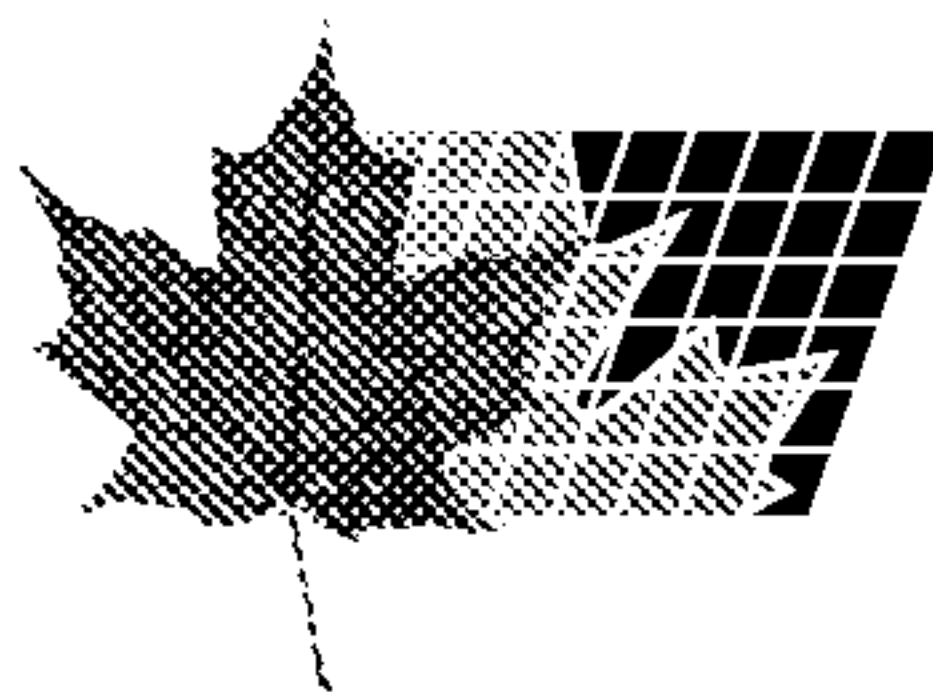




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(54) **VIRUS CHIMERES DE RECOMBINAISON ET LEURS
UTILISATIONS**

(54) **RECOMBINANT CHIMERIC VIRUSES AND USES THEREOF**

(57) Cette invention se rapporte à un virus chimère de recombinaison de l'herpèsvirus des dindes et du virus de la maladie de Marek. Ce virus chimère de recombinaison comprend une région génomique virale, longue, unique de l'herpèsvirus des dindes et une région génomique virale, courte, unique du virus de la maladie de Marek.

(57) This invention provides a recombinant herpesvirus of turkeys-Marek's disease virus chimera comprising a herpesvirus of turkeys unique long viral genome region and a Marek's disease virus unique short viral genome region.

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(54) Title: RECOMBINANT CHIMERIC VIRUSES AND USES THEREOF (57) Abstract This invention provides a recombinant herpesvirus of turkeys-Marek's disease virus chimera comprising a herpesvirus of turkeys unique long viral genome region and a Marek's disease virus unique short viral genome region.		

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RECOMBINANT CHIMERIC VIRUSES AND USES THEREOF

This application claims priority of U.S. serial No. 08/804,372, filed February 21, 1997.

Throughout this application various publications are referenced by Arabic numerals in parenthesis. Full citations for these publications may be found at the end of the specification preceding the claims. The disclosures of these publications are in their entirety hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

The ability to isolate DNA and clone such isolated DNA into bacterial plasmids has greatly expanded the approaches available to make viral vaccines. The methods used to make the present invention involve modifying cloned DNA sequences from various viral pathogens of animals, by insertions, deletions, single or multiple base changes, and subsequent insertions of these modified sequences into the genome of the virus. One utility of the addition of a foreign sequence is achieved when the foreign sequence encodes a foreign protein that is expressed during viral infection of the animal. The resulting live virus may then be used in a vaccine to elicit an immune response in a host animal and provide protection to the animal against disease. A virus with these characteristics is referred to as a viral vector, because it becomes a living vector that will carry and

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express the foreign protein in the host animal. In effect it becomes an elaborate delivery system for the foreign protein(s).

5 The application of recombinant DNA techniques to animal viruses has a relatively recent history. The first viruses to be engineered have been those with the smallest genomes. In the case of the papovaviruses, because these viruses are so small and cannot accommodate much extra DNA, their use in
10 genetic engineering has been as defective replicons. Foreign gene expression from these viruses requires a wild-type helper virus and is limited to cell culture systems. For adenoviruses, there is a small amount of nonessential DNA that can be replaced by foreign sequences. The only
15 foreign DNA that seems to have been expressed in adenoviruses are the T-antigen genes from papovaviruses (Mansour, et al., Proc. Natl. Acad. Sci. US, 1985; Thummel, et al., Cell, 1983; Scolnick, et al., Cell, 1981; Thummel, et al., Cell, 1981), and the herpes simplex virus (HSV)
20 thymidine kinase gene (Haj-Ahmed and Graham, J. of Virology, 1986). These publications do not identify the nonessential regions in HVT wherein foreign DNA may be inserted, nor do they teach how to achieve the expression of foreign genes in HVT, e.g., which promoter sequence and termination sequence
25 to use.

Another group of viruses that have been engineered are the poxviruses. One member of this group, vaccinia, has been the subject of much research on foreign gene expression.
30 Poxviruses are large DNA-containing viruses that replicate in the cytoplasm of the infected cell. They have a structure that is unique in that they do not contain any capsid that is based upon icosahedral symmetry or helical symmetry. The poxviruses are most likely to have evolved
35 from bacterial-like microorganisms through the loss of

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function and degeneration. In part due to this uniqueness, the advances made in the genetic engineering of poxviruses cannot be directly extrapolated to other viral systems, including herpesviruses and HVT. Vaccinia recombinant virus constructs have been made in a number of laboratories that express the following inserted foreign genes: HSV thymidine kinase gene (Mackett, et al., Proc. Natl. Acad. Sci. USA, 1982; Panicali and Paoletti, Proc. Natl. Acad. Sci. USA, 1982, hepatitis B surface antigen (Paoletti, et al., Proc. Natl. Acad. Sci. USA, 1984; Smith et al., Nature, 1983), HSV glycoprotein D gene, influenzae hemagglutinin gene (Panicali, et al., Proc. Natl. Acad. Sci. USA, 1983; Smith, et al., Proc. Natl. Acad. Sci. USA, 1983), malaria antigen gene (Smith, et al., Science, 1984, and vesicular stomatitis glycoprotein G gene (Mackett, et al., Science, 1986). The general overall features of vaccinia recombinant DNA work are similar to the techniques used for all the viruses, especially as they relate to the techniques in reference (Maniatis, et al., Molecular Cloning, 1982). However in detail, the vaccinia techniques are not applicable to herpesviruses and HVT. The utility of vaccinia as a vaccine vector is in question because of its close relationship to human smallpox and its known pathogenicity to humans. Thus, the use of the host-specific herpesvirus HVT is a better solution to vaccination of poultry.

Among the primate herpesviruses, only HSV of humans and, to a limited extent, herpes saimiri of monkeys have been engineered to contain foreign DNA sequences. The first use of recombinant DNA to manipulate HSV involved cloning a piece of DNA from the L-S junction region into the unique long region of HSV DNA, specifically into the thymidine kinase gene (Mocarski, et al., Cell, 1980). This insert was not a foreign piece of DNA, rather it was a naturally occurring piece of herpesvirus DNA that was duplicated at

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another place in the genome. This piece of DNA was not engineered to specifically express a protein, and thus this work does not involve expression of protein in herpesviruses. The next manipulation of HSV involved the creation of deletions in the virus genome by a combination of recombinant DNA techniques and thymidine kinase selection. Using this approach, the HSV alpha-22 gene has been deleted (Post, et al., Cell, 1981), and a 15,000 basepair sequence of DNA has been deleted from the internal repeat of HSV (Poffenberger, et al., Proc. Natl. Acad. Sci. USA, 1981).

The following cases involve insertion of genes that encode protein into herpesviruses: the insertion of HSV glycoprotein C into a naturally occurring deletion mutant of this gene in HSV (Gibson and Spear, J. of Virology, 1983); the insertion of glycoprotein D of HSV type 2 into HSV type 1 (Lee, et al., Proc. Natl. Acad. Sci. USA, 1982), with no manipulation of promoter sequences since the gene is not 'foreign'; the insertion of hepatitis B surface antigen into HSV under the control of the HSV ICP4 promoter (Shih, et al., Proc. Natl. Acad. Sci. USA, 1984); and the insertion of bovine growth hormone into herpes saimiri virus with an SV40 promoter (the promoter did not work in this system and an endogenous upstream promoter served to transcribe the gene) (Desrosiers, et al., 1984). Two additional foreign genes (chicken ovalbumin gene and Epstein-Barr virus nuclear antigen) have been inserted into HSV (Arsenakis and Roizman, 1984), and glycoprotein X of pseudorabies virus has been inserted into HSV (Post, et al., 1985).

These cases of deletion or insertion of genes into herpesviruses demonstrate that it is possible to genetically engineer herpesvirus genomes by recombinant DNA techniques. The methods that have been used to insert genes involve

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homologous recombination between the viral DNA cloned in plasmids and purified viral DNA transfected into the same animal cell. However, the extent to which one can generalize the location of the deletion and the sites for insertion of foreign genes is not known from these previous studies.

One object of the present invention is a vaccine for Marek's disease. Marek's disease virus (MDV) is the causative agent of Marek's disease which encompasses fowl paralysis, a common lymphoproliferative disease of chickens. The disease occurs most commonly in young chickens between 2 and 5 months of age. The prominent clinical signs are progressive paralysis of one or more of the extremities, incoordination due to paralysis of legs, drooping of the limb due to wing involvement, and a lowered head position due to involvement of the neck muscles. In acute cases, severe depression may result. In the case of highly oncogenic strains, there is characteristic bursal and thymic atrophy. In addition, there are lymphoid tumors affecting the gonads, lungs, liver, spleen, kidney and thymus (Mohanty and Dutta, 1981).

Most chickens are vaccinated against MDV at one day of age to protect the bird against MDV for life. Prior to the present invention, the principal vaccination method for MDV involved using naturally occurring strains of turkey herpesvirus (HVT). It would be advantageous to incorporate other antigens into this vaccination at one day of age, but efforts to combine conventional vaccines have not proven satisfactory to date due to competition and immunosuppression between pathogens. The multivalent HVT-based vaccines engineered in this invention represent a novel way to simultaneously vaccinate against a number of different pathogens. For the first time, a recombinant HVT with a foreign gene inserted into a non-essential region of

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the HVT genome is disclosed.

5 The types of genetic engineering that have been performed on these herpesviruses consist of cloning parts of the virus DNA into plasmids in bacteria, reconstructing the virus DNA while in the cloned state so that the DNA contains deletions of certain sequences, and furthermore adding foreign DNA sequences either in place of the deletions or at sites removed from the deletions.

10

A foreign gene of interest targeted for insertion into the genome of HVT may be obtained from any pathogenic organism of interest. Typically, the gene of interest will be derived from pathogens that in poultry cause diseases that have an economic impact on the poultry industry. The genes may be derived from organisms for which there are existing vaccines, and because of the novel advantages of the vectoring technology the HVT derived vaccines will be superior. Also, the gene of interest may be derived from pathogens for which there is currently no vaccine but where there is a requirement for control of the disease. Typically, the gene of interest encodes immunogenic polypeptides of the pathogen, and may represent surface proteins, secreted proteins and structural proteins.

25

A relevant avian pathogen that is a target for HVT vectoring is Infectious Laryngotracheitis virus (ILTV). ILTV is a member of the herpesviridae family, and this pathogen causes an acute disease of chickens which is characterized by respiratory depression, gasping and expectoration of bloody exudate. Viral replication is limited to cells of the respiratory tract, where in the trachea the infection gives rise to tissue erosion and hemorrhage. In chickens, no drug has been effective in reducing the degree of lesion formation or in decreasing clinical signs. Vaccination of

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birds with various modified forms of the ILT virus derived by cell passage and/or tedious regimes of administration have conferred acceptable protection in susceptible chickens. Because of the degree of attenuation of current
5 ILT vaccines care must be taken to assure that the correct level of virus is maintained; enough to provide protection, but not enough to cause disease in the flock.

An additional target for the HVT vectoring approach is
10 Newcastle disease, an infectious, highly contagious and debilitating disease that is caused by the Newcastle disease virus (NDV). NDV is a single-stranded RNA virus of the paramyxovirus family. The various pathotypes of NDV
15 (velogenic, mesogenic, lentogenic) differ with regard to the severity of the disease, the specificity and symptoms, but most types seem to infect the respiratory system and the nervous system. NDV primarily infects chickens, turkeys and other avian species. Historically vaccination has been used to prevent disease, but because of maternal antibody
20 interferences, life-span of the bird and route of administration, the producer needs to adapt immunization protocols to fit specific needs.

The therapeutic agent that is delivered by a viral vector of
25 the present invention must be a biological molecule that is a by-product of swinepox virus replication. This limits the therapeutic agent in the first analysis to either DNA, RNA, or protein. There are examples of therapeutic agents from each of these classes of compounds in the form of anti-sense
30 DNA, anti-sense RNA (S. Joshi, et al., J. of Virology, 1991), ribozymes (M. Wachsman, et al., J. of General Virology, 1989), suppressor tRNAs (R.A. Bhat, et al., Nucleic Acids Research, 1989), interferon-inducing double stranded RNA and numerous examples of protein therapeutics,
35 from hormones, e.g., insulin, to lymphokines, e.g.,

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interferons and interleukins, to natural opiates. The discovery of these therapeutic agents and the elucidation of their structure and function does not make obvious the ability to use them in a viral vector delivery system.

5

A number of genes encoding cytokines have been cloned in mammalian species. Fewer cytokine genes have been cloned in avian species. The genes encoding type I and type II interferons have been cloned and characterized in several mammalian species. The gene for chicken interferon-a was cloned, and the amino acid sequence was compared to mammalian interferon type I and shown to have 20-24% amino acid sequence identity. ChIFN-a is unrelated to mammalian IFN-g (59, 65). The gene for chicken interferon-g was cloned, and the amino acid sequence shares 15% identity to ChIFN-a. ChIFN-g is 35 and 32% identical to the equine and human IFN-g, respectively (62, 63, 66). A gene for duck interferon was cloned and is 50% identical at the amino acid level to ChIFN-a (64). The chicken has more than 20 interferon genes which have not yet been classified. It is not known whether the cloned duck IFN should be classified as IFN-a, -b, or -g. The avian interferon activities are species specific. An avian interferon is not active in a different avian or mammalian species or a different avian or mammalian cell line. Due to the lack of cross species interferon activities and the low percentage identity of amino acid sequences between interferons of different species, it is not obvious that one can clone and express genes from different avian or mammalian species.

25
30

SUMMARY OF THE INVENTION

This invention provides a recombinant herpesvirus of turkeys -Marek's disease virus chimera comprising a herpesvirus of turkeys unique long viral genome region and a Marek's disease virus unique short viral genome region.

35

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C: Details of HVT Construction and Map Data.

5 Figure 1A shows BamHI restriction fragment map of the HVT genome. Fragments are numbered in order of decreasing size; letters refer to small fragments whose comparative size has not been determined.

10 Figure 1B shows BamHI #16 fragment of the HVT genome showing location of β -galactosidase gene insertion in S-HVT-001.

15 Figure 1C shows BamHI #19 fragment of the HVT genome showing location of β -galactosidase gene insertion.

Legend: B = BamHI; X = XhoI; H = HindIII; P = PstI; S = Sall; N = NdeI; R = EcoRI.

20 Figure 2: BamHI, NotI restriction map of the HVT genome. The unique long (UL) and unique short (US) regions are shown. The long and short region repeats are indicated by boxes. The BamHI fragments are numbered in decreasing order of size. The location of probes P1-P4 are indicated. The origin of each probe is as follows: P1 - BamHI #6, P2 - BamHI #2, P3 - BamHI #13, and P4 - 4.0 kb BgIII to StuI sub-fragment of HVT genomic XbaI fragment #5 (8.0 kb).

30

Figures 3A-3B:

35 Show how the unique XhoI site of the BamHI #10 fragment of the HVT genome was converted into a PacI site and a NotI site by insertion of the synthetic DNA sequence at the XhoI site

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(Nucleotides #1333-1338; SEQ ID NO. 12). Figure 3A shows the Xho site converted into a PacI site to generate Plasmid 654-45.1 (SEQ ID NO. 17) and Figure 3B shows the XhoI site converted into a NotI site to generate Plasmid 686-63.A1 (SEQ ID NO. 18).

Figure 4:

Restriction map and open reading frames of the sequence surrounding the insertion site within the unique long of HVT (SEQ ID NO. 12). This map shows the XhoI restriction site (SEQ ID NO. 12; Nucl. 1333-1338) used for insertion of foreign genes. Also shown are four open reading frames within this sequence. ORF A is interrupted by insertion of DNA into the XhoI site. The ORF A amino acid sequence (SEQ ID NO. 14; Nucl. 1402 to 602; 267 amino acids) shows no significant sequence identity to any known amino acid sequence in the protein databases. UL 54 (SEQ ID NO. 13; Nucl. 146 to 481; 112 amino acids) and UL55 (SEQ ID NO. 15; Nucl. 1599 to 2135; 179 amino acids) show significant sequence identity to the herpes simplex virus type I UL54 and UL55 proteins, respectively. ORF B (SEQ ID NO. 16; Nucl. 2634 to 2308; 109 amino acids) shows no significant sequence identity to any known amino acid sequence in the protein databases. Searches were performed on NCBI databases using Blast software.

Figure 5:

Restriction map of cosmids 407-32.1C1, 672-01.A40, 672-07.C40, and 654-45.1. The overlap of HVT genomic DNA fragments EcoRI #9 and BamHI #10 is illustrated. A unique XhoI site within the EcoRI

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#9 and BamHI #10 fragments has been converted to a unique PacI site in Plasmid 654-45.1 or a unique NotI site in Plasmid 686-63.A1.

5 Figure 6:

Expression of β -galactosidase from chicken anemia virus promoter and HCMV immediate early promoter in transient transfection assays in BES-treated chicken embryo fibroblast. Expression of β -galactosidase was measured by ONPG assay at 0, 20, 40, and 60 minutes and expressed as OD₄₁₅. The protocol is described in TRANSIENT TRANSFECTION ASSAY. Plasmids 388-65.2 contains the immediate early promoter. Plasmid 850-80.2, 850-25.18 and 850-69.1 contain chicken anemia virus promoters as described in Materials and Methods.

Figure 7:

DNA sequence of the infectious laryngotracheitis virus (ILTV) glycoprotein D (gD) which is useful for the expression of foreign DNA in recombinant herpesvirus of turkeys or in recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. (SEQ ID NO. __)

Figure 8:

DNA sequence of the infectious laryngotracheitis virus (ILTV) glycoprotein I (gI) which is useful for the expression of foreign DNA in recombinant herpesvirus of turkeys or in recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. (SEQ. ID NO. __)

35

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a recombinant herpesvirus of turkeys -Marek's disease virus chimera comprising a herpesvirus of turkeys long viral genome region and a Marek's disease virus short viral genome region. This invention provides a recombinant herpesvirus of turkeys -Marek's disease virus chimera comprising a herpesvirus of turkeys unique long viral genome region and a Marek's disease virus unique short viral genome region.

In one embodiment the foreign DNA sequence is inserted within a non-essential region of the turkeys - Marek's disease virus chimera viral genome, and is capable of being expressed in a host cell. In another embodiment the foreign DNA sequence is inserted within an EcoR1 #9 fragment of the unique long region of the turkeys - Marek's disease virus chimera viral genome.

In another embodiment the foreign DNA sequence encodes a polypeptide. For example, the foreign DNA sequence may encode a cytokine such as chicken myelomonocytic growth factor (cMGF), chicken interferon (cIFN) or quail interferon (qIFN). Alternatively the foreign DNA sequence may encode an antigenic polypeptide selected from the group consisting of: Marek's disease virus, Newcastle disease virus, Infectious laryngotracheitis virus, Infectious bronchitis virus and Infectious bursal disease virus.

In another embodiment the foreign DNA sequence is under control of an endogenous upstream herpesvirus promoter. Examples of promoters include but are not limited to: heterologous upstream promoters such as PRV gX, HSV-1 alpha 4, HCMV immediate early, MDV gA, MDV gB, MDV gD, ILT gB, BHV-1.1 VP8, ILT gD and chicken anemia virus (CAV) promoter. Examples of the recombinant chimeric virus include but are not limited to: S-HVY-145, S-HVY-149, and S-HVY-152.

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This invention provides a vector which comprises the of the isolated nucleic acid molecule encoding chicken Interferon. This invention provides a host cell containing the vector. This invention provides a recombinant DNA which comprises
5 the complementary sequence of the isolated nucleic acid molecule encoding chicken Interferon. The complementary sequence is the anti-sense strand. In one embodiment the antisense nucleic acid molecule hybridizes to the mRNA of the chicken interferon.

10

This invention provides a method for enhancing the immune response of an animal by vaccinating the animal with the recombinant virus which comprises a foreign DNA. Examples of foreign DNA include but are not limited to; cytokines, such
15 as the sense strand of the nucleic acid sequence encoding quail interferon and chicken interferon, and viruses, such as chicken anemia virus, MDV, NDV, ILT, or IBDV. Alternatively, it is contemplated that an isolated polypeptide which is expressed from the recombinant virus
20 may be used in combination with a vaccine of a killed or attenuated virus so as to enhance the immune response of the animal.

25

This invention provides a method for enhancing the immune response of an animal by vaccinating the animal with a vaccine containing a vector which comprises the complementary sequence of the sense strand of the isolated nucleic acid molecule encoding chicken Interferon.

30

This invention provides a vector which comprises the isolated nucleic acid molecule encoding chicken Interferon.

35

This invention provides an isolated nucleic acid comprising a promoter sequence normally associated with the transcription of a gene of the chicken anemia virus (CAV). This invention provides an isolated nucleic acid encoding a chicken anemia virus promoter. In one embodiment the

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isolated nucleic acid encoding a chicken anemia virus promoter has the nucleic acid sequence as set forth in SEQ. ID. NO. 23.

5 This invention provides a vector which comprises the isolated nucleic acid encoding a chicken anemia virus promoter. This invention provides a recombinant herpesvirus which comprises a foreign DNA sequence under the control of the chicken anemia virus promoter.

10

This invention provides a recombinant herpesvirus or recombinant pox virus comprising a foreign DNA sequence inserted into a non-essential site in the HVT genome, wherein the foreign DNA sequence is capable of being
15 expressed in a host cell infected with the recombinant HVT and its expression is under the control of chicken anemia virus promoter.

This invention provides a recombinant chimeric virus
20 comprising a foreign DNA sequence inserted into a non-essential site in the HVT genome. The foreign DNA sequence is capable of being expressed in a host cell infected with the recombinant chimeric virus and its expression is under the control of a promoter located upstream of the foreign
25 DNA sequence.

As defined herein "a non-essential site" in the recombinant chimeric virus genome or the HVT genome means a region in the viral genome which is not necessary for the viral
30 infection or replication.

As defined herein, "viral genome" or "genomic DNA" means the entire DNA which the naturally occurring in the virus. As defined herein, "foreign DNA sequence" or "gene" means any
35 DNA or gene that is exogenous to the genomic DNA.

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As defined herein, an "open reading frame" is a segment of DNA which contains codons that can be transcribed into RNA which can be translated into an amino acid sequence and which does not contain a termination codon.

5

The invention further provides several appropriate insertion sites in the virus, either HVT or MDV genome, useful for constructing the recombinant chimeric virus of the present invention. Insertion sites include the EcoRI #9 fragment and the BamHI #10 fragment of the HVT genome, a preferred
10 insertion site within both of those fragments being a XhoI restriction endonuclease.

This invention provides a recombinant chimeric virus
15 comprising a foreign DNA sequence inserted within the EcoRI #9 fragment of the herpesvirus of turkeys viral genome, and the foreign DNA sequence is capable of being expressed in a host cell infected with the herpesvirus of turkeys.

20 In one embodiment, the foreign DNA sequence is inserted within an Open Reading Frame A (ORFA) of the EcoRI #9 fragment. Insertion of foreign DNA sequences into the XhoI site of EcoRI #9 interrupts ORFA indicated that the entire ORFA region is non-essential for replication of the
25 recombinant.

For purposes of this invention, "a recombinant chimeric virus" and "a recombinant herpesvirus of turkeys" are live viruses which have been generated by the recombinant methods
30 well known to those of skill in the art, e.g., the methods set forth in DNA TRANSFECTION FOR GENERATING RECOMBINANT in Materials and Methods, and the virus has not had genetic material essential for the replication of the recombinant chimera viurs or the recombinant herpesvirus of turkeys
35 deleted. The purified recombinant chimera virus and the

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recombinant herpesvirus of turkeys results in stable insertion of foreign DNA sequences or a gene in the EcoR1 #9 fragment or BamH1 #10 fragment.

- 5 The invention further provides recombinant chimeric virus where the foreign DNA sequence encodes a polypeptide which is antigenic in an animal into which the recombinant chimeric virus is introduced.
- 10 In one embodiment the polypeptide is a detectable marker. For purposes of this invention, a "polypeptide which is a detectable marker" includes the dimer, trimer and tetramer form of the polypeptide. E. coli B-galactosidase is a tetramer composed of four polypeptides or monomer subunits.
- 15 In one embodiment the polypeptide is E. coli beta-galactosidase.

This invention provides a recombinant herpesvirus of turkeys (HVT) comprising a foreign DNA sequence inserted into a non-essential site in the HVT genome. The foreign DNA sequence is capable of being expressed in a host cell infected with the recombinant HVT and its expression is under the control of a promoter located upstream of the foreign DNA sequence.

20

25 In another embodiment the foreign DNA sequence encodes a cytokine. In another embodiment the cytokine is chicken myelomonocytic growth factor (cMGF), chicken interferon (cIFN) or quail interferon. In a preferred embodiment the recombinant herpesvirus of turkeys is designated S-HVT-144.

30 The invention further provides a recombinant herpesvirus of turkeys whose viral genome contains foreign DNA encoding an antigenic polypeptide which is from Marek's disease virus (MDV), Newcastle disease virus (NDV), infectious laryngotracheitis virus (ILTV), infectious bronchitis virus

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(IBV) or infectious bursal disease virus (IBDV).

This invention provides a recombinant herpesvirus of turkeys with a foreign DNA sequence insertion in the EcoR1 #9
5 fragment which further comprises a foreign DNA sequence encoding the antigenic polypeptide selected from the group consisting of: Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, infectious bronchitis virus and infectious bursal disease virus.

10

In one embodiment the foreign DNA sequence encoding the antigenic polypeptide is from Marek's disease virus and encodes Marek's disease virus glycoprotein gA, Marek's disease virus glycoprotein gB or Marek's disease virus
15 glycoprotein gD. In another embodiment the foreign DNA sequences encoding the Marek's disease virus glycoprotein gA, glycoprotein gB or glycoprotein gD are inserted into the unique StuI site of the US2 gene coding region of the herpesvirus of turkeys.

20

The invention further provides recombinant herpesvirus of turkeys whose genomic DNA contains foreign DNA encoding antigenic polypeptide from Marek's disease virus. Preferably, the antigenic polypeptide is Marek's disease
25 virus glycoprotein gB, gA or gD.

In one embodiment a recombinant HVT containing a foreign DNA sequence encodes IBDV VP2, MDV gA, and MDV gB. Preferably, such recombinant virus is designated S-HVT-137 and S-HVT-
30 143.

The present invention provides a recombinant chimeric virus containing a foreign DNA sequence encoding an antigenic polypeptide from Newcastle disease virus (NDV). In such
35 case, it is preferred that the antigenic polypeptide is

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Newcastle disease virus fusion (F) protein or Newcastle disease virus hemagglutinin-neuraminidase (HN), or a recombinant protein comprising E. coli B-galactosidase fused to Newcastle disease virus hemagglutinin-neuraminidase (HN).

The present invention also provides recombinant chimeric viruses engineered to contain one or more foreign DNA sequence encoding an antigenic polypeptide from MDV as well as one or more foreign DNA sequences encoding an antigenic polypeptide from NDV. Preferably, the MDV antigenic polypeptide is MDV gB, gD, or gA and the NDV F or HN.

The invention further provides recombinant chimeric virus whose genomic DNA contains foreign DNA encoding antigenic polypeptide from Marek's disease virus and further comprising foreign DNA encoding antigenic polypeptide from Newcastle disease virus.

Further, in one embodiment the foreign DNA sequence encodes the antigenic polypeptide from an infectious laryngotracheitis virus and encodes infectious laryngotracheitis virus glycoprotein gB, infectious laryngotracheitis virus glycoprotein gI or infectious laryngotracheitis virus glycoprotein gD.

In another embodiment the foreign DNA sequence encodes an antigenic polypeptide which is derived or derivable from a group consisting of: MDV gA, MDV gB, MDV gD, NDV HN, NDV F, ILT gB, ILT gI, ILT gD, IBV, IBDV VP2, IBDV VP3, IBDV VP4, avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus (agent), Salmonella spp. E. coli, Pasteurella spp., Bordetella spp., Eimeria spp., Histomonas spp., Trichomonas

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spp., Poultry nematodes, cestodes, trematodes, poultry mites/lice, poultry protozoa. In a preferred embodiment the recombinant herpesvirus of turkeys is designated S-HVT-136.

5 The invention further provides a recombinant herpesvirus of turkeys which contains a foreign DNA sequence encoding an antigenic polypeptide from infectious laryngotracheitis virus. It is preferred that the antigenic polypeptide is ILTV glycoprotein gB, ILTV gD or ILTV gI.

10

In one embodiment the foreign DNA sequence is from an infectious laryngotracheitis virus and encodes infectious laryngotracheitis virus glycoprotein gD, or laryngotracheitis virus glycoprotein gI.

15

This invention provides a recombinant herpesvirus of turkeys containing a foreign DNA sequence inserted into the EcoR1 #9 fragment herpesvirus of turkeys viral genome wherein the foreign DNA sequence is from an Newcastle disease virus and
20 encodes a Newcastle disease virus HN or Newcastle disease virus F.

Such antigenic polypeptide may be derived or derivable from the following: feline pathogen, canine pathogen, equine
25 pathogen, bovine pathogen, avian pathogen, porcine pathogen, or human pathogen.

In another embodiment, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, herpes simplex
30 virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicell-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, human immunodeficiency virus, rabies virus, measles virus, hepatitis B virus and hepatitis C virus. Furthermore, the
35 antigenic polypeptide of a human pathogen may be associated

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with malaria or malignant tumor from the group consisting of Plasmodium falciparum, Bordetella pertussis, and malignant tumor.

5 The invention further provides recombinant herpes virus of turkeys whose genomic DNA contains foreign DNA encoding Newcastle disease virus fusion (F) protein and further comprising foreign DNA encoding a recombinant protein, wherein E. coli B-galactosidase is fused to Newcastle
10 disease virus hemagglutinin-neuraminidase (HN).
The invention further provides recombinant chimeric virus whose genomic DNA contains foreign DNA encoding Marek's disease virus glycoprotein gB and Marek's disease virus glycoprotein gA and further comprising foreign DNA encoding
15 Newcastle disease virus hemagglutinin-neuraminidase (HN).

This invention provides a recombinant herpesvirus of turkeys-Marek's disease virus chimera comprising a herpesvirus of turkeys unique long viral genome region and
20 a Marek's disease virus unique short region. In one embodiment the recombinant herpesvirus of turkeys-Marek's disease virus chimera contains a foreign DNA sequence inserted within the EcoR1 #9 fragment of the herpesvirus of turkeys viral genome, and the foreign DNA sequence capable
25 of being expressed in a host cell infected with the herpesvirus of turkeys.

In one embodiment the recombinant herpesvirus of turkeys contains a foreign DNA sequence which encodes a polypeptide.
30 The polypeptide may be antigenic in an animal into which the recombinant herpesvirus is introduced.

In another embodiment the polypeptide is E. coli beta-galactosidase. In another embodiment the foreign DNA
35 sequence encodes a cytokine. In another embodiment the

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cytokine is chicken myelomonocytic growth factor (cMGF), chicken interferon (cIFN) or quail interferon.

5 The invention further provides recombinant herpesvirus of turkeys where the foreign DNA sequence encodes a polypeptide which is antigenic in an animal into which the recombinant herpesvirus is introduced.

10 Further, the recombinant herpesvirus of turkeys further comprises a foreign DNA sequence encoding the antigenic polypeptide selected from the group consisting of: Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, infectious bronchitis virus and infectious bursal disease virus.

15 This invention provides a recombinant herpesvirus of turkeys wherein the foreign DNA sequence is under control of an endogenous upstream herpesvirus promoter. In one embodiment the foreign DNA sequence is under control of a heterologous
20 upstream promoter. In another embodiment the promoter is selected from CAV, PRV gX, HSV-1 alpha 4, HCMV immediate early, MDV gA, MDV gB, MDV gD, ILT gB, BHV-1.1 VP8 and ILT gD.

25 This invention provides a homology vector for producing a recombinant chimeric virus by inserting foreign DNA into the viral genome. Examples of homology vectors include: 301-07.YD1, 852-52.II4, 864-74.18, 881-23.28, and 739-27.16.

30 This invention provides a homology vector for producing a recombinant herpesvirus of turkeys by inserting foreign DNA into the viral genome of a herpesvirus of turkey which comprises a double-stranded DNA molecule consisting essentially of: a) double stranded foreign DNA not usually
35 present within the herpesvirus of turkeys viral genome; b) at

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one end the foreign DNA, double-stranded herpesvirus of turkeys DNA homologous to the viral genome located at one side of the EcoR1 #9 site the coding region of the herpesvirus of turkeys viral genome; and c) at the other end of the foreign DNA, double-stranded herpesvirus of turkeys DNA homologous to the viral genome located at the other side of the EcoR1 #9 fragment of the coding region of the herpesvirus of turkeys viral genome. Examples of the homology vectors are designated 751-87.A8.

10

In one embodiment the polypeptide is antigenic in the animal into which the recombinant herpesvirus of turkeys is introduced. In another embodiment the antigenic polypeptide is from a cytokine, Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, or infectious bronchitis virus. In a preferred embodiment the antigenic polypeptide is a chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN), quail interferon, infectious bursal disease virus polyprotein, infectious bursal disease virus VP2 protein, Marek's disease virus glycoprotein gB, Marek's disease virus glycoprotein gA, Marek's disease virus glycoprotein gD, Newcastle disease virus fusion protein, Newcastle disease virus hemagglutinin-neuraminidase, infectious laryngotracheitis virus glycoprotein gB, infectious laryngotracheitis virus glycoprotein gD, infectious bronchitis virus spike protein, or infectious bronchitis virus matrix protein.

In another embodiment the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from an equine pathogen. The antigenic polypeptide of an equine pathogen can be derived from equine influenza virus or equine herpesvirus. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type

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5 A/Prague 56 neuraminidase, equine influenza virus type
A/Miami 63 neuraminidase, equine influenza virus type
A/Kentucky 81 neuraminidase, equine herpesvirus type 1
glycoprotein B, and equine herpesvirus type 1 glycoprotein
D.

10 In another embodiment the double stranded foreign DNA
sequence of the homology vector encodes an antigenic
polypeptide derived from bovine respiratory syncytial virus
or bovine parainfluenza virus. The antigenic polypeptide of
derived from bovine respiratory syncytial virus equine
pathogen can derived from equine influenza virus is bovine
respiratory syncytial virus attachment protein (BRSV G),
bovine respiratory syncytial virus fusion protein (BRSV F),
15 bovine respiratory syncytial virus nucleocapsid protein
(BRSV N), bovine parainfluenza virus type 3 fusion protein,
and the bovine parainfluenza virus type 3 hemagglutinin
neuraminidase.

20 In another embodiment the double stranded foreign DNA
sequence in the homology vector encodes a cytokine capable
of stimulating human immune response. For example, the
cytokine may be, but is not limited to, interleukin-1 -
interleukin-15, interferons, quail interferon, chicken
25 intereferon, granulocyte-macrophage colony stimulating
factors, and interleukin receptors.

For purposes of this invention, a "homology vector" is a
plasmid constructed to insert foreign DNA in a specific site
30 on the genome of a herpesvirus of turkeys.

In one embodiment of the invention, the double-stranded
herpesvirus of turkeys DNA is homologous to DNA sequences
present within the EcoR1 #9 fragment of the herpesvirus of
35 turkeys genome. Preferably, this homology vector is

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designated 172-63.1.

In another embodiment the foreign DNA sequence encodes a screenable marker. Examples of screenable markers, include
5 but are not limited to: E. coli B-galactosidase or E. coli B-glucuronidase.

The invention further provides a vaccine which comprises an effective immunizing amount of a recombinant herpesvirus of
10 turkeys of the present invention and a suitable carrier.

This invention provides a vaccine useful for immunizing a bird against Marek's disease virus which comprises an effective immunizing amount of the recombinant herpesvirus
15 of turkeys and a suitable carrier.

This invention provides a vaccine useful for immunizing a bird against Newcastle disease virus which comprises an effective immunizing amount of the recombinant herpesvirus
20 of turkeys and a suitable carrier.

This invention provides a vaccine useful for immunizing a bird against infectious laryngotracheitis virus which comprises an effective immunizing amount of the recombinant
25 herpesvirus of turkeys and a suitable carrier.

This invention provides a vaccine useful for immunizing a bird against infectious bronchitis virus which comprises an effective immunizing amount of the recombinant herpesvirus
30 of turkeys and a suitable carrier.

This invention provides a vaccine useful for immunizing a bird against infectious bursal disease virus which comprises an effective immunizing amount of the recombinant
35 herpesvirus of turkeys and a suitable carrier.

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This invention provides a multivalent vaccine useful for immunizing a bird against Marek's disease virus and Newcastle disease virus which comprises an effective immunizing amount of the recombinant herpesvirus of turkeys.

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This invention provides a multivalent vaccine useful for immunizing a bird against Marek's disease virus and infectious laryngotracheitis virus which comprises an effective immunizing amount of the recombinant herpesvirus of turkeys and a suitable carrier.

10

This invention provides a multivalent vaccine useful for immunizing a bird against Marek's disease virus and infectious bronchitis virus which comprises an effective immunizing amount of the recombinant herpesvirus of turkeys and a suitable carrier.

15

This invention provides a multivalent vaccine useful for immunizing a bird against Marek's disease virus and infectious bursal disease virus which comprises an effective immunizing amount of the recombinant herpesvirus of turkeys and a suitable carrier.

20

The present invention also provides a method of immunizing a fowl. For purposes of this invention, this includes immunizing a fowl against infectious bursal disease virus, Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, or infectious bronchitis virus. The method comprises administering to the fowl an effective immunizing dose of the vaccine of the present invention. The vaccine may be administered by any of the methods well known to those skilled in the art, for example, by intramuscular, subcutaneous, intraperitoneal or intravenous injection. Alternatively, the vaccine may be administered intranasally or orally.

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This invention provides a host cell infected with the recombinant herpesvirus of turkey. In one embodiment the host cell is an avian cell.

5 For purposes of this invention, a "host cell" is a cell used to propagate a vector and its insert. Infecting the cell was accomplished by methods well known to those skilled in the art, for example, as set forth in DNA TRANSFECTION FOR GENERATING RECOMBINANT HERPESVIRUS in Materials and Methods.
10 Methods for constructing, selecting and purifying recombinant herpesvirus of turkeys are detailed below in

This invention provides a method of distinguishing chickens or other poultry which are vaccinated with the above vaccine
15 from those which are infected with a naturally-occurring Marek's disease virus which comprises analyzing samples of body fluids from chickens or other poultry for the presence of glycoprotein gG and at least one other antigen normally expressed in chickens or other poultry infected by a
20 naturally-occurring Marek's disease virus, the presence of those antigens normally expressed in infected chickens but the absence of glycoprotein gG being indicative of vaccination with the above vaccine and not infection with a naturally-occurring Marek's disease virus.

25 This invention provides a recombinant herpesvirus of turkeys which expresses foreign DNA sequences is useful as vaccines in avian or mammalian species including but not limited to chickens, turkeys, ducks, feline, canine, bovine, equine,
30 and primate, including human. This vaccine may contain either inactivated or live recombinant virus.

For purposes of this invention, an "effective immunizing amount" of the recombinant feline herpes virus of the
35 present invention is within the range of 10^3 to 10^9 PFU/dose.

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In another embodiment the immunizing amount is 10^5 to 10^7 PFU/dose. In a preferred embodiment the immunizing amount is 10^6 PFU/dose.

5 The method comprises administering to the animal an effective immunizing dose of the vaccine of the present invention. The vaccine may be administered by any of the methods well known to those skilled in the art, for example, by intramuscular, subcutaneous, intraperitoneal or
10 intravenous injection. Alternatively, the vaccine may be administered intranasally or orally.

Suitable carriers for the recombinant virus are well known to those skilled in the art and include but are not limited
15 to proteins, sugars, etc. One example of such a suitable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as hydrolyzed proteins, lactose, etc. Preferably, the live vaccine is created by taking tissue culture fluids and adding
20 stabilizing agents such as stabilizing, hydrolyzed proteins. Preferably, the inactivated vaccine uses tissue culture fluids directly after inactivation of the virus.

This invention provides an isolated nucleic acid molecule
25 encoding Quail Interferon Type 1. In one embodiment, the isolated nucleic acid molecule encoding Quail Interferon Type 1 has the nucleic acid sequence as set forth in SEQ. ID. NO. 31.

30 In one embodiment the isolated nucleic acid molecule is genomic DNA. In another embodiment the isolated nucleic acid molecule is cDNA. In another embodiment RNA is derived from the isolated nucleic acid molecule or is capable of hybridizing with the isolated nucleic acid molecule.

35 "Nucleic acid sequence" refers to a single- or double-

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stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes both self-replicating plasmids, infectious polymers of DNA or RNA and nonfunctional DNA or RNA.

5

It will be readily understood by those skilled in the art and it is intended here, that when reference is made to particular sequence listings, such reference includes sequences which substantially correspond to the listing and it's complement, including allowances for minor sequencing errors, single base changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence of the pathogenic organism or disease marker to which the relevant sequence listing relates.

15

This invention provides a nucleic acid molecule of at least 14 nucleotides capable of specifically hybridizing to the isolated nucleic acid molecule encoding Quail Interferon Type 1. This invention provides a nucleic acid molecule of at least 14 nucleotides capable of specifically hybridizing to a complementary sequence of the sense strand of isolated nucleic acid molecule encoding Quail Interferon Type 1. A complementary sequence is the antisense strand of the isolated nucleic acid molecule encoding Quail Interferon Type 1.

20

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In one embodiment the molecule is 8 to 36 nucleotides. In another embodiment the molecule is 12 to 25 nucleotides. In another embodiment the molecule is 14 nucleotides. In one embodiment the molecule is DNA. In another embodiment the molecule is RNA.

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This invention provides an antisense molecule capable of hybridizing to the isolated nucleic acid molecule. In one embodiment the antisense molecule is DNA. In another embodiment the antisense molecule is RNA. In another

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embodiment, the antisense molecule is a nucleic acid derivative (e.g., DNA or RNA with a protein backbone).

5 The present invention extends to the preparation of antisense nucleic acids or fragments thereof and ribozymes that may be used to interfere with the expression of a polypeptide either by masking the mRNA with an antisense nucleic acid or cleaving it with a ribozyme, respectively. In one embodiment the antisense nucleic acid molecule
10 hybridizes to the mRNA of a quail interferon.

Approaches targeting DNA fall into several categories. Nucleic acids can be designed to bind to the major groove of the duplex DNA to form a triple helical or "triplex"
15 structure. Alternatively, inhibitory nucleic acids are designed to bind to regions of single stranded DNA resulting from the opening of the duplex DNA during replication or transcription.

20 More commonly, inhibitory nucleic acids are designed to bind to mRNA or mRNA precursors. Inhibitory nucleic acids are used to prevent maturation of pre-mRNA. Inhibitory nucleic acids may be designed to interfere with RNA processing, splicing or translation. The complementary strand of the
25 isolated nucleic acid molecule is also called the anti-sense strand.

Lastly, the inhibitory nucleic acids can be used to induce chemical inactivation or cleavage of the target genes or
30 mRNA. Chemical inactivation can occur by the induction of crosslinks between the inhibitory nucleic acid and the target nucleic acid within the cell. Other chemical modifications of the target nucleic acids induced by appropriately derivatized inhibitory nucleic acids may also
35 be used.

High stringency hybridization conditions are selected at

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about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, i.e. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. For example, high stringency may be attained by overnight hybridization at about 68°C in a 6X SSC solution, washing at room temperature with 6X SSC solution, followed by washing at about 68°C in a 0.6X SSC solution.

Hybridization with moderate stringency may be attained for example by: 1) filter pre-hybridizing and hybridizing with a solution of 3X SSC, 50% formamide, 0.1M Tris buffer at pH 7.5, 5X Denhardt's solution; 2.) pre-hybridization at 37°C for 4 hours; 3) hybridization at 37°C with amount of labeled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in x SSC and 0.1% SDS solution; 5) wash 4X for 1 minute each at room temperature in 4X SSC at 60°C for 30 minutes each; and 6) dry and expose to film.

Nucleic acid probe technology is well known to those skilled in the art who readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule having the full-length or a fragment of the isolated nucleic acid molecule of the DNA virus into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed

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bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

5 RNA probes may be generated by inserting the full length or a fragment of the isolated nucleic acid molecule of the DNA virus downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with a linearized
10 isolated nucleic acid molecule of the DNA virus or its fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

As defined herein nucleic acid probes may be DNA or RNA
15 fragments. DNA fragments can be prepared, for example, by digesting plasmid DNA, or by use of PCR, or synthesized by either the phosphoramidite method described by Beaucage and Carruthers, 1981, *Tetrahedron Lett.* 22, 1859-1862 or by the triester method according to Matteucci et al., 1981, *Am.*
20 *Chem. Soc.* 103:3185. A double stranded fragment may then be obtained, if desired, by annealing the chemically synthesized single strands together under appropriate conditions or by synthesizing the complementary strand using DNA polymerase with an appropriate primer sequence. Where
25 a specific sequence for a nucleic acid probe is given, it is understood that the complementary strand is also identified and included. The complementary strand will work equally well in situations where the target is a double-stranded nucleic acid. It is also understood that when a specific
30 sequence is identified for use as a nucleic probe, a subsequence of the listed sequence which is 25 base pairs (bp) or more in length is also encompassed for use as a probe.

35 The nucleic acid molecules of the subject invention also include molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from

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naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the polypeptide, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms.

10

The term "SSC" refers to a citrate-saline solution of 0.15 M sodium chloride and 20 mM sodium citrate. Solutions are often expressed as multiples or fractions of this concentration. For example, 6XSSC refers to a solution having a sodium chloride and sodium citrate concentration of 6 times this amount or 0.9 M sodium chloride and 120 mM sodium citrate. 0.2XSSC refers to a solution 0.2 times the SSC concentration or 0.03 M sodium chloride and 4 mM sodium citrate.

20

The phrase "specifically hybridizing" describes a nucleic acid probe that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. By selectively hybridizing it is meant that a probe binds to a given target in a manner that is detectable in a different manner from non-target sequence under high stringency conditions of hybridization.

25

The phrase "nucleic acid molecule encoding" refers to a nucleic acid molecule which directs the expression of a specific polypeptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA, the complementary DNA strand, and the RNA sequence that is translated into protein. The nucleic acid molecule includes both the full length nucleic acid sequence as well as non-full length sequences. It being further understood that the

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sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

5 This invention provides an isolated DNA operatively linked to a promoter of RNA transcription. The term "operably linked" as used herein refers to linkage of a promoter upstream from a DNA sequence such that the promoter mediates transcription of the DNA sequence.

10

This invention provides a vector which comprises the isolated nucleic acid molecule encoding Quail Interferon Type 1. This invention provides a recombinant DNA which comprises the isolated nucleic acid molecule encoding Quail
15 Interferon Type 1.

This invention provides a vector which comprises the complementary sequence of the sense strand of the isolated nucleic acid molecule encoding Quail Interferon Type 1. This
20 invention provides a recombinant DNA which comprises the complementary sequence of the sense strand of isolated nucleic acid molecule encoding Quail Interferon Type 1.

The vector includes, but is not limited to: a plasmid,
25 cosmid, λ phage, yeast artificial chromosome (YAC), or a recombinant virus which contains the isolated nucleic acid molecule.

To obtain the vector, for example, insert and vector DNA can
30 both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with DNA ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA,
35 which is then digested with the restriction enzyme which cuts at that site. Other means are available and well-known to those skilled in the art.

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This invention provides a host cell containing the vector. Suitable host cells include, but are not limited to, bacteria (such as *E. coli*), yeast, fungi, plant, insect and mammalian cells. Suitable animal cells include, but are not limited to CEF, QT-35, ESK-4, Vero cells, HeLa cells, Cos cells, CV1 cells and various primary mammalian cells.

This invention provides an isolated polypeptide having the biological activity of Quail Interferon Type I. In another embodiment, the isolated polypeptide encodes Quail Interferon Type 1 which has the amino acid sequence as set forth in SEQ. ID. NO. 32.

The term "polypeptide", as used herein, refers to either the full length gene product encoded by the nucleic acid, or portions thereof. Thus, "polypeptide" includes not only the full-length protein, but also partial-length fragments, including peptides less than fifty amino acid residues in length.

Further, the isolated polypeptide may be linked to a second polypeptide to form a fusion protein by linking the isolated nucleic acid molecule to a second nucleic acid molecule and expression in a suitable host cell. In one embodiment the second nucleic acid molecule encodes beta-galactosidase. Other nucleic acid molecules which are used to form a fusion protein are known to those skilled in the art.

This invention provides an antibody which specifically binds to the polypeptide encoded by the isolated nucleic acid molecule. In one embodiment the antibody is a monoclonal antibody. In another embodiment the antibody recognizes an epitope of the polypeptide. In another embodiment the antibody is a polyclonal antibody. In another embodiment the antibody recognizes more than one epitope of the polypeptide. In another embodiment the antibody is an anti-idiotypic antibody.

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An antibody, polypeptide or isolated nucleic acid molecule may be labeled with a detectable marker including, but not limited to: a radioactive label, or a colorimetric, a luminescent, or a fluorescent marker, or gold. Radioactive labels include, but are not limited to: ^3H , ^{14}C , ^{32}P , ^{33}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{59}Co , ^{59}Fe , ^{125}I , ^{131}I , ^{186}Re . Fluorescent markers include, but are not limited to: fluorescein, rhodamine and auramine. Colorimetric markers include, but are not limited to: biotin, and digoxigenin. Methods of producing the polyclonal or monoclonal antibody are known to those of ordinary skill in the art.

Further, the antibody, polypeptide or nucleic acid molecule may be detected by a second antibody which may be linked to an enzyme, such as alkaline phosphatase or horseradish peroxidase. Other enzymes which may be employed are well known to one of ordinary skill in the art.

This invention provides a method of producing a polypeptide encoded by the isolated nucleic acid molecule, which comprises growing a host-vector system under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced. Suitable host cells include bacteria, yeast, filamentous fungal, plant, insect and mammalian cells. Host-vector systems for producing and recovering a polypeptide are well known to those skilled in the art and include, but are not limited to, E. coli and pMAL (New England Biolabs), the Sf9 insect cell-baculovirus expression system, and mammalian cells (such as HeLa, COS, NIH 3T3 and HEK293) transfected with a mammalian expression vector by Lipofectin (Gibco-BRL) or calcium phosphate precipitation or other methods to achieve vector entry into the cell.

This invention provides a method to select specific regions on the polypeptide encoded by the isolated nucleic acid molecule of the polypeptide to generate antibodies. Amino

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acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the polypeptides which they build. In the case of a cell membrane polypeptide, hydrophobic regions are well known to form the part of the polypeptide that is inserted into the lipid bilayer of the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Usually, the hydrophilic regions will be more immunogenic than the hydrophobic regions. Therefore the hydrophilic amino acid sequences may be selected and used to generate antibodies specific to polypeptide encoded by the isolated nucleic acid molecule encoding the DNA virus. The selected peptides may be prepared using commercially available machines. As an alternative, nucleic acid may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen.

In addition, enzymes may be used as labels. Suitable enzymes include alkaline phosphatase, beta-galactosidase, glucose-6-phosphate dehydrogenase, maleate dehydrogenase and peroxidase. Two principal types of enzyme immunoassay are the enzyme-linked immunosorbent assay (ELISA), and the homogeneous enzyme immunoassay, also known as enzyme-multiplied immunoassay (EMIT, Syva Corporation, Palo Alto, CA). In the ELISA system, separation may be achieved, for example, by the use of antibodies coupled to a solid phase. The EMIT system depends on deactivation of the enzyme in the tracer-antibody complex; activity is thus measured without the need for a separation step.

Additionally, chemiluminescent compounds may be used as labels. Typical chemiluminescent compounds include luminol, isoluminol, aromatic acridinium esters, imidazoles, acridinium salts, and oxalate esters. Similarly, bioluminescent compounds may be utilized for labelling, the bioluminescent compounds including luciferin, luciferase,

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and aequorin.

A description of a radioimmunoassay (RIA) may be found in:
Laboratory Techniques in Biochemistry and Molecular Biology
5 (1978) North Holland Publishing Company, New York, with
particular reference to the chapter entitled "An
Introduction to Radioimmune Assay and Related Techniques" by
T. Chard. A description of general immunometric assays of
various types can be found in the following U.S. Pat. Nos.
10 4,376,110 (David et al.) or 4,098,876 (Piasio).

This invention provides a recombinant virus which comprises
a foreign DNA inserted into a non-essential region of a
viral genome which is capable of being expressed in a host
15 cell, wherein the foreign DNA encodes Quail Interferon Type
I.

The virus is selected from the group consisting of:
herpesvirus of turkeys, swinepox virus, pseudorabies virus,
20 infectious bovine rhinotracheitis virus, Equine herpes
virus, feline herpesvirus, fowlpox virus, infectious
laryngotracheitis virus, mareck's disease virus, pox virus,
canary pox, raccoon pox, vaccinia, adeno-associated virus,
adeno virus, canine herpesvirus, infectious bursal disease
25 virus, herpes simplex virus, and alpha virus.

This invention provides a vaccine which comprises an
effective immunizing amount of the Quail Interferon Type I
polypeptide and a suitable pharmaceutical carrier.

30

The vaccines may be administered by any conventional method
for the administration of vaccines including oral and
parenteral (e.g., subcutaneous or intra-muscular) injection.
Intramuscular administration is preferred. The treatment
35 may consist of a single dose of vaccine or a plurality of
doses over a period of time. It is preferred that the dose
be given to a human patient within the first 8 months of

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life. The antigen of the invention can be combined with appropriate doses of compounds including influenza antigens, such as influenza type A antigens. Also, the antigen could be a component of a recombinant vaccine which could be adaptable for oral administration.

Vaccines of the invention may be combined with other vaccines for other diseases to produce multivalent vaccines. A pharmaceutically effective amount of the antigen can be employed with a pharmaceutically acceptable carrier such as a protein or diluent useful for the vaccination of mammals, particularly humans. Other vaccines may be prepared according to methods well-known to those skilled in the art.

Those of skill will readily recognize that it is only necessary to expose a mammal to appropriate epitopes in order to elicit effective immunoprotection. The epitopes are typically segments of amino acids which are a small portion of the whole protein. Using recombinant genetics, it is routine to alter a natural protein's primary structure to create derivatives embracing epitopes that are identical to or substantially the same as (immunologically equivalent to) the naturally occurring epitopes. Such derivatives may include peptide fragments, amino acid substitutions, amino acid deletions and amino acid additions of the amino acid sequence for the polypeptide. For example, it is known in the protein art that certain amino acid residues can be substituted with amino acids of similar size and polarity without an undue effect upon the biological activity of the protein.

This invention provides a method of growing a recombinant virus to a high titre by growing the recombinant virus in a cell line which contains the complementary sequence of the sense strand of the isolated nucleic acid molecule which encodes quail interferon, which is expressed. In another embodiment the virus is killed or attenuated. In one

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embodiment the nucleic acid is the sense strand. In another embodiment the nucleic acid is the anti-sense strand

5 This invention provides a method of enhancing the replication of a recombinant virus. In one embodiment the virus is killed or attenuated. This invention provides a method of inhibiting IFN production of an avian species. In one embodiment the nucleic acid is the sense strand. In another embodiment the nucleic acid is the anti-sense
10 strand.

This invention provides a transgenic avian species which expresses quail interferon. Avian species include but are not limited to: quail, turkey, chicken, geese, ducks,
15 ostrich, dove, or fowl.

This invention provides a method for enhancing the immune response of an animal by vaccinating the animal with a vaccine containing a vector or plasmid which comprises the
20 isolated nucleic acid molecule encoding quail Interferon. This invention provides a method for enhancing the immune response of an animal by vaccinating the animal with a vaccine containing a vector or plasmid which comprises the complementary sequence of the sense strand of the isolated
25 nucleic acid molecule encoding quail Interferon. It is contemplated by this invention that the vector may be administered concurrently, subsequently, or after the recombinant virus which is used to vaccinate the animal.

30 This invention provides a method of increasing the weight of an animal which comprises vaccinating the avian animal with a vaccine which comprises the isolated nucleic acid molecule which encodes the quail interferon. In one embodiment the nucleic acid is the sense strand. In another embodiment the
35 nucleic acid is the anti-sense strand.

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This invention is further illustrated in the Experimental Details section which follows. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

EXPERIMENTAL DETAILS:**Materials and Methods:****PREPARATION OF HERPESVIRUS OF TURKEYS STOCK SAMPLES.**

Herpesvirus of turkeys stock samples were prepared by infecting tissue culture cells at a multiplicity of infection of 0.01 PFU/cell in Dulbecco's Modified Eagle Medium (DMEM) containing 2 mM glutamine, 100 units/ml penicillin, 100 units/ml streptomycin (these components are obtained from Irvine Scientific or an equivalent supplier, and hereafter are referred to as complete DME medium) plus 1% fetal bovine serum. After cytopathic effect was complete, the medium and cells were harvested and the cells were pelleted at 3000 rpm for 5 minutes in a clinical centrifuge. Infected cells were resuspended in complete medium containing 20% fetal bovine serum, 10% DMSO and stored frozen at -70°C.

PREPARATION OF HERPESVIRUS OF TURKEY DNA. All manipulations of herpesvirus of turkey (HVT) were made using strain FC-126 (ATCC #584-C). For the preparation of HVT viral DNA from the cytoplasm of infected cells, primary chicken embryo fibroblasts were infected at a MOI sufficient to cause extensive cytopathic effect before the cells overgrew. All incubations were carried out at 39°C in a humidified incubator with 5% CO₂ in air. Best DNA yields were obtained by harvesting monolayers which were maximally infected, but

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showing incomplete cell lysis (typically 5-7 days). Infected cells were harvested by scraping the cells into the medium using a cell scraper (Costar brand). The cell suspension was centrifuged at 3000 rpm for 10 minutes at 5°C in a GS-3 rotor (Sorvall Instruments). The resultant pellet was resuspended in cold PBS (20 ml/Roller Bottle) and subjected to another centrifugation for 10 minutes at 3000 rpm in the cold. After decanting the PBS, the cellular pellet was resuspended in 4 ml/roller bottle of RSB buffer (10 mM Tris pH 7.5, 1 mM EDTA, and 1.5 mM MgCl₂). NP40 (Nonidet P-40™;Sigma) was added to the sample to a final concentration of 0.5% minutes with occasional mixing. The sample was centrifuged for 10 minutes at 3000 rpm in the cold to pellet the nuclei and remove cellular debris. The supernatant fluid was carefully transferred to a 15 ml Corex centrifuge tube. Both EDTA (0.5M pH 8.0) and SDS (sodium dodecyl sulfate; stock 20%) were added to the sample to final concentrations of 5 mM and 1%, respectively. One hundred µl of proteinase-K (10 mg/ml; Boehringer Mannheim) was added per 4 ml of sample, mixed, and incubated at 45°C for 1-2 hours. After this period, an equal volume of water-saturated phenol was added to the sample and gently mixed by hand. The sample was spun in a clinical centrifuge for 5 minutes at 3000 rpm to separate the phases. NaAc was added to the aqueous phase to a final concentration of 0.3M (stock solution 3M pH 5.2), and the nucleic acid precipitated at -70°C for 30 minutes after the addition of 2.5 volumes of cold absolute ethanol. DNA in the sample was pelleted by spinning for 20 minutes to 8000 rpm in an HB-4 rotor at 5°C. The supernatant was carefully removed and the DNA pellet washed once with 25 ml of 80% ethanol. The DNA pellet was dried briefly by vacuum (2-3 minutes), and resuspended in 50 µl/roller bottle of infected cells of TE buffer (10 mM Tris pH 7.5, 1 mM EDTA). Typically, yields of viral DNA ranged between 5-10 µg/roller bottle of infected cells. All viral

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DNA was stored at approximately 10°C.

POLYMERASE FILL-IN REACTION. DNA was resuspended in buffer containing 50 mM Tris pH 7.4, 50 mM KCl, 5 mM MgCl₂, and 400 micromolar each of the four deoxynucleotides. Ten units of Klenow DNA polymerase (BRL) were added and the reaction was allowed to proceed for 15 minutes at room temperature. The DNA was then phenol extracted and ethanol precipitated as above.

DNA SEQUENCING. Sequencing was performed using the USB Sequenase Kit and ³⁵S-dATP (NEN). Reactions using both the dGTP mixes and the dTTP mixes were performed to clarify areas of compression. Alternatively, compressed areas were resolved on formamide gels. Templates were double-stranded plasmid subclones or single stranded M13 subclones, and primers were either made to the vector just outside the insert to be sequenced, or to previously obtained sequence. Sequence obtained was assembled and compared using Dnastar software. Manipulation and comparison of sequences obtained was performed with Superclone and Supersee programs from Coral Software.

MOLECULAR BIOLOGICAL TECHNIQUES. Techniques for the manipulation of bacteria and DNA, including such procedures as digestion with restriction endonucleases, gel electrophoresis, extraction of DNA from gels, ligation, phosphorylation with kinase, treatment with phosphatase, growth of bacterial cultures, transformation of bacteria with DNA, and other molecular biological methods are described by Maniatis et al (1982) and Sambrook et al (1989). The polymerase chain reaction (PCR) was used to introduce restriction sites convenient for the manipulation of various DNAs. The procedures used are described by Innis et al (1990). In general amplified fragments were less than

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500 base pairs in size and critical regions of amplified fragments were confirmed by DNA sequencing. Except as noted, these techniques were used with minor variation.

5 **SOUTHERN BLOTTING OF DNA.** The general procedure for Southern blotting was taken from Maniatis et al. (1982). DNA was blotted to nitrocellulose filters (S&S BA85) in 20X SSC (1X ssc = 0.15M NaCl, 0.015M sodium citrate, pH 7.0), and prehybridized in hybridization solution consisting of
10 30% formamide, 1X Denhardt's solution (0.02% polyvinylpyrrolidone (PVP), 0.02% bovine serum albumin (BSA), 0.02% Ficoll), 6X SSC, 50 mM NaH₂PO₄, pH 6.8, 200 µg/ml salmon sperm DNA for 4-24 hours at 55°C. Labeled probe DNA was added that had been labeled by nick translation
15 using a kit from Bethesda Research Laboratories (BRL) and one 32P-labeled nucleotide. The probe DNA was separated from the unincorporated nucleotides by NACS column (BRL) or on a Sephadex G50 column (Pharmacia). After overnight hybridization at 55°C, the filter was washed once with 2X SSC
20 at room temperature followed by two washes with 0.1X SSC, 0.1% sodium dodecyl sulfate (SDS) for 30 minutes at 55°C. The filter was dried and autoradiographed.

cDNA CLONING PROCEDURE. cDNA cloning refers to the methods
25 used to convert RNA molecules into DNA molecules following state of the art procedures. Applicants' methods are described in (Gubler and Hoffman, 1983). Bethesda Research Laboratories (Gaithersburg, MD) have designed a cDNA Cloning Kit that is very similar to the procedures used by
30 applicants, and contains a set of reagents and protocols that may be used to duplicate our results.

For cloning virus mRNA species, a host cell line sensitive to infection by the virus was infected at 5-10 plaque
35 forming units per cell. When cytopathic effect was evident,

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but before total destruction, the medium was removed and the cells were lysed in 10 mls lysis buffer (4 M guanidine thiocyanate, 0.1% antifoam A, 25 mM sodium citrate pH 7.0, 0.5% N-lauroyl sarcosine, 0.1 M beta-mercaptoethanol). The cell lysate was poured into a sterilized Dounce homogenizer and homogenized on ice 8-10 times until the solution was homogenous. For RNA purification, 8 mls of cell lysate were gently layered over 3.5 mls of CsCl solution (5.7 M CsCl, 25 mM sodium citrate pH 7.0) in Beckman SW41 centrifuge tube. The samples were centrifuged for 18 hrs at 20° C at 36000 rpm in a Beckman SW41 rotor. The tubes were put on ice and the supernatants from the tubes were carefully removed by aspiration to leave the RNA pellet undisturbed. The pellet was resuspended in 400 µl glass distilled water, and 2.6 mls of guanidine solution (7.5 M guanidine-HCL, 25 mM sodium citrate pH 7.0, 5 mM dithiothreitol) were added. The 0.37 volumes of 1 M acetic acid were added, followed by 0.75 volumes of cold ethanol and the sample was put at -20° C for 18 hrs to precipitate RNA. The precipitate was collected by centrifugation in a Sorvall centrifuge for 10 min at 4° C at 10000 rpm in an SS34 rotor. The pellet was dissolved in 1.0 ml distilled water, recentrifuged at 13000 rpm, and the supernatant saved. RNA was re-extracted from the pellet 2 more times as above with 0.5 ml distilled water, and the supernatants were pooled. A 0.1 volume of 2 M potassium acetate solution was added to the sample followed by 2 volumes of cold ethanol and the sample was put at -20° C for 18 hrs. The precipitated RNA was collected by centrifugation in the SS34 rotor at 4° C for 10 min at 10000 rpm. The pellet was dissolved in 1 ml distilled water and the concentration taken by absorption at A260/280. The RNA was stored at -70°C.

mRNA containing polyadenylate tails (poly-A) was selected using oligo-dT cellulose (Pharmacia #27 5543-0). Three mg

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of total RNA was boiled and chilled and applied to the 100 mg oligo-dT cellulose column in binding buffer (0.1 M Tris pH 7.5, 0.5 M LiCl, 5mM EDTA pH 8.0, 0.1% lithium dodecyl sulfate). The retained poly-A RNA was eluted from the
5 column with elution buffer (5mM Tris pH 7.5, 1mM EDTA pH 8.0, 0.1% sodium dodecyl sulfate). This mRNA was reapplied to an oligo-dT column in binding buffer and eluted again in elution buffer. The sample was precipitated with 200 mM sodium acetate and 2 volumes cold ethanol at -20°C for 18
10 hrs. The RNA was resuspended in 50 µl distilled water.

Ten µg poly-A RNA was denatured in 20 mM methyl mercury hydroxide for 6 min at 22°C. β-mercaptoethanol was added to 75 mM and the sample was incubated for 5 min at 22°C. The
15 reaction mixture for first strand cDNA synthesis in 0.25 ml contained 1 µg oligo-dT primer (P-L Bio-chemicals) or 1 µg synthetic primer, 28 units placental ribonuclease inhibitor (Bethesda Research Labs #5518SA), 100 mM Tris pH 8.3, 140 mM KCl, 10mM MgCl₂, 0.8 mM dATP, dCTP, dGTP, and dTTP
20 (Pharmacia), 100 microcuries 32p-labeled dCTP (New England Nuclear #NEG-013H), and 180 units AMV reverse transcriptase (Molecular Genetics Resources #MG 101). The reaction was incubated at 42°C for 90 min, and then was terminated with 20mM EDTA pH 8.0. The sample was extracted with an equal
25 volume of phenol/chloroform (1:1) and precipitated with 2 M ammonium acetate and 2 volumes of cold ethanol -20°C for 3 hrs. After precipitation and centrifugation, the pellet was dissolved in 100 µl distilled water. The sample was loaded onto a 15 ml G-100 Sephadex column (Pharmacia) in buffer
30 (100 mM Tris pH 7.5, 1 mM EDTA pH 8.0, 100 mM NaCl). The leading edge of the eluted DNA fractions was pooled, and DNA was concentrated by lyophilization until the volume was about 100 µl, then the DNA was precipitated with ammonium acetate plus ethanol as above.

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The entire first strand sample was used for second strand reaction which followed the Gubler and Hoffman (1983) method except that 50 µg/ml dNTP's, 5.4 units DNA polymerase I (Boehringer Mannheim #642-711), and 100 units/ml E. coli DNA
5 ligase (New England Biolabs #205) in a total volume of 50 microliters were used. After second strand synthesis, the cDNA was phenol/chloroform extracted and precipitated. The DNA was resuspended in 10 µl distilled water, treated with 1 µg RNase A for 10 min at 22°C, and electrophoresed through
10 a 1% agarose gel (Sigma Type II agarose) in 40 mM Tris-acetate pH 6.85. The gel was stained with ethidium bromide, and DNA in the expected size range was excised from the gel and electroeluted in 8 mM Tris-acetate pH 6.85. Electroeluted DNA was lyophilized to about 100 microliters,
15 and precipitated with ammonium acetate and ethanol as above. The DNA was resuspended in 20 µl water.

Oligo-dC tails were added to the DNA to facilitate cloning. The reaction contained the DNA, 100 mM potassium cacodylate
20 pH 7.2, 0.2 mM dithiothreitol, 2mM CaCl₂, 80 µmoles dCTP, and 25 units terminal deoxynucleotidyl transferase (Molecular Genetic Resources #S1001) in 50 µl. After 30 min at 37°C, the reaction was terminated with 10mM EDTA, and the sample was phenol/chloroform extracted and precipitated as
25 above.

The dC-tailed DNA sample was annealed to 200 ng plasmid vector pBR322 that contained oligo-dG tails (Bethesda
Research Labs #5355 SA/SB) in 200 µl of 0.01 M Tris pH 7.5,
30 0.1 M NaCl, 1 mM EDTA pH 8.0 at 65°C for 2 min and then 57°C for 2 hrs. Fresh competent E. coli DH-1 cells were prepared and transformed as described by Hanahan (1983) using half the annealed cDNA sample in twenty 200 µl aliquots of cells. Transformed cells were plated on L-broth agar plates plus 10
35 µg/ml tetracycline. Colonies were screened for the presence

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of inserts into the ampicillin gene using Ampscreen (Bethesda Research Labs #5537 UA), and the positive colonies were picked for analysis.

5 **DNA TRANSFECTION FOR GENERATING RECOMBINANT HERPESVIRUS.**

The method is based upon the polybrene-DMSO procedure of Kawai and Nishizawa (1984) with the following modifications. Generation of recombinant HVT virus is dependent upon homologous recombination between HVT viral DNA and the
10 plasmid homology vector containing the desired foreign DNA flanked by the appropriate herpesvirus cloned sequences. Transfections were carried out in 6 cm plates (Corning plastic) of 50% confluent primary chick embryo fibroblast (CEF) cells. The cells were plated out the day before in
15 CEF growth media (1X F10/199, 5% fetal calf serum, 2% glutamine, 1% non-essential amino acids, and 2% penicillin/streptomycin) containing 4 µg/ml polybrene (stock 4 mg/ml in 1X HBSS). For cotransfections into CEF cells, 5 µg of intact HVT DNA, and suspended in 1 ml of CEF media
20 containing 30 µg/ml polybrene (stock 4 mg/ml in 1X HBSS). The DNA-polybrene suspension (1 ml) was then added to a 6 cm plate of CEF cells from which the media had been aspirated, and incubated at 39°C for 30 minutes. The plates were rocked periodically during this time to redistribute the inoculum.
25 After this period, 4 ml of CEF growth media was added directly to wash plate, and incubated an additional 2.5 hours at 39°C. At this time, the media was removed from each plate, and the cells shocked with 2 ml of 30% DMSO (Dimethyl Sulfoxide, J.T. Baker Chemical Co.) in 1X HBSS for 4 minutes
30 at room temperature. The 30% DMSO was carefully removed and the monolayers washed once with 1X HBSS at room temperature. The cells were then incubated at 39°C after the addition of 5 mls of CEF growth media. The next day, the media was changed to remove any last traces of DMSO and to stimulate
35 cell growth. Cytopathic effect from the virus becomes

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apparent within 6 days. Generation of a high titer stock (80%-90% CPE) can usually be made within 1 week from this date. HVT stock samples were prepared by resuspending the infected cells in CEF growth media containing 20% fetal calf serum, 10% DMSO and stored at -70°C.

PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM SUBGENOMIC DNA FRAGMENTS. The ability to generate herpesviruses by cotransfection of cloned overlapping subgenomic fragments has been demonstrated for pseudorabies virus (Zijl et al., 1988). If deletions and/or insertions are engineered directly into the subgenomic fragments prior to the cotransfection, this procedure results in a high frequency of viruses containing the genomic alteration, greatly reducing the amount of screening required to purify the recombinant virus. This procedure was used to construct recombinant HVT.

A library of subclones containing overlapping HVT subgenomic fragments was generated as follows. HVT DNA was obtained from the American Type Culture Collection (FC-126("Calnek")). It was sheared and then size selected on a glycerol gradient as described by van Zijl et al., (1988) with 40-50 kb fragments chosen as the insert population. The pooled fractions were diluted twofold with TE, one-tenth volume of 3M NaAc and 2.5 volumes of ethanol were added, and the DNA was precipitated at 30K rpm in a Beckman SW41 rotor for 1 hr. The sheared fragments were given blunt ends by initial treatment with T4 DNA polymerase, using low dNTP concentrations to promote 3' overhang removal, followed by treatment with Klenow polymerase to fill in recessed 3' ends. These insert fragments were then ligated to a pWE15 (Stratagene) cosmid vector, which had been digested with BamHI, treated with calf intestinal phosphatase, and made blunt by treatment with Klenow polymerase. The ligated

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mixture was then packaged using Gigapack XL packaging extracts (Stratagene). Ligation and packaging was as recommended by the manufacturer.

5 Published restriction maps for the enzymes BamHI, HindIII, and XhoI permitted the use of subcloned fragments as specific probes to screen the cosmid library for subclones spanning the genome. Probes were generated from subcloned restriction fragments. The fragments were then labeled
10 using a non-radioactive system (Genius, Boehringer Mannheim). Screening was facilitated by picking colonies to media followed by growth overnight. Sets of five filters and a master plate were stamped from microtiter dish and again grown overnight. Glycerol was added to the wells to
15 15% and the plates were frozen at -20°C to provide stock cultures of each colony. Filters were BioRad Colony Lift Membranes and were treated and hybridized per manufacturer's instructions, and washed in 0.1X SSC, 0.1% SDS, 65°C. Clones which hybridized with the non-radioactive probe were
20 detected according to the Genius kit directions.

Colonies were selected for further analysis on the basis of their hybridization to two or more of the specific probes. These were then digested with BamHI, and compared to
25 published maps of HVT (Buckmaster et al., 1988). The three cosmids (407-32.2C3, 407-32.IG7, and 407-32.5G6) were obtained in this manner. A detailed description of each clone is given below. It was found that chloramphenicol amplification (Maniatis et al., 1982) was necessary to
30 achieve reasonable yields of DNA from these clones. In addition, one cosmid clone (407-32.5G6) was unstable and had to be grown from the original frozen stock in order to obtain satisfactory DNA preparations.

35 The pWE15 vector allows the inserts to be excised with NotI.

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However, four NotI sites are present in the HVT genome, so that inserts spanning these sites cannot be excised with NotI. Two of the NotI sites are present in the BamHI #2 fragment of HVT, this fragment was cloned directly in pSP64. 5 The other two sites are present in the unique short region within the BamHI #1 fragment. This fragment was cloned directly in the pWE15 vector. The three sheared cosmids and the two BamHI fragments cover all but a small portion of the ends of the HVT genome. Because these regions are repeated 10 in the internal portions of the genome, all of the genetic information is available.

A StuI site within the HVT US2 gene was established as a useful site for foreign DNA insertion utilizing the 15 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUSES (see Example 6). The HVT US2 gene is located within the BamHI #1 fragment which contains five StuI sites. To facilitate the use of this site for insertion of foreign DNA by the StuI site within the US2 gene was 20 converted to a unique HindIII site. This was accomplished by partially digesting the BamHI #1 subclone with StuI, and then inserting a 10 kb fragment conferring kanomycin resistance (NeoR) into the site using HindIII linkers. The kanomycin resistance gene allowed positive selection of 25 recombinant clones. The NeoR fragment was removed by digestion with HindIII followed by religation generating clone 430-84.215.

DNA was prepared for reconstruction experiments by 30 restriction digestion with enzymes which cut the subclones outside or flanking the HVT insertions. In some instances, one cosmid in a reconstruction was used undigested. Digested DNAs were extracted once with phenol and precipitated with ethanol. DNA was resuspended at a 35 concentration of 0.5 to 1 ug/ml. Viral reconstruction

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experiments were performed using Lipofectin (BRL) to mediate transfection. Two to three micrograms each of subclone were added to 0.5 ml of MEM media (Earle's salts) supplemented with 1% non-essential amino acids and 2% penicillin/Streptomycin (MEM+). Controls consisted of MEM+ with no DNA, with several ug of HVT DNA, or with 4 out of 5 of the subclones. Separately, 30 µl of the Lipofectin were added to another 0.5 ml. of MEM+. These two mixtures were then combined and incubated at RT for 15 minutes.

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Chick embryo fibroblast (CEF) cells were prepared for transfection in the following manner. CEFs (Spafas) were grown in 6 well dishes at 39°C in F10/M199 (1:1) media containing 1% non-essential amino acids, 2% penicillin/streptomycin, and 5% fetal calf serum (CEF+). Cells were transfected at a confluence of 90 - 95%. For transfection, wells were aspirated and rinsed 3 times with MEM+, and then incubated 4 hours at 39°C with the 1 ml lipofectin/DNA mixture above. One ml more of CEF+ was then added to the wells, and cells were incubated overnight and fed with CEF+. Plates were then examined daily for the appearance of plaques.

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Lipofectin with control HVT DNA resulted in the appearance of plaques within 5 days. When only four of the five subclones were used, no plaques were obtained. When the five overlapping genomic fragments of HVT were used to reconstruct the virus, plaques appeared anywhere from 5 to 19 days after the initial lipofection. In the case of plaques appearing late, plaques were not initially seen on the infected monolayer, and it was only after passaging the monolayer and replating on a larger surface that plaques appeared. After passaging, plaques generally appeared within 3 days. Recombinant viruses were plaque purified approximately three and then analyzed for insertion of

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foreign DNAs.

BLUOGAL SCREEN OR CPRG SCREEN FOR RECOMBINANT HERPESVIRUS.

When the foreign gene encoded the enzyme β -galactosidase, the plaques that contained the gene were visualized more easily. The chemical BluogalTM (Bethesda Research Labs) for blue plaques was incorporated at the level of 200-300 μ g/ml into the agarose overlay during the plaque assay, and the plaques that expressed active β -galactosidase turned blue. The chemical CPRG (chlorophenol Red Galactopyranoside, Boehringer Mannheim) for red plaques was incorporated into the agarose overlay during the plaque assay, and the plaques that expressed active β -galactosidase turned red. The blue or red plaques were then picked and purified by further blue or red plaque isolations. Other foreign genes were inserted by homologous recombination such that they replaced the β -galactosidase gene; in this instance non-blue plaques were picked for purification of the recombinant virus.

SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT HVT USING BLACK PLAQUE ASSAYS.

To analyze expression of foreign antigens expressed by recombinant HVT viruses, monolayers of CEF cells are infected with recombinant HVT, overlaid with nutrient agarose media and incubated for 4-5 days at 39°C. Once plaques have developed, the agarose overlay is removed from the dish, the monolayer rinsed 1x with PBS, fixed with 100% methanol for 10 minutes at room temperature and the cells air dried. After re-hydrating the plate with PBS, the primary antibody is diluted to the appropriate dilution with PBS and incubated with the cell monolayer for 2 hours to overnight at room temperature. Unbound antibody is then removed from the cells by washing three times with PBS at room temperature. An alkaline phosphatase conjugated secondary antibody is diluted with PBS and incubated with the cells for 2 hours at room temperature. Unbound

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secondary antibody is then removed by washing the cells three times with PBS at room temperature. Next, the monolayer is rinsed in color development buffer (100mM Tris pH 9.5/ 100mM NaCl/ 5mM MgCl₂), and then incubated 10 minutes to overnight at room temperature with freshly prepared substrate solution (0.3 mg/ml Nitro Blue tetrazolium + 0.15 mg/ml 5-Bromo-4-Chloro-3-Indolyl Phosphatase in color development buffer.) Finally, the reaction is stopped by replacing the substrate solution with TE (10mM Tris, pH7.5/ 1 mM EDTA). Plaques expressing the correct antigen will stain black.

PLAQUE HYBRIDIZATION PROCEDURE FOR ASSESSING THE PURITY OF RECOMBINANT HVT STOCKS. When no suitable immunological reagent exists to detect the presence of a particular antigen in a recombinant HVT virus, plaque hybridization can be used to assess the purity of a stock. Initially, CEF cell monolayers are infected with various dilutions of the viral stocks to give ~50-100 plaques/10 cm.dish, overlaid with nutrient agarose media and incubated for 4-5 days at 39°C. Once plaque development occurs, the position of each plaque is marked on bottom of the dish. The agarose overlay is then removed, the plate washed with PBS, and the remaining CEF monolayer is transferred to a NC membrane or BioRad nylon membrane pre-wetted with PBS (making note of the membrane position relative to the dish). Cells contained on the NC membranes are then lysed by placing the membranes in 1.5 mls of 1.5M NaCl and 0.5M NaOH for five minutes. The membranes are neutralized by placing them in 1.5 mls of 3M Sodium acetate (pH 5.2) for five minutes. DNA from the lysed cells is then bound to the NC membranes by baking at 80°C for one hour. After this period the membranes are prehybridized in a solution containing 6X SSC, 3% skim milk, 0.5% SDS, (±) salmon sperm DNA (50 µg/ml) for one hour at 65°C. Radio-labeled probe DNA (alpha 32P-dCTP) is then

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added and the membranes incubated at 65°C overnight (~12 hours). After hybridization the NC membranes are washed two times (30 minutes each) with 2X SSC at 65°C, followed by two additional washes at 65°C with 0.5X SSC. The NC membranes are then dried and exposed to X-ray film (Kodak X-OMAT,AR) at -70°C for 12 hours. Positive signals are then aligned with the position of the plaques on the dish and purity of the stock is recorded as the percentage of positive plaques over the total.

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CONSTRUCTION OF HOMOLOGY VECTOR FOR INSERTION OF THE BETA-GALACTOSIDASE GENE INTO HVT US2 GENE.

The beta-galactosidase (lacZ) gene was inserted into the HVT EcoRI # 7 fragment at the unique StuI site. The marker gene is oriented in the same direction as the US2 gene. It is constructed utilizing standard recombinant DNA techniques (Maniatis et al, 1982 and Sambrook et al, 1989), by joining restriction fragments from the following sources with the synthetic DNA sequences. Fragment 1 is an approximately 413 base pair SalI to BamHI restriction sub-fragment of the PRV BamHI restriction fragment 10 (Lomniczi et al., 1984). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984).

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RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN

CELLS: Chicken spleens were dissected from 3 week old chicks from SPAFAS, Inc., washed, and disrupted through a syringe/needle to release cells. After allowing stroma and debris to settle out, the cells were pelleted and washed twice with PBS. The cell pellet was treated with a hypotonic lysis buffer to lyse red blood cells, and

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splenocytes were recovered and washed twice with PBS. Splenocytes were resuspended at 5×10^6 cells/ml in RPMI containing 5% FBS and 5 μ g/ml Concanavalin A and incubated at 39° for 48 hours. Total RNA was isolated from the cells using guanidine isothionate lysis reagents and protocols from the Promega RNA isolation kit (Promega Corporation, Madison WI). 4 μ g of total RNA was used in each 1st strand reaction containing the appropriate antisense primers and AMV reverse transcriptase (Promega Corporation, Madison WI). cDNA synthesis was performed in the same tube following the reverse transcriptase reaction, using the appropriate sense primers and Vent® DNA polymerase (Life Technologies, Inc. Bethesda, MD).

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV OR SPV. This method relies upon the homologous recombination between FPV or SPV DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both FPV or SPV DNA and transfected plasmid homology vector. For homologous recombination to occur, monolayers of CEF cells are infected with S-FPV 001 (A mild fowlpox vaccine strain available as Bio-Pox™ from Agri-Bio Corporation, Gainesville, Georgia) or or SPV-001 (Kasza strain) at a multiplicity of infection of 0.01 PFU/cell to introduce replicating FPV (i.e. DNA synthesis) or SPV into the cells. The plasmid homology vector DNA is then transfected into these cells according to the "Infection-Transfection Procedure".

INFECTION-TRANSFECTION PROCEDURE. CEF cells in 6 cm plates (about 80% confluent) were infected with S-FPV-001 at a multiplicity of infection of 0.01 PFU/cell in CEF negative medium and incubated at 37°C in a humidified 5% CO₂ incubator for five hours. ESK-4 cells CEF cells in 6 cm plates (about 80% confluent) were infected with S-SPV-001 at a

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multiplicity of infection of 0.01 PFU/cell in CEF negative medium and incubated at 37°C in a humidified 5% CO₂ incubator for five hours. The transfection procedure used is essentially that recommended for LipofectinTM Reagent (BRL).
5 Briefly, for each 6 cm plate, 15 micrograms of plasmid DNA were diluted up to 100 microliters with H₂O. Separately, 50 micrograms of LipofectinTM Reagent were diluted to 100 microliters with H₂O. The 100 microliters of diluted LipofectinTM Reagent were added dropwise to the diluted
10 plasmid DNA contained in a polystyrene, 5 ml, snap cap tube and mixed gently. The mixture was then incubated for 15-20 minutes at room temperature. During this time, the virus inoculum was removed from the 6 cm plates and the cell monolayers washed once with CEF negative medium. Three mls
15 of CEF negative medium were added to the plasmid DNA/lipofectin mixture and the contents pipetted onto the cell monolayer. Following overnight (about 16 hours) incubation at 37°C in a humidified 5% CO₂ incubator, the medium was removed and replaced with 5 ml CEF complete
20 medium. The cells were incubated at 37°C in 5% CO₂ for 3-7 days until cytopathic effect from the virus was 80-100%. Virus was harvested as described above for the preparation of virus stocks. This stock was referred to as a transfection stock and was subsequently screened for
25 recombinant virus by the "Plaque Hybridization Procedure For Purifying Recombinant FPV".

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT AVIAN CHIMERIC HVT/MDV HERPESVIRUS: Transfect
30 subconfluent 1° or 2° chicken embryo fibroblast monolayer (70-80% confluent). Day of use, change media to maintenance media (F10 media + M199 media (1:1 mix), 1% fetal bovine serum; 2% glutamine; 1% NEAA; 1% Penn-Strep). (1) Mix DNA: 20 ul parental viral DNA (amount visible on gel); 2 - 3 ug
35 insertion vector ; dH₂O to 300 ul. (2) Calcium Phosphate

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ppt.: 300 ul DNA; 37 ul 2.5 M CaCl_2 ; 340 ul 2X HEPES-buffered saline. (3) Incubate mixture 1 min. at room temperature, than add to cells dropwise (into media), splitting reaction mix evenly onto 2 x 35 mm dishes (2
5 wells of a 6-well dish). (4) Incubate cells at 39°C for 3 hours. (5) Glycerol shock cells 1 min, by replacing media/reaction mix with 1 ml of 15% glycerol in PBS. After 1 min., remove glycerol, and wash monolayer 3x with PBS. (6)
10 Feed cells with maintenance media, and incubate at 39°C overnight. (7) Next day, replace with fresh media and continue incubation until CPE is observed, changing media every 2 - 3 days. The infected cells are passaged, one or more times, to larger dish until CPE is reached.

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SUBGENOMIC CLONE 172-07.BA2. Plasmid 172-07.BA2 was constructed for the purpose of generating recombinant HVT. It contains an approximately 25,000 base pair region of genomic HVT DNA. It may be used in conjunction with other
20 subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT. This plasmid may be constructed utilizing standard recombinant DNA techniques (Maniatis et al, 1982 and Sambrook et al,
25 1989), by joining two restriction fragments from the following sources. The first fragment is an approximately 2999 base pair BamHI to BamHI restriction fragment of pSP64 (Promega). The second fragment is the approximately 25,000 base pair BamHI #2 fragment of HVT (Buckmaster et al.,
30 1988).

HOMOLOGY VECTOR 172-29.31. The plasmid 172-29.31 was constructed for the purpose of inserting foreign DNA into HVT. It contains a unique XhoI restriction enzyme site into
35 which foreign DNA may be inserted. When a plasmid

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containing a foreign DNA insert at the XhoI site is used according to the DNA COTRANSFECTION FOR GENERATING RECOMBINANT HERPESVIRUSES or the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS a virus containing the foreign DNA will result. This plasmid may be constructed utilizing standard recombinant DNA techniques (Maniatis et al, 1982 and Sambrook et al, 1989), by joining two restriction fragments from the following sources. The first fragment is an approximately 2999 base pair BamHI to BamHI restriction fragment of pSP64 (Promega). The second fragment is the approximately 3300 base pair BamHI #16 fragment of HVT (Buckmaster et al., 1988). The complete sequence of the BamHI #16 fragment is given in SEQ ID NO:1. Note that the fragment was cloned such that the UL43 ORF is in the opposite transcriptional orientation to the pSP64 β -lacatamase gene.

HOMOLOGY VECTOR 172-63.1. The plasmid 172-63.1 was constructed for the purpose of inserting foreign DNA into HVT. It contains a unique XhoI restriction enzyme site into which foreign DNA may be inserted. When a plasmid containing a foreign DNA insert at the XhoI site is used according to the DNA COTRANSFECTION FOR GENERATING RECOMBINANT HERPESVIRUSES or the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS a virus containing the foreign DNA will result. This plasmid may be constructed utilizing standard recombinant DNA techniques (Maniatis et al, 1982 and Sambrook et al, 1989), by joining two restriction fragments from the following sources. The first fragment is an approximately 2999 base pair EcoRI to EcoRI restriction fragment of pSP64 (Promega). The second fragment is the approximately 5500 base pair EcoRI #9 fragment of HVT. Note that the EcoRI fragment was cloned such that the unique XhoI

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site is closest to the unique HindIII site in the pSP64 vector.

SUBGENOMIC CLONE 407-32.1C1. Cosmid 407-32.1C1 was constructed for the purpose of generating recombinant HVT and recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. It contains an approximately 38,850 base pair region of genomic HVT DNA (see Figure 2). This region includes BamHI fragments 11, 7, 8, 21, 6, 18, approximately 1250 base pairs of fragment 13, and approximately 6,700 base pairs of fragment 1. It may be used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT. This cosmid maybe constructed as described above in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. It was isolated from the sheared DNA library by screening with the probes P1 and P4 (described in Figure 2). A bacterial strain containing this cosmid has been deposited on March 3, 1993 pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. 75428.

SUBGENOMIC CLONE 407-32.2C3. Cosmid 407-32.2C3 was constructed for the purpose of generating recombinant HVT. It contains an approximately 40,170 base pair region of genomic HVT DNA (see Figure 2). This region includes BamHI fragments 10, 14, 19, 17, 5, and approximately 2,100 base pairs of fragment 2. It may be used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING

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SUBGENOMIC FRAGMENTS for the construction of recombinant HVT. This cosmid may be constructed as described above in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. It was isolated from the sheared DNA library by screening with the probes P1 and P2 (described in Figure 2). A bacterial strain containing this cosmid has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. 75430.

SUBGENOMIC CLONE 407-32.5G6. Cosmid 407-32.5G6 was constructed for the purpose of generating recombinant HVT. It contains an approximately 40,000 base pair region of genomic HVT DNA (see Figure 2). This region includes BamHI fragments 9, 3, 20, 12, 16, 13, approximately 1,650 base pairs of fragment 2, and approximately 4,000 base pairs of fragment 11. It may be used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT. This cosmid may be constructed as described above in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. It was isolated from the sheared DNA library by screening with the probes P2 and P3 (described in Figure 2). A bacterial strain containing this cosmid has been deposited on March 3, 1993 pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. 75427.

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HOMOLOGY VECTOR 435-47.1. The plasmid 435-47.1 was constructed for the purpose of inserting foreign DNA into HVT. It contains a unique HindIII restriction enzyme site into which foreign DNA may be inserted. When a plasmid containing a foreign DNA insert at the HindIII site is used according to the DNA COTRANSFECTION FOR GENERATING RECOMBINANT HERPESVIRUSES or the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS a virus containing the foreign DNA will result. This plasmid may be constructed utilizing standard recombinant DNA techniques (Maniatis et al, 1982 and Sambrook et al, 1989), by joining two restriction fragments from the following sources. The first fragment is an approximately 2999 base pair EcoRI to EcoRI restriction fragment of pSP64 (Promega). The second fragment is the approximately 7300 base pair EcoRI #7 fragment of HVT. Note that the HindIII site of the pSP64 vector was removed by digesting the subclone with HindIII followed by a Klenow fill in reaction and religation. A synthetic HindIII linker (CAAGCTTG) was then inserted into the unique StuI site of the EcoRI #7 fragment.

SUBGENOMIC CLONE 437-26.26. Plasmid 437-26.26 was constructed for the purpose of generating recombinant HVT. It contains an approximately 15,300 base pair region of genomic HVT DNA. It may be used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT. This plasmid may be constructed utilizing standard recombinant DNA techniques (Maniatis et al, 1982 and Sambrook et al, 1989), by joining two restriction fragments from the following sources. The first fragment is an approximately 2970 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). The second fragment is the approximately

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15,300 base pair BamHI to StuI sub-fragment of the BamHI #2 fragment of HVT (Buckmaster et al., 1988). Note that the BamHI #2 fragment contains five StuI sites, the site utilized in this subcloning was converted to a HindIII site as described in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS.

SUBGENOMIC CLONE 415-09.BA1. Cosmid 415-09.BA1 was constructed for the purpose of generating recombinant HVT. It contains an approximately 29,500 base pair BamHI #1 fragment of genomic HVT DNA. It was used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT. This cosmid was constructed by joining two restriction fragments (Sambrook, et al., 1989) from the following sources. The vector is an approximately 4430 base pair BamHI to BamHI restriction fragment of pSY1005 derived from pH79 (Bethesda Research Labs, Inc.) and pWE15 (Stratagene, Inc.). The first fragment is the approximately 29,500 base pair BamHI #1 fragment of the HVT genome (Buckmaster et al., 1988).

SUBGENOMIC CLONE 672-01.A40. Cosmid 672-01.A40 was constructed for the purpose of generating recombinant HVT. It was isolated as a subclone of cosmid 407-32.1C1 (see Figures 2 and 5). Cosmid 672-01.A40 contains an approximately 14,000 base pair NotI to AscI subfragment and an approximately 1300 base pair AscI to BamHI subfragment of cosmid 407-32.1C1. The cosmid was constructed by joining restriction fragments (Sambrook, et al., 1989) from the following sources. The vector is an approximately 2700 base pair NotI to BamHI fragment constructed from pNEB193 (New England Biolabs, Inc.) which contains a NotI linker inserted into the SmaI site. Fragment 1 is an approximately 15,300

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base pair region of genomic HVT DNA. This region includes BamHI fragments 11 and 7, and approximately 1250 base pairs of fragment 13. It was used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT.

SUBGENOMIC CLONE 654-45.1. Plasmid 654-45.1 was constructed for the purpose of generating recombinant HVT. It was isolated as an AscI subclone of cosmid 407-32.1C1 (see Figures 2 and 5). The cosmid was constructed by joining restriction fragments (Sambrook, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB 193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. Fragment 1 is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) has been converted to a unique PacI site using synthetic DNA linkers. The PacI site was used in insertion and expression of foreign genes in HVT. (See Figure 3A). It was used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT.

SUBGENOMIC CLONE 686-63.A1. Plasmid 686-63.A1 was constructed for the purpose of generating recombinant HVT. It was isolated as an AscI subclone of cosmid 407-32.1C1 (see Figures 2 and 5). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000

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base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. Fragment 1 is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) has been converted to a unique NotI site using synthetic DNA linkers. The NotI site was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). It was used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT.

SUBGENOMIC CLONE 672-07.C40. Cosmid 672-07.C40 was constructed for the purpose of generating recombinant HVT. It was isolated as a subclone of cosmid 407-32.1C1 (see Figures 2 and 5). Cosmid 672-07.C40 contains an approximately 1100 base pair BamHI to AscI subfragment and an approximately 13,000 base pair AscI to NotI subfragment of cosmid 407-32.1C1. The cosmid was constructed by joining restriction fragments (Sambrook, et al., 1989) from the following sources. The vector is an approximately 2700 base pair NotI to BamHI fragment constructed from pNEB193 (New England Biolabs, Inc.) which contains a NotI linker inserted into the SmaI site. Fragment 1 is an approximately 14,100 base pair region of genomic HVT DNA. This region includes BamHI fragments 6 and 18, and an approximately 2600 base pair BamHI to NotI fragment within BamHI fragment #1. It was used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT.

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SUBGENOMIC CLONE 720-51.3. Cosmid 720-51.3 was constructed for the purpose of generating recombinant HVT and generating recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. It contains an approximately 34,700 base pair region of genomic HVT DNA (see Figure 2). This region includes BamHI fragments 11, 7, 8, 21, 6, 18, approximately 1250 base pairs of BamHI fragment 13, and approximately 2,600 base pairs of BamHI fragment 1. It is used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT. Cosmid 720-51.3 was constructed from cosmid 407-32.1C1 in the following procedure. Cosmid 407-32.1C1 was digested with restriction endonuclease Not I to yield an approximately 34,700 base pair HVT genomic fragment, an approximately 4,100 base pair HVT genomic fragment, and an approximately 8,000 base pair pWE15 cosmid vector. The 34,700 base pair HVT genomic fragment and the 8,000 base pair pWE15 cosmid vector were ligated to form cosmid 720-51.3. Cosmid 720-51.3 is missing approximately 4,100 base pairs from HVT BamHI 1 genomic fragment which is present in cosmid 407-32.1C1. Cosmid 720-51.3 contains an XhoI site within the HVT genomic fragment EcoRI #9 which is useful for insertion of foreign DNA into recombinant HVT and into recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region.

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SUBGENOMIC CLONE 721-38.1J. Cosmid 721-38.1J was constructed for the purpose of inserting the MDV gA, gD, and gB genes into the unique short of HVT and for the purpose of generating recombinant HVT. Cosmid 721-38.1J contains the MDV gA, gD and gB genes inserted into a StuI site in the HVT US2 gene converted to a unique HindIII site within the

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BamHI #1 fragment of the unique short region of HVT. This region of the HVT BamHI #1 fragment containing the MDV genes was derived from S-HVT-062. Cosmid 721-38.1J was constructed by a partial restriction digest with BamHI of S-HVT-062 DNA and isolation of an approximately 39,300 base pair fragment. The cosmid was constructed utilizing standard recombinant DNA techniques (Sambrook, et al., 1989) by joining restriction fragments from the following sources. The vector is an approximately 8200 base pair BamHI fragment from cosmid vector pWE15. The first fragment is an approximately 900 base pair BamHI fragment from the repeat region of the HVT genome. The second fragment is an approximately 15,500 base pair BamHI to StuI subfragment of BamHI #1 of HVT. The third fragment is an approximately 8400 base pair cassette containing the MDV gA, gD, and gB genes. The fourth fragment is an approximately 14,500 base pair HindIII to BamHI subfragment of the BamHI #1 of HVT.

SUBGENOMIC CLONE 722-60.E2. Cosmid 722-60.E2 was constructed for the purpose of inserting the MDV gA, gD, and gB genes and the NDV HN and F genes into the unique short of HVT and for the purpose of generating recombinant HVT. Cosmid 722-60.E2 contains the MDV gA, gD and gB genes and the NDV HN and F genes inserted into a StuI site in the HVT US2 gene converted to a unique HindIII site within the BamHI #1 fragment of the unique short region of HVT. All five genes were inserted in the same transcriptional orientation as the HVT US2 gene. This region of the HVT BamHI #1 fragment containing the MDV and NDV genes was derived from S-HVT-106. Cosmid 722-60.E2 was constructed by a partial restriction digest with BamHI of S-HVT-106 and isolation of an approximately 46,300 base pair fragment. The cosmid was constructed utilizing standard recombinant DNA techniques (Sambrook, et al., 1989) by joining restriction fragments from the following sources. The vector is an approximately

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6100 base pair BamHI fragment from cosmid vector pSY1626 derived from pH79 (Bethesda Research Labs, Inc.) and pWE15 (Strategene, Inc.). The first fragment is an approximately 900 base pair BamHI fragment from the repeat region of the HVT genome. The second fragment is an approximately 15,500 base pair BamHI to StuI subfragment of BamHI #1 of HVT. The third fragment is an approximately 15,400 base pair cassette containing the MDV gA gene (SEQ ID NO: 6), the PRV gX promoter (Lomniczi et al., 1984), the NDV HN gene (SEQ ID NO: 8), the PRV gX polyadenylation site (Lomniczi et al., 1984), the HCMV immediate early promoter (D.R. Thomsen, et al., 1981), the NDV F gene (SEQ ID NO: 10), the HSV TK polyadenylation site (McGeoch, et al., 1985), the MDV gD gene, the approximately 450 base pair ILTV US3 polyadenylation site, and the MDV gB gene. The fourth fragment is an approximately 14,500 base pair StuI to BamHI subfragment of the BamHI #1 of HVT.

SUBGENOMIC CLONE 739-27.16. Cosmid 739-27.16 was constructed for the purpose of constructing a chimeric HVT/MDV virus containing the HVT genes of the unique long region and the MDV type 1 genes of the unique short region. Cosmid 739-27.16 contains the complete unique short region of MDV type 1. This region contains the entire SmaI B fragment and two SmaI K fragments. Cosmid 739-27.16 was constructed by a partial restriction digest with SmaI of MDV DNA and isolation of an approximately 29,000 to 33,000 base pair fragment. The cosmid was constructed utilizing standard recombinant DNA techniques (Sambrook, et al., 1989) by joining restriction fragments from the following sources. The vector is an approximately 8200 base pair BamHI fragment (made blunt-ended with Lenov DNA polymerase) from cosmid vector pWE15. The first fragment is an approximately 4050 base pair SmaI K fragment from the short internal repeat region of the MDV genome. The second fragment is an

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approximately 21,000 base pair fragment SmaI B of MDV. The third fragment is an approximately 3,650 base pair SmaI K fragment from the short terminal repeat region of the MDV genome (Fukuchi, et al., 1984, 1985).

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SUBGENOMIC CLONE 751-87.A8. Plasmid 751-87.A8 was constructed for the purpose of generating recombinant HVT. Plasmid 751-87.A8 contains the chicken myelomonocytic growth factor (cGMF) gene inserted into the PacI site of plasmid 654-45.1. The cMGF gene uses the HCMV immediate early promoter and HSV-1 TK polyadenylation signal. The cosmid was constructed utilizing standard recombinant DNA techniques (Sambrook, et al., 1989). The following fragments were inserted into the PacI site of HVT subgenomic clone 654-45.1. The first fragment is an approximately 1191 base pair PstI to AvaII restriction subfragment of the HCMV genomic XbaI E fragment (D.R. Thomsen, et al., 1981). The second fragment is an approximately 640 base pair fragment coding for the cMGF gene (58) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer used for reverse transcription and PCR was 5'-CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3' (SEQ ID NO: 19). The sense primer used for PCR was 5'-GAGCGGATCCTGCAGGAGGAGACACAGAGCTG-3' (SEQ ID NO: 20). The cMGF fragment was subcloned next to the HCMV IE promoter using BamHI sites generated by the PCR primers. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 201 of the cMGF protein (58) which includes a 23 amino acid leader sequence at the amino terminus and 178 amino acids of the mature cMGF protein. The third fragment is an approximately 784 base pair SmaI to SmaI restriction subfragment of the HSV-1 BamHI restriction fragment Q (McGeoch, et al., 1985).

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SUBGENOMIC CLONE 761-07.A1. Plasmid 761-07.A1 was constructed for the purpose of generating recombinant HVT. Plasmid 761-07.A1 contains the chicken interferon gene inserted into the PacI site of plasmid 654-45.1. The chicken
5 interferon gene uses the HCMV immediate early promoter and HSV-1 TK polyadenylation signal. The cosmid was constructed utilizing standard recombinant DNA techniques (Sambrook, et al., 1989). The following fragments were inserted into the PacI site of HVT subgenomic clone 654-45.1. The first
10 fragment is an approximately 1191 base pair PstI to AvaII restriction subfragment of the HCMV genomic XbaI E fragment (D.R. Thomsen, et al., 1981). The second fragment is an approximately 577 base pair fragment coding for the chicken interferon gene (59) derived by reverse transcription and
15 polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer used for reverse transcription and PCR was 5'-TGTAGAGATCTGGCTAAGTGCGCGTGTTCCTG-3' (SEQ ID NO: 21). The sense primer used for PCR was 5'-
20 TGTACAGATCTCACCATGGCTGTGCCTGCAAGC-3' (SEQ ID NO: 22). The chicken interferon gene fragment was subcloned next to the HCMV IE promoter using BglII sites generated by the PCR primers. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 193 of the chicken interferon
25 protein (59) which includes a 31 amino acid signal sequence at the amino terminus and 162 amino acids of the mature protein encoding chicken interferon. The third fragment is an approximately 784 base pair SmaI to SmaI restriction subfragment of the HSV-1 BamHI restriction fragment Q
30 (McGeoch, et al., 1985).

HOMOLOGY VECTOR 301-07.Y#D1: Plasmid 301-07.Y#D1 was constructed for the purpose of generating recombinant chimeric HVT/MDV vaccine expressing a foreign DNA sequence.
35 The E. coli lacZ gene is expressed under the control of the

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PRV gX promoter. The HVT DNA is an AscI subclone of cosmid 407-32.1C1 (see Figures 2 and 5). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is an approximately 413 base pair SalI to BamHI restriction sub-fragment of the PRV BamHI restriction fragment 10 (Lomniczi et al., 1984). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Plasmid 301-07.Y#D1 was used in conjunction with S-HVY-145 according to the DNA TRANSFECTION FOR GENERATING RECOMBINANT VIRUS for the construction of recombinant chimeric HVT/MDV vaccine.

SUBGENOMIC CLONE 852-52.II4. Plasmid 852-52.II4 was constructed for the purpose of generating recombinant chimeric HVT/MDV vaccine expressing a foreign DNA sequence. The infectious laryngotracheitis (ILT) virus glycoprotein D (gD) and glycoprotein I (gI) genes are expressed under the control of the ILT virus gD and gI promoters. The HVT DNA is an AscI subclone of cosmid 407-32.1C1 (see Figures 2 and 5). The cosmid was constructed by joining restriction fragments

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(Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: The fragment is an approximately 3556 base pair SalI to HindIII restriction subfragment of the ILTV Asp718I genomic fragment #8 (10.6 kb). Plasmid 852-52.II4 was used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT.

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SUBGENOMIC CLONE 854-33.6. Plasmid 854-33.6 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence and generating recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region expressing a foreign DNA sequence. The Newcastle disease virus (NDV) hemagglutinin (HN) gene is expressed under the control of the PRV gX promoter. The NDV fusion (F) gene is expressed under the control of the HCMV immediate early promoter. The NDV HN and F genes under the control of their respective promoters are transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA. The HVT DNA is derived from cosmid 720-51.3, which is a subclone of cosmid 407-32.1C1 (see Figure 2, 5). The HVT fragment is an approximately 34,700 base pair fragment of genomic HVT DNA. This region includes

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BamHI fragments 11, 7, 8, 21, 6, 18, and approximately 1250 base pairs of fragment 13 and approximately 2600 base pairs of fragment 1. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 8000 base pair NotI fragment of pWE15. The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is an approximately 413 base pair SalI to BamHI restriction subfragment of the PRV BamHI fragment #10 (Lomniczi, et al., 1984). Fragment 2 is an approximately 1811 base pair AvaII to NaeI restriction fragment of the full length NDV HN cDNA clone (B1 strain). Fragment 3 is an approximately 754 base pair NdeI to SalI subfragment of PRV BamHI #7 (B. Lomniczi et al.). Fragment 4 is an approximately 1191 base pair PstI to AvaII restriction subfragment of the HCMV genomic XbaI E fragment (D.R. Thomsen, et al., 1981). Fragment 5 is an approximately 1812 base pair BamHI to PstI restriction fragment of the full length NDV F cDNA clone (B1 strain; Reference: WO 96/05291). Fragment 6 is an approximately 784 base pair SmaI to SmaI restriction sub-fragment of the HSV-1 BamHI restriction fragment Q (McGeoch, et al., 1985).

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HOMOLOGY VECTOR 864-74.18: Plasmid 864-74.18 was constructed for the purpose of generating recombinant chimeric HVT/MDV vaccine expressing a foreign DNA sequence. The E. coli lacZ gene is expressed under the control of the PRV gX promoter. The HVT DNA is an AscI subclone of cosmid 407-32.1C1 (see Figures 2 and 5). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers

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inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is an approximately 3556 base pair SalI to HindIII restriction subfragment of the ILTV Asp718I genomic fragment #8 (10.6 kb). Fragment 2 is an approximately 413 base pair SalI to BamHI restriction sub-fragment of the PRV BamHI restriction fragment 10 (Lomniczi et al., 1984). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 4 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Plasmid 864-74.18 was used in conjunction with S-HVY-145 according to the DNA TRANSFECTION FOR GENERATING RECOMBINANT VIRUS for the construction of recombinant chimeric HVT/MDV vaccine.

SUBGENOMIC CLONE 867-96.B9. Plasmid 867-96.B9 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence. The E. coli β -galactosidase gene is expressed under the control of a novel chicken anemia virus promoter. The HVT DNA is an AscI subclone of cosmid 407-32.1C1 (see Figures 2 and 5). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of

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genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the

5 insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is the CAV promoter synthesized by PCR as a 386 bp EcoRI to BamHI fragment from the CAV strain CL-1 (NVSL; Dr. D.B. Snyder, Univ. Maryland;

10 SEQ ID NO. 23). Fragment 2 is an approximately 3001 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984).

15 Plasmid 867-96.B9 was used in conjunction with other subgenomic clones according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS for the construction of recombinant HVT.

20 **SUBGENOMIC CLONE 890-77.10.** Plasmid 890-77.10 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence and generating recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys

25 long region expressing a foreign DNA sequence. The E. coli lacZ gene is expressed under the control of the PRV gX promoter. The Newcastle disease virus (NDV) fusion (F) gene is expressed under the control of the HCMV immediate early promoter. The HVT DNA is an AscI subclone of cosmid 407-

30 32.1C1 (see Figure 2, 5). The E. coli lacZ gene and the NDV F gene under the control of their respective promoters are transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA. The cosmid was constructed by joining restriction fragments

35 (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment

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constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is an approximately 413 base pair SalI to BamHI restriction subfragment of the PRV BamHI fragment #10 (Lomniczi, et al., 1984). Fragment 2 is an approximately 3006 base pair PvuII to BamHI subfragment of pJF751 (Ferrari, et al.). Fragment 3 is an approximately 754 base pair NdeI to SalI subfragment of PRV BamHI #7 (B. Lomniczi et al.). Fragment 4 is an approximately 1191 base pair PstI to AvaII restriction subfragment of the HCMV genomic XbaI E fragment (D.R. Thomsen, et al., 1981). Fragment 5 is an approximately 1812 base pair BamHI to PstI restriction fragment of the full length NDV F cDNA clone (B1 strain; Reference: WO 96/05291). Fragment 6 is an approximately 784 base pair SmaI to SmaI restriction subfragment of the HSV-1 BamHI restriction fragment Q (McGeoch, et al., 1985).

SUBGENOMIC CLONE 900-87.H8. Plasmid 900-87.H8 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence and generating recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region expressing a foreign DNA sequence. The infectious bursal disease virus (IBDV) VP2 gene is expressed under the control of a chicken anemia virus (CAV) promoter. The IBDV VP2 gene under the control of the CAV promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA. The HVT

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DNA is an AscI subclone of cosmid 407-32.1C1 (see Figure 2, 5). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is the CAV promoter synthesized by PCR as a 386 bp EcoRI to BamHI fragment from the CAV strain CL-1 (NVSL; SEQ ID NO. ____). The BamHI site was altered to an EcoRV site in this construct. Fragment 2 is an approximately 1356 base pair HincII to EcoRI fragment coding for the IBDV VP2 gene derived from plasmid 779-54.1. IBDV Bursvac M large segment RNA was first cloned as cDNA in two overlapping fragments using Okayama-Berg first strand/second strand cDNA synthesis. These clones were joined at a unique restriction site to create plasmid 2.40/84#3. The plasmid 779-54.1 was created by PCR in two parts using plasmid 2.40/84#3 as a template. The sense and antisense primers for the first half were 5'-GCCGGCGGCCGCGGATACGATCGGTCTGACCCCGG-3' and 5'-TTGTGTGCACCGCGGAGTACCCC-3', respectively. The sense and antisense primers for the second half were 5'-TCGCGAATCTATTCCAGGTGCCCC-3' and 5'-CGGAATTCTCCAATTTGGGATGTTGTAAGGCCGA-3', respectively. The two halves were joined at a unique SalI site. Fragment 3 is the ILTV PK polyadenylation signal synthesized by PCR as a 144 bp SalI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-GGTGCCACGGTCGACGAGTGAAGGTTAAT-3' (SEQ ID NO. ____)

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AGACCGAGCAGAGTCGACGCGCGAAAG-3' (SEQ ID NO. ____),
respectively.

SUBGENOMIC CLONE 928-47.1. Cosmid 928-47.1 was constructed
5 for the purpose of generating recombinant HVT expressing a
foreign DNA sequence and generating recombinant chimeric
viral vaccine comprising a chimera of the Marek's disease
virus short region and the herpesvirus of turkeys long
region expressing a foreign DNA sequence. The vector is an
10 approximately 8000 base pair NotI fragment of pWE15. The
viral DNA insert is composed of two segments. Fragment 1 is
an approximately 8.6 kb AscI to ScaI fragment containing the
junction region between the HVT long repeat region and the
MDV short region from the recombinant chimeric virus HVY-
15 145. Fragment 2 is a 28.8 kb ScaI to NotI fragment derived
from 739-27.16, and contains the remainder of the MDV short
region from that clone.

SUBGENOMIC CLONE 928-58.J2. Plasmid 928-58.J2 was
20 constructed for the purpose of generating recombinant HVT
expressing a foreign DNA sequence and generating recombinant
chimeric viral vaccine comprising a chimera of the Marek's
disease virus short region and the herpesvirus of turkeys
long region expressing a foreign DNA sequence. The E.coli
25 lacZ gene is expressed under the control of an infectious
laryngotracheitis virus (ILTV) glycoprotein I (gI) promoter.
The E. coli lacZ gene under the control of the ILTV gI
promoter is transcribed in the opposite direction from the
HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic
30 DNA. The HVT DNA is an AscI subclone of cosmid 407-32.1C1
(see Figure 2, 5). The cosmid was constructed by joining
restriction fragments (Sambrooks, et al., 1989) from the
following sources. The vector is an approximately 2000 base
pair AscI fragment constructed from a 2000 base pair AatII
35 to PvuII fragment of pNEB193 (New England Biolabs, Inc.)
blunt ended with Klenow DNA polymerase and AscI linkers
inserted. The HVT fragment is an approximately 8600 base

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pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is the ILTV gI promoter synthesized by PCR as a 279 bp PmeI to BamHI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-CAGTTTAAACTCGGATTCTGACTATTAC-3' (SEQ ID NO. __) and 5'-CGGATCCATGCTTTTCGAACGTCC-3' (SEQ ID NO. __), respectively. (See Figure 8). Fragment 2 is an approximately 3006 base pair PvuII to BamHI subfragment of pJF751 (Ferrari, et al.). Fragment 3 is the ILTV PK polyadenylation signal synthesized by PCR as a 144 bp SalI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-GGTGCCACGGTCGACGAGTGAAGGTTAAT-3' (SEQ ID NO. __) and 5'-AGACCGAGCAGAGTCGACGCGCGAAAG-3' (SEQ ID NO. XX), respectively.

SUBGENOMIC CLONE 928-58.K7. Plasmid 928-58.K7 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence and generating recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region expressing a foreign DNA sequence. The E.coli lacZ gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein D (gD) promoter. The E. coli lacZ gene under the control of the ILTV gD promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA. The HVT DNA is an AscI subclone of cosmid 407-32.1C1 (see Figure 2, 5). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII

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to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is the ILTV gD promoter synthesized by PCR as a 530 bp PmeI to BamHI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for reverse transcription and PCR were 5'-GGTTTAAACAGCTGTACTACAGAGTAAC-3' (SEQ ID NO. 15) and 5'-CGGATCCATAGCGTTGCGTACAAAG-3' (SEQ ID NO. 16), respectively. (See Figure 7). Fragment 2 is an approximately 3006 base pair PvuII to BamHI subfragment of pJF751 (Ferrari, et al.). Fragment 3 is the ILTV PK polyadenylation signal synthesized by PCR as a 144 bp SalI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-GGTGCCACGGTCGACGAGTGAAGGTTAAT-3' (SEQ ID NO. 17) and 5'-AGACCGAGCAGAGTCGACGCGCGAAAG-3' (SEQ ID NO. 18), respectively.

SUBGENOMIC CLONE 949-01.I2. Plasmid 949-01.I2 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence and generating recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region expressing a foreign DNA sequence. The infectious bursal disease virus (IBDV) VP2 gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein I (gI) promoter. The IBDV VP2 gene under the control of the ILTV gI promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA. The HVT DNA is an AscI subclone of cosmid 407-32.1C1 (see Figure 2, 5). The cosmid

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was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is the ILTV gI promoter synthesized by PCR as a 279 bp PmeI to BamHI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'- CAGTTTAAACTCGGATTCTGACTATTAC-3' (SEQ ID NO. __) and 5'- CGGATCCATGCTTTTCGAACGTCC-3' (SEQ ID NO. __), respectively. (See Figure 8). Fragment 2 is an approximately 1356 base pair HincII to EcoRI fragment coding for the IBDV VP2 gene derived from plasmid 779-54.1. IBDV Bursvac M large segment RNA was first cloned as cDNA in two overlapping fragments using Okayama-Berg first strand/second strand cDNA synthesis. These clones were joined at a unique restriction site to create plasmid 2.40/84#3. The plasmid 779-54.1 was created by PCR in two parts using plasmid 2.40/84#3 as a template. The sense and antisense primers for the first half were 5'-GCCGGCGGCCGCGGATACGATCGGTCTGACCCCGG-3' and 5'-TTGTGTGCACCGCGGAGTACCCC-3', respectively. The sense and antisense primers for the second half were 5'-TCGCGAATCTATTCCAGGTGCCCC-3' and 5'-CGGAATTCTCCAATTGTTGTAAGGCCGA-3', respectively. The two halves were joined at a unique SalI site. Fragment 3 is the ILTV PK polyadenylation signal synthesized by PCR as a 144 bp SalI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-GGTGCCACGGTCGACGAGTGAAGGTTAAT-3' (SEQ ID NO. __) and 5'-

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AGACCGAGCAGAGTCGACGCGCGAAAG-3' (SEQ ID NO. ____),
respectively.

SUBGENOMIC CLONE 949-19.2. Plasmid 949-19.2 was constructed
5 for the purpose of generating recombinant HVT expressing a
foreign DNA sequence and generating recombinant chimeric
viral vaccine comprising a chimera of the Marek's disease
virus short region and the herpesvirus of turkeys long
region expressing a foreign DNA sequence. The infectious
10 bursal disease virus (IBDV) VP2 gene is expressed under the
control of an infectious laryngotracheitis virus (ILTV)
glycoprotein I (gI) promoter. The IBDV VP2 gene under the
control of the ILTV gI promoter is transcribed in the same
direction as the HVT UL52, UL54 and UL55 genes in the
15 adjacent HVT genomic DNA. The HVT DNA is derived from
cosmid 720-51.3, which is a subclone of cosmid 407-32.1C1
(see Figure 2, 5). The HVT fragment is an approximately
34,700 base pair fragment of genomic HVT DNA. This region
includes BamHI fragments 11, 7, 8, 21, 6, 18, and
20 approximately 1250 base pairs of fragment 13 and
approximately 2600 base pairs of fragment 1. The XhoI site
(Nucleotide #1339-1344; SEQ ID NO. 12) was used for the
insertion and expression of foreign genes in HVT. (See
Figure 3B). The cosmid was constructed by joining
25 restriction fragments (Sambrooks, et al., 1989) from the
following sources. The vector is an approximately 8000 base
pair NotI fragment of pWE15. The foreign DNA inserted into
the XhoI site of HVT is as follows: Fragment 1 is the ILTV
gI promoter synthesized by PCR as a 279 bp PmeI to BamHI
30 fragment from the USDA challenge strain of ILTV. The sense
and antisense primers used for PCR were 5'-
CAGTTTAAACTCGGATTCTGACTATTAC-3' (SEQ ID NO. ____)
and 5'-CGGATCCATGCTTTTTCGAACGTCC-3' (SEQ ID NO. ____),
respectively. (See Figure 8). The BamHI site was then altered to an EcoRV
35 site using a linker. Fragment 2 is an approximately 1356
base pair HincII to EcoRI fragment coding for the IBDV VP2
gene derived from plasmid 779-54.1. IBDV Bursvac M large

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segment RNA was first cloned as cDNA in two overlapping fragments using Okayama-Berg first strand/second strand cDNA synthesis. These clones were joined at a unique restriction site to create plasmid 2.40/84#3. The plasmid 779-54.1 was created by PCR in two parts using plasmid 2.40/84#3 as a template. The sense and antisense primers for the first half were 5'-GCCGGCGGCCGCGGATACGATCGGTCTGACCCCGG-3' and 5'-TTGTGTGCACCGCGGAGTACCCC-3', respectively. The sense and antisense primers for the second half were 5'-TCGCGAATCTATTCCAGGTGCCCC-3' and 5'-CGGAATTCTCCAATTTGGGATGTTGTAAGGCCGA-3', respectively. The two halves were joined at a unique SalI site. Fragment 3 is the ILTV PK polyadenylation signal synthesized by PCR as a 144 bp SalI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-GGTGCCACGGTCGACGAGTGAAGGTTAAT-3' (SEQ ID NO. __) and 5'-AGACCGAGCAGAGTCGACGCGCGAAAG-3' (SEQ ID NO. __), respectively.

SUBGENOMIC CLONE 949-24.D1. Plasmid 949-24.D1 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence and generating recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region expressing a foreign DNA sequence. The infectious bursal disease virus (IBDV) VP2 gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein D (gD) promoter. The IBDV VP2 gene under the control of the ILTV gD promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA. The HVT DNA is derived from cosmid 720-51.3, which is a subclone of cosmid 407-32.1C1 (see Figure 2, 5). The HVT fragment is an approximately 34,700 base pair fragment of genomic HVT DNA. This region includes BamHI fragments 11, 7, 8, 21, 6, 18, and approximately 1250 base pairs of fragment 13 and approximately 2600 base pairs of fragment 1. The XhoI site

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(Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 8000 base pair NotI fragment of pWE15. The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is the ILTV gD promoter synthesized by PCR as a 530 bp PmeI to BamHI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for reverse transcription and PCR were 5'-GGTTTAAACAGCTGTACTACAGAGTAAC-3' (SEQ ID NO. __) and 5'-CGGATCCATAGCGTTGCGTACAAAG-3' (SEQ ID NO. __), respectively. (See Figure 7). Fragment 2 is an approximately 1356 base pair HincII to EcoRI fragment coding for the IBDV VP2 gene derived from plasmid 779-54.1. IBDV Bursvac M large segment RNA was first cloned as cDNA in two overlapping fragments using Okayama-Berg first strand/second strand cDNA synthesis. These clones were joined at a unique restriction site to create plasmid 2.40/84#3. The plasmid 779-54.1 was created by PCR in two parts using plasmid 2.40/84#3 as a template. The sense and antisense primers for the first half were 5'-GCCGGCGGCCGCGGATACGATCGGTCTGACCCCGG-3' and 5'-TTGTGTGCACCGCGGAGTACCCC-3', respectively. The sense and antisense primers for the second half were 5'-TCGCGAATCTATTCCAGGTGCCCC-3' and 5'-CGGAATTCTCCAATTTGGGATGTTGTAAGGCCGA-3', respectively. The two halves were joined at a unique SalI site. Fragment 3 is the ILTV PK polyadenylation signal synthesized by PCR as a 144 bp SalI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-GGTGCCACGGTCGACGAGTGAAGGTTAAT-3' (SEQ ID NO. __) and 5'-AGACCGAGCAGAGTCGACGCGCGAAAG-3' (SEQ ID NO. __), respectively.

35 SUBGENOMIC CLONE 949-24.I2. Plasmid 949-24.I2 was constructed for the purpose of generating recombinant HVT expressing a foreign DNA sequence and generating recombinant

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chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region expressing a foreign DNA sequence. The infectious bursal disease virus (IBDV) VP2 gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein I (gI) promoter. The IBDV VP2 gene under the control of the ILTV gI promoter is transcribed in the opposite direction as the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA. The HVT DNA is derived from cosmid 720-51.3, which is a subclone of cosmid 407-32.1C1 (see Figure 2, 5). The HVT fragment is an approximately 34,700 base pair fragment of genomic HVT DNA. This region includes BamHI fragments 11, 7, 8, 21, 6, 18, and approximately 1250 base pairs of fragment 13 and approximately 2600 base pairs of fragment 1. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 8000 base pair NotI fragment of pWE15. The foreign DNA inserted into the XhoI site of HVT is as follows: Fragment 1 is the ILTV gI promoter synthesized by PCR as a 279 bp PmeI to BamHI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-CAGTTTAAACTCGGATTCTGACTATTAC-3' (SEQ ID NO. __) and 5'-CGGATCCATGCTTTTCGAACGTCC-3' (SEQ ID NO. __), respectively. (See Figure 8). Fragment 2 is an approximately 1356 base pair HincII to EcoRI fragment coding for the IBDV VP2 gene derived from plasmid 779-54.1. IBDV Bursvac M large segment RNA was first cloned as cDNA in two overlapping fragments using Okayama-Berg first strand/second strand cDNA synthesis. These clones were joined at a unique restriction site to create plasmid 2.40/84#3. The plasmid 779-54.1 was created by PCR in two parts using plasmid 2.40/84#3 as a template. The sense and antisense primers for the first half were 5'-GCCGGCGGCCGCGGATACGATCGGTCTGACCCCGG-3' and 5'-

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TTGTGTGCACCGCGGAGTACCCC-3', respectively. The sense and antisense primers for the second half were 5'-TCGCGAATCTATTCCAGGTGCCCC-3' and 5'-CGGAATTCTCCAATTTGGGATGTTGTAAGGCCGA-3', respectively. The two halves were joined at a unique SalI site. Fragment 3 is the ILTV PK polyadenylation signal synthesized by PCR as a 144 bp SalI fragment from the USDA challenge strain of ILTV. The sense and antisense primers used for PCR were 5'-GGTGCCACGGTCGACGAGTGAAGGTTAAT-3' (SEQ ID NO. __) and 5'-AGACCGAGCAGAGTCGACGCGCGAAAG-3' (SEQ ID NO. __), respectively.

HOMOLOGY VECTOR 881-23.#28: Plasmid 881-23.#28 was constructed for the purpose of generating recombinant chimeric HVT/MDV vaccine expressing a foreign DNA sequence. The E. coli lacZ gene is expressed under the control of the HSV-1 TK promoter. The Newcastle disease virus (NDV) hemagglutinin (HN) gene is under the control of the PRV gX promoter, and the Newcastle disease virus (NDV) fusion (F) gene is under the control of the HCMV immediate early promoter. The HVT DNA is an AscI subclone of cosmid 407-32.1C1 (see Figures 2 and 5). The cosmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AscI fragment constructed from a 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) blunt ended with Klenow DNA polymerase and AscI linkers inserted. The HVT fragment is an approximately 8600 base pair AscI to AscI fragment of genomic HVT DNA. This region includes BamHI fragments 10 and 21, and approximately 1100 base pairs of fragment 6 and approximately 1300 base pairs of fragment 7. The XhoI site (Nucleotide #1339-1344; SEQ ID NO. 12) was used for the insertion and expression of foreign genes in HVT. (See Figure 3B). The foreign DNA inserted into the XhoI site of HVT genomic DNA is as

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follows: Fragment 1 is an approximately 266 base pair *RsaI* to *HaeIII* restriction sub-fragment of the HSV-1 *BamHI* restriction fragment N. Fragment 2 is an approximately 3330 base pair *BamHI* to *BalI* restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 413 base pair *SalI* to *BamHI* restriction sub-fragment of the PRV *BamHI* restriction fragment 10 (Lomniczi et al., 1984). Fragment 4 is an approximately 1811 base pair *AvaII* to *NaeI* restriction fragment of the full length NDV HN cDNA clone (B1 strain). Fragment 5 is an approximately 754 base pair *NdeI* to *SalI* restriction sub-fragment of the PRV *BamHI* restriction fragment #7 (Lomniczi et al., 1984). Fragment 6 is an approximately 1191 base pair *PstI* to *AvaII* restriction sub-fragment of the HCMV genomic *XbaI* E fragment (D.R. Thomsen, et al., 1981). Fragment 7 is an approximately 1812 base pair *BamHI* to *PstI* restriction fragment of the full length NDV F cDNA clone (B1 strain). Fragment 8 is an approximately 784 base pair *SmaI* to *SmaI* restriction sub-fragment of the HSV-1 *BamHI* restriction fragment Q (McGeoch, et al., 1985). Plasmid 881-23.#28 was used in conjunction with S-HVY-145 according to the DNA TRANSFECTION FOR GENERATING RECOMBINANT VIRUS for the construction of recombinant chimeric HVT/MDV vaccine.

HOMOLOGY VECTOR 883-10.A1: The homology vector 883-10.A1 was constructed for the purpose of inserting the *lacZ* marker gene under the control of a novel chicken anemia virus promoter into fowlpox virus (FPV) at a unique *NotI* site. The 2.8 KB FPV genomic *EcoRI* fragment was used for the insertion of foreign DNA into FPV. The *NotI/SfiI* linker was inserted into a unique *SnaBI* site in the FPV fragment. The cassette was constructed utilizing standard recombinant DNA techniques (Maniatis et al., 1982 and Sambrook et al., 1989), by joining restriction fragments from the following

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sources with the synthetic DNA sequences indicated. The foreign DNA inserted into the NotI site of FPV is as follows: Fragment 1 is the CAV promoter synthesized by PCR as a 386 bp EcoRI to BamHI fragment from the CAV strain CL-1 (NVSL; Dr. D.B. Snyder, Univ. Maryland; SEQ ID NO. 23). Fragment 2 is an approximately 3001 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Recombinant fowlpox virus was generated by the procedure of HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV

TRANSIENT TRANSFECTION ASSAY: Chick embryo fibroblast cells or QT-35 cells at 80-90% confluence in 6 cm dishes have growth media removed, and 5 ml of maintenance media is added. 10-20 µg of plasmid DNA is diluted in water and CaCl₂ is added to a concentration of 0.25 M and a volume of 600 µl. To this, 600 µl of 2X HEPES buffer (0.28 M NaCl, 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 1.5 mM Na₂HPO₄, pH 7.05) is added, allowed to incubate 1' at room temperature, and then this solution is split in two 600 µl aliquots and dropped onto two 6 cm dishes. After 3 hours, the solution is removed and a 10% glycerol in PBS solution is added and allowed to remain on the cells for 1 minute. This is removed, cells are washed once with PBS, and refed with 5 ml of maintenance media. After 24-48 hours, cells were harvested, pelleted at 2.8K in a Sorvall RT7, and resuspended in 500 µl PBS. Samples were subjected to three cycles of freezing and thawing and cellular debris was spun down in a microfuge. Duplicate samples consisting of 11 µl of each supernatant were tested in a microtiter plate with 100 µl of ONPG assay solution (0.8 mg/ml ortho-nitro-phenyl-galactoside, 60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 50 mM β-mercaptoethanol, pH 7.5), incubated

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at 37°C, and readings at 415 nm were taken with a BioRad Model 450 microplate reader every fifteen minutes.

PLASMID 388-65.2. Plasmid 388-65.2 was constructed for the purpose of generating an HCMV immediate early (IE) promoter to express a foreign DNA sequence. The *E. coli* β -galactosidase gene is expressed under the control of the HCMV IE promoter. The plasmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) Fragment 1 is an 1149 base pair PstI to AvaII subfragment of the XbaI E fragment of the HCMV Towne strain. Fragment 2 is an approximately 3001 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Plasmid 388-65.2 was used according to the TRANSIENT TRANSFECTION ASSAY to measure the strength of the CAV promoter.

PLASMID 850-25.18. Plasmid 850-25.18 was constructed for the purpose of generating a novel CAV promoter to express a foreign DNA sequence. The *E. coli* β -galactosidase gene is expressed under the control of a modified chicken anemia virus promoter. The plasmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.). Fragment 1 is the CAV promoter synthesized by PCR as a 854 bp EcoRI to BamHI fragment from the CAV strain CL-1 (NVSL; Dr. D.B. Snyder, Univ. Maryland;) using PCR primers 5'- ATCGAATTCCGAGTGGTTACTATTCC -3' (SEQ ID NO 24) and 5'- CGTGGATCCATCTTACAGTCTTATAC -3' (SEQ ID NO 25). Fragment 2 is an approximately 3001 base pair BamHI to PvuII

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restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Plasmid 850-25.18 was used according to the TRANSIENT TRANSFECTION ASSAY to measure the strength of the CAV promoter.

PLASMID 850-69.1. Plasmid 850-69.1 was constructed for the purpose of generating a novel CAV promoter to express a foreign DNA sequence. The E. coli β -galactosidase gene is expressed under the control of a modified chicken anemia virus promoter. The plasmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) Fragment 1 is the CAV promoter synthesized by PCR as a 858 bp EcoRI to BamHI fragment from the CAV strain CL-1 (NVSL; Dr. D.B. Snyder, Univ. Maryland;) using PCR primers 5'- ATCGAATTCCGAGTGGTTACTATTCC -3' (SEQ ID NO 24) and 5'- CGTGGATCCATCTTACAGTCTTATAC -3' (SEQ ID NO 25). The HindIII site near the BamHI site is within the CAV apoptin reading frame. The HindIII site was filled in to destroy the apoptin reading frame. Fragment 2 is an approximately 3001 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Plasmid 850-69.1 was used according to the TRANSIENT TRANSFECTION ASSAY to measure the strength of the CAV promoter.

PLASMID 850-80.2. Plasmid 850-80.2 was constructed for the purpose of generating a novel CAV promoter to express a foreign DNA sequence. The E. coli β -galactosidase gene is

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expressed under the control of a modified chicken anemia virus promoter. The plasmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) Fragment 1 is the CAV promoter synthesized by PCR as a 381 bp EcoRI to BamHI fragment (SEQ ID NO. 23) from the CAV strain CL-1 (NVSL; Dr. D.B. Snyder, Univ. Maryland) using PCR primers 5'- GTTCGGATCCATCCTCCCGGACCGCCTTG-3' (SEQ ID NO 26) and 5'- GCGGAAGAGCGCCAATACG-3' (SEQ ID NO 27). Fragment 2 is an approximately 3001 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Plasmid 850-80.2 was used according to the TRANSIENT TRANSFECTION ASSAY to measure the strength of the CAV promoter.

PLASMID 883-11.A5. Plasmid 883-11.A5 was constructed for the purpose of generating a novel CAV promoter to express a foreign DNA sequence. The E. coli β -galactosidase gene is expressed under the control of a modified chicken anemia virus promoter. The plasmid was constructed by joining restriction fragments (Sambrooks, et al., 1989) from the following sources. The vector is an approximately 2000 base pair AatII to PvuII fragment of pNEB193 (New England Biolabs, Inc.) Fragment 1 is the CAV promoter synthesized by PCR as a 381 bp EcoRI to BamHI fragment from the CAV strain CL-1 (NVSL; Dr. D.B. Snyder, Univ. Maryland) using PCR primers 5'- GTTCGGATCCATCCACCGGACCGCCTTG -3' (SEQ ID NO 28) and 5'- GCGGAAGAGCGCCAATACG -3' (SEQ ID NO 27). Fragment 2 is an approximately 3001 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (Ferrari et al., 1985). Fragment 3 is an approximately 754 base pair NdeI

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to SalI restriction sub-fragment of the PRV BamHI restriction fragment #7 (Lomniczi et al., 1984). Plasmid 883-11.A5 was used according to the TRANSIENT TRANSFECTION ASSAY to measure the strength of the CAV promoter.

5

HOMOLOGY VECTOR 849-69.A1: Plasmid 849-69.A1 was constructed for the purpose of generating recombinant swinepox viral vector expressing a foreign DNA sequence. The E. Coli lacZ gene is expressed under the control of the LP1 promoter. The quail interferon type 1 (qIFN-1) gene is expressed under the control of the LP2EP2 promoter. The qIFN-1 ORF (594 base pairs) was generated by PCR (as described in Example 23B) with compatible EcorRI and BamHI ends for cloning into SPV homology vector 752-22.1 (see WO 96/22363). Plasmid 849-69.A1 was used in conjunction with S-SPV-001 according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV or SPV for the construction of recombinant SPV viral vector.

20 Example 19A S-HVY-145

Recombinant HVT/MDV chimeric virus

S-HVY-145 is a recombinant chimeric virus containing MDV and HVT genomic sequences which in a vaccine formulation protects against Marek's disease is produced by combining cosmids of MDV genomic DNA containing genes coding for the relevant protective antigens of virulent MDV serotype 1 and cosmids of HVT genomic DNA according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The resulting virus is a vaccine that has the protective immune response to virulent MDV serotype 1 and the attenuated growth characteristics of the HVT. In one embodiment, the chimeric virus contains the MDV genes of the unique short region and the HVT genes of the unique long region and is useful as a vaccine against Marek's disease in

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chickens. In another embodiment, the chimeric virus contains the MDV genes of the short region and the HVT genes of the long region and is useful as a vaccine against Marek's disease in chickens.

5

The MDV protective antigens within the unique short (gD, gE, and gI) elicit a protective immune response to MDV, while the virulence elements present in the unique long of MDV (55,56, 57) are replaced by the attenuating unique long sequences of HVT. The result is an attenuated virus vaccine which protects against Marek's disease. Multivalent protection against Marek's disease, infectious laryngotracheitis, infectious bursal disease, Newcastle's disease, or another poultry pathogen is achieved by inserting the ILTV gB,gD, and gI genes, the IBDV VP2 gene, the NDV HN and F genes, or an antigen gene from a poultry pathogen into an XhoI site converted to a PacI site or NotI site in the EcoRI #9 (BamHI #10) fragment within the unique long region of HVT/MDV recombinant virus (Figures 2 and 5).

20

A cosmid was constructed containing the entire MDV short region. MDV genomic DNA contains several SmaI sites in the unique long and internal and terminal repeats of the virus, but no SmaI sites within the unique short of the virus. The entire short region of MDV was isolated by a partial restriction digestion of MDV genomic DNA with SmaI. A DNA fragment approximately 29,000 to 33,000 base pairs was isolated and cloned into a blunt ended site of the cosmid vector pWE15. To generate S-HVY-145, a recombinant HVT/MDV chimeric virus, the cosmid containing the MDV short region was combined with cosmids containing the HVT long region according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The following combination of subgenomic clones and enzymes were used: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-

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32.5G6 with NotI, 407-32.1C1 with NotI, and 739-27.16 with NotI.

5 The resulting virus vaccine provides superior protection against Marek's disease or as a multivalent vaccine against Marek's disease and infectious laryngotracheitis, infectious bursal disease, Newcastle's disease, or another poultry pathogen. This vaccine is superior because expression of MDV genes in the HVT/MDV chimera vaccine is safer and
10 provides better protection against Marek's disease than vaccines presently available containing HVT and MDV type 2 (SB-1) or HVT alone. Secondly, one can demonstrate expression of the MDV glycoprotein genes in the absence of the homologous HVT genes for both diagnostic and regulatory
15 purposes. This is useful since antibodies to an MDV glycoprotein will cross react with the homologous HVT glycoprotein. Finally, a recombinant HVT/MDV virus which contains a single copy of each glycoprotein gene is more stable than a recombinant virus containing two copies of a
20 homologous glycoprotein gene from HVT and MDV which may delete by homologous recombination.

In an alternative embodiment, cosmids containing MDV protective antigen genes from the unique long (MDV gB and
25 gC) are combined with cosmids containing HVT gene sequences from the short and the long, effectively avoiding the MDV virulence genes at the unique long/internal repeat junction and the unique long/terminal repeat junction (55, 56, and 57).

30

SB-1 strain is an MDV serotype 2 with attenuated pathogenicity. Vaccination with a combination of HVT and SB-1 live viruses protects against virulent MDV challenge better than vaccination with either virus alone. In an
35 alternative embodiment of the present invention, a

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recombinant virus vaccine comprises protective antigen genes of the virulent MDV serotypes 2 combined with the attenuating genes of the non-virulent MDV serotypes 1 and 3, such as SB-1 and HVT. The genomic DNA corresponding to the unique long region is contributed by the SB-1 serotype. The genomic DNA corresponding to the unique short region is contributed by the HVT serotype. Three major glycoprotein antigens (gB, gA and gD) from the MDV serotype 1 are inserted into the unique short region of the virus.

10

The recombinant virus is constructed utilizing HVT subgenomic clone 721-38.1J to reconstruct the unique short region. Subgenomic clone 721-38.1J contains an insertion of the MDV gB, gA, and gD genes. A large molar excess of these clones is cotransfected with a sub-infectious dose of Sb-1 genomic DNA. To determine the appropriate sub-infectious dose, transfection of the SB-1 is titrated down to a dose which no longer yields virus plaques in cell culture. Such a dose contains sub-genomic fragments spanning the unique long region of SB-1 which recombine with the HVT unique short subgenomic clones. Therefore, a virus resulting from recombination between overlapping homologous regions of the SB-1 and HVT subgenomic fragments is highly favored. Alternatively, SB-1 genomic fragments from the unique long region are subcloned into cosmid vectors. A recombinant virus containing the SB-1 unique long the HVT unique short with the MDV, gB, gA, and gD genes were produced using the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. This procedure is also used with an HVT subgenomic clone to insert antigen genes from other avian pathogens including but not limited to infectious laryngotracheitis virus, Newcastle's disease virus and infectious bursal disease virus.

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Example 19B

S-HVY-149

5 S-HVY-149 is a recombinant chimeric virus comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. S-HVY-149 is a recombinant chimeric viral vaccine that comprises foreign DNA from the infectious laryngotracheitis virus glycoprotein D (gD) and glycoprotein I (gI) genes inserted into an XhoI site in the EcoRI #9 fragment within the unique long region
10 of the chimeric virus. The ILT virus gD and gI genes are under the control of the ILT virus gD and gI promoters. The recombinant chimeric viral vaccine is useful against challenge with virulent Marek's disease virus and infectious laryngotracheitis virus.

15

To generate S-HVY-149, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with
20 BamHI, 407-32.5G6 with NotI, 852-52.II4 with NotI, and 739-27.16 with NotI.

S-HVY-149 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVY-149 was
25 100% pure by BLACK PLAQUE ASSAY using convalescent ILT virus antisera.

Example 19C

S-HVY-151

30 S-HVY-151 is a recombinant chimeric virus comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. S-HVY-151 is a recombinant chimeric viral vaccine that comprises foreign DNA from E. coli lacZ gene inserted into an XhoI site in the
35 EcoRI #9 fragment within the unique long region of the

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chimeric virus. The E. coli lacZ gene is under the control of the PRV gX promoter. The recombinant chimeric viral vaccine is useful against challenge with virulent Marek's disease virus.

5

S-HVY-151 was derived from S-HVY-145. This was accomplished using homology vector 301-07.Y#D1 and virus S-HVY-145 in the DNA TRANSFECTION FOR GENERATING RECOMBINANT VIRUS procedure into primary chick embryo fibroblast (CEF) cells. A blue virus obtained from the transfection stock is purified by successive plaque purifications using the BLUOGAL SCREEN FOR RECOMBINANT HERPESVIRUS procedure.

10

S-HVY-151 is useful as a vaccine against challenge with Marek's disease virus.

15

Example 19D

S-HVY-152

S-HVY-152 is a recombinant chimeric virus comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. S-HVY-152 is a recombinant chimeric viral vaccine that comprises foreign DNA from E. coli lacZ gene and the infectious laryngotracheitis (ILT) virus glycoprotein D (gD) and glycoprotein I (gI) genes inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of the chimeric virus. The E. coli lacZ gene is under the control of the PRV gX promoter. The ILT virus gD and gI genes are under the control of the ILT virus gD and gI promoters, respectively.

20

25

30

S-HVY-152 was derived from S-HVY-145. This was accomplished using homology vector 864-74.18 and virus S-HVY-145 in the DNA TRANSFECTION FOR GENERATING RECOMBINANT VIRUS procedure into primary chick embryo fibroblast (CEF) cells.

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S-HVY-152 was tested for purity by BLACK PLAQUE ASSAY using convalescent antisera to ILT virus and rabbit anti- β -galactosidase antisera and was purified by successive plaque purifications and BLACK PLAQUE ASSAY.

5

S-HVY-152 is useful as a vaccine against challenge with Marek's disease virus and infectious laryngotracheitis virus.

10 Example 19E S-HVY-153

15 S-HVY-153 is a recombinant chimeric virus comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. S-HVY-153 is a recombinant chimeric viral vaccine that comprises foreign DNA from E. coli lacZ gene and the Newcastle disease virus (NDV) hemagglutinin (HN) and fusion (F) genes inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of the chimeric virus. The E. coli lacZ gene is under
20 the control of the HSV-1 TK promoter. The NDV HN gene is under the control of the PRV gX promoter, and the NDV F gene is under the control of the HCMV immediate early promoter.

25 S-HVY-153 was derived from S-HVY-145. This was accomplished using homology vector 881-23.#28 and virus S-HVY-145 in the DNA TRANSFECTION FOR GENERATING RECOMBINANT VIRUS procedure into primary chick embryo fibroblast (CEF) cells. A red plaque virus obtained from the transfection stock was purified by successive plaque purifications using the
30 BLUOGAL OR CPRG SCREEN FOR RECOMBINANT HERPESVIRUS procedure and selecting red or blue plaques.

S-HVY-153 is tested for purity by BLACK PLAQUE ASSAY using anti- β -galactosidase and anti-NDV antibodies.

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S-HVY-153 is useful as a vaccine against challenge with Marek's disease virus and Newcastle disease virus.

Example 20

5

Recombinant HVT expressing chicken myelomonocytic growth factor (cMGF), chicken interferon (cIFN) or quail interferon (qIFN) are useful as vaccines against Marek's disease virus and are also useful to enhance the immune response against other diseases of poultry. Chicken myelomonocytic growth factor (cMGF) is related to mammalian G-CSF and interleukin-6 protein (58), and chicken interferon Type 1 (cIFN) is homologous to mammalian type 1 interferon (59). When used in combination with vaccines described in previous examples, S-HVT-144 or HVT expressing cIFN are useful to provide enhanced mucosal, humoral, or cell mediated immunity against avian disease-causing viruses including, but not limited to, Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, infectious bronchitis virus, infectious bursal disease virus. Recombinant HVT expressing cMGF or cIFN are useful provide enhanced immunity against avian disease causing organisms described in Example 15.

25 Example 20A

S-HVT-144

S-HVT-144 is a recombinant herpesvirus of turkeys that contains the chicken myelomonocytic growth factor (cMGF) gene inserted into an XhoI site converted to a PacI site in the EcoRI #9 fragment within the unique long region of HVT. The cMGF gene is in the opposite transcriptional orientation to the open reading frame (ORF A) within the EcoRI #9 fragment of the HVT genome (Figure 4; SEQ ID NOs: 12 and

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13). The cmGF gene is expressed from a human cytomegalovirus immediate early promoter. S-HVT-144 is useful as a vaccine in poultry against Marek's Disease.

5 S-HVT-144 was constructed according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The following combination of subgenomic clones and enzymes were used: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-
10 07.C40 with NotI, 672-01.A40 with NotI, 751-87.A8 with Asc I, 415-09.BA1 with BamHI.

Example 20B

15 Recombinant HVT expressing chicken interferon

A recombinant herpesvirus of turkeys contains the chicken interferon Type 1 (cIFN) gene inserted into an XhoI site converted to a PacI site in the EcoRI #9 fragment within the
20 unique long region of HVT. The cIFN gene is expressed from a human cytomegalovirus immediate early promoter. Recombinant HVT expressing cIFN is useful as a vaccine in poultry against Marek's Disease.

25 Recombinant HVT expressing cIFN is constructed according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The following combination of subgenomic clones and enzymes were used: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-
30 07.C40 with NotI, 672-01.A40 with NotI, 761-07.A1 with Asc I, 415-09.BA1 with BamHI.

Recombinant HVT expressing avian cytokines is combined with HVT expressing genes for avian disease antigens to enhance
35 immune response. Additional cytokines that are expressed in

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HVT and have immune stimulating effects include, but not limited to, transforming growth factor beta, epidermal growth factor family, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factors, B-nerve growth factor, platelet-derived growth factor, vascular endothelial growth factor, interleukin 1, IL-1 receptor antagonist, interleukin 2, interleukin 3, interleukin 4, interleukin 5, interleukin 6, IL-6 soluble receptor, interleukin 7, interleukin 8, interleukin 9, interleukin 10, interleukin 11, interleukin 12, interleukin 13, angiogenin, chemokines, colony stimulating factors, granulocyte-macrophage colony stimulating factors, erythropoietin, interferon, interferon gamma, leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, and soluble TNF receptors. These cytokines are from avian species or other animals including humans, bovine, equine, feline, canine or porcine.

20 Example 20C

Recombinant HVT expressing Marek's disease virus genes and chicken interferon gene.

25 A recombinant herpesvirus of turkeys contains the chicken interferon Type 1 (cIFN) gene inserted into an XhoI site converted to a PacI site in the EcoRI #9 fragment within the unique long region of HVT and further contains the MDV gA, gD, and gB genes inserted into a unique StuI site converted
30 into a HindIII site in the HVT US2 gene. The cIFN gene is expressed from an human cytomegalovirus immediate early promoter. The MDV genes are expressed from the endogenous MDV promoters. Recombinant HVT expressing cIFN and MDV gA, gB, and gD is useful as a vaccine with an enhanced immune
35 response in poultry against Marek's Disease.

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Recombinant HVT expressing MDV genes and the cIFN gene is constructed according to the PROCEDURE FROM GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The following combination of subgenomic clones and enzymes are used: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-07.C40 with NotI, 672-01.A40 with NotI, 761-07.A1 with Asc I, 721-38.1J uncut.

Example 20D

10

Recombinant HVT expressing Marek's disease virus genes, Newcastle disease virus genes and chicken interferon gene.

A recombinant herpesvirus of turkeys contains the chicken interferon Type 1 (cIFN) gene inserted into an XhoI site converted to a PacI site in the EcoRI #9 fragment within the unique long region of HVT and further contains the MDV gA, gD, and gB genes and NDV HN and F genes inserted into a unique StuI site converted into a HindIII site in the HVT US2 gene. The cIFN gene is expressed from an human cytomegalovirus immediate early promoter. The MDV genes are expressed from the endogenous MDV promoters. The NDV HN gene is under the control of the PRV gX promoter, and the NDV F gene is under the control of the HCMV immediate early promoter. Recombinant HVT expressing cIFN and MDV gA, gB, and gD is useful as a vaccine with an enhanced immune response in poultry against Marek's Disease and Newcastle disease.

Recombinant HVT expression MDV genes, NDV genes and cIFN is constructed according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The following combination of subgenomic clones and enzymes are used: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-07.C40 with NotI, 672-

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01.A40 with NotI, 761-07.A1 with Asc I, 722-60.E2 uncut.

Example 20E

- 5 Recombinant HVT expressing Marek's disease virus genes and chicken myelomonocytic growth factor gene.

10 A recombinant herpesvirus of turkeys contains the chicken myelomonocytic growth factor (cMGF) gene inserted into and XhoI site converted to a PacI site in the EcoRI #9 fragment within the unique long region of HVT and further contains the MDV gA, gD, and gB genes inserted into a unique StuI site converted into a HindIII site in the HVT US2 gene. The cMGF gene is expressed from a human cytomegalovirus
15 immediate early promoter. The MDV genes are expressed from the endogenous MDV promoters. Recombinant HVT expression cMGF and MDV gA, gB, and gD is useful as a vaccine with an enhanced immune response in poultry against Marek's Disease.

- 20 Recombinant HVT expressing the cMGF gene and MDV genes is constructed according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The following combination of subgenomic clones and enzymes are used: 407-32.2C3 with NotI, 172-07.BA2 with
25 BamHI, 407-32.5G6 with NotI, 672-07.C40 with NotI, 672-01.A40 with NotI, 751-87.A8 with Asc I, 721-38.1J uncut.

Example 20F

- 30 Recombinant HVT expressing Marek's disease virus genes, Newcastle disease virus genes and chicken myelomonocytic growth factor gene.

35 A recombinant herpesvirus of turkeys contains the chicken myelomonocytic growth factor (cMGF) gene inserted into an

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XhoI site converted to a PacI site in the EcoRI #9 fragment within the unique long region of HVT and further contains the MDV gA, gD, and gB genes and NDV HN and F genes inserted into a unique StuI site converted into a HindIII site in the HVT US2 gene. The cGMF gene is expressed from an human cytomegalovirus immediate early promoter. The MDV genes are expressed from the endogenous MDV promoters. The NDV HN gene is under the control of the PRV gX promoter, and the NDV F gene is under the control of the HCMV immediate early promoter. Recombinant HVT expressing cIFN and MDV gA, gB and gD is useful as a vaccine with an enhanced immune response in poultry against Marek's Disease and Newcastle disease.

Recombinant HVT expressing MDV genes, NDV genes and the cGMF gene is constructed according to the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS. The following combination of subgenomic clones and enzymes are used: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-07.C40 with NotI, 672-01.A40 with NotI, 751-87.A8 uncut, 722-60.E2 uncut.

Example 21

25

Recombinant herpesvirus of turkeys expressing antigens from disease causing microorganisms

Recombinant herpesvirus of turkeys (HVT) is useful for expressing antigens from disease causing microorganisms from animals in addition to avian species. Recombinant HVT is useful as a vaccine in animals including but not limited to humans, equine, bovine, porcine, canine and feline.

Recombinant HVT is useful as a vaccine against equine

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diseases when foreign antigens from diseases or disease organisms are expressed in the HVT vector, including but not limited to: equine influenza, equine herpesvirus-1 and equine herpesvirus-4. Recombinant HVT is useful as a vaccine against bovine diseases when foreign antigens from the following diseases or disease organisms are expressed in the HVT vector, including, but not limited to: bovine herpesvirus type 1, bovine viral diarrhea virus, bovine respiratory syncytial virus, bovine parainfluenza virus. Recombinant HVT is useful as a vaccine against swine diseases when foreign antigens from the following diseases or disease organisms are expressed in the HVT vector, including but not limited to: pseudorabies virus, porcine reproductive and respiratory syndrome (PRRS/SIRS), hog cholera virus, swine influenza virus, swine parvovirus, swine rotavirus. Recombinant HVT is useful as a vaccine against feline or canine diseases when foreign antigens from the following diseases or disease organisms are expressed in the HVT vector, including but not limited to feline herpesvirus, feline leukemia virus, feline immunodeficiency virus and *Dirofilaria immitis* (heartworm). Disease causing microorganisms in dogs include, but are not limited to canine herpesvirus, canine distemper, canine adenovirus type 1 (hepatitis), adenovirus type 2 (respiratory disease), parainfluenza, *Leptospira canicola*, icterohemorrhagia, parvovirus, coronavirus, *Borrelia burgdorferi*, canine herpesvirus, *Bordetella bronchiseptica*, *Dirofilaria immitis* (heartworm) and rabies virus.

30 Example 22

Human vaccines using recombinant herpesvirus of turkeys as a vector

35 Recombinant herpesvirus of turkeys (HVT) is useful as a

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vaccine against human diseases. For example, human influenza is a rapidly evolving virus whose neutralizing viral epitopes are rapidly changing. A useful recombinant HVT vaccine is one in which the influenza neutralizing epitopes are quickly changed to protect against new strains of influenza. Human influenza HA and NA genes are cloned using polymerase chain reaction into the recombinant HVT. Recombinant HVT is useful as a vaccine against other human diseases when foreign antigens from the following diseases or disease organisms are expressed in the HVT vector: hepatitis B virus surface and core antigens, hepatitis C virus, human immunodeficiency virus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, measles virus, hantaan virus, pneumonia virus, rhinovirus, poliovirus, human respiratory syncytial virus, retrovirus, human T-cell leukemia virus, rabies virus, mumps virus, malaria (*Plasmodium falciparum*), *Bordetella pertussis*, *Diphtheria*, *Rickettsia prowazekii*, *Borrelia burgdorferi*, Tetanus toxoid, malignant tumor antigens,

Recombinant HVT expressing human cytokines is combined with HVT expressing genes for human disease antigens to enhance immune response. Additional cytokines, including, but not limited to, transforming growth factor beta, epidermal growth factor family, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factors, B-nerve growth factor, platelet-derived growth factor, vascular endothelial growth factor, interleukin 1, IL-1 receptor antagonist, interleukin 2, interleukin 3, interleukin 4, interleukin 5, interleukin 6, IL-6 soluble receptor, interleukin 7, interleukin 8, interleukin 9, interleukin 10, interleukin 11, interleukin 12, interleukin 13, angiogenin, chemokines, colony stimulating factors, granulocyte-macrophage colony

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stimulating factors, erythropoietin, interferon, interferon gamma, leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, and soluble TNF
5 receptors from human and other animals are expressed in HVT and have immune stimulating effects.

Example 23A

10 Improved production of a recombinant herpesvirus of turkeys vaccine.

Cytokines, such as interferons and interleukins, inhibit the replication of viruses in cell culture and in the animal.
15 Inhibition of the production of cellular interferon or interleukin improves the growth of recombinant HVT in cell culture. Chicken interferon type 1 (cIFN) expressed from a recombinant swinepox vector was added to chick embryo fibroblast (CEF) cell cultures and infected with S-HVT-012
20 which expresses β -galactosidase. cIFN added to the cell culture media reduced both the expression of β -galactosidase and S-HVT-012 titer in a dose dependent manner. This result indicates that growth of HVT is limited by exogenous addition of chicken interferon. Several strategies are
25 utilized to improve growth of HVT in CEF cells by removing or inactivating chicken interferon activity in the CEF cells.

In one embodiment, a chicken interferon neutralizing
30 antibody is added to the culture medium to inhibit the chicken interferon activity and improve the growth of recombinant HVT in CEF cell culture. The anti-cIFN antibody is derived from mouse or rabbit sera of animals injected with chicken interferon protein, preferably the cIFN is from
35 a recombinant swinepox virus expressing chicken interferon.

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Poxviruses secrete cytokine-inhibiting proteins as an immune evasion strategy. One type of poxvirus immune evasion mechanism involves poxvirus soluble receptors for interleukins, interferon, or tumor necrosis factors which
5 inactive the cytokines and allow viral replication (60). In an embodiment of the invention, fowlpox virus is useful as a source of chicken interferon-inhibiting proteins and other immune evasion proteins. Conditioned media from FPV infected CEF cell cultures is added to the HVT infected CEF
10 cells to inhibit interferon activity and increase the HVT titer. In a further embodiment, the recombinant chicken interferon inhibiting protein or another poxvirus immune evasion protein is expressed in a vector in combination with an HVT vaccine composition to increase the HVT titer.

15 Chicken embryo fibroblast cells have been engineered to express foreign genes (61). In a further embodiment, an interferon-negative CEF cell line is constructed by the introduction of a vector expressing a gene encoding
20 antisense RNA for chicken interferon into the CEF cell line. Recombinant HVT grown in an interferon-negative CEF cell line demonstrate improved virus titers compared to HVT grown in an interferon producing CEF cell line. In a further embodiment, a chicken myelomonocytic growth factor (cMGF) -
25 positive CEF cell line is constructed by the introduction of a vector expressing the cMGF gene into the CEF cells. Recombinant HVT grown in a cMGF-positive CEF cell line demonstrates improved virus titers compared to HVT grown in a cMGF negative CEF cell line.

30 Recombinant HVT of the present invention is useful as a vaccine against Marek's disease and against other diseases as outlined in previous examples. An increased efficiency in growth of recombinant HVT in CEF cells is useful in
35 production of the vaccine.

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Example 23BCloning of Quail Interferon, Type 1

5 Southern blot analysis showed that a ³²P -labeled chicken
interferon-1 gene fragment hybridized to a 5.0 kb BamHI
genomic DNA fragment from quail cell line QT-35. Based on
this information, the PCR method was utilized with
homologous primers to chicken interferon, type 1, to clone
10 the gene for quail interferon, type 1. (The PCR primers
were: 5'-TGTACAGATCTCACCATGGCTGTGCCTGCAAGC-3' (SEQ ID NO.
29) from the 5' end of the quail IFN gene; 5'-
GGCGAATTCGGCTAAGTGCGCGTGTG-3' (SEQ ID NO. 30) from the 3'
end of the quail IFN gene.) A blunt 594 bp QT-35 genomic PCR
15 DNA fragment was generated by PCR and inserted into a
unique SmaI site in the multiple cloning site of pBluescript
II KS- plasmid (Stratagene, La Jolla, CA). The resultant
plasmid is, 832-71.B11. DNA and amino acid sequences were
analyzed and determined to be the complete open reading
20 frame of quail interferon, type 1 (SEQ ID NO. 31 and 32),
based on sequence homology and conservation of significant
structural genetic motifs among three published avian
interferon, type 1 genes, e.g. duck, turkey and chicken. The
quail IFN-1 ORF contains 594 nucleotides encoding 198 amino
25 acids, including a translation initiation start codon, ATG,
and an amber translational stop signal, TAG. The sequence
contains 6 cysteine residues, which are conserved motifs
among other avian IFN, type 1 genes. The amino acid sequence
predicts a 31 amino acid hydrophobic signal sequence and 2
30 putative N-glycosylation sites. Amino acid sequence
homology (Jotun Hein Method, DNASTAR MegAlign Program) of
quail IFN-1 compared to chicken, turkey and duck IFN-1 is
82.0%, 76.0% and 49.0%, respectively. Protein
hydrophobicity Kyte-Doolittle plots show the Quail IFN-1
35 protein structure and charge characteristics to be similar

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to chicken, turkey and duck IFN-1 proteins. A plasmid pSY832-71.B11 containing Quail DNA has been deposited on February 21, 1997 pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. 97892.

Expression plasmid 832-71.B11 is useful as a vaccine against avian diseases in avian species such as chickens, ducks, quail, turkeys, bantams, guinea fowl and others. Quail IFN-1 improves an immune response against disease causing microorganisms when supplied in a viral vector alone or in combination with other avian disease-causing antigens. Expression plasmid 832-71.B11 is useful as a vaccine to improve weight gain in avian species.

Example 23C

20

Expression plasmid for antisense quail interferon: 866-79.2

A bicistronic expression plasmid, pIRES1neo, (Clontech, Palo Alto, CA) was used as the cloning vector. This expression cassette contains the human cytomegalovirus (CMV) major immediate early protein/enhancer followed by a multiple cloning site (MCS); a synthetic intron; and the encephalomyocarditis virus internal ribosome entry site (IRES), followed by the neomycin phosphotransferase gene, with a downstream bovine growth hormone polyadenylation signal. The antisense for the DNA open reading frame of quail interferon, type 1, was inserted into a unique BamHI site in the MCS downstream of the CMV promoter and transcription start site of pIRES1neo. The resultant expression pIRES1neo plasmid containing quail interferon,

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type1, was 866-79.2.

Stably transformed cell line QT-35 with antisense quail interferon type 1 plasmid 866-79.2.

5

Because a stably transformed chicken cell line is not readily available to make chicken interferon minus cell lines, a QT-35 cell line transformed with a Quail IFN-1 antisense expression plasmid was designed, to make new and improved cell substrates for the growth of avian viruses, such as HVT. The QT-35 stably transformed cell line and a vector expressing the antisense cDNA to quail interferon, type 1 were utilized.

15 QT-35 cells were transfected with a plasmid containing antisense DNA for quail IFN, type 1 and a selectable marker gene, neomycin phosphotransferase. The DNA plasmid, 866-79.2, was precipitated in the presence of calcium and HEPES buffer, mixed with QT-35 growth media and incubated with
20 monolayers of QT-35 cells at 39C. After 5-6 hours, the cells were shocked with 15% glycerol for 3 minutes, washed with PBS and fed with growth media. Cells were allowed to form a confluent monolayer and then trypsinized and plated onto 6 cm dishes at subconfluent concentrations. After cells had
25 attached and acclimated to the plastic, the media was replaced with growth media containing 400ug/ml G418. Cells resistant to G418 were allowed to form colonies in the presence of 400-800ug/ml of G418 for 2-3 weeks, or at least until untransfected QT-35 cells not resistant to G418 had
30 died. G418 resistant clones were trypsinized and plated onto 10cm culture dishes. Clones were subcultured and expanded as needed once a week in media containing 800ug/ml G418.

35 QT-35 clones were selected on the basis of their growth

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stability in the presence of 800ug/ml G418. Southern blot analysis of genomic DNA from the G418 resistant clones and the parent QT-35 cells was performed. A neomycin phosphotransferase DNA probe labeled with digoxigenin dUTP (Boeringer Mannheim) was used to identify the presence of the genetic construct stably integrated into the genomic DNA. Cell clones resistant to G418 and positive for the neomycin gene by southern blot are considered to have the entire expression plasmid, 866-79.2 stably integrated into the chromosomal genomic cellular DNA.

The improved QT-35 cell line transformed with a Quail IFN-1 antisense expression plasmid is useful for production of avian viruses such as HVT, HVT/MDV chimeric viral vector, fowlpox virus, avipox virus, and other viruses. The avian viruses grow to higher titer in the improved QT-35 cell line due to inhibition of interferon production in the cell line.

S-SPV-129

20

S-SPV-129 is a recombinant swinepox virus that contains 2 genes, the quail interferon type 1 (qIFN-1) gene and the lacZ gene, inserted into a unique AccI site (within the O1L ORF) within the HindIII to BglII subfragment of the SPV Hind III M genomic fragment. The qIFN-1 gene is under the control of the late promoter 2 early promoter 2 (LP2EP2), and the lacZ gene is under the control of the late promoter 1 (LP1).

S-SPV-129 was created by HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV OR SPV between S-SPV-001 and homology vector 849069.A1 in ESK-4 cell line (See WO 96/22363). The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification was the recombinant virus designated S-SPV-129. This virus was

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assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in the materials and methods. After the initial three rounds of purification all plaques observed
5 were blue indicating that the virus was pure, stable and expressing the marker gene.

To verify the biological activity of quail IFN-1, supernatants from ESK-4 cells infected with S-SPV-129 were
10 collected and tested for their ability to inhibit vesicular stomatitis virus (VSV) infection of CEF cells.

S-SPV-129 is useful as a vaccine against avian diseases in avian species such as chickens, ducks, quail, turkeys,
15 bantams, guinea fowl and others. Quail IFN-1 improves an immune response against disease causing microorganisms when supplied in a viral vector alone or in combination with other avian disease-causing antigens. S-SPV-129 is useful as a vaccine to improve weight gain in avian species.

20 Expression of quail IFN-1 in S-SPV-129 or in a eukaryotic expression plasmid such as, pIRES1neo, (Clontech, Palo Alto, CA) (see example 23C, above) is useful when injected into the germline of chickens or other avian species to produce
25 transgenic chickens or other avian species. Chickens transgenic for qIFN-1 demonstrate improved weight gain and growth and improved resistance to disease.

Example 24

30 Transient expression assays comparing a novel chicken anemia virus (CAV) promoter to the HMCV immediate early promoter.

Chicken anemia virus (CAV) is a single stranded DNA virus of
35 2300 nucleotides, containing one major open reading frame (51 kdal capsid) and several smaller ORFs. CAV infects lymphoblastoid cells of the chicken and produces a 2100

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nucleotide RNA transcript from one distinct promoter region.
(References 67-72))

Four different CAV promoter constructs expressing β -
5 galactosidase have been assessed for activity in transient
assays. These promoter constructs are plasmids 850-25.18,
850-69.1, 850-80.2, and 883-11.A5, all of which use
different CAV promoters to drive expression of β -
galactosidase. A number of transient assays in CEFs and
10 other cell lines have been performed with these constructs
and plasmid 388-65.2, which uses the HCMV-IE promoter to
express β -galactosidase.

Plasmid 850-25.18 contains an 854 bp version of the CAV
15 promoter which extends through the first two translational
starts for ORFs 1 and 2 up to the translational start for
ORF 3, the CAV capsid protein gene. This promoter would
include a functional coding sequence for the apoptin gene
(ORF 2).

20 Plasmid 850-69.1 contains a novel CAV promoter which is
similar to 850-25.18, except that a Hind III site within the
apoptin reading frame has been filled in to destroy the
apoptin reading frame, albeit only near the 3' end. This
25 creates an 858 bp version of the CAV promoter.

Plasmid 883-11.A5 contains a CAV promoter which starts at
the same upstream site as the previous two promoters, but is
only 381 bp long, extending to the translational start of
30 ORF1.

Plasmid 850-80.2 contains a CAV promoter (SEQ ID NO 23)
which is similar to 883-11.A5, but the -3 position relative
to the translational start has been altered from nucleotide
35 T to an A (Nucleotide 377 of SEQ ID NO 23), which is closer

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to a consensus ribosome entry site as described for other eukaryotic promoters by Kozak.

5 The results of the transient transfection assay show that CAV promoter plasmid 850-80.2 expresses high levels of β -galactosidase comparable to the levels observed with plasmid 388-65.2, containing the highly active HMCV IE promoter. The CAV promoter plasmid 850-80.2, which has a nucleotide change of T to A at the proposed ribosome entry site
10 expresses higher transient levels of β -galactosidase than the CAV promoter plasmids, 850-25.18, 850-69.1, and 883-11.A5 (see Figure 6). The results of transient transfection assays were observed in both chicken embryo fibroblast cells and in QT-35 (quail) cells. The CAV promoter in plasmid 850-
15 80.2 is useful as a vaccine to express high levels of viral or bacterial antigens, cytokines, immune modulating proteins and cytoplasmic or cell surface receptors making it an excellent promoter for use in recombinant viruses. The CAV promoter in plasmid 850-80.2 was used to make recombinant S-
20 HVT-148 and S-FPV-106.

Example 25

25 Recombinant HVT and recombinant FPV expressing foreign DNA from a chicken anemia virus promoter

S-HVT-148

30 S-HVT-148 is a recombinant herpesvirus of turkeys that contains *E. coli* β -galactosidase gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of HVT. This plasmid contains the *E. coli* β -galactosidase gene under the control of a chicken anemia virus (CAV) promoter. The CAV promoter is a 381 bp fragment containing
35 the CAV promoter sequence up to the first ORF of CAV, with

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the -3 position changed from T to A (Nucleotide 377 of SEQ ID NO 23).

5 S-HVT-148 was constructed according to the PROCEDURE FOR
GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING
SUBGENOMIC FRAGMENTS. The following combination of
subgenomic clones and enzymes are used: 407-32.2C3 with
NotI, 407-32.5G6 with NotI, 172-07.BA2 with BamHI, 415-
09.BA1 with BamHI, 672-01.A40 with NotI, 672-07.C40 with
10 NotI, and homology vector 867-96.B9 uncut. HVT-148 is a pure
virus and expresses beta-galactosidase as evidenced by ONPG
and blue plaque assays.

15 S-HVT-148 is useful as a vaccine in poultry against Marek's
Disease. Other foreign DNA of interest are inserted under
the control of the CAV promoter for use as a vaccine in
poultry. The CAV promoter is useful in HVT, chimeric HVT/MDV
viral vaccine, and other herpesviruses and upon insertion of
foreign DNA from disease-causing microorganisms is useful as
20 a vaccine in canine, feline, bovine, porcine, equine, and
human species.

S-FPV-106

25 S-FPV-106 is a recombinant fowlpox virus that contains E.
coli β -galactosidase gene inserted into a unique SnaBI site
(converted to a NotI site) in the 2.8 KB FPV genomic EcoRI
fragment. S-FPV-106 contains the E. coli β -galactosidase
gene under the control of a novel chicken anemia virus (CAV)
30 promoter. The CAV promoter is a 381 bp fragment containing
the CAV promoter sequence up to the first ORF of CAV, with
the -3 position changed from T to A (Nucleotide 377 of SEQ
ID NO 23).

35 S-FPV-106 was created by HOMOLOGOUS RECOMBINATION PROCEDURE

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FOR GENERATING RECOMBINANT FPV between 883-10.A1 and
homology vector S-FPV-001. (See WO 94/19015). The
transfection stock was screened by the SCREEN FOR
RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final
5 result of blue plaque purification was the recombinant virus
designated S-FPV-106. This virus was assayed for β -
galactosidase expression, purity, and insert stability by
multiple passages monitored by the blue plaque assay as
described in the materials and methods. After the initial
10 three rounds of purification all plaques observed were blue
indicating that the virus was pure, stable and expressing
the marker gene.

S-FPV-106 is useful as a vaccine in poultry against fowlpox
15 disease. Other foreign DNA of interest are inserted under
the control of the CAV promoter for use as a vaccine in
poultry. The CAV promoter is useful in FPV, swinepox virus,
raccoonpox virus and other poxviruses as a vaccine and upon
insertion of foreign DNA from disease-causing microorganisms
20 is useful as a vaccine in canine, feline, bovine, porcine,
equine, and human species.

Example 26

S-HVY-148

25 S-HVY-148 is a recombinant chimeric viral vaccine comprising
a chimera of the Marek's disease virus short region and the
herpesvirus of turkeys long region. S-HVT-148 is a
recombinant chimeric viral vaccine that comprises foreign
DNA from the Newcastle disease virus (NDV) hemagglutinin
30 (HN) and fusion (F) genes inserted into an XhoI site in the
EcoRI #9 fragment within the unique long region of the
chimeric virus. The NDV HN gene is under the control of the
PRV gX promoter, and the NDV F gene is under the control of
the HCMV immediate early promoter. The NDV HN and F genes
35 under the control of their respective promoters are
transcribed in the opposite direction from the HVT UL52,

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UL54 and UL55 genes in the adjacent HVT genomic DNA.

To generate S-HVY-148, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR
5 GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 854-33.6 with SfiI, and 739-27.16 with NotI.

10 S-HVY-148 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVY-148 was 100% pure by BLACK PLAQUE ASSAY using anti-NDV-F monoclonal antisera (#3-1G5) and anti-NDV-HN monoclonal antisera (#15c4).

15 To confirm the expression of the NDV HN and F gene products, cells were infected with S-HVY-148 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the
20 WESTERN BLOTTING PROCEDURE. Antisera to NDV HN and F was used to detect expression of NDV HN and F protein. The lysate from S- HVY-148 infected cells exhibited a band at the size expected for native NDV HN and F.

25 The recombinant chimeric viral vaccine, S-HVY-148, is useful for protection against challenge with virulent Marek's disease virus and Newcastle disease virus. The recombinant virus S-HVY-148 is useful for production of recombinant protein, NDV HN and F, for diagnostic assay or as a vaccine.

30

Example 27

S-HVY-154

S-HVY-154 is a recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the
35 herpesvirus of turkeys long region. S-HVT-154 is a recombinant chimeric viral vaccine that comprises foreign DNA from the Newcastle disease virus (NDV) fusion (F) gene

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and E. coli lacZ gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of the chimeric virus. The NDV F gene is under the control of the HCMV immediate early promoter, and the E. coli lacZ gene is under the control of the PRV gX promoter. The E. coli lacZ gene and the NDV F gene under the control of their respective promoters are transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

10

S-HVY-154 was derived from S-HVY-145. This was accomplished using homology vector 890-77.10 and virus S-HVY-145 in the DNA TRANSFECTION FOR GENERATING RECOMBINANT VIRUS procedure into primary chick embryo fibroblast (CEF) cells. A blue virus obtained from the transfection stock is purified by successive plaque purifications using the CPRG SCREEN FOR RECOMBINANT HERPESVIRUS procedure.

15

To confirm the expression of the NDV HN and F gene products, cells were infected with S-HVY-154 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Antisera to NDV HN and F was used to detect expression of NDV HN and F protein. The lysate from S- HVY-154 infected cells exhibited a band at the size expected for native NDV HN and F.

20

25

S-HVY-154 is useful as a vaccine against challenge with virulent Marek's disease virus and Newcastle disease virus. The recombinant virus S-HVY-154 is useful for production of recombinant protein, NDV F, for diagnostic assay or as a vaccine.

30

Example 28

S-HVT-149

35

S-HVT-149 is a recombinant herpesvirus of turkeys vaccine that comprises foreign DNA from the infectious bursal

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disease virus (IBDV) VP2 gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of HVT. The IBDV VP2 gene is expressed under the control of a chicken anemia virus (CAV) promoter. The IBDV VP2 gene under
5 the control of the CAV promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

To generate S-HVT-149, the following combination of
10 subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-01.C40 with NotI, 900-87.H8 with NotI, 672-01.A40 with Not I and 415-09.BA1 with BamHI..

15 S-HVT-149 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVT-149 was 100% pure by BLACK PLAQUE ASSAY using anti-IBDV antisera.

20 To confirm the expression of the IBDV VP2 gene product, cells were infected with S-HVT-149 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Antisera to IBDV VP2 was used to
25 detect expression of IBDV VP2 protein. The lysate from S-HVT-149 infected cells exhibited a band at the size expected for native IBDV VP2.

The recombinant viral vaccine, S-HVT-149, is useful for
30 protection against challenge with Marek's disease virus and infectious bursal disease virus. The recombinant virus S-HVT-149 is useful for production of recombinant protein, IBDV VP2, for diagnostic assay or as a vaccine. The recombinant virus S-HVT-149 illustrates the utility of the
35 CAV promoter to express foreign DNA in a recombinant virus.

Example 29

S-HVT-154

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S-HVT-149 is a recombinant herpesvirus of turkeys vaccine that comprises foreign DNA from the infectious bursal disease virus (IBDV) VP2 gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of HVT. The IBDV VP2 gene is expressed under the control of a chicken anemia virus (CAV) promoter. The IBDV VP2 gene under the control of the CAV promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

10

To generate S-HVT-149, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-01.C40 with NotI, 900-87.H8 with NotI, 672-01.A40 with Not I and 415-09.BA1 with BamHI..

15

S-HVT-149 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVT-149 was 100% pure by BLACK PLAQUE ASSAY using anti-IBDV antisera. The recombinant protein, IBDV VP2, produced by S-HVT-149 was shown by WESTERN BLOT ASSAY to be similar in size to native IBDV VP2.

20

S-HVT-154 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVT-154 was 100% pure by BLACK PLAQUE ASSAY using anti IBDV antisera.

25

The recombinant viral vaccine, S-HVT-154, is useful for protection against challenge with Marek's disease virus and infectious bursal disease virus. The recombinant virus S-HVT-154 is useful for production of recombinant protein, IBDV VP2, for diagnostic assay or as a vaccine. The recombinant virus S-HVT-154 illustrates the utility of the ILTV gI promoter to express foreign DNA in a recombinant virus.

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Example 30

S-HVT-155

S-HVT-155 is a recombinant herpesvirus of turkeys vaccine that comprises foreign DNA from the infectious bursal disease virus (IBDV) VP2 gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of HVT. The IBDV VP2 gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein I (gI) promoter. The IBDV VP2 gene under the control of the ILTV gI promoter is transcribed in the same direction as the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

To generate S-HVT-155, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 949-19.2 with I-SceI, and 415-09.BA1 with BamHI.

S-HVT-155 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVT-155 was 100% pure by BLACK PLAQUE ASSAY using anti IBDV antisera.

The recombinant viral vaccine, S-HVT-155, is useful for protection against challenge with Marek's disease virus and infectious bursal disease virus. The recombinant virus S-HVT-155 is useful for production of recombinant protein, IBDV VP2, for diagnostic assay or as a vaccine. The recombinant virus S-HVT-155 illustrates the utility of the ILTV gI promoter to express foreign DNA in a recombinant virus.

Example 31

S-HVT-156

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S-HVT-156 is a recombinant herpesvirus of turkeys vaccine that comprises foreign DNA from the infectious bursal

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disease virus (IBDV) VP2 gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of HVT. The IBDV VP2 gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein D (gD) promoter. The IBDV VP2 gene under the control of the ILTV gD promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

To generate S-HVT-156, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 949-24.D1 with I-SceI, and 415-09.BA1 with BamHI.

S-HVT-156 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVT-156 was 100% pure by BLACK PLAQUE ASSAY using anti IBDV antisera.

The recombinant viral vaccine, S-HVT-156, is useful for protection against challenge with Marek's disease virus and infectious bursal disease virus. The recombinant virus S-HVT-156 is useful for production of recombinant protein, IBDV VP2, for diagnostic assay or as a vaccine. The recombinant virus S-HVT-156 illustrates the utility of the ILTV gD promoter to express foreign DNA in a recombinant virus.

30 Example 32 S-HVY-159

S-HVY-159 is a recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. S-HVY-159 is a recombinant chimeric viral vaccine that comprises foreign DNA from the infectious bursal disease virus (IBDV) VP2 gene inserted into an XhoI site in the EcoRI #9 fragment within

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the unique long region of the chimeric virus. The IBDV VP2 gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein I (gI) promoter. The IBDV VP2 gene under the control of the ILTV gI promoter is transcribed in the same direction as the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

To generate S-HVY-159, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 949-19.2 with I-SceI, and 928-47.1 with NotI.

S-HVY-159 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVY-159 was 100% pure by BLACK PLAQUE ASSAY using anti IBDV antisera.

The recombinant viral vaccine, S-HVY-159, is useful for protection against challenge with Marek's disease virus and infectious bursal disease virus. The recombinant virus S-HVY-159 is useful for production of recombinant protein, IBDV VP2, for diagnostic assay or as a vaccine. The recombinant virus S-HVY-159 illustrates the utility of the ILTV gI promoter to express foreign DNA in a recombinant virus.

Example 33

S-HVY-160

S-HVY-160 is a recombinant chimeric viral vaccine comprising a chimera of the Marek's disease virus short region and the herpesvirus of turkeys long region. S-HVY-160 is a recombinant chimeric viral vaccine that comprises foreign DNA from the infectious bursal disease virus (IBDV) VP2 gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of the chimeric virus. The IBDV VP2 gene is expressed under the control of an infectious

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laryngotracheitis virus (ILTV) glycoprotein I (gI) promoter. The IBDV VP2 gene under the control of the ILTV gI promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

5

To generate S-HVY-160, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 949-24.I2 with I-SceI, and 928-47.1 with NotI.

10

S-HVY-160 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVY-160 was 100% pure by BLACK PLAQUE ASSAY using anti IBDV antisera.

15

The recombinant viral vaccine, S-HVY-160, is useful for protection against challenge with Marek's disease virus and infectious bursal disease virus. The recombinant virus S-HVY-160 is useful for production of recombinant protein, IBDV VP2, for diagnostic assay or as a vaccine. The recombinant virus S-HVY-160 illustrates the utility of the ILTV gI promoter to express foreign DNA in a recombinant virus.

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Example 34

S-HVT-150

S-HVT-150 is a recombinant herpesvirus of turkeys vaccine that comprises foreign DNA from the E. coli lacZ gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of HVT. The E. coli lacZ gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein I (gI) promoter. The E. coli lacZ gene under the control of the ILTV gI promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

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To generate S-HVT-150, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-07.C40 with NotI, 928-58.J2 with NotI, 672-01.A40 with Not I, and 415-09.BA1 with BamHI.

S-HVT-150 was purified by plating and plaque purification, and tested for purity by BLACK PLAQUE ASSAY. S-HVT-150 was 100% pure by BLACK PLAQUE ASSAY using anti- β -galactosidase antisera.

The recombinant viral vaccine, S-HVT-150, is useful for protection against challenge with Marek's disease virus. The recombinant virus S-HVT-150 is useful for diagnostic assay or as a vaccine. The recombinant virus S-HVT-150 illustrates the utility of the ILTV gI promoter to express foreign DNA in a recombinant virus.

20 Example 35 S-HVT-151

S-HVT-151 is a recombinant herpesvirus of turkeys vaccine that comprises foreign DNA from the E. coli lacZ gene inserted into an XhoI site in the EcoRI #9 fragment within the unique long region of HVT. The E. coli lacZ gene is expressed under the control of an infectious laryngotracheitis virus (ILTV) glycoprotein D (gD) promoter. The E. coli lacZ gene under the control of the ILTV gD promoter is transcribed in the opposite direction from the HVT UL52, UL54 and UL55 genes in the adjacent HVT genomic DNA.

To generate S-HVT-151, the following combination of subgenomic clones and enzymes were used in the PROCEDURE FOR GENERATING RECOMBINANT HERPESVIRUS FROM OVERLAPPING SUBGENOMIC FRAGMENTS: 407-32.2C3 with NotI, 172-07.BA2 with BamHI, 407-32.5G6 with NotI, 672-07.C40 with NotI, 928-58.K7

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with NotI, 672-01.A40 with Not I, and 415-09. 415-09.BA1
with BamHI.

5 S-HVT-151 was purified by plating and plaque purification,
and tested for purity by BLACK PLAQUE ASSAY. S-HVT-151 was
100% pure by BLACK PLAQUE ASSAY using anti- β -galactosidase
antisera.

10 The recombinant viral vaccine, S-HVT-151, is useful for
protection against challenge with Marek's disease virus. The
recombinant virus S-HVT-151 is useful for diagnostic assay
or as a vaccine. The recombinant virus S-HVT-151 illustrates
the utility of the ILTV gD promoter to express foreign DNA
in a recombinant virus.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Wild, Martha A
Barbara J. Winslow
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Thereof
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 - (B) COMPUTER: IBM PC compatible
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5426 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
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 - (D) OTHER INFORMATION: /product= "HVT UL42"
- (ix) FEATURE:

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(ix) FEATURE:

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 (B) LOCATION: 4701..5339
 (D) OTHER INFORMATION: /product= "HVT UL45"

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ACA	ATA	TTG	GCT	ATA	AAT	ATC	GCT	AGA	GAT	GCA	AGC	GCT	ACA	TTA	TTA	2004
Thr	Ile	Leu 220	Ala	Ile	Asn	Ile	Ala 225	Arg	Asp	Ala	Ser	Ala 230	Thr	Leu	Leu	
TCC	ACT	TGC	TAT	TAT	CGC	AAT	TGC	CGC	GAG	AGG	ACT	ATA	CTT	CGC	CCT	2052
Ser	Thr	Cys	Tyr	Tyr	Arg	Asn 240	Cys	Arg	Glu	Arg	Thr 245	Ile	Leu	Arg	Pro	
TCT	CGT	CTC	GGA	CAT	GGT	TAC	ACA	ATC	CCT	TCT	CCC	GGT	GCC	GAT	ATG	2100
Ser	Arg	Leu	Gly	His	Gly 255	Tyr	Thr	Ile	Pro	Ser 260	Pro	Gly	Ala	Asp	Met 265	
CTT	TAT	GAA	GAA	GAC	GTA	TAT	AGT	TTT	GAC	GCA	GCT	AAA	GGC	CAT	TAT	2148
Leu	Tyr	Glu	Glu	Asp 270	Val	Tyr	Ser	Phe	Asp 275	Ala	Ala	Lys	Gly	His 280	Tyr	

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TCG TCA ATA TTT CTA TGT TAT GCC ATG GGG CTT ACA ACA CCG CTG ATT	2196
Ser Ser Ile Phe Leu Cys Tyr Ala Met Gly Leu Thr Thr Pro Leu Ile	
285 290 295	
ATT GCG CTC CAT AAA TAT ATG GCG GGC ATT AAA AAT TCG TCA GAT TGG	2244
Ile Ala Leu His Lys Tyr Met Ala Gly Ile Lys Asn Ser Ser Asp Trp	
300 305 310	
ACT GCT ACA TTA CAA GGC ATG TAC GGG CTT GTC TTG GGA TCG CTA TCG	2292
Thr Ala Thr Leu Gln Gly Met Tyr Gly Leu Val Leu Gly Ser Leu Ser	
315 320 325	
TCA CTA TGT ATT CCA TCC AGC AAC AAC GAT GCC CTA ATT CGT CCC ATT	2340
Ser Leu Cys Ile Pro Ser Ser Asn Asn Asp Ala Leu Ile Arg Pro Ile	
330 335 340 345	
CAA ATT TTG ATA TTG ATA ATC GGT GCA CTG GCC ATT GCA TTG GCT GGA	2388
Gln Ile Leu Ile Leu Ile Ile Gly Ala Leu Ala Ile Ala Leu Ala Gly	
350 355 360	
TGT GGT CAA ATT ATA GGG CCT ACA TTA TTT GCC GCG AGT TCG GCT GCG	2436
Cys Gly Gln Ile Ile Gly Pro Thr Leu Phe Ala Ala Ser Ser Ala Ala	
365 370 375	
ATG TCA TGT TTT ACA TGT ATC AAT ATT CGC GCT ACT AAT AAG GGT GTC	2484
Met Ser Cys Phe Thr Cys Ile Asn Ile Arg Ala Thr Asn Lys Gly Val	
380 385 390	
AAC AAA TTG GCA GCA GCC AGT GTC GTG AAA TCT GTA CTG GGC TTC ATT	2532
Asn Lys Leu Ala Ala Ala Ser Val Val Lys Ser Val Leu Gly Phe Ile	
395 400 405	
ATT TCC GGG ATG CTT ACT TGC GTG CTA TTA CCA CTA TCG TGATAGATCG	2581
Ile Ser Gly Met Leu Thr Cys Val Leu Leu Pro Leu Ser	
410 415 420	
TCGGTCTGCG CATCGCCCAT GCTGGCGGAA CGCTCTTTTCG AACCGTGAAT AAAACTTTGT	2641
ATCTACTAAA CAATAACTTT GTGTTTTATT GAGCGGTCGA AAACAATGAG GAGCTGCAAT	2701
TTAAAGCTAA CCGCATACGC CGGGCGGGTA AAGACCATTT TATACCATAT TACGCATCTA	2761
TCGAAACTTG TTCGAGAACC GCAAGTAT ATG GTT TCC AAC ATG CGC GTT CTA	2813
Met Val Ser Asn Met Arg Val Leu	
1 5	
CGC GTA CTG CGC CTG ACG GGA TGG GTG GGC ATA TTT CTA GTT CTG TCT	2861
Arg Val Leu Arg Leu Thr Gly Trp Val Gly Ile Phe Leu Val Leu Ser	
10 15 20	
TTA CAG CAA ACC TCT TGT GCC GGA TTG CCC CAT AAC GTC GAT ACC CAT	2909
Leu Gln Gln Thr Ser Cys Ala Gly Leu Pro His Asn Val Asp Thr His	
25 30 35 40	
CAT ATC CTA ACT TTC AAC CCT TCT CCC ATT TCG GCC GAT GGC GTT CCT	2957
His Ile Leu Thr Phe Asn Pro Ser Pro Ile Ser Ala Asp Gly Val Pro	
45 50 55	
TTG TCA GAG GTG CCC AAT TCG CCT ACG ACC GAA TTA TCT ACA ACT GTC	3005
Leu Ser Glu Val Pro Asn Ser Pro Thr Thr Glu Leu Ser Thr Thr Val	
60 65 70	

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GCC	ACC	AAG	ACA	GCT	GTA	CCG	ACG	ACT	GAA	AGC	ACT	AGT	TCC	TCC	GAA	3053
Ala	Thr	Lys	Thr	Ala	Val	Pro	Thr	Thr	Glu	Ser	Thr	Ser	Ser	Ser	Glu	
		75					80					85				
GCG	CAC	CGC	AAC	TCT	TCT	CAC	AAA	ATA	CCT	GAT	ATA	ATC	TGC	GAC	CGA	3101
Ala	His	Arg	Asn	Ser	Ser	His	Lys	Ile	Pro	Asp	Ile	Ile	Cys	Asp	Arg	
	90					95					100					
GAA	GAA	GTA	TTC	GTA	TTC	CTT	AAC	AAT	ACA	GGA	AGA	ATT	TTG	TGT	GAC	3149
Glu	Glu	Val	Phe	Val	Phe	Leu	Asn	Asn	Thr	Gly	Arg	Ile	Leu	Cys	Asp	
105					110					115					120	
CTT	ATA	GTC	GAC	CCC	CCT	TCA	GAC	GAT	GAA	TGG	TCC	AAC	TTC	GCT	CTT	3197
Leu	Ile	Val	Asp	Pro	Pro	Ser	Asp	Asp	Glu	Trp	Ser	Asn	Phe	Ala	Leu	
				125					130					135		
GAC	GTC	ACG	TTC	AAT	CCA	ATC	GAA	TAC	CAC	GCC	AAC	GAA	AAG	AAT	GTA	3245
Asp	Val	Thr	Phe	Asn	Pro	Ile	Glu	Tyr	His	Ala	Asn	Glu	Lys	Asn	Val	
			140					145					150			
GAG	GTT	GCC	CGA	GTG	GCC	GGT	CTA	TAC	GGA	GTA	CCG	GGG	TCG	GAT	TAT	3293
Glu	Val	Ala	Arg	Val	Ala	Gly	Leu	Tyr	Gly	Val	Pro	Gly	Ser	Asp	Tyr	
		155					160					165				
GCA	TAC	CCT	AGG	AAA	TCG	GAA	TTA	ATA	TCC	TCC	ATT	CGA	CGG	GAT	CCC	3341
Ala	Tyr	Pro	Arg	Lys	Ser	Glu	Leu	Ile	Ser	Ser	Ile	Arg	Arg	Asp	Pro	
	170					175					180					
CAG	GGT	TCT	TTC	TGG	ACT	AGT	CCT	ACA	CCC	CGT	GGA	AAT	AAA	TAT	TTC	3389
Gln	Gly	Ser	Phe	Trp	Thr	Ser	Pro	Thr	Pro	Arg	Gly	Asn	Lys	Tyr	Phe	
185					190					195					200	
ATA	TGG	ATT	AAT	AAA	ACA	ATG	CAC	ACC	ATG	GGC	GTG	GAA	GTT	AGA	AAT	3437
Ile	Trp	Ile	Asn	Lys	Thr	Met	His	Thr	Met	Gly	Val	Glu	Val	Arg	Asn	
				205					210					215		
GTC	GAC	TAC	AAA	GAC	AAC	GGC	TAC	TTT	CAA	GTG	ATA	CTG	CGT	GAT	AGA	3485
Val	Asp	Tyr	Lys	Asp	Asn	Gly	Tyr	Phe	Gln	Val	Ile	Leu	Arg	Asp	Arg	
			220					225					230			
TTT	AAT	CGC	CCA	TTG	GTA	GAA	AAA	CAT	ATT	TAC	ATG	CGT	GTG	TGC	CAA	3533
Phe	Asn	Arg	Pro	Leu	Val	Glu	Lys	His	Ile	Tyr	Met	Arg	Val	Cys	Gln	
		235					240					245				
CGA	CCC	GCA	TCC	GTG	GAT	GTA	TTG	GCC	CCT	CCA	GTT	CTC	AGC	GGA	GAA	3581
Arg	Pro	Ala	Ser	Val	Asp	Val	Leu	Ala	Pro	Pro	Val	Leu	Ser	Gly	Glu	
	250					255					260					
AAC	TAC	AAA	GCA	TCT	TGC	ATC	GTT	AGA	CAT	TTT	TAT	CCC	CCG	GGA	TCT	3629
Asn	Tyr	Lys	Ala	Ser	Cys	Ile	Val	Arg	His	Phe	Tyr	Pro	Pro	Gly	Ser	
265					270					275					280	
GTC	TAC	GTA	TCT	TGG	AGA	CGT	AAC	GGA	AAC	ATT	GCC	ACA	CCC	CGC	AAG	3677
Val	Tyr	Val	Ser	Trp	Arg	Arg	Asn	Gly	Asn	Ile	Ala	Thr	Pro	Arg	Lys	
				285					290					295		
GAC	CGT	GAC	GGG	AGT	TTT	TGG	TGG	TTC	GAA	TCT	GGC	CGC	GGG	GCC	ACA	3725
Asp	Arg	Asp	Gly	Ser	Phe	Trp	Trp	Phe	Glu	Ser	Gly	Arg	Gly	Ala	Thr	
			300					305					310			
CTA	GTA	TCC	ACA	ATA	ACC	CTC	GGA	AAC	TCT	GGA	CTC	GAA	TCT	CCT	CCA	3773
Leu	Val	Ser	Thr	Ile	Thr	Leu	Gly	Asn	Ser	Gly	Leu	Glu	Ser	Pro	Pro	
		315					320					325				

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AAG	GTT	TCC	TGC	TTG	GTA	GCG	TGG	AGG	CAA	GGC	GAT	ATG	ATA	AGC	ACA	3821
Lys	Val	Ser	Cys	Leu	Val	Ala	Trp	Arg	Gln	Gly	Asp	Met	Ile	Ser	Thr	
	330					335					340					
TCG	AAT	GCT	ACA	GCT	GTA	CCG	ACG	GTA	TAT	TAT	CAC	CCC	CGT	ATC	TCT	3869
Ser	Asn	Ala	Thr	Ala	Val	Pro	Thr	Val	Tyr	Tyr	His	Pro	Arg	Ile	Ser	
	345				350					355					360	
CTG	GCA	TTT	AAA	GAT	GGG	TAT	GCA	ATA	TGT	ACT	ATA	GAA	TGT	GTT	CCC	3917
Leu	Ala	Phe	Lys	Asp	Gly	Tyr	Ala	Ile	Cys	Thr	Ile	Glu	Cys	Val	Pro	
				365					370					375		
TCT	GGG	ATT	ACT	GTG	AGG	TGG	TTA	GTT	CAT	GAT	GAA	CCC	CAG	CCT	AAC	3965
Ser	Gly	Ile	Thr	Val	Arg	Trp	Leu	Val	His	Asp	Glu	Pro	Gln	Pro	Asn	
			380					385					390			
ACA	ACT	TAT	GAT	ACT	GTG	GTT	ACA	GGT	CTC	TGC	AGG	ACC	ATC	GAT	CGT	4013
Thr	Thr	Tyr	Asp	Thr	Val	Val	Thr	Gly	Leu	Cys	Arg	Thr	Ile	Asp	Arg	
		395					400					405				
TAT	AGA	AAT	CTC	GCC	AGT	CGG	ATT	CCA	GTC	CAG	GAC	AAC	TGG	GCG	AAA	4061
Tyr	Arg	Asn	Leu	Ala	Ser	Arg	Ile	Pro	Val	Gln	Asp	Asn	Trp	Ala	Lys	
	410					415					420					
ACG	AAG	TAT	ACG	TGC	AGA	CTA	ATT	GGA	TAT	CCG	TTC	GAC	GTG	GAT	AGA	4109
Thr	Lys	Tyr	Thr	Cys	Arg	Leu	Ile	Gly	Tyr	Pro	Phe	Asp	Val	Asp	Arg	
	425				430					435					440	
TTT	CAA	AAT	TCC	GAA	TAT	TAT	GAT	GCA	ACG	CCG	TCG	GCA	AGA	GGA	ATG	4157
Phe	Gln	Asn	Ser	Glu	Tyr	Tyr	Asp	Ala	Thr	Pro	Ser	Ala	Arg	Gly	Met	
				445					450					455		
CCG	ATG	ATT	GTA	ACA	ATT	ACG	GCC	GTT	CTA	GGA	CTG	GCC	TTG	TTT	TTA	4205
Pro	Met	Ile	Val	Thr	Ile	Thr	Ala	Val	Leu	Gly	Leu	Ala	Leu	Phe	Leu	
			460					465					470			
GGT	ATT	GGT	ATC	ATT	ATC	ACA	GCC	CTA	TGC	TTT	TAC	CTA	CCG	GGG	CGG	4253
Gly	Ile	Gly	Ile	Ile	Ile	Thr	Ala	Leu	Cys	Phe	Tyr	Leu	Pro	Gly	Arg	
		475					480					485				
AAT	TAAGATTAAC	CATCGTATGT	GATATAAAAA	TTATTAAGTG	TTATAACCGA											4306
Asn																
	490															
TCGCATTCTT	CTGTTTCGAT	TCACAATAAA	TAAATGGTA	TTGTAATCAG	CACCATCGCA											4366
TTGTTTCGTA	GATGACTCAT	GTTCACTCCG	CGTGATGTCA	AAAATACGTA	TTTTTGGTAT											4426
CACGCAGCGG	CCAAAATGCC	CATTATGTTA	TTTTTACTCC	AAACGCGGTA	TTTAAACAT											4486
CGGGACGTAC	ATCATGTGGC	GCACGTTAAT	CGTATACGGT	GCCGCTACAT	TAAAAATCGC											4546
AAGTCTCCGA	ATATCAAGCT	CACGGCCAAA	ACGTCGGTAA	TAATCTTACG	CATCGAATGT											4606
GATACGGATA	CCGTACAATC	GCTGAGTAGA	TTTCCTATAT	AGTTACTCAG	TAGTGATACA											4666
CAATCACAAA	ATCGCTGGGG	TATATCATAT	AAGA	ATG	ATG	TCG	CCC	ACC	CCT							4718
							Met	Met	Ser	Pro	Thr	Pro				
							1				5					
GAA	GAT	GAT	CGC	GAT	CTC	GTT	GTG	GTT	CGT	GGA	CGT	CTC	CGA	ATG	ATG	4766
Glu	Asp	Asp	Arg	Asp	Leu	Val	Val	Val	Arg	Gly	Arg	Leu	Arg	Met	Met	

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10						15						20						
GAT	AGC	GGC	ACG	GAA	ACA	GAT	AGA	GAG	CAA	CGA	CAT	CCA	CGT	ACG	ACT	4814		
Asp	Ser	Gly	Thr	Glu	Thr	Asp	Arg	Glu	Gln	Arg	His	Pro	Arg	Thr	Thr			
		25					30					35						
TGG	CGA	TCG	ATC	TGT	TGT	GGG	TGT	ACG	ATA	GGA	ATG	GTA	TTT	ACC	ATA	4862		
Trp	Arg	Ser	Ile	Cys	Cys	Gly	Cys	Thr	Ile	Gly	Met	Val	Phe	Thr	Ile			
	40					45					50							
TTC	GTT	CTC	GTA	GCG	GCA	GTA	TTG	TTG	GGA	TCA	CTA	TTC	ACT	GTT	TCA	4910		
Phe	Val	Leu	Val	Ala	Ala	Val	Leu	Leu	Gly	Ser	Leu	Phe	Thr	Val	Ser			
	55				60					65					70			
TAC	ATG	GCC	ATG	GAA	TCG	GGA	ACA	TGT	CCC	GAT	GAA	TGG	ATT	GGT	TTG	4958		
Tyr	Met	Ala	Met	Glu	Ser	Gly	Thr	Cys	Pro	Asp	Glu	Trp	Ile	Gly	Leu			
				75					80					85				
GGT	TAT	AGT	TGC	ATG	CGC	GTG	GCC	GGG	AAA	AAT	GCA	ACT	GAT	CTT	GAG	5006		
Gly	Tyr	Ser	Cys	Met	Arg	Val	Ala	Gly	Lys	Asn	Ala	Thr	Asp	Leu	Glu			
			90					95					100					
GCG	TTG	GAT	ACA	TGT	GCT	CGG	CAT	AAC	AGC	AAA	CTT	ATT	GAC	TTC	GCA	5054		
Ala	Leu	Asp	Thr	Cys	Ala	Arg	His	Asn	Ser	Lys	Leu	Ile	Asp	Phe	Ala			
		105					110					115						
AAC	GCC	AAA	GTT	CTG	GTT	GAA	GCT	ATC	GCC	CCA	TTC	GGT	GTG	CCA	AAT	5102		
Asn	Ala	Lys	Val	Leu	Val	Glu	Ala	Ile	Ala	Pro	Phe	Gly	Val	Pro	Asn			
	120					125					130							
GCA	GCA	TAT	GGG	GAA	GTC	TTC	CGG	TTA	AGG	GAC	AGC	AAA	ACC	ACG	TGT	5150		
Ala	Ala	Tyr	Gly	Glu	Val	Phe	Arg	Leu	Arg	Asp	Ser	Lys	Thr	Thr	Cys			
	135				140					145					150			
ATA	CGA	CCT	ACC	ATG	GGA	GGA	CCC	GTG	TCG	GCA	GAC	TGT	CCT	GTA	ACA	5198		
Ile	Arg	Pro	Thr	Met	Gly	Gly	Pro	Val	Ser	Ala	Asp	Cys	Pro	Val	Thr			
				155				160						165				
TGT	ACC	GTT	ATA	TGT	CAG	CGA	CCC	AGG	CCT	CTA	AGT	ACC	ATG	TCT	TCC	5246		
Cys	Thr	Val	Ile	Cys	Gln	Arg	Pro	Arg	Pro	Leu	Ser	Thr	Met	Ser	Ser			
			170					175					180					
ATC	ATT	AGA	GAT	GCC	CGC	GTG	TAT	CTT	CAT	TTA	GAA	CGA	CGC	GAT	TAT	5294		
Ile	Ile	Arg	Asp	Ala	Arg	Val	Tyr	Leu	His	Leu	Glu	Arg	Arg	Asp	Tyr			
		185					190					195						
TAT	GAA	GTC	TAC	GCC	TCT	GTC	CTC	TCT	AAT	GCG	ATG	AGT	AAA	TAAAAACGCA	5346			
Tyr	Glu	Val	Tyr	Ala	Ser	Val	Leu	Ser	Asn	Ala	Met	Ser	Lys					
	200					205					210							
CCTCTAACGG TTACTGTGTT TATTATCCAA TCACACCATA GACATTATTA CAATAATATG 5406																		
GATCTTTATT TCATATAATG 5426																		

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 369 amino acids

(B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Met Ala Gly Ile Thr Val Ala Cys Asp His Thr Ala Gly Glu Ala
 1           5           10           15
His Thr Pro Glu Asp Met Gln Lys Lys Trp Arg Ile Ile Leu Ala Gly
          20           25           30
Glu Lys Phe Met Thr Ile Ser Ala Ser Leu Lys Ser Ile Val Ser Cys
          35           40           45
Val Lys Asn Pro Leu Leu Thr Phe Gly Ala Asp Gly Leu Ile Val Gln
          50           55           60
Gly Thr Val Cys Gly Gln Arg Ile Phe Val Pro Ile Asp Arg Asp Ser
 65           70           75           80
Phe Ser Glu Tyr Glu Trp His Gly Pro Thr Ala Met Phe Leu Ala Leu
          85           90           95
Thr Asp Ser Arg Arg Thr Leu Leu Asp Ala Phe Lys Cys Glu Lys Arg
          100          105          110
Arg Ala Ile Asp Val Ser Phe Thr Phe Ala Gly Glu Pro Pro Cys Arg
          115          120          125
His Leu Ile Gln Ala Val Thr Tyr Met Thr Asp Gly Gly Ser Val Ser
          130          135          140
Asn Thr Ile Ile Lys Tyr Glu Leu Trp Asn Ala Ser Thr Ile Phe Pro
145           150           155           160
Gln Lys Thr Pro Asp Val Thr Phe Ser Leu Asn Lys Gln Gln Leu Asn
          165          170          175
Lys Ile Leu Ala Val Ala Ser Lys Leu Gln His Glu Glu Leu Val Phe
          180          185          190
Ser Leu Lys Pro Glu Gly Gly Phe Tyr Val Gly Thr Val Cys Thr Val
          195          200          205
Ile Ser Phe Glu Val Asp Gly Thr Ala Met Thr Gln Tyr Pro Tyr Asn
          210          215          220
Pro Pro Thr Ser Ala Thr Leu Ala Leu Val Val Ala Cys Arg Lys Lys
225           230           235           240
Lys Ala Asn Lys Asn Thr Ile Leu Thr Ala Tyr Gly Ser Gly Lys Pro
          245          250          255
Phe Cys Val Ala Leu Glu Asp Thr Ser Ala Phe Arg Asn Ile Val Asn
          260          265          270
Lys Ile Lys Ala Gly Thr Ser Gly Val Asp Leu Gly Phe Tyr Thr Thr
          275          280          285
Cys Asp Pro Pro Met Leu Cys Ile Arg Pro His Ala Phe Gly Ser Pro
          290          295          300
Thr Ala Phe Leu Phe Cys Asn Thr Asp Cys Met Thr Ile Tyr Glu Leu

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305		310		315		320									
Glu	Glu	Val	Ser	Ala	Val	Asp	Gly	Ala	Ile	Arg	Ala	Lys	Arg	Ile	Asn
				325					330					335	
Glu	Tyr	Phe	Pro	Thr	Val	Ser	Gln	Ala	Thr	Ser	Lys	Lys	Arg	Lys	Gln
			340					345					350		
Ser	Pro	Pro	Pro	Ile	Glu	Arg	Glu	Arg	Lys	Thr	Thr	Arg	Ala	Asp	Thr
		355					360					365			
Gln															

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 422 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Pro	Ser	Gly	Ala	Ser	Ser	Ser	Pro	Pro	Pro	Ala	Tyr	Thr	Ser	Ala
1				5				10						15	
Ala	Pro	Leu	Glu	Thr	Tyr	Asn	Ser	Trp	Leu	Ser	Ala	Phe	Ser	Cys	Ala
		20						25					30		
Tyr	Pro	Gln	Cys	Thr	Ala	Gly	Arg	Gly	His	Arg	Gln	Asn	Gly	Lys	Lys
		35					40					45			
Cys	Ile	Arg	Cys	Ile	Val	Ile	Ser	Val	Cys	Ser	Leu	Val	Cys	Ile	Ala
	50					55					60				
Ala	His	Leu	Ala	Val	Thr	Val	Ser	Gly	Val	Ala	Leu	Ile	Pro	Leu	Ile
65				70						75					80
Asp	Gln	Asn	Arg	Ala	Tyr	Gly	Asn	Cys	Thr	Val	Cys	Val	Ile	Ala	Gly
				85					90					95	
Phe	Ile	Ala	Thr	Phe	Ala	Ala	Arg	Leu	Thr	Ile	Arg	Leu	Ser	Glu	Thr
			100					105					110		
Leu	Met	Leu	Val	Gly	Lys	Pro	Ala	Gln	Phe	Ile	Phe	Ala	Ile	Ile	Ala
	115					120						125			
Ser	Val	Ala	Glu	Thr	Leu	Ile	Asn	Asn	Glu	Ala	Leu	Ala	Ile	Ser	Asn
	130					135					140				
Thr	Thr	Tyr	Lys	Thr	Ala	Leu	Arg	Ile	Ile	Glu	Val	Thr	Ser	Leu	Ala
145					150					155					160
Cys	Phe	Val	Met	Leu	Gly	Ala	Ile	Ile	Thr	Ser	His	Asn	Tyr	Val	Cys
			165						170					175	
Ile	Ser	Thr	Ala	Gly	Asp	Leu	Thr	Trp	Lys	Gly	Gly	Ile	Phe	His	Ala
			180					185					190		

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Tyr His Gly Thr Leu Leu Gly Ile Thr Ile Pro Asn Ile His Pro Ile
 195 200 205
 Pro Leu Ala Gly Phe Leu Ala Val Tyr Thr Ile Leu Ala Ile Asn Ile
 210 215 220
 Ala Arg Asp Ala Ser Ala Thr Leu Leu Ser Thr Cys Tyr Tyr Arg Asn
 225 230 235 240
 Cys Arg Glu Arg Thr Ile Leu Arg Pro Ser Arg Leu Gly His Gly Tyr
 245 250 255
 Thr Ile Pro Ser Pro Gly Ala Asp Met Leu Tyr Glu Glu Asp Val Tyr
 260 265 270
 Ser Phe Asp Ala Ala Lys Gly His Tyr Ser Ser Ile Phe Leu Cys Tyr
 275 280 285
 Ala Met Gly Leu Thr Thr Pro Leu Ile Ile Ala Leu His Lys Tyr Met
 290 295 300
 Ala Gly Ile Lys Asn Ser Ser Asp Trp Thr Ala Thr Leu Gln Gly Met
 305 310 315 320
 Tyr Gly Leu Val Leu Gly Ser Leu Ser Ser Leu Cys Ile Pro Ser Ser
 325 330 335
 Asn Asn Asp Ala Leu Ile Arg Pro Ile Gln Ile Leu Ile Leu Ile Ile
 340 345 350
 Gly Ala Leu Ala Ile Ala Leu Ala Gly Cys Gly Gln Ile Ile Gly Pro
 355 360 365
 Thr Leu Phe Ala Ala Ser Ser Ala Ala Met Ser Cys Phe Thr Cys Ile
 370 375 380
 Asn Ile Arg Ala Thr Asn Lys Gly Val Asn Lys Leu Ala Ala Ala Ser
 385 390 395 400
 Val Val Lys Ser Val Leu Gly Phe Ile Ile Ser Gly Met Leu Thr Cys
 405 410 415
 Val Leu Leu Pro Leu Ser
 420

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 489 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Ser Asn Met Arg Val Leu Arg Val Leu Arg Leu Thr Gly Trp
 1 5 10 15
 Val Gly Ile Phe Leu Val Leu Ser Leu Gln Gln Thr Ser Cys Ala Gly

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20							25					30				
Leu	Pro	His	Asn	Val	Asp	Thr	His	His	Ile	Leu	Thr	Phe	Asn	Pro	Ser	
		35					40					45				
Pro	Ile	Ser	Ala	Asp	Gly	Val	Pro	Leu	Ser	Glu	Val	Pro	Asn	Ser	Pro	
	50					55					60					
Thr	Thr	Glu	Leu	Ser	Thr	Thr	Val	Ala	Thr	Lys	Thr	Ala	Val	Pro	Thr	
65					70					75					80	
Thr	Glu	Ser	Thr	Ser	Ser	Ser	Glu	Ala	His	Arg	Asn	Ser	Ser	His	Lys	
				85					90					95		
Ile	Pro	Asp	Ile	Ile	Cys	Asp	Arg	Glu	Glu	Val	Phe	Val	Phe	Leu	Asn	
			100					105					110			
Asn	Thr	Gly	Arg	Ile	Leu	Cys	Asp	Leu	Ile	Val	Asp	Pro	Pro	Ser	Asp	
		115						120					125			
Asp	Glu	Trp	Ser	Asn	Phe	Ala	Leu	Asp	Val	Thr	Phe	Asn	Pro	Ile	Glu	
	130					135					140					
Tyr	His	Ala	Asn	Glu	Lys	Asn	Val	Glu	Val	Ala	Arg	Val	Ala	Gly	Leu	
145					150					155					160	
Tyr	Gly	Val	Pro	Gly	Ser	Asp	Tyr	Ala	Tyr	Pro	Arg	Lys	Ser	Glu	Leu	
				165					170					175		
Ile	Ser	Ser	Ile	Arg	Arg	Asp	Pro	Gln	Gly	Ser	Phe	Trp	Thr	Ser	Pro	
			180					185					190			
Thr	Pro	Arg	Gly	Asn	Lys	Tyr	Phe	Ile	Trp	Ile	Asn	Lys	Thr	Met	His	
		195						200					205			
Thr	Met	Gly	Val	Glu	Val	Arg	Asn	Val	Asp	Tyr	Lys	Asp	Asn	Gly	Tyr	
	210					215					220					
Phe	Gln	Val	Ile	Leu	Arg	Asp	Arg	Phe	Asn	Arg	Pro	Leu	Val	Glu	Lys	
225					230					235					240	
His	Ile	Tyr	Met	Arg	Val	Cys	Gln	Arg	Pro	Ala	Ser	Val	Asp	Val	Leu	
				245					250					255		
Ala	Pro	Pro	Val	Leu	Ser	Gly	Glu	Asn	Tyr	Lys	Ala	Ser	Cys	Ile	Val	
			260					265					270			
Arg	His	Phe	Tyr	Pro	Pro	Gly	Ser	Val	Tyr	Val	Ser	Trp	Arg	Arg	Asn	
		275						280					285			
Gly	Asn	Ile	Ala	Thr	Pro	Arg	Lys	Asp	Arg	Asp	Gly	Ser	Phe	Trp	Trp	
	290					295					300					
Phe	Glu	Ser	Gly	Arg	Gly	Ala	Thr	Leu	Val	Ser	Thr	Ile	Thr	Leu	Gly	
305					310					315					320	
Asn	Ser	Gly	Leu	Glu	Ser	Pro	Pro	Lys	Val	Ser	Cys	Leu	Val	Ala	Trp	
				325					330					335		
Arg	Gln	Gly	Asp	Met	Ile	Ser	Thr	Ser	Asn	Ala	Thr	Ala	Val	Pro	Thr	
			340					345					350			
Val	Tyr	Tyr	His	Pro	Arg	Ile	Ser	Leu	Ala	Phe	Lys	Asp	Gly	Tyr	Ala	

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355		360		365
Ile Cys Thr Ile Glu Cys Val Pro Ser Gly Ile Thr Val Arg Trp Leu				
370		375		380
Val His Asp Glu Pro Gln Pro Asn Thr Thr Tyr Asp Thr Val Val Thr				
385		390		395
Gly Leu Cys Arg Thr Ile Asp Arg Tyr Arg Asn Leu Ala Ser Arg Ile				
		405		410
Pro Val Gln Asp Asn Trp Ala Lys Thr Lys Tyr Thr Cys Arg Leu Ile				
		420		425
Gly Tyr Pro Phe Asp Val Asp Arg Phe Gln Asn Ser Glu Tyr Tyr Asp				
		435		440
				445
Ala Thr Pro Ser Ala Arg Gly Met Pro Met Ile Val Thr Ile Thr Ala				
450		455		460
Val Leu Gly Leu Ala Leu Phe Leu Gly Ile Gly Ile Ile Ile Thr Ala				
465		470		475
				480
Leu Cys Phe Tyr Leu Pro Gly Arg Asn				
		485		

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 212 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Met Ser Pro Thr Pro Glu Asp Asp Arg Asp Leu Val Val Val Arg	
1 5 10 15	
Gly Arg Leu Arg Met Met Asp Ser Gly Thr Glu Thr Asp Arg Glu Gln	
20 25 30	
Arg His Pro Arg Thr Thr Trp Arg Ser Ile Cys Cys Gly Cys Thr Ile	
35 40 45	
Gly Met Val Phe Thr Ile Phe Val Leu Val Ala Ala Val Leu Leu Gly	
50 55 60	
Ser Leu Phe Thr Val Ser Tyr Met Ala Met Glu Ser Gly Thr Cys Pro	
65 70 75 80	
Asp Glu Trp Ile Gly Leu Gly Tyr Ser Cys Met Arg Val Ala Gly Lys	
85 90 95	
Asn Ala Thr Asp Leu Glu Ala Leu Asp Thr Cys Ala Arg His Asn Ser	
100 105 110	
Lys Leu Ile Asp Phe Ala Asn Ala Lys Val Leu Val Glu Ala Ile Ala	
115 120 125	

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Pro Phe Gly Val Pro Asn Ala Ala Tyr Gly Glu Val Phe Arg Leu Arg
 130 135 140

Asp Ser Lys Thr Thr Cys Ile Arg Pro Thr Met Gly Gly Pro Val Ser
 145 150 155 160

Ala Asp Cys Pro Val Thr Cys Thr Val Ile Cys Gln Arg Pro Arg Pro
 165 170 175

Leu Ser Thr Met Ser Ser Ile Ile Arg Asp Ala Arg Val Tyr Leu His
 180 185 190

Leu Glu Arg Arg Asp Tyr Tyr Glu Val Tyr Ala Ser Val Leu Ser Asn
 195 200 205

Ala Met Ser Lys
 210

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1506 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1506

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATG	CTC	ACG	CCG	CGT	GTG	TTA	CGA	GCT	TTG	GGG	TGG	ACT	GGA	CTC	TTT	48
Met	Leu	Thr	Pro	Arg	Val	Leu	Arg	Ala	Leu	Gly	Trp	Thr	Gly	Leu	Phe	
1				5					10					15		
TTT	TTG	CTT	TTA	TCT	CCG	AGC	AAC	GTC	CTA	GGA	GCC	AGC	CTT	AGC	CGG	96
Phe	Leu	Leu	Leu	Ser	Pro	Ser	Asn	Val	Leu	Gly	Ala	Ser	Leu	Ser	Arg	
			20					25					30			
GAT	CTC	GAA	ACA	CCC	CCA	TTT	CTA	TCC	TTT	GAT	CCA	TCC	AAC	ATT	TCA	144
Asp	Leu	Glu	Thr	Pro	Pro	Phe	Leu	Ser	Phe	Asp	Pro	Ser	Asn	Ile	Ser	
		35					40					45				
ATT	AAC	GGC	GCG	CCT	TTA	ACT	GAG	GTA	CCT	CAT	GCA	CCT	TCC	ACA	GAA	192
Ile	Asn	Gly	Ala	Pro	Leu	Thr	Glu	Val	Pro	His	Ala	Pro	Ser	Thr	Glu	
	50					55				60						
AGT	GTG	TCA	ACA	AAT	TCG	GAA	AGT	ACC	AAT	GAA	CAT	ACC	ATA	ACA	GAA	240
Ser	Val	Ser	Thr	Asn	Ser	Glu	Ser	Thr	Asn	Glu	His	Thr	Ile	Thr	Glu	
65				70				75							80	
ACG	ACG	GGC	AAG	AAC	GCA	TAC	ATC	CAC	AAC	AAT	GCG	TCT	ACG	GAC	AAG	288
Thr	Thr	Gly	Lys	Asn	Ala	Tyr	Ile	His	Asn	Asn	Ala	Ser	Thr	Asp	Lys	

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85								90				95				
CAA	AAT	GCG	AAC	GAC	ACT	CAT	AAA	ACG	CCC	AAT	ATA	CTC	TGC	GAT	ACG	336
Gln	Asn	Ala	Asn	Asp	Thr	His	Lys	Thr	Pro	Asn	Ile	Leu	Cys	Asp	Thr	
			100					105					110			
GAA	GAA	GTT	TTT	GTT	TTC	CTT	AAC	GAA	ACG	GGA	AGA	TTT	GTT	TGT	ACT	384
Glu	Glu	Val	Phe	Val	Phe	Leu	Asn	Glu	Thr	Gly	Arg	Phe	Val	Cys	Thr	
		115					120					125				
CTC	AAA	GTC	GAC	CCC	CCC	TCG	GAT	AGT	GAA	TGG	TCC	AAC	TTT	GTT	CTA	432
Leu	Lys	Val	Asp	Pro	Pro	Ser	Asp	Ser	Glu	Trp	Ser	Asn	Phe	Val	Leu	
	130					135					140					
GAT	CTG	ATC	TTT	AAC	CCA	ATT	GAA	TAC	CAC	GCC	AAC	GAA	AAG	AAT	GTG	480
Asp	Leu	Ile	Phe	Asn	Pro	Ile	Glu	Tyr	His	Ala	Asn	Glu	Lys	Asn	Val	
	145				150					155					160	
GAA	GCG	GCG	CGT	ATC	GCT	GGT	CTC	TAT	GGA	GTC	CCC	GGA	TCA	GAC	TAT	528
Glu	Ala	Ala	Arg	Ile	Ala	Gly	Leu	Tyr	Gly	Val	Pro	Gly	Ser	Asp	Tyr	
				165					170					175		
GCA	TAC	CCA	CGT	CAA	TCT	GAA	TTA	ATT	TCT	TCG	ATT	CGA	CGA	GAT	CCC	576
Ala	Tyr	Pro	Arg	Gln	Ser	Glu	Leu	Ile	Ser	Ser	Ile	Arg	Arg	Asp	Pro	
			180					185					190			
CAG	GGC	ACA	TTT	TGG	ACG	AGC	CCA	TCA	CCT	CAT	GGA	AAC	AAG	TAC	TTC	624
Gln	Gly	Thr	Phe	Trp	Thr	Ser	Pro	Ser	Pro	His	Gly	Asn	Lys	Tyr	Phe	
		195					200					205				
ATA	TGG	ATA	AAC	AAA	ACA	ACC	AAT	ACG	ATG	GGC	GTG	GAA	ATT	AGA	AAT	672
Ile	Trp	Ile	Asn	Lys	Thr	Thr	Asn	Thr	Met	Gly	Val	Glu	Ile	Arg	Asn	
	210					215					220					
GTA	GAT	TAT	GCT	GAT	AAT	GGC	TAC	ATG	CAA	GTC	ATT	ATG	CGT	GAC	CAT	720
Val	Asp	Tyr	Ala	Asp	Asn	Gly	Tyr	Met	Gln	Val	Ile	Met	Arg	Asp	His	
	225				230					235				240		
TTT	AAT	CGG	CCT	TTA	ATA	GAT	AAA	CAT	ATT	TAC	ATA	CGT	GTG	TGT	CAA	768
Phe	Asn	Arg	Pro	Leu	Ile	Asp	Lys	His	Ile	Tyr	Ile	Arg	Val	Cys	Gln	
				245					250					255		
CGA	CCT	GCA	TCA	GTG	GAT	GTA	CTG	GCC	CCT	CCA	GTC	CTC	AGC	GGA	GAA	816
Arg	Pro	Ala	Ser	Val	Asp	Val	Leu	Ala	Pro	Pro	Val	Leu	Ser	Gly	Glu	
			260					265					270			
AAT	TAC	AAG	GCA	TCT	TGT	ATC	GTT	AGA	CAC	TTT	TAT	CCC	CCT	GGA	TCT	864
Asn	Tyr	Lys	Ala	Ser	Cys	Ile	Val	Arg	His	Phe	Tyr	Pro	Pro	Gly	Ser	
		275					280					285				
GTC	TAT	GTA	TCT	TGG	AGA	CAG	AAT	GGA	AAC	ATT	GCA	ACT	CCT	CGG	AAA	912
Val	Tyr	Val	Ser	Trp	Arg	Gln	Asn	Gly	Asn	Ile	Ala	Thr	Pro	Arg	Lys	
	290					295					300					
GAT	CGC	GAT	GGA	AGT	TTT	TGG	TGG	TTC	GAA	TCT	GGT	AGA	GGA	GCT	ACG	960
Asp	Arg	Asp	Gly	Ser	Phe	Trp	Trp	Phe	Glu	Ser	Gly	Arg	Gly	Ala	Thr	
	305				310					315				320		
TTG	GTT	TCT	ACA	ATA	ACA	TTG	GGA	AAT	TCA	GGA	ATT	GAT	TTC	CCC	CCC	1008
Leu	Val	Ser	Thr	Ile	Thr	Leu	Gly	Asn	Ser	Gly	Ile	Asp	Phe	Pro	Pro	
				325					330					335		
AAA	ATA	TCT	TGT	CTG	GTT	GCC	TGG	AAG	CAG	GGT	GAT	ATG	ATC	AGC	ACG	1056

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Lys	Ile	Ser	Cys	Leu	Val	Ala	Trp	Lys	Gln	Gly	Asp	Met	Ile	Ser	Thr		
			340					345					350				
ACG	AAT	GCC	ACA	GCT	ATC	CCG	ACG	GTA	TAT	CAT	CAT	CCC	CGT	TTA	TCC	1104	
Thr	Asn	Ala	Thr	Ala	Ile	Pro	Thr	Val	Tyr	His	His	Pro	Arg	Leu	Ser		
		355					360					365					
CTG	GCT	TTT	AAA	GAT	GGG	TAT	GCA	ATA	TGT	ACT	ATA	GAA	TGT	GTC	CCC	1152	
Leu	Ala	Phe	Lys	Asp	Gly	Tyr	Ala	Ile	Cys	Thr	Ile	Glu	Cys	Val	Pro		
	370					375					380						
TCT	GAG	ATT	ACT	GTA	CGG	TGG	TTA	GTA	CAT	GAT	GAA	GCG	CAG	CCT	AAC	1200	
Ser	Glu	Ile	Thr	Val	Arg	Trp	Leu	Val	His	Asp	Glu	Ala	Gln	Pro	Asn		
385					390					395					400		
ACA	ACT	TAT	AAT	ACT	GTG	GTT	ACA	GGT	CTC	TGC	CGG	ACC	ATC	GAT	CGC	1248	
Thr	Thr	Tyr	Asn	Thr	Val	Val	Thr	Gly	Leu	Cys	Arg	Thr	Ile	Asp	Arg		
				405					410					415			
CAT	AGA	AAT	CTC	CTC	AGC	CGC	ATT	CCA	GTA	TGG	GAC	AAT	TGG	ACG	AAA	1296	
His	Arg	Asn	Leu	Leu	Ser	Arg	Ile	Pro	Val	Trp	Asp	Asn	Trp	Thr	Lys		
			420					425					430				
ACA	AAA	TAT	ACG	TGC	AGA	CTC	ATA	GGC	TAC	CCC	TTC	GAT	GAA	GAT	AAA	1344	
Thr	Lys	Tyr	Thr	Cys	Arg	Leu	Ile	Gly	Tyr	Pro	Phe	Asp	Glu	Asp	Lys		
		435					440					445					
TTT	CAA	GAT	TCG	GAA	TAT	TAC	GAT	GCA	ACT	CCA	TCT	GCA	AGA	GGA	ACA	1392	
Phe	Gln	Asp	Ser	Glu	Tyr	Tyr	Asp	Ala	Thr	Pro	Ser	Ala	Arg	Gly	Thr		
	450					455					460						
CCC	ATG	GTT	ATT	ACG	GTT	ACG	GCA	GTT	TTG	GGA	TTG	GCT	GTA	ATT	TTA	1440	
Pro	Met	Val	Ile	Thr	Val	Thr	Ala	Val	Leu	Gly	Leu	Ala	Val	Ile	Leu		
465					470					475					480		
GGG	ATG	GGG	ATA	ATC	ATG	ACT	GCC	CTA	TGT	TTA	TAC	AAC	TCC	ACA	CGA	1488	
Gly	Met	Gly	Ile	Ile	Met	Thr	Ala	Leu	Cys	Leu	Tyr	Asn	Ser	Thr	Arg		
				485					490					495			
AAA	AAT	ATT	CGA	TTA	TAA											1506	
Lys	Asn	Ile	Arg	Leu													
			500														

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 501 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Leu	Thr	Pro	Arg	Val	Leu	Arg	Ala	Leu	Gly	Trp	Thr	Gly	Leu	Phe		
1				5					10					15			
Phe	Leu	Leu	Leu	Ser	Pro	Ser	Asn	Val	Leu	Gly	Ala	Ser	Leu	Ser	Arg		
	20						25						30				
Asp	Leu	Glu	Thr	Pro	Pro	Phe	Leu	Ser	Phe	Asp	Pro	Ser	Asn	Ile	Ser		

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35					40					45					
Ile	Asn	Gly	Ala	Pro	Leu	Thr	Glu	Val	Pro	His	Ala	Pro	Ser	Thr	Glu
	50					55					60				
Ser	Val	Ser	Thr	Asn	Ser	Glu	Ser	Thr	Asn	Glu	His	Thr	Ile	Thr	Glu
	65					70					75				80
Thr	Thr	Gly	Lys	Asn	Ala	Tyr	Ile	His	Asn	Asn	Ala	Ser	Thr	Asp	Lys
				85					90					95	
Gln	Asn	Ala	Asn	Asp	Thr	His	Lys	Thr	Pro	Asn	Ile	Leu	Cys	Asp	Thr
			100					105					110		
Glu	Glu	Val	Phe	Val	Phe	Leu	Asn	Glu	Thr	Gly	Arg	Phe	Val	Cys	Thr
		115					120					125			
Leu	Lys	Val	Asp	Pro	Pro	Ser	Asp	Ser	Glu	Trp	Ser	Asn	Phe	Val	Leu
	130					135					140				
Asp	Leu	Ile	Phe	Asn	Pro	Ile	Glu	Tyr	His	Ala	Asn	Glu	Lys	Asn	Val
	145					150					155				160
Glu	Ala	Ala	Arg	Ile	Ala	Gly	Leu	Tyr	Gly	Val	Pro	Gly	Ser	Asp	Tyr
				165					170					175	
Ala	Tyr	Pro	Arg	Gln	Ser	Glu	Leu	Ile	Ser	Ser	Ile	Arg	Arg	Asp	Pro
			180					185					190		
Gln	Gly	Thr	Phe	Trp	Thr	Ser	Pro	Ser	Pro	His	Gly	Asn	Lys	Tyr	Phe
		195					200					205			
Ile	Trp	Ile	Asn	Lys	Thr	Thr	Asn	Thr	Met	Gly	Val	Glu	Ile	Arg	Asn
	210					215					220				
Val	Asp	Tyr	Ala	Asp	Asn	Gly	Tyr	Met	Gln	Val	Ile	Met	Arg	Asp	His
	225					230					235				240
Phe	Asn	Arg	Pro	Leu	Ile	Asp	Lys	His	Ile	Tyr	Ile	Arg	Val	Cys	Gln
				245					250					255	
Arg	Pro	Ala	Ser	Val	Asp	Val	Leu	Ala	Pro	Pro	Val	Leu	Ser	Gly	Glu
			260					265					270		
Asn	Tyr	Lys	Ala	Ser	Cys	Ile	Val	Arg	His	Phe	Tyr	Pro	Pro	Gly	Ser
		275					280					285			
Val	Tyr	Val	Ser	Trp	Arg	Gln	Asn	Gly	Asn	Ile	Ala	Thr	Pro	Arg	Lys
	290					295					300				
Asp	Arg	Asp	Gly	Ser	Phe	Trp	Trp	Phe	Glu	Ser	Gly	Arg	Gly	Ala	Thr
	305					310					315				320
Leu	Val	Ser	Thr	Ile	Thr	Leu	Gly	Asn	Ser	Gly	Ile	Asp	Phe	Pro	Pro
				325					330					335	
Lys	Ile	Ser	Cys	Leu	Val	Ala	Trp	Lys	Gln	Gly	Asp	Met	Ile	Ser	Thr
			340					345					350		
Thr	Asn	Ala	Thr	Ala	Ile	Pro	Thr	Val	Tyr	His	His	Pro	Arg	Leu	Ser
		355					360					365			
Leu	Ala	Phe	Lys	Asp	Gly	Tyr	Ala	Ile	Cys	Thr	Ile	Glu	Cys	Val	Pro

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370		375		380
Ser Glu Ile Thr Val Arg Trp Leu Val His Asp Glu Ala Gln Pro Asn				
385		390		395
Thr Thr Tyr Asn Thr Val Val Thr Gly Leu Cys Arg Thr Ile Asp Arg				
	405		410	415
His Arg Asn Leu Leu Ser Arg Ile Pro Val Trp Asp Asn Trp Thr Lys				
	420		425	430
Thr Lys Tyr Thr Cys Arg Leu Ile Gly Tyr Pro Phe Asp Glu Asp Lys				
	435		440	445
Phe Gln Asp Ser Glu Tyr Tyr Asp Ala Thr Pro Ser Ala Arg Gly Thr				
	450		455	460
Pro Met Val Ile Thr Val Thr Ala Val Leu Gly Leu Ala Val Ile Leu				
	465		470	475
Gly Met Gly Ile Ile Met Thr Ala Leu Cys Leu Tyr Asn Ser Thr Arg				
	485		490	495
Lys Asn Ile Arg Leu				
	500			

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1734 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1734

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATG GAC CGC GCC GTT AGC CAA GTT GCG TTA GAG AAT GAT GAA AGA GAG	48
Met Asp Arg Ala Val Ser Gln Val Ala Leu Glu Asn Asp Glu Arg Glu	
1 5 10 15	
GCA AAA AAT ACA TGG CGC TTG ATA TTC CGG ATT GCA ATC TTA TTC TTA	96
Ala Lys Asn Thr Trp Arg Leu Ile Phe Arg Ile Ala Ile Leu Phe Leu	
20 25 30	
ACA GTA GTG ACC TTG GCT ATA TCT GTA GCC TCC CTT TTA TAT AGC ATG	144
Thr Val Val Thr Leu Ala Ile Ser Val Ala Ser Leu Leu Tyr Ser Met	
35 40 45	
GGG GCT AGC ACA CCT AGC GAT CTT GTA GGC ATA CCG ACT AGG ATT TCC	192

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Gly	Ala	Ser	Thr	Pro	Ser	Asp	Leu	Val	Gly	Ile	Pro	Thr	Arg	Ile	Ser	
50						55					60					
AGG	GCA	GAA	GAA	AAG	ATT	ACA	TCT	ACA	CTT	GGT	TCC	AAT	CAA	GAT	GTA	240
Arg	Ala	Glu	Glu	Lys	Ile	Thr	Ser	Thr	Leu	Gly	Ser	Asn	Gln	Asp	Val	
65					70					75					80	
GTA	GAT	AGG	ATA	TAT	AAG	CAA	GTG	GCC	CTT	GAG	TCT	CCA	TTG	GCA	TTG	288
Val	Asp	Arg	Ile	Tyr	Lys	Gln	Val	Ala	Leu	Glu	Ser	Pro	Leu	Ala	Leu	
				85					90					95		
TTA	AAT	ACT	GAG	ACC	ACA	ATT	ATG	AAC	GCA	ATA	ACA	TCT	CTC	TCT	TAT	336
Leu	Asn	Thr	Glu	Thr	Thr	Ile	Met	Asn	Ala	Ile	Thr	Ser	Leu	Ser	Tyr	
			100					105					110			
CAG	ATT	AAT	GGA	GCT	GCA	AAC	AAC	AGC	GGG	TGG	GGG	GCA	CCT	ATT	CAT	384
Gln	Ile	Asn	Gly	Ala	Ala	Asn	Asn	Ser	Gly	Trp	Gly	Ala	Pro	Ile	His	
		115					120					125				
GAC	CCA	GAT	TAT	ATA	GGG	GGG	ATA	GGC	AAA	GAA	CTC	ATT	GTA	GAT	GAT	432
Asp	Pro	Asp	Tyr	Ile	Gly	Gly	Ile	Gly	Lys	Glu	Leu	Ile	Val	Asp	Asp	
	130					135					140					
GCT	AGT	GAT	GTC	ACA	TCA	TTC	TAT	CCC	TCT	GCA	TTT	CAA	GAA	CAT	CTG	480
Ala	Ser	Asp	Val	Thr	Ser	Phe	Tyr	Pro	Ser	Ala	Phe	Gln	Glu	His	Leu	
145					150					155					160	
AAT	TTT	ATC	CCG	GCG	CCT	ACT	ACA	GGA	TCA	GGT	TGC	ACT	CGA	ATA	CCC	528
Asn	Phe	Ile	Pro	Ala	Pro	Thr	Thr	Gly	Ser	Gly	Cys	Thr	Arg	Ile	Pro	
				165				170						175		
TCA	TTT	GAC	ATG	AGT	GCT	ACC	CAT	TAC	TGC	TAC	ACC	CAT	AAT	GTA	ATA	576
Ser	Phe	Asp	Met	Ser	Ala	Thr	His	Tyr	Cys	Tyr	Thr	His	Asn	Val	Ile	
			180					185					190			
TTG	TCT	GGA	TGC	AGA	GAT	CAC	TCA	CAC	TCA	CAT	CAG	TAT	TTA	GCA	CTT	624
Leu	Ser	Gly	Cys	Arg	Asp	His	Ser	His	Ser	His	Gln	Tyr	Leu	Ala	Leu	
		195					200					205				
GGT	GTG	CTC	CGG	ACA	TCT	GCA	ACA	GGG	AGG	GTA	TTC	TTT	TCT	ACT	CTG	672
Gly	Val	Leu	Arg	Thr	Ser	Ala	Thr	Gly	Arg	Val	Phe	Phe	Ser	Thr	Leu	
	210					215					220					
CGT	TCC	ATC	AAC	CTG	GAC	GAC	ACC	CAA	AAT	CGG	AAG	TCT	TGC	AGT	GTG	720
Arg	Ser	Ile	Asn	Leu	Asp	Asp	Thr	Gln	Asn	Arg	Lys	Ser	Cys	Ser	Val	
225					230					235					240	
AGT	GCA	ACT	CCC	CTG	GGT	TGT	GAT	ATG	CTG	TGC	TCG	AAA	GCC	ACG	GAG	768
Ser	Ala	Thr	Pro	Leu	Gly	Cys	Asp	Met	Leu	Cys	Ser	Lys	Ala	Thr	Glu	
				245				250					255			
ACA	GAG	GAA	GAA	GAT	TAT	AAC	TCA	GCT	GTC	CCT	ACG	CGG	ATG	GTA	CAT	816
Thr	Glu	Glu	Glu	Asp	Tyr	Asn	Ser	Ala	Val	Pro	Thr	Arg	Met	Val	His	
			260					265					270			
GGG	AGG	TTA	GGG	TTC	GAC	GGC	CAA	TAT	CAC	GAA	AAG	GAC	CTA	GAT	GTC	864
Gly	Arg	Leu	Gly	Phe	Asp	Gly	Gln	Tyr	His	Glu	Lys	Asp	Leu	Asp	Val	
		275					280					285				
ACA	ACA	TTA	TTC	GGG	GAC	TGG	GTG	GCC	AAC	TAC	CCA	GGA	GTA	GGG	GGT	912
Thr	Thr	Leu	Phe	Gly	Asp	Trp	Val	Ala	Asn	Tyr	Pro	Gly	Val	Gly	Gly	
		290				295					300					

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GGA Gly 305	TCT Ser	TTT Phe	ATT Ile	GAC Asp	AGC Ser	CGC Arg	GTG Val	TGG Trp	TTC Phe	TCA Ser	GTC Val	TAC Tyr	GGA Gly	GGG Gly	TTA Leu	960
AAA Lys	CCC Pro	AAT Asn	ACA Thr	CCC Pro	AGT Ser	GAC Asp	ACT Thr	GTA Val	CAG Gln	GAA Glu	GGG Gly	AAA Lys	TAT Tyr	GTG Val	ATA Ile	1008
TAC Tyr	AAG Lys	CGA Arg	TAC Tyr	AAT Asn	GAC Asp	ACA Thr	TGC Cys	CCA Pro	GAT Asp	GAG Glu	CAA Gln	GAC Asp	TAC Tyr	CAG Gln	ATT Ile	1056
CGA Arg	ATG Met	GCC Ala	AAG Lys	TCT Ser	TCG Ser	TAT Tyr	AAG Lys	CCT Pro	GGA Gly	CGG Arg	TTT Phe	GGT Gly	GGG Gly	AAA Lys	CGC Arg	1104
ATA Ile	CAG Gln	CAG Gln	GCT Ala	ATC Ile	TTA Leu	TCT Ser	ATC Ile	AAA Lys	GTG Val	TCA Ser	ACA Thr	TCC Ser	TTA Leu	GGC Gly	GAA Glu	1152
GAC Asp	CCG Pro	GTA Val	CTG Leu	ACT Thr	GTA Val	CCG Pro	CCC Pro	AAC Asn	ACA Thr	GTC Val	ACA Thr	CTC Leu	ATG Met	GGG Gly	GCC Ala	1200
GAA Glu	GGC Gly	AGA Arg	ATT Ile	CTC Leu	ACA Thr	GTA Val	GGG Gly	ACA Thr	TCC Ser	CAT His	TTC Phe	TTG Leu	TAT Tyr	CAG Gln	CGA Arg	1248
GGG Gly	TCA Ser	TCA Ser	TAC Tyr	TTC Phe	TCT Ser	CCC Pro	GCG Ala	TTA Leu	TTA Leu	TAT Tyr	CCT Pro	ATG Met	ACA Thr	GTC Val	AGC Ser	1296
AAC Asn	AAA Lys	ACA Thr	GCC Ala	ACT Thr	CTT Leu	CAT His	AGT Ser	CCT Pro	TAT Tyr	ACA Thr	TTC Phe	AAT Asn	GCC Ala	TTC Phe	ACT Thr	1344
CGG Arg	CCA Pro	GGT Gly	AGT Ser	ATC Ile	CCT Pro	TGC Cys	CAG Gln	GCT Ala	TCA Ser	GCA Ala	AGA Arg	TGC Cys	CCC Pro	AAC Asn	TCA Ser	1392
TGT Cys	GTT Val	ACT Thr	GGA Gly	GTC Val	TAT Tyr	ACA Thr	GAT Asp	CCA Pro	TAT Tyr	CCC Pro	CTA Leu	ATC Ile	TTC Phe	TAT Tyr	AGA Arg	1440
AAC Asn	CAC His	ACC Thr	TTG Leu	CGA Arg	GGG Gly	GTA Val	TTC Phe	GGG Gly	ACA Thr	ATG Met	CTT Leu	GAT Asp	GGT Gly	GAA Glu	CAA Gln	1488
GCA Ala	AGA Arg	CTT Leu	AAC Asn	CCT Pro	GCG Ala	TCT Ser	GCA Ala	GTA Val	TTC Phe	GAT Asp	AGC Ser	ACA Thr	TCC Ser	CGC Arg	AGT Ser	1536
CGC Arg	ATA Ile	ACT Thr	CGA Arg	GTG Val	AGT Ser	TCA Ser	AGC Ser	AGC Ser	ATC Ile	AAA Lys	GCA Ala	GCA Ala	TAC Tyr	ACA Thr	ACA Thr	1584
TCA Ser	ACT Thr	TGT Cys	TTT Phe	AAA Lys	GTG Val	GTC Val	AAG Lys	ACC Thr	AAT Asn	AAG Lys	ACC Thr	TAT Tyr	TGT Cys	CTC Leu	AGC Ser	1632
ATT Asn	GCT Thr	GAA Lys	ATA Ile	TCT Ser	AAT Thr	ACT Thr	CTC Leu	TTC Gly	GGA Gly	GAA Gly	TTC Gly	AGA Arg	ATC Thr	GTC Val	CCG Ala	1680

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Ile	Ala	Glu	Ile	Ser	Asn	Thr	Leu	Phe	Gly	Glu	Phe	Arg	Ile	Val	Pro	
545					550					555					560	
TTA	CTA	GTT	GAG	ATC	CTC	AAA	GAT	GAC	GGG	GTT	AGA	GAA	GCC	AGG	TCT	1728
Leu	Leu	Val	Glu	Ile	Leu	Lys	Asp	Asp	Gly	Val	Arg	Glu	Ala	Arg	Ser	
				565					570					575		
GGC	TAG															1734
Gly																

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 577 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met	Asp	Arg	Ala	Val	Ser	Gln	Val	Ala	Leu	Glu	Asn	Asp	Glu	Arg	Glu	
1				5					10					15		
Ala	Lys	Asn	Thr	Trp	Arg	Leu	Ile	Phe	Arg	Ile	Ala	Ile	Leu	Phe	Leu	
			20					25					30			
Thr	Val	Val	Thr	Leu	Ala	Ile	Ser	Val	Ala	Ser	Leu	Leu	Tyr	Ser	Met	
		35				40						45				
Gly	Ala	Ser	Thr	Pro	Ser	Asp	Leu	Val	Gly	Ile	Pro	Thr	Arg	Ile	Ser	
	50					55					60					
Arg	Ala	Glu	Glu	Lys	Ile	Thr	Ser	Thr	Leu	Gly	Ser	Asn	Gln	Asp	Val	
65				70						75					80	
Val	Asp	Arg	Ile	Tyr	Lys	Gln	Val	Ala	Leu	Glu	Ser	Pro	Leu	Ala	Leu	
			85						90					95		
Leu	Asn	Thr	Glu	Thr	Thr	Ile	Met	Asn	Ala	Ile	Thr	Ser	Leu	Ser	Tyr	
		100						105					110			
Gln	Ile	Asn	Gly	Ala	Ala	Asn	Asn	Ser	Gly	Trp	Gly	Ala	Pro	Ile	His	
		115				120						125				
Asp	Pro	Asp	Tyr	Ile	Gly	Gly	Ile	Gly	Lys	Glu	Leu	Ile	Val	Asp	Asp	
	130					135					140					
Ala	Ser	Asp	Val	Thr	Ser	Phe	Tyr	Pro	Ser	Ala	Phe	Gln	Glu	His	Leu	
145					150					155					160	
Asn	Phe	Ile	Pro	Ala	Pro	Thr	Thr	Gly	Ser	Gly	Cys	Thr	Arg	Ile	Pro	
			165					170						175		
Ser	Phe	Asp	Met	Ser	Ala	Thr	His	Tyr	Cys	Tyr	Thr	His	Asn	Val	Ile	
		180						185					190			
Leu	Ser	Gly	Cys	Arg	Asp	His	Ser	His	Ser	His	Gln	Tyr	Leu	Ala	Leu	
		195				200					205					
Gly	Val	Leu	Arg	Thr	Ser	Ala	Thr	Gly	Arg	Val	Phe	Phe	Ser	Thr	Leu	

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210					215					220					
Arg 225	Ser	Ile	Asn	Leu	Asp 230	Asp	Thr	Gln	Asn	Arg 235	Lys	Ser	Cys	Ser	Val 240
Ser	Ala	Thr	Pro	Leu 245	Gly	Cys	Asp	Met	Leu 250	Cys	Ser	Lys	Ala	Thr	Glu 255
Thr	Glu	Glu	Glu	Asp 260	Tyr	Asn	Ser	Ala 265	Val	Pro	Thr	Arg	Met	Val	His
Gly	Arg	Leu 275	Gly	Phe	Asp	Gly	Gln 280	Tyr	His	Glu	Lys	Asp 285	Leu	Asp	Val
Thr	Thr 290	Leu	Phe	Gly	Asp	Trp 295	Val	Ala	Asn	Tyr	Pro 300	Gly	Val	Gly	Gly
Gly 305	Ser	Phe	Ile	Asp	Ser 310	Arg	Val	Trp	Phe	Ser 315	Val	Tyr	Gly	Gly	Leu 320
Lys	Pro	Asn	Thr	Pro 325	Ser	Asp	Thr	Val	Gln 330	Glu	Gly	Lys	Tyr	Val	Ile 335
Tyr	Lys	Arg	Tyr 340	Asn	Asp	Thr	Cys 345	Pro	Asp	Glu	Gln	Asp	Tyr 350	Gln	Ile
Arg	Met	Ala 355	Lys	Ser	Ser	Tyr	Lys 360	Pro	Gly	Arg	Phe	Gly 365	Gly	Lys	Arg
Ile 370	Gln	Gln	Ala	Ile	Leu	Ser 375	Ile	Lys	Val	Ser	Thr 380	Ser	Leu	Gly	Glu
Asp 385	Pro	Val	Leu	Thr	Val 390	Pro	Pro	Asn	Thr	Val 395	Thr	Leu	Met	Gly	Ala 400
Glu	Gly	Arg	Ile	Leu 405	Thr	Val	Gly	Thr	Ser 410	His	Phe	Leu	Tyr	Gln	Arg 415
Gly	Ser	Ser	Tyr 420	Phe	Ser	Pro	Ala 425	Leu	Leu	Tyr	Pro	Met	Thr 430	Val	Ser
Asn	Lys	Thr 435	Ala	Thr	Leu	His	Ser 440	Pro	Tyr	Thr	Phe	Asn 445	Ala	Phe	Thr
Arg 450	Pro	Gly	Ser	Ile	Pro	Cys 455	Gln	Ala	Ser	Ala 460	Arg	Cys	Pro	Asn	Ser
Cys 465	Val	Thr	Gly	Val	Tyr 470	Thr	Asp	Pro	Tyr	Pro 475	Leu	Ile	Phe	Tyr	Arg 480
Asn	His	Thr	Leu	Arg 485	Gly	Val	Phe	Gly	Thr 490	Met	Leu	Asp	Gly	Glu 495	Gln
Ala	Arg	Leu	Asn 500	Pro	Ala	Ser	Ala 505	Val	Phe	Asp	Ser	Thr	Ser 510	Arg	Ser
Arg	Ile	Thr 515	Arg	Val	Ser	Ser	Ser 520	Ser	Ile	Lys	Ala	Ala 525	Tyr	Thr	Thr
Ser 530	Thr	Cys	Phe	Lys	Val	Val 535	Lys	Thr	Asn	Lys	Thr 540	Tyr	Cys	Leu	Ser

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Ile Ala Glu Ile Ser Asn Thr Leu Phe Gly Glu Phe Arg Ile Val Pro
545 550 555 560

Leu Leu Val Glu Ile Leu Lys Asp Asp Gly Val Arg Glu Ala Arg Ser
565 570 575

Gly

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1662 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1662

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATG	GGC	TCC	AGA	CCT	TCT	ACC	AAG	AAC	CCA	GCA	CCT	ATG	ATG	CTG	ACT	48
Met	Gly	Ser	Arg	Pro	Ser	Thr	Lys	Asn	Pro	Ala	Pro	Met	Met	Leu	Thr	
1				5					10					15		
ATC	CGG	GTC	GCG	CTG	GTA	CTG	AGT	TGC	ATC	TGT	CCG	GCA	AAC	TCC	ATT	96
Ile	Arg	Val	Ala	Leu	Val	Leu	Ser	Cys	Ile	Cys	Pro	Ala	Asn	Ser	Ile	
			20					25					30			
GAT	GGC	AGG	CCT	CTT	GCA	GCT	GCA	GGA	ATT	GTG	GTT	ACA	GGA	GAC	AAA	144
Asp	Gly	Arg	Pro	Leu	Ala	Ala	Ala	Gly	Ile	Val	Val	Thr	Gly	Asp	Lys	
		35					40					45				
GCA	GTC	AAC	ATA	TAC	ACC	TCA	TCC	CAG	ACA	GGA	TCA	ATC	ATA	GTT	AAG	192
Ala	Val	Asn	Ile	Tyr	Thr	Ser	Ser	Gln	Thr	Gly	Ser	Ile	Ile	Val	Lys	
	50					55					60					
CTC	CTC	CCG	AAT	CTG	CCA	AAG	GAT	AAG	GAG	GCA	TGT	GCG	AAA	GCC	CCC	240
Leu	Leu	Pro	Asn	Leu	Pro	Lys	Asp	Lys	Glu	Ala	Cys	Ala	Lys	Ala	Pro	
65				70					75					80		
TTG	GAT	GCA	TAC	AAC	AGG	ACA	TTG	ACC	ACT	TTG	CTC	ACC	CCC	CTT	GGT	288
Leu	Asp	Ala	Tyr	Asn	Arg	Thr	Leu	Thr	Thr	Leu	Leu	Thr	Pro	Leu	Gly	
				85					90					95		
GAC	TCT	ATC	CGT	AGG	ATA	CAA	GAG	TCT	GTG	ACT	ACA	TCT	GGA	GGG	GGG	336
Asp	Ser	Ile	Arg	Arg	Ile	Gln	Glu	Ser	Val	Thr	Thr	Ser	Gly	Gly	Gly	
			100					105					110			
AGA	CAG	GGG	CGC	CTT	ATA	GGC	GCC	ATT	ATT	GGC	GGT	GTG	GCT	CTT	GGG	384
Arg	Gln	Gly	Arg	Leu	Ile	Gly	Ala	Ile	Ile	Gly	Gly	Val	Ala	Leu	Gly	

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115						120						125						
GTT	GCA	ACT	GCC	GCA	CAA	ATA	ACA	GCG	GCC	GCA	GCT	CTG	ATA	CAA	GCC			432
Val	Ala	Thr	Ala	Ala	Gln	Ile	Thr	Ala	Ala	Ala	Ala	Leu	Ile	Gln	Ala			
	130					135					140							
AAA	CAA	AAT	GCT	GCC	AAC	ATC	CTC	CGA	CTT	AAA	GAG	AGC	ATT	GCC	GCA			480
Lys	Gln	Asn	Ala	Ala	Asn	Ile	Leu	Arg	Leu	Lys	Glu	Ser	Ile	Ala	Ala			
	145				150					155					160			
ACC	AAT	GAG	GCT	GTG	CAT	GAG	GTC	ACT	GAC	GGA	TTA	TCG	CAA	CTA	GCA			528
Thr	Asn	Glu	Ala	Val	His	Glu	Val	Thr	Asp	Gly	Leu	Ser	Gln	Leu	Ala			
				165					170						175			
GTG	GCA	GTT	GGG	AAG	ATG	CAG	CAG	TTC	GTT	AAT	GAC	CAA	TTT	AAT	AAA			576
Val	Ala	Val	Gly	Lys	Met	Gln	Gln	Phe	Val	Asn	Asp	Gln	Phe	Asn	Lys			
			180					185							190			
ACA	GCT	CAG	GAA	TTA	GAC	TGC	ATC	AAA	ATT	GCA	CAG	CAA	GTT	GGT	GTA			624
Thr	Ala	Gln	Glu	Leu	Asp	Cys	Ile	Lys	Ile	Ala	Gln	Gln	Val	Gly	Val			
		195				200						205						
GAG	CTC	AAC	CTG	TAC	CTA	ACC	GAA	TCG	ACT	ACA	GTA	TTC	GGA	CCA	CAA			672
Glu	Leu	Asn	Leu	Tyr	Leu	Thr	Glu	Ser	Thr	Thr	Val	Phe	Gly	Pro	Gln			
	210					215					220							
ATC	ACT	TCA	CCT	GCC	TTA	AAC	AAG	CTG	ACT	ATT	CAG	GCA	CTT	TAC	AAT			720
Ile	Thr	Ser	Pro	Ala	Leu	Asn	Lys	Leu	Thr	Ile	Gln	Ala	Leu	Tyr	Asn			
	225				230					235					240			
CTA	GCT	GGT	GGG	AAT	ATG	GAT	TAC	TTA	TTG	ACT	AAG	TTA	GGT	ATA	GGG			768
Leu	Ala	Gly	Gly	Asn	Met	Asp	Tyr	Leu	Leu	Thr	Lys	Leu	Gly	Ile	Gly			
				245				250							255			
AAC	AAT	CAA	CTC	AGC	TCA	TTA	ATC	GGT	AGC	GGC	TTA	ATC	ACC	GGT	AAC			816
Asn	Asn	Gln	Leu	Ser	Ser	Leu	Ile	Gly	Ser	Gly	Leu	Ile	Thr	Gly	Asn			
			260					265					270					
CCT	ATT	CTA	TAC	GAC	TCA	CAG	ACT	CAA	CTC	TTG	GGT	ATA	CAG	GTA	ACT			864
Pro	Ile	Leu	Tyr	Asp	Ser	Gln	Thr	Gln	Leu	Leu	Gly	Ile	Gln	Val	Thr			
		275				280							285					
CTA	CCT	TCA	GTC	GGG	AAC	CTA	AAT	AAT	ATG	CGT	GCC	ACC	TAC	TTG	GAA			912
Leu	Pro	Ser	Val	Gly	Asn	Leu	Asn	Asn	Met	Arg	Ala	Thr	Tyr	Leu	Glu			
	290					295				300								
ACC	TTA	TCC	GTA	AGC	ACA	ACC	AGG	GGA	TTT	GCC	TCG	GCA	CTT	GTC	CCA			960
Thr	Leu	Ser	Val	Ser	Thr	Thr	Arg	Gly	Phe	Ala	Ser	Ala	Leu	Val	Pro			
	305				310					315					320			
AAA	GTG	GTG	ACA	CGG	GTC	GGT	TCT	GTG	ATA	GAA	GAA	CTT	GAC	ACC	TCA			1008
Lys	Val	Val	Thr	Arg	Val	Gly	Ser	Val	Ile	Glu	Glu	Leu	Asp	Thr	Ser			
				325				330							335			
TAC	TGT	ATA	GAA	ACT	GAC	TTA	GAT	TTA	TAT	TGT	ACA	AGA	ATA	GTA	ACG			1056
Tyr	Cys	Ile	Glu	Thr	Asp	Leu	Asp	Leu	Tyr	Cys	Thr	Arg	Ile	Val	Thr			
			340					345							350			
TTC	CCT	ATG	TCC	CCT	GGT	ATT	TAC	TCC	TGC	TTG	AGC	GGC	AAT	ACA	TCG			1104
Phe	Pro	Met	Ser	Pro	Gly	Ile	Tyr	Ser	Cys	Leu	Ser	Gly	Asn	Thr	Ser			
		355					360						365					

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GCC	TGT	ATG	TAC	TCA	AAG	ACC	GAA	GGC	GCA	CTT	ACT	ACA	CCA	TAT	ATG	1152
Ala	Cys	Met	Tyr	Ser	Lys	Thr	Glu	Gly	Ala	Leu	Thr	Thr	Pro	Tyr	Met	
	370					375					380					
ACT	ATC	AAA	GGC	TCA	GTC	ATC	GCT	AAC	TGC	AAG	ATG	ACA	ACA	TGT	AGA	1200
Thr	Ile	Lys	Gly	Ser	Val	Ile	Ala	Asn	Cys	Lys	Met	Thr	Thr	Cys	Arg	
	385				390					395					400	
TGT	GTA	AAC	CCC	CCG	GGT	ATC	ATA	TCG	CAA	AAC	TAT	GGA	GAA	GCC	GTG	1248
Cys	Val	Asn	Pro	Pro	Gly	Ile	Ile	Ser	Gln	Asn	Tyr	Gly	Glu	Ala	Val	
				405					410					415		
TCT	CTA	ATA	GAT	AAA	CAA	TCA	TGC	AAT	GTT	TTA	TCC	TTA	GGC	GGG	ATA	1296
Ser	Leu	Ile	Asp	Lys	Gln	Ser	Cys	Asn	Val	Leu	Ser	Leu	Gly	Gly	Ile	
			420					425					430			
ACT	TTA	AGG	CTC	AGT	GGG	GAA	TTC	GAT	GTA	ACT	TAT	CAG	AAG	AAT	ATC	1344
Thr	Leu	Arg	Leu	Ser	Gly	Glu	Phe	Asp	Val	Thr	Tyr	Gln	Lys	Asn	Ile	
		435					440					445				
TCA	ATA	CAA	GAT	TCT	CAA	GTA	ATA	ATA	ACA	GGC	AAT	CTT	GAT	ATC	TCA	1392
Ser	Ile	Gln	Asp	Ser	Gln	Val	Ile	Ile	Thr	Gly	Asn	Leu	Asp	Ile	Ser	
		450				455					460					
ACT	GAG	CTT	GGG	AAT	GTC	AAC	AAC	TCG	ATC	AGT	AAT	GCC	TTG	AAT	AAG	1440
Thr	Glu	Leu	Gly	Asn	Val	Asn	Asn	Ser	Ile	Ser	Asn	Ala	Leu	Asn	Lys	
	465				470					475					480	
TTA	GAG	GAA	AGC	AAC	AGA	AAA	CTA	GAC	AAA	GTC	AAT	GTC	AAA	CTG	ACC	1488
Leu	Glu	Glu	Ser	Asn	Arg	Lys	Leu	Asp	Lys	Val	Asn	Val	Lys	Leu	Thr	
				485					490					495		
AGC	ACA	TCT	GCT	CTC	ATT	ACC	TAT	ATC	GTT	TTG	ACT	ATC	ATA	TCT	CTT	1536
Ser	Thr	Ser	Ala	Leu	Ile	Thr	Tyr	Ile	Val	Leu	Thr	Ile	Ile	Ser	Leu	
			500					505					510			
GTT	TTT	GGT	ATA	CTT	AGC	CTG	ATT	CTA	GCA	TGC	TAC	CTA	ATG	TAC	AAG	1584
Val	Phe	Gly	Ile	Leu	Ser	Leu	Ile	Leu	Ala	Cys	Tyr	Leu	Met	Tyr	Lys	
		515				520						525				
CAA	AAG	GCG	CAA	CAA	AAG	ACC	TTA	TTA	TGG	CTT	GGG	AAT	AAT	ACC	CTA	1632
Gln	Lys	Ala	Gln	Gln	Lys	Thr	Leu	Leu	Trp	Leu	Gly	Asn	Asn	Thr	Leu	
	530					535					540					
GAT	CAG	ATG	AGA	GCC	ACT	ACA	AAA	ATG	TGA							1662
Asp	Gln	Met	Arg	Ala	Thr	Thr	Lys	Met								
	545				550											

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 553 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met	Gly	Ser	Arg	Pro	Ser	Thr	Lys	Asn	Pro	Ala	Pro	Met	Met	Leu	Thr
1				5					10					15	

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Ile	Arg	Val	Ala	Leu	Val	Leu	Ser	Cys	Ile	Cys	Pro	Ala	Asn	Ser	Ile	20	25	30	
Asp	Gly	Arg	Pro	Leu	Ala	Ala	Ala	Gly	Ile	Val	Val	Thr	Gly	Asp	Lys	35	40	45	
Ala	Val	Asn	Ile	Tyr	Thr	Ser	Ser	Gln	Thr	Gly	Ser	Ile	Ile	Val	Lys	50	55	60	
Leu	Leu	Pro	Asn	Leu	Pro	Lys	Asp	Lys	Glu	Ala	Cys	Ala	Lys	Ala	Pro	65	70	75	80
Leu	Asp	Ala	Tyr	Asn	Arg	Thr	Leu	Thr	Thr	Leu	Leu	Thr	Pro	Leu	Gly	85	90	95	
Asp	Ser	Ile	Arg	Arg	Ile	Gln	Glu	Ser	Val	Thr	Thr	Ser	Gly	Gly	Gly	100	105	110	
Arg	Gln	Gly	Arg	Leu	Ile	Gly	Ala	Ile	Ile	Gly	Gly	Val	Ala	Leu	Gly	115	120	125	
Val	Ala	Thr	Ala	Ala	Gln	Ile	Thr	Ala	Ala	Ala	Ala	Leu	Ile	Gln	Ala	130	135	140	
Lys	Gln	Asn	Ala	Ala	Asn	Ile	Leu	Arg	Leu	Lys	Glu	Ser	Ile	Ala	Ala	145	150	155	160
Thr	Asn	Glu	Ala	Val	His	Glu	Val	Thr	Asp	Gly	Leu	Ser	Gln	Leu	Ala	165	170	175	
Val	Ala	Val	Gly	Lys	Met	Gln	Gln	Phe	Val	Asn	Asp	Gln	Phe	Asn	Lys	180	185	190	
Thr	Ala	Gln	Glu	Leu	Asp	Cys	Ile	Lys	Ile	Ala	Gln	Gln	Val	Gly	Val	195	200	205	
Glu	Leu	Asn	Leu	Tyr	Leu	Thr	Glu	Ser	Thr	Thr	Val	Phe	Gly	Pro	Gln	210	215	220	
Ile	Thr	Ser	Pro	Ala	Leu	Asn	Lys	Leu	Thr	Ile	Gln	Ala	Leu	Tyr	Asn	225	230	235	240
Leu	Ala	Gly	Gly	Asn	Met	Asp	Tyr	Leu	Leu	Thr	Lys	Leu	Gly	Ile	Gly	245	250	255	
Asn	Asn	Gln	Leu	Ser	Ser	Leu	Ile	Gly	Ser	Gly	Leu	Ile	Thr	Gly	Asn	260	265	270	
Pro	Ile	Leu	Tyr	Asp	Ser	Gln	Thr	Gln	Leu	Leu	Gly	Ile	Gln	Val	Thr	275	280	285	
Leu	Pro	Ser	Val	Gly	Asn	Leu	Asn	Asn	Met	Arg	Ala	Thr	Tyr	Leu	Glu	290	295	300	
Thr	Leu	Ser	Val	Ser	Thr	Thr	Arg	Gly	Phe	Ala	Ser	Ala	Leu	Val	Pro	305	310	315	320
Lys	Val	Val	Thr	Arg	Val	Gly	Ser	Val	Ile	Glu	Glu	Leu	Asp	Thr	Ser	325	330	335	
Tyr	Cys	Ile	Glu	Thr	Asp	Leu	Asp	Leu	Tyr	Cys	Thr	Arg	Ile	Val	Thr	340	345	350	

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Phe Pro Met Ser Pro Gly Ile Tyr Ser Cys Leu Ser Gly Asn Thr Ser
 355 360 365

Ala Cys Met Tyr Ser Lys Thr Glu Gly Ala Leu Thr Thr Pro Tyr Met
 370 375 380

Thr Ile Lys Gly Ser Val Ile Ala Asn Cys Lys Met Thr Thr Cys Arg
 385 390 395 400

Cys Val Asn Pro Pro Gly Ile Ile Ser Gln Asn Tyr Gly Glu Ala Val
 405 410 415

Ser Leu Ile Asp Lys Gln Ser Cys Asn Val Leu Ser Leu Gly Gly Ile
 420 425 430

Thr Leu Arg Leu Ser Gly Glu Phe Asp Val Thr Tyr Gln Lys Asn Ile
 435 440 445

Ser Ile Gln Asp Ser Gln Val Ile Ile Thr Gly Asn Leu Asp Ile Ser
 450 455 460

Thr Glu Leu Gly Asn Val Asn Asn Ser Ile Ser Asn Ala Leu Asn Lys
 465 470 475 480

Leu Glu Glu Ser Asn Arg Lys Leu Asp Lys Val Asn Val Lys Leu Thr
 485 490 495

Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu Thr Ile Ile Ser Leu
 500 505 510

Val Phe Gly Ile Leu Ser Leu Ile Leu Ala Cys Tyr Leu Met Tyr Lys
 515 520 525

Gln Lys Ala Gln Gln Lys Thr Leu Leu Trp Leu Gly Asn Asn Thr Leu
 530 535 540

Asp Gln Met Arg Ala Thr Thr Lys Met
 545 550

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2681 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 146..481

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: complement (602..1402)

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(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1599..2135

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: complement (2308..2634)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTTATCGGAC CTTGGGTATT CAGGGGAACC CATCTGGTTG AAATGCATCC GACCCTGCAC 60
TTGATCCTGG TTACCCCGAC CCAANTTTTA AGCCGGCTGG CGCGGTCCCT AGATAACCCC 120
CCGCTTAAAA CTAGCCCCAA TATTGATGTG CAGATATAAC ACAGNNANCC GATCAATGGA 180
AGACATGCTA CGGCGGTCAT CTCCCGAAGA CATCACCGAT TCCCTAACAA TGTGCCTGAT 240
TATGTTATCG CGCATTCGTC GTACCATGCG CACCGCAGGA AATAAATATA GCTATATGAT 300
AGATCCAATG AATCGTATGT CTAATTACAC TCCAGGCGAA TGTATGACAG GTATATTGCG 360
ATATATTGAC GAACATGCTA GAAGGTGTCC TGATCACATA TGTAATTTGT ATATCACATG 420
TACACTTATG CCGATGTATG TGCACGGGCG ATATTTCTAT TGTAATTCAT TTTTTTGKTA 480
GTAAACTACC ACAGGCTGTC CGGAAATCTA AGTTAATGAA TAAAGTAGAT GGTAAATACT 540
CATTGCTTAG AATTGGACTA CTTTAAATYC TCTTAAATGT TCGTATTAAA TAAAAACATC 600
TTTAATAAAC TTCAGCCTCT TCGCTTATTG TAGAAATTGA GTATTCAMAA TCATGTTCAA 660
AGCCGTCTTC GGAGAGTGTA CTCGCCACGG TGGTTGGAAC ATCACTATGT CTACACGTCA 720
AATTTAAGCA CGTCAGGTCT GTCGAGGACA AGAAATGGTT AACTAGTGTT TCAATTATTC 780
TTATAAACGT TAAGCATTGT AAGCCCCCGG GCCGTCCGCA GCAACAATTT ACTAGTATGC 840
CGTGGGCTCC GGGACTATCA CGGATGTCCA ATTCGCACAT GCATATAATT TTTCTAGGGT 900
CTCTCATTTT GAGAAATCTT CGGGGATCCA TCAGCAATGC GGGCTGTAGT CCCGATTCCC 960
GTTTCAAATG AAGGTGCTCC AACACGGTCT TCAAAGCAAC CGGCATACCA GCAAACACAG 1020
ACTGCAACTC CCCGCTGCAA TGATTGGTTA TAAACAGTAA TCTGTCTTCT GGAAGTATAT 1080
TTCGCCCCGAC AATCCACGGC GCCCCCAAAG TTAAAAACCA TCCATGTGTA TTTGCGTCTT 1140
CTCTGTTAAA AGAATATTGA CTGGCATTTC CCCGTTGACC GCCAGATATC CAAAGTACAG 1200
CACGATGTTG CACGGACGAC TTTGCAGTCA CCAGCCTTCC TTTCCACCCC CCCACCAACA 1260
AAATGTTTAT CGTAGGACCC ATATCCGTAA TAAGGATGGG TCTGGCAGCA ACCCCATAGG 1320
CGCCTCGGCG TGGTAGTTCT CGAGGATACA TCCAAAGAGG TTGAGTATTC TCTCTACACT 1380
TCTTGTTAAA TGGAAAGTGC ATTTGCTTGT TCTTACAATC GGCCCGAGTC TCGTTCACAG 1440
CGCCTCGTTC ACACTTAAAC CACAAATAGT CTACAGGCTA TATGGGAGCC AGACTGAAAC 1500
TCACATATGA CTAATATTTC GGGGTGTTAG TCACGTGTAG CCCATTGTGT GCATATAACG 1560

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ATGTTGGACG CGTCCTTATT CGCGGTGTAC TTGATACTAT GGCAGCGAGC ATGGGATATT 1620
 CATCCTCGTC ATCGTTAACA TCTCTACGGG TTCAGAATGT TTGGCATGTC GTCGATCCTT 1680
 TGCCCATCGT TGCAAATTAC AAGTCCGATC GCCATGACCG CGATAAGCCT GTACCATGTG 1740
 GCATTAGGGT GACATCTCGA TCATACATTA TAAGACCAAC GTGCGAGTCT TCCAAAGACC 1800
 TGCACGCCTT CTTCTTCGGA TTGTCAACGG GTTCTTCAGA ATCTATGCCC ATATCTGGCG 1860
 TTGAGACCAT TGTGCGTTTA ATGAACAATA AAGCGGCATG CCATGGAAAG GAGGGCTGCA 1920
 GATCTCCATT TTCTCACGCC ACTATCCTGG ACGCTGTAGA CGATAATTAT ACCATGAATA 1980
 TAGAGGGGGT ATGTTTCCAC TGCCACTGTG ATGATAAGTT TTCTCCAGAT TGTTGGATAT 2040
 CTGCATTTTC TGCTGCCGAA CAAACTTCAT CGCTATGCAA AGAGATGCGT GTGTACACGC 2100
 GCCGGTGGAG TATACGGGAA ACTAAATGTT CATAGAGGTC TTTGGGCTAT ATGTTATTAA 2160
 ATAAAATAAT TGACCAGTGA ACAATTTGTT TAATGTTAGT TTATTCAATG CATTGGTTGC 2220
 AAATATTCAT TACTTCTCCA ATCCCAGGTC ATTCTTTAGC GAGATGATGT TATGACATTG 2280
 CTGTGAAAAT TACTACAGGA TATATTTTTA AGATGCAGGA GTAACAATGT GCATAGTAGG 2340
 CGTAGTTATC GCAGACGTGC AACGCTTCGC ATTTGAGTTA CCGAAGTGCC CAACAGTGCT 2400
 GCGGTTATGG TTTATGCGCA CAGAATCCAT GCATGTCCTA ATTGAACCAT CCGATTTTTC 2460
 TTTTAATCGC GATCGATGTT TGGGCAACTG CGTTATTTCA GATCTAAAAA ATTTACCCTY 2520
 TATGACCATC ACATCTCTCT GGYTCATACC CCGCTTGGGN TAAGATATCA TGTAGATTCC 2580
 GCCCCTAAGA AATTGCAAAC TAACATNATT GNCGGGTTCC ATATACAATC CCATCTTGTC 2640
 CNCTCGAAAT TACAACTCG CGCAATAGAC CCCC GTACAT T 2681

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Cys Arg Tyr Asn Thr Xaa Xaa Arg Ser Met Glu Asp Met Leu Arg
 1 5 10 15

Arg Ser Ser Pro Glu Asp Ile Thr Asp Ser Leu Thr Met Cys Leu Ile
 20 25 30

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Met Leu Ser Arg Ile Arg Arg Thr Met Arg Thr Ala Gly Asn Lys Tyr
 35 40 45

Ser Tyr Met Ile Asp Pro Met Asn Arg Met Ser Asn Tyr Thr Pro Gly
 50 55 60

Glu Cys Met Thr Gly Ile Leu Arg Tyr Ile Asp Glu His Ala Arg Arg
 65 70 75 80

Cys Pro Asp His Ile Cys Asn Leu Tyr Ile Thr Cys Thr Leu Met Pro
 85 90 95

Met Tyr Val His Gly Arg Tyr Phe Tyr Cys Asn Ser Phe Phe Xaa
 100 105 110

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 266 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met His Phe Pro Phe Asn Lys Lys Cys Arg Glu Asn Thr Gln Pro Leu
 1 5 10 15

Trp Met Tyr Pro Arg Glu Leu Pro Arg Arg Gly Ala Tyr Gly Val Ala
 20 25 30

Ala Arg Pro Ile Leu Ile Thr Asp Met Gly Pro Thr Ile Asn Ile Leu
 35 40 45

Leu Val Gly Gly Trp Lys Gly Arg Leu Val Thr Ala Lys Ser Ser Val
 50 55 60

Gln His Arg Ala Val Leu Trp Ile Ser Gly Gly Gln Arg Glu Asn Ala
 65 70 75 80

Ser Gln Tyr Ser Phe Asn Arg Glu Asp Ala Asn Thr His Gly Trp Phe
 85 90 95

Leu Thr Leu Gly Ala Pro Trp Ile Val Gly Arg Asn Ile Leu Pro Glu
 100 105 110

Asp Arg Leu Leu Phe Ile Thr Asn His Cys Ser Gly Glu Leu Gln Ser
 115 120 125

Val Phe Ala Gly Met Pro Val Ala Leu Lys Thr Val Leu Glu His Leu
 130 135 140

His Leu Lys Arg Glu Ser Gly Leu Gln Pro Ala Leu Leu Met Asp Pro
 145 150 155 160

Arg Arg Phe Leu Glu Met Arg Asp Pro Arg Lys Ile Ile Cys Met Cys

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165					170					175					
Glu	Leu	Asp	Ile	Arg	Asp	Ser	Pro	Gly	Ala	His	Gly	Ile	Leu	Val	Asn
			180					185					190		
Cys	Cys	Cys	Gly	Arg	Pro	Gly	Gly	Leu	Gln	Cys	Leu	Thr	Phe	Ile	Arg
		195				200						205			
Ile	Ile	Glu	Thr	Leu	Val	Asn	His	Phe	Leu	Ser	Ser	Thr	Asp	Leu	Thr
	210					215					220				
Cys	Leu	Asn	Leu	Thr	Cys	Arg	His	Ser	Asp	Val	Pro	Thr	Thr	Val	Ala
225					230					235					240
Ser	Thr	Leu	Ser	Glu	Asp	Gly	Phe	Glu	His	Asp	Xaa	Glu	Tyr	Ser	Ile
				245					250					255	
Ser	Thr	Ile	Ser	Glu	Glu	Ala	Glu	Val	Tyr						
			260					265							

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 178 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met	Ala	Ala	Ser	Met	Gly	Tyr	Ser	Ser	Ser	Ser	Ser	Leu	Thr	Ser	Leu
1				5				10						15	
Arg	Val	Gln	Asn	Val	Trp	His	Val	Val	Asp	Pro	Leu	Pro	Ile	Val	Ala
			20					25					30		
Asn	Tyr	Lys	Ser	Asp	Arg	His	Asp	Arg	Asp	Lys	Pro	Val	Pro	Cys	Gly
		35					40					45			
Ile	Arg	Val	Thr	Ser	Arg	Ser	Tyr	Ile	Ile	Arg	Pro	Thr	Cys	Glu	Ser
	50					55					60				
Ser	Lys	Asp	Leu	His	Ala	Phe	Phe	Phe	Gly	Leu	Ser	Thr	Gly	Ser	Ser
65					70					75				80	
Glu	Ser	Met	Pro	Ile	Ser	Gly	Val	Glu	Thr	Ile	Val	Arg	Leu	Met	Asn
			85					90					95		
Asn	Lys	Ala	Ala	Cys	His	Gly	Lys	Glu	Gly	Cys	Arg	Ser	Pro	Phe	Ser
		100						105					110		
His	Ala	Thr	Ile	Leu	Asp	Ala	Val	Asp	Asp	Asn	Tyr	Thr	Met	Asn	Ile
		115					120					125			
Glu	Gly	Val	Cys	Phe	His	Cys	His	Cys	Asp	Asp	Lys	Phe	Ser	Pro	Asp
	130					135					140				
Cys	Trp	Ile	Ser	Ala	Phe	Ser	Ala	Ala	Glu	Gln	Thr	Ser	Ser	Leu	Cys

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145		150		155		160									
Lys	Glu	Met	Arg	Val	Tyr	Thr	Arg	Arg	Trp	Ser	Ile	Arg	Glu	Thr	Lys
				165					170					175	
Cys	Ser														

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 108 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met	Gly	Leu	Tyr	Met	Glu	Pro	Xaa	Asn	Xaa	Val	Ser	Leu	Gln	Phe	Leu
1				5					10					15	
Arg	Gly	Gly	Ile	Tyr	Met	Ile	Ser	Xaa	Pro	Lys	Arg	Gly	Met	Xaa	Gln
			20					25					30		
Arg	Asp	Val	Met	Val	Ile	Xaa	Gly	Lys	Phe	Phe	Arg	Ser	Glu	Ile	Thr
		35					40					45			
Gln	Leu	Pro	Lys	His	Arg	Ser	Arg	Leu	Lys	Glu	Lys	Ser	Asp	Gly	Ser
	50					55					60				
Ile	Arg	Thr	Cys	Met	Asp	Ser	Val	Arg	Ile	Asn	His	Asn	Arg	Ser	Thr
65					70					75				80	
Val	Gly	His	Phe	Gly	Asn	Ser	Asn	Ala	Lys	Arg	Cys	Thr	Ser	Ala	Ile
				85					90					95	
Thr	Thr	Pro	Thr	Met	His	Ile	Val	Thr	Pro	Ala	Ser				
				100				105							

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTCGGCGTGG TAGTTCTCGA GGCCTTAATT AAGGCCCTCG AGGATACATC CAAAGAG 57

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGGCGTGGTA GTTCTCGAGG CCTTAAGCGG CCGCTTAAGG CCCTCGAGGA TACATCCAAA 60
GAG 63

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGCAGGATCC GGGGCGTCAG AGGCGGGCG AGGTG 35

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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GAGCGGATCC TGCAGGAGGA GACACAGAG CTG

33

(2) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TG TAGAGATC TGGCTAAGTG CGCGTGTTG CCTG

34

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGTACAGATC TCACCATGGC TGTGCCTGC AAGC

34

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 387 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GAATTCCGAG TGGTTACTAT TCCATCACCA TTCTAGCCTG TACACAGAAA GTCAAGATGG 60

ACGAATCGCT CGACTTCGCT CGCGATTCGT CGAAGGCGGG GGGCCGGAGG CCCCCCGGTG 120

GCCCCCCTCC AACGAGTGGA GCACGTACAG GGGGGTACGT CATCCGTACA GGGGGGTACG 180

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TCATCCGTAC AGGGGGGTAC GTCACAAAGA GGC GTTCCCG TACAGGGGGG TACGTCACGC 240
GTACAGGGGG GTACGTCACA GCCAATCAAA AGCTGCCACG TTGCGAAAGT GACGTTTCGA 300
AAATGGGCGG CGCAAGCCTC TCTATATATT GAGCGCACAT ACCGGTCGGC AGTAGGTATA 360
CGCAAGGCGG TCCGGGAGGA TGGATCC 387

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATCGAATTCC GAGTGGTTAC TATTCC 26

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGTGGATCCA TCTTACAGTC TTATAC 26

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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GTTCGGATCC ATCCTCCCGG ACCGCCTTG

29

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCGGAAGAGC GCCAATACG

19

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTTCGGATCC ATCCACCCG GACCGCCTTG

30

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGACAGATC TCACCATGGC TGTGCCTGCA AGC

33

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGCGAATTCG GCTAAGTGC GCGTGTTG

28

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 594 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..594
- (D) OTHER INFORMATION: /product= "Quail Interferon type 1"

ATG	GCT	GTG	CCT	GCA	AGC	CCA	CAG	CAC	CCA	CGG	GGG	TAC	GGC	ATC	CTG	CTC	51
M	A	V	P	A	S	P	Q	H	P	R	G	Y	G	I	L	L	17
CTC	ACG	CTC	CTT	CTG	AAA	GCT	CTC	GCC	ACC	ACC	GCC	ACC	GCC	TCC	GCC	TGC	102
L	T	L	L	L	K	A	L	A	T	T	A	T	A	S	A	C	34
AGC	CAC	CTT	CGC	CCC	CAC	GAC	GCC	ACC	TTC	TCT	CGC	GAC	AGC	CTC	CAG	CTC	153
S	H	L	R	P	H	D	A	T	F	S	R	D	S	L	Q	L	51
CTA	GGG	GAC	ATG	GCT	CCC	AGC	CCA	CCC	CAG	CTG	TGC	CCA	CAG	CAC	AGC	GCG	204
L	G	D	M	A	P	S	P	P	Q	L	C	P	Q	H	S	A	68
TCG	CCT	TGC	TCC	TTC	AAC	GAC	ACC	ATC	CTG	GAC	ACC	AGC	AAC	ATC	TGG	CAA	255
S	P	C	S	F	N	D	T	I	L	D	T	S	N	I	W	Q	85
ACT	GAC	AAA	ACC	ACC	CAC	GAC	ATT	CTT	CAG	GAC	CTC	TTC	AGT	ATC	CTC	AGC	306
T	D	K	T	T	H	D	I	L	Q	D	L	F	S	I	L	S	102
GGA	CCA	AGC	ACT	CCA	CCC	CAC	TGG	ATC	GAA	AGC	CAA	CGC	CAA	AGC	CTC	CTC	357
G	P	S	T	P	P	H	W	I	E	S	Q	R	Q	S	L	L	119
AGC	CAC	ATC	CAG	CGC	TAC	ACC	CAG	CAC	CTC	GAG	CAG	TGC	CTG	GAA	AAA	AAC	408
S	H	I	Q	R	Y	T	Q	H	L	E	Q	C	L	E	K	N	136
AGC	GAC	ACG	CGC	TCC	CGG	ACA	CGA	CGG	CCT	CGA	AAC	CTT	CAC	CTC	ACC	ATC	459
S	D	T	R	S	R	T	R	R	P	R	N	L	H	L	T	I	153

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AGC	AAA	CAC	TTC	AGC	TGC	CTC	CGC	ACC	TTC	CTC	AGC	GAT	AAC	GAC	TAC	AGC	510	
S	K	H	F	S	C	L	R	T	F	L	S	D	N	D	Y	S	170	
GAC	TGC	GCC	TGG	GAC	CTC	GTC	CTC	CTG	CAA	GCT	CGT	GAA	TGG	TTC	CGG	CGC	561	
D	C	A	W	D	L	V	L	L	Q	A	R	E	W	F	R	R	187	
ATC	AAC	AAC	CTC	ACA	GGC	AAC	ACG	CGC	ACT	TAG								594
I	N	N	L	T	G	N	T	R	T									197

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 197 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

M	A	V	P	A	S	P	Q	H	P	R	G	Y	G	I	L	L	17
L	T	L	L	L	K	A	L	A	T	T	A	T	A	S	A	C	34
S	H	L	R	P	H	D	A	T	F	S	R	D	S	L	Q	L	51
L	G	D	M	A	P	S	P	P	Q	L	C	P	Q	H	S	A	68
S	P	C	S	F	N	D	T	I	L	D	T	S	N	I	W	Q	85
T	D	K	T	T	H	D	I	L	Q	D	L	F	S	I	L	S	102
G	P	S	T	P	P	H	W	I	E	S	Q	R	Q	S	L	L	119
S	H	I	Q	R	Y	T	Q	H	L	E	Q	C	L	E	K	N	136
S	D	T	R	S	R	T	R	R	P	R	N	L	H	L	T	I	153
S	K	H	F	S	C	L	R	T	F	L	S	D	N	D	Y	S	170
D	C	A	W	D	L	V	L	L	Q	A	R	E	W	F	R	R	187
I	N	N	L	T	G	N	T	R	T								197

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What is claimed is:

1. A recombinant herpesvirus of turkeys -Marek's disease virus chimera comprising a herpesvirus of turkeys unique long viral genome region and a Marek's disease virus unique short viral genome region.
2. The recombinant herpesvirus of turkeys- Marek's disease virus chimera of claim 1, wherein a foreign DNA sequence is inserted within a non-essential region of the herpesvirus of turkeys - Marek's disease virus chimera viral genome, and is capable of being expressed in a host
3. The recombinant herpesvirus of turkeys- Marek's disease virus chimera of claim 2, wherein the foreign DNA sequence is inserted within an EcoR1 #9 fragment of the unique long region of the herpesvirus of turkeys - Marek's disease virus chimera viral genome.
4. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 2, wherein the foreign DNA sequence encodes a polypeptide.
5. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 2, wherein the foreign DNA sequence encodes a cytokine.
6. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 5, wherein the cytokine is a chicken myelomonocytic growth factor (CMGF), chicken interferon (cIFN) or quail

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interferon Type I (qIFN).

7. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 2, wherein the foreign DNA sequence encodes an antigenic polypeptide selected from the group consisting of: Marek's disease virus, Newcastle disease virus, Infectious laryngotracheitis virus, Infectious bronchitis virus and Infectious bursal disease virus.
8. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 2, wherein the polypeptide is *E. coli* beta-galactosidase.
9. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 7, wherein the antigenic polypeptide is Marek's disease virus glycoprotein A (gA), Marek's disease virus glycoprotein B (gB), or Marek's disease virus glycoprotein D (gD).
10. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 7, wherein the antigenic polypeptide is Newcastle disease virus fusion protein or Newcastle disease virus hemagglutinin-neuraminidase.
11. The recombinant herpesvirus of turkeys - Marek's disease virus chimera of claim 7, wherein the antigenic polypeptide is infectious laryngotracheitis virus glycoprotein B (gB), infectious laryngotracheitis virus glycoprotein I (gI), or glycoprotein D (gD).

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12. The recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 7, wherein the antigenic polypeptide is infectious bronchitis virus spike protein or infectious bronchitis virus matrix protein.
13. The recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 7, wherein the antigenic polypeptide is infectious bursal disease virus VP2, infectious bursal disease virus VP3 or infectious bursal disease virus VP4.
14. The recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 7, wherein the antigenic polypeptide is selected form the group consisting of: avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus (agent), *Salmonella* spp., *E. coli*, *Pasteurella* spp., *Bordetella* spp., *Eimeria* spp., *Histomonas* spp., *Trichomonas* spp., Poultry nematodes, cestodes, trematodes, and poultry mites/lice, poultry protozoa.
15. The recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 2, wherein the foreign DNA sequence is under control of an endogenous upstream herpesvirus promoter.
16. The recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 2, wherein the foreign DNA sequence is under control of a heterologous upstream promoter.

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17. The recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 16 wherein the promoter is selected from the group consisting of: chicken anemia virus promoter, psuedorabies virus gX promoter, herpes simplex virus-1 alpha 4 promoter, human cytomegalovirus immediate early promoter, Marek's disease virus gA promoter, gB promoter, Marek's disease virus gD promoter, infectious laryngotracheitis gB promoter, bovine herpesvirus-1.1 VP8 promoter and infectious laryngotracheitis gD promoter.
18. A recombinant herpesvirus of turkeys - Marek's disease virus chimera designated S-HVY-149.
19. A recombinant herpesvirus of turkeys - Marek's disease virus chimera designated S-HVY-152.
20. A vaccine which comprises an effective immunizing amount of the recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 2 and a suitable carrier.
21. A multivalent vaccine which comprises an effective immunizing amount of the recombinant herpesvirus of turkeys-Marek's disease virus chimera of claim 2 and a suitable carrier.
22. A method of immunizing a bird against an avian pathogen which comprises administering to the bird an effecting immunizing dose of the vaccine of claim 20.

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FIGURE 1A
BamHI fragments

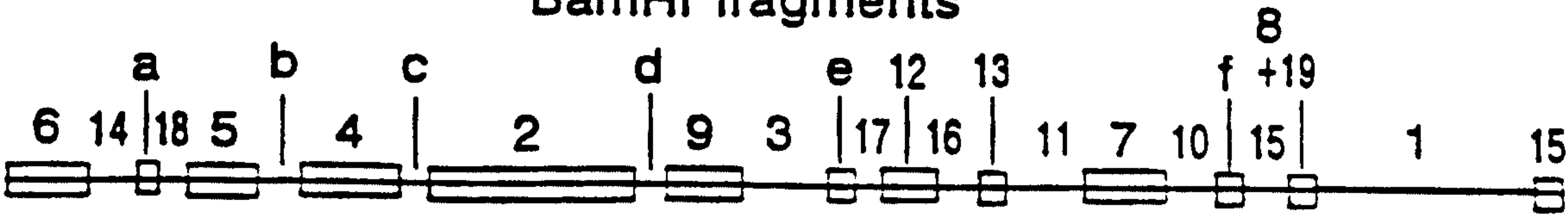


FIGURE 1B
BamHI #16

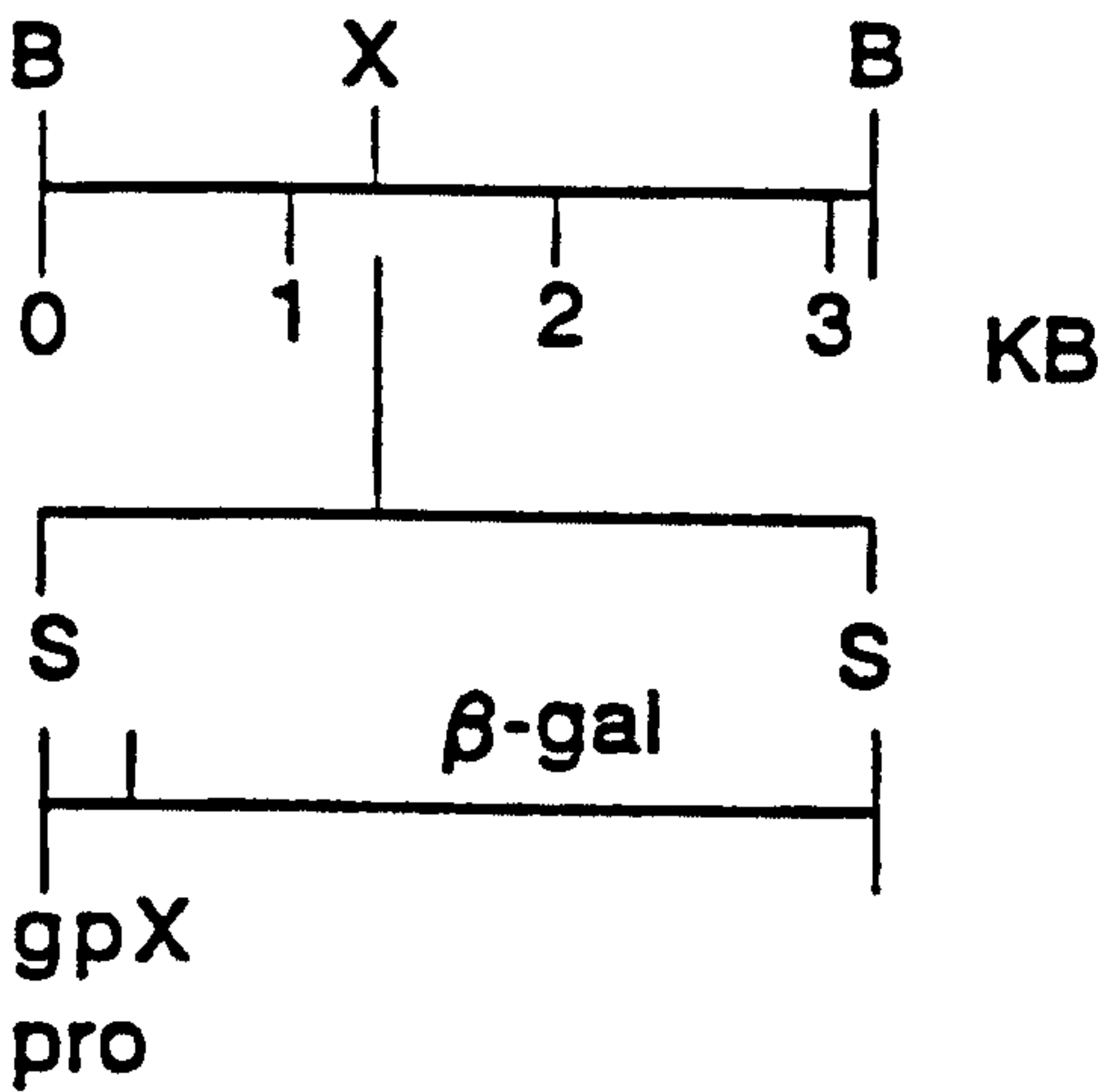


FIGURE 1C

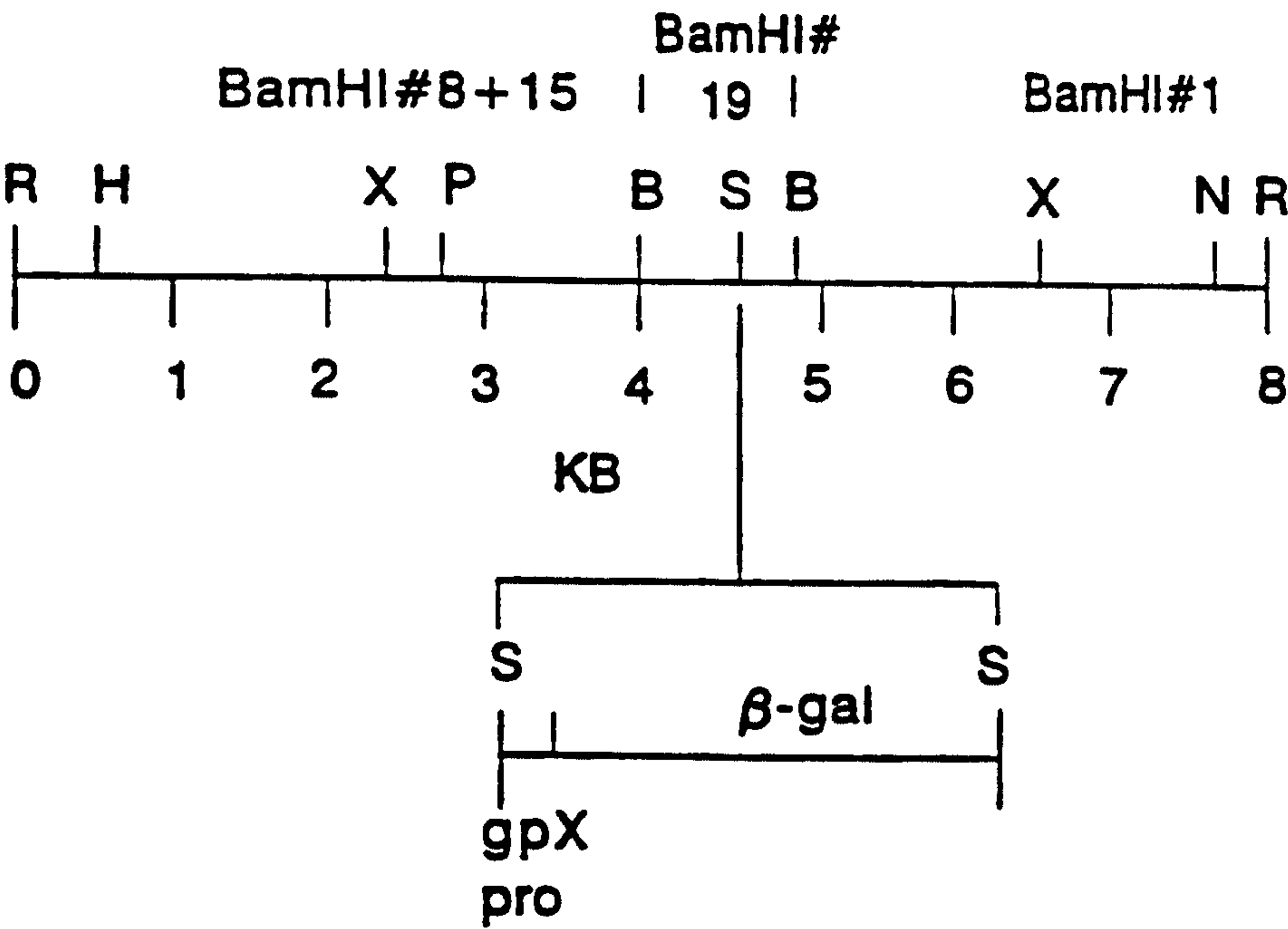


FIGURE 2

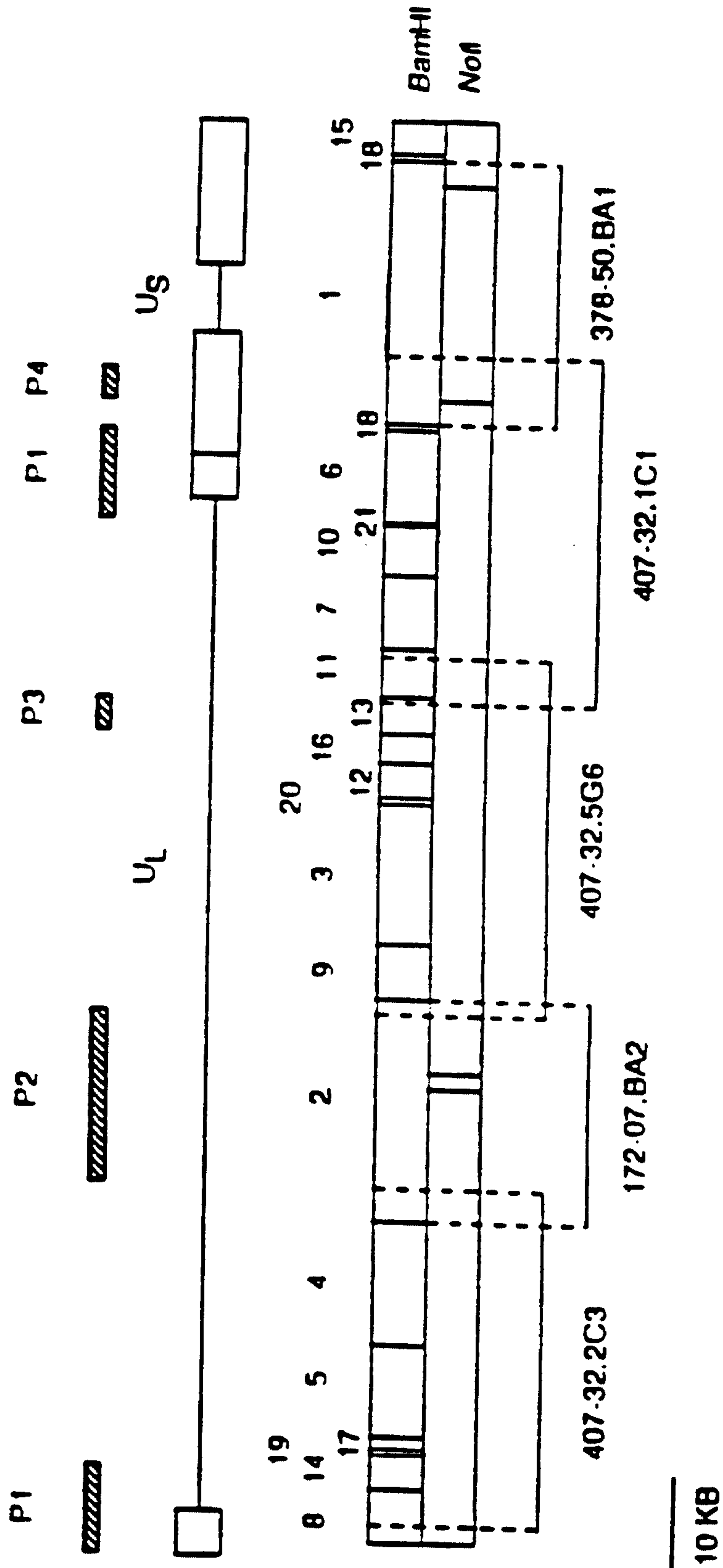


FIGURE 3A

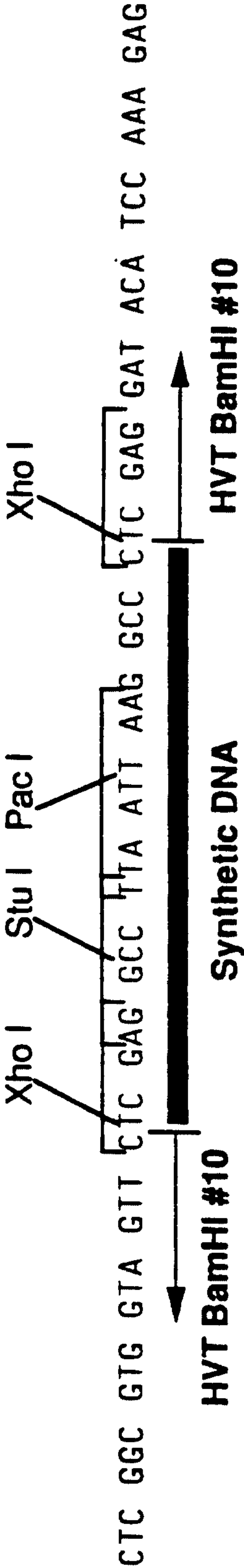


FIGURE 3B

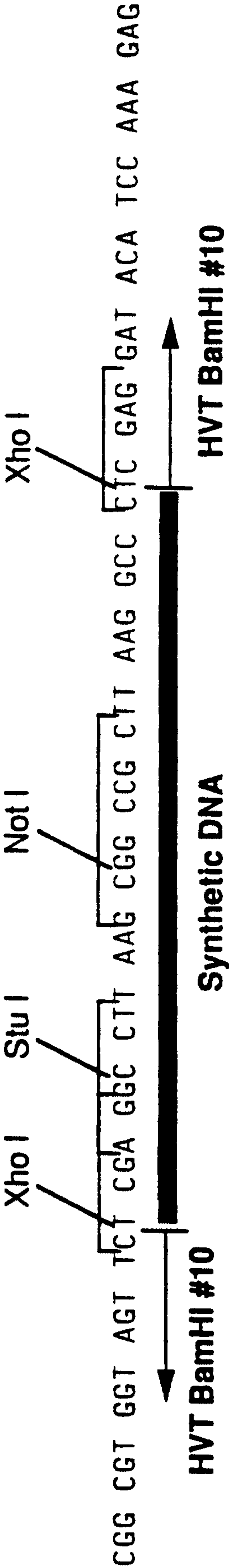


FIGURE 4

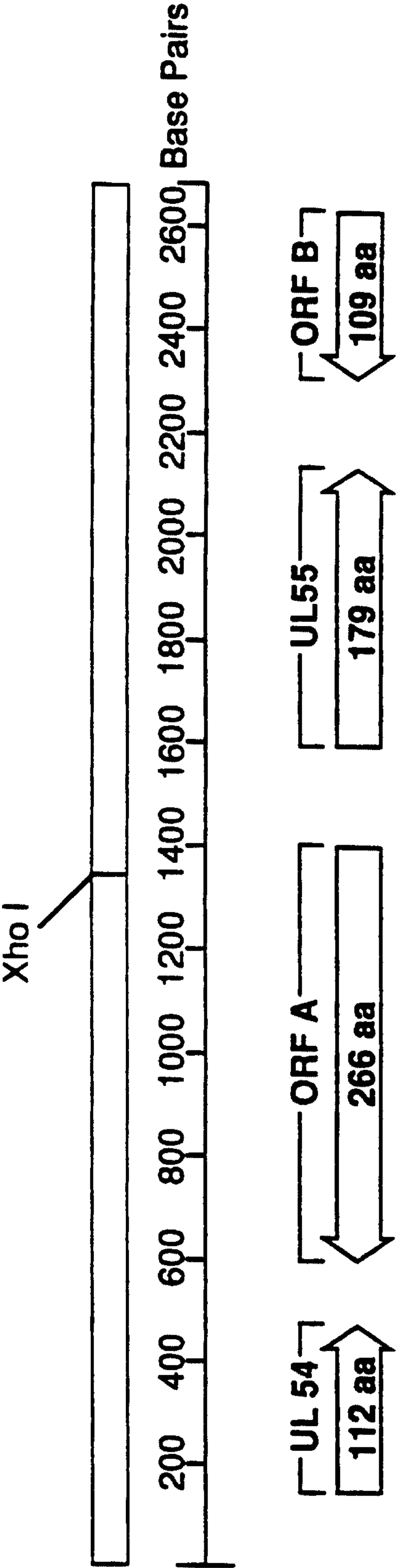
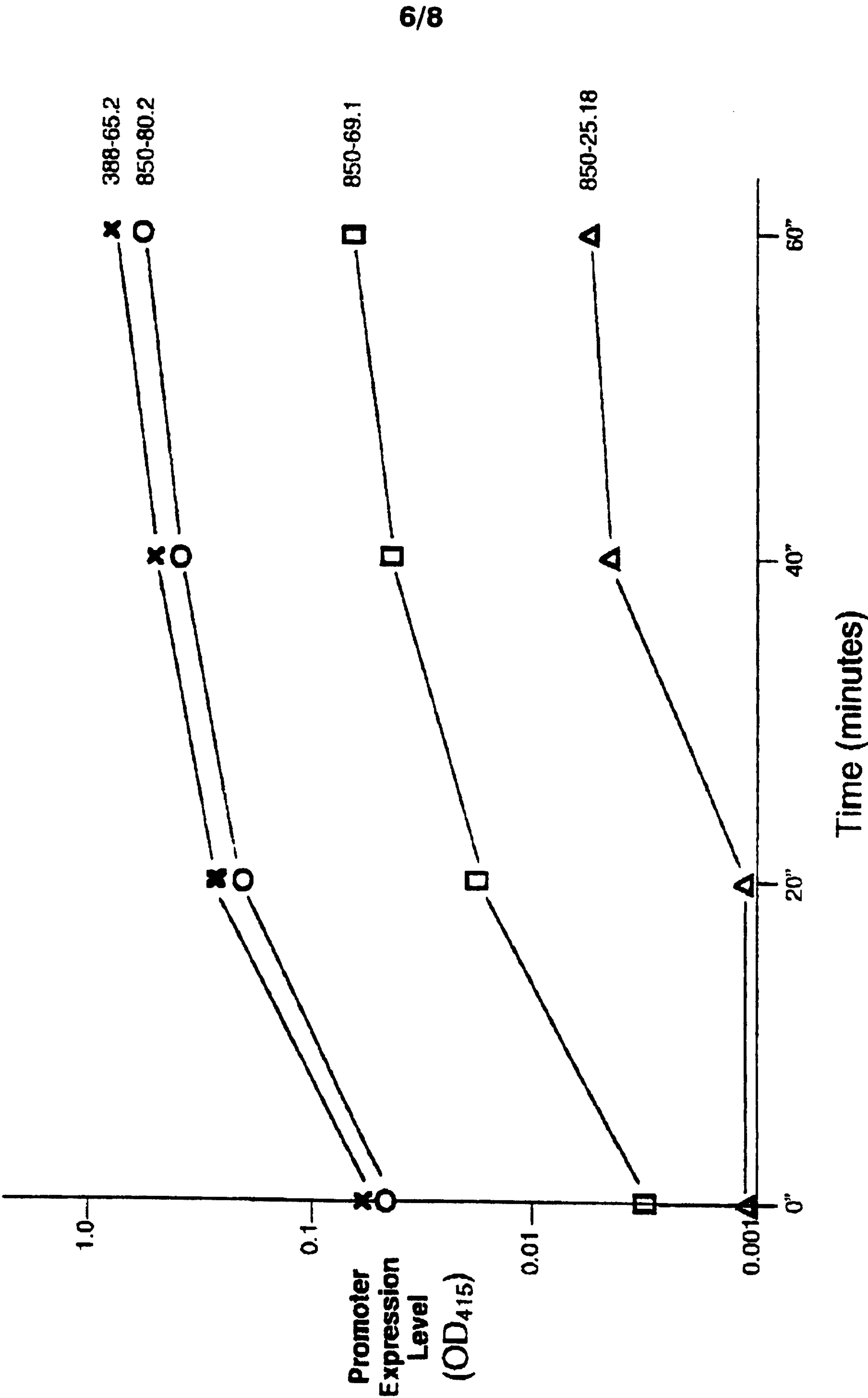


FIGURE 6



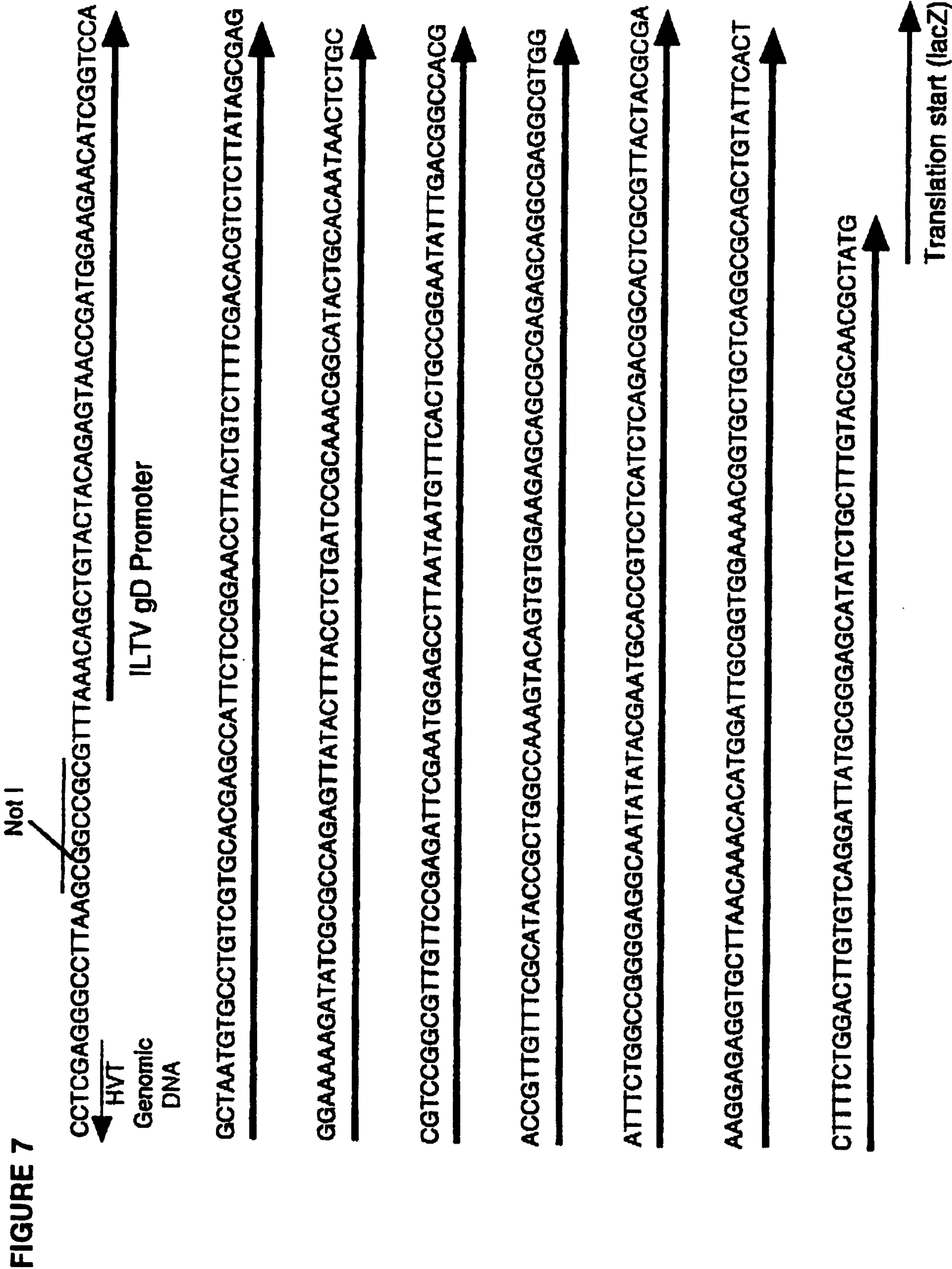


FIGURE 8

