CPMV114, when X-160, 155, 150, or 114, respectively.

[0055] The nucleotide sequence of interest may be fused (operatively linked) to the enhancer sequence comprising a plant regulatory region, using a variety of approaches. For example, which are not to be considered limiting:

1) A nucleotide sequence of interest encoding a protein of interest may be fused to the 3' end of the expression enhancer immediately after the 5'UTR sequence, for example CPMVX, where X=160, 155, 150, 114 nucleotides of SEQ ID NO:1. In this example, the nucleotide sequence of interest is fused to the 5'UTR without a multiple cloning site, and the nucleotide sequence of interest may include at its 5' end a plant kozak sequence immediately upstream from an ATG initiation site of the nucleotide sequence of interest (see Figure 1B). If X=160 (i.e. CPMV160), then a nucleotide sequence of interest that is operatively linked to CPMV160 may not require a plant kozak sequence fused to its 5' end, as nucleotides 150–160, or 155-160, of SEQ ID NO:1 comprise a kozak-like sequence. However, a plant kozak sequence may be included in constructs comprising CPMV160 if desired (see Figure 1B: "+/- plant kozak"). If X-155, 150, or 114, then including a plant kozak sequence that is fused to the 5'end of the nucleotide sequence of interest in constructs comprising CPMV155, CPMV150, or CPMV114 is recommended for optimal expression of the nucleotide sequence of interest.

2) The nucleotide sequence of interest, may be fused to a CMPVX+ expression enhancer (where X=160, 155, 150, 114 of SEQ ID NO:1) that comprises a plant kozak sequence at the 3' end of the expression enhancer, so that the nucleotide sequence of interest is positioned immediately after the plank kozak sequence. In this example, the nucleotide sequence of interest that is fused to CPMVX+ would not include a multiple cloning site or plant kozak sequence (the resulting construct would .be analogous to those as presented in Figure 1B).

3) The nucleotide sequence of interest may be fused to a CPMVX+ expression enhancer (where X=160, 155, 150, 114 of SEQ ID NO:1), comprising a multiple cloning site (MCS) at the 3'end of the expression enhancer, using the multiple cloning site. In this example, the nucleotide sequence of interest may include at its 5' end a

corresponding sequence to permit fusion with the multiple cloning sire of the expression enhancer, and a plant kozak sequence immediately upstream from the ATG initiation site of the nucleotide sequence of interest (see figure 1C).

[0056] The overall result using any of the above methods, is a construct (or expression cassette) comprising a plant regulatory region in operative association (operatively linked) with a CPMV 5'UTR sequence comprising nucleotides X, where X=160, 155, 150, 114 of SEQ ID NO:1 (or an enhancer sequence that comprises 80% sequence similarity with CPMV 5'UTR sequence), the 3' end of the CPMV 5'UTR sequence is fused to the 5'end of a plant kozak sequence, the 3' end of the plant kozak sequence fused and adjacent to the 5' end of the nucleotide sequence of interest comprising an ATG initiation sequence. The construct may, or may not, comprise a multiple cloning site located between the 5'UTR and the plant kozak sequence. The construct may further comprise a 3' untranslated region (UTR) sequence, for example, a comovirus 3'UTR, or a plastocyanin 3' UTR, and a terminator sequence, for example a NOS terminator, operatively linked to the 3'end of the nucleotide sequence of interest (see Figure 1A).

[0057] A plant expression system comprising a nucleic acid comprising a regulatory region, operatively linked with one or more than one expression enhancer as described herein (e.g. CPMVX), and a nucleotide sequence of interest. is also provided. Furthermore, a nucleic acid comprising a promoter (regulatory region) sequence, operatively linked with an expression enhancer comprising a CPMV 5'UTR and a modified or deleted stuffer sequence (e.g. CPMVX+) and a nucleotide sequence of interest is described. The nucleic acid may further comprise a sequence encoding a 3'UTR, for example a comovirus 3' UTR, or a plastocyanin 3' UTR, and a terminator sequence, for example a NOS terminator, so that the nucleotide sequence of interest is inserted upstream from the 3'UTR.

[0058] By "operatively linked" it is meant that the particular sequences interact either directly or indirectly to carry out an intended function, such as mediation or modulation of expression of a nucleic acid sequence. The interaction of operatively linked sequences may, for example, be mediated by proteins that interact with the operatively linked sequences.

[0059] "Expression enhancer(s)", "enhancer sequence(s)" or "enhancer element(s)", as referred to herein, include sequences derived from, or that share sequence similarity with, portions of the CPMV 5'UTR from the RNA-2 genome segment. An enhancer sequence can enhance expression of a downstream heterologous open reading frame (ORF) to which they are attached.

[0060] The term "5'UTR" or "5' untranslated region" or "5' leader sequence" refers to regions of an mRNA that are not translated. The 5'UTR typically begins at the transcription start site and ends just before the translation initiation site or start codon (usually AUG in an mRNA, ATG in a DNA sequence) of the coding region. The length of the 5'UTR may be modified by mutation for example substitution, deletion or insertion of the 5'UTR. The 5'UTR may be further modified by mutating a naturally occurring start codon or translation initiation site such that the codon no longer functions as start codon and translation may initiate at an alternate initiation site.

[0061] 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).

[0062] The expression enhancer may comprise a CPMV 5' untranslated region (UTR) comprising nucleotides from X of SEQ ID NO:1, where X=160, 155, 150, or 114 of SEQ ID NO:1 (CPMVX), or a sequence that comprises 80% sequence similarity with CPMVX (where X=160, 155, 150, or 114 of SEQ ID NO:1; see Figures 1A and 1B) and exhibits the property of enhancing expression of a nucleotide sequence encoding a heterologous open reading frame that is operatively linked to the expression enhancer, when compared to the expression of the same nucleotide sequence

encoding a heterologous open reading frame operatively linked to the prior art CPMV HT enhancer sequence comprising an incomplete M protein (as described in Sainsbury F., and Lomonossoff G.P., 2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference).

[0063] The CPMVX enhancer sequence may also be fused to a stuffer sequence, for example a multiple cloning site (MCS), or an MCS linked to a plant kozak sequence, wherein the CMPVX comprises nucleotides from X of SEQ ID NO:1, where X=160, 155, 150, or 114 of SEQ ID NO:1, or a sequence that comprises 80% sequence similarity with CPMVX (where X=160, 155, 150, or 114 of SEQ ID NO:1), and exhibits the property of enhancing the expression of nucleotide sequence encoding a heterologous open reading frame operatively linked to the expression enhancer, when compared to the expression of the same sequence encoding a heterologous open reading frame operatively linked to the prior art CPMV HT enhancer sequence comprising an incomplete M protein (as described in Sainsbury F., and Lomonossoff G.P., 2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference). The stuffer sequence comprises from 0-500 nucleotides fused to the 3' end of the CMPVX sequence. Preferably, the stuffer sequence comprises an multiple cloning site (MCS), or an MCS linked to a plant kozak sequence, and does not include an M protein. If the CMPVX sequence comprises a stuffer fragment (without an M protein), then this expression enhancer may be referred to as "CPMVX+" (see Figures 1A and 1C), as "CMPVX comprising a stuffer sequence and a plant kozak sequence", or as "CMPVX comprising an MCS along with a plant kozak sequence".

[0064] 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)
```

If the expression enhancer consists of nucleotide 1-160 of SEQ ID NO:1 (CPMV160), then a nucleotide sequence of interest with or without a 5'plant kozak sequence located at the 5' end adjacent to an initiation sequence (ATG), may be fused to the 3' end of the 5'UTR (after nucleotide 160 of SEQ ID NO:1), so that the overall construct

resembles that as shown in Figure 1B (CPMV160). The construct comprising CPMV160 may further comprise a regulatory region operatively linked to the 5'end of the expression enhancer, and a sequence encoding a 3'UTR, for example a comovirus 3' untranslated region (UTR) or a plastocyanin 3' UTR, and a terminator sequence, for example a NOS terminator, fused to the 3' end of the nucleotide sequence of interest. Without wishing to be bound by theory, CPMV160 may not require the addition of a plant kozak sequence to the 5' end of the nucleotide sequence of interest, since the sequence at positions 150-155, 155-160, or 150-160 of SEQ ID NO:1 may function as an active (native) kozak sequence in a plant. Construct number 1935 (see Example 3) and construct number 1885 (see Example 6) are examples of CPMV160 (CPMVX, where X=160 ) based constructs.

[0065] The expression enhancer may comprise CPMVX+, where X=160. A non-limiting example of such an enhancer is CPMV160+ (see figure 1C) comprising the sequence of SEQ ID NO:2 (5'UTR: nucleotide 1-160; multiple cloning site in italics nucleotides 161-176; plant kozak sequence in caps and bold, nucleotides 177-181):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca *gggcccaata ccgcgg*A***GAA***

181 ***A*** (SEQ ID NO:2)

[0066] Examples of constructs using SEQ ID NO:2 as an expression enhancer include constructs 1800, 1897, 1880, 2168, 2188, 1937, 1977, 2050, 2060, 1975, 1893, 2100, 2109, 2120, 2129 (see Examples 3, and 5-18, respectively).

[0067] As would be evident to one of skill in the art, any multiple cloning site (MCS), or an MCS of different length (either shorter or longer) may used in place of the sequence at nucleotides 161-176 of SEQ ID NO:2. Furthermore, the plant kozak sequence of SEQ ID NO:2 (shown at nucleotides 177-181) may be any plant kozak sequence, including but not limited, one of the sequences selected from SEQ ID NO's:5-17 (also see Figure 4A; the construct of Figure 4 includes SEQ ID NO:2, with variations of the plant kozak sequence as indicated, and comprises a plant regulatory region attached to the 5' end of the 5'UTR, and the transcription initiation site, ATG, of a nucleotide sequence of interest, located 3' to the plant kozak sequence).

[0068] The expression enhancer CPMVX, may include an "A" in position 115 (115A), so that CMPVX, 115A, where X=160, 155 or 150, comprises the sequence of the wild-type CPMV RNA2 genome (see WO 2009/087391, which is incorporated herein by reference, for the complete sequence of the wild type CPMV RNA-2 genome segment). An example of an expression enhancer CPMVX, 115A is "CPMV160, 115A", as defined by SEQ ID NO: 69 (the "A" is shown in bold and underline):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc**a**tgagc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca (SEQ ID NO:69)

[0069] The expression enhancer CPMVX+, may also include an "A" in position 115 (115A), so that CMPVX+, 115A, where X=160, 155 or 150, comprises the sequence of the wild-type CPMV RNA2 genome (WO 2009/087391, which is incorporated herein by reference). A non-limiting example of an expression enhancer CPMVX+, 115A is "CPMV160+, 115A", as defined by SEQ ID NO: 75 (the "A" is shown in bold and underline):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttg**ca**tgagc

121 gatcttcaac gttgtcagat cgtgcttcgg caccagtaca *gggcccaata ccgcgg*A***GAA***

181 ***A*** (SEQ ID NO:75)

[0070] As noted above for SEQ ID NO:2, any MCS, or an MCS of different length, may used in place of the MCS sequence of SEQ ID NO:75, and the plant kozak sequence may be any plant kozak sequence.

[0071] If the expression enhancer consists of nucleotide 1-155 of SEQ ID NO:1 (CPMV155):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc

121 gatcttcaac gttgtcagat cgtgcttcgg cacca (SEQ ID NO:24),

then a nucleotide sequence of interest with a plant kozak sequence located at the 5' end, adjacent an initiation sequence (ATG), may be fused to the 3' end of the 5'UTR (after nucleotide 155 of SEQ ID NO:1), so that the overall construct resembles that as shown in Figure 1B (CPMV155). The construct comprising CPMV155 may further comprise a regulatory region operatively linked to the 5'end of the expression enhancer, and a sequence encoding a 3'UTR, for example a comovirus 3' untranslated region (UTR) or a plastocyanin 3' UTR, and a terminator sequence, for example a NOS terminator, fused to the 3' end of the nucleotide sequence of interest. In this example, the nucleotide sequence of interest comprises a plant kozak sequence at its 5' end, since the native kozak sequence or a portion of this sequence (nucleotides 155-160 of SEQ ID NO:1), is removed.

[0072] The expression enhancer may comprise CPMV155+, comprising the sequence of SEQ ID NO:72 (5'UTR: nucleotide 1-155; multiple cloning site in italics nucleotides 156-171; plant kozak sequence in caps and bold, nucleotides 172-176):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc

121 gatcttcaac gttgtcagat cgtgcttcgg cacca*gggcc caataccgcg g***AGAAA**

(SEQ ID NO:72)

[0073] As noted above for CPMV160+ (SEQ ID NO:2), any MCS, including an MCS's of different length, may used in place of the MCS sequence of SEQ ID NO:72, and the plant kozak sequence may be any plant kozak sequence.

[0074] The expression enhancer CPMV155, may include an "A" in position 115 (115A), so that "CMPV155, 115A" comprises the sequence of the wild-type CPMV RNA2 genome (see WO 2009/087391, which is incorporated herein by reference), as defined by SEQ ID NO: 70 ("A" is bolded and underlined):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc**a**tgagc

121 gatcttcaac gttgtcagat cgtgcttcgg cacca (SEQ ID NO:70)

[0075] The expression enhancer CPMV155+, may also include an "A" in position 115 (115A), so that "CMPV155+, 115a" comprises the sequence of the wild-type

CPMV RNA2 genome (WO 2009/087391, which is incorporated herein by reference), as defined by SEQ ID NO: 76 (the "A" is shown in bold and underline):

```
1   tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc
61  ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcatgagc
121 gatcttcaac gttgtcagat cgtgcttcgg caccagggcc caataccgcg gAGAA
181 A (SEQ ID NO:76)
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[0076] As noted above for SEQ ID NO:2, any MCS, or an MCS of different length, may used in place of the MCS sequence of SEQ ID NO:76, and the plant kozak sequence may be any plant kozak sequence.

[0077] If the expression enhancer consists of nucleotide 1-150 of SEQ ID NO:1 (CPMV150):

```
1   tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc
61  ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc
121 gatcttcaac gttgtcagat cgtgcttcgg (SEQ ID NO:27),
```

then a nucleotide sequence of interest with a plant kozak sequence located at the 5' end, adjacent an initiation sequence (ATG), may be fused to the 3' end of the 5'UTR (after nucleotide 150 of SEQ ID NO:1), so that the overall construct resembles that as shown in Figure 1B (CPMV150). The construct comprising CPMV150 may further comprise a regulatory region operatively linked to the 5'end of the expression enhancer, and a sequence encoding a 3'UTR, for example a comovirus 3' untranslated region (UTR) or a plastocyanin 3' UTR, and a terminator sequence, for example a NOS terminator, fused to the 3' end of the nucleotide sequence of interest. In this example, the nucleotide sequence of interest comprises a plant kozak sequence at its 5' end, since the native kozak sequence at position 150-160 of SEQ ID NO:1, is removed.

[0078] The expression enhancer may comprise CPMV150+, comprising the sequence of SEQ ID NO:73 (5'UTR: nucleotide 1-150; multiple cloning site in italics nucleotides 156-166; plant kozak sequence in caps and bold, nucleotides 167-171):

```
1   tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc
61  ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgcgtgagc
```

121 gatcttcaac gttgtcagat cgtgcttcgg *gggcccaata ccgcgg***AGAA A**

(SEQ ID NO:73)

[0079] As noted above for CPMV160+ (SEQ ID NO:2), any MCS, including an MCS's of different length, may used in place of the MCS sequence of SEQ ID NO:73, and the plant kozak sequence may be any plant kozak sequence.

[0080] The expression enhancer CPMV150, may include an "A" in position 115 (115A), so that "CMPV150, 115A" comprises the sequence of the wild-type CPMV RNA2 genome (see WO 2009/087391, which is incorporated herein by reference) as defined by SEQ ID NO: 71 (the "A" is shown in bold and underline):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc**a**tgagc

121 gatcttcaac gttgtcagat cgtgcttcgg (SEQ ID NO:71)

[0081] The expression enhancer CPMV150+, may also include an "A" in position 115 (115A), so that "CMPV150+, 115A" comprises the sequence of the wild-type CPMV RNA2 genome (WO 2009/087391, which is incorporated herein by reference), as defined by SEQ ID NO: 77 (the "A" is shown in bold and underline):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc**a**tgagc

121 gatcttcaac gttgtcagat cgtgcttcgg *gggcccaata ccgcgg*A***GAA***

181 ***A*** (SEQ ID NO:77)

[0082] As noted above for SEQ ID NO:2, any MCS, or an MCS of different length, may used in place of the MCS sequence of SEQ ID NO:77, and the plant kozak sequence may be any plant kozak sequence.

[0083] If the expression enhancer consists of nucleotide 1-114 of SEQ ID NO:1:

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttgc

(SEQ ID NO:68)

then a nucleotide sequence of interest with a plant kozak sequence located at the 5' end, adjacent an initiation sequence (ATG), may be fused to the 3' end of the 5'UTR (after nucleotide 114 of SEQ ID NO:1), so that the overall construct resembles that as shown in Figure 1B (CPMV114). The construct comprising CPMV1114 may further comprise a regulatory region operatively linked to the 5'end of the expression enhancer, and a sequence encoding a 3'UTR, for example a comovirus 3' untranslated region (UTR) or a plastocyanin 3' UTR, and a terminator sequence, for example a NOS terminator, fused to the 3' end of the nucleotide sequence of interest. In this example, the nucleotide sequence of interest comprises a plant kozak sequence at its 5' end, since there is kozak-like sequence 5' to nucleotide 114 of SEQ ID NO:1.

[0084] The expression enhancer may comprise CPMV114+, comprising the sequence of SEQ ID NO:74 (5'UTR: nucleotide 1-114; multiple cloning site in italics nucleotides 115-130; plant kozak sequence in caps and bold, nucleotides 131-135):

1 tattaaaatc ttaataggtt ttgataaaag cgaacgtggg gaaacccgaa ccaaaccttc

61 ttctaaactc tctctcatct ctcttaaagc aaacttctct cttgtctttc ttg**c***gggccc*

121 *aataccgcgg* **AGAAA**

(SEQ ID NO:74)

[0085] As noted above for CPMV160+ (SEQ ID NO:2), any MCS, including an MCS's of different length, may used in place of the MCS sequence of SEQ ID NO:73, and the plant kozak sequence may be any plant kozak sequence.

[0086] The expression enhancer may also comprise nucleotides 1-160 of SEQ ID NO: 1, fused with a plant kozak sequence located downstream from position 160 of SEQ ID NO:1. The plant kozak sequence may be located immediately adjacent to nucleotide 160 of SEQ ID NO:1, or the expression enhancer may comprise a stuffer fragment of about 0 to about 500 nucleotides, or any amount therebetween, located immediately adjacent to nucleotide 160 of SEQ ID NO:1 (CPMVX+) and the plant kozak sequence linked to 3' end of the stuffer fragment. The stuffer fragment may comprise a multiple cloning site (MCS) of from about 4 to 100 nucleotides or any amount therebetween, and a nucleotide sequence of interest comprising a plant kozak sequence and a corresponding cloning site at its 5' end may be operatively linked to the CMPVX expression enhancer using the MCS, or the stuffer fragment may

comprise a multiple cloning site of from about 4 to 100 nucleotides fused to a plant kozak sequence, and a nucleotide sequence of interest may be fused to the expression enhancer immediately downstream of the plant kozak sequence. Preferably, the stuffer fragment does not comprise a sequence encoding an M protein.

[0087] An example, which is not to be considered limiting, of a construct, comprising in series, a plant regulatory region fused to a CPMV 5'UTR consisting of nucleotides 1-160 of SEQ ID NO:1, that is fused to a stuffer fragment is CPMV160+ as shown in Figure 1C (in Figure 1C, the ATG start site of the nucleotide sequence of interest "GOI", is also shown for clarity). In this example, the stuffer fragment is fused to the 3' end of the CPMV 1-160 sequence and comprises, in series, a multiple cloning site fused to a plant kozak sequence (in this example which is not to be considered limiting, the plant kozak sequence is: AGAAA). The stuffer fragment does not comprise any sequence encoding an M protein If the CPMV160+ construct is fused to a nucleotide sequence of interest (as shown in Figure 1C), then the plant kozak sequence is located 5' to the nucleotide sequence of interest, and adjacent to the ATG initiation site of the nucleotide sequence of interest. As would be appreciated by one of skill in the art, the multiple cloning site may comprise one or more than one suitable restriction sites, and the sequence of the multiple cloning site is not limited to the example shown in Figure 1C. Furthermore, the plant kozak sequence may be any plant kozak sequence and not limited to the sequence shown in Figure 1C. Construct numbers 1800, 1897, 1880, 2168, 2188, 1937, 1977, 2050, 2060, 1975, 1893, 2100, 2109, 2120, 2129 (see Examples 3, and 5-18, respectively) are examples of CPMV160+ (CPMVX+, where X=160 ) based constructs.

[0088] Also shown in Figure 1C are example of expression enhancers CPMV155+, CPMV150+, and CPMV114+ each comprising nucleotides 1-155, 1-150, or 1-114 of SEQ ID NO:1, respectively, fused to a stuffer fragment in a similar manner as that described for CPMV160+, above. In Figure 1C, the ATG start site of the nucleotide sequence of interest (GOI) is also shown for each of CPMV155+, CPMV150+, and CPMV114+. In these examples, the stuffer fragment is fused to the 3' end of the CPMV enhancer sequence comprises, in series, a multiple cloning site fused to a plant kozak sequence. The stuffer fragment does not comprise any sequence encoding an M protein. As would be appreciated by one of skill in the art, the multiple cloning

site may comprise one or more than one suitable restriction sites, and the sequence of the multiple cloning site is not limited to the examples shown in Figure 1C. Furthermore, the plant kozak sequence may be any plant kozak sequence and not limited to the sequence shown in Figure 1C (AGAAA).

[0089] The expression enhancer may also comprise the expression enhancer CPMVX, where X=160, 155, 150, or 114 of SEQ ID NO: 1, in combination with a multiple cloning site (polylinker, restriction site; cloning site) fused to the 3' end of the 5'UTR sequence, and lacking a plant kozak sequence (i.e. CPMVX+, where X=160, 155, 150, or 114 of SEQ ID NO: 1). In these cases the nucleic acid sequence encoding a protein of interest (nucleotide sequence of interest) to be joined to the enhancer, will comprises, in series from the 5' end to the 3' end of the nucleotide sequence of interest, a multiple cloning site (complimentary with that of the stuffer fragment; the stuffer fragment does not comprise any sequence encoding an M protein.) fused to a plant kozak sequence located upstream from and adjacent to an ATG initiation site (transcriptional start site) of the nucleotide sequence of interest.

[0090] The expression enhancer may further comprise one or more "kozak consensus sequence" or "kozak sequence". Kozak sequences play a major role in the initiation of translation. The rate of translation can be optimized by ensuring that any mRNA instability sequences are eliminated from the transgene construct, and that the translational start site or initiation site matches the Kozak consensus for plants (Gutierrrez, R.A. et al., 1999, Trends Plant Sci. 4, 429–438; Kawaguchi, R. and Bailey-Serres, J., 2002, Curr. Opin. Plant Biol. 5, 460–465). The most highly conserved position in this motif is the purine (which is most often an A) three nucleotides upstream of the ATG codon, which indicates the start of translation (Kozak, M., 1987, J. Mol. Biol. 20:947-950, herein incorporated by reference). 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.

[0091] The plant kozak sequence may be any known plant kozak sequences (see for example L. Rangan et. al. Mol. Biotechnol., 2008, July 39(3), pp. 207-213), including, but not limited to the following plant consensus sequences:

caA(A/C)a (SEQ ID NO:5; plant kingdom)

aaA(A/C)a (SEQ ID NO:6; dicots)

aa(A/G)(A/C)a (SEQ ID NO:7; arabidopsis)

The plant kozak sequence may also be selected from the group of (see Figure 4):

AGAAA (SEQ ID NO: 8)
AGACA (SEQ ID NO: 9)
AGGAA (SEQ ID NO: 10)
AAAAA (SEQ ID NO: 11)
AAACA (SEQ ID NO: 12)
AAGCA (SEQ ID NO: 13)
AAGAA (SEQ ID NO: 14)
AAAGAA (SEQ ID NO: 15)
AAAGAA (SEQ ID NO: 16)

(A/-)A(A/G)(A/G)(A/C)A. (SEQ ID NO: 3; Consensus sequence)

[0092] The expression enhancer may further comprise one or more "restriction site(s)" or "restriction recognition site(s)", "multiple cloning site", "MCS", "cloning site(s)" "polylinker sequence" or "polylinker' to facilitate the insertion of the nucleotide of interest into the plant expression system. Restrictions sites are specific sequence motifs that are recognized by restriction enzymes as are well known in the art. The expression enhancer may comprise one or more restriction sites or cloning sites that are located downstream (3') of the 5'UTR. The one or more restriction sites or cloning sites may further be located up-stream (5') of one or more kozak sequences, and located between a 5' UTR and a kozak sequence. The polylinker sequence (multiple cloning site) may comprise any sequence of nucleic acids that are useful for adding and removing nucleic acid sequences, including a nucleotide sequence encoding a protein of interest, to the 3' end of the 5'UTR. A polylinker sequence may comprise from 4 to about 100 nucleic acids, or any amount therebetween.

[0093] The expression enhancer may also comprise the sequence of SEQ ID NO:1 in operative association with a plant regulatory region and a transcriptional start site (ATG) fused to a nucleotide sequence of interest (GOI), as shown in Figure 1B

(CPMVX; where X=160, 155, 150 or 114). CPMVX may also comprise any plant kozak sequence including but not limited to, one of the sequences of SEQ ID NO's:5-17.

[0094] The 5'UTR for use in the expression enhancer described herein (CPMVX or CPMVX+, where X=160, 155, 150 or 144), may be derived from a bipartite RNA virus, e.g. from the RNA-2 genome segment of a bipartite RNA virus such as a comovirus, provided that it exhibits 100%, 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to the sequence as set forth in either SEQ ID NO's: 1 and 2. For example the enhancer sequence may have from about 80% to about 100% identity to the sequence of SEQ ID NO's: 1 and 2, or any amount therebetween, from about 90% to about 100% identity to the sequence of SEQ ID NO's: 1 and 2, or any amount therebetween, about 95% to about 100%, identity to the sequence of SEQ ID NO's: 1 and 2, or any amount therebetween, or about 98% to about 100%, identity to the sequence of SEQ ID NO's: 1 and 2, or any amount therebetween wherein the expression enhancer, when operatively linked to a plant regulatory region and a plant kozak sequence as described herein, increases the level of expression of a nucleotide sequence of interest that is operatively linked to the expression enhancer when compared to the level of expression of the nucleotide sequence of interest fused to the CMPV HT (SEQ ID NO:4; prior art enhancer sequence comprising an incomplete M protein as described in Sainsbury F., and Lomonossoff G.P., 2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference) using the same plant regulatory region.

[0095] SEQ ID NO:4 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:4 with modified nucleotides at positions 115 (cgt) and 162 (acg), and an incomplete M protein, and lacks a plant kozak sequence (5'UTR: nucleotides 1-160; incomplete M protein underlined, nucleotides 161 – 509). SEQ ID NO:4 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
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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 cgggccc* SEQ ID NO: 4

[0096] Constructs comprising CPMV HT are used herein as reference constructs, so that the expression levels of a nucleotide sequence of interest, or a product encoded by the nucleotide sequence of interest produced using a construct comprising CPMVX or CPMVX+, may be compared. Constructs 1391, 484, 489, 2140, 2130, 1039, 1067, 2072, 2074, 1445, 1454, 5001, 5002, 5021 and 5022 (see Examples 1 and 5-18, respectively) comprise the reference construct CPMV HT.

[0097] As shown in Figures 2-5, the use of the expression enhancers as described herein resulted in an increase of expression of the nucleotide sequence of interest, when compared to the expression of the same nucleotide sequence of interest using the same promoter and 3'UTR and terminator sequences. For example, with reference to Figures 2, 3 and 5, there is shown a comparison of expression of proteins produced in plants comprising CPMV-HT (prior art) expression constructs and CPMV160+ based expression constructs, operatively linked with:

H1 A/California/07/2009 ("PDI-H1 Cal", or "H1 A/California/07/2009"): CPMV160+ based construct number 1897, CPMV HT based construct number 484 (see Example 5);

H3 A/Victoria/361/2011 ("PDI-H3 Vic", or "H3 A/Victoria/361/2011"): CPMV160+ based construct number 1800; CPMV HT based construct number 1391 (see Examples 1 and 2, respectively);

H5 from Influenza A/Indonesia/5/2005 with a native signal peptide (WtSp-H5 Indo): CPMV160+ based construct number 1880; CPMV HT based construct number 489 (see Example 6);

B/Wisconsin/1/2010 with deleted proteolytic loop and with a native signal peptide ("WtSp-B Wis-PrL", or "B/Wisconsin/1/2010"): CPMV160+ based construct number 1975; CPMV HT based construct number 1445 (see Example 13);

B Brisbane/60/08 with deleted proteolytic loop and with a PDI signal peptide ("B Brisbane/60/08"): CPMV160+ based construct number 1937; CMPV HT based construct number 1039 (see Example 9);

B Brisbane/60/08+H1Tm, with deleted proteolytic loop fused to the transmembrane domain and cytoplasmic tail and with a PDI signal peptide ("B Brisbane/60/08+H1Tm"): CPMV160+ based construct number 1977; CMPV HT based construct 1067 (see Example 10),

B Massachusetts/2/2012 2012 with deleted proteolytic loop and with a PDI signal peptide ("B Massachusetts/2/2012 2012"): CPMV160+ based construct number 2050; CPMV HT based construct number 2072 ( see Example 11),

B Massachusetts/2/2012+H1Tm with deleted proteolytic loop fused to the transmembrane domain and cytoplasmic tail and with a PDI signal peptide ("B Massachusetts/2/2012+H1Tm"): CPMV160+ based construct number 2060; CPMV HT based construct 2074 (see Example 12),

B Wisconsin/1/2010+H1Tm with deleted proteolytic loop fused to the transmembrane domain and cytoplasmic tail and with the native signal peptide ("B Wisconsin/1/2010+H1Tm"): CPMV160+ based construct number 1893; CPMV HT based construct 1454 (see Example 14);

Rituximab (Rituxan) under the control of CPMV-HT with a native or PDI signal peptide ("CPMV-HT/wild-type SP" and "CPMV-HT/PDISP"; construct numbers 5001 and 5002, respectively, see examples 15 and 16), or CPMV160+ ("CPMV160+/wile-typeSP" and "CPMV160+/PDISP"; construct numbers 2100 and 2109, respectively, see example 15 and 16).

[0098] In each case, the expression (determined as hemagglutination activity or rituximab (Rituxan) expression as the case may be) is increased in the CMPV160+ based construct when compared to that for the prior art CPMV based construct.

Furthermore, several of the nucleotide sequences of interest encoded chimeric or modified proteins, for example comprising heterologous signal peptides (e.g. PDI), heterologous transmembrane domain cytoplasmic tail sequences (TDCT), and/or modified sequences including a deleted proteolytic loop (PrL-).

[0099] The increase in expression observed using CPMV160+ based constructs is also observed if the plant kozak sequence used in the CPMV160+ based constructs above is replaced with other plant kozak sequences for example, one of those plant kozak sequences defined in SEQ ID NO:8-16. For example, with reference to Figure 4, there is shown a comparison of the expression of proteins produced in plants comprising CPMV160+ based expression constructs, operatively linked with a nucleotide sequence of interest (H3 A/Victoria/361) each fused to various plant kozak sequences. In each case, the expression (determined as hemagglutination titre) the CMPV160+ based construct demonstrates significant expression levels and greater than the prior art CMPV HT based construct.

[00100] The terms "percent similarity", or "percent identity" when referring to a particular sequence are used for example as set forth in the University of Wisconsin GCG software program, or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology, Ausubel et al., eds. 1995 supplement). Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, using for example the algorithm of Smith & Waterman, (1981, Adv. Appl. Math. 2:482), by the alignment algorithm of Needleman & Wunsch, (1970, J. Mol. Biol. 48:443), by the search for similarity method of Pearson & Lipman, (1988, Proc. Nat'l. Acad. Sci. USA 85:2444), by computerized implementations of these algorithms (for example: GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis.).

[00101] An example of an algorithm suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977, Nuc. Acids Res. 25:3389-3402) and Altschul et al., (1990, J. Mol. Biol. 215:403-410 ), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for

the nucleic acids and proteins of the invention. For example the BLASTN program (for nucleotide sequences) may use as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program may use as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, 1989, Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (see URL: ncbi.nlm.nih.gov/).

[00102] A nucleotide sequence interest that encodes a protein requires the presence of a "translation initiation site" or "initiation site" or "translation start site" or "start site" or "start codon" located upstream of the gene to be expressed. Such initiation sites may be provided either as part of an enhancer sequence or as part of a nucleotide sequence encoding the protein of interest.

[00103] "Expression cassette" refers to a nucleotide sequence comprising a nucleic acid of interest under the control of, and operably (or operatively) linked to, an appropriate promoter or other regulatory elements for transcription of the nucleic acid of interest in a host cell.

[00104] By "proteolytic loop" or "cleavage site" is meant the consensus sequence of the proteolytic site that is involved in precursor HA0 cleavage. "Consensus" or "consensus sequence" as used herein means a sequence (either amino acid or nucleotide sequence) that comprises the sequence variability of related sequences based on analysis of alignment of multiple sequences, for example, subtypes of a particular influenza HA0 sequence. Consensus sequence of the influenza HA0 cleavage site may include influenza A consensus hemagglutinin amino acid sequences, including for example consensus H1, consensus H3, consensus H5, or influenza B consensus hemagglutinin amino acid sequences, for example but not limited to B Florida, B Malaysia, B Wisconsin and B Massachusetts. Non limiting examples of sequences of the proteoloytic loop region are shown in Figure 15 and 18B of US provisional application No.61/806,227 (filed March 28, 2013, which is

incorporated herein by reference; also see Bianchi et al., 2005, Journal of Virology, 79:7380-7388; incorporated herein by reference).

[00105] Residues in the proteolytic loop or cleavage site might be either mutated, for example but not limited to point mutation, substitution, insertion, or deletion. The term "amino acid mutation" or “amino acid modification” as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made as described in US provisional application No.61/806,227 (filed March 28, 2013, which is incorporated herein by reference) to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., reduced or abolished cleavage of the proteolytic loop or cleavage site by a protease.

[00106] As described herein, there is provided a nucleic acid construct (expression system) comprising an expression enhancer sequence operatively linked to a nucleotide sequence of interest encoding a protein of interest. Also provided are plant expression systems comprising an enhancer sequence as described herein . Also provided is a plant expression system comprising a plant regulatory region, in operative association with an enhancer sequence that is operatively linked to a nucleotide sequence of interest, the nucleotide sequence of interest encoding a protein of interest. The enhancer sequence may be selected from any one of SEQ ID NO’s:1, 2, 24, 27, 68, 69 and 70-77, or a .nucleotide sequence that exhibits 100%, 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to the sequence as set forth in any one of SEQ ID NO’s:1, 2, 24, 27, 68, 69 and 70-77, wherein the expression enhancer, when operatively linked to a plant regulatory region and a plant kozak sequence as described herein, increases the level of expression of a nucleotide sequence of interest that is operatively linked to the expression enhancer when compared to the level of expression of the nucleotide sequence of interest fused to the CMPV HT (SEQ ID NO:4; prior art enhancer sequence comprising an incomplete M protein as described in Sainsbury F., and Lomonossoff G.P., 2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference) using the same plant regulatory region.

[00107] The enhancer sequence of the present invention may be used to express a protein of interest in a host organism for example a plant. In this case, the protein of

interest may also be heterologous to the host organism in question and introduced into the plant cells using transformation techniques know in the art. A heterologous gene in an organism may replace an endogenous equivalent gene, i.e. one which normally performs the same or a similar function, or the inserted sequence may be additional to the endogenous gene or other sequence.

[00108] The enhancer sequence operatively linked to a nucleotide sequence of interest may also be operatively linked to promoter, or plant regulatory region, and a 3'UTR and terminator sequences. The enhancer sequence may be defined by, for example, any one of SEQ ID NO's:1, 2, 24, 27, 68, 69 and 70-77, or a .nucleotide sequence that exhibits 100%, 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to the sequence as set forth in any one of SEQ ID NO's:1, 2, 24, 27, 68, 69 and 70-77. Thus, the nucleotide sequence of interest is located between the enhancer sequence and the termination sequence (see Figure 1A). Either the expression enhancer or the nucleotide sequence of interest may comprise a plant kozak sequence.

[00109] The invention further provides an expression cassette comprising in series, a promoter or plant regulatory region, operatively linked to an expression enhancer sequence as described herein which is fused with a nucleotide sequence of interest, a 3'UTR sequence, and a terminator sequence. The enhancer sequence may be defined by, for example, any one of SEQ ID NO's:1, 2, 24, 27, 68, 69 and 70-77, or a .nucleotide sequence that exhibits 100%, 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to the sequence as set forth in any one of SEQ ID NO's:1, 2, 24, 27, 68, 69 and 70-77. Either the expression enhancer or the nucleotide sequence of interest may comprise a plant kozak sequence.

[00110] As one of skill in the art would appreciate, the termination (terminator) sequence may be any sequence that is active the plant host, for example the termination sequence may be derived from the RNA-2 genome segment of a bipartite RNA virus, e.g. a comovirus, or the termination sequence may be a NOS terminator.

[00111] The constructs of the present invention can further comprise a 3' untranslated region (UTR). A 3' untranslated region contains a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by effecting the

addition of polyadenylic acid tracks to the 3' end of the mRNA precursor. Polyadenylation signals are commonly recognized by the presence of homology to the canonical form 5' AATAAA-3' although variations are not uncommon. Non-limiting examples of suitable 3' regions are the 3' transcribed non-translated regions containing a polyadenylation signal of *Agrobacterium* tumor inducing (Ti) plasmid genes, such as the nopaline synthase (Nos gene) and plant genes such as the soybean storage protein genes, the small subunit of the ribulose-1, 5-bisphosphate carboxylase gene (ssRUBISCO; US 4,962,028; which is incorporated herein by reference), the promoter used in regulating plastocyanin expression (Pwee and Gray 1993; which is incorporated herein by reference). The termination (terminator) sequence may be obtained from the 3'UTR of the alfalfa plastocyanin gene.

[00112] By "nucleotide (or nucleic acid) sequence of interest", or "coding region of interest", it is meant any nucleotide sequence, or coding region (these terms may be used interchangeably) that is to be expressed within a host organism, for example a plant, to produce a protein of interest. Such a nucleotide sequence of interest may encode, but is not limited to, native or modified proteins, an industrial enzyme or a modified industrial enzyme, an agricultural protein or a modified agricultural protein, a helper protein, a protein supplement, a pharmaceutically active protein, a nutraceutical, a value-added product, or a fragment thereof for feed, food, or both feed and food use.

[00113] The protein of interest may comprise a native, or a non-native signal peptide; the non-native signal peptide may be of plant origin. For example, the signal peptide may be a protein disulfide isomerase signal peptide (PDI). The native signal peptide may correspond to that of the protein of interest being expressed.

[00114] The nucleotide sequence of interest, or coding region of interest may also include a nucleotide sequence that encodes a pharmaceutically active protein, for example growth factors, growth regulators, antibodies, antigens, and fragments thereof, or their derivatives useful for immunization or vaccination and the like. Such proteins include, but are not limited to a protein that is a human pathogen, a viral protein, for example but not limited to VLP-forming antigens, one or more proteins from Respiratory syncytial virus (RSV), Rotavirus, influenza virus, human immunodeficiency virus (HIV), Rabies virus, human papiloma virus (HPV),

Enterovirus 71 (EV71), or interleukins, for example one or more than one of IL-1 to IL-24, IL-26 and IL-27, cytokines, Erythropoietin (EPO), insulin, G-CSF, GM-CSF, hPG-CSF, M-CSF or combinations thereof, interferons, for example, interferon-alpha, interferon-beta, interferon-gama, blood clotting factors, for example, Factor VIII, Factor IX, or tPA hGH, receptors, receptor agonists, antibodies for example but not limited to rituximab (Rituxan), neuropolypeptides, insulin, vaccines, growth factors for example but not limited to epidermal growth factor, keratinocyte growth factor, transformation growth factor, growth regulators, antigens, autoantigens, fragments thereof, or combinations thereof.

[00115] The protein of interest may also include an influenza hemagglutinin (HA; see WO 2009/009876, which is incorporated herein by reference). HA is a homotrimeric membrane type I glycoprotein, generally comprising a signal peptide, an HA1 domain, and an HA2 domain comprising a membrane-spanning anchor site at the C-terminus and a small cytoplasmic tail. Nucleotide sequences encoding HA are well known and are available (see, for example, the BioDefense and Public Health Database (Influenza Research Database; Squires et al., 2008 Nucleic Acids Research 36:D497-D503) at URL: biohealthbase.org/GSearch/home.do?decorator=Influenza; or the databases maintained by the National Center for Biotechnology Information (see URL: ncbi.nlm.nih.gov), both of which are incorporated herein by reference).

[00116] An HA protein may be of a type A influenza, a type B influenza, or is a subtype of type A influenza HA selected from the group of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, and H16. In some aspects of the invention, the HA may be from a type A influenza, selected from the group H1, H2, H3, H5, H6, H7 and H9. Fragments of the HAs listed above may also be considered a protein of interest. Furthermore, domains from an HA type or subtype listed above may be combined to produce chimeric HA's (see for example WO2009/076778 which is incorporated herein by reference).

[00117] Examples of subtypes comprising HA proteins include A/New Caledonia/20/99 (H1N1), A/Indonesia/5/2006 (H5N1), A/chicken/New York/1995, A/herring gull/DE/677/88 (H2N8), A/Texas/32/2003, A/mallard/MN/33/00, A/duck/Shanghai/1/2000, A/northern pintail/TX/828189/02,

A/Turkey/Ontario/6118/68(H8N4), A/shoveler/Iran/G54/03, A/chicken/Germany/N/1949(H10N7), A/duck/England/56(H11N6), A/duck/Alberta/60/76(H12N5), A/Gull/Maryland/704/77(H13N6), A/Mallard/Gurjev/263/82, A/duck/Australia/341/83 (H15N8), A/black-headed gull/Sweden/5/99(H16N3), B/Lee/40, C/Johannesburg/66, A/PuertoRico/8/34 (H1N1), A/Brisbane/59/2007 (H1N1), A/Solomon Islands 3/2006 (H1N1), A/Brisbane 10/2007 (H3N2), A/Wisconsin/67/2005 (H3N2), B/Malaysia/2506/2004, B/Florida/4/2006, A/Singapore/1/57 (H2N2), A/Anhui/1/2005 (H5N1), A/Vietnam/1194/2004 (H5N1), A/Teal/HongKong/W312/97 (H6N1), A/Equine/Prague/56 (H7N7), A/HongKong/1073/99 (H9N2)).

[00118] The HA protein may be an H1, H2, H3, H5, H6, H7 or H9 subtype. For example, the H1 protein may be from the A/New Caledonia/20/99 (H1N1), A/PuertoRico/8/34 (H1N1), A/Brisbane/59/2007 (H1N1), A/Solomon Islands 3/2006 (H1N1), A/California/04/2009 (H1N1) or A/California/07/2009 (H1N1) strain. The H3 protein may also be from the A/Brisbane 10/2007 (H3N2), A/Wisconsin/67/2005 (H3N2), A/Victoria/361/2011 (H3N2), A/Texas/50/2012 (H3N2), A/Hawaii/22/2012 (H3N2), A/New York/39/2012 (H3N2), or A/Perth/16/2009 (H3N2) strain. In a further aspect of the invention, the H2 protein may be from the A/Singapore/1/57 (H2N2) strain. The H5 protein may be from the A/Anhui/1/2005 (H5N1), A/Vietnam/1194/2004 (H5N1), or A/Indonesia/5/2005 strain. In an aspect of the invention, the H6 protein may be from the A/Teal/HongKong/W312/97 (H6N1) strain. The H7 protein may be from the A/Equine/Prague/56 (H7N7) strain, or H7 A/Hangzhou/1/2013, A/Anhui/1/2013 (H7N9), or A/Shanghai/2/2013 (H7N9) strain. In an aspect of the invention, the H9 protein is from the A/HongKong/1073/99 (H9N2) strain. In a further aspect of the invention, the HA protein may be from an influenza virus may be a type B virus, including B/Malaysia/2506/2004, B/Florida/4/2006, B/Brisbane/60/08, B/Massachusetts/2/2012 –like virus (Yamagata lineage), or B/Wisconsin/1/2010 (Yamagata lineage). Non-limiting examples of amino acid sequences of the HA proteins from H1, H2, H3, H5, H6, H7, H9 or B subtypes include sequences as described in WO 2009/009876, WO 2009/076778, WO 2010/003225 (which are incorporated herein by reference). The influenza virus HA protein may be H5 Indonesia.

[00119] The HA may also be a chimeric HA, wherein a native transmembrane domain of the HA is replaced with a heterologous transmembrane domain. The transmembrane domain of HA proteins is highly conserved (see for example Figure 1C of WO 2010/148511; which is incorporated herein by reference). The heterologous transmembrane domain may be obtained from any HA transmembrane domain, for example but not limited to the transmembrane domain from H1 California, B/Florida/4/2006 (GenBank Accession No. ACA33493.1), B/Malaysia/2506/2004 (GenBank Accession No. ABU99194.1), H1/Bri (GenBank Accession No. ADE28750.1), H1 A/Solomon Islands/3/2006 (GenBank Accession No. ABU99109.1), H1/NC (GenBank Accession No. AAP34324.1), H2 A/Singapore/1/1957 (GenBank Accession No. AAA64366.1), H3 A/Brisbane/10/2007 (GenBank Accession No. ACI26318.1), H3 A/Wisconsin/67/2005 (GenBank Accession No. ABO37599.1), H5 A/Anhui/1/2005 (GenBank Accession No. ABD28180.1), H5 A/Vietnam/1194/2004 (GenBank Accession No. ACR48874.1), H5-Indo (GenBank Accession No. ABW06108.1),. The transmembrane domain may also be defined by the following consensus amino acid sequence:

iLXiYystvAiSslXlXXmlagXsXwmcs (SEQ ID NO:78)

[00120] The HA may comprise a native, or a non-native signal peptide; the non-native signal peptide may be of plant origin. The native signal peptide may correspond to that of the hemagglutinin being expressed, or may correspond to a second hemagglutinin. Additionally, the signal peptide may be from a structural protein or hemagglutinin of a virus other than influenza. Non-limiting examples of a signal peptide that may be used is that of alfalfa protein disulfide isomerase (PDI SP; nucleotides 32-103 of Accession No. Z11499), or the patatin signal peptide (PatA SP; located nucleotides 1738 - 1806 of GenBank Accession number A08215). The nucleotide sequence of PatA SP for this accession number is:

ATGGCAACTACTAAAACTTTTTTAATTTTATTTTTTATGATATTAGCAACTACTAGTTCAACATGTGCT (SEQ ID NO:79)

the amino acid sequence of patatin A signal peptide is :

MATTKTFLILFFMILATTSSTCA (SEQ ID NO:80)

[00121] The present invention also provides nucleic acid molecules comprising sequences encoding an HA protein. The nucleic acid molecules may further comprise one or more regulatory regions operatively linked to the sequence encoding an HA protein. The nucleic acid molecules may comprise a sequence encoding an H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16 or HA from type B influenza. For example, the HA protein encoded by the nucleic acid molecule may be an H1, H2, H3, H5, H6, H7, H9 subtype an HA from type B. The H1 protein encoded by the nucleic acid may be from the A/New Caledonia/20/99 (H1N1), A/PuertoRico/8/34 (H1N1), A/Brisbane/59/2007 (H1N1), A/Solomon Islands 3/2006 (H1N1), A/California/04/2009 (H1N1) or A/California/07/2009 (H1N1) strain. The H3 protein encoded by the nucleic acid molecule may be from the A/Brisbane 10/2007 (H3N2), A/Wisconsin/67/2005 (H3N2), A/Victoria/361/2011 (H3N2), A/Texas/50/2012 (H3N2), A/Hawaii/22/2012 (H3N2), A/New York/39/2012 (H3N2), or A/Perth/16/2009 (H3N2) strain. The H2 protein encoded by the nucleic acid molecule may be from the A/Singapore/1/57 (H2N2) strain. The H5 protein encoded by the nucleic acid molecule A/Anhui/1/2005 (H5N1), A/Vietnam/1194/2004 (H5N1), or A/Indonesia/5/2005 strain. The H6 protein encoded by the nucleic acid molecule may be from the A/Teal/HongKong/W312/97 (H6N1) strain. The H7 protein encoded by the nucleic acid molecule may be from the A/Equine/Prague/56 (H7N7) strain, or H7 A/Hangzhou/1/2013, A/Anhui/1/2013 (H7N9), or A/Shanghai/2/2013 (H7N9) strain. Additional, the H9 protein encoded by the nucleic acid molecule may be from the A/HongKong/1073/99 (H9N2) strain. The HA protein encoded by the nucleic acid molecule may be from an influenza virus type B virus, including B/Malaysia/2506/2004, B/Florida/4/2006, B/Brisbane/60/08, B/Massachusetts/2/2012–like virus (Yamagata lineage), or B/Wisconsin/1/2010 (Yamagata lineage). Non-limiting examples of amino acid sequences of the HA proteins from H1, H2, H3, H5, H6, H7, H9 or B subtypes include sequences as described in WO 2009/009876, WO 2009/076778, WO 2010/003225 (which are incorporated herein by reference). The influenza virus HA protein may be H5 Indonesia.

[00122] Table 1: Examples of constructs that have been prepared as described herein:

**CMPV-HT based constructs**

(constructs comprising SEQ ID NO:4; prior art)

| **Construct #** | **SP[1]** | **Sequence of Interest** | **Example** |
|---|---|---|---|
| 484 | PDI[2] | H1 California | 5 |
| 489 | WT[3] | H5 Indonesia | 6 |
| 2140 | PDI | H7 Hangzhou | 7 |
| 2130 | PDI | H7 Hangzhou+H5 Indonesia TMCT[4] | 8 |
| 1039 | PDI | B Brisbane(Prl-) | 9 |
| 1067 | PDI | B Brisbane(PrL-)+Hi California TMCT | 10 |
| 2072 | PDI | B Massachussetts (PrL-) | 11 |
| 2074 | PDI | B Massachussetts (PrL-)+H1 California TMCT | 12 |
| 1445 | WT | B Wisconsin (PrL-) | 13 |
| 1454 | WT | B Wisconsin (PrL-)+H1 California TMCT | 14 |
| 5001 | WT | HC rituximab (Rituxan) | 15 |
| 5002 | PDI | HC rituximab (Rituxin) | 16 |
| 5021 | WT | LC rituximab (Rituxin) | 17 |
| 5022 | PDI | LC rituximab (Rituxin) | 18 |

**CPMV160+ based constructs**

(constructs comprising SEQ ID NO:2)

| Construct # | SP | Sequence of Interest | Example |
|---|---|---|---|
| 1800 | PDI | H3 Victoria | 2 |
| 1897 | PDI | H1 California | 5 |
| 1880 | WT | H5 Indonesia | 6 |
| 2168 | PDI | H7 Hangzhou | 7 |
| 2188 | PDI | H7 Hangzhou+ H5 Indonesia TMCT | 8 |
| 1937 | PDI | B Brisbane(Prl-) | 9 |
| 1977 | PDI | B Brisbane(PrL-)+Hi California TMCT | 10 |
| 2050 | PDI | B Massachussetts (PrL-) | 11 |
| 2060 | PDI | B Massachussetts (PrL-)+H1 California TMCT | 12 |
| 1975 | WT | B Wisconsin (PrL-) | 13 |
| 1893 | WT | B Wisconsin (PrL-)+H1 California TMCT | 14 |
| 2100 | WT | HC rituximab (Rituxan) | 15 |
| 2109 | PDI | HC rituximab (Rituxin) | 16 |
| 2120 | WT | LC rituximab (Rituxin) | 17 |
| 2129 | PDI | LC rituximab (Rituxin) | 18 |

**CPMV160 based constructs**

(constructs comprising SEQ ID NO:1)

| Construct # | SP | Sequence of Interest | Example |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1935 | PDI | H3 Victoria | 3 |
| 1885 | WT | H5 Indonesia | 6 |

[1]: SP - signal peptide

[2]: PDI - alfalfa protein disulfide isomerise

[3]: WT – wild type or native

[4]: TMCT - transmembrane domain and cytoplasmic tail

[00123] If the nucleic acid sequence of interest encodes a product that is directly or indirectly toxic to the plant, then such toxicity may be reduced by selectively expressing the nucleotide sequence of interest within a desired tissue or at a desired stage of plant development.

[00124] The coding region of interest or the nucleotide sequence of interest may be expressed in any suitable plant host which is either transformed or comprises the nucleotide sequences, or nucleic acid molecules, or genetic constructs, or vectors of the present invention. Examples of suitable hosts include, but are not limited to, *Arabidopsis*, agricultural crops including for example canola, *Brassica* spp., maize, *Nicotiana* spp., (tobacco) for example, *Nicotiana benthamiana*, alfalfa, potato, sweet potato (*Ipomoea batatus*), ginseng, pea, oat, rice, soybean, wheat, barley, sunflower, cotton, corn, rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), safflower (*Carthamus tinctorius*).

[00125] The terms "biomass" and "plant matter" as used herein refer to any material derived from a plant. Biomass or plant matter may comprise an entire plant, or part of plant including the leaf, root, stem, flower, seed, it may also include any tissue of the plant, any cells of the plant, or any fraction of the plant, part or the plant, tissue or cell. Further, biomass or plant matter may comprise intracellular plant components, extracellular plant components, liquid or solid extracts of plants, or a combination thereof. Further, biomass or plant matter may comprise plants, plant cells, tissue, a liquid extract, or a combination thereof, from plant leaves, stems, fruit, roots or a combination thereof. A portion of a plant may comprise plant matter or biomass.

[00126] By "regulatory region" "regulatory element" or "promoter" it is meant a portion of nucleic acid typically, but not always, upstream of the protein coding region of a gene, which may be comprised of either DNA or RNA, or both DNA and RNA. When a regulatory region is active, and in operative association, or operatively linked, with a 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" includes promoter elements, core promoter elements exhibiting a basal promoter activity, elements that are inducible in response to an external stimulus, elements that mediate promoter activity such as negative regulatory elements or transcriptional enhancers. "Regulatory region", as used herein, also includes elements that are active following transcription, for example, regulatory elements that modulate gene expression such as translational and transcriptional enhancers, translational and transcriptional repressors, upstream activating sequences, and mRNA instability determinants. Several of these latter elements may be located proximal to the coding region.

[00127] 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 may comprise a basal promoter element, responsible for the initiation of transcription, as well as other regulatory elements (as listed above) that modify gene expression.

[00128] 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).

[00129] An inducible regulatory region is one that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor that binds specifically to an inducible regulatory region to activate transcription may be present in an inactive form, which is then directly or indirectly converted to the active form by the inducer. However, the protein factor may also be absent. The inducer can be a chemical agent such as a protein, metabolite, growth regulator, herbicide or phenolic compound or a physiological stress imposed directly by heat, cold, salt, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus. A plant cell containing an inducible regulatory region may be exposed to an inducer by externally applying the inducer to the cell or plant such as by spraying, watering, heating or similar methods. Inducible regulatory elements may be derived from either plant or non-plant genes (e.g. Gatz, C. and Lenk, I.R.P., 1998, Trends Plant Sci. 3, 352-358; 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. Biol. 48, 89-108; which is incorporated by reference), steroid inducible promoter (Aoyama, T. and Chua, N.H.,1997, Plant J. 2, 397-404; which is incorporated by reference) and ethanol-inducible promoter (Salter, M.G., et al, 1998, Plant Journal 16, 127-132; Caddick, M.X., et al,1998, Nature Biotech. 16, 177-180, which are incorporated by reference) cytokinin inducible IB6

and CKI1 genes (Brandstatter, I. and Kieber, J.J.,1998, Plant Cell 10, 1009-1019; Kakimoto, T., 1996, Science 274, 982-985; which are incorporated by reference) and the auxin inducible element, DR5 (Ulmasov, T., et al., 1997, Plant Cell 9, 1963-1971; which is incorporated by reference).

[00130] A constitutive regulatory region directs the expression of a gene throughout the various parts of a plant and continuously throughout plant development. Examples of known constitutive regulatory elements include promoters associated with the CaMV 35S transcript. (p35S; Odell et al., 1985, Nature, 313: 810-812), the rice actin 1 (Zhang et al, 1991, Plant Cell, 3: 1155-1165), actin 2 (An *et al.*, 1996, *Plant J.*, 10: 107-121), or tms 2 (U.S. 5,428,147, which is incorporated herein by reference), and triosephosphate isomerase 1 (Xu et. al., 1994, Plant Physiol. 106: 459-467) genes, the maize ubiquitin 1 gene (Cornejo et al, 1993, Plant Mol. Biol. 29: 637-646), the *Arabidopsis* ubiquitin 1 and 6 genes (Holtorf et al, 1995, Plant Mol. Biol. 29: 637-646), the tobacco translational initiation factor 4A gene (Mandel et al, 1995 Plant Mol. Biol. 29: 995-1004). the Cassava Vein Mosaic Virus promoter, pCAS, (Verdaguer et al., 1996); the promoter of the small subunit of ribulose biphosphate carboxylase, pRbcS: (Outchkourov et al., 2003), the pUbi (for monocots and dicots ).

[00131] As described herein, regulatory regions comprising enhancer sequences with demonstrated efficiency in leaf expression, have been found to be effective in transient expression. Without wishing to be bound by theory, attachment of upstream regulatory elements of a photosynthetic gene by attachment to the nuclear matrix may mediate strong expression. For example up to -784 from the translation start site of pea plastocyanin (US 7,125,978, which is incorporated herein by reference) may be used mediate strong reporter gene expression.

[00132] The term "constitutive" as used herein does not necessarily indicate that a nucleotide sequence under control of the constitutive regulatory region is expressed at the same level in all cell types, but that the sequence is expressed in a wide range of cell types even though variation in abundance is often observed.

[00133] The expression constructs as described above may be present in a vector. The vector may comprise border sequences which permit the transfer and

integration of the expression cassette into the genome of the organism or host. The construct may be a plant binary vector, for example a binary transformation vector based on pPZP (Hajdukiewicz, et al. 1994). Other example constructs include pBin19 (see Frisch, D. A., L. W. Harris-Haller, et al. 1995, *Plant Molecular Biology* 27: 405-409).

[00134] If desired, the constructs of this invention may be further manipulated to include selectable markers. However, this may not be required. 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.

[00135] A vector may also include a expression enhancer as described herein. The expression enhancer may be positioned on a T-DNA which also contains a suppressor of gene silencing and NPTII. The polylinker may also encode one or two sets of 6 x Histidine residues to allow the inclusion of N- or C-terminal His-tags to the protein of interest to facilitate protein purification.

[00136] Post-transcriptional gene silencing (PTGS) may be involved in limiting expression of transgenes in plants, and co-expression of a suppressor of silencing from the potato virus Y (HcPro) may be used to counteract the specific degradation of transgene mRNAs (Brigneti et al., 1998, *EMBO J.* **17**, 6739-6746, which is incorporated herein by reference). 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, TEV-p1/HC-Pro (Tobacco etch virus-p1/HC-Pro), BYV -p21, p19 of Tomato bushy stunt virus (TBSV p19; the construction of p19 is described in described in WO 2010/0003225, which is incorporated herein by reference), 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 tristeza 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).

[00137] Therefore, one or more suppressors of silencing, for example, but not limited to, HcPro, TEV -p1/HC-Pro, BYV-p21, TBSV p19, TCV-CP, CMV-2b, PVX-p25, rgscam, B2 protein from FHV, the small coat protein of CPMV, and coat protein from TCV, PVM-p11, PVS-p11, BScV-p16, CTV-p23, GLRaV-2 p24, GBV-p14, HLV-p10, GCLV-p16, or GVA-p10 may be co-expressed along with the comovirus-based expression cassette, geminivirus-derived amplification element, and the nucleic acid sequence encoding the protein of interest to further ensure high levels of protein production within a plant.

[00138] The constructs of the present invention can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, micro-injection, electroporation, etc. For reviews of such techniques see for example Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academy Press, New York VIII, pp. 421-463 (1988); Geierson and Corey, *Plant Molecular Biology*, 2d Ed. (1988); and Miki and Iyer, Fundamentals of Gene Transfer in Plants. In *Plant Metabolism*, 2d Ed. DT. Dennis, DH Turpin, DD Lefebrve, DB Layzell (eds), Addison Wesly, Langmans Ltd. London, pp. 561-579 (1997). Other methods include direct DNA uptake, the use of liposomes, electroporation, for example using protoplasts, micro-injection, microprojectiles or whiskers, and vacuum infiltration. See, for example, Bilang, et al. (1991, *Gene* 100: 247-250), Scheid et al. (1991, *Mol. Gen. Genet*. 228: 104-112), Guerche et al. (1987, *Plant Science* 52: 111-116), Neuhause et al. (1987, *Theor. Appl Genet*. 75: 30-36), Klein et al., (2987, *Nature* 327: 70-73); Freeman et al. (1984, *Plant Cell Physiol*. 29: 1353), Howell et al. (1980, *Science* 208: 1265), Horsch et al. (1985, *Science* 227: 1229-1231), DeBlock et al., (1989, *Plant Physiology* 91: 694-701), Methods for Plant Molecular Biology (Weissbach and Weissbach, eds., Academic Press Inc., 1988), Methods in Plant Molecular Biology (Schuler and Zielinski, eds., Academic Press Inc., 1989), WO 92/09696, WO 94/00583, EP 331083, EP 175966, Liu and Lomonossoff (2002, *J Virol Meth*, 105:343-348), EP 290395; WO 8706614; U.S. Pat. Nos. 4,945,050; 5,036,006; and 5,100,792, U.S. patent application Ser. Nos. 08/438,666, filed May 10,

1995, and 07/951,715, filed Sep. 25, 1992, (all of which are hereby incorporated by reference).

[00139] Transient expression methods may be used to express the constructs of the present invention (see D'Aoust et al., 2009, *Methods in molecular biology*, Vol 483, pages41-50; Liu and Lomonossoff, 2002, *Journal of Virological Methods*, 105:343-348; which is incorporated herein by reference). Alternatively, a vacuum-based transient expression method, as described by Kapila et al., (1997, *Plant Sci.* **122**, 101-108; which is incorporated herein by reference), or WO 00/063400, WO 00/037663 (which are 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.

[00140] Also considered part of this invention are transgenic plants, plant cells or seeds containing the gene construct of the present invention that may be used as a platform plant suitable for transient protein expression described herein. Methods of regenerating whole plants from plant cells are also known in the art (for example see Guerineau and Mullineaux (1993, Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148). In general, transformed plant cells are cultured in an appropriate medium, which may contain selective agents such as antibiotics, where selectable markers are used to facilitate identification of transformed plant cells. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be used to establish repetitive generations, either from seeds or using vegetative propagation techniques.

Transgenic plants can also be generated without using tissue culture. Methods for stable transformation, and regeneration of these organisms are established in the art and known to one of skill in the art. Available techniques are reviewed in Vasil et al., (Cell Culture and Somatic Cell Genetics of Plants, VoI I, Il and III, Laboratory Procedures and Their Applications, Academic Press, 1984), and Weissbach and Weissbach, (Methods for Plant Molecular Biology, Academic Press, 1989). The method of obtaining transformed and regenerated plants is not critical to the present invention.

[00141] If plants, plant portion or plant cell are to be transformed or co-transformed by two or more nucleic acid constructs, the nucleic acid construct may be introduced into the *Agrobacterium* in a single transfection event the nucleic acids are pooled, and the bacterial cells transfected as described. Alternately, the constructs may be introduced serially. In this case, a first construct is introduced to the *Agrobacterium* as described, the cells grown under selective conditions (e.g. in the presence of an antibiotic) where only the singly transformed bacteria can grow. Following this first selection step, a second nucleic acid construct is introduced to the *Agrobacterum* as described, and the cells grown under doubly-selective conditions, where only the doubly-transformed bacteria can grow. The doubly-transformed bacteria may then be used to transform a plant, plant portion or plant cell as described herein, or may be subjected to a further transformation step to accommodate a third nucleic acid construct.

[00142] Alternatively, if plants, a plant portion, or a plant cell are to be transformed or co-transformed by two or more nucleic acid constructs, the nucleic acid construct may be introduced into the plant by co-infiltrating a mixture of *Agrobacterium* cells with the plant, plant portion, or plant cell, each *Agrobacterium* cell may comprise one or more constructs to be introduced within the plant. In order to vary the relative expression levels within the plant, plant portion or plant cell, of a nucleotide sequence of interest within a construct, during the step of infiltration, the concentration of the various *Agrobacteria* populations comprising the desired constructs may be varied.

[00143] The present disclosure further provides a transgenic plant comprising the expression system as defined herein, wherein the heterologous nucleic acid of interest in the cassette is expressed at an enhanced level when compared to other analogous expression systems that lack one or more components of the expression system as described herein, for example CMPV HT (SEQ ID NO:4).

[00144] The present disclosure further comprises a method for generating a protein of interest, comprising the steps of providing a plant, or plant part, that expresses the expression system as described herein, harvesting, at least, a tissue in which the protein of interest has been expressed and optionally, isolating the protein of interest from the tissue.

[00145] Thus in various aspects, and without limitation, the invention provides:

- an expression enhancer, comprising a comovirus 5'UTR selected from any one of SEQ ID NO's:1, 2, 24, 27, 68, 69 and 70-77, or a .nucleotide sequence that exhibits 100%, 99%, 98%, 97%, 96%, 95%, 90%, 85% or 80% identity to the sequence as set forth in any one of SEQ ID NO's:1, 2, 24, 27, 68, 69 and 70-77, wherein the expression enhancer, when operatively linked to a plant regulatory region and a plant kozak sequence as described herein, increases the level of expression of a nucleotide sequence of interest that is operatively linked to the expression enhancer when compared to the level of expression of the nucleotide sequence of interest fused to the CMPV HT (SEQ ID NO:4; prior art enhancer sequence comprising an incomplete M protein as described in Sainsbury F., and Lomonossoff G.P., 2008, Plant Physiol. 148: pp. 1212-1218; which is incorporated herein by reference) using the same plant regulatory region.

- one or more expression systems comprising a comovirus-based expression enhancer or expression cassette as defined above, a promoter (regulatory region), optionally a polylinker, a kozak sequence, a nucleic acid encoding a protein of interest, and a terminator.

- methods of expressing a protein of interest, in a host organism such as a plant using one or more expression systems or vectors as described herein.

- host cells and organisms expressing proteins of interest from the one or more expression systems or vectors of the invention and methods of producing the hosts and organisms.

[00146] Table 2: list of sequences

| SEQ ID NO | Description | SEQ ID NO | Description |
|---|---|---|---|
| 1 | CPMV160 | 41 | Nucleotide sequence of native H5 Indonesia |
| 2 | CPMV160+ | 42 | Amino acid sequence of native H5 Indonesia |
| 3 | Consensus kozak sequence<br><br>(A/-)A(A/G)(A/G)(A/C)A | 43 | Nucleotide sequence of PDISP/H7 Hangzhou |
| 4 | CPMV HT (prior art 5'UTR) | 44 | Amino acid sequence of PDISP/H7 Hangzhou |
| 5 | Consensus plant kingdom kozak sequence | 45 | Nucleotide sequence of PDISP/H7 Hangzhou+H5 Indonesia TMCT |
| 6 | Consensus dicot kozak sequence | 46 | Amino acid sequence of PDISP/H7 Hangzhou+H5 Indonesia TMCT |
| 7 | Consensus Arabidopsis kozak sequence | 47 | Nucleotide sequence of PDISP/HA B Brisbane (PrL-) |
| 8 | kozak sequence AGAAA | 48 | Amino acid sequence of PDISP/HA B Brisbane (PrL-) |
| 9 | kozak sequence AGACA | 49 | Nucleotide sequence of PDISP/HA B Brisbane (PrL-)+H1 California TMCT |
| 10 | kozak sequence AGGAA | 50 | Amino acid sequence of PDISP/HA B Brisbane (PrL-)+H1 California TMCT |
| 11 | kozak sequence AAAAA | 51 | Nucleotide sequence of PDISP/HA B Massachussetts (PrL-) |
| 12 | kozak sequence AAACA | 52 | Amino acid sequence of PDISP/HA B Massachussetts |

| | | | |
|---|---|---|---|
| | | | (PrL-) |
| 13 | kozak sequence AAGCA | 53 | Nucleotide sequence of PDISP/HA B Massachussetts (PrL-)+H1 California TMCT |
| 14 | kozak sequence AAGAA | 54 | Amino acid sequence of PDISP/HA B Massachussetts (PrL-)+H1 California TMCT |
| 15 | kozak sequence AAAGAA | 55 | Nucleotide sequence of HA B Wisconsin (PrL-) |
| 16 | kozak sequence AAAAGAA | 56 | Amino acid sequence of HA B Wisconsin (PrL-) |
| 17 | IF-H3V36111.s1-4r | 57 | Nucleotide sequence of HA B Wisconsin (PrL-)+H1 California TMCT |
| 18 | Nucleotide sequence of PDISP/H3 Victoria. | 58 | Amino acid sequence of HA B Wisconsin (PrL-)+H1 California TMC |
| 19 | Nucleotide sequence of construct 1191 | 59 | Nucleotide sequence of HC rituximab (Rituxan) |
| 20 | Nucleotide sequence of expression cassette number 1391 | 60 | Amino acid sequence of HC Rituxan |
| 21 | Amino acid sequence of PDISP/H3 Victoria | 61 | Nucleotide sequence of PDISP/HC rituximab (Rituxan) |
| 22 | IF**(SacII)-PDI.s1+4c | 62 | Amino acid sequence of PDISP/HC rituximab (Rituxan) |
| 23 | IF-H3V36111.s1-4r | 63 | Nucleotide sequence of LC rituximab (Rituxan) |
| 24 | CPMV155 | 64 | Amino acid sequence of LC rituximab (Rituxan) |
| 25 | Nucleotide sequence of construct 2171 | 65 | Nucleotide sequence of PDISP/LC rituximab (Rituxan) |
| 26 | Nucleotide sequence of expression cassette number 1800 from 2X35S promoter to NOS terminator | 66 | Amino acid sequence of PDISP/LC rituximab (Rituxan) |
| 27 | CPMV150 | 67 | IF-PDI.S1+3c |
| 28 | IF-CPMV(fl5'UTR)_SpPDI.c | 68 | CPMV114 |

| | | | |
|---|---|---|---|
| 29 | Nucleotide sequence of construct 1190 | 69 | CPMV160, 115A |
| 30 | Nucleotide sequence of expression cassette number 1935 from 2X35S promoter to NOS terminator | 70 | CPMV155, 115A |
| 31 | IF-HT1*(-Mprot)-PDI.c | 71 | CPMV150,115A |
| 32 | IF-HT2*(-Mprot)-PDI.c | 72 | CPMV155+ |
| 33 | IF-HT3*(-Mprot)-PDI.c | 73 | CPMV150+ |
| 34 | IF-HT4*(-Mprot)-PDI.c | 74 | CPMV114+ |
| 35 | IF-HT5*(-Mprot)-PDI.c | 75 | CPMV160+, 115A |
| 36 | IF-HT6*(-Mprot)-PDI.c | 76 | CPMV155+, 115A |
| 37 | IF-HT7*(-Mprot)-PDI.c | 77 | CPMV150+, 115A |
| 38 | IF-HT8*(-Mprot)-PDI.c | 78 | Transmembrane domain consensus amino acid |
| 39 | Nucleotide sequence of PDISP/H1 California | 79 | Patatin signal peptide; nucleic acid sequence |
| 40 | Amino acid sequence of PDISP/H1 California | 80 | Patatin signal peptide; amino acid sequence |

[00147] Example 1 - 2X35S/CPMV-HT/PDISP/H3 Victoria/ NOS (Construct number 1391)

[00148] A sequence encoding H3 from Influenza A/Victoria/361/2011 in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/H3 Victoria) was cloned into 2X35S-CPMV-HT-NOS expression system (original CMPV-HT) using the following PCR-based method. A fragment containing the PDISP/H3 Victoria coding sequence was amplified using primers IF-PDI.S1+3c (Figure 6A, SEQ ID NO: 67) and IF-H3V36111.s1-4r (Figure 6B, SEQ ID NO: 17), using PDISP/H3 Victoria sequence (Figure 6C, SEQ ID NO :18) 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 6D) 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 6E (SEQ ID NO: 19). The resulting construct was given number 1391 (Figure 6F, SEQ ID NO: 20). The amino acid sequence of mature H3 from Influenza A/Victoria/361/2011 fused with PDISP is presented in Figure 6G (SEQ ID NO: 21). A representation of plasmid 1391 is presented in Figure 6H.

[00149] Example 2 - 2X35S/CPMV160+/PDISP/H3 Victoria/ NOS (Construct number 1800)

[00150] A sequence encoding H3 from Influenza A/Victoria/361/2011 in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/H3 Victoria) was cloned into 2X35S/CPMV160+/NOS expression system (CPMV160+) using the following PCR-based method. A fragment containing the PDISP/H3 Victoria coding sequence was amplified using primers IF**(SacII)-PDI.s1+4c (Figure 7A, SEQ ID NO: 22) and IF-H3V36111.s1-4r (Figure 7B, SEQ ID NO: 23), using PDISP/H3 Victoria sequence (Figure 7C, SEQ ID NO: 24) as template. The PCR product was cloned in 2X35S/CPMV160+/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 2171 (Figure 7D) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 2171 is an acceptor plasmid intended for "In Fusion" cloning of genes of interest in a CPMV160+ 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: 25). The resulting construct was given number 1800 (Figure 7F, SEQ ID NO: 26). The amino acid sequence of mature H3 from Influenza A/Victoria/361/2011 fused with PDISP is presented in Figure 7G (SEQ ID NO: 27). A representation of plasmid 1800 is presented in Figure 7H.

[00151] Example 3 - 2X35S/CPMV160/PDISP/H3 Victoria/ NOS (Construct number 1935)

[00152] A sequence encoding H3 from Influenza A/Victoria/361/2011 in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/H3 Victoria) was cloned into 2X35S-CPMV160-NOS expression using the following PCR-based method. A fragment containing the PDISP/H3 Victoria coding sequence was amplified using primers IF-CPMV(fl5'UTR)_SpPDI.c (Figure 8A, SEQ ID NO: 28) and IF-H3V36111.s1-4r (Figure 7B, SEQ ID NO: 23), using PDISP/H3 Victoria sequence (Figure 7C, SEQ ID NO : 24) as template. The PCR product was cloned in 2X35S/CPMV160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figure 8B) 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 CPMV160-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 8C (SEQ ID NO: 29). The resulting construct was given number 1935 (Figure 8D, SEQ ID NO: 30). The amino acid sequence of mature H3 from Influenza A/Victoria/361/2011 fused with PDISP is presented in Figure 7G (SEQ ID NO: 27). A representation of plasmid 1935 is presented in Figure 8E.

[00153] Example 4 - Variation of sequence between SacII restriction site and ATG of PDISP/H3 Victoria in 2X35S/CPMV160+/NOS expression system (Constructs number 1992 to 1999)

[00154] Eight constructs comprising sequence variations between SacII restriction site and the ATG of PDISP/H3 Victoria in 2X35S/CPMV160+/NOS expression system were created using the same PCR-based method as for construct no 1800 (see Example 2) using a modified forward primer and keeping all other components the same. Variant HT1* to HT8* were amplified using the primers listed in Figures 9A – 9H, primers:

IF-HT1*(-Mprot)-PDI.c (Figure 9A, SEQ ID NO: 31),

IF-HT2*(-Mprot)-PDI.c (Figure 9B, SEQ ID NO: 32),

IF-HT3*(-Mprot)-PDI.c (Figure 9C, SEQ ID NO: 33)

IF-HT4*(-Mprot)-PDI.c (Figure 9D, SEQ ID NO: 34)

IF-HT5*(-Mprot)-PDI.c (Figure 9E, SEQ ID NO: 35)

IF-HT6*(-Mprot)-PDI.c (Figure 9F, SEQ ID NO: 36)

IF-HT7*(-Mprot)-PDI.c (Figure 9G, SEQ ID NO: 37) and

IF-HT8*(-Mprot)-PDI.c (Figure 9H, SEQ ID NO: 38),

to create construct no 1992 to 1999, respectively. Representations of plasmid 1992 is presented in Figure 9I. Analogous features were used to prepare constructs 1993 -1999.

[00155] Example 5 - 2X35S/CPMV HT (construct no 484) and 2X35S/CPMV160+ (construct no 1897) for PDISP/H1 California

[00156] A coding sequence corresponding to H1 from Influenza A/California/7/2009 in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/H1 California) (Figure 10A, SEQ ID NO: 39) was cloned into original CPMV-HT and CPMV160 using the same PCR-based method as construct 1391 (see Example 1) and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for PDISP/H1 California. The amino acid sequence of mature H1 from Influenza A/California/7/2009 fused with PDISP is presented in Figure 10B (SEQ ID NO: 40). Representations of plasmid 484 and 1897 are presented in Figure 10C and 10D.

[00157] Example 6 - 2X35S/CPMV HT (construct no 489), 2X35S/CPMV160+ (construct no 1880) and 2X35S/CPMV160 (construct no 1885) for H5 Indonesia

[00158] A coding sequence corresponding to native H5 from Influenza A/Indonesia/5/2005 (Figure 11A, SEQ ID NO: 41) was cloned into original CPMV-

HT, CPMV160+ and CPMV160 using the same PCR-based method as construct 1391 (see Example 1), 1800 (see Example 2) and 1935 (see Example 3), respectively but with modified PCR primers specifically designed for H5 Indonesia. The amino acid sequence of native H5 from Influenza A/Indonesia/5/2005 is presented in Figure 11B (SEQ ID NO: 42). Representations of plasmid 489, 1880 and 1885 are presented in Figure 11C to Figure 11E.

[00159] Example 7 - 2X35S/CPMV HT (construct no 2140) and 2X35S/CPMV160+ (construct no 2168) for PDISP-H7 Hangzhou

[00160] A coding sequence corresponding to H7 from Influenza A/Hangzhou/1/2013 in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/H7 Hangzhou) (Figure 12A, SEQ ID NO:43) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1) and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for PDISP/H7 Hangzhou. The amino acid sequence of mature H7 from Influenza A/Hangzhou/1/2013 fused with PDISP is presented in Figure 12B (SEQ ID NO:44). Representations of plasmid 2140 and 2168 are presented in Figure 12C and 12D.

[00161] Example 8 - 2X35S/CPMV HT (construct no 2130) and 2X35S/CPMV160+ (construct no 2188) for PDISP/H7 Hangzhou+H5 Indonesia TMCT

[00162] A chimer hemagglutinin coding sequence corresponding to the ectodomain of H7 from Influenza A/Hangzhou/1/2013 fused to the transmembrane domain and cytoplasmic tail (TMCT) of H5 from influenza A/Indonesia/5/2005 and with the signal peptide of alfalfa protein disulfide isomerase (PDISP/H7 Hangzhou+H5 Indonesia TMCT) (Figure 13A, SEQ ID NO:45) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1) and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for the PDISP/H7 Hangzhou+H5 Indonesia TMCT. The amino acid sequence of H7 Hangzhou+H5 Indonesia TMCT fused with PDISP is presented in Figure 13B (SEQ ID NO: 46). Representations of plasmid 2130 and 2188 are presented in Figure 13C and 13D.

[00163] Example 9 - 2X35S/CPMV HT (construct no 1039) and 2X35S/CPMV160+ (construct no 1937) for PDISP/HA B Brisbane (PrL-)

[00164] A coding sequence corresponding to HA from Influenza B/Brisbane/60/2008 with deleted proteolytic loop (PrL-) (see US provisional application No.61/806,227 Filed March 28, 2013, which is incorporated herein by reference, for additional information re: deleted proteolytic loop regions in HA sequences) in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/HA B Brisbane (PrL-)) (Figure 14A, SEQ ID NO: 47) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1) and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for PDISP/HA B Brisbane (PrL-). The amino acid sequence of mature HA B Brisbane (PrL-) fused with PDISP is presented in Figure 14B (SEQ ID NO: 48). Representations of plasmid 1039 and 1937 are presented in Figure 14C and Figure 14D.

[00165] Example 10 - 2X35S/CPMV HT (construct no 1067) and 2X35S/CPMV160+ (construct no 1977) for PDISP/HA B Brisbane (PrL-)+H1 California TMCT

[00166] A chimer hemagglutinin coding sequence corresponding to the ectodomain of HA from Influenza B/Brisbane/60/08 with deleted proteolytic loop (PrL-) (see US provisional application No.61/806,227 Filed March 28, 2013, which is incorporated herein by reference, for additional information re: deleted proteolytic loop regions in HA sequences) fused to the transmembrane domain and cytoplasmic tail (TMCT) of H1 from influenza A/California/7/2009 and with the signal peptide of alfalfa protein disulfide isomerase (PDISP/HA B Brisbane (PrL-)+H1 California TMCT) (Figure 15A, SEQ ID NO: 49) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1) and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for PDISP/HA B Brisbane (PrL-)+H1 California TMCT. The amino acid sequence of mature HA B Brisbane (PrL-)+H1 California TMCT fused with PDISP is presented in Figure 15B (SEQ ID NO: 50). Representations of plasmid 1067 and 1977 are presented in Figure 15C and Figure 15D.

[00167] Example 11 - 2X35S/CPMV HT (construct no 2072) and 2X35S/CPMV160+ (construct no 2050) for PDISP/HA B Massachussetts (PrL-)

[00168] A coding sequence corresponding to HA from Influenza B/Massachussetts/2/2012 with deleted proteolytic loop (PrL-) (see US provisional application No.61/806,227 Filed March 28, 2013 for additional information re: deleted proteolytic loop regions in HA sequences, which is incorporated herein by reference) in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/HA B Massachussetts (PrL-)) (Figure 16A, SEQ ID NO: 51) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1) and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for PDISP/HA B Massachussetts (PrL-). The amino acid sequence of mature HA B Massachussetts (PrL-) fused with PDISP is presented in Figure 16B (SEQ ID NO: 52). Representations of plasmid 2072 and 2050 are presented in Figure 16C and Figure 16D.

[00169] Example 12 - 2X35S/CPMV HT (construct no 2074) and 2X35S/CPMV160+ (construct no 2060) for PDISP/HA B Massachussetts (PrL-)+H1 California TMCT

[00170] A chimer hemagglutinin coding sequence corresponding to the ectodomain of HA from Influenza B/Massachussetts/2/2012 with deleted proteolytic loop (PrL-) (see US provisional application No.61/806,227 Filed March 28, 2013 for additional information re: deleted proteolytic loop regions in HA sequences, which is incorporated herein by reference) fused to the transmembrane domain and cytoplasmic tail (TMCT) of H1 from influenza A/California/7/2009 and with the signal peptide of alfalfa protein disulfide isomerase (PDISP/HA B Massachussetts (PrL-)+H1 California TMCT) (Figure 17A, SEQ ID NO: 53) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1) and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for PDISP/HA B Massachussetts (PrL-)+H1 California TMCT. The amino acid sequence of mature HA B Massachussetts (PrL-)+H1

California TMCT fused with PDISP is presented in Figure 17B (SEQ ID NO: 54). Representations of plasmid 2074 and 2060 are presented in Figure 17C and 17D.

[00171] Example 13 - 2X35S/CPMV HT (construct no 1445), 2X35S/CPMV160+ (construct no 1820) and CPMV160 (construct no 1975) for HA B Wisconsin (PrL-)

[00172] A coding sequence corresponding to HA from Influenza B/Wisconsin/1/2010 with deleted proteolytic loop (PrL-) (see US provisional application No.61/806,227 Filed March 28, 2013 for additional information re: deleted proteolytic loop regions in HA sequences, which is incorporated herein by reference) with his native signal peptide (HA B Wisconsin (PrL-)) (Figure 18A, SEQ ID NO: 55) was cloned into original CPMV-HT, CPMV160+, and CPMV160 using the same PCR-based method as construct 1391 (see Example 1), 1800 (see Example 2) and 1935 (see Example 3), respectively, but with modified PCR primers specifically designed for HA B Wisconsin (PrL-). The amino acid sequence of HA B Wisconsin (PrL-) with his native signal peptide is presented in Figure 18B (SEQ ID NO: 56). Representations of plasmid 1445, 1820 and 1975 are presented in Figures 18C, 18D and 18E, respectively.

[00173] Example 14 - 2X35S/CPMV HT (construct no 1454) and 2X35S/CPMV160+ (construct no 1893) for HA B Wisconsin (PrL-)+H1 California TMCT

[00174] A chimer hemagglutinin coding sequence corresponding to the ectodomain of HA from Influenza B/ Wisconsin /2/2012 with deleted proteolytic loop (PrL-) (see US provisional application No.61/806,227 Filed March 28, 2013 for additional information re: deleted proteolytic loop regions in HA sequences, which is incorporated herein by reference) fused to the transmembrane domain and cytoplasmic tail (TMCT) of H1 from influenza A/California/7/2009 with the native signal peptide of HA B Wisconsin (HA B Wisconsin (PrL-)+H1 California TMCT) (Figure 19A, SEQ ID NO: 57) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1), and 1800 (see Example 2), respectively, but with modified PCR primers specifically designed for HA B Wisconsin (PrL-)+H1 California TMCT. The amino acid sequence of HA B

Wisconsin (PrL-)+H1 California TMCT is presented in Figure 19B (SEQ ID NO: 58). Representations of plasmid 1454 and 1893 are presented in Figure 19C and 19D.

[00175] Example 15 - 2X35S/CPMV HT (construct no 5001) and 2X35S/CPMV160+ (construct no 2100) for HC rituximab (Rituxan)

[00176] A coding sequence corresponding to the heavy chain of monoclonal IgG1 antibody Rituximab (HC rituximab (Rituxan); Figure 20A, SEQ ID NO: 59) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 (see Example 1), and 1800 (see Example 2), respectively but with modified PCR primers specifically designed for HC rituximab (Rituxan). The amino acid sequence of HC rituximab (Rituxan) is presented in Figure 20B (SEQ ID NO:60). Representations of plasmid 5001 and 2100 are presented in Figure 20C and Figure 20D.

[00177] Example 16 - 2X35S/CPMV HT (construct no 5002) and 2X35S/CPMV160+ (construct no 2109) for PDISP/HC rituximab (Rituxan)

[00178] A coding sequence corresponding to the heavy chain of monoclonal IgG1 antibody Rituximab in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/HC rituximab (Rituxan); Figure 21A, SEQ ID NO: 61) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 and 1800, respectively but with modified PCR primers specifically designed for PDISP/HC rituximab (Rituxan). The amino acid sequence of mature HC rituximab (Rituxan) fused with PDISP is presented in Figure 21B (SEQ ID NO: 62). Representations of plasmid 5002 and 2109 are presented in Figure 21C and Figure 21D.

[00179] Example 17 - 2X35S/CPMV-HT (construct no 5021) and 2X35S/CPMV160+ (construct no 2120) for LC rituximab (Rituxan)

[00180] A coding sequence corresponding to the light chain of monoclonal IgG1 antibody Rituximab (LC rituximab (Rituxan; Figure 22A, SEQ ID NO: 63) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 and 1800, respectively but with modified PCR primers specifically designed for LC rituximab (Rituxan). The amino acid sequence of LC rituximab

(Rituxan) is presented in Figure 22B (SEQ ID NO: 64). Representations of plasmid 5021 and 2120 are presented in Figure 22C and Figure 22D.

[00181] Example 18 - 2X35S/CPMV-HT (construct no 5022) and 2X35S/CPMV160+ (construct no 2129) for PDISP/LC rituximab (Rituxan)

[00182] A coding sequence corresponding to the light chain of monoclonal IgG1 antibody Rituximab in which the native signal peptide has been replaced by that of alfalfa protein disulfide isomerase (PDISP/LC rituximab (Rituxan; Figure 23A, SEQ ID NO: 65) was cloned into original CPMV-HT and CPMV160+ using the same PCR-based method as construct 1391 and 1800, respectively but with modified PCR primers specifically designed for PDISP/LC rituximab (Rituxan). The amino acid sequence of mature LC rituximab (Rituxan) fused with PDISP is presented in Figure 23B (SEQ ID NO: 66). Representations of plasmid 5022 and 2129 are presented in Figure 23C and Figure 23D.

[00183] Example 19 - Agrobacterium transfection

[00184] *Agrobacterium* strain AGL1 was transfected by electroporation with the DNA constructs using the methods described by D'Aoust et al 2008 (Plant Biotechnology Journal 6:930-940). Transfected *Agrobacterium* were grown in YEB medium supplemented with 10 mM 2-(N-morpholino)ethanesulfonic acid (MES), 20 µM acetosyringone, 50 µg/ml kanamycin and 25 µg/ml of carbenicillin pH5.6 to an $OD_{600}$ between 0.6 and 1.6. *Agrobacterium* suspensions were centrifuged before use and resuspended in infiltration medium (10 mM $MgCl_2$ and 10 mM MES pH 5.6).

Preparation of plant biomass, inoculum and agroinfiltration

[00185] *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 25ºC day/20ºC 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.

[00186] *Agrobacteria* transfected with each construct were grown in a YEB medium supplemented with 10 mM 2-(N-morpholino)ethanesulfonic acid (MES), 20 μM acetosyringone, 50 μg/ml kanamycin and 25 μg/ml of carbenicillin pH5.6 until they reached an $OD_{600}$ between 0.6 and 1.6. *Agrobacterium* suspensions were centrifuged before use and resuspended in infiltration medium (10 mM $MgCl_2$ and 10 mM MES pH 5.6) and stored overnight at 4°C. On the day of infiltration, culture batches were diluted in 2.5 culture volumes and allowed to warm before use. Whole plants of *N. benthamiana* were placed upside down in the bacterial suspension in an air-tight stainless steel tank under a vacuum of 20-40 Torr for 2-min. Plants were returned to the greenhouse for a 2-6 day incubation period until harvest.

Leaf harvest and total protein extraction

[00187] Following incubation, the aerial part of plants was harvested, frozen at -80ºC and crushed into pieces. Total soluble proteins were extracted by homogenizing (Polytron) each sample of frozen-crushed plant material in 3 volumes of cold 50 mM Tris pH 8.0, 0.15 M NaCl, 0.1% Triton X-100 and 1 mM phenylmethanesulfonyl fluoride. After homogenization, the slurries were centrifuged at 10,000 g for 10 min at 4ºC and these clarified crude extracts (supernatant) kept for analyses.

[00188] Example 20 - Protein analysis and immunoblotting

[00189] The total protein content of clarified crude extracts was determined by the Bradford assay (Bio-Rad, Hercules, CA) using bovine serum albumin as the reference standard. Proteins were separated by SDS-PAGE and electrotransferred onto polyvinylene difluoride (PVDF) membranes (Roche Diagnostics Corporation, Indianapolis, IN) for immunodetection. Prior to immunoblotting, the membranes were blocked with 5% skim milk and 0.1% Tween-20 in Tris-buffered saline (TBS-T) for 16-18h at 4ºC.

[00190] Immunoblotting was performed with a first incubation with a primary antibody (Table 4 presents the antibodies and conditions used for the detection of each HA), in 2 μg/ml 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).

[00191] Table 4: Electrophoresis conditions, antibodies, and dilutions for immunoblotting of expressed proteins.

| HA subtype | Influenza strain | Electro-phoresis condition | Primary antibody | Dilution | Secondary antibody | Dilution |
|---|---|---|---|---|---|---|
| B | B/Brisbane/60/2008 | Non-reducing | NIBSC 10/146 | 1:20000 | Rabbit anti-sheep (JIR 313-035-045) | 1:10 000 |
| B | B/Wisconsin/1/2010 | Non-reducing | NIBSC 07/356 | 1 :2000 | Rabbit anti-sheep (JIR 313-035-045) | 1:10 000 |
| B | B/ Massachussetts/2/2012 | Non-reducing | NIBSC 07/356 | 1 :2000 | Rabbit anti-sheep (JIR 313-035-045) | 1:10 000 |
| H7 | A/ Hangzhou/1/2013 (H7N9)) | Non-reducing | ITC, IT-003-008M6 | 1:5000 | Goat anti-mouse (JIR 115-035-146) | 1:5 000 |
| H3 | A/Victoria/361/2011 | Non-reducing | TGA, AS400 | 1 :20000 | Rabbit anti-sheep (JIR 313-035-045) | 1:10 000 |
| H1 | A/California/07/2009 (H1N1) | Reducing | NIBSC 11/110 | 1 µg/ml | Rabbit anti-sheep (JIR 313-035-045 | 1:7 500 |
| H5 | A/Indonesia/05/2005 (H5N1) | Reducing | CBER, S-7858 | 1:4000 | Rabbit anti-sheep (JIR 313-035-045) | 1:10 000 |

JIR: Jackson ImmunoResearch, West Grove, PA, USA;

CBER: Center for Biologics Evaluation and Research, Rockville, MD, USA.

Sino: Sino Biological inc., Beijing, China.

TGA: Therapeutic Goods Administration, Australia.

NIBSC: National Institute for Biological Standards and Control, United Kingdom

ITC: Immune Technology Corp., New York, NY, USA

[00192] Example 21 - Hemagglutination assay

[00193] Hemagglutination assay was based on a method described by Nayak and Reichl (2004). Briefly, serial double dilutions of the test samples (100 µL) were made in V-bottomed 96-well microtiter plates containing 100 µL PBS, leaving 100

μL of diluted sample per well. One hundred microliters of a 0.25% turkey red blood cells suspension (Bio Link Inc., Syracuse, NY; for all B strains, H1, H5 and H7) or 0.5% guinea pig red blood cells suspension (for H3) were added to each well, and plates were incubated for 2h at room temperature. The reciprocal of the highest dilution showing complete hemagglutination was recorded as HA activity.

[00194] All citations are hereby incorporated by reference.

[00195] 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. An expression enhancer comprising a CPMV 5'UTR nucleotide sequence consisting of X nucleotides (CMPVX), where X=160, 155, 150, or 114 of SEQ ID NO:1, or consisting of a nucleotide sequence comprising from about 80% to 100% sequence similarity with CMPVX, nucleotides X, where X=160, 155, 150, or 114 of SEQ ID NO:1SEQ ID NO:1.

2. The expression enhancer of claim 1, further comprising a stuffer sequence of from about 1 to 100 nucleotides in length, fused to the 3' end of the CMPV 5'UTR nucleotide sequence (CMPVX+, where X=160, 155, 150, or 114 of SEQ ID NO:1).

3. The expression enhancer of claim 2, wherein the stuffer sequence comprises a plant kozak sequence.

4. The expression enhancer of claim 3, wherein the stuffer sequence further comprises a multiple cloning site.

5. The expression enhancer of claim 3, wherein the kozak sequence is selected from the group of sequences as shown in SEQ ID NO's: 5 - 17.

6. The expression enhancer of claim 2 comprising a nucleotide sequence of SEQ ID NO: 2.

7. The expression enhancer of claim 1 comprising a nucleotide sequence selected from the group of SEQ ID NO: 24, 27, 68, 69, 70 and 71.

8. The expression enhancer of claim 2 comprising a nucleotide sequence selected from the group of SEQ ID NO: 72, 73, 74, 75, 76 and 77.

9. A plant expression system comprising a nucleic acid sequence comprising a regulatory region, operatively linked with the expression enhancer of claim 1and a nucleotide sequence of interest.

10. The plant expression system of claim 9, further comprising a comovirus 3' UTR.

11. The plant expression system of claim 9, further comprising a second nucleic acid sequence, the second nucleic acid sequence encoding a suppressor of silencing.

12. The plant expression system of claim 11 wherein the suppressor of silencing is selected from the group HcPro and p19.

13. The plant expression system of claim 9, wherein the regulatory region is selected from a plastocyanin promoter, a CaMV 35S promoter, a 2x CaMV35S promoter, a CAS promoter, a RbcS promoter, a Ubi promoter, or an actin promoter.

14. The plant expression system of claim 9, wherein the nucleotide sequence of interest encodes a viral protein or an antibody.

15. The plant expression system of claim 14, wherein the viral protein is an influenza hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, and influenza type B hemagglutinin.

16. The plant expression system of claim 14, wherein the nucleotide sequence encoding the viral protein or the antibody comprises a native signal peptide sequence, or a non-native signal peptide.

17.The plant expression system of claim 16, wherein the non-native signal peptide is from protein disulfide isomerase (PDI).

18. A method of producing a protein of interest in a plant or in a portion of a plant comprising, introducing into the plant or in the portion of a plant the plant expression system of claim 9, and incubating the plant or the portion of a plant under conditions that permit expression of the nucleotide sequence encoding the protein of interest.

19. A plant or portion of a plant transiently transfected or stably transformed with the plant expression system of claim 9.

20. A nucleic acid comprising the expression enhancer of claim 1, operatively linked to a nucleotide sequence of interest.

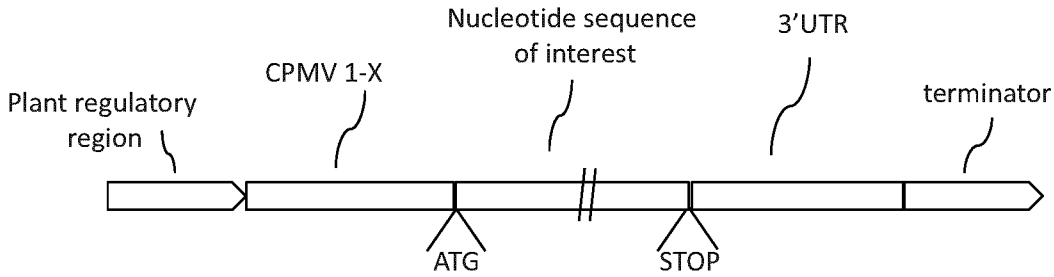
21. The nucleic acid of claim 20, wherein the nucleotide sequence of interest is a influenza hemagglutinin (HA), selected from B HA, C, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, and H16.

22. The nucleic acid of claim 21, wherein the HA is a chimeric HA, wherein a native

trans-membrane domain of the HA is replaced with a heterologous trans-membrane domain.

23. The nucleic acid of claim 22, wherein the heterologous trans-membrane domain is obtained from H1 California.

## Construct comprising CPMV1-X




## Construct comprising CPMV1-X+

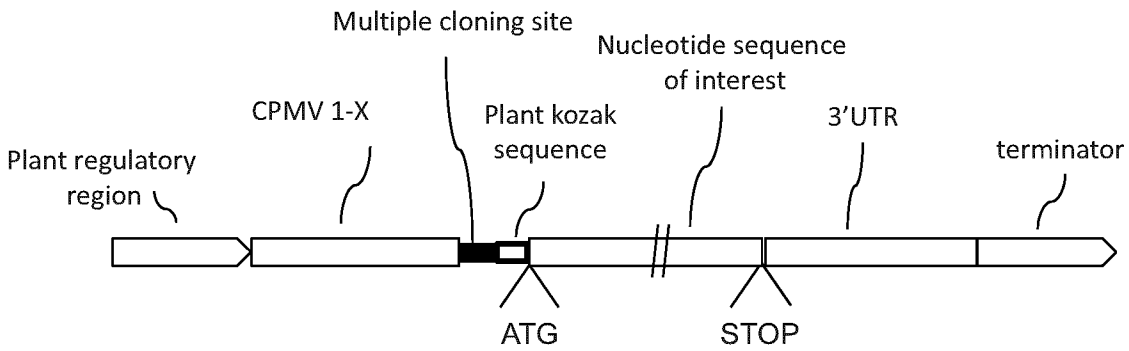



**Figure 1a**

**Constructs comprising CPMV1-X,**

when X=160; **CPMV160**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...CTTCGGCACCAGTACA**[+/-Plant Kozak ATG...GOI]
160

when X=155; **CPMV155**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...CTTCGGCACCA**[Plant Kozak - ATG...GOI]
155

when X=150; **CPMV150**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...CTTCGG**[plant kozak - ATG...GOI]
150

when X=114; **CPMV114**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...CTTTCTTGC**[plant kozak - ATG...GOI]
114

**Figure 1b**

**Constructs comprising CPMV1-X+,**

when X=160; **CPMV160+**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...ACCAGTACA***GGGCCCAATACCGCGGAG*AAAAATG...(GOI)

when X=155; **CPMV155+**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...CTTCGGCACCA***GGGCCCAATACCGCGGAG*AAAAATG...GOI

155

when X=150; **CPMV150+**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...CTTCGG***GGGCCCAATACCGCGGAG*AAAAATG...GOI

150

when X=114; **CPMV114+**

[2X35S...]TTCATTTGGAGAGGTATTAAAA...**(CPMV_5'UTR)...CTTTCTTGC***GGGCCCAATACCGCGGAG*AAAAATG...GOI

**Figure 1c**

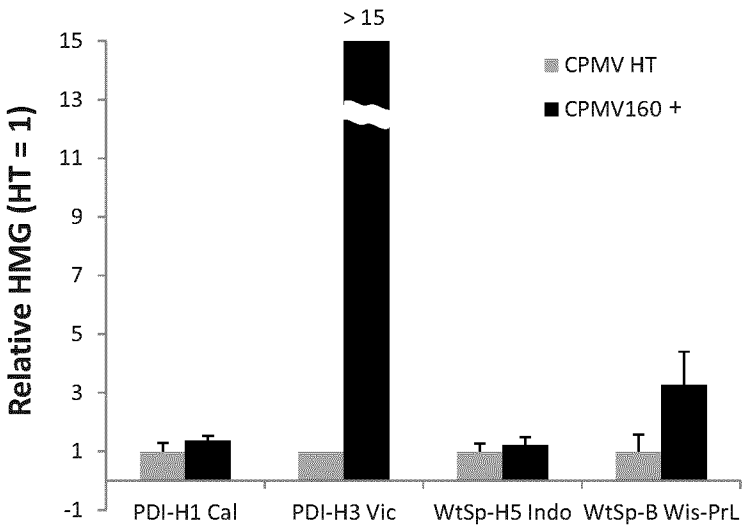

> 15
15
13
11
9
7
5
3
1
-1
Relative HMG (HT = 1)
CPMV HT
CPMV160 +
PDI-H1 Cal
PDI-H3 Vic
WtSp-H5 Indo
WtSp-B Wis-PrL

**Figure 2**

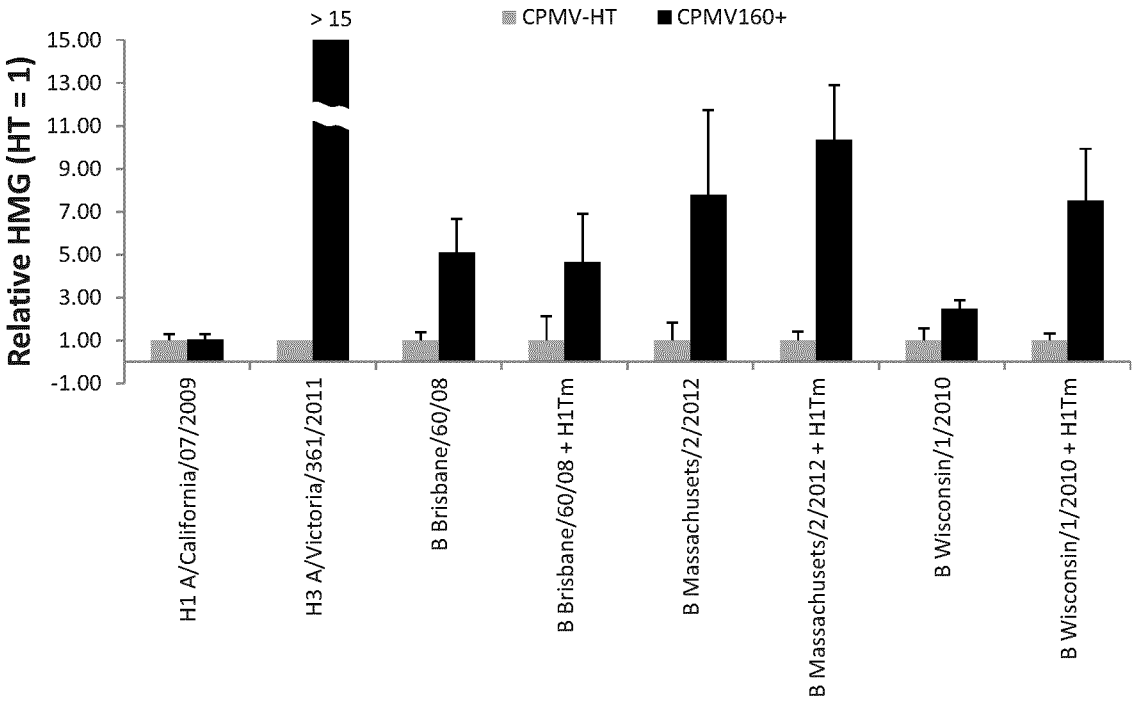

> 15
CPMV-HT
CPMV160+
Relative HMG (HT = 1)
15.00
13.00
11.00
9.00
7.00
5.00
3.00
1.00
-1.00
H1 A/California/07/2009
H3 A/Victoria/361/2011
B Brisbane/60/08
B Brisbane/60/08 + H1Tm
B Massachusets/2/2012
B Massachusets/2/2012 + H1Tm
B Wisconsin/1/2010
B Wisconsin/1/2010 + H1Tm

**Figure 3**

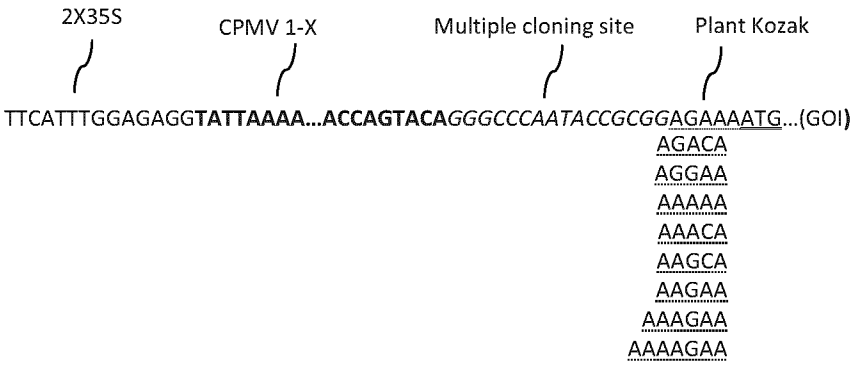



**Figure 4a**

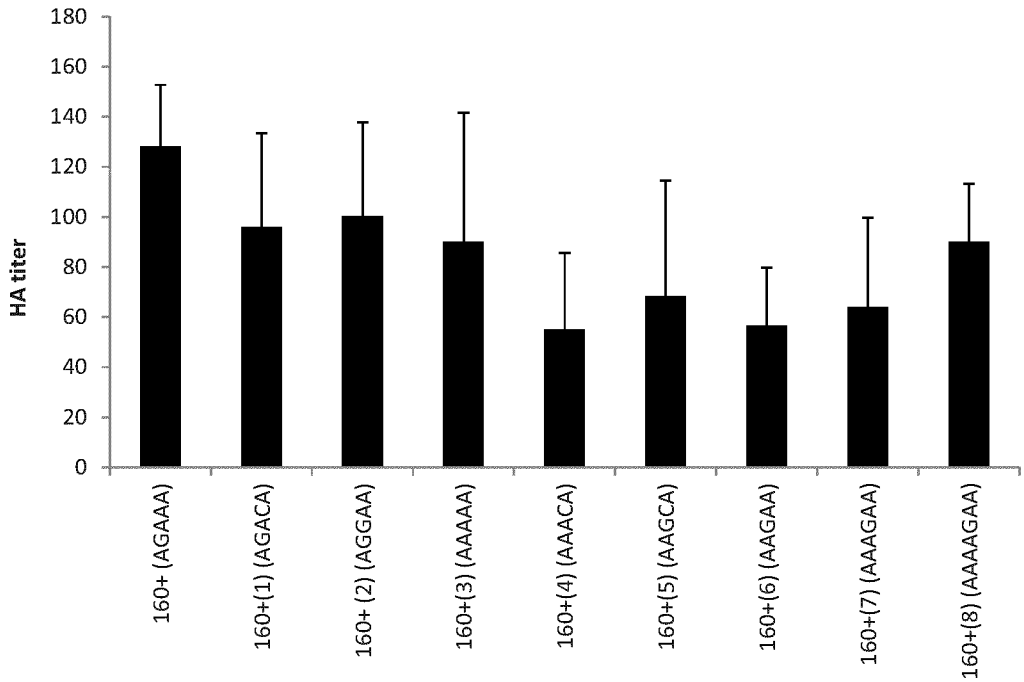



**Figure 4b**

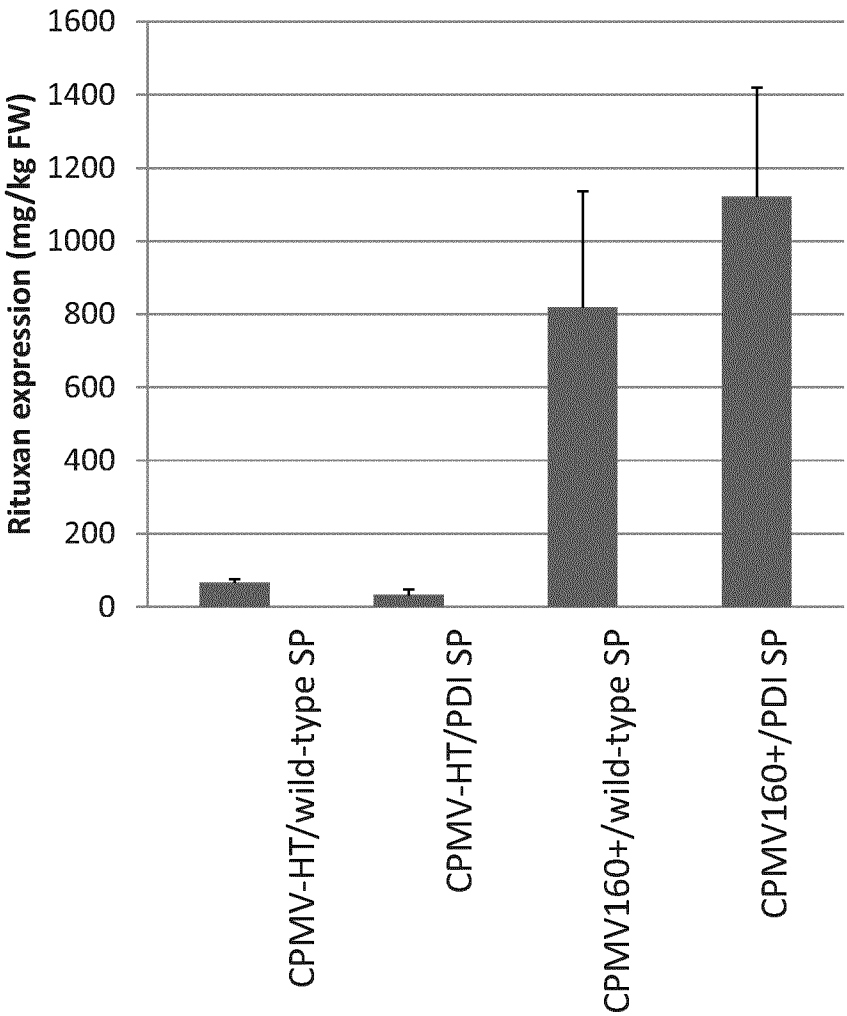

Rituxan expression (mg/kg FW)
1600
1400
1200
1000
800
600
400
200
0
CPMV-HT/wild-type SP
CPMV-HT/PDI SP
CPMV160+/wild-type SP
CPMV160+/PDI SP

**Figure 5**

**Figure 6:** A-2X35S/CPMV-HT/ PDISP/H3 Victoria/ NOS (Construct number 1391)

**Figure 6A** (SEQ ID NO: 67) IF-PDI.S1+3c

AAATTTGTCGGGCCCATGGCGAAAAACGTTGCGATTTTCGGCTTATTG

**Figure 6B** (SEQ ID NO: 17) IF-H3V36111.s1-4r

ACTAAAGAAAATAGGCCTTCAAATGCAAATGTTGCACCTAATGTTGCCCTT

**Figure 6C** (SEQ ID NO :18) Nucleotide sequence of PDISP/H3 Victoria.

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
CCAAAAACTTCCTGGAAATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAA
CGATAGTGAAAACAATCACGAATGACCAAATTGAAGTTACTAATGCTACTGAGCTGGTTCAGAATTCCTCA
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GGGAGACCCTCAGTGTGATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACA
GCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGGCACACTG
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GACCAAATCTTCCTGTATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAGCTGT
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TAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATA
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AAATGGAAGCATTCCCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGAT
ATGTTAAGCAAAGCACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAGGCATA
TTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCA
TCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATG
GGAAGCTGAATCGATTGATCGGGAAAACCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTC
GAAGGGAGAATTCAGGACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGA
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GACAATGCCTGCATAGGATCAATCAGAAATGGAACTTATGACCACGATGTATACAGAGATGAAGCATTAAA
CAACCGGTTCCAGATCAAGGGAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATCCTATGGATTTCCTTTG
CCATATCATGTTTTTTGCTTTGTGTTGCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAGGGCAACATT
AGGTGCAACATTTGCATTTGA

**Figure 6D** Schematic representation of construct 1191.

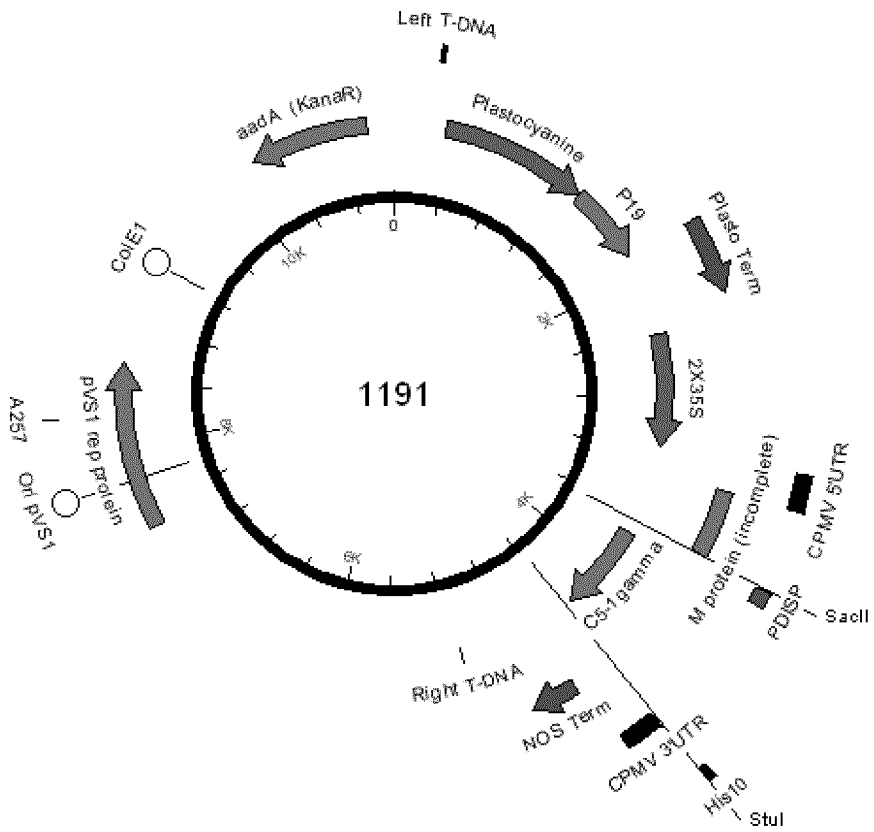

**Figure 6E** (SEQ ID NO: 19) Construct 1191 from left to right t-DNA borders (underlined). 2X35S/CPMV-HT/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAA
TGTACTGAATTAACGCCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTCAAAAACCATAAAAG
TTTAAGTTAGCAAGTGTGTACATTTTTACTTGAACAAAAATATTCACCTACTACTGTTATAAATCATTATT
AAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAGTGATATTTTGACAACAATTTTGTTGCAA
CATTTGAGAAAATTTTGTTGTTCTCTCTTTTCATTGGTCAAAAACAATAGAGAGAGAAAAAGGAAGAGGGA
GAATAAAAACATAATGTGAGTATGAGAGAGAAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATAT
CATTGAGGAATTTGACAAAAGCTACACAAATAAGGGTTAATTGCTGTAAATAAATAAGGATGACGCATTAG
AGAGATGTACCATTAGAGAATTTTTGGCAAGTCATTAAAAAGAAAGAATAAATTATTTTTAAAATTAAAAG
TTGAGTCATTTGATTAAACATGTGATTATTTAATGAATTGATGAAAGAGTTGGATTAAAGTTGTATTAGTA
ATTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATATATTGCCCCATAGAGTCAGTTAA
CTCATTTTTATATTTCATAGATCAAATAAGAGAAATAACGGTATATTAATCCCTCCAAAAAAAAAAAAACGG
TATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTAGGAGGATAACATCCAATCCA
ACCAATCACAACAATCCTGATGAGATAACCCACTTTAAGCCCACGCATCTGTGGCACATCTACATTATCTA
AATCACACATTCTTCCACACATCTGAGCCACACAAAAACCAATCCACATCTTTATCACCCATTCTATAAAA
AATCACACTTTGTGAGTCTACACTTTGATTCCCTTCAAACACATACAAAGAGAAGAGACTAATTAATTAAT
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TTGGGATGGAGGATCAGGAGGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGAGT
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GGGAAAGTTGTATTTAAGAGATATCTCAGATACGACAGGACGGAAGCTTCACTGCACAGAGTCCTTGGATC
TTGGACGGGAGATTCGGTTAACTATGCAGCATCTCGATTTTTCGGTTTCGACCAGATCGGATGTACCTATA
GTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGTCGCGAACTCTTCAGCATCTCTGTGAGATG
GCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAAGTGGAAAGTAATGTATCAAGAGGATG
CCCTGAAGGTACTCAAACCTTCGAAAAAGAAAGCGAGTAAGTTAAAATGCTTCTTCGTCTCCTATTTATAA
TATGGTTTGTTATTGTTAATTTTGTTCTTGTAGAAGAGCTTAATTAATCGTTGTTGTTATGAAATACTATT
TGTATGAGATGAACTGGTGTAATGTAATTCATTTACATAAGTGGAGTCAGAATCAGAATGTTTCCTCCATA
ACTAACTAGACATGAAGACCTGCCGCGTACAATTGTCTTATATTTGAACAACTAAAATTGAACATCTTTTG
CCACAACTTTATAAGTGGTTAATATAGCTCAAATATATGGTCAAGTTCAATAGATTAATAATGGAAATATC
AGTTATCGAAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCCCTTTGATAAA
TGATAGTACACCAATTAGGAAGGAGCATGCTCGCCTAGGAGATTGTCGTTTCCCGCCTTCAGTTTGCAAGC
TGCTCTAGCCGTGTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGGAATTACTAGCGCGTGTCGACA
AGCTTGCATGCCGGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCT
CAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGC
CCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGA
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TCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGA
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CACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATC
CTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTT
TGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAA
ACTTCTCTCTTGTCTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACAACG
TTTTCTTTCACTGAAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTA
CTTGTCCTATTCTTGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGTTCAGCCCCATACA
TTACTTGTTACGATTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTTGC
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TTCCCGTGGTTTTCGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTGTCGGGCCCG
CGGATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTT
CGCCTGCAGGCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAA
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CTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTGAGCA
GCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGC

AGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGA
AGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCA
CGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAG
GTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCC
CATCATGCACCAGGACTGGCTCAATGGCAAGGAGCGATCGCTCACCATCACCATCACCATCACCATCACCA
TTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTT
TCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCT
TCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAA
GCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCT
TGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGT
TATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATA
TAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCTAGAGTCTCAAGCT
TGGCGCGCCCACGTGACTAGTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTA
CCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGAT
CGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGATTGTCGT
TTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAACCTAAGAGAAAAGAGCG
TTTA

**Figure 6E** (SEQ ID NO: 19) con't

**Figure 6F** (SEQ ID NO: 20) Expression cassette number 1391 from 2X35S promoter to NOS terminator. PDISP/H3 Victoria nucleotide sequence is underlined; CPMV 5'UTR in bold; incomplete M protein in italics

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAG
GGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTC
ACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCC
ATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAA
AGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTG
TCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTA
ATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGA
AGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAG
CAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCC
TTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGG**TATTAAAATCTTAATAGGTTTTGATAAAAGCGAA**
**CGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGT**
**CTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACA***ACGTTTTCTTTCACTG*
*AAGCGAAATCAAAGATCTCTTTGTGGACACGTAGTGCGGCGCCATTAAATAACGTGTACTTGTCCTATTCT*
*TGTCGGTGTGGTCTTGGGAAAAGAAAGCTTGCTGGAGGCTGCTGTTCAGCCCCATACATTACTTGTTACGA*
*TTCTGCTGACTTTCGGCGGGTGCAATATCTCTACTTCTGCTTGACGAGGTATTGTTGCCTGTACTTCTTTC*
*TTCTTCTTCTTGCTGATTGGTTCTATAAGAAATCTAGTATTTTCTTTGAAACAGAGTTTTCCCGTGGTTTT*
*CGAACTTGGAGAAAGATTGTTAAGCTTCTGTATATTCTGCCCAAATTTG*TCGGGCCCATGGCGAAAAACGT
TGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCCAAAAACTTCCTG
GAAATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACA
ATCACGAATGACCAAATTGAAGTTACTAATGCTACTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATG
CGACAGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGT
GTGATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACAGCAACTGTTACCCT
TATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTTTAACAATGA
AAGCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAGATCTAATAATAGTT
TCTTTAGTAGATTAAATTGGTTGACCCACTTAAACTTCAAATACCCAGCATTGAACGTGACTATGCCAAAC
AATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCCT
GTATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAGCTGTAATCCCGAATATCG
GATCTAGACCCAGAATAAGGAATATCCCTAGCAGAATAAGCATCTATTGGACAATAGTAAAACCGGGAGAC
ATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAG
CTCAATAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTC
CCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAGC
ACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATAGC
GGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCATCAAAATTCTGAGG
GAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGA
TTGATCGGGAAAACCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCA
GGACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCC
TGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAA
CTAAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAAAATATACCACAAATGTGACAATGCCTGCAT
AGGATCAATCAGAAATGGAACTTATGACCACGATGTATACAGAGATGAAGCATTAAACAACCGGTTCCAGA
TCAAGGGAGTTGAGCTGAAGTCAGGGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTT
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CATTTGAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCATTTCTATGTTTGGTGAGCGG
TTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTC
CCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATAT
CAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGG
TCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGA
CGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAA
ATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

**Figure 6G** ( SEQ ID NO: 21) Amino acid sequence of PDISP/H3 Victoria

MAKNVAIFGLLFSLLVLVPSQIFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQNSS
IGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTL
EFNNESFNWTGVTQNGTSSACIRRSNNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDK
DQIFLYAQSSGRITVSTKRSQQAVIPNIGSRPRIRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKI
RSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQSTLKLATGMRNVPEKQTRGI
FGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEV
EGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKC
DNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVALLGFIMWACQKGNI
RCNICI*

**Figure 6H**

Schematic representation of construct number 1391

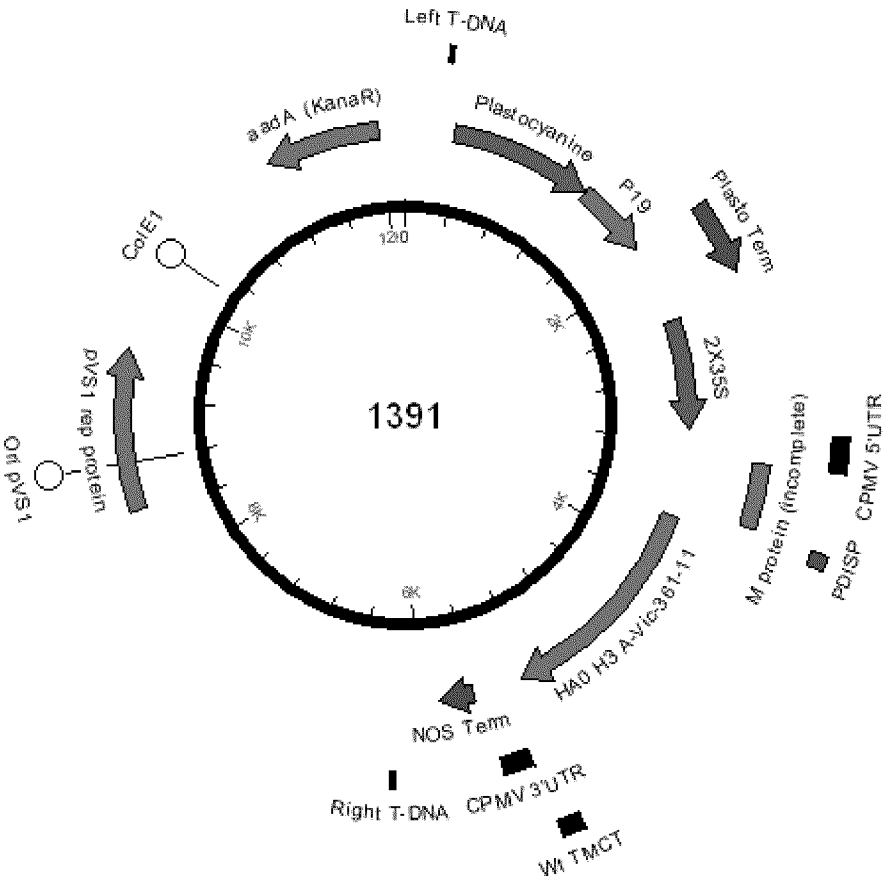

**Figure 7:** components for 2X35S/CPMV160/PDISP/H3 Victoria/ NOS (Construct number 1800)

**Figure 7A** (SEQ ID NO: 22) IF**(SacII)-PDI.s1+4c

ACAGGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 7B** (SEQ ID NO: 23) IF-H3V36111.s1-4r

ACTAAAGAAAATAGGCCTTCAAATGCAAATGTTGCACCTAATGTTGCCCTT

**Figure 7C**

Schematic representation of construct 2171. SacII and StuI restriction enzyme sites used for plasmid linearization are annotated on the representation.

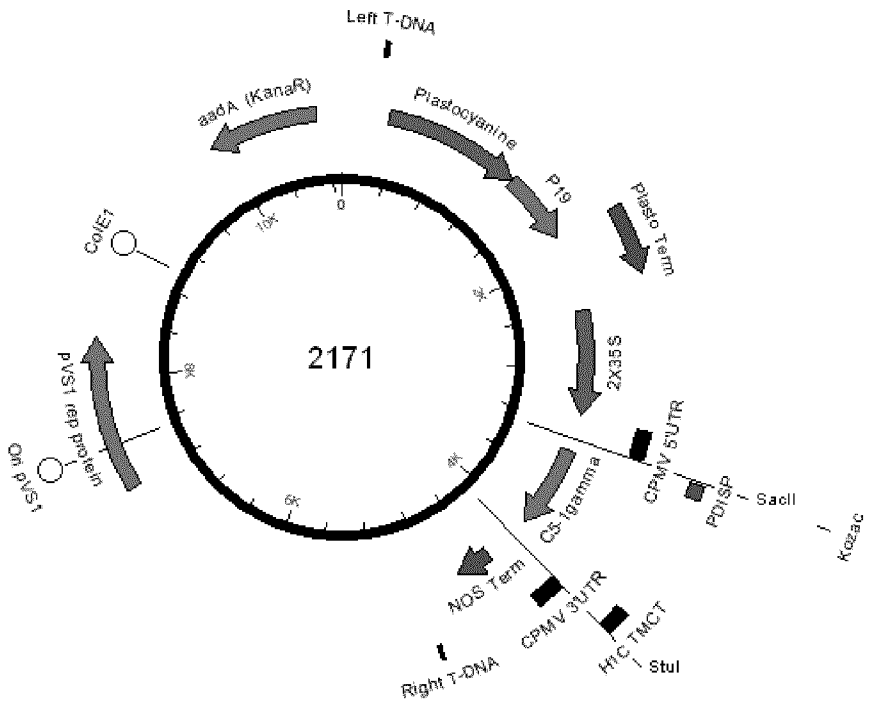

**Figure 7D** (SEQ ID NO: 25) Construct 2171 from left to right t-DNA borders (underlined). 2X35S/CPMV160/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAA
TGTACTGAATTAACGCCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTCAAAAACCATAAAAG
TTTAAGTTAGCAAGTGTGTACATTTTTACTTGAACAAAAATATTCACCTACTACTGTTATAAATCATTATT
AAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAGTGATATTTTGACAACAATTTTGTTGCAA
CATTTGAGAAAATTTTGTTGTTCTCTCTTTTCATTGGTCAAAAACAATAGAGAGAGAAAAAGGAAGAGGGA
GAATAAAAACATAATGTGAGTATGAGAGAGAAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATAT
CATTGAGGAATTTGACAAAAGCTACACAAATAAGGGTTAATTGCTGTAAATAAATAAGGATGACGCATTAG
AGAGATGTACCATTAGAGAATTTTTGGCAAGTCATTAAAAAGAAAGAATAAATTATTTTTAAAATTAAAAG
TTGAGTCATTTGATTAAACATGTGATTATTTAATGAATTGATGAAAGAGTTGGATTAAAGTTGTATTAGTA
ATTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATATATTGCCCCATAGAGTCAGTTAA
CTCATTTTTATATTTCATAGATCAAATAAGAGAAATAACGGTATATTAATCCCTCCAAAAAAAAAAAACGG
TATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTAGGAGGATAACATCCAATCCA
ACCAATCACAACAATCCTGATGAGATAACCCACTTTAAGCCCACGCATCTGTGGCACATCTACATTATCTA
AATCACACATTCTTCCACACATCTGAGCCACACAAAAACCAATCCACATCTTTATCACCCATTCTATAAAA
AATCACACTTTGTGAGTCTACACTTTGATTCCCTTCAAACACATACAAAGAGAAGAGACTAATTAATTAAT
TAATCATCTTGAGAGAAAATGGAACGAGCTATACAAGGAAACGACGCTAGGGAACAAGCTAACAGTGAACG
TTGGGATGGAGGATCAGGAGGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGAGT
GGCGGCTACATAACGATGAGACGAATTCGAATCAAGATAATCCCCTTGGTTTCAAGGAAAGCTGGGGTTTC
GGGAAAGTTGTATTTAAGAGATATCTCAGATACGACAGGACGGAAGCTTCACTGCACAGAGTCCTTGGATC
TTGGACGGGAGATTCGGTTAACTATGCAGCATCTCGATTTTTCGGTTTCGACCAGATCGGATGTACCTATA
GTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGTCGCGAACTCTTCAGCATCTCTGTGAGATG
GCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAAGTGGAAAGTAATGTATCAAGAGGATG
CCCTGAAGGTACTCAAACCTTCGAAAAAGAAAGCGAGTAAGTTAAAATGCTTCTTCGTCTCCTATTTATAA
TATGGTTTGTTATTGTTAATTTTGTTCTTGTAGAAGAGCTTAATTAATCGTTGTTGTTATGAAATACTATT
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AGTTATCGAAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCCCTTTGATAAA
TGATAGTACACCAATTAGGAAGGAGCATGCTCGCCTAGGAGATTGTCGTTTCCCGCCTTCAGTTTGCAAGC
TGCTCTAGCCGTGTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGGAATTACTAGCGCGTGTCGACA
AGCTTGCATGCCGGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCT
CAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGC
CCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGA
TAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGA
GCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAG
CACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTT
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TCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAAC
CACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATC
CTTCGCAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGTATTAAAATCTTAATAGGTTT
TGATAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAA
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GGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGT
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TCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTT
GCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCAT
ATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATTACTC
TGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTT
GTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTC
AGTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGACGTCCAGATTTTGGCGATCTA
TTCAACTGTCGCCAGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATG
GGTCTCTACAGTGTAGAATATGTATTTAAAGGCCTATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTG
CATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTG
AGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAA
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ATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACAT
TTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGT
TACTAGATCTCTAGAGTCTCAAGCTTGGCGCGCCCACGTGACTAGTGGCACTGGCCGTCGTTTTACAACGT
CGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCG
TAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCA
GCTTGAGCTTGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGG
CGGGTAAACCTAAGAGAAAAGAGCGTTTA

**Figure 7D** (SEQ ID NO: 25) con't

**Figure 7E** (SEQ ID NO: 26) Expression cassette number 1800 from 2X35S promoter to NOS terminator. PDISP/H3 Victoria nucleotide sequence is underlined; 5'UTR in bold; plant kozak sequence double underline

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTG
AGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGAT
AGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGAC
AGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAG
TGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCA
AAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTT
ATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATG
CCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTC
TTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT
TCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGG**TATTAAAATCTTAATAGGTTTTGATAAAAGCGAACGTGGGGAA**
**ACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGCGTGAGCGA**
**TCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACA**GGGCCCAATACCGCGGAGAAAATGGCGAAAAACGTTGCGAT
TTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCCAAAAACTTCCTGGAAATGACAACAGC
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AAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGTGATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTT
GAACGAAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCAT
CCGGCACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAG
GAGATCTAATAATAGTTTCTTTAGTAGATTAAATTGGTTGACCCACTTAAACTTCAAATACCCAGCATTGAACGTGACT
ATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCC
TGTATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAGCTGTAATCCCGAATATCGGATCTAG
ACCCAGAATAAGGAATATCCCTAGCAGAATAAGCATCTATTGGACAATAGTAAAACCGGGAGACATACTTTTGATTAAC
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AAACAAACTAGAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACG
GTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAA
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AGAATTCAGGACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCC
TGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTAAGGGA
AAATGCTGAGGATATGGGCAATGGTTGTTTCAAAATATACCACAAATGTGACAATGCCTGCATAGGATCAATCAGAAAT
GGAACTTATGACCACGATGTATACAGAGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGCTGAAGTCAG
GGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTTTTGCTTTGTGTTGCTTTGTTGGGGTTCATCAT
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TTCGGTGTGCATTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGT
GAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAAAAAAAAAAAAAAAAGA
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TTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGAC
GTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGC
GCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

**Figure 7F**

Schematic representation of construct number 1800

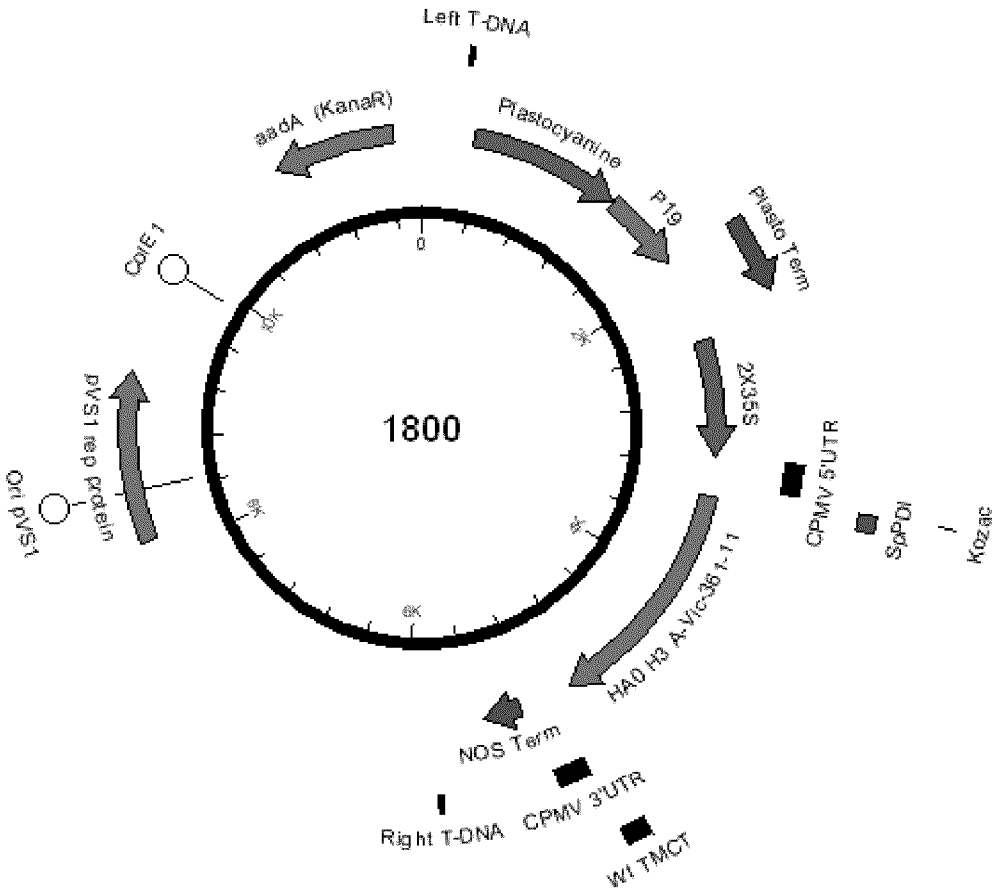

**Figure 8** Components for 2X35S/CPMV160 PDISP/H3 Victoria/ NOS (Construct number 1935)

**Figure 8A** (SEQ ID NO: 28) IF-CPMV(fl5'UTR)_SpPDI.c

TCGTGCTTCGGCACCAGTACAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 8B**

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

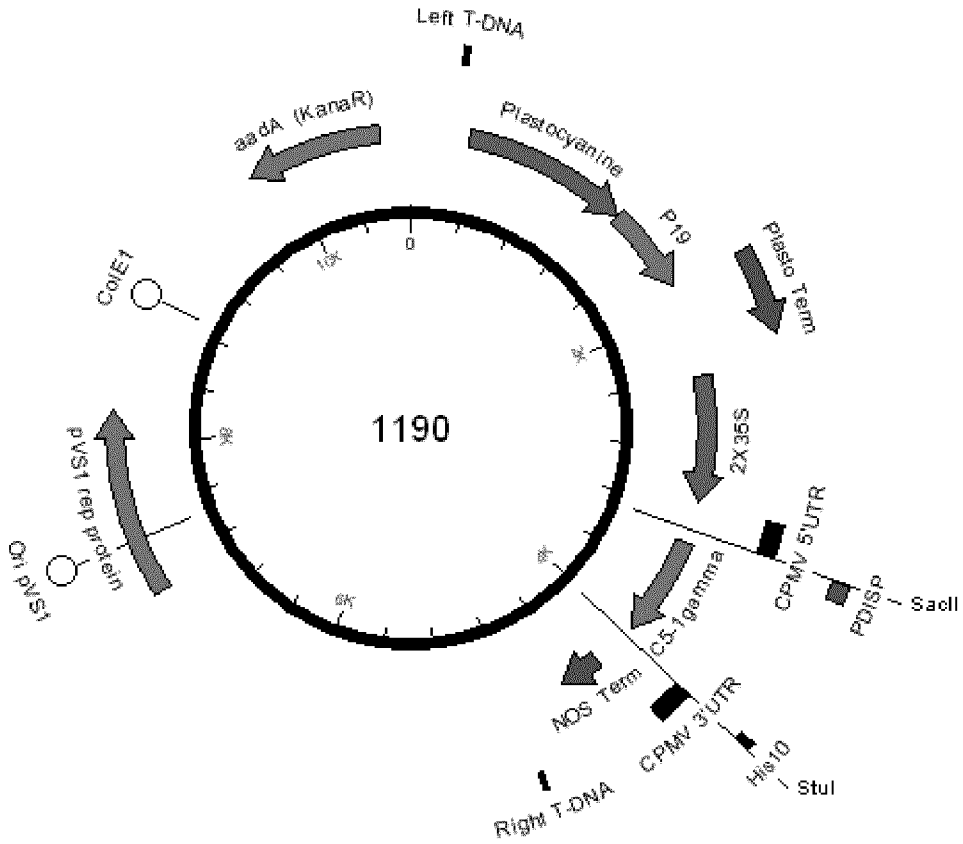

**Figure 8C** (SEQ ID NO: 29) Construct 1190 from left to right t-DNA borders (underlined). 2X35S/CPMV-HT(fl5'UTR)/NOS with Plastocyanine-P19-Plastocyanine silencing inhibitor expression cassette

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAA
TGTACTGAATTAACGCCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTCAAAAACCATAAAAG
TTTAAGTTAGCAAGTGTGTACATTTTTACTTGAACAAAAATATTCACCTACTACTGTTATAAATCATTATT
AAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAGTGATATTTTGACAACAATTTTGTTGCAA
CATTTGAGAAAATTTTGTTGTTCTCTCTTTTCATTGGTCAAAAACAATAGAGAGAGAAAAAGGAAGAGGGA
GAATAAAAACATAATGTGAGTATGAGAGAGAAAGTTGTACAAAAGTTGTACCAAAATAGTTGTACAAATAT
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ATTAGAATTTGGTGTCAAATTTAATTTGACATTTGATCTTTTCCTATATATTGCCCCATAGAGTCAGTTAA
CTCATTTTTATATTTCATAGATCAAATAAGAGAAATAACGGTATATTAATCCCTCCAAAAAAAAAAAACGG
TATATTTACTAAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCCCGTAGGAGGATAACATCCAATCCA
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TAATCATCTTGAGAGAAAATGGAACGAGCTATACAAGGAAACGACGCTAGGGAACAAGCTAACAGTGAACG
TTGGGATGGAGGATCAGGAGGTACCACTTCTCCCTTCAAACTTCCTGACGAAAGTCCGAGTTGGACTGAGT
GGCGGCTACATAACGATGAGACGAATTCGAATCAAGATAATCCCCTTGGTTTCAAGGAAAGCTGGGGTTTC
GGGAAAGTTGTATTTAAGAGATATCTCAGATACGACAGGACGGAAGCTTCACTGCACAGAGTCCTTGGATC
TTGGACGGGAGATTCGGTTAACTATGCAGCATCTCGATTTTTCGGTTTCGACCAGATCGGATGTACCTATA
GTATTCGGTTTCGAGGAGTTAGTATCACCGTTTCTGGAGGGTCGCGAACTCTTCAGCATCTCTGTGAGATG
GCAATTCGGTCTAAGCAAGAACTGCTACAGCTTGCCCCAATCGAAGTGGAAAGTAATGTATCAAGAGGATG
CCCTGAAGGTACTCAAACCTTCGAAAAAGAAAGCGAGTAAGTTAAAATGCTTCTTCGTCTCCTATTTATAA
TATGGTTTGTTATTGTTAATTTTGTTCTTGTAGAAGAGCTTAATTAATCGTTGTTGTTATGAAATACTATT
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CCACAACTTTATAAGTGGTTAATATAGCTCAAATATATGGTCAAGTTCAATAGATTAATAATGGAAATATC
AGTTATCGAAATTCATTAACAATCAACTTAACGTTATTAACTACTAATTTTATATCATCCCCTTTGATAAA
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TGCTCTAGCCGTGTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGGAATTACTAGCGCGTGTCGACA
AGCTTGCATGCCGGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCT
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AGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGC
ACCAGGACTGGCTCAATGGCAAGGAGCGATCGCTCACCATCACCATCACCATCACCATCACCATTAAAGGC
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CAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAG
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ACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGA
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AAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCTAGAGTCTCAAGCTTGGCGCGC
CCACGTGACTAGTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTT
AATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTC
CCAACAGTTGCGCAGCCTGAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGATTGTCGTTTCCCGCC
TTCAGTTTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAACCTAAGAGAAAAGAGCGTTTA

**Figure 8C** (SEQ ID NO: 29) con't

**Figure 8D** (SEQ ID NO: 30) Expression cassette number 1935 from 2X35S promoter to NOS terminator. PDISP/H3 Victoria nucleotide sequence is underlined. 5"UTR is shown in bold

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAG
GGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTC
ACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCC
ATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAA
AGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTG
TCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTA
ATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGA
AGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTG
GTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAG
CAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCC
TTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGG**TATTAAAATCTTAATAGGTTTTGATAAAAGCGAA**
**CGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTCTCTTAAAGCAAACTTCTCTCTTGT**
**CTTTCTTGCGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGGCACCAGTACA**ATGGCGAAAAACGTTG
CGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGCCCAAAAACTTCCTGGA
AATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAAT
CACGAATGACCAAATTGAAGTTACTAATGCTACTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATGCG
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GATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACAGCAACTGTTACCCTTA
TGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGGCACACTGGAGTTTAACAATGAAA
GCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAGATCTAATAATAGTTTC
TTTAGTAGATTAAATTGGTTGACCCACTTAAACTTCAAATACCCAGCATTGAACGTGACTATGCCAAACAA
TGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCCTGT
ATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAGCTGTAATCCCGAATATCGGA
TCTAGACCCAGAATAAGGAATATCCCTAGCAGAATAAGCATCTATTGGACAATAGTAAAACCGGGAGACAT
ACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCT
CAATAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTCCC
AATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAGCAC
TCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATAGCGG
GTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCATCAAAATTCTGAGGGA
AGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATT
GATCGGGAAAACCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCAGG
ACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTGCCCTG
GAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACT
AAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAAAATATACCACAAATGTGACAATGCCTGCATAG
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TTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCC
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TTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACG
TTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAAT
ATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

**Figure 8E**

Schematic representation of construct number 1935

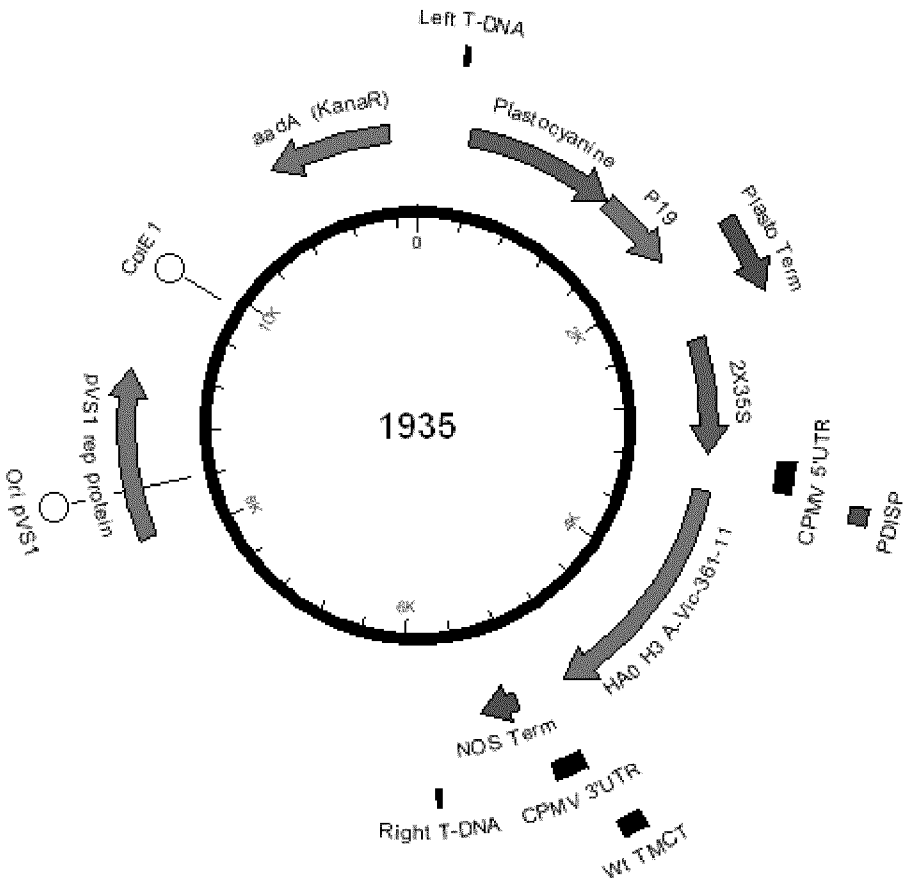

**Figure 9** Variation of sequence between SacII restriction site and ATG of PDISP/H3 Victoria in 2X35S/CPMV HT*(-Mprot)/NOS expression system

**Figure 9A** (SEQ ID NO: 31) IF-HT1*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAGACAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9B** (SEQ ID NO: 32) IF-HT2*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAGGAAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9C** (SEQ ID NO: 33) IF-HT3*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAAAAAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9D** (SEQ ID NO: 34) IF-HT4*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAAACAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9E** (SEQ ID NO: 35) IF-HT5*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAAGCAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9F** (SEQ ID NO: 36) IF-HT6*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAAGAAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9G** (SEQ ID NO: 37) IF-HT7*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAAAGAAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9H** (SEQ ID NO: 38) IF-HT8*(-Mprot)-PDI.c (modified sequence from original 1800 construct underlined)

ACAGGGCCCAATACCGCGGAAAAGAAATGGCGAAAAACGTTGCGATTTTCGGCT

**Figure 9I**

Schematic representation of construct number 1992. Analogous features were used to prepare constructs 1993 -1999.

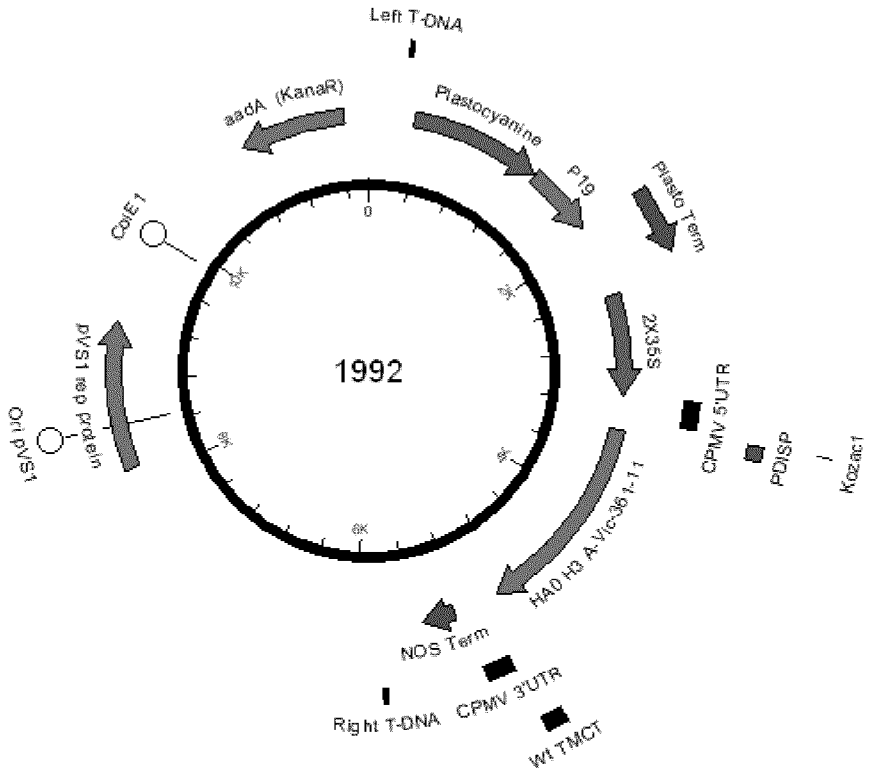

**Figure 10** 2X35S/CPMV HT (construct no 484), and HT*(-Mprot) (construct no 1897) for PDISP/H1 California/NOS

**Figure 10A** (SEQ ID NO: 39) Nucleotide sequence of PDISP/H1 California.

```
ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
TGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAGAATG
TAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCATAACGGGAAACTATGCAAACTAAGAGGGGTA
GCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAATCACTCTC
CACAGCAAGCTCATGGTCCTACATTGTGGAAACACCTAGTTCAGACAATGGAACGTGTTACCCAGGAGATT
TCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCATCATTTGAAAGGTTTGAGATATTCCCC
AAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCTCATGCTGGAGCAAA
AAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAGCTCAGCAAATCCTACA
TTAATGATAAAGGGAAAGAAGTCCTCGTGCTATGGGGCATTCACCATCCATCTACTAGTGCTGACCAACAA
AGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGTCATCAAGATACAGCAAGAAGTTCAAGCCGGA
AATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACTATTACTGGACACTAGTAGAGCCGG
GAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGATATGCATTCGCAATGGAAAGAAAT
GCTGGATCTGGTATTATCATTTCAGATACACCAGTCCACGATTGCAATACAACTTGTCAAACACCCAAGGG
TGCTATAAACACCAGCCTCCCATTTCAGAATATACATCCGATCACAATTGGAAAATGTCCAAAATATGTAA
AAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATATCCCGTCTATTCAATCTAGAGGACTATTTGGG
GCCATTGCCGGTTTCATTGAAGGGGGGTGGACAGGGATGGTAGATGGATGGTACGGTTATCACCATCAAAA
TGAGCAGGGGTCAGGATATGCAGCCGACCTGAAGAGCACACAGAATGCCATTGACGAGATTACTAACAAAG
TAAATTCTGTTATTGAAAAGATGAATACACAGTTCACAGCAGTAGGTAAAGAGTTCAACCACCTGGAAAAA
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GGTTCTATTGGAAAATGAAAGAACTTTGGACTACCACGATTCAAATGTGAAGAACTTATATGAAAAGGTAA
GAAGCCAGCTAAAAAACAATGCCAAGGAAATTGGAAACGGCTGCTTTGAATTTTACCACAAATGCGATAAC
ACGTGCATGGAAAGTGTCAAAAATGGGACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAAACAG
AGAAGAAATAGATGGGGTAAAGCTGGAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCG
CCAGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAG
TGTAGAATATGTATTTAA
```

**Figure 10B** (SEQ ID NO: 40) Amino acid sequence of PDISP/H1 California.

```
MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGV
APLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERFEIFP
KTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQ
SLYQNADAYVFVGSSRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERN
AGSGIIISDTPVHDCNTTCQTPKGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRGLFG
AIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADLKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEK
RIENLNKKVDDGFLDIWTYNAELLVLLENERTLDYHDSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHKCDN
TCMESVKNGTYDYPKYSEEAKLNREEIDGVKLESTRIYQILAIYSTVASSLVLVVSLGAISFWMCSNGSLQ
CRICI*
```

**Figure 10C**

Schematic representation of construct number 484 (2X35S/CPMV HT)

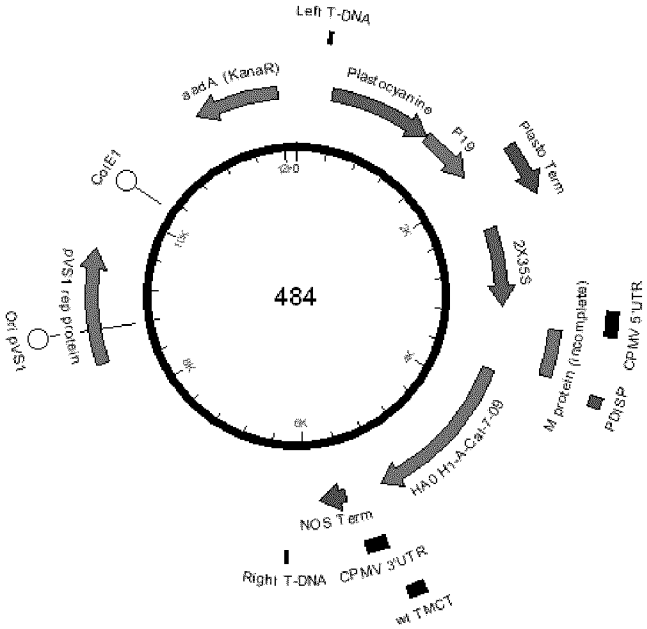



**Figure 10D**

Schematic representation of construct number 1897 (2X35S/CPMV HT*(-Mprot))

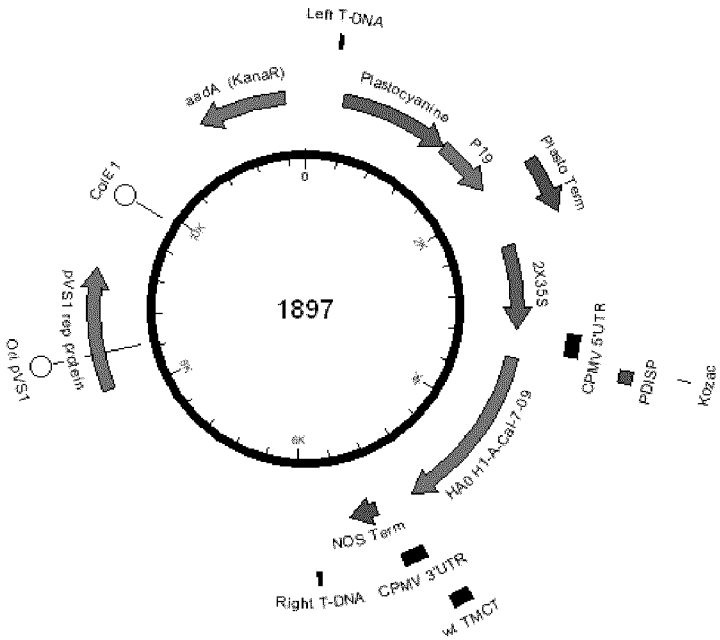


**Figure 11:** 2X35S/CPMV HT (construct no 489), HT*(-Mprot) (construct no 1880) and HT(fl5'UTR) (construct no 1885) for H5 Indonesia

**Figure 11A** (SEQ ID NO: 41) Nucleotide sequence of native H5 Indonesia.

```
ATGGAGAAAATAGTGCTTCTTCTTGCAATAGTCAGTCTTGTTAAAAGTGATCAGATTTGCATTGGTTACCA
TGCAAACAATTCAACAGAGCAGGTTGACACAATCATGGAAAAGAACGTTACTGTTACACATGCCCAAGACA
TACTGGAAAAGACACACAACGGGAAGCTCTGCGATCTAGATGGAGTGAAGCCTCTAATTTTAAGAGATTGT
AGTGTAGCTGGATGGCTCCTCGGGAACCCAATGTGTGACGAATTCATCAATGTACCGGAATGGTCTTACAT
AGTGGAGAAGGCCAATCCAACCAATGACCTCTGTTACCCAGGGAGTTTCAACGACTATGAAGAACTGAAAC
ACCTATTGAGCAGAATAAACCATTTTGAGAAAATTCAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAA
GCCTCATCAGGAGTTAGCTCAGCATGTCCATACCTGGGAAGTCCCTCCTTTTTTAGAAATGTGGTATGGCT
TATCAAAAAGAACAGTACATACCCAACAATAAAGAAAAGCTACAATAATACCAACCAAGAGGATCTTTTGG
TACTGTGGGGAATTCACCATCCTAATGATGCGGCAGAGCAGACAAGGCTATATCAAAACCCAACCACCTAT
ATTTCCATTGGGACATCAACACTAAACCAGAGATTGGTACCAAAAATAGCTACTAGATCCAAAGTAAACGG
GCAAAGTGGAAGGATGGAGTTCTTCTGGACAATTTTAAAACCTAATGATGCAATCAACTTCGAGAGTAATG
GAAATTTCATTGCTCCAGAATATGCATACAAAATTGTCAAGAAAGGGGACTCAGCAATTATGAAAAGTGAA
TTGGAATATGGTAACTGCAACACCAAGTGTCAAACTCCAATGGGGGCGATAAACTCTAGTATGCCATTCCA
CAACATACACCCTCTCACCATCGGGGAATGCCCCAAATATGTGAAATCAAACAGATTAGTCCTTGCAACAG
GGCTCAGAAATAGCCCTCAAAGAGAGAGCAGAAGAAAAAAGAGAGGACTATTTGGAGCTATAGCAGGTTTT
ATAGAGGGAGGATGGCAGGGAATGGTAGATGGTTGGTATGGGTACCACCATAGCAATGAGCAGGGGAGTGG
GTACGCTGCAGACAAAGAATCCACTCAAAAGGCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTG
ACAAAATGAACACTCAGTTTGAGGCCGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAATAGAGAATTTA
AACAAGAAGATGGAAGACGGGTTTCTAGATGTCTGGACTTATAATGCCGAACTTCTGGTTCTCATGGAAAA
TGAGAGAACTCTAGACTTTCATGACTCAAATGTTAAGAACCTCTACGACAAGGTCCGACTACAGCTTAGGG
ATAATGCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCTATCACAAATGTGATAATGAATGTATGGAAAGT
ATAAGAAACGGAACGTACAACTATCCGCAGTATTCAGAAGAAGCAAGATTAAAAAGAGAGGAAATAAGTGG
GGTAAAATTGGAATCAATAGGAACTTACCAAATACTGTCAATTTATTCAACAGTGGCGAGTTCCCTAGCAC
TGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGTTACAATGCAGAATTTGCATT
TAA
```

**Figure 11B** (SEQ ID NO: 42) Amino acid sequence of native H5 Indonesia.

```
MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLILRDC
SVAGWLLGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDYEELKHLLSRINHFEKIQIIPKSSWSDHE
ASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIKKSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTY
ISIGTSTLNQRLVPKIATRSKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSE
LEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRESRRKKRGLFGAIAGF
IEGGWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENL
NKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMES
IRNGTYNYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLALAIMMAGLSLWMCSNGSLQCRICI
*
```

**Figure 11C**

Schematic representation of construct number 489 (2X35S/CPMV HT)

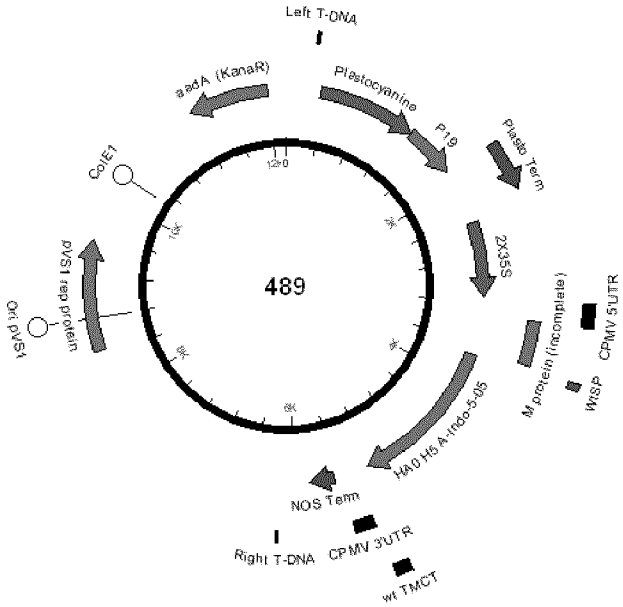



**Figure 11D**

Schematic representation of construct number 1880 (2X35S/CPMV HT*(-Mprot))

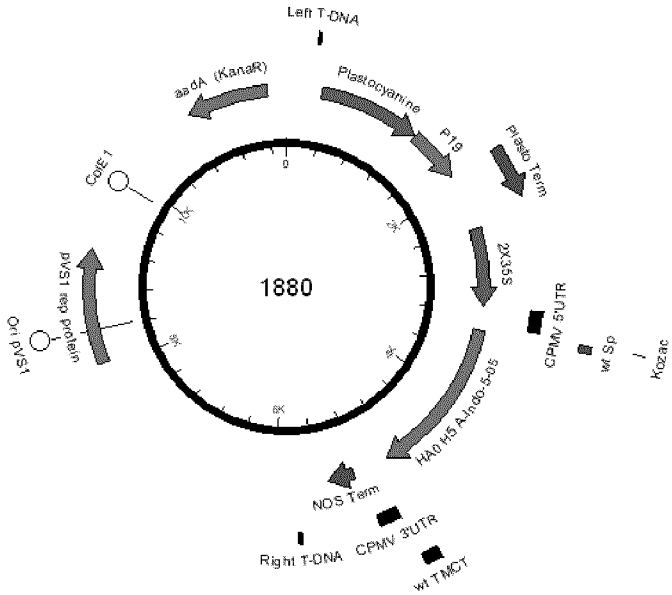


**Figure 11E**

Schematic representation of construct number 1885 (2X35S/CPMV HT(fl5'UTR))

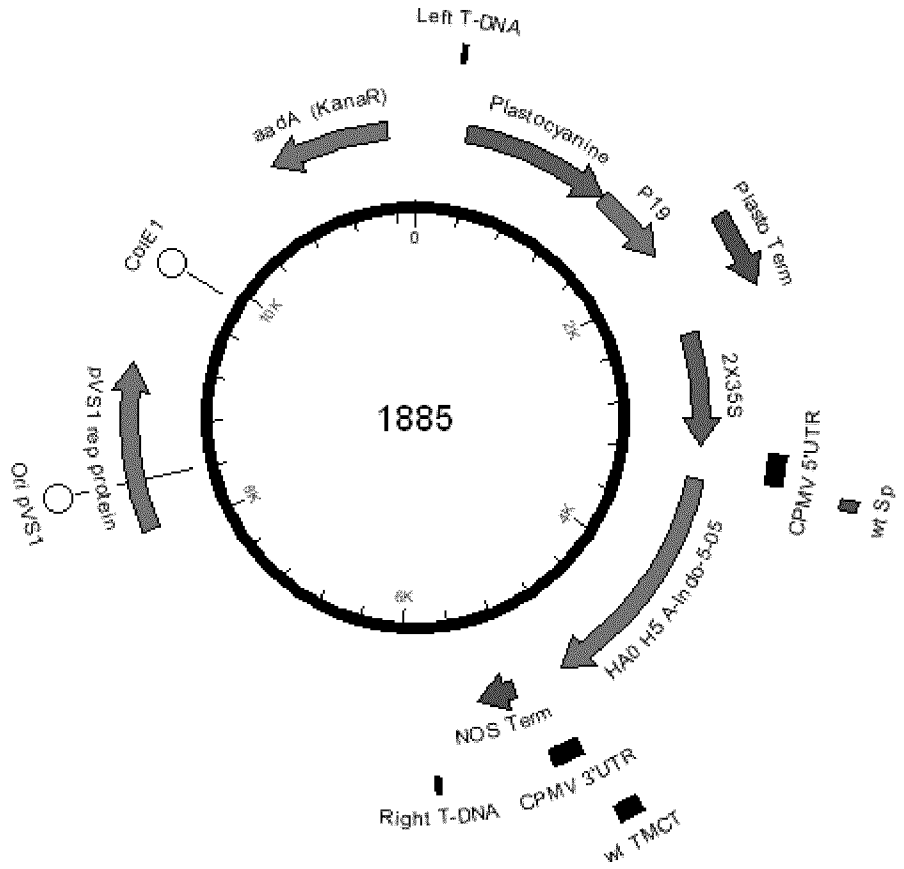


**Figure 12:** 2X35S/CPMV HT (construct no 2140) and HT*(-Mprot) (construct no 2168) for PDISP/H7 Hangzhou

**Figure 12A** (SEQ ID NO: 43) Nucleotide sequence of PDISP/H7 Hangzhou.

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
GGACAAAATCTGCCTCGGACATCATGCCGTGTCAAACGGAACCAAAGTAAACACATTAACTGAAAGAGGAG
TGGAAGTCGTCAATGCAACTGAAACAGTGGAACGAACAAACATCCCCAGGATCTGCTCAAAAGGGAAAAGG
ACAGTTGACCTCGGTCAATGTGGACTCCTGGGGACAATCACTGGACCACCTCAATGTGACCAATTCCTAGA
ATTTTCAGCCGATTTAATTATTGAGAGGCGAGAAGGAAGTGATGTCTGTTATCCTGGGAAATTCGTGAATG
AAGAAGCTCTGAGGCAAATTCTCAGAGAATCAGGCGGAATTGACAAGGAAGCAATGGGATTCACATACAGT
GGAATAAGAACTAATGGAGCAACCAGTGCATGTAGGAGATCAGGATCTTCATTCTATGCAGAAATGAAATG
GCTCCTGTCAAACACAGATAATGCTGCATTCCCGCAGATGACTAAGTCATATAAAAATACAAGAAAAAGCC
CAGCTCTAATAGTATGGGGGATCCATCATTCCGTATCAACTGCAGAGCAAACCAAGCTATATGGGAGTGGA
AACAAACTGGTGACAGTTGGGAGTTCTAATTATCAACAATCTTTTGTACCGAGTCCAGGAGCGAGACCACA
AGTTAATGGTATATCTGGAAGAATTGACTTTCATTGGCTAATGCTAAATCCCAATGATACAGTCACTTTCA
GTTTCAATGGGGCTTTCATAGCTCCAGACCGTGCAAGCTTCCTGAGAGGAAAATCTATGGGAATCCAGAGT
GGAGTACAGGTTGATGCCAATTGTGAAGGGGACTGCTATCATAGTGGAGGGACAATAATAAGTAACTTGCC
ATTTCAGAACATAGATAGCAGGGCAGTTGGAAAATGTCCGAGATATGTTAAGCAAAGGAGTCTGCTGCTAG
CAACAGGGATGAAGAATGTTCCTGAGATTCCAAAGGGAAGAGGCCTATTTGGTGCTATAGCGGGTTTCATT
GAAAATGGATGGGAAGGCCTAATTGATGGTTGGTATGGTTTCAGACACCAGAATGCACAGGGAGAGGGAAC
TGCTGCAGATTACAAAAGCACTCAATCGGCAATTGATCAAATAACAGGAAAATTAAACCGGCTTATAGAAA
AAACCAACCAACAATTTGAGTTGATCGACAATGAATTCAATGAGGTAGAGAAGCAAATCGGTAATGTGATA
AATTGGACCAGAGATTCTATAACAGAAGTGTGGTCATACAATGCTGAACTCTTGGTAGCAATGGAGAACCA
GCATACAATTGATCTGGCTGATTCAGAAATGGACAAACTGTACGAACGAGTGAAAAGACAGCTGAGAGAGA
ATGCTGAAGAAGATGGCACTGGTTGCTTTGAAATATTTCACAAGTGTGATGATGACTGTATGGCCAGTATT
AGAAATAACACCTATGATCACAGCAAATACAGGGAAGAGGCAATGCAAAATAGAATACAGATTGACCCAGT
CAAACTAAGCAGCGGCTACAAAGATGTGATACTTTGGTTTAGCTTCGGGGCATCATGTTTCATACTTCTAG
CCATTGTAATGGGCCTTGTCTTCATATGTGTAAAGAATGGAAACATGCGGTGCACTATTTGTATATAA

**Figure 12B** (SEQ ID NO: 44) Amino acid sequence of PDISP/H7 Hangzhou.

MAKNVAIFGLLFSLLVLVPSQIFADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKGKR
TVDLGQCGLLGTITGPPQCDQFLEFSADLIIERREGSDVCYPGKFVNEEALRQILRESGGIDKEAMGFTYS
GIRTNGATSACRRSGSSFYAEMKWLLSNTDNAAFPQMTKSYKNTRKSPALIVWGIHHSVSTAEQTKLYGSG
NKLVTVGSSNYQQSFVPSPGARPQVNGISGRIDFHWLMLNPNDTVTFSFNGAFIAPDRASFLRGKSMGIQS
GVQVDANCEGDCYHSGGTIISNLPFQNIDSRAVGKCPRYVKQRSLLLATGMKNVPEIPKGRGLFGAIAGFI
ENGWEGLIDGWYGFRHQNAQGEGTAADYKSTQSAIDQITGKLNRLIEKTNQQFELIDNEFNEVEKQIGNVI
NWTRDSITEVWSYNAELLVAMENQHTIDLADSEMDKLYERVKRQLRENAEEDGTGCFEIFHKCDDDCMASI
RNNTYDHSKYREEAMQNRIQIDPVKLSSGYKDVILWFSFGASCFILLAIVMGLVFICVKNGNMRCTICI*

**Figure 12C**

Schematic representation of construct number 2140 (2X35S/CPMV HT)

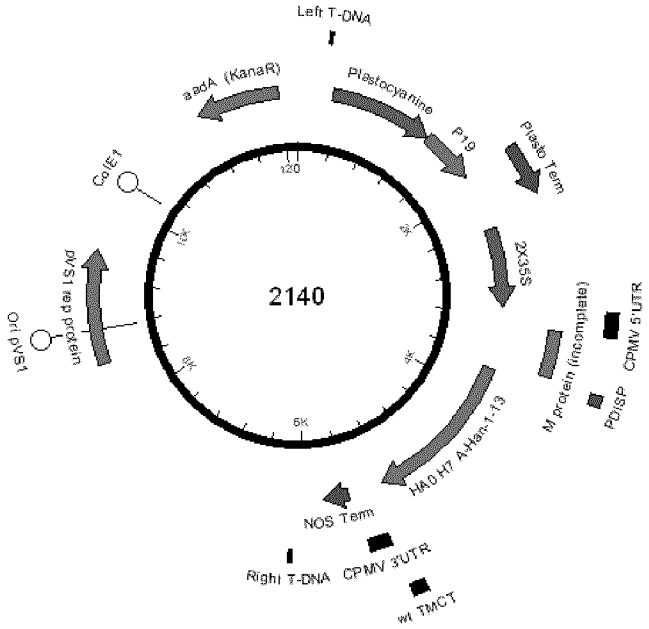



**Figure 12D**

Schematic representation of construct number 2168 (2X35S/CPMV HT*(-Mprot))

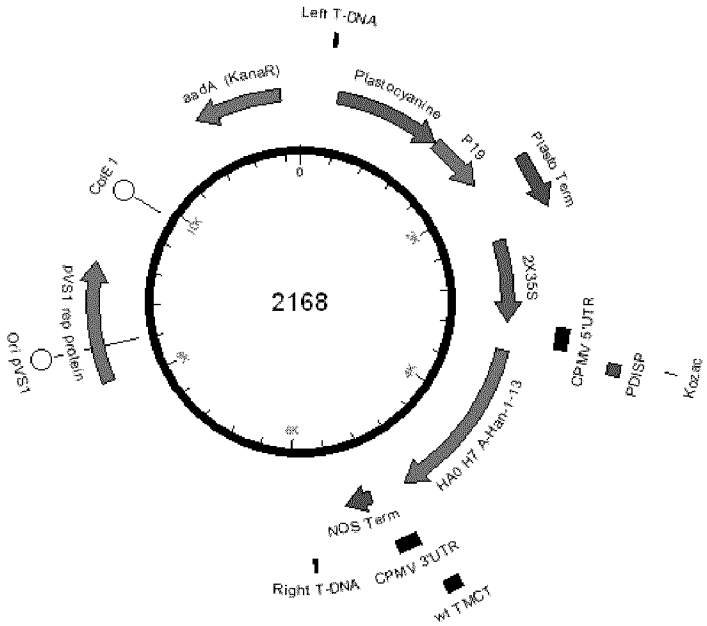


**Figure 13:** 2X35S/CPMV HT (construct no 2130) and HT*(-Mprot) (construct no 2188) for PDISP/H7 Hangzhou+H5 Indonesia TMCT

**Figure 13A** (SEQ ID NO: 45) Nucleotide sequence of PDISP/H7 Hangzhou+H5 Indonesia TMCT.

```
ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
GGACAAAATCTGCCTCGGACATCATGCCGTGTCAAACGGAACCAAAGTAAACACATTAACTGAAAGAGGAG
TGGAAGTCGTCAATGCAACTGAAACAGTGGAACGAACAAACATCCCCAGGATCTGCTCAAAAGGGAAAAGG
ACAGTTGACCTCGGTCAATGTGGACTCCTGGGGACAATCACTGGACCACCTCAATGTGACCAATTCCTAGA
ATTTTCAGCCGATTTAATTATTGAGAGGCGAGAAGGAAGTGATGTCTGTTATCCTGGGAAATTCGTGAATG
AAGAAGCTCTGAGGCAAATTCTCAGAGAATCAGGCGGAATTGACAAGGAAGCAATGGGATTCACATACAGT
GGAATAAGAACTAATGGAGCAACCAGTGCATGTAGGAGATCAGGATCTTCATTCTATGCAGAAATGAAATG
GCTCCTGTCAAACACAGATAATGCTGCATTCCCGCAGATGACTAAGTCATATAAAAATACAAGAAAAAGCC
CAGCTCTAATAGTATGGGGGATCCATCATTCCGTATCAACTGCAGAGCAAACCAAGCTATATGGGAGTGGA
AACAAACTGGTGACAGTTGGGAGTTCTAATTATCAACAATCTTTTGTACCGAGTCCAGGAGCGAGACCACA
AGTTAATGGTATATCTGGAAGAATTGACTTTCATTGGCTAATGCTAAATCCCAATGATACAGTCACTTTCA
GTTTCAATGGGGCTTTCATAGCTCCAGACCGTGCAAGCTTCCTGAGAGGAAAATCTATGGGAATCCAGAGT
GGAGTACAGGTTGATGCCAATTGTGAAGGGGACTGCTATCATAGTGGAGGGACAATAATAAGTAACTTGCC
ATTTCAGAACATAGATAGCAGGGCAGTTGGAAAATGTCCGAGATATGTTAAGCAAAGGAGTCTGCTGCTAG
CAACAGGGATGAAGAATGTTCCTGAGATTCCAAAGGGAAGAGGCCTATTTGGTGCTATAGCGGGTTTCATT
GAAAATGGATGGGAAGGCCTAATTGATGGTTGGTATGGTTTCAGACACCAGAATGCACAGGGAGAGGGAAC
TGCTGCAGATTACAAAAGCACTCAATCGGCAATTGATCAAATAACAGGAAAATTAAACCGGCTTATAGAAA
AAACCAACCAACAATTTGAGTTGATCGACAATGAATTCAATGAGGTAGAGAAGCAAATCGGTAATGTGATA
AATTGGACCAGAGATTCTATAACAGAAGTGTGGTCATACAATGCTGAACTCTTGGTAGCAATGGAGAACCA
GCATACAATTGATCTGGCTGATTCAGAAATGGACAAACTGTACGAACGAGTGAAAAGACAGCTGAGAGAGA
ATGCTGAAGAAGATGGCACTGGTTGCTTTGAAATATTTCACAAGTGTGATGATGACTGTATGGCCAGTATT
AGAAATAACACCTATGATCACAGCAAATACAGGGAAGAGGCAATGCAAAATAGAATACAGATTGACCCAGT
CAAACTAAGCAGCGGCTACCAAATACTGTCAATTTATTCAACAGTGGCGAGTTCCCTAGCACTGGCAATCA
TGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGTTACAATGCAGAATTTGCATTTAA
```

**Figure 13B** (SEQ ID NO: 46) Amino acid sequence of PDISP/H7 Hangzhou+H5 Indonesia TMCT.

```
MAKNVAIFGLLFSLLVLVPSQIFADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKGKR
TVDLGQCGLLGTITGPPQCDQFLEFSADLIIERREGSDVCYPGKFVNEEALRQILRESGGIDKEAMGFTYS
GIRTNGATSACRRSGSSFYAEMKWLLSNTDNAAFPQMTKSYKNTRKSPALIVWGIHHSVSTAEQTKLYGSG
NKLVTVGSSNYQQSFVPSPGARPQVNGISGRIDFHWLMLNPNDTVTFSFNGAFIAPDRASFLRGKSMGIQS
GVQVDANCEGDCYHSGGTIISNLPFQNIDSRAVGKCPRYVKQRSLLLATGMKNVPEIPKGRGLFGAIAGFI
ENGWEGLIDGWYGFRHQNAQGEGTAADYKSTQSAIDQITGKLNRLIEKTNQQFELIDNEFNEVEKQIGNVI
NWTRDSITEVWSYNAELLVAMENQHTIDLADSEMDKLYERVKRQLRENAEEDGTGCFEIFHKCDDDCMASI
RNNTYDHSKYREEAMQNRIQIDPVKLSSGYQILSIYSTVASSLALAIMMAGLSLWMCSNGSLQCRICI*
```

**Figure 13C**

Schematic representation of construct number 2130 (2X35S/CPMV HT)

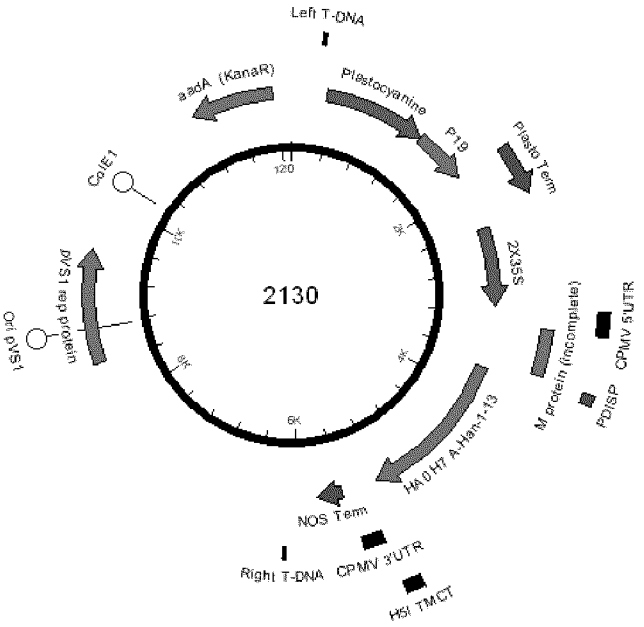



**Figure 13D**

Schematic representation of construct number 2188 (2X35S/CPMV HT*(-Mprot))

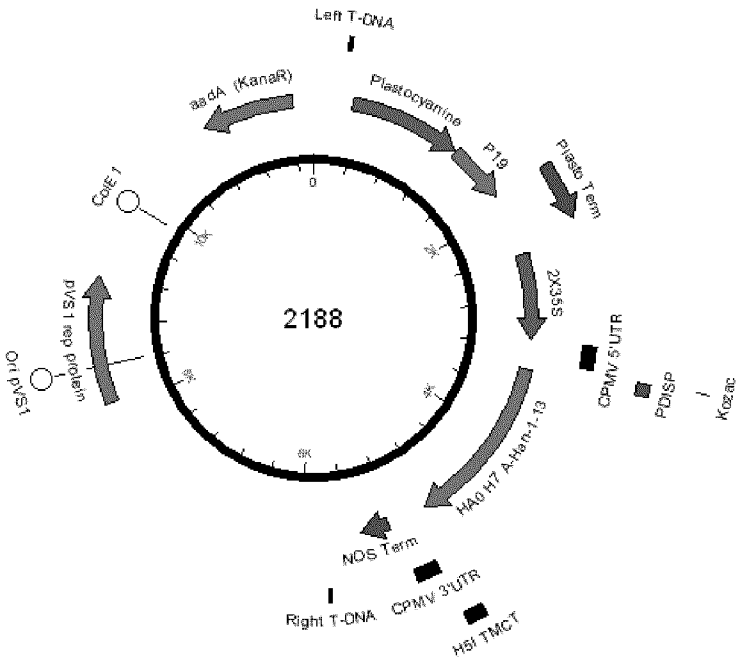


**Figure 14:** 2X35S/CPMV HT (construct no 1039) and HT*(-Mprot) (construct no 1937) for PDISP/HA B Brisbane (PrL-)

**Figure 14A** (SEQ ID NO: 47) Nucleotide sequence of PDISP/HA B Brisbane (PrL-).

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
CGATCGAATCTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGG
TCAATGTGACTGGTGTAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACA
GAAACCAGGGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTTGGGCAGACCAAA
ATGCACGGGGAAAATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTACATCTGGGTGCT
TTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAACCTTCTCCGAGGATACGAACATATCAGG
TTATCAACCCATAACGTTATCAATGCAGAAAATGCACCAGGAGGACCCTACAAAATTGGAACCTCAGGGTC
TTGCCCTAACATTACCAATGGAAACGGATTTTTCGCAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAA
ACAAAACAGCAACAAATCCATTAACAATAGAAGTACCATACATTTGTACAGAAGGAGAAGACCAAATTACC
GTTTGGGGGTTCCACTCTGACAACGAGACCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTT
CACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAG
ACGGAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGGAACA
ATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAA
AGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAAACAAAAGCAAGC
CTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAATATGGGTGAAAACACCCTTGAAGCTG
GCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACAC
ATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATAA
CAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAA
CTCCACAACGAAATACTAGAACTAGATGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTCACAAAT
AGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAA
GAAAGCTGAAGAAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAG
TGCAACCAGACCTGTCTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTT
TGATTCACTGAATATTACTGCTGCATCTTTAAATGACGATGGATTGGATAATCATACTATACTGCTTTACT
ACTCAACTGCTGCCTCCAGTTTGGCTGTAACACTGATGATAGCTATCTTTGTTGTTTATATGGTCTCCAGA
GACAATGTTTCTTGCTCCATCTGTCTATAA

**Figure 14B** (SEQ ID NO : 48) Amino acid sequence of PDISP/HA B Brisbane (PrL-).

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGT
ETRGKLCPKCLNCTDLDVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIR
LSTHNVINAENAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQIT
VWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGT
ITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKL
ANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDE
LHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHK
CNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFVVYMVSR
DNVSCSICL*

**Figure 14C**

Schematic representation of construct number 1039 (2X35S/CPMV HT)

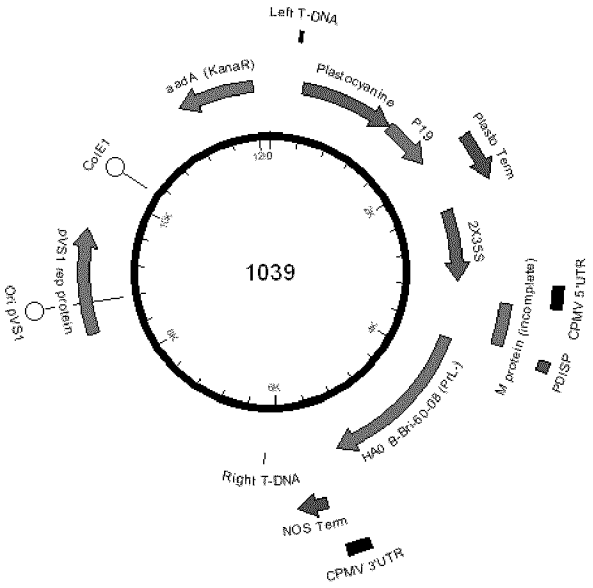



**Figure 14D**

Schematic representation of construct number 1937 (2X35S/CPMV HT*(-Mprot))

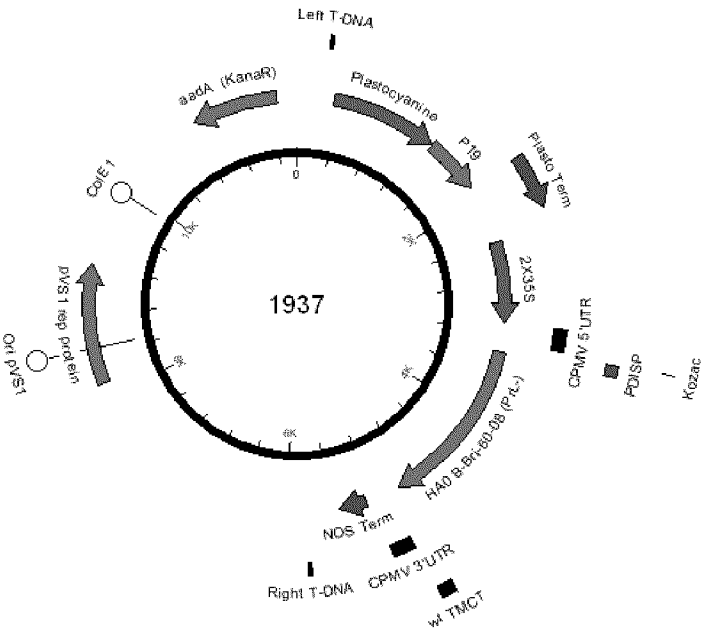


**Figure 15:** 2X35S/CPMV HT (construct no 1067) and HT*(-Mprot) (construct no 1977) for PDISP/HA B Brisbane (PrL-)+H1 California TMCT

**Figure 15A** (SEQ ID NO :49) Nucleotide sequence of PDISP/HA B Brisbane (PrL-)+H1 California TMCT.

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
CGATCGAATCTGCACTGGAATAACATCGTCAAACTCACCACATGTCGTCAAAACTGCTACTCAAGGGGAGG
TCAATGTGACTGGTGTAATACCACTGACAACAACACCCACCAAATCTCATTTTGCAAATCTCAAAGGAACA
GAAACCAGGGGGAAACTATGCCCAAAATGCCTCAACTGCACAGATCTGGACGTAGCCTTGGGCAGACCAAA
ATGCACGGGGAAAATACCCTCGGCAAGAGTTTCAATACTCCATGAAGTCAGACCTGTTACATCTGGGTGCT
TTCCTATAATGCACGACAGAACAAAAATTAGACAGCTGCCTAACCTTCTCCGAGGATACGAACATATCAGG
TTATCAACCCATAACGTTATCAATGCAGAAAATGCACCAGGAGGACCCTACAAAATTGGAACCTCAGGGTC
TTGCCCTAACATTACCAATGGAAACGGATTTTTCGCAACAATGGCTTGGGCCGTCCCAAAAAACGACAAAA
ACAAAACAGCAACAAATCCATTAACAATAGAAGTACCATACATTTGTACAGAAGGAGAAGACCAAATTACC
GTTTGGGGGTTCCACTCTGACAACGAGACCCAAATGGCAAAGCTCTATGGGGACTCAAAGCCCCAGAAGTT
CACCTCATCTGCCAACGGAGTGACCACACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAACAGAAG
ACGGAGGACTACCACAAAGTGGTAGAATTGTTGTTGATTACATGGTGCAAAAATCTGGGAAAACAGGAACA
ATTACCTATCAAAGGGGTATTTTATTGCCTCAAAAGGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAA
AGGATCCTTGCCTTTAATTGGAGAAGCAGATTGCCTCCACGAAAAATACGGTGGATTAAACAAAAGCAAGC
CTTACTACACAGGGGAACATGCAAAGGCCATAGGAAATTGCCCAATATGGGTGAAAACACCCTTGAAGCTG
GCCAATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACAC
ATCCCATGGGGCACATGGAGTAGCGGTGGCAGCAGACCTTAAGAGCACTCAAGAGGCCATAAACAAGATAA
CAAAAAATCTCAACTCTTTGAGTGAGCTGGAAGTAAAGAATCTTCAAAGACTAAGCGGTGCCATGGATGAA
CTCCACAACGAAATACTAGAACTAGATGAGAAAGTGGATGATCTCAGAGCTGATACAATAAGCTCACAAAT
AGAACTCGCAGTCCTGCTTTCCAATGAAGGAATAATAAACAGTGAAGATGAACATCTCTTGGCGCTTGAAA
GAAAGCTGAAGAAAATGCTGGGCCCCTCTGCTGTAGAGATAGGGAATGGATGCTTTGAAACCAAACACAAG
TGCAACCAGACCTGTCTCGACAGAATAGCTGCTGGTACCTTTGATGCAGGAGAATTTTCTCTCCCCACCTT
TGATTCACTGAATATTACTGCTGCATCTTTAAATGACGATGGATTGGATAATTACCAGATTTTGGCGATCT
ATTCAACTGTCGCCAGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAAT
GGGTCTCTACAGTGTAGAATATGTATTTAA

**Figure 15B** (SEQ ID NO : 50) Amino acid sequence of PDISP/HA B Brisbane (PrL-)+H1 California TMCT.

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGT
ETRGKLCPKCLNCTDLDVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIR
LSTHNVINAENAPGGPYKIGTSGSCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQIT
VWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGT
ITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKL
ANGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDE
LHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHK
CNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVASSLVLVVSLGAISFWMCSN
GSLQCRICI*

**Figure 15C**

Schematic representation of construct number 1067 (2X35S/CPMV HT)

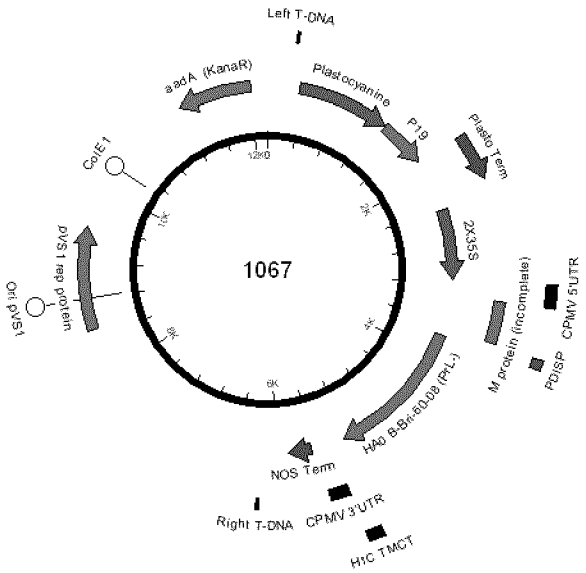



**Figure 15D**

Schematic representation of construct number 1977 (2X35S/CPMV HT*(-Mprot))

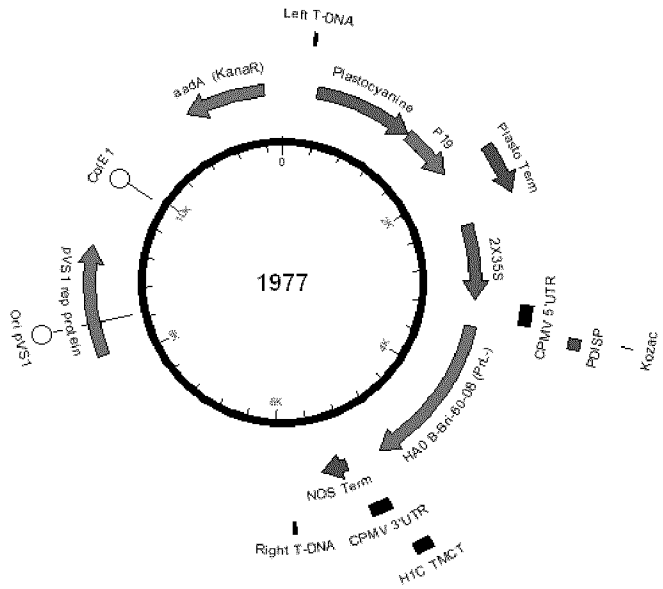


**Figure 16:** 2X35S/CPMV HT (construct no 2072) and HT*(-Mprot) (construct no 2050) for PDISP/HA B Massachussetts (PrL-)

**Figure 16A** (SEQ ID NO : 51) Nucleotide sequence of PDISP/HA B Massachussetts (PrL-).

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
CGATCGAATCTGCACTGGGATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGG
TCAATGTGACTGGTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACA
AAGACCAGAGGGAAACTATGCCCAGACTGTCTCAACTGTACAGATCTGGATGTGGCCCTGGGCAGGCCAAT
GTGTGTGGGAACTACACCTTCTGCGAAAGCTTCAATACTTCACGAAGTCAGACCTGTTACATCCGGGTGCT
TCCCTATAATGCACGACAGAACAAAAATCAGGCAACTAGCCAATCTTCTCAGAGGATATGAAAATATCAGG
TTATCAACCCAAAACGTTATCGATGCAGAAAAGGCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATC
TTGCCCTAACGCTACCAGTAAAAGCGGATTTTTCGCAACAATGGCTTGGGCTGTCCCAAAGGACAACAACA
AAAATGCAACGAACCCATTAACAGTAGAAGTACCATACATTTGTGCAGAAGGGGAAGACCAAATTACTGTT
TGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCAAATCCTCAAAAGTTCAC
CTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCGGCTTCCCAGATCAAACAGAAGACG
GAGGACTACCACAAAGCGGCAGAATTGTCGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATT
GTCTATCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGG
GTCCTTGCCTTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTT
ACTACACAGGAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTTGCC
AATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATC
TCACGGAGCACATGGAGTGGCAGTTGCTGCAGACCTTAAGAGCACACAAGAAGCTATAAACAAGATAACAA
AAAATCTCAACTCTTTGAGTGAGCTAGAAGTAAAGAATCTTCAAAGGCTAAGTGGTGCCATGGATGAACTC
CACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGACCTCAGAGCTGACACTATAAGTTCACAAATAGA
ACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAA
AACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAATGGATGCTTCGAAACCAAACACAAATGC
AACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAGTTTTCTCTCCCCACTTTTGA
TTCATTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACT
CAACTGCTGCTTCTAGTTTGGCTGTAACATTGATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGAC
AACGTTTCATGCTCCATCTGTCTATAA

**Figure 16B** (SEQ ID NO : 52) Amino acid sequence of PDISP/HA B Massachussetts (PrL-).

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGT
KTRGKLCPDCLNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLANLLRGYENIR
LSTQNVIDAEKAPGGPYRLGTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICAEGEDQITV
WGFHSDNKTQMKNLYGDSNPQKFTSSANGVTTHYVSQIGGFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTI
VYQRGVLLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLA
NGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDEL
HNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKC
NQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMLAIFIVYMVSRD
NVSCSICL*

**Figure 16C**

Schematic representation of construct number 2072 (2X35S/CPMV HT)

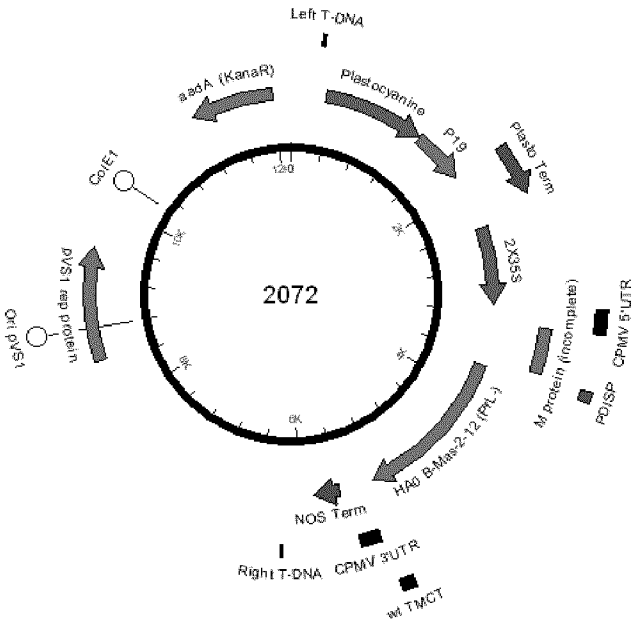



**Figure 16D**

Schematic representation of construct number 2050 (2X35S/CPMV HT*(-Mprot))

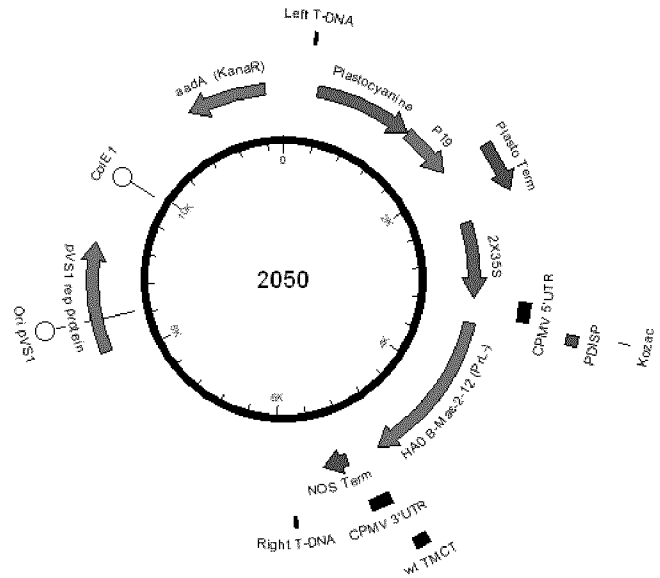


**Figure 17:** 2X35S/CPMV HT (construct no 2074) and HT*(-Mprot) (construct no 2060) for PDISP/HA B Massachussetts (PrL-)+H1 California TMCT

**Figure 17A** (SEQ ID NO : 53) Nucleotide sequence of PDISP/HA B Massachussetts (PrL-)+H1 California TMCT.

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
CGATCGAATCTGCACTGGGATAACATCTTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGG
TCAATGTGACTGGTGTGATACCACTAACAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACA
AAGACCAGAGGGAAACTATGCCCAGACTGTCTCAACTGTACAGATCTGGATGTGGCCCTGGGCAGGCCAAT
GTGTGTGGGAACTACACCTTCTGCGAAAGCTTCAATACTTCACGAAGTCAGACCTGTTACATCCGGGTGCT
TCCCTATAATGCACGACAGAACAAAAATCAGGCAACTAGCCAATCTTCTCAGAGGATATGAAAATATCAGG
TTATCAACCCAAAACGTTATCGATGCAGAAAAGGCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATC
TTGCCCTAACGCTACCAGTAAAAGCGGATTTTTCGCAACAATGGCTTGGGCTGTCCCAAAGGACAACAACA
AAAATGCAACGAACCCATTAACAGTAGAAGTACCATACATTTGTGCAGAAGGGGAAGACCAAATTACTGTT
TGGGGGTTCCATTCAGATAACAAAACCCAAATGAAGAACCTCTATGGAGACTCAAATCCTCAAAAGTTCAC
CTCATCTGCTAATGGAGTAACCACACATTATGTTTCTCAGATTGGCGGCTTCCCAGATCAAACAGAAGACG
GAGGACTACCACAAAGCGGCAGAATTGTCGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATT
GTCTATCAAAGAGGTGTTTTGTTGCCTCAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGG
GTCCTTGCCTTTAATTGGTGAAGCAGATTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTT
ACTACACAGGAGAACATGCAAAAGCCATAGGAAATTGCCCAATATGGGTGAAAACACCTTTGAAGCTTGCC
AATGGAACCAAATATAGACCTCCTGGTGGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATC
TCACGGAGCACATGGAGTGGCAGTTGCTGCAGACCTTAAGAGCACACAAGAAGCTATAAACAAGATAACAA
AAAATCTCAACTCTTTGAGTGAGCTAGAAGTAAAGAATCTTCAAAGGCTAAGTGGTGCCATGGATGAACTC
CACAACGAAATACTCGAGCTGGATGAGAAAGTGGATGACCTCAGAGCTGACACTATAAGTTCACAAATAGA
ACTTGCAGTCTTGCTTTCCAACGAAGGAATAATAAACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAA
AACTAAAGAAAATGCTGGGTCCCTCTGCTGTAGACATAGGAAATGGATGCTTCGAAACCAAACACAAATGC
AACCAGACCTGCTTAGACAGGATAGCTGCTGGCACCTTTAATGCAGGAGAGTTTTCTCTCCCCACTTTTGA
TTCATTGAACATTACTGCTGCATCTTTAAATGATGATGGATTGGATAACTACCAGATTTTGGCGATCTATT
CAACTGTCGCCAGTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGG
TCTCTACAGTGTAGAATATGTATTTAA

**Figure 17B** (SEQ ID NO :54) Amino acid sequence of PDISP/HA B Massachussetts (PrL-)+H1 California TMCT.

MAKNVAIFGLLFSLLVLVPSQIFADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGT
KTRGKLCPDCLNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLANLLRGYENIR
LSTQNVIDAEKAPGGPYRLGTSGSCPNATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYICAEGEDQITV
WGFHSDNKTQMKNLYGDSNPQKFTSSANGVTTHYVSQIGGFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTI
VYQRGVLLPQKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLA
NGTKYRPPGGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDEL
HNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKC
NQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVASSLVLVVSLGAISFWMCSNG
SLQCRICI*

**Figure 17C**

Schematic representation of construct number 2074 (2X35S/CPMV HT)

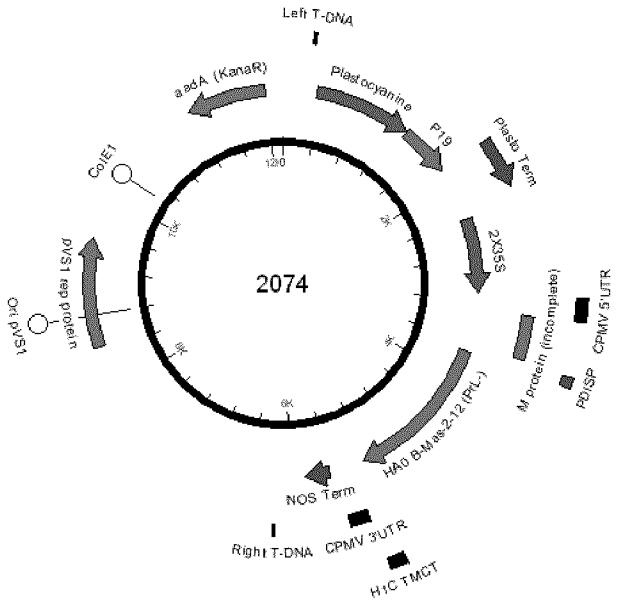



**Figure 17D**

Schematic representation of construct number 2060 (2X35S/CPMV HT*(-Mprot))

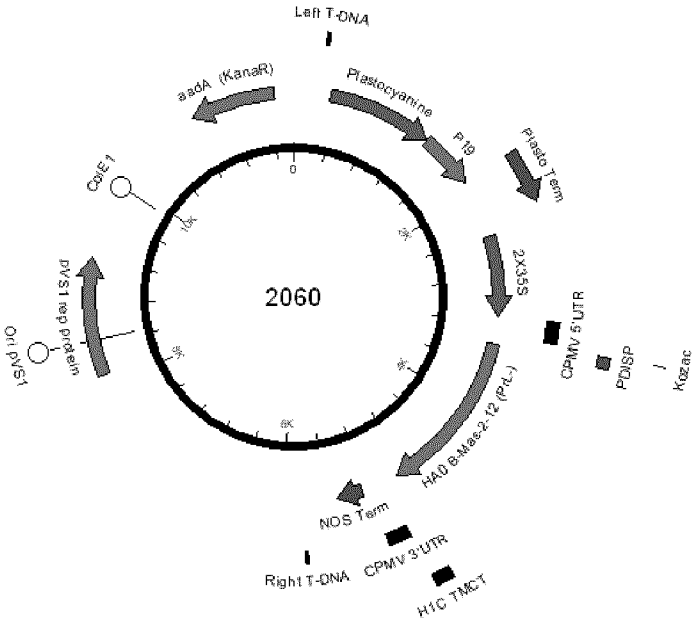


**Figure 18:** 2X35S/CPMV HT (construct no 1445), HT*(-Mprot) (construct no 1820) and HT(fl5'UTR) (construct no 1975) for HA B Wisconsin (PrL-)

**Figure 18A** ( SEQ ID NO : 55) Nucleotide sequence of HA B Wisconsin (PrL-).

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGGATAACATC
TTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGGCGTGATACCACTGA
CAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCGGAC
TGTCTCAACTGTACAGATCTGGATGTGGCCTTGGGCAGGCCAATGTGTGTGGGGACCACACCTTCTGCTAA
AGCTTCAATACTCCACGAGGTCAGACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAA
TCAGGCAACTACCCAATCTTCTCAGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCA
GAAAAAGCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAATCGG
ATTTTTTGCAACAATGGCTTGGGCTGTCCCAAAGGACAACTACAAAAATGCAACGAACCCACTAACAGTAG
AAGTACCATACATTTGTACAGAAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACC
CAAATGAAGAGCCTCTATGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACA
TTATGTTTCTCAGATTGGCGACTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTG
TTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTATCAAAGAGGTGTTTTGTTGCCT
CAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCATTGCCTTTAATTGGTGAAGCAGA
TTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCA
TAGGAAATTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAATGGAACCAAATATAGACCTCCTGGT
GGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGC
GGCAGACCTTAAGAGTACACAAGAAGCTATAAATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAG
AAGTAAAGAACCTTCAAAGACTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAG
AAAGTGGATGATCTCAGAGCTGACACTATAAGCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGG
AATAATAAACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTG
CTGTAGACATAGGAAACGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATAGCT
GCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCATCTTT
AAATGATGATGGATTGGATAACCATACTATACTGCTCTATTACTCAACTGCTGCTTCTAGTTTGGCTGTAA
CATTAATGCTAGCTATTTTTATTGTTTATATGGTCTCCAGAGACAACGTTTCATGCTCCATCTGTCTATAA

**Figure 18B**, (SEQ ID NO : 56) Amino acid sequence of HA B Wisconsin (PrL-).

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPD
CLNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDA
EKAPGGPYRLGTSGSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKT
QMKSLYGDSNPQKFTSSANGVTTHYVSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLP
QKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPG
GGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDE
KVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIA
AGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMLAIFIVYMVSRDNVSCSICL*

**Figure 18C**

Schematic representation of construct number 1445 (2X35S/CPMV HT)

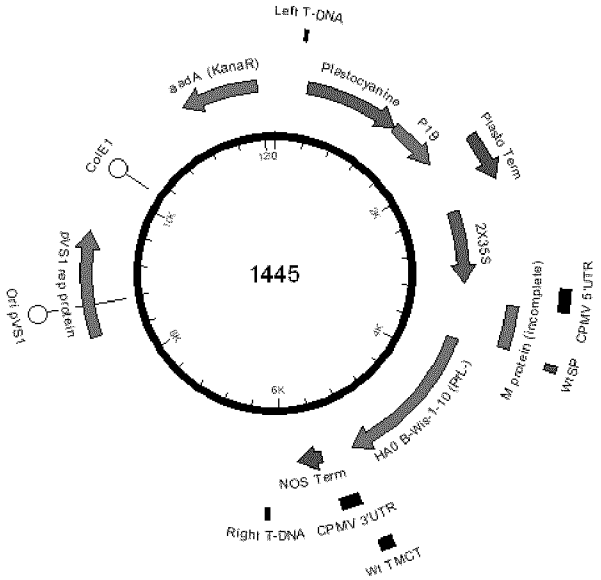



**Figure 18D**

Schematic representation of construct number 1820 (2X35S/CPMV HT*(-Mprot))

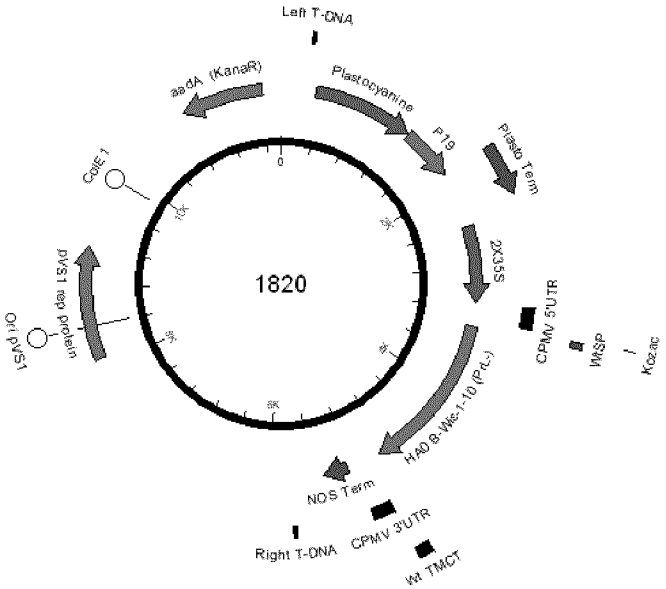


**Figure 18E**

Schematic representation of construct number 1975 (2X35S/CPMV HT*(fl5'UTR))

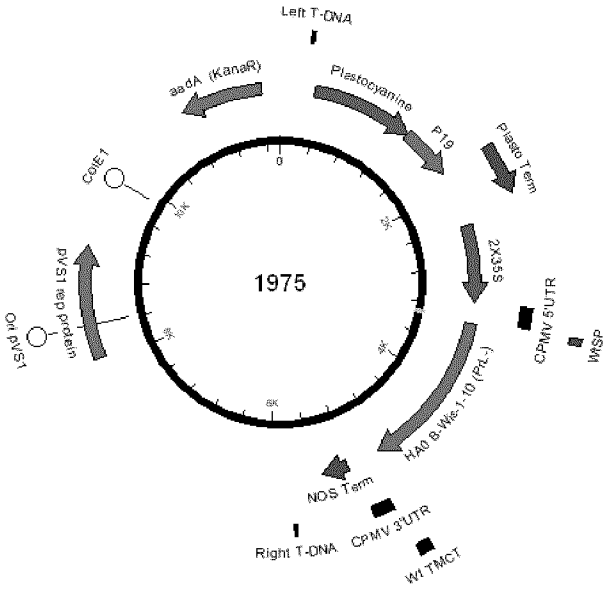


**Figure 19:** 2X35S/CPMV HT (construct no 1454) and HT*(-Mprot) (construct no 1893) for HA B Wisconsin (PrL-)+H1 California TMCT

**Figure 19A** (SEQ ID NO : 57) Nucleotide sequence of HA B Wisconsin (PrL-)+H1 California TMCT

ATGAAGGCAATAATTGTACTACTCATGGTAGTAACATCCAATGCAGATCGAATCTGCACTGGGATAACATC
TTCAAACTCACCTCATGTGGTCAAAACAGCTACTCAAGGGGAGGTCAATGTGACTGGCGTGATACCACTGA
CAACAACACCAACAAAATCTTATTTTGCAAATCTCAAAGGAACAAGGACCAGAGGGAAACTATGCCCGGAC
TGTCTCAACTGTACAGATCTGGATGTGGCCTTGGGCAGGCCAATGTGTGTGGGGACCACACCTTCTGCTAA
AGCTTCAATACTCCACGAGGTCAGACCTGTTACATCCGGGTGCTTTCCTATAATGCACGACAGAACAAAAA
TCAGGCAACTACCCAATCTTCTCAGAGGATATGAAAATATCAGGTTATCAACCCAAAACGTTATCGATGCA
GAAAAAGCACCAGGAGGACCCTACAGACTTGGAACCTCAGGATCTTGCCCTAACGCTACCAGTAAAATCGG
ATTTTTTGCAACAATGGCTTGGGCTGTCCCAAAGGACAACTACAAAAATGCAACGAACCCACTAACAGTAG
AAGTACCATACATTTGTACAGAAGGGGAAGACCAAATTACTGTTTGGGGGTTCCATTCAGATAACAAAACC
CAAATGAAGAGCCTCTATGGAGACTCAAATCCTCAAAAGTTCACCTCATCTGCTAATGGAGTAACCACACA
TTATGTTTCTCAGATTGGCGACTTCCCAGATCAAACAGAAGACGGAGGACTACCACAAAGCGGCAGAATTG
TTGTTGATTACATGATGCAAAAACCTGGGAAAACAGGAACAATTGTCTATCAAAGAGGTGTTTTGTTGCCT
CAAAAGGTGTGGTGCGCGAGTGGCAGGAGCAAAGTAATAAAAGGGTCATTGCCTTTAATTGGTGAAGCAGA
TTGCCTTCATGAAAAATACGGTGGATTAAACAAAAGCAAGCCTTACTACACAGGAGAACATGCAAAAGCCA
TAGGAAATTGCCCAATATGGGTAAAAACACCTTTGAAGCTTGCCAATGGAACCAAATATAGACCTCCTGGT
GGAGGATGGGAAGGAATGATTGCAGGTTGGCACGGATACACATCTCACGGAGCACATGGAGTGGCAGTGGC
GGCAGACCTTAAGAGTACACAAGAAGCTATAAATAAGATAACAAAAAATCTCAATTCTTTGAGTGAGCTAG
AAGTAAAGAACCTTCAAAGACTAAGTGGTGCCATGGATGAACTCCACAACGAAATACTCGAGCTGGATGAG
AAAGTGGATGATCTCAGAGCTGACACTATAAGCTCACAAATAGAACTTGCAGTCTTGCTTTCCAACGAAGG
AATAATAAACAGTGAAGACGAGCATCTATTGGCACTTGAGAGAAAACTAAAGAAAATGCTGGGTCCCTCTG
CTGTAGACATAGGAAACGGATGCTTCGAAACCAAACACAAATGCAACCAGACCTGCTTAGACAGGATAGCT
GCTGGCACCTTTAATGCAGGAGAATTTTCTCTCCCCACTTTTGATTCATTGAACATTACTGCTGCATCTTT
AAATGATGATGGATTGGATAACTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTGG
TAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

**Figure 19B** (SEQ ID NO : 58) Amino acid sequence of HA B Wisconsin (PrL-)+H1 California TMC.

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPD
CLNCTDLDVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYENIRLSTQNVIDA
EKAPGGPYRLGTSGSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKT
QMKSLYGDSNPQKFTSSANGVTTHYVSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLP
QKVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPG
GGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDE
KVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIA
AGTFNAGEFSLPTFDSLNITAASLNDDGLDNYQILAIYSTVASSLVLVVSLGAISFWMCSNGSLQCRICI*

**Figure 19C**

Schematic representation of construct number 1454 (2X35S/CPMV HT)

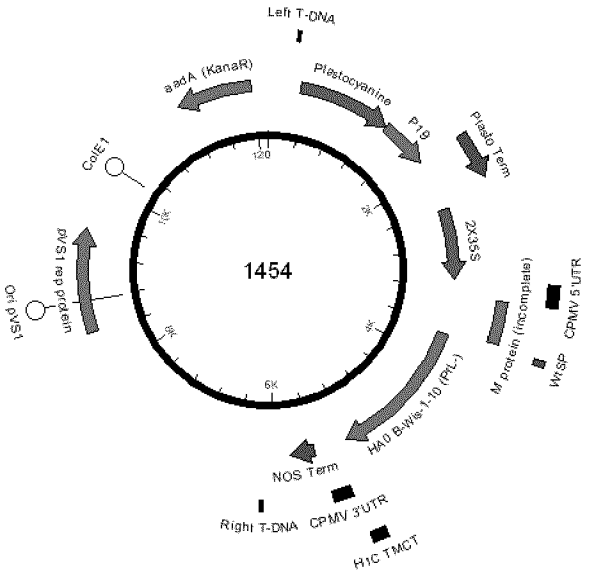



**Figure 19D**

Schematic representation of construct number 1893 (2X35S/CPMV HT*(-Mprot))

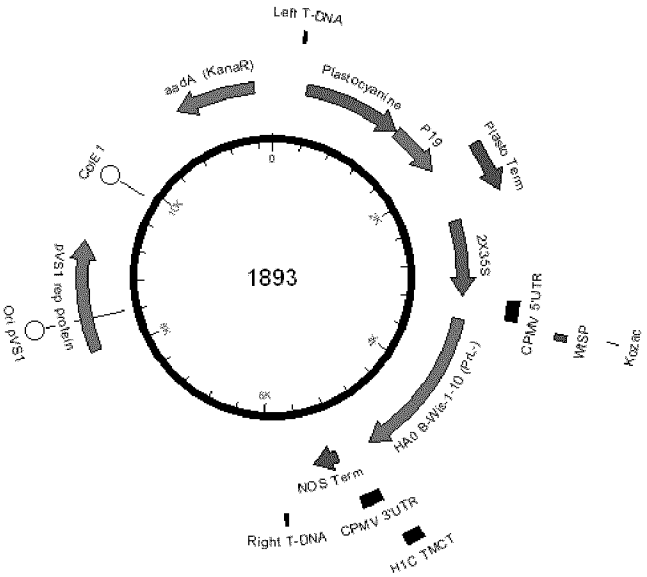


**Figure 20:** 2X35S/CPMV HT (construct no 5001) and HT*(-Mprot) (construct no 2100) for HC Rituxan

**Figure 20A** (SEQ ID NO : 59) Nucleotide sequence of HC Rituxan.

ATGGGTTGGAGCCTCATCTTGCTCTTCCTTGTCGCTGTTGCTACGCGTGTCCTGTCCCAGGTACAACTGCA
GCAGCCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCTGGCTACACAT
TTACCAGTTACAATATGCACTGGGTAAAACAGACACCTGGTCGGGGCCTGGAATGGATTGGAGCTATTTAT
CCCGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACATTGACTGCAGACAAATCCTC
CAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGATCGA
CTTACTACGGCGGTGACTGGTACTTCAATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCTGCAGCTAGC
ACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGG
CTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCG
TGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA
AGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGAC
CGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCC
TGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC
GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCTAGGGAACCACAAGTGTACACTCTTCCACCATCTAGGGA
TGAGCTTACTAAGAACCAAGTTTCTCTTACTTGTCTTGTGAAGGGATTTTATCCATCTGACATCGCCGTGG
AATGGGAATCCAACGGACAACCAGAGAACAATTACAAGACTACTCCACCAGTTCTTGATTCTGATGGATCC
TTCTTTCTTTATTCCAAGCTTACTGTTGATAAGTCCAGATGGCAGCAAGGAAATGTGTTCTCTTGTTCTGT
TATGCACGAAGCTCTTCATAATCATTATACTCAAAAGTCCCTTTCTCTTTCTCCTGGAAAGTGA

**Figure 20B** (SEQ ID NO : 60) Amino acid sequence of HC Rituxan.

MGWSLILLFLVAVATRVLSQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIY
PGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**Figure 20C**

Schematic representation of construct number 5001 (2X35S/CPMV HT)

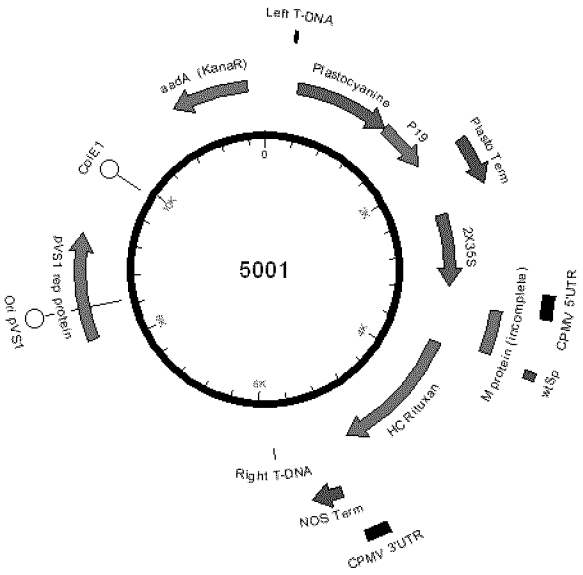



**Figure 20D**

Schematic representation of construct number 2100 (2X35S/CPMV HT*(-Mprot))

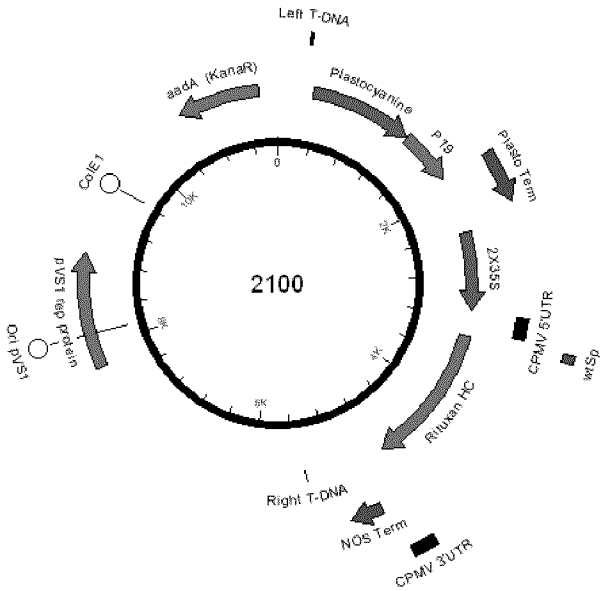


**Figure 21:** 2X35S/CPMV HT (construct no 5002) and HT*(-Mprot) (construct no 2109) for PDISP/HC Rituxan

**Figure 21A**, (SEQ ID NO : 61) Nucleotide sequence of PDISP/HC Rituxan.

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
CCAGGTACAACTGCAGCAGCCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTCAGTGAAGATGTCCTGCAAGG
CTTCTGGCTACACATTTACCAGTTACAATATGCACTGGGTAAAACAGACACCTGGTCGGGGCCTGGAATGG
ATTGGAGCTATTTATCCCGGAAATGGTGATACTTCCTACAATCAGAAGTTCAAAGGCAAGGCCACATTGAC
TGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATT
ACTGTGCAAGATCGACTTACTACGGCGGTGACTGGTACTTCAATGTCTGGGGCGCAGGGACCACGGTCACC
GTCTCTGCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG
CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCG
CCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG
GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACAC
CAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTG
AACTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACC
CCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCA
GCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCC
CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCTAGGGAACCACAAGTGTACACTCT
TCCACCATCTAGGGATGAGCTTACTAAGAACCAAGTTTCTCTTACTTGTCTTGTGAAGGGATTTTATCCAT
CTGACATCGCCGTGGAATGGGAATCCAACGGACAACCAGAGAACAATTACAAGACTACTCCACCAGTTCTT
GATTCTGATGGATCCTTCTTTCTTTATTCCAAGCTTACTGTTGATAAGTCCAGATGGCAGCAAGGAAATGT
GTTCTCTTGTTCTGTTATGCACGAAGCTCTTCATAATCATTATACTCAAAAGTCCCTTTCTCTTTCTCCTG
GAAAGTGA

**Figure 21B** (SEQ ID NO : 62) Amino acid sequence of PDISP/HC Rituxan.

MAKNVAIFGLLFSLLVLVPSQIFAQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEW
IGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVT
VSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**Figure 21C**

Schematic representation of construct number 5002 (2X35S/CPMV HT)

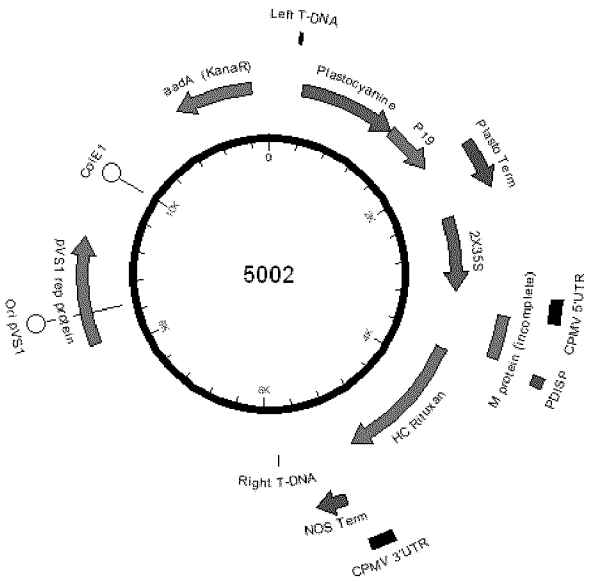



**Figure 21D**

Schematic representation of construct number 2109 (2X35S/CPMV HT*(-Mprot))

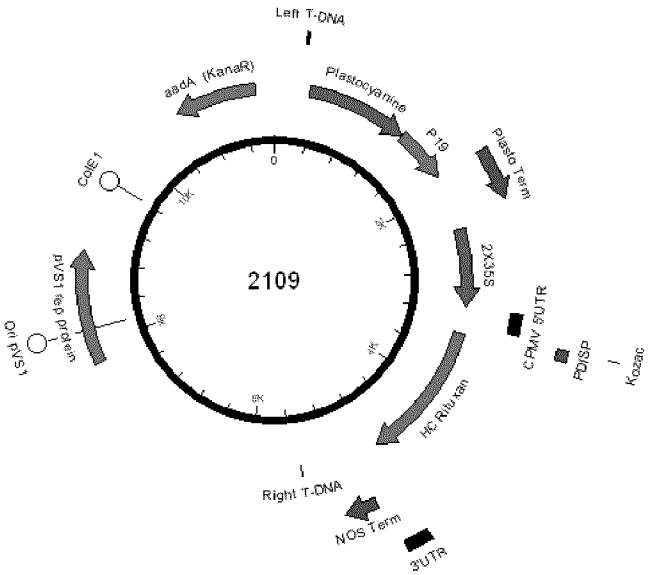


**Figure 22:** 2X35S/CPMV HT (construct no 5021) and HT*(-Mprot) (construct no 2120) for LC Rituxan

**Figure 22A** (SEQ ID NO : 63) Nucleotide sequence of LC Rituxan.

```
ATGGATTTTCAGGTGCAGATTATCAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTCCAGAGGACAAAT
TGTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGCAGGGCCA
GCTCAAGTGTAAGTTACATCCACTGGTTCCAGCAGAAGCCAGGATCCTCCCCCAAACCCTGGATTTATGCC
ACATCCAACCTGGCTTCTGGAGTCCCTGTTCGCTTCAGTGGCAGTGGGTCTGGGACTTCTTACTCTCTCAC
AATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGACTAGTAACCCACCCACGT
TCGGAGGGGGGACCAAGCTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCT
GATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCA
AGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA
```

**Figure 22B** (SEQ ID NO : 64) Amino acid sequence of LC Rituxan.

```
MDFQVQIISFLLISASVIMSRGQIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYA
TSNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC*
```

**Figure 22C**

Schematic representation of construct number 5021 (2X35S/CPMV HT)

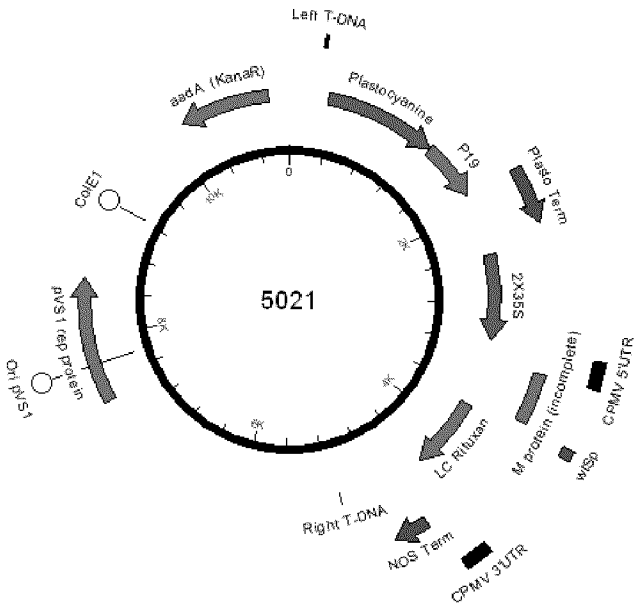



**Figure 22D**

Schematic representation of construct number 2120 (2X35S/CPMV HT*(-Mprot))

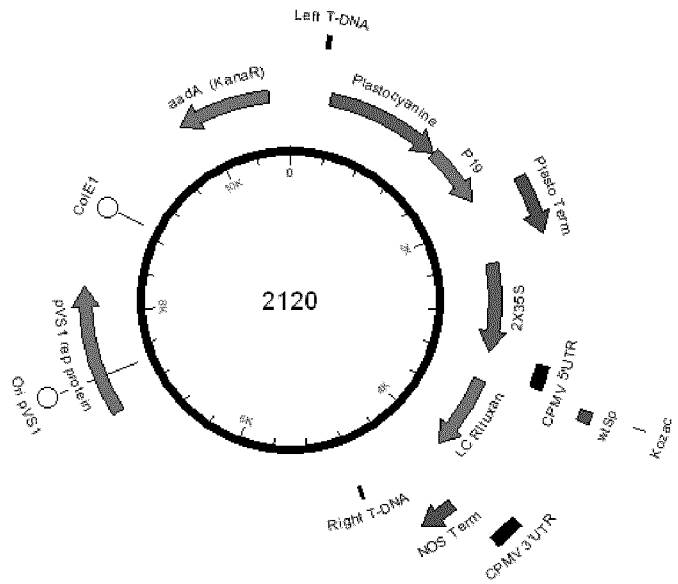


**Figure 23:** 2X35S/CPMV HT (construct no 5022) and HT*(-Mprot) (construct no 2129) for PDISP/LC Rituxan

**Figure 23A** (SEQ ID NO : 65) Nucleotide sequence of PDISP/LC Rituxan.

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTCGC
CCAAATTGTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGCA
GGGCCAGCTCAAGTGTAAGTTACATCCACTGGTTCCAGCAGAAGCCAGGATCCTCCCCCAAACCCTGGATT
TATGCCACATCCAACCTGGCTTCTGGAGTCCCTGTTCGCTTCAGTGGCAGTGGGTCTGGGACTTCTTACTC
TCTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGACTAGTAACCCAC
CCACGTTCGGAGGGGGGACCAAGCTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGA
GGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGG
ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAA
GTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTG
TTGA

**Figure 23B** (SEQ ID NO : 66) Amino acid sequence of PDISP/LC Rituxan.

MAKNVAIFGLLFSLLVLVPSQIFAQIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWI
YATSNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC*

**Figure 23C**

Schematic representation of construct number 5022 (2X35S/CPMV HT)

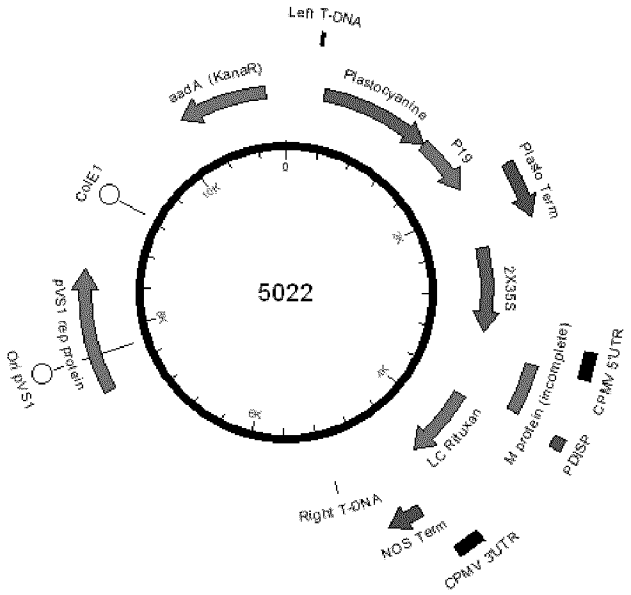
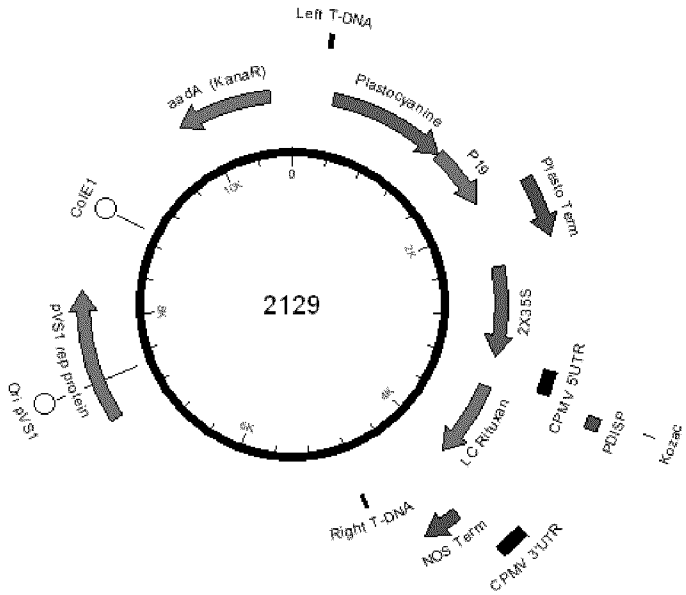



**Figure 23D**

Schematic representation of construct number 2129 (2X35S/CPMV HT*(-Mprot))



**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/CA2015/050009**

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 15/113*** (2010.01), ***A01H 5/00*** (2006.01), ***C07H 21/04*** (2006.01), ***C12N 15/13*** (2006.01), ***C12N 15/33*** (2006.01), ***C12N 15/44*** (2006.01), ***C12N 15/82*** (2006.01), *C12N 15/41* (2006.01)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***C12N 15/113*** (2010.01), ***A01H 5/00*** (2006.01), ***C07H 21/04*** (2006.01), ***C12N 15/13*** (2006.01), ***C12N 15/33*** (2006.01), ***C12N 15/44*** (2006.01), ***C12N 15/82*** (2006.01), *C12N 15/41* (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)
Biosis, CAplus, Medline, FAMPAT, GenomeQuest (SEQ ID NO:1, 2, 24, 27, 68-77 at >80% identity), Canadian Patent Database; Keywords: cowpea mosaic virus, CPMV, enhancer(s), expression, recombinant, 5'-UTR, transgen(e, ic)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO2009087391A1 (SAINSBURY, F. & LOMONOSSOFF, G.) 16 July 2009 (16-07-2009)<br>- the entire document; SEQ ID NO:10; | 1-4, 7, 9-12, 14, 16, 18-20 |
| A | - cited in the description | 6, 8, 13, 15, 17, 21-23 |
| X | WO2010148511A1 (COUTURE, M. et al) 29 December 2010 (29-12-2010)<br>- the entire document; SEQ ID NO:62 | 1-4, 8-22 |
| A | - cited in the description | 6, 7, 23 |
| X | WO2012058762A1 (D'AOUST, M.-A. et al) 10 May 2012 (10-05-2012)<br>- entire document; SEQ ID NO:23 | 1-4, 8-22 |
| A | | 6, 7, 23 |

☑ 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<br>10 April 2015 (10-04-2015) | Date of mailing of the international search report<br>17 April 2015 (17-04-2015) |
| Name and mailing address of the ISA/CA<br>Canadian Intellectual Property Office<br>Place du Portage I, C114 - 1st Floor, Box PCT<br>50 Victoria Street<br>Gatineau, Quebec K1A 0C9<br>Facsimile No.: 001-819-953-2476 | Authorized officer<br>Michael W. De Vouge (819) 997-2952 |

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO2013044390A1 (D'AOUST, M.-A. et al) 04 April 2013 (04-04-2013)<br>-the entire document; SEQ ID NO:10,13 | 1-4, 8-22 |
| A | | 6, 7, 23 |
| P, X | WO2014153674A1 (COUTURE, M. et al) 02 October 2014 (02-10-2014)<br>- the entire document | 1-23 |

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/CA2015/050009**

**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.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☑ Claim Nos.: 5
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:

The Sequence Listing provided lacks sequences for SEQ ID NO:5-17, thus precluding a search of the claimed subject matter.

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/CA2015/050009**

| Patent Document Cited in Search Report | Publication Date | Patent Family Member(s) | Publication Date |
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| | | CN101952446B | 11 June 2014 (11-06-2014) |
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| | | EP2635684A4 | 21 May 2014 (21-05-2014) |
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| | | US2013295609A1 | 07 November 2013 (07-11-2013) |

…continued on **Extra Sheet**

| Patent Document Cited in Search Report | Publication Date | Patent Family Member(s) | Publication Date |
|---|---|---|---|
| WO2013044390A1 | 04 April 2013 (04-04-2013) | AU2012315421A1 | 10 April 2014 (10-04-2014) |
| | | CA2850407A1 | 04 April 2013 (04-04-2013) |
| | | CN103930435A | 16 July 2014 (16-07-2014) |
| | | EP2760882A1 | 06 August 2014 (06-08-2014) |
| | | IL231587D0 | 28 May 2014 (28-05-2014) |
| | | JP2014530006A | 17 November 2014 (17-11-2014) |
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| | | MX2014003776A | 21 August 2014 (21-08-2014) |
| | | TW201329235A | 16 July 2013 (16-07-2013) |
| WO2014153674A1 | 02 October 2014 (02-10-2014) | None | |