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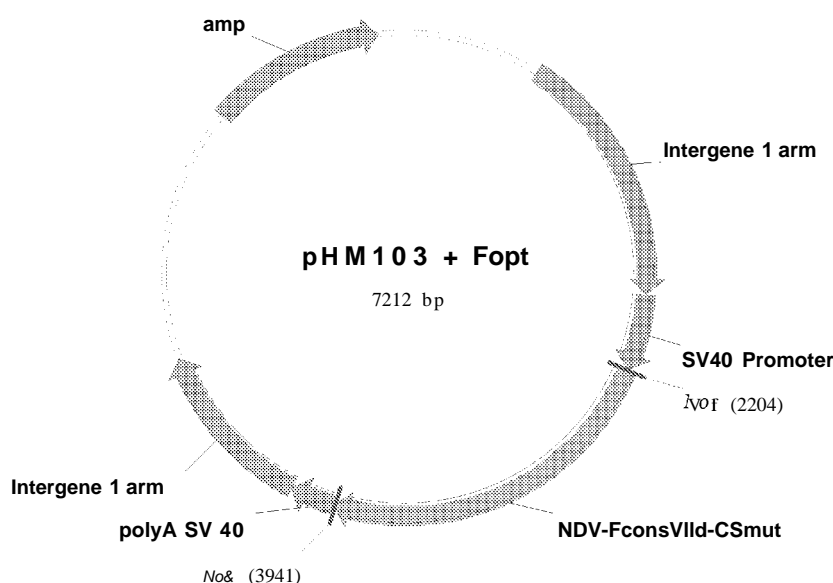
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[Continued on nextpage]

(54) Title: RECOMBINANT HVT VECTORS EXPRESSING ANTIGENS OF AVIAN PATHOGENS AND USES THEREOF

Figure 3

Plasmid map of pHM103 containing codon-optimized NDV-F gene



(57) Abstract: The present invention provides recombinant herpesvirus of turkeys (HVT) vectors that contain and express antigens of avian pathogens, compositions comprising the recombinant HVT vectors, polyvalent vaccines comprising the recombinant HVT vectors and one or more wild type viruses or recombinant vectors. The present invention further provides methods of vaccination against a variety of avian pathogens and method of producing the recombinant HVT vectors.



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## RECOMBINANT HVT VECTORS EXPRESSING ANTIGENS OF AVIAN PATHOGENS AND USES THEREOF

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application 61/564,877 filed on November 30, 2011 and U.S. provisional application 61/694,957 filed on August 30, 2012.

### FIELD OF THE INVENTION

[0002] The invention relates to recombinant viral vectors for the insertion and expression of foreign genes for use as safe immunization vehicles to protect against a variety of pathogens. It also relates to multivalent composition or vaccine comprising one or more recombinant viral vectors for protection against a variety of pathogens. The present invention relates to methods of making and using the recombinant viral vectors.

### BACKGROUND OF THE INVENTION

[0003] Poultry vaccination is widely used to protect poultry flocks against devastating diseases including Newcastle disease (ND), infectious bursal disease (IBD), Marek's disease (MD), infectious bronchitis (IB), infectious laryngotracheitis (ILT) and avian influenza (AI). ND is caused by the avian paramyxovirus 1 (APMV-1) also designated ND virus (NDV) belonging to the *Paramyxoviridae* family. MD is caused by Gallid herpesvirus 2 (*Herpesviridae* family) also designated as MD virus serotype 1 (MDV1). IB is caused by IB virus (IBV) belonging to the *Coronaviridae* family, ILT is caused by Gallid herpesvirus 1 (*Herpesviridae* family) also designated ILT virus (ILTV) and AI is caused by AI virus (AIV) belonging to the *Orthomyxoviridae* family.

[0004] A number of recombinant avian viral vectors have been proposed with a view to vaccinating birds against these avian pathogens. The viral vectors used comprise avipox viruses, especially fowlpox (EP-A-0,517,292), Marek's virus, such as serotypes 2 and 3 (HVT) (WO-A-87/04463), or alternatively the ITLV, NDV and avian adenovirus. When some of these recombinant avian viral vectors were used for vaccination, they display variable levels of protection.

**[0005]** Several recombinant herpesvirus of turkeys (HVT, also designated Meleagrid herpesvirus 1 or MDV serotype 3) vectors expressing antigens from various pathogens (US patent Nos. 5,980,906, 5,853,733, 6,183,753, 5,187,087) including IBDV, NDV, ILTV and AIV have been developed and licensed. Of particular interest is a HVT vector-expressing IBDV VP2 protective gene that has shown clear advantages over classical IBD vaccines (Bublöt et al J.Comp. Path.2007, Vol.137, S81-S84; US 5,980,906). Other HVT vectors of interest are those expressing either NDV (Morgan et al 1992, Avian dis. 36, 858-70; US 6,866,852; US5,650,153) or ILTV (Johnson et al, 2010 Avian Dis 54, 1251-1259; US6,299,882; US5,853,733) protective gene(s). One of the practical problems of using several HVT-based recombinant vaccines together is their interference. Lower protection is induced at least against one of the disease when two HVT recombinants expressing different antigens are mixed (Rudolf Heine 2011; Issues of the Poultry Recombinant Viral Vector Vaccines which May Cause an Effect on the Economic Benefits of those Vaccines; paper presented at the XVII World Veterinary Poultry Association (WVPA) Congress in Canciín, Mexico, August 14-18, 2011; Slacum G, Hein R. and Lynch P., 2009, The compatibility of HVT recombinants with other Marek's disease vaccines, 58<sup>th</sup> Western Poultry Disease Conference, Sacramento, CA, USA, March 23<sup>rd</sup>-25<sup>th</sup>, p 84).

**[0006]** The combination of HVT and SB-1, a Gallid herpesvirus 3 (MDV serotype 2 or MDV-2) vaccine strain, has shown a synergistic effect on MD protection (Witter and Lee, 1984, Avian Pathology 13, 75-92). To address the interference problem, it is of interest to evaluate the HVT virus as a vaccine vector to express one or more protective antigen(s) against a variety of avian pathogens.

**[0007]** The SB-1 genome was cloned and characterized in bacterial artificial chromosome (BAC) (Petherbridge, et al, J. Virol. Methods 158, 11-17, 2009; Singh et al, Research in Veterinary Science 89, 140-145, 2010). The MDV2 SB-1 sequence was recently obtained and analyzed (Spatz and Schat, Virus Gene 42, 331-338, 2011). A glycoprotein E deletion of SB-1 virus was described by Petherbridge, et al. (J. Virol. Methods 158, 11-17, 2009). However, no research has been reported using SB-1 as a viral vector expressing foreign protective genes.

[0008] Considering the potential effect of animal pathogens, such as NDV and IBDV on veterinary public health and the economy, efficient methods of preventing infection and protecting animals are needed. There is a need for a solution of combined effective vector vaccines and a suitable method for making the vaccine that could alleviate the problem of interference observed between two HVT-based vector vaccines.

### **SUMMARY OF THE INVENTION**

[0009] The present invention showed surprising result when polyvalent compositions or vaccines comprising single or double HVT vector were effective to protect animals against a variety of avian pathogens without interference. Surprising results were also observed when various combinations of promoters, codon-optimized gene, polyA tails and insertion sites conferred different levels of efficacy and stability to the expression of one or more heterologous genes *in vivo*.

[0010] The present invention relates to a recombinant HVT vector comprising one or more heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.

[0011] The present invention provides a composition or vaccine comprising one or more recombinant HVT vectors comprising one or more heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.

[0012] The present invention provides a polyvalent composition or vaccine comprising one or more recombinant HVT vectors comprising heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen and one or more recombinant SB l vectors comprising heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.

[0013] The present invention relates to a method of vaccinating an animal, or inducing an immunogenic or protective response in an animal, comprising at least one administration of the composition or vector of the present invention.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

[0014] The following detailed description, given by way of example, and which is not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, incorporated herein by reference, in which:

[0015] Figure 1 is a table showing the SEQ ID NO assigned to each DNA and protein sequence.

[0016] Figure 2 depicts the genome structure of HVT and its insertion sites.

[0017] Figure 3 depicts the plasmid map of pHM103.

[0018] Figure 4 depicts the PCR analysis results of vHVTI 14.

[0019] Figure 5 shows the dual immunofluorescent assay results.

[0020] Figure 6 depicts the Southern blot results of vHVTI 14.

[0021] Figure 7 depicts the immunoprecipitation and Western blot analysis results of vHVTI 14.

[0022] Figure 8 depicts the Western blot analysis of immunoprecipitated sample from vHVT306 infected cells.

[0023] Figure 9 depicts the Western blot analysis of immunoprecipitated sample from vSB 1-009 infected cells.

[0024] Figure 10 depicts the result of challenge study of vHVT304 and vHVTI 14 against NDV ZJ1 and CA02.

[0025] Figure 11 depicts the viral shedding result after NDV CA02 and ZJ1 challenge.

[0026] Figure 12 depicts the viral shedding result after NDV Chimalhuacan challenge.

[0027] Figure 13 shows the sequence alignment and percentage identity.

[0028] Figure 14 shows the DNA and protein sequences.

#### **DETAILED DESCRIPTION OF THE INVENTION**

[0029] It is noted that in this disclosure and particularly in the claims, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

**[0030]** Unless otherwise noted, technical terms are used according to conventional usage.

Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

**[0031]** The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicate otherwise. The word "or" means any one member of a particular list and also includes any combination of members of that list.

**[0032]** The term "animal" is used herein to include all mammals, birds and fish. The animal as used herein may be selected from the group consisting of equine (e.g., horse), canine (e.g., dogs, wolves, foxes, coyotes, jackals), feline (e.g., lions, tigers, domestic cats, wild cats, other big cats, and other felines including cheetahs and lynx), bovine (e.g., cattle), swine (e.g., pig), ovine (e.g., sheep, goats, lamas, bison), avian (e.g., chicken, duck, goose, turkey, quail, pheasant, parrot, finches, hawk, crow, ostrich, emu and cassowary), primate (e.g., prosimian, tarsier, monkey, gibbon, ape), humans, and fish. The term "animal" also includes an individual animal in all stages of development, including embryonic and fetal stages.

**[0033]** The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of consecutive amino acid residues.

**[0034]** The term "nucleic acid", "nucleotide", and "polynucleotide" are used interchangeably and refer to RNA, DNA, cDNA, or cRNA and derivatives thereof, such as those containing modified backbones. It should be appreciated that the invention provides polynucleotides comprising sequences complementary to those described herein. The "polynucleotide" contemplated in the present invention includes both the forward strand (5' to 3') and reverse complementary strand (3' to 5'). Polynucleotides according to the invention can be prepared in different ways (e.g. by chemical synthesis, by gene cloning etc.) and can take various forms (e.g. linear or branched, single or double stranded, or a hybrid thereof, primers, probes etc.).

[0035] The term "genomic DNA" or "genome" is used interchangeably and refers to the heritable genetic information of a host organism. The genomic DNA comprises the DNA of the nucleus (also referred to as chromosomal DNA) but also the DNA of the plastids (e.g., chloroplasts) and other cellular organelles (e.g., mitochondria). The genomic DNA or genome contemplated in the present invention also refers to the RNA of a virus. The RNA may be a positive strand or a negative strand RNA. The term "genomic DNA" contemplated in the present invention includes the genomic DNA containing sequences complementary to those described herein. The term "genomic DNA" also refers to messenger RNA (mRNA), complementary DNA (cDNA), and complementary RNA (cRNA).

[0036] The term "gene" is used broadly to refer to any segment of polynucleotide associated with a biological function. Thus, genes or polynucleotides include introns and exons as in genomic sequence, or just the coding sequences as in cDNAs, such as an open reading frame (ORF), starting from the start codon (methionine codon) and ending with a termination signal (stop codon). Genes and polynucleotides can also include regions that regulate their expression, such as transcription initiation, translation and transcription termination. Thus, also included are promoters and ribosome binding regions (in general these regulatory elements lie approximately between 60 and 250 nucleotides upstream of the start codon of the coding sequence or gene; Doree S M *et al*; Pandher K *et al*; Chung J Y *et al*), transcription terminators (in general the terminator is located within approximately 50 nucleotides downstream of the stop codon of the coding sequence or gene; Ward C K *et al*). Gene or polynucleotide also refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

[0037] The term "heterologous DNA" as used herein refers to the DNA derived from a different organism, such as a different cell type or a different species from the recipient. The term also refers a DNA or fragment thereof on the same genome of the host DNA wherein the heterologous DNA is inserted into a region of the genome which is different from its original location.

[0038] As used herein, the term "antigen" or "immunogen" means a substance that induces a specific immune response in a host animal. The antigen may comprise a whole



organism, killed, attenuated or live; a subunit or portion of an organism; a recombinant vector containing an insert with immunogenic properties; a piece or fragment of DNA capable of inducing an immune response upon presentation to a host animal; a polypeptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

[0039] The term "immunogenic protein or peptide" as used herein includes polypeptides that are immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral and/or cellular type directed against the protein. Preferably the protein fragment is such that it has substantially the same immunological activity as the total protein. Thus, a protein fragment according to the invention comprises or consists essentially of or consists of at least one epitope or antigenic determinant. An "immunogenic" protein or polypeptide, as used herein, includes the full-length sequence of the protein, analogs thereof, or immunogenic fragments thereof. By "immunogenic fragment" is meant a fragment of a protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance.

[0040] The term "immunogenic protein or peptide" further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. The term "conservative variation" denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change or is another biologically similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic—aspartate and glutamate; (2) basic—

lysine, arginine, histidine; (3) non-polar~alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar—glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, or the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like; or a similar conservative replacement of an amino acid with a structurally related amino acid that will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the definition of the reference polypeptide. All of the polypeptides produced by these modifications are included herein. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

[0041] The term "epitope" refers to the site on an antigen or hapten to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site". Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

[0042] An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, and/or cytotoxic T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be

demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered viral titer in the infected host.

[0043] The terms "recombinant" and "genetically modified" are used interchangeably and refer to any modification, alteration or engineering of a polynucleotide or protein in its native form or structure, or any modification, alteration or engineering of a polynucleotide or protein in its native environment or surrounding. The modification, alteration or engineering of a polynucleotide or protein may include, but is not limited to, deletion of one or more nucleotides or amino acids, deletion of an entire gene, codon-optimization of a gene, conservative substitution of amino acids, insertion of one or more heterologous polynucleotides.

[0044] The term "double HVT construct" or "double HVT vector" refers to an HVT viral vector comprising two heterologous polynucleotides.

[0045] The terms "polyvalent vaccine or composition", "combination or combo vaccine or composition" and "multivalent vaccine or composition" are used interchangeably to refer to a composition or vaccine containing more than one composition or vaccines. The polyvalent vaccine or composition may contain two, three, four or more compositions or vaccines. The polyvalent vaccine or composition may comprise recombinant viral vectors, active or attenuated or killed wild-type viruses, or a mixture of recombinant viral vectors and wild-type viruses in active or attenuated or killed forms.

[0046] One embodiment of the invention provides a recombinant HVT viral vector comprising one or more heterologous polynucleotides coding for and expressing at least one antigen or polypeptide of an avian pathogen. The HVT strains used for the recombinant viral vector may be any HVT strains, including, but not limited to, the HVT strain FC126 (Igarashi T. et al, J. Gen. Virol. 70, 1789-1804, 1989).

[0047] Another embodiment of the invention provides a recombinant SB-1 viral vector comprising one or more heterologous polynucleotides coding for and expressing at least one antigen or polypeptide of an avian pathogen. The SB-1 strains may be any SB-1 strains, including, but not limited to, the commercial Marek's Disease Vaccine (SB-1 vaccine) (Merial Select Inc., Gainesville, GA 30503, USA), the SB-1 strain having the genome sequence as defined by GenBank Accession Number HQ840738. 1.

[0048] The genes coding for antigen or polypeptide may be those coding for Newcastle Disease Virus fusion protein (NDV-F), Newcastle Disease Virus hemagglutinin neuraminidase (NDV-HN), Marek's Disease Virus glycoprotein C (gC), Marek's Disease Virus glycoprotein B (gB), Marek's Disease Virus glycoprotein E (gE), Marek's Disease Virus glycoprotein I (gI), Marek's Disease Virus glycoprotein H (gH) or Marek's Disease Virus glycoprotein L (gL), Infectious Bursal Disease Virus (IBDV) VP2, IBDV VPX, IBDV VP3, IBDV VP4, ILTV glycoprotein B, ILTV glycoprotein I, ILTV UL32, ILTV glycoprotein D, ILTV glycoprotein E, ILTV glycoprotein C, influenza hemagglutinin (HA), influenza neuraminidase (NA), protective genes derived from *Mycoplasma gallisepticum* (MG), or *Mycoplasma synoviae* (MS), or combinations thereof. The antigen or polypeptide may be any antigen from the poultry pathogen selected from the group consisting of avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian metapneumovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus, avian astrovirus, avian parvovirus, coccidiosis (*Eimeria* sp.), *Campylobacter* sp., *Salmonella* sp., *Pasteurella* sp., *Avibacterium* sp., *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Clostridium* sp., and *E. coli*.

[0049] Moreover, homologs of aforementioned antigen or polynucleotides are intended to be within the scope of the present invention. As used herein, the term "homologs" includes orthologs, analogs and paralogs. The term "analogs" refers to two polynucleotides or polypeptides that have the same or similar function, but that have evolved separately in unrelated organisms. The term "orthologs" refers to two polynucleotides or polypeptides from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides having the same or similar functions. The term "paralogs" refers to two polynucleotides or polypeptides that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related. Analogs, orthologs, and paralogs of a wild-type polypeptide can differ from the wild-type polypeptide by post-translational modifications, by amino acid sequence differences, or by both. In particular, homologs of the invention will generally exhibit at least 80-85%, 85-90%, 90-95%, or 95%, 96%,

97%, 98%, 99%, sequence identity, with all or part of the polynucleotide or polypeptide sequences of antigens described above, and will exhibit a similar function.

[0050] In one embodiment, the present invention provides a recombinant HVT or SB-1 viral vector comprising one or more heterologous polynucleotides coding for and expressing the NDV-F antigen or polypeptide. In one aspect of the embodiment, the NDV-F antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%, sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. In another aspect of the embodiment, the heterologous polynucleotide encoding an NDV-F antigen or polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37. In yet another aspect of the embodiment, the heterologous polynucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1, 3, 5, 32, 34, or 36.

[0051] Variants include allelic variants. The term "allelic variant" refers to a polynucleotide or a polypeptide containing polymorphisms that lead to changes in the amino acid sequences of a protein and that exist within a natural population (e.g., a virus species or variety). Such natural allelic variations can typically result in 1- 5% variance in a polynucleotide or a polypeptide. Allelic variants can be identified by sequencing the nucleic acid sequence of interest in a number of different species, which can be readily carried out by using hybridization probes to identify the same gene genetic locus in those species. Any and all such nucleic acid variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity of gene of interest, are intended to be within the scope of the invention.

[0052] The term "identity" with respect to sequences can refer to, for example, the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm

(Wilbur and Lipman). The sequence identity or sequence similarity of two amino acid sequences, or the sequence identity between two nucleotide sequences can be determined using Vector NTI software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

**[0053]** The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code, e.g., optimized codon usage for a specific host. As used herein, "optimized" refers to a polynucleotide that is genetically engineered to increase its expression in a given species. To provide optimized polynucleotides coding for NDV-F polypeptides, the DNA sequence of the NDV-F protein gene can be modified to 1) comprise codons preferred by highly expressed genes in a particular species; 2) comprise an A+T or G+C content in nucleotide base composition to that substantially found in said species; 3) form an initiation sequence of said species; or 4) eliminate sequences that cause destabilization, inappropriate polyadenylation, degradation and termination of RNA, or that form secondary structure hairpins or RNA splice sites. Increased expression of NDV F protein in said species can be achieved by utilizing the distribution frequency of codon usage in eukaryotes and prokaryotes, or in a particular species. The term "frequency of preferred codon usage" refers to the preference exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the NDV-F polypeptide encoded by the nucleotide sequence is functionally unchanged.

**[0054]** Successful expression of the heterologous polynucleotides by the recombinant/modified infectious virus requires two conditions. First, the heterologous polynucleotides must be inserted or introduced into a region of the genome of the virus in order that the modified virus remains viable. The second condition for expression of inserted heterologous polynucleotides is the presence of a regulatory sequences allowing

expression of the gene in the viral background (for instance: promoter, enhancer, donor and acceptor splicing sites and intron, Kozak translation initiation consensus sequence, polyadenylation signals, untranslated sequence elements).

[0055] The insertion site may be any non-essential region of the HVT genome, including, but not limited to, the region between the ATG of ORF UL55 and the junction of UL with the adjacent repeat region (US5,980,906), the IG1 locus, the IG2 locus, the IG3 locus, the UL43 locus, the US10 locus, the SORF3/US2 locus (see FIG. 2)

[0056] In general, it is advantageous to employ a strong promoter functional in eukaryotic cells. The promoters include, but are not limited to, an immediate early cytomegalovirus (CMV) promoter, guinea pig CMV promoter, an SV40 promoter, Pseudorabies Virus promoters such as that of glycoprotein X promoter, Herpes Simplex Virus- 1 such as the alpha 4 promoter, Marek's Disease Viruses (including MDV-1, MDV-2 and HVT) promoters such as those driving glycoproteins gC, gB, gE, or gI expression, Infectious Laryngotracheitis Virus promoters such as those of glycoprotein gB, gE, gI, gD genes, or other herpesvirus promoters.

[0057] One embodiment of the invention provides a recombinant HVT vector comprising a heterologous polynucleotide coding for and expressing the NDV-F antigen or polypeptide. In one aspect of the embodiment, the polynucleotide encoding the NDV-F polypeptide is operably linked to the SV40 promoter having the sequence as set forth in SEQ ID NO:9 and therefore the expression of the NDV-F antigen or polypeptide is regulated by the SV40 promoter. In another aspect of the embodiment, the expression of NDV-F antigen or polypeptide is regulated by the SV40 polyA signal having the sequence as set forth in SEQ ID NO: 11. In yet another aspect of the embodiment, the polynucleotide encoding the NDV-F polypeptide is operably linked to the MDV gB promoter having the sequence as set forth in SEQ ID NO:38 and therefore the expression of the NDV-F antigen or polypeptide is regulated by the MDV gB promoter.

[0058] Another embodiment of the invention provides a recombinant double HVT vector comprising a first heterologous polynucleotide coding for and expressing the NDV-F antigen or polypeptide and a second polynucleotide coding for and expressing the IBDV VP2 antigen or polypeptide. In one aspect of the embodiment, the NDV-F antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%

sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37. In another aspect of the embodiment, the IBDV VP2 antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:8 or 42. In another aspect, the polynucleotide encoding the NDV-F polypeptide is operably linked to the SV40 promoter having the sequence as set forth in SEQ ID NO:9 and the expression of NDV-F antigen or polypeptide is regulated by the SV40 promoter. In yet another aspect, the expression of NDV-F antigen or polypeptide is regulated by the SV40 polyA signal having the sequence as set forth in SEQ ID NO:11, or the synthetic polyA signal having the sequence as set forth in SEQ ID NO:12. In another aspect, the expression of IBDV VP2 antigen or polypeptide is regulated by the CMV-IE promoter having the sequence as set forth in SEQ ID NO:10 and the SV40 polyA signal having the sequence as set forth in SEQ ID NO:11.

**[0059]** Yet another embodiment of the invention provides a recombinant double HVT vector comprising two polynucleotides coding for and expressing the IBDV VP2 antigens or polypeptides. In one aspect of the embodiment, the IBDV VP2 antigen or polypeptide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:8 or 42. In one aspect, the polynucleotide encoding a first IBDV VP2 antigen or polypeptide is operably linked to the CMV-IE promoter having the sequence as set forth in SEQ ID NO:10, and the polynucleotide encoding a second IBDV VP2 antigen or polypeptide is operably linked to the guinea pig CMV promoter having the sequence as set forth in SEQ ID NO:43. In another aspect, the expression of a first IBDV VP2 antigen or polypeptide is regulated by the CMV-IE promoter having the sequence as set forth in SEQ ID NO:10 and the SV40 polyA signal having the sequence as set forth in SEQ ID NO:11, and the expression of a second IBDV VP2 antigen or polypeptide is regulated by the guinea pig CMV promoter having the sequence as set forth in SEQ ID NO:43 and the synthetic polyA signal having the sequence as set forth in SEQ ID NO:12. In yet another aspect of the embodiment, the polynucleotides encoding the IBDV VP2 antigen or polypeptide may be inserted in one or more locus regions selected from the group consisting of IG1, IG2, US10, SORF3-US2 and gD of HVT genome. In one embodiment, the present invention relates to a



pharmaceutical composition or vaccine comprising one or more recombinant HVT or SB-1 rival vectors of the present invention and a pharmaceutically or veterinarily acceptable carrier, excipient, vehicle or adjuvant.

[0060] In another embodiment, the present invention provides a composition or vaccine comprising an HVT viral vector comprising a polynucleotide encoding an NDV-F antigen, an SV40 promoter, and optionally a pharmaceutically or veterinarily acceptable carrier, excipient, vehicle or adjuvant. In another embodiment, the present invention provides a pharmaceutical composition or vaccine comprising a first HVT vector comprising a polynucleotide encoding an NDV-F antigen, a second HVT vector comprising a polynucleotide encoding an IBDV VP2 antigen, and optionally a pharmaceutically or veterinarily acceptable carrier, excipient, vehicle or adjuvant. In another embodiment, the present invention provides a pharmaceutical composition or vaccine comprising an HVT vector comprising a polynucleotide encoding an NDV-F antigen, an SB-1 vector comprising a polynucleotide encoding an NDV-F antigen, optionally a pharmaceutically or veterinarily acceptable carrier, excipient, vehicle or adjuvant. The pharmaceutical composition or vaccine of the present invention may comprise a first HVT vector comprising a polynucleotide encoding an NDV-F antigen, a second HVT vector comprising a polynucleotide encoding an IBDV VP2 antigen, an SB-1 vector comprising a polynucleotide encoding an NDV-F antigen, optionally a pharmaceutically or veterinarily acceptable carrier, excipient, vehicle or adjuvant.

[0061] In yet another embodiment, the present invention provides a composition or vaccine comprising a double HVT viral vector comprising: i) a first heterologous polynucleotide coding for and expressing an NDV-F antigen or polypeptide; ii) a second polynucleotide coding for and expressing an IBDV VP2 antigen or polypeptide; and iii) optionally a pharmaceutically or veterinarily acceptable carrier, excipient, vehicle or adjuvant. In another embodiment, the present invention provides a composition or vaccine comprising a double HVT viral vector comprising two polynucleotides coding for and expressing the IBDV VP2 antigens or polypeptides, and optionally a pharmaceutically or veterinarily acceptable carrier, excipient, vehicle or adjuvant. In yet another embodiment, the composition comprising the double HVT viral vector further comprises an HVT vector comprising a polynucleotide encoding an IBDV VP2 antigen,

or an SB-1 vector comprising a polynucleotide encoding an NDV-F antigen, or a combination thereof. The pharmaceutically or veterinarily acceptable carriers or adjuvant or vehicles or excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarily acceptable carrier or adjuvant or vehicle or excipient can be Marek's disease vaccine diluent used for MD vaccines. Other pharmaceutically or veterinarily acceptable carrier or adjuvant or vehicle or excipients that can be used for methods of this invention include, but are not limited to, 0.9% NaCl (e.g., saline) solution or a phosphate buffer, poly-(L-glutamate) or polyvinylpyrrolidone. The pharmaceutically or veterinarily acceptable carrier or vehicle or excipients may be any compound or combination of compounds facilitating the administration of the vector (or protein expressed from an inventive vector *in vitro*), or facilitating transfection or infection and/or improve preservation of the vector (or protein). Doses and dose volumes are herein discussed in the general description and can also be determined by the skilled artisan from this disclosure read in conjunction with the knowledge in the art, without any undue experimentation.

**[0062]** Optionally other compounds may be added as pharmaceutically or veterinarily acceptable carriers or adjuvant or vehicles or excipients, including, but not limited to, alum; CpG oligonucleotides (ODN), in particular ODN 2006, 2007, 2059, or 2135 (Pontarollo R.A. *et al.*, *Vet. Immunol. Immunopath.*, 2002, 84: 43-59; Wernette CM. *et al.*, *Vet. Immunol. Immunopath.*, 2002, 84: 223-236; Mutwiri G. *et al.*, *Vet. Immunol. Immunopath.*, 2003, 91: 89-103); polyA-polyU, dimethyldioctadecylammonium bromide (DDA) ("Vaccine Design The Subunit and Adjuvant Approach", edited by Michael F. Powell and Mark J. Newman, *Pharmaceutical Biotechnology*, 6: p.03, p.157); N,N-dioctadecyl-N',N'-bis(2-hydroxy ethyl) propanediamine (such as AVRIDINE®) (*Ibid*, p. 148); carbomer, chitosan (*see* US Patent Serial No. 5,980,912 for example).

**[0063]** The pharmaceutical compositions and vaccines according to the invention may comprise or consist essentially of one or more adjuvants. Suitable adjuvants for use in the practice of the present invention are (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), such as oligodeoxyribonucleotide sequences having one or more non-methylated CpG units (Klinman *et al.*, 1996; W098/16247), (3) an oil in water emulsion, such as the

SPT emulsion described on p 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, and the emulsion MF59 described on p 183 of the same work, (4) cation lipids containing a quaternary ammonium salt, e.g., DDA (5) cytokines, (6) aluminum hydroxide or aluminum phosphate, (7) saponin or (8) other adjuvants discussed in any document cited and incorporated by reference into the instant application, or (9) any combinations or mixtures thereof.

[0064] Another aspect of the invention relates to a method for inducing an immunological response in an animal against one or more antigens or a protective response in an animal against one or more avian pathogens, which method comprises inoculating the animal at least once with the vaccine or pharmaceutical composition of the present invention. Yet another aspect of the invention relates to a method for inducing an immunological response in an animal to one or more antigens or a protective response in an animal against one or more avian pathogens in a prime-boost administration regimen, which is comprised of at least one primary administration and at least one booster administration using at least one common polypeptide, antigen, epitope or immunogen. The immunological composition or vaccine used in primary administration may be same, may be different in nature from those used as a booster.

[0065] The avian pathogens may be Newcastle Disease Virus (NDV), Infectious Bursal Disease Virus (*i.e.*, IBDV or Gumboro Disease virus), Marek's Disease Virus (MDV), Infectious Laryngotracheitis Virus (ILTV), avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian metapneumovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, avian parvovirus, avian astrovirus and chick anemia virus coccidiosis (*Eimeria sp.*), *Campylobacter sp.*, *Salmonella sp.*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Pasteurella sp.*, *Avibacterium sp.*, *E. coli* or *Clostridium sp.*

[0066] Usually, one administration of the vaccine is performed either at one day-of-age by the subcutaneous or intramuscular route or *in ovo* in 17-19 day-old embryo. A second administration can be done within the first 10 days of age. The animals are preferably at least 17 day-embryo or one day old at the time of the first administration.

[0067] A variety of administration routes in day-old chicks may be used such as subcutaneously or intramuscularly, intradermally, transdermally. The *in ovo* vaccination can be performed in the amniotic sac and/or the embryo. Commercially available *in ovo* and SC administration devices can be used for vaccination.

[0068] The invention will now be further described by way of the following non-limiting examples.

### **EXAMPLES**

[0069] Construction of DNA inserts, plasmids and recombinant viral vectors was carried out using the standard molecular biology techniques described by J. Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

#### **Example 1 Construction of recombinant vHVT114 expressing NDV-F**

##### **Preparation of donor plasmid pHM103+Fopt**

[0070] The plasmid pHM103 (Merial Limited) containing the Intergenic I arms of HVT FC126 (see FIG.2), SV40 promoter and SV40 poly A was digested with NotI, dephosphorylated, and the 5.6kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized codon-optimized genotype V1ld NDV-F gene (SEQ ID NO:1, coding for SEQ ID NO:2) was also NotI digested and the 1.7kb fragment was gel extracted. The 5.6 and 1.7kb fragments were ligated to create pHM103+Fopt (FIG. 3).

##### **Generation of recombinant HVT viral vector**

[0071] An in vitro recombination (IVR) was performed by co-electroporation of secondary chicken embryo fibroblast cells (2° CEF cells) using pHM103+Fopt as the donor plasmid and viral DNA isolated from the HVT strain FC126. Co-electroporation was performed using  $1 \times 10^7$  2° CEF in 300ul Opti-MEM and shocked at 150 volts with 950 capacitance in a 2mm electroporation cuvette. The transfected cells were seeded into 96-well plate and incubated for 5 days. The cells grown in the 96-well plate were then duplicated into two 96-well plates. One set of 96-well plates was used for IFA using chicken polyclonal sera against NDV-F to identify positive wells containing

recombinants and another set of 96-well plates was used for recovering the infected cells from the positive wells.

[0072] The recombinant viral purification was performed first by 96-well plate duplication and IFA selection for the wells containing the most IFA positive plaques with the least amount of IFA negative plaques. Wells matching those criteria were then harvested and adjusted to 1ml in DMEM+2% FBS. From the 1ml stock, 5-20ul were removed and mixed with  $1 \times 10^7$  CEFs in 10ml DMEM+2% FBS and aliquoted onto a new 96-well plate to have single HVT plaques per well. The supernatant of the wells that contained single plaques were tested for the absence of parental virus by PCR. After five rounds of plaque purification, a recombinant virus designated as vHVT1 14 was isolated and the purity was tested by IFA and PCR to confirm NDV-F expression and the absence of parental virus.

#### PCR analysis of recombinant vHVT1 14

[0073] DNA was extracted from vHVT1 14 by phenol/chloroform extraction, ethanol precipitated, and was resuspended in 20mM HEPES. PCR primers (shown in Table 1) were designed to specifically identify the presence of the codon optimized NDV-F, the SV40 promoter, as well as, the purity of the recombinant virus from FC126 CL2 parental virus. PCR was performed using 200ng of DNA template along with the specified primers pairs indicted in Table 1. PCR cycling conditions are as follows: 94°C for 2 mins; 30 cycles of 94°C for 30 sees, 55°C for 30 sees, 68°C for 3 mins; 68°C for 5 mins. The expected PCR products are shown in Table 2. The PCR results are shown in Figure 4. As shown in Figure 4, the sizes of PCR products after gel electrophoresis correspond well with the expected sizes and the banding patterns.

Table 1

primer	SEQ ID NO	Sequence 5'-3'
MB080	13	CGA ACA AAC TTC ATC GCT ATG C
MB081	14	TAA CTC AAA TGC GAA GCG TTG C
optF	15	ACT GAC AAC ACC CTA CAT GGC
VlloptF RP	16	GCC AGC ACC AGG CTC AGG G
SV40promoterF	17	AGC TTG GCT GTG GAA TGT

Table 2

Primer pairs	Expected size (bp)	
	FC126 CL21	vHVTI 14
MB081 + VIIoptF.RP	—	2138
SV40promoterF + MB080	—	2368
OptFprimer + MB080	—	872
MB080 + MB081	323	2578

#### Expression analysis of recombinant vHVTI 14

[0074] Immunofluorescence testing was performed using the vHVTI 14 which was passaged over ten times beyond an experimental pre-master seed (pre-MSV). The pre-MSV and pre-MSV+12 materials were diluted 1:100 in media. Fifty microliters of the diluted virus was added to 10 ml of DMEM+2% FBS with  $1 \times 10^7$  CEFs and then aliquoted onto a 96 well plate (100ul/well). The plates were incubated for 3 days at  $37^\circ\text{C}+5\%\text{CO}_2$  until viral plaques were visible. The plates were fixed with 95% ice-cold acetone for three minutes and washed three times with PBS. Chicken anti-sera against Newcastle Disease Virus (lot#C0139, Charles Rivers Laboratory) at 1:1000 were added along with monoclonal antibody L-78 (Merial Limited) at 1:3000 and the plates were incubated at  $37^\circ\text{C}$  for 1 hour. After the 1 hour incubation the plates were washed three times with PBS and FITC anti-chicken (cat# F8888, Sigma) was added along with Alexz Fluor 568 donkey anti-mouse (IgG) (cat# A 10037, Molecular Probe) at 1:500. Again the plates were incubated at  $37^\circ\text{C}$  for 1 hour. After the 1 hour incubation the cells were rinsed three times with PBS. A small amount of PBS was added to prevent the monolayer from drying and causing auto fluorescence. The cells were then visualized with a fluorescent microscope using both the tetramethylrhodamine isothiocyanate (TRITC) and fluorescein isothiocyanate (FITC) filters in combination.

[0075] The vHVTI 14 viral plaques were visualized using both the TRITC and FITC filters for the dual staining. The FITC test showed the NDV-F expression and the TRITC test showed the HVT expression. Because of the small wells of the 96 well plates, each well was recorded with the plaques first counted with the TRITC filter and then recounted with the FITC filter. Over 500 plaques were counted for the pre-MSV and pre-

MSV+12 passage. All the plaques were positive for both the FITC and TRITC on both plates. (FIG. 5)

Southern blot analysis of recombinant vHVTI 14

[0076] Total genomic DNA was extracted from HVT FC126 and vHVTI 14 according to the standard genomic DNA extraction protocol. For each restriction digest, 3 µg of genomic DNA (1 ng for the donor plasmid) was used with a total digestion volume of 20 µl for each sample. The genomic DNA of HVT FC126 (negative control), pHM103+Fopt donor plasmid, and vHVTI 14 were each digested overnight at 37°C with *BamHI*, *PstI*, *SphI*, and *NcoI* restriction endonucleases. The restriction fragments of HVT FC126 (negative control), pHM103+Fopt donor plasmid, and vHVTI 14 genomic DNA were separated by a 1% agarose gel and transferred to a positively charged Nylon membrane. Following the North2South Chemiluminescent Hybridization and Detection Kit (Thermo Scientific) manufacturers' instructions, the membrane was pre-hybridized for 1 hr and then hybridized with a biotinylated NDV-F probe overnight at 55°C. Following the overnight hybridization, several stringency washes were performed until the membrane was placed in blocking buffer with the addition of Streptavidin-HRP. After rinsing the membrane of any unbound Streptavidin-HRP the substrate solution of Luminal and peroxide were added. The membrane was then exposed to X-ray film and developed. Areas where the biotinylated probe bound to the DNA were chemiluminescent and were captured by the X-ray film. Table 3 shows the expected Southern blot bands using the NDV-F probe. The Southern blot results showed the digestion patterns as expected (Fig. 6).

Table 3

## NDV-F Probe

Restriction Endonuclease	Donor plasmid pHM103+Fopt	vHVT114	FC126 CL2
BamHI	7.014 0.198	6.630 1.259 0.198	---
PstI	5.481 0.947 0.784	6.359 0.947 0.784	---
SphI	4.763 2.377 0.072	2.377 2.119 0.072	---
NcoI	4.931 2.157 0.124	3.753 2.157 0.124	---

Sequence analysis of the inserted region in recombinant vHVTI 14

[0077] Analysis of vHVTI 14 genomic DNA region was performed by PCR amplification. Total of 10 primers were used to amplify the entire cassette, as well as, beyond the flanking BamHI-I arms used in the donor plasmid. The 4.727 kb PCR product was gel purified and the entire fragment was sequenced using the sequencing primers. The sequence result confirmed that the vHVTI 14 contains the correct SV40 promoter, the codon-optimized NDV-F and the SV40 polyA sequences that match exactly the sequence described for the donor plasmid pHM103+Fopt in SEQ ID NO: 18.

Western blot analysis of recombinant vHVTI 14

[0078] Approximately  $2 \times 10^6$  chicken fibroblast cells were infected at ~0.1 MOI with vHVTI 14 Pre-MSV. After two days of incubation at 37°C, infected as well as uninfected cells were harvested using a cell scraper after removing the media and rinsing with PBS. The cells were harvested with 1ml of PBS and centrifuged. The cell pellets were lysed by following the Pierce Classic IP Kit (cat#26146, Thermo Scientific). 100 µl of the anti-NDV-F monoclonal antibody 001C3 (Merical Limited) was used to form the immune



complex. The antibody/lysate sample was added to Protein A/G Plus Agarose to capture the immune complex. The immune complex was washed three times to remove non-bound material and then eluted in 50ul volume using sample buffer elution under non-reducing condition. After boiling for 5 minutes, 10 µl of the samples were loaded into a 10% Acrylamide gel (Invitrogen). The PAGE gel was run in MOPS buffer (Invitrogen) at 200volts for 1 hour. Then the gel was transferred onto a PVDF membrane.

[0079] The Protein Detector Western Blot Kit TMB System (KPL, cat# 54-11-50) was used for blotting the PVDF membrane by using the reagents and following manufacturer's directions. After blocking the membrane for 1 hour at room temperature, the membrane was then rinsed three times in IX Wash Buffer, five minutes each and then soaked in blocking buffer containing 1:1000 dilution of chicken serum raised against NDV virus (Lot # C0139, Charles River Laboratories). After washing three times in a washing buffer, the membrane was incubated with a peroxidase labeled goat anti-chicken IgG (KPL, cat# 14-24-06) at a dilution of 1:2000 for 1 hour at room temperature. The membrane was then rinsed three times in IX Wash Buffer, five minutes each. 5ml of TMB membrane peroxidase substrate was added to the membrane and gently rocked for about 1 minute. The developing reaction was stopped by placing the membrane into water.

[0080] The immunoprecipitation and Western blot technique detected an approximately 55 kD protein in vHVTI 14 sample that corresponds to the expected size of F1 component of the NDV-F protein (FIG. 7).

## **Example 2 Construction of recombinant vHVTHO, vHVTHI, vHVT112, VHVT113 and vHVT116 expressing NDV-F**

[0081] Generation and characterization of HVT recombinants vHVT 110, vHVT 111, vHVTI 12, vHVTI 13, and vHVTI 16 was essentially done in the same way as for vHVTI 14 described in example 1. Table 4 shows the features unique to each construct around the expression cassettes, including the respective sequences.

Table 4 Characteristics of the expression cassettes  
of single HVT recombinants

Name	Parental	Promoter	F gene	Poly-A	Locus
------	----------	----------	--------	--------	-------

	virus				
vHVT039	HVT	MDV gB	Wtnm-Texas	SV40	IG1
vHVTHO	HVT	mCMV IE	Wt-VIIId	SV40	IG1
vHVTII 1	HVT	SV40	Wt-VIIId	SV40	IG1
vHVTI 12	HVT	MCMV IE	Wt-YZCQ	SV40	IG1
vHVT113	HVT	MCMV IE	Wt-Texas	SV40	IG1
vHVT114	HVT	SV40	Opt-VIIId	SV40	IG1
vHVT116	HVT	SV40	Opt-Ca02	SV40	IG1

#### vHVTI 10

[0082] The plasmid pCD046 (Merial proprietary material) containing the Intergenic I arms of HVT FC126, mouse CMV promoter and SV40 poly A was digested with NotI, dephosphorylated, and a 6.6kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized NDV-F gene containing wild-type F sequence (SEQ ID NO:3, coding for SEQ ID NO:4) was also NotI digested and the 1.7 kb fragment was gel extracted. The 6.6 and 1.7 kb fragments were ligated to create a donor plasmid pCD046+NDV-F wt (SEQ ID NO:21 for vHVTI 10) used in transfection to generate recombinant vHVTI 10. Sequencing of the insert region confirmed that vHVTI 10 contains the correct sequences of mCMV promoter, the wildtype NDV-F gene and the SV40 polyA. The sequence also exactly matches the sequence described for the donor plasmid pCD046+NDV-F wt in SEQ ID NO:21.

#### vHVTI 11

[0083] The plasmid pHM103 plasmid (Merial proprietary material) containing the Intergenic I arms of HVT FC126, SV40 promoter and SV40 polyA was digested with NotI, dephosphorylated, and the 5.6 kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized NDV-F gene containing wildtype F sequence (SEQ ID NO:3, coding for SEQ ID NO:4) was also NotI digested and a 1.7kb fragment was gel extracted. The 5.6 and 1.7 kb fragments were ligated to create a donor plasmid (SEQ ID NO:22 for vHVTI 110) used in transfection to generate recombinant vHVTI 11. Sequencing of the insert region confirmed that vHVTI 11 contains the correct sequences of SV40 promoter, the wildtype NDV-F gene and the SV40 polyA as shown in the sequence of the donor plasmid pHM103+NDV-F wt (SEQ ID NO:22).

#### vHVTI 12

[0084] A fragment encompassing the synthetic NDV-F YZCQ wild type gene (SEQ ID NO:34 encoding SEQ ID NO:35) was excised from pUC57 NDV-F YZCQ plasmid (synthesized by GeneScript) using NotI and inserted into the same site of pCD046 plasmid containing mCMV promoter and SV40 polyA tail. Ligated material was transformed using Top 10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagen MiniSpin Prep kit, and screened for insert orientation. The correct donor plasmid was designated pCD046+NDV-F VII YZCQ. Large scale cultures were grown and plasmid extraction was done by using Qiagen Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against NDV.

[0085] Plasmid pCD046+NDV-F VII YZCQ (SEQ ID NO:29) was used in transfection to generate recombinant vHVTI 12. Sequencing of the insert region confirmed that vHVTI 12 contains the correct sequences of mCMV promoter, the wildtype NDV-F YZCQ gene and the SV40 polyA. The sequence also exactly matches the sequence described for the donor plasmid pCD046+NDV-F VII YZCQ in SEQ ID NO:29.

vHVTI 13

[0086] A fragment encompassing the synthetic NDV Texas F gene (SEQ ID NO:36 encoding SEQ ID NO:37) was excised from pUC57 NDV Texas F plasmid (synthesized by GeneScript) using NotI and inserted into the same site of pCD046 plasmid containing mCMV promoter and SV40 polyA tail. Ligated material was transformed using Top 10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagen MiniSpin Prep kit, and screened for insert orientation. The correct donor plasmid was designated pCD046+Texas NDV-F. Large scale cultures were grown and plasmid extraction was done by using Qiagen Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against NDV.

[0087] Plasmid pCD046+Texas NDV-F (SEQ ID NO:30) was used in transfection to generate recombinant vHVTI 13. Sequencing of the insert region confirmed that vHVTI 13 contains the correct sequences of mCMV promoter, the wild-type NDV-F

Texas F gene and the SV40 polyA. The sequence also exactly matches the sequence described for the donor plasmid pCD046+Texas NDV-F in SEQ ID NO:30.

#### VHVT039

[0088] The MDV gB promoter (SEQ ID NO:38) was amplified from MDV1 RB1B strain extracted DNA by PCR using the primers HM101 (5'-CCG-GAA-TTC-CGA-TGT-TTA-GTC-ACG-ATA-GAC-3') (SEQ ID NO:44) and HM102 (5'-ATA-AGA-GCG-GCC-GCA-GTG-AGA-TGA-TCT-TAA-TGA-TG-3') (SEQ ID NO:45). The former contains an EcoRI site and the latter contains a NotI site for ligation of the EcoRI/NotI digested 630 bp PCR product into EcoRI/NotI digested pCD046 plasmid. The ligation product was used to transform DH5a competent cells. Colonies were picked and screened for the presence of the inserted PCR fragment by restriction analysis with EcoRI and NotI. The resulting plasmid was designated pHM102.

[0089] The velogenic NDV Texas strain (genotype IV) was grown on 11-day-old SPF embryonated eggs and semi-purified. Total RNA was extracted and an RT PCR was performed using two primers F-ATG (5' TAT-AGC-GGC-CGC-AAG-ATG-GGC-TCC-AGA-TCT-TCT-ACC-AG 3') (SEQ ID NO:46) and F-STOP (5' CGA-GGC-GGC-CGC-TCA-TAT-TTT-TGT-AGT-GGC-TCT-C 3') (SEQ ID NO:47). They allow the whole amplification of the NDV F gene with addition of NotI site upstream ATG and downstream STOP codons. The 1.7 kb PCR fragment was digested with NotI and ligated into NotI-digested pHM102. The resulting plasmid was designated pHMI 19 and was used as a donor plasmid in *in vitro* recombination study by co-transfection of CEF cells with HVT parental DNA to generate vHVT039 as described above. Sequencing of the insert region confirmed that vHVT039 contains the correct sequences of MDV gB promoter, the wildtype unmodified NDV-F gene from Texas strain (SEQ ID NO:32 encoding SEQ ID NO:33) and the SV40 polyA as shown in the partial sequence of the donor plasmid pHMI 19 (SEQ ID NO:31).

#### vHVT116

[0090] The plasmid pHM103 plasmid (Merial proprietary material) containing the Intergenic I arms of HVT FC126, SV40 promoter and SV40 polyA was digested with NotI, dephosphorylated, and the 5.6kb fragment was gel extracted. A NotI flanked 1.7 kb fragment of a chemically synthesized, codon-optimized, CA02 genotype V NDV-F gene

(SEQ ID NO:5, coding for SEQ ID NO:6) was also NotI digested and the 1.7 kb fragment was gel extracted. The 5.6 and 1.7kb fragments were ligated to create pHM103 + NDV-F CA02 (SEQ ID NO:23 for vHVTI 16) used in transfection to generate recombinant vHVTI 16. Sequencing of the insert region confirmed that vHVTI 16 contains the correct sequences of SV40 promoter, the codon-optimized CA02 NDV-F gene and the SV40 polyA as shown in the sequence of the donor plasmid pHM103+NDV-F wt (SEQ ID NO:23).

#### Discussion

[0091] Various cassettes under mCMV or non-CMV promoter were inserted at different loci of HVT genome (Table 4). Despite repeated attempts, generating a construct with a combination of mCMV and codon-optimized F sequence was not successful beyond passage 2. However, when wild-type sequence was driven by mCMV a stable construct, vHVTI 10 could be generated. In addition, recombinant vHVTI 11 with wild-type F sequence under SV40 promoter was also stable for more than 10 *in vitro* passages. Surprisingly, a codon-optimized F sequence under SV40 promoter was similarly found to be stable for more than 10 *in vitro* passages (e.g. vHVTI 14 and vHVTI 16). These results indicate the delicate balance between the strength of the promoter and the nature of the gene they control (codon-optimized or not optimized) in generating a genetically stable HVT construct.

### **Example 3 Construction of vHVT306, a double HVT vector expressing NDV-F and IBDV VP2**

[0092] The donor plasmid pHVT US2 SV- Fopt-synPA was constructed containing SV40 promoter, synthetic NDV F codon optimized VII gene, synthetic polyA tail flanked by the SORF3 and US2 arm sequences of HVT FC126.

#### Generation of recombinant virus

[0093] A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using donor plasmid pHVT US2 SV-Fopt-synPA and viral DNA isolated from vHVT13 (an HVT vector expressing the IBDV VP2 gene, Meril Limited). Essentially the procedure described in example 1 for vHVTI 14 was

followed to generate, plaque purify and characterize recombinants by immunofluorescence.

[0094] After five rounds of plaque purification, pure recombinant virus (vHVT306) was isolated and the purity of vHVT306 was tested and confirmed by IFA and PCR.

#### PCR analysis

[0095] Viral DNA was extracted from vHVT306 pre-master seed virus (pre-MSV) stock by QIA DNeasy Blood & Tissue Kit (Qiagen cat#69506). PCR primers were designed to identify the presence of the NDV F optimized, the NDV F wild type, the SV40 promoter, the mCMV promoter, the flanking arms of US2 HVT virus and SB-1 virus.

[0096] PCR amplification with various primers confirmed that the vHVT306 has the expected amplification patterns and amplicons.

#### Expression analysis

[0097] Indirect immunofluorescent assay (IFA) was performed on the vHVT306 pre-MSV stock. The CEFs that were inoculated with vHVT306 were fixed with ice-cold 95% acetone for three minutes at room temperature and air-dried for 10 min. After three washes with PBS, two primary antibodies, chicken anti-Newcastle Disease Virus sera (Charles Rivers Laboratories cat#10100641, lot#C01 17A) at 1:500 dilution and L78 monoclonal antibody against HVT (Merial Select, Gainesville, GA) at 1:3000 dilution were added and incubated for 45 min at 37°C. After three washes with PBS, two secondary antibodies, goat anti-chicken IgG~fluorescein (KPL cat#.02-24-06, lot#l 10020) at 1:500 dilution and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe #A10037, lot#989784) at 1:300 dilution were added. The plates were incubated at 37°C for 45 min and followed by three washes with PBS. The cells were observed to identify the IFA positive plaques with a fluorescent microscope using fluorescein isothiocyanate (FITC)- and tetramethylrhodamine isothiocyanate (TRITC)-filters of Nikon Eclipse Ti inverted microscope.

[0098] Similarly the expression of IBDV VP2 protein (SEQ ID NO:8 encoded by SEQ ID NO:7) of vHVT306 were examined by IFA using chicken anti-IBDV sera (Charles River Laboratories cat#10100610 lot#G01 17) (1:500 dilution) and anti-NDV F monoclonal antibody 001C3 (Asceitic fluid, Batch 10/09/044, 02/1 1/2010) (1:300 dilution) as primary antibodies; followed by goat anti-chicken IgG-fluorescein (KPL

cat#.02-24-06, lot#1 10020) (1:500 dilution) and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe #A10037, lot#989784) (1:300 dilution) as secondary antibodies.

**[0099]** IFA results indicate that vHVT306 expresses the NDV F genes in virus-infected CEFs.

**[0100]** Over 400 vHVT306 plaques were counted using the FITC-filter and TRITC-filter of microscope. The overall expression of NDV F gene and IBDV VP2 match with the HVT plaques (Table 5).

Table 5 Dual IFA of vHVT306

Virus	IFA #1 (total 453 plaques)		IFA#2 (total 478 plaques)	
	Anti-NDV serum positive plaques	Anti-HVT MAb positive plaques	Anti-NDV F MAb positive plaques	Anti-IBDV serum positive plaques
vHVT306 pre-MSV	453	453	478	478

#### Southern Blot analysis

**[0101]** Total genomic DNA was extracted from vHVT306 pre-MSV stock infected CEFs. The Southern blot analysis was performed according to the standard protocol.

**[0102]** A total 3 probes were used to confirm the NDV F cassette (SV40 promoter, NDV F codon optimized gene, synthetic polyA tail) between SORF3 and US2 of vHVT306 as well as retention of IBDV VP2 cassette (mCMV promoter, IBDV VP2 gene, SV40 poly A tail).

**[0103]** The Southern blot results showed the digestion patterns as expected based on Vector NTI (Invitrogen, 1600 Faraday Ave., Carlsbad, CA) map analysis. The NDV F cassette (SV40 promoter, NDV F codon optimized gene, synthetic poly A tail) is located between SORF3 and US2, and IBDV VP2 cassette (mCMV promoter, IBDV VP2 gene, SV40 poly A tail) is intact like the parent virus (vHVT13).

#### Genomic analysis

**[0104]** The genomic DNA of vHVT306 pre-MSV stock was sequenced to verify the sequence of the recombination arm region as well as inserted gene cassette.

**[0105]** Primers were designed to amplify the entire inserted gene cassette including recombination arm used in donor plasmid. Analysis of vHVT306 genomic DNA was performed by PCR amplification and followed by nucleotide sequence determination.

[0106] The vHVT306 (donor plasmid pHVT US2 SV- Fopt-synPA) containing the recombinant arms, SV40 promoter and NDV F codon-optimized gene was confirmed to be correct as shown in SEQ ID NO:20.

#### Western blot analysis

[0107] The CEF monolayer was infected with vHVT306 pre-MSV at MOI ~ 0.1. After a 4-day incubation, the CEFs were pelleted and washed with PBS followed by lysis with IP Lysis/Wash buffer of Pierce Classic IP Kit (Thermo Scientific cat#26146) according to the manufacturer's protocols. The lysate was pre-cleared and incubated with 100 ul of anti-NDV F monoclonal antibody 001C3 to make the immune complex. The immune complex was captured by Protein A/G Plus Agarose and after removing of the un-bound immune complex by washing steps, the 50 ul of sample buffer was used to elute under non-reducing conditions. The uninfected CEFs were included as controls. The 20 ul of eluted samples were separated in a 10% Bis-Tris Gels by electrophoresis. After the electrophoresis, the separated proteins were transferred onto PVDF membrane. The Protein Detection TMB Western Blot Kit (KPL cat#54-1 1-50) was used to detect the NDV antigens on PVDF membrane with chicken anti-NDV serum (Charles River Laboratories cat#10100641, lot#C01 17A), and goat anti-chicken IgG-peroxidase conjugate (KPL cat# 14-24-06) following the manufacturers' protocols.

[0108] The NDV F protein expression of vHVT306 was confirmed by two-step immunodetection. First, the expressed NDV F proteins from vHVT306 infected CEF were captured by the immunoprecipitation using anti-NDV F monoclonal antibody 001C3. Subsequently Western blot analysis using anti-NDV polyclonal serum (Charles River Laboratories cat#10100641, lot#C01 17A) was applied to detect the NDV F protein in the captured samples (NDV F protein-monoclonal antibody complex) (FIG.8). A 55 kDa protein in vHVT306 pre-MSV lysates was detected by anti-NDV serum which corresponds to the expected size of NDV F1 fusion protein (FIG.8).

#### **Example 4 Construction of double HVT vectors vHVT301, vHVT302, vHVT303, vHVT304 and vHVT307 expressing NDV-F and IBDV VP2, and double HVT vector vHVT202 expressing IBDV VP2 variants**

Example 4.1 Construction of vHVT301, vHVT302, vHVT303,



## VHVT304 and vHVT307

[0109] Generation and characterization of double HVT recombinants vHVT301, vHVT302, vHVT303, vHVT304, and vHVT307 were essentially done in the same way as for vHVT306 described in example 3. Table 6.1 shows the features unique to each construct around the expression cassettes, including the respective sequences.

Table 6.1 Characteristics of the expression cassettes  
of double HVT recombinants

Name	Parental virus	Promoter	NDV-F gene	Poly-A	Locus
vHVT301	vHVT13	SV40	Wt-VIId NDV-F	SV40	IG2
vHVT302	vHVT13	US10	Opt-VIId NDV-F	US10	US10
vHVT303	vHVT13	US10	Opt-V NDV-F	US10	US10
vHVT304	vHVT13	SV40	Opt-VIId NDV-F	Synthetic	IG2
vHVT306	vHVT13	SV40	Opt-VIId NDV-F	Synthetic	SORF3-US2
vHVT307	vHVT13	SV40	Opt-V NDV-F	Synthetic	SORF3-US2

VHVT301

[0110] The plasmid pHVT IG2 SbfI (Merial proprietary material) containing the Intergenic 2 arm sequences of vHVT13 was digested with *SmaI*, dephosphorylated, and the 4.3kb fragment was gel extracted. The donor plasmid pHM103+NDV-F wt containing an SV40 promoter, wildtype NDV-F genotype VIId, SV40 poly A tail was *EcoRI* and *SacI* digested, klenow treated, and the 2.3kb fragment was gel extracted. The two fragments were ligated to create a donor plasmid pHVT IG2 SV Fwt SbfI (SEQ ID NO: 24) used in transfection to generate recombinant vHVT301.

VHVT302

[0111] A synthetically synthesized plasmid, pHVT US10 cds, containing the US10 arm sequences of vHVT13 was digested with *NotI*, dephosphorylated, and the 4.7kb fragment was gel extracted. A *NotI* flanked 1.7kb fragment of a chemically synthesized, codon-optimized, NDV-F genotype VIId was *NotI* digested and gel extracted. The two fragments were ligated to create a donor plasmid pHVT US10 cds F opt used in

transfection to generate recombinant vHVT302. The transcription of the inserted F gene should be driven by the native US10 promoter and be stopped by the native US10 polyA signal. No exogenous promoter or polyA is added to express this insert. Sequencing of the insert region confirmed that vHVT302 contains the correct sequence of the codon-optimized Vld NDV-F gene as shown in the sequence of the donor plasmid pHVT US10 cds F opt (SEQ ID NO: 25).

#### VHVT303

[0112] The synthetically synthesized plasmid pHVT US10 cds containing the US10 arm sequences of vHVT13 was digested with *NotI*, dephosphorylated, and the 4.7kb fragment was gel extracted. A *NotI* flanked 1.7kb fragment of a chemically synthesized, codon-optimized, NDV-F genotype V was *NotI* digested and gel extracted. The two fragments were ligated to create a donor plasmid pHVT US10 cds F CA02 opt used in transfection to generate recombinant vHVT303. As with vHVT302, the transcription of this inserted F gene should also be driven by the native US10 promoter and be stopped by the native US10 polyA signal. No exogenous promoter or polyA is added to express this insert. Sequencing of the insert region confirmed that vHVT303 contains the correct sequence of the codon-optimized NDV-F genotype V as shown in the sequence of the donor plasmid pHVT US10 cds F CA02 (SEQ ID NO: 26).

#### VHVT304

[0113] The donor plasmid pHVT IG2 *SbfI* containing the Intergenic 2 arm sequences of vHVT13 was digested with *SbfI*, dephosphorylated, and the 4.3 kb fragment was gel extracted. A synthetically synthesized plasmid containing an SV40 promoter + codon optimized NDV-F genotype Vld + synthetic polyA tail flanked by *SbfI* was digested with *SbfI* and the 2.3kb fragment was gel extracted. The two fragments were ligated to create a donor plasmid pHVT IG2 SV Fopt syn tail used in transfection to generate recombinant vHVT304. Sequencing of the insert region confirmed that vHVT304 contains the correct sequences of SV40 promoter, the codon-optimized Vld NDV-F gene, and the synthetic poly A tail as shown in the sequence of the donor plasmid pHVT IG2 SV Fopt syn tail (SEQ ID NO:27).

#### VHVT307

[0114] The donor plasmid pHVT US2-SORF3 containing the US2 and SORF3 arm sequences of vHVT13 was digested with *Sbf*I, dephosphorylated, and the 5.1kb fragment was gel extracted. The plasmid SB-1 UL55 SV CaF syn tail *Sbf*I containing an SV40 promoter + codon optimized NDV-F genotype V + synthetic polyA tail flanked by *Sbf*I was digested with *Sbf*I and the 2.3kb fragment was gel extracted. The two fragments were ligated to create a donor plasmid pHVT US2 SV-FCA02 opt-synPA used in transfection to generate recombinant vHVT307. Sequencing of the insert region confirmed that vHVT307 contains the correct sequences of SV40 promoter, the codon-optimized VIRD NDV-F gene, and the synthetic poly A tail as shown in the sequence of the donor plasmid pHVT US2 SV-FCA02 opt-synPA (SEQ ID NO: 28).

#### Discussion

[0115] One of the main goals of this work was to develop a multivalent avian Herpesvirus-based vector by incorporating multiple protective genes of interest to one avian Herpesvirus backbone (e.g. HVT). A prerequisite for this approach is to define expression cassettes containing appropriate promoter-gene-polyA combinations and evaluate for their genetic stability and ability to protect against the specific disease.

[0116] For the purpose of creating an efficacious MD-IBD-ND trivalent vector vaccine, either codon-optimized or non-optimized Newcastle Disease Virus (NDV)-F gene sequences were cloned into vHVT13 backbone (HVT-IBD, a licensed vaccine to simultaneously protect chickens against MD and IBD) under human CMV (mouse CMV is already used in vHVT13). All vHVT-IBD-F constructs under human CMV promoter lost F-protein expression within six passages whether or not the NDV-F sequence is codon-optimized and regardless of the insertion site. The loss of F protein expression was rapid (within two passes) when hCMV was combined with codon-optimized F protein as compared to a combination of hCMV with wild-type F-sequence (loss of F protein expression within 6 passages). Taken together, the data shows that human CMV is not an ideal promoter for the generation of stable HVT recombinants expressing NDV-F protein. Surprisingly, this example shows that SV40 promoter and HVT endogenous promoter (US 10 promoter) generated stable HVT recombinants expressing NDV-F protein.

#### Example 4.2 Construction of vHVT202

##### Donor plasmid HVT SORF3-US2 gpVar-Ewtsvn construction

[0117] A fragment encompassing the synthetic Variant E wild type IBDV VP2 gene (SEQ ID NO:41 encoding SEQ ID NO:42) was excised from pUC57 Variant E wt plasmid (synthesized by GeneScript) using NotI and inserted into the same site of SORF3 and US2 plasmid containing gpCMV promoter and synthetic polyA tail. Ligated material was transformed using Top 10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagen MiniSpin Prep kit, and screened for insert orientation using *SacI*+*HindIII* digestion. The correct donor plasmid was designated pHVT SORF3-US2 gpVar-Ewt Syn. Table 6.2 shows the features unique to the construct around the expression cassettes, including the respective sequences. Large scale cultures were grown and plasmid extraction was done by using Qiagen Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against IBDV.

Table 6.2 Characteristics of the expression cassettes  
of double HVT recombinants

Name	Parental virus	Promoter	IBDV VP2 gene	Poly-A	Locus
vHVT202	vHVT306	Guinea pig CMV	IBDV E VP2	Synthetic	SORF3-US2

#### Recombinant generation

[0118] A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using pHVTSORF3-US2 gpVar-Ewt Syn donor plasmid and viral DNA isolated from vHVT306 and digested with *SbfI*. vHVT306, expressing classical VP2 of IBDV and NDV-F, was chosen as a parent to simplify the section process as described below. The variant E VP2 donor plasmid was designed to replace the F gene and recombinants were initially selected for the absence of F gene expression and later by PCR for the presence of variant E VP2. Co-electroporation was performed using  $1 \times 10^7$  CEF in 300  $\mu$ l Opti-MEM and shocked at 150 volts with 950 capacitance in a 2 mm electroporation cuvette. The transfected cells were seeded into 96-well plate and incubated for 5-7 days. The cells grown in the 96-well plate were then duplicated into two 96-well plates and incubated for 5 more days. One set of 96-well

plates was used for IFA using chicken polyclonal sera against NDV-F to identify positive wells containing the vHVT306 parents and another set of 96-well plates was used for recovering the infected cells from the IFA negative wells.

[0119] The recombinant viral purification methods were preformed first by 96-well plate duplication and IFA selection for the wells containing the most IFA negative (against NDV-F) plaques with the least amount of IFA positive plaques. Wells matching those criteria were then harvested and adjusted to 1ml in DMEM+2% FBS. From the 1ml stock 5-20  $\mu$ l (depending on the number of visible plaques) were removed and mixed with  $1 \times 10^7$  CEFs in 10ml DMEM+2% FBS and aliquoted onto a new 96-well plate in an attempt to have single HVT plaques per well. The 96-well plates were duplicated after 4 days of incubation and wells that contained plaques were tested for the presence of recombinant HVT and absence of parental virus by IFA and PCR. Again the wells that appeared to have more recombinant virus and less parent virus, by comparing the PCR banding results, were harvested and adjusted to 1ml and aliquoted onto new 96-well plates (the same as before). After five rounds of purification of virus infected cells, recombinant HVT carrying two IBDV VP2 proteins was isolated and the purity of the recombinant virus was tested by PCR to confirm the absence of parental virus.

[0120] Sequencing of the insert region confirmed that vHVT202 contains the correct sequences of guinea pig CMV promoter, the IBDV Variant E wildtype VP2 gene, and the synthetic poly A tail as shown in the sequence of the donor plasmid HVT SORF3-US2 gpVar-Ewtsyn (SEQ ID NO:39).

#### Analysis of recombinant by PCR

[0121] DNA was extracted from a stock virus by phenol/chloroform extraction, ethanol precipitated, and resuspended in 20mM HEPES. PCR primers were designed to specifically identify the Variant E wt gene, the promoter, the polyA, as well as, the purity of the recombinant virus from HVT parental virus. PCR was performed using 200  $\mu$ g of DNA template along with the specified primers pairs indicated in Table 1. PCR cycling conditions are as follows (unless otherwise noted): 94°C - 2 min; 30 cycles of 94°C - 30 sec, 55°C - 30 sec, 68°C - 3 min; 68°C - 5 min.

[0122] Purity of recombinant virus was verified by PCR using primer pairs that are specific to the HVT flanking arms, the gpCMV promoter, the Variant E gene and the syn

tail. Primers, specific to SB1, MDV serotype 2 (SB1US1.FP +SB1Sorf4.RP) were also included in the analysis. The PCR results demonstrate that recombinant virus vHVT202 carries the intended expression cassette and the virus stock is free from detectable amounts of parental HVT virus.

Immunofluorescent staining of recombinant vHVT202 virus expressing two VP2 proteins of IBDV

[0123] For immunofluorescence testing, the P3 material was diluted 1:100 in media. Approximately 50  $\mu$ l of the diluted virus was added to 10 ml of DMEM+2% FBS with  $1 \times 10^7$  CEFs and then aliquoted onto a 96 well plate (100  $\mu$ l/well). The plates were incubated for 4 days at 37°C+5% CO<sub>2</sub> until viral plaques were visible. The plates were fixed with 95% ice-cold acetone for three minutes and washed three times with PBS. One well was used for chicken anti-sera against Newcastle Disease Virus (lot#C0139, Charles Rivers Laboratory) at 1:1000 was added and the plates were incubated at 37°C for 1 hour. The other well was used for chicken anti-sera against IBDV (lot#G0117) After one hour incubation, the plates were washed three times with PBS and FITC anti-chicken (cat# F8888, Sigma) was added at 1:500. Again the plates were incubated at 37°C for 1 hour. After one hour incubation the cells were rinsed three times with PBS and visualized with a fluorescent microscope using fluorescein isothiocyanate (FITC) filter.

[0124] The immunofluorescent staining results indicate that vHVT202 exhibited a very strong expression of the VP2 protein when the polyclonal sera against both classical and variant E VP2 proteins were used.

Conclusion

[0125] Based on PCR and immunofluorescence analysis, vHVT202 is a recombinant HVT in which a VP2 gene of variant E IBDV under the control of gpCMV promoter was successfully inserted into a recombinant HVT background that already expresses the VP2 gene of classical IBDV. Consequently vHVT202 carries both VP2 genes of variant E and classical IBDV and it is free of any detectable parental vHVT306 virus.

**Example 5 Construction of recombinant vSBI-009, vSBI-004, vSBI-006, vSBI-007, vSBI-008, and vSBI-010 expressing NDV-F**

**Example 5.1 Construction of vSBI-009, vSBI-004, vSBI-006, vSBI-007,  
and vSBI-008**

[0126] The aim of the study is to construct a recombinant SB-1 viral vector vSB 1-009 in which an expression cassette containing SV40 promoter and Newcastle disease virus fusion protein (NDV-F) is inserted to replace UL44 coding sequence (gC) of SB-1 .

[0127] A donor plasmid pSBI 44 cds SV FCAopt was constructed containing UL44 flanking arms of SB1 virus, SV40 promoter and NDV F codon optimized gene sequence (SEQ ID NO:5, coding for SEQ ID NO:6).

Generation of recombinant virus

[0128] A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using donor plasmid pSBI 44 cds SV FCAopt and viral DNA isolated from SB-1 virus infected CEFs. Essentially the procedure described in example 1 for vHVT1 14 was followed to generate, plaque purify and characterize recombinants by immunofluorescence.

[0129] After five rounds of plaque purification, pure recombinant virus (vSB 1-009) was isolated and the purity of vSB 1-009 was tested by IFA and PCR to validate the appropriate insertion as well as no remnant parental virus.

PCR analysis

[0130] Viral DNA was extracted from vSB 1-009 pre-master seed virus (pre-MSV) stock by QIA DNeasy Blood & Tissue Kit (Qiagen cat#69506). PCR primers were designed to identify the presence of the NDV F optimized, the NDV F wild type, the SV40 promoter, the mCMV promoter, the UL44 flanking arms of SB-1 virus and HVT virus. PCR amplifications were performed using approximately 200ng of DNA template along with the primer pairs.

[0131] PCR amplification with various primers confirmed that the vSBI-009 has the expected amplification patterns and amplicons.

Expression analysis

[0132] Indirect immunofluorescent assay (IFA) was performed on the vSB 1-009 pre-MSV stock to examine the expression of NDV F gene and SB-1 virus antigen. The CEFs that were inoculated with vSB 1-009 were fixed with ice-cold 95% acetone for three minutes at room temperature and air-dried for 10 min. The plates were washed with PBS,

then two primary antibodies, chicken anti-Newcastle Disease Virus sera (Charles Rivers Laboratories cat#10100641, lot#C01 17A) at 1:500 dilution and Y5.9 monoclonal antibody against SB-1 virus (Merial Select, Gainesville, GA) at 1:3000 dilution were added and the plates were incubated for 45 min at 37°C. After three washes with PBS, two secondary antibodies, goat anti-chicken IgG-fluorescein (KPL cat#.02-24-06, lot#1 10020) at 1:500 dilution and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe #A10037, lot#989784) at 1:250 dilution were added. The plates were incubated at 37°C for 45 min and followed by three washes with PBS. The wells were screened for IFA positive plaques with a fluorescent microscope using fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC)-filters of Nikon Eclipse Ti inverted microscope. Similarly, reactivity of vSB 1-009 with NDV F Mab was examined by Dual IFA using anti-MDV serum (Charles River Laboratories, cat#10100628, lot#D01 11) (1/300 dilution) and anti-NDV F monoclonal antibody (1/300 dilution) as primary antibody. The goat anti-chicken IgG-fluorescein (KPL cat#.02-24-06, lot#1 10020) (1:500 dilution) and donkey anti-mouse IgG-Alexa Fluor 568 (Molecular Probe #A10037, lot#989784) (1:250 dilution) were used as secondary antibodies. The wells were observed to identify the IFA positive plaques with a fluorescent microscope using FITC- and TRITC-filters of Nikon Eclipse Ti inverted microscope.

[0133] IFA results indicate that vSB 1-009 expresses the NDV F protein in virus-infected CEF. Over 500 vSB 1-009 plaques were counted for NDV F protein expression as well as SB-1 virus specific protein expression with dual IFA. The expression of NDV F protein completely matched with SB-1 virus antigen expression in each virus plaque (Table 7).

Table 7 Dual IFA of vSB 1-009

Virus	Dual IFA plate#1(total 189 plaques)		Dual IFA plate#2(total 361 plaques)	
	Anti-NDV serum positive plaques	Anti-SB-1 MAb positive plaques	Anti-NDV serum positive plaques	Anti-SB-1 MAb positive plaques
vSB1-009 pre-MSV	189	189	361	361

[0134] NDV F Mab reactivity was confirmed by Dual IFA. Over 200 vSB 1-009 plaques were examined for NDV F Mab reactivity as well as anti-MDV serum reactivity. The reactivity with NDV F Mab completely matched with anti-MDV serum reactivity in each virus plaque (Table 8).



Table 8 Reactivity of vSB 1-009 with anti-NDV F Mab

Virus	Dual IFA (total 254 plaques)	
	Anti-MDV serum positive plaques	Anti-NDV F MAb positive plaques
vSB1-009 pre-MSV	254	254

#### Southern Blot analysis

[0135] Total genomic DNA was extracted from vSB 1-009 pre-MSV stock infected CEFs. The genomic DNA of vSB 1-009, SB-1 virus (negative control), pSBI 44 cds SV FCA opt donor plasmid were digested at 37°C with EcoRI, NcoI, and KpnI restriction endonucleases separately. The restriction fragments were separated by a 0.8 % agarose gel electrophoresis and transferred onto a positively charged Nylon membrane. After transfer, the membrane was treated with 0.4M NaOH and then neutralized with 2XSSC-HCl buffer. The membrane was then air dried and UV crosslinked.

[0136] Following the Nottfi2South Chemiluminescent Hybridization and Detection Kit (Thermo Scientific cat#89880) manufacturers' instructions, the membrane was pre-hybridized for 1 hr and then hybridized with the probe at 55°C for overnight. For hybridization, two probes were used; 1) the SbfI fragment of pSBI 44 cds SV FCA opt as NDV F cassette probe, 2) the SmaI-EcoRI fragment of pUC57 SB1 44 arm (GenScript) as recombination arm probe. After the overnight hybridization, several stringency washes were conducted until the membrane was placed in blocking buffer with the addition of Streptavidin-HRP. After rinsing the membrane of any unbound Streptavidin-HRP, the substrate solution of Luminal and peroxide were added. The membrane was then exposed to X-ray film and the film was developed.

[0137] The Southern blot results were as expected based on Vector NTI map analysis. The NDV F cassette (SV40 promoter, NDV-F CA02 codon optimized gene) replaced the UL44 coding sequences of SB-1 virus.

#### Genomic analysis

[0138] The genomic DNA of vSB 1-009 pre-MSV stock was conducted by nucleotide sequence determination of the region of recombination arm as well as inserted gene

cassette. Primers were designed and used to amplify the entire NDV-F gene cassette including the recombination arms.

**[0139]** The vSB 1-009 sequence (donor plasmid pSB 1 44 cds SV FCAopt) containing the recombinant arms, SV40 promoter and NDV F codon-optimized gene was confirmed to be correct as shown in SEQ ID NO: 19.

#### Western blot analysis

**[0140]** The CEF monolayer was infected with vSB 1-009 pre-MSV at MOI ~ 0.1. After a 5-day incubation, the CEFs were pelleted and washed with PBS followed by lysis with IP Lysis/Wash buffer of Pierce Classic IP Kit (Thermo Scientific cat#26146) according to the manufacturers' protocols. The lysate was pre-cleared and incubated with 100 $\mu$ l of anti-NDV F monoclonal antibody to make the immune complex. The immune complex was captured by Protein A/G Plus Agarose and after removing of the un-bounded immune complex by washing steps, the 50 $\mu$ l of sample buffer was used to elute under non-reducing conditions. The uninfected CEFs were included as a control. The 20 $\mu$ l of eluted samples were separated in 10% Bis-Tris gels by electrophoresis. After the electrophoresis, the separated proteins in a gel were transferred onto PVDF membrane. The Protein Detection TMB Western Blot Kit (KPL cat#54-11-50) was used to detect the NDV antigens onto PVDF membrane with chicken anti-NDV serum (Charles River Laboratories cat#1 0100641, lot#C01 17A), and goat anti-chicken IgG-peroxidase conjugate (KPL cat#14-24-06) following the manufacturers' protocols.

**[0141]** The NDV F protein expression of vSB 1-009 was confirmed by two-step immunodetection. First, the expressed NDV F proteins from vSB 1-009 infected CEF lysate were captured by the immunoprecipitation using anti-NDV F monoclonal antibody 001C3. Subsequently Western blot analysis using anti-NDV polyclonal serum (Charles River Laboratories cat#1 0100641, lot#C01 17A) was applied to detect the NDV F protein in the captured samples (NDV F protein-monoclonal antibody complex) (FIG.9). An approximately 55 kDa protein in vSB 1-007 pre-MSV lysates was detected by anti-NDV serum that corresponding the expected size of NDV F1 fusion protein (FIG.9).

**[0142]** Generation and characterization of HVT recombinants vSB 1-004, vSB 1-006, vSB 1-007 and vSB 1-008 were essentially done in the same way as for vSB 1-009 described in this example. Table 9.1 shows the features unique to each construct around

the expression cassettes, including the respective sequences. The generation and characterization of recombinant SB 1 viral vectors were also described in US patent application serial No. US \_\_\_\_\_ filed on \_\_\_\_\_ (Merial limited), which is incorporated herein by reference in their entirety.

Table 9.1 Characteristics of the expression cassettes  
of SB1 recombinants

Name	Parental virus	Promoter	F gene	Locus
vSB1-009	SB1	SV40	Opt-CA02	UL44 (gC)
vSB1-004	SB1	mCMV IE	Wt-VIId	US10
vSB1-006	SB1	SV40	Opt-VIId	UL55/LORF5
vSB1-007	SB1	SV40	Opt-VIId	UL44 (gC)
vSB1-008	SB1	SV40	Opt-CA02	UL55/LORF5

### Example 5.2 Construction of double construct vSB1-010

#### Donor plasmid SB1US2 gpVIIIdwtsyn construction

[0143] Using the plasmid HVT SOrf3-US2 gpVar-Ewt Syn, the gpCMV, Varient E, Syn tail was removed by *SbfI* digestion. This fragment was ligated into the SB1 US2 donor plasmid. The Varient E gene was cut out by *NotI* and replaced by NDV-F VIId wt. The synthetic NDV-F VIId wild type gene (SEQ ID NO:3 encoding SEQ ID NO:4) was excised from pUC57 NDV-F VIId wt plasmid (synthesized by GeneScript) using *NotI* digestion. Ligated material was transformed using Top10 Oneshot kit (cat#C404002, Invitrogen). Bacterial colonies were grown in LBamp broth, plasmid extracted by using Qiagen MiniSpin Prep kit, and screened for insert orientation using *NcoI*+*Sall* digestion. The correct donor plasmid was designated pSB1 US2 gpVIIdwt Syn. Table 9.2 shows the features unique to the construct around the expression cassettes, including the respective sequences. Large scale cultures were grown and plasmid extraction was done by using Qiagen Maxi Prep kit. Transient expression of the maxi preps was verified using Fugene Transfection Reagent in Chicken Embryo Fibroblast Cells (CEF's) and chicken polyclonal sera against NDV-F.

#### Recombinant generation

[0144] A standard homologous recombination procedure was followed by co-electroporation of secondary CEF cells using pSB1 US2 gpVIIdWt Syn donor plasmid

and viral DNA isolated from vSB 1-009 (vSB 1-009 is already a recombinant virus expressing CA02 F gene of NDV). Essentially the procedure described in example 1 for vHVT1 14 was followed to generate, plaque purify and characterize recombinants by immunofluorescence.

[0145] After five rounds of plaque purification, pure recombinant virus (vSB 1-010) was isolated and the purity of vSB1-010 was tested by IFA and PCR to validate the appropriate insertion as well as no remnant parental virus.

Table 9.2 Characteristics of the expression cassette of vSB1-010

Name	Parental virus	Promoter	F gene	Locus
vSB1-010	vSB1-009	Guinea pig CMV	NDV-F Vlld	SORF4-US2

[0146] Sequencing of the insert region confirmed that vSB 1-010 contains the correct sequences of guinea pig CMV promoter and the NDV-F Vlld wt gene as shown in the sequence of the donor plasmid SB1US2 gpVIIIdwtsyn (SEQ ID NO:40).

#### Analysis of recombinant by PCR

[0147] DNA was extracted from a stock virus by phenol/chloroform extraction, ethanol precipitated, and resuspended in 20mM HEPES. PCR primers were designed to specifically identify the NDV-F Vlld wt gene, the promoter, the polyA, as well as, the purity of the recombinant virus from SB1 parental virus. PCR was performed using 200 µg of DNA template along with the specified primers pairs indicted in Table 1. PCR cycling conditions are as follows (unless otherwise noted): 94°C - 2 min; 30 cycles of 94°C - 30 sec, 55°C - 30 sec, 68°C - 3 min; 68°C - 5 min.

[0148] Purity of recombinant virus was verified by PCR using primer pairs that are specific to the SB1 flanking arms, the gpCMV promoter, the NDV-F Vlld wt gene and the syn tail. Primers, specific to HVT, MDV serotype 3 (MB080 +MB081) were also included in the analysis. The PCR results demonstrate that recombinant virus vSB1-010 carries the intended expression cassette and the virus stock is free from detectable amounts of parental SB 1-009 virus.

#### Immunofluorescent staining of recombinant vSB1-010 virus expressing two NDV-F proteins

- [0149] For immunofluorescence testing, the P3 material was diluted 1:100 in media. Approximately 50  $\mu$ l of the diluted virus was added to 10 ml of DMEM+2% FBS with  $1 \times 10^7$  CEFs and then aliquoted onto a 96 well plate (100  $\mu$ l/well). The plates were incubated for 5 days at 37°C+5% CO<sub>2</sub> until viral plaques were visible. The plates were fixed with 95% ice-cold acetone for three minutes and washed three times with PBS. Chicken anti-sera against Newcastle Disease Virus (lot#C0139, Charles Rivers Laboratory) at 1:1000 was added and the plates were incubated at 37°C for 1 hour. After one hour incubation, the plates were washed three times with PBS and FITC anti-chicken (cat# F8888, Sigma) was added at 1:500. Again the plates were incubated at 37°C for 1 hour. After one hour incubation the cells were rinsed three times with PBS and visualized with a fluorescent microscope using fluorescein isothiocyanate (FITC) filter.
- [0150] The immunofluorescent staining results indicate that vSBI-010 exhibited a very strong expression of the NDV-F protein when the polyclonal sera against both CA02 and VIRD F proteins of NDV were used.

#### Conclusion

- [0151] Based on PCR testing and immunofluorescence analysis, vSBI-010 is a recombinant SB-1 in which VIRD-F gene of NDV under the control of gpCMV promoter was successfully inserted into a vSB 1-009, which already expresses the CA02-F gene of NDV. Consequently vSBI-010 carries both VIRD and CA02 F genes of NDV genotypes and it is free of any detectable parental vSB 1-009.

#### **Example 6 Efficacy of vHVTHO, vHVT11, vHVT114 and vSBI-004 expressing the NDV F gene against challenges with NDV Chimalhuacan and Malaysian (MAL04-01) strains at 14 days of age in SPF chickens**

- [0152] The aim of the study was to assess the efficacy of three HVT recombinant constructs (vHVT1 10, vHVT1 11 and vHVT1 14) and one SB1 recombinant construct (vSB 1-004) expressing the NDV F gene against Newcastle disease challenges (Chimalhuacan and Malaysian virus strains) performed at 14 days of age in SPF chickens.
- [0153] The characteristics of these 5 vaccine candidates are described in Table 10 below.
- Table 10 Characteristics of the vectors used in the challenge study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVTI 10	HVT	mCMV IE	Wt-VIId	SV40	IG1
vHVTII 1	HVT	SV40	Wt-VIId	SV40	IG1
vHVT114	HVT	SV40	Opt-VIId	SV40	IG1
vSB 1-004	SB-1	mCMV IE	Wt-VIId	SV40	US10

[0154] On DO, 100 one-day-old SPF chickens were randomly allocated into 10 groups of 10 birds. The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in Table 11 below. It should be mentioned that the titer of vSB 1-004 (3 1600 pfu) administered to birds of groups 6 was well above the target. The birds were challenged by the intramuscular route on D14 with velogenic ND Malaysia (genotype VIId) strain (sub-groups "a") or with virulent ND Chimalhuacan (genotype V) strain (sub-groups "b").

Table 11 Challenge study with vHVT 110, vHVT 111, vHVT 114 and vSB 1-004

Group	Vaccine at day-old (DO)	NDV serology at D14*	% protection against mortality/morbidity after Newcastle challenge at 14 days of age (D14)	
			Malaysian strain	Chimalhuacan strain
<b>G1a</b>	-	0/10	0%/0%	-
<b>G1b</b>	-		-	0%/0%
<b>G2a</b>	vHVTI 10	7/10	100%/89%	-
<b>G2b</b>	vVHTHO		-	100%/70%
<b>G3a</b>	vHVTI 11	2/10	30%/20%	-
<b>G3b</b>	vHVTI 11		-	67%/11%
<b>G4a</b>	vHVTI 14	9/10	100%/100%	-
<b>G4b</b>	vHVTI 14		-	89%/89%
<b>G5a</b>	vSB 1-004	3/10	70%/50%	-
<b>G5b</b>	vSB 1-004		-	40%/30%

\*Number of birds positive by NDV HI test/total tested

[0155] Each group was monitored before and after challenge. Clinical signs after challenge were scored daily as follows: healthy / with specific symptoms (ruffled feathers, prostration, torticollis, tremor) / dead. On D14, serum samples were taken in each group for serology (Newcastle Disease virus haemagglutination inhibition (HI) test).

[0156] As expected, the unvaccinated animals (G1a and G1b) displayed no NDV antibodies on D14. A low titer seroconversion (mean HI titer <0.6 log<sub>10</sub>) was obtained in each vaccinated group (sub-groups "a" and "b" of G2 to G5) confirming the vaccine

takes. The number of positive birds/total tested was group-dependent and was the highest (90%) in vHVTI 14 vaccinated birds (see Table above).

[0157] Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the control groups Gla and Gib thus validating the high severity of both challenges. Lowest protection levels were observed in the groups vaccinated with vHVTI 11 or vSB 1-004. Highest protection rates against morbidity and mortality were obtained in the groups vaccinated with vHVTI 10 or vHVTI 14 whatever the challenge strain used (homologous strain *i.e.* Malaysian genotype Vlld or heterologous strain *i.e.* Chimalhuacan genotype V). There was a correlation between the % of birds positive by HI test before challenge and the % protection.

[0158] The difference of protection obtained between vHVTI 10 and vHVTI 11 clearly illustrates the importance of the promoter, the mCMV IE promoter being more potent than the SV40 promoter for the transcription of the wild type (wt) genotype Vlld F gene. The difference of protection obtained between vHVTI 11 and vHVTI 14 illustrates the importance of the nucleotide sequence of the F gene, the optimized sequence being more potent than the wild type (or native) sequence.

[0159] In conclusion, the results of this study showed the importance of the promoter and the nucleotide sequence of the F gene in the ND protection induced by Marek's disease vector vaccines. An optimal combination of these factors needs to be found to reach the best efficacy performances as for vHVTI 14.

**Example 7 Efficacy of vHVT114, vHVT116, vHVT301, vHVT302 and vHVT303  
expressing the NDV F gene against challenges with NDV Texas GB strain at 14 days  
of age in SPF chickens**

[0160] The aim of the study was to assess the efficacy of 2 single HVT recombinant constructs (vHVTI 14 and vHVTI 16) expressing the NDV F gene and 3 double HVT recombinant constructs (vHVT-301, vHVT302 and vHVT303) expressing both NDV F and IBDV VP2 genes against Newcastle disease challenge (Texas GB strain, genotype II) performed at 14 days of age in SPF chickens.

[0161] The characteristics of these 4 vaccine candidates are described in Table 12 below.

Table 12 Characteristics of the vectors used in the challenge study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VIIId	SV40	IG1
vHVT116	HVT	SV40	Opt-V	SV40	IG1
vHVT301	vHVT13*	SV40	Wt-VIIId	SV40	IG2
vHVT302	vHVT13	US10	Opt-VIIId	US10	US10
vHVT303	vHVT13	US10	Opt-V	US10	US10

\*vHVT13 is the active ingredient of the licensed Vaxxitek HVT-IBD vaccine based on an HVT vector expressing the IBDV VP2 gene (see US 5,980,906 and EP 0 719 864).

[0162] On DO, 120 one-day-old SPF chickens were randomly allocated into 6 groups of 20 birds. The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 1000 pfu as described in Table 13 below. The birds were challenged by the intramuscular route on D14 with 4.5 log<sub>10</sub> EID<sub>50</sub> velogenic ND Texas GB (genotype II) strain.

Table 13 Results of efficacy

Group	Vaccine at day-old (DO)	% clinical protection (number infected/total) after Newcastle challenge at 14 days of age (D14)
G1	-	0% (20/20)
G2	vHVTI 14	80% (4/20)
G3	vHVTI 16	70% (6/20)
G4	vHVT301	15% (17/20)
G5	vHVT302	52.6% (9/19)*
G6	vHVT303	15% (17/20)

\* 1 bird died before challenge

[0163] Each group was monitored before and after challenge. NDV clinical signs and mortality were recorded after challenge.

[0164] Percentages of clinical protection are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of both challenges. Partial protection was observed for the 5 vaccine candidates, the best performances being obtained with vHVTI 14 and vHVTI 16. Among the double HVT recombinants, the vHVT302 was the most protective. It performed better than vHVT303 suggesting that the optimized genotype VIIId NDV F gene may be better cross-protective against genotype II challenge than the optimized genotype V NDV F gene. A similar tendency was observed with single HVT, the



vHVT1 14 (VlId gene) performing slightly better than vHVT1 16 (V gene) but the difference was less pronounced. These results indicated that both genotypes VlId and V NDV F genes inserted in the HVT vector provide cross-protection against a heterologous genotype II NDV challenge; the VlId gene may potentially be more cross-protective. The vHVT302 induced a better ND protection than vHVT301 confirming the importance of the promoter, poly-A and locus of insertion. In conclusion, the results of this study showed the very good early ND protection induced by tested Marek's disease vector vaccines, especially for the tested single HVT-ND.

**Example 8 Efficacy of vHVT114, vHVT116, vSB1-007, vSB1-008 (alone or with vHVT13) and vHVT 304 against challenges with NDV ZJ1 (genotype VlId) and California/02 (genotype V) at 21 days of age in SPF chickens**

[0165] The aim of the study was to assess the efficacy of 2 single HVT recombinant constructs (vHVT1 14 and vHVT1 16), 2 SB1 recombinant constructs (vSB 1-007 & vSB 1-008) expressing the NDV F gene and a double HVT recombinant (vHVT304) against Newcastle disease challenge with NDV ZJ1 (genotype VlId) and California/02 (genotype V) performed at 21 days of age in SPF chickens.

[0166] The characteristics of these 5 vaccine candidates are described in Table 14 below.

Table 14 Characteristics of the vectors used in the challenge study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VlId	SV40	IG1
vHVT116	HVT	SV40	Opt-V	SV40	IG1
vSB1-007	SB-1	SV40	Opt-VlId	gC	UL44 (gC)
vSB1-008	SB-1	SV40	Opt-V	SV40	IG1
vHVT304	vHVT13*	SV40	Opt-VlId	Synth	IG2

\*vHVT13 is the active ingredient of the licensed Vaxxitek HVT-IBD vaccine based on an HVT vector expressing the IBDV VP2 gene (see US 5,980,906 and EP 0 719 864).

[0167] On DO, 158 one-day-old SPF chickens were randomly allocated into 6 groups of 24 birds (vaccinated) and 1 group of 12 birds (non-vaccinated controls). The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 1000 pfu as described in Table 15 below. The birds were then separated into two sub-groups, each sub-group being challenged by the

intramuscular route on D21 with 5 log<sub>10</sub> EID<sub>50</sub> of either NDV ZJ1 (genotype Vlld) or California/02 (genotype V) velogenic strain.

Table 15 Results of efficacy

Group	Vaccine at day-old (DO)	% clinical protection	
		CA/02 (genotype V)	ZJ1 (genotype Vlld)
<b>G1</b>	-	0%	0%
<b>G2</b>	vHVTI 14	100%	100%
<b>G3</b>	vHVTI 16	100%	90%
<b>G4</b>	vSB 1-007	92%	100%
<b>G5</b>	vSB 1-008	100%	100%
<b>G6</b>	vSBI-008+vHVT13	100%	83%
<b>G7</b>	vHVT304	92%	75%

[0168] Each group was monitored before and after challenge. Technical problems observed with isolators reduced the number of birds in group 2 (vHVTI 14: from 24 to 14) and in group 3 (vHVTI 16: from 24 to 20). NDV clinical signs were recorded after challenge. Serum was collected from blood samples taken from birds of groups 2 and 7 before challenge (D21) for NDV serology by HI test using each challenge strains as antigen.

[0169] Mean serologic HI titers in G2 and G7 before challenge are shown in Figure 10. HI titers were higher with the ZJ1 antigen in both groups. The HI titers induced by vHVTI 14 were higher than those induced by vHVT304.

[0170] Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of both challenges. All vaccines induced high levels (>75%) of protection against both challenges. Full clinical protection against both challenges was induced by vHVTI 14 and vSB 1-008. Following a similar tendency as the HI titers, the ND protection induced by vHVT304 was slightly lower than that induced by vHVTI 14.

[0171] The shedding was evaluated after challenge by real time RT-PCR in oral and cloacal swabs taken 2 and 4 days post-challenge. Percentage of positive (Ct<40) birds are shown for both challenges in FIG. 11A and 11B. Note that all 6 birds were dead at 4 dpch in the control group challenged with the CA/02 isolate and only one bird (out of 6) was

still alive at 4 dpch in the control group challenged with ZJ1. Shedding was detected in all control birds. Reduction of the percentage of birds positive for shedding was observed in all vaccinated groups.

[0172] In conclusion, the results of this study showed the very good ND protection at 3 weeks of age induced by tested Marek's disease vector vaccines.

**Example 9 Efficacy of vHVTI 14, vSBI-007, vSBI-009, vHVT306 and vHVT307 vaccines against challenges with NDV Texas GB strain at 28 days of age in SPF chickens**

[0173] The aim of the study was to assess the efficacy of combinations of different Marek's disease vector vaccines expressing the NDV F and/or the IBDV VP2 gene against Newcastle disease challenge (Texas GB strain, genotype II) performed at 28 days of age in SPF chickens.

[0174] The characteristics of the 5 recombinant vaccine candidates tested in this study are described in Table 16 below.

Table 16 Characteristics of the vectors used in the challenge study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VIIId	SV40	IG1
vSB1-007	SB-1	SV40	Opt-VIIId	gC	UL44 (gC)
vSB1-009	SB-1	SV40	Opt-V	gC	UL44 (gC)
vHVT306	vHVT13	SV40	Opt-VIIId	Synth	SORF3-US2
vHVT307	vHVT13	SV40	Opt-V	Synth	SORF3-US2

[0175] The Marek's disease virus serotype 1 (CVI988 (or Rispens) strain; Gallid herpesvirus 2) and serotype 2 (SB-1 strain; gallid herpesvirus 3) vaccines were used also in combination with recombinant viruses in some of the groups.

[0176] On DO, 135 one-day-old SPF chickens were randomly allocated into 9 groups of 15 birds. The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL containing a target dose of 2000 pfu for recombinant vaccines (vSB 1-007, vSB 1-009, vHVT13, vHVT306, vHVT307, vHVTI 14), and 1000 pfu for parental Marek's disease vaccine strains (SB-1 and CVI988). The design of the 9 groups is shown in Table 17 below. The birds were challenged by the intramuscular route on D28 with 4.0 log<sub>10</sub> EID<sub>50</sub> velogenic ND Texas GB (genotype II) strain.

Table 17 Results of efficacy

Group	Vaccine at day-old (DO)	% ND protection after Newcastle disease challenge at 28 days of age
G1	-	0%
G2	vSBI-007+vHVT13	80%
G3	vSB 1-009	100%
G4	vSBI-009+vHVT13	86%
G5	vSB 1-009+vHVT 13+CVI988	93%
G6	vHVT306+SB-1	100%
G7	vHVT307	100%
G8	vHVT307+SB-1	93%
G9	vHVT1 14+vHVT13+SB-1	100%

[0177] Each group was monitored before and after challenge. NDV clinical signs after challenge were recorded.

[0178] Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Excellent levels of protection were observed in all vaccinated groups. Birds from G3, G6, G7 and G9 were fully protected. This study shows that the vSBI-ND candidates can be co-administered with vHVT13 and CVI988 and still provide a very good ND protection. Similarly, double HVT-IBD+ND are compatible with SB-1 and vHVT-ND (vHVT1 14) is compatible with vHVT13 and SB-1.

[0179] In conclusion, the results of this study showed the lack of interference on ND protection induced by the tested Marek's disease parental and vector vaccines.

**Example 10 Efficacy of vHVT114, vHVT307, vSBI-007 and vSBI-009 in combination with vHVT13 against challenges with NDV Chimalhuacan strain (genotype V) at D28 in SPF chickens**

[0180] The aim of the study was to assess the efficacy of 1 HVT recombinant construct (vHVT1 14) and 2 SBI recombinant constructs (vSB 1-007 and vSB 1-009) expressing the NDV F gene in combination with vHVT-IBD (vHVT13), as well as a double HVT vHVT307 expressing both NDV F and IBV VP2 against Newcastle disease challenge (Chimalhuacan, genotype V) performed at 28 days of age in SPF chickens.

[0181] The characteristics of these 4 vaccine candidates are described in Table 18 below.

Table 18 Characteristics of the vectors used in the challenge study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT114	HVT	SV40	Opt-VIIId	SV40	IG1
vSB1-007	SB-1	SV40	Opt-VIIId	gC	UL44 (gC)
vSB1-009	SB-1	SV40	Opt-V	gC	UL44 (gC)
vHVT307	vHVT13	SV40	Opt-V	Synth	SORF3-US2

[0182] On DO, 45 one-day-old SPF chickens were randomly allocated into 4 groups of 10 birds and 1 group of 5 birds (unvaccinated control group). The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in Table 19 below. The birds were challenged by the intramuscular route on D28 with 5.0 log<sub>10</sub> EID<sub>50</sub> velogenic Chimalhuacan (genotype V) strain.

Table 19 Results of efficacy

Group	Vaccine at day-old (DO)	% protection against mortality	% protection against morbidity
<b>G1</b>	-	0%	0%
<b>G2</b>	vHVT1 14+vHVT13	100%	100%
<b>G3</b>	vHVT307	80%	80%
<b>G4</b>	vSB1-007+vHVT13	90%	90%
<b>G5</b>	vSB1-009+vHVT13	90%	90%

[0183] Each group was monitored before and after challenge. NDV clinical signs were recorded after challenge. Oropharyngeal swabs were taken in the vaccinated groups at 5 and 7 days post-challenge to evaluate the viral load by real time RT-PCR.

[0184] Percentages of protection against mortality and morbidity are reported in the table above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Very good protection was observed in all 4 vaccinated groups, a full clinical protection being induced by vHVT1 14+vHVT13.

[0185] The percentage of positive birds and the mean shedding titer (expressed as log<sub>10</sub> EID<sub>50</sub> equivalent per mL) are shown in FIG. 12A and 12B. Surprisingly, no shedding was detected in G2 indicating a complete (against both clinical signs and shedding) ND protection induced by vHVT1 14 even if co-administered with vHVT13, in the tested

conditions. The shedding levels detected in the other vaccinated groups were low with a slightly higher level detected in G3 (vHVT307) at 5 days post-infection (pi) only.

[0186] In conclusion, this example further illustrates the excellent ND protection induced by double HVT-IBD+ND recombinant or a combination of SB1-ND or HVT-ND and HVT-IBD (vHVT13) recombinant viruses. Contrary to the general belief in the field that a second HVT vaccine (regular HVT vaccines or recombinant HVT vaccines) interferes with the immunity to the foreign genes inserted into the first recombinant HVT vaccine, the present invention showed surprising result that vHVT1 14 in combination with vHVT13 offered excellent protection against NDV and no interference effect was observed.

**Example 11 Efficacy of vHVT306, vSB1-008 in combination with vHVT13 administered by SC or *in ovo* route against challenge with NDV Chimalhuacan strain (genotype V) at D28 in SPF chickens**

[0187] The aim of the study was to assess the efficacy of the vHVT306 double HVT expressing both NDV F and IBDV VP2 genes, and the vSB 1-008 SB1 recombinant expressing the NDV F gene in combination with vHVT-IBD (vHVT13), administered by the *in ovo* or by the subcutaneous route against Newcastle disease challenge (Chimalhuacan, genotype V) performed at 28 days of age in SPF chickens.

[0188] The characteristics of these 2 ND vaccine candidates are reported in the table 14 (vSB 1-008) and in table 16 (vHVT306).

[0189] The design of the groups is shown on Table 20. Sixty SPF embryonated eggs (after approximately 18 days and 18 hours of incubation; D-3) were used for the *in ovo* administration (20 per group for G1, G2 and G3). Fifty microliters of vaccine containing 2000 PFU were administered by the *in ovo* route using the IntelliLab System device from AviTech LLC (Salisbury, MD, USA). Hatchability and survival were recorded after *in ovo* administration. On DO, 20 one-day-old SPF chickens were randomly allocated into 2 groups of 10 birds (G4 and G5). The birds were injected by subcutaneous (SC) injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in Table 20 below. Ten birds per group were challenged by the

intramuscular route on D28 with 5.0 log<sub>10</sub> EID<sub>50</sub> velogenic Chimalhuacan (genotype V) strain.

Table 20 Study design and results of ND efficacy

Group	Vaccine at day-old (DO)	Admin, route	% protection against mortality	% protection against morbidity
G1	vHVT13	<i>In ovo</i>	0%	0%
G2	vHVT306	<i>In ovo</i>	100%	100%
G3	vSBI-008+vHVT13	<i>In ovo</i>	78%	68%
G4	vHVT306	SC	100%	100%
G5	vSBI-008+vHVT13	SC	100%	70%

[0190] Each group was monitored before and after challenge. NDV clinical signs were recorded after challenge. Oropharyngeal swabs were taken in the vaccinated groups at 5 and 7 days post-challenge to evaluate the viral load by real time RT-PCR.

[0191] Full hatchability and viability were recorded up to D28 (challenge day) for birds of groups G1 and G2. Hatchability in G3 was 85% and one additional bird died after hatching in this group. The lower hatchability of that group may be due to egg incubator problems. Body weights of males and females in G1, G2 and G3 were similar at D1 and at D28.

[0192] Percentages of protection against mortality and morbidity are reported in the table 20. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Very good protection was observed in all 4 vaccinated groups, a full clinical protection being induced by vHVT306 administered by both routes.

[0193] The percentage of positive birds and the mean shedding titer (expressed as log<sub>10</sub> EID<sub>50</sub> equivalent per mL) are shown in Table 21. Absence of detectable or very low shedding was observed in G2 and G4 vaccinated with vHVT306. The shedding levels detected in the groups vaccinated with vSBI-008+vHVT13 were higher especially at 5 days post-infection (pi).

Table 21 Results of protection against shedding (percentage of birds with detectable shedding and mean viral load in log<sub>10</sub>) evaluated at D5 and D7 after NDV challenge

Group	Vaccine at day-old (DO)	Admin. Route	Percent of positive birds (D5/D7 pi)	Mean viral load* (D5/D7 pi)
G2	vHVT306	<i>In ovo</i>	0/0%	2.7/2.7
G3	vSB1-008+vHVT13	<i>In ovo</i>	100/38%	5.2/3.2
G4	vHVT306	SC	20/10%	3.2/2.9
G5	vSB1-008+vHVT13	SC	80/50%	4.6/3.4

\* Mean quantitative real time PCR value expressed in equivalent log<sub>10</sub> EID<sub>50</sub>; the threshold is set at 2.7 log<sub>10</sub>.

[0194] In conclusion, this example shows excellent ND protection induced by vHVT306 double HVT recombinant administered either by *in ovo* or by SC routes. The performance of vSB1-008+vHVT13 was slightly lower especially after *in ovo* administration, but it may be at least partially due to egg incubator problems. Indeed, the *in ovo* safety testing of another SB1-ND recombinant (vSB 1-009) at 1000 or 4000 PFU associated with 6000 PFU of vHVT13 did not show any difference in hatchability and early survival with a group receiving 6000 PFU of vHVT13 only.

**Example 12 Efficacy of vHVT304, vHVT306, vSB1-007 and vSB1-008 in combination with vHVT13 against challenge with NDV Chimalhuacan strain (genotype V) at D42 in commercial broiler chickens**

[0195] The aim of the study was to assess the efficacy of two double HVT (vHVT304 and vHVT306) expressing both NDV F and IBDV VP2 genes, and two SB1 recombinants (vSB 1-007 and vSB 1-008) expressing the NDV F gene in combination with vHVT-IBD (vHVT13) against Newcastle disease challenge (Chimalhuacan, genotype V) performed at 42 days of age in commercial broiler chickens.

[0196] The characteristics of these 4 ND vaccine candidates are reported in tables 14 and 16. The design of the groups is shown on Table 22. On DO, 55 one-day-old commercial broiler chickens were randomly allocated into 5 groups of 11 birds. The birds were injected by subcutaneous (SC) injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in Table 22 below. Ten birds per group were challenged by the intramuscular route on D42 with 5.0 log<sub>10</sub> EID<sub>50</sub> velogenic Chimalhuacan (genotype V) strain.

Table 22 Study design and results of ND efficacy



Group	Vaccine at day-old (DO)	% protection against mortality	% protection against morbidity
G1	vHVT13	0%	0%
G2	vHVT304	82%	82%
G3	vHVT306	100%	100%
G4	vSBI-007+vHVT13	100%	100%
G5	vSBI-008+vHVT13	91%	91%

[0197] Each group was monitored before and after challenge. NDV clinical signs were recorded during 14 days after challenge. Oropharyngeal swabs were taken in the vaccinated groups at 5 and 7 days post-challenge to evaluate the viral load by real time RT-PCR.

[0198] Percentages of protection against mortality and morbidity are reported in the table 22. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of challenge. Very good protection was observed in all 4 vaccinated groups, a full clinical protection being induced by vHVT306 and by vSBI-007+vHVT13.

[0199] The percentage of positive birds and the mean shedding titer (expressed as log<sub>10</sub> EID<sub>50</sub> equivalent per mL) are shown in Table 23. The best reduction of shedding was induced by vHVT306 and vSBI-007+vHVT13, which were also the best candidates for clinical protection.

Table 23 Results of protection against shedding (percentage of birds with detectable shedding and mean viral load in log<sub>10</sub>) evaluated at D5 and D7 after NDV challenge (pi)

Group	Vaccine at day-old (DO)	Percent of positive birds (D5/D7 pi)	Mean viral load* (D5/D7 pi)
G2	vHVT304	100/100%	5.4/4.6
G3	vHVT306	40/50%	3.5/3.7
G4	vSBI-007+vHVT13	80/70%	3.8/4.8
G5	vSBI-008+vHVT13	100/100%	4.8/4.3

\* Mean quantitative real time PCR value expressed in equivalent log<sub>10</sub> EID<sub>50</sub>; the threshold was set at 2.7 log<sub>10</sub>.

[0200] The vHVT306 ND protection was found to be better than that of vHVT304. These two double HVT contain the same NDV F expression cassette but inserted in two

different loci, the IBDV VP2 one being inserted at the same position. This example therefore illustrates the importance of the locus of insertion in the design of HVT recombinants. The vSB1-007+vHVT13 was better than vSB1-008+vHVT13. The vSB1-007 genomic structure differs from that of vSB 1-008 in different aspects: locus of insertion, promoter, poly-adenylation signal and F gene origin. The combination of these foreign sequences and locus of insertion in vSB 1-007 were likely responsible for its better ND protection performances.

[0201] In summary, this example illustrates the importance of the locus of insertion and other regulatory sequences of the NDV expression cassette in the ND protection induced by HVT and MDV serotype 2 vectors.

**Example 13 Efficacy of double HVT-ND+IBD (vHVT304 and vHVT306) or SB1-ND (vSB1-008) in combination with vHVT13 recombinant vaccines, against challenge with a classical IBDV isolate on D14 in SPF chickens**

[0202] The aim of the study was to assess the early IBD efficacy of double HVT recombinants vHVT304 and vHVT306 as well as that of vHVT13 co-administered with a SB1-ND (vSB 1-008) recombinant constructs against a virulent infectious bursal disease virus (vIBDV) challenge (Faragher 52/70 strain) performed at 14 days of age in SPF chickens.

[0203] The characteristics of the double HVT and SB1 recombinants used in this study are shown in Tables 14 and 16.

[0204] On DO, 95 one-day-old SPF chickens were randomly allocated into 9 groups of 10 birds and 1 group of 5 birds (unvaccinated unchallenged control group). The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 300 or 1000 pfu as described in the Table 24 below. On D14, blood sample was collected from 5 birds per group for serological testing with the Kit ProFLOK® plus IBD (Synbiotics Corp). The birds (10 birds per group except for group 7 in which 1 bird died before challenge) were challenged by the eye drop (0.05 mL per bird) on D14 with 2.5 log<sub>10</sub> EID<sub>50</sub>.

Table 24 Study design and results of IBD efficacy

Group	Vaccine at day-old (dose in PFU)	IBD+ ELISA titer at D14 <sup>1</sup>	Number Dead /Sick <sup>2</sup>	% protection <sup>3</sup>	Mean bursal/body weight ratio <sup>4</sup>
G1	vSBI-008 (1000)	0.2	7/10	0%	0.0013
G2	vHVT 13 (300)	2.7	0/0	100%	0.0051
G3	vHVT 13 (1000)	2.7	0/0	90%	0.0049
G4	vHVT13+vSB 1-008 (300)	1.9	1/1	60%	0.0041
G5	vHVT13+vSB 1-008 (1000)	2.4	0/0	70%	0.0041
G6	vHVT304 (300)	2.9	0/0	60%	0.0037
G7	VHVT304 (1000)	2.2	0/0	67%	0.0047
G8	vHVT306 (300)	2.4	0/0	80%	0.0033
G9	vHVT306 (1000)	2.7	0/0	40%	0.0026

<sup>1</sup> Mean IBD+ ELISA titers expressed in log 10 in the serum of 5 birds per group sampled at D14 before challenge;

<sup>2</sup> Birds sick for more than 2 days or still sick on D25 were considered as sick.

<sup>3</sup> Protection against clinical signs and severe bursal lesion (bursal score <3)

<sup>4</sup> The bursal/body weight ratio of the unvaccinated/unchallenged group was 0.0047.

[0205] Each group was monitored before and after challenge. IBDV clinical signs were recorded for 11 days after challenge (from D15 to D25). At the end of the post-challenge observation period (D33), all the surviving birds were euthanized and necropsied. Body and bursal weights were recorded. Each bursa of Fabricius (BF) was weighted then stored in individual recipients containing 4% formaldehyde for histology. Histological lesions of the bursa were scored according to the scale presented in Table 25.

Table 25 Scoring scale of histological lesions of the bursa of Fabricius\*

Score	Histology observation/lesions
0	No lesion, normal bursa
1	1%, to 25% of the follicles show lymphoid depletion (i.e. less than 50% of depletion in 1 affected follicle), influx of heterophils in lesions
2	26% to 50% of the follicles show nearly complete lymphoid depletion (i.e. more than 75% of depletion in 1 affected follicle), affected follicles show necrosis and severe influx of heterophils may be detected
2	51%, to 75%, of the follicles show lymphoid depletion; affected follicles show necrosis lesions and a severe influx of heterophils is detected
4	76%, to 100%, of the follicles show nearly complete lymphoid depletion; hyperplasia and cyst structures are detected; affected follicles show necrosis and severe influx of heterophils is detected
5	100%, of the follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, fibrosis of

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bursal tissue

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\* sourced from Monograph No. 01/2008:0587 of EU Pharmacopoeia "Avian Infectious Bursal Disease vaccine (live)

- [0206] A bird was considered as affected if it died and/or showed notable sign of disease and/or severe lesions of the bursa of Fabricius (*i.e.*, histology score >3).
- [0207] The mean ELISA IBD+ antibody titer expressed in log 10 before challenge is shown in Table 24. Significant titers were detected in all vaccinated groups that were significantly higher than that of the control group G1. The serology titer was not dose-dependent.
- [0208] Severe clinical signs were observed after challenge in all birds of the control group G1. Seven out of 10 birds of that group died within the 11 days observation period indicating the high severity of challenge. None of the vaccinated birds showed severe clinical signs after challenge except 1 bird of G4 that died. Percentages of protection against severe bursal lesions are shown in the table above. Significant IBD protection was observed in all groups, the best protection being observed in G2 and G3 (vHVT13 alone). The co-administration of vSB1-008+vHVT13 and the double vHVT304 and vHVT306 constructs induced similar levels of IBD protection. The protection was not dose-dependent at the tested doses. The mean bursal/body weight ratios are also shown in Table 24. Ratios in all vaccinated groups were higher than those of the challenged control group.
- [0209] In conclusion, these data indicate that both the combination of a SB1-ND vector with a single HVT-IBD or double HVT expressing both NDV-F and IBDV-VP2 induce IBD antibodies and early IBD protection in a severe IBDV challenge model.

**Example 14 Efficacy of single HVT-ND (vHVT114) or SB1-ND (vSB1-007 and vSB1-009) in combination with vHVT13 recombinant vaccines, against challenge with a very virulent IBDV isolate on D23 in commercial broiler chickens**

- [0210] The aim of the study was to assess the IBD efficacy of vHVT13 co-administered with an HVT-ND (vHVT1 14) or SB1-ND (vSB 1-007 and vSB 1-009) recombinant constructs against a very virulent infectious bursal disease virus (vIBDV) challenge (91-168/980702) performed at 23 days of age in commercial broiler chickens.

[0211] The characteristics of these 4 vaccine candidates are described in Tables 14 and 16. On DO, 90 one-day-old broiler chickens were randomly allocated into 7 groups of 12 birds and 1 group of 6 birds (unvaccinated unchallenged control group). The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 3000 pfu as described in the Table 26. On D14, blood sample was collected from 5 birds per group for serological testing with the Kit ProFLOK® plus IBD (Synbiotics Corp). The serum of 10 extra one-day-old broiler chickens was tested at DO with the same kit to evaluate the level of IBDV maternal antibody. The birds (10 birds per group) were challenged by the eye drop (0.05 mL per bird) on D23 with 4.3 log<sub>10</sub> EID<sub>50</sub> of the wIBDV 91-168 isolate.

[0212] Each group was monitored before and after challenge. IBDV clinical signs were recorded for 11 days after challenge (from D23 to D33). At the end of the post-challenge observation period (D33), all the surviving birds were euthanized and necropsied. Body and bursal weights were recorded. Each bursa of Fabricius (BF) was weighted then stored in individual recipients containing 4% formaldehyde for histology. Histological lesions of the bursa were scored according to the scale presented in Table 25.

[0213] A bird was considered as affected if it died and/or showed notable signs of disease and/or severe lesions of the bursa of Fabricius (*i.e.*, histology score >3).

Table 26 Study design and serology results

Group	Vaccine at day-old (DO)	IBD+ ELISA titer at D23 <sup>1</sup>	Mean bursal/body weight ratio <sup>2</sup>
G1	-	3.9	0.0007
G2	vHVT13	4.0	0.0015
G3	vHVT1 14+vHVT13	4.1	0.0015
G4	vSBI-007+vHVT13	3.8	0.0018
G5	vSBI-009+vHVT13	4.0	0.0019

<sup>1</sup> Mean IBD+ ELISA titers expressed in log<sub>10</sub> in the serum of 5 birds per group sampled at D23 before challenge;

<sup>2</sup> The bursal/body weight ratio of the unvaccinated/unchallenged group was 0.0047

[0214] The mean ELISA IBD+ serological titer at DO was 4.36±0.01 log<sub>10</sub> indicating a very high level of IBD maternal antibody at hatch. At D23, the mean ELISA IBD+ titer

was still high (3.9) in the control G1. ELISA mean titers in the vaccinated groups were not significantly different from those of the control group.

[0215] Neither morbidity nor mortality was observed in any of the groups after challenge. Percentages of protection against severe bursal lesions are shown in the table 26 above. The result showed that co-administration of vHVT1 14, vSB 1-007 or vSB 1-009 did not interfere with vHVT 13-induced IBD protection indicating a lack of interference. Similarly, the mean bursal/body weight ratios of the vaccinated groups were similar and clearly higher than that of the control group, indicating IBD protection and no difference between the vaccination regimens.

[0216] In conclusion, the data indicate the compatibility between vHVT1 14, vSB1-007 or vSB 1-009 and vHVT13 for IBD protection. The lack of interference between the two HVT vectors for IBD protection was again surprising and confirmed the results observed for ND protection (see example 10),

**Example 15 Efficacy of double HVT-ND+IBD (vHVT304 and vHVT306) associated or not with SB-1 and of SB1-ND (vSB1-007 and vSB1-008) in combination with vHVT13 recombinant vaccines, against challenge with a variant E IBDV isolate on D28 in SPF chickens**

[0217] The aim of the study was to assess the efficacy of two double HVT (HVT-ND+IBD: vHVT304 and vHVT306) or two vSB-1-NDV in combination with vHVT13 (vSB1-007+vHVT13, vSB1-008+vHVT13) vectored vaccines administered subcutaneously (SC) to day-old SPF chicks and challenged with IBDV-Variant (VAR-E) 28 days post-vaccination.

[0218] On DO, 105 one-day-old SPF chickens were randomly allocated into 7 groups of 15 birds including a group of challenged controls (G6) and unchallenged controls (G7). The birds of groups G1 to G5 were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant and/or SB-1 vaccines containing each a target dose of 2000 pfu. The design of the study is shown in Table 27 below. On D28, all birds from groups G1 to G6 were challenged by the eye drop (0.03 mL containing 3 log<sub>10</sub> EID<sub>50</sub> per bird) of the IBDV variant E isolate from University of Delaware (USA). Each group was monitored before and after challenge. Eleven days post-challenge, birds were weighed

and necropsied. The bursa were collected and weighed. The bursal/body weight ratios (bursa weight/body weight ratio X 100) were calculated.

Table 27 Study design and results of IBD efficacy

Group	Vaccine at day-old	Mean bursal/body weight ratio (*100)
G1	vHVT304	0.33
G2	vHVT304+SB-1	0.33
G3	vHVT306	0.29
G4	vHVT13+vSB 1-007	0.49
G5	vHVT13+vSB 1-008	0.47
G6	- (challenged)	0.13
G7	- (unchallenged)	0.46

[0219] The mean bursal/body weight ratios are shown in the Table 27. The challenged control birds had a severe bursal atrophy compared to unchallenged ones. The vSB 1-007 and vSB 1-008 vaccines did not interfere on vHVT 13-induced protection (G4 and G5). The bursal/body weight ratios of birds vaccinated with the double HVT (HVT-ND+IBD) were slightly lower than the unchallenged control group but were clearly higher than the challenged control groups. Furthermore, the SB-1 serotype 2 Marek's disease vaccine did not interfere with vHVT304-induced IBD protection.

[0220] In conclusion, these data indicate that both the combination of a SB1-ND vector with a single HVT-IBD or double HVT expressing both NDV-F and IBDV-VP2 induce IBD protection in a variant E IBDV challenge model.

**Example 16 Lack of interference of vHVT114, vSBI-009 and/or SB-1 on vHVT13 induced variant E IBD protection in SPF chickens**

[0221] The aim of the study was to assess the IBD efficacy of vHVT13 when administered by SC or in ovo route concomitantly with vHVT1 14, vSB 1-009 and/or SB-1 in SPF chicks in an IBDV-Variant (VAR-E) at D28 challenge model.

[0222] 75 one-day-old SPF chickens and 75 SPF 18 to 19 day-old chicken embryo were randomly allocated into 5 groups (G1 to G5 and G6 to G10, respectively) including a group of challenged controls (G4 and G9, respectively) and unchallenged controls (G5

and G10, respectively). The birds of groups G1 to G3 were injected by subcutaneous injection in the neck at DO with 0.2 mL of vaccines containing each a target dose of 3000 pfu except for SB-1 which had a target dose of 1000 PFU. Birds from G6 to G8 received the same vaccine doses but in 0.05 mL volume by the *in ovo* route 2-3 days before hatch. The design of the study is shown in Table 28 below. At 28 days of age, all birds from groups G1 to G4 and G6 to G9 were challenged by the eye drop (0.03 mL containing 3 log<sub>10</sub> EID<sub>50</sub> per bird) of the IBDV variant E isolate from University of Delaware (USA). Each group was monitored before and after challenge. Eleven days post-challenge, birds were weighed and necropsied. The bursa were collected and weighed. The bursal/body weight ratios (bursa weight/body weight ratio X 100) were calculated.

Table 28 Study design and results of IBD efficacy

Group	Vaccine at day-old	Administration route	Mean bursal/body weight ratio (*100)
G1	vHVT13+vHVT1 14+SB-1	SC	0.56
G2	vHVT13+vHVT1 14+vSB 1-009	SC	0.58
G3	vHVT13+vSB 1-009	SC	0.52
G4	- (challenged)	SC	0.13
G5	- (unchallenged)	SC	0.51
G6	vHVT13+vHVT1 14+SB-1	<i>In ovo</i>	0.54
G7	vHVT13+vHVT1 14+vSB 1-009	<i>In ovo</i>	0.47
G8	vHVT13+vSB 1-009	<i>In ovo</i>	0.53
G9	- (challenged)	<i>In ovo</i>	0.14
G10	- (unchallenged)	<i>In ovo</i>	0.58

[0223] The mean bursal/body weight ratios are shown in the Table 28. The challenged control birds (G4 and G9) had a severe bursal atrophy compared to unchallenged ones. The bursal/body weight ratios of the vaccinated groups (G1 to G3 and G6 to G8) were similar to those of the unchallenged control groups (G5 and G10) and well above those of the challenged control groups (G4 and G9). The lack of interference of vHVT1 14 on vHVT 13-induced IBD protection after both SC or *in ovo* routes was surprising and confirmed data obtained in examples 10 and 14.

[0224] In conclusion, these data indicate clearly the compatibility of vHVT1 14+vSB 1-009 or +SB-1 and of vSB 1-009 with vHVT13 when administered by SC or *in ovo* route in a variant E IBDV challenge model.



**Example 17 Efficacy of vHVT114 and vHVT13 and SB1 or vSB1-009 vectors  
against very virulent plus Marek's disease challenge**

[0225] The aim of this study was to evaluate the Marek's disease efficacy induced by different combinations of vaccines including vHVT1 14, vHVT13, SB-1 and/or vSB 1-009 administered by the SC route to one-day-old SPF chicks and challenged 4 days later with the very virulent plus Marek's disease virus (w+MDV) T-King isolate.

[0226] On DO, 100 one-day-old SPF chickens were randomly allocated into 5 groups of 20 birds. The birds from groups 1 to 3 were injected by subcutaneous injection in the neck at DO with 0.2 mL of vaccines containing a target dose of 2000 pfu for each vaccine except for SB-1 for which the target dose was 1000 pfu. Birds from groups 4 and 5 were non-vaccinated and were used as sham controls challenged (group 4) or unchallenged (group 5). The study design is shown in the Table 29. On D4, All birds from groups 1 to 4 were challenged with 0.2 mL of the w+MDV T-King isolate using the intraperitoneal route of administration.

Table 29 Study design and MD protection results

Group	Vaccine at day-old (DO)	Number of MD positive/total	Percentage of protection
<b>G1</b>	vHVm+SB-1	7/20	65%
<b>G2</b>	vHVT1 14+SB-1	7/20	65%
<b>G3</b>	vHVT13+vHVT1 14+vSB 1-009	7/20	65%
<b>G4</b>	- (challenged)	20/20	0%
<b>G5</b>	- (unchallenged)	0/20	100%

[0227] Each group was monitored daily for any unfavourable reactions before and after challenge. At day 49, all live birds were terminated and necropsied to examine for gross lesions associated with Marek's disease. Chickens were classified as positive for infection with Marek's disease if nervous signs, such as paralysis, locomotive signs attributable to the disease, and severe emaciation or depression are observed, if mortality directly attributable to Marek's Disease occurs, or if gross lesions are observed at necropsy. Lesions might include, but not be limited to, the following: liver, heart, spleen, gonads, kidneys, and muscle lesions

[0228] Results of protection are shown in the Table 29 above. All vaccinated groups (G1 to G3) performed equally, inducing a partial (65%) MD protection as expected in this very severe and early challenge model. These results indicated that the vector vaccine candidates retain their ability to protect against Marek's disease.

**Example 18 Efficacy of recombinant HVT and SB1 vectors against Marek's disease**

[0229] Marek's disease efficacy is also demonstrated for the HVT vectored recombinants and the SB-1 vectored recombinants either alone or in combination. The challenge strains include a virulent Marek's disease (vMD) challenge such as GA22, a very virulent Marek's disease (wMD) challenge such as RB1B and/or a very virulent plus Marek's disease (w+MD) challenge such as the T. King virus. One-day-old chickens are inoculated subcutaneously or 18- 19-day-old embryonated eggs are inoculated with a 0.2 ml dose or 0.05 ml dose, respectively, of the test viruses. At five days of age the vaccinated chickens and naïve controls are challenged with the relevant Marek's challenge virus (v, vv, or w + MDV). The challenged birds are observed until seven weeks of age. All birds are terminated and necropsied to observe for grossly visible lesions associated with Marek's disease as described in Example 17.

**Example 19 Interference of HVT on vHVT13-induced IBDV antibodies in commercial pullets**

[0230] The objective of this study was to determine if co-administration of HVT with vHVT13 had an impact on vHVT 13-induced IBDV antibody response in commercial pullets.

[0231] Eighty day-old commercial brown pullets were used in three isolation units. Fifteen were blood sampled at day-old to test IBD maternally derived antibodies (MDA). The remaining birds were split into three groups as shown in Table 30. Birds from group 2 and 3 were vaccinated by the SC route in the nape of the neck with commercial doses of vHVT13 (VAXXITEK HVT+IBD; Merial SAS, Lyon, France) and/or HVT cell-associated Bio HVT (Merial S.p.A., Noventa, Italy). Blood sampling was performed at the age of 25, 35 and 45 days of age. The ELISA kit used to evaluate IBDV serological

response was the PROFLOK PLUS IBD (IBD+) Ab ELISA kit from Synbiotics (Synbiotics Corp., Kansas City, MO, USA).

Table 30 Study design and serology results

Group	Vaccine at day-old (DO)	ELISA titre D1	ELISA titre D25	ELISA titre D35	ELISA titre D45
G1	-	10,502	7,814	6,237	3,664
G2	vHVT13	10,502	8,023	9,360	9,486
G3	vHVT13+HVT	10,502	6,896	4,763	3,795

[0232] Mean ELISA titers are shown in Table 30. Titers in the unvaccinated group G1 decreased from D1 to D45, which corresponded to the decline of IBDV maternal antibodies. As expected; ELISA titers in the vHVT13 group G2, remains high up to D45 indicating maternal antibodies were progressively replaced by vHVT 13-induced antibodies. The addition of HVT to vHVT13 had a clear negative impact since the antibody titers observed in G3 were similar to G1. These results contrast with those obtained with vHVT1 14+vHVT13 since the vHVT1 14 did not decrease vHVT13-induced IBD+ ELISA titers (see example 14, Table 26). They confirm the unexpected property of vHVT1 14 in not interfering with vHVT13 immunogenicity.

[0233] In conclusion, in contrast to what was observed with vHVT1 14, the addition of HVT to vHVT13 had a clear negative impact on vHVT 13-induced IBDV humoral immunity.

#### **Example 20 Interference of commercial HVT-ND on vHVT13-induced IBD protection**

[0234] The objective of this study was to determine if co-administration of commercial HVT-ND vector vaccines with vHVT13 had an impact on vHVT 13-induced IBD protection in SPF chickens.

[0235] Seventy five SPF chickens (3 groups (G2, G3 and G4) of 25) were vaccinated at one day-of-age by the SC route with a commercial dose of vHVT13 (VAXXITEK HVT+IBD) with or without one commercial dose of licensed HVT-vectored ND vaccine (vHVT-ND1 and vHVT-ND2) as shown in the Table 31. Fifteen birds were kept as non-vaccinated controls (G1). Three weeks post-vaccination, birds (20 chickens in G2, G3 and G4 and 10 chickens in G1) were challenged with at least 2.0 log<sub>10</sub> EID<sub>50</sub> in 0.05ml

of IBD virus Ph/B1 strain (isolated in the Philippines) administered via ocular route. All chickens were observed for 5 days for clinical signs or death from causes attributable to IBD challenge virus and euthanatized humanely at end of post-challenge observation for necropsy examination of IBD lesion, especially from the bursa of Fabricius. Birds were considered as protected if their bursa did not show bursal lesions typical of IBD: bursal atrophy, peri-bursa edema and/or hemorrhages in bursa tissues.

Table 31 Study design and IBD protection data

Group	Vaccine at day-old (DO)	Number of sick (dead)/total	Number of positive bursa/total	Percent of protection
G1	-	10(8)/10	10/10	0%
G2	vHVT13+vHVT-ND1	3(3)/20	9/20	55%
G3	vHVT13+vHVT-ND2	3(1)/20	7/20	65%
G4	vHVT13	0(0)/20	0/20	100%

[0236] Results are shown in Table 31. All 10 challenged control birds showed clinical signs and 8 out of 10 died 4 or 5 dpi indicating that the IBDV challenge was very severe. All of them had severe lesions of bursa including severe atrophy and haemorrhagic patches. The vHVT13 alone induced full protection whereas both combinations with vHVT-ND induced partial clinical and bursal protection.

[0237] In conclusion, these results clearly indicate that the 2 commercial HVT-vectored ND vaccines interfere with vHVT 13-induced IBD protection.

**Example 21 Efficacy of vSBI-004, vSBI-006, vSBI-007, vSBI-008, SBI-vectored ND vaccine alone or in association with vHVT13 HVT-vectored IBD vaccine, and the vHVT302 and vHVT304 vaccines against challenges with NDV Texas GB strain at 14 and/or 28 days of age in SPF chickens**

[0238] The aim of the study was to assess the efficacy of combinations of different Marek's disease vector vaccines expressing the NDV F and/or the IBDV VP2 gene against Newcastle disease challenge (Texas GB strain, genotype II) performed at 14 and/or 28 days of age in SPF chickens.

[0239] The characteristics of the 6 NDV recombinant vaccine candidates tested in this study are described in the Table 32 below.

Table 32 characteristics of the 6 NDV recombinant vaccine candidates tested in this study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vSB1-004	SB-1*	mCMV IE	Wt-VIId	SV40	SORF4/US10
vSB1-006	SB-1	SV40	Opt-VIId	Synthetic	UL55/LORF5
vSB1-007	SB-1	SV40	Opt-VIId	(endogeneous from gC gene)	gC
vSB1-008	SB-1	SV40	Opt-CA02	Synthetic	UL55/LORF5
vHVT302	vHVT13	US10	Opt-VIId	US10	US10
vHVT304	vHVT13	SV40	Opt-VIId	Synthetic	IG2

[0240] On DO, 225 one-day-old SPF chickens were randomly allocated into 9 groups of 15 birds (G1a to G9a challenged at D14) and 6 groups of 15 birds (G1b, G3b, G4b, G5b, G8b, G9b challenged at D28). The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL containing a target dose of 2000 pfu for recombinant vaccines. The design of the study is shown in Table 33 below. The birds were challenged by the intramuscular route on D14 or D28 with 4.3 and 4.2 log<sub>10</sub> EID<sub>50</sub> (0.1 mL) velogenic ND Texas GB (genotype II) strain, respectively.

Table 33 Results of ND efficacy

Group	Vaccine at day-old (DO)	% ND protection after ND challenge at 14 days of age	% ND protection after ND challenge at 28 days of age
G1a & 1b	-	0%	0%
G2a	vSB 1-004	20%	ND*
G3a & 3b	vSB 1-006	26.6%	73.3%
G4a & 4b	vSB 1-007	33.3%	93.3%
G5a & 5b	vSB 1-008	46.6%	86.6%
G6a	vSB1-006+vHVT13	14%	ND
G7a	vSB1-008+vHVT13	21.4%	ND
G8a & 8b	vHVT302	13.3%	80%
G9a & 9b	vHVT304	33.3%	93.3%

\*ND = not done

[0241] Each group was monitored before and after challenge. NDV clinical signs after challenge were recorded. One bird died in G6 and G7 before challenge reducing the number of birds from 15 to 14 in these groups.

[0242] Percentages of clinical protection (including protection against both mortality and morbidity) are reported in Table 33 above. Full susceptibility was observed in the non-vaccinated challenged control group Gla and Gib thus validating the high severity of challenge. Partial protections ranging from 13.3 to 46.6% were observed after challenge at D14, the highest levels of protection being induced by vSBI-008, vSBI-007 and vHVT304. Protection levels after ND challenge at D28 were much higher for all vaccinated groups and were again slightly higher in the groups vaccinated with vSBI-008, vSB 1-007 or vHVT304. These results indicated that ND protection levels were dependent on the date of challenge and on the construct. The vSB 1-008 and vSB 1-007 constructs performed slightly better than vSB 1-004 and vSB 1-006, and the vHVT304 performed slightly better than vHVT302, indicating that different characteristics of the constructs are playing a role in the performances of MDV-based vector vaccines.

[0243] In conclusion, the results of this study showed that ND protection levels induced by Marek's disease vectors expressing NDV F gene may depend on different parameters including the vector, the locus of insertion, the F gene, the promoter, the poly-adenylation site and the challenge conditions.

**Example 22 Efficacy of double HVT-ND+IBD vHVT304 and vHVT306 vaccines  
against challenges with NDV Texas GB strain at 14 and/or 28  
days of age in SPF chickens**

[0244] The aim of the study was to assess the efficacy of HVT-vectored vaccine expressing both NDV F and IBDV VP2 genes against Newcastle disease challenge (Texas GB strain, genotype II) performed at 14 and/or 28 days of age in SPF chickens.

[0245] The characteristics of the 2 recombinant vaccine candidates tested in this study are described in the Table 34 below.

Table 34 Characteristics of the recombinant vaccine candidates  
used in this study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT304	vHVT13	SV40	Opt-VIIId	Synthetic	IG2
vHVT306	vHVT13	SV40	Opt-VIIId	Synthetic	SORF3-US2

[0246] On DO, 90 one-day-old SPF chickens were randomly allocated into 3 groups of 15 birds (G1a to G3a challenged at D14) and 3 groups of 15 birds (G1b to G3b challenged at D28). The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL containing a target dose of 2000 pfu for recombinant vaccines. The design of the study is shown in Table 35 below. The birds were challenged by the intramuscular route on D14 or D28 with a target dose of 4.0 log<sub>10</sub> EID<sub>50</sub> (0.1 mL) velogenic ND Texas GB (genotype II) strain.

Table 35 Results of ND efficacy

Group	Vaccine at day-old (DO)	% ND protection after ND challenge at 14 days of age	% ND protection after ND challenge at 28 days of age
G1a & 1b	-	0%	0%
G2a & 2b	vHVT304	26.7%	92.9%
G3a & 3b	vHVT306	33.3%	86.7%

[0247] Each group was monitored before and after challenge. NDV clinical signs after challenge were recorded. One bird died in G2b before challenge reducing the number of birds from 15 to 14 in this group.

[0248] Percentages of clinical protection (including protection against both mortality and morbidity) are reported in Table 35 above. Full susceptibility was observed in the non-vaccinated challenged control group G1a and G1b thus validating the high severity of challenge. Protection levels after challenge at D14 were much lower than those obtained after challenge at D28. These vaccine candidates had the same NDV F expression cassette inserted into 2 different loci of vHVT13 genome. They performed equally in terms of ND protection in the tested conditions, indicating that both insertion loci (IG2 and SORF3-US2) are equally suitable for NDV F cassette insertion.

[0249] In conclusion, the results of this study showed that ND protection levels induced by Marek's disease vectors expressing NDV F gene depend on different parameters including the vector, the locus of insertion, the F gene, the promoter, the poly-adenylation site and the challenge conditions.

**Example 23 ND early efficacy induced by double HVT-ND+IBD (vHVT302, VHVT303, and vHVT304) or SBI-vectors (vSBI-006 and vSBI-007) in one day-old SPF chickens against a velogenic genotype V NDV challenge**

[0250] The objective of the study was to evaluate the efficacy of three double HVT-ND+IBD (vHVT302, vHVT303, and vHVT304) and two SB1-ND vectors (vSBI-006 and vSB 1-007) in one day-old SPF chickens against a velogenic genotype V (Chimalhuacan) NDV challenge performed at D14.

[0251] The characteristics of the 5 recombinant vaccine candidates tested in this study are described in Table 36 below.

Table 36 Characteristics of the recombinant vaccine candidates used in this study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT302	vHVT13	US10	Opt-VIIId	US10	US10
vHVT303	vHVT13	US10	Opt-V (CA02)	US10	US10
vHVT304	vHVT13	SV40	Opt-VIIId	Synthetic	IG2
vSBI-006	SB-1	SV40	Opt-VIIId	Synthetic	UL55/LORF5
vSBI-007	SB-1	SV40	Opt-VIIId	(endogeneous from gC gene)	gC

[0252] Six groups (1 and 2) of ten one-day-old specific pathogen free (SPF) white Leghorn chicks were randomly constituted. Birds from groups 2 to 6 were vaccinated by the subcutaneous route (nape of the neck) with a target dose of 2000 PFU as shown in the Table 37 below. Chickens from group 1 were not vaccinated and were kept as control birds. At 2 week-of-age, all birds were challenged with the genotype V Mexican Chimalhuacan (Mex V) velogenic NDV strain. The challenge was performed by the intramuscular (IM) route using  $10^5$  Egg Infectious Dose 50 (EID50) diluted in 0.2 ml of physiological sterile water. All birds were monitored until 14 days post-challenge. After challenge, health status of each bird was scored daily as follows: healthy / with specific symptoms (ruffled feathers, prostration, torticollis, tremor) / dead. Any bird that showed specific symptoms for more than 2 days or was noted sick on D28 was taken into account for calculation of morbidity.

Table 37 Results of early ND protection induced by different MDV vectored candidates expressing NDV F gene in SPF day-old chicks



Group	Vaccine	Target dose (PFU) under 0.2 mL (actual dose)	Protection against mortality	Protection against morbidity
G1	-	-	0%	0%
G2	vHVT302	2000 (4427)	50%	10%
G3	vHVT303	2000 (ND)	10%	0%
G4	vHVT304	2000 (1169)	80%	60%
G5	vSB 1-006	2000 (1720)	60%	40%
G6	vSB 1-007	2000 (1564)	80%	50%

[0253] Results of protection are summarized in Table 37. All control birds died after ND challenge. Variable levels of ND protection were induced by the different tested vaccines ranging from 10% to 80% and from 0% and 60% in terms of protection against mortality and morbidity, respectively. The vHVT304 candidate induced a better protection than the vHVT303 and vHVT302 candidates; this may be due to the exogenous SV40 promoter placed in front of the NDV F gene. The vSB 1-007 performed slightly better than the vSB 1-006. Furthermore, performances obtained with vHVT304 were comparable to those obtained with vSB 1-007 indicating that different Marek's disease vectors can reach the same level of ND protection.

[0254] In conclusion, this study demonstrates that both double HVT-ND+IBD and SB1-ND vectored vaccines can reach significant levels of ND protection in a very severe and early NDV challenge model.

**Example 24 ND efficacy induced by the double HVT-ND+IBD vHVT306 administered by *in ovo* or SC route to one day-old SPF chickens against a velogenic genotype V NDV challenge performed at D28**

[0255] The objective of the study was to evaluate the efficacy of one double HVT-ND+IBD (vHVT306) administered by the *in ovo* or SC route to SPF chickens against a velogenic genotype V (Chimalhuacan) NDV challenge performed at 28 days of age.

[0256] The characteristics of the vHVT306 recombinant vaccine candidate tested in this study are described in Table 38 below. The single HVT-IBD vector vaccine vHVT13 was used as a control.

Table 38 Characteristics of the recombinant vaccine candidate used in this study

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT306	vHVT13	SV40	Opt-VIIId	Synthetic	SORF3-US2

[0257] On day -3, 40 SPF embryonated eggs aged around 18 days and 18 hours of incubation were randomly allocated into 2 groups of 20 eggs each. On DO, one group of 12 day-old SPF chicks was added. The definition of groups is given in Table 39 below. The vaccination was performed on D-3 (*in ovo* route) or on DO (SC route, in the back of the neck) and the target dose of vHVT306 and vHVT13 was 2000PFU/bird. For the *in ovo* route, hatchability, viability (until D28) and growth of the birds (between hatching and D28) were monitored.

[0258] On D28, 10 birds per group were challenged with virulent ND Chimalhuacan strain. The challenge was performed by the intramuscular (IM) route using  $10^5$  Egg Infectious Dose 50 (EID50) diluted in 0.2 ml of physiological sterile water. Birds were monitored until 14 days post-challenge. Specific clinical signs and mortality were recorded. Any bird that showed specific symptoms for more than 2 days or was noted sick on D42 was taken into account for calculation of morbidity. Five and seven days post-challenge (i.e. on D33 and D35), oropharyngeal swab was taken from each surviving bird. All the swabs were analyzed by specific NDV qRT-PCR.

Table 39 Results of ND protection induced by vHVT306 MDV vectored candidate expressing both NDV F and IBDV VP2 genes administered by the SC or *in ovo* route into SPF chicks

Group	Vaccine/route	Hatchability/ viability (%)	Protection against mortality/morbidity	% birds shedding at 5 dpi/7 dpi (mean log10 titer*)
G1	vHVT13/ <i>in ovo</i>	100%/100%	0%/0%	(not tested)
G2	vVHT306/ <i>in ovo</i>	100%/100%	100%/100%	0% (2.7)/0% (2.7)

G3	vHVT306/SC	-	100%/100%	20% (3.2)/10% (2.9)
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\* The threshold titer of the real time RT PCR was set at 2.7 log<sub>10</sub> equivalent EID<sub>50</sub>

[0259] Full hatchability was recorded after *in ovo* vaccination in groups 1 and 2 and all hatched birds survived up to D28. No difference in body weights was detected between the two groups at both D0 and D28 confirming the perfect safety of vHVT306 when administered *in ovo*. Results of protection are summarized in Table 39. All vHVT13-vaccinated control birds died by 4 days after ND challenge. Full clinical ND protection was induced by vHVT306 administered by both routes. Furthermore, no shedding was detected after *in ovo* administration whereas only a few birds shed detectable amount of challenge virus after SC administration.

[0260] In conclusion, this study demonstrates that the double HVT-ND+IBD vHVT306 induce excellent level of ND protection by SC or *in ovo* administration routes in a very severe heterologous NDV challenge model.

**Example 25 Efficacy of double HVT-ND+IBD (vHVT302, vHVT303 and vHVT304) recombinant vaccines, against challenge with a classical IBDV isolate on D15 in SPF chickens**

[0261] The aim of the study was to assess the early IBD efficacy of double HVT recombinants vHVT302, vHVT303 and vHVT304 recombinant constructs against a virulent infectious bursal disease virus (vIBDV) challenge (Faragher 52/70 strain) performed at 15 days of age in SPF chickens.

[0262] The characteristics of the 3 double HVT-ND+IBD recombinant vaccine candidates tested in this study are described in the Table 40 below.

Table 40 Characteristics of the expression cassettes  
of double HVT recombinants

Name	Parental virus	Promoter	F gene	Poly-A	Locus
vHVT302	vHVT13	US10	Opt-VIIId	US10	US10
vHVT303	vHVT13	US10	Opt-V (CA02)	US10	US10
vHVT304	vHVT13	SV40	Opt-VIIId	Synthetic	IG2

[0263] On D0, 40 one-day-old SPF chickens were randomly allocated into 4 groups of 10 birds including one control groups (G1) that was vaccinated with vSB 1-004, a SB-1 vector expressing NDV F gene. Five other SPF birds were kept unvaccinated and unchallenged for bursal/body weights evaluation. The birds were injected by subcutaneous injection in the neck at D0 with 0.2 mL of recombinant vaccines containing a target dose of 2000 pfu as described in the Table 41 below. On D15, blood sample was collected from all birds per group (10 birds per group except for groups 1 and 3 in which 1 bird died before blood sampling) for serological testing with the Kit ProFLOK® plus IBD (Synbiotics Corp). On D15, birds from all 4 groups were challenged by the eye drop (0.05 mL per bird) with 2.5 log<sub>10</sub> EID<sub>50</sub>.

Table 41 Study design and results of IBD efficacy

Group	Vaccine at day-old	ELISA IBD+ titer (log <sub>10</sub> )	Number Dead /Sick (total) <sup>1</sup>	% protection <sup>2</sup>	Mean bursal/body weight ratio <sup>4</sup>
G1	vSB 1-004	0.25	1/9 (9)	0%	0.0014
G2	vHVT302	2.6	0/1 (10)	80%	0.0043
G3	vHVT303	3.0	0/0 (9)	100%	0.0053
G4	vHVT304	2.4	0/0 (10)	80%	0.0034

<sup>1</sup> Birds sick for more than 2 days or still sick on D25 were considered as sick. The number in brackets is the total number of birds in the group that were challenged.

<sup>2</sup> Protection against clinical signs and severe bursal lesion (bursal score <3)

<sup>4</sup> The bursal/body weight ratio of the unvaccinated/unchallenged group was 0.0043.

[0264] Each group was monitored before and after challenge. IBDV clinical signs were recorded for 11 days after challenge (from D15 to D25). At the end of the post-challenge observation period (D25), all the surviving birds were euthanized and necropsied. Body and bursal weights were recorded. Each bursa of Fabricius (BF) was weighted then stored in individual recipients containing 4% formaldehyde for histology. Histological lesions of the bursa were scored according to the scale presented in Table 42.

Table 42 Scoring scale of histological lesions of the bursa of Fabricius\*

Score	Histology observation/lesions
0	No lesion, normal bursa
j	1%, to 25% of the follicles show lymphoid depletion (i.e. less than 50% of depletion in 1 affected follicle), influx of heterophils in lesions

2	26% to 50% of the follicles show nearly complete lymphoid depletion (i.e. more than 75% of depletion in 1 affected follicle), affected follicles show necrosis and severe influx of heterophils may be detected
2	51% to 75% of the follicles show lymphoid depletion; affected follicles show necrosis lesions and a severe influx of heterophils is detected
4	76% to 100% of the follicles show nearly complete lymphoid depletion; hyperplasia and cyst structures are detected; affected follicles show necrosis and severe influx of heterophils is detected
5	100% of the follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, fibrosis of bursal tissue

\* sourced from Monograph No. 01/2008:0587 of EU Pharmacopoeia "Avian Infectious Bursal Disease vaccine (live)"

[0265] A bird was considered as affected if it died and/or showed notable sign of disease and/or severe lesions of the bursa of Fabricius (*i.e.*, histology score >3).

[0266] The mean ELISA IBD+ antibody titer expressed in log 10 before challenge is shown in Table 41. Significant titers were detected in all vaccinated groups that were significantly higher than that of the control group G1. The serology titer was slightly higher in G3 (vHVT303).

[0267] Severe clinical signs were observed after challenge in all 9 birds of the control group G1, which lead to the death of 1 bird. Only one vaccinated bird in G2 (vHVT302) showed clinical signs after challenge. Percentages of protection against severe bursal lesions are shown in Table 41 above. Significant IBD protection was observed in all vaccinated groups, a full protection being observed in G3 (vHVT303). The mean bursal/body weight ratios are also shown in Table 41. Ratios in all vaccinated groups were higher than those of the challenged control group G1 and not significantly different from the unvaccinated and unchallenged control group.

[0268] In conclusion, these data indicate that the three double HVT-IBD+ND tested in this study induced IBD antibodies and early IBD protection in a severe IBDV challenge model.

**Example 26 Efficacy of five different HVT-ND vaccine candidates against challenges with velogenic NDV ZJI (genotype Vlld) isolate at 14 days of age in SPF chickens**

[0269] The aim of the study was to assess the efficacy of 5 single HVT recombinant constructs (vHVT39, vHVT1 10, vHVT1 11, vHVT1 12 and vHVT1 13) expressing the NDV F gene against Newcastle disease challenge with velogenic NDV ZJ1 (genotype Vlld) isolate performed at 14 days of age in SPF chickens.

[0270] The characteristics of these 5 vaccine candidates are described in Table 43 below.

Table 43 Characteristics of the HVT-ND recombinant viruses  
used in the challenge study

Name	Parental virus	Promoter	F gene*	Poly-A	Locus
vHVT039	HVT	MDV gB	Wtnm-Texas	SV40	IG1
vHVT110	HVT	MCMV IE	Wt-Vlld	SV40	IG1
vHVT111	HVT	SV40	Wt-Vlld	SV40	IG1
vHVT112	HVT	MCMV IE	Wt-YZCQ	SV40	IG1
vHVT113	HVT	MCMV IE	Wt-Texas	SV40	IG1

\*Wt means that the wild type velogenic F gene sequence was used but the cleavage site was modified to that of a lentogenic virus. Wtnm means that the cleavage site of the wild type sequence was not modified. The Texas velogenic strain belongs to genotype IV and YZCQ to the genotype Vlld.

[0271] On DO, 72 one-day-old SPF chickens were randomly allocated into 5 groups of 12 birds (vaccinated) and 1 group of 12 birds (non-vaccinated controls). The birds were injected by subcutaneous injection in the neck at DO with 0.2 mL of recombinant vaccines containing a target dose of 6000 pfu as described in Table 44 below. The birds were challenged by the intramuscular route on D14 with 5 log<sub>10</sub> EID<sub>50</sub> of NDV ZJ1/2000 (genotype Vlld) velogenic strain.

Table 44 Results of ND efficacy

Group	Vaccine at day-old (DO)	% clinical protection	
		Protection against mortality/morbidity	Mean shedding titer (log <sub>10</sub> ) at 2/4 dpi
G1	-	0%/0%	3.5/- (all dead)
G2	vHVT039	25%/8%	2.5/4.8
G3	vHVT1 10	100%/83%	1.8/2.0
G4	vHVT1 11	100%/67%	1.8/2.8
G5	vHVT1 12	75%/42%	1.7/3.4
G6	vHVT1 13	83%/25%	1.4/3.3

[0272] Each group was monitored before and after challenge. NDV clinical signs and mortality were recorded after challenge. Oropharyngeal swabs were taken at 2 and 4 days

post-infection (dpi) for evaluation of viral load by real time RT-PCR using the method described by Wise et al. (2004; Development of a Real-Time Reverse-Transcription PCR for Detection of Newcastle Disease Virus RNA in Clinical Samples. J Clin Microbiol 42, 329-338).

[0273] Percentages of protection against mortality and morbidity are reported in the table 44 above. Full susceptibility was observed in the non-vaccinated challenged control group G1 thus validating the high severity of the challenge. Vaccines induced variable levels of protection against mortality (25-100%) or against morbidity (8%-83%). The best protection level was induced by vHVT1 10 whereas the lowest one was induced by vHVT039, the other candidates giving intermediate results. Results of oropharyngeal shedding at 2 and 4 dpi are also shown in Table 44 above and are in line with those of clinical protection. These vaccine candidates differ in their promoter and F gene sequence. These results show that both of these parameters are important for the design of optimal HVT-ND vaccine candidate.

[0274] In conclusion, the results of this study showed the importance of promoter and F gene sequence in the ND efficacy induced by HVT-vectored ND vaccine candidates.

**Example 27 Evaluation of the Newcastle disease efficacy induced by double SB1 constructs expressing IBDV VP2 and NDV F.**

[0275] The aim of the study is to assess the efficacy of double SB1 constructs expressing IBDV VP2 and NDV F against Newcastle disease challenge.

[0276] On DO, one-day-old SPF chickens are randomly allocated into several groups of 10-20 birds, including vaccinated and non-vaccinated groups. The birds of the vaccinated groups are injected by subcutaneous injection in the neck at DO with 0.2 mL containing a target dose of 1000 to 5000 pfu of recombinant vaccines. Alternatively, the same dose in 0.05 mL may be administered *in ovo* 2 or 3 days before hatch. The birds (at least one vaccinated and one non vaccinated group) are challenged by the intramuscular route at different time after vaccination: for instance, D14, D28 or D42 with about 4.0 log<sub>10</sub> EID<sub>50</sub> (0.1 mL) of a velogenic NDV strain such as Texas GB (genotype II), ZJ1 (genotype V<sub>l</sub>ld), Chimalhuacan (genotype V) strain.

[0277] Each group is monitored clinically before and after challenge. NDV clinical signs (morbidity) and mortality are recorded after challenge. Percentages of clinical protection in all groups are calculated. At least 90% of non-vaccinated challenged SPF birds should die or be severely sick after challenge to validate the severity of challenge.

Oropharyngeal and cloacal swabs can be samples at different times after challenge such as 3, 5, 7 and 9 days post-challenge and the viral load can be estimated by real-time RT-PCR. The best candidates will be those who induced the highest level of clinical protection and the lowest level of viral load in the swabs. A similar study can be performed in broilers containing NDV maternal antibodies; however, these maternal antibodies may potentially protect the non-vaccinated birds if the challenge is performed early. The double SB1 construct may also be tested in combination with other Marek's disease vaccine or vector vaccines.

**Example 28 Evaluation of the infectious bursal disease efficacy induced by double SB1 constructs expressing IBDV VP2 and NDV F**

[0278] The aim of the study is to assess the IBD efficacy of double SB 1 expressing both the IBDV VP2 and the NDV F.

[0279] One-day-old SPF chickens are randomly allocated into several groups of 10 to 20 birds including vaccinated and non-vaccinated controls. Non-vaccinated controls will be separated into 2 subgroups including challenged and unchallenged birds. The birds of vaccinated groups are injected by subcutaneous injection in the neck at DO with 0.2 mL of vaccines containing each a target dose of 1000 to 5000 pfu. Alternatively, the same dose in 0.05 mL may be administered *in ovo* 2 or 3 days before hatch. At different times after vaccination such as 14, 21, 28 or 42 days post-vaccination, all birds from vaccinated groups and the challenged controls are challenged by the eye drop (0.03 mL containing 2 to 4 log<sub>10</sub> EID<sub>50</sub> per bird) of a virulent IBDV (such as the Faragher or the US standard strain), a very virulent IBDV such as the 91-168 isolate or a variant IBDV isolate such as the US Delaware variant E isolate. Each group is clinically monitored before and after challenge. Birds can be necropsied 4 or 5 days post-challenge for bursal gross lesions evaluation. They can also be necropsied 10 to 11 days post-challenge. Gross and/or histological lesions can be evaluated. Furthermore, birds and bursa are weighed the



bursal/body weight ratios (bursa weight/body weight ratio X 100) are calculated compared to those of the non-vaccinated unchallenged group. Control SPF challenged birds must show clinical signs and/or have significant gross and/or histological lesions, and/or should have a bursal/body weight ratio significantly lower than the unvaccinated unchallenged control birds to validate the severity of challenge. The efficacy of the vaccine is evaluated by comparing these parameters with unvaccinated/challenged and unvaccinated/unchallenged groups. Such study may be performed in broiler chickens containing IBDV maternal antibodies; however, these maternal antibodies may potentially protect the non-vaccinated birds if the challenge is performed early. The double SB1 construct may also be tested in combination with other Marek's disease vaccine or vector vaccines.

**Example 29 Evaluation of the Marek's disease efficacy induced by double SB1 constructs expressing IBDV VP2 and NDV F**

[0280] The aim of the study is to evaluate Marek's disease efficacy induced by the SB1 vectors expressing both IBDV VP2 and NDVF.

[0281] One-day-old SPF chickens are randomly allocated into several groups of 20 to 50 birds including vaccinated and non-vaccinated controls. Non-vaccinated controls may be separated into 2 subgroups including challenged and unchallenged birds. The birds of vaccinated groups are injected by subcutaneous injection in the neck at DO with 0.2 mL of vaccines containing each a target dose of 1000 to 5000 pfu. Alternatively, the same dose in 0.05 mL may be administered *in ovo* 2 or 3 days before hatch. At different times after vaccination such as 3 to 10 days post-vaccination, all birds from vaccinated groups and the challenged controls are challenged by the intraperitoneal route with 0.2 mL of a Marek's disease virus (MDV) strain. MDV strain may be of several pathotypes such as virulent MDV (vMDV) including the JM or GA22 isolate, very virulent MDV (wMDV) such as the RB-1B or Md5 isolate, very virulent plus (w+MDV) such as the T-King or 648A isolate. MDV challenge strain inoculum are prepared by infecting chickens, harvesting and freezing their blood cells into liquid nitrogen in presence of a cryopreservative such as DMSO. The chicken infectious dose 50 (CID50) is established for each challenge batch before performing vaccination/challenge studies. Each group is

clinically monitored before and after challenge. Birds are necropsied after at least 7 weeks post-vaccination and the presence Marek's disease gross lesions is checked in each bird. Lesions might include, but not be limited to, the following: liver, heart, spleen, gonads, kidneys, nerve and muscle lesions. Such study may be performed in broiler chickens containing MDV maternal antibodies. The double SB1 construct may also be tested in combination with other Marek's disease vaccine (for instance HVT and or CVI988 Rispens strains) or MD vector vaccines. MD challenge may also be performed by contact between vaccinated birds and MDV infected non-vaccinated SPF chicks.

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**[0282]** Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above examples is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

**[0283]** All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

## CLAIMS

What we claim is:

1. A composition or vaccine comprising one or more recombinant herpesvirus of turkeys (HVT) vectors comprising at least one or more heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.
2. The composition or vaccine of claim 1, wherein the HVT vector comprises a heterologous polynucleotide coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37.
3. The composition or vaccine of claim 2, wherein the polynucleotide encoding the NDV-F polypeptide is operably linked to a promoter selected from the group consisting of SV40 promoter, MDV gB promoter and guinea pig CMV promoter.
4. The composition or vaccine of claim 3, wherein the polynucleotide encoding the NDV-F polypeptide is operably linked to an SV40 polyA signal.
5. The composition or vaccine of claim 4, wherein the polynucleotide encoding the NDV-F polypeptide, the operably linked promoter, and the SV40 polyA signal are inserted in the IG1 locus of HVT genome.
6. The composition of claim 1, wherein the composition further comprises a second recombinant HVT vector comprising a heterologous polynucleotide coding for and expressing IBDV VP2 antigen.
7. The composition of claim 6, wherein the second recombinant HVT vector is vHVT 13.
8. The composition of claim 1 or 6, wherein the composition further comprises one or more recombinant SB1 vectors.
9. The composition of claim 8, wherein the recombinant SB1 vector comprises one or more heterologous polynucleotides coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen.

10. The composition of claim 9, wherein the SB1 vector is selected from the group consisting of vSB 1-009, vSB1-010, vSB 1-004, vSB 1-006, vSB 1-007 and vSB1-008.
11. The composition or vaccine of claim 1, wherein the HVT vector comprises a first heterologous polynucleotide coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37 and a second heterologous polynucleotide coding for and expressing an Infectious Bursal Disease Virus (IBDV) VP2 antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO:8 or 42.
12. The composition or vaccine of claim 1, wherein the HVT vector comprises two heterologous polynucleotides coding for and expressing two IBDV VP2 antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO:8 or 42.
13. The composition or vaccine of claim 11, wherein the polynucleotide encoding the NDV-F antigen is operably linked to a promoter selected from the group consisting of SV40 promoter, MDV gB promoter, and guinea pig CMV promoter, and wherein the polynucleotide encoding the IBDV VP2 antigen is operably linked to a CMV promoter.
14. The composition or vaccine of claim 13, wherein the polynucleotide encoding the NDV-F antigen is operably linked to an SV40 polyA signal and the polynucleotide encoding the IBDV VP2 antigen is operably linked to an SV40 polyA signal.
15. The composition or vaccine of claim 14, wherein the polynucleotide encoding the NDV-F antigen, the operably linked promoter, and the SV40 polyA signal are inserted in the IG1 locus of the HVT genome, and wherein the polynucleotide encoding the IBDV VP2 antigen, the operably linked CMV promoter, and the SV40 polyA signal are inserted in the IG1 locus or the SORF3-US2 locus of the HVT genome.

16. The composition of claim 11, wherein the composition further comprises a recombinant HVT vector comprising a heterologous polynucleotide coding for and expressing IBDV VP2 antigen.
17. The composition of claim 16, wherein the recombinant HVT vector is vHVT13.
18. The composition of claim 11 or 12, wherein the composition further comprises one or more recombinant SBI vectors.
19. The composition of claim 18, wherein the recombinant SBI vector comprises one or more heterologous polynucleotides coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen.
20. The composition of claim 19, wherein the SBI vector is selected from the group consisting of vSB 1-009, vSB 1-004, vSB 1-006, vSB 1-007, vSB 1-008 and vSBI-010.
21. A recombinant HVT vector comprising one or more heterologous polynucleotides coding for and expressing at least one antigen of an avian pathogen.
22. The recombinant HVT vector of claim 21, wherein the vector comprises a heterologous polynucleotide coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37.
23. The recombinant HVT vector of claim 22, wherein the polynucleotide encoding the NDV-F polypeptide is operably linked to a promoter selected from the group consisting of SV40 promoter, MDV gB promoter, and guinea pig CMV promoter.
24. The recombinant HVT vector of claim 23, wherein the polynucleotide encoding the NDV-F polypeptide is operably linked to an SV40 polyA signal.
25. The recombinant HVT vector of claim 24, wherein the polynucleotide encoding the NDV-F polypeptide, the operably linked promoter, and SV40 polyA signal are inserted in the locus selected from the group consisting of IG1 , IG2, US 10, SORF3-US2 and gD of HVT genome.
26. The recombinant HVT vector of claim 21 wherein the vector comprises a first heterologous polynucleotide coding for and expressing a Newcastle Disease Virus F (NDV-F) antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO:2, 4, 6, 33, 35, or 37 and a second

- heterologous polynucleotide coding for and expressing an Infectious Bursal Disease Virus (IBDV) VP2 antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 8 or 42.
27. The recombinant HVT vector of claim 21, wherein the HVT vector comprises two heterologous polynucleotides coding for and expressing two IBDV Vp2 antigen having at least 80% sequence identity to the amino acid sequence as set forth in SEQ ID NO:8 or 42.
28. The recombinant HVT vector of claim 26, wherein the polynucleotide encoding the NDV-F antigen is operably linked to a promoter selected from the group consisting of SV40 promoter, MDV gB promoter, and guinea pig CMV promoter, and wherein the polynucleotide encoding the IBDV VP2 antigen is operably linked to a CMV promoter.
29. The recombinant HVT vector of claim 28, wherein the polynucleotide encoding the NDV-F antigen is operably linked to an SV40 polyA signal and the polynucleotide encoding the IBDV VP2 antigen is operably linked to an SV40 polyA signal.
30. The recombinant HVT vector of claim 29, wherein the polynucleotide encoding the NDV-F antigen, the operably linked promoter, and SV40 polyA signal are inserted in the IG1 locus of the HVT genome, and wherein the polynucleotide encoding the IBDV VP2 antigen and the operably linked CMV promoter and SV40 polyA signal are inserted in the IG1 locus or the SORF3-US2 locus of the HVT genome.
31. A method of vaccinating an animal comprising at least one administration of the composition or vector of any one of claims 1-30.
32. A method for inducing an immunogenic or protective response in an animal against one or more avian pathogens comprising at least one administration of the composition or vector of any one of claims 1-30.
33. The method of claim 32, wherein the avian pathogen is selected from the group consisting of Newcastle Disease Virus (NDV), Infectious Bursal Disease Virus (*i.e.*, IBDV or Gumboro Disease virus), Marek's Disease Virus (MDV), Infectious Laryngotracheitis Virus (ILT), avian encephalomyelitis virus, avian

reovirus, avian paramyxovirus, avian metapneumovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, avian parvovirus, avian astrovirus and chick anemia virus coccidiosis (*Eimeria sp.*), *Campylobacter sp.*, *Salmonella sp.*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Pasteurella sp.*, *Avibacterium sp.*, *E. coli* and *Clostridium sp.*

34. The method of claim 31 or 32, wherein the animal is avian.

Figure 1 (1/2)

SEQ ID NO	Type	Gene
1	DNA	NDV-F VIIId codon optimized DNA sequence
2	Protein	NDV-F protein sequence from codon-optimized VIIId strain
3	DNA	NDV-F VIIId wildtype sequence
4	Protein	NDV-F protein sequence from wildtype VIIId strain
5	DNA	NDV-F Ca02 codon optimized DNA sequence
6	Protein	NDV-F protein sequence from codon-optimized Ca02 strain
7	DNA	IBDV DNA encoding VP2 protein
8	Protein	IBDV VP2 protein
9	DNA	SV40 promoter
10	DNA	CMV-IE promoter
11	DNA	SV40 polyA signal
12	DNA	Synthetic polyA signal
13	oligo	MB080 primer
14	oligo	MB081 primer
15	oligo	optF primer
16	oligo	VlloptF RP primer
17	oligo	SV40promoterF primer
18	DNA	Partial plasmid pHM103+Fopt DNA sequence (for vHVT114)
19	DNA	Partial plasmid pSB1 44 cds SV FCAopt (for vSB1-009)
20	DNA	Partial plasmid pHVT US2 SV- Fopt-synPA (for vHVT306)
21	DNA	Partial plasmid pCD046+NDV-F wt (for vHVT110)
22	DNA	Partial plasmid pHM103+NDV-F wt (for vHVT111)
23	DNA	Partial plasmid pHM103 + NDV-F CA02 (for vHVT116)
24	DNA	Partial plasmid HVTIG2 SV Fwt SbfI sequence (for vHVT301)
25	DNA	Partial plasmid pHVTUS10 cds F opt plasmid (for vHVT302)
26	DNA	Partial plasmid pHVTUS10 cds F CA02 opt sequence (for vHVT303)
27	DNA	Partial plasmid HVT IG2 SVFopt syn tail sequence (for vHVT304)
28	DNA	Partial plasmid pHVT US2 SV-FCA02 opt-synPA (for vHVT307)
29	DNA	Partial plasmid pCD046+NDV-F VII YZCQ sequence (vHVT112)
30	DNA	Partial plasmid pCD046+NDV Texas F sequence (for vHVT113)
31	DNA	Partial plasmid pHM119 sequence (for vHVT039)
32	DNA	NDV-F Wtnm-Texas wildtype DNA sequence
33	protein	NDV-F protein from Wtnm-Texas wildtype
34	DNA	NDV-F YZCQ wildtype DNA sequence
35	protein	NDV-F protein from wildtype YZCQ strain
36	DNA	NDV-F Texas wildtype DNA sequence
37	protein	NDV-F protein from wildtype Texas strain
38	DNA	MDV gB promoter



Figure 1 (2/2)

SEQ ID NO	Type	Gene
39	DNA	Partial plasmid HVT SORF3-US2 gpVar-Ewtsyn sequence (for vHVT202)
40	DNA	Partial plasmid SB1US2 gpVII dwtsyn sequence (for vSB1-010)
41	DNA	IBDV DNA encoding VP2 protein of IBDV E strain
42	protein	IBDV VP2 protein of IBDV E strain
43	DNA	Guinea pig CMV promoter
44	oligo	primer HM101
45	oligo	Primer HM102
46	oligo	primer F-ATG
47	oligo	Primer F-STOP

Figure 2

Genomic Structure of HVT, ORFs of the *Bam*HI fragment,  
and Insertion/Replacement Locations  
(GenBank accession number for HVT FC126 sequence: AF291866.1)

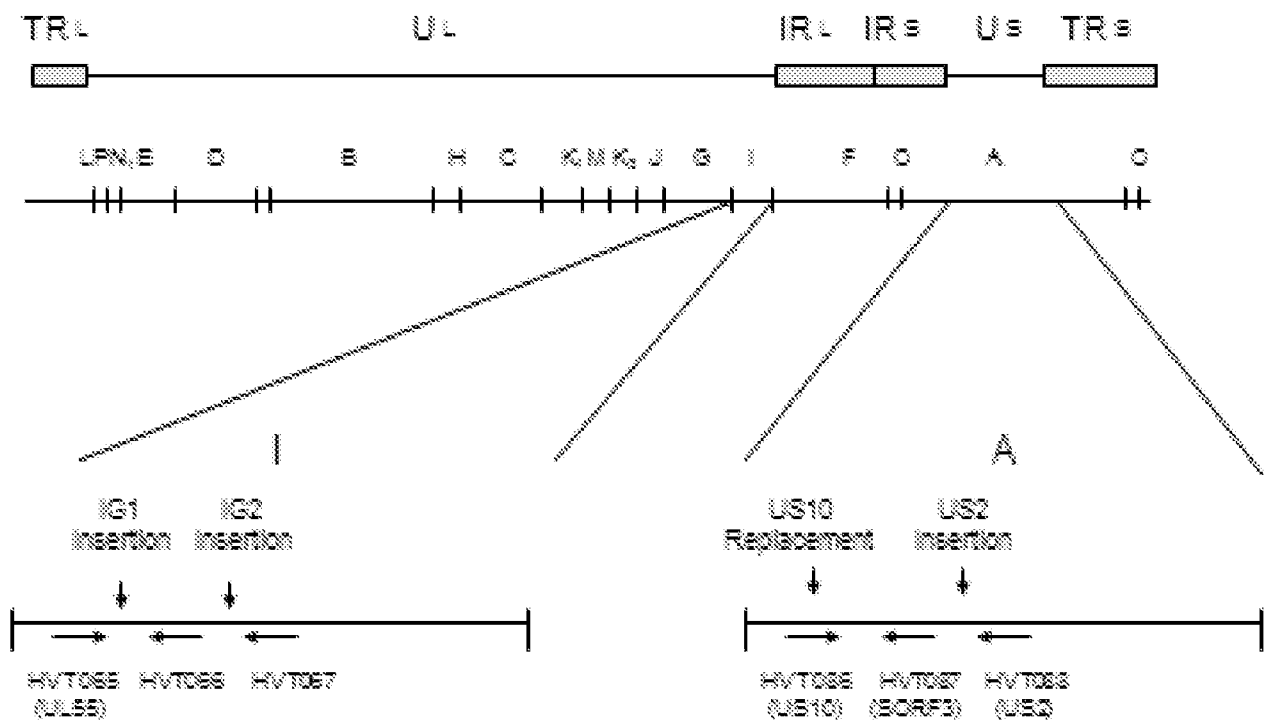


Figure 3

Plasmid map of pHM103 containing codon-optimized NDV-F gene

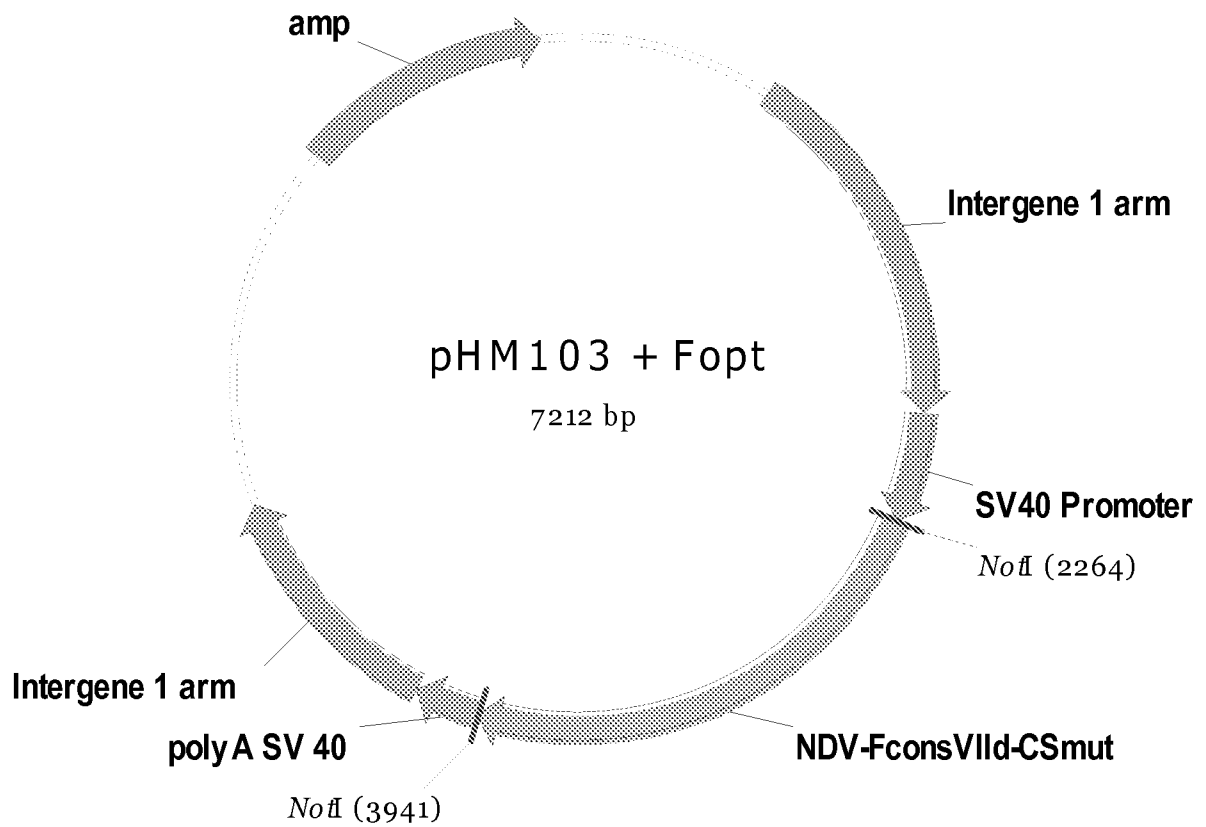
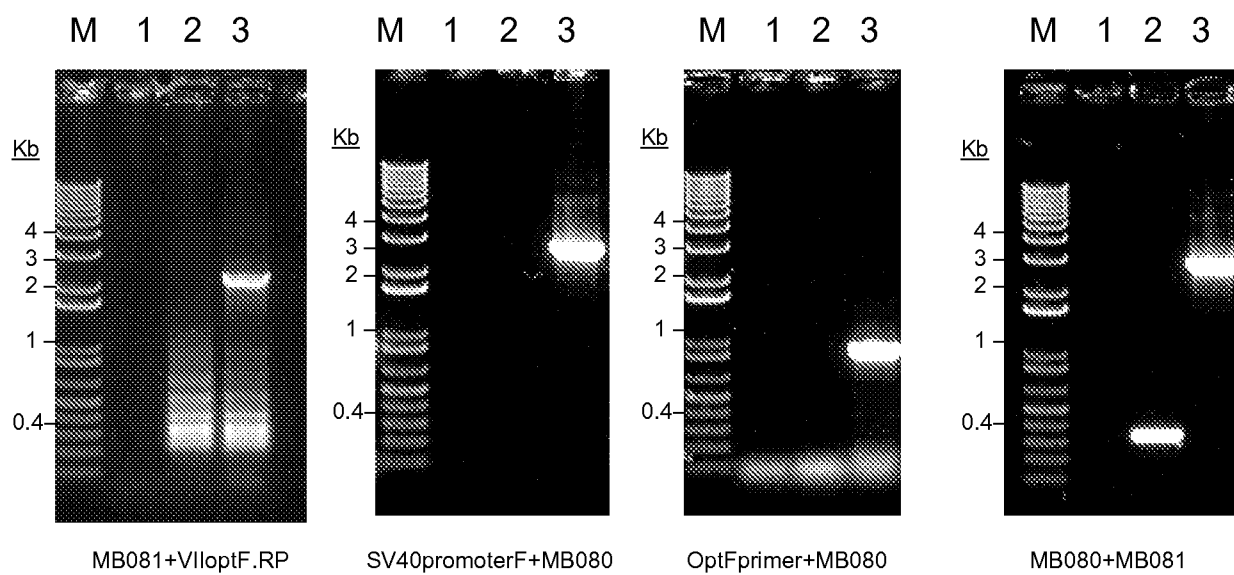


Figure 4

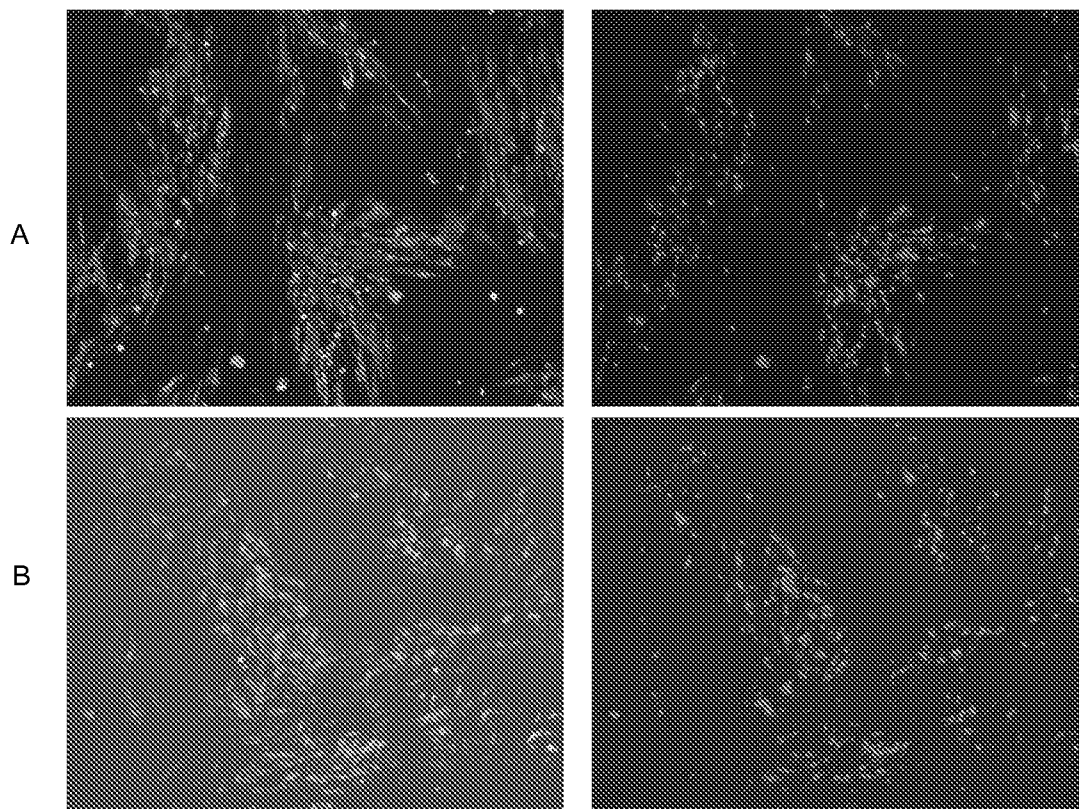
vHVT114 identity PCR



Lane 1: no template  
Lane 2: FC126 cl2  
Lane 3: vHVT114

Figure 5

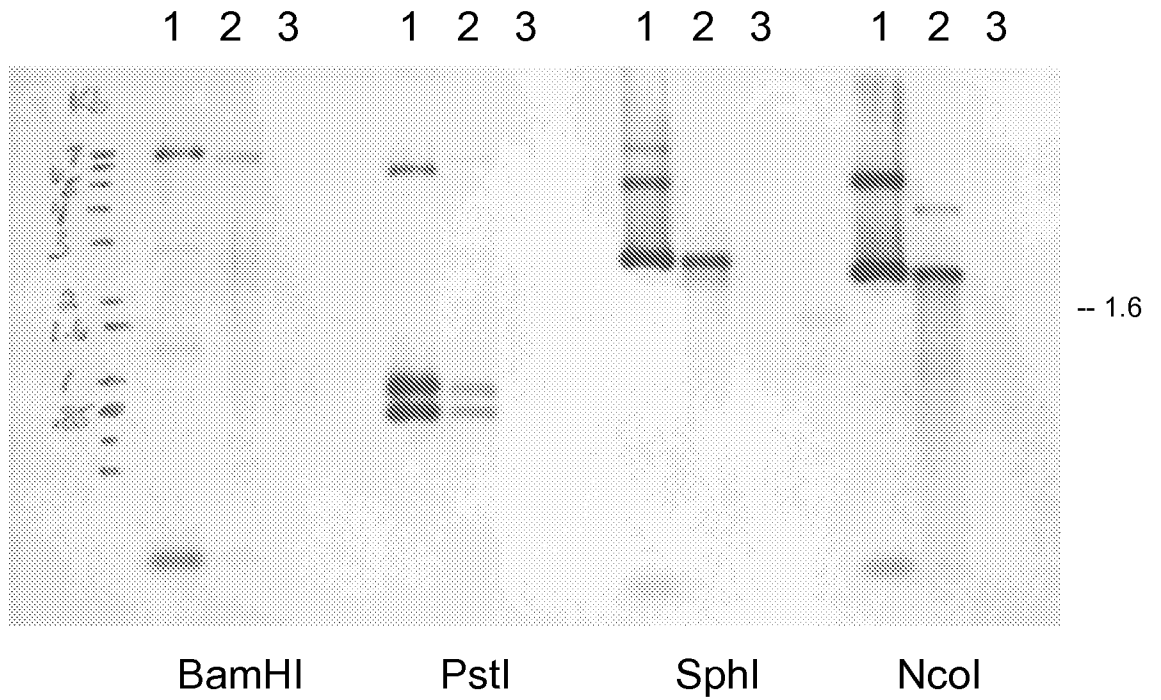
## Dual Immunofluorescent Assay



Panel A is from the pre-MSV passage  
Panel B is from the pre-MSV+12 passage

Figure 6

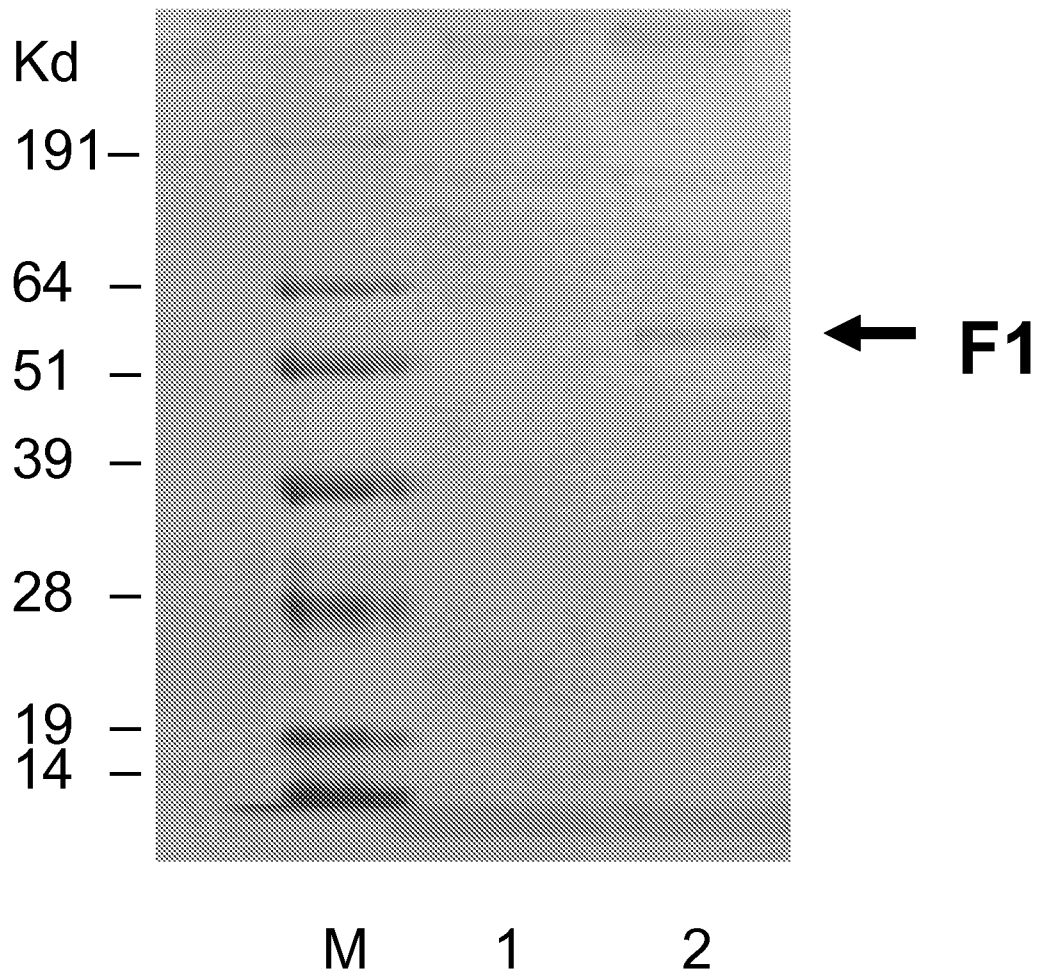
Southern blot using the NDV-F probe



1 = pHM103+Fopt donor  
2 = vHVT114  
3 = FC126 cl2

Figure 7

## Immunoprecipitation and Western Blot of vHVT114



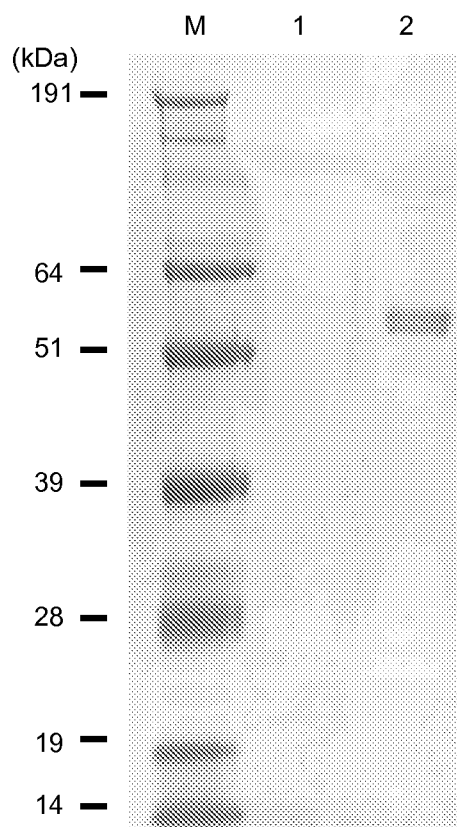
Lane M: Pre-Stained Standard (SeeBlue, Invitrogen);

Lane 1: CEF;

Lane 2: vHVT114.

Figure 8

Western blot analysis of immunoprecipitated sample from vHVT306 infected cells



Lane M: pre-stained protein standard (Invitrogen, SeeBlue)

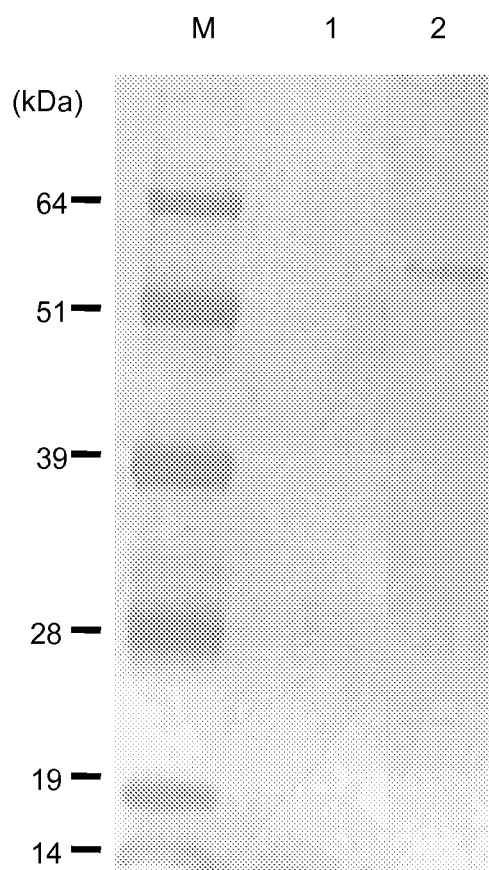
Lane 1: uninfected CEF

Lane 2: vHVT306



Figure 9

Western blot analysis of immunoprecipitated sample  
from vSB1-009 infected cells



Lane M: pre-stained protein standard (Invitrogen, SeeBlue)

Lane 1: uninfected CEF

Lane 2: vSB1-009 pre-MSV stock

Figure 10

Challenge study of vHVT304 and vHVT114 against NDV ZJ1 and CA02

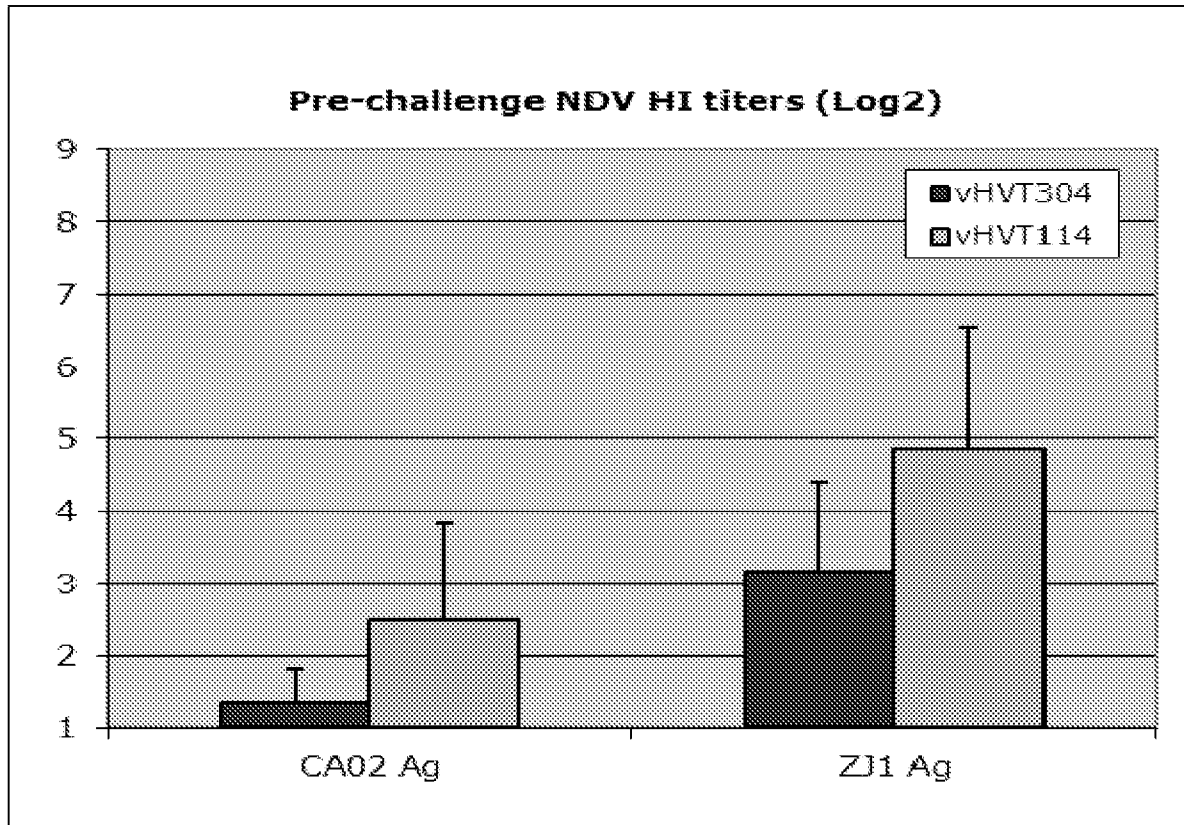


Figure 11

FIG. 11A

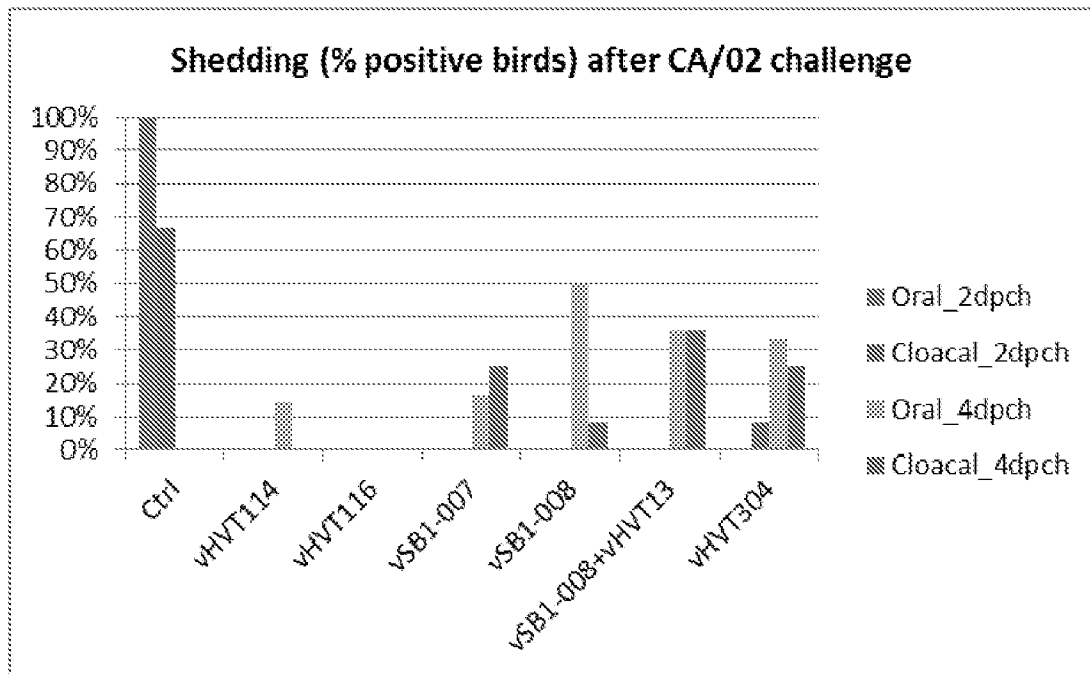


FIG. 11B

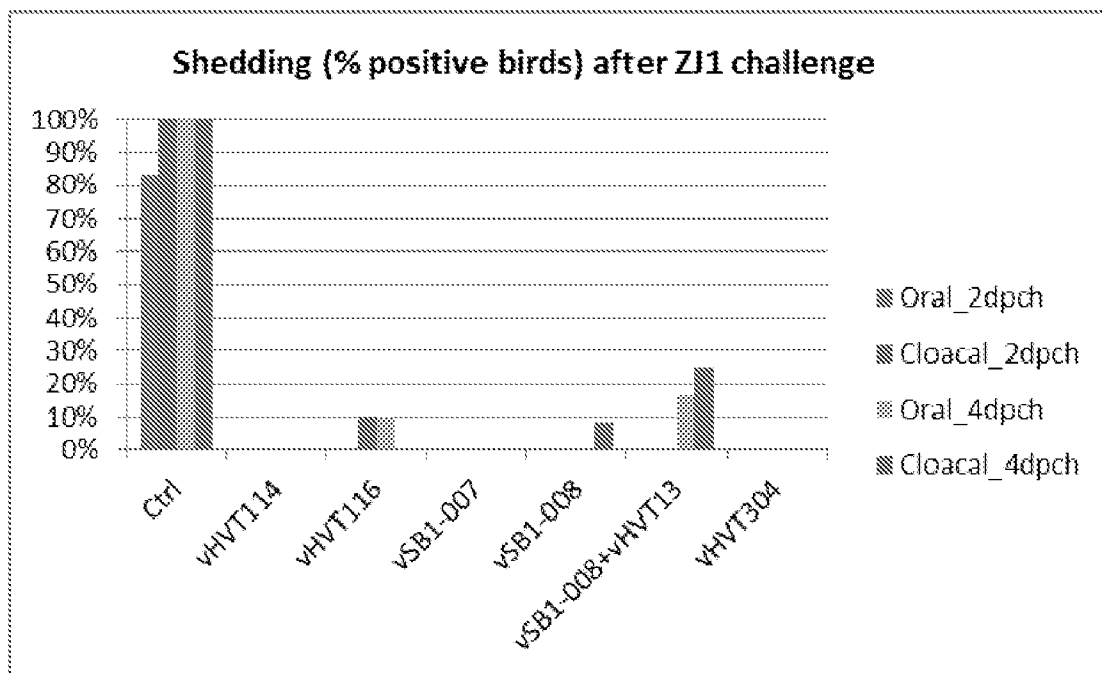


Figure 12

FIG. 12A

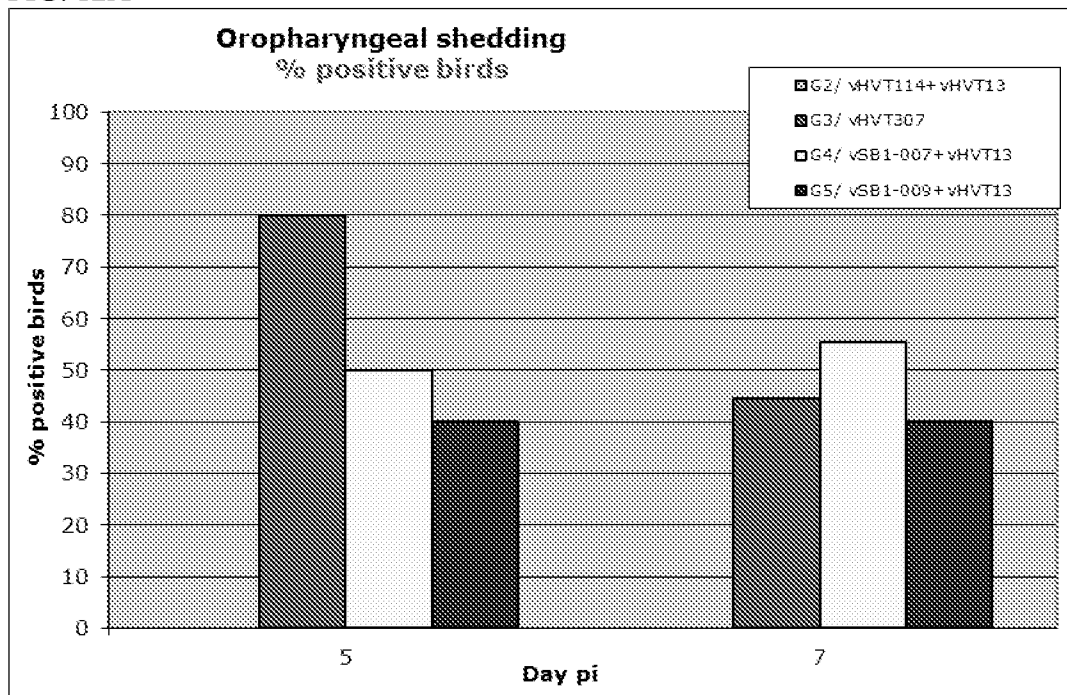


FIG. 12B

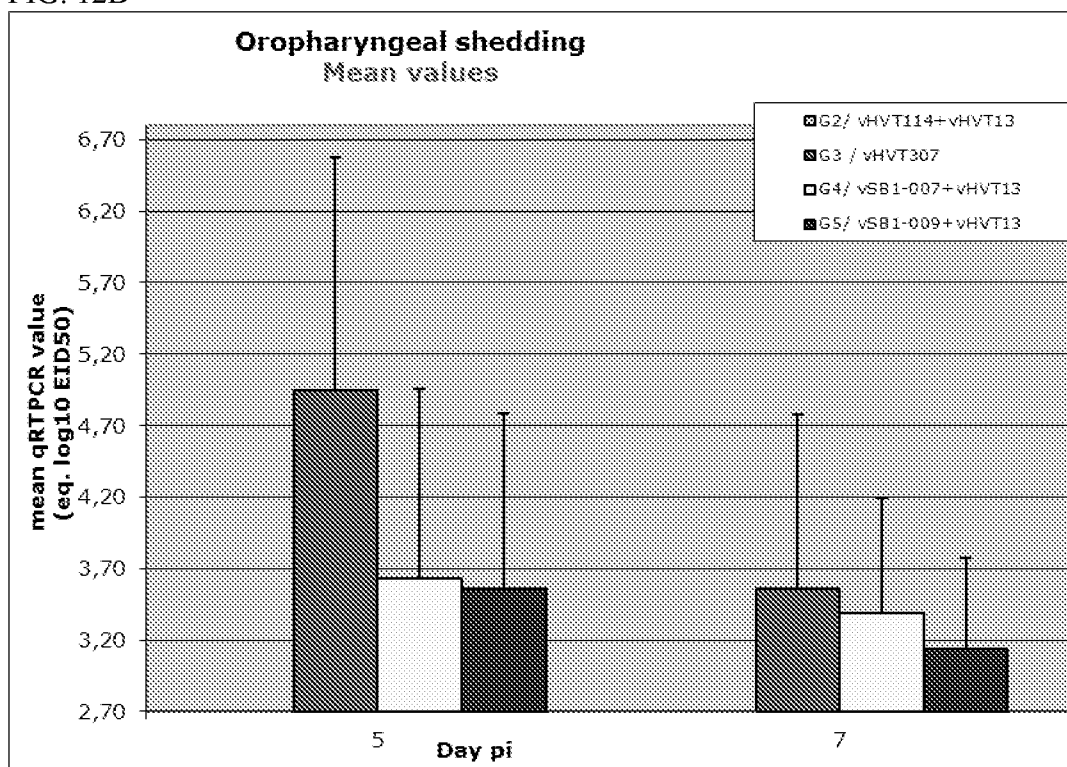


Figure 13

## Protein sequence alignment of NDV-F

		1	50
SEQ ID NO:2	(1)	MGSKPSTRIPAPIMLITRIMLILGCIPTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:33	(1)	MGSRSSSTRIPVPEMLLIIRTAITLSCTILTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:35	(1)	MGSRSSSTRIPVPEMLLIIRTAITLSCTILTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:37	(1)	MGSKPSTRIPAPIMLITRIMLILDCIRPTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:4	(1)	MGSKPSTRIPAPIMLITRIMLILGCIPTSSLDGRPLAAAGIVVTGDKAV	
SEQ ID NO:6	(1)	MGSKPSTWISVTIMLITRIMLILSCICPTSSLDGRPLAAAGIVVTGDKAV	
		51	100
SEQ ID NO:2	(51)	NVYTSSQTGSIIIVKLLPNMPKDKKACAKAPLEAYNRTLTTLTPLGDSIR	
SEQ ID NO:33	(51)	NIYTSSQTGSIIIVKLLPNMPKDKKACAKAPLEAYNRTLTTLTPLGDSIR	
SEQ ID NO:35	(51)	NIYTSSQTGSIIIVKLLPNMPKDKKACAKAPLEAYNRTLTTLTPLGDSIR	
SEQ ID NO:37	(51)	NVYTSSQTGSIIIVKLLPNMPKDKKACAKAPLEAYNRTLTTLTPLGDSIR	
SEQ ID NO:4	(51)	NVYTSSQTGSIIIVKLLPNMPKDKKACAKAPLEAYNRTLTTLTPLGDSIR	
SEQ ID NO:6	(51)	NIYTSSQTGSIIIVKLLPNMPKDKKACAKAPLEAYNRTLTTLTPLGDSIR	
		101	150
SEQ ID NO:2	(101)	KIQGSVSTSGGGKQGRLLIGAVIGSVALGVATAAQITAAALIQANQNAAN	
SEQ ID NO:33	(101)	RIQESVITSGGRRQRRFIGALIGSVALGVATAAQITAAALIQANQNAAN	
SEQ ID NO:35	(101)	RIQESVITSGGKKQGRLLIGALIGSVALGVATAAQITAAALIQANQNAAN	
SEQ ID NO:37	(101)	KIQGSVSTSGGGKQGRLLIGAVIGSVALGVATAAQITAAALIQANQNAAN	
SEQ ID NO:4	(101)	KIQGSVSTSGGGKQGRLLIGAVIGSVALGVATAAQITAAALIQANQNAAN	
SEQ ID NO:6	(101)	RIQGSATTSGGGKQGRLLIGALIGSVALGVATAAQITAAALIQANQNAAN	
		151	200
SEQ ID NO:2	(151)	ILRLKESIAATNEAVHEVTDGLSOLSVAVGKMQQFVNDQFNNTARELDCI	
SEQ ID NO:33	(151)	ILRLKESIAATNEAVHEVTDGLSOLAVAVGKMQQFVNDQFNNTAQELDCI	
SEQ ID NO:35	(151)	ILRLKESIAATNEAVHEVTDGLSOLAVAVGKMQQFVNDQFNNTAQELDCI	
SEQ ID NO:37	(151)	ILRLKESIAATNEAVHEVTDGLSOLSVAVGKMQQFVNDQFNNTARELDCI	
SEQ ID NO:4	(151)	ILRLKESIAATNEAVHEVTDGLSOLSVAVGKMQQFVNDQFNNTARELDCI	
SEQ ID NO:6	(151)	ILRLKESIAATNDVAVHEVTNGLSOLAVAVGKMQQFVNNQFNNTARELDCI	
		201	250
SEQ ID NO:2	(201)	KITQOVGVNELNLYLTELTTFVFGPQITSPALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:33	(201)	KIAQQVGVNELNLYLTELTTFVFGPQITSPALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:35	(201)	KIAQQVGVNELNLYLTELTTFVFGPQITSPALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:37	(201)	KITQOVGVNELNLYLTELTTFVFGPQITSPALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:4	(201)	KITQOVGVNELNLYLTELTTFVFGPQITSPALTQLTIQALYNLAGGNMDYLL	
SEQ ID NO:6	(201)	KIAQQVGVNELNLYLTELTTFVFGPQITSPALTQLTIQALYNLAGGNMDYLL	
		251	300
SEQ ID NO:2	(251)	TKLGIQNNQLSSLIGSGLITGYPILYDSQTQILGIQVNLPSVGNLNNMRA	
SEQ ID NO:33	(251)	TKLGVGNNQLSSLIGSGLITGNPILYDSQTQILGIQVTLPSVGNLNNMRA	
SEQ ID NO:35	(251)	TKLGVGNNQLSSLIGSGLITGNPILYDSQTQILGIQVTLPSVGNLNNMRA	
SEQ ID NO:37	(251)	TKLGIQNNQLSSLIGSGLITGYPILYDSQTQILGIQVNLPSVGNLNNMRA	
SEQ ID NO:4	(251)	TKLGIQNNQLSSLIGSGLITGYPILYDSQTQILGIQVNLPSVGNLNNMRA	
SEQ ID NO:6	(251)	TKLGVGNNQLSSLIGSGLITGNPILYDSQTQILGIQVNLPSVGSLLNNMRA	

Figure 13 (continued)

		301		350
SEQ ID NO:2	(301)	TYLETLSVSTTKGYASALVPKVVTVQVGSVIEELDTSYCIESDLDLYCTRI		
SEQ ID NO:33	(301)	TYLETLSVSTTKGYASALVPKVVTVQVGSVIEELDTSYCIGTDLDLYCTRI		
SEQ ID NO:35	(301)	TYLETLSVSTTKGYASALVPKVVTVQVGSVIEELDTSYCIGTDLDLYCTRI		
SEQ ID NO:37	(301)	TYLETLSVSTAKGYASALVPKVVTVQVGSVIEELDTSYCIESDLDLYCTRI		
SEQ ID NO:4	(301)	TYLETLSVSTTKGYASALVPKVVTVQVGSVIEELDTSYCIESDLDLYCTRI		
SEQ ID NO:6	(301)	TYLETLSVSTTKGYASALVPKVVTVQVGSVIEELDTSYCIESDLDLYCTRV		
		351		400
SEQ ID NO:2	(351)	VTFFMSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSVIANCKITTCR		
SEQ ID NO:33	(351)	VTFFMSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSVIANCKITTCR		
SEQ ID NO:35	(351)	VTFFMSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSVIANCKITTCR		
SEQ ID NO:37	(351)	VTFFMSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSVIANCKITTCR		
SEQ ID NO:4	(351)	VTFFMSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSVIANCKITTCR		
SEQ ID NO:6	(351)	VTFFMSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSVIANCKITTCR		
		401		450
SEQ ID NO:2	(401)	CTDPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI		
SEQ ID NO:33	(401)	CADPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI		
SEQ ID NO:35	(401)	CADPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI		
SEQ ID NO:37	(401)	CTDPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI		
SEQ ID NO:4	(401)	CTDPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISI		
SEQ ID NO:6	(401)	CADPPGIISQNYGEAVSLIDRHSCSVLSLDGITLRLSGEFDATYQKNISI		
		451		500
SEQ ID NO:2	(451)	LDSQVIVTGNLDISTELGNVNNSISNALDRLAESNSKLEKVNVLSTSA		
SEQ ID NO:33	(451)	LDSQVIVTGNLDISTELGNVNNSISNALNKLKLEESNSKLDKVNVLSTSA		
SEQ ID NO:35	(451)	LDSQVIVTGNLDISTELGNVNNSISNALNKLKLEESNSKLDKVNVLSTSA		
SEQ ID NO:37	(451)	LDSQVIVTGNLDISTELGNVNNSISNALDKLAKSNSKLEKVNVLSTSA		
SEQ ID NO:4	(451)	LDSQVIVTGNLDISTELGNVNNSISNALDRLAESNSKLEKVNVLSTSA		
SEQ ID NO:6	(451)	LDSQVIVTGNLDISTELGNVNNSISSTLDKLAESNNKLNKVNVLSTSA		
		501		550
SEQ ID NO:2	(501)	LITYIVLTVISLVFGALSIVLACYLMYKQKAQOKTLLWLGNNTLDQMRAT		
SEQ ID NO:33	(501)	LITYIVLTVISLVFGVLSLVLACYLMYKQKAQOKTLLWLGNNTLDQMRAT		
SEQ ID NO:35	(501)	LITYIVLTVISLVFGVLSLVLACYLMYKQKAQOKTLLWLGNNTLDQMRAT		
SEQ ID NO:37	(501)	LITYIVLTVISLVFGALSGLTCYLMYKQKAQOKTLLWLGNNTLDQMRAT		
SEQ ID NO:4	(501)	LITYIVLTVISLVFGALSIVLACYLMYKQKAQOKTLLWLGNNTLDQMRAT		
SEQ ID NO:6	(501)	LITYIVLAIVSLAFGVISLVLACYLMYKQKAQOKTLLWLGNNTLDQMRAT		
		551		
SEQ ID NO:2	(551)	TRA--		
SEQ ID NO:33	(551)	TKI--		
SEQ ID NO:35	(551)	TKI--		
SEQ ID NO:37	(551)	TRA--		
SEQ ID NO:4	(551)	TRA--		
SEQ ID NO:6	(551)	TRT--		

Figure 13 (continued)

	SEQ ID NO:2	SEQ ID NO:33	SEQ ID NO:35	SEQ ID NO:37	SEQ ID NO:4	SEQ ID NO:6
SEQ ID NO:2		92%	93%	98%	100%	92%
SEQ ID NO:33			99%	92%	92%	91%
SEQ ID NO:35				92%	93%	92%
SEQ ID NO:37					98%	91%
SEQ ID NO:4						92%
SEQ ID NO:6						

Figure 13 (continued)

## DNA sequence alignment of NDV-F genes

		1	50
SEQ ID NO:1	(1)	ATGGGCAGCAAGCCAGCACAAAGATCCAGCCGCCCTGATGCTGATCAG	
SEQ ID NO:3	(1)	ATGGGCTCCAAACCTTCTACCAGGATCCAGCACCTCTGATGCTGATCAG	
SEQ ID NO:32	(1)	ATGGGCTCCAGATCTTCTACCAGGATCCCGGTACCTCTAATGCTGATCAT	
SEQ ID NO:34	(1)	ATGGGCTCCAGATCTTCTACCAGGATCCCGGTACCTCTAATGCTGATCAT	
SEQ ID NO:36	(1)	ATGGGCTCTAAACCTTCTACCAGGATCCAGCACCTCTGATGCTGATCAG	
SEQ ID NO:5	(1)	ATGGGCAGCAAGCCAGCACCTGGATCAGCGTGACCTGATGCTGATCAG	
		51	100
SEQ ID NO:1	(51)	CEGCATCATGCTGATCCTGGGCTGCATCAGACCCACAAGCTCCCTGGATG	
SEQ ID NO:3	(51)	CEGGATTATGCTGATATTGGGCTGTATCGTCCGACAAGCTCTCTTGACG	
SEQ ID NO:32	(51)	CCGAACCGCGCTGACACTGAGCTGTATCGTCTGACAAGCTCTCTTGATG	
SEQ ID NO:34	(51)	CCGAACCGCGCTGACACTGAGCTGTATCGTCTGACAAGCTCTCTTGATG	
SEQ ID NO:36	(51)	CEGGATTATGCTGATATTGGAAGTGTATCGTCCGACAAGCTCTCTTGACG	
SEQ ID NO:5	(51)	CAGAACCATGCTGATCCTGAGCTGCATCTGCCCCACAAGCAGCCTGGACG	
		101	150
SEQ ID NO:1	(101)	GACGCCCCCTGGCCGCTGCCCGCATCGTGCTGACCGGCGACAAGGCCGTG	
SEQ ID NO:3	(101)	GCAGGCCTCTTGGCAGCTGCAGGAATTGTAGTAACAGGAGATAAGGCAGTC	
SEQ ID NO:32	(101)	GCAGGCCTCTTGGCGCTGCAGGGATCGTGCTAACAGGAGATAAAGCAGTC	
SEQ ID NO:34	(101)	GCAGGCCTCTTGGCGCTGCAGGGATCGTGCTAACAGGAGATAAAGCAGTC	
SEQ ID NO:36	(101)	GCAGGCCTCTTGGCAGCTGCAGGAATTGTAGTAACAGGAGATAAGGCAGTC	
SEQ ID NO:5	(101)	GCAGACCCCTGGCCGCTGCCCGCATCGTGCTGACCGGCGACAAGGCCGTG	
		151	200
SEQ ID NO:1	(151)	AACGTGTACACCAGCAGCCAGACCGGCAGCATCATCGTGAAGCTGCTGCC	
SEQ ID NO:3	(151)	AATGTATACACTTCGTCTCAGACAGGGTCAATCATAGTCAAGTTGCTCCC	
SEQ ID NO:32	(151)	AACATATACACCTCATCCAGACAGGGTCAATCATAGTTAAGTTACTCCC	
SEQ ID NO:34	(151)	AACATATACACCTCATCCAGACAGGGTCAATCATAGTTAAGTTACTCCC	
SEQ ID NO:36	(151)	AATGTATATACCTCGTCTCAGACAGGGTCAATCATAGTCAAGTTGCTCCC	
SEQ ID NO:5	(151)	AACATCTACACCAGCAGCCAGACCGGCAGCATCATCATCAAGCTGCTGCC	
		201	250
SEQ ID NO:1	(201)	CAACATGCCCAAGGACAAAGAGGGCTGCGCCAAAGGCCCCCTGGAAGCCT	
SEQ ID NO:3	(201)	GAATATGCCCAAGGATAAGGAGGCGTGTGCAAAAGCCCCATTAGAGGCAT	
SEQ ID NO:32	(201)	GAATATGCCCAAGGACAAAGAGGTGTGTGCAAAAGCCCCATTGAGGCAT	
SEQ ID NO:34	(201)	GAATATGCCCAAGGACAAAGAGGTGTGTGCAAAAGCCCCATTGAGGCAT	
SEQ ID NO:36	(201)	GAATATGCCCAAGGATAAGGAGGCGTGTGCAAAAGACCCATTAGAGGCAT	
SEQ ID NO:5	(201)	CAACATGCCCAAGGACAAAGAGGGCTGCGCCAAAGGCCCCCTGGAAGCCT	
		251	300
SEQ ID NO:1	(251)	ACAACAGAACCTGACCACCCCTGCTGACCCCTGGGGGACAGCATCAGA	
SEQ ID NO:3	(251)	ATAACAGAACACTGACTACTTTGCTCACTCCTCTTGGGGACTCCATCCGC	
SEQ ID NO:32	(251)	ACAACAGGACACTGACTACTTTACTCAACCCCTTGGTGATTCTATCCGC	
SEQ ID NO:34	(251)	ACAACAGGACACTGACTACTTTACTCAACCCCTTGGTGATTCTATCCGC	
SEQ ID NO:36	(251)	ATAACAGAACACTGACTACTTTGCTCACTCCTCTTGGGGAAATCCATCCGC	
SEQ ID NO:5	(251)	ACAACAGAACCTGACCACCCCTGCTGACCCCTGGGGGACAGCATCAGA	



Figure 13 (continued)

		301		350
SEQ ID NO:1	(301)	AAGATCCAGGGCTCCGTGAGCACAAGCGGCGGAGCAAGCAGGCCCACT		
SEQ ID NO:3	(301)	AAGATCCAGGGTCTGTGTCCACATCTGGAGGAGCCAAAGCAAGCCCGCCT		
SEQ ID NO:32	(301)	AGGATACAAGAGTCTGTGACTACTTCCGGAGGAAGGAGACAGAGCGCTT		
SEQ ID NO:34	(301)	AGGATACAAGAGTCTGTGACTACTTCCGGAGGAGGCAAGCAAGCCCGCCT		
SEQ ID NO:36	(301)	AAGATCCAGGGTCTGTGTCCACGTCTGGAGGAGGCAAGCAAGCCCGCCT		
SEQ ID NO:5	(301)	AGAATCCAGGGCAGGCCACCACAAGCGGCGGAGGAAGCAGGCCCACT		
		351		400
SEQ ID NO:1	(351)	GATCGGCGCGTGATCGGCAGCGTGGCCCTGGGAGTGGCTACAGCTGCC		
SEQ ID NO:3	(351)	GATAGGTGCTGTATTATGGCAGTGTAGCTCTTGGGGTTGCAACAGCGGCAC		
SEQ ID NO:32	(351)	TATAGGTGCCATTATCGGCAGTGTAGCTCTTGGGGTTGCGACAGCTGCAC		
SEQ ID NO:34	(351)	GATAGGTGCCATTATCGGCAGTGTAGCTCTTGGGGTTGCGACAGCTGCAC		
SEQ ID NO:36	(351)	GATAGGTGCTGTATTATGGTAGTGTAGCTCTTGGGGTTGCAACAGCGGCAC		
SEQ ID NO:5	(351)	GGTGGCGCTATCATCGGGAGCGTGGCCCTGGGCGTGGCCACAGCTGCC		
		401		450
SEQ ID NO:1	(401)	AGATTACCGCTGCAGCGGCCCTGATCCAGGCCAACAGAACCGCCGCCAAC		
SEQ ID NO:3	(401)	AGATAACAGCAGCTGGCGGCCCTAATAACAAGCCAAACAGAAATGCCGCCAAC		
SEQ ID NO:32	(401)	AGATAACAGCAGCTTCCGGCCCTGATACAAGCCAAACAGAAATGCTGCCAAC		
SEQ ID NO:34	(401)	AGATAACAGCAGCTTCCGGCCCTGATACAAGCCAAACAGAAATGCTGCCAAC		
SEQ ID NO:36	(401)	AATAACAGCAGCTGGCGGCCCTAATAACAAGCCAAACAGAAATGCTGCCAAC		
SEQ ID NO:5	(401)	AGATTACCGCTGCAGCGGCCCTGATTCAGGCCAATCAGAACCGCCGCCAAC		
		451		500
SEQ ID NO:1	(451)	ATCCTGAGACTGAAGAGAGCATTGCGCCACCAACGAGGCCGTGCACGA		
SEQ ID NO:3	(451)	ATCCTCGGGCTTAAGGAGAGCATTGCTGCAACCAATGAAGCTGTGCATGA		
SEQ ID NO:32	(451)	ATCCTCGGGCTTAAGGAGAGCATTGCTGCAACCAATGAAGCTGTGCACGA		
SEQ ID NO:34	(451)	ATCCTCGGGCTTAAGGAGAGCATTGCTGCAACCAATGAAGCTGTGCACGA		
SEQ ID NO:36	(451)	ATCCTTCGGCTTAAGGAGAGCATTGCTGCAACCAATGAAGCTGTGCATGA		
SEQ ID NO:5	(451)	ATCCTGAGACTGAAGAGAGCATTGCGCCACCAACGACGCCCGTGCACGA		
		501		550
SEQ ID NO:1	(501)	AGTGACCGACGGCCTGAGCCAGCTGTCCGTGGCGTGGGCAAGATGCAGC		
SEQ ID NO:3	(501)	AGTCACCGACGGATTATCACAACCTATCAGTGGCAGTTGGGAAGATGCAGC		
SEQ ID NO:32	(501)	GGTCACTGACGGATTATCACAACCTAGCAGTGGCAGTASGGAAGATGCAC		
SEQ ID NO:34	(501)	GGTCACTGACGGATTATCACAACCTAGCAGTGGCAGTASGGAAGATGCAC		
SEQ ID NO:36	(501)	AGTCACCGACGGATTATCACAACCTATCAGTGGCAGTTGGGAAGATGCAGC		
SEQ ID NO:5	(501)	AGTGACAAACGGACTGTCCAGCTGGCTGTCTGCTGTCCGCAAGATGCAGC		
		551		600
SEQ ID NO:1	(551)	AGTTTCGTGAACGACCAGTTCAACAACACCGCCAGAGAGCTGGACTGCATC		
SEQ ID NO:3	(551)	AGTTTGTCAATGACCAGTTTAATAAATACGGCGGAGAAATTGGACTGTATA		
SEQ ID NO:32	(551)	AGTTTGTCAATGACCAGTTCAATAAATACAGCGCAAGAAATTGGACTGTATA		
SEQ ID NO:34	(551)	AGTTTGTCAATGACCAGTTCAATAAATACAGCGCAAGAAATTGGACTGTATA		
SEQ ID NO:36	(551)	AGTTTGTCAATGACCAGTTTAATAAATACAGCGGAGAAATTGGACTGTATA		
SEQ ID NO:5	(551)	AGTTTCGTGAACAACAGTTCAACAACACCGCCAGAGAGCTGGACTGCATC		

Figure 13 (continued)

		601		650
SEQ ID NO:1	(601)	AAGATCAACCCAGCAGGTGGGCGTGGAGCTGAACCTGTACCTGACCGAGCT		
SEQ ID NO:3	(601)	AAAATCACACACACAGGTGGGTGTAGAACTCAACCTATACCTAACTGAATT		
SEQ ID NO:32	(601)	AAAATTGCACAGCAGGTGGGTGTAGAACTCAACTTGTACCTAACTGAATT		
SEQ ID NO:34	(601)	AAAATTGCACAGCAGGTGGGTGTAGAACTCAACTTGTACCTAACTGAATT		
SEQ ID NO:36	(601)	AAAATCACACACACAGGTGGGTGTAGAACTCAACCTATACCTAACTGAATT		
SEQ ID NO:5	(601)	AAGATGCGCCAGCAGGTGGGCGTGGAGCTGAACCTGTACCTGACCGAGCT		
		651		700
SEQ ID NO:1	(651)	GACCACAGTGTTCGGCCCCCAGATCACAAAGCCCCGCTGACACAGCTGA		
SEQ ID NO:3	(651)	GACTACAGTATTCGGGCCACAGATCACCTCCCTGCAATTAACCTCAGCTGA		
SEQ ID NO:32	(651)	GACTACAGTATTCGGGCCACAAATCACTTCCCTGCAATTAACCTCAGCTGA		
SEQ ID NO:34	(651)	GACTACAGTATTCGGGCCACAAATCACTTCCCTGCAATTAACCTCAGCTGA		
SEQ ID NO:36	(651)	GACTACAGTATTCGGGCCACAGATCACCTCCCTGCAATTAACCTCAGCTGA		
SEQ ID NO:5	(651)	GACCACAGTGTTCGGCCCCCAGATCACAAAGCCCCGCTCTGACCCAGCTGA		
		701		750
SEQ ID NO:1	(701)	CCATCCAGGCCCTGTACAACCTGGCTGGCGGCAACATGGACTATCTGCTG		
SEQ ID NO:3	(701)	CCATCCAGGCCACTTTATAATTTAGCTGGTGGCAATATGGATTACTTATTA		
SEQ ID NO:32	(701)	CTATCCAGCGCTTTACAATCTAGCTGGTGGTAAATATGGATTACTTGGTG		
SEQ ID NO:34	(701)	CTATCCAGCGCTTTACAATCTAGCTGGTGGTAAATATGGATTACTTGGTG		
SEQ ID NO:36	(701)	CCATCCAGGCCACTTTATAATTTAGCTGGTGGCAATATGGATTACTTATTA		
SEQ ID NO:5	(701)	CAATCCAGGCCCTGTACAACCTGGCTGGCGGCAACATGGACTATCTGCTG		
		751		800
SEQ ID NO:1	(751)	ACAAAGCTGGGAATCGGCAACAACAGCTGTCCAGCCTGATCGGAAAGCGG		
SEQ ID NO:3	(751)	ACTAAGTTAGGTATAGGGAACAATCAACTCAGCTCGTTAATTGGTAGCGG		
SEQ ID NO:32	(751)	ACTAAGTTAGGTGTAGGGAACAACCAACTCAGCTCATTAAATTGGTAGCGG		
SEQ ID NO:34	(751)	ACTAAGTTAGGTGTAGGGAACAACCAACTCAGCTCATTAAATTGGTAGCGG		
SEQ ID NO:36	(751)	ACTAAGTTAGGTATAGGGAACAATCAACTCAGCTCATTAAATTGGCAGCGG		
SEQ ID NO:5	(751)	ACTAAGCTGGGAGTGGGCAACAACAGCTGTCCAGCCTGATCGGGTCCGG		
		801		850
SEQ ID NO:1	(801)	CCTGATCACCGGCTACCCCATCCTGTACGACAGCCAGACACAGCTGCTGG		
SEQ ID NO:3	(801)	CCTGATCACTGGTTACCCATATCTGTATGACTCAGACTCAACTCTTGG		
SEQ ID NO:32	(801)	CTTGATCACCGGCAACCCATATCTGTACGACTCAGACTCAGATCTTGG		
SEQ ID NO:34	(801)	CTTGATCACCGGCAACCCATATCTGTACGACTCAGACTCAGATCTTGG		
SEQ ID NO:36	(801)	CCTGATCACTGGTTACCCATATTTGTATGACTCAGACTCAACTCTTGG		
SEQ ID NO:5	(801)	GCTGATCACAGGCAACCCCATCTGTACGACAGCCAGACACAGCTGCTGG		
		851		900
SEQ ID NO:1	(851)	GCATCCAGGTGAACCTGCCCAGCGTGGGCAACCTGAACAACATGGCGGCC		
SEQ ID NO:3	(851)	GCATACAAGTGAATTTACCCCTCAGTCGGGAACCTTAATAAATATGGGTGCC		
SEQ ID NO:32	(851)	GTATACAGGTAACTTTGCCTTCAGTTGGGAACCTGAATAAATATGGGTGCC		
SEQ ID NO:34	(851)	GTATACAGGTAACTTTGCCTTCAGTTGGGAACCTGAATAAATATGGGTGCC		
SEQ ID NO:36	(851)	GCATACAAGTGAATTTGCCCTCAGTCGGGAACCTTAATAAATATGGGTGCC		
SEQ ID NO:5	(851)	GCATCCAGATCAACCTGCCATCCGTGGGAAGCCTGAACAACATGAGAGCC		

Figure 13 (continued)

		901		950
SEQ ID NO:1	(901)	ACCTACCTGGAAACCCCTGAGCGTGTCCACCAACCAAGGGCTACGCCAGCSC		
SEQ ID NO:3	(901)	ACCTATTTGGAGACCTTATCTGTAAGTACAAACCAAGGATATGCCTCAGC		
SEQ ID NO:32	(901)	ACCTACCTGGAGACCTTATCTGTAAGCACAACCAAGGGATTGGCTCAGC		
SEQ ID NO:34	(901)	ACCTACCTGGAGACCTTATCTGTAAGCACAACCAAGGGATTGGCTCAGC		
SEQ ID NO:36	(901)	ACCTATTTAGAGACCTTATCTGTAAGTACAGCCAAAGGATATGCCTCAGC		
SEQ ID NO:5	(901)	ACCTACCTGGAAACCCCTGAGCGTGTCCACCAACCAAGGGCTTCGCCAGCSC		
		951		1000
SEQ ID NO:1	(951)	CCTGGTGCCCAAGGTGGTGACACAGGTGGGCAGCGTGATCGAGGAAGTGG		
SEQ ID NO:3	(951)	ACTTGTCCCGAAAGTAGTGACACAAGTCGGTTCCGTTGATAGAAAGAGCTTG		
SEQ ID NO:32	(951)	ACTTGTCCCGAAAGTAGTGACACAGGTGGTTCCGTTGATAGAAAGACTTG		
SEQ ID NO:34	(951)	ACTTGTCCCGAAAGTAGTGACACAGGTGGTTCCGTTGATAGAAAGACTTG		
SEQ ID NO:36	(951)	ACTTGTTCCTAAAAGTAGTGACACAAGTCGGTTCTGTGATAGAAAGAGCTTG		
SEQ ID NO:5	(951)	CCTGGTGCCCAAGGTGGTGACACAGGTGGGCAGCGTGATCGAGGAAGTGG		
		1001		1050
SEQ ID NO:1	(1001)	ACACCAGCTACTGCATCGAGAGCGACCTGGACCTGTAAGTGCACCAGAAATC		
SEQ ID NO:3	(1001)	ACACCTCATACTGTATAGAGTCGGATCTGGATTATATTGTACTAGAATA		
SEQ ID NO:32	(1001)	ACACCTCATACTGTATAGGACCGACTTGGATTATATTGTACTAGAATA		
SEQ ID NO:34	(1001)	ACACCTCATACTGTATAGGACCGACTTGGATTATATTGTACTAGAATA		
SEQ ID NO:36	(1001)	ACACCTCATACTGTATAGAGTCCGATCTGGATTATATTGTACTAGAATA		
SEQ ID NO:5	(1001)	ACACCAGCTACTGCATCGAGAGCGACATCGACCTGTAAGTGCACCAGAGTG		
		1051		1100
SEQ ID NO:1	(1051)	GTGACCTTCCCAATGAGCCCCGGCATCTACAGCTGCCTGAGCGGCAACAC		
SEQ ID NO:3	(1051)	GTGACATTCCCATGTCCTCCAGGTATTTATTCCTGTTGAGCGGCAACAC		
SEQ ID NO:32	(1051)	GTGACATTCCCTATGTCTCCTGGTATTTATTCCTGTTGAGCGGTAATAC		
SEQ ID NO:34	(1051)	GTGACATTCCCTATGTCTCCTGGTATTTATTCCTGTTGAGCGGTAATAC		
SEQ ID NO:36	(1051)	GTGACATTCCCATGTCCTCCAGGTATTTATTCCTGTTAAGCGGCAACAC		
SEQ ID NO:5	(1051)	GTGACCTTCCCAATGAGCCCCGGCATCTACAGCTGCCTGAGCGGCAACAC		
		1101		1150
SEQ ID NO:1	(1101)	CAGCGCCTGCATGTACAGCAAGACCGAAGGCGCACTGACAACACCCCTACA		
SEQ ID NO:3	(1101)	ATCAGCTTGCATGTATTCAAAGACTGAAGGCGCACTCACTACGCCGTATA		
SEQ ID NO:32	(1101)	ATCGGCTTGCATGTATTCAAAGACTGAAGGCGCACTTACTACGCCATATA		
SEQ ID NO:34	(1101)	ATCGGCTTGCATGTATTCAAAGACTGAAGGCGCACTTACTACGCCATATA		
SEQ ID NO:36	(1101)	ATCAGCTTGCATGTATTCAAAGACTGAAGGCGCACTCACTACGCCGTATA		
SEQ ID NO:5	(1101)	CAGCGCCTGCATGTACAGCAAGACCGAAGGAGCACTGACAACACCCCTACA		
		1151		1200
SEQ ID NO:1	(1151)	TGGCCCTGAAGGGAAGCSTGATCGCCAACTGCAAGATCACCACCTGCAGA		
SEQ ID NO:3	(1151)	TGGCCCTTAAAGGCTCAGTTATTGCCAATTGTAAATAACAACATGTAGA		
SEQ ID NO:32	(1151)	TGGCTCTCAAAGGCTCAGTTATTGCCAATTGCAAGCTGACAACATGTAGA		
SEQ ID NO:34	(1151)	TGGCTCTCAAAGGCTCAGTTATTGCCAATTGCAAGCTGACAACATGTAGA		
SEQ ID NO:36	(1151)	TGGCCCTTAAAGGCTCAGTTATTGCCAATTGTAAAGATAACAACATGTAGA		
SEQ ID NO:5	(1151)	TGGCCCTGAAGGGAAGCSTGATCGCCAACTGCAAGATCACCACCTGCAGA		



Figure 13 (continued)

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1501                                     1550
SEQ ID NO:1 (1501) CTGATCACCTACATCGTGGCTGACCGTGATCAGCCTGGTGTGGGCGCCCT
SEQ ID NO:3 (1501) CTCATTACCTATATTGTTCTAACTGTCAATTCTCTAGTTTTGGGTGCACT
SEQ ID NO:32 (1501) CTCATTACCTACATCGTTTTAACTGTCAATCTCTTGTGTTTTGGGTGTA
SEQ ID NO:34 (1501) CTCATTACCTACATCGTTTTAACTGTCAATCTCTTGTGTTTTGGGTGTA
SEQ ID NO:36 (1501) CTCATTACCTATATTGTTCTGACTGTCAATTCTCTAGTTTTGGGTGCACT
SEQ ID NO:5 (1501) CTGATCACCTACATCGTGGCTGGCCATCGTGTCCCTGGCCTTGGGCGTGAT

1551                                     1600
SEQ ID NO:1 (1551) GAGCCTGGTGGCTGGCCTGCTACCTGATGTACAAGCAGAAGGCCAGCAGA
SEQ ID NO:3 (1551) TAGTCTGGTGGTGGCCTGCTACCTGATGTACAAACAGAAGGCCACAACAAA
SEQ ID NO:32 (1551) TAGCCTGGTGGTGGCCTGCTACCTGATGTACAAGCAAAAGGCCACAACAAA
SEQ ID NO:34 (1551) TAGCCTGGTGGTGGCCTGCTACCTGATGTACAAGCAAAAGGCCACAACAAA
SEQ ID NO:36 (1551) AAGTCTGGGTTTAAATGTTACCTGATGTACAAACAAAGGCCACAACAAA
SEQ ID NO:5 (1551) CAGCCTGGTGGCTGGCCTGCTACCTGATGTACAAGCAGAGAGGCCAGCAGA

1601                                     1650
SEQ ID NO:1 (1601) AAACCCCTGCTGTGGCTGGGCAACAACACCCTGGACCAGATGAGAGGCCACC
SEQ ID NO:3 (1601) AGACCTTGCTATGGCTTGGGAATAATACCCTCGATCAGATGAGAGGCCACT
SEQ ID NO:32 (1601) AGACCTTGTTATGGCTTGGGAATAATACCCTTGATCAGATGAGAGGCCACT
SEQ ID NO:34 (1601) AGACCTTGTTATGGCTTGGGAATAATACCCTTGATCAGATGAGAGGCCACT
SEQ ID NO:36 (1601) AGACCTTGCTATGGCTTGGGAATAATACCCTCGATCAGATGAGAGGCCACT
SEQ ID NO:5 (1601) AAACCCCTGCTGTGGCTGGGCAATAACACCCTGGACCAGATGAGAGGCCACC

1651                                     1665
SEQ ID NO:1 (1651) ACCAGAGCCTGATGA
SEQ ID NO:3 (1651) ACAAGAGCATGA---
SEQ ID NO:32 (1651) ACAAAAATATGA---
SEQ ID NO:34 (1651) ACAAAAATATGA---
SEQ ID NO:36 (1651) ACAAGAGCATGA---
SEQ ID NO:5 (1651) ACCAGAACCTGATGA

```

	SEQ ID NO:1	SEQ ID NO:3	SEQ ID NO:32	SEQ ID NO:34	SEQ ID NO:36	SEQ ID NO:5
SEQ ID NO:1		72%	71%	71%	71%	92%
SEQ ID NO:3			88%	89%	98%	69%
SEQ ID NO:32				99%	88%	70%
SEQ ID NO:34					88%	71%
SEQ ID NO:36						69%
SEQ ID NO:5						

Figure 13 (continued)

## Protein sequence alignment of IBDV VP2

		1	50
SEQ ID NO: 8	(1)	MTNLQDQTQQIVPFIRSLLMPTTGPASIPDDTLEKHTLRSETSTYNLTVG	
SEQ ID NO: 42	(1)	MTNLQDQTQQIVPFIRSLLMPTTGPASIPDDTLEKHTLRSETSTYNLTVG	
		51	100
SEQ ID NO: 8	(51)	DTGSGLVFFPGFPGSIVGAHYTLQSNNGYKFDQMLLTAQNLPASYNLYCR	
SEQ ID NO: 42	(51)	DTGSGLVFFPGFPGSIVGAHYTLQSNNGYKFDQMLLTAQNLPASYNLYCR	
		101	150
SEQ ID NO: 8	(101)	LVSRSALTVRSSSTLPGGVYALNGTINAVTFQGSLSSELTDVSYNGLMSATAN	
SEQ ID NO: 42	(101)	LVSRSALTVRSSSTLPGGVYALNGTINAVTFQGSLSSELTDVSYNGLMSATAN	
		151	200
SEQ ID NO: 8	(151)	INDKIGNVLVGEVTVLSLPTSYDLGYVRLGDFIPAIGLDPKMWATCDSS	
SEQ ID NO: 42	(151)	INDKIGNVLVGEVTVLSLPTSYDLGYVRLGDFIPAIGLDPKMWATCDSS	
		201	250
SEQ ID NO: 8	(201)	DRPRVYTITAADDYQFSSQYQPGGVTTITLFSANIDAITSLSTGGELVFQT	
SEQ ID NO: 42	(201)	DRPRVYTITAADNYQFSSQYQTGGVTTITLFSANIDAITSLSTGGELVFKT	
		251	300
SEQ ID NO: 8	(251)	SVQGLVLGATIYLLIGFDGTAVITRAVAADNGLTAGTDNLMPFNLVIPTNE	
SEQ ID NO: 42	(251)	SVQSLVLGATIYLLIGFDGTAVITRAVAANNGLTAGIDNLMPFNLVIPTNE	
		301	350
SEQ ID NO: 8	(301)	ITQPITSIKLEIVTSKSGGQAGDQMSWSASGSLAVTIHGGNYPGALRPVT	
SEQ ID NO: 42	(301)	ITQPITSIKLEIVTSKSDGQAGEQMSWSASGSLAVTIHGGNYPGALRPVT	
		351	400
SEQ ID NO: 8	(351)	LVAYERVATGSVVTVAGVSNFELIPNPELAKNLVTEYGRFDPGAMNYTKL	
SEQ ID NO: 42	(351)	LVAYERVATGSVVTVAGVSNFELIPNPELAKNLVTEYGRFDPGAMNYTKL	
		401	450
SEQ ID NO: 8	(401)	ILSERDRLGIKTVWPTREYTDFREYFMEVADLNSPLKIAGAFGFKDIIRA	
SEQ ID NO: 42	(401)	ILSERDRLGIKTVWPTREYTDFREYFMEVADLNSPLKIAGAFGFKDIIRA	
		451	
SEQ ID NO: 8	(451)	IRR-	
SEQ ID NO: 42	(451)	IRR-	

SEQ ID NO:8 is 98% identical to SEQ ID NO:42

Figure 13 (continued)

## DNA sequence alignment of IBDV VP2 gene

		1	50
SEQ ID NO: 7	(1)	ATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTTCATACGGAG	
SEQ ID NO: 41	(1)	ATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTTCATACGGAG	
		51	100
SEQ ID NO: 7	(51)	CCTTCTGATGCCAACAACCGGACCGGCGTCCATTCCGGACGACACCCTGG	
SEQ ID NO: 41	(51)	CCTTCTGATGCCAACAACCGGACCGGCGTCCATTCCGGACGACACCCTGG	
		101	150
SEQ ID NO: 7	(101)	AGAAGCACACTCTCAGGTCAGAGACCTCGACCTACAATTTGACTGTGGGG	
SEQ ID NO: 41	(101)	AGAAGCACACTCTCAGGTCAGAGACCTCGACCTACAATTTGACTGTGGGG	
		151	200
SEQ ID NO: 7	(151)	GACACAGGGTCAGGGCTAATTGTCTTTTTCCCTGGATTCCCTGGCTCAAT	
SEQ ID NO: 41	(151)	GACACAGGGTCAGGGCTAATTGTCTTTTTCCCTGGATTCCCTGGCTCAAT	
		201	250
SEQ ID NO: 7	(201)	TGTGGGTGCTCACTACACACTGCAGAGCAATGGGAAC TACAAGTTTCGATC	
SEQ ID NO: 41	(201)	TGTGGGTGCTCACTACACACTGCAGAGCAATGGGAAC TACAAGTTTCGATC	
		251	300
SEQ ID NO: 7	(251)	AGATGCTCCTGACTGCCCAGAACCTACCGGCCAGCTACAAC TACTGCAGAG	
SEQ ID NO: 41	(251)	AGATGCTCCTGACTGCCCAGAACCTACCGGCCAGCTACAAC TACTGCAGAG	
		301	350
SEQ ID NO: 7	(301)	CTAGTGAGTCGGAGTCTCACAGTGAAGGTCAAGCACACTCCCTGGTGGCGT	
SEQ ID NO: 41	(301)	CTAGTGAGTCGGAGTCTCACAGTAAGGTCAAGCACACTCCCTGGTGGCGT	
		351	400
SEQ ID NO: 7	(351)	TTATGCACTAAACGGCACCATAAACGCCGTGACCTTCCAAGGAAGCCTGA	
SEQ ID NO: 41	(351)	TTATGCACTAAACGGCACCATAAACGCCGTGACCTTCCAAGGAAGCCTGA	
		401	450
SEQ ID NO: 7	(401)	GTGAACTGACAGATGTTAGCTACAATGGGTTGATGTCTGCAACAGCCAAC	
SEQ ID NO: 41	(401)	GTGAACTGACAGATGTTAGCTACAACGGGTTGATGTCTGCAACAGCCAAC	
		451	500
SEQ ID NO: 7	(451)	ATCAACGACAAAATTGGGAATGTCCTGGTAGGGGAAGGGGTCACTGTCTCT	
SEQ ID NO: 41	(451)	ATCAACGACAAAATTGGGAACGTCCTAGTAGGGGAAGGGGTAAACGTCCT	
		501	550
SEQ ID NO: 7	(501)	CAGCCTACCCACATCATATGATCTTGGGTATGTGAGGCTTGGTGACCCCA	
SEQ ID NO: 41	(501)	CAGCTTACCCACATCATATGATCTTGGGTATGTGAGGCTTGGTGACCCCA	
		551	600
SEQ ID NO: 7	(551)	TTCCCGCTATAGGGCTTGACCCAAAAATGGTAGCTACATGCGACAGCAGT	
SEQ ID NO: 41	(551)	TACCCGCTATAGGGCTTGACCCAAAAATGGTAGCAACATGTGACAGCAGT	

Figure 13 (continued)

		601		650
SEQ ID NO: 7	(601)	GACAGGCCCCAGAGTCTACACCATAACTGCAGCCGATGATTACCAATTCTC		
SEQ ID NO: 41	(601)	GACAGGCCCCAGAGTCTACACCATAACTGCAGCCGAT <b>A</b> ATTACCAATTCTC		
		651		700
SEQ ID NO: 7	(651)	ATCACAGTACCAAC <b>C</b> AGGTGGGGTAACAATCACACTGTTCTCAGCCAACA		
SEQ ID NO: 41	(651)	ATCACAGTACCAAC <b>A</b> CAGGTGGGGTAACAATCACACTGTTCTCAGCCAACA		
		701		750
SEQ ID NO: 7	(701)	TTGATGCTATCACAAG <b>C</b> CTCAGCATTGGGGGAGAGCTCGTGTT <b>T</b> CAAAACA		
SEQ ID NO: 41	(701)	TTGATGCCATCACAAG <b>T</b> CTCAGC <b>G</b> TTGGGGGAGAGCTCGTGTT <b>C</b> AAACA		
		751		800
SEQ ID NO: 7	(751)	AGCGTCCAAG <b>G</b> CCCTTGTA <b>C</b> TGGGCGCCACCATCTACCTTATAGGCTTTGA		
SEQ ID NO: 41	(751)	AGCGTCCAAG <b>A</b> GCCTTGTA <b>C</b> TGGGCGCCACCATCTACCTTATAGGCTTTGA		
		801		850
SEQ ID NO: 7	(801)	TGGGACTGCGGTAATCACCAGAGCTGT <b>A</b> GCCGCA <b>G</b> ATAATGGGCTGACGG		
SEQ ID NO: 41	(801)	TGGGACTGCGGTAATCACCAGAGCTGT <b>G</b> SCCGCA <b>A</b> CAATGGGCTGACGG		
		851		900
SEQ ID NO: 7	(851)	CCGGCACC <b>G</b> ACAATCTTATGCCATTCAATCTTGT <b>C</b> ATTCCAACCAATGAG		
SEQ ID NO: 41	(851)	CCGGCATC <b>G</b> ACAATCTTATGCCATTCAATCTTGT <b>G</b> ATTCCAACCAATGAG		
		901		950
SEQ ID NO: 7	(901)	ATAACCCAGCCAATCACATCCATCAAACTGGAGATAGTGACCTCCAAAAG		
SEQ ID NO: 41	(901)	ATAACCCAGCCAATCACATCCATCAAACTGGAGATAGTGACCTCCAAAAG		
		951		1000
SEQ ID NO: 7	(951)	TGGTGGTCAGGCAGGGGAT <b>C</b> AGATGTCATGGTCGGCAAGTGGGAGCCTAG		
SEQ ID NO: 41	(951)	TG <b>A</b> TGGTCAGGCAGGGGA <b>A</b> CAGATGTCATGGTCGGCAAGTGGGAGCCTAG		
		1001		1050
SEQ ID NO: 7	(1001)	CAGTGACGATCCATGGTGGCAACTATCCAGG <b>G</b> SCCCTCCGTCCCGTCACA		
SEQ ID NO: 41	(1001)	CAGTGACGATCCATGGTGGCAACTATCCAGG <b>A</b> GCCCTCCGTCCCGTCACA		
		1051		1100
SEQ ID NO: 7	(1051)	CTAGT <b>A</b> GCCTACGAAAGAGTGGCAACAGGAT <b>C</b> GTCTGTTACGGTCGCTGG		
SEQ ID NO: 41	(1051)	CTAGT <b>G</b> CCCTACGAAAGAGTGGCAACAGGAT <b>T</b> GTCGTTACGGTCGCTGG		
		1101		1150
SEQ ID NO: 7	(1101)	GGTGAGTAACTTCGAGCTGAT <b>T</b> CCAAATCCTGAAGTAGCAAAGAACCTGG		
SEQ ID NO: 41	(1101)	GGTGAGCAACTTCGAGCTGAT <b>C</b> CCAAATCCTGAAGTAGCAAAGAACCTGG		
		1151		1200
SEQ ID NO: 7	(1151)	TTACAGAATACGGCCGATTTGACCCAGGAGCCATGAAGTACAC <b>A</b> AAATTG		
SEQ ID NO: 41	(1151)	TTACAGAATATGGCCGATTTGACCCAGGAGCCATGAAGTACAC <b>G</b> AAATTG		
		1201		1250
SEQ ID NO: 7	(1201)	ATACTGAGTGAGAGGGACCG <b>T</b> CTTGGCATCAAGACCGTCTGGCCAACAAG		
SEQ ID NO: 41	(1201)	ATACTGAGTGAGAGGGACCG <b>C</b> CTTGGCATCAAGACCGTCTGGCCAACAAG		



Figure 13 (continued)

		1251		1300
SEQ ID NO:7	(1251)	GGAGTACACTGATTTTCGTGAGTACTTCATGGAGGTGGCCGACCTCAACT		
SEQ ID NO:41	(1251)	GGAGTACACTGACTTTTCGTGAGTACTTCATGGAGGTGGCCGACCTCAACT		
		1301		1350
SEQ ID NO:7	(1301)	CTCCCCTGAAGATTGCAGGAGCATTTGGCTTCAAAGACATAATCCGGGCT		
SEQ ID NO:41	(1301)	CTCCCCTGAAGATTGCAGGAGCATTTGGCTTCAAAGACATAATCCGGGCC		
		1351	1362	
SEQ ID NO:7	(1351)	ATAAGGAGGTAA		
SEQ ID NO:41	(1351)	ATAAGGAGGTGA		

SEQ ID NO:7 is 97% identical to SEQ ID NO:41

Figure 14

## DNA and protein sequences

**NDV-F codon optimized DNA (SEQ ID NO:1)**

atgggcagcaagcccagcacaagaatcccagccccctgatgctgatcaccgcgcatcat  
gctgatecctgggctgcatcagaccacaagctccctggatggacgccccctggcgcgtg  
ccggcatcgtgggtgaccggcgacaaggccgtgaacgtgtacaccagcagccagaccggc  
agcatcatcgtgaagctgctgcccacatgccagagacaaagaggcctgcgccaaggc  
ccccctggaagcctacaacagaaccctgaccaccctgctgacccccctgggcgacagca  
tcagaaagatccagggtccgtgagcacaagcggcgaggaaagcaggggcagactgac  
ggcgccgtgatcggcagcgtggccctgggagtggttacagctgccagattaccgctgc  
agccgcccctgatccaggccaaccagaacgcgcgccaacatcctgagactgaaagagagca  
ttgccgccaccaacgaggccgtgcacgaagtgaccgacggcctgagccagctgtccgtg  
gccgtgggcaagatgcagcagttcgtgaacgaccagttcaacaacaccgccagagagct  
ggactgcatcaagatcaccagcaggtgggctggagctgaacctgtacctgaccgagc  
tgaccacagtgttcggccccccagatcacaagcccagccctgacacagctgaccatccag  
gccctgtacaacctggctggcggaacatggactatctgctgacaaagctgggaatcgg  
caacaaccagctgtccagcctgatcggaagcggcctgatcaccggctacccccatcctgt  
acgacagccagacacagctgctgggcatccaggtgaacctgccagcgtgggcaacctg  
aacaacatgcgcgccacctacctggaaacctgagcgtgtccaccaccaagggtacgc  
cagcgccctgggtgcccaaggtggtgacacaggtgggcagcgtgatcgaggaactggaca  
ccagctactgcatcgagagcgacctggacctgtactgcaccagaatcgtgaccttccca  
atgagccccggcatctacagctgcctgagcggcaacaccagcgcctgcatgtacagcaa  
gaccgaaggcgactgacaacacctacatggccctgaagggaagcgtgatcgccaact  
gcaagatcaccacctgcagatgcaccgacccccagggcatcatcagccagaactacggc  
gaggccgtgagcctgatcgatcgccattcctgtaacgtgctgtccctggacggcatcac  
actgagactgagcggcgagttcgatgccacctaccagaagaacatcagcatcctggaca  
gccaggtgatcgtgaccggcaacctggacatcagcaccgagctgggcaacgtgaataac  
agcatcagcaacgccctggacagactggccgagagcaacagcaagctggaaaaagtga  
cgtgcgccctgacatccacttccgctctgatcacctacatcgtgctgaccgtgatcagcc  
tggtgttcggcgccctgagcctggtgctggcctgctacctgatgtacaagcagaaggcc  
cagcagaaaacctgctgtggctgggcaacaacacctggaccagatgagagccaccac  
cagagcctgatga

**NDV-F protein (SEQ ID NO:2)**

MGSKPSTRIPAPLMLITRIMLILGCIRPTSSLDGRPLAAAGIVVTGDKAVNVYTSSQTGSIIVKL  
LPNMPRDKEACAKAPLEAYNRTLTTLLTPLGDSIRKIQGSVSTSGGGKQGRLLIGAVIGSVALGVA  
TAAQITAAAALIQANQNAANILRLKESIAATNEAVHEVTDGLSQLSVAVGKMQQFVNDQFNNTAR  
ELDCIKITQQVGVELNLYLTELTTFVGPQITSPALTQLTIQALYNLAGGNMDYLLTKLGIGNNQL  
SSLIGSGLITGYPILYDSQTQLLGIQVNLPSVGNLNNMRATYLETSLVSTTKGYASALVPKVVTQ  
VGSVIEELDTSYCIESDLDYCTRIVTFPMSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSV  
IANCKITTCRCTDPPGIISQNYGEAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISILDSQV  
IVTGNLDISTELGNVNNSISNALDRLAESNSKLEKVNVRILTSTALITYIVLTVISLVFGALS LV  
LACYLMYKQKAQQKTLLWLGNNTLDQMRATTRA\*

Figure 14 (Continued)

**DNA sequence of NDV-F VIId wildtype (SEQ ID NO:3)**

atgggctccaaaccttctaccaggatcccagcacctctgatgctgatcaccggtattat  
gctgatattgggctgtatccgtccgacaagctctcttgacggcaggcctcttgacagctg  
caggaattgtagtaacaggagataaggcagtcattgtatacacttcgtctcagacaggg  
tcaatcatagtcaagttgctcccgaaatattgccagggataaggaggcgtgtgcaaaagc  
cccattagaggcatataacagaacactgactactttgctcactcctcttggcgactcca  
tccgcaagatccaagggtctgtgtccacatctggaggaggcaagcaaggccgcctgata  
gggtgctgttattggcagtgtagctcttgggggttgcaacagcggcacagataacagcagc  
tgcgccctaataacaagccaaccagaatgccgccaacatcctccggcttaaggagagca  
ttgctgcaaccaatgaagctgtgcatgaagtcaccgacggattatcacactatcagtg  
gcagttgggaagatgcagcagtttgtcaatgaccagtttaataatacggcgcgagaatt  
ggactgtataaaaaatcacacaacagggttggtgtagaactcaacctatacctaactgaat  
tgactacagtattcggggccacagatcacctccctgcatttaactcagctgacctccag  
gcactttataatttagctggtggcaatatggattacttattaactaagttaggtatagg  
gaacaatcaactcagctcgtaattggtagcggcctgatcactgggtaccctatactgt  
atgactcacagactcaactcttgggcatacaagtgaatttaccctcagtcgggaactta  
aataatatgctgcccacctaatttggagaccttatctgtaagtacaaccaaggatagc  
ctcagcacttgctcccgaaagtagtgacacaagtcgggtccgtgatagaagagcttgaca  
cctcatactgtatagagtcggatctggatttatattgtactagaatagtgacattcccc  
atgtccccaggtattttattcctgtttgagcggcaacacatcagcttgcatgtattcaaa  
gactgaaggcgcaactcactacgccgtatatggcccttaaaggctcagttattgccaat  
gtaaaataacaacatgtagatgtacagaccctcctgggtatcatatcgcaaaattatgga  
gaagctgtatccctgatagatagacattcgtgcaatgtcttatcattagacgggataac  
tctaaggctcagtggggaatttgatgcaacttatcaaaagaacatctcaactagatt  
ctcaagtcacgtgacaggcaatcttgatataatcaactgaacttggaacgtcaacaat  
tcaatcagcaatgccttgataggttggcagaaagcaacagcaagctagaaaaagtcaa  
tgtcagactaaccagcacatctgctctcattacctaattgttctaactgtcattttctc  
tagttttcgggtgcacttagtctggtgttagcgtgttacctgatgtacaaacagaaggca  
caacaaaagaccttgctatggcctgggaataataccctcgatcagatgagagccactac  
aagagcatga

**Amino Acid sequence of NDV-F VIId wildtype (SEQ ID NO:4)**

1	MGSKPSTRIP	APLMLITRIM	LILGCIRPTS	SLDGRPLAAA	GIVVTGDKAV
51	NVYTSSQTGS	IIVKLLPNMP	RDKEACAKAP	LEAYNRTLTT	LLTPLGDSIR
101	KIQGSVSTSG	GGKQGRLLIGA	VIGSVALGVA	TAAQITAAAA	LIQANQNAAN
151	ILRLKESIAA	TNEAVHEVTD	GLSQLSVAVG	KMQQFVNDQF	NNTARELDCI
201	KITQQVGVEL	NLYLTELTTV	FGPQITSPAL	TQLTIQALYN	LAGGNMDYLL
251	TKLGIGNNQL	SSLIGSGLIT	GYPILYDSQT	QLLGIQVNLP	SVGNLNNMRA
301	TYLETLSVST	TKGYASALVP	KVVTQVGSVI	EELDTSYCIE	SDLDLYCTRI
351	VTFPMSPGIY	SCLSGNTSAC	MYSKTEGALT	TPYMALKGSV	IANCKITTCR
401	CTDPPGIISQ	NYGEAVSLID	RHSCNVLSLD	GITLRLSGEF	DATYQKNISI
451	LDSQVIVTGN	LDISTELGNV	NNSISNALDR	LAESNSKLEK	VNVRLTSTSA
501	LITYIVLTVI	SLVFGALSLV	LACYLMYKQK	AQQKTLLWLW	NNTLDQMRAT
551	TRA*				

Figure 14 (Continued)

**DNA sequence of NDV-F-CAO2-CSmut (SEQ ID NO:5) for HVT116**

atgggcagcaagcccagcacctggatcagcgtgacctgatgctgatcaccagaaccat  
gctgacacctgagctgcatctgccccacaagcagcctggacggcagacccctggccgctg  
ccggcatcgtggtgaccggcgacaaggccgtgaacatctacaccagcagccagaccggc  
agcatcatcatcaagctgctgccccaacatgccccaggacaaagaggcctgcgccaaggc  
ccccctggaagcctacaacagaaccctgaccaccctgctgacccccctgggcgacagca  
tcagaagaatccagggcagcgcaccacacaagcggcgaggaaagcagggcagactggtg  
ggcgctatcatcgggagcgtggccctgggcgtggccacagctgccagattaccgctgc  
agccgccctgattcaggccaatcagaacgcgcgaacatcctgagactgaaagagagca  
ttgccgccaccaacgacgccgtgcacgaagtgacaaacggactgtcccagctggctgtc  
gctgtcggcaagatgcagcagttcgtgaacaaccagttcaacaacaccgccagagagct  
ggactgcatcaagatcgcccagcaggtgggcgtggagctgaacctgtacctgaccgagc  
tgaccacagtgttcggccccccagatcacaagccccgctctgaccacgtgacaatccag  
gccctgtacaacctggctggcggaacatggactatctgctgactaagctgggagtggtg  
caacaaccagctgtccagcctgatcgggtccgggctgatcacaggcaacccccatcctgt  
acgacagccagacacagctgctgggcattccagatcaacctgccatccgtgggaagcctg  
aacaacatgagagccacctacctggaaacctgagcgtgtccaccaccaagggttcgc  
cagcgcacctgggtgcccaagggtggtgacacaggtgggcagcgtgatcgaggaactggaca  
ccagctactgcatcgagagcgacatcgacctgtactgcaccagagtgggtgaccttccca  
atgagccccggcatctacagctgcctgagcggcaacaccagcgcctgcatgtacagcaa  
gaccgaaggagcactgacaacacctacatggccctgaagggaagcgtgatcgccaact  
gcaagatgaccacctgcagatgcgcgcgacccccccaggcatcatcagccagaactacggc  
gaggccgtgagcctgatcgacaaacattcctgtagcgtgctgtccctggatggcatcac  
actgagactgagcggcgagttcgacgccacctaccagaagaacatcagcatcctggaca  
gccaggtgatcgtgaccggcaacctggacatcagcaccgagctgggcaacgtgaacaac  
agcatcagcagcaccttgacaagctggccgagtccaacaacaagctgaacaaagtga  
cgtgaacctgaccagcacaagcgcacctgatcacctacatcgtgctggccatcgtgtccc  
tggccttcggcgctgatcagcctggtgctggcctgctacctgatgtacaagcagagagcc  
cagcagaaaacctgctgtggctgggcaataaacacctggaccagatgagggccaccac  
cagaacctgatga

**Amino Acid sequence of NDV-F-CAO2-CSmut (SEQ ID NO:6) for HVT116**

1	MGSKPSTWIS	VTLMILITRM	LILSCICPTS	SLDGRPLAAA	GIVVTGDKAV
51	NIYTSSQTGS	IIIKLLPNMP	KDKEACAKAP	LEAYNRTLTT	LLTPLGDSIR
101	RIQGSATTSG	GGKQGRVLGA	IIGSVALGVA	TAAQITAAAA	LIQANQNAAN
151	ILRLKESIAA	TNDVHEVTN	GLSQLAVAVG	KMQQFVNNQF	NNTARELDCI
201	KIAQQVGVEL	NLYLTELTIV	FGPQITSPAL	TQLTIQALYN	LAGGNMDYLL
251	TKLGVGNNQL	SSLIGSGLIT	GNPILYDSQT	QLLGIQINLP	SVGSLNNMRA
301	TYLETLSVST	TKGFASALVP	KVVTQVGSVI	EELDTSYCIE	SDIDLCTRV
351	VTFFMSPGIY	SCLSGNTSAC	MYSKTEGALT	TPYMALKGSV	IANCKMTTCR
401	CADPPGIISQ	NYGEAVSLID	KHSCSVLSLD	GITLRLSGEF	DATYQKNISI
451	LDSQVIVTGN	LDISTELGNV	NNSISSTLDK	LAESNNKLNK	VNVNLTSTSA
501	LITYIVLAIV	SLAFGVISLV	LACYLMYKQR	AQOKTLLWLW	NNTLDQMRAT
551	TRT*				

Figure 14 (Continued)

**DNA coding for IBDV VP2 protein (SEQ ID NO:7)**

ATGACAAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTTCATACGGAGCCTTCTGAT  
GCCAACAAACCGGACCGGGCGTCCATTCCGGACGACACCCTGGAGAAGCACACTCTCAGGT  
CAGAGACCTCGACCTACAATTTGACTGTGGGGGACACAGGGTCAGGGCTAATTGTCTTT  
TTCCCTGGATTCCCTGGCTCAATTGTGGGTGCTCACTACACACTGCAGAGCAATGGGAA  
CTACAAGTTCGATCAGATGCTCCTGACTGCCCAGAACCTACCGGCCAGCTACAACACT  
GCAGACTAGTGAGTCGGAGTCTCACAGTGAGGTCAAGCACACTCCCTGGTGGCGTTTAT  
GCACTAAACGGCACCATAAACGCCGTGACCTTCCAAGGAAGCCTGAGTGAACCTGACAGA  
TGTTAGCTACAATGGGTTGATGTCTGCAACAGCCAACATCAACGACAAAATTGGGAATG  
TCCTGGTAGGGGAAGGGGTCAGTGTCTCAGCCTACCCACATCATATGATCTTGGGTAT  
GTGAGGCTTGGTGACCCCATTTCCCGCTATAGGGCTTGACCCAAAAATGGTAGCTACATG  
CGACAGCAGTGACAGGCCAGAGTCTACACCATAACTGCAGCCGATGATTACCAATTCT  
CATCACAGTACCAACCAGGTGGGGTAACAATCACACTGTTCTCAGCCAACATTGATGCT  
ATCACAAGCCTCAGCATTGGGGGAGAGCTCGTGTTCAAACAAGCGTCCAAGGCCTTGT  
ACTGGGCGCCACCATCTACCTTATAGGCTTTGATGGGACTGCGGTAATCACCAGAGCTG  
TAGCCGCAGATAATGGGCTGACGGCCGGCACCGACAATCTTATGCCATTCAATCTTGTC  
ATTCCAACCAATGAGATAACCCAGCCAATCACATCCATCAAACCTGGAGATAGTGACCTC  
CAAAAGTGGTGGTCAGGCAGGGGATCAGATGTCATGGTCGGCAAGTGGGAGCCTAGCAG  
TGACGATCCATGGTGGCAACTATCCAGGGGGCCCTCCGTCCCGTCACACTAGTAGCCTAC  
GAAAGAGTGGCAACAGGATCCGTGCTTACGGTCGCTGGGGTGAGTAACTTCGAGCTGAT  
TCCAATCCTGAACTAGCAAAGAACCTGGTTACAGAATACGGCCGATTTGACCCAGGAG  
CCATGAACTACACAAAATTGATACTGAGTGAGAGGGACCGTCTTGGCATCAAGACCGTC  
TGGCCAACAAGGGAGTACACTGATTTTCGTGAGTACTTCATGGAGGTGGCCGACCTCAA  
CTCTCCCCTGAAGATTGCAGGAGCATTTGGCTTCAAAGACATAATCCGGGCTATAAGGA  
GGTAA

**IBDV VP2 protein (SEQ ID NO:8)**

MTNLQDQTQQIVPFIRSLMPTTGPASIPDDTLEKHTLRSETSTYNLTVGDTGSGLIVF  
FPGFPGSIVGAHYTLQSNGNYKFDQMLLTAQNLPASYNLCRLVSRSLTVRSSTLPGGVY  
ALNGTINAVTFQGSLSSELTDVSYNGLMSATANINDKIGNVLVGEVTVLSLPTSIDLGY  
VRLGDPIPAIGLDPKMOVATCDSSDRPRVYTTITAADDYQFSSQYQPGGVTITLFSANIDA  
ITSLSIGGELVFQTSVQGLVLGATIYLIIGFDGTAVITRAVAADNGLTAGTDNLMPFNLV  
IPTNEITQPITSIKLEIVTSKSGGQAGDQMSWSASGSLAVTIHGGNYPGALRPVTLVAY  
ERVATGSVVTVAGVSNFELIPNPELAKNLVTEYGRFDPGAMNYTKLILSERDRLGIKTV  
WPTREYTDREYFMEVADLNSPLKIAGAFGFKDIIRAIR

**Sv40 Promoter (SEQ ID NO:9)**

gaattcgagctcggtacagcttggtgtggaatgtgtgtcagttaggggtgtggaaagtc  
cccaggctccccagcaggcagaagtatgcaaagcatgcatctcaattagtcagcaacca  
gggtgtggaaagtcccccaggctccccagcaggcagaagtatgcaaagcatgcatctcaat  
tagtcagcaaccatagtcctcgccccctaactccgccccatccccgccccctaactccgccccag  
ttccgccccattctccgccccatggctgactaattttttttatattatgcagaggccgagg  
ccgcctcggcctctgagctattccagaagtagtgaggaggcttttttggaggcctaggc  
ttttgcaaaaagct

Figure 14 (Continued)

**CMV-IE promoter (SEQ ID NO:10)**

aactccgcccgttttatgactagaaccaatagtttttaatgccaaatgcactgaaatcc  
cctaatttgcaaagccaaacgccccctatgtgagtaatacggggactttttacccaatt  
tcccaagcggaaagccccctaatacactcatatggcatatgaatcagcacgggtcatgca  
ctctaattggcggcccatagggactttccacatagggggcggttcaccatttcccagcata  
gggggtggtgactcaatggcctttacccaagtacattgggtcaatgggaggtaagccaat  
gggtttttccattactggcaagcacactgagtc aaatgggactttccactgggttttg  
cccaagtacattgggtcaatgggagggtgagccaatgggaaaaaccattgctgccaaagt  
acactgactcaatagggactttccaatgggtttttccattggttggaagcatataaggt  
caatgtgggtgagtc aatagggactttccattgtattctgccagtacataaggtcaat  
aggggggtgaatcaacaggaaagtcccatgggagccaagtacactgcgtcaatagggact  
ttccattgggttttgcccagtacataaggtcaataggggatgagtc aatgggaaaaacc  
cattggagccaagtacactgactcaatagggactttccattgggttttgcccagtacat  
aaggtcaataggggggtgagtc aacaggaaagtcccatgggagccaagtacattgagtc a  
atagggactttccaatgggttttgcccagtacataaggtcaatgggaggtaagccaatg  
ggtttttccattactggcacgtatactgagtc attagggactttccaatgggttttgcc  
ccagtacataaggtcaataggggtgaatcaacaggaaagtcccatgggagccaagtaca  
ctgagtc aatagggactttccattgggttttgcccagtacaaaaggtcaataggggggtg  
agtc aatgggtttttccattattggcacgtacataaggtcaataggggtgagtc attg  
ggtttttccagccaatttaattaaaacgccatgtactttcccaccattgacgtcaatgg  
gctattgaaactaatgcaacgtgaccttaaacgggtactttcccatagctgattaatgg  
gaaagtaccgttctcgagccaatacacgtcaatgggaagtgaaggggcagccaaaacgt  
aacaccgccccgggttttccctggaaattccatattggcacgcattctattgggtgagc  
tgcgttctacgtgggtataagaggcgcgaccagcgtcgggtaccgtcgcagtccttcggtc  
tgaccaccgtagaacgcagagctcctcgctgcag

**SV40 polyA signal (SEQ ID NO:11)**

Ggggatccagcatgataagatacattgatgagtttgacaaaccacaactagaatgca  
gtgaaaaaaaaatgctttatttgtgaaatttgtgatgctattgctttatttgtaaccatta  
taagctgcaataaacaagttaacaacaacaattgcattgattttatgttttcagggttcag  
ggggagggtgtgggagggttttttcggatcctctagagtcgac

**Synthetic polyA signal (SEQ ID NO:12)**

aataaaatatctttattttcattacatctgtgtgttggtttttttgtgtgaatcgatagt  
actaacatacgtctctccatcaaaaacaaaacgaaacaaaacaaactagcaaaataggctg  
tccccagtgcaagtgcagggtgccagaacatttctctt

Figure 14 (Continued)

**The nucleotide sequence of the cloned NDV Texas F gene (wild type non-modified) (SEQ ID NO:32)**

ATGGGCTCCAGATCTTCTACCAGGATCCCGGTACCTCTAATGCTGATCATCCGAACCGC  
GCTGACACTGAGCTGTATCCGTCTGACAAGCTCTCTTGATGGCAGGCCTCTTGCGGCTG  
CAGGGATCGTGGTAACAGGAGATAAAGCAGTCAACATATACACCTCATCCCAGACAGGG  
TCAATCATAGTTAAGTTACTCCCGAATATGCCCAAGGACAAAGAGGTGTGTGCAAAAGC  
CCCATTGGAGGCATACAACAGGACACTGACTACTTTACTCACCCCCCTTGGTGATTCTA  
TCCGCAGGATACAAGAGTCTGTGACTACTTCCGGAGGAAGGAGACAGAGACGCTTTATA  
GGTGCCATTATCGGCAGTGTAGCTCTTGGGGTTGCGACAGCTGCACAGATAACAGCAGC  
TTCGGCCCTGATACAAGCCAACCAGAATGCTGCCAACATCCTCCGGCTTAAAGAGAGCA  
TTGCTGCAACCAATGAAGCTGTGCACGAGGTCAGTGACGGATTATCACAACCTAGCAGTG  
GCAGTAGGGAAGATGCAACAGTTTGTCAATGACCAGTTCAATAATACAGCGCAAGAATT  
GGACTGTATAAAAAATTGCACAGCAGGTCGGTGTAGAACTCAACTTGTACCTAACTGAAT  
TGACTACAGTATTTGGGCCACAAATCACTTCCCCTGCCTTAACTCAGCTGACTATCCAA  
GCGCTTTACAATCTAGCTGGTGGTAATATGGATTACTTGCTGACTAAGTTAGGTGTAGG  
GAACAACCAACTCAGCTCATTAATTGGTAGCGGCTTGATCACCGGCAACCCTATTCTGT  
ACGACTCACAGACTCAGATCTTGGGTATACAGGTAACTTTGCCTTCAGTTGGGAACCTG  
AATAATATGCGTGCCACCTACCTGGAGACCTTATCTGTAAGCACAAACCAAGGGATTTGC  
CTCAGCACTTGTCCCAAAAGTGGTGACACAGGTCGGTTCCGTGATAGAAGAACTTGACA  
CCTCATACTGTATAGGGACCGACTTGGATTTATACTGTACAAGAATAGTGACATTCCCT  
ATGTCTCCTGGTATTTATTCTTGTCTGAGCGGTAATACATCGGCTTGCATGTATTCAA  
GACTGAAGGCGCACTTACTACGCCATATATGGCTCTCAAAGGCTCAGTTATTGCCAATT  
GCAAGCTGACAACATGTAGATGTGCAGATCCCCCAGGTATCATATCGCAAAATTATGGA  
GAAGCTGTGTCCTTAATAGATAGGCACTCATGCAACGCTTATCCTTAGACGGGATAAC  
TCTGAGGCTCAGTGGGGAATTTGATGCAACCTATCAAAAGAATATCTCTATACTAGATT  
CTCAAGTTATAGTGACAGGCAATCTTGATATATCAACTGAGCTTGGGAATGTCAACAAC  
TCAATAAGTAATGCCCTGAATAAGTTAGAGGAAAGCAACAGCAAACCTAGACAAAGTCAA  
TGTCAAACTGACCAGCACATCTGCTCTCATTACCTACATCGTTTTAACTGTCATATCTC  
TTGTTTTTGGTGTACTTAGCCTGGTTCTAGCATGCTACCTGATGTACAAGCAAAAGGCA  
CAACAAAAGACCTTGTTATGGCTTGGGAATAATACCCTTGATCAGATGAGAGCCACTAC  
AAAAATATGA

**The amino acid sequence of the cloned NDV Texas F gene (wild type non-modified; cleavage site underlined) (SEQ ID NO:33)**

MGSRSSSTRIPVPLMLIIRTALTLSCIRLTSSLDGRPLAAAGIVVTGDKAVNIYTSSQTG  
SIIIVKLLPNMPKDKEVCAKAPLEAYNRTLTTLLTPLGDSIRRIQESVTTSGRRQRRFI  
GAIIGSVALGVATAAQITAASALIQANQNAANILRLKESIAATNEAVHEVTDGLSQLAV  
AVGKMQQFVNDQFNNTAQELDCIKIAQQVGVELNLYLTELTTFVFGPQITSPALTQLTIQ  
ALYNLAGGNMDYLLTKLGVGNQNLSSLIGSLITGNPILYDSQTQILGIQVTLPSVGNL  
NNMRATYLETLVSSTTKGFASALVPKVVTQVGSVIEELDTSYCI GTDLDLYCTRIVTFP  
MSPGIYSCLSGNTSACMYSKTEGALTTPYMALKGSVIANCKLTTCRCADPPGIISQNYG  
EAVSLIDRHSCNVLSLDGITLRLSGEFDATYQKNISILDSQVIVTGNLDISTELGNVNN  
SISNALNKLEESNSKLDKVNKLTSTSA LITYIVLTVISLVFGVLSLV LACYLMYKQKA  
QQKTLLWLGNNTLDQMRATTKI

Figure 14 (Continued)

**NDV-F YZCQ wildtype DNA sequence (SEQ ID NO:34)**

atgggctccagatcttctaccaggatcccgggtacctctaatactgatcatccgaaccgc  
gctgacactgagctgtatccgtctgacaagctctcttgatggcaggcctcttgccggtg  
cagggatcgtggtaacaggagataaagcagtcacatatacacctcatcccagacaggg  
tcaatcatagtttaagttactcccgaatatgcccaggacaaagaggtgtgtgcaaaagc  
cccattggaggcatacaacaggacactgactactttactcaccccccttggtgattcta  
tccgcaggatacaagagtctgtgactacttccggaggaggcaagcaaggccgcctgata  
ggtgccattatcggcagtgtagctcttgggggtgacagctgcacagataaacagcagc  
ttcggccctgatacaagccaaccagaatgctgccaacatcctccggcttaaagagagca  
ttgctgcaaccaatgaagctgtgacagaggtcactgacggattatcacaaactagcagtg  
gcagtagggaagatgcaacagtttgtcaatgaccagttcaataatacagcgcaagaatt  
ggactgtataaaaaattgcacagcaggtcgggtgtagaactcaacttgtaactgaat  
tgactacagtatttggggccacaaatcacttccccctgccttaactcagctgactatccaa  
gcgctttacaatctagctgggtggtaatatggattacttgctgactaagttaggtgtagg  
gaacaaccaactcagctcattaattggtagcggcttgatcacgggcaaccctattctgt  
acgactcacagactcagatcttgggtatacaggttaactttgccttcagttgggaacctg  
aataatatgctgcccacctacctggagaccttatctgtaagcacaaccaagggtattgc  
ctcagcacttgctcccaaaagtgggtgacacaggtcgggttcctgtatagaagaacttgaca  
cctcatactgtatagggaccgacttggtattatactgtacaagaatagtgacattccct  
atgtctcctgggtatttattcttctgtctgagcggtaatacatcggcttgcatgtattcaaa  
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tcaataagtaatgccctgaataagtttagaggaaagcaacagcaaaactagacaaaagtcaa  
tgtcaaaactgaccagcacatctgctctcattacctacatcgttttaactgtcatatctc  
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caacaaaagaccttggttatggcttggaataataacccttgatcagatgagagccactac  
aaaaatatga

**NDV-F protein from wildtype YZCQ strain (Amino Acid Sequence of NDV-F of Texas strain with lentogenic cleavage site sequence) (SEQ ID NO:35)**

mgsrsstripvplmliirtaltlscirltssldgrplaaagivvtgdkavniytssqtg  
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mspgiysclsgntsacmysktegaltpymalkgsviancklttcrcadppgiisqnyg  
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qqktllwlgnntldqmrattki\*



Figure 14 (Continued)

**NDV-F Texas wildtype DNA sequence (SEQ ID NO:36)**

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caggaattgtagtaacaggagataaggcagtcattgtatatacctcgtctcagacaggg  
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aagagcatga

**NDV-F protein from wildtype Texas strain (Amino Acid Sequence of NDV-F VII<sub>d</sub> wt YZCQ with lentogenic cleavage site sequence) (SEQ ID NO:37)**

mgskpstripaplmiltrimlildcirptssldgrplaaagivvtgdkavnvytssqtg  
siivklpnpkdkkeacakdpleaynrtlttlltplgesirkiqgsvstsgggkqgrli  
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avgkmqqfvndqfnntareldcikitqqvgvelnlyltelttvfqpqitspaltqltiq  
alynlaggnmdylltklgiggnqlssligsglitgypilydsqtqllgiqvnlpvgnl  
nnmratyletlsvstakgyasalvpkvvtqvgsvieeldtsyciesdldlyctrivtfp  
mspgiysclsgntsacmysktegalttptymalkgsvianckittcrctdppgiisqnyg  
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qqktllwlgnntldqmrattr\*

Figure 14 (Continued)

**MDV gB promoter (SEQ ID NO:38)**

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ATTTTTGCAACAGCTTTATTTTCTTCTGTATTTAAATGTGGCGAATTGCACATCTGTCTG  
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GAAACGCAAAATATGACTGCAATTATTGATACAGATGTTTTTTCGTTGCTTTATTCTAT  
TTTGCAGTATATGGCCCCCGTTACGGCAGATCAGGTGCGAGTAGAACAGATTACCAACA  
GCCACGCCCCCATCTGACCCGCTCCAATATTCTTGTGTCCCTGCATTTTATCTCACACAA  
TTTATGAACAGCATCATTAAGATCATCTCACT

**IBDV DNA encoding VP2 protein of IBDV E strain (SEQ ID NO:41)**

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ggtga

Figure 14 (Continued)

**IBDV VP2 protein of IBDV E strain (SEQ ID NO:42)**

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ervatgsvvtvagvsnfelipnpelaknlvteygrfdpgamnytklilserdrlgiktv  
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**Guinea pig CMV promoter (SEQ ID NO:43)**

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Figure 14 (Continued)

Partial plasmid pHM103+Fopt DNA sequence (SEQ ID NO:18)

*Green and Italic* = Arms

**Black and bold** = NDV Fopt

BLUE AND UPPERCASE = SV40 PROMOTER

Red and Italic and underlined = SV40 polyA

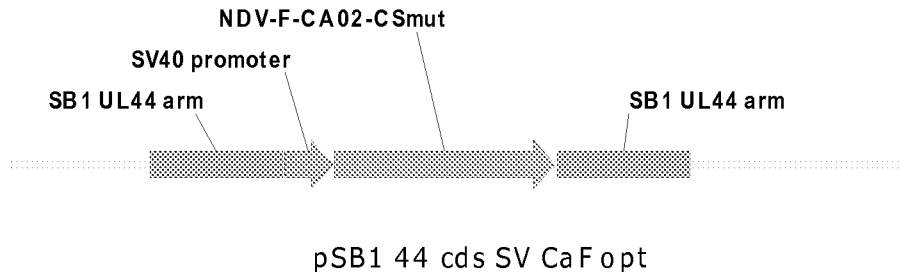
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Figure 14 (Continued)

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Figure 14 (Continued)

Partial plasmid pSB1 44cds SV FCAopt sequence for vSB1-009  
(SEQ ID NO:19)



*Green and Italic = UL44 Recombination Arms*

BLUE AND UPPERCASE = SV40 PROMOTER

**Black and Bold = NDV-F-CA02-CSmut sequence**

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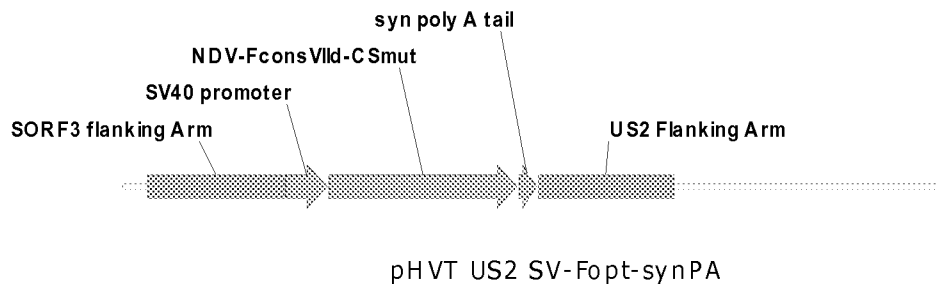
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid pHVT US2 SV-Fopt-SynPA for vHVT306 (SEQ ID NO:20)**

*Green and Italic = SORF3 and US2 Recombination Arms*

BLUE AND UPPERCASE = SV40 PROMOTER

**Black and Bold = NDV-FconsVIId-CSmut sequence**

Red and Italic and Underlined = Synthetic Poly A tail

```

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Figure 14 (Continued)

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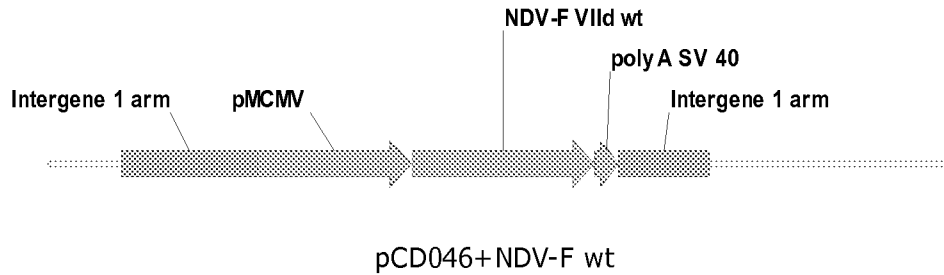
Figure 14 (Continued)

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Figure 14 (Continued)

**plasmid pCD046+NDV-F wt for vHVT110 (SEQ ID NO:21)***Green and Italic = BamHI fragment I intergenic**Recombination Arms*

BLUE AND UPPERCASE = MCMV PROMOTER

**Black and Bold = NDV-F VIIId wildtype consensus sequence***Red and Italic and Underlined = SV40 Poly A tail*

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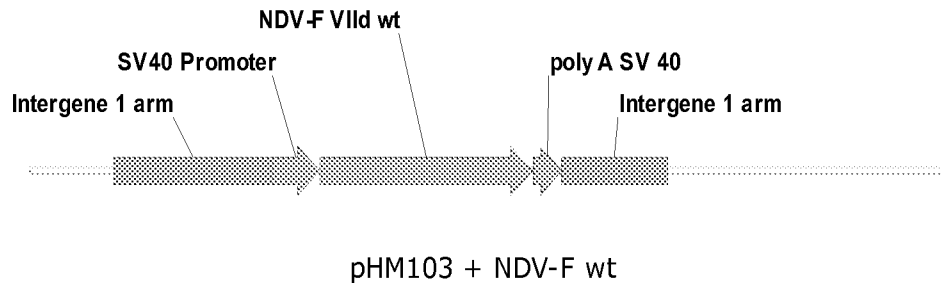
Figure 14 (Continued)

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Figure 14 (Continued)

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**Figure 14 (Continued)**  
**Partial plasmid pHM103+NDV-F wt sequence for vHVT111 (SEQ ID NO:22)**



*Green and Italic = BamHI fragment I intergenic  
 Recombination Arms*

*BLUE AND UPPERCASE = SV40 PROMOTER*

**Black and Bold = NDV-F VIId wildtype consensus sequence**

*Red and Italic and Underlined = SV40 Poly A tail*

```

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Figure 14 (Continued)

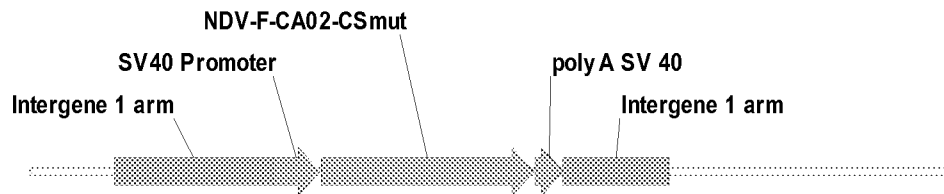
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid pHM103+NDV-F CA02 for vHVT116 (SEQ ID NO:23)**

pHM103 + NDV-F CA02

*Green and Italic = BamHI fragment I intergenic Recombination Arms*

BLUE AND UPPERCASE = SV40 PROMOTER

**Black and Bold = NDV-F-CA02-CSmut sequence**

*Red and Italic and Underlined = SV40 Poly A tail*

```

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Figure 14 (Continued)

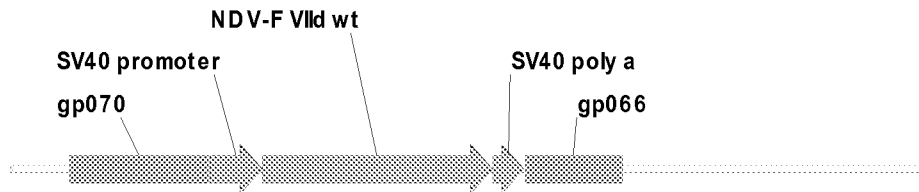
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid HVTIG2 SV Fwt SbfI sequence for vHVT301 (SEQ ID NO:24)**



**HVT IG2 SV Fwt SbfI**

*Green and Italic = gp070 and gp066 Recombination Arms*

**BLUE AND UPPERCASE = SV40 PROMOTER**

**Black and Bold = NDV-F VIId wildtype consensus sequence**

Red and Underlined = SV40 Poly A tail

```

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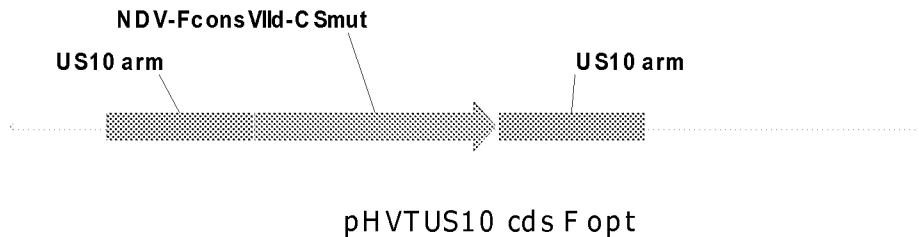
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid pHVTUS10 cds F opt plasmid for  
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**Black and Bold = NDV-FconsVIIId-C Smut sequence**

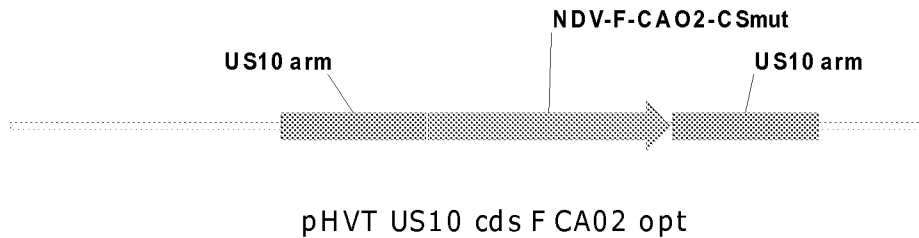
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid pHVT US10 cds F CA02 opt sequence  
for vHVT303 (SEQ ID NO:26)**



Green and Italic = US10 cds Recombination Arms

**Black and Bold = NDV-F-CA02-CSmut sequence**

tcccttacggcggatcgaaaacgacattagggcatactcgggtaccatctttgcattccgat  
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**ccagcagccagaccggcagcatcatcatcaagctgctgcccacatgcccaggacaaa**  
**gaggcctgcgccaaggccccctggaagcctacaacagaaccctgaccaccctgctgac**  
**ccccctggggcagacatcagaagaatccagggcagcgccaccacaagcggcgaggaa**  
**agcagggcagactggtgggcgtatcatcgggagcgtggccctgggcgtggccacagct**  
**gccagattaccgctgcagccgccctgattcaggccaatcagaacgccgccaacatcct**  
**gagactgaaagagagcattgccgccaccaacgacgccgtgcacgaagtgacaaacggac**  
**tgtcccagctggctgtcgtgtcggcaagatgcagcagttcgtgaacaaccagttcaac**  
**aacaccgccagagagctggactgcatcaagatcgcccagcaggtgggcgtggagctgaa**  
**cctgtacctgaccgagctgaccacagtggttcggccccagatcacaagccccgctctga**  
**cccagctgacaatccaggccctgtacaacctggctggcggaacatggactatctgctg**

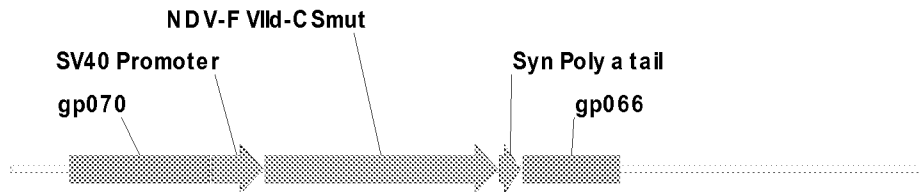


Figure 14 (Continued)

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gatcgaggaactggacaccagctactgcatcgagagcgacatcgacctgtactgcacca  
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gcctgcatgtacagcaagaccgaaggagcactgacaacacctacatggccctgaaggg  
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Figure 14 (Continued)

**Partial plasmid HVT IG2 SVFopt syn tail sequence  
for vHVT304 (SEQ ID NO:27)**



HVT IG2 SVFopt syn tail.

*Green and italic = gp070 and gp066 Recombination Arms*

**BLUE AND UPPERCASE = SV40 PROMOTER**

**Black and Bold = NDV-FconsVld-CSmut sequence**

Red and Underlined = Synthetic Poly A tail

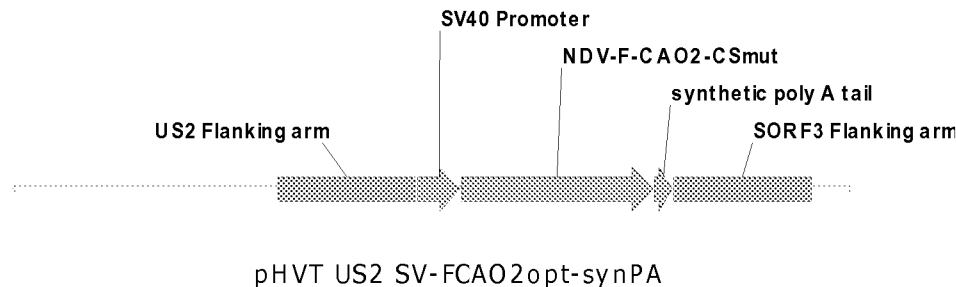
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cataagaacgctagagatgctatttaacgatgtgctgtcgtctaaagaatttgtgcatt  
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ggataaagggtcgtttgggtctgtcctagcgatataaatttatatgaacgatatacattaaa  
catctgtgtgcagtaacttaggtatttaatcatgtcgatgaaatgttatgtgtaaatatac  
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ctaatagtgtggatgatgtatacagtataattacaaaacggaaatgatacgtataataaacct  
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GTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCA  
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AGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTG  
GAGGCCTAGGCTTTTGCAAAAAGCTcccgggggcgccgccaccatggggcagcaagccca  
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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid pHVT US2 SV-FCA02 opt-synPA for vHVT307 (SEQ ID NO:28)**



*Green and Italic = US2 and SORF3 Recombination Arms*

BLUE AND UPPERCASE = SV40 PROMOTER

**Black and Bold = NDV-F-CA02-CSmut sequence**

Red and underlined = Synthetic Poly A tail

```
tatctccacatcgtatttcaggcccccacggaagtcttcggttatcgaagctattgttactag
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```

Figure 14 (Continued)

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Figure 14 (Continued)

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Figure 14 (Continued)

**Partial plasmid pCD046+NDV-F VII YZCQ for vHVT112 (SEQ ID NO:29)**Green and *Italic* = Flanking Arms

BLUE AND UPPERCASE = mCMV IE

**Black and Bold = NDV-F VIIId wt YZCQ**Red and underlined = SV40 Poly A

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 tggagggtatctggggatttttgccaatgctggagcgacgactgtacgattcgtcccat  
 cgggatctagcagacccaatgatgttgacacacatcgggccatgcatgtacggacggtcta  
 ttgcgcgagttttgttatttttcgaaggacaagatggaagtgtatatggaaccgacaataa  
 tgtagttttgcatttcttagggcggaatctacatgatatcttatccaagcgggggtatga  
 gccagagagatgtgatgggtcataaagggttaaattttttagatctgaaataacgcagttg  
 cccaaacaacgatcgcgattaaaagaaaaaatcggatgggttcaattaggacatgcattgga  
 ttctgtgcgcataaaaccataaccgcagcactgtttgggcaacttcggtaactcaaatgcga  
 agcgtttgcacgtctgcgataactacgcctactatgcacattggttactcctgcatcttaa  
 aaatataatcctgttagtaatttttcacagcaatgtcataacatcatctcgctaaagaatga  
 cctgggatttggaagaagtaaatgaatattttgcaaccaatgcattgaataaaactaactaa  
 acGAATTCAATAGTGGATCCCCCACTCCGCCCGTTTTATGACTAGAACCAATAGTTTT  
 TAATGCCAAATGCACTGAAATCCCCTAATTTGCAAAGCCAAACGCCCCCTATGTGAGTA  
 ATACGGGGACTTTTTACCCAATTTCCACGCGGAAAGCCCCCTAATACTCATATGGC  
 ATATGAATCAGCACGGTCATGCACTCTAATGGCGGCCCATAGGGACTTTCCACATAGGG  
 GGCGTTACCATTTCCCAGCATAGGGGTGGTGACTCAATGGCCTTTACCCAAGTACATT  
 GGGTCAATGGGAGGTAAGCCAATGGGTTTTTCCCATTACTGGCAAGCACACTGAGTCAA  
 ATGGGACTTTCCACTGGGTTTTTGCCCAAGTACATTGGGTCAATGGGAGGTGAGCCAATG  
 GGAAAAACCCATTGCTGCCAAGTACACTGACTCAATAGGGACTTTCCAATGGGTTTTTC  
 CATTGTTGGCAAGCATATAAGGTCAATGTGGGTGAGTCAATAGGGACTTTCCATTGTAT  
 TCTGCCCAGTACATAAGGTCAATAGGGGGTGAATCAACAGGAAAGTCCCATTGGAGCCA  
 AGTACACTGCGTCAATAGGGACTTTCCATTGGGTTTTGCCCAGTACATAAGGTCAATAG  
 GGGATGAGTCAATGGGAAAAACCCATTGGAGCCAAGTACACTGACTCAATAGGGACTTT  
 CCATTGGGTTTTTGCCCAGTACATAAGGTCAATAGGGGGTGAGTCAACAGGAAAGTTCCA  
 TTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTTGCCCAGTACATAA  
 GGTCAATGGGAGGTAAGCCAATGGGTTTTTCCCATTACTGGCACGTATACTGAGTCATT

Figure 14 (Continued)

AGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATAGGGGTGAATCAACAGGA  
 AAGTCCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCA  
 GTACAAAAGGTCAATAGGGGGTGAGTCAATGGGTTTTTCCATTATTGGCACGTACATA  
 AGGTCAATAGGGGTGAGTCATTGGGTTTTTCCAGCCAATTTAATTAAAACGCCATGTAC  
 TTTCCCACCATTGACGTCAATGGGCTATTGAAACTAATGCAACGTGACCTTTAAACGGT  
 ACTTTCCCATAGCTGATTAATGGGAAAGTACCGTTCTCGAGCCAATACACGTCAATGGG  
 AAGTGAAAGGGCAGCCAAAACGTAAACACCGCCCCGGTTTTTCCCTGGAAATTCCATATT  
 GGCACGCATTCTATTGGCTGAGCTGCGTTCTACGTGGGTATAAGAGGCGCGACCAGCGT  
 CGGTACCGTCGCAGTCTTCGGTCTGACCACCGTAGAACGCAGAGCTCCTCGCTGCAGgc  
 ggcgcgcattgggctctaaaccttctaccaggatcccagcacctctgatgctgatcacccg  
 gattatgctgatattggactgtatccgtccgacaagctctcttgacggcaggcctcttg  
 cagctgcaggaattgtagtaacaggagataaggcagtcattgtatatacctcgtctcag  
 acagggtcaatcatagtcaagttgctcccgaatatgcccaaggataaggaggcgtgtgc  
 gaaagaccattagaggcatataacagaacactgactactttgctcactcctcttggcg  
 aatccatccgcaagatccaagggtctgtgtccacgtctggaggaggcaagcaaggccgc  
 ctgatagggtgctgttattggtagtgtagctcttggggttgcaacagcggcacaataac  
 agcagctgcggccctaatacaagccaaccagaatgctgccaacatccttcggcttaagg  
 agagcattgctgcaaccaatgaagctgtgcatgaagtcaccgacggattatcacaacta  
 tcagtggcagttgggaagatgcagcagtttgtcaatgaccagtttaataatacagcgcg  
 agaattggactgtataaaaaatcacacaacagggttggtgtagaactcaacctatacctaa  
 ctgaattgactacagtattcgggccacagatcacctcccctgcattaactcagctgacc  
 atccaggcactttataaatttagctgggtggcaatatggattacttattaactaagttagg  
 tatagggaacaatcaactcagctcattaattggcagcggcctgatcactgggtacccta  
 tattgtatgactcacagactcaactccttgggcatacaagtgaatttgccctcagtcggg  
 aacttaaataatatgcgtgccacctatttagagaccttatctgtaagtacagccaaagg  
 atatgcctcagcacttggtccaaaagtagtgacacaagtcggttctgtgatagaagagc  
 ttgacacctcatactgtatagagtccgatctggatttatattgtactagaatagtgcaca  
 tccccatgtccccagggtattttattcctgtttaagcggcaacacatcagcttgcatgta  
 ttcaaagactgaaggcgcactcactacgccgtatatggcccttaaaggctcagttattg  
 ccaattgtaagataacaacatgtagatgtacagaccctcctgggtatcatatcgcaaaat  
 tatggagaagctgtatccctgatagatagacattcgtgcaatgtcttatcattagacgg  
 gataactctgaggctcagtgaggagaatttgatgcaacttatcaaaagaacatctcaatac  
 tagattctcaagtcacgtgacaggcaatcttgatataatcaactgaacttggaacgctc  
 aacaattcaatcagcaatgccttgataagttggcaaaaagcaacagcaagctagaaaa  
 agtcaatgtcagactaaccagcacatccgctctcattacctataattgttctgactgtca  
 tttctctagttttcgggtgcactaagctctgggtttaacatgttacctgatgtacaaaca  
 aaggcacaacaaaagaccttgctatggcttggaataataaccctcgatcagatgagagc  
 cactacaagagcatgagcggcgcggggatccagacatgataagatacattgatgagtt  
 tggacaaaccacaactagaatgcagtgaaaaaaatgctttatttggtgaaatttgatgag  
 ctattgctttatttgtaaccattataagctgcaataaacaagtttaacaacaacaattgc  
 attgatttttatgtttcagggttcagggggagggtgtgggagggttttttoggatcctctaga  
 gtogacaattattttatttaataacatatagcccaaagacctctatgaacatttagttt  
 cccgtataactcaacgggcggtgtacacacgcacatctctttgcatagcgatgaagtttgtt  
 cggcagcagaaaaatgcagatatccaacaatctggagaaaaacttatcatcacagtggcag



Figure 14 (Continued)

tggaaacataccccctctatatattcatggtataattatcgtctacagcgtccaggatagt  
ggcgtgagaaaaatggagatctgcagccctcctttccatggcatgcccgtttattgttca  
ttaaacgcacaaatggtctcaacgccagatatgggcatagattctgaagaacccggttgac  
aatccgaagaagaaggcgtgcaggtctttggaagactcgcacgttggtcttataatgta  
tgatcgagatgtcacccctaattgccacatggtacaggcttatcgcgggtcatggcgatcgg  
acttgtaattttgcaacgatgggcaaaggatcgacgacatgocaaacattctgaacccgt  
agagatgttaacgatgacgaggatgaatatcccatgctcgctgccatagtatcaagtac  
accgcgaataaggacgcgtccaacatcgttatatgcacacaatgggctacacgtgacta  
acacccccgaatattagtcataatgtgagtttcagtcctggctcccatatagcctgtagac  
tatttgtggttttaagtgtgaacgaggcgtgtgaaacgagactcgggcgattgtlaagaa  
caagcaaatgcactttccatttaacaagaagtgtagagagaataactcaacctctttgga  
tgtatcctcgag

Figure 14 (Continued)

**Partial plasmid pCD046 + NDV Texas F for vHVT113 (SEQ ID NO:30)***Green and Italic = Flanking Arms*

BLUE AND UPPERCASE = mCMV IE

**Black and Bold = NDV Texas F**Red and underlined = SV40 Poly A

gagctcaggggtatgatactcagctggttattgtggccgaccaggaggactccaatgctta  
gcattcstaagaacgctagagatgctattttaaaggatgtgctgtcgtctaaagaatttgt  
gcatttagccttttaaattgtaaaaccaaagacgcattcactacgctcgtgctgcaattt  
ctggggccaggggtatgcatattccataacagaaatcgacacttgagaagaggatctgact  
gtttgggataaaaggtcgttttgggtctgtccctagcgatataattttatatgacgatataca  
ttaaacaatctgtgtgcagtaacttaggtattttaatcatgtcgatgaaaatgttatgtgttaa  
atatcggacaatatagataacggggcagcgtgctatttgaacgtgcgccccgcgcgctagt  
gctgactaataagtgtggatgatgtatatacagtataattacaaacggaaaatgatacgtataa  
aattatgtactcttatttgattttataaaaaacatacatgcagtggttgctatgtcacataat  
tagcctcgcgcgtctacgctccactgaagataatggggtcccgctgttcaaaaaaatca  
gcgtgcgtcgataaagacttttgggtgcagtcctcttcgggggtcgcaatttagatttgcgcga  
tggagggtatctggggatttttgccaatgctggagcgacgactgtacgattcgtcccat  
cgggatctagcagaccaatgaigtgttgacacacacatcggccatgcatgtacggacgggtcta  
ttgcgcgagtttgtttattttcgaaggacaagatgggaagtgtatatggaaccgacaataa  
tgttagtttgcatttcttagggcggaatctacatgatattcttatccaagcgggggtatga  
gccagagagatgtgatgggtcataaaaggggtaaaatttttttagatctgaaataaacgcagttg  
cccaaacaacgatcgcgattaaaaagaaaaaatcggatgggttcaattaggacatgcatgga  
ttctgtgcgcataaaaccataaaccgcagcactgttgggcacttcggtaactcaaatgcga  
agcgttgcagctctgcgataactacgcctactatgcacattgttactcctgcactcttaa  
aaatataatcctgttagtaatttttcacagcaatgtcataacatcatctcgtctaaagaatga  
cctgggatttggagaagtaaatgaatattttgcaaccaatgcattgaataaactaacattaa  
acGAATTCAATAGTGGATCCCCAACTCCGCCCGTTTTATGACTAGAACCAATAGTTTT  
TAATGCCAAATGCACTGAAATCCCCTAATTTGCAAAGCCAAACGCCCCCTATGTGAGTA  
ATACGGGGACTTTTTACCCAATTTCCCACGCGGAAAGCCCCCTAATACACTCATATGGC  
ATATGAATCAGCACGGTCATGCACTCTAATGGCGGCCCATAGGGACTTTCCACATAGGG  
GGCGTTACCATTTCCCAGCATAGGGGTGGTGACTCAATGGCCTTTACCCAAGTACATT  
GGGTCAATGGGAGGTAAAGCCAATGGGTTTTTTCCATTACTGGCAAGCACACTGAGTCAA  
ATGGGACTTTCCACTGGGTTTTTGCCCAAGTACATTGGGTCAATGGGAGGTGAGCCAATG  
GGAAAAAACCATTGCTGCCAAGTACACTGACTCAATAGGGACTTTCCAATGGGTTTTTC  
CATTGTTGGCAAGCATATAAGGTCAATGTGGGTGAGTCAATAGGGACTTTCCATTGTAT  
TCTGCCCAGTACATAAGGTCAATAGGGGGTGAATCAACAGGAAAGTCCCATTGGAGCCA  
AGTACACTGCGTCAATAGGGACTTTCCATTGGGTTTTTGCCCAGTACATAAGGTCAATAG  
GGGATGAGTCAATGGGAAAAAACCATTGGAGCCAAGTACACTGACTCAATAGGGACTTT  
CCATTGGGTTTTTGCCCAGTACATAAGGTCAATAGGGGGTGAATCAACAGGAAAGTTCCA  
TTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTTGCCCAGTACATAA

Figure 14 (Continued)

GGTCAATGGGAGGTAAGCCAATGGGTTTTTCCCATTACTGGCACGTATACTGAGTCATT  
AGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATAGGGGTGAATCAACAGGA  
AAGTCCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCA  
GTACAAAAGGTCAATAGGGGGTGAGTCAATGGGTTTTTCCCATTATTGGCACGTACATA  
AGGTCAATAGGGGTGAGTCATTGGGTTTTTCCAGCCAATTTAATTAAAACGCCATGTAC  
TTTCCCACCATTGACGTCAATGGGCTATTGAAACTAATGCAACGTGACCTTTAAACGGT  
ACTTTCCCATAGCTGATTAATGGGAAAGTACCGTTCTCGAGCCAATACACGTCAATGGG  
AAGTGAAAGGGCAGCCAAAACGTAAACACCGCCCCGGTTTTCCCTGGAAATTCCATATT  
GGCACGCATTCTATTGGCTGAGCTGCGTTCTACGTGGGTATAAGAGGCGCGACCAGCGT  
CGGTACCGTCGCAGTCTTCGGTCTGACCACCGTAGAACGCAGAGCTCCTCGCTGCAGgc  
ggcgcgc**atgggctccagatcttctaccaggatcccggtaacctctaattgctgatcatccg**  
**aaccgcgctgacactgagctgtatccgtctgacaagctctcttgatggcaggcctcttg**  
**cggctgcagggatcgtggtaacaggagataaagcagtcacatatacacctcatcccag**  
**acagggtcaatcatagtttaagttactcccgaatatgcccaaggacaaagaggtgtgtgc**  
**aaaagccccattggaggcatacaacaggacactgactactttactcaccccccttggtg**  
**attctatccgcaggatacaagagtctgtgactacttccggaggaggcaagcaaggccgc**  
**ctgataggtgccattatcggcagtgtagctcttggggttgcgacagctgcacagataac**  
**agcagcttcggccctgatacaagccaaccagaatgctgccaacatcctccggcttaaag**  
**agagcattgctgcaaccaatgaagctgtgcacgaggtcactgacggattatcacaacta**  
**gcagtggcagtagggaagatgcaacagtttgtcaatgaccagttcaataatacagcgca**  
**agaattggactgtataaaaaattgcacagcaggtcgggtgtagaactcaacttgtacctaa**  
**ctgaattgactacagtatttgggccacaaatcacttcccctgccttaactcagctgact**  
**atccaagcgctttacaatctagctggtggttaatatggattacttgctgactaagttagg**  
**tgtagggaacaaccaactcagctcattaattggtagcggcttgatcacccggcaacccta**  
**ttctgtacgactcacagactcagatcttgggtatacaggtaactttgccttcagttggg**  
**aacctgaataatatgcgtgccacctacctggagaccttatctgtaagcacaccaaggg**  
**atttgcctcagcacttggtcccaaaagtgggtgacacaggtcgggtccgtgatagaagaac**  
**ttgacacctcatactgtatagggaccgacttggtttatactgtacaagaatagtgaca**  
**ttccctatgtctcctggtattttattcttgtctgagcggtaatacatcggttgcatgta**  
**ttcaaagactgaaggcgcacttactacgccatatatggctctcaaaggctcagttattg**  
**ccaattgcaagctgacaacatgtagatgtgcagatccccagggtatcatatcgcaaaat**  
**tatggagaagctgtgtccttaatagataggcactcatgcaacgtcttatccttagacgg**  
**gataactctgaggctcagtggggaatttgatgcaacctatcaaaagaatatctctatac**  
**tagattctcaagttatagtgacaggcaatcttgatatatcaactgagcttgggaaatgtc**  
**aacaactcaataagtaatgccctgaataagttagaggaaagcaacagcaaaactagacaa**  
**agtcaatgtcaaactgaccagcacatctgtctctcattacctacatcgttttaactgtca**  
**tatctcttgttttttgggtgtacttagcctggttctagcatgctacctgatgtacaagcaa**  
**aaggcacaacaaaagaccttggttatggcttggggaataatacccttgatcagatgagagc**  
cactacaaaaatatgagcgggcgcggggatccagacatgataagatacattgatgagtt  
tggacaaaaccacaactagaatgcagtgaaaaaaatgcctttatttggtaaaatttgatg  
ctattgcctttatttggtaaccattataagctgcaataaacaagttaacaacaacaattgc  
attgatttttatgttttcagggttcagggggaggtgtgggaggttttttccggatcctctaga  
gtcgacaattatttttatttaatacatatagcccaaagacctctatgaacatttagttt  
cccgataactcaacgggcgcgtgtacacacgcacatctctttgcatagcgatgaagtttgtt

Figure 14 (Continued)

cggcagcagaaaaatgcagatatccaacaatctggagaaaaacttatcatcacagtggcag  
tggaaacataccccctctatatattcatgggtataattatcgtctacagcgtccaggatagt  
ggcgtgagaaaaatggagatctgcagocctcctttccatggcatgocgctttattgttca  
ttaaacgcacaatgggtctcaacgccagatatgggcatagattctgaagaacccggtgac  
aatccgaagaagaaggcgtgcaggtctttggaagactcgacacgttggtcttataatgta  
tgatcgagatgtcacccctaattgccacatggtacaggccttatcgcggtcatggcgatcgg  
acttgtaatttgcaacgatgggcaaaggatcgacgacatgccaaacattctgaacccgt  
agagatgttaacgatgacgaggatgaatatcccatgctcgctgccatagtatcaagtac  
accgcgaataaggacgcgtccaacatcggttatatgcacacaatgggctacacgtgacta  
acacccccgaataattagtcatatgtgagtttcagtcctggctcccatatagcctgtagac  
tatttggtggtttaagtgtgaacgaggcgctgtgaaacgagactcgggcccattgtgaagaa  
caagcaaatgcactttccatttaacaagaagtgtagagagaataactcaacctctttgga  
tgtatcctcgag

Figure 14 (Continued)

Partial plasmid pHM119 sequence for vHVT039 (SEQ ID NO:31)

*Green and Italic = BamHI fragment I intergenic  
Recombination Arms*

BLUE AND UPPERCASE = MDV gB PROMOTER

**Black and Bold = NDV-F wild type unmodified Texas strain  
sequence**

*Red and Italic and Underlined = SV40 Poly A tail*

gagctcaggggtatgatactcagctgttattgtggccgaccaggaggactccaatgotta  
gcattcctaagaacgctagagatgctattttaaagcatgtgctgtogtctaaagaatttgt  
gcatttagccttttaaatgtaaaaaccaatgaacgcattcactacgctcgtgogtgcaattt  
ctgggccaggggtatgcataattccataaacagaaatcgacacttgagaagaggatctgact  
gtttgggataaaaggtcgtttgggtctgtcctagcgatataattttatatgacgatataca  
ttaaacatctgtgtgcagtaacttaggtattttaatcatgtcgatgaaaatgttatgtgtaa  
atatcggacaatatagataaacgggcacgctgctattgtaacgtgogccccgcgcgctagt  
gctgactaataagtgtggatgatgtatacagtatattacaaaacggaaatgatagcgaata  
aattatgtactcttatttgattttataaaaaacatacatgcagtgttgctatgtcacataat  
tagcctcgcgcgtctacgctccactgaagataaatgggctcccgcgtgttcaaaaaaatca  
gcgtgcgtcgataaagacttttggtgcagtcctcttcggggctcgcaatttagatttgccgca  
tggaggggtatctggggatttttgccaatgctggagcgacgactgtacgattcgtcccat  
cgggatctagcagaccaatgatgttgacacacatcggccatgcatgtacggacgggtcta  
ttgcgcgagtttgttatttttcgaaggacaagatgggaagtgtatatggaaccgacaataa  
tgttagtttgcatttcttagggcggaatctacatgatattcttatccaagcgggggtatga  
gccagagagatgtgatgggtcataaaagggtaaaatttttttagatctgaaataaacgcagttg  
cccaaacaacgatcgcgattataaaagaaaaaatcggatgggttcaatttaggacatgcatgga  
ttctgtgcgcataaaaccataaacgcagcactgttggggcacttcgggtaactcaaatgcga  
agcgttgcagctctgcgataactacgcctactatgcacattgttactcctgcattcttaa  
aaatataatcctgtagtaatttttcacagcaatgtcataacatcatctcgctaaagaatga  
cctgggattggagaagtaaatgaatattttgcaaccaatgcattgaataaactaascattaa  
acgaattcCGATGTTTAGTCACGATAGACATCGGTTCGCCCAGCCGTCGAATACAGCAT  
TATATTTTAGTGTTGAAAATGTAGGGCTGCTTCCTCACTTAAAGGAGGAAATGGCTCGA  
TTCATGTTTCATAGCAGTAGAAAAACAGATTGGACCGTCAGTAAGTTTAGAGGGTTTTA  
TGACTTTAGCACTATAGATAATGTAACCTGCGGCCCATCGCATGGCTTGGAATATATCA  
AAGAACTGATTTTTGCAACAGCTTTATTTTCTTCTGTATTTAAATGTGGCGAATTGCAC  
ATCTGTCTGCGCCGACAGTTTGCAGATCAACAGCAATGGAGACTATGTATGGAAAAATGG  
AATATATATAACATATGAAACCGAATATCCACTTATAATGATTCTGGGGTCAGAATCAA  
GCACTTCAGAAACGCAAAATATGACTGCAATTATTGATACAGATGTTTTTTTCGTTGCTT  
TATTCTATTTTGCAGTATATGGCCCCCGTTACGGCAGATCAGGTGCGAGTAGAACAGAT  
TACCAACAGCCACGCCCCCATCTGACCCGTCCAATATTCTTGTGTCCCTGCATTTTATC  
TCACACAATTTATGAACAGCATCATTAAGATCATCTCACTgcggccgcaag**atggggctc  
cagatcttctaccaggatcccgggtacctctaatactgatcatccgaaccgcgctgacac  
tgagctgtatccgtctgacaagctctcttgatggcaggcctcttgcggtgcagggatc**

Figure 14 (Continued)

gtggtaacaggagataaaagcagtcaacatatacacctcatcccagacaggggtcaatcat  
 agttaagttactcccgaatatgcccgaaggacaaagaggtgtgtgcaaaagccccattgg  
 aggcatacaacaggacactgactactttactcaccccccttggtgattctatccgcagg  
 atacaagagtctgtgactacttccggaggaaggagacagagacgctttataggtgccat  
 tatcggcagtgtagctcttgggggttgcgacagctgcacagataacagcagcttcggccc  
 tgatacaagccaaccagaatgctgccaacatcctccggcttaaagagagcattgctgca  
 accaatgaagctgtgcacgaggtcactgacggattatcacactagcagtggcagtagg  
 gaagatgcaacagtttgtcaatgaccagttcaataatacagcgcagaattggactgta  
 taaaaattgcacagcaggtcgggtgtagaactcaacttgtaactgaattgactaca  
 gtatttggggccacaaatcacttcccctgccttaactcagctgactatccaagcgttta  
 caatctagctgggtggtaatatggattacttgctgactaagttaggtgtagggaacaacc  
 aactcagctcattaattggtagcggcttgatcacccgcaaccctattctgtacgactca  
 cagactcagatcttgggtatacaggtaactttgccttcagttgggaacctgaataatat  
 gcgtgccacctacctggagaccttatctgtaagcacaaccaagggatttgcctcagcac  
 ttgtcccaaaagtgggtgacacaggtcgggtccgtgatagaagaacttgacacctcatac  
 tgtatagggaccgacttggtttatactgtacaagaatagtgcattccctatgtctcc  
 tgggtattttattcttgtctgagcggtaatacatcggcttgcatgtattcaaagactgaag  
 gcgcacttactacgccatatatggctctcaaaggctcagttattgccaattgcaagctg  
 acaacatgtagatgtgcagatccccaggtatcatatcgcaaaattatggagaagctgt  
 gtccttaatagataggcactcatgcaacgtcttatccttagacgggataactctgaggc  
 tcagtggggaatttgatgcaacctatcaaaagaatatctctatactagattctcaagtt  
 atagtgcaggcaatcttgatatatcaactgagcttggaatgtcaacaactcaataag  
 taatgccctgaataagtttagaggaaagcaacagcaaaactagacaaagtcaatgtcaaac  
 tgaccagcacatctgctctcattacctacatcggttttaactgtcatactctctgttttt  
 ggtgtacttagcctgggttctagcatgctacctgatgtacaagcaaaaggcacaacaaaa  
 gaccttggttatggcttggaataatacccttgatcagatgagagccactacaaaaatat  
 gagcgccgcgggggatccagacatgataagatacattgatgagtttggacaaaaccacaa  
 ctagaatgcagtgaaaaaaatgcttttatttggtaaatttggtagctattgcttttattt  
 gtaaccattataagctgcaataaacaagtttaacaacaacaattgcattcattttatgtt  
 tcaggttcagggggaggtgtgggaggttttttcggatcccttagagtcgacaattattt  
 tatttaataacatatagcccaagacctctatgaacatttagtttcccgatatactcaac  
 ggcgcgtgtacacacgcacatctctttgcatagcgatgaagtttggttcggcagcagaaaaat  
 gcagatatccaacaatctggagaaaaacttatcatcacagtggcagtggaacatacccc  
 ctctatatattcatgggtataattatogtctacagcgtccaggatagtgggcgtgagaaaaatg  
 gagatctgcagccctcctttccatggcatgocgctttattgttcattaaacgcacaatg  
 gtctcaacgccagatatgggcatagattctgaagaacccggttgacaatccgaagaagaa  
 ggcggtgcaggtctttggaagactcgcacggttggtctttataatgtatgatcgagatgtca  
 cctaatgccacatgggtacaggcttatcgcggtcatggcgatcggacttgtaatttgca  
 acgatgggcaaaaggatcgacgacatgccaaacattctgaacccgtagagatgtttaacga  
 tgacgaggatgaatatcccatgctcgctgocatagtatcaagtacaccgcogaataagga  
 cgcgtccaacatcggttataatgcacacaatgggctacacgctgactaacacccccgaatat  
 tagtcatatgtgagtttcagttctggctcccatatagcctgtagaactatttgggttttaa  
 gtgtgaacgagggcgctgtgaacgagactcggggcgattgtgaagaacaagcaaatgcact  
 ttccatttaacaagaagtgttagagagaataactcaacctctttggatgtatcctcgag

Figure 14 (Continued)

**Partial plasmid SORF3-US2 gpVar-Ewtsyn sequence (for vHVT202) (SEQ ID NO:39)***Green and Italic* = Flanking Arms

BLUE AND UPPERCASE = GPCMV

**Black and Bold** = Variet E wtRed and Italic and Underlined = Syn Poly A

taaaatgggatctatcattacattcgttaagagttotggataaattttactgtttgccago  
 ttogattcttggaaacgtactgtggatagtgcccttaacttggaaatcgtgaaaatttgaaaacg  
 tocattatatttggaatatcttccgggttggtcccatatcccgccctgggtaccgctcgggatacc  
 ttgcccgtatggattcgtattgacagtcgogcaatcgggggaccaacaacgcgtgggtcc  
 acactcattcggaaaattttcogattgattctgaatatatttattgcccgtcgttaocgagtcg  
 ttggacatatctgtaatacattttcttctctgaaggatcgtgcacatttgatctatac  
 attggccaggatggttcaagttctcagatggttgccattctggcacagcacaactttatggca  
 tttccgatgtaatcgtccggcagccctgggggagttctatatattcgcataattgggatggt  
 aaggacaatagcagatctcgcaacctccaggagggtataataacggttttttaaaggatg  
 gatttctcataaaaaatctgtcgcgaattacactgagaatatcctttactagcgcgcgatt  
 gagagcatcgtcgttccaattttctaaaatggaaagaaaaacaaggcggggcaagagtggttcc  
 aaacatttttcatttttcggcggaatctctcctcaaatcccattggcggtgcaattgattgcaaaat  
 tggcactttccggttcaagtttgatctcctcaaatcttaagacactttttaattgaaaaacta  
 cgttctagtggtggaaagaaaacctataggcagaccatagaactattttgacaccacatatc  
 tttttgtatgtcaaaactgaccatgatogtatggttgctgaatgcactaggggcaattcgcgt  
 cgcgcgactccatacattgaataattccacacgtcagctcatcggttagcaagggtccag  
 tagttgaagtcattttatttttcccgcggttggtccaaatctacctctgggaatatccaa  
 gtttgctgaatatgatcgcacccggtctctggtcatggtgaagggaacttgtagcataaagac  
 gcagggtatcataggggtaatatatttttttattcaactcacatactaaaagtaacgcgatatt  
 agcaccatgtatgggtatcaattgacattttgcgttagcaactacatcaagattatgtaca  
 acataatgggacaacatatgcctgcaggTTAGTCATATGTTACTTTGGCAGAGGCCGCAT  
 GGAAAGTCCCTGGACGTGGGACATCTGATTAATACGTGAGGAGGTCAGCCATGTTCTTT  
 TTGGCAAAGGACTACGGTCATTGGACGTTTGATTGGCATGGGATAGGGTCAGCCAGAGT  
 TAACAGTGTTCTTTTGGCAAAGGGATACGTGGAAAGTCCCGGGCCATTTACAGTAAACT  
 GATACGGGGACAAAGCACAGCCATATTTAGTCATGTATTGCTTGGCAGAGGGTCTATGG  
 AAAGTCCCTGGACGTGGGACGTCTGATTAATATGAAAGAAGGTCAGCCAGAGGTAGCTG  
 TGTCTTTTGGCAAAGGGATACGGTTATGGGACGTTTGATTGGACTGGGATAGGGTCA  
 GCCAGAGTTAACAGTGTTCTTTTGGCAAAGGAAACGTGGAAAGTCCCGGGCCATTTACA  
 GTAAACTGATACTGGGACAAAGTACACCCATATTTAGTCATGTTCTTTTGGCAAAGAG  
 CATCTGGAAAGTCCCGGGCAGCATTATAGTCACTTGGCAGAGGGAAAGGGTCACTCAGA  
 GTTAAGTACATCTTTCCAGGGCCAATATTCAGTAAATTACACTTAGTTTTATGCAAAT  
 CAGCCACAAAGGGGATTTTCCCGGTCAATTATGACTTTTTCCTTAGTCATGCGGTATCC  
 AATTACTGCCAAATTGGCAGTACATACTAGGTGATTCACTGACATTTGGCCGTCTCTG  
 GAAAGTCCCTGGAAACCGCTCAAGTACTGTATCATGGTGACTTTGCATTTTGGAGAGC  
 ACGCCCCACTCCACCATTGGTCCACGTACCCTATGGGGGAGTGTTTATGAGTATATAA  
 GGGGCTCCGGTTTAGAAGCCGGGCAGAgcggccgc**atgacaaacctgcaagatcaaacc**

Figure 14 (Continued)

caacagattgttccggttcatacggagccttctgatgccacaacccggaccggcggtccat  
tccggacgacaccctggagaagcacactctcagggtcagagacctcgacctacaatttga  
ctgtgggggacacaggggtcagggctaattgtctttttccctggattccctggctcaatt  
gtgggtgctcactacacactgcagagcaatgggaactacaagttcgatcagatgctcct  
gactgccagaacctaccggccagctacaactactgcaggctagttagtgcggagtctca  
cagtaagggtcaagcacactccctgggtggcggtttatgcactaaacggcaccataaacgcc  
gtgaccttccaaggaagcctgagtgaactgacagatgttagctacaacgggttgatgtc  
tgcaacagccaacatcaacgacaaaattgggaacgtcctagtaggggaaggggtaaccg  
tcctcagcttaccacatcatatgatcttgggtatgtgaggcttggtgacccataccc  
gctatagggttgacccaaaaatggtagcaacatgtgacagcagtgacaggcccagagt  
ctacaccataactgcagccgataattaccaattctcatcacagtaccaaacagggtgggg  
taacaatcacactgttctcagccaacattgatgccatcacaagtctcagcgttggggga  
gagctcgtgttcaaaacaagcgtccaaagccttgactgggcgccaccatctaccttat  
aggctttgatgggactgcggtaatcaccagagctgtggccgcaaacaatgggctgacgg  
ccggcatcgacaatcttatgccattcaatcttgtgattccaaccaatgagataaccag  
ccaatcacatccatcaaactggagatagtgacctccaaaagtgatggtcaggcagggga  
acagatgtcatggtcggcaagtgggagcctagcagtgacgatccatggtggcaactatc  
caggagccctccgtcccgtcacactagtggcctacgaaagagtggaacaggatctgtc  
gttacggctcgtgggggtgagcaacttcgagctgatcccaaactcctgaactagcaaagaa  
cctggttacagaatatggccgatttgaccaggagccatgaactacacgaaattgatac  
tgagtgagagggaccgccttggtcatcaagaccgtctggccaacaaggagtagactgac  
tttcgtgagtacttcatggaggtggccgacctcaactctcccctgaagattgcaggagc  
at ttggcttcaaagacataatccggggccataaggaggtgagcgggcgcgatatacaataa  
aatatctttat ttttcattacatctgtgtgttttgggttttttgtgtgaatcgatagtactaa  
catacgtctotccatcaaaaacaaaacgaaacaaaacaaactagcaaaataggctgtcccc  
agtgcgaagtgcagggtgcagaaacattttcttcttagacctgcaggcccggggcaagtag  
atgcaatttctcactagttgggtttatctactattgaattttccctatctgtgat  
acacttggggagcctctacaagcatattgccatcatgtacgtttttatctactgtcttaa  
cgcccatgggaacggaggcgctcgtcgtcatgtattgggacggcaacataggcagcaaac  
aaattgcgttttaggtgggggtgcatgtggactcgataccaagcccctgcagctggggaa  
gtctgggtggagagccgataat ttgataacgcacgccatattactgtcgttgaaagtacg  
ccttatcttctatgtttttcaaat tttaggttcccaagtggaagtgagaagtgtttgtatc  
tcacatgggaatggcccaaggcattccagcccagggtgccttggtacttttaatggcaaa  
acgtttttggtagaggtattgattotattgcagttctgcagatatctgcagccccgagta  
tccacaggctatacagatacgttatcggaggcctccgattctagcattacatagccggtc  
agtagatcctgcccattcggtagcgcaaccgggtacatcttcaaacagttctcacaataaa  
tgcattctctcgttctcgtcccaatccgggaacccgggcataccactcccgcctgcccatttaa  
ttctcacaattggggcgatgcgggcggggcaaaaacgaatgtggattttggcaaacogacac  
aggctctgctgtacggactaatatggggcacacccacatcattcttcagatgctccatgca  
ttgttctatgagaaaagatccatagggtggaggcagcgtcacgagatcgcccaggcaatc  
gatcgcaattcgtctagtaaaagtgaogagagttatcatgcacacacccatgcccacgcct  
tccgaataaactggagctgtggaagatcggaacgtcttttttgactgccgggtctcgtact  
actttcgcacagggtgtatacccggaacgcgtactatataat ttttatatcatccaacgtccc  
gaaattacatacgtggcgccgatggaagtagatgttgagtcttcgaaagtaagtgcctc



Figure 14 (Continued)

gaatatgggtatttgtctgtgaaaaatatcgaaagcgggtacgacggttgcagaaccgtcga  
tgtcgccagataactagtaacaatagcttcgataacgaagacttccgtgggcctgaatac  
gatgtggagata

Figure 14 (Continued)

**Partial plasmid SB1US2 gpVII dwtsyn sequence (for vSB1-010) (SEQ ID NO:40)***Green and Italic* = Flanking Arms

BLUE AND UPPERCASE = GPCMV

**Black and Bold** = NDV-F VIIId wtRed and Italic and Underlined = *Syn Poly A*

tctcgtctaaaaacgctccagtgctttacagttcgataatctggacctggggacgcggtat  
 aggatcggttcctccacatgcgctgctgtcgggtatctcgaatccccgggtattcagttgaa  
 tggttggcggagtgctcctcctggaactctgcaaatgttccctagccgtcttcactatctcg  
 tgcaaggctctataataacagttcctctgcagaccggtcggttgctcttccctctctgcgctc  
 gttagttattttctgtaggctccagacgattttgcctgcattttgtgogcaacataatctga  
 ttgcattccctatctcgtcttccggtaatcccataggtgttcgggtattcgcagataggt  
 agagaaagcaccactgcaaactcgtgcaattttccattgcccccaaccaatatttttttaa  
 gaacggcatcgccgtttaatgtacctcgggcattgtgacgatcgaaaacccttatggatgc  
 cttaaagagagcattgcgggtccagttctccaggtgaaaaagagaatagcgcgggtagaaaac  
 gggccgattagttttatcttcgcgcgcgtccctaataatcccaagttctgcagttataactt  
 ccatcgtccgtttttcgacaagggtccggcgcgcacatagtttgaaatgtcatctatcagaa  
 acatctcgcccatcgtagaaaaaaacctgtacgcagaccataaaaaccattcgggtaccac  
 atatccttgggtatatacaaacgatatgtttgggttatgtcgtttggcgggatgtttgatgaaa  
 tagagctaagcgttctctggtattccacgcactgaaacgattccggttagtcaattcatctg  
 ctaacataggccaaaagttttattcgtgttacttttctcggcgggtttggcaaaaacgcccc  
 cttggcacatccatgtcattaaaatacagcggcataaactcctactcatgtgttccatagc  
 ccaggttttctgttcgggtctgtactacgatcagatcagtgggcgcgatcagatgcgtggg  
 atgaatgaagtgtatccgaaagcagttttgagatatacgcataaactgtacgacgattgt  
 ggcactaaacgaagcttttgccgcgacccccatcccaacgcccctgcaggTTAGTCATATGTT  
 ACTTGGCAGAGGCCGCATGGAAAGTCCCTGGACGTGGGACATCTGATTAATACGTGAGG  
 AGGTCAGCCATGTTCTTTTTGGCAAAGGACTACGGTCATTGGACGTTTGATTGGCATGG  
 GATAGGGTCAGCCAGAGTTAACAGTGTTCTTTTTGGCAAAGGGATACGTGGAAAGTCCCCG  
 GGCCATTTACAGTAACTGATACGGGGACAAAGCACAGCCATATTTAGTCATGTATTGC  
 TTGGCAGAGGGTCTATGGAAAGTCCCTGGACGTGGGACGTCTGATTAATATGAAAGAAG  
 GTCAGCCAGAGGTAGCTGTGTCTTTTTGGCAAAGGGATACGGTTATGGGACGTTTGAT  
 TGGACTGGGATAGGGTCAGCCAGAGTTAACAGTGTTCTTTTTGGCAAAGGAAACGTGGAA  
 AGTCCCGGGCCATTTACAGTAACTGATACTGGGACAAAGTACACCCATATTTAGTCAT  
 GTTCTTTTTGGCAAAGAGCATCTGGAAAGTCCCGGGCAGCATTATAGTCACTTGGCAGA  
 GGGAAAGGGTCACTCAGAGTTAAGTACATCTTTCCAGGGCCAATATTTCCAGTAAATTAC  
 ACTTAGTTTTATGCAATCAGCCACAAAGGGGATTTTCCCGGTCAATTATGACTTTTTTC  
 CTTAGTCATGCGGTATCCAATTACTGCCAAATTGGCAGTACATACTAGGTGATTCCTG  
 ACATTTGGCCGTCTCTGGAAAGTCCCTGGAAACCGCTCAAGTACTGTATCATGGTGAC  
 TTTGCATTTTTGGAGAGCACGCCCCACTCCACCATTGGTCCACGTACCCTATGGGGGAG  
 TGGTTTATGAGTATATAAGGGGCTCCGGTTTAGAAGCCGGGCAGAgcgccgc**atgggc**  
**tccaaaccttctaccaggatcccgacacctctgatgctgatcaccggattatgctgat**  
**attgggctgtatccgtccgacaagctctcttgacggcaggcctcttgacgctgcaggaa**  
**ttgtagtaacaggagataaggcagtcgaatgtatacacttcgtctcagacagggtcaatc**

Figure 14 (Continued)

atagtcaagttgctcccgaatatgccaggggataaggaggcgtgtgcaaaagccccatt  
 agaggcatataacagaacactgactactttgtcactcctcttggcgactccatccgca  
 agatccaagggctctgtgtccacatctggaggaggcaagcaaggccgcctgataggtgct  
 gttattggcagtgtagctcttgggggttgcaacagcggcacagataacagcagctgcggc  
 cctaatacaagccaaccagaatgccgccaacatcctccggcttaaggagagcattgctg  
 caaccaatgaagctgtgcatgaagtcaccgacggattatcacaaactatcagtggcagtt  
 gggaagatgcagcagtttgtcaatgaccagtttaataatacggcgcgagaattggactg  
 tataaaaatcacacaacaggttggtgtagaactcaacctatacctaactgaattgacta  
 cagtattcggggccacagatcacctcccctgcattaactcagctgaccatccaggcactt  
 tataatttagctggtggcaatatggattacttattaactaagtttaggtatagggaaaca  
 tcaactcagctcgttaattggttagcggcctgatcactggttaccctatactgtatgact  
 cacagactcaactcttgggcatacaagtgaatttaccctcagtcgggaacttaaataat  
 atgcgtgccacctatttggagaccttatctgtaagtacaaccaaaggatatgcctcagc  
 acttgtcccgaagtagtgacacaagtcggttccgtgatagaagagccttgacacctcat  
 actgtatagagtccgatcttgatttatattgtactagaatagtgcattccccatgtcc  
 ccaggtatttattcctgtttgagcggcaacacatcagcttgcatgtattcaaagactga  
 aggcgcactcactacgccgtatatggcccttaaaggctcagttattgccaattgtaaaa  
 taacaacatgtagatgtacagaccctcctgggtatcatatcgcaaaattatggagaagct  
 gtatccctgatagatagacattcgtgcaatgtcttatcattagacgggataactctaag  
 gctcagtggggaatttgatgcaacttatcaaaagaacatctcaatactagattctcaag  
 tcatcgtgacaggcaatcttgatataatcaactgaacttggaacgtcaacaattcaatc  
 agcaatgccttggaataggttggcagaaagcaacagcaagctagaaaaagtcaatgtcag  
 actaaccagcacatctgctctcattacctataattgtttctaactgtcattttctctagttt  
 tcggtgcacttagtctggtgtagcgtgttacctgatgtacaaacagaaggcacaacaa  
 aagaccttgctatggcttgggaataataccctcgatcagatgagagccactacaagagc  
atgagcggccgcgatatacaataaaaatatctttatttttcattacatctgtgtgtttggttt  
tttgtgtgaatcgatagtaactaacatacgcctctccatcaaaaacaaaacgaaacaaaaca  
aactagcaaaaataggctgtccccagtgcaagtgcaaggtgccagaacattttctcttctag  
 acctgcaggggagtcctgtgcaagggttaatgaacctcgcagttcattcgggaagttataac  
 tgccgccttcgcacattttcttttgtcctgttttgtattgccataacagataggaattg  
 aaacctgatcctcctgttttttgcagcatggccagcaacagaataactttgtcggatcga  
 ctacttgcgcgagatgggttcctgttggagggttccggcgggtcgggtggagaacctat  
 tattttatacacacaogtcataaccgttgtcgogaaaaatgttcttttgtctttotgcogtct  
 cgaacgtcgggttcccacgttagacgttaggagcgttggaatggtatcaggaagagocccac  
 ggcattgcgggaaccaagtaccgcctactttgaccgcgagcagctctcttcggtaattgggat  
 gtattccagagcagcgcggcagagatcagcgggccccactatccacagactgtatgaag  
 tgttttctgaaacatcggactccaacatcaaatatccagacataacatcttgccattcg  
 gaagcacatccgcccagatcttcaaataagcctaactataaaacgagtcctctagttcctgc  
 taacctcagtaacctcgaatgccagtcocatccgggtgggttcgtcctgataatcgggtctct  
 gacgcgcgaggaagaactaaaaggggtctggaaaagcggaacagatctgcagacogaacg  
 actacagacacgcccacatcatcatgtatctgttccatgcattgctttatgagaaaaat  
 ccataaggccgagggcggcatctctagatctcccggggagtcctctogcaotcatctagga  
 gagtgcgcagcagttatcatagacacgcccattttgtgcacccaaacgaaaagttcctgtac  
 tgggtggagcgtcggcgcggggaatcgggtccgtgctctgaaaccagtggtctagacagaaga

Figure 14 (Continued)

ccatccggtaaattctgggtgtatgaactgacgggtctccagacgaacgctogaagacatta  
acgatggaaaactaacgagctttcttcaaaaagtgtctgattacaacgctaatagacctta  
cgaaactatacgcagcgataccagtgacacagatccgctcgggtgtcg

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2012/067135

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K39/12  
ADD.

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , BIOSIS, Sequence Search , EMBASE, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 980 906 A (AUDONNET JEAN-CHRISTOPHE FRANC [FR] ET AL) 9 November 1999 (1999-11-09) cited in the applicati on	1-5 , 11 , 13-15 , 21-26 , 28-34
Y	col umn 1, line 5 - line 14 col umn 3, line 20 - line 32 col umn 3, line 49 - line 54 col umn 2, line 5 - line 24 examples 9, 12, 13 col umn 12, line 24 - line 26 ----- -/- .	8-10, 18-20



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 March 2013

Date of mailing of the international search report

20/03/2013

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/067135

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WO 2008/038845 AI (KBNP INC [KR] ; BIOPOA INC [KR] ; CHO SUN-HEE [KR] ; KWON HYUK-JOON [KR] ; ) 3 April 2008 (2008-04-03)</p> <p>The query sequence SEQ ID NO:2 has 98.00 % identity (99.10 % similarity) over 553 positions in a common overlap (range (q: s): 1-553 : 1-553) with subject GSP:ARL55197 (length : 553) from W02008038845-A1 published on 2008-04-03 .</p> <p>The query sequence SEQ ID NO:4 has 98.00 % identity (99.10 % similarity) over 553 positions in a common overlap (range (q: s): 1-553 : 1-553) with subject GSP:ARL55197 (length : 553) from W02008038845-A1 published on 2008-04-03 .</p> <p>The query sequence SEQ ID NO:37 has 97.10 % identity (99.30 % similarity) over 553 positions in a common overlap (range (q: s): 1-553 : 1-553) with subject GSP:ARL55197 (length : 553) from W02008038845-A1 published on 2008-04-03 .</p> <p>-----</p>	2-5 , 11-20, 22-34
Y	<p>FR 2 751 225 AI (RHONE MERIEUX [FR] ) 23 January 1998 (1998-01-23)</p> <p>The query sequence SEQ ID NO:8 has 100.00 % identity (100.00 % similarity) over 453 positions in a common overlap (range (q: s): 1-453 : 1-453) with subject GSP:AAW44942 (length : 453) from FR2751225-A1 published on 1998-01-23 .</p> <p>The query sequence SEQ ID NO:33 has 100.00 % identity (100.00 % similarity) over 553 positions in a common overlap (range (q: s): 1-553 : 1-553) with subject GSP:AAW44941 (length : 553) from FR2751225-A1 published on 1998-01-23 .</p> <p>The query sequence SEQ ID NO:35 has 99.30 % identity (99.60 % similarity) over 553 positions in a common overlap (range (q: s): 1-553 : 1-553) with subject GSP:AAW44941 (length : 553) from FR2751225-A1 published on 1998-01-23 .</p> <p>-----</p>	2-5 , 11-20, 22-34
Y	<p>DATABASE Uni Prot [Online]</p> <p>1 November 1996 (1996-11-01) , "SubName: Full=Polypeptide n; Flags : Fragment; ", XP002693064, retrieved from EBI accession no. UNIPROT:Q64957 Database accession no. Q64957 sequence</p> <p>-/--</p>	2-5 , 11-20, 22-34

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/067135

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>- &amp; VAKHARIA V N ET AL: "USE OF POLYMERASE CHAIN REACTION FOR EFFICIENT CLONING OF DSRNA SEGMENTS OF INFECTIOUS BURSAL DISEASE VIRUS", AVIAN DISEASES, AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS, KENNET SQ. , PA, US, vol . 36, no. 3, 1 January 1992 (1992-01-01) , pages 736-742 , XP000872361 , ISSN: 0005-2086</p> <p>The query sequence SEQ ID N0:42 has 100.00 % identity (100.00 % similarity) over 453 positions in a common overlap (range (q: s): 1-453 : 1-453) with subject UNI PR0T:Q64957 (length : 473) .</p> <p>-----</p> <p>DATABASE Uni Prot [Online]</p> <p>23 October 2007 (2007-10-23) , "RecName: Full=Fusion glycoprotein F0; ", XP002693065 , retrieved from EBI accession no. UNI PR0T:A7XL40</p> <p>Database accession no. A7XL40</p> <p>The query sequence SEQ ID N0:6 has 99.30 % identity (99.60 % similarity) over 553 positions in a common overlap (range (q: s): 1-553 : 1-553) with subject UNI PR0T:A7XL40 (length : 553) . ; sequence</p> <p>- &amp; MILLER ET AL: "Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge", VACCINE, ELSEVIER LTD, GB, vol . 25, no. 41, 27 September 2007 (2007-09-27) , pages 7238-7246, XP022276678, ISSN: 0264-410X, DOI : 10.1016/J.VACCINE.2007.07.017</p> <p>The query sequence SEQ ID N0:6 has 99.30 % identity (99.60 % similarity) over 553 positions in a common overlap (range (q: s): 1-553 : 1-553) with subject UNI PR0T:A7XL40 (length : 553) .</p> <p>-----</p> <p>-/--</p>	2-5 , 11-20, 22-34

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/067135

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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