

Influenza Virus Hemagglutinin Mutants

FIELD OF INVENTION

[0001] The present invention relates to producing mutant viral proteins in plants. More specifically, the present invention relates to producing and increasing influenza virus-like particle production in plants.

BACKGROUND OF THE INVENTION

[0002] Influenza viruses are enveloped, single-stranded-RNA viruses of the Orthomyxoviridae family. Influenza viruses are highly contagious and can cause mild to serious illness across all age groups.

[0003] Vaccination remains the most effective method to prevent influenza infection. Conventionally, vaccination is accomplished using live attenuated or whole inactivated forms of the virus, which elicit an immune response when administered to a patient. To eliminate the potential risk of live attenuated and whole inactivated viruses re-acquiring the competency to replicate and become infectious, vaccines comprising recombinant viral proteins have also been used to elicit protective immunity to influenza infection.

[0004] However, the use of recombinant viral proteins as the immunogenic component of vaccines is subject to a number of limitations. Firstly, in the absence of the full complement of viral proteins and genetic components required for optimal expression and proper protein folding, the yield of recombinant viral proteins in standard *in vitro* expression systems may be insufficient for the purpose of vaccine production. Second, recombinant viral protein vaccines may exhibit poor immunogenicity, owing to improper folding, poor antigen presentation, and/or the generation of a primarily humoral immune response that is ineffective in conferring long-lasting, protective immunity.

[0005] There are four types of influenza virus: A, B, C and D, of which influenza A and B are the causative organism for seasonal disease epidemics in humans.

[0006] Influenza A viruses are further divided based on the expression of hemagglutinin (HA) and neuraminidase (NA) glycoprotein subtypes on the surface of the virus. There are 18 different HA subtypes (H1-H18).

[0007] HA is a trimeric lectin that facilitates binding of the influenza virus particle to sialic acid-containing proteins on the surface of target cells and mediates release of the viral genome into the target cell. HA proteins comprise two structural elements: the head, which is the primary target of seroprotective antibodies; and the stalk. A publication by Ha et al. 2002 (EMBO J. 21:865-875; which is incorporated herein by reference) illustrates the relative orientation of the various subdomains of the stem domain cluster (SDC) and head domain cluster (HDC) in several influenza subtypes, based on Xray crystallographic structures.

[0008] HA is translated as a single polypeptide, HA0 (assembled as trimers), that must be cleaved by a serine endoprotease between the HA1 (~40 kDa) and HA2 (~20 kDa) subdomains. After cleavage, the two disulfide-bonded protein domains adopt the requisite conformation necessary for viral infectivity. HA1 forms the globular head domain containing vestigial esterase domains E1' and E2 and a receptor-binding site (RBS), and the RBS the least conserved segment of influenza virus. HA2 is a single-pass integral membrane protein with fusion peptide (FP), soluble ectodomain (SE), transmembrane (TM), and cytoplasmic tail (CT) with respective lengths of approximately 25, 160, 25, and 10 residues. HA2 together with the N and C terminal HA1 residues forms a stalk domain, which includes the transmembrane region, and is relatively conserved.

[0009] Various mutations in influenza virus proteins, particularly influenza HA, have been investigated.

[0010] For example, Castelán-Vega *et al.* (*Adv Appl Bioinform Chem.* 2014;7:37-44) used a stability prediction algorithm to compare 7,479 full-length amino acid sequences of HA from the influenza A (H1N1)pdm09 virus and identified that D104N, A259T, S124N, and E172K mutations resulted in a predicted enhancement of influenza HA stability. In contrast, S206T, K285E, and E47K mutations had a predicted destabilizing effect on HA.

[0011] In comparing the sequences of the original influenza A (H1N1)pdm [A/California/7/2009] and a later-emerging influenza strain [A/Brisbane/10/2010], Cotter *et al.* (*PLoS Pathog.* 2014;10(1):e1003831) identified that a E47K mutation in the stalk region of A/California/7/2009 HA stabilized the trimer structure, lowered the pH for membrane fusion, and increased the thermal and acid stability of the virus. Cotter *et al.* additionally observed that A/California/7/2009 E47K mutant HA was more infectious in ferrets than its wildtype counterpart.

[0012] Antanasijevic *et al.* (*J Biol Chem.* 2014;289(32):22237-45) investigated the structure-function properties of H5 HA stem loop region by site directed mutagenesis at 14 different positions. A/Vietnam/1203/04 (H5N1) mutants were expressed in HEK 293T cells and Antanasijevic reported that most mutations in the stem loop region did not disrupt expression, proteolytic processing, viral assembly, or receptor binding. However, Antanasijevic observed that HA1-D26K, HA1-M102L, HA2-V52A and HA2-I55A mutants (based on H3 numbering) exhibited significantly reduced levels of total HA, suggesting reduced expression and/or assembly of HA into viral particles. HA1-D26K, HA2-T49A and HA2-M102L mutants also exhibited lower hemagglutination titers as compared to wildtype virus. Antanasijevic additionally observed that all single mutants exhibited decreased entry into A549 lung cells, with the most pronounced impairment shown in HA1-D26K and HA2-I55A mutants. Antanasijevic further demonstrated that the HA2-L99A mutant was more sensitive to A549 lung cell inhibition by C179 neutralizing antibody as compared to wildtype virus, suggesting that the mutation enhances antibody binding and/or the mode of neutralizing action. In contrast, HA1-I28A, HA1-M31A, HA1-M31L, HA2-I45A, and HA2-I55V mutants were rendered less sensitive to entry inhibition by C179 neutralizing antibody.

[0013] WO2013/177444 and its companion publication Lu *et al.* (*Proc Natl Acad Sci USA.* 2014;111(1):125-30) reported a method for the production of properly folded HA stem domain from A/California/05/2009 (H1N1) using an *Escherichia coli*-based cell-free protein expression system and a simple refolding protocol. For inducing the trimerization of HA stem domain, either a chloramphenicol acetyl transferase (CAT) or foldon domain was fused to the C terminus of the HA. To mitigate newly exposed hydrophobicity and/or intermolecular ion pairing causing aggregation of expressed

HA stem protein, five groups of mutations were evaluated: M1 (I69T + I72E + I74T + C77T); M2 (I69T + I72E + I74T + C77T + F164D); M3 (I69T + I72E + I74T + C77T + F164D + L174D); M4 (F164D); and M5 (F164D + L174D). Lu observed that the M5 (F164D + L174D) mutations appeared to be the most influential mutations for improving HA stem protein solubility. Additional deletions (H38 to C43 and C49 to N61) and a C77T mutation were made to M5 mutants to avoid the formation of undesirable disulfide bonds, reduce surface hydrophobicity and pI, and avoid regions with disordered structure.

[0014] US Application No. 13/838,796 and its companion publication by Holtz *et al.* (*BMC Biotechnology*. 2014;14:111) teach the improved stability and maintained potency of recombinant HA by the mutation of cysteine residues in the carboxy terminal region of the HA protein including the transmembrane (TM) and cytosolic domain (CT). Specifically, Holtz *et al.* demonstrate C539A, C546A, C549A, C524S and C528A mutations in recombinant Perth/16/2009 HA (H3N2). Mutation of all five cysteine residues, or different subsets thereof, resulted in HA yields, purities, particle size, hemagglutination activity, and thermostability comparable to recombinant wildtype HA protein. In contrast, C64S and C76S mutations resulted in significantly reduced HA expression, indicating the critical role of these residues in proper HA folding. By using a single radial immune-diffusion assay (SRID), Holtz *et al.* also show that the five cysteine residue mutations improve potency of recombinant HA as compared to wildtype protein, by preventing disulfide cross-linking in the TM and CT domains. The mutant HA proteins maintain potency for at least 12 months at 25°C, whereas wildtype HA protein exhibited less than 40% potency after only 50 days post purification.

[0015] WO2015/020913 teaches the mutation of specific residues at one or more positions selected from the group of 403, 406, 411, 422, 429, 432, 433, and 435 of influenza A/Puerto Rico/8/1934 (H1N1) to tyrosine. These mutations facilitate the formation of di-tyrosine cross-links that stabilize or “lock” the stalk domain of influenza HA in its native trimeric conformation.

[0016] WO2013/079473 discloses a modified influenza HA lacking a globular head domain. The polypeptide taught in WO2013/079473 comprises an HA1 domain

where amino acids 53 to 620 (with reference to A/Brisbane/59/2007 [H1N1] numbering) are deleted and replaced with covalently linked sequence of 0 to 10 amino acids, and an HA2 domain, wherein the C-terminal amino acid of the HA1 domain is an amino acid other than arginine or lysine, and wherein one or more amino acids at position 406, 409, 413 and 416 are mutated to an amino acid selected from the group consisting of: serine, threonine, asparagine, glutamine, arginine, histidine, lysine, aspartic acid, glutamic acid, and glycine.

[0017] WO2014/191435 similarly teaches a modified influenza HA comprising an HA1 domain having a deleted segment replaced with a covalently linked sequence of 0 to 50 amino acids, and an HA2 domain, wherein the HA is resistant to cleavage at the junction between HA1 and HA2 and wherein one or more amino acids at positions 337, 340, 352, 353, 402, 406, 409, 413 and/or 416 have been mutated.

[0018] Virus-like particles (VLPs) are potential candidates for inclusion in immunogenic compositions. VLPs closely resemble mature virions, but they do not contain viral genomic material. Therefore, VLPs are non-replicative in nature, which make them safe for administration as a vaccine. In addition, VLPs can be engineered to express viral glycoproteins on the surface of the VLP, which is their most native physiological configuration. Moreover, since VLPs resemble intact virions and are multivalent particulate structures, VLPs may be more effective in inducing neutralizing antibodies to the glycoprotein than soluble envelope protein antigens.

[0019] VLPs have been produced in plants (see for example WO2009/076778; WO2009/009876; WO 2009/076778; WO 2010/003225; WO 2010/003235; WO2010/006452; WO2011/03522; WO 2010/148511; and WO2014153674, which are incorporated herein by reference).

[0020] WO2009/076778 teaches a method of producing influenza VLPs in plants comprising introducing a nucleic acid having a regulatory region active in the plant operatively linked to a nucleotide sequence encoding an influenza HA from a type A or type B influenza.

[0021] WO2009/009876 teaches a method of producing influenza HA VLPs in plants, wherein influenza HA self-assembles into VLPs in plant cells and bud from plant cell membranes.

5 [0022] WO2010/003225 discloses a method of producing influenza HA VLPs in plants comprising introducing a nucleic acid having a regulatory region active in the plant, operatively linked to a nucleotide sequence encoding an influenza HA from A/California/04/09 (H1N1).

10 [0023] WO2010/006452 teaches the production of VLPs comprising modified influenza HA proteins, wherein glycosylation sites at positions 154, 165, 286, or combinations thereof (with reference to A/Vietnam/1194/04 [H5N1] numbering), have been abolished by mutating the residues at said positions to amino acids other than asparagine. WO2010/006452 further teaches that amino acids at positions 156, 167, 288, or combinations thereof, may be mutated to residues other than serine or threonine to similarly abolish the N-linked glycosylation signal triad "N-X-S/T". By
15 selectively deleting glycosylation sites located in the globular head of the HA protein, WO2010/006452 demonstrates that the resulting HA protein has increased antigenicity and broader cross-reactivity.

[0024] WO2011/035422 teaches a method of preparing plant-derived VLPs comprising: obtaining a plant or plant matter comprising apoplast-localized VLPs;
20 producing a protoplast/spheroplast fraction and an apoplast fraction; and recovering the apoplast fraction comprising the plant-derived VLPs.

[0025] WO2010/148511 discloses a method for producing influenza VLPs in plants, wherein the VLPs comprise chimeric HA proteins. The chimeric HA proteins comprise a stem domain cluster having an F'1, F'2 and F subdomain; a head domain
25 cluster having an RB, E1 and E2 subdomain; and a transmembrane domain cluster having a transmembrane domain and a C-terminal tail domain, wherein at least one subdomain is derived from a first influenza strain and the other subdomains are derived from one or more second influenza strain.

[0026] WO2014/153674 teaches a method of producing influenza VLPs in a plant,
30 wherein the VLPs comprise modified influenza HA having a modified proteolytic

loop. The modified proteolytic loop comprises the removal of the proteolytic cleavage site between HA1 and HA2 domains of the HA0 precursor. The HA protein is thus stabilized and increased protein yields are achieved as compared to native HA protein.

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SUMMARY OF THE INVENTION

[0027] The present invention relates to the production of modified influenza viral proteins in plants. More specifically, the present invention relates to producing and increasing influenza virus-like particle (VLP) production in plants, wherein the VLPs comprise the modified influenza viral proteins for example a modified hemagglutinin (HA) protein.

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[0028] It is an object of the invention to provide an improved method to increase influenza VLP production in plants.

[0029] According to the present invention, there is provided:

[0030] A nucleic acid comprising a nucleotide sequence encoding a modified influenza H1 hemagglutinin (HA) protein, the HA protein comprising an amino acid sequence comprising at least one substitution when compared to a corresponding wildtype amino acid sequence, said at least one substitution being at one or more than one amino acid corresponding to amino acids at position 97, 374, 390 or 429 of H1 A/Michigan/45/15 HA.

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[0031] The HA protein may comprise an amino acid sequence with a substitution to a non-asparagine at the amino acid corresponding to the amino acid at position 97 of H1 A/Michigan/45/15 HA. The HA protein may comprise an amino acid sequence with a substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to the amino acid at position 97 of H1 A/Michigan/45/15 HA.

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[0032] The HA protein may comprise an amino acid sequence with a substitution to a non-lysine at the amino acid corresponding to the amino acid at position 374 of H1 A/Michigan/45/15 HA. The HA protein may comprise an amino acid sequence with a substitution to a glutamic acid or a conserved substitution of glutamic acid at the

amino acid corresponding to the amino acid at position 374 of H1 A/Michigan/45/15 HA.

5 [0033] The HA protein may comprise an amino acid sequence with a substitution to a non-phenylalanine at the amino acid corresponding to the amino acid at position 390 of H1 A/Michigan/45/15 HA. The HA protein may comprise an amino acid sequence with a substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to the amino acid at position 390 of H1 A/Michigan/45/15 HA.

10 [0034] The HA protein may comprise an amino acid sequence with a substitution to a non-leucine at the amino acid corresponding to the amino acid at position 429 of H1 A/Michigan/45/15 HA. The HA protein may comprise an amino acid sequence with a substitution to a methionine or a conserved substitution of methionine at the amino acid corresponding to the amino acid at position 429 of H1 A/Michigan/45/15 HA.

15 [0035] The HA protein may further comprise an amino acid sequence with a substitution to a non-asparagine at the amino acid corresponding to the amino acid at position 380 of H1 A/Michigan/45/15 HA. The HA protein may comprise an amino acid sequence with a substitution to an alanine or a conserved substitution of alanine at the amino acid corresponding to the amino acid at position 380 of H1 A/Michigan/45/15 HA.

20 [0036] The HA protein may further comprise an amino acid sequence with a first substitution to a non-phenylalanine at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a second substitution to a non-leucine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA. The HA protein may further comprise an amino acid sequence
25 with a first substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a second substitution to a methionine or a conserved substitution of methionine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA.

[0037] The HA protein may further comprise an amino acid sequence with a first substitution to a non-asparagine at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA and a second substitution to a non-lysine at the amino acid corresponding to amino acid at position 374 of H1 A/Michigan/45/15 HA. The HA protein may further comprise an amino acid sequence with a first substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA and a second substitution to glutamic acid or a conserved substitution of glutamic acid at the amino acid corresponding to amino acid at position 374 of H1 A/Michigan/45/15 HA.

[0038] The HA protein may further comprise an amino acid sequence with a first substitution to a non-asparagine at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA, a second substitution to a non-phenylalanine at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a third substitution to a non-leucine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA. The HA protein may further comprise an amino acid sequence with a first substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA, a second substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a third substitution to a methionine or a conserved substitution of methionine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA.

[0039] The HA protein may further comprise an amino acid sequence with a first substitution to a non-lysine at the amino acid corresponding to amino acid at position 374 of H1 A/Michigan/45/15 HA, a second substitution to a non-phenylalanine at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a third substitution to a non-leucine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA. The HA protein may further comprise an amino acid sequence with a first substitution to a glutamic acid or a conserved substitution of glutamic acid at the amino acid corresponding to amino acid at position

374 of H1 A/Michigan/45/15 HA, a second substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a third substitution to a methionine or a conserved substitution of methionine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA.

[0040] The HA protein may further comprise an amino acid sequence with a first substitution a non-asparagine at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA, a second substitution to a non-lysine at the amino acid corresponding to amino acid at position 374 of H1 A/Michigan/45/15 HA, a third substitution to a non-phenylalanine at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a fourth substitution to a non-leucine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA. The HA protein may further comprise an amino acid sequence with a first substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA, a second substitution to a glutamic acid or a conserved substitution of glutamic acid at the amino acid corresponding to amino acid at position 374 of H1 A/Michigan/45/15 HA, a third substitution to an aspartic acid or a conserved substitution of aspartic acid at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA and a fourth substitution to a methionine or a conserved substitution of methionine at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA.

[0041] Further provided are HA protein encoded by the recombinant nucleic acids as described above and virus-like particle (VLP) comprising the HA protein encoded by the recombinant nucleic acids as described above.

[0042] It is therefore provided a modified influenza H1 hemagglutinin (HA) protein, the HA protein comprising an amino acid sequence comprising at least one substitution when compared to a corresponding wildtype amino acid sequence, said at least one substitution being at one or more than one amino acid corresponding to amino acid at position 97, 374, 390 or 429 of H1 A/Michigan/45/15 HA.

[0043] Furthermore a method of producing an influenza virus like particle (VLP) in a plant, portion of a plant, or a plant cell, is provided, the method comprising:

a) introducing the recombinant nucleic acid as described above into the plant, portion of the plant, or plant cell; and

5 b) incubating the plant, portion of the plant, or plant cell under conditions that permit expression of the HA protein encoded by the recombinant nucleic acid, thereby producing the VLP. The method may further comprises a step c) of harvesting the plant, portion of the plant, or plant cell, and purifying the VLP.

10 [0044] It is further provided a method of producing an influenza virus like particle (VLP) in a plant, portion of a plant, or a plant cell, comprising:

a) providing a plant, portion of a plant, or plant cell comprising the recombinant nucleic acid as described above; and

15 b) incubating the plant, portion of the plant, or plant cell under conditions that permit expression of the HA protein encoded by the recombinant nucleic acid, thereby producing the VLP. The method may further comprises a step c) of harvesting the plant, portion of the plant, or plant cell, and purifying the VLP.

20 [0045] Furthermore, it is provided a method of increasing yield of production of an influenza virus like particle (VLP) in a plant, portion of a plant, or a plant cell, comprising: a) introducing the recombinant nucleic acid into the plant, portion of the plant, or plant cell; or providing a plant, portion of a plant, or plant cell comprising the recombinant nucleic acid; and b) incubating the plant, portion of the plant, or plant cell under conditions that permit expression of the HA protein encoded by the recombinant nucleic acid, thereby producing the VLP at a higher yield compared to plant, portion of the plant, or plant cell expressing an unmodified influenza HA
25 protein. The method may further comprises a step c) of harvesting the plant, portion of the plant, or plant cell, and purifying the VLP.

[0046] The methods may further comprise introducing a second nucleic acid encoding a proton channel protein; wherein the plant, portion of the plant, or plant cell is incubated under conditions that permit expression of the proton channel protein

encoded by the second nucleic acid. The proton channel protein may be an influenza A subtype M2 protein.

[0047] It is further provided a VLP produced by the method as described herewith.

5 [0048] The VLP may comprise one or more than one lipid derived from the plant, portion of the plant, or plant cell, plant-specific N-glycans, modified N-glycans or a combination thereof.

10 [0049] In addition a method of producing an antibody or antibody fragment is provided, the method comprising administering the VLP as described to a subject, or a host animal, thereby producing the antibody or the antibody fragment. Antibodies or the antibody fragments produced by the method are also provided.

15 [0050] Furthermore it is provided a plant, portion of the plant, or plant cell comprising the recombinant nucleic acid or HA protein encoded by the recombinant nucleic acid. The HA protein may form VLP. Accordingly, a plant, portion of the plant, or plant cell comprising VLP comprising HA protein encoded by the recombinant nucleic acid are also provided.

20 [0051] In addition, it is provided a composition for inducing an immune response comprising, an effective dose of the VLP as described herewith, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient. A method for inducing immunity to an influenza infection in a subject, the method comprising administering the VLP as described is also provided. The VLP may be administered to the subject orally, intranasally, intramuscularly, intraperitoneally, intravenously or subcutaneously.

25 [0052] Furthermore, it is provided a modified influenza hemagglutinin (HA) protein comprising an amino acid sequence having from about 30% to about 100%, sequence identity or sequence similarity with a sequence of the sequences of SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 4, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55,
30 SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65,

SEQ ID NO: 67, SEQ ID NO: 72, SEQ ID NO: 77, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 105, SEQ ID NO: 108, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 140, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, provided that the influenza HA protein comprises at least on substitution as described herewith and is able to form VLPs, induces an immune response when administered to a subject, induces hemagglutination or a combination thereof.

[0053] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0054] 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:

[0055] **Figure 1** shows a sequence alignment of the amino acid sequences of hemagglutinin (HA) of A/California/7/09 (H1N1) (SEQ ID NO: 130); A/Honduras/17734/16 (H1N1) (SEQ ID NO: 131); A/Darwin/11/15 (H1N1) (SEQ ID NO: 132); A/Costa Rica/0513/16 (H1N1) (SEQ ID NO: 133); A/Michigan/45/15 (H1N1) (SEQ ID NO: 134); A/Massachusetts/06/17 (H1N1) (SEQ ID NO: 135). Outlined residues align with amino acids D97, E374, F390, and L429 of HA from influenza H1 strains (H1N1) for example A/California/7/09 (H1N1).

[0056] **Figure 2A** shows the hemagglutination titers of wildtype A/California/07/09 H1, L429M A/California/07/09 mutant H1, N380A A/California/7/09 mutant H1 and F390D A/California/7/09 mutant H1. **Figure 2B** shows the hemagglutination titers of wildtype A/California/07/09 H1, F390D A/California/07/09 mutant H1, L429M A/California/7/09 mutant H1, and F390D + L429M A/California/07/09 mutant H1, expressed as percentages relative to wildtype A/California/07/09. **Figure 2C** shows the post-density gradient VLP yields of wildtype A/California/07/09 H1, N380A A/California/07/09 mutant H1, L429M A/California/07/09 mutant H1, and F390D A/California/07/09 mutant H1.

[0057] **Figure 3A** shows the hemagglutination titers of wildtype A/California/07/09 H1, wildtype A/Michigan/45/15 H1, K374E A/Michigan/45/15 mutant H1 and N97D A/Michigan/45/15 mutant H1. **Figure 3B** shows the hemagglutination titers of wildtype A/California/07/09 H1, F390D A/California/07/09 mutant H1, wildtype A/Michigan/45/15 H1, N97D A/Michigan/45/15 mutant H1, K374E A/Michigan/45/15 mutant H1, N380A A/Michigan/45/15 mutant H1, F390D A/Michigan/45/15 mutant H1, L429M A/Michigan/45/15 mutant H1, N97D + K374E A/Michigan/45/15 mutant H1, F390D + L429M A/Michigan/45/15 mutant H1, F390D + N380A A/Michigan/45/15 mutant H1, L429M + N380A A/Michigan/45/15 mutant H1, K374E + F390D + L429M A/Michigan/45/15 mutant H1, N97D + F390D + L429M A/Michigan/45/15 mutant H1 and N97D + K374E + F390D + L429M A/Michigan/45/15 mutant H1.

[0058] **Figure 4A** shows the hemagglutination titers of wildtype A/Michigan/45/15 H1, N97D A/Michigan/45/15 mutant H1, K374E A/Michigan/45/15 mutant H1, F390D A/Michigan/45/15 mutant H1, L429M A/Michigan/45/15 mutant H1, F390D + L429M A/Michigan/45/15 mutant H1 and N97 + F390D + L429M A/Michigan/45/15 mutant H1; hemagglutination titers of wildtype A/Honduras/17734/16 H1, N97D A/Honduras/17734/16 mutant H1, K374E A/Honduras/17734/16 mutant H1, F390D A/Honduras/17734/16 mutant H1, L429M A/Honduras/17734/16 mutant H1, F390D + L429M A/Honduras/17734/16 mutant H1, and N97D + F390D + L429M A/Honduras/17734/16 mutant H1; and hemagglutination titers of wildtype A/Darwin/11/15 H1, N97D A/Darwin/11/15 mutant H1, K374E A/Darwin/11/15 mutant H1, F390D A/Darwin/11/15 mutant H1, L429M A/Darwin/11/15 mutant H1, F390D + L429M A/Darwin/11/15 mutant H1 and N97D + F390D + L429M A/Darwin/11/15 mutant H1. **Figure 4B** shows the hemagglutination titers of F390D + L429M A/Michigan/45/15, F390D + L429M A/Massachusetts/06/17 mutant H1, K374E + F390D + L429M A/Massachusetts/06/17 mutant H1, N97D + F390D + L429M A/Massachusetts/06/17 mutant H1, and N97D + K374E + F390D + L429M A/Massachusetts/06/17 mutant H1; hemagglutination titers of F390D + L429M A/Costa Rica/0513/16 mutant H1, K374E + F390D + L429M A/Costa Rica/0513/16 mutant H1, N97D + F390D + L429M A/Costa Rica/0513/16 mutant H1, and N97D + K374E + F390D + L429M A/Costa Rica/0513/16 mutant H1. **Figure 4C** shows the

hemagglutination titers of F390D + L429M A/Michigan/45/15, F390D + L429M A/Paris/1227/2017 mutant H1, K374E + F390D + L429M A/Paris/1227/2017 mutant H1, N97D + F390D + L429M A/Paris/1227/2017 mutant H1, and N97D + K374E + F390D + L429M A/Paris/1227/2017 mutant H1; hemagglutination titers of F390D + L429M A/Norway/2147/2017 mutant H1, K374E + F390D + L429M A/Norway/2147/2017 mutant H1, N97D + F390D + L429M A/Norway/2147/2017 mutant H1, and N97D + K374E + F390D + L429M A/Norway/2147/2017 mutant H1.

[0059] **Figure 5** shows the hemagglutination titers of wildtype A/Indonesia/5/2005 H5, wildtype A/Egypt/N04915/2014 H5, F393D A/Indonesia/5/2005 mutant H5, F393D A/Egypt/N04915/2014 mutant H5, N383A A/Indonesia/5/2005 mutant H5, and N383A A/Egypt/N04915/2014 mutant H5. The numbering is in accordance with A/Indonesia/5/2005.

[0060] **Figure 6A** shows a schematic representation of vector 1314 (H1 A-Cal-7-2009). **Figure 6B** shows a schematic representation of vector 2980 (H1 A-Cal-7-09 (F390D)). **Figure 6C** shows a schematic representation of vector 2962 (H1 A-Cal-7-09 (L429M)). **Figure 6D** shows a schematic representation of vector 2995 (H1 A-Cal-7-09 (F390D+L429M)). **Figure 6E** shows a schematic representation of vector 3640 (H1 A-Mich-45-2015). **Figure 6F** shows a schematic representation of vector 3774 (H1 A-Mich-45-2015 (N97D)). **Figure 6G** shows a schematic representation of vector 3771 (H1 A-Mich-45-2015 (K374E)). **Figure 6H** shows a schematic representation of vector 3641 (H1 A-Mich-45-2015 (F390D)). **Figure 6I** shows a schematic representation of vector 3643 (H1 A-Mich-45-2015 (L429M)). **Figure 6J** shows a schematic representation of vector 3880 (H1 A-Mich-45-2015 (N97D+K374E)). **Figure 6K** shows a schematic representation of vector 3703 (H1 A-Mich-45-2015 (F390D+L429M)). **Figure 6L** shows a schematic representation of vector 3879 (H1 A-Mich-45-2015 (N97D+F390D+L429M)). **Figure 6M** shows a schematic representation of vector 3878 (H1 A-Mich-45-2015 (K374E+F390D+L429M)). **Figure 6N** shows a schematic representation of vector 3881 (H1 A-Mich-45-2015 (N97D+K374E+F390D+L429M)). **Figure 6O** shows a schematic representation of vector 4091 (H1 A-Mass-06-2017 (F390D+L429M)). **Figure 6P** shows a schematic representation of vector 4093 (H1 A-Mass-06-2017

(N97D+F390D+L429M)). **Figure 6Q** shows a schematic representation of vector 4092 (H1 A-Mass-06-2017 (K374E+F390D+L429M)). **Figure 6R** shows a schematic representation of vector 4094 (H1 A-Mass-06-2017 (N97D+K374E+F390D+L429M)). **Figure 6S** shows a schematic representation of vector 4715 (H1 A-CostaRica-0513-2016 (F390D+L429M)). **Figure 6T** shows a schematic representation of vector 4717 (H1 A-CostaRica-0513-2016 (N97D+F390D+L429M)). **Figure 6U** shows a schematic representation of vector 4716 (H1 A-CostaRica-0513-2016 (K374E+F390D+L429M)). **Figure 6V** shows a schematic representation of vector 4718 (H1 A-CostaRica-0513-2016 (N97D+K374E+F390D+L429M)). **Figure 6W** shows a schematic representation of vector 3944 (H1 A-Hond-17734-16). **Figure 6X** shows a schematic representation of vector 3950 (H1 A-Hond-17734-16 (N97D)). **Figure 6Y** shows a schematic representation of vector 3948 (H1 A-Hond-17734-16 (K374E)). **Figure 6Z** shows a schematic representation of vector 3945 (H1 A-Hond-17734-16 (F390D)). **Figure 6AA** shows a schematic representation of vector 3949 (H1 A-Hond-17734-16 (L429M)). **Figure 6BB** shows a schematic representation of vector 3946 (H1 A-Hond-17734-16 (F390D+L429M)). **Figure 6CC** shows a schematic representation of vector 3951 (H1 A-Hond-17734-16 (N97D+F390D+L429M)). **Figure 6DD** shows a schematic representation of vector 3984 (H1 A-Darw-11-15). **Figure 6EE** shows a schematic representation of vector 3990 (H1 A-Darw-11-15 (N97D)). **Figure 6FF** shows a schematic representation of vector 3988 (H1 A-Darw-11-15 (K374E)). **Figure 6GG** shows a schematic representation of vector 3985 (H1 A-Darw-11-15 (F390D)). **Figure 6HH** shows a schematic representation of vector 3989 (H1 A-Darw-11-15 (L429M)). **Figure 6II** shows a schematic representation of vector 3986 (H1 A-Darw-11-15 (F390D+L429M)). **Figure 6JJ** shows a schematic representation of vector 3991 (H1 A-Darw-11-15 (N97D+F390D+L429M)). **Figure 6KK** shows a schematic representation of vector 3644 (H1 A-Mich-45-2015 (N380A)). **Figure 6LL** shows a schematic representation of vector 3704 (H1 A-Mich-45-2015 (F390D+N380A)). **Figure 6MM** shows a schematic representation of vector 4765 (A/Paris/1227/17 (F390D+L429M)). **Figure 6NN** shows a schematic representation of vector 4766 (A/Paris/1227/17 (K374E+F390D+L429M)). **Figure 6OO** shows a schematic representation of vector 4767 (A/Paris/1227/17 (N97D+F390D+L429M)).

Figure 6PP shows a schematic representation of vector 4768 (A/Paris/1227/17 (N97D+K374E+F390D+L429M)). **Figure 6QQ** shows a schematic representation of vector 4775 (A/Norway/2147/17 (F390D+L429M)). **Figure 6RR** shows a schematic representation of vector 4776 (A/Norway/2147/17 (K374E+F390D+L429M)). **Figure 6SS** shows a schematic representation of vector 4777 (A/Norway/2147/17 (N97D+F390D+L429M)). **Figure 6TT** shows a schematic representation of vector 4778 (A/Norway/2147/17 (N97D+K374E+F390D+L429M)).

[0061] **Figure 7A** shows a schematic representation of vector 2295 (H5 A-Indo-5-05). **Figure 7B** shows a schematic representation of vector 3680 (H5 A-Indo-5-05 (F393D)). **Figure 7C** shows a schematic representation of vector 3645 (H5 A-Egypt-N04915-14). **Figure 7D** shows a schematic representation of vector 3690 (H5 A-Egypt-N04915-14 (F392D)).

[0062] **Figure 8** shows a schematic representation of vector 1190 (Vector for In-Fusion cloning into CPMV 160-based expression cassette).

DETAILED DESCRIPTION

[0063] The following description is of a preferred embodiment.

[0064] As used herein, the terms “comprising”, “having”, “including”, “containing”, and grammatical variations thereof, are inclusive or open-ended and do not exclude additional, un-recited elements and/or method steps. The term “consisting essentially of” when used herein in connection with a product, use or method, denotes that additional elements and/or method steps may be present, but that these additions do not materially affect the manner in which the recited method or use functions. The term “consisting of” when used herein in connection with a product, use or method, excludes the presence of additional elements and/or method steps. A product, use or method described herein as comprising certain elements and/or steps may also, in certain embodiments, consist essentially of those elements and/or steps, and in other embodiments consist of those elements and/or steps, whether or not these embodiments are specifically referred to. In addition, the use of the singular includes the plural, and “or” means “and/or” unless otherwise stated. Unless otherwise defined herein, all technical and scientific terms used herein have the same meaning as

commonly understood by one of ordinary skill in the art. As used herein, the term “about” refers to an approximately $\pm 10\%$ variation from a given value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to. The use of the word “a” or “an” when used herein in conjunction with the term “comprising” may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one” and “one or more than one.”

[0065] The term “plant”, “portion of a plant”, “plant portion”, “plant matter”, “plant biomass”, “plant material”, “plant extract”, or “plant leaves”, as used herein, may comprise an entire plant, tissue, cells, or any fraction thereof, intracellular plant components, extracellular plant components, liquid or solid extracts of plants, or a combination thereof, that are capable of providing the transcriptional, translational, and post-translational machinery for expression of one or more than one nucleic acids described herein, and/or from which an expressed protein or VLP may be extracted and purified. Plants may include, but are not limited to, herbaceous plants. Furthermore plants may include, but are not limited to, agricultural crops including for example canola, Brassica spp., maize, Nicotiana spp., (tobacco) for example, *Nicotiana benthamiana*, *Nicotiana rustica*, *Nicotiana*, *tabacum*, *Nicotiana glauca*, *Arabidopsis thaliana*, alfalfa, potato, sweet potato (*Ipomoea batatas*), ginseng, pea, oat, rice, soybean, wheat, barley, sunflower, cotton, corn, rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), safflower (*Carthamus tinctorius*).

[0066] The term “plant portion”, as used herein, refers to any part of the plant including but not limited to leaves, stem, root, flowers, fruits, a plant cell obtained from leaves, stem, root, flowers, fruits, a plant extract obtained from leaves, stem, root, flowers, fruits, or a combination thereof. The term “plant extract”, as used herein, refers to a plant-derived product that is obtained following treating a plant, a portion of a plant, a plant cell, or a combination thereof, physically (for example by freezing followed by extraction in a suitable buffer), mechanically (for example by grinding or homogenizing the plant or portion of the plant followed by extraction in a suitable buffer), enzymatically (for example using cell wall degrading enzymes), chemically (for example using one or more chelators or buffers), or a combination thereof. A plant extract may be further processed to remove undesired plant

components for example cell wall debris. A plant extract may be obtained to assist in the recovery of one or more components from the plant, portion of the plant or plant cell, for example a protein (including protein complexes, protein suprastructures and/or VLPs), a nucleic acid, a lipid, a carbohydrate, or a combination thereof from the plant, portion of the plant, or plant cell. If the plant extract comprises proteins, then it may be referred to as a protein extract. A protein extract may be a crude plant extract, a partially purified plant or protein extract, or a purified product, that comprises one or more proteins, protein complexes, protein suprastructures, and/or VLPs, from the plant tissue. If desired a protein extract, or a plant extract, may be partially purified using techniques known to one of skill in the art, for example, the extract may be subjected to salt or pH precipitation, centrifugation, gradient density centrifugation, filtration, chromatography, for example, size exclusion chromatography, ion exchange chromatography, affinity chromatography, or a combination thereof. A protein extract may also be purified, using techniques that are known to one of skill in the art.

[0067] The term “construct”, “vector” or “expression vector”, as used herein, refers to a recombinant nucleic acid for transferring exogenous nucleic acid sequences into host cells (e.g. plant cells) and directing expression of the exogenous nucleic acid sequences in the host cells. “Expression cassette” refers to a nucleotide sequence comprising a nucleic acid of interest under the control of, and operably (or operatively) linked to, an appropriate promoter or other regulatory elements for transcription of the nucleic acid of interest in a host cell. As one of skill in the art would appreciate, the expression cassette may comprise a termination (terminator) sequence that is any sequence that is active the plant host. For example the termination sequence may be derived from the RNA-2 genome segment of a bipartite RNA virus, e.g. a comovirus, the termination sequence may be a NOS terminator, or terminator sequence may be obtained from the 3'UTR of the alfalfa plastocyanin gene.

[0068] The constructs of the present disclosure may further comprise a 3' untranslated region (UTR). A 3' untranslated region contains a polyadenylation signal and any other regulatory signals capable of effecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by effecting the addition of

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

[0069] By "regulatory region" "regulatory element" or "promoter" it is meant a portion of nucleic acid typically, but not always, upstream of the protein coding region of a gene, which may be comprised of either DNA or RNA, or both DNA and RNA. When a regulatory region is active, and in operative association, or operatively linked, with a nucleotide sequence of interest, this may result in expression of the nucleotide sequence of interest. A regulatory element may be capable of mediating organ specificity, or controlling developmental or temporal gene activation. A "regulatory region" includes promoter elements, core promoter elements exhibiting a basal promoter activity, elements that are inducible in response to an external stimulus, elements that mediate promoter activity such as negative regulatory elements or transcriptional enhancers. "Regulatory region", as used herein, also includes elements that are active following transcription, for example, regulatory elements that modulate gene expression such as translational and transcriptional enhancers, translational and transcriptional repressors, upstream activating sequences, and mRNA instability determinants. Several of these latter elements may be located proximal to the coding region.

[0070] 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 that modify gene expression.

[0071] 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).

[0072] An inducible regulatory region is one that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor that binds specifically to an inducible regulatory region to activate transcription may be present in an inactive form, which is then directly or indirectly converted to the active form by the inducer. However, the protein factor may also be absent. The inducer can be a chemical agent such as a protein, metabolite, growth regulator, herbicide or phenolic compound or a physiological stress imposed directly by heat, cold, salt, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus. A plant cell containing an inducible regulatory region may be exposed to an inducer by externally

applying the inducer to the cell or plant such as by spraying, watering, heating or similar methods. Inducible regulatory elements may be derived from either plant or non-plant genes (e.g. Gatz, C. and Lenk, I.R.P., 1998, Trends Plant Sci. 3, 352-358). Examples, of potential inducible promoters include, but not limited to, tetracycline-inducible promoter (Gatz, C., 1997, Ann. Rev. Plant Physiol. Plant Mol. Biol. 48, 89-108), steroid inducible promoter (Aoyama, T. and Chua, N.H., 1997, Plant J. 2, 397-404) and ethanol-inducible promoter (Salter, M.G., et al, 1998, Plant Journal 16, 127-132; Caddick, M.X., et al, 1998, Nature Biotech. 16, 177-180) cytokinin inducible IB6 and CKII genes (Brandstatter, I. and Kieber, J.J., 1998, Plant Cell 10, 1009-1019; Kakimoto, T., 1996, Science 274, 982-985) and the auxin inducible element, DR5 (Ulmasov, T., et al., 1997, Plant Cell 9, 1963-1971).

[0073] 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; which is incorporated herein by reference), the rice actin 1 (Zhang et al, 1991, Plant Cell, 3: 1155-1165), actin 2 (An *et al.*, 1996, *Plant J.*, 10: 107-121), or tms 2 (U.S. 5,428,147), and triosephosphate isomerase 1 (Xu et. al., 1994, Plant Physiol. 106: 459-467) genes, the maize ubiquitin 1 gene (Cornejo et al, 1993, Plant Mol. Biol. 29: 637-646), the *Arabidopsis* ubiquitin 1 and 6 genes (Holtorf et al, 1995, Plant Mol. Biol. 29: 637-646), the tobacco translational initiation factor 4A gene (Mandel et al, 1995 Plant Mol. Biol. 29: 995-1004), the Cassava Vein Mosaic Virus promoter, pCAS, (Verdaguer et al., 1996); the promoter of the small subunit of ribulose biphosphate carboxylase, pRbcS: (Outchkourov et al., 2003), the pUbi (for monocots and dicots).

[0074] 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.

[0075] 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).

5 [0076] The term “native”, “native protein” or “native domain”, as used herein, refers to a protein or domain having a primary amino acid sequence identical to wildtype. Native proteins or domains may be encoded by nucleotide sequences having 100% sequence similarity to the wildtype sequence. A native amino acid sequence may also be encoded by a human codon (hCod) optimized nucleotide sequence or a nucleotide
10 sequence comprising an increased GC content when compared to the wild type nucleotide sequence provided that the amino acid sequence encoded by the hCod-nucleotide sequence exhibits 100% sequence identity with the native amino acid sequence.

[0077] By a nucleotide sequence that is “human codon optimized” or a “hCod”
15 nucleotide sequence, it is meant the selection of appropriate DNA nucleotides for the synthesis of an oligonucleotide sequence or fragment thereof that approaches the codon usage generally found within an oligonucleotide sequence of a human nucleotide sequence. By “increased GC content” it is meant the selection of appropriate DNA nucleotides for the synthesis of an oligonucleotide sequence or
20 fragment thereof in order to approach codon usage that, when compared to the corresponding native oligonucleotide sequence, comprises an increase of GC content, for example, from about 1 to about 30%, or any amount therebetween, over the length of the coding portion of the oligonucleotide sequence. For example, from about 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30%, or any amount therebetween, over
25 the length of the coding portion of the oligonucleotide sequence. As described below, a human codon optimized nucleotide sequence, or a nucleotide sequence comprising an increased GC content (when compared to the wild type nucleotide sequence) exhibits increased expression within a plant, portion of a plant, or a plant cell, when compared to expression of the non-human optimized (or lower GC content) nucleotide
30 sequence.

[0078] Modified influenza hemagglutinin (HA) proteins (also termed modified HA protein, modified influenza HA protein, modified HA, modified influenza HA, mutant HA, influenza mutant HA, influenza HA variants or HA variants) and methods of producing modified influenza HA proteins in plants are described herein. The modified influenza HA proteins disclosed herewith comprise modifications or mutations that have been found to result in improved HA characteristics as compared to the wildtype HA or unmodified HA proteins. For example, the modified influenza HA protein may have an amino acid sequence with at least one substitution of an amino acid when compared to a corresponding wildtype amino acid sequence.

[0079] Examples of improved characteristics of the modified HA protein include, increased HA protein yield when expressed in plant cells as compared to the wildtype or unmodified HA of the same strain or subtype of influenza that does not comprise the modification(s) or mutation(s); improved hemagglutination titer of the modified HA protein when compared to the wildtype or unmodified HA protein; improved integrity, stability, or both integrity and stability, of virus like particles (VLPs) that are comprised of the modified HA proteins as compared to the integrity, stability or both of VLPs comprising wildtype HA that does not comprise the modification(s) or mutation(s); increased VLP yield when expressed in plant cells as compared to the wildtype level of VLP production that does not comprise the modification(s) or mutation(s); and a combination thereof.

Influenza Subtypes and Strain

[0080] The term “influenza virus subtype” as used herein refers to influenza A virus variants that are characterized by various combinations of the hemagglutinin (H or HA) and neuramidase (N) viral surface proteins. According to the present specification, influenza virus subtypes and hemagglutinin (HA) from such virus subtypes may be referred to by their H number, such as, for example, “HA of the H1 subtype”, “H1 HA”, or “H1 influenza”. The term “subtype” specifically includes all individual “strains” within each subtype, which usually result from mutations and may show different pathogenic profiles. Such strains may also be referred to as various “isolates” of a viral subtype. Accordingly, as used herein, the terms “strains” and “isolates” may be used interchangeably.

[0081] Traditionally, different strains of influenza have been categorized based upon, e.g., the ability of influenza to agglutinate red blood cells (RBCs or erythrocytes). Antibodies specific for particular influenza strains may bind to the virus and, thus, prevent such agglutination. Assays determining strain types based on such inhibition are typically known as hemagglutinin inhibition assays (HI assays or HAI assays) and are standard and well known methods in the art to characterize influenza strains.

[0082] However, HA proteins from different virus strains also show significant sequence similarity at both the nucleic acid and amino acid levels. This level of similarity varies when strains of different subtypes are compared, with some strains clearly displaying higher levels of similarity than others (Air, Proc. Natl. Acad. Sci. USA, 1981, 78:7643). The levels of amino acid similarity vary between virus strains of one subtype and virus strains of other subtypes (Air, Proc. Natl. Acad. Sci. USA, 1981, 78:7643). This variation is sufficient to establish discrete subtypes and the evolutionary lineage of the different strains, but the DNA and amino acid sequences of different strains are still readily aligned using conventional bioinformatics techniques (Air, Proc. Natl. Acad. Sci. USA, 1981, 78:7643; Suzuki and Nei, Mol. Biol. Evol. 2002, 19:501).

[0083] Multiple nucleotide sequences, or corresponding polypeptide sequences of hemagglutinin (HA), may be aligned to determine a “consensus” or “consensus sequence” of a subtype (see Figure 1).

[0084] Based on sequence similarities, influenza virus subtypes can further be classified by reference to their phylogenetic group. Phylogenetic analysis (Fouchier et al., J Virol. 2005 Mar;79(5):2814-22) has demonstrated a subdivision of HAs that falls into two main groups (Air, Proc. Natl. Acad. Sci. USA, 1981, 78:7643): inter alia the H1, H2, H5 and H9 subtypes in phylogenetic group 1 and inter alia the H3, H4 and H7 subtypes in phylogenetic group 2.

[0085] New influenza HA proteins, HA modifications, HA protein variants and mutants are created by introducing changes to the amino acid sequence of HA protein that results in an improved characteristic of the HA as described above. Isolation of nucleic acids encoding such HA molecules is routine, as is modification of the nucleic

acid to introduce changes in the amino acid sequence, e.g., by site-directed mutagenesis.

[0086] Modified influenza HA proteins and methods of producing modified influenza HA proteins in plants are described herein. It has been observed that the modification for example by substitution of specific amino acids in HA proteins for example HA from subtype H1 results in improved characteristics of the modified HA protein when compared to the wildtype HA protein or unmodified HA protein.

[0087] The one or more than one modification, mutation or substitution of the HA protein as described herein are not located in known epitopic regions of the HA protein nor do these modifications, mutations or substitutions add or remove glycosylation sites within the HA protein.

[0088] The HA protein, mutant HA protein or modified HA protein as described herein is modified and comprises one or more than one mutation, modification, or substitution in its amino acid sequence at any one or more amino acid that correspond with amino acids at positions 97, 374, 380, 390 or 429 of A/Michigan/45/15 HA (SEQ ID NO: 134; see Fig 1) or A/California/07/09 HA (SEQ ID NO: 130; see Figure 1).

[0089] By “correspond to an amino acid” or “corresponding to an amino acid”, it is meant that an amino acid corresponds to an amino acids in a sequence alignment with an influenza reference strain as described below.

[0090] The amino acid residue number or residue position of HA is in accordance with the numbering of the HA of an influenza reference strain. For example in the case of influenza H1 the reference strain may be A/Michigan/45/15 HA (SEQ ID NO: 134; see Fig 1) or A/California/07/09 HA (SEQ ID NO: 130 see Fig 1). The corresponding amino acid positions may be determined by aligning the sequences of the HA (for example H1 HA) with the sequence of HA of their respective reference strain. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA

85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)). An amino acid sequence alignment of several influenza A HA domains, which are not to be considered limiting, is shown in Figure 1.

[0091] When referring to modifications, mutants or variants, the wild type amino acid residue (also referred to as simply 'amino acid') is followed by the residue number and the new or substituted amino acid. For example, substitution of Aspartic Acid (D, Asp) for Asparagine (N, Asn) in residue or amino acid at position 97 is denominated N97D (see Table 1).

[0092] The modified HA, HA mutants or variants for example modified H1 HA are designated in the same manner by using the single letter amino acid code for the wild-type residue followed by its position and the single letter amino acid code of the replacement residue. Multiple mutants are indicated by component single mutants separated by slashes (/) or pluses (+). Thus for example the H1 HA mutant N380A/L429M is a di-substituted mutant in which Alanine (A, Ala) replaces Asparagine (N, Asp) at residue position 380 and Methionine (M, Met) replaces Leucine (L, Leu) at residue position 429 and the H1 HA mutant protein N380A/F390D is a di-substituted variant in which Alanine (A, Ala) replaces Asparagine (N, Asn) at position 380 and Aspartic Acid (D, Asp) replaces Phenylalanine (F, Phe) at position in the H1 HA protein.

[0093] **Table 1.** Positions of modification in HA and the corresponding amino acid/residue position in reference strains of influenza H1 and H5. The exemplified modification is shown in brackets.

Position of Modification in HA (exemplification)	H1 HA ¹	H5 HA ²
97 (N97D)	D97	D97
374 (K374E)	E374	G377
382 (N382A)	N380	N383
390 (F390D)	F390	F393
429 (L429M)	L429	M432

¹A/Michigan/45/15 or A/California/07/09

²A/Indonesia /05/05

5 [0094] The modified influenza hemagglutinin (HA) protein may comprise an amino acid sequence having at least one amino acid substitution when compared to a corresponding wildtype amino acid sequence.

10 [0095] By “amino acid substitution” or “substitution” it is meant the replacement of an amino acid in the amino acid sequence of a protein with a different amino acid. The terms amino acid, amino acid residue or residue are used interchangeably in the disclosure. One or more amino acids may be replaced with one or more amino acids that are different than the original or wildtype amino acid at this position, without changing the overall length of the amino acid sequence of the protein. The substitution or replacement may be experimentally induced by altering the codon sequence in a nucleotide sequence encoding the protein to the codon sequence of a
15 different amino acid compared to the original or wildtype amino acid. The resulting protein is a modified protein, for example a modified influenza HA protein. The modified influenza HA protein does not occur naturally.

20 [0096] The modified HA includes non-naturally occurring HA protein, having at least one modification to naturally occurring HA and having improved characteristics compared to naturally occurring HA protein from which the amino acid sequence of the modified HA is derived. Modified HA proteins have an amino acid sequence, not found in nature, which is derived by replacement of one or more amino acid residues of an HA protein with one or more different amino acids.

25 [0097] Accordingly, modified HA, mutant HA or recombinant HA refers to an HA in which the DNA sequence encoding the naturally-occurring HA is modified to produce a modified or mutant DNA sequence which encodes the modification, mutation or substitution of one or more amino acids in the HA amino acid sequence.

30 [0098] Some of the residues identified for modification, mutation or substitution correspond to conserved residues whereas others are not. In the case of residues which are not conserved, the replacement of one or more amino acids is limited to substitutions which produce a modified HA which has an amino acid sequence that does not correspond to one found in nature. In the case of conserved residues, such

modification, substitution or replacements should also not result in a naturally-occurring HA sequences.

Conservative substitutions

5 [0099] As described herein, residues in HA proteins may be identified and modified, substituted or mutated to produce modified HA protein or HA protein variants. The substitutions or mutations at specific positions are not limited to the amino acid substitutions described herewith or as given in the examples. For example, the HA variants may contain conserved or conservative substitutions of describes amino acid substitutions.

10 [00100] As used herein, the term “conserved substitution” or “conservative substitution” and grammatical variations thereof, refers to the presence of an amino acid residue in the sequence of the HA protein that is different from, but is in the same class of amino acid as the described substitution or described residue (i.e., a nonpolar residue replacing a nonpolar residue, an aromatic residue replacing an aromatic
15 residue, a polar-uncharged residue replacing a polar-uncharged residue, a charged residue replacing a charged residue). In addition, conservative substitutions can encompass a residue having an interfacial hydropathy value of the same sign and generally of similar magnitude as the residue that is replacing the wildtype residue.

[00101] As used herein, the term “nonpolar residue” refers to glycine (G, Gly),
20 alanine (A, Ala), valine (V, Val), leucine (L, Leu), isoleucine (I, Ile), and proline (P, Pro); the term “aromatic residue” refers to phenylalanine (F, Phe), tyrosine (Y, Tyr), and tryptophan (W, Trp); the term “polar uncharged residue” refers to serine (S, Ser), threonine (T, Thr), cysteine (C, Cys), methionine (M, Met), asparagine (N, Asn) and glutamine (Q, Gln); the term “charged residue” refers to the negatively charged amino
25 acids aspartic acid (D, Asp) and glutamic acid (E, Glu), as well as the positively charged amino acids lysine (K, Lys), arginine (R, Arg), and histidine (H, His). Other classification of amino acids may be as follows:

- amino acids with hydrophobic side chain (aliphatic): Alanine (A, Ala), Isoleucine (I, Ile), Leucine (L, Leu), Methionine (M, Met) and Valine (V, Val);

- amino acids with hydrophobic side chain (aromatic): Phenylalanine (F, Phe), Tryptophan (W, Trp), Tyrosine (Y, Tyr);
- amino acids with polar neutral side chain: Asparagine (N, Asn), Cysteine (C, Cys), Glutamine (Q, Gln), Serine (S, Ser) and Threonine (T, Thr);
- 5 • amino acids with electrically charged side chains (acidic): Aspartic acid (D, Asp), Glutamic acid (E, Glu);
- amino acids with electrically charged side chains (basic): Arginine (R, Arg); Histidine (H, His); Lysine (K, Lys), Glycine (G, Gly) and Proline (P, Pro).

10 [00102] Conservative amino acid substitutions are likely to have a similar effect on the activity of the resultant HA protein variant or modified HA protein, as the original substitution or modification. Further information about conservative substitutions can be found, for instance, in Ben Bassat et al. (J. Bacteriol, 169:751-757, 1987), O'Regan et al. (Gene, 77:237-251, 1989), Sahin-Toth et al. (Protein ScL, 3:240-247, 1994), Hochuli et al (Bio/Technology, 6:1321-1325, 1988) and in widely used textbooks of

15 genetics and molecular biology.

[00103] The Blosum matrices are commonly used for determining the relatedness of polypeptide sequences. The Blosum matrices were created using a large database of trusted alignments (the BLOCKS database), in which pairwise sequence alignments related by less than some threshold percentage identity were counted (Henikoff et al.,

20 Proc. Natl. Acad. Sci. USA, 89:10915-10919, 1992). A threshold of 90% identity was used for the highly conserved target frequencies of the BLOSUM90 matrix. A threshold of 65% identity was used for the BLOSUM65 matrix. Scores of zero and above in the Blosum matrices are considered "conservative substitutions" at the percentage identity selected. The following table shows exemplary conservative

25 amino acid substitutions: Table 2.

Table 2. Exemplary conservative amino acid substitutions.

Original Residue	Very Highly - Conserved Substitutions	Highly Conserved Substitutions (from the Blosum90 Matrix)	Conserved Substitutions (from the Blosum65 Matrix)
Ala	Ser	Gly, Ser, Thr	Cys, Gly, Ser, Thr, Val
Arg	Lys	Gln, His, Lys	Asn, Gln, Glu, His, Lys
Asn	Gln; His	Asp, Gln, His, Lys, Ser, Thr	Arg, Asp, Gln, Glu, His, Lys, Ser, Thr
Asp	Glu	Asn, Glu	Asn, Gln, Glu, Ser
Cys	Ser	None	Ala
Gln	Asn	Arg, Asn, Glu, His, Lys, Met	Arg, Asn, Asp, Glu, His, Lys, Met, Ser
Glu	Asp	Asp, Gln, Lys	Arg, Asn, Asp, Gln, His, Lys, Ser
Gly	Pro	Ala	Ala, Ser
His	Asn; Gln	Arg, Asn, Gln, Tyr	Arg, Asn, Gln, Glu, Tyr
Ile	Leu; Val	Leu, Met, Val	Leu, Met, Phe, Val
Leu	Ile; Val	Ile, Met, Phe, Val	Ile, Met, Phe, Val
Lys	Arg; Gln; Glu	Arg, Asn, Gln, Glu	Arg, Asn, Gln, Glu, Ser,
Met	Leu; Ile	Gln, Ile, Leu, Val	Gln, Ile, Leu, Phe, Val
Phe	Met; Leu; Tyr	Leu, Trp, Tyr	Ile, Leu, Met, Trp, Tyr
Ser	Thr	Ala, Asn, Thr	Ala, Asn, Asp, Gln, Glu, Gly, Lys, Thr
Thr	Ser	Ala, Asn, Ser	Ala, Asn, Ser, Val
Trp	Tyr	Phe, Tyr	Phe, Tyr
Tyr	Trp; Phe	His, Phe, Trp	His, Phe, Trp
Val	Ile; Leu	Ile, Leu, Met	Ala, Ile, Leu, Met, Thr

[00104] The nucleotide sequence encoding the modified HA protein may be optimized for human codon usage, for increased GC content, or a combination thereof. The modified HA protein may be expressed in a plant, portion of a plant, or plant cell.

H1 HA MODIFICATIONS

[00105] Modified influenza H1 HA proteins and methods of producing modified influenza H1 HA proteins in plants are described herein. It has been observed that the modification of specific amino acids in HA proteins from subtype H1 results in improved characteristics of the modified H1 HA protein when compared to the wildtype H1 HA protein or unmodified H1 HA protein.

[00106] A total of 42 single, double, and/or triple modifications were tested to improve the characteristics of the H1 HA protein. As described herewith and as shown in the Examples, only modifications or combinations of modifications at specific positions improved the characteristics of the H1 HA protein. Modifications at 32 positions or combinations of positions had negative effects on the characteristics of the H1 HA protein (data not shown).

[00107] Examples of improved characteristics of the H1 HA mutant protein include, increased HA protein yield or accumulation when expressed in plant cells as

compared to the wildtype or unmodified H1 HA of the same strain or subtype of influenza that does not comprise the modification(s) or mutation(s); improved hemagglutination titer of the modified or mutated HA protein when compared to the wildtype or unmodified H1 HA protein; improved integrity, stability, or both integrity and stability, of VLPs that are comprised of the modified H1 HA proteins as compared to the integrity, stability or both of VLPs comprising wildtype HA that does not comprise the mutation(s); increased VLP yield when expressed in plant cells as compared to the wildtype level of VLP production that does not comprise the modification(s) or mutation(s); and a combination thereof.

[00108] The modified H1 HA protein or mutant H1 HA protein as described herein is modified and comprises one or more than one mutation, or modification, at any one or more residues in sequence alignment with positions 97, 374, 380, 390 and/or 429 of A/California/07/09 HA (SEQ ID NO: 130; see Figure 1). It is therefore provided influenza H1 HA polypeptides, proteins, and/or protein complexes such as for example virus-like particle (VLP) that comprise modifications or mutations at one or more of amino acid positions 97, 374, 380, 390 and/or 429, where such amino acid numbering is based upon the sequence of H1 A/California/07/09 HA as shown in Figure 1 (SEQ ID NO: 130), or at amino acid positions that correspond to such amino acid positions, for example as determined by alignment of an H1 HA amino acid sequence to SEQ ID NO: 130. Non-limiting examples of influenza H1 HA amino acid sequences that comprise one or more of such mutations include SEQ ID NOs: 131, 132, 133, 134, 135, 138 and 139.

[00109] The modified H1 HA protein described herewith includes H1 HA protein with amino acid sequences that have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence encoding HA from H1 (SEQ ID NO: 130, 131, 132, 133, 134, 135, 138 or 139), wherein the amino acid sequence has one or more than one mutation, or modification, at any one or more residues in sequence alignment with positions 97, 374, 380, 390 and 429 of A/California/07/09 HA (SEQ ID NO: 130) and wherein the HA proteins when expressed form VLP.

[00110] Furthermore, the H1 HA protein may be encoded by a nucleotide sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 (SEQ ID NO: : 130, 131, 132, 133, 134, 135, 138 or 139), wherein the H1 HA protein has one or more than one mutation, or modification, at any one or more residues in sequence alignment with positions 97, 374, 380, 390 and 429 of A/California/07/09 HA and wherein the nucleotide sequence encodes HA proteins that when expressed form VLP.

[00111] Non-limiting examples of strains from which the H1 HA might be derived are A/California/07/09 (H1N1, SEQ ID NO: 130), A/Michigan/45/15 (H1N1, SEQ ID NO: 134), A/Massachusetts/06/17 (H1N1, SEQ ID NO: 135), A/Costa Rica/0513/16 (H1N1, SEQ ID NO: 133), A/Honduras/17734/16 (H1N1, SEQ ID NO: 131), A/Darwin/11/15 (H1N1, SEQ ID NO: 132), A/Paris/1227/2017 (SEQ ID NO: 138), or A/Norway/2147/2017 (SEQ ID NO: 139)

[00112] The modified or mutant H1 HA may be mono-substituted, di-substituted, tri-substituted or quadruple-substituted at residues at position 97, 374, 380, 390 or 429. In the mono-substituted H1 HA, one residue may be mutated at position 97, 374, 380, 390 or 429. In the di-substituted H1 HA, two residues may be substituted, for example residues at position 380 and 429, 97 and 374 or 390 and 429 may be substituted. In the tri-substituted H1 HA mutant, three residues may be substituted. For example residues at position 97, 390 and 429 or residues at position 97, 374 and 429 may be substituted. In the quadruple-substituted H1 HA mutant, four residues may be substituted. For example residues at position 97, 374, 390 and 429 may be substituted. (All H1 HA numbering is accordance with sequence alignment to reference strain A/California/07/09 HA).

[00113] Non limiting examples of modified H1 HA proteins include the following modifications or mutations in the HA sequence when compared with the H1 HA wildtype sequence (numbering is in accordance with A/California/07/09 HA):

- mono-substituted H1 HA mutants: N97D, K374E, F390D or L429M;

- di-substituted H1 HA mutant: N390A/L429M, N97D/K374E or N380/L429M;
- tri-substituted H1 HA mutant: N97D/F390D/L429M or K374E/F390D/L429M;
- quadruple-substituted H1 HA mutant: N97D/K374E/F390D/L429M.

The one or more than one mutations described herein specifically increase influenza HA protein production and VLP yield in plants. It was observed that mutations at other positions significantly reduced, or had no significant effect, on influenza HA protein accumulation or VLP production in plant cells.

Mono-substituted H1 HA

Modification at position 97

[00114] In one aspect of the disclosure, the modified H1 HA may have at least residue at position 97 modified. This residue is not involved in receptor binding of the HA and it has been shown that the residue is located in one of the vestigial esterase (VE) subdomains in the globular head of the HA. Xray crystallography showed that the residue is buried inside the HA trimer. Therefore, the residue is not part of the antigenic sites and is not involved in antigenic change, nor is it recognized by broadly neutralizing antibodies.

[00115] In influenza A (H1N1)pdm09 this residue has been predicted to be involved in the stability of HA (see Castelán-Vega *et al.* 2014). However, Castelán-Vega *et al.* points out that single point mutations seem to have little impact on HA stability and cites Yang *et al.* (Structural stability of influenza A(H1N1)pdm09 virus hemagglutinins, J. Virol. 2014;88(9):4828-4838). Yang *et al.* used size exclusion chromatography analysis of recombinant HA ectodomain to compare the differences among recombinant trimeric HA proteins from early 2009 pandemic H1N1 viruses, which dissociate to monomers, with those of more recent virus HAs that can be expressed as trimers. Yang *et al.* found that A/Texas/1/2011 (Tex 11) has a unique Asp97Asn (D97N) substitution in HA compared to the four other A(H1N1)pdm09

virus strains that were examined for sequences differences. However, influenza H1 HA strains having evolved since then have an Asparagine (N, Asn) at position 97 (see Figure 1, sequence alignment of H1), suggesting an evolutionary advantage for H1 HA strains having Asparagine (N, Asn) at this position. Accordingly, it was unexpected that the modification from an Asparagine (N, Asn) at position 97 to a non-Asparagine lead to improved characteristics of the H1 HA protein as described herein.

[00116] As shown in Figures 3A, 3B, 4A and 4B, H1 HA having the residue at position 97 changed from for example Asparagine (N, Asn) to Aspartic Acid (Asp; D), hereinafter referred to as N97D, showed an increase of up to 1200% in hemagglutination titer as compared to an H1 HA that has Asparagine (N, Asn) at this position (see also Table 5A). Figure 3A shows that HA from A/Michigan/45/15 with the N97D substitution exhibited an approximate 1100% increase in hemagglutination titer when compared to A/Michigan/45/15 HA wildtype (also referred to as H1 Michigan). HA from A/Honduras/17734/16 with the N97D substitution showed an approximate 375% increase in hemagglutination titer as compared to wildtype A/Honduras/17734/16 HA (see Figures 4A and 4B). Furthermore, as shown in Figures 4A and 4B, HA from A/Darwin/11/15 with a N97D substitution exhibited an approximate 300% increase in hemagglutination titer when compared wildtype A/Darwin/11/15 HA.

[00117] In one aspect it is therefore provided that the residue at position 97 (numbering in accordance with A/California/07/09 HA numbering) of an H1 HA may be modified to replace a charged amino acid at position 97 with a polar amino acid at position 97 to produce a modified H1 HA with a non-naturally occurring sequence. For example the H1 HA protein may be modified to contain an Aspartic Acid (D, Asp) or any other polar amino acid for example Glutamine (Q, Gln.), Histidine (H, His), Serine (S, Ser), Threonine (T, Thr), Tyrosine (Y, Tyr), Cysteine (C, Cys), or Tryptophane (W, Trp) at position 97.

[00118] The H1 HA may be modified to replace an Asparagine (N, Asn) at position 97 with a non-Asparagine at position 97. For example the HA protein may be mutated to contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) at position 97. The conserved

substitution may for example be Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore, the H1 HA may be modified to replace a non-Aspartic Acid (D, Asp) with an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) at position 97. The conserved substitution of Aspartic Acid may for example be Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser).

[00119] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 (SEQ ID NO: 134), wherein the amino acid sequence has Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 97, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00120] The present specification also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 97 as described above operatively linked to a regulatory region active in a plant.

[00121] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 (SEQ ID NO: 134), wherein the nucleotide sequence encodes a modified H1 HA protein that has Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 97, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00122] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 97 of the modified H1

HA, encodes Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 97 and wherein the modified H1 HA sequence does not occur naturally.

5 [00123] For example the modified H1 HA may have one or more modification; wherein at least residue 97 of H1 HA is modified as described herewith. For example the modified H1 HA may be a mono-substituted, di-substituted, tri-substituted or quadruple-substituted H1 HA wherein at least the residue at position 97 is modified. In non-limiting examples the modified H1 HA may have a substituted
10 residue at position 97 and one or more substitutions at positions 374, 390, 429 or a combination thereof, wherein the modified H1 HA sequence does not occur naturally.

[00124] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at position 97 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA
15 with a substitution at position 97 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00125] In addition, it is provided a method of increasing yield of VLPs
20 that comprise a modified H1 HA with a substitution at position 97 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 97 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby
25 producing the VLPs.

[00126] The present specification further provides for a VLP comprising a H1 HA with a substitution at position 97. The VLP may be produced by the method as provided by the present disclosure. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1
30 HA protein.

Modification at position 374

[00127] In one aspect of the disclosure, the residue at position 374 in H1 HA (numbering in accordance with A/California/07/09 HA numbering) may be substituted. This residue is located in the stem portion of HA of H1.

5 [00128] Cotter *et al.* (*PLoS Pathog.* 2014;10(1):e1003831) identified that a E47K (HA2 numbering) mutation in the stalk region of A/California/7/2009 HA stabilized the trimer structure, lowered the pH for membrane fusion, and increased the thermal and acid stability of the virus. Position 47 of the HA2 stalk region of H1N1 in Cotter is equivalent to position 374 (HA0 numbering of A/California/7/2009) in the present disclosure. Cotter *et al.* additionally observed that A/California/7/2009 E47K mutant HA was more infectious in ferrets than its wildtype counterpart. Similar results were obtained by Yang et al. 2014 (*J. Virol.* May 2014 vol. 88 no. 94828-4838), who showed that the introduction of a basic side change at position 374 by changing Glutamic Acid to Lysine at this position, potentially forms a new salt bridge across monomer interface to Glu 21 on the adjacent chain and thus improving stability at a lower pH. Yang et al. found that the presence of Lysine at position 374 enhances the ability of the mutant Tex09 ectodomain trimer to withstand changes in both heat and acidity to levels equivalent to those of Wash11 recHA.

20 [00129] However, it was unexpectedly found that the replacement of for example Lysine (K, Lys) with a Glutamic Acid (E, Glu) at position 374 (K374E) of HA of influenza H1 Michigan (A/Michigan/45/15 (H1N1)) leads to an approximate 1200% increase in hemagglutination titer as compared to extracts from plants expressing the H1 HA wildtype (see Figures 3A, 3B, 4A, 4B and Table 5A).

25 [00130] In one aspect it is therefore provided that the residue at position 374 (numbering in accordance with A/California/07/09 HA numbering) of an H1 HA may be modified to replace a non-Glutamic Acid with Glutamic acid (E, Glu) at position 374 to produce a modified H1 HA with a non-naturally occurring sequence. The H1 HA may be modified to replace a non-Glutamic Acid with Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg),

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Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser). Furthermore, a Lysine (K, Lys) at position 374 may be substituted with a non-Lysine at position 374 to produce a modified H1 HA with a non-naturally occurring sequence. For example, Lysine (K, Lys) at position 374 may be substituted with Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser).

[00131] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 Michigan (A/Michigan/45/15, SEQ ID NO: 134), wherein the amino acid sequence has Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00132] The present specification also provides a nucleic acid comprising a nucleotide sequence encoding a H1 HA with a substitution at position 374 operatively linked to a regulatory region active in a plant.

[00133] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 Michigan (A/Michigan/45/15, SEQ ID NO: 136), wherein the nucleotide sequence encodes a hemagglutinin protein that has Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00134] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 374, encodes Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00135] For example the modified H1 HA may have one or more modification; wherein at least residue 374 of H1 HA is modified as described herewith. For example the modified H1 HA may be a mono -substituted, di-substituted, tri-substituted or quadruple-substituted H1 HA wherein at least the residue at position 374 is modified. In non-limiting examples the modified H1 HA may have a substituted residue at position 374 and one or more substitutions at positions 97, 390, 429 or a combination thereof wherein the modified H1 HA sequence does not occur naturally.

[00136] Furthermore, the present specification provides a method of producing VLPs that comprise a modified H1 HA with a substitution at position 374 in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 374 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00137] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with a substitution at position 374 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 374 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00138] The present specification further provides for a VLP comprising a H1 HA with a substitution at position 374. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Modification at position 390

[00139] In one aspect of the disclosure, the residue at position 390 in H1 HA (numbering in accordance with A/California/07/09 HA numbering) may be substituted.

[00140] WO2013/177444 and its companion publication Lu et al. (Proc Natl Acad Sci USA. 2014;111(1):125-30) reported a method for the production of properly folded HA stem domain from A/California/05/2009 (H1N1) using an Escherichia coli-based cell-free protein expression system and a simple refolding protocol. For inducing the trimerization of HA stem domain, either a chloramphenicol acetyl transferase (CAT) or foldon domain was fused to the C terminus of the HA. To mitigate newly exposed hydrophobicity and/or intermolecular ion pairing causing aggregation of expressed HA stem protein, five groups of mutations were evaluated: M1 (I69T + I72E + I74T + C77T); M2 (I69T + I72E + I74T + C77T + F164D); M3 (I69T + I72E + I74T + C77T + F164D + L174D); M4 (F164D); and M5 (F164D + L174D). Lu notes that the soluble yield of the mutants was low and that insoluble inclusion bodies were formed. Lu further notes that mutants M3 and M5 produced much fewer aggregates than the wild-type of other variants and therefore developed mutant M5 (F164D + L174D) further. Lu observed that the M5 (F164D + L174D) mutations appeared to be the most influential mutations for improving HA stem protein solubility. Position 164 of Lu is equivalent to position 390 in the present disclosure. Lu's M4 (F164D) mutation showed no advantage over the other mutation tested and in fact was inferior to mutations M3 (I69T + I72E + I74T + C77T + F164D + L174D) and M5 (F164D + L174D).

[00141] When Phenylalanine (F, Phe) at position 390 and Leucine (L, Leu) at position 400 (H1 HA numbering) which corresponds to Phenylalanine at position

164 and Leucine at position 174 of M5 (F164D + L174D) of Lu, were altered in the H1 HA of the current disclosure no increases VLP yield was observed and the H1 F390D+L400D mutant showed a complete loss of hemagglutination activity (data not shown). Therefore the equivalent mutation of the M5 mutant in Lu did not lead to an improvement of characteristics in H1 HA that was expressed in plants.

[00142] Unexpectedly it was found that when a hydrophobic amino acid at position 390 in H1 HA was substituted with a charged amino acid, an approximate 60% increase in VLP yield following iodixanol gradient purification was observed from plants expressing the H1 HA with the substitution at position 390 when compared to plants that had been infiltrated with wildtype construct (see Figure 3B, 4A, 4B, Tables 5A, 5B). In addition, as shown in Table 5C, the full process yield increased to 226%. However, the equivalent modification in HA from H5 (F393D) lead to a decrease in hemagglutination titer (see Figure 5, Table 6).

[00143] In one aspect it is therefore provided that the residue at position 390 (numbering in accordance with A/California/07/09 HA numbering) of an H1 HA may be modified to replace a hydrophobic amino acid at position 390 with a charged amino acid at position 390 to produce a modified H1 HA with a non-naturally occurring sequence. For example the H1 HA protein may be modified to contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) at position 390. The conserved substitution of Aspartic Acid may for example be Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser).

[00144] The H1 HA may be modified to replace a non-Aspartic Acid with an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) at position 390. The conserved substitution of Aspartic Acid may for example be Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore, the H1 HA may be modified to replace a Phenylalanine (F, Phe) at position 390 with a non-Phenylalanine at position 390. For example the HA protein may be modified to contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) at position 390. The conserved substitution of Aspartic Acid may for example be Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser).

[00145] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 A/Michigan/45/15 (SEQ ID NO: 134), wherein the amino acid sequence has Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

10 [00146] The present disclosure also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 390 as described above operatively linked to a regulatory region active in a plant.

[00147] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

20 [00148] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 390 of the modified H1 HA, encodes Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00149] For example the modified H1 HA may have one or more modification; wherein at least residue 390 of H1 HA is modified as described herewith. For example the modified H1 HA may be a mono-substituted, di-substituted, tri-substituted or quadruple-substituted H1 HA wherein at least the residue at position 390 is modified. In non-limiting examples the modified H1 HA may have a substituted residue at position 390 and one or more substitutions at positions 97, 374, 380, 429 or a combination thereof.

[00150] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at position 390 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 390 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00151] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with a substitution at position 390 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 390 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00152] The present specification further provides for a VLP comprising a H1 HA with a substitution at position 390. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Modification at position 429

[00153] In one aspect of the disclosure, the residue at position 429 in H1 HA (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)) may be modified.

[00154] Antanasijevic *et al.* (*J Biol Chem.* 2014;289(32):22237-45) investigated the structure-function properties of H5 HA stem loop region by site directed mutagenesis at 14 different positions. Antanasijevic observed that HA1-D26K, HA1-M102L, HA2-V52A and HA2-I55A mutants (based on H3 numbering) exhibited significantly reduced levels of total HA, suggesting reduced expression and/or assembly of HA into viral particles. HA1-D26K, HA2-T49A and HA2-M102L mutants also exhibited lower hemagglutination titers as compared to wildtype virus. Position 102 in the HA of H5 of Antanasijevic corresponds to position 429 in H1 HA of the current specification.

[00155] When HA of H1 was modified to introduce alterations at V19I (HA1-I28V), L20M (HA1-M31L), T368A (HA2-T41A), N380A (HA2-N53A) or L429M (HA2-M102L), it was found that the T368A resulted in complete loss of activity, V19I and L20M were found to have lower activity while N380A and L429M displayed higher activity than H1 A/California wildtype HA.

[00156] It appears therefore that the majority of residues identified by Antanasijevic as being of importance to the expression and/or assembly or hemagglutination titer of HA from H5 do not translate to a similar importance in HA from H1.

[00157] However, as shown in Figures 2A, 2B, 2C, 3B, 4A and 4B, when residue 429 in H1 HA was mutated from a Leucine (L, Leu) to a Methionine (M, Met), the modified H1 HA exhibited increase in hemagglutination titer (100-160%) as compared to wildtype H1 HA (also see Table 5A). Furthermore, as seen in Figure 2C, plants expressing H1 HA with a substitution at position 429 show an approximate 30% increase in VLP yield following sucrose gradient purification, in comparison to plants infiltrated with wildtype construct (also see Table 5B). In addition, as shown in Table 5C, the full process yield increased to 260%.

[00158] In addition, di-substituted H1 HA, wherein phenylalanine at position 390 was modified to an aspartic acid and leucine at position 429 was modified to a methionine exhibited an approximate 60% increase in hemagglutination titer when compared to the unmodified H1 HA (see Figure 2B).

[00159] In one aspect it is therefore provided that the residue at position 429 (numbering in accordance with H1 A/Michigan/45/15 amino acid sequence (SEQ ID NO: 134)) of an H1 HA may be modified to replace a Leucine (L, Leu) at position 429 with another hydrophobic amino acid that is not Leucine to produce a modified H1 HA with a non-naturally occurring sequence. For example the H1 HA protein may be modified to contain a Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429.

[00160] Furthermore, the H1 HA may be modified to replace a Leucine (L, Leu) at position 429 with a non-Leucine at position 429. For example the HA protein may be mutated to contain a Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) at position 429. The conserved substitution of Methionine may for example be Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe). Furthermore, the H1 HA may be modified to replace a non-Methionine at position 429 with Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) at position 429. The conserved substitution of Methionine may for example be Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe).

[00161] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 Michigan (A/Michigan/45/15 (H1N1), SEQ ID NO: 134), wherein the amino acid sequence has a Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00162] The present disclosure also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 429 as described above operatively linked to a regulatory region active in a plant.

[00163] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has a Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00164] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 429 of the modified H1 HA, encodes a Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00165] For example the modified H1 HA may have one or more modification; wherein at least residue 429 of H1 HA is modified as described herewith. For example the modified H1 HA may be a mono-substituted, di-substituted, tri-substituted or quadruple-substituted H1 HA wherein at least the residue at position 429 is modified. In non-limiting examples the modified H1 HA may have a substituted residue at position 429 and one or more substitutions at positions 97, 374, 380, 390 or a combination thereof.

[00166] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at position 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of

the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00167] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with a substitution at position 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00168] The present specification further provides for a VLP comprising a H1 HA with a substitution at position 429. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Di-substituted H1 HA

[00169] It is further provided H1 HA proteins that comprise at least a di-substitution or di-modification. Accordingly, the H1 HA protein has at least two modifications from the wildtype H1 HA protein. For example the H1 HA may have any two combinations of the following residues modified: 97, 374, 380, 390 and 429 (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)).

Modification of position 380 and 429

[00170] In one aspect of the specification, the modified H1 HA may have at least residues at position 380 and 429 modified.

[00171] As shown in Figures 3B H1 HA having the residue at position 380 modified from an Asparagine to Alanine, and the residue at position 429 modified from leucine to a methionine exhibited an approximate 800% increase in hemagglutination titer as compared to wildtype H1 HA (also see Table 5A).

[00172] It is therefore provided in one aspect that the residues at position 380 and 429 (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)) of an H1 HA may be modified to replace an Asparagine (N, Asn) at position 380 with a non-Asparagine at position 380 and to replace Leucine (L, Leu) at position 429 with a non-Leucine at position 429 to produce a modified H1 HA with a non-naturally occurring sequence. For example H1 HA may be modified to replace a polar amino acid at position 380 with a hydrophobic amino acid at position 380 and to replace Leucine (L, Leu) at position 429 with another hydrophobic amino acid that is not Leucine to produce a modified H1 HA with a non-naturally occurring sequence.

[00173] For example the H1 HA protein may be modified to contain an Alanine (A, Ala) or a conserved substitution of Alanine at position 380. The conserved substitution of Alanine may for example be Serine (S, Ser), Glycine (G, Gly), Threonine (T, Thr), Cysteine (C, Cys) or Valine (V, Val). Furthermore the H1 HA protein may be modified to contain a Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429.

[00174] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO:134), wherein the amino acid sequence has Alanine, or a conserved substitution of Alanine for example Serine (S, Ser), Glycine (G, Gly), Threonine (T, Thr), Cysteine (C, Cys) or Valine (V, Val) at position 380 and the amino acid sequence has Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00175] The present specification also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 380 and 429 as described above operatively linked to a regulatory region active in a plant.

[00176] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has Alanine, or a conserved substitution of Alanine for example Serine (S, Ser), Glycine (G, Gly), Threonine (T, Thr), Cystein (C, Cys) or Valine (V, Val) at position 380 and Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00177] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encodes amino acid residue 380 of the modified H1 HA encodes Alanine, or a conserved substitution of Alanine, for example Serine (S, Ser), Glycine (G, Gly), Threonine (T, Thr), Cystein (C, Cys) or Valine (V, Val) and the nucleotide codon that encodes amino acid residue 429 of the modified H1 HA, encodes an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00178] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at positions 380 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 380 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00179] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with a substitution at position 380 and 429 as

described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 380 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00180] The present specification further provides for a VLP comprising a H1 HA with a substitution at position 380 and 429. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Modification of position 390 and 429

[00181] In one aspect of the disclosure, the modified H1 HA may have at least residues at position 390 and 429 modified.

[00182] As shown in Figures 2B, 3B, 4A, 4B and Table 5A, H1 HA having the residue at position 390 modified from a phenylalanine to aspartic acid, and the residue at position 429 modified from leucine to a methionine exhibited an approximate 400-1200% increase in hemagglutination titer as compared to wildtype H1 HA. In addition, as shown in Table 5C, the full process yield increased to 633%.

[00183] It is therefore provided in one aspect that the residues at position 390 and 429 (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)) of an H1 HA may be modified to replace a Phenylalanine (F, Phe) at position 390 with a non- Phenylalanine at position 390 and to replace Leucine (L, Leu) at position 429 with a non-Leucine at position 429 to produce a modified H1 HA with a non-naturally occurring sequence. For example H1 HA may be modified to replace a hydrophobic amino acid at position 390 with a charged amino acid at position 390 and to replace Leucine (L, Leu) at position 429 with another hydrophobic amino acid that is not Leucine to produce a modified H1 HA with a non-naturally occurring sequence.

[00184] For example the H1 HA protein may be modified to contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) at

position 390. The conserved substitution may for example be Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore the H1 HA protein may be modified to contain an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429.

[00185] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 A/Michigan/45/15 (H1N1)(SEQ ID NO: 134), wherein the amino acid sequence has Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390 and the amino acid sequence has Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00186] The present specification also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 390 and 429 as described above operatively linked to a regulatory region active in a plant.

[00187] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (H1N1)(SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390 and Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00188] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encodes amino acid residue 390 of the modified H1 HA encodes Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the nucleotide codon that encodes amino acid residue 429 of the modified H1 HA, encodes an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00189] For example the modified H1 HA may have one or more modification; wherein at least residues 390 and 429 of H1 HA is modified as described herewith. For example the modified H1 HA may be a di-substituted, tri-substituted or quadruple-substituted H1 HA wherein at least the residue at position 390 and 429 are modified. In non-limiting examples the modified H1 HA may have a substituted residue at positions 390 and 429 and one or more substitutions at positions 97, 374, 380 or a combination thereof.

[00190] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at positions 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00191] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with a substitution at position 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and

incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00192] The present specification further provides for a VLP comprising a H1 HA with a substitution at position 390 and 429. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Modification of position 97 and 374

[00193] In one aspect of the disclosure, the modified H1 HA may have at least residues at position 97 and 374 modified.

[00194] As shown in Figure 3B and Table 5A, H1 HA having residue at position 97 modified from asparagine to an aspartic acid and residue at position 374 modified from a lysine to a glutamic acid exhibit an approximate 1200% increase in hemagglutination titer as compared to wildtype H1 HA.

[00195] In one aspect it is therefore provided that the residues at position 97 and 374 (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)) of an H1 HA may be modified to replace a Asparagine (N, Asn) at position 97 with a non- Asparagine at position 97 and to replace Lysine (K, Lys) at position 374 with a non-Lysine at position 374 to produce a modified H1 HA with a non-naturally occurring sequence. For example H1 HA may be modified to replace a charged amino acid at position 97 with a polar amino acid at position 97 and to replace a charged amino acid at position 374 with another charged amino acid that is not Lysine (K, Lys) to produce a modified H1 HA with a non-naturally occurring sequence.

[00196] For example the H1 HA protein may be modified to contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not at Asparagine (N, Asn) position 97. The conserved substitution may for example be Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore the H1 HA protein may be modified to contain Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example

Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374.

[00197] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 134), wherein the amino acid sequence at position 97 has Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn); for example, Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 97 and the amino acid sequence has at position 374 Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00198] The present specification also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 97 and 374 as described above operatively linked to a regulatory region active in a plant.

[00199] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has at position 97 Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn); for example, Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the amino acid sequence has at position 374 Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00200] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence

identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 97 of the modified H1 HA encodes Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn); for example, Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the nucleotide codon that encode amino acid residue 374 of the modified H1 HA, encodes Glutamic acid (E, Glu), or a conserved substitution of Glutamic acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00201] For example the modified H1 HA may have one or more modification; wherein at least residues 97 and 374 of H1 HA are modified as described herewith. For example the modified H1 HA may be a di-substituted, tri-substituted or quadruple-substituted H1 HA wherein at least the residue at position 97 and 374 are modified. In non-limiting examples the modified H1 HA may have a substituted residue at positions 97 and 374 and one or more substitutions at positions 380, 390 and 429 or a combination thereof.

[00202] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at positions 97 and 374 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 97 and 374 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00203] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with a substitution at position 97 and 374 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with a substitution at position 97 and 374 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00204] The present specification further provides for a VLP comprising a H1 HA with a substitution at position 97 and 374. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Tri-substituted H1 HA

Modification at position 97, 390 and 429

[00205] It is further provided H1 HA proteins that comprise at least a tri-substitution or tri-modification. Accordingly, the H1 HA protein has at least three modifications from the wildtype H1 HA protein. For example the H1 HA may have any three combinations of the following residues modified: 97, 374, 390 and 429 (numbering in accordance H1 A/Michigan/45/15 (SEQ ID NO: 134)).

[00206] In one aspect of the specification, the modified H1 HA may have residues at least at position 97, 390 and 429 modified.

[00207] As shown for example in Figures 3B, 4A and 4B, H1 HA having the residue at position 97 modified from Asparagine to Aspartic Acid, residue at position 390 modified from phenylalanine to aspartic acid and residue 429 modified from leucine to a methionine exhibited an approximate 2600% increase in hemagglutination titer as compared to wildtype H1 HA. In addition, as shown in Table 5C, the full process yield increased to 647%.

[00208] It is therefore provided in one aspect that the residues at position 97, 390 and 429 (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)) of an H1 HA may be modified to replace Asparagine (N, Asn) at position 97 with a non-Asparagine, Phenylalanine (F, Phe) at position 390 with a non-Phenylalanine and to replace Leucine at position 429 with a non-Leucine (L, Leu) to produce a modified H1 HA with a non-naturally occurring sequence. For example H1 HA may be modified to replace a polar amino acid with a charged amino acid at position 97, a hydrophobic amino acid at position 390 with a charged amino acid and to replace leucine at position 429 with another hydrophobic amino acid that is not Leucine to produce a modified H1 HA with a non-naturally occurring sequence.

[00209] For example the H1 HA protein may be modified to contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) at position 97. The conserved substitution of Aspartic Acid may for example be Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser).
5 The modified H1 HA may further contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) at position 390. The conserved substitution of Aspartic Acid may for example be Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore the H1 HA protein may be modified to contain an Methionine (M, Met) or a conserved substitution of
10 Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429.

[00210] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino
15 acid sequence of HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 134), wherein the amino acid sequence has at position 97 Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser), the amino acid sequence has at position 390 Aspartic Acid (D, Asp), or a conserved substitution of
20 Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the amino acid sequence has at position 429 Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe), wherein the modified H1 HA sequence does not occur
25 naturally and wherein the HA proteins when expressed form VLP.

[00211] The present disclosure also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 97, 390 and 429 as described above operatively linked to a regulatory region active in a plant.

30 [00212] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween,

sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 97, Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390 and Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00213] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 97 of the modified H1 HA encodes Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser), the nucleotide codon that encode amino acid residue 390 of the modified H1 HA encodes Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the nucleotide codon that encode amino acid residue 429 of the modified H1 HA, encodes an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00214] For example the modified H1 HA may have one or more modification; wherein at least residues 97, 390 and 429 of H1 HA is modified as described herewith. For example the modified H1 HA may be a tri-substituted or quadruple-substituted H1 HA wherein the residue at least at position 97, 390 and 429

are modified. In non-limiting examples the modified H1 HA may have substituted residues at positions 97, 390, 429 and 374.

[00215] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at positions 97, 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with substitutions at position 97, 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00216] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with substitution at position 97, 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with substitution at position 97, 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00217] The present specification further provides for a VLP comprising a H1 HA with substitution at position 97, 390 and 429. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Modification of positions 374, 390 and 429

[00218] In one aspect of the disclosure, the modified H1 HA may have residues at least at position 374, 390 and 429 modified.

[00219] As shown for example in Figure 3B, H1 HA having the residue at position 374 modified from Lysine to glutamic acid, residue at position 390 modified from phenylalanine to aspartic acid and residue 429 modified from leucine to a methionine exhibited an approximate 2500% increase in hemagglutination titer as

compared to wildtype H1 HA. In addition, as shown in Table 5C, the full process yield increased to 689%.

[00220] It is therefore provided in one aspect that the residues at position 374, 390 and 429 (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)) of an H1 HA may be modified to replace Lysine (K, Lys) at position 374 with a non- Lysine, Phenylalanine (F, Phe) at position 390 with a non- Phenylalanine and to replace Leucine at position 429 with a non-Leucine to produce a modified H1 HA with a non-naturally occurring sequence. For example H1 HA may be modified to replace Lysine with a charged amino acid that is not- Lysine at position 374, a hydrophobic amino acid at position 390 with a charged amino acid and to replace Leucine (L, Leu) at position 429 with another hydrophobic amino acid that is not Leucine to produce a modified H1 HA with a non-naturally occurring sequence.

[00221] For example the H1 HA protein may be modified to contain Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374. The modified H1 HA may further contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) at position 390. The conserved substitution may for example be Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore the H1 HA protein may be modified to contain an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429.

[00222] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 134), wherein the amino acid sequence has at position 374 Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser), the amino acid sequence has at position 390

Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the amino acid sequence has at position 429 Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00223] The present specification also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 374, 390 and 429 as described above operatively linked to a regulatory region active in a plant.

[00224] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (H1N1)(SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374, Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390 and Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429, wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00225] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 374 of the modified H1 HA encodes Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q,

Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser), the nucleotide codon that encode amino acid residue 390 of the modified H1 HA encodes Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the nucleotide codon that encode amino acid residue 429 of the modified H1 HA, encodes an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00226] For example the modified H1 HA may have one or more modification; wherein at least residues 374, 390 and 429 of H1 HA is modified as described herewith. For example the modified H1 HA may be a tri-substituted or quadruple-substituted H1 HA wherein at least the residue at position 374, 390 and 429 are modified. In non-limiting examples the modified H1 HA may have substituted residues at positions 374, 390, 429 and 97.

[00227] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at positions 374, 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with substitutions at position 374, 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00228] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with substitution at position 374, 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with substitution at position 374, 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00229] The present specification further provides for a VLP comprising a H1 HA with substitution at position 374, 390 and 429. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

Quadruple-substituted H1 HA

Modification at positions 97, 374, 390 and 429

[00230] It is further provided H1 HA proteins that comprise at least a quadruple-substitution or quadruple-modification. Accordingly, the H1 HA protein has at least four modifications from the wildtype H1 HA protein. For example the H1 HA may have modifications at positions 97, 374, 390 and 429 (numbering in accordance with H1 A/Michigan/45/15 (SEQ ID NO: 134)).

[00231] Accordingly, in one aspect of the specification, the modified H1 HA may have residues at least at position 97, 374, 390 and 429 modified.

[00232] As shown for example in Figure 3B, H1 HA having residue at position 97 modified from Asparagine to Aspartic Acid, residue at position 374 modified from lysine to glutamic acid, residue at position 390 modified from phenylalanine to aspartic acid and residue at position 429 modified from leucine to a methionine exhibited an approximate 3300% increase in hemagglutination titer as compared to wildtype H1 HA.

[00233] In one aspect it is therefore provided that the residues at position 97, 374, 390 and 429 (numbering in accordance with A/California/07/09 HA) of an H1 HA may be modified to replace Asparagine (N, Asn) at position 97 with a non-Asparagine, Lysine at position 374 with a non-Lysine, Phenylalanine (F, Phe) at position 390 with a non- Phenylalanine and to replace Leucine at position 429 with a non-Leucine to produce a modified H1 HA with a non-naturally occurring sequence. For example H1 HA may be modified to replace a polar amino acid with a charged amino acid at position 97, replace Lysine with a charged amino acid that is not- Lysine at position 374, replace a hydrophobic amino acid at position 390 with a charged

amino acid and to replace Leucine (L, Leu) at position 429 with another hydrophobic amino acid that is not Leucine to produce a modified H1 HA with a non-naturally occurring sequence.

[00234] For example the H1 HA protein may be modified to contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) at position 97. The conserved substitution of Aspartic Acid may for example be Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore, the H1 HA protein may be modified to contain Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374. The modified H1 HA may further contain an Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) at position 390. The conserved substitution of Aspartic Acid may for example be Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser). Furthermore the H1 HA protein may be modified to contain an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe) at position 429.

[00235] For example the modified H1 HA protein may have an amino acid sequence that has about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the amino acid sequence of HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 134), wherein the amino acid sequence has at position 97 Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser), the amino acid sequence has at position 374 Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) , the amino acid sequence has at position 390 Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the amino acid sequence has at position 429 Methionine (M, Met) or a conserved

substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

5 [00236] The present specification also provides a nucleic acid comprising a nucleotide sequence encoding a modified H1 HA with a substitution at position 97, 374, 390 and 429 as described above operatively linked to a regulatory region active in a plant.

10 [00237] For example the nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence encoding HA from H1 A/Michigan/45/15 (H1N1) (SEQ ID NO: 136), wherein the nucleotide sequence encodes a modified H1 HA protein that has Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for
15 example Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 97, Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374, Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for
20 example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) at position 390 and Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when
25 expressed form VLP.

[00238] The nucleotide sequences may have about 70, 75, 80, 85, 87, 90, 91, 92, 93 94, 95, 96, 97, 98, 99, 100% or any amount therebetween, sequence identity, or sequence similarity, with the nucleotide sequence of SEQ ID NO: 136, wherein the nucleotide codon that encode amino acid residue 97 of the modified H1
30 HA encodes Aspartic Acid (D, Asp) or a conserved substitution of Aspartic Acid (D, Asp) that is not Asparagine (N, Asn) for example Glutamic Acid (E, Glu), Glutamine

(Q, Gln) or Serine (S, Ser), the nucleotide codon that encode amino acid residue 374 of the modified H1 HA encodes Glutamic Acid (E, Glu) or a conserved substitution of Glutamic Acid (E, Glu) that is not Lysine (K, Lys) for example Aspartic acid (D, Asp), Glutamine (Q, Gln), Arginine (R, Arg), Asparagine (N, Asn), Histidine (H, His) or Serine (S, Ser) at position 374, the nucleotide codon that encode amino acid residue 390 of the modified H1 HA encodes Aspartic Acid (D, Asp), or a conserved substitution of Aspartic Acid (D, Asp) for example Asparagine (N, Asn), Glutamic Acid (E, Glu), Glutamine (Q, Gln) or Serine (S, Ser) and the nucleotide codon that encode amino acid residue 429 of the modified H1 HA, encodes an Methionine (M, Met) or a conserved substitution of Methionine (M, Met) that is not Leucine (L, Leu) for example Isoleucine (I, Ile), Glutamine (Q, Gln), Valine (V, Val) or Phenylalanine (F, Phe), wherein the modified H1 HA sequence does not occur naturally and wherein the HA proteins when expressed form VLP.

[00239] For example the modified H1 HA may have one or more modification; wherein at least residues 97, 374, 390 and 429 of H1 HA is modified as described herewith. For example the modified H1 HA may be a quadruple-substituted H1 HA wherein the residue at position 97, 374, 390 and 429 are modified.

[00240] Furthermore, it is provided a method of producing VLPs that comprise a modified H1 HA with a substitution at positions 97, 374, 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with substitutions at position 97, 374, 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00241] In addition, it is provided a method of increasing yield of VLPs that comprise a modified H1 HA with substitution at position 97, 374, 390 and 429 as described above in a plant. The method involves introducing a nucleic acid encoding a modified H1 HA with substitution at position 97, 374, 390 and 429 operatively linked to a regulatory region active in the plant, into the plant, or portion of the plant, and incubating the plant or portion of the plant under conditions that permit the expression of the nucleic acid, thereby producing the VLPs.

[00242] The present specification further provides for a VLP comprising a H1 HA with substitution at position 97, 374, 390 and 429. The VLP may be produced by the method as provided by the present specification. The VLP comprising the modified H1 HA show improved characteristics when compared to VLPs that comprise the unmodified H1 HA protein.

[00243] Also provided herein are methods of increasing production or yield of VLPs comprising mutant influenza HAs in plants. For example, a method may involve introducing a nucleic acid encoding a mutant influenza HA, as described herein, into the plant, portion of the plant, or plant cell. The nucleic acid encoding the mutant influenza HA may be optimized for human codon usage, increased GC content, or a combination thereof. One or more than one mutant influenza HA protein may be expressed in a plant, portion of the plant, or plant cell, in order to produce a VLP comprising one or more than one mutant influenza HA protein. Alternatively, the method may comprise providing a plant, portion of the plant, or plant cell that comprises the nucleic acid encoding the mutant influenza HA protein in order to produce a VLP comprising the one or more than one mutant influenza HA protein.

[00244] The methods of producing a VLP comprising a mutant influenza HA may further comprise a step of introducing a second nucleic acid sequence into the plant, portion of the plant, or plant cell, wherein the second nucleic acid encodes a proton channel protein that is co-expressed with the mutant influenza HA. For example, the proton channel protein may be an influenza A subtype M2 protein, such as A/New Caledonia/20/99 M2. The co-expression of the proton channel protein may lead to an increased accumulation of mutant influenza HA protein and/or VLP comprising the mutant influenza HA protein as for example described in WO 2013/044390 which is incorporated herein by reference.

[00245] Furthermore, the mutant influenza HA might further comprise a modified proteolytic loop or cleavage site as described in WO 2013/044390 and WO 2014/153674 and which are incorporated herein by reference.

[00246] By “co-expression”, it is meant the introduction and expression of two or more nucleotide sequences, each of the two or more nucleotide sequences encoding a

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

[00247] The nucleic acid encoding a mutant influenza HA as described herein may further comprise sequences that enhance expression of the mutant influenza HA in a plant, portion of the plant, or plant cell. Sequences that enhance expression may include, a cowpea mosaic virus (CPMV) enhancer element in operative association with the nucleic acid encoding the mutant influenza HA protein.

[00248] The nucleic acid comprising a nucleotide sequence encoding a modified influenza hemagglutinin (HA) protein, as described herein may further comprise sequences that enhance expression of the HA protein in the plant, portion of the plant, or plant cell. Sequences that enhance expression may include, a CPMV enhancer element, or a plant-derived expression enhancer, in operative association with the nucleic acid encoding the modified influenza hemagglutinin (HA) protein. The sequence encoding the modified influenza hemagglutinin (HA) may also be optimized for human codon usage, increased GC content, or a combination thereof.

[00249] The term “CPMV enhancer element”, as used herein, refers to a nucleotide sequence encoding the 5'UTR regulating the Cowpea Mosaic Virus (CPMV) RNA2 polypeptide or a modified CPMV sequence as is known in the art. For example, a CPMV enhancer element or a CPMV expression enhancer, includes a

nucleotide sequence as described in WO2015/14367; WO2015/103704; WO2007/135480; WO2009/087391; Sainsbury F., and Lomonossoff G.P., (2008, Plant Physiol. 148: pp. 1212-1218), each of which is incorporated herein by reference. A CPMV enhancer sequence can enhance expression of a downstream heterologous open reading frame (ORF) to which they are attached. The CPMV expression enhancer may include CPMV HT, CPMVX (where X=160, 155, 150, 114), for example CPMV 160, CPMVX+ (where X=160, 155, 150, 114), for example CPMV 160+, CPMV-HT+, CPMV HT+[WT115], or CPMV HT+ [511] (WO2015/143567; WO2015/103704 which are incorporated herein by reference).

The CPMV expression enhancer may be used within a plant expression system comprising a regulatory region that is operatively linked with the CPMV expression enhancer sequence and a nucleotide sequence of interest.

[00250] The term “CPMV enhancer element”, as used herein, refers to a nucleotide sequence encoding the 5'UTR regulating the Cowpea Mosaic Virus (CPMV) RNA2 polypeptide or a modified CPMV sequence as is known in the art. For example, a CPMV enhancer element or a CPMV expression enhancer, includes a nucleotide sequence as described in WO2015/14367; WO2015/103704; WO2007/135480; WO2009/087391; Sainsbury F., and Lomonossoff G.P., (2008, Plant Physiol. 148: pp. 1212-1218), each of which is incorporated herein by reference.

A CPMV enhancer sequence can enhance expression of a downstream heterologous open reading frame (ORF) to which they are attached. The CPMV expression enhancer may include CPMV HT, CPMVX, CPMVX+, CPMV-HT+, CPMV HT+[WT115], or CPMV HT+ [511] (WO2015/14367; WO2015/103704 which are incorporated herein by reference). The CPMV expression enhancer may be used within a plant expression system comprising a regulatory region that is operatively linked with the CPMV expression enhancer sequence and a nucleotide sequence of interest.

[00251] The term “5'UTR” or “5' untranslated region” or “5' leader sequence” refers to regions of an mRNA that are not translated. The 5'UTR typically begins at the transcription start site and ends just before the translation initiation site or start codon of the coding region. The 5' UTR may modulate the stability and/or translation of an mRNA transcript.

[00252] The term “plant-derived expression enhancer”, as used herein, refers to a nucleotide sequence obtained from a plant, the nucleotide sequence encoding a 5'UTR. Examples of a plant derived expression enhancer are described in US Provisional Patent Application No.62/643,053 (Filed March 14, 2018; which is incorporated herein by reference) or in Diamos A.G. et al. (2016, Front Plt Sci. 7:1-15; which is incorporated herein by reference). The plant-derived expression enhancer may be selected from nbMT78, nbATL75, nbDJ46, nbCHP79, nbEN42, atHSP69, atGRP62, atPK65, atRP46, nb30S72, nbGT61, nbPV55, nbPPI43, nbPM64 and nbH2A86 as described in US 62/643,053). The plant derived expression enhancer may be used within a plant expression system comprising a regulatory region that is operatively linked with the plant-derived expression enhancer sequence and a nucleotide sequence of interest.

[00253] 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.

[00254] When one or more than one mutant influenza HA protein is expressed in a plant, portion of the plant, or plant cell, the one or more than one mutant influenza HA proteins self-assemble into VLPs. The plant, portion of the plant, or plant cell, may be harvested under suitable extraction and purification conditions to maintain the integrity of the VLP, and the VLP comprising the one or more than one mutant influenza HA may be purified.

[00255] The present invention also provides the use of a mutant influenza HA, or VLP comprising the mutant influenza HA, as described herein, for inducing immunity to an influenza infection in a subject. Also disclosed herein is an antibody or antibody fragment, prepared by administering the mutant influenza HA or VLP comprising the mutant influenza HA, to a subject or a host animal. Further provided is a composition comprising an effective dose of a mutant influenza HA or VLP comprising the mutant influenza HA, as described herein, and a pharmaceutically acceptable carrier, adjuvant, vehicle, or excipient, for inducing an immune response in

a subject. Also provided is a vaccine for inducing an immune response in a subject, wherein the vaccine comprises an effective dose of the mutant influenza HA.

[00256] Also provided herein are methods for inducing immunity to an influenza infection in a subject comprising of administering the mutant influenza HA or VLP comprising the mutant influenza HA, to a subject orally, intranasally, intramuscularly, intraperitoneally, intravenously, or subcutaneously.

[00257] The term “influenza virus”, as used herein, refers to an enveloped viral strain of the family *Orthomyxoviridae* that is characterized as having a negative sense single-stranded RNA genome. The influenza virus genome comprises eight gene segments coding for 12-14 proteins depending on the strain.

[00258] There are four types of influenza virus: A, B, C and D, of which influenza A and B are the causative organism for seasonal disease epidemics in humans. Influenza A is further classified based on the expression of HA and neuraminidase (NA) glycoprotein subtypes.

[00259] The term “hemagglutinin” or “HA”, as used herein, refers to a trimeric lectin that facilitates binding of the influenza virus particle to sialic acid-containing proteins on the surface of target cells and mediates release of the viral genome into the target cell. There are 18 different HA subtypes (H1-H18). HA proteins comprise two structural elements: the head, which is the primary target of seroprotective antibodies; and the stalk. HA is translated as a single polypeptide, HA0 (assembled as trimers), that must be cleaved by a serine endoprotease between the HA1 (~40 kDa) and HA2 (~20 kDa) subdomains. After cleavage, the two disulfide-bonded protein domains adopt the requisite conformation necessary for viral infectivity.

[00260] Influenza A HA proteins or modified influenza A HA proteins as disclosed herein, include any known HA proteins derived from any known influenza A strain, but also modifications to known influenza A strains that develop over time. For example, influenza HA may be derived from A/California/07/09 (H1N1), A/Michigan/45/15 (H1N1), A/Massachusetts/06/17 (H1N1), A/Costa Rica/0513/16 (H1N1), A/Honduras/17734/16 (H1N1), or A/Darwin/11/15 (H1N1). Influenza A HA may include HA derived from strains, wherein the HA has about 30-100%, or any

amount therebetween, amino acid sequence identity to any HA derived from the influenza A strains listed above, provided that the influenza HA protein comprises at least one substitution as described herewith and is able to form VLPs, induces an immune response when administered to a subject, induces hemagglutination or a combination thereof.

[00261] For example, influenza HA proteins may have 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100%, or any amount therebetween, amino acid sequence identity (sequence similarity, percent identity, percent similarity) to any HA derived from the influenza A strains listed above and comprises at least one substitution as described herewith and is able to form VLPs, induces an immune response when administered to a subject, induces hemagglutination or a combination thereof. An amino acid sequence alignment of several influenza A HA domains, which are not to be considered limiting, is shown in Figure 1.

[00262] The terms “percent similarity”, “sequence similarity”, “percent identity”, or “sequence identity”, when referring to a particular sequence, are used for example as set forth in the University of Wisconsin GCG software program, or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology, Ausubel et al., eds. 1995 supplement). Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, using for example the algorithm of Smith & Waterman, (1981, Adv. Appl. Math. 2:482), by the alignment algorithm of Needleman & Wunsch, (1970, J. Mol. Biol. 48:443), by the search for similarity method of Pearson & Lipman, (1988, Proc. Natl. Acad. Sci. USA 85:2444), by computerized implementations of these algorithms (for example: GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis.).

[00263] An example of an algorithm suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977, Nuc. Acids Res. 25:3389-3402) and Altschul et al., (1990, J. Mol. Biol. 215:403-410), respectively. BLAST and BLAST 2.0 are used,

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

[00264] The term “virus-like particle”, VLP, “virus like particles”, or “VLPs”, as used herein, refers to influenza particles that comprise one or more than one influenza HA protein, and that self-assemble into non-replicating, non-infectious viral capsid structures lacking all parts of the influenza genome.

Influenza HA Protein Production in Plants

Influenza A HA protein includes any HA protein comprising an amino acid sequence having from about 30 to about 100%, from about 40 to about 100%, from about 50 to about 100%, from about 60 to about 100%, from about 70 to about 100%, from about 80 to about 100%, from about 85 to about 100%, from about 90 to about 100%, from 95 to about 100%, or from about 97 to about 100% from about 98 to about 100%, or any amount therebetween, sequence identity or sequence similarity with influenza A HA sequence from a A/California/07/09 (H1N1, SEQ ID NO: 130), A/Michigan/45/15 (H1N1, SEQ ID NO: 134), A/Massachusetts/06/17 (H1N1, SEQ ID NO: 135), A/Costa Rica/0513/16 (H1N1, SEQ ID NO: 133), A/Honduras/17734/16 (H1N1, SEQ ID NO: 131), A/Darwin/11/15 (H1N1, SEQ ID NO: 132), A/Paris/1227/2017 (SEQ ID NO: 138), and A/Norway/2147/2017 (SEQ ID NO: 139), provided that the influenza HA protein comprises at least one substitution as described herewith and is able to form VLPs, induces an immune response when administered to a subject, induces hemagglutination or a combination thereof.

[00265] Furthermore the modified influenza HA protein includes any HA protein comprising an amino acid sequence having from about 30% to about 100%,

from about 40% to about 100%, from about 50% to about 100%, from about 60% to about 100%, from about 70% to about 100%, from about 80% to about 100%, from about 85% to about 100%, from about 90% to about 100%, from 95% to about 100%, or from about 97% to about 100% from about 98% to about 100%, or any amount therebetween, sequence identity or sequence similarity with a sequence of the sequences of SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 4, SEQ ID NO: 28, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 105, SEQ ID NO: 108, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 140, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 148, provided that the influenza HA protein comprises at least one substitution as described herewith and is able to form VLPs, induces an immune response when administered to a subject, induces hemagglutination or a combination thereof.

[00266] As described herein, one or more than one specific mutation or modification in influenza HA results in increased accumulation of HA protein and increased VLP production in plants, as compared to wildtype influenza HA.

[00267] Examples of mutant influenza A HA proteins having enhanced influenza HA and/or VLP production in plants include, but are not limited to the following:

F390D A/California/07/09 Mutant H1 (Construct #2980, SEQ ID NO: 18); L429M A/California/07/09 Mutant H1 (Construct #2962, SEQ ID NO: 22); F390D + L429M A/California/07/09 Mutant H1 (Construct #2995, SEQ ID NO: 24); N380A A/Michigan/45/15 Mutant H1 (Construct #3644, SEQ ID NO: 105); F390D+N380A A/Michigan/45/15 Mutant H1 (Construct #3704, SEQ ID NO: 108); N97D A/Michigan/45/15 Mutant H1 (Construct #3774, SEQ ID NO: 28); K374E A/Michigan/45/15 Mutant H1 (Construct #3771, SEQ ID NO: 32); F390D A/Michigan/45/15 Mutant H1 (Construct #3641, SEQ ID NO: 36); L429M

A/Michigan/45/15 Mutant H1 (Construct #3643, SEQ ID NO: 39); N97D + K374E
 A/Michigan/45/15 Mutant H1 (Construct #3880, SEQ ID NO: 41); F390D + L429M
 A/Michigan/45/15 Mutant H1 (Construct #3703, SEQ ID NO: 43); N97D + F390D +
 L429M A/Michigan/45/15 Mutant H1 (Construct #3879, SEQ ID NO: 45); K374E +
 5 F390D + L429M A/Michigan/45/15 Mutant H1 (Construct #3878, SEQ ID NO:47);
 N97D +K374E + F390D + L429M A/Michigan/45/15 Mutant H1 (Construct #3881,
 SEQ ID NO:49); F390D + L429M A/Massachusetts/06/17 Mutant H1 (Construct
 #4091, SEQ ID NO:51); N97D + F390D + L429M A/Massachusetts/06/17 Mutant H1
 (Construct #4093, SEQ ID NO:53); K374E + F390D + L429M
 10 A/Massachusetts/06/17 Mutant H1 (Construct #4092, SEQ ID NO: 55); N97D +
 K374E + F390D + L429M A/Massachusetts/06/17 Mutant H1 (Construct #4094, SEQ
 ID NO: 57); F390D + L429M A/Costa Rica/0513/16 Mutant H1 (Construct #4715,
 SEQ ID NO:59); N97D + F390D + L429M A/Costa Rica/0513/16 Mutant H1
 (Construct #4717, SEQ ID NO:61); K374E + F390D + L429M A/Costa Rica/0513/16
 15 Mutant H1 (Construct #4716, SEQ ID NO:63); N97D + K374E + F390D + L429M
 A/Costa Rica/0513/16 Mutant H1 (Construct #4718, SEQ ID NO:65); N97D
 A/Honduras/17734/16 Mutant H1 (Construct #3950, SEQ ID NO: 68); K374E
 A/Honduras/17734/16 Mutant H1 (Construct #3948, SEQ ID NO: 72); F390D
 A/Honduras/17734/16 Mutant H1 (Construct #3945, SEQ ID NO: 75); L429M
 20 A/Honduras/17734/16 Mutant H1 (Construct #3949, SEQ ID NO: 80); F390D +
 L429M A/Honduras/17734/16 Mutant H1 (Construct #3946, SEQ ID NO:82); N97D
 + F390D + L429M A/Honduras/17734/16 Mutant H1 (Construct #3951, SEQ ID
 NO:84); N97D A/Darwin/11/15 Mutant H1 (Construct #3990, SEQ ID NO: 86);
 K374E A/Darwin/11/15 Mutant H1 (Construct #3988, SEQ ID NO:89); F390D
 25 A/Darwin/11/15 Mutant H1 (Construct #3985, SEQ ID NO: 91); L429M
 A/Darwin/11/15 Mutant H1 (Construct #3989, SEQ ID NO:93); F390D + L429M
 A/Darwin/11/15 Mutant H1 (Construct #3986, SEQ ID NO:95); N97D + F390D +
 L429M A/Darwin/11/15 Mutant H1 (Construct #3991, SEQ ID NO: 97); F390D +
 L429M A/Paris/1227/2017 Mutant H1 (Construct #4765, SEQ ID NO: 124), K374E +
 30 F390D + L429M A/Paris/1227/2017 Mutant H1 (Construct #4766, SEQ ID NO:
 126), N97D + F390D + L429M A/Paris/1227/2017 Mutant H1 (Construct #4767,
 SEQ ID NO: 128), N97D + K374E + F390D + L429M A/Paris/1227/2017 Mutant
 H1(Construct #4768, SEQ ID NO: 140); F390D + L429M A/Norway/2147/2017

mutant H1 (Construct #4775, SEQ ID NO:142), K374E + F390D + L429M
 A/Norway/2147/2017 mutant H1 (Construct #4776, SEQ ID NO: 144), N97D +
 F390D + L429M A/Norway/2147/2017 mutant H1 (Construct #4777, SEQ ID NO:
 146), and N97D + K374E + F390D + L429M A/Norway/2147/2017 mutant H1
 (Construct #4778, SEQ ID NO: 148).

Induction of Immunity Against Influenza Infection

[00268] An “immune response” generally refers to a response of the adaptive immune system of a subject. The adaptive immune system generally comprises a humoral response, and a cell-mediated response. The humoral response is the aspect of immunity that is mediated by secreted antibodies, produced in the cells of the B lymphocyte lineage (B cell). Secreted antibodies bind to antigens on the surfaces of invading microbes (such as viruses or bacteria), which flags them for destruction. Humoral immunity is used generally to refer to antibody production and the processes that accompany it, as well as the effector functions of antibodies, including Th2 cell activation and cytokine production, memory cell generation, opsonin promotion of phagocytosis, pathogen elimination and the like. The terms “modulate” or “modulation” or the like refer to an increase or decrease in a particular response or parameter, as determined by any of several assays generally known or used, some of which are exemplified herein.

[00269] A cell-mediated response is an immune response that does not involve antibodies but rather involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Cell-mediated immunity is used generally to refer to some Th cell activation, Tc cell activation and T-cell mediated responses. Cell mediated immunity may be of particular importance in responding to viral infections.

[00270] For example, the induction of antigen specific CD8 positive T lymphocytes may be measured using an ELISPOT assay; stimulation of CD4 positive T-lymphocytes may be measured using a proliferation assay. Anti-influenza HA antibody titres may be quantified using an ELISA assay; isotypes of antigen-specific or cross reactive antibodies may also be measured using anti-isotype antibodies (e.g.

anti -IgG, IgA, IgE or IgM). Methods and techniques for performing such assays are well-known in the art.

[00271] Cytokine presence or levels may also be quantified. For example a T-helper cell response (Th1/Th2) will be characterized by the measurement of IFN- γ and IL-4 secreting cells using by ELISA (e.g. BD Biosciences OptEIA kits). Peripheral blood mononuclear cells (PBMC) or splenocytes obtained from a subject may be cultured, and the supernatant analyzed. T lymphocytes may also be quantified by fluorescence-activated cell sorting (FACS), using marker specific fluorescent labels and methods as are known in the art.

[00272] A microneutralization assay may also be conducted to characterize an immune response in a subject, see for example the methods of Rowe *et al.*, 1973. Virus neutralization titers may be quantified in a number of ways, including: enumeration of lysis plaques (plaque assay) following crystal violet fixation/coloration of cells; microscopic observation of cell lysis in *in vitro* culture; and 2) ELISA and spectrophotometric detection of influenza virus.

[00273] The term “epitope” or “epitopes”, as used herein, refers to a structural part of an antigen to which an antibody specifically binds.

[00274] Immune responses elicited in response to administration of plant-produced wildtype influenza HA proteins or VLPs, or mutant influenza HA proteins or VLPs may for example be observed in Balb/C mice. Serum samples from blood collected from animals may be analyzed by ELISA for H1-specific total IgG and IgA antibodies. Mice immunized with either plant-produced wildtype influenza HA or mutant influenza HA proteins may exhibit HA-specific IgG antibody titers in sera for each treatment group.

[00275] Plant expression

[00276] The constructs of the present invention may 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); Geier 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 Lefebvre, DB Layzell (eds), Addison Wesley, 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), Neuhauser et al. (1987, *Theor. Appl. Genet.* 75: 30-36), Klein et al. (1987, *Nature* 327: 70-73); Freeman et al. (1984, *Plant Cell Physiol.* 29: 1353), Howell 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 Lomonosoff (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).

[00277] 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, pages 41-50; Liu and Lomonosoff, 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.

[00278] Also considered part of this invention are transgenic plants, plant cells or seeds containing the gene construct of the present invention that may be used as a platform plant suitable for transient protein expression described herein. Methods of regenerating whole plants from plant cells are also known in the art (for example see Guerineau and Mullineaux (1993, Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148). In general, transformed plant cells are cultured in an appropriate medium, which may contain selective agents such as antibiotics, where selectable markers are used to facilitate identification of transformed plant cells. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be used to establish repetitive generations, either from seeds or using vegetative propagation techniques. Transgenic plants can also be generated without using tissue culture. Methods for stable transformation, and regeneration of these organisms are established in the art and known to one of skill in the art. Available techniques are reviewed in Vasil et al. (Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications, Academic Press, 1984), and Weissbach and Weissbach (Methods for Plant Molecular Biology, Academic Press, 1989). The method of obtaining transformed and regenerated plants is not critical to the present invention.

[00279] If plants, plant portions or plant cells are to be transformed or co-transformed by two or more nucleic acid constructs, the nucleic acid construct may be introduced into the *Agrobacterium* in a single transfection event so that the nucleic acids are pooled, and the bacterial cells transfected. Alternatively, the constructs may be introduced serially. In this case, a first construct is introduced into the *Agrobacterium* as described, the cells are grown under selective conditions (e.g. in the presence of an antibiotic) where only the singly transformed bacteria can grow. Following this first selection step, a second nucleic acid construct is introduced into

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

[00280] Alternatively, if plants, plant portions, or plant cells are to be transformed or co-transformed by two or more nucleic acid constructs, the nucleic acid construct may be introduced into the plant by co-infiltrating a mixture of *Agrobacterium* cells with the plant, plant portion, or plant cell, each *Agrobacterium* cell may comprise one or more constructs to be introduced within the plant. In order to vary the relative expression levels within the plant, plant portion or plant cell, of a nucleotide sequence of interest within a construct, during the step of infiltration, the concentration of the various *Agrobacteria* populations comprising the desired constructs may be varied.

Table 3: SEQ ID NOs and Description of Sequences

SEQ ID NO:	Description of Sequence	SEQ ID NO:	Description of Sequence
SEQ ID NO: 1	PDI-H1 Cal DNA	SEQ ID NO: 20	H1Cal(L429M).c
SEQ ID NO: 2	PDI-H1 Cal AA	SEQ ID NO: 21	PDI-H1 Cal-L429M DNA
SEQ ID NO: 3	PDI-H1 Mich DNA	SEQ ID NO: 22	PDI-H1 Cal-L429M AA
SEQ ID NO: 4	PDI-H1 Mich AA	SEQ ID NO: 23	PDI-H1 Cal-F390D+L429M DNA
SEQ ID NO: 5	PDI-H1 Mass DNA	SEQ ID NO: 24	PDI-H1 Cal-F390D+L429M AA
SEQ ID NO: 6	PDI-H1 Mass AA	SEQ ID NO: 25	H1Mich(N97D).r
SEQ ID NO: 7	PDI-H1 CostaR DNA	SEQ ID NO: 26	H1Mich(N97D).c
SEQ ID NO: 8	PDI-H1 CostaR AA	SEQ ID NO: 27	PDI-H1 Mich-N97D DNA
SEQ ID NO: 9	PDI-H1 Hond DNA	SEQ ID NO: 28	PDI-H1 Mich-N97D AA
SEQ ID NO: 10	PDI-H1 Hond AA	SEQ ID NO: 29	H1Mich(K374E).r
SEQ ID NO: 11	PDI-H1 Darw DNA	SEQ ID NO: 30	H1Mich(K374E).c
SEQ ID NO: 12	PDI-H1 Darw AA	SEQ ID NO: 31	PDI-H1 Mich-K374E DNA
SEQ ID NO: 13	IF-CPMV(fl5'UTR)_SpPDI.c	SEQ ID NO: 32	PDI-H1 Mich-K374E AA
SEQ ID NO: 14	IF-H1cTMCT.S1-4r	SEQ ID NO: 33	H1Mich(F390D).r
SEQ ID NO: 15	H1Cal(F390D).r	SEQ ID NO: 34	H1Mich(F390D).c
SEQ ID NO: 16	H1Cal(F390D).c	SEQ ID NO: 35	PDI-H1 Mich-F390D DNA
SEQ ID NO: 17	PDI-H1 Cal-F390D DNA	SEQ ID NO: 36	PDI-H1 Mich-F390D AA
SEQ ID NO: 18	PDI-H1 Cal-F390D AA		
SEQ ID NO: 19	H1Cal(L429M).r		

SEQ ID NO:	Description of Sequence
SEQ ID NO: 37	H1Mich(L429M).c
SEQ ID NO: 38	PDI-H1 Mich-L429M DNA
SEQ ID NO: 39	PDI-H1 Mich-L429M AA
SEQ ID NO: 40	PDI-H1 Mich-N97D+K374E DNA
SEQ ID NO: 41	PDI-H1 Mich-N97D+K374E AA
SEQ ID NO: 42	PDI-H1 Mich-F390D+L429M DNA
SEQ ID NO: 43	PDI-H1 Mich-F390D+L429M AA
SEQ ID NO: 44	PDI-H1 Mich-N97D+F390D+L429M DNA
SEQ ID NO: 45	PDI-H1 Mich-N97D+F390D+L429M AA
SEQ ID NO: 46	PDI-H1 Mich-K374E+F390D+L429M DNA
SEQ ID NO: 47	PDI-H1 Mich-K374E+F390D+L429M AA
SEQ ID NO: 48	PDI-H1 Mich-N97D+K374E+F390D+L429M DNA
SEQ ID NO: 49	PDI-H1 Mich-N97D+K374E+F390D+L429M AA
SEQ ID NO: 50	PDI-H1 Mass-F390D+L429M DNA
SEQ ID NO: 51	PDI-H1 Mass-F390D+L429M AA
SEQ ID NO: 52	PDI-H1 Mass-N97D+F390D+L429M DNA
SEQ ID NO: 53	PDI-H1 Mass-N97D+F390D+L429M AA
SEQ ID NO: 54	PDI-H1 Mass-K374E+F390D+L429M DNA
SEQ ID NO: 55	PDI-H1 Mass-K374E+F390D+L429M AA
SEQ ID NO: 56	PDI-H1 Mass-N97D+K374E+F390D+L429M DNA
SEQ ID NO: 57	PDI-H1 Mass-N97D+K374E+F390D+L429M AA
SEQ ID NO: 58	PDI-H1 CR-F390D+L429M DNA

SEQ ID NO:	Description of Sequence
SEQ ID NO: 59	PDI-H1 CR-F390D+L429M AA
SEQ ID NO: 60	PDI-H1 CR-N97D+F390D+L429M DNA
SEQ ID NO: 61	PDI-H1 CR-N97D+F390D+L429M AA
SEQ ID NO: 62	PDI-H1 CR-K374E+F390D+L429M DNA
SEQ ID NO: 63	PDI-H1 CR-K374E+F390D+L429M AA
SEQ ID NO: 64	PDI-H1 CR-N97D+K374E+F390D+L429M DNA
SEQ ID NO: 65	PDI-H1 CR-N97D+K374E+F390D+L429M AA
SEQ ID NO: 66	H1Hond(N97D).c
SEQ ID NO: 67	PDI-H1 Hond-N97D DNA
SEQ ID NO: 68	PDI-H1 Hond-N97D AA
SEQ ID NO: 69	H1Hond(K374E).r
SEQ ID NO: 70	H1Hond(K374E).c
SEQ ID NO: 71	PDI-H1 Hond-K374E DNA
SEQ ID NO: 72	PDI-H1 Hond-K374E AA
SEQ ID NO: 73	H1Hond(F390D).r
SEQ ID NO: 74	H1Hond(F390D).c
SEQ ID NO: 75	PDI-H1 Hond-F390D DNA
SEQ ID NO: 76	PDI-H1 Hond-F390D AA
SEQ ID NO: 77	H1Hond(L429M).r
SEQ ID NO: 78	H1Hond(L429M).c
SEQ ID NO: 79	PDI-H1 Hond-L429M DNA
SEQ ID NO: 80	PDI-H1 Hond-L429M AA
SEQ ID NO: 81	PDI-H1 Hond-F390D+L429M DNA
SEQ ID NO: 82	PDI-H1 Hond-F390D+L429M AA
SEQ ID NO: 83	PDI-H1 Hond-N97D+F390D+L429M DNA
SEQ ID NO: 84	PDI-H1 Hond-N97D+F390D+L429M AA
SEQ ID NO: 85	PDI-H1 Darw-N97D DNA

SEQ ID NO:	Description of Sequence
SEQ ID NO: 86	PDI-H1 Darw-N97D AA
SEQ ID NO: 87	H1Darw(K374E).r
SEQ ID NO: 88	PDI-H1 Darw-K374E DNA
SEQ ID NO: 89	PDI-H1 Darw-K374E AA
SEQ ID NO: 90	PDI-H1 Darw-F390D DNA
SEQ ID NO: 91	PDI-H1 Darw-F390D AA
SEQ ID NO: 92	PDI-H1 Darw-L429M DNA
SEQ ID NO: 93	PDI-H1 Darw-L429M AA
SEQ ID NO: 94	PDI-H1 Darw-F390D+L429M DNA
SEQ ID NO: 95	PDI-H1 Darw-F390D+L429M AA
SEQ ID NO: 96	PDI-H1 Darw-N97D+F390D+L429M DNA
SEQ ID NO: 97	PDI-H1 Darw-N97D+F390D+L429M AA
SEQ ID NO: 98	Cloning vector 1190 from left to right T-DNA
SEQ ID NO: 99	Construct 1314 from 2X35S prom to NOS term
SEQ ID NO: 100	Construct 2980 from 2X35S prom to NOS term
SEQ ID NO: 101	Construct 2995 from 2X35S prom to NOS term
SEQ ID NO: 102	H1Mich(N380A).r
SEQ ID NO: 103	H1Cal(N380A).c
SEQ ID NO: 104	PDI-H1 Mich-N380A DNA
SEQ ID NO: 105	PDI-H1 Mich-N380A AA
SEQ ID NO: 106	H1Mich(N380A+F390D).r
SEQ ID NO: 107	PDI-H1 Mich-F390D+N380A DNA
SEQ ID NO: 108	PDI-H1 Mich-F390D+N380A AA
SEQ ID NO: 109	IF-H5ITMCT.s1-4r
SEQ ID NO: 110	PDI-H5 Indo DNA
SEQ ID NO: 111	PDI-H5 Indo AA
SEQ ID NO: 112	H5Ind(F393D).r
SEQ ID NO: 113	H5Ind(F393D).c

SEQ ID NO:	Description of Sequence
SEQ ID NO: 114	PDI-H5 Indo-F393D DNA
SEQ ID NO: 115	PDI-H5 Indo-F393D AA
SEQ ID NO: 116	IF-H5_Egy.r
SEQ ID NO: 117	PDI-H5 Egypt DNA
SEQ ID NO: 118	PDI-H5 Egypt AA
SEQ ID NO: 119	H5Egy(F392D).r
SEQ ID NO: 120	H5Egy(F392D).c
SEQ ID NO: 121	PDI-H5 Egypt-F392D DNA
SEQ ID NO: 122	PDI-H5 Egypt-F392D AA
SEQ ID NO: 123	PDI-H1 Par-F390D+L429M DNA
SEQ ID NO: 124	PDI-H1 Par-F390D+L429M AA
SEQ ID NO: 125	PDI-H1 Par-K374E+F390D+L429M DNA
SEQ ID NO: 126	PDI-H1 Par-K374E+F390D+L429M AA
SEQ ID NO: 127	PDI-H1 Par-N97D+F390D+L429M DNA
SEQ ID NO: 128	PDI-H1 Par-N97D+F390D+L429M AA
SEQ ID NO: 129	PDI-H1 Par-N97D+K374E+F390D+L429M DNA
SEQ ID NO: 130	A/California/7/09 (H1N1) (aa)
SEQ ID NO: 131	A/Honduras/17734/16 (H1N1) (aa)
SEQ ID NO: 132	A/Darwin/11/15 (H1N1) (aa)
SEQ ID NO: 133	A/Costa Rica/0513/16 (H1N1) (aa)
SEQ ID NO: 134	A/Michigan/45/15 (H1N1) (aa)
SEQ ID NO: 135	A/Massachusetts/06/17 (H1N1) (aa)
SEQ ID NO: 136	A/Michigan/45/15 (nt)
SEQ ID NO: 137	A/California/7/09 (H1N1) (nt)
SEQ ID NO: 138	A/Paris/1227/2017 (aa)
SEQ ID NO: 139	A/Norway/2147/2017
SEQ ID NO: 140	PDI-H1 Par-N97D+K374E+F390D+L429M AA
SEQ ID NO: 141	PDI-H1 Nor-F390D+L429M DNA

SEQ ID NO:	Description of Sequence
SEQ ID NO: 142	PDI-H1 Nor-F390D+L429M AA
SEQ ID NO: 143	PDI-H1 Nor-K374E+F390D+L429M DNA
SEQ ID NO: 144	PDI-H1 Nor-K374E+F390D+L429M AA
SEQ ID NO: 145	PDI-H1 Nor-N97D+F390D+L429M DNA

SEQ ID NO:	Description of Sequence
SEQ ID NO: 146	PDI-H1 Nor-N97D+F390D+L429M AA
SEQ ID NO: 147	PDI-H1 Nor-N97D+K374E+F390D+L429M DNA
SEQ ID NO: 148	PDI-H1 Nor-N97D+K374E+F390D+L429M AA

[00281] The present invention will be further illustrated in the following examples.

5 Example 1: Influenza HA Constructs

[00282] The influenza HA constructs were produced using techniques well known within the art. For example wildtype A-California-07-09 HA, F390D A-California-07-09 HA and F390D+L429M A-California-07-09 HA were cloned as described below. Other H1 mutants were obtained using similar techniques and the HA sequences primers, templates and products are described in Example 3 (Influenza HA and VLP Production in Plants) and Table 4.

[00283] A summary of the wildtype and mutated HA proteins, primers, templates and products is provided in Table 4 below.

15 **A. Modification of H1 HA**

2X35S/CPMV 160/ PDISP-HA0 H1 A-California-07-09/ NOS (Construct number 1314)

[00284] A sequence encoding mature HA0 from influenza HA from A/California/07/09 fused to alfalfa PDI secretion signal peptide (PDISP) was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. A fragment containing the PDISP-A/California/07/09 coding sequence was amplified using primers IF-CPMV(f5'UTR)_SpPDI.c (SEQ ID NO: 13) and IF-H1cTMCT.S1-4r (SEQ ID NO: 14), using PDISP-H1 A/California/7/09 gene

sequence (SEQ ID NO: 1) as template. The PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figure 8) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for “In Fusion” cloning of genes of interest in a 2X35S/CPMV 160/NOS-based expression cassette. It also incorporates a gene construct for the co-expression of the TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCAMBIA binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 98. The resulting construct was given number 1314 (SEQ ID NO: 99). The amino acid sequence of mature HA0 from influenza HA from A/California/07/09 fused to alfalfa PDI secretion signal peptide (PDISP) is presented in SEQ ID NO: 2. A representation of plasmid 1314 is presented in Figure 6A.

2X35S/CPMV 160/ PDISP-HA0 H1 A-California-07-09 (F390D)/ NOS (Construct number 2980)

[00285] A sequence encoding mature HA0 from influenza HA from A/California/07/09 (F390D) fused to alfalfa PDI secretion signal peptide (PDISP) was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. In a first round of PCR, a fragment containing the PDISP-H1 A/California/07/09 with the mutated F390D amino acid was amplified using primers IF-CPMV(fl5'UTR)_SpPDI.c (SEQ ID NO: 13) and H1Cal(F390D).r (SEQ ID NO: 15), using PDISP-H1 A/California/7/09 gene sequence (SEQ ID NO: 1) as template. A second fragment containing the F390D mutation with the remaining of the H1 A/California/07/09 was amplified using H1Cal(F390D).c (SEQ ID NO: 16) and IF-H1cTMCT.S1-4r (SEQ ID NO: 14), using PDISP-H1 A/California/07/09 gene sequence (SEQ ID NO: 1) as template. The PCR products from both amplifications were then mixed and used as template for a second round of amplification using IF-CPMV(fl5'UTR)_SpPDI.c (SEQ ID NO: 13) and IF-H1cTMCT.S1-4r (SEQ ID NO: 14) as primers. The final PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figure 8) was digested with SacII and StuI restriction enzyme

and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for “In Fusion” cloning of genes of interest in a 2X35S/CPMV 160/NOS-based expression cassette. It also incorporates a gene construct for the co-expression of the TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCambia binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 98. The resulting construct was given number 2980 (SEQ ID NO: 100). The amino acid sequence of mutated PDISP-HA from A/California/07/09 (F390D) is presented in SEQ ID NO: 18. A representation of plasmid 2980 is presented in Figure 6B.

2X35S/CPMV 160/ PDISP-HA0 H1 A-California-07-09 (F390D+L429M)/NOS
(Construct number 2995)

[00286] A sequence encoding mature HA0 from influenza HA from A/California/07/09 (F390D+L429M) fused to alfalfa PDI secretion signal peptide (PDISP) was cloned into 2X35S/CPMV 160/NOS expression system using the following PCR-based method. In a first round of PCR, a fragment containing the PDISP-H1 A/California/07/09 with the mutated F390D and L429M amino acids was amplified using primers IF-CPMV(fl5'UTR)_SpPDI.c (SEQ ID NO: 13) and H1Cal(L429M).r (SEQ ID NO: 19), using PDISP-H1 A/California/7/09 (F390D) gene sequence (SEQ ID NO: 17) as template. A second fragment containing the L429M mutation with the remaining of the H1 A/California/07/09 was amplified using H1Cal(L429M).c (SEQ ID NO: 20) and IF-H1cTMCT.S1-4r (SEQ ID NO: 14), PDISP-H1 A/California/7/09 (F390D) gene sequence (SEQ ID NO: 17) as template. The PCR products from both amplifications were then mixed and used as template for a second round of amplification using IF-CPMV(fl5'UTR)_SpPDI.c (SEQ ID NO: 13) and IF-H1cTMCT.S1-4r (SEQ ID NO: 14) as primers. The final PCR product was cloned in 2X35S/CPMV 160/NOS expression system using In-Fusion cloning system (Clontech, Mountain View, CA). Construct number 1190 (Figure 8) was digested with SacII and StuI restriction enzyme and the linearized plasmid was used for the In-Fusion assembly reaction. Construct number 1190 is an acceptor plasmid intended for “In Fusion” cloning of genes of interest in a 2X35S/CPMV 160/NOS-based expression cassette. It also incorporates a gene construct for the co-expression of the

TBSV P19 suppressor of silencing under the alfalfa Plastocyanin gene promoter and terminator. The backbone is a pCambia binary plasmid and the sequence from left to right t-DNA borders is presented in SEQ ID NO: 98. The resulting construct was given number 2995 (SEQ ID NO: 101). The amino acid sequence of mutated PDISP-HA from A/California/07/09 (F390D+L429M) is presented in SEQ ID NO: 24. A representation of plasmid 2995 is presented in Figure 6D.

Example 2: Methods

Agrobacterium tumefaciens Transfection

[00287] *Agrobacterium tumefaciens* strain AGL1 was transfected by electroporation with the wildtype influenza HA or mutant influenza HA expression vectors using the methods described by D'Aoust *et al.*, 2008 (*Plant Biotech. J.* 6:930-40). Transfected *Agrobacterium* were grown in YEB medium supplemented with 10 mM 2-(N-morpholino)ethanesulfonic acid (MES), 20 μ M acetosyringone, 50 μ g/ml kanamycin and 25 μ g/ml of carbenicillin pH5.6 to an OD₆₀₀ between 0.6 and 1.6. *Agrobacterium* suspensions were centrifuged before use and resuspended in infiltration medium (10 mM MgCl₂ and 10 mM MES pH 5.6).

Preparation of Plant Biomass, Inoculum and Agroinfiltration

[00288] *N. benthamiana* plants were grown from seeds in flats filled with a commercial peat moss substrate. The plants were allowed to grow in the greenhouse under a 16/8 photoperiod and a temperature regime of 25° C. day/20° C. night. Three weeks after seeding, individual plantlets were picked out, transplanted in pots and left to grow in the greenhouse for three additional weeks under the same environmental conditions.

[00289] *Agrobacteria* transfected with each wildtype influenza HA or mutant influenza HA expression vector were grown in a YEB medium supplemented with 10 mM 2-(N-morpholino)ethanesulfonic acid (MES), 20 μ M acetosyringone, 50 μ g/ml kanamycin and 25 μ g/ml of carbenicillin pH 5.6 until they reached an OD₆₀₀ between 0.6 and 1.6. *Agrobacterium* suspensions were centrifuged before use and resuspended in infiltration medium (10 mM MgCl₂ and 10 mM MES pH 5.6) and stored overnight at 4° C. On the day of infiltration, culture batches were diluted in 2.5

culture volumes and allowed to warm before use. Whole plants of *N. benthamiana* were placed upside down in the bacterial suspension in an air-tight stainless steel tank under a vacuum of 20-40 Torr for 2-min. Plants were returned to the greenhouse for a 6 or 9 day incubation period until harvest.

5 Leaf Harvest and Total Protein Extraction

[00290] Proteins were extracted from fresh biomass cut into ~1 cm² pieces by an overnight enzymatic extraction at room temperature using an orbital shaker. The slurry was then filtered through a large pore nylon filter to remove coarse undigested vegetal tissue.

10 [00291] To obtain the “Full Process yields”, the slurry was centrifuged to remove protoplasts and intracellular contaminants. The supernatant was clarified by depth-filtration. The clarified fraction was then loaded over a cation exchange column with a step-elution step with increasing concentrations of NaCl. The purified VLPs were concentrated by TFF, diafiltered against a formulation buffer and passed through
15 a filter. Protein content of purified VLP was analysed by BCA assay and activity was analysed by a hemagglutination assay. Relative yields were obtained by comparing the protein yields from the new construct to the native construct used as control.

[00292] To obtain the “Post-Density Gradient Yields”, the slurry was centrifuged to remove protoplasts and intracellular contaminants. The supernatant was
20 centrifuged further to remove additional debris. The supernatant was the clarified by depth-filtration using glass fiber filter. The clarified fraction was then loaded on a discontinuous iodixanol density gradient. Separation density gradient centrifugation was performed as follows: 38 ml tubes containing discontinuous iodixanol density gradient in Tris buffer (successive layers of 35%, 30%, 25%, 20%, 15, 10% and 5%)
25 were prepared and overlaid with clarified extract. The gradients were centrifuged at 120 000 g for 2 hours (4°C). After centrifugation, the first 5 mL collected from the bottom to the top were discarded while the next 5 mL were collected for protein content analysis (BCA), activity measurement (hemagglutination assay) and intensity measurement of the HA0 band on a reduced SDS-PAGE (densitometry). Relative

yields were obtained by comparing the HA0 band intensity from the new construct to the native construct used as control.

Hemagglutination Assay

[00293] Hemagglutination assay was based on a method described by
5 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 (for
H1) red blood cells suspension (Bio Link Inc., Syracuse, NY) were added to each
well, and plates were incubated for 2h at room temperature. The reciprocal of the
10 highest dilution showing complete hemagglutination was recorded as HA activity. In
parallel, a recombinant HA standard (A/Vietnam/1203/2004 H5N1) (Protein Science
Corporation, Meriden, CT) was diluted in PBS and run as a control on each plate.

Protein Analysis and Immunoblotting

Immunoblotting was performed with a first incubation with a primary mAb, diluted
15 1/500 in 2% skim milk in TBS-Tween 20 0.1%. Peroxydase-conjugated goat anti-
mouse (Jackson ImmunoResearch, cat #115-035-146) diluted 1/10000 was used as
secondary antibody for chemiluminescence detection in 2% skim milk in TBS-Tween
20 0.1%. Immunoreactive complexes were detected by chemiluminescence using
luminol as the substrate (Roche Diagnostics Corporation). Horseradish peroxidase-
20 enzyme conjugation of human IgG antibody was carried out by using the EZ-Link
Plus® Activated Peroxidase conjugation kit (Pierce, Rockford, Ill.).

Example 3: Influenza HA and VLP Production in Plants

A. Modification of H1 HA

25 [00294] The influenza HA constructs were produced using techniques well
known within the art (see Example 1). A summary of the wildtype and mutated HA
proteins, primers, templates and products is provided in Table 4 below. The sequences
used are provided in Example 4 and in the sequence listing.

30 *F390D A/California/07/09 Mutant H1*

[00295] F390D A/California/07/09 Mutant H1 was constructed by mutating the phenylalanine residue at position 390 of wildtype A/California/07/09 H1 to aspartic acid (Construct# 2980). As shown in Figure 2A and 2B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 2980 exhibited an approximate 60% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/California/07/09 H1 (Construct #1314). Furthermore, as seen in Figure 2C, *N. benthamiana* plants agroinfiltrated with Construct# 2980 exhibited an approximate 60% increase in VLP yield following iodixanol gradient purification, in comparison to plants infiltrated with wildtype construct.

L429M A/California/07/09 Mutant H1

[00296] L429M A/California/07/09 Mutant H1 was constructed by mutating the leucine residue at position 429 of wildtype A/California/07/09 H1 to methionine (Construct# 2962). As shown in Figure 2A and 2B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 2962 exhibited an approximate 20% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/California/07/09 H1 (Construct #1314). Furthermore, as seen in Figure 2C, *N. benthamiana* plants agroinfiltrated with Construct# 2962 exhibited an approximate 30% increase in VLP yield following iodixanol gradient purification, in comparison to plants infiltrated with wildtype construct.

F390D + L429M A/California/07/09 Mutant H1

[00297] F390D + L429M A/California/07/09 Mutant H1 was constructed by introducing a double mutation to the wildtype sequence of A/California/07/09 H1, wherein the phenylalanine at position 390 was mutated to an aspartic acid and the leucine at position 429 was mutated to a methionine (Construct# 2995). As shown in Figure 2B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 2995 exhibited an approximate 60% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/California/07/09 H1 (Construct #1314).

N97D A/Michigan/45/15 Mutant H1

[00298] N97D A/Michigan/45/15 Mutant H1 was constructed by mutating the asparagine residue at position 97 of wildtype A/Michigan/45/15 H1 to aspartic acid (Construct# 3774). As shown in Figure 3A and 3B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3774 exhibited an approximate 1100% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

K374E A/Michigan/45/15 Mutant H1

[00299] K374E A/Michigan/45/15 Mutant H1 was constructed by mutating the lysine residue at position 374 of wildtype A/Michigan/45/15 H1 to glutamic acid (Construct# 3771). As shown in Figure 3A and 3B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3771 exhibited an approximate 1100% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

F390D A/Michigan/45/15 Mutant H1

[00300] F390D A/Michigan/45/15 Mutant H1 was constructed by mutating the phenylalanine residue at position 390 of wildtype A/Michigan/45/15 H1 to aspartic acid (Construct# 3641). As shown in Figure 3B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3641 exhibited a similar activity in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640). However, when full process yield for the purified VLPs with H1 was measured and increase to 172% compared to the wildtype was observed (see Table 5C).

L429M A/Michigan/45/15 Mutant H1

[00301] L429M A/Michigan/45/15 Mutant H1 was constructed by mutating the leucine residue at position 429 of wildtype A/Michigan/45/15 H1 to aspartic acid (Construct# 3643). As shown in Figure 3 B, purified extracts from *N.*

benthamiana plants agroinfiltrated with Construct# 3643 exhibited an approximate 500% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

N97D + K374E A/Michigan/45/15 Mutant H1

5 [00302] N97D + K374E A/Michigan/45/15 Mutant H1 was constructed by introducing a double mutation to the wildtype sequence of wildtype A/Michigan/45/15, wherein the asparagine at position 97 was replaced with an aspartic acid residue, and the lysine at position 374 was replaced with a glutamic acid residue (Construct# 3880). As shown in Figure 3B, purified extracts from *N.*
 10 *benthamiana* plants agroinfiltrated with Construct# 3880 exhibited an approximate 1100% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

F390D + L429M A/Michigan/45/15 Mutant H1

15 [00303] F390D + L429M A/Michigan/45/15 Mutant H1 was constructed by introducing a double mutation to the wildtype sequence of wildtype A/Michigan/45/15, wherein the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 3703). As shown in Figure 3B, purified extracts from *N.*
 20 *benthamiana* plants agroinfiltrated with Construct# 3703 exhibited an approximate 1100% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

N97D + F390D + L429M A/Michigan/45/15 Mutant H1

25 [00304] N97D + F390D + L429M A/Michigan/45/15 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Michigan/45/15, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue

(Construct# 3879). As shown in Figure 3B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3879 exhibited an approximate 2300% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

5 *K374E + F390D + L429M A/Michigan/45/15 Mutant H1*

[00305] K374E + F390D + L429M A/Michigan/45/15 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Michigan/45/15, wherein the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 3878). As shown in Figure 3B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3878 exhibited an approximate 2500% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

15 *N97D + K374E + F390D + L429M A/Michigan/45/15 Mutant H1*

[00306] N97D + K374E + F390D + L429M A/Michigan/45/15 Mutant H1 was constructed by introducing a quadruple mutation to the wildtype sequence of wildtype A/Michigan/45/15, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 3881). As shown in Figure 3B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3881 exhibited an approximate 3300% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

N97D + F390D + L429M A/Massachusetts/06/17 Mutant H1

[00307] N97D + F390D + L429M A/Massachusetts/06/17 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Massachusetts/06/17, wherein the asparagine at position 97 was mutated to an

aspartic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4093). As shown in Figure 4B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4093 exhibited an approximate 40% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with the double mutant construct, F390D + L429M A/Massachusetts/06/17 Mutant H1 (Construct #4091).

K374E + F390D + L429M A/Massachusetts/06/17 Mutant H1

[00308] K374E + F390D + L429M A/Massachusetts/06/17 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Massachusetts/06/17, wherein the lysine at position 374 was mutated to an glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4092). As shown in Figure 4B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4092 exhibited an approximate 50% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with the double mutant construct, F390D + L429M A/Massachusetts/06/17 Mutant H1 (Construct #4091).

N97D + K374E + F390D + L429M A/Massachusetts/06/17 Mutant H1

[00309] N97D + K374E + F390D + L429M A/Massachusetts/06/17 Mutant H1 was constructed by introducing a quadruple mutation to the wildtype sequence of wildtype A/Massachusetts/06/17, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the lysine at position 374 was mutated to an glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4094). As shown in Figure 4B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4094 exhibited an approximate 70% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with the double mutant construct, F390D + L429M A/Massachusetts/06/17 Mutant H1 (Construct #4091).

N97D + F390D + L429M A/Costa Rica/0513/16 Mutant H1

[00310] N97D + F390D + L429M A/Costa Rica/0513/16 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Costa Rica/0513/16, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4717). As shown in Figure 4B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4717 exhibited an approximate 100% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with the double mutant construct, F390D + L429M A/Costa Rica/0513/16 Mutant H1 (Construct #4715).

K374E + F390D + L429M A/Costa Rica/0513/16 Mutant H1

[00311] K374E + F390D + L429M A/Costa Rica/0513/16 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Costa Rica/0513/16, wherein the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4716). As shown in Figure 4B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4716 exhibited an approximate 10% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with the double mutant construct, F390D + L429M A/Costa Rica/0513/16 Mutant H1 (Construct #4715).

N97D + K374E + F390D + L429M A/Costa Rica/0513/16 Mutant H1

[00312] N97D + K374E + F390D + L429M A/Costa Rica/0513/16 Mutant H1 was constructed by introducing a quadruple mutation to the wildtype sequence of wildtype A/Costa Rica/0513/16, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4718). As shown in Figure 4B, purified extracts from *N. benthamiana* plants agroinfiltrated

with Construct# 4718 exhibited an approximate 140% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with the double mutant construct, F390D + L429M A/Costa Rica/0513/16 Mutant H1 (Construct #4715).

5 *N97D A/Honduras/17734/16 Mutant H1*

[00313] *N97D A/Honduras/17734/16 Mutant H1* was constructed by mutating the asparagine residue at position 97 of wildtype A/Honduras/17734/16 H1 to aspartic acid (Construct# 3950). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3950 exhibited an approximate
10 300% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Honduras/17734/16 H1 (Construct #3944).

K374E A/Honduras/17734/16 Mutant H1

[00314] *K374E A/Honduras/17734/16 Mutant H1* was constructed by mutating the lysine residue at position 374 of wildtype A/Honduras/17734/16 H1 to glutamic acid (Construct# 3948). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3948 exhibited an approximate
15 100% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Honduras/17734/16 H1 (Construct #3944).

F390D A/Honduras/17734/16 Mutant H1

20 [00315] *F390D A/Honduras/17734/16 Mutant H1* was constructed by mutating the phenylalanine residue at position 390 of wildtype A/Honduras/17734/16 H1 to aspartic acid (Construct# 3945). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3945 exhibited an approximate
25 30% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Honduras/17734/16 H1 (Construct #3944).

L429M A/Honduras/17734/16 Mutant H1

[00316] *L429M A/Honduras/17734/16 Mutant H1* was constructed by mutating the leucine residue at position 429 of wildtype A/Honduras/17734/16 H1 to

methionine (Construct# 3949). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3949 exhibited an approximate 400% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Honduras/17734/16 H1 (Construct #3944).

5 *F390D + L429M A/Honduras/17734/16 Mutant H1*

[00317] F390D + L429M A/Honduras/17734/16 Mutant H1 was constructed by introducing a double mutation to the wildtype sequence of wildtype A/Honduras/17734/16, wherein the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 3946). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3946 exhibited an approximate 300% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Honduras/17734/16 H1 (Construct #3944).

N97D + F390D + L429M A/Honduras/17734/16 Mutant H1

15 [00318] N97D + F390D + L429M A/Honduras/17734/16 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Honduras/17734/16, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 3951). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3951 exhibited an approximate 600% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Honduras/17734/16 H1 (Construct #3944).

N97D A/Darwin/11/15 Mutant H1

25 [00319] N97D A/Darwin/11/15 Mutant H1 was constructed by mutating the asparagine residue at position 97 of wildtype A/Darwin/11/15 H1 to aspartic acid (Construct# 3990). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3990 exhibited an approximate 200% increase

in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Darwin/11/15 H1 (Construct #3984).

K374E A/Darwin/11/15 Mutant H1

[00320] K374E A/Darwin/11/15 Mutant H1 was constructed by mutating the lysine residue at position 374 of wildtype A/Darwin/11/15 H1 to glutamic acid (Construct# 3988). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3988 exhibited an approximate 300% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Darwin/11/15 H1 (Construct #3984).

F390D A/Darwin/11/15 Mutant H1

[00321] F390D A/Darwin/11/15 Mutant H1 was constructed by mutating the phenylalanine residue at position 390 of wildtype A/Darwin/11/15 H1 to aspartic acid (Construct# 3985). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3985 exhibited an approximate 50% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Darwin/11/15 H1 (Construct #3984).

L429M A/Darwin/11/15 Mutant H1

[00322] L429M A/Darwin/11/15 Mutant H1 was constructed by mutating the leucine residue at position 429 of wildtype A/Darwin/11/15 H1 to methionine (Construct# 3989). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3989 exhibited an approximate 500% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Darwin/11/15 H1 (Construct #3984).

F390D + L429M A/Darwin/11/15 Mutant H1

[00323] F390D + L429M A/Darwin/11/15 Mutant H1 was constructed by introducing a double mutation to the wildtype sequence of wildtype A/Darwin/11/15, wherein the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 3986).

As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3986 exhibited an approximate 300% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Darwin/11/15 H1 (Construct #3984).

5 *N97D + F390D + L429M A/Darwin/11/15 Mutant H1*

[00324] N97D + F390D + L429M A/Darwin/11/15 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Darwin/11/15, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 3991). As shown in Figure 4A, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3991 exhibited an approximate 1100% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Darwin/11/15 H1 (Construct #3984).

15 *F390D + L429M A/Paris/1227/2017 Mutant H1*

[00435] F390D + L429M A/Paris/1227/2017 Mutant H1 was constructed by introducing a double mutation to the wildtype sequence of wildtype A/Paris/1227/2017, wherein the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4765). As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4765 exhibited an approximate 117% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with F390D + L429M A/Massachusetts/06/17 Mutant H1 (Construct# 4091).

25 *K374 + F390D + L429M A/ Paris/1227/2017*

[00325] K374E + F390D + L429M A/Paris/1227/2017 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Paris/1227/2017, wherein the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and

the leucine at position 429 was replaced with a methionine residue (Construct# 4766).

As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4766 exhibited an approximate 127% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with F390D + L429M A/Paris/1227/2017 Mutant H1 (Construct# 4765).

N97D + F390D + L429M A/Paris/1227/2017

[00326] N97D + F390D + L429M A/Paris/1227/2017 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Paris/1227/2017, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4767). As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4767 exhibited an approximate 171% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with F390D + L429M A/Paris/1227/2017 Mutant H1 (Construct# 4765).

N97D + K374E + F390D + L429M A/Paris/1227/2017

[00327] N97D + K374E + F390D + L429M A/Paris/1227/2017 Mutant H1 was constructed by introducing a quadruple mutation to the wildtype sequence of wildtype A/Paris/1227/2017, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4768). As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4768 exhibited an approximate 230% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with F390D + L429M A/Paris/1227/2017 Mutant H1 (Construct# 4765).

F390D + L429M A/Norway/2147/2017 Mutant H1

[00435] F390D + L429M A/Norway/2147/2017 Mutant H1 was constructed by introducing a double mutation to the wildtype sequence of wildtype A/Norway/2147/2017, wherein the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4775). As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4775 exhibited an approximate 112% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with F390D + L429M A/Massachusetts/06/17 Mutant H1 (Construct# 4091).

K374 + F390D + L429M A/Norway/2147/2017

[00328] K374E + F390D + L429M A/Norway/2147/2017 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Norway/2147/2017, wherein the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4776). As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4776 exhibited an approximate 128% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with F390D + L429M A/Norway/2147/2017 Mutant H1 (Construct# 4775).

N97D + F390D + L429M A/Norway/2147/2017

[00329] N97D + F390D + L429M A/Norway/2147/2017 Mutant H1 was constructed by introducing a triple mutation to the wildtype sequence of wildtype A/Norway/2147/2017, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4777). As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4777 exhibited an approximate 200% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants

agroinfiltrated with F390D + L429M A/Norway/2147/2017 Mutant H1 (Construct# 4775).

N97D + K374E + F390D + L429M A/Norway/2147/2017

[00330] N97D + K374E + F390D + L429M A/Norway/2147/2017 Mutant H1 was constructed by introducing a quadruple mutation to the wildtype sequence of wildtype A/Norway/2147/2017, wherein the asparagine at position 97 was mutated to an aspartic acid residue, the lysine at position 374 was mutated to a glutamic acid residue, the phenylalanine at position 390 was mutated to an aspartic acid residue, and the leucine at position 429 was replaced with a methionine residue (Construct# 4778). As shown in Figure 4C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 4778 exhibited an approximate 213% increase in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with F390D + L429M A/Norway/2147/2017 Mutant H1 (Construct# 4775).

N380A A/Michigan/45/15

[00331] N380A A/Michigan/45/15 Mutant H1 was constructed by mutating the asparagine at position 380 of wildtype A/Michigan/45/15 H1 to an alanine residue (Construct #3644). As seen in Figure 3B and 3C, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3644 exhibited an approximate 80% decrease in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640).

N380A + F390D A/Michigan/45/15

[00332] A N380A + F390D A/Michigan/45/15 Mutant H1 was also constructed, wherein a double mutation was introduced into wildtype A/Michigan/45/15 H1 by replacing the asparagine at position 380 with an alanine residue, and replacing the phenylalanine at position 390 with an aspartic acid residue (Construct #3704). As seen in Figure 3B, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3704 exhibited an approximate 50% decrease in hemagglutination titer as compared to extracts from *N. benthamiana* plants

agroinfiltrated with wildtype A/Michigan/45/15 H1 (Construct #3640). In view of the reduced hemagglutination titer observed with the N380A A/Michigan/45/15 Mutant H1, these results suggest that mutation of the asparagine residue at position 380 negatively affects the expression of influenza HA protein and/or the stability of influenza HA protein in plants.

[00333] The one or more than one mutations described herein specifically increase influenza HA protein production and VLP yield in plants. It was observed that mutations at other positions significantly reduced, or had no significant effect, on influenza HA protein accumulation or VLP production in plant cells.

[00334] The increased hemagglutination titers achieved with the influenza HA proteins comprising the one or more than one mutation described herein was also observed to be specific to influenza H1 HAs. Similar enhancements were not observed in plants agroinfiltrated with constructs encoding mutant influenza HAs derived from non-H1 strains.

[00335] For example, F393D A/Indonesia/5/2005 Mutant H5 was constructed by mutating the phenylalanine at position 393 of wildtype A/Indonesia/5/2005 H5 to aspartic acid (Construct #3680). As shown in Figure 5, purified extracts from *N. benthamiana* plants agroinfiltrated with Construct# 3680 exhibited an approximate 98% reduction in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Indonesia/5/2005 H5(Construct #2295).

[00336] Similarly, purified extracts from *N. benthamiana* plants agroinfiltrated with F392D A/Egypt/N04915/2014 Mutant H5 (Construct #3690), exhibited an approximate 99% reduction in hemagglutination titer as compared to extracts from *N. benthamiana* plants agroinfiltrated with wildtype A/Egypt/N04915/2014 H5 (Construct #3645) (see Figure 5, Table 6).

[00337] The one or more than one mutations described herein specifically increase influenza HA protein production and VLP yield in plants. It was observed that mutations at other positions significantly reduced, or had no significant effect, on influenza HA protein accumulation or VLP production in plant cells.

Table 4: Examples of constructs that have been prepared as described herein. Sequences are provided in Example 4 and the sequence listing.

Construct Name	TMCT	Construct #	Fig	Primer 1	Primer 2	Primer 3	Primer 4	Template for PCR	Resulting Gene	Resulting Protein
H1 A-Cal-7-2009	Wt	1314	6A	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 1	SEQ ID NO: 1	SEQ ID NO: 2
H1 A-Cal-7-09 (F390D)	Wt	2980	6B	SEQ ID NO: 13	SEQ ID NO: 15	SEQ ID NO: 16	SEQ ID NO: 14	SEQ ID NO: 1	SEQ ID NO: 17	SEQ ID NO: 18
H1 A-Cal-7-09 (L429M)	Wt	2962	6C	SEQ ID NO: 13	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 14	SEQ ID NO: 1	SEQ ID NO: 21	SEQ ID NO: 22
H1 A-Cal-7-09 (F390D+L429M)	Wt	2995	6D	SEQ ID NO: 13	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 14	SEQ ID NO: 17	SEQ ID NO: 23	SEQ ID NO: 24
H1 A-Mich-45-2015	Wt	3640	6E	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 3	SEQ ID NO: 3	SEQ ID NO: 4
H1 A-Mich-45-2015 (N97D)	Wt	3774	6F	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 27	SEQ ID NO: 28
H1 A-Mich-45-2015 (K374E)	Wt	3771	6G	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 31	SEQ ID NO: 32
H1 A-Mich-45-2015 (F390D)	Wt	3641	6H	SEQ ID NO: 13	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 35	SEQ ID NO: 36
H1 A-Mich-45-2015 (L429M)	Wt	3643	6I	SEQ ID NO: 13	SEQ ID NO: 19	SEQ ID NO: 37	SEQ ID NO: 14	SEQ ID NO: 3	SEQ ID NO: 38	SEQ ID NO: 39
H1 A-Mich-45-2015 (N97D+K374E)	Wt	3880	6J	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 27	SEQ ID NO: 40	SEQ ID NO: 41
H1 A-Mich-45-2015 (F390D+L429M)	Wt	3703	6K	SEQ ID NO: 13	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 14	SEQ ID NO: 38	SEQ ID NO: 42	SEQ ID NO: 43
H1 A-Mich-45-2015 (N97D+F390D+L429M)	Wt	3879	6L	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 42	SEQ ID NO: 44	SEQ ID NO: 45
H1 A-Mich-45-2015 (K374E+F390D+L429M)	Wt	3878	6M	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 42	SEQ ID NO: 46	SEQ ID NO: 47
H1 A-Mich-45-2015 (N97D+K374E+F390D+L429M)	Wt	3881	6N	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 46	SEQ ID NO: 48	SEQ ID NO: 49
H1 A-Mass-06-2017 (F390D+L429M)	Wt	4091	6O	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 50	SEQ ID NO: 50	SEQ ID NO: 51
H1 A-Mass-06-2017 (N97D+F390D+L429M)	Wt	4093	6P	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 50	SEQ ID NO: 52	SEQ ID NO: 53
H1 A-Mass-06-2017 (K374E+F390D+L429M)	Wt	4092	6Q	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 50	SEQ ID NO: 54	SEQ ID NO: 55
H1 A-Mass-06-2017 (N97D+K374E+F390D+L429M)	Wt	4094	6R	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 54	SEQ ID NO: 56	SEQ ID NO: 57
H1 A-Costa Rica-0513-2016 (F390D+L429M)	Wt	4715	6S	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 58	SEQ ID NO: 58	SEQ ID NO: 59
H1 A-Costa Rica-0513-2016 (N97D+F390D+L429M)	Wt	4717	6T	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 58	SEQ ID NO: 60	SEQ ID NO: 61
H1 A-Costa Rica-0513-2016 (K374E+F390D+L429M)	Wt	4716	6U	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 58	SEQ ID NO: 62	SEQ ID NO: 63
H1 A-Costa Rica-0513-2016 (N97D+K374E+F390D+L429M)	Wt	4718	6V	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 62	SEQ ID NO: 64	SEQ ID NO: 65
H1 A-Hond-17734-16	Wt	3944	6W	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 9	SEQ ID NO: 10	SEQ ID NO: 11
H1 A-Hond-17734-16 (N97D)	Wt	3950	6X	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 66	SEQ ID NO: 14	SEQ ID NO: 9	SEQ ID NO: 67	SEQ ID NO: 68

Construct Name	TMCT	Construct #	Fig	Primer 1	Primer 2	Primer 3	Primer 4	Template for PCR	Resulting Gene	Resulting Protein
H1 A-Hond-17734-16 (K374E)	Wt	3948	6Y	SEQ ID NO: 13	SEQ ID NO: 69	SEQ ID NO: 70	SEQ ID NO: 14	SEQ ID NO: 9	SEQ ID NO: 71	SEQ ID NO: 72
H1 A-Hond-17734-16 (F390D)	Wt	3945	6Z	SEQ ID NO: 13	SEQ ID NO: 74	SEQ ID NO: 75	SEQ ID NO: 14	SEQ ID NO: 9	SEQ ID NO: 76	SEQ ID NO: 77
H1 A-Hond-17734-16 (L429M)	Wt	3949	6AA	SEQ ID NO: 13	SEQ ID NO: 77	SEQ ID NO: 78	SEQ ID NO: 14	SEQ ID NO: 9	SEQ ID NO: 79	SEQ ID NO: 80
H1 A-Hond-17734-16 (F390D+L429M)	Wt	3946	6BB	SEQ ID NO: 13	SEQ ID NO: 74	SEQ ID NO: 75	SEQ ID NO: 14	SEQ ID NO: 79	SEQ ID NO: 81	SEQ ID NO: 82
H1 A-Hond-17734-16 (N97D+F390D+L429M)	Wt	3951	6CC	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 66	SEQ ID NO: 14	SEQ ID NO: 81	SEQ ID NO: 83	SEQ ID NO: 84
H1 A-Darw-11-15	Wt	3984	6DD	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 11	SEQ ID NO: 11	SEQ ID NO: 12
H1 A-Darw-11-15 (N97D)	Wt	3990	6EE	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 11	SEQ ID NO: 85	SEQ ID NO: 86
H1 A-Darw-11-15 (K374E)	Wt	3988	6FF	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 87	SEQ ID NO: 14	SEQ ID NO: 11	SEQ ID NO: 88	SEQ ID NO: 89
H1 A-Darw-11-15 (F390D)	Wt	3985	6GG	SEQ ID NO: 13	SEQ ID NO: 74	SEQ ID NO: 75	SEQ ID NO: 14	SEQ ID NO: 11	SEQ ID NO: 90	SEQ ID NO: 91
H1 A-Darw-11-15 (L429M)	Wt	3989	6HH	SEQ ID NO: 13	SEQ ID NO: 77	SEQ ID NO: 78	SEQ ID NO: 14	SEQ ID NO: 11	SEQ ID NO: 92	SEQ ID NO: 93
H1 A-Darw-11-15 (F390D+L429M)	Wt	3986	6II	SEQ ID NO: 13	SEQ ID NO: 77	SEQ ID NO: 78	SEQ ID NO: 14	SEQ ID NO: 90	SEQ ID NO: 94	SEQ ID NO: 95
H1 A-Darw-11-15 (N97D+F390D+L429M)	Wt	3991	6JJ	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 94	SEQ ID NO: 96	SEQ ID NO: 97
H1 A-Mich-45-2015 (N380A)	Wt	3644	6KK	SEQ ID NO: 13	SEQ ID NO: 102	SEQ ID NO: 103	SEQ ID NO: 14	SEQ ID NO: 104	SEQ ID NO: 3	SEQ ID NO: 105
H1 A-Mich-45-2015 (F390D+N380A)	Wt	3704	6LL	SEQ ID NO: 13	SEQ ID NO: 106	SEQ ID NO: 103	SEQ ID NO: 14	SEQ ID NO: 35	SEQ ID NO: 107	SEQ ID NO: 108
H5 A-Indo-5-05	Wt	2295	7A	SEQ ID NO: 13	SEQ ID NO: 109	-	-	SEQ ID NO: 110	SEQ ID NO: 110	SEQ ID NO: 111
H5 A-Indo-5-05 (F393D)	Wt	3680	7B	SEQ ID NO: 13	SEQ ID NO: 112	SEQ ID NO: 113	SEQ ID NO: 109	SEQ ID NO: 110	SEQ ID NO: 114	SEQ ID NO: 115
H5 A-Egypt-N04915-14	Wt	3645	7C	SEQ ID NO: 13	SEQ ID NO: 116	-	-	SEQ ID NO: 117	SEQ ID NO: 117	SEQ ID NO: 118
H5 A-Egypt-N04915-14 (F392D)	Wt	3690	7D	SEQ ID NO: 13	SEQ ID NO: 119	SEQ ID NO: 120	SEQ ID NO: 116	SEQ ID NO: 117	SEQ ID NO: 121	SEQ ID NO: 122
A/Paris/1227/17 (F390D+L429M)	Wt	4765	6MM	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 123	SEQ ID NO: 127	SEQ ID NO: 1
A/Paris/1227/17 (K374E+F390D+L429M)	Wt	4766	6NN	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 123	SEQ ID NO: 127	SEQ ID NO: 1
A/Paris/1227/17 (N97D+F390D+L429M)	Wt	4767	6OO	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 123	SEQ ID NO: 127	SEQ ID NO: 1
A/Paris/1227/17 (N97D+K374E+F390D+L429M)	Wt	4768	6PP	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 127	SEQ ID NO: 127	SEQ ID NO: 1
A/Norway/2147/17 (F390D+L429M)	Wt	4775	6QQ	SEQ ID NO: 13	SEQ ID NO: 14	-	-	SEQ ID NO: 141	SEQ ID NO: 141	SEQ ID NO: 1
A/Norway/2147/17 (K374E+F390D+L429M)	Wt	4776	6RR	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 141	SEQ ID NO: 143	SEQ ID NO: 1

Construct Name	TMCT	Construct #	Fig	Primer 1	Primer 2	Primer 3	Primer 4	Template for PCR	Resulting Gene	Resulting Protein
A/Norway/2147/17 (N97D+F390D+L429M)	Wt	4777	6SS	SEQ ID NO: 13	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 14	SEQ ID NO: 141	SEQ ID NO: 145	SEQ ID NO: 146
A/Norway/2147/17 (N97D+K374E+F390D+L429M)	Wt	4778	6TT	SEQ ID NO: 13	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 14	SEQ ID NO: 145	SEQ ID NO: 147	SEQ ID NO: 148

Example 3: Hemagglutination Titer, Post-density Gradient Yields and Full Process Yields

[00338] A Summary of the measured Hemagglutination Titer is given in Table 5A. Hemagglutination Titer were measured as described in Example 2. The relative hemagglutination titer were obtained by comparing the hemagglutination titer of the mutated or modified HA protein to wildtype HA (Tables 5A).

[00339] A Summary of the measured Post-Density Gradient Yields is given in Table 5B . Post-Density Gradient Yields were measured as described in Example 2. Relative yields were obtained by comparing the HA0 band intensity from the mutated or modified HA protein to the HA0 band intensity of wildtype HA (Table 5B) .

[00340] A Summary of the measured Full Process Yield is given in Table 5C . Full Process Yield were obtained as described above in Example 2. Relative yields were obtained by comparing the protein yield from the mutated or modified HA protein to the protein yield of wildtype HA (Table 5C).

Table 5A: Summary of relative Hemagglutination Titer for H1. Numbering is in accordance with A/Michigan/45/15 HA.

	WT	N97D	K374E	N380A	F390D	L429M	N380A + L429M	N97D +K374E	F390D +L429M	N97D +F390D +L429M	K374E +F390D +L429M	N97D +K374E +F390D +L429M
H1 A/California/07/09	100%			120%	100-160%	100-120%			140-160%			
H1 A/Michigan/45/15	100%	400-1200%	400-1200%	20%	80-100%	400-600%	800%	1200%	400-1200%	800-2400%	2400-2600%	3200-3400%
H1 A/Massachusetts/06/17									100%	140%	150%	170%
H1 A/Costa Rica/0513/16									100%	200%	110%	240%
H1 A/Honduras/17734/16	100%	400%	200%		120-130%	500%			400%	500-700%		
H1 A/Darwin/11/15	100%	300%	400%		140-150%	600%			400%	1200%		
H1 A/Paris/1227/2017							117%			171%	127%	2
A/Norway/2147/2017							112%			200%	128%	2

Table 5B: Summary of relative Post-Density Gradient Yields for H1. Numbering is in accordance with A/Michigan/45/15 HA.

	WT	N380A	F390D	L429M
H1 A/California/07/09	100%	100%	157%	130%

Table 5C: Summary of relative Full Process Yield for H1. Numbering is in accordance with A/Michigan/45/15 HA.

	WT	F390D	L429M	F390D +L429M	N97D +F390D +L429M	K374E +F390D +L429M
H1 A/California/07/09	100%	226%				
H1 A/Michigan/45/15	100%	172%	260%	633%	647%	689%

Table 6 Summary of relative Hemagglutination Titer for H5. Numbering in accordance with H5 A/Indonesia/5/2005

	WT	N383A	F393D
H5 A/Indonesia/5/2005	100%	50%	2%
H5 A/Egypt/N04915/2014	100%	50%	1%

Example 4: Sequences

The following sequences were used (also see Table 4):

PDI-H1 Cal DNA (SEQ ID NO: 1)

5 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTTCCTTCTCAGATCTTC
GCTGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTAGAAAGACAAGCATAACGGGAACTATGCAAACTAAGA
GGGGTAGCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGCTCATGGTCTACATTGTGGAACACCTAGTTCAGACAATGGAACGTGTTAC
10 CCAGGAGATTTTCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTGCATTTTGAAAGGTTT
GAGATATTCCTCCAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGGCAGCATGTCTT
CATGCTGGAGCAAAAAGCTTCTACAAAAATTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTCAGCAAAATCCTACATTAATGATAAAGGAAAGAAAGTCTCTGCTGCTATGGGGCATTCCACCATCCATCT
ACTAGTGTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGTTCATCAAGATAC
15 AGCAAGAGTTTCAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCGCAATGGAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACCAAGTCCAGATTGC
AATACAACCTGTCAAACACCCAAGGGTCTATAAACACCAAGCTCCCATTTTTCAGAAATATACATCCGATC
ACAATTTGAAAAATGTCCAAATATGTAAGAACACAAAATGAGACTGGCCACAGGATTGAGGAATATC
20 CCGTCTATTCAATCTAGAGGACTATTGGGGCCATTGCCGGTTTCATTGAAGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATTCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAGATGCCATTGACGAGATTACTAACAAGTAAATCTGTTATTGAAAAGATGAATACACAGTTTC
ACAGCAGTAGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTTCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTATTGGAAAAATGAAAGAACCTTGGAC
25 TACCACGATTCAATGTGAAGAATTATATGAAAAGGTAAGAAGCCAGCTAAAAACAATGCCAAGGAA
ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATCTCAGAGGAAGCAAAATTAACAGAGAAGAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTACAGATTTTGGCGATCTATTCAACTGTCCGACGTTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Cal AA (SEQ ID NO: 2)

30 MAKNVAFGLLFSLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
GVAPLHLGKCNIAIWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELEQLSSVSSFEF
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLKKGNSYPKLSKSYINDKGKEVLVLWGIHPS
TSADQQSLYQNADAYVFVGSRSYSKKFKPEIAIRPKVRDQEGRMNYYWTLPVPGDKITFEATGNLVVPR
YAFAMERNAGSGIIISDTPVHDCNNTCQT PKGAINSTLFPQNIHPITIGKCPKYVKSTKLRLATGLRNI
35 PSIQSRGLFGAIAIGFIEGGWTGMVDGWYGHQNEQSGSYAADLKSTQNAIDEITNKNVSNVIEKMNTQF
TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLENERLTDYHDSNVKNLYEKVRSQKLNNAKE
IGNGCFEFYHKCDNTECMESVKNGTIDYDYPKYSEEAKLNRREEIDGVKLESTRYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI

PDI-H1 Mich DNA (SEQ ID NO: 3)

40 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGGAACTATGCAAACTAAGA
GGGGTAGCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
45 TCACTCTCCACAGCAAGTTCATGGTCTACATTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTGCATTTTGAAAGGTTT
GAGATATTCCTCCAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGGCAGCATGTCTT
CACGCTGGAGCAAAAAGCTTCTACAAAAATGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAATCCTACATTAATGATAAAGGAAAGAAAGTCTCTGCTGCTGTGGGGCATTCCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
50 AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACCAAGTCCAGATTGC
AATACAACCTGTGAGACACCCGAGGGTGTCTATAAACACCAAGCTCCCATTTTTCAGAAATATACATCCGATC
ACAATTTGAAAAATGTCCAAAGTATGTAAGAACACAAAATGAGACTGGCCACAGGATTGAGGAATGTT
55 CCGTCTATTCAATCTAGAGGCTATTTCGGGGCCATTGCCGGTTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATTCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACAAGATTACTAACAAGTAAATCTGTTATTGAAAAGATGAATACACAGTTTC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTTCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTATTGGAAAAATGAAAGAACCTTGGAC
60 TATCAGATTCAAATGTGAAGAATTGTATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATCTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTACAGATTTTGGCGATCTATTCAACTGTCCGACGTTTTCATTGGTACTGGTAGTC

TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mich AA (SEQ ID NO: 4)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 5 GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSFFERF
 EIFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQF
 10 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNRKIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGLQCRICI

PDI-H1 Mass DNA (SEQ ID NO: 5)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCCTTCTCAGATCTTC
 15 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGAAAAACATTGCAAACTAAGA
 GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGATCATGGTCTACATTGTGGAACATCTAATTGAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 20 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAGGTGTAACGGCAGCATGTCTCT
 CACGCTGGAGCAAAAAGCTTCTACAAAACCTGATATGGCTAGTTAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCC TACATTATGATAAGGGGAAAGAAGTCCCTCGTGTGTGGGGCATTCACCATCCATCT
 ACTACTGCTGACCAACAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGACATCAAGATAC
 AGCAAGAAGTTC AAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 25 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGCAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
 AATAACAATTTGT CAGACACCCGAGGGTGCTATAAACACCAAGCCTCCCATTTGAGAATGTGCATCCGAT
 ACAATTGGAATAATGTCCAAGTATGTA AAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATATCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 30 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTCAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAGAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTGGGTAAGAGTTCAACCACCTGGA AAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTCCTCGGACATTTGGACTTACATGCCGAACCTGTTGGTTCTATTGGGAAATGAAAGAACTTTGGAC
 35 TATCACGATTCAAAATGTGAAGAAC TTGTATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTAC CAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mass AA (SEQ ID NO: 6)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 40 GVAPLHLGKCNIAWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSFFERF
 EIFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNVHPITIGKCPKYVSTKLRLATGLRNV
 45 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNRKIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGLQCRICI

PDI-H1 CostaR DNA (SEQ ID NO: 7)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCCTTCTCAGATCTTC
 50 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGAAAAACATTGCAAACTAAGA
 GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGTTTCATGGTCTACATTGTGGAACATCTAATTGAGACAATGGAACGTGTTAC
 55 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAGGTGTAACGGCAGCATGTCTCT
 CACGCTGGAGCAAAAAGCTTCTACAAAACCTGATATGGCTAGTTAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCC TACGTGAATGATAAAGGGAAAGAAGTCCCTCGTGTGTGGGGCATTCACCATCCACCC
 ACTACTGCTGACCAACAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGTAC
 60 AGCAAGAAGTTC AAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGCAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
 AATAACAATTTGT CAGACACCCGAGGGTGCTATAAACACCAAGCCTCCCATTTGAGAATATACATCCGATC
 ACAATTGGAATAATGTCCAAGTATGTA AAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATATCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG

GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTCAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAAAGATTACTAACAAAGTAAATCTGTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 5 GGTTCCTCGGACATTTGGACTTACAATGCCGAACCTGTGGTTCTATTGGAAAATGAAAGAACTTTGGAC
 TATCACGATTCAAATGTGAAGAACTTGTATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 ATTGGAACGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 CostaR AA (SEQ ID NO: 8)

MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERF
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYVNDKGKEVLVLWGIHHP
 10 TTADQQSILYQNADAYVFGVTSKYSKKFKPEIATRPKVRDQEGRMNYWTVLVEPGDKITFEATGNLVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKRLRLATGLRNV
 15 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKINKNVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNCGFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLLVLVV
 SLGAISFWMCSNGSLQCRICI

PDI-H1 Hond DNA (SEQ ID NO: 9)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCATAACGGGAACTATGCAAACTAAGA
 20 GGGGTACCCCATTTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 CCATCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAGTTCAGACAATGGAACGTGTTC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGGGAGCAATTGAGCTCAGTGTCTCATTTTGAGAGATT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAAACAGCAGCATGTCT
 CACGCTGGGGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCCAATCCTACATTAATGATAAAGGAAAAAGTCTCTGTGCTGTGGGGCATTCCACATCCATCT
 30 ACTACTGCTGACCAACAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACAGTCCAGATTGC
 AATACAACCTGTGAGACACCCGAGGGTGTCTATAAACACCAGCCTCCCATTTTTCAGAAATATACATCCGATC
 35 ACAATTTGGGAAATGTCCAAAGTATGTAAGAACACAAATTTGAGACTGGCCACAGGATTGAGGAATGT
 CCATCTATTCAATCTAGAGGCCATTTCCGGGGCGATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCCAGAAGAGC
 ACACAAAGTGCCATTGACAAAATTACTAACAAAGTAAATCTGTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTTGGAAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
 40 GGTTCCTCGGACATTTGGACTTACAATGCCGAACCTGCTGGTTCTATTGAAAAATGAAAGAACTTTGGAC
 TACCAGCTCAATGTGAAGAACTTGTATGAAAAGGTAAGAAACCAGTTAAAAAACAACTGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTTGGTACTGGTAGTC
 45 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Hond AA (SEQ ID NO: 10)

MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVPPLHLGKCNIAGWILGNPECEPLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERF
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLSQSYINDKGKEVLVLWGIHHP
 50 TTADQQSILYQNADAYVFGVTSRYSKKFKPEIATRPKVRDQEGRMNYWTVLVEPGDKITFEATGNLVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKRLRLATGLRNV
 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQSAIDKINKNVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNCGFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLLVLVV
 55 SLGAISFWMCSNGSLQCRICI

PDI-H1 Darw DNA (SEQ ID NO: 11)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCACAACGGGAACTATGCAAACTAAGA
 60 GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAACCCAGAGTGTGAA
 TCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAGTTCAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTCATTTTGAAGATTT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAAACGGCAGCATGTCT
 CACGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAACTAAAAAAGGAAATTCATACCCAAAG

CTCAGCCAATCCTACATTAATGATAAAGGGAAAGAAATCCTCGTGCTGTGGGGCATTACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCAGGTGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
 5 TATGCATTCAATGGAAGAAATGCTGGATCTGGTATTATCATTTCAGATACACCAAGTCCACGATTGC
 AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTCAGAATATACATCCGATC
 ACAATTGGAAGAAATGTCCAAAGTATGTGAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTC
 CCATCTATTCAATCTAGAGGCCATTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGGTATGCAGCCGACCTGAAGAGC
 10 ACACAAAATGCCATTGACAAAATTACTAACAAAGTAAATTCTGTATTGAAAAGATGAATACACAGTTT
 ACAGCAGTGGGTAAAGAGTTCAACCACTTGGAAAAAAGAAATAGAGAATTTAAATAAAAAAGTTGATGAT
 GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGCTGGTTCTATTGGAAAATGAAAGAACCTTTGGAC
 TACCACGATTCAATGTGAAGAACCTGTATGAAAAGGTAAGAAACCAGTTAAAAAACAAATGCCAAGGAA
 ATTGGAATGGTTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 15 ACTTATGACTACCCAAAATCTCAGGGGAAGCAAAATTAACAGAGAAAAAATAGAAGGGGTAAAGCTG
 GAATCAACAAGAATTTACCAATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTAA
PDI-H1 Darw AA (SEQ ID NO: 12)
 MAKNVAI FGLLFSLVLVPSQIFADTL CIGYHANNSTDTVDTVLEKNVTVTHSVNILEDKHNKGLCKLR
 20 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVS SFERF
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKS FYKNLIWLTKKGN SYPKLSQSYINDKKEILVLWGIIHPS
 TTADQQSLYQNADAYVVFVGTSTRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTEMRNAGSGIILSDTPVHDCNTTCQTPEGAINTSLFFQNIHPITIGKC PKYVKSTKLRATGLRNV
 25 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGSYAADLKSTQNAIDKITNKVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWITYNAELLVLENERLTDYHDSNVKNLYEVRNQLKNNAKE
 IGNGCFEYHKCDNTCMESVKNGTYDYPKYSGEAKLNREKIEGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCNSGLQCRICI

IF-CPMV(fl5'UTR)_SpPDI.c (SEQ ID NO: 13)
 30 TCGTGCTTCGGCACCAAGTACAATGGCGAAAAACGTTGCGATTTTCGGCT

IF-H1cTMCT.S1-4r (SEQ ID NO: 14)
 ACTAAAGAAAATAGGCCTTTAAATACATATTCTACACTGTAGAGAC

H1Cal(F390D).r (SEQ ID NO: 15)
 CTCTTTACCTACTGCTGTGCTCTGTGATTTCATCTTTTCAATAACAGAATTTA

H1Cal(F390D).c (SEQ ID NO: 16)
 35 TTATTGAAAAGATGAATACACAGGACACAGCAGTAGGTAAAGAGTTCAAC

PDI-H1 Cal-F390D DNA sequence (SEQ ID NO: 17)
 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTC AACAGACACTGTAGACACAGTACTAGAAAAAG
 40 AATGTAACAGTAACACACTCTGTAACTTCTAGAAAGACAAGCATAACGGGAACTATGCAAACTAAGA
 GGGGTAGCCCCCATTTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGA
 TCACTCTCCACAGCAAGCTCATGGTCTTACATTGTGGAACACCTAGTTAGACAAATGGAACGTGTTAC
 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTCT
 45 CATGCTGGAGCAAAAAGCTTCTACAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCAAATCCTACATTAATGATAAAGGGAAAGAAAGTCCCTCGTGCTATGGGGCATTACCATCCATCT
 ACTAGTGCTGACCAACAAAGTCTCTATCAGAAATGCAGATGCATATGTTTTTGTGGGGTCATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
 50 TATGCATTCGCAATGGAAAGAAATGCTGGATCTGGTATTATCATTTCAGATACACCAAGTCCACGATTGC
 AATACAACCTTGTCAAACACCCAAGGGTGCTATAAACACCAGCCTCCCATTTCAGAATATACATCCGATC
 ACAATTGGAAAATGTCCAAAATATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATATC
 CCGTCTATTCAATCTAGAGGACTATTGGGGCCATTGCCGGTTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 55 ACACAGAATGCCATTGACGAGATTACTAACAAAGTAAATTCTGTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTAGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATTTAAATAAAAAAGTTGATGAT
 GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTATTGGAAAATGAAAGAATTTGGAC
 TACCACGATTCAATGTGAAGAATTTATATGAAAAGGTAAGAAGCCAGCTAAAAACAATGCCAAGGAA
 ATTGGAACGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAATGTCAAAAATGGG
 60 ACTTATGACTACCCAAAATCTCAGAGGAAGCAAAATTAACAGAGAAGAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTAA
PDI-H1 Cal-F390D AA sequence (SEQ ID NO: 18)

MAKNVAIFGLLFLSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHP
 TSADQQSLYQNADAYVFGSSRYSKKFKPEIAIRPKVRDQEGRMNYWTVLVEPGDKITFEATGNLVVPR
 YAFAMERNAGSGIIISDTPVHDCNTTCQT PKGAINSTLFPQNIHPITIGKCPKYVKSTKRLRLATGLRNI
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSYAADLKSTQNAIDEITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLDYHDSNVKNLYEKVRSQKLNNAKE
 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKNLREEIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGSLQCRICI

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10 **H1Cal(L429M).r (SEQ ID NO: 19)**
 GTTCTTTTCATTTTCCATTAGAACCACAGTTCGGCATTGTAAGTCCAA
H1Cal(L429M).c (SEQ ID NO: 20)
 CGAACTGTTGGTCTAATGGAAATGAAAGAACTTTGGACTACCACGA

15 **PDI-H1 Cal-L429M DNA sequence (SEQ ID NO: 21)**
 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCATAACGGGAACTATGCAAACCTAAGA
 GGGGTAGCCCATTCGATTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGCTCATGGTCTTACATTGTGGAACACCTAGTTCAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAGGTGTAACGGCAGCATGTCCT
 CATGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCAAAATCCACATTAATGATAAAGGAAAGAAAGTCTCGTGTATGGGGCATTACCATCCATCT
 ACTAGTGTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGTCATCAAGATAC
 AGCAAGAGTTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTTCGCAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACAGTCCACGATTGCT
 AATACAACCTTGTCAAACACCCAAGGTGCTATAAACACACAGCTCCCATTTAGAAATATACATCCGATC
 ACAATTTGGAATATGTCCAAATATGTAAAAAGCACAAATTTAGACTGGCCACAGGATTGAGGAATATC
 CCGTCTATTCAATCTAGAGGACTATTTGGGGCCATTGCCGGTTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATTCAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAGATGCCATTGACGAGATTACTAACAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTAGGTAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
 GGTTTTCTGGACATTTGGACTTACAAATGCCGAACCTGTTGGTTCTAATGGAAAAATGAAAGAACTTTGGAC
 TACCACGATTCAAATGTGAAGAACTTATATGAAAAGGTAAGAAGCCAGCTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCCGATAACACGTGCATGGAAAGTGTCAAATATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAGAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATGGGTACTGGTAGTC
 TCCCTGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

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40 **PDI-H1 Cal-L429M AA sequence (SEQ ID NO: 22)**
 MAKNVAIFGLLFLSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHP
 TSADQQSLYQNADAYVFGSSRYSKKFKPEIAIRPKVRDQEGRMNYWTVLVEPGDKITFEATGNLVVPR
 YAFAMERNAGSGIIISDTPVHDCNTTCQT PKGAINSTLFPQNIHPITIGKCPKYVKSTKRLRLATGLRNI
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSYAADLKSTQNAIDEITNKVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLDYHDSNVKNLYEKVRSQKLNNAKE
 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKNLREEIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGSLQCRICI*

45

50 **PDI-H1 Cal-F390D+L429M DNA sequence (SEQ ID NO: 23)**
 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTAACTTCTAGAAGACAAGCATAACGGGAACTATGCAAACCTAAGA
 GGGGTAGCCCATTCGATTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGCTCATGGTCTTACATTGTGGAACACCTAGTTCAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAGGTGTAACGGCAGCATGTCCT
 CATGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCAAAATCCACATTAATGATAAAGGAAAGAAAGTCTCGTGTATGGGGCATTACCATCCATCT
 ACTAGTGTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGTCATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTTCGCAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACAGTCCACGATTGC
 AATACAACCTTGTCAAACACCCAAGGTGCTATAAACACACAGCTCCCATTTAGAAATATACATCCGATC

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ACAATTGGAAAAATGTCCAAAATATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATATC
 CCGTCTATTTCAATCTAGAGGACTATTTGGGGCCATTGCGGGTTTCATTGAAGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTCAGGATATGCAGCCGACCTGAAGAGC
 ACACAGAATGCCATTGACGAGATTACTAACAAAGTAAATTTCTGTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTAGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
 5 GGTTCCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACCTTGGAC
 TACCACGATTCAAAATGTGAAGAACCTTATATGAAAAGGTAAAGAAGCCAGCTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAAACAGAGAAGAAATAGATGGGGTAAAGCTG
 10 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Cal-F390D+L429M AA sequence (SEQ ID NO: 24)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLKL
 GVAPLHLGKCNIAIWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSFFERF
 EIFFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHP
 15 TSADQQSLYQNADAYVVFVGSRRYSKKFKPEIAIRPKVRDQEGRMNYWTLVEPGDKITFEATGNLVVPR
 YAFAMERNAGSGIIISDTPVHDCNNTCQT PKGAINSLPFFQNIHPITIGKCPKYVKSTKLRLATGLRNI
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSYAADLKSTQNAIDETITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLDYHDSNVKNLYEKVRSQKLKNNAKE
 20 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNRREDIGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGSLQCRICI*

H1Mich(N97D).r (SEQ ID NO: 25)

TAGCTCCTCATAATCGATGAAATCTCCTGGGTAACACGTTCCATTGTCTGAA

H1Mich(N97D).c (SEQ ID NO: 26)

25 CCCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAG

PDI-H1 Mich-N97D DNA (SEQ ID NO: 27)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACATATGCAAACTAAGA
 30 GGGGTAGCCCATTTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGTTTCATGGTCTTACATTGTGGAACATCTAATTGAGCAATGGAACGTTGAC
 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTGATCATTTTAAAAGGTTT
 GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGGCAGCATGTCCCT
 CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 35 CTTAACCAATCCTTACATTAATGATAAAGGGAAGAAAGTCCCTCGTGTGTGGGGCAATCCACCATCTAT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 AGCAAGAGTTTCAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGCAAGCAACTGGAATCTAGTGGTACCGAGA
 40 TATGCAATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACAGTCCACGATTG
 AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTTCAGAATATACATCCGATC
 ACAATTTGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCGGGCTTCATTGAAGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTCAGGATATGCAGCCGACCTGAAGAGC
 45 ACACAAAATGCCATTGACAAAGATTACTAACAAAGTAAATTTCTGTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTCCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTATTGGAAAATGAAAGAACCTTGGAC
 TATCACGATTCAAAATGTGAAGAACCTGTATGAAAAGTAAGAACCAGTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAAACAGAGAAAAAATAGATGGGGTAAAGCTG
 50 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mich-N97D AA (SEQ ID NO: 28)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLKL
 GVAPLHLGKCNIAIWILGNPECESLSTASSWSYIVETNSDNGTCYPGDFIDYEELREQLSSVSFFERF
 55 EIFFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYWTLVEPGDKITFEATGNLVVPR
 YAFAMERNAGSGIIISDTPVHDCNNTCQTPEGAINSLPFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSYAADLKSTQNAIDKITNKVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLDYHDSNVKNLYEKVRNQLKNNAKE
 60 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNRKIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGSLQCRICI*

H1Mich(K374E).r (SEQ ID NO: 29)

TTTGTTAGTAATCTCGTCAATGGCATTTTGTGTGCTCTTCAGGTCGGCTGCATATCC

H1Mich(K374E).c (SEQ ID NO: 30)

ACAAAAATGCCATTGACGAGATTACTAACAAAGTAAATCTGTTATTGAAA

PDI-H1 Mich-K374E DNA (SEQ ID NO: 31)

5 ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTTGTGTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAAATCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACCTAAGA
GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGTTCATGGTCTTACATTTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC
10 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGGTTT
GAGATATTTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
CAGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCCAAAG
CTTAACCAATCCTACATTAATGATAAAGGGAAGAAGTCTCTGCTGTGGGGCATTACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
15 TACTGGACACTAGTAGAGCCGGGAGACAAAAAACAATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACCAAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACACAGCCTCCCATTTTCAGAATATACATCCGATC
ACAATTTGGAAGTGTCCAAAGTATGTAAGAACACAAAAATGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCTTATTCGGGGCCATTGCGGGCTTCATTGAAGGGGGTGGACAGGGATG
20 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGTTT
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTATTGGAAAATGAAAGAAGTTTGGAC
TATCAGGATTCAAATGTGAAGAATTTGTATGAAAAAGTAAGAAACAGTTAAAAAACAATGCCAAGGAA
25 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mich-K374E AA (SEQ ID NO: 32)

30 MAKNVAI FGLLFSLVLVPSQIFADTL CIGYHANNSTDTVDTVLEKNVTVTHSVNLEEDKHNGKCLKLR
GVAPLHLGKCNIA GWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
E FPKTSSWPNHDSNKGVTAAACPHAGAKS FYKNLIWL VKKGNS YPKLNQSYINDKKEVLVLWGIIHPS
TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATR PKVRDQEGRMNYWTVLVEPGDKITFEATGNLVVPR
YAFTMERNAGSGIILSDTPVHDCNTTCQTPEGAINSTLFPQNIHPITIGKCPKYVKSTKLRLATGLRNV
35 PSIQSRGLFGAIAFGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDEITNKVNSVIEKMNTQF
TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLENERLTDYHDSNVKNLYEVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

H1Mich(F390D).r (SEQ ID NO: 33)

CCCCTGCTGTGCTCTGTGTATTTCATCTTTTCAATAACAGAATTTACTT

H1Mich(F390D).c (SEQ ID NO: 34)

AAAGATGAATACACAGGACACAGCAGTGGGTAAAGAGTTCAACCACCTG

PDI-H1 Mich-F390D DNA (SEQ ID NO: 35)

45 ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTTGTGTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAAATCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACCTAAGA
GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGTTCATGGTCTTACATTTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC
50 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGGTTT
GAGATATTTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
CAGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCCAAAG
CTTAACCAATCCTACATTAATGATAAAGGGAAGAAGTCTCTGCTGTGGGGCATTACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
55 TACTGGACACTAGTAGAGCCGGGAGACAAAAAACAATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACCAAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACACAGCCTCCCATTTTCAGAATATACATCCGATC
ACAATTTGGAAGTGTCCAAAGTATGTAAGAACACAAAAATGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCTTATTCGGGGCCATTGCGGGCTTCATTGAAGGGGGTGGACAGGGATG
60 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTATTGGAAAATGAAAGAAGCTTTGGAC
TATCAGGATTCAAATGTGAAGAATTTGTATGAAAAAGTAAGAAACAGTTAAAAAACAATGCCAAGGAA

ATTGGAACGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTTCAACTGTCCGAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAAATATGTATTTAA

5 **PDI-H1 Mich-F390D AA (SEQ ID NO: 36)**

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLDKHNGKLCCLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVFGTSRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 10 YAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKNRNLKNNAKE
 IGNGCFEYFHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGSLQCRICI*

15 **H1Mich(L429M).c (SEQ ID NO: 37)**

CGAACTGTTGGTTCTAATGGAAAATGAAAGAACTTTGGACTATCACGATTCAA

PDI-H1 Mich-L429M DNA (SEQ ID NO: 38)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 20 AATGTAACAGTAACACACTCTGTAAACCTTCTGGAAGACAAGCATAACGGAAAACATGCAAACTAAGA
 GGGGTAGCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACCTCCACAGCAAGTTCATGGTCTTACATTTGTGGAACATCTAATTGAGACAAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATTTGAAAGGTTT
 25 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAGGTGTAACGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACATTAATGATAAAGGGAAGAAGTCTCGTGTGTGGGGCATTCCACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCAGAGA
 30 TATGCATTACAAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACAGTCCACGATTGC
 AATACAACTTGTGAGACACCCGAGGTGCTATAAACACCCAGCCTCCCATTTGAGATATACATCCGATC
 ACAATTGGAAAATGTCCAAAGTATGTA AAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 35 ACACAAAATGCCATTGACAAAGATTACTAACAAAGTAAATCTGTTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTTCTCGGACATTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGA AAAATGAAAGAACTTTGGAC
 TATCAGGATTCAATGTGAGAAGCTTGATGAAAAAGTAAGAAACCAAGTTAAAAACCAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 40 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCCGAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAAATATGTATTTAA

PDI-H1 Mich-L429M AA (SEQ ID NO: 39)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLDKHNGKLCCLR
 45 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVFGTSRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 50 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKNRNLKNNAKE
 IGNGCFEYFHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Mich-N97D+K374E DNA (SEQ ID NO: 40)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTTGTGTGGTTCCTTCTCAGATCTTC
 55 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 AATGTAACAGTAACACACTCTGTAAACCTTCTGGAAGACAAGCATAACGGAAAACATGCAAACTAAGA
 GGGGTAGCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACCTCCACAGCAAGTTCATGGTCTTACATTTGTGGAACATCTAATTGAGACAAATGGAACGTGTTAC
 60 CCAGGAGATTTATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGGTTT
 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAGGTGTAACGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACATTAATGATAAAGGGAAGAAGTCTCGTGTGTGGGGCATTCCACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGAGAATGAACATAT

TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCTGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTACACAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACCACTCCACGATTGC
 AATACAACCTTGTGAGACACCCGAGGGTCTATAAACACCAGCCTCCCATTTAGAAATATACATCCGATC
 ACAAATGGAAGATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 5 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTCTGTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 10 GGTTCCTGGACATTTGGACTTACAATGCCGAACTGTTGGTTCTATTGGAAAATGAAAGAACTTTGGAC
 TATCAGGATTCAAATGTGAAGAACCTGTATGAAAAAGTAAGAAACCACTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
 15 **PDI-H1 Mich-N97D+K374E AA (SEQ ID NO: 41)**
 MAKNVAFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVSSFERE
 EIFPKTSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKNSYPKLNQSYINDKKEVLVLWGIIHPS
 TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 20 YAFTEMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDEITKNVNSVIEKMNTQF
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNCGFEFYHKCDNTCMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*
 25 **PDI-H1 Mich-F390D+L429M DNA (SEQ ID NO: 42)**
 ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACATATGCAAACTAAGA
 GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCGGAATCCAGAGTGTGAA
 30 TCACTCTCCACAGCAAGTTCATGGTCTACATTTGTGGAACATCTAATTAGACAAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTGCATCATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAGGTGTAACGGCAGCATGTCCT
 CACGCTGGAGCAAAAAGCTTCTACAAAAAATTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCATCAATTATGATAAAGGGAAGAAGTCTCGTGCTGTGGGGCATTACCATCCATCT
 35 ACTACTGCTGACCAACAAAGTCTCTATCAGAAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTACAAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACCACTCCACGATTGC
 AATACAACCTTGTGAGACACCCGAGGGTCTATAAACACCAGCCTCCCATTTAGAAATATACATCCGATC
 40 ACAATTGGAAGATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCTTATTCGGGGCCATTGCGGGCTTCATTGAAGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAGATTACTAACAAAGTAAATTCTGTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 45 GGTTCCTGGACATTTGGACTTACAATGCCGAACTGTTGGTTCTAATGGAAAATGAAAGAATTTGGAC
 TATCAGGATTCAAATGTGAAGAACCTGTATGAAAAAGTAAGAAACCACTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 50 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
PDI-H1 Mich-F390D+L429M AA (SEQ ID NO: 43)
 MAKNVAFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
 EIFPKTSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKNSYPKLNQSYINDKKEVLVLWGIIHPS
 55 TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTEMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITKNVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNCGFEFYHKCDNTCMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 60 SLGAISFWMCSNGSLQCRICI*
PDI-H1 Mich-N97D+F390D+L429M DNA (SEQ ID NO: 44)
 ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACATATGCAAACTAAGA

GGGGTAGCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGTTCATGGTCTACATTTGTGGAAACATCTAATTGAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTGCATCATTTGAAAGGTTT
5 GAGATATTTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAATCCTACATTAATGATAAAGGGAAGAAGTCTCTGCTGTGTGGGGCATTACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
AGCAAGAGTTTCAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
10 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTGAGAATATACATCCGATC
ACAATTTGGAATGTCCAAAGTATGTAAGGACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCGGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
15 ACACAAAATGCCATTGACAGATTACTAACAAGTAAATTTCTGTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACCTTTGGAC
TATCAGGATTCAAAATGTGAAGAATTTGTATGAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
20 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mich-N97D+F390D+L429M AA (SEQ ID NO: 45)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGLCKLKR
25 GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVSFFERF
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKGNSTPKLNQSYINDKGKEVLVLWGIHHP
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIATRPKVRDQEGRMNYWTVLVEPGDKITFEATGNLVVPR
YAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
30 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGSYAADLKSTQNAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEYFHKCDNTCMESVKNGTDYDPKYSEEAKLNRKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGLQCRICI*

PDI-H1 Mich-K374E+F390D+L429M DNA (SEQ ID NO: 46)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTTCCTTCTCAGATCTTC
35 GCGGACACATTATGTATAGGTTATCATGCGAACAAATTCACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
GGGGTAGCCCATTTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGTTCATGGTCTACATTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTGCATCATTTGAAAGGTTT
40 GAGATATTTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAATCCTACATTAATGATAAAGGGAAGAAGTCTCTGCTGTGTGGGGCATTACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
45 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTGAGAATATACATCCGATC
ACAATTTGAAAAATGTCCAAAGTATGTAAGGACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCGGGCTTCATTGAAGGGGGGTGGACAGGGATG
50 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAGTAAATTTCTGTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAATTTGGAC
TATCAGGATTCAAATGTGAAGAACTTGATGAAAAAGTAAGAAACCAAGTTAAAAAACATGCCAAGGAA
55 ATTTGGAACCGGCTGCTTTGAATTTTACCACAAATGCCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mich-K374E+F390D+L429M AA (SEQ ID NO: 47)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGLCKLKR
60 GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSFFERF
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKGNSTPKLNQSYINDKGKEVLVLWGIHHP
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIATRPKVRDQEGRMNYWTVLVEPGDKITFEATGNLVVPR
YAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
65 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGSYAADLKSTQNAIDEITNKVNSVIEKMNTQD

TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEYHKCDNTCMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Mich-N97D+K374E+F390D+L429M DNA (SEQ ID NO: 48)

5 ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAAATTCACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAACATATGCAAACTAAGA
 GGGGTAGCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGTTCATGGTCTACATTTGTGGAACATCTAATTCAGACAAATGGAACGTGTTAC
 10 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTCT
 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACATTAATGATAAAGGGAAAGAAGTCTCGTGCTGTGGGGCATTACCATCCATCT
 15 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAAGATACACAGTCCACGATTGC
 AATAACAATTTGTAGAGCCCGAGGGTGCTATAAACACACAGCCTCCCATTTCAAGATATACATCCGATC
 20 ACAATTGGAAGATGTCCAAAGTATGTAAGAACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATCTGTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 25 GGTTCCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAGATGAAAGAAGTTTGGAC
 TATCACGATTCAATGTGAAGAAGTTGATGAAAAAGTAAGAAACAGTTAAAAAACAAATGCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATCTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTAA

PDI-H1 Mich-N97D+K374E+F390D+L429M AA (SEQ ID NO: 49)

30 MAKNVAI FGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVSSFERE
 ELFPKTSWPNHDSNKGVTAAACPHAGAKSFYKNLIWL VKKGN SYPKLNQSYINDKAEVLVLWGIIHPS
 TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 35 YAFTEMRNAGSGIILSDTPVHDCNTTCQTPEGAINTSLFFQNIHPITIGKCPKYVSTKLRLATGLRNV
 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDEITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEYHKCDNTCMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Mass-F390D+L429M DNA (SEQ ID NO: 50)

40 ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAAATTCACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAACATATGCAAACTAAGA
 GGGGTAGCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 45 TCACTCTCCACAGCAAGATCATGGTCTACATTTGTGGAACATCTAATTCAGACAAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTCT
 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACATTAATGATAAAGGGAAAGAAGTCTCGTGCTGTGGGGCATTACCATCCATCT
 50 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAAGATACACAGTCCACGATTGC
 AATAACAATTTGTAGAGCCCGAGGGTGCTATAAACACACAGCCTCCCATTTCAAGATGTGCATCCGATC
 55 ACAATTGGAAGATGTCCAAAGTATGTAAGAACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAGATTACTAACAAAGTAAATCTGTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 60 GGTTCCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAGATGAAAGAAGTTTGGAC
 TATCACGATTCAATGTGAAGAAGTTGATGAAAAAGTAAGAAACAGTTAAAAAACAAATGCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATCTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 65 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTAA

PDI-H1 Mass-F390D+L429M AA (SEQ ID NO: 51)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSFFERF
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 5 TTADQQSLYQNADAYVFGVTSRYSKKFKPEIATRPKVRDQEGRMNYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNVHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 10 IGNGCFEYFHKCDNTCMESVKNGTYDYPKYSEEAFLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Mass-N97D+F390D+L429M DNA (SEQ ID NO: 52)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
 15 GGGGTAGCCCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGA
 TCACTCTCCACAGCAAGATCATGGTCTACATTGTGGAAACATCTAATTCAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTACCGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 20 CTTAACCAATCC TACATTAATGATAAAGGGAAAGAAGTCTCTGCTGTGGGGCATTACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAAGATACACAGTCCACGATTGC
 25 AATACAATCTGTGAGACACCCGAGGGTGTCTATAAACACACAGCTCCCATTTCAAGATGTGCATCCGATC
 ACAATTTGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAAGATTACTAACAAGTAAATCTGTTATTGAAAAGATGAATACACAGGAC
 30 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTCCTGGACATTGGAATTACAAATGCCGAACCTGTTGGTTCTAATGGAATGAAAGAACTTTGGAC
 TATCACGATTCAATGTGAAGAACTGTGATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
 ATTTGGAACCGCTGCTTTGAATTTTACCAAAATGCCGATAACAGTGCATGGAAGTGTCAAAAATGGG
 35 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mass-N97D+F390D+L429M AA (SEQ ID NO: 53)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVSFFERF
 40 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVFGVTSRYSKKFKPEIATRPKVRDQEGRMNYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNVHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 45 IGNGCFEYFHKCDNTCMESVKNGTYDYPKYSEEAFLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Mass-K374E+F390D+L429M DNA (SEQ ID NO: 54)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 50 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
 GGGGTAGCCCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGATCATGGTCTACATTGTGGAAACATCTAATTCAGACAATGGAACGTGTTAC
 CGAGGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTACCGGCAGCATGTCTT
 55 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCC TACATTAATGATAAAGGGAAAGAAGTCTCTGCTGTGGGGCATTACCATCCATCT
 ACTACTGCTGACCAACAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 60 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAAGATACACAGTCCACGATTGC
 AATACAATCTGTGAGACACCCGAGGGTGTATAAACACACAGCTCCCATTTCAAGATGTGCATCCGATC
 ACAATTTGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC

ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTTCCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACTTTGGAC
 TATCACGATTCAAATGTGAAGAACCTGTATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 5 ATTGGAACCGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTAA
PDI-H1 Mass-K374E+F390D+L429M AA (SEQ ID NO: 55)
 MAKNVAFGLLFSLLVLPVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLDKHNGKLCCLR
 10 GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTEMERNAGSGIISDTPVHDCNTCQTPEGAINTSLPFQNVHPITIGKCPKYVSTKLRLATGLRNV
 15 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDEITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Mass-N97D+K374E+F390D+L429M DNA (SEQ ID NO: 56)
 20 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTTCTTGTGTGGTTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACATGCAAACTAAGA
 GGGGTAGCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACCTCTCCACAGCAAGATCATGGTCTTACATTGTGGAACATCTAATTGAGACAATGGAACGTGTTAC
 25 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCT
 CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACATTATGATAAAGGGAAAGAAGTCCCTCGTGCTGTGGGGCATTACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
 30 AGCAAGAAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAAGAAATGCTGGATCTGGTATATCATTTTTCAGATACACAGTCCACGATTGC
 AATACAACTTGTGAGACACCCGAGGGTGCTATAAACACCAAGCCTCCATTTCAGAATGTGCATCCGATC
 35 ACAATTGGAAAATGTCCAAAGTATGTAAAAAGCACAAAATGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATATTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACGAGATTACTAACAAGTAAATTCGTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 40 GGTTTCCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACTTTGGAC
 TATCACGATTCAAATGTGAAGAACTTGATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 ATTGAAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 45 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTAA

PDI-H1 Mass-N97D+K374E+F390D+L429M AA (SEQ ID NO: 57)
 MAKNVAFGLLFSLLVLPVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLDKHNGKLCCLR
 50 GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 YAFTMERNAGSGIISDTPVHDCNTCQTPEGAINTSLPFQNVHPITIGKCPKYVSTKLRLATGLRNV
 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDEITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLV
 55 SLGAISFWMCSNGSLQCRICI*

PDI-H1 CR-F390D+L429M DNA (SEQ ID NO: 58)
 60 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTTCTTGTGTGGTTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACATGCAAACTAAGA
 GGGGTAGCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACCTCTCCACAGCAAGTTTCATGGTCTTACATTGTGGAACATCTAATTGAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACGTGAATGATAAAGGGAAAGAAGTCCCTCGTGCTGTGGGGCATTACCATCCACCC

ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAAGTAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCTGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTTCACAATGGAAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACCAGTCCACGATTGC
 5 AATACAACCTTGT CAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTTCAGAATATACATCCGATC
 ACAATTTGGAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 10 ACACAAAATGCCATTGACAAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACCTTTGGAC
 TATCACGATTCAAATGTGAAGAACCTGTATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 ATTTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAATAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 15 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 CR-F390D+L429M AA (SEQ ID NO: 59)

MAKNVAFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
 20 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYVNDKKEVLVLWGIHHP
 TTADQQSLYQNADAYVFGTSKYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLMENERTLDYHDSNVKNLYEKVRNQLKNNAKE
 25 IGNCGFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 CR-N97D+F390D+L429M DNA (SEQ ID NO: 60)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTTCCTTCTCAGATCTTC
 CGCGACACATTATGTATAGGTTATCATGCGAACAAATTCACAGACACTGTAGACACAGTACTAGAAAAAG
 30 AATGTAACAGTAACACACTCTGTTAACTTCTGGAAAGACAAGCATAACCGAAAACTATGCAAACTAAGA
 GGGGTAGCCCCATTGCATTTGGGTAATGTAAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACCTCACCACAGCAAGTTTCATGGTCTTACATTTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCAATTTGAAAGGTTT
 35 GAGATATTTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACGTGAATGATAAAGGGAAGAAGTCTCTGCTGTGGGGCATTACCATCCACCC
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAAGTAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCTGAAGCAACTGGAAATCTAGTGGTACCGAGA
 40 TATGCATTTCACAATGGAAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACCAGTCCACGATTGC
 AATACAACCTTGT CAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTTCAGAATATACATCCGATC
 ACAATTTGGAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 45 ACACAAAATGCCATTGACAAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACCTTTGGAC
 TATCACGATTCAAATGTGAAGAACCTGTATGAAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 50 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 CR-N97D+F390D+L429M AA (SEQ ID NO: 61)

MAKNVAFGLLFSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 55 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYVNDKKEVLVLWGIHHP
 TTADQQSLYQNADAYVFGTSKYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
 60 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLMENERTLDYHDSNVKNLYEKVRNQLKNNAKE
 IGNCGFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 CR-K374E+F390D+L429M DNA (SEQ ID NO: 62)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTTCCTTCTCAGATCTTC

GCGGACACATTATGTATAGGTTATCATGCGAACAAATTCACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
GGGGTAGCCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGTTCATGGTCTACATTGTGGAAACATCTAATTGAGACAATGGAACGTGTTAC
5 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGGTTT
GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCT
CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAATCCTACGTGAATGATAAAGGGAAAGAAGTCTCGTGCTGTGGGGCATTCACCATCCACCC
10 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAAGTAC
AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCTGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
AATACAACTTGTGAGACACCCGAGGGTGCTATAAACACCAAGCCTCCCATTTGAGAATATACATCCGATC
ACAATTGGAAAATGTCCAAAGTATGTAAGAACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
15 CCGTCTATTCAATCTAGAGGCCATATTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTTCTGGACATTTGGACTTACATGCGCAACTGTTGGTTCTAATGGAATGAAAGAATTTGGAC
20 TATCAGGATTCAAATGTGAAGAACTTGATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
25 **PDI-H1 CR-K374E+F390D+L429M AA (SEQ ID NO: 63)**
MAKNVAIFGLLFLSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLR
GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVVSFFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNYPKLNQSYVNDKGKEVLVLWGIHHP
TTADQQSLYQNADAYVFGVTSKYSKKFKPEIATRPKVRDQEGRMNYWTLVEBPGDKITFEATGNLVPR
30 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNV
PSIQSRGLFGAIGFIEGGWTGMVDGWYGYHHQNEQSSGYAADLKSTQNAIDEITNKVNSVTEKMNTQD
TATGKCFNHLKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLVEKVRNLKNNAKE
IGNGCFEYHKCDNTCMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

35 **PDI-H1 CR-N97D+K374E+F390D+L429M DNA (SEQ ID NO: 64)**
ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTCTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAAATTCACAGACACTGTAGACACAGTACTAGAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
GGGGTAGCCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
40 TCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAAACATCTAATTGAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGTTT
GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCT
CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAATCCTACGTGAATGATAAAGGGAAAGAAGTCTCGTGCTGTGGGGCATTCACCATCCACCC
45 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAAGTAC
AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCTGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAAGCCTCCCATTTGAGAATATACATCCGATC
50 ACAATTGGAAAATGTCCAAAGTATGTAAGAACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCCATATTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
55 GGTTTTCTGGACATTTGGACTTACAAATGCCGAACCTGTTGGTTCTAATGGAATGAAAGAATTTGGAC
TATCAGGATTCAAATGTGAAGAACTTGATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
60 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
PDI-H1 CR-N97D+K374E+F390D+L429M AA (SEQ ID NO: 65)
MAKNVAIFGLLFLSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLR
GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVVSFFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNYPKLNQSYVNDKGKEVLVLWGIHHP
65 TTADQQSLYQNADAYVFGVTSKYSKKFKPEIATRPKVRDQEGRMNYWTLVEBPGDKITFEATGNLVPR

YAF TMERNAGSGIIISDTPVHDCNTTCQTPEGAIN TSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGSYAADLKSTQNAIDEITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLMENERTLDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEA KLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

H1Hond(N97D).c (SEQ ID NO: 66)

CCCAGGAGATTT CATCGATTATGAGGAGCTAAGGGAGCAATTGAGCTCAG

PDI-H1 Hond-N97D DNA (SEQ ID NO: 67)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTCCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTAGAAGACAAGCATAACGGGAACTATGCAAACTAAGA
GGGGTACCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
CCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAGTTTACAGCAATGGAACGTGTTAC
CCAGGAGATTTTCATCGATTATGAGGAGCTAAGGGAGCAATTGAGCTCAGTGTCTATCATTTGAGAGATTT
GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACAGCAGCATGTGCTT
15 CACGCTGGGGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTCAGCCAATCCTACATTAAATGATAAAGGAAAAAGAGTCCCTCGTGTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
20 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTACAGATACACAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGTATAAACACACAGCCTCCCATTTCAGATAACATCCGATC
ACAATTTGGGAAATGTCCAAAGTATGTA AAAAGCACAAAATGAGACTGGCCACAGGATTGAGGAATGTC
CCATCTATTCAATCTAGAGGCCATTCGCGGGCGATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
25 GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGATATGCAGCCGACCAGAAGAGC
ACAAAAAGTGCCATTGACAAAATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGTTT
ACAGCAGTGGGTAAAGAGTTCAACCACCTTGGAAAAAAGAAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTTCTGGACATTTGGACTTACATGCGCAACTGCTGGTTCTATTGGAAAATGAAAGAATTTTGGAC
TACCACGACTCAAATGTGAAGAACTTGTATGAAAAGGTAAAGAAACAGTTAAAAACAATGCCAAGGAA
30 ATTTGGAAACGGCTGCTTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTTCAAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTTGGTACTGGTAGTC
TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Hond-N97D AA (SEQ ID NO: 68)

MAKNVAIFGLLFLSLVLVPSQIFADTLICIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLR
GVPLHLGKCNIAWILGNPECEPLSTASSWSYIVETSSSDNGTCYPGDFIDYEELREQLSSVVSFFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNYPKLSQSYINDKGKEVLVLWGIHHP
TTADQSLYQNADAYVFVGTSTRYSKFKPEIAIRPKVRDQEGRMNYWTLVEPGDKITFEATGNLVPR
YAF TMERNAGSGIIISDTPVHDCNTTCQTPEGAIN TSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGSYAADLKSTQNAIDKITNKVNSVIEKMNTQF
40 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLENERLDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEA KLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

H1Hond(K374E).r (SEQ ID NO: 69)

TTTGTTAGTAATCTCGTCAATGGCACTTTGTGTGCTCTTCTGGTCGGCTGCATATCC

H1Hond(K374E).c (SEQ ID NO: 70)

ACAAAGTGCCATTGACGAGATTACTAACAAAGTAAATCTGTTATTGAAA

PDI-H1 Hond-K374E DNA (SEQ ID NO: 71)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTCCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTAGAAGACAAGCATAACGGGAACTATGCAAACTAAGA
GGGGTACCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
50 CCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAGTTTACAGCAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGGGAGCAATTGAGCTCAGTGTCTATCATTTGAGAGATTT
GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACAGCAGCATGTGCTT
55 CACGCTGGGGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTCAGCCAATCCTACATTAAATGATAAAGGAAAAAGAGTCCCTCGTGTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
60 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTACAGATACACAGTCCACGATTGC
AATAACAACCTTGTGAGACACCCGAGGGTGTATAAACACACAGCCTCCCATTTCAGAAATATACATCCGATC
ACAATTTGGGAAATGTCCAAAGTATGTA AAAAGCACAAAATTGAGACTGGCCACAGGATGAGGAATGTC
CCATCTATTCAATCTAGAGGCCATTCGCGGGCGATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG

GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTCAGGATATGCAGCCGACCAGAAGAGC
ACACAAAGTGCCATTGACGAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGTTC
ACAGCAGTGGGTAAAGAGTTCAACCACTTGGAAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGCTGGTTCTATTGGAAAATGAAAGAACTTTGGAC
5 TACCACGACTCAAATGTGAAGAACTTGTATGAAAAGGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
ATTGGAACGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAATAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
10 **PDI-H1 Hond-K374E AA (SEQ ID NO: 72)**
MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
GVPPHLHLGKCNIAGWILGNPECEPLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLSQSYINDKGKEVLVLWGIHPS
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
15 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFPQNIHPITIGKCPKYVKSTKRLATGLRNV
PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQSAIDKITNKVNSVIEKMNTQF
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

20 **H1Hond(F390D).r (SEQ ID NO: 73)**
ACCCACTGCTGTGTCTCTGTATTTCATCTTTTCAATAACAGAATTTACTTT
H1Hond(F390D).c (SEQ ID NO: 74)
AAAGATGAATACACAGGACACAGCAGTGGGTAAAGAGTTCAACCACTTGG
PDI-H1 Hond-F390D DNA (SEQ ID NO: 75)
25 ATGGCGAAAAACGTTGCGATTTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTC AACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCATAACGGGAACTATGCAAACTAAGA
GGGGTACCCCAATGTCATTTGGGTAAATGTAAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
CCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAGTTCAGACAATGGAACGTGTTAC
30 CCAGGAGATTTTCATCAATATGAGGAGCTAAGGGAGCAATTGAGCTCAGTGTTCATTTGAGAGATTT
GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACAGCAGCATGTCTC
CACGCTGGGGCAAAAAGCTTCTACAAAAATTAATATGGCTAGTTAAAAAAGGAAATTCATACCCCAAAG
CTCAGCCCAATCCATCAATATGATAAAGGAAAAGAAGTCTCTGCTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
35 AGCAAGAAAGTTCAAGCCGGAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACCAAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTAGAAATATACATCCGATC
ACAATTTGGGAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTC
40 CCATCTATTCAATCTAGAGGCCATTTCGGGGCGATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCAGAAGAGC
ACACAAAGTGCCATTGACAAAATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCATGGGTAAAGAGTTCAACCACTTGGAAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGCTGGTTCTATTGGAAAATGAAAGAACTTTGGAC
45 TACCACGACTCAAATGTGAAGAACTTGTATGAAAAGGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
ATTGGAACGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAATAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
50 **PDI-H1 Hond-F390D AA (SEQ ID NO: 76)**
MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
GVPPHLHLGKCNIAGWILGNPECEPLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLSQSYINDKGKEVLVLWGIHPS
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
55 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFPQNIHPITIGKCPKYVKSTKRLATGLRNV
PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQSAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

60 **H1Hond(L429M).r (SEQ ID NO: 77)**
TCTTTTCATTTTCATTAGAACCAAGTTCGGCATTGTAAGTCCAAATGTCCAGG
H1Hond(L429M).c (SEQ ID NO: 78)
CGAACTGCTGGTTCTAATGGAAAATGAAAGAACTTTGGACTACCACGA

PDI-H1 Hond-L429M DNA (SEQ ID NO: 79)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTGTGTTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAAATCAACAGACACTGTAGACACAGTACTAGAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTAGAAAGACAAGCATAACGGGAAACTATGCAAACTAAGA
5 GGGGTACCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
CCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAGTTCAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGGGAGCAATTGAGCTCAGTGTCTCATTTTGAGAGATTT
GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAAACAGCAGCATGTCTT
10 CACGCTGGGGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTCAGCCCAATCCTACATTAATGATAAAGGAAAAGAAGTCTCTGCTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
15 ATGCAAGAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
AATACAACCTTGTGAGACACCCGAGGGTGTCTATAAACACACAGCTCCCATTTCAGAATATACATCCGATC
ACAATTTGGGAAATGTCCAAAGTATGTAAAAAGCACAAATTTGAGACTGGCCACAGGATTGAGGAATGTC
CCATCTATTCAATCTAGAGGCCCTATTCTGGGGCGATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCCAGAGAAGAGC
20 ACACAAAGTGCCATTGACAAAATTACTAACAAAGTAAATTTCTGTATTGAAAAGATGAATACACAGTTC
ACAGCAGTGGGTAAAGAGTTCAACCCTTGGAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGCTGGTTCTAATGGAATGAAAGAACCTTTGGAC
TACCACGACTCAATGTGAAGAATCTGTATGAAAAGGTAAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
25 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Hond-L429M AA (SEQ ID NO: 80)

MAKNVAIFGLLFLSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLR
GVFPLHLGKCNIAGWILGNPECEPLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERE
30 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLSQSYINDKKEVLVLWGIHHS
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIAIRPKVRDQEGRMNYYWTVLVEPGDKITFEATGNLVVPR
YAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKRLATGLRNV
PSIQSRGLFGAIAGFIEGWTGMVDGWYGHQNEQSGYAADQKSTQSAIDKITKNVNSVIEKMNQF
35 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLMENERLDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEYHKCDNTECMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCNSGSLQCRICI*

PDI-H1 Hond-F390D+L429M DNA (SEQ ID NO: 81)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAAATCAACAGACACTGTAGACACAGTACTAGAAAAG
40 AATGTAACAGTAACACACTCTGTAACTTCTAGAAAGACAAGCATAACGGGAAACTATGCAAACTAAGA
GGGGTACCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
CCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAGTTCAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGGGAGCAATTGAGCTCAGTGTCTCATTTTGAGAGATTT
GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAAACAGCAGCATGTCTT
45 CACGCTGGGGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTCAGCCAATCCTACATTAATGATAAAGGAAAAGAAGTCTCTGCTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
50 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACACAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGTCTATAAACACACAGCTCCCATTTTTCAGAATATACATCCGATC
ACAATTTGGGAAATGTCCAAAGTATGTAAAAAGCACAAATTTGAGACTGGCCACAGGATTGAGGAATGTC
CCATCTATTCAATCTAGAGGCCCTATTCTGGGGCGATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
55 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCCAGAGAAGAGC
ACACAAAGTGCCATTGACAAAATTACTAACAAAGTAAATTTCTGTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCCTTGGAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGCTGGTTCTAATGGAATGAAAGAACCTTTGGAC
TACCACGACTCAATGTGAAGAATCTGTATGAAAAGGTAAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
60 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Hond-F390D+L429M AA (SEQ ID NO: 82)

MAKNVAIFGLLFLSLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLR

GVPPLHLGKCNIAWGILGNPECEPLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVVSFFER
 EIFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVVFVGTSTRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLEFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 5 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQSAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Hond-N97D+F390D+L429M DNA (SEQ ID NO: 83)

10 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCATAACGGGAAACTATGCAAACTAAGA
 GGGGTACCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 CCATCTCCACAGCAAGTTCATGGTCTACATTGTGGAAACATCTAGTTAGACAAATGGAACGTGTTAC
 15 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGGGAGCAATTGAGCTCAGTGTGCATCATTTGAGAGATTT
 GAGATATTCCTCCAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACAGCAGCATGTCTCT
 CACGCTGGGGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCCAAATCCTACATTAATGATAAAGGAAAAGAAAGTCTCTGCTGCTGTGGGGCATTCACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGCACATCAAGATAC
 20 AGCAAGAAGTTCAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGAGACAAAATAACATTGGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACCAAGTCCACGATTGCT
 AATACAACCTTGTGAGACACCCGAGGGTGTCTATAAACACCAGCCTCCCATTTAGAAATATACATCCGATC
 ACAATTTGGGAAATGTCCAAAGTATGTAAGGACACAAAATGAGACTGGCCACAGGATTGAGGAATGTC
 25 CCATCTAATCAATCTAGAGGCCATATCGGGGGCATTGCGGGCTTCATTGAAGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCAAGAGC
 ACACAAAGTGCCATTGACAAAATTACTAACAAAGTAAATCTGTTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTTGGAAAAAGAAATAGAGAATTTAAATAAAAAAGTTGATGAT
 GGTTCCTGGACATTTGGACTTACATGCGGAACCTGCTGGTTCTAATGGAAAATGAAAGAACCTTGGAC
 30 TACCACGACTCAAAATGTGAAGAACCTGTATGAAAAGGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Hond-N97D+F390D+L429M AA (SEQ ID NO: 84)

35 MAKNVAFGLLFSLLVLVPSQIFADTLICIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVPPLHLGKCNIAWGILGNPECEPLSTASSWSYIVETSSSDNGTCYPGDFIDYEELREQLSSVVSFFER
 EIFPKTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVVFVGTSTRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 40 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLEFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQSAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Darw-N97D DNA (SEQ ID NO: 85)

45 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCACAACGGGAAACTATGCAAACTAAGA
 GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAACCCAGAGTGTGAA
 50 TCACTCTCCACAGCAAGTTCATGGTCTACATTGTGGAAACATCTAGTTAGACAAATGGAACGTGTTAC
 CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTGCATCATTTGAAAGATTT
 GAGATATTCCTCCAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTCT
 CACGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAACTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCCAAATCCTACATTAATGATAAAGGAAAAGAAATCCTCTGCTGCTGTGGGGCATTCACCATCCATCT
 55 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGCACATCAAGATAC
 AGCAAGAAGTTCAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCAGGTGACAAAATAACATTGGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACCAAGTCCACGATTGCT
 AATACAACCTTGTGAGACACCCGAGGGTGTCTATAAACACCAGCCTCCCATTTAGAAATATACATCCGATC
 60 ACAATTTGGAAGATGTCCAAAGTATGTGAAAAGCACAATAATGAGACTGGCCACAGGATTGAGGAATGTC
 CCATCTATTCAATCTAGAGGCCATTTGCGGGCCATTGCGGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGGTATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAAAATTACTAACAAAGTAAATCTGTTATTGAAAAGATGAATACACAGTTTC
 65 ACAGCAGTGGGTAAAGAGTTCAACCCTTGGAAAAAGAAATAGAGAATTTAAATAAAAAAGTTGATGAT
 GGTTCCTGGACATTTGGACTTACAATGCGGAACCTGCTGGTTCTATTGGAAAATGAAAGAACCTTGGAC

TACCACGATTCAAATGTGAAGAACTTGTATGAAAAGGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
ATTGGAAATGGTTGCTTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAATAAGG
ACTTATGACTACCCAAAATACTCAGGGGAAGCAAAATTAACAGAGAAAAAATAGAAGGGGTAAAGCTG
GAATCAACAAGAATTTACCAAATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTGGTAGTC
TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Darw-N97D AA (SEQ ID NO: 86)

MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLR
GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFIDYEELREQLSSVSSFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLTKKGNSYPKLSQSYINDKGKEILVLWGIHHP
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQF
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSGEAKLNREKIEGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGLQCRICI*

H1Darw(K374E).r (SEQ ID NO: 87)

TTTGTTAGTAATCTCGTCAATGGCATTCTTGTGTGCTCTTCAGGTCGGCTGCATACC

PDI-H1 Darw-K374E DNA (SEQ ID NO: 88)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCACAACGGGAAACTATGCAAACTAAGA
GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAACCCAGAGTGTGAA
TCACCTCTCCACAGCAAGTTCATGGTCTACATTGTGGAACACATCTAGTTCAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGATTT
GAGATATTTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGGCAGCATGTCTT
CACGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAACTAAAAAGGAAATTCATACCCAAAG
CTCAGCCAATCCTACATTAATGATAAAGGGAAAGAAATCCTCGTGCTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
AGCAAGAGTTTCAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCAGGTGACAAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCTTACACAATGGAAAGAAATGCTGGATCTGGTATATCATTTTTCAGATACACAGTCCACAGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTTCAGAATATACATCCGATC
ACAATTTGAAAAATGTCCAAAGTATGTGAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTC
CCATCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCGGGCTTCATTGAAGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGGTATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGTTC
ACAGCAGTGGGTAAAGAGTTCAACCACTTGGAAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTTCTGGACATTTGGACTTACAATGCCGAACCTGCTGGTTCTATTGGAAAAATGAAGAAGCTTTGGAC
TACCACGATTCAAAATGTGAAGAACTTGTATGAAAAGGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
ATTGGAATGGTTGCTTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGGGGAAGCAAAATTAACAGAGAAAAAATAGAAGGGGTAAAGCTG
GAATCAACAAGAATTTACCAAATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Darw-K374E AA (SEQ ID NO: 89)

MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLR
GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLTKKGNSYPKLSQSYINDKGKEILVLWGIHHP
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGGYAADLKSTQNAIDEITNKVNSVIEKMNTQF
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSGEAKLNREKIEGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGLQCRICI*

PDI-H1 Darw-F390D DNA (SEQ ID NO: 90)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCACAACGGGAAACTATGCAAACTAAGA
GGGGTAGCCCCATTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAACCCAGAGTGTGAA
TCACTCTCCACAGCAAGTTCATGGTCTACATTGTGGAACATCTAGTTCAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGATTT
GAGATATTTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGGCAGCATGTCTT
CACGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAACTAAAAAGGAAATTCATACCCAAAG
CTCAGCCAATCCTACATTAATGATAAAGGGAAAGAAATCCTCGTGCTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC

AGCAAGAAGTTCAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCAGGTGACAAAATAACATTGCAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTACAAATGGAAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACCAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTTCAGAAATATACATCCGATC
ACAATTGGAAAATGTCCAAAGTATGTGAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTC
CCATCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCGCGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGGTATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACAAAATTACTAACAAAGTAAATTTCTGTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACTTGGAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTCTTGGACATTTGGACTTACAAATGCCGAACCTGCTGGTTCTATTGGAAAATGAAAGAACTTTGGAC
TACCACGATTCAAATGTGAAGAAGTTGTATGAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGAAAATGGTTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGGGGAAGCAAAAATTAACAGAGAAAAATAGAAGGGGTAAAGCTG
GAATCAACAAGAATTTACCAAATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Darw-F390D AA (SEQ ID NO: 91)

MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLDKHNGKLCCLR
GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVVSFFERF
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLTKKGNSYPKLSQSYINDKGKEILVLWGIHHP
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTSLPFQNIHPITIGKCPKYVKS TKLRLATGLRNV
PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSGEAKLNREKIEGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

PDI-H1 Darw-L429M DNA (SEQ ID NO: 92)

ATGGCGAAAAACGTTGCGATTTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTAGAAAGACAAGCACAACGGGAACTATGCAAACTAAGA
GGGTAGCCCCCATTTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAACCCAGAGTGTGAA
TCACTCTCCACAGCAAGTTCATGGTCTACATTTGTGGAACATCTAGTTCAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCAATTTGAAAGATTT
GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCT
CACGCTGGAGCAAAAAGCTTCTACAAAATTTAATATGGCTAACTAAAAAAGGAAATTCATACCCAAAG
CTCAGCCAATCCTACATTATGATAAGGGGAAAGAAATCCTCGTGCTGTGGGGCATTCACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAAATGCAAT
TACTGGACACTAGTAGAGCCAGGTGACAAAATAACATTGCAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACCAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTTCAGAAATATACATCCGATC
ACAATTTGAAAAATGTCCAAAGTATGTGAAAAGCACAAAATTTGAGACTGGCCACAGGATTGAGGAATGTC
CCATCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCGCGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGGTATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACAAAATTACTAACAAAGTAAATTTCTGTATTGAAAAGATGAATACACAGTTTC
ACAGCAGTGGGTAAAGAGTTCAACCACTTGGAAAAAGAATAGAGAATTTAAATAAAAAAGTTGATGAT
GGTTTTCTGGACATTTGGACTTACAAATGCCGAACCTGCTGGTTCTAATGGAAAATGAAAGAATTTGGAC
TACCACGATTCAAATGTGAAGAAGTTGTATGAAAAGGTAAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAATGGTTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGGGGAAGCAAAATTAACAGAGAAAAAATAGAAGGGGTAAAGCTG
GAATCAACAAGAATTTACCAAATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Darw-L429M AA (SEQ ID NO: 93)

MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLDKHNGKLCCLR
GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVVSFFERF
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLTKKGNSYPKLSQSYINDKGKEILVLWGIHHP
TTADQQSLYQNADAYVFGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTSLPFQNIHPITIGKCPKYVKS TKLRLATGLRNV
PSIQSRGLFGAIAAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQF
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSGEAKLNREKIEGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

PDI-H1 Darw-F390D+L429M DNA (SEQ ID NO: 94)

ATGGCGAAAAACGTTGCGATTTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG

AATGTAACAGTAACACACTCTGTAACTTCTAGAAGACAAGCACAACGGGAACTATGCAAACTAAGA
 GGGGTAGCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAACCCAGAGTGTGAA
 TCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAAACATCTAGTTCAGACAAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGATTT
 5 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAATTTAATATGGCTAACTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCCAATCC TACATTAATGATAAAGGGAAAGAAATCCTCGTGCTGTGGGGCATTCCACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 10 TACTGGACACTAGTAGAGCCAGGTGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTCACAAATGGAAAGAAATGCTGGATCTGGTATTATCATTTT CAGATACACAGTCCACGATTGC
 AATACAACCTTGT CAGACACCCGAGGGTGCTATAAACACACAGCCTCCCATTT CAGAATATACATCCGATC
 ACAATTTGGAAAATGTCCAAGTATGTGAAAAGCACAATAATGAGACTGGCCACAGGATTGAGGAATGTC
 CCATCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 15 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGGTATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAAAATTACTAACAAAGTAAATCTGTTATTGAAAAGATGAATACACAGGAC
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 GGTTCCTCGGACATTGGACTTACAATGCCGAACCTGCTGGTTCTAATGGAAAATGAAAGAATTTGGAC
 TACCACGATTCAATGTGAAGAATCTGTATGAAAAGGTAAAGAAACAGTTAAAAACAATGCCAAGGAA
 20 ACTTGAAAATGGTTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGGGGAAGCAAAAATAAACAGAGAAAAAATAGAGGGGTAAAGCTG
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 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
PDI-H1 Darw-F390D+L429M AA (SEQ ID NO: 95)
 25 MAKNVAFGLLFLSLVLPVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLTKKGNYPKLSQSYINDKGKEILVLWGIHHP
 TTADQQSLYQNDAYVVFVGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 30 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAE
 IGNCFEFYHKDNCTCMESVKNGTYDPKYSGBAKLNRKIEGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*
PDI-H1 Darw-N97D+F390D+L429M DNA (SEQ ID NO: 96)
 35 ATGGCGAAAAACGTGCGATTTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTTCTTCTCAGATCTTC
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 TCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAAACATCTAGTTCAGACAAATGGAACGTGTTAC
 40 CCAGGAGATTTCTCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCATTTGAAAGATTT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAATTTAATATGGCTAACTAAAAAAGGAAATTCATACCCAAAG
 CTCAGCCAATCC TACATTAATGATAAAGGGAAAGAAATCCTCGTGCTGTGGGGCATTCCACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 45 AGCAAGAAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCAGGTGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTCACAAATGGAAAGAAATGCTGGATCTGGTATTATCATTTT CAGATACACAGTCCACGATTGC
 AATACAACCTTGT CAGACACCCGAGGGTGCTATAAACACACAGCCTCCCATTT CAGAATATACATCCGATC
 ACAATTTGAAAATGTCCAAGTATGTGAAAAGCACAATAATGAGACTGGCCACAGGATTGAGGAATGTC
 50 CCATCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGGTATGCAGCCGACCTGAAGAGC
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 55 TACCACGATTCAATGTGAAGAATTTGTATGAAAAGGTAAAGAAACAGTTAAAAACAATGCCAAGGAA
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 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
PDI-H1 Darw-N97D+F390D+L429M AA (SEQ ID NO: 97)
 60 MAKNVAFGLLFLSLVLPVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFIDYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLTKKGNYPKLSQSYINDKGKEILVLWGIHHP
 TTADQQSLYQNDAYVVFVGTSTRYSKKFKPEIAIRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVVPR
 65 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV

PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFEYHKCDNTCMESVKNGTYDYPKYSGEAKLNREKIEGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNSLQCRICI*

5

Cloning vector 1190 from left to right T-DNA (SEQ ID NO: 98)

TGGCAGGATATATTGTGGTGTAAACAAATTGACGCTTAGACAACCTTAATAACACATTGCGGACGTTTTT
AATGTACTGAATTAACGCCGAATCCCGGGCTGGTATATTTATATGTTGTCAAATAACTCAAAAACCATA
AAAGTTTAAAGTTAGCAAGTGTGTACATTTTACTTGACAAAAATATTCACCTACTACTGTTATAAATC
10 ATTATTTAAACATTAGAGTAAAGAAATATGGATGATAAGAACAAGAGTAGTGATATTTTGACACAATTT
TGTTGCAACATTTGAGAAAAATTTTGTGTTCTCTCTTTTCATTGGTCAAAAACAATAGAGAGAGAAAA
GGAAGAGGGAGAATAAAAACATAATGTGAGTATGAGAGAGAAAGTTGTACAAAAGTTGTACAAAATAG
TTGTACAAATATCATTGAGGAATTTGACAAAAGCTACACAAATAAGGGTTAATTGCTGTAAATAAATAA
GGATGACGCATTAGAGAGATGTACCATTTAGAGAATTTTGGCAAGTCATTAAAAAGAAAGATAAATTA
15 TTTTTAAATTAAGAGTTGAGTCATTTGATTAAACATGTGATTATTTAATGAATTGATGAAGAGTTGG
ATTAAAGTTGTATTAGTAATTAGAAATTTGGTGTCAAATTTAATTGACATTTGATCTTTTCTATATAT
TGCCCCATAGAGTCAGTTAACTCATTTTATATTTTATAGATCAAATAAGAGAAATAACGGTATATTAA
GCCCTCCAAAAAAGAAACGGTATATTTTACTAAAAATCTAAGCCACGTAGGAGGATAACAGGATCCC
CGTAGGAGGATAACATCCAATCCAACCAATCACAAACATCTGTATGAGATAACCCACTTTAAGCCACG
20 CATCTGTGGCACATCTACATTTATCTAAATCACACATTTCTCCACACATCTGAGCCACACAAAAACCAAT
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25 GATAATCCCTTGGTTTCAAGGAAAGCTGGGGTTTCGGGAAAGTTGTATTTAAGAGATATCTCAGATAC
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30 AAAGAAAGCGAGTAAGTTAAATGCTTCTTCTCTCTCTATTTATAATATGGTTTGTATTGTTAATTTT
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35 TTAACAATCAACTTAACGTTATTAACCTACTAATTTTATATCATCCCCCTTTGATAAATGATAGTACACCA
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GCCGGTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAATATCAAAGATACAGTCTCAGAAGA
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40 TATCTGTCACTTTATTGTGAAGATAGTGGAAAGGAAGGTGGCTCCTACAAATGCCATTGCGATATA
AGGAAAGGCCATCGTTGAAGATGCCCTTGCCGACAGTGGTCCCAAAGATGGACCCCAACCCACGAGGAG
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45 TTTTCAACAAAGGTAATATCCGGAACCTCCTCGGATTCATTGCCCAGCTATCTGTCACTTTATTGT
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AGATGCCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCAACCCACGAGGAGCATCTGGAAAAAGAAGA
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50 TCTCTCTTAAAGCAAACCTTCTCTTGTCTTTCTTGGCTGAGCGATCTTCAACGTTGTGATCGTGTCT
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55 CCTCTACACTCTGAGCAGCTCAGTGACTGTCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGC
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TTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTATCTTCCCCCAAGCCCAAGGATGTGCTCAC
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60 CACTTTCCGCTCAGTCAGTGAACCTCCCATCATGCACCAGGACTGGCTCAATGGCAGGAGCGATCGCT
CACCATCACCATCACCATCACCATCACCATTAAAGGCCATTTTCTTTAGTTTGAATTTACTGTTATTC
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AAAAAAGGAAAGACCGGGAATTCGATATCAAGCTTATCGACCTGCGATCGTTCAAACATTTGGC
65 AATAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCATGATTATCATATAATTTCTGTTGAATT

ACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAG
TCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAAATTATCGC
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5 ATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCA
GCCTGAATGGCGAATGCTAGAGCAGCTTGAGCTTGGATCAGATTGTGCTTCCCGCCTTCAGTTTAAAC
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Construct 1314 from 2X35S prom to NOS term (SEQ ID NO: 99)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAA
10 AGGCCAATTGAGACTTTTTCAACAAAGGGTAATATCCGGAACCTCCTCGGATTCCATTGCCAGCTATC
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15 CAACAAAGGGTAATATCCGGAACCTCCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTGTGAAG
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CCAACACGCTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCGACAATCC
CACTATCCTTCGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGGTATTAAATCTT
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20 TCTTAAAGCAAACTTCTCTCTTGTCTTTCTTGGCTGAGCGATCTTCAACGTTGTGTAGATCGTCTTCGG
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25 CAACTAAGAGGGGTAGCCCCATTGGCATTTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCC
AGAGTGTGAATCACTCTCCACAGCAAGCTCATGGTCTACATTGTGGAACACCTAGTTTCAGACAATGG
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30 ATACCCAAAGCTCAGCAAATCCTACATTAATGATAAAGGGAAGAAGTCTCTGCTATGGGGCATTCA
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35 CCACGATTGCAATACAACCTTGTCAAACACCCAAAGGTGCTATAAACACCAGCCTCCCATTTTCAGAAT
ACATCCGATCACAAATTGGAAAATGTCCAAAATATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATT
GAGGAATATCCCCTCTATTCAATCTAGAGGACTATTTGGGGCCATTGCCGGTTTCATTGAAGGGGGTG
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CCTGAAGAGCACACAGAATGCCATTGACGAGATTACTAACAAGTAAATTCGTATTGAAAAGATGAA
40 TACACAGTTACAGCAGTAGGTAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAAATTTAAATAAAAA
AGTTGATGATGGTTTCTGGACATTTGGACTTACAATGCCGAAGTGTGGTTCTATTGAAAAATGAAAG
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45 CAAAAATGGGACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAGAAATAGATGG
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50 CGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCT
GTTGCCGGTCTTTCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATG
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Construct 2980 from 2X35S prom to NOS term (SEQ ID NO: 100)

GTCAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAA
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60 GACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTT
CAACAAAGGGTAATATCCGGAACCTCCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTGTGAAG
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65 CACTATCCTTCGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGGTATTAAATCTT

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CACCAGTACAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTTCTTGTGTTGGTTCTTCTC
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5 ACTAGAAAAGAAATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCATAACGGGAAACTATG
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10 TGAAAGGTTTGAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGC
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15 GGTACCGAGATATGCATTGCAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACCAGT
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ACATCCGATCACAAATTGGAAAATGTCCAAAATATGTAAGAAAGCACAATAATGAGACTGGCCACAGGATT
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20 CCTGAAGAGCACACAGAATGCCATTGACGAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAA
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AGTTGATGATGGTTTCTTGGACATTGGACCTTACAATGCCGAACGTTGGTTCTATTGGAAATGAAAG
AACTTTGGACTACCACGATTCAAATGTGAAGAACTTATATGAAAAGGTAAGAAGCCAGCTAAAAAACAA
25 TGCCAAGGAAATGGAAACGGCTGCTTTGAAATTTTACCACAAATGCGATAACACGTCGATGGAAAGTGT
CAAAAAATGGGACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAGAAATAGATGG
GGTAAAGCTGGAATCAACAGGATTACACAGATTTTGGCGATCTATTCAACTGTCCGCGAGTTTCATTGGT
ACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATG
TATTTAAAGGCCATTTTTCTTTAGTTTGAATTTACTGTTATTTCGGTGTGCATTTCTATGTTTTGGTGAGC
30 GGTTTTCTGTGCTCAGAGTGTGTTTTATTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGT
CGTCCCTTCAGCAAGGACACAAAAAGATTTTAATTTTATTAAGGAGGAGGAGGAGGAGGAGGAGGAGG
CGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTGGCAATAAGTTTCTTAAGATTGAATCTT
GTTGCCGGTCTTTCGATGATTTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATTAACATG
TAATGCATGACGTTATTTATGAGATGGGTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG
ATAGAAAACAAAATATAGCGCGCAACTAGGATAAATATCGCGCGCGGTGTCATCTATGTTACTAGAT
35 Construct 2995 from 2X35S prom to NOS term (SEQ ID NO: 101)
GTCAACATGGTGAGCACGACACACTTGTCTACTTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAA
AGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAACCTCCTCGGATTCCATTGCCAGCTATC
TGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGA
AAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATC
40 GTGGAAAAAGAAAGACGTTTCCAACACGCTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTTGAGCAC
GACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTT
CAACAAAGGGTAATATCCGGAACCTCCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTTGTGAAG
ATAGTTGAAAAGGAAGGTGGCTCTCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGAT
GCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAAGACGTT
45 CCAACCAGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCC
CACTATCCTTCGCAAGACCTTCTCTATATAAGGAAGTTTCATTTCATTGAGAGGTATTAATCTT
AATAGGTTTTTGATAAAAAGCGAACGTGGGGAAACCCGAACCAAACCTTCTTCTAAACTCTCTCTCATCTC
TCTTAAAGCAAACCTTCTCTTGTCTTTCTTTCGGTGAGCGATCTTCAACGTTGTCAGATCGTGCTTCGG
CACCAGTACAATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCTTCTC
50 TCAGATCTTCGCGGACACATTATGTATAGGTTATCATGCGAACAAATTCACAGACACTGTAGACACAGT
ACTAGAAAAGAAATGTAACAGTAACACACTCTGTTAACCTTCTAGAAGACAAGCATAACCGGAAACTATG
CAAACCTAAGAGGGGTAGCCCCATTGTCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCC
AGAGTGTGAATCACTCTCCACAGCAAGCTCATGGTCTTACATTGTGGAACACCTAGTTTCAGACAATGG
AACGTGTTACCCAGGAGATTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATCAT
55 TGAAAGGTTTGAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGC
AGCATGTCTCTATGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAAATTC
ATACCCAAAGCTCAGCAAATCCTACATTAATGATAAAGGGAAAGAAGTCTCTGCTGCTATGGGGCATTCA
CCATCCATCTACTAGTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGTCT
ATCAAGATACAGCAAGAAGTTCAAGCCGGAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAG
60 AATGAACATTACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGGAAGCAACTGGAATCTAGT
GGTACCGAGATATGCATTGCAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACCAGT
CCACGATTGCAATACAACCTTGTCAAACACCCAAAGGGTGTATAAACACCAAGCCTCCCATTTTCAGAAAT
ACATCCGATCACAAATGGAAAATGTCCAAAATATGTAAGCAACAAATTCAGACTGGCCACAGGATT
GAGGAATATCCGCTATTTCAATCTAGAGGACTATTTGGGGCCATTGCCGGTTTCATTGAAGGGGGGTG
65 GACAGGGATGGTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGA

CCTGAAGAGCACACAGAATGCCATTGACGAGATTACTAACAAAGTAAATTCGTATTGAAAAGATGAA
 TACACAGGACACAGCAGTAGGTAAAGAGTTCAACACCTGGAAAAAAGAATAGAGAATTTAAATAAAAA
 AGTTGATGATGGTTTCTGGACATTTGGACTTACAATGCCGAACGTGTGGTTCTAATGGAAAAATGAAAG
 AACTTTGGACTACCACGATTCAAATGTGAAGAACTTATATGAAAAGGTAAGAAGCCAGCTAAAAAACAA
 5 TGCCAAGGAAATTGAAACGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGT
 CAAAAATGGGACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAAACAGAGAAGAAATAGATGG
 GGTAAGCTGGAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGT
 ACTGGTAGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATG
 10 TATTTAAAGGCCATTTTCTTAGTTTGAATTTACTGTTATTTCGGTGTGCATTTCTATGTTTGGTGAGC
 GGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTTAATTTCTTTGTGAGCTCCTGTTTAGCAGGT
 CGTCCCTTCAGCAAGGACACAAAAAGATTTTAAATTTTATTAATAAAAAAAAAAAAAAAAAAGACCGGGAAT
 CGATATCAAGCTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCT
 GTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATG
 15 TAATGCATGACGTTATTTATGAGATGGGTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCG
 ATAGAAAACAAAATATAGCGCGCAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT

H1Mich(N380A).r (SEQ ID NO: 102)

TTCAATAACAGAAGCTACTTTGTTAGTAATCTTGTCATGGCATTGTTGT

H1Cal(N380A).c (SEQ ID NO: 103)

20 GATTACTAACAAAGTAGCTTCTGTTATTGAAAAGATGAATACACAGTT

PDI-H1 Mich-N380A DNA (SEQ ID NO: 104)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
 25 GGGGTAGCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAATTCAGACAATGGAACGTGTTC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATTTGAAAGGTTT
 GAGATATTCCTCCAAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTACGGCAGCATGTCT
 CACGCTGGAGCAAAAAGCTTCTACAAAACCTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 30 CTTAACCAATCC TACATTAATGATAAAGGGAAGAGTCTCTGCTGTGGGGCATTCCACATCCATCT
 ACTACTGCTGACCAACAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACC CAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCAGGA
 35 TATGCATTACAAATGGAAGAAATGCTGGATCTGGTATTATCATTTAGATACACCAAGTCCAGATTGC
 AATACAATTTGT CAGACACCCGAGGGTGTATAAACACCAGCCTCCCATTTAGAAATATACATCCGATC
 ACAATTTGAAAATGTCCAAAGTATGTAAAAAGCACAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATTGGATGGTACGGTTATCACCATTCAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 40 ACACAAAATGCCATTGACAAGATTACTAACAAAGTAGCTTCTGTTATTGAAAAGATGAATACACAGTTC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTCCTGGACATTTGGACTTACAATGCCGAACGTGTGGTTCTATTGGAAAATGAAAGAACTTTGGAC
 TATCAGGATTCAAATGTGAAGAACTTGTATGAAAAAGTAAGAAACCAGTTAAAAAACAAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 45 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mich-N380A AA (SEQ ID NO: 105)

MAKNVAFGLLFSLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
 50 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKNSYPKLNQSYINDKGKVLVLWGIIHPS
 TTADQQSLYQNADAYVFGTSRYSKKFKPEIATRPKVRDQEGRMNYWTVLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNIHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGSYAADLKSQTQNAIDKITNKVASVIEKMNTQF
 55 TAVGKEFNHLEKRIENLNKKVDDGFLDIWYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNCGFEFYHKCDNTCMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVVLVV
 SLGAISFWMCNSNGSLQCRICI*

H1Mich(N380A+F390D).r (SEQ ID NO: 106)

CCCactGCTGTGTCCTGTGTATTTCATCTTTTCAATAACAGAAGCTACTT

PDI-H1 Mich-F390D+N380A DNA (SEQ ID NO: 107)

60 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
 AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
 GGGGTAGCCCCCATTCGATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGTTCATGGTCTTACATTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC

CCAGGAGATTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTTCATCATTTGAAAGGTTT
 GAGATATTCCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCGAGCATGTCCCT
 CACGCTGGAGCAAAAAGCTTCTACAAAACCTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCCTACATTATGATAAAGGGAAAGAAGTCCCTCGTGCTGTGGGGCATTCACCATCCATCT
 5 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTGGAAGCAACTGGAAATCTAGTGGTACCGAGA
 TATGCATTCACAATGGAAAGAAATGCTGGATCTGGTATTATCATTTTCAGATACACCAAGTCCACGATTGC
 AATACAACCTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTCAGAATATACATCCGATC
 10 ACAATTGGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAAGATTACTAACAAAGTAGCTTCTGTTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 15 GGTTTTCTGGACATTTGGACTTACATGCGCAACTGTTGGTTCTATTGGAAAATGAAAGAATTTTGGAC
 TATCAGGATTCAAATGTGAAGAAGTTGATGAAAAAGTAAGAAACCAAGTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAGATGTCAAATAAGGG
 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 20 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Mich-F390D+N380A AA (SEQ ID NO: 108)

MAKNVAIFGLLFSLVLVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNKGLCKLR
 GVAPLHLGKCNIAWILGNPECESLSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKNSYPKLNQSYINDKGKEVLVLWGIHPS
 25 TTADQQSLYQNADAYVFVGTSTRYSKFKPEIATRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVPR
 YAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINSTLFPQNIHPITIGKCPKYVKS TKLRLATGLRNV
 PSIQSRGLFGAIGFIEGGWTGMVDGWYGYHHQNEQSSGYAADLKSTQNAIDKITNKVASVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNCGFEFYHKCDNTCMESVKNGTYPKYSEEA KLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 30 SLGAISFWMCSNGSLQCRICI*

IF-H5ITMCT.s1-4r (SEQ ID NO: 109)

ACTAAAGAAAATAGGCCTTTAAATGCAAAATTCGCAATTGTAACGATCCAT

PDI-H5 Indo DNA (SEQ ID NO: 110)

ATGGCGAAAAACGTTGCGATTTTCGCGCTTATGTTTTCTCTTCTGTGTTGGTTCTTCTCAGATCTTC
 35 GCCGATCAGATTTGCATTGGTTACCATGCAACAATTCACAGAGCAGGTTGACACAATCATGGAAAAG
 AACGTTACTGTTACACATGCCCAAGACATACTGGAAAAGACACACAACGGGAAGCTCTGCCATCTAGAT
 GGAGTGAAGCCTCTAATTTTAAGAGATTGTAGTGTAGCTGGATGGCTCCTCGGGAACCCAAATGTGTGAC
 GAATTCATCAATGTACCGGAATGGTCTTACATAGTGGAGAAGGCCAATCCAACCAATGACCTCTGTTAC
 40 CCAGGGAGTTTCAACGACTATGAAGAACTGAAACACCTATTGAGCAGAATAAACCATTTTGGAGAAAT
 CAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAAGCCTCATCAGGAGTTAGCTCAGCATGTCCATAC
 CTGGGAAGTCCCCTCTTTTGTAGAAATGTGGTATGGCTTATCAAAAAGAACAGTACATACCCAACAATA
 AAGAAAAGCTACATAATACCAACCAAGAGGATCTTTTGGTACTGTGGGGAATTCACCATCTAATGAT
 45 GCGGCAGAGCAGACAAGGCTATATCAAAACCCAACCCTATATTTCCATTGGGACATCAACACTAAAC
 CAGAGATTGGTACCAAAAATAGCTACTAGATCCAAAGTAAACGGGCAAAGTGGAAGGATGGAGTTCTTC
 TGGACAATTTTAAACCTAATGATGCAATCAACTTCGAGAGTAATGGAATTTTCATTGCTCCAGAATAT
 GCATACAAAATTGTCAAGAAAGGGGACTCAGCAATTATGAAAAGTGAATTGGAATATGGTAACTGCAAC
 ACCAAGTGTCAAATCCAAATGGGGGCGATAAACTCTAGTATGCCATTCCACAACATACACCCCTCTCACC
 50 ATCGGGGAATGCCCAATATGTGAATCAACAGATTAGTCCTTGCAACAGGGCTCAGAAATAGCCCT
 CAAAGAGAGAGCAGAAGAAAAAAGAGAGGACTATTTGGAGCTATAGCAGGTTTTATAGAGGGAGGATGG
 CAGGGAATGGTAGATGGTTGGTATGGGTACCAACCATAGCAATGAGCAGGGGAGTGGGTACGCTGCAGAC
 AAAGAATCCACTCAAAGGCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTGACAAAATGAAC
 ACTCAGTTTGAGGCCGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAAATAGAGAATTTAAACAAGAAG
 55 ATGGAAGACGGGTTTCTAGATGCTGGACTTATAATGCCGAACCTCTGGTTCTCATGGAAAATGAGAGA
 ACTCTAGACTTTTCATGACTCAAATGTTAAGAACCTCTACGACAAGGTCCGACTACAGCTTAGGGATAAT
 GCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCTATCAGAAATGTGATAATGAATGTATGGAAAGTATA
 AGAAACGGAACGTACAACATATCCGCAGTATTGAGAAGAAGCAAGATTAAAAAGAGAGGAAATAAGTGGG
 GTAAAATTGGAATCAATAGGAACCTTACCAATACTGTCAATTTATTCAACAGTGGCGAGTTCCCTAGCA
 CTGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGTTACATGCAAGATTTGCA
 60 ATTTAA

PDI-H5 Indo AA (SEQ ID NO: 111)

MAKNVAIFGLLFSLVLVPSQIFADQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDDLD
 GVKPLILRDCSVAGWLLGNPMCMDEFINPEWSYIVEKANPTNDLCYPGSFNDYEELKHLISRINHFEKI
 QIIPKSSWSDHEASSGVSSACPYLGSFSFFRNVLVWL IKKNSTYPTIKKS YNNTNQEDLLVLWGIHHPND
 AAEQTRLYQNPTTYISIGTSTLNLQRLVPKIASTRSKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPFY

AYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNNRLVLATGLRNSP
 QRESRRKKRGLFGAIAAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKESTQKAIDVNTKNVNSIIDKMN
 TQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDN
 AKELGNGCFEFYHKCDNECMESIRNGTYNYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLA
 LAIMMAGLSLWMCNLSLQCRICI*
H5Ind(F393D).r (SEQ ID NO: 112)
 CTTCCAACGGCCTCGTCCTGAGTGTTTCATTTGTCAATGATTGAGTTGA
H5Ind(F393D).c (SEQ ID NO: 113)
 CAAATGAACACTCAGGACGAGGCCGTTGGAAGGGAATTTAATAACTTA
PDI-H5 Indo-F393D DNA (SEQ ID NO: 114)
 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTC
 GCGGATCAGATTTGCATTGGTTACCATGCAAACAATTCAACAGAGCAGGTTGACACAATCATGGAAAAG
 AACGTTACTGTTACACATGCCCCAAGACATACTGAAAAGACACACAACGGGAAGCTCTGCGATCTAGAT
 GGAGTGAAAGCCTCTAATTTTAAGAGATTGTAGTGTAGCTGGATGGCTCCTCGGGAACCCAAATGTGTGAC
 GAATTCATCAATGTACCGGAATGGTCTTACATAGTGGAGAAGGCCAATCCAACCAATGACCTCTGTTAC
 CCAGGGAGTTTCAACGACTATGAAGAAGTCAAACACCTATTGAGCAGAATAAACCAATTTTGAGAAAAAT
 CAAATCATCCCCAAAAGTTCTTGGTCCGATCATGAAGCCTCATCAGGAGTTAGCTCAGCATGTCCATAC
 CTGGGAAGTCCCTCCTTTTGTAGAAATGTGGTATGGCTTATCAAAAAGAAGTACATACCCAACAATA
 AAGAAAAGCTACAATAATACCAACCAAGAGGATCTTTTGGTACTGTGGGAATTCACCATCCTAATGAT
 GCGGCAGAGCAGACAAGGCTATATCAAAACCAACCACTATATTTCCATTGGGACATCAACACTAAAC
 CAGAGATTGGTACCAAAATAGCTACTAGATCCAAAGTAAACGGGCAAGTGGAAGGATGGAGTTCTTTC
 TGGACAATTTTAAACCTAATGATGCAATCAACTTCGAGAGTAATGGAATTTTCATTGCTCCAGAATAT
 GCATACAAAATTTGTCAGAAAGGGGCTCAGCAATTATGAAAAGTGAATTGGAAATGTTGTAATGCAAC
 ACCAAGTGTCAAACCTCAATGGGGGCGATAAACTCTAGTATGCCATTCCACAACATACACCTCTCACC
 ATCGGGGAATGCCCAAAATATGTGAATCAAACAGATTAGTCTTGCAACAGGGCTCAGAAATAGCCCT
 CAAAGAGAGAGCAGAAGAAAAAGAGAGGACTATTTGGAGCTATAGCAGGTTTATAGAGGGAGGATGG
 CAGGGAATGGTAGATGGTTGGTATGGGTACCAACCATAGCAATGAGCAGGGGAGTGGGTACGCTGCAGAC
 AAAGAATCCACTCAAAGGCAATAGATGGAGTCACCAATAAGGTCAACTCAATCATTGACAAAATGAAC
 ACTCAGGACGAGGCCGTTGGAAGGGGAATTTAATAACTTAGAAAGGAGAAATAGAGAATTTAAACAAGAAG
 ATGGAAGACGGGTTTCTAGATGTCTGGACTTATAATGCCGAATCTCTGGTTCTCATGGAATAAGAGAGA
 ACTCTAGACTTTTATGACTCAAATGTTAAGAACCTCTACGACAAAGGTCGACTACAGCTTAGGGATAAT
 GCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCTATCACAATGTGATAATGAATGTATGGAAGATATA
 AGAAACGGAACGTACAACATATCCGCAGTATTGAGAAGAAGCAAGATTAAAAAGAGAGGAAATAAGTGGG
 GTAAAAATTGGAATCAATAGGAACCTACCAATAGTGTCAATTTATTCAACAGTGGCGAGTTCCCTAGCA
 CTGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGTTACAATGCAGAATTTGC
 ATTTAA
PDI-H5 Indo-F393D AA (SEQ ID NO: 115)
 MAKNVIAIFGLLFSLVLVPSQIFADQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCIDL
 GVKNLILRDCSVAGWLLGNPMCDEFINVPWYSYIVEKANPTNDLCYPGSFNDYEECLKHLLSRINHFEKI
 QIIPKSSWSDHEASSGVSSACPYLGSFSPFFRNVVWL IKKNSTYPTIKKSYNNTNQEDLLVLWGIHHPND
 AAEQTRL YQNPTTYISIGTSTLNLQRLVPKIATRSKVNQSGRMEFFWTILKPNDAINFESNGNFIAPY
 AYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNNRLVLATGLRNSP
 QRESRRKKRGLFGAIAAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKESTQKAIDVNTKNVNSIIDKMN
 TQDEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDN
 AKELGNGCFEFYHKCDNECMESIRNGTYNYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLA
 LAIMMAGLSLWMCNLSLQCRICI*
IF-H5 Egy.r (SEQ ID NO: 116)
 ACTAAAGAAAATAGGCCTTTAATGCAAAATCTGCATTGTAGCGATCCATT
PDI-H5 Egypt DNA (SEQ ID NO: 117)
 ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTTGTGTTGGTTCCTTCTCAGATCTTC
 GCGGATCAGATTTGCATTGGTTACCATGCAAACAACCTCGACAGAGCAGGTTGACACAATAATGGAAAAG
 AATGTCACCTGTTACACACGCCCAAGACATACTGGAAAAGACACACAACGGGAAACTCTGCAATCTAGAT
 GGAGTGAAAGCCTCTCATTTTGAGAGATTGTAGTGTAGCTGGATGGCTCCTCGGGAACCCAAATGTGCGAT
 GAATTCCTCAATGTGCCGGAATGGTCTTACATAGTGGAGAAAATCAATCCAGCCAATGACCTCTGTTAT
 CCAGGGAATTTCAACGACTATGAAGAAGTCAAACACCTATTGAGCAGAATAAACCAATTTTGAGAAAAAT
 CAGATCATTCCCAAGATTTCTTGGTCAGATCATGAAGCCTCGGGAGTGAGCTCAGCATGCCCATACCAA
 GGAAGATCCTCCTTTTGTAGAAATGTTGTATGGCTTACCAAAAAGAAGCATGCATACCCAACAATAAAG
 AAAAGTTACAATAATACTAACCAAGAAGATCTTTTGGTACTATGGGGGATTCACCATCCAATGATGCT
 GCAGAGCAGACAAGGCTTTATCAAAACCAACTACCTATATCTCCGTTGGGACATCAACACTAAACCAAG
 AGATTGGTACCCAAAATAGTACTAGATCTAAGGTAAACGGGCAAGTGGAAGGATGGAGTTCTTTTGG
 ACAATTTTAAATCGAATGATGCAATAAATTTGAGAGCAATGGAACTTCATTGCTCCAGAAAATGCA
 TACAAAATTGTCAGAAAGGAGATTCAACAATATGAAAAGTGAGTTGGAATATAGTAACCTGCAACACC
 AAGTGTGAGACTCCAATAGGGGCGATAAACTCCAGTATGCCATTCCACAACATCCACCTCACCACATC
 GGGGAATGCCCAATATGTGAATCAAACAGATTAGTCTTGTACTGGGCTCAGGAATAGCCCTCAA

GGAGAGAAAAGAAGAAAAAGAGAGGACTATTTCGGAGCCATAGCAGGCTTTATAGAGGGAGGATGGCAG
GGAATGGTAGATGGTTGGTATGGGTACCACTAGCAACGAGCAGGGGAGTGGGTACGCTGCAGACAAA
GAATCCACTCAAAGGGCTATAGATGGAGTCACCAATAAGGTCAATTCGATCATTGACAAAATGAACACT
CAGTTTGAGGCTGTTGGAAGGGAATTAATAACTTAGAAAGGAGAATAGAAAATTTAAACAAGAAGATG
5 GAAGACGGATTCTAGATGTCTGGACTTATAATGCTGAACCTTCTGGTTCATGGAAAATGAGAGAACT
CTAGACTTTTCATGACTCAAATGTCAAGAATCTTTATGACAAGGTCCGACTACAGCTTAGGGATAATGCA
AAGGAGCTTGGTAACGGTTGTTTCGAGTTCTATCACAGATGTGATAATGAATGTATGGAAAGTGTAAGA
AACGGAACGTATGACTACCTCAATATTCAGAAGAAGCAAGATTAAGGAGAGAGGAAATAAGTGGAGTA
AAATTGGAGTCAATAGGAACTTACCAATACTGTCAATTTATTCAACAGTGGCGAGCTCCCTAGCACTG
10 GCAATCATGGTGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGCTACAATGCAGAATTTGCATT
TAA

PDI-H5 Egypt AA (SEQ ID NO: 118)

MAKNVAIFGLLFSLLVLVPSQIFADQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCNLD
GVKPLILRDCSVAGWLLGNPMCDFLNVPEWSYIVEKINPANDLCYPGNFNDYEELKHLLSRINHFEKI
15 QIIPKDSWSDHEASGVSSACPYQGRSSFFRNVVWLTKKNDAYPTIKKS YNNTNQEDLLVLWGIIHPNDA
AEQTRL YQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSGRMEFFWTILKSNDAINFESNGNFIAPENA
YKIVKKGDSTIMKSELEYSNCNTKCQTPIGAINSSMPFHNHPLTIGEC PKYVKS NRLVLATGLRNS PQ
GEKRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKESTQRAIDGVTNKVNSIIDKMNT
QFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELVLMMENERTLDFHDSNVKNLYDKVRLQLRDNA
20 KELGNGCFEFYHRCNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLAL
AIMVAGLSLWMCNNGSLQCRICI*

H5Egy(F392D).r (SEQ ID NO: 119)

CTTCCAACAGCCTCGTCCGAGTGTTTCATTTTGTCAATGATCGAATTGA

H5Egy(F392D).c (SEQ ID NO: 120)

25 CAAAATGAACACTCAGGACGAGGCTGTTGGAAGGGAATTTAATAACTTA

PDI-H5 Egypt-F392D DNA (SEQ ID NO: 121)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTCTTCTCAGATCTTTC
GCGGATCAGATTTGCAATTGGTTACCATGCAACAACCTCGACAGAGCAGGTTGACACAATAATGGAAAAG
AATGTCACCTGTTACACACGCCAAGACATACTGAAAAGACACACAACGGGAACTCTGCAATCTAGAT
30 GGAGTGAGCCTCTCATTTTGAGAGATTGTAGTGTAGCTGGATGGCTCCTCGGGAAACCCAAATGTGCGAT
GAATTCCTCAATGTGCCGAATGGTCTTACATAGTGGAGAAAATCAATCCAGCCAAATGACCTCTGTTAT
CCAGGGAATTTCAACGACTATGAAGAAGTGAACACCTATTTGAGCAGAAATAAACCATTTTGAGAAAAT
CAGATCATTTCCCAAAGATTCTTGGTCAGATCATGAAGCCTCGGGAGTGAGCTCAGCATGCCCATACCAA
GGAAGATCCTCCTTTTTTAGAAATGTTGTATGGCTTACCAAAAAGAACGATGCATACCCAACAATAAAG
35 AAAAGTTACAATAATACTAACAAGAGATCTTTTGGTACTATGGGGGATTACCATGCAATGATGCT
GCAGAGCAGACAAGGCTTTATCAAAACCCAACTACCTATATCTCCGTTGGGACATCAACACTAAACCAG
AGATTGTTACCCAAAATAGCTACTAGATCTAAGGTAACGGGCAAGTGGAAGGATGGAGTTCTTTTTGG
ACAATTTTAAATCGAATGATGCAATAAATTTGAGAGCAATGGAACTTCATTGCTCCAGAAAATGCA
TACAAAATTTGTCAGAAAAGGAGATTCAACAATTTATGAAAAGTGAGTTGGAATATAGTAACCTGCAACACC
40 AAGTGTGAGACTCCAATAGGGGCGATAAATCCAGTATGCCATTCCACAACATCCACCCTCTCACCATC
GGGGAATGCCCAAATATGTGAAATCAAACAGATTAGTCTTGTACTGGGCTCAGGAATAGCCCTCAA
GGAGAGAAAAGAAGAAAAAGAGAGGACTATTCCGAGCCATAGCAGGCTTTATAGAGGGAGGATGGCAG
GGAATGGTAGATGGTTGGTATGGGTACCACTAGCAACGAGCAGGGGAGTGGGTACGCTGCAGACAAA
GAATCCACTCAAAGGGCTATAGATGGAGTCACCAATAAGGTCAATTCGATCATTGACAAAATGAACACT
45 CAGGACGAGGCTGTTGGAAGGGAATTTAATAACTTAGAAAGGAGAATAGAAAATTTAAACAAGAAGATG
GAAGACGGATTCTAGATGTCTGGACTTATAATGCTGAACCTTCTGGTTCTCATGGAAAATGAGAGAACT
CTAGACTTTTCATGACTCAAATGTCAAGAATCTTTATGACAAGGTCCGACTACAGCTTAGGGATAATGCA
AAGGAGCTTGGTAACGGTTGTTTCGAGTTCTATCACAGATGTGATAATGAATGTATGGAAAGTGTAAGA
AACGGAACGTATGACTACCTCAATATTCAGAAGAAGCAAGATTAAGGAGAGAGGAAATAAGTGGAGTA
50 AAATTGGAGTCAATAGGAACTTACCAATACTGTCAATTTATTCAACAGTGGCGAGCTCCCTAGCACTG
GCAATCATGGTGGCTGGTCTATCTTTATGGATGTGCTCCAATGGATCGCTACAATGCAGAATTTGCATT
TAA

PDI-H5 Egypt-F392D AA (SEQ ID NO: 122)

MAKNVAIFGLLFSLLVLVPSQIFADQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCNLD
55 GVKPLILRDCSVAGWLLGNPMCDFLNVPEWSYIVEKINPANDLCYPGNFNDYEELKHLLSRINHFEKI
QIIPKDSWSDHEASGVSSACPYQGRSSFFRNVVWLTKKNDAYPTIKKS YNNTNQEDLLVLWGIIHPNDA
AEQTRL YQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSGRMEFFWTILKSNDAINFESNGNFIAPENA
YKIVKKGDSTIMKSELEYSNCNTKCQTPIGAINSSMPFHNHPLTIGEC PKYVKS NRLVLATGLRNS PQ
GEKRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKESTQRAIDGVTNKVNSIIDKMNT
60 QDEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELVLMMENERTLDFHDSNVKNLYDKVRLQLRDNA
KELGNGCFEFYHRCNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLAL
AIMVAGLSLWMCNNGSLQCRICI*

PDI-H1 Par-F390D+L429M (nt) (SEQ ID NO: 123)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTCTTCTCAGATCTTTC

GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
GGGGTAGCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGATCATGGTCTACATTGTGGAACATCTAATTGAGACAATGGAACGTGTTAC
5 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTT
GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCT
CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAAACCTACATTATGATAAAGGGAAAGAGTCTCTGCTGTGGGGCATTCACCATCCATCT
10 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTTCGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
AATACAACTTGTGAGACACCCGAGGTGCTATAAACACCAAGCCTCCCATTTGAGAATGTGCATCCGATC
ACAATTGGAAAATGTCCAAGTATGTAAGAACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
15 CCGTCTATTCAATCTAGAGGCCATTCGCGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAATGCCATTGACAGATTACTAACAAGTAAATTCGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTTCTGGACATTTGGACTTACATGCGCAACTGTTGGTTCTAATGGAATGAAAGAATCTTGGAC
20 TATCAGGATTCAAATGTGAAGAACTTGATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
25 **PDI-H1 Par-F390D+L429M (aa) (SEQ ID NO: 124)**
MAKNVAIFGLLFLSLVLPVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLR
GVAPLHLGKCNIAWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVVSFFERF
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNYPKLNQTYINDKGKEVLVLWGIHHP
TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVPR
30 YAFTMERNAGSGIIISDTPVHDCNNTCQTPEGAINSTLFPQNVHPITIGKCPKYVKSTKLRLATGLRNV
PSIQSRGLFGAIGFIEGWTGMVDGWYGYHHQNEQSSGYAADLKSTQNAIDKIYNKVNSTVEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNLKNNAKE
IGNGCFEYHKCDNTCMESVKNGTYDYPKYSEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

35 **PDI-H1 Par-K374E+F390D+L429M (nt) (SEQ ID NO: 125)**
ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
GGGGTAGCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
40 TCACTCTCCACAGCAAGATCATGGTCTTACATTGTGGAACATCTAATTGAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAAGTTT
GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCT
CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAAACCTACATTAATGATAAAGGGAAAGAGTCTCTGCTGTGGGGCATTCACCATCCATCT
45 ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTTCGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTGAGATACACCAAGTCCACGATTGC
AATACAACCTTGTGAGACACCCGAGGTGCTATAAACACCAAGCCTCCCATTTGAGAATGTGCATCCGATC
50 ACAATTGGAAAATGTCCAAGTATGTAAGAACACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCCATTCGCGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAGTAAATTCGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATCTAAATAAAAAAGTTGATGAT
55 GGTTTTCTGGACATTTGGACTTACAAATGCGCAACTGTTGGTTCTAATGGAATGAAAGAATCTTGGAC
TATCAGGATTCAAATGTGAAGAACTTGATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
60 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
PDI-H1 Par-K374E+F390D+L429M (aa) (SEQ ID NO: 126)
MAKNVAIFGLLFLSLVLPVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLR
GVAPLHLGKCNIAWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVVSFFERF
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNYPKLNQTYINDKGKEVLVLWGIHHP
65 TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYWTLVPEPGDKITFEATGNLVPR

YAFTEMRNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNVHPITIGKCPKYVKSTKLRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERTLDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFFEFYHKCDNTCMESVKNGTYDYPKYSEEAALNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

PDI-H1 Par-N97D+F390D+L429M (nt) (SEQ ID NO: 127)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACATATGCAAACTAAGA
GGGGTAGCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGATCATGGTCTTACATTGTGGAAACATCTAATTGAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATTTTGAAGGTTT
GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTCT
CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAAACTTACATTAATGATAAAGGAAAGAGTCTCTGCTGTGGGGCATTACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAAAATACATTGCAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAAGATACACAGTCCACGATTGC
AATAACAATTGTGAGACACCCGAGGGTGCTATAAACACACAGCTCCCATTTGAGAATGTGCATCCGATC
ACAATTTGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTTGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCCATTTCCGGGGCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACAAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAGTGAAGAACTTTGGAC
TATCACGATTCAATGTGAAGAATTTGTATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGCTGCTTTGAATTTTACCACAAATGCCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACACAGAGAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCCGAGTTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTTAATGGGTCTTACAGTGTAGAATATGTATTTAA

PDI-H1 Par-N97D+F390D+L429M (aa) (SEQ ID NO: 128)

MAKNVAIFGLLFLSLLVLPVPSQIFADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLDKHNGKCLKLR
GVAPLHLGKCNIAWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVVSFFERF
ELFPKTSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQTYINDKKEVLVLWGIIHPS
YTADQQSLSYQADAYVVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYWTLVEPGDKITFEATGNLVVPR
YAFTEMRNAGSGIIISDTPVHDCNTTCQTPEGAINSTLFFQNVHPITIGKCPKYVKSTKLRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERTLDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFFEFYHKCDNTCMESVKNGTYDYPKYSEEAALNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

PDI-H1 Par-N97D+K374E+F390D+L429M (nt) (SEQ ID NO: 129)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTGGTTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACATATGCAAACTAAGA
GGGGTAGCCCCATTGCATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACTCTCCACAGCAAGATCATGGTCTTACATTGTGGAAACATCTAATTGAGACAATGGAACGTGTTAC
CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATTTTGAAGGTTT
GAGATATTTCCCAAGACAAGTTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCTCT
CACGCTGGAGCAAAAAGCTTCTACAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
CTTAACCAAACTTACATTAATGATAAAGGAAAGAGTCTCTGCTGTGGGGCATTACCATCCATCT
ACTACTGCTGACCAACAAAGTCTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAAAATACATTGCAAGCAACTGGAAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAAGATACACAGTCCACGATTGC
AATAACAATTGTGAGACACCCGAGGGTGCTATAAACACACAGCTCCCATTTGAGAATGTGCATCCGATC
ACAATTTGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTTGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCCATTTCCGGGGCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAGTGAAGAACTTTGGAC
TATCACGATTCAATGTGAAGAATTTGTATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGCTGCTTTGAATTTTACCACAAATGCAGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACACAGAGAAAAATAGATGGGGTAAAGCTG

GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTGGTAGTC
TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

A/California/7/09 (H1N1) (aa) (SEQ ID NO: 130) GenBank: FJ969540.1

DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLRGVAPLHLGKCNIAGWILGNPECES
LSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
AGAKSFYKNIWLKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQSLYQNADAYVFGSSRYS
KKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERNAGSGIIISDTPVHDCN
TTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRGLFGAIAAGFIEGGWTGMV
DGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRSQKLNNAKEIGNGCFFEFYHKCDNTCMESVKNGT
YDYPKYSEEAKLNREEIDGVKLESTRIYQILAIYSTVASSLVLVVS LGAISFWMCSNGSLQCRICI

A/Honduras/17734/16 (H1N1) (aa) (SEQ ID NO: 131)

DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLRGVPPPLHLGKCNIAGWILGNPECEP
LSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
AGAKSFYKNIWLKKGNSYPKLSQSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFGTSRYS
KKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFMTMERNAGSGIIISDTPVHDCN
TTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNVPISIQSRGLFGAIAAGFIEGGWTGMV
DGWYGYHHQNEQSGGYAADQKSTQSAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKLNNAKEIGNGCFFEFYHKCDNTCMESVKNGT
YDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVVS LGAISFWMCSNGSLQCRICI

A/Darwin/11/15 (H1N1) (aa) (SEQ ID NO: 132)

DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLRGVAPLHLGKCNIAGWILGNPECES
LSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
AGAKSFYKNIWLTKKGNSYPKLSQSYINDKGKEILVLWGIHHPSTTADQQSLYQNADAYVFGTSRYS
KKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFMTMERNAGSGIIISDTPVHDCN
TTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNVPISIQSRGLFGAIAAGFIEGGWTGMV
DGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKLNNAKEIGNGCFFEFYHKCDNTCMESVKNGT
YDYPKYSGEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVVS LGAISFWMCSNGSLQCRICI

A/Costa Rica/0513/16 (H1N1) (aa) (SEQ ID NO: 133)

DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLRGVAPLHLGKCNIAGWILGNPECES
LSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
AGAKSFYKNIWLKKGNSYPKLNQSYVNDKGKEVLVLWGIHHPPTTADQQSLYQNADAYVFGTSKYS
KKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFMTMERNAGSGIIISDTPVHDCN
TTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNVPISIQSRGLFGAIAAGFIEGGWTGMV
DGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKLNNAKEIGNGCFFEFYHKCDNTCMESVKNGT
YDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVVS LGAISFWMCSNGSLQCRICI

A/Michigan/45/15 (H1N1) (aa) (SEQ ID NO: 134) GenBank: KY117023.1

DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLRGVAPLHLGKCNIAGWILGNPECES
LSTASSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
AGAKSFYKNIWLKKGNSYPKLNQSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFGTSRYS
KKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFMTMERNAGSGIIISDTPVHDCN
TTCQTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNVPISIQSRGLFGAIAAGFIEGGWTGMV
DGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKLNNAKEIGNGCFFEFYHKCDNTCMESVKNGT
YDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVVS LGAISFWMCSNGSLQCRICI

A/Massachusetts/06/17 (H1N1) (aa) (SEQ ID NO: 135)

DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGKLCCLRGVAPLHLGKCNIAGWILGNPECES
LSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
AGAKSFYKNIWLKKGNSYPKLNQSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFGTSRYS
KKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFMTMERNAGSGIIISDTPVHDCN
TTCQTPEGAINTSLPFQNVHPITIGKCPKYVKSTKLRLATGLRNVPISIQSRGLFGAIAAGFIEGGWTGMV
DGWYGYHHQNEQSGGYAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKLNNAKEIGNGCFFEFYHKCDNTCMESVKNGT
YDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVVS LGAISFWMCSNGSLQCRICI

A/Michigan/45/15 (H1N1) (nt) (SEQ ID NO: 136) GenBank: KY117023.1

ATGAAGGCAATACTAGTAGTTCTGCTATATACATTTACAACCGCAAATGCAGACACATTATGTATAGGT
TATCATCGGAACAAATCAACAGACACTGTAGACACAGTACTAGAAAAGAATGTAACAGTAACACACTCT
GTTAACCTTCTGGAAGACAAGCATAACGGAAACTATGCAAACCTAAGAGGGGTAGCCCCATTGCATTTG
GGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAATCACTCTCCACAGCAAGTTCA
TGGTCTTACATTGTGGAACATCTAATTACAGACAATGGAACGTGTTACCCAGGAGATTTTCATCAATTAT

GAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATCATTGAAAGGTTTGGAGATATCCCCAAGACAAGT
 TCATGGCCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCTCACGCTGGAGCAAAAAGCTTC
 TACAAAAAAGCTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAGCTTAACCAATCCTACATTAAT
 5 GATAAAGGGAAAGAGTCCCTCGTGCTGTGGGGCATTACCATCCATCTACTACTGCTGACCAACAAAGT
 CTCTATCAGAATGCAGATGCATATGTTTTGTGGGGACATCAAGATACAGCAAGAAGTTCAAGCCGGAA
 ATAGCAACCAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACTATTACTGGACACTAGTAGAGCCG
 GGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCAGATATGCATTCACAATGGAAGA
 AATGCTGGATCTGGTATTATCATTTTCAGATACACCAAGTCCACGATTGCAATACAACCTTGTTCAGACACCC
 10 GAGGGTGCTATAAACACCAAGCCTCCCATTTTCAGAATATACATCCGATCACAATGGAAAATGTCCAAAG
 TATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTTCCGCTCATTTCAATCTAGAGGC
 CTATTTCGGGGCCATTGCGCGGCTTCATTGAAGGGGGGTGGACAGGGATGGTAGATGGATGGTACGGTTAT
 CACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGCACACAAAATGCCATTGACAAG
 ATTACTACAAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGTTTCACAGCAGTGGGTAAAGAGTTTC
 AACCACCTGGAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGATGGTTTCCCTGGACATTTGGACT
 15 TACAATGCCGAACCTGTTGGTTCTATTGGAAAATGAAAGAACTTTGGACTATCACGATTCAAAATGTGAAG
 AACTTGTATGAAAAAGTAAGAAACAGTTAAAAAACAAATGCCAAGGAAATTTGGAACCGGCTGCTTTGAA
 TTTTACCACAAATGCGATAACACGTCATGGAAAGTGTCAAAAATGGGACTTATGACTACCCAAAGATAC
 TCAGAGGAAGCAAAATTAACAGAGAAAAATAGATGGGGTAAAGCTGGAATCAACAAGGATTTACCAG
 ATTTTGGCGATCTATTCAACTGTGCGCCAGTTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGCTTC
 20 TGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
A/California/7/09 (H1N1) (nt) (SEQ ID NO: 137) GenBank: FJ969540.1
 ATGAAGGCAATACTAGTAGTTCTGTATATACATTTGCAACCGCAAATGCAGACACATTATGTATAGGT
 TATCATGCGAACAAATTCACAGACACTGTAGACACAGTACTAGAAAAGAATGTAACAGTAACACACTCT
 GTTAACCTTCTAGAAGACAAGCATAACGGGAACTATGCAAACTAAGAGGGGTAGCCCCATTGCATTTG
 25 GGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAATCACTCTCCACAGCAAGAGTTC
 TGGTCTTACATTGTGGAACACCTAGTTTCAGACAATGGAACGTGTTACCCAGGAGATTTTCATCGATTAT
 GAGGAGCTAAGAGAGCAATTGAGCTCAGTGTATCATTTGAAAGGTTTGGAGATATTTCCCAAGACAAGT
 TCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGTCCTCATGCTGGAGCAAAAAGCTTC
 TACAAAATTTAATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAGCTCAGCAAAATCCTACATTAAT
 30 GATAAAGGGAAAGAAGTCTCGTGCTATGGGGCATTACCATCCATCTACTAGTGTGACCAACAAAGT
 CTCTATCAGAATGCAGATGCATATGTTTTGTGGGGTCATCAAGATACAGCAAGAAGTTCAAGCCGGAA
 ATAGCAATAAGACCCAAAGTGAGGGRTCRAGAAGGGGAGAATGAACTATTACTGGACACTAGTAGAGCCG
 GGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCAGATATGCATTGCAATGGAAAGA
 AATGCTGGATCTGGTATTATCATTTTCAGATACACCAAGTCCACGATTGCAATACAACCTTGTCAACACCC
 35 AAGGGTGCTATAAACACCAAGCCTCCCATTTTCAGAATATACATCCGATCACAATTTGGAATGTCAAAAA
 TATGTAAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATATCCCGTCTATTCAATCTAGAGGC
 CTATTTGGGGCCATTGCGCGGTTTCATTGAAGGGGGGTGGACAGGGATGGTAGATGGATGGTACGGTTAT
 CACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGCACACAGAATGCCATTGACGAG
 ATTTACTAACAAAGTAAATTTCTGTTATTGAAAAGATGAATACACAGTTTCACAGCAGTAGGTTAAAGAGTTC
 40 AACCACTGGAAAAAAGAAATAGAGATTTAAATAAAAAAGTTGATGATGGTTTCCCTGGACATTTGGACT
 TACAATGCCGAACCTGTTGGTTCTATTGGAAAATGAAAGAACTTTGGACTACCACGATTCAAAATGTGAAG
 AACTTATATGAAAGGTAAGAAGCCAGCTAAAAAACAAATGCCAAGGAAATTTGGAACCGGCTGCTTTGAA
 TTTTACCACAAATGCGATAACACGTCATGGAAAGTGTCAAAAATGGGACTTATGACTACCCAAAATAC
 TCAGAGGAAGCAAAATTAACAGAGAAAAATAGATGGGGTAAAGCTGGAATCAACAAGGATTTACCAG
 45 ATTTTGGCGATCTATTCAACTGTGCGCCAGTTTCATTGGTACTGGTAGTCTCCCTGGGGGCAATCAGTTTC
 TGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

A/Paris/1227/2017 (H1N1) (aa) (SEQ ID NO: 138)
 DTLICIGYHANNSTDTVDTVLEKNVTVTHSVNLLDKHNGKLCCLRGVAPLHLGKNCIAGWILGNPECES
 LSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
 50 AGAKSFYKNLIWLVKKGNSYPKLNQTYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFVGTSTRYS
 KKFKEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFTMERNAGSGIIISDTPVHDCN
 TTCQTPEGAINTSLPFQNVHPITIGKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAIAAGFIEGGWTGMV
 DGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
 55 FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGT
 YDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVSLGAISFWMCNLSLQCRICI

A/Norway/2147/2017 (H1N1) (aa) (SEQ ID NO: 139)
 DTLICIGYHANNSTDTVDTVLEKNVTVTHSVNLLDKHNGKLCCLRGVAPLHLGKNCIAGWILGNPECES
 LSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPH
 60 AGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFVGTSTRYS
 KKFKEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFTMERNAGSGIIISDTPVQDCN
 TTCQTPEGAINTSLPFQNVHPITIGKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAIAAGFIEGGWTGMV
 DGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDG
 FLDIWTYNAELLVLENERLTDYHDSNVKNLYEKVRNQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGT
 YDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVSLGAISFWMCNLSLQCRICI

PDI-H1 Par-N97D+K374E+F390D+L429M (aa) (SEQ ID NO: 140)

MAKNVAIFGLLFSLLVLPVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVVSFFERF
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQTYINDKGKEVLVLWGIHHP
 5 TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINTSLEFFQNVHPITIGKCPKYVKSTKLRLATGLRNV
 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSYAADLKSTQNAIDEITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLDYHDSNVKNLYEKVRNQLKNNAKE
 10 IGNGCFEYFHKCDNTCMESVKNGTYDYPKYSEEAKNREKIDGVKLESTRIYQILAIYSTVASSLLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Nor-F390D+L429M (nt) (SEQ ID NO: 141)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
 15 GGGGTAGCCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGATCATGGTCTTACATTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTACGGCAGCATGTCTT
 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 20 CTTAACCAATCC TACATTAATGATAAAGGGAAGAAGTCTCTGCTGTGGGCGATTCCACATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACAGTCCAGGATTGC
 25 AATACAACCTTGTGAGACACCCGAGGGTCTATAAACACCAGCCTCCCATTTTCAGAAATGTGCATCCGATC
 ACAATTTGGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACAAGATTACTAACAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
 30 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 GGTTCCTGGACATTGTTGACTTACAATGCCGAACCTGTTGGTTCTAATGGAATGAAAGAACTTTGGAC
 TATCACGATTCAAATGTGAAGAACTTGTATGAAAAAGTAAGAAACAGTTAAAAACAATGCCAAGGAA
 ATTTGGAACGGCTGCTTTGAATTTTACCACAAATGCCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 35 ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTAGTC
 TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA

PDI-H1 Nor-F390D+L429M (aa) (SEQ ID NO: 142)

MAKNVAIFGLLFSLLVLPVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNGLCKLR
 40 GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVVSFFERF
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHP
 TTADQQSLYQNADAYVFVGTSTRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
 YAFTMERNAGSGIISDTPVQDCNTTCQTPEGAINTSLEFFQNVHPITIGKCPKYVKSTKLRLATGLRNV
 45 PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSYAADLKSTQNAIDKITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEYFHKCDNTCMESVKNGTYDYPKYSEEAKNREKIDGVKLESTRIYQILAIYSTVASSLLVLVV
 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Nor-K374E+F390D+L429M (nt) (SEQ ID NO: 143)

ATGGCGAAAAACGTTGCGATTTTCGGCTTATTGTTTTCTCTTCTGTGTGGTTCCTTCTCAGATCTTC
 GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAAG
 AATGTAACAGTAACACACTCTGTAACTTCTGGAAGACAAGCATAACGGAAAACTATGCAAACTAAGA
 50 GGGGTAGCCCCATTCGATTTGGGTAAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
 TCACTCTCCACAGCAAGATCATGGTCTTACATTGTGGAACATCTAATTCAGACAATGGAACGTGTTAC
 CCAGGAGATTTTCATCAATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTATTTGAAAGGTTT
 GAGATATTTCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTACGGCAGCATGTCTT
 55 CACGCTGGAGCAAAAAGCTTCTACAAAAACTTGATATGGCTAGTTAAAAAAGGAAATTCATACCCAAAG
 CTTAACCAATCC TACATTAATGATAAAGGGAAGAAGTCTCTGCTGTGGGCGATTCCACCATCCATCT
 ACTACTGCTGACCAACAAAGTCTCTATCAGAAATGCAGATGCATATGTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 60 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
 TATGCATTTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTTCAGATACACAGTCCAGGATTGC
 AATACAACCTTGTGAGACACCCGAGGGTCTATAAACACCAGCCTCCCATTTTCAGAAATGTGCATCCGATC
 ACAATTTGAAAAATGTCCAAAGTATGTAAAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATTTCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG

GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTCAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACTTTGGAC
5 TATCAGCATCAAAATGTGAAGAACTTGTATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
ATTGGAACGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTTGGTACTGGTAGTC
TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
10 **PDI-H1 Nor-K374E+F390D+L429M (aa) (SEQ ID NO: 144)**
MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFINYEELREQLSSVSSFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHPS
TTADQQSLSYQNADAYVFGTSRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
15 YAFTMERNAGSGIIISDTPVQDCNTTCQTPEGAINTSLFPQNVHPITIGKCPKYVKSTKRLRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFFEYHKCDNTCMESVKNGTYDYPKYSEEAKNLREKIDGVKLESTRIYQILAIYSTVASSLVLVV
SLGAISFWMCSNGSLQCRICI*

20 **PDI-H1 Nor-N97D+F390D+L429M (nt) (SEQ ID NO: 145)**
ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACATGCAAACTAAGA
GGGGTAGCCCATTTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
25 TCACCTCCACAGCAAGATCATGGTCTTACATTGTGGAACATCTAATTGAGACATGGAACGTGTTC
CCAGGAGATTTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTCATTTTGAAGGTTT
GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTACGGCAGCATGTCTT
CACGCTGGAGCAAAAAGCTTCTACAAAACCTTGATATGGCTAGTTAAAAAGGAAATTCATACCCCAAAG
CTTAACCAATCC TACATTAATGATAAAGGGAAGAAGTCTCTGCTGTGGGGCATTCCACATCCATCT
30 ACTACTGCTGACCAACAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGACATCAAGATAC
AGCAAGAAGTTCAAGCCGGAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGAGAATGAACATAT
TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAATCTAGTGGTACCGAGA
TATGCATTCACAATGGAAGAAATGCTGGATCTGGTATTATCATTTTCAAGATACACAGTCCAGGATTGC
AATACAACCTGTGAGACACCCGAGGGTGTCTATAAACACCAGCCTCCCATTTGAGATGTGCATCCGATC
35 ACAATTTGAAAAATGTCCAAAGTATGTAAGAACACAAATGAGACTGGCCACAGGATTGAGGAATGTT
CCGTCTATTCAATCTAGAGGCCATTTCCGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
TATGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTGAGGATATGCAGCCGACCTGAAGAGC
ACACAAAATGCCATTGACAAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
40 GGTTTCTCGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACTTTGGAC
TATCAGATTCAATGTGAAGAACTTGTATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
ACTTATGACTACCCAAAATACTCAGAGGAAGCAAAATTAAACAGAGAAAAAATAGATGGGGTAAAGCTG
GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTTGGTACTGGTAGTC
45 TCCCTGGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
PDI-H1 Nor-N97D+F390D+L429M (aa) (SEQ ID NO: 146)
MAKNVAIFGLLFSLLVLVPSQIFADTLCIGYHANNSTDVDTVLEKNVTVTHSVNLLLEDKHNKGLCKLR
GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSNSDNGTCYPGDFIDYEELREQLSSVSSFERE
EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHPS
50 TTADQQSLSYQNADAYVFGTSRYSKKFKPEIATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPR
YAFTMERNAGSGIIISDTPVQDCNTTCQTPEGAINTSLFPQNVHPITIGKCPKYVKSTKRLRLATGLRNV
PSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADLKSTQNAIDKITNKVNSVIEKMNTQD
TAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
IGNGCFFEYHKCDNTCMESVKNGTYDYPKYSEEAKNLREKIDGVKLESTRIYQILAIYSTVASSLVLVV
55 SLGAISFWMCSNGSLQCRICI*

PDI-H1 Nor-N97D+K374E+F390D+L429M (nt) (SEQ ID NO: 147)
ATGGCGAAAAACGTTGCGATTTTCGGCTTATGTTTTCTCTTCTGTGTTGGTTCCTTCTCAGATCTTC
GCGGACACATTATGTATAGGTTATCATGCGAACAATTCAACAGACACTGTAGACACAGTACTAGAAAAG
AATGTAACAGTAACACACTCTGTTAACCTTCTGGAAGACAAGCATAACGGAAAACATGCAAACTAAGA
60 GGGGTAGCCCATTTGCATTTGGGTAATGTAACATTGCTGGCTGGATCCTGGGAAATCCAGAGTGTGAA
TCACCTCCACAGCAAGATCATGGTCTTACATTGTGGAACATCTAATTGAGACATGGAACGTGTTAC
CCAGGAGATTTCATCGATTATGAGGAGCTAAGAGAGCAATTGAGCTCAGTGTCTCATTTTGAAGGTTT
GAGATATTCCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTACGGCAGCATGTCTT
CACGCTGGAGCAAAAAGCTTCTACAAAACCTTGATATGGCTAGTTAAAAAGGAAATTCATACCCCAAAG

5 CTTAACCAATCCTACATTAATGATAAAGGGAAAGAAGTCCTCGTGCTGTGGGGCATTACCCATCCATCT
 ACTACTGCTGACCAACAAAAGTCTCTATCAGAATGCAGATGCATATGTTTTTGTGGGGACATCAAGATAC
 AGCAAGAAGTTCAAGCCGGAAATAGCAACAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATGAACATAT
 TACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAAATCTAGTGGTACCGAGA
 10 TATGCATTACAAATGGAAGAAATGCTGGATCTGGTATTATCATTTCAGATACACCAAGTCCAGGATTGC
 AATACAACTTGTGAGACACCCGAGGGTGCTATAAACACCAGCCTCCCATTTCAGAATGTGCATCCGATC
 ACAATTGGAAGAAATGTCCAAAGTATGTAAGAAAGCACAAAATTGAGACTGGCCACAGGATTGAGGAATGTT
 CCGTCTATTCAATCTAGAGGCCATATCGGGGCCATTGCCGGCTTCATTGAAGGGGGGTGGACAGGGATG
 GTAGATGGATGGTACGGTTATCACCATCAAAATGAGCAGGGGTCAGGATATGCAGCCGACCTGAAGAGC
 ACACAAAATGCCATTGACGAGATTACTAACAAAGTAAATTCTGTTATTGAAAAGATGAATACACAGGAC
 ACAGCAGTGGGTAAAGAGTTCAACCACCTGGAAAAAAGAATAGAGAATCTAAATAAAAAAGTTGATGAT
 15 GGTTTCTGGACATTTGGACTTACAATGCCGAACCTGTTGGTTCTAATGGAAAATGAAAGAACCTTGGAC
 TATCACGATTCAATGTGAAGAACCTGTATGAAAAAGTAAGAAACCAGTTAAAAACAATGCCAAGGAA
 ATTGGAACCGGCTGCTTTGAATTTTACCACAAATGCGATAACACGTGCATGGAAAGTGTCAAAAATGGG
 ACTTATGACTACCCAAAATCTCAGAGGAAGCAAAATTAACAGAGAAAAAATAGATGGGGTAAAGCTG
 GAATCAACAAGGATTTACCAGATTTTGGCGATCTATTCAACTGTCGCCAGTTTCATTGGTACTGGTATGC
 TCCCTGGGGCAATCAGCTTCTGGATGTGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTAA
PDI-H1 Nor-N97D+K374E+F390D+L429M (aa) (SEQ ID NO: 148)
 20 MAKNVAI FGLLFSLVLVPSQIFADTL CIGYHANNSTDTVDTVLEKNVTVTHSVNILEDKHNKGLCKLR
 GVAPLHLGKCNIAGWILGNPECESLSTARSWSYIVETSDNGTCYPGDFIDYEELREQLSSVS SFERF
 EIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWL VKKNSY PKLNQSYINDK GKEVLVLWG IHHPS
 TTADQQSLYQNADAYVFVGT SRYSKKFKPEIATRPKVRDQEGRMNYWT LVEPGDKITFEATGNLVVPR
 YFTMERNAGSGIIISDTPVQDCNTTCQTPEG AINTSLFFQNVHPITIGKCPKYVKSTKLRLATGLRNV
 25 PSIQSRGLFGAIA GFIEGGWTGMVDGWYGYHHQNEQSGSYAADL KSTQNAIDEITNKVNSVIEKMNTQD
 TAVGKEFNHLEKRIENLNKKVDDGFLDIW TYNAELLVLMENERLTDYHDSNVKNLYEKVRNQLKNNAKE
 IGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVKLESTRIYQILAIYSTVASSLVLVV
 SLGAISFWMCSNGSLQCRICI*

All citations are hereby incorporated by reference.

30 [00341] 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.

Claims

1. A nucleic acid comprising a nucleotide sequence encoding a modified influenza H1 hemagglutinin (HA) protein, the HA protein comprising an amino acid sequence comprising at least one substitution when compared to a corresponding wildtype amino acid sequence, said at least one substitution being at one or more than one amino acid corresponding to amino acid at position 97, 374, 390 or 429 of H1 A/Michigan/45/15 HA.
2. The nucleic acid of claim 1, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 97 of H1 A/Michigan/45/15 HA is to a non-asparagine.
3. The nucleic acid of claim 2, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 97 of the H1 A/Michigan/45/15 HA is to an aspartic acid or a conserved substitution of aspartic acid.
4. The nucleic acid of claim 1, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 374 of H1 A/Michigan/45/15 HA is to a non-lysine.
5. The nucleic acid of claim 4, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 374 of H1 A/Michigan/45/15 HA is to a glutamic acid or a conserved substitution of glutamic acid.
6. The nucleic acid of claim 1, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 390 of H1 A/Michigan/45/15 HA is to a non-phenylalanine.
7. The nucleic acid of claim 6, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 390 of H1 A/Michigan/45/15 HA is to an aspartic acid or a conserved substitution of aspartic acid.
8. The nucleic acid of claim 1, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 429 of H1 A/Michigan/45/15 HA is to a non-leucine.

9. The nucleic acid of claim 8, wherein the at least one substitution of the amino acid corresponding to the amino acid at position 429 of H1 A/Michigan/45/15 HA is to a methionine or a conserved substitution of methionine.

5 10. The nucleic acid of claim 8 or 9, further comprising a second substitution, said second substitution being at an amino acid corresponding to amino acid at position 380 of H1 A/Michigan/45/15 HA, said second substitution being to a non-asparagine.

11. The nucleic acid of claim 10, wherein the second substitution of the amino acid corresponding to amino acid at position 380 of H1 A/Michigan/45/15 HA is to an alanine or a conserved substitution of alanine.

10 12. The nucleic acid of claim 1, comprising
a first substitution at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA, said substitution being to a non-phenylalanine, and
a second substitution at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA, said substitution being to a non-leucine.

15 13. The nucleic acid of claim 12, wherein
the first substitution is to an aspartic acid or a conserved substitution of aspartic acid, and
the second substitution is to a methionine or a conserved substitution of methionine.

20 14. The nucleic acid of claim 1, comprising
a first substitution at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA, said substitution being to a non-asparagine, and
a second substitution at the amino acid corresponding to amino acid at position 374 of the H1 A/Michigan/45/15 HA, said substitution being to a non-lysine.

25 15. The nucleic acid of claim 14, wherein

the first substitution is to an aspartic acid or a conserved substitution of aspartic acid, and

the second substitution is to a glutamic acid or a conserved substitution of glutamic acid.

5 16. The nucleic acid of claim 1, comprising

a first substitution at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA, said substitution being to a non-asparagine,

a second substitution at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA, said substitution being to a non-phenylalanine, and

10 a third substitution at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA, said substitution being to a non-leucine.

17. The nucleic acid of claim 16, wherein

the first substitution is to an aspartic acid or a conserved substitution of aspartic acid,

15 the second substitution is to an aspartic acid or a conserved substitution of aspartic acid, and

the third substitution is to a methionine or a conserved substitution of methionine.

18. The nucleic acid of claim 1, comprising

20 a first substitution at the amino acid corresponding to amino acid at position 374 of H1 A/Michigan/45/15 HA, said substitution being to a non-lysine,

a second substitution at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA, said substitution being to a non-phenylalanine, and

a third substitution at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA, said substitution being to a non-leucine.

19. The nucleic acid of claim 18, wherein

the first substitution is to a glutamic acid or a conserved substitution of glutamic acid,

the second substitution is to an aspartic acid or a conserved substitution of aspartic acid, and

the third substitution is to a methionine or a conserved substitution of methionine.

20. The nucleic acid of claim 1, comprising

a first substitution at the amino acid corresponding to amino acid at position 97 of H1 A/Michigan/45/15 HA, said substitution being to a non-asparagine,

a second substitution at the amino acid corresponding to amino acid at position 374 of H1 A/Michigan/45/15 HA, said substitution being to a non-lysine,

a third substitution at the amino acid corresponding to amino acid at position 390 of H1 A/Michigan/45/15 HA, said substitution being to a non-phenylalanine, and

a fourth substitution at the amino acid corresponding to amino acid at position 429 of H1 A/Michigan/45/15 HA, said substitution being to a non-leucine.

21. The nucleic acid of claim 20, wherein

the first substitution is to an aspartic acid or a conserved substitution of aspartic acid,

the second substitution is to a glutamic acid or a conserved substitution of glutamic acid,

the third substitution is to an aspartic acid or a conserved substitution of aspartic acid, and

the fourth substitution is to a methionine or a conserved substitution of methionine.

22. The nucleic acid of any one of claim 3, 7, 13, 15, 17, 19 or 21, wherein the conserved substitution of aspartic acid is glutamic acid, glutamine, or serine.

5 23. The nucleic acid of claim 5, 15, 19 or 21 wherein the conserved substitution of glutamic acid is aspartic acid, glutamine, arginine, asparagine, histidine or serine.

24. The nucleic acid of claim 9, 13, 17, 19 or 21 wherein the conserved substitution of methionine is isoleucine, glutamine, valine or phenylalanine.

10 25. The nucleic acid of claim 11, wherein the conserved substitution of alanine is serine, glycine, threonine, cysteine, or valine.

26. A modified influenza H1 hemagglutinin (HA) protein encoded by the nucleic acid of any one of claims 1 to 25.

27. A virus-like particle (VLP) comprising the modified influenza H1 hemagglutinin (HA) protein encoded by the nucleic acid of any one of claims 1 to 25.

15 28. A method of producing an influenza virus like particle (VLP) in a plant, portion of a plant, or a plant cell, comprising:

a) introducing the nucleic acid of any one of claims 1 to 25 into the plant, portion of the plant, or plant cell; and

20 b) incubating the plant, portion of the plant, or plant cell under conditions that permit expression of the HA protein encoded by the nucleic acid, thereby producing the VLP.

29. The method of claim 28, wherein the method further comprises step c), harvesting the plant, portion of the plant, or plant cell, and purifying the VLP.

30. A method of producing an influenza virus like particle (VLP) in a plant, portion of a plant, or a plant cell, comprising:

25 a) providing a plant, portion of a plant, or plant cell comprising the nucleic acid of any one of claims 1 to 25; and

b) incubating the plant, portion of the plant, or plant cell under conditions that permit expression of the HA protein encoded by the nucleic acid, thereby producing the VLP.

31. The method of claim 30, wherein the method further comprises step c), harvesting the plant, portion of the plant, or plant cell, and purifying the VLP.

5 32. A VLP produced by the method of any one of claims 28 to 31.

33. The VLP of claim 32, further comprising one or more than one lipid derived from the plant, portion of the plant, or plant cell, plant-specific N-glycans, modified N-glycans or a combination thereof.

10 34. A method of producing an antibody or antibody fragment comprising, administering the VLP of any one of claims 27, 32 or 33 to a subject, or a host animal, thereby producing the antibody or the antibody fragment.

35. An antibody produced by the method of claim 34.

36. A plant, portion of the plant, or plant cell comprising the nucleic acid of any one of claims 1 to 25.

15 37. A plant, portion of the plant, or plant cell comprising the HA protein of claim 26 or the VLP of claim 27 or 32.

38. A composition for inducing an immune response comprising, an effective dose of the VLP of any one of claims 27, 32 or 33, and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

20 39. A method for inducing immunity to an influenza infection in a subject, the method comprising administering the VLP of any one of claims 27, 32 or 33 to the subject.

40. The method of claim 39, wherein the VLP is administered to the subject orally, intranasally, intramuscularly, intraperitoneally, intravenously or subcutaneously.

25 41. A method of increasing yield of production of an influenza virus like particle (VLP) in a plant, portion of a plant, or a plant cell, comprising:

a) introducing the nucleic acid of any one of claims 1 to 25 into the plant, portion of the plant, or plant cell; or providing a plant, portion of a plant, or plant cell comprising the nucleic acid of any one of claims 1 to 25; and

5 b) incubating the plant, portion of the plant, or plant cell under conditions that permit expression of the HA protein encoded by the nucleic acid, thereby producing the VLP at a higher yield compared to plant, portion of the plant, or plant cell expressing an unmodified HA protein.

42. The method of claim 41, wherein the method further comprises step c), harvesting the plant, portion of the plant, or plant cell, and purifying the VLP.

10 43. A modified influenza H1 hemagglutinin (HA) protein, the HA protein comprising an amino acid sequence comprising at least one substitution when compared to a corresponding wildtype amino acid sequence, said at least one substitution being at one or more than one amino acid corresponding to amino acid at position 97, 374, 390 or 429 of H1 A/Michigan/45/15 HA.

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