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(54) Title: RECOMBINANT FOWLPOX VIRUSES AND USES THEREOF

(57) Abstract

This invention provides a recombinant fowlpox virus comprising a foreign DNA sequence inserted into the fowlpox virus genomic DNA, wherein the foreign DNA sequence is inserted within a non-essential region of the fowlpox virus genomic DNA and is capable of being expressed in a fowlpox virus infected host cell. The invention further provides homology vectors, vaccines and methods of immunization.

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RECOMBINANT FOWLPOX VIRUSES AND USES THEREOF

Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

The present invention relates to recombinant fowlpox virus useful in live vaccine to protect fowl against Newcastle disease virus and fowlpox virus.

The ability to isolate DNA and clone this isolated DNA into bacterial plasmids has greatly expanded the approaches available to make viral vaccines. The method used to make the present invention involve modifying cloned DNA sequences by insertions, deletions and single or multiple base changes. The modified DNA is then inserted into a viral genome, and the resulting 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.

Fowlpox virus (FPV) is a member of the poxviridiae family of viruses. There are two subfamilies in this classification, and they are differentiated based upon

the host range (vertebrate or invertebrate) of the virus. Among the vertebrate poxviruses, there is serological cross reactivity to group specific antigens that has aided in classification of the viruses into six genera, and FPV has been placed in the avipoxvirus genera along with seven additional poxviruses that primarily infect birds. In general, poxviruses are the largest of the animal viruses and can be visualized with the light microscope. Under the electron microscope, the virus takes on a biscuit like or oval shaped appearance. The principal chemical components of the poxviruses are protein (90% by weight), deoxyribonucleic acid (DNA) (3%) and lipid (5%), but in FPV the lipid component is -1/3 of the dry weight. Polyacrylamide gel electrophoresis (PAGE) of solubilized virions indicates that there are >100 different proteins associated with the viruses that include: structural polypeptides, enzymes associated with translation of messenger ribonucleic acid (mRNA), enzymes involved in RNA synthesis, and enzymes associated with DNA replication. The genome of poxviruses consists double-stranded DNA that varies in base composition (32% G+C to 64% G+C) and length (140 kilobasepairs [kb] to 280 kb for FPV) depending upon individual virus. The complete nucleotide sequence of the vaccina virus (VV) genome has recently been determined, and most of the essential genes have been found to lie within the highly conserved middle region of the genome while nonessential functions seem to map nearer to the termini of the DNA. The poxviruses are unique in their propensity to replicate within the cytoplasmic space of the infected cell, and in the case of VV, mature virus particles are moved out of the assembly areas and into the periphery of the cell where additional membrane encapsulation occurs. With FPV, the assembled viral particles become associated with a dense viral-derived protein matrix that occludes the virus in the form of cellular inclusions that may help protect the virion from lytic activities. Depending

upon the specific poxvirus and strain (from 1% to 30% of different mature VV strains) varying levels of mature virus can be found extracellularly, but the majority of the virus population remains associated with the cell at the end of the growth cycle.

Fowlpox is unique throughout the world, but because its host-range is limited to birds it is not considered to be a public health hazard. All chickens can be infected by the virus with a resulting decline in the growth rate of the bird and temporary decreases in egg production. Usually, transmission of FPV occurs through physical contact of injured skin, but there are reports that the virus is also transmitted via arthropod vectors. After an incubation period of four to ten days, the disease is typically manifested in the following ways: skin lesions in non-feathered areas, lesions of the nasal passages, and lesions of the mouth. A normal FPV infection usually lasts three to four weeks, and afterward the bird is conferred life-long immunity to the disease.

Currently, conventionally derived FPV vaccines are being used in commercial settings to provide protection to chickens and turkeys. Typically, the vaccine viruses are attenuated by serial passage in cell culture selecting for strains that have altered growth and/or virulence properties. The modified live vaccine is prepared by growth in vitro in chicken embryo fibroblast cells or by growth on the chorioallantoic membrane of the chicken embryo. The vaccine virus is given to birds subcutaneously.

The present invention concerns the use of FPV as a vector for the delivery of specific vaccine antigens to poultry. The idea of using live viruses as delivery systems for antigens (vectoring) has a long history that is associated with introduction of the first live viral

vaccines. The antigens that were delivered were not foreign but were naturally expressed by the live virus in the vaccine. The use of viruses to deliver foreign antigens in the modern sense became obvious with the recombinant DNA studies. The vaccinia virus was the vector and various antigens from other disease causing pathogens were the foreign antigens, and the vaccine was created by genetic engineering. While the concept became obvious with these disclosures, what was not obvious were the answers to more practical questions concerning what makes the best candidate viral vector and what constitutes the best foreign gene or gene to deliver. In answering these questions, details of the pathogenicity, site of replication or growth, the kind of elicited immune response, expression levels for the virus and foreign gene of interest (GOI), its suitability for genetic engineering, its probability of being licensed by regulatory agencies, etc. are all factors in the configuration. The prior art does not teach these questions of utility.

The presently preferred method for creating recombinant poxviruses uses a plasmid of bacterial origin that contains at least one cassette consisting of a poxvirus promoter followed by the gene of interest. The cassette(s) is flanked by poxvirus genomic DNA sequences that direct the gene of interest to the corresponding homologous nonessential region of the viral genome by homologous recombination. Cells are initially infected with the wild-type virus, and shortly thereafter the plasmid DNA is introduced into the infected cells. Since poxviruses have their own RNA polymerase and transcriptional apparatus, it is necessary that the gene of interest be regulated by a promoter of poxvirus origin. There are three characteristic poxvirus promoters that are differentiated based upon their temporal regulation of gene expression relative to the

infective cycle of the virus: early, intermediate and late expression. Each promoter type can be identified by a typical consensus sequence that is ~30 bp in length and specific to each promoter type. In vaccinia virus, some viral genes are regulated by tandem early/late promoters that can be used by the virus to continually express the downstream gene throughout the infective cycle.

It is generally agreed that poxviruses contain non-essential regions of DNA in various parts of the genome, and that modifications of these regions can either attenuate the virus, leading to a non-pathogenic strain from which a vaccine may be derived, or give rise to genomic instabilities that yield mixed populations of virus. The degree of attenuation of the virus is important to the utility of the virus as a vaccine. Insertions or deletions which cause too much attenuation or genetic deletions which cause too much attenuation or genetic instability of the virus will result in a vaccine that fails to elicit an adequate immune response. Although several examples of deletions/insertions are known for poxviruses, the appropriate configuration is not readily apparent.

Thus far, gene expression from foreign genes of interest have been inserted into the genome of poxviruses has been obtained for five different pox viruses: vaccinia, canary pox, pigeon pox, raccoon pox and fowlpox. Vaccinia virus is the classically studied poxvirus, and it has been used extensively to vector foreign genes of interest; it is the subject of U.S. Patents 4,603,112 and 4,722,848. Raccoon pox (Esposito, et al., 1988) and Canary pox (Taylor, et al., 1991) have bene used to express antigens from the rabies virus. More recently, FPV has been used to vector a number of different foreign gene of interest, and is the subject of patent applications (EPA 0 284 416, PCT WO 89/03429, PCT WO 89/12684, PCT WO 91/02072, PCT WO

89/03879, PCT etc.). However, these publications do not teach the vectored antigen configuration, the FPV insertion sites, or the promoter sequences and the arrangement of the present invention.

A foreign gene of interest targeted for insertion into the genome of FPV can be obtained from any pathogenic organism of interest. Typically, the gene of interest will be derived from pathogens that cause diseases in poultry that have an economic impact on the poultry industry. The genes can be derived from organisms for which there are existing vaccines, and because of the novel advantages of the vectoring technology the FPV derived vaccines will be superior. Also, the gene of interest may be derived from pathogens for which thee 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.

One relevant avian pathogen that is a target for FPV vectoring in the present invention is Infectious Laryngotracheitis virus (ILT). ILT is a member of the herpesviridiae 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 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 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 FPV vectoring approach is Newcastle disease, an infectious, highly contagious and debilitating disease that is caused by the Newcastle disease virus (NDV), a single-stranded RNA virus of the paramyxovirus family. The various pathotypes of NDV (velongic, 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 interference, life-span of the bird and route of administration, the producer needs to adapt immunization protocols to fit specific needs.

Marek's disease of poultry is a lymphoproliferative tumor producing disease of poultry that primarily affects the peripheral nervous system and other visceral tissues and organs. Marek's disease exists in poultry producing countries throughout the world, and is an additional target described by the present invention for a FPV-based vectored vaccine. The causative agent of Marek's disease is a cell associated gammaherpesvirus that has been designated as Marek's disease virus (MDV). Three classes of viruses have been developed as conventional vaccines for protecting chickens against Marek's disease: attenuated serotype 1 MDV, herpesvirus of turkeys (HVT), and naturally avirulent serotype 2 isolates of MDV. Protection obtained with these vaccines is principally directed toward the tumorigenic aspect of the disease. The occurrence of excessive Marek's disease losses in such conventionally vaccinated flocks has led to the requirement for forming admixtures of the various vaccine

types. Such polyvalent vaccines while generally ore effective in disease control, complicate the vaccine regime.

SUMMARY OF THE INVENTION

This invention provides a recombinant fowlpox virus comprising a foreign DNA sequence inserted into the fowlpox virus genomic DNA, wherein the foreign DNA sequence is inserted within a non-essential region of the fowlpox virus genomic DNA and is capable of being expressed in a fowlpox virus infected host cell.

The invention further provides homology vectors, vaccines and methods of immunization.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C:

Detailed description of the *Sfi*I fragment insert in Homology Vector 502-26.22. The diagram shows the orientation of DNA fragments assembled in the cassette. The origin of each fragment is described in the Materials and Methods section. The sequences located at the junctions between each fragment and at the ends of the marker gene are shown, including junction A (SEQ ID NO: 15), junction B (SEQ ID NO: 16), junction C (SEQ ID NO: 17), and junction D (SEQ ID NO: 18). The restriction sites used to generate each fragment are indicated at the appropriate junction. The location of the NDV F and HN genes is shown. Numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction.

Figures 2A-2D:

Detailed description of fowlpox virus S-FPV-099 and S-FPV-101 and the DNA insertion in Homology Vector 751-07.D1. Diagram showing the orientation of DNA fragments assembled in plasmid 751-07.D1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 2A-2D show the sequences located at Junction A (SEQ ID NO:), (SEQ ID NO:), C (SEQ ID NO:), D (SEQ ID NO:) and E (SEQ ID NO:) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The

location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: fowlpox virus (FPV), chicken interferon (cIFN), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic late promoter 1 (LP1), base pairs (BP), polymerase chain reaction (PCR).

Figures 3A-3D:

Detailed description of fowlpox virus S-FPV-100 and the DNA insertion in Homology Vector 751-56.C1. Diagram showing the orientation of DNA fragments assembled in plasmid 751-56.C1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 3A-3D show the sequences located at Junction A (SEQ ID NOS:), (SEQ ID NO:), C (SEQ ID NO:), D (SEQ ID NO:) and E (SEQ ID NO:) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: fowlpox virus (FPV), chicken myelomoncytic growth factor (cMGF), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic late promoter 1 (LP1), base pairs

(BP), polymerase chain reaction (PCR).

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a recombinant fowlpox virus comprising a foreign DNA sequence inserted into the fowlpox virus genomic DNA, wherein the foreign DNA sequence is inserted within a 2.8 kB EcoRI fragment of the fowlpox virus genomic DNA and is capable of being expressed in a fowlpox virus infected host cell.

In one embodiment the foreign DNA sequence is inserted within a SnaBI restriction endonuclease site within the approximately 2.8 kB EcoRI fragment of the fowlpox virus genomic DNA.

This invention provides a recombinant fowlpox virus comprising a foreign DNA sequence inserted into the fowlpox virus genomic DNA, wherein the foreign DNA sequence is inserted within a 3.5 kB EcoRI fragment of the fowlpox virus genomic DNA and is capable of being expressed in a fowlpox virus infected host cell.

In one embodiment the recombinant fowlpox virus the foreign DNA sequence is inserted within a HpaI restriction endonuclease site within the approximately 3.5 kB EcoRI fragment of the fowlpox virus genomic DNA.

The present invention provides a recombinant fowlpox virus comprising a foreign DNA sequence inserted into the fowlpox virus genomic DNA, wherein the foreign DNA sequence is inserted within a 4.2 kB EcoRI fragment of the fowlpox virus genomic DNA and is capable of being expressed in a fowlpox virus infected host cell.

In one embodiment of the recombinant fowlpox virus foreign DNA sequence is inserted within a MluI restriction endonuclease site within the approximately 4.2 kB EcoRI fragment of the fowlpox virus genomic DNA.

The invention provides a recombinant fowlpox virus comprising a foreign DNA sequence inserted into the fowlpox virus genomic DNA, wherein the foreign DNA sequence is inserted within a non-essential region of the fowlpox virus genomic DNA and is capable of being expressed in a fowlpox virus infected host cell.

In one embodiment this invention provides a recombinant fowlpox virus wherein the foreign DNA sequence is inserted into an open reading frame within the non-essential region the fowlpox virus genomic DNA.

For purposes of this invention, "a recombinant fowlpox virus capable of replication" is a live fowlpox virus which has been generated by the recombinant methods well known to those of skill in the art, e.g., the methods set forth in HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV in Materials and Methods and has not had genetic material essential for the replication of the recombinant fowlpox virus deleted.

The invention further provides a foreign DNA sequence or foreign RNA which encodes a polypeptide. Preferably, the polypeptide is antigenic in the animal. Preferably, this antigenic polypeptide is a linear polymer of more than 10 amino acids linked by peptide bonds which stimulates the animal to produce antibodies.

The invention further provides a recombinant fowlpox virus capable of replication which contains a foreign DNA encoding a polypeptide which is a detectable marker. Preferably the detectable marker is the polypeptide *E. coli β-galactosidase* or *E. coli beta-glucuronidase*.

In one embodiment of the recombinant fowlpox virus the foreign DNA sequence encodes a cytokine. In another

embodiment the cytokine is chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). Cytokines include, but are not limited to: transforming growth factor beta, epidermal growth factor family, fibroblast growth factors, hepatocyte growth factor, insulin-like 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, c-kit ligand, leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, and soluble TNF receptors. These cytokines are from humans, bovine, equine, feline, canine, porcine or avian.

This invention provides a recombinant fowlpox virus further comprising a newcastle disease virus hemagglutinin (NDV HN), or a newcastle disease virus fusion (NDV F).

Antigenic polypeptide of a human pathogen which are derived from human herpesvirus include, but are not limited to: hepatitis B virus and hepatitis C virus 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, Diptheria, *Rickettsia prowazekii*, *Borrelia berfdorferi*, Tetanus toxoid, malignant tumor antigens.

The antigenic polypeptide of an equine pathogen can derived from equine influenza virus, or equine herpesvirus. In one embodiment the antigenic polypeptide is equine influenza neuraminidase or hemagglutinin. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine influenza virus type A/Kentucky 92 neuraminidase equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, *Streptococcus equi*, equine infectious anemia virus, equine encephalitis virus, equine rhinovirus and equine rotavirus.

The present invention further provides an antigenic polypeptide which includes, but is not limited to: hog cholera virus gE1, hog cholera virus gE2, swine influenza virus hemagglutinin, neuromanidase, matrix and nucleoprotein, pseudorabies virus gB, gC and gD, and PRRS virus ORF7.

For example, the antigenic polypeptide of derived from infectious bovine rhinotracheitis virus gE, 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), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase

The present invention provides a recombinant fowlpox virus wherein the foreign DNA sequence encodes an antigenic polypeptide which is derived or derivable from a group consisting of: feline immunodeficiency virus gag, feline immunodeficiency virus env, infectious laryngotracheitis virus glycoprotein B, infectious laryngotracheitis virus gI, infectious laryngotracheitis virus gD, infectious bovine rhinotracheitis virus glycoprotein G, infectious bovine rhinotracheitis virus glycoprotein E, pseudorabies virus glycoprotein 50, pseudorabies virus II glycoprotein B, pseudorabies virus III glycoprotein C, pseudorabies virus glycoprotein E, pseudorabies virus glycoprotein H, marek's disease virus glycoprotein A, marek's disease virus glycoprotein B, marek's disease virus glycoprotein D, newcastle disease virus hemagglutinin or neuraminadase, newcastle disease virus fusion, infectious bursal disease virus VP2, infectious bursal disease virus VP3, infectious bursal disease virus VP4, infectious bursal disease virus polyprotein, infectious bronchitis virus spike, infectious bronchitis virus matrix, and chick anemia virus.

The present invention provides a recombinant fowlpox virus wherein the foreign DNA sequence is under control of a promoter. In one embodiment the foreign DNA sequence is under control of an endogenous upstream poxvirus promoter. In another embodiment the foreign DNA sequence is under control of a heterologous upstream promoter. In another embodiment the promoter is selected from a group consisting of: synthetic pox viral promoter, pox synthetic late promoter 1, pox synthetic late promoter 2 early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, pox E10R promoter, HCMV immediate early, BHV-1.1 VP8, marek's disease virus glycoprotein A, marek's disease virus glycoprotein B, marek's disease virus glycoprotein D,

laryngotracheitis virus glycoprotein I, infectious laryngotracheitis virus glycoprotein B, and infectious laryngotracheitis virus gD.

The present invention also provides a recombinant fowlpox virus designated S-FPV-097. The S-FPV-097 has been deposited on February 25, 1994 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. VR 2446.

The present invention also provides a vaccine which comprises an effective immunizing amount of the recombinant virus designated S-FPV-097 and a suitable carrier. The vaccine may contain either inactivated or live fowlpox virus S-FPV-097, although live virus is presently preferred. The present invention also provides a method of immunizing an animal, particularly poultry, against disease caused by fowlpox virus, Newcastle disease virus and infectious laryngotracheitis virus. This 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, intraperitoneal, intravenous or intradermal injection. Alternatively, the vaccine may be administered intranasally, orally, or ocularly.

The present invention also provides a recombinant fowlpox virus designated S-FPV-095. The present invention also provides a vaccine which comprises an effective immunizing amount of the recombinant virus designated S-FPV-095 and a suitable carrier. The vaccine may contain either inactivated or live fowlpox virus S-FPV-095, although live virus is presently preferred. The present

invention also provides a method of immunizing an animal, particularly poultry, against disease caused by fowlpox virus, Newcastle disease virus and infectious laryngotracheitis virus. This 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, intraperitoneal, intravenous or intradermal injection. Alternatively, the vaccine may be administered intranasally, orally, or ocularly.

The present invention also provides a recombinant fowlpox virus designated S-FPV-074. The present invention also provides a vaccine which comprises an effective immunizing amount of the recombinant virus designated S-FPV-074 and a suitable carrier. The vaccine may contain either inactivated or live fowlpox virus S-FPV-074, although live virus is presently preferred. The present invention also provides a method of immunizing an animal, particularly poultry, against disease caused by fowlpox virus and Newcastle disease virus. This 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, intraperitoneal, intravenous or intradermal injection. Alternatively, the vaccine may be administered intranasally, orally, or ocularly.

The present invention also provides a recombinant fowlpox virus designated S-FPV-081. The present invention also provides a vaccine which comprises an effective immunizing amount of the recombinant virus designated S-FPV-081 and a suitable carrier. The vaccine may contain either inactivated or live fowlpox virus S-FPV-081, although live virus is presently preferred. The present

invention also provides a method of immunizing an animal, particularly poultry, against disease caused by fowlpox virus and Marek's disease virus. This 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, intraperitoneal, intravenous or intradermal injection. Alternatively, the vaccine may be administered intranasally, orally, or ocularly.

The present invention also provides a recombinant fowlpox virus designated S-FPV-085. The present invention also provides a vaccine which comprises an effective immunizing amount of the recombinant virus designated S-FPV-085 and a suitable carrier. The vaccine may contain either inactivated or live fowlpox virus S-FPV-085, although live virus is presently preferred. The present invention also provides a method of immunizing an animal, particularly poultry, against disease caused by fowlpox virus, Newcastle disease virus, infectious laryngotracheitis virus and Marek's disease virus. This 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, intraperitoneal, intravenous or intradermal injection. Alternatively, the vaccine may be administered intranasally, orally, or ocularly.

The present invention also provides a recombinant fowlpox virus designated S-FPV-082, S-FPV-083, S-FPV-099, S-FPV-100, and S-FPV-101.

Suitable carriers for use with the recombinant fowlpox virus vaccines of the present invention are those well

known in the art and include proteins, sugars, etc. One example of such a suitable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc.

An "effective immunizing amount" of the recombinant viruses of the present invention is an amount within the range of 10^2-10^9 PFU/dose. Preferably, the effective immunizing amount is from about 10^3-10^5 PFU/dose for the live virus vaccine. Preferable, the live vaccine is created by taking tissue culture fluids and adding stabilizing agents such as stabilized, hydrolyzed proteins.

MATERIAL AND METHODS

PREPARATION OF FOWLPOX VIRUS STOCK SAMPLES. Fowlpox virus samples were prepared by infecting chicken embryo fibroblast (CEF) cells at a multiplicity of infection of 0.01 PFU/cell in a 1:1 mixture of HAM's F10 medium and Medium 199 (F10/199) containing 2 mM glutamine and antibiotics (referred to as CEF negative medium). Prior to infection, the cell monolayers were washed once with CEF negative medium to remove fetal bovine serum. The FPV contained in the initial inoculum (0.5 ml for 10 cm plate; 10 ml for T175 cm flask) was allowed to absorb onto the cell monolayer for two hours, being redistributed every half hour. After this period, the original inoculum was brought up to an appropriate final volume by the addition of complete CEF medium (CEF negative medium plus 2% fetal bovine serum). The plates were incubated at 37°C in 5% CO_2 until cytopathic effect was complete. The medium and cells were harvested, frozen at -70°C, thawed and dispensed into 1.0 ml vials and refrozen at -70°C. Virus titers typically range between 10^8 and 10^7 PFU/ml.

PREPARATION OF FPV DNA. For fowlpox virus DNA isolation, a confluent monolayer of CEF cells in a T175 cm^2 flask was infected at a multiplicity of 0.1 and incubated 4-6 days until the cells were showing 100% cytopathic effect. The infected cells were harvested by scraping into the medium and centrifuging at 3000 rpm for 5 minutes in a clinical centrifuge. The medium was decanted, and the cell pellet was gently resuspended in 1.0 ml PBS (per T175) and subjected to two successive freeze-thaws (-70°C to 37°C). After the last thaw, the cells (on ice) were sonicated two times for 30 seconds each with 45 seconds cooling time in between. Cellular debris was removed by centrifuging (Sorvall RC-5B Superspeed Centrifuge) at 3000 rpm for 5 minutes in an HB4 rotor at 4°C. FPV

virions, present in the supernatant, were pelleted by centrifugation at 15,000 rpm for 20 minutes at 4°C in a SS34 rotor (Sorvall) and resuspended in 10mM Tris (pH 7.5). This fraction was then layered onto a 36% sucrose gradient (w/v in 10 mM Tris pH 7.5) and centrifuged (Beckman L8-70M Ultracentrifuge) at 18,000 rpm for 60 minutes in a SW41 rotor at 4°C. The virion pellet was resuspended in 1.0 ml of 10 mM Tris pH 7.5 and sonicated on ice for 30 seconds. This fraction was layered onto a 20% to 50% continuous sucrose gradient and centrifuged at 16,000 rpm for 60 minutes in a SW41 rotor at 4°C. The FPV virion band located about three quarters down the gradient was harvested, diluted with 20% sucrose and pelleted by centrifugation at 18,000 rpm for 60 minutes in a SW41 rotor at 4°C. The resultant pellet was then washed once with 10 mM Tris pH 7.5 to remove traces of sucrose and finally resuspended in 10mM Tris pH 7.5. FPV DNA was then extracted from the purified virions by lysis (four hours at 60°C) following the addition of EDTA, SDS, and proteinase K to final concentrations of 20 mM, 0.5% and 0.5 mf/ml, respectively. After digestion, three phenol-chloroform (1:1) extractions were conducted and the sample precipitated by the addition of two volumes of absolute ethanol and incubated at -20°C for 30 minutes. The sample was then centrifuged in an Eppendorf minifuge for five minutes at full speed. The supernatant was decanted, and the pellet air dried and rehydrated in 0.01 M Tris pH 7.5, 1mM EDTA at 4°C.

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). Except as noted, these were used with minor variation.

DNA SEQUENCING. Sequencing was performed using the BRL Sequenase Kit and ^{35}S-dATP (NEN). Reactions using both the dGTP mixes and the dITP 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.

STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS. For recombinant fowlpox vectors synthetic pox promoters offer several advantages including the ability to control the strength and timing of foreign gene expression. We chose to design four promoter cassettes EP1 (SEQ ID NO:8, LP1 (SEQ ID NO:9), EP2 (SEQ ID NO:10), and LP2 (SEQ ID NO:11) based on promoters that have been defined in the vaccinia virus (Bertholet *et al.* 1986, Davidson and Moss, 1989a, and Davidson and Moss, 1989b). Each cassette was designed to contain the DNA sequences defined in vaccina flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were also designed into each cassette such that inframe fusions could be made at either *Eco*RI or *Bam*Hi sites. A set of translational stop codons in all three reading frames and an early transcriptional termination signal (Earl, *et al.*, 1990) was also engineered downstream of the inframe fusion site. DNA encoding each cassette was synthesized according to standard techniques and cloned into the appropriate homology vectors.

cDNA CLONING PROCEDURE. cDNA cloning refers to the methods 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 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 forming units per cell. When cytopathic effect was evident, 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, 8mls 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 a 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. Then 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 adsorption 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 of total RNA was boiled and chilled and applied to a 100 mg oligo-dT cellulose column in binding buffer (0.1 M Tris pH 7.5, 0.5 M LiCl, 5 mM EDTA pH 8.0, 0.1% lithium dodecyl sulfate). The retained poly-A+ RNA was eluted from the column with elution buffer (5 mM Tris pH 7.5, 1 mM 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 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 reaction mixture for first strand cDNA synthesis in 0.25 ml contained 1 μg oligo-dT primer (P-L Biochemicals) or 1 μg synthetic primer, 28 units placental ribonuclease inhibitor (Bethesda Research Labs #5518SA), 100 mM Tris pH 8.3, 140 mM KCl, 10 mM MgCl2, 0.8 mM dATP, dCTP, dGTP, and dTTP (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 20 mM EDTA pH 8.0. The sample was extracted with an equal 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 (100 mM Tris pH 7.5, 1 mM EDTA pH 8.0, 100 mM NaCl). The leading edge of the eluted DNA fractions were 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.

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 (Boerhinger Mannheim #642-711), and 100 units/ml *E. coli* DNA 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 a 1% agarose gel (Sigma Type II agarose) in 40 mM Tris-acetate buffer pH 6.85. The gel was strained 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, 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 pH 7.2, 0.2 mM dithiothreitol, 2 mM $CaCl_2$, 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 10 mM EDTA, and the sample was phenol/chloroform extracted and precipitated as 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, 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 μg/ml tetracycline. Colonies were screened for the presence of inserts into the ampicillin gene using Ampscreen\ (Bethesda Research Labs #5537 UA), and the positive colonies were picked for analysis.

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. This method relies upon the homologous recombination between FPV DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both FPV 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, Gainsville, Georgia) at a multiplicity of infection of 0.01 PFU/cell to introduce replicating FPV (i.e. DNA synthesis) 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

horseradish peroxidase conjugated secondary antibody was diluted with PBS and incubated on the cell monolayer for two hours at room temperature. Unbound secondary antibody was then removed by washing the cells three times with PBS at room temperature. The cells were incubated 15-30 minutes at room temperature with freshly prepared substrate solution (100 μg/ml 4-chloro-1-naphthol, 0.003% H_2O_2 in PBS). Plaques expressing the correct antigen stain black.

SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. When the *E. coli* β-galactosidase (lacZ) or β-glucuronidase (uidA) marker gene was incorporated into a recombinant virus the plaques containing recombinants were visualized by a simple assay. The enzymatic substrate was incorporated (300 μg/ml) into the agarose overlay during the plaque assay. For the *lac*Z marker gene the substrates Bluogal™ (halogenated indolyl-β-D-galactosidase, Bethesda Research Labs) for blue plaques or CPRG (chlorophenol Red Galactopyranoside, Boehringer mannheim) for red plaques were used. For the *uid*A marker gene the substrate X-Glucuro Chx (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid Cyclohexylammonium salt, Biosynth AG) was used. Plaques that expressed active marker enzyme turned either red or blue. The plaques were then picked onto fresh cells and purified by further plaque isolation.

RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. Chicken spleens were dissected from 3 week old SPAFAS hatched chicks, washed, and disrupted through a syringe/needle to release cells. After allowing stroma and debri 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 splenocytes were recovered and washed twice with PBS. Splenocytes were resuspended at 5 x 106 cells/ml in RPMI

containing 5% FBS and 5 μg/ml Concanavalin A and incubated at 39o 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). 4μg 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).

HOMOLOGY VECTOR 451-79.95. The plasmid 451-79.95 was constructed for the purpose of inserting the NDV HN gene into FPV. A *lac*Z marker gene followed by the NDV HN gene was inserted as a cassette into the homology vector 443-88.14 at the unique *Sfi*I site. The cassette may be 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 indicated. The first fragment is the synthetic late promoter LP1 (SEQ ID NO:9). The second fragment contains the coding region of *E. coli lac*Z and is derived from plasmid pJF751 (Ferrari *et al.*, 1985). Note that the promoter and *lac*Z gene are fused so as to express a hybrid protein consisting of 4 amino acids derived from the synthetic promoter followed by amino acids 10 to 1024 of the *lac*Z gene. The third fragment is another copy of the synthetic late promoter LP1. the fourth fragment contains the coding region of the NDV HN gene and was derived from the full length HN cDNA clone. Note that the promoter and HN gene are fused so as to express a hybrid protein consisting of 4 amino acids derived from the synthetic promoter followed by amino acids 2 to 577 of the HN gene. Both genes are in the opposite transcriptional orientation relative to the

negative medium and incubated at 37°C in a humidified 5% CO_2 incubator for five hours. The transfection procedure used is essentially that recommended for Lipofectin™ Reagent (BRL). Briefly, for each 6 cm plate, 15 micrograms of plasmid DNA were diluted up to 100 microliters with H_2O. Separately, 50 micrograms of Lipofectin™ Reagent were diluted to 100 microliters with H_2O. The 100 microliters of diluted Lipofectin™ Reagent were added dropwise to the diluted 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 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% CO_2 incubator, the medium was removed and replaced with 5 ml CEF complete medium. The cells were incubated at 37°C in 5% CO_2 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 recombinant virus by the "Plaque Hybridization Procedure For Purifying Recombinant FPV".

PLAQUE HYBRIDIZATION PROCEDURE FOR PURIFYING RECOMBINANT FPV. CEF cell monolayers were infected with various dilutions of the infection/transfection viral stocks, overlaid with nutrient agarose media (equal volumes of 1.2%-1.4% agarose and 2X M199) and incubated 6-7 days for plaque development to occur. The agarose overlay and plate were marked with the same three asymmetrical dots (India ink) to aid in positioning the Nitrocellulose (NC) membrane (cell monolayer) and agarose overlay. The agarose overlay was transferred to the lid of the 10 cm

dish and stored at 4°C. The CEF monolayer was overlaid with a pre-wetted (PBS) NC membrane and pressure applied to transfer the monolayer to the NC membrane. Cells contained on the NC membrane were then lysed by placing the membranes in 1.5 ml of 1.5 M NaCl and 0.5 M NaOH for five minutes. The membranes were placed in 1.5 ml of 3 M sodium acetate (pH 5.2) for five minutes. DNA from the lysed cells was bound to the NC membrane by baking at 80°C for one hour. After this period the membranes were prehybridized with a solution containing 6X SSC, 3% skim milk, 0.5% SDS, salmon sperm DNA (50 μg/ml) and incubated at 65°C for one hour. Radio-labeled probe DNA (alpha^{32}P-dCTP) was added and incubated at 65°C overnight (12 hours). After hybridization the NC membranes were 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 were dried and exposed to X-ray film (Kodak X-OMAT, AR) at -70°C for 12 hours. Plaques corresponding to positive signals seen on the autoradiogram were picked from the agarose overlay, using a pasteur pipette, and were resuspended into 1 ml of CEF media and stored at -70°C. Typically, 5-6 rounds of plaque purification were required to ensure purity of the recombinant virus.

SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV USING BLACK PLAQUE ASSAYS. To analyze expression of foreign antigens expressed by recombinant fowlpox viruses, monolayers of CEF cells were infected with recombinant FPV, overlaid with nutrient agarose media and incubated for 6-7 days at 37°C for plaque development to occur. The agarose overlay was removed from the dish, the cells fixed with 100% methanol for 10 minutes at room temperature and air dried. The primary antibody was diluted to an appropriate concentration with PBS and incubated on the cell monolayer for two hours at room temperature. Unbound antibody was removed from the cells by washing three times with PBS at room temperature. A

ORF1 gene in the parental homology vector.

HOMOLOGY VECTOR 489-21.1. The plasmid 489-21.1 was constructed for the purpose of inserting the NDV HN gene into FPV. The NDV HN gene was inserted as a cassette into the homology vector 443-88.8 at the unique *Sfi*I site. The cassette may be 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 indicated. The first fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO:8/SEQ ID NO:11). The second fragment contains the coding region of the NDV HN gene and was derived from the full length HN cDNA clone. Note that the promoter and HN gene are fused so as to express a hybrid protein consisting of 4 amino acids derived from the synthetic promoter followed by amino acids 2 to 577 of the HN gene. The HN gene is in the opposite transcriptional orientation relative to the ORF in the parental homology vector.

HOMOLOGY VECTORS 502-26.22. The plasmid 502-26.22 was constructed for the purpose of inserting the NDV HN and F genes into FPV. The NDV HN and F genes were inserted as a *Sfi*I fragment (SEQ ID NO:12) into the homology vector 443-88.8 at the unique *Sfi*I site. The NDV HN and F genes were inserted in the same transcriptional orientation as the ORF in the parental homology vector. A detailed description of the *Sfi*I is shown in Figures 1A-1C. The inserted *Sfi*I fragment may be constructed utilizing standard recombinant DNA techniques (Maniatis *et al.* and Sambrook *et al.*, 1989), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 1A-1C. Fragment 1 is approximately 1811 base pair *Ava*II to *Nae*I restriction fragment of the full length NDV HN cDNA clone (B1 strain). Fragment 2 is an approximately 1812 base pair

*Bam*HI to *Pst*I restriction fragment of the full length NDV F cDNA (B1 strain). Fragment 3 is an approximately 235 base pair *Pst*I and *Sca*I restriction fragment of the plasmid pBR322.

HOMOLOGY VECTOR 502-27.5. The plasmid 502-27.5 was constructed for the purpose of inserting the NDV F gene into FPV. A *Lac*Z marker gene followed by the NDV F gene was inserted as a cassette into the homology vector 443-88.14 at the unique *Sfi*I site. The cassette may be constructed utilizing standard recombinant DNA techniques (Maniatis *et al.*, 1982 and Sambrook *et al.*, 1989), joining restriction fragments from the following sources with the synthetic DNA sequences indicated. The first fragment is the synthetic late promoter LP1 (SEQ ID NO:9). The second fragment contains the coding region of *E. coli Lac*Z and is derived from plasmid pJF751 (Ferrari *et al.*, 1985). Note that the promoter and *Lac*Z gene are fused so as to express a hybrid protein consisting of 4 amino acids derived from the synthetic promoter followed by amino acids 10 to 1024 of the *Lac*Z gene. The third fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO:8/SEQ ID NO:11). The fourth fragment contains the coding region of the NDV F gene and was derived from the full length F cDNA clone. Note that the promoter and F gene are fused so as to express a hybrid protein consisting of 4 amino acids dervied from the synthetic promoter followed by 10 amino acids derivied from the F gene 5' untranslated region followed by amino acid 1 to 544 of the F gene. Both genes are in the opposite transcriptional orientation relative to the ORF in the parental homology vector.

HOMOLOGY VECTOR 586-36.6. The plasmid 586-36.6 was constructed for the purpose of inserting the infectious laryngotracheitis virus (ILT) gB and gD genes into the FPV. An *E. coli* ß-glucuronidase *uid*A marker gene

preceeded by the ILT gB and gD genes was inserted as a cassette into the homology vector 451-08.22 at the unique *Sfi*I site. The cassette may be 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 indicated. The first fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO:8/SEQ ID NO:11). The second fragment contains the coding region of ILT gB and is dervied from an approximately 3000 base pair ILT virus genomic *Eco*RI fragment. Note that the promoter and gB gene are fused so as to express the complete coding region of the gB gene (amino acids 1-883). The third fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO:8/SEQ ID NO:11). The fourth fragment contains the coding region of the ILT gD gene (SEQ ID NO:19) and was derived from an approximately 2060 base pair *Eco*RI to *Bcl*I restriction sub-fragment of the ILT *Kpn*I genomic restriction fragment #8 (10.6 KB). Note that the promoter and gD gene are fused so as to express a hybrid protein consisting of 3 amino acids dervied from the synthetic promoter followed by amino acids 3 to 434 of the gD gene. The fifth fragment is the synthetic late promoter LP1 (SEQ ID NO:9). The last fragment contains the coding region of *E. coli uid*A and is derived from plasmid pRAJ260 (Clonetech). Note that the promoter and *uid*A gene are fused so as to express a hybrid protein consisting of 3 amino acids derived from the synthetic promoter followed by amino acids 1 to 602 of the *uid*A gene. All three genes are in the opposite transcriptional orientation relative to ORF1 in the parental homology vector.

HOMOLOGY VECTOR 608-10.3. The plasmid 608-10.3 was constructed for the purpose of inserting the Marek's Disease virus (MDV) gD and gB genes into FPV. A *LacZ* marker gene preceeded by the MDV gD and gB genes was

inserted as a cassette into the homology vector 443-88.14 at the unique *Sfi*I site. The cassette may be 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 indicated. The first fragment is the synthetic late/early promoter LP2EP2 (SEQ ID NO:11/SEQ ID NO:10). The second fragment contains the coding region of MDV gD and is derived from an approximately 2177 base pair *Nco*I to *Sal*I sub-fragment of the MDV *Bgl*II 4.2 KB genomic restriction fragment (Ross, *et al.*, 1991). Note that the promoter and gD are fused so as to express a hybrid protein consisting of 3 amino acids derived from the synthetic promoter followed by amino acids 3 to 403 of the gD gene. The third fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO:8/SEQ ID NO:11). The fourth fragment contains the coding region of the MDV gB gene and was derived from an approximately 3898 base pair *Sal*I to *Eco*RI genomic MDV fragment (Ross, *et al.*, 1989). Note that the promoter and gB gene are fused so as to express a hybrid protein consisting of 3 amino acids derived from the synthetic promoter followed by amino acids 3 to 865 of the gB gene. The fifth fragment is the synthetic late promoter LP1 (SEQ ID NO:9). The sixth fragment contains the coding region of *E. coli LacZ* and is derived from plasmid pJF751 (Ferrari, *et al.*, 1985). Note that the promoter and *LacZ* gene are fused so as to express a hybrid protein consisting of 4 amino acids derived from the synthetic promoter followed by amino acids 10 to 1024 of the *LacZ* gene. All three genes are in the opposite transcriptional orientation relative to ORF1 in the parental homology vector.

HOMOLOGY VECTOR 538-51.27. The plasmid 538-51.27 was constructed for the purpose of inserting the genes for Infectious Bronchitis virus (IBV) Massachusetts Spike

protein (Mass Spike) and Massachusetts Matrix protein (Mass Matrix) into FPV. A lacZ marker gene and the genes for IBV Mass Spike and Mass Matrix were inserted as a cassette into the homology vector 443-88.14 at the unique *SfiI* site. The inserted *SfiI* fragment is constructed utilizing standard recombinant DNA techniques (Maniatis et al., 1982 and Sambrook et al., 1989), by joining restriction fragments from the following sources. The first fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO: 8/ SEQ ID NO: 11). The second fragment contains the coding region for the IBV Mass Spike gene and (amino acids 3-1162) is derived from an approximately 3500 base pair BsmI to PvuI IBV cDNA fragment. The third fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO: 8/ SEQ ID NO: 11). The fourth fragment contains the coding region for the IBV Mass Matrix gene (amino acids 1-232) and is derived from an approximately 1500 base pair XbaI to SpeI IBV cDNA fragment. The fifth fragment is the synthetic late promoter LP1 (SEQ ID NO: 9). The sixth fragment contains the coding region of E. coli lacZ and is derived from plasmid pJF751 (Ferrari, et al. 1985).

HOMOLOGY VECTOR 622-49.1. The plasmid 622-49.1 was constructed for the purpose of inserting the IBV Massachusetts (Mass) Nucleocapsid gene into FPV. A uidA marker gene and the IBV Mass Nucleocapsid gene was inserted as a cassette into the homology vector 451-08.22 at the unique *SfiI* site. The inserted *SfiI* fragment was constructed utilizing standard recombinant DNA techniques (Maniatis et al., 1982 and Sambrook et al., 1989), by joining restriction fragments from the following sources. The first fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO: 8/ SEQ ID NO: 11). The second fragment contains the coding region for the IBV Mass Nucleocapsid gene and is derived from an approximately 3800 base pair PstI to IBV cDNA fragment. The third fragment is the synthetic late promoter LP1 (SEQ ID NO:

9). The fourth fragment contains the coding region of E. coli uidA and is derived from plasmid pRAJ260 (Clonetech).

HOMOLOGY VECTORS 584-36.12. The plasmid 584-36.12 was constructed for the purpose of inserting the NDV HN and F genes into FPV. The NDV HN and F genes were inserted as a *Sfi*I fragment into the homology vector 443-88.14 (see example 1B) at the unique *Sfi*I site. The NDV HN and F genes were inserted in the same transcriptional orientation as the ORF in the parental homology vector. A detailed description of the *Sfi*I fragment is shown in Figures 1A-1C. The inserted *Sfi*I fragment was 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 indicated in Figures 1A-1C. Fragment 1 is an approximately 1811 base pair *Ava*II to *Nae*I restriction fragment of the full length NDV HN cDNA clone (B1 strain). Fragment 2 is an approximately 1812 base pair *Bam*HI to *Pst*I restriction fragment of the full length NDV F cDNA (B1 strain). Fragment 3 is an approximately 235 base pair *Pst*I to *Sca*I restriction fragment of the plasmid pBR322.

HOMOLOGY VECTOR 694-10.4. The plasmid 694-10.4 was constructed for the purpose of inserting the infectious laryngotracheitis virus (ILTV) gB and gD genes into FPV. An *E.coli* β-glucuronidase *uid*A marker gene preceded by the ILTV gB and gD genes was inserted as a cassette into the homology vector 451-08.22 at the unique *Sfi*I site. 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 sources with the synthetic DNA sequences indicated. The first fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO:8/SEQ ID NO:11).

The second fragment contains the coding region of ILTV gB and is derived from an approximately 3000 base pair ILT virus genomic *Eco*RI fragment. Note that the promoter and gB gene are fused so as to express the complete coding region of the gB gene (animo acids 1-883). The third fragment is the synthetic early/late promoter EP1LP2 (SEQ ID NO:8/SEQ ID NO:11). The fourth fragment contains the coding region of the ILTV gD gene and was derived from an approximately 2060 base pair *Eco*RI to *Bcl*I restriction sub-fragment of the ILTV *Kpn*I genomic restriction fragment #8 (10.6 KB). Note that the promoter and gD gene are fused so as to express a hybrid protein consisting of 3 amino acids derived from the synthetic promoter followed by amino acids 3 to 434 of the gD gene. The fifth fragment is the synthetic late promoter LP1 (SEQ ID NO:9). The last fragment contains the coding region of *E.coli uid*A and is derived from plasmid pRAJ260 (Clonetech). Note that the promoter and *uid*A gene are fused so as to express a hybrid protein consisting of 3 amino acids derived from the synthetic promoter followed by amino acids 1 to 602 of the *uid*A gene.

HOMOLOGY VECTOR 749-75.82. The plasmid 749-75.82 was used to insert foreign DNA into FPV. It incorporates an E. coli β-galactosidase (lacZ) marker gene and the infectious bursal disease virus (IBDV) polymerase gene flanked by FPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV a virus containing DNA coding for the foreign genes results. Note that the β-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the IBDV polymerase gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (11 and 14), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid

vector was derived from an approximately 2999 base pair EcoRI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1184 base pair EcoRI to SnaBI restriction sub-fragment of the 2.8 kb EcoRI FPV genomic fragment (SEQ ID NO. 5). Fragment 2 is an approximately 2700 EcoRI to AscI restriction fragment synthesized by cDNA cloning and polymerase chain reaction (PCR) from an IBDV RNA template. cDNA and PCR primers (5'-CACGAATTCTGACATTTTCAACAGTCCACAGGCGC-3'; 12/93.4) (SEQ ID NO:) and 5'-GCTGTTGGACATCACGGGCCAGG-3'; 9/93.28) (SEQ ID NO:) were used to synthesize an approximately 1100 base pair EcoRI to BclI fragment at the 5' end of the IBDV polymerase gene. cDNA and PCR primers (5'-ACCCGGAACATATGGTCAGCTCCAT-3'; 12/93.2) (SEQ ID NO:) and 5'-GGCGCGCCAGGCGAAGGCCGGGGATACGG-3'; 12/93.3) (SEQ ID NO:) were used to synthesize an approximately 1700 base pair BclI to AscI fragment at the 3' end of the IBDV polymerase gene. The two fragments were ligated at the BclI site to form the approximately 2800 base pair EcoRI to BclI fragment. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (7). Fragment 4 is an approximately 1626 base pair SnaBI to EcoRI restriction sub-fragment of the 2.8 kb EcoRI FPV genomic fragment (SEQ ID NO. 5).

HOMOLOGY VECTOR 751-07.D1. The plasmid 751-07.D1 was used to insert foreign DNA into FPV. It incorporates an E. coli β-galactosidase (lacZ) marker gene and the chicken interferon (cIFN) gene flanked by FPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV a virus containing DNA coding for the foreign genes results. Note that the β-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the cIFN gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA

techniques (17), by joining restriction fragmen
ts from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair EcoRI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1626 base pair EcoRI to SnaBI restriction sub-fragment of the 2.8 kb EcoRI FPV genomic fragment (SEQ ID NO. 5). Fragment 2 is an approximately 577 base pair EcoRI to BglII fragment coding for the cIFN gene (17) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.13) used for reverse transcription and PCR was 5' CGACGGATCCGAGGTGCGTTTGGGGCTAAGTGC-3' (SEQ ID NO:). The sense primer (6/94.12) used for PCR was 5' CCACGGATCCAGCACAACGCGAGTCCCACCATGGCT-3' (SEQ ID NO:). The BamHI fragment resulting from reverse transcription and PCR was gel purified and used as a template for a second PCR reaction to introduce a unique EcoRI site at the 5' end and a unique BglII site at the 3' end. The second PCR reaction used primer 6/94.22 (5' CCACGAATTCGATGGCTGTGCCTGCAAGCCCACAG-3'; SEQ ID NO:) at the 5' end and primer 6/94.34 (5'-CGAAGATCTGAGGTGCGTTTGGGGCTAAGTGC-3'; SEQ ID NO:) at the 3' end to yield an approximately 577 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 193 of the chicken interferon protein (17) which includes a 31 amino acid signal sequence at the amino terminus and 162 amino acids of the mature protein encoding chicken interferon. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (7). Fragment 4 is an approximately 1184 base pair SnaBI to EcoRI restriction sub-fragment of the 2.8 kb EcoRI FPV genomic fragment (SEQ ID NO. 5).

HOMOLOGY VECTOR 751-56.C1. The plasmid 751-56.C1 was used to insert foreign DNA into FPV. It incorporates an E. coli β-galactosidase (lacZ) marker gene and the chicken myelomonocytic growth factor (cMGF) gene flanked by FPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV a virus containing DNA coding for the foreign genes results. Note that the β-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the cMGF gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (11 and 14), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair EcoRI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1184 base pair EcoRI to SnaBI restriction sub-fragment of the 2.8 kb EcoRI FPV genomic fragment (SEQ ID NO. 5). Fragment 2 is an approximately 640 base pair EcoRI to BamHI fragment coding for the cMGF gene (16) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.20) used for reverse transcription and PCR was 5' CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3' (SEQ ID NO:). The sense primer (5/94.5) used for PCR was 5' GAGCGGATCCTGCAGGAGGAGACACAGAGCTG-3' (SEQ ID NO:). The BamHI fragment derived from PCR was subcloned into a plasmid and used as a template for a second PCR reaction using primer 6/94.16 (5'-GCGCGAATTCCATGTGCTGCCTCACCCCTGTG 3'; SEQ ID NO:) at the 5' end and primer 6/94.20 (5' CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3'; SEQ ID NO:) at the 3' end to yield an approximately 640 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 201 of the cMGF protein

(16) which includes a 23 amino acid signal sequence at the amino terminus and 178 amino acids of the mature protein encoding cMGF. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (7). Fragment 4 is an approximately 1626 base pair SnaBI to EcoRI restriction sub-fragment of the 2.8 kb EcoRI FPV genomic fragment (SEQ ID NO. 5).

Example 1

Sites for Insertion of Foreign DNA into FPV

In order to define appropriate insertion sites, a library of FPV *Eco*RI restriction fragments was generated in the plasmid vector pSP64 (Promega). Several of these restriction fragments were subjected to restriction mapping analysis. Unique blunt cutting restriction endonuclease sites were identified and mapped within the cloned FPV DNA regions. The blunt restriction sites were converted to *Not* I and *Sfi* I sites through the use of synthetic DNA linkers (oligo 66.04; 5'-GGCGGCCGCGGCCCTCGAGGCCA-3' SEQ ID NO: 1 and oligo 66.05; 5' TGGCCTCGAGGGCCGCGGCCGCC 3' SEQ ID NO: 2). A β-galactosidase (*lacZ*) marker gene was inserted in each of the potential sites. A plasmid containing such a foreign DNA insert may be used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV to construct a FPV containing the foreign DNA. For this procedure to be successful it is important that the insertion site be in a region non-essential to the replication of the FPV and that the site be flanked with FPV DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. The plasmids containing the *lacZ* marker gene were utilized in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The generation of recombinant virus was determined by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. Three sites were successfully used to generate a recombinant viruses. In each case the resulting virus was easily purified to 100%, clearly defining an appropriate site for the insertion of foreign DNA. The three homology vectors used to define these sites are described below.

Example 1A

Homology Vector 443-88.8

The homology vector 443-88.8 contains a 3.5 KB FPV genomic *Eco*RI fragment and is useful for the insertion of foreign DNA into FPV. This *Eco*RI fragment maps to the approximately 5.5 KB overlap of FPV genomic fragments *Sal*I C and *Pst*I F (Coupar *et al.*, 1990). The *Not*I/*Sfi*I linker described above was inserted into a unique *Hpa*I site in this fragment. This site is designated the 680 insertion site.

The homology vector 443-88.8 was characterized by DNA sequence analysis. Approximately 1495 base pairs of DNA sequence flanking the HpaI site was determined (SEQ ID NO: 3). This sequence indicates that the open reading frame of 383 amino acids spans the *Hpa*I insertion site. The *Hpa*I site interrupts this ORF at amino acid 226. This ORF shows no amino acid sequence homology to any known pox virus genes.

Example 1B

Homology Vector 443-88.14

The homology vector 443-88.14 contains a 2.8 KB FPV genomic *Eco*RI fragment and is useful for the insertion of foreign DNA into FPV. The *Not*I/*Sfi*I linker described above was inserted into a unique *Sna*BI site in this fragment. This site is designated the 681 insertion site.

The homology vector 443-88.14 was characterized by DNA sequence analysis. The entire sequence of the 2.8 KB fragment was determined (SEQ ID NO: 5). This sequence indicates that the *Sna*BI site is flanked on one side by a complete ORF of 422 amino acids (ORF1) reading toward the restriction site and on the other side by an

incomplete ORF of 387 amino acids (ORF2) also reading toward the restriction site. Both ORF1 and ORF2 share homology with the vaccinia virus M1L gene (ref). The M1L gene shares homology with the vaccinia virus K1L gene which has been shown to be involved in viral host-range functions.

Example 1C

Homology Vector 451-08.22

The homology vector 451-08.22 contains a 4.2 KB FPV genomic *Eco*RI fragment and is useful for the insertion of foreign DNA into FPV. The *Not*I/*Sfi*I linker described above was inserted into a unique *Stu*I site in this fragment. A unique *Mlu*I site is located approximately 500 base pairs away from the *Stu*I insertion site. This site is designated the 540 insertion site.

Example 2

Bivalent Vaccines Against Newcastle Disease and Fowlpox

Recombinant FPV expressing proteins from NDV make bivalent vaccines protecting against both Marek's Disease and Newcastle disease. We have constructed several recombinant FPV expressing NDV proteins: S-FPV-013 (example 2A), S-FPV-035 (example 2B), S-FPV-041 (example 2C), S-FPV-042 (example 2D), and S-FPV-043 (example 2E).

Example 2A

S-FPV-013

S-FPV-013 is a recombinant fowlpox virus that expresses two foreign genes. The gene for *E. coli* β-galactosidase (*lac*Z gene) and the gene for Newcastle Disease virus

hemagglutinin-neuraminidase (HN) protein were inserted into the 681 insertion site. The *lac*Z gene is under the control of a synthetic late promoter LP1 and the HN gene is under the control of the synthetic late promoter LP2.

S-FPV-013 was derived from S-FPV-001. This was accomplished utilizing the homology vector 451-79.95 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-013. 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 three rounds of purification all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-013 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. An NDV HN specific monoclonal antibody (3-1G-5) was shown to react specifically with S-FPV-013 plaques and not with S-FPV-001 negative control plaques. All S-FPV-013 observed plaques reacted with the monoclonal antibody antiserum indicating that the virus was stably expressing the NDV foreign gene.

Example 2B

S-FPV-035

S-FPV-035 is a recombinant fowlpox virus that express a foreign gene. The Newcastle Disease virus HN gene was inserted at the 680 insertion site (see example 1A). The

HN gene is under the control of the synthetic early/late promoter EP1LP2.

S-FPV-035 was derived from S-FPV-001. This was accomplished utilizing the homology vector 489-21.1 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the PLAQUE HYBRIDIZATION PROCEDURE FOR PURIFYING RECOMBINANT FPV. The final result of plaque hybridization purification was the recombinant virus designated S-FPV-035.

S-FPV-035 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. An NDV HN specific monoclonal antibody (3-1G-5) was shown to react specifically with S-FPV-035 plaques and not with S-FPV-001 negative control plaques. All S-FPV-035 observed plaques reacted with the monoclonal antibody indicating that the virus was stably expressing the NDV foreign gene.

Example 2C

S-FPV-041

S-FPV-041 is a recombinant fowlpox virus that expresses two foreign genes. The gene for *E. coli* β-galactosidase (*lac*Z gene) and the gene for Newcastle Disease virus fusion (F) protein were inserted into the 681 insertion site. The *lac*Z gene is under the control of a synthetic late promoter LP1 and the F gene is under the control of the synthetic early/late promoter EP1LP2.

S-FPV-041 was derived from S-FPV-001. This was accomplished utilizing the homology vector 502-27.5 (see

Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-041. 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 three rounds of purification all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-041 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. An NDV F specific monoclonal antibody (5-3F-2) was shown to react specifically with S-FPV-041 plaques and not with S-FPV-001 negative control plaques. All S-FPV-041 observed plaques reacted with the monoclonal antibody indicating that the virus was stably expressing the NDV foreign gene.

Example 2D

S-FPV-042

S-FPV-042 is a recombinant fowlpox virus that expresses three foreign genes. The gene for *E. coli* β-galactosidase (lacZ gene) and the gene for Newcastle Disease virus fusion (F) protein was inserted into the 681 insertion site. The *lac*Z gene is under the control of a synthetic late promoter LP1 and the F gene is under the control of the synthetic early/late promoter EP1LP2. The Newcastle Disease virus hemagglutinin (HN) gene were inserted at the 680 insertion site. The HN gene is under the control of the synthetic early/late promoter EP1LP2.

S-FPV-042 was derived from S-FPV-035. This was accomplished utilizing the homology vector 502-27.5 (see Materials and Methods) and virus S-FPV-035 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-042. 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 three rounds of purification all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-042 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Monoclonal antibodies specific for both HN (3-1G-5) and F (5-3F-2) were shown to react specifically with S-FPV-042 plaques and not with S-FPV-001 negative control plaques. All S-FPV-042 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes.

Example 2E

S-FPV-043

S-FPV-043 is a recombinant fowlpox virus that expresses two foreign genes. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 680 insertion site. The F and HN genes are each under the control of a synthetic early/late promoter EP1LP2.

S-FPV-043 was derived from S-FPV-001. This was accomplished utilizing the homology vector 502-26.22 (see

Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the PLAQUE HYBRIDIZATION PROCEDURE FOR PURIFYING RECOMBINANT FPV. The final result of plaque hybridization purification was the recombinant virus designated S-FPV-043. The S-FPV-043 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. VR 2395.

S-FPV-043 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Monoclonal antibodies specific for both HN (3-1G-5) and F (5-3F-2) were shown to react specifically with S-FPV-043 plaques and not with S-FPV-001 negative control plaques. All S-FPV-043 observed plaques reacted with the monoclonal antibodies antiserum indicating that the virus was stably expressing the NDV foreign genes.

TESTING OF RECOMBINANT FPV EXPRESSING NDV ANTIGENS

Groups of one day old SPF chicks (HyVac Inc.) were immunized with recombinant fowlpox viruses S-FPV-035, S-FPV-041, or S-FPV-043. Non vaccinated controls were also included. Three weeks post-vaccination, the birds were challenged intramuscularly with either virulent NDV or virulent FPV (Table 1). The challenged chicks were observed daily for 14 days for clinical signs and death due to NDV. Non vaccinated control birds showed 100% mortality. S-FPV-043 vaccinated birds showed 100% protection against FPV challenge. Birds vaccinated with S-FPV-035 showed 95% protection compared with 85% seen

with birds immunized with S-FPV-041. These results suggest that recombinants expressing HN or F alone provide only partial protection. When both NDV proteins are combined into the same virus S-FPV-043, an enhancement of protection against lethal NDV challenge is obtained, resulting in a lower protective dose. The chicks that were challenged with FPV were scored for pox lesions. Non vaccinated control birds showed no protection against FPV lesions. Birds vaccinated with S-FPV-043 were completely protected from FPV lesions.

The duration of immunity conferred by vaccination with S-FPV-043 was examined. A group of SPF chicks was immunized with S-FPV-043 at one day of age and then challenged six weeks post-vaccination with either NDV or FPV. Complete protection was observed against both NDV and FPV challenge in S-FPV-043 vaccinated birds, whereas non vaccinated controls were totally susceptible to both challenge viruses. These results suggest that the duration of immunity afforded by vaccination with S-FPV-043 would span the life of a broiler bird (~ 6 weeks).

The effect of vaccinating hens in lay with the recombinant S-FPV-043 was evaluated by assessing egg production post-vaccination. One group of 50 hens was vaccinated and a second group of 50 hens, housed under conditions identical to the vaccinated group, served as non vaccinated controls. Daily egg production was monitored for four weeks post-vaccination. No differences were observed in egg production between the two groups of hens, indicating this vaccine will not adversely affect egg production in laying hens.

A study was conducted to determine whether S-FPV-043 could actively immunize chicks in the presence of maternal antibodies to both NDV and FPV. Chicks obtained from NDV and FPV immunized flocks were vaccinated with S-

FPV-043 and three weeks after vaccination, they were challenged with either virulent NDV or virulent FPV. Clinical responses were compared with non vaccinated chicks from the same flock and with non-vaccinated chicks from an antibody negative flock (Table 2). Chicks derived from antibody negative flocks showed 100% mortality after NDV challenge. Protection against NDV challenge, in non-vaccinated chicks known to have maternally derived antibody against NDV, ranged from 30 to 60%. Protection levels increased, to a range of 75 to 85%, when the maternal antibody positive chicks were vaccinated with S-FPV-043 suggesting an active immunization. The increase in NDV protection from 30% to 75% (flock 1) and 55% to 85% (flock 2) clearly demonstrate the ability of S-FPV-043 to partially overcome maternal antibody to both NDV and FPV. A decrease in FPV protection (90%) was observed in flock 1, suggesting some inhibition of FPV replication.

Table 1. Immunity conferred by Fowlpox recombinant vaccines vectoring different genes from Newcastle disease virus.

		Challenge[a]	
VIRUS	DOSE[b]	NDV	FPV
FPV/NDV-HN	8×10^5	95	NT[c]
FPV/NDV-F	2×10^4	85	NT
FPV/NDV-HN+F	2×10^3	100	100
Controls	none	0	0

[a] Percent protection following challenge 3 weeks post-vaccination
[b] PFU/0.1 ml dose
[c] Not tested

Table 2. Ability of recombinant vaccine FPV/NDV-HN+F (S-FPV-043) to vaccinate chicks with maternal antibody.

History Flock Vaccination	Hen Antibody[b]			Challenge[a] NDV		Challenge[a] FPV	
	NDV-HI[c]	NDV ELISA	FPV-AGP[d]	Vacc.	Con.	Vacc.	Con.
1 NDV + FPV	1:36	1:1738	Neg	75	30	90	0
2 NDV + FPV	1:64	1:2852	Neg	85	55	100	0
3 NDV only	1:92	1:4324	Neg	80	60	95	0
4 None	Neg	Neg	Neg	--	0	--	0

[a] Percent protection following challenge 3 weeks post-vaccination.
[b] Every flock antibody.
[c] HI - Hemagglutination Inhibition Assay
[d] AGP - Agar Gel Precipitation Assay

Example 2F

S-FPV-074

S-FPV-074 is a recombinant fowlpox virus that expresses two foreign genes. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 681 insertion site. The F and HN genes are each under the control of a synthetic late/early promoter LP2EP2.

S-FPV-074 was derived from S-FPV-001. This was accomplished utilizing the homology vector 584-36.12 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the PLAQUE HYBRIDIZATION PROCEDURE FOR PURIFYING RECOMBINANT FPV. The final result of plaque hybridization purification was the recombinant virus designated S-FPV-074.

S-FPV-074 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Monoclonal antibodies specific for NDV HN (3-1G-5) and F (5-3F-2) were shown to react specifically with S-FPV-074 plaques and not with S-FPV-001 negative control plaques. All S-FPV-074 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes.

S-FPV-074 expresses foreign antigens from NDV. This virus is useful as a multi-valent vaccine against Newcastle Diseases and Fowlpox.

Example 3

Recombinant fowlpox viruses expressing proteins from Marek's disease virus (MDV) make vaccines protecting against both fowlpox virus and Marek's disease virus. We have constructed several recombinant FPV expressing MDV proteins: S-FPV-081, S-FPV-082 and S-FPV-085. Of these S-FPV-082 and S-FPV-085 also express proteins from Newcastle disease virus. These viruses are useful for vaccinating against fowlpox virus, Marek's disease virus, and Newcastle disease virus.

S-FPV-085 further expresses proteins from infectious laryngotracheitis virus (ILTV), making them useful as vaccines against ILTV.

Example 3A

S-FPV-081

S-FPV-081 is a recombinant fowlpox virus that expresses three foreign genes. The gene for E.coli β-galactosidase (lacZ gene) and the genes for Marek's Disease virus (MDV) glycoprotein D (gD) and glycoprotein B (gB) were inserted into the 681 insertion site. The lac Z gene is under the control of a synthetic late promoter LP1 and the MDV gD and gB genes are under the control of the synthetic early/late promoters LP2EP2 and EP1LP2 respectively.

S-FPV-081 was derived from S-FPV-001. This was accomplished utilizing the homology vector 608-10.3 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-081. 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 three rounds of purification all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-081 was assayed for expression of MDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Convalescent sera from MDV infected chickens was shown to react specifically with S-FPV-081 plaques and not with S-FPV-001 negative control plaques. All S-FPV-081 observed plaques reacted with the chicken antiserum indicating that the virus was stably expressing the MDV foreign genes. Western blot assays of infected cell lysates using convalescent sera from MDV-infected chickens indicated that S-FPV-081 was expressing a MDV glycoprotein B and MDV glycoprotein D.

S-FPV-081 expresses foreign antigens from MDV. This virus is useful as a multi-valent vaccine against Marek's Disease and Fowlpox.

Example 3B

S-FPV-082

S-FPV-082 is a recombinant fowlpox virus that expresses five foreign genes. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 680 insertion site. The F and HN genes are each under the control of a synthetic early/late promoter EP1LP2. The gene for *E. coli* β-galactosidase (lacZ gene) and the genes for Marek's Disease virus (MDV) gD and gB were inserted into the 681 insertion site. The *lacZ* gene is under the control of a synthetic late promoter LP1 and the MDV gD and gB genes are under the control of the

synthetic early/late promoters LP2EP2 and EP1LP2 respectively.

S-FPV-082 was derived from S-FPV-043. This was accomplished utilizing the homology vector 608-10.3 (see Materials and Methods) and virus S-FPV-043 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-082. 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 three rounds of purification all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-082 was assayed for expression of MDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Convalescent sera from MDV infected chickens was shown to react specifically with S-FPV-082 plaques and not with S-FPV-001 negative control plaques. All S-FPV-082 observed plaques reacted with the chicken antiserum indicating that the virus was stably expressing the MDV foreign genes.

S-FPV-082 expresses foreign antigens from NDV and MDV. This virus will be valuable as a multi-valent vaccine against Newcastle Disease, Marek's Disease and Fowlpox.

Example 3C

S-FPV-085

S-FPV-085 is a recombinant fowlpox virus that expresses eight foreign genes. The genes for Newcastle Disease

virus F protein and HN protein are inserted at the 680 insertion site. The F and HN genes are each under the control of a synthetic early/late promoter EP1LP2. The gene for E.coli β-galactosidase (lacZ gene) and the genes for Marek's Disease virus (MDV) gD and gB are inserted into the 681 insertion site. The lac Z gene is under the control of a synthetic late promoter LP1 and the MDV gD and gB genes are under the control of the synthetic early/late promoters LP2EP2 and EP1LP2 respectively. The gene for E.coli β-glucuronidase (*uid*A gene) and the genes for Infectious Laryngotracheitis virus (ILTV) gD and gB are inserted into the 540 insertion site. The *uid*A gene is under the control of a synthetic late promoter LP1 and the ILTV gD and gB genes are each under the control of a synthetic early/late promoter EP1LP2.

S-FPV-085 is derived from S-FPV-082. This is accomplished utilizing the homology vector 586-36.6 (see Materials and Methods) and virus S-FPV-082 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque (β-glucuronidase) purification is the recombinant virus designated S-FPV-085. This virus is assayed for β-glucuronidase 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 are blue indicating that the virus is pure, stable and expressing the marker gene.

S-FPV-085 is assayed for expression of MDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. S-FPV-085 expresses foreign antigens from NDV, MDV and ILTV. This virus is useful as a multi-valent vaccine against Newcastle

Disease, Marek's Disease, Infectious Laryngotracheitis and Fowlpox.

Example 4

Recombinant fowlpox virus (FPV) expressing proteins from infectious laryngotracheitis virus (ILTV) make vaccines protecting against both FPV and ILTV. We have constructed several recombinant FPV expressing ILTV proteins: S-FPV-095, S-FPV-083, and S-FPV-097. Of these, S-FPV-083 and S-FPV-097 also express proteins from Newcastle disease virus (NDV), making them useful as vaccines against NDV as well.

Example 4A

S-FPV-095

S-FPV-095 is a recombinant fowlpox virus that expresses three foreign genes. The gene for E.coli β-glucuronidase (*uid*A gene) and the genes for Infectious Laryngotracheitis virus (ILTV) glycoprotein D (gD) and glycoprotein B (gB) were inserted into the 540 insertion site. The *uid*A gene is under the control of a synthetic late promoter LP1 and the ILTV gD and gB genes are each under the control of a synthetic early/late promoter EP1LP2.

S-FPV-095 was derived from S-FPV-001. This was accomplished utilizing the homology vector 694-10.4 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification (β-glucuronidase) was the recombinant virus designated S-FPV-095. This virus was assayed for β-

glucuronidase 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 were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-095 was assayed for expression of ILTV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Antibodies to ILTV gB and gD was shown to react specifically with S-FPV-095 plaques and not with S-FPV-001 negative control plaques. All S-FPV-095 observed plaques reacted with the antiserum indicating that the virus was stably expressing the ILTV foreign genes.

S-FPV-095 expresses foreign antigens from ILTV. This virus is useful as a multi-valent vaccine against Infectious Laryngotracheitis and Fowlpox.

Example 4B

S-FPV-083

S-FPV-083 is a recombinant fowlpox virus that expresses five foreign genes. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 680 insertion site. The F and HN genes are each under the control of a synthetic early/late promoter EP1LP2. The gene for *E. coli* β-glucuronidase (*uid*A gene) and the genes for Infectious Laryngotracheitis virus (ILT) gD and gB were inserted into the 540 insertion site. The *uid*A gene is under the control of a synthetic late promoter LP1 and the ILT gD and gB genes are each under the control of a synthetic early/late promoter (EP1LP2).

S-FPV-083 was derived from S-FPV-043. This was

accomplished utilizing the homology vector 586-36.6 (see Materials and Methods) and virus S-FPV-043 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification was the recombinant virus designated S-FPV-083. This virus was assayed for β-glucuronidase 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 were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-083 was assayed for expression of ILTV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Convalescent sera from ILTV infected chickens was shown to react specifically with S-FPV-083 plaques and not with S-FPV-001 negative control plaques. All S-FPV-083 observed plaques reacted with the chicken antiserum indicating that the virus was stably expressing the ILTV foreign genes.

S-FPV-083 expresses foreign antigens from NDV and ILTV. This virus will be valuable as a multi-valent vaccine against Newcastle Disease, Infectious Laryngotracheitis and Fowlpox.

Example 4C

S-FPV-097

S-FPV-097 is a recombinant fowlpox virus that expresses five foreign genes. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 680 insertion site. The F and HN genes are each under the

control of a synthetic early/late promoter EP1LP2. The gene for E.coli β-glucuronidase (*uid*A gene) and the genes for Infectious Laryngotracheitis virus (ILTV) glycoprotein D (gD) and glycoprotein B (gB) were inserted into the 540 insertion site. The *uid*A gene is under the control of a synthetic late promoter LP1 and the ILTV gD and gB genes are each under the control of a synthetic early/late promoter EP1LP2.

S-FPV-097 was derived from S-FPV-043. This was accomplished utilizing the homology vector 694-10.4 (see Materials and Methods) and virus S-FPV-043 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification was the recombinant virus designated S-FPV-097. This virus was assayed for β-glucuronidase 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 were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-097 was assayed for expression of ILTV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Antibodies to ILTV gB and gD was shown to react specifically with S-FPV-097 plaques and not with S-FPV-001 negative control plaques. All S-FPV-097 observed plaques reacted with the antiserum indicating that the virus was stably expressing the ILTV foreign genes. All S-FPV-097 observed plaques reacted with the chicken antiserum to ILTV indicating that the virus was stably expressing the ILTV foreign genes. Monoclonal antibodies specific for NDV HN (3-1G-5) and F (5-3F-2) were shown to react specifically with S-FPV-097

plaques and not with S-FPV-001 negative control plaques. All S-FPV-097 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes.

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S-FPV-097 expresses foreign antigens from NDV and ILTV. This virus is useful as a multi-valent vaccine against Newcastle Disease, Infectious Laryngotracheitis and Fowlpox.

Example 5

Recombinant fowlpox virus (FPV) expressing proteins from infectious bronchitis virus (IBV) make vaccines protecting against both FPV and IBV. We have constructed two recombinant FPV expressing IBV proteins: S-FPV-072 and S-FPV-079. Both of these viruses also express proteins from Newcastle disease virus (NDV), making them useful as vaccines against NDV.

Example 5A

S-FPV-072

S-FPV-072 is a recombinant fowlpox virus that expresses five foreign genes. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 680 insertion site. The F and HN genes are each under the control of a synthetic early/late promoter EP1LP2. The gene for E.coli β-galactosidase (lacZ gene) and the genes for Infectious Bronchitis virus (IBV) Massachusetts Spike protein (Mass Spike) and Massachusetts Matrix protein (Mass Matrix) were inserted into the 681 insertion site. The lac Z gene is under the control of a synthetic late promoter LP1 and the IBV Mass Spike and Mass Matrix genes are each under the control of the synthetic early/late promoter EP1LP2.

S-FPV-072 was derived from S-FPV-043. This was accomplished utilizing the homology vector 538-51.27 (see Materials and Methods) and virus S-FPV-043 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-072. This virus was assayed for B-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 were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-072 was assayed for expression of NDV and IBV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Monoclonal antibody 15-88 to the IBV Mass Spike protein was shown to react specifically with S-FPV-072 plaques and not with S-FPV-001 negative control plaques. All S-FPV-072 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the IBV foreign gene. Western blot assays of infected cell lysates using monoclonal antibody 15-88 to the IBV Mass Spike protein indicated that S-FPV-072 was expressing a 90 kD IBV Mass Spike protein. Monoclonal antibodies specific for both HN (3-1G-5) and F (5-3F-2) were shown to react specifically with S-FPV-072 plaques and not with S-FPV-001 negative control plaques. All S-FPV-072 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes.

S-FPV-072 expresses foreign antigens from NDV and IBV. This virus is useful as a multi-valent vaccine against Newcastle Diseases, Infectious Bronchitis, and Fowlpox.

Example 5B

S-FPV-079 is a recombinant fowlpox virus that expresses seven foreign genes. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 680 insertion site. The F and HN genes are each under the control of a synthetic early/late promoter EP1LP2. The gene for E.coli β-galactosidase (lacZ gene) and the genes for Infectious Bronchitis virus (IBV) Massachusetts Spike

protein (Mass Spike) and Massachusetts Matrix protein (Mass Matrix) were inserted into the 681 insertion site. The lac Z gene is under the control of a synthetic late promoter LP1 and the IBV Mass Spike and Mass Matrix genes are each under the control of the synthetic early/late promoter EP1LP2. The gene for the E. coli β-glucuronidase (uidA) gene and the gene for the IBV Mass Nucleocapsid protein were inserted into the 540 insertion site. The uidA gene is under the control of the synthetic late/early promoter LP2EP2 and the IBV Mass Nucleocapsid gene is under the control of the synthetic early/late promoter EP1LP2.

S-FPV-079 was derived from S-FPV-072. This was accomplished utilizing the Homology Vector 611-49.1 (see Materials and Methods) and virus S-FPV-072 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-079. This virus was assayed for B-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 were blue indicating that the virus was pure, stable and expressing the marker gene.

S-FPV-079 was assayed for expression of NDV and IBV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT FPV. Monoclonal antibody 15-88 to the IBV Mass Spike protein was shown to react specifically with S-FPV-072 plaques and not with S-FPV-001 negative control plaques. All S-FPV-079 observed plaques reacted with the monoclonal antibody antiserum to IBV indicating that the virus was stably expressing the

IBV foreign gene. Western blot assays of infected cell lysates using monoclonal antibody 15-88 to the IBV Mass Spike protein indicated that S-FPV-079 was expressing a 90 kD IBV Mass Spike protein. Monoclonal antibodies specific for both HN (3-1G-5) and F (5-3F-2) were shown to react specifically with S-FPV-079 plaques and not with S-FPV-001 negative control plaques. All S-FPV-079 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes.

S-FPV-079 expresses foreign antigens from NDV and IBV. This virus is useful as a multi-valent vaccine against Newcastle Diseases, Infectious Bronchitis, and Fowlpox.

Example 6

Recombinant fowlpox virus, S-FPV-099 or S-FPV-101, expressing chicken interferon (cIFN) or S-FPV-100, expressing chicken myelomonocytic growth factor (cMGF), are useful to enhance the immune response when added to vaccines against diseases of poultry. Chicken myelomonocytic growth factor (cMGF) is homologous to mammalian interleukin-6 protein, and chicken interferon (cIFN) is homologous to mammalian interferon Type I. When used alone or in combination with vaccines against specific avian diseases, S-FPV-099, S-FPV-100 and S-FPV-101 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.

S-FPV-099

S-FPV-099 is a recombinant fowlpox virus that expresses

two foreign genes. The genes for chicken interferon (cIFN) and *E. coli* lacZ were inserted at the uniqe SnaBI restriction endonuclease site in the 2.8 kB EcoRI FPV genomic fragment (681 insertion site). The cIFN gene is under the control of a synthetic late/early promoter LP2EP2, and the *E. coli* lacZ gene is under the control of a synthetic late promoter LP1.

S-FPV-099 was derived from S-FPV-001. This was accomplished utilizing the homology vector 751-07.D1 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-099. This virus was assayed for β-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus S-FPV-099 was pure, stable, and expressing the foreign gene.

Supernatants from S-FPV-099 have interferon activity in cell culture. Addition of S-FPV-099 conditioned media to chicken embryo fibroblast (CEF) cell culture inhibits infection of the CEF cells by vesicular stomatitis virus or by herpesvirus of turkeys. S-FPV-099 is useful to enhance the immune response alone or when added to vaccines against diseases of poultry.

S-FPV-100

S-FPV-100 is a recombinant fowlpox virus that expresses two foreign genes. The genes for chicken myelomonocytic growth factor (cMGF) and *E. coli* lacZ were inserted at

the uniqe SnaBI restriction endonuclease site in the 2.8 kB EcoRI FPV genomic fragment (681 insertion site). The cMGF gene is under the control of a synthetic late/early promoter LP2EP2, and the *E. coli* lacZ gene is under the control of a synthetic late promoter LP1.

S-FPV-100 was derived from S-FPV-001. This was accomplished utilizing the homology vector 751-56.C1 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-100. This virus was assayed for β-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus S-FPV-100 was pure, stable, and expressing the foreign gene.

S-FPV-100 is useful to enhance the immune response alone or when added to vaccines against diseases of poultry.

S-FPV-101

S-FPV-101 is a recombinant fowlpox virus that expresses four foreign genes. The genes for chicken interferon (cIFN) and *E. coli* lacZ were inserted at the uniqe SnaBI restriction endonuclease site in the 2.8 kB EcoRI FPV genomic fragment (681 insertion site). The cIFN gene is under the control of a synthetic late/early promoter LP2EP2, and the *E. coli* lacZ gene is under the control of a synthetic late promoter LP1. The genes for Newcastle Disease virus F protein and HN protein were inserted at the 680 insertion site. The F and HN genes are each under

the control of a synthetic early/late promoter EP1LP2.

S-FPV-101 was derived from S-FPV-043. This was accomplished utilizing the homology vector 751-07.D1 (see Materials and Methods) and virus S-FPV-043 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-101. This virus was assayed for β-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus S-FPV-101 was pure, stable, and expressing the foreign gene.

Supernatants from S-FPV-101 have interferon activity in cell culture. Addition of S-FPV-101 conditioned media to chicken embryo fibroblast (CEF) cell culture inhibits infection of the CEF cells by vesicular stomatitis virus or by herpesvirus of turkeys. S-FPV-101 is useful to enhance the immune response alone or when added to vaccines against diseases of poultry. S-FPV-101 is useful as a multi-valent vaccine against Newcastle Diseases and Fowlpox.

Example 7

Recombinant fowlpox virus expressing Newcastle's disease virus HN and F proteins lacking the membrane anchor sequences is a superior vaccine against fowlpox and Newcastle's disease.

Day old chicks from hens which have been exposed to or vaccinated against Newcastle's disease virus carry

antibodies to NDV which may neutralize a vaccine containing a recombinant fowlpox virus expressing the NDV HN and F proteins. *In vitro* virus neutralization (VN) assays using VN monoclonal antibodies specific for either NDV HN or F proteins have been shown to neutralize recombinant fowlpox virus expressing the NDV HN and F proteins. These results suggest that the NDV HN and F glycoproteins are incorporated into the fowlpox virus virion. To increase the efficacy of a vaccine in the presence on maternal antibodies against Newcastle's disease virus, a recombinant fowlpox virus is constructed which expresses the NDV HN and F proteins lacking the membrane anchor domains of each protein. The resulting recombinant virus produces NDV HN and F proteins secreted into the serum of the vaccinated animal producing a strong humoral and cell mediated immune response to the Newcastle's disease virus. The NDV HN and F proteins are not presented on the surface of the FPV particle and thus evade neutralization by maternal antibodies present in the vaccinated day old chicks.

The hemagglutinin-Neuraminidase (HN) and Fusion (F) genes from the B1 strain of Newcastle Disease Virus (ATCC VR-108) were isolated as cDNA clones, using oligo dT primed poly A selected mRNA.

The fusion (F) protein mediates penetration of NDV into host cells by fusion of the viral envelope with the host cell plasma membrane. A posttranslational cleavage of inactive precursors F_0 into two disulfide-bonded polypeptides, F1 and F2, is necessary to produce fusion active F protein and thereby yield infectious virions. The new hydrophobic N-terminus of F1 generated after cleavage of F_0 is responsible for the fusion characteristic of paramyxoviruses and thus determines virulence. The required proteolytic cleavage signal (paired basic residues) in the NDV B1 strain is altered,

thereby preventing cleavage of F_0 into F1 and F2, resulting in an attenuated NDV strain.

The addition of the NDV F signal sequence (aa1-25) to VP2 (vFP147), resulted in the secretion of VP2 in the TC fluid, but abolished its protective response (Paoletti, et. al WO 93/03145). Three hydrophobic domains exist within the F glycoprotein which interact with the lipid bilayer : 1). The signal sequence at the N-terminus of the primary translation product F_0; 2). the N-terminus of F1; and 3). the transmembrane anchor domain near the C-terminus of F1. The F glycoprotein of the B1 strain of NDV is 544 amino acids in length with the transmembrane anchor domain spanning 27 amino acids from position 500 to 526 (LITYIVLTIISLVFGILSLILACYLMY). Amino acids 1-499 of the NDV F protein are expressed under the control of a synthetic promoter element which functions as both an early and late promoter, such as EP1LP2 or LP2EP2, directing expression throughout the reproduction cycle. This results in the deletion of amino acids 527-544, the cytoplasmic tail, thought to interact with the inner membrane protein (M) before or during virus assembly. A recombinant fowlpox virus is constructed which expresses the NDV F protein lacking the C-terminal membrane anchor domain from a synthetic early/late promoter.

The hemagglutinin-neuraminidase (HN) glycoprotein provides NDV with the ability to agglutinate and elute erythrocytes. The process consists of two stages: attachment of the virus to the receptor on the red blood cell surface (agglutination) and destruction of the receptor by the neuraminidase enzyme activity (elution). The major hydrophobic anchor domain is present near the N-terminus of HN, supporting the view that the N-terminus is anchored to the lipid bilayer. The HN glycoprotein of the B1 strain of NDV is 577 amino acids in length with the transmembrane anchor domain spanning 28 amino acids

from position 27 to 54 (IAILFLTVVTLAISVASLLYSMGASTPS). The extreme N-terminal amino acids (1 to 26) are relatively hydrophilic. Amino acids 55 to 577 of the HN protein are expressed under the control of a synthetic promoter element which functions as both an early and late promoter, such as EP1LP2 or LP2EP2, directing expression throughout the reproduction cycle. THE NDV HN polypeptide has a membrane transport signal sequence, such as the PRV gX signal sequence, at its amino terminus to direct the protein to be secreted into the serum of a vaccinated animal. A recombinant fowlpox virus is constructed which expresses the NDV HN protein lacking the N-terminal membrane anchor domain and containing an N-terminal PRV gX signal sequence from a synthetic early/late promoter. Alternatively the NDV HN polypeptide contains a deletion of the transmembrane anchor domain spanning 28 amino acids from position 27 to 54 and retains amino acids 1 to 26 and 55 to 577. A recombinant fowlpox virus is constructed which expresses the NDV HN protein lacking the membrane anchor domain (amino acids 27 to 54) from a synthetic early/late promoter.

A recombinant fowlpox virus is constructed which expresses both the NDV HN and F proteins lacking the membrane anchor domains of each protein from a synthetic early/late promoter. The resulting recombinant virus produces NDV HN and F proteins secreted into the serum of the vaccinated animal producing a strong humoral and cell mediated immune response to the Newcastle's disease virus. The NDV HN and F proteins are not presented on the surface of the FPV particle and thus evade neutralization by maternal antibodies present in the vaccinated day old chicks.

Example 8

Recombinant fowlpox virus expressing cell surface

receptors on the surface of the FPV viral particle useful for targeting gene products to specific tissues or organs.

Serum from chickens carrying maternal antibodies to Newcastle's disease virus inhibits productive infection and plaque formation by S-FPV-043 on chicken embryo fibroblasts in cell culture. One explanation for this result is that the antigenic epitopes of the NDV HN and F proteins expressed in S-FPV-043 are displayed on the surface of the fowlpox viral particle. Display of proteins on the surface of the FPV particle is useful to target specific gene products to specific normal cell types or tumor cell types. Proteins which are displayed on the surface of the FPV particle include but are not limited to integrins which would target the virus to integrin receptors on the cell surface; erythropoetin which would target the virus to erythropoetin receptors on the surface of red blood cells; antibodies or other proteins which would target to specific proteins or receptors on the surface of normal or tumor cells. The fowlpox virus also delivers cytokines, interleukins, interferons, or colony stimulating factors which stimulate a strong humoral or cell mediated immune response against a tumor or disease causing organism. The proteins displayed on the surface of the fowlpox virus are expressed from the fowlpox genome as fusion proteins to the membrane anchor domains of the NDV HN or F proteins, or to other proteins containing membrane anchor domains. The cytokines, interleukins, interferons, or colony stimulating factors are expressed as fusion proteins to PRV gX, E. coli β-galactosidase or another protein in a soluble, not membrane bound, form. The fusion protein stabilizes the cytokine protein and allows it to diffuse in the serum of the animal to reach its cellular target.

Example 9

S-FPV-098

S-FPV-098 is a recombinant fowlpox virus that expresses two foreign genes. The genes for infectious bursal disease virus (IBDV) polymerase gene and *E. coli* lacZ were inserted at the 681 insertion site. The IBDV polymerase gene is under the control of a synthetic late/early promoter LP2EP2, and the *E. coli* lacZ gene is under the control of a synthetic late promoter LP1.

S-FPV-098 was derived from S-FPV-001. This was accomplished utilizing the homology vector 749-75.82 (see Materials and Methods) and virus S-FPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT FPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT FPV EXPRESSING ENZYMATIC MARKER GENES. The final result of red plaque purification was the recombinant virus designated S-FPV-098. This virus was assayed for β-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus S-FPV-098 was pure, stable, and expressing the foreign gene.

S-FPV-098 is useful for expression of IBDV polymerase protein. S-FPV-098 is useful in an *in vitro* approach to a recombinant IBDV attenuated vaccine. RNA strands from the attenuated IBDV strain are synthesized in a bacterial expression system using T3 or T7 promoters (pBlueScript plasmid; Stratagene, Inc.) to synthesize double stranded short and long segments of the IBDV genome. The IBDV double stranded RNA segments and S-FPV-098 are transfected into Vero cells. The fowlpox virus expresses the IBDV polymerase but does not replicate in Vero cells.

The IBDV polymerase produced from S-FPV-098 synthesizes infectious attenuated IBDV virus from the double stranded RNA genomic templates. The resulting attenuated IBDV virus is useful as a vaccine against infectious bursal disease in chickens.

As an alternative to the construction of a IBD vaccine using a viral vectored delivery system and/or subunit approaches, IBD virus RNA is directly manipulated re-constructing the virus using full length RNA derived from cDNA clones representing both the large (segment A) and small (segment B) double-stranded RNA subunits. Generation of IBD virus is this manner offers several advantages over the first two approaches. First, if IBD virus is re-generated using RNA templates, one is able to manipulate the cloned cDNA copies of the viral genome prior to transcription (generation of RNA). Using this approach, it is possible to either attenuate a virulent IBD strain or replace the VP2 variable region of the attenuated vaccine backbone with that of virulent strains. In doing so, the present invention provides protection against the virulent IBDV strain while providing the safety and efficacy of the vaccine strain. Furthermore, using this approach, the present invention constructs and tests temperature sensitive IBD viruses generated using the RNA polymerase derived from the related birnavirus infectious pancreatic necrosis virus (IPNV) and the polyprotein derived from IBDV. The IPNV polymerase has optimum activity at a temperature lower than that of IBDV. If the IPNV polymerase recognizes the regulatory signals present on IBDV, the hybrid virus is expected to be attenuated at the elevated temperature present in chickens. Alternatively, it is possible to construct and test IBD viruses generated using the RNA polymerase derived from IBDV serotype 2 viruse and the polyprotein derived from IBDVserotype 1 virus.

cDNA clones representing the complete genome of IBDV (double stranded RNA segments A and B) is constructed, initially using the BursaVac vaccine strain (Sterwin Labs). Once cDNA clones representing full length copies of segment A and B are constructed, template RNA is prepared. Since IBDV exists as a bisegmented double-stranded RNA virus, both the sense and anti-sense RNA strands of each segment are produced using the pBlueScript plasmid; Stratagene, Inc.). These vectors utilize the highly specific phage promoters SP6 or T7 to produce substrate amounts of RNA *in vitro*. A unique restriction endonuclease site is engineered into the 3' PCR primer to linearize the DNA for the generation of run-off transcripts during transcription.

The purified RNA transcripts (4 strands) are transfected into Vero cells to determine whether the RNA is infectious. If IBD virus is generated, as determined by black plaque assays using IBDV specific Mabs, no further manipulations are required and engineering of the vaccine strain can commence. The advantage of this method is that engineered IBD viruses generated in this manner will be pure and require little/no purification, greatly decreasing the time required to generate new vaccines. If negative results are obtained using the purified RNA's, functional viral RNA polymerase is required by use of a helper virus. Birnaviruses replicate their nucleic acid by a strand displacement (semi-conservative) mechanism, with the RNA polymerase binding to the ends of the double-stranded RNA molecules forming circularized ring structures (Muller & Nitschke, Virology 159, 174-177, 1987). RNA polymerase open reading frame of about 878 amino acids in fowlpox virus is expressed and this recombinant virus (S-FPV-098) is used to provide functional IBDV RNA polymerase *in trans*. Fowlpox virus expressed immunologically recognizable foreign antigens in non-avian cells (Vero cells), where there are no signs

of productive replication of the viral vector (Paoletti *et al.*, Technological Advances in Vaccine Development, 321-334, 1988, Alan R. Liss, Inc.). In the present invention the IBDV polymerase protein is expressed in the same cells as the transfected RNA using the fowlpox virus vector without contaminating the cells with FPV replication.

With the demonstration that IBD virus is generated *in vitro* using genomic RNA, an improved live attenuated virus vaccines against infectious bursal disease is developed. Using recombinant DNA technology along with the newly defined system of generating IBD virus, specific deletions within the viral genome, facilitating the construction of attenuated viruses are made. Using this technology, the region of IBDV responsible for virulence and generate attenuated, immunogenic IBDV vaccines are identified. The present invention provides a virulent IBD strain or replacement of the VP2 variable region of the attenuated vaccine backbone with that of a virulent strain, thus protecting against the virulent strain while providing the safety and efficacy of the vaccine strain.

Example 10

The chicken interferon (cIFN) gene was cloned into wild type (FPV) viruses by homologous recombinant techniques. Briefly, the entire coding region of cIFN was isolated from activated chicken spleen cell RNA by RT/PCR using primer sequences from the recently published cIFN sequence (Sekellick, M., et al., 1994). Recombinant FPV viruses containing cIFN, and FPV/cIFN (S-FPV-099), were engineered to contain the entire cIFN ORF under the control of a synthetic pox virus promoter (LP2EP2), which functions as both an early and late promoter, directing expression throughout the entire viral replication cycle.

A third recombinant virus, FPV/cIFN+NDV, (S-FPV-101) was made in a similar manner, except that a FPV virus previously engineered to contain the Newcastle Disease (NDV) antigens HN and F was used as the parent virus during homologous recombination, thus yielding a recombinant fowlpox virus co-expressing the cIFN and NDV genes. All recombinant viruses contain the *lac Z* gene engineered in tandem with cIFN under the control of a synthetic late (LP1) pox promoter. All promoter/gene constructs were sequenced at the promoter/cIFN junction to confirm the integrity of the proper DNA coding frame. Co-expression of β-galactosidase facilitated the isolation and plaque purification of the recombinant viruses. Independent viral insertion sites were used for insertion of the cIFN gene and the NDV genes in the fowlpox virus. The insertion sites were found to interrupt nonessential virus genes in both SPV and FPV.

To confirm the presence of the cIFN gene, recombinant viral DNAs were analyzed by PCR, using cIFN specific primers flanking the coding region. All viral DNA's yielded the expected 600 bp amplified cIFN DNA product. In addition, southern blot analysis on the viral DNA was performed using a non-radioactive labeled cIFN cDNA probe. Plasmid constructs containing the cIFN gene cassettes were sequenced across the transcriptional and translational initiation/termination signals, to confirm the integrity of the ORF.

Growth Properties of Recombinant Viruses in Cell Culture.

Recombinant FPV/cIFN and FPV/cIFN+NDV were found to be attenuated with respect to their growth in chicken embryo fibroblast (CEF) cells. Plaque size was decreased significantly and viral titers were 0.9-1.4 logs less when compared to wild type FPV. We suggest that fowlpox virus has anti-IFN mechanisms, similar to anti-IFN

mechanisms reported for other pox viruses, e.g. vaccinia, cowpox. And that these mechanisms help the virus to overcome the inhibitory effects of exogenously expressed cIFN. Therefore, fowlpox virus is able to infect, replicate and retain a productive infectious state.

In Vivo Properties of Recombinant FPV/cIFN Virus in Chicks.

10-day old chicks were inoculated, subcutaneously, with recombinant FPV/cIFN (S-FPV-099) virus at increasing dosages. At 10 days post inoculation, all chicks were inoculated with a mixture of sheep red blood cells (SRBC) and *Brucella abortus* (BA). At 15 days post FPV/cIFN virus inoculation, sera was collected, total body weights and antibody responses to SRBC's and BA were measured, and chicks were sacrificed for necropsy analysis. These data show that there were no significant differences in chick body weight, SRBC and BA antibody responses or gross pathology[c] associated with inoculation of recombinant FPV/cIFN virus, as compared to chicks inoculated with PBS alone. Therefore, this virus appears to be safe in 10-day old chicks.

Table 3. Determination of safety of recombinant FPV/cIFN virus in 10-day old chicks.

FPV/cIFN (pfu/chick)	Total body weight (grams)[a,b]	Antibody titers [a,d]	
		BA	SRBC
0 (PBS)	438	4.66	2.16
600	460	4.00	2.00
6,000	461	4.25	2.00

60,000	460	4.62	2.00

[a]. Measured 15 days post FPV/cIFN virus inoculation

[b]. Mean body weight (n=8).

[c]. There were no detectable gross pathological changes in any of the groups.

[d]. Mean antibody titers were determined by agglutination assay and expressed as $\log_2$ (n=8).

One-day old chicks were inoculated intranasally/intraocularly with NDV B1 (10^6 ELD_{50}/chick) alone or in addition to subcutaneous inoculation with FPV/cIFN (10^3 pfu/chick). Chick mortality was recorded 2 weeks post vaccination. Chicks vaccinated with NDV B1 alone or with NDV B1 plus FPV wild-type virus showed 20-30% mortality compared to chickens co-vaccinated with NDV-B1 and FPV/cIFN, in which group, all chicks remained alive. Subsequently, all chicks were challenged at 4 weeks post vaccination with a pathogenic strain of NDV (GB-TX). All chicks were protected, except for those in the "no treatment " control group. These data show that NDV B1 vaccine induced mortality was reduced without affecting the vaccine's protective ability.

Table 4. Effect of recombinant FPV/cIFN virus on NVD B1 vaccine induced chick mortality and NDV B1 induced protection from NDV challenge.

Treatment	Vaccine induced mortality.[a]	Challenge induced mortality.[b,c]	Post vaccination anti-NDV antibody responses.	
	Dead/Total	Dead/Total	2 weeks[d]	4 weeks
No treatment	0/25	15/15	<1	<1
NDVB1 alone	7/30	0/12	1.87 (0.31)	2.15 (0.32)
NDVB1 + FPV	9/30	0/10	1.96 (0.54)	1.99 (0.35)
NDVB1 + FPV/cIFN	0/30	0/19	2.00 (0.42)	2.15 (0.37)

a. Mortality was measured 2 weeks post vaccination.

b. Chicks were challenged 4 weeks post vaccination, intramuscularly, with 10,000 ELD_{50}NDV GB-TX.

c. Mortality was measured 2 weeks post challenge

d. Antibody titers were determined by NDV virus neutralization and expressed as group mean (log_{10}).

17-day-old chicken embryos were inoculated with 500 pfu/embryo with FPV/cIFN/NDV virus, FPV wild-type virus or PBS diluent (0.2 ml). Chicks were allowed to hatch and then placed in an isolation unit and observed for mortality for one week. These data show that inoculation of chicken embryos with FPV/cIFN+NDV or FPV wild-type does not interfere with normal hatching.

Table 5. Effect of FPV/cIFN/NDV virus *in ovo*.

Treatment	Number of Eggs Hatched/Total	Mortality (Dead/Total)[a]
Diluent (PBS)	15/17	1/15
FPV (wild-type)	15/17	3/15
FPV/cIFN/NDV	14/18	0/14

[a] 1 week post hatch

Three week old SPF chicks were vaccinated, subcutaneously, with 500 pfu/chick of FPV/cIFN/NDV recombinant virus. Sera were collected 9 days and 28 days post vaccination to measure neutralizing antibody responses raised against NDV. All chickens were challenged 28 days post vaccination with a pathogenic strain of NDV and observed for NDV induced mortality for 15 days. These data show that vaccinated chicks developed detectable anti-NDV antibody responses as little as 9 days post vaccination with FPV/NDV/cIFN recombinant virus. These antibody levels were maintained for at least 28 days. In addition, chickens vaccinated with FPV/cIFN/NDV recombinant virus were all protected against challenge with a virulent strain of NDV.

Table 6. Protective efficacy of FPV/cIFN/NDV vaccine in 3-week-old-chickens.

Vaccine	Post Challenge Mortality[a]	Post Vaccination Antibody Responses	
	Dead/Total	9 days	28 days
None	19/19	< 1[b]	<1[c]
FPV-IFN-NDV	0/20	1.36 (0.12)	1.33 (0.31)

a. Chicks were challenged intramuscularly, 28 days post vaccination, with 10,000 ELD_{50}NDV GB-TX.

b. Antibody responses were determined by VN test and expressed as geometric mean titer (log10) of 5 chickens

c. Antibody responses were determined by VN test and expressed as geometric mean titer (log10) of 10 chickens

One day old SPF chicks were vaccinated, subcutaneously, with 500 pfu/chick of FPV/cIFN/NDV recombinant virus. Chicks were challenged intranasally/intraocularly at 4 , 7 and 15 days post vaccination with virulent NDV (GB-TX), and observed for NDV induced mortality for 15 days in each case. These data show that vaccinated chicks are resistant to virulent NDV when challenged at 7 days post vaccination, but not as early as 4 days post vaccination. Thus, onset of immunity to NDV following vaccination with FPV/cIFN/NDV recombinant virus occurs between 4 and 7 days post vaccination.

Table 6. Protective efficacy of FPV/cIFN/NDV vaccine in one day old chicks.

		Mortality following challenge at 4, 7, and 15 days post vaccination.		
Experiment No.	Vaccine	4-days	7-days	15-days
		Dead/Total	Dead/Total	Dead/Total
1	None	ND[a]	10/10	10/10
	FPV-IFN-NDV	ND	0/10	0/10
2	None	10/10	10/10	10/10
	FPV-IFN-NDV	10/10	1/10	0/10
	NDV-B1	4/10	0/10	0/10

[a] Not Done

Conclusions

1. Recombinant fowlpox viruses express biologically active chicken interferon into the supernatants of infected cells, as measured by protection of CEF cells from VSV infection.

2. Chicken interferon expressed in supernatants from recombinant SPV/cIFN infected cells has been shown to protect CEF cells against infection with HVT in a dose dependent manner.

3. Chicken interferon expressed from SPV/cIFN acted synergistically with LPS to activate chicken macrophages as detected by nitric oxide induction.

4. Recombinant FPV/cIFN virus was found to be safe in 10 day old chicks at a dosage of 6×10^4 pfu/chick.

5. Recombinant FPV/cIFN virus was shown to reduce NDV B1 vaccine induced mortality without affecting the vaccine's ability to protect chicks against NDV infection.

6. Inoculation of recombinant FPV/cIFN/NDV virus *in ovo* does not appear to interfere with normal hatching.

7. Recombinant FPV/cIFN/NDV virus was shown to induce anti-NDV neutralizing antibody in 3-week-old chicks as early as 9 days post vaccination with sustained immunity thru 28 days post vaccination. Furthermore, three-week-old chicks were fully protected against virulent NDV challenge at 28 days post vaccination.

8. Recombinant FPV/cIFN/NDV virus was shown to protect one-day-old chicks from virulent NDV challenge as early as 7 days post vaccination.

9. The foregoing data indicate that recombinant fowlpox viruses expressing chicken IFN may have beneficial applications as immune modulating agents *in vitro*, *in vivo* and *in ovo*.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Mark D. Cochran and David E. Junker

(ii) TITLE OF INVENTION: Recombinant Fowlpox Viruses and Uses Thereof

(iii) NUMBER OF SEQUENCES: 20

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(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:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(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:2:

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(2) INFORMATION FOR SEQ ID NO:3:

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(A) LENGTH: 1507 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: 260..1411

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(A) LENGTH: 383 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

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(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: 300..1568

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: complement (1685..2848)

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

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ATAAGAGGGA AGGAATATAT CTATCTACAT AAACATCATA AGGTTCTTTG ATAGATTTAT 240

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AAT ACT AAA ATT CCT AAT CCC TTG TTA ACA GCT ATC AAA ATA GGA TCA      539
Asn Thr Lys Ile Pro Asn Pro Leu Leu Thr Ala Ile Lys Ile Gly Ser
65                  70                  75                  80

CAC GAT ATA GTA AAA CTG CTG TTG ATT AAC GGA GTT GAT ACT TCT ATT      587
His Asp Ile Val Lys Leu Leu Leu Ile Asn Gly Val Asp Thr Ser Ile
                85                  90                  95

TTG CCA GTC CCC TGC ATA AAT AAA GAA ATG ATA AAA ACT ATA TTA GAT      635
Leu Pro Val Pro Cys Ile Asn Lys Glu Met Ile Lys Thr Ile Leu Asp
            100                 105                 110

AGT GGT GTG AAA GTA AAC ACA AAA AAT GCT AAA TCT AAA ACT TTC TTG      683
Ser Gly Val Lys Val Asn Thr Lys Asn Ala Lys Ser Lys Thr Phe Leu
        115                 120                 125

CAT TAC GCG ATT AAG AAT AAT GAC TTA GAG GTT ATC AAA ATG CTT TTT      731
His Tyr Ala Ile Lys Asn Asn Asp Leu Glu Val Ile Lys Met Leu Phe
    130                 135                 140

GAG TAT GGA GCT GAT GTT AAT ATA AAA GAT GAT AAC ATA TGT TAT TCT      779
Glu Tyr Gly Ala Asp Val Asn Ile Lys Asp Asp Asn Ile Cys Tyr Ser
145                 150                 155                 160

ATA CAC ATA GCT ACT AGG AGT AAT TCA TAT GAA ATC ATA AAA TTA CTA      827
Ile His Ile Ala Thr Arg Ser Asn Ser Tyr Glu Ile Ile Lys Leu Leu
                165                 170                 175

TTA GAA AAA GGT GCT TAT GCA AAC GTA AAA GAC AAT TAT GGT AAT TCT      875
Leu Glu Lys Gly Ala Tyr Ala Asn Val Lys Asp Asn Tyr Gly Asn Ser
            180                 185                 190

CCG TTA CAT AAC GCG GCT AAA TAT GGC GAT TAT GCT TGT ATT AAA TTA      923
Pro Leu His Asn Ala Ala Lys Tyr Gly Asp Tyr Ala Cys Ile Lys Leu
        195                 200                 205

GTT TTA GAC CAT ACT AAT AAC ATA AGC AAT AAG TGC AAC AAC GGT GTT      971
Val Leu Asp His Thr Asn Asn Ile Ser Asn Lys Cys Asn Asn Gly Val
    210                 215                 220

ACA CCG TTA CAT AAC GCT ATA CTA TAT AAT AGA TCT GCC GTA GAA TTA     1019
Thr Pro Leu His Asn Ala Ile Leu Tyr Asn Arg Ser Ala Val Glu Leu
225                 230                 235                 240

CTG ATT AAC AAT CGA TCT ATT AAT GAT ACG GAT GTA GAC GGA TAT ACT     1067
Leu Ile Asn Asn Arg Ser Ile Asn Asp Thr Asp Val Asp Gly Tyr Thr
                245                 250                 255

CCA CTA CAT TAT GCT TTG CAA CCT CCG TGT AGT ATA GAT ATT ATA GAT     1115
Pro Leu His Tyr Ala Leu Gln Pro Pro Cys Ser Ile Asp Ile Ile Asp
            260                 265                 270
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ATA CTA CTA TAT AAC AAC GCC GAT ATA TCT ATA AAA GAT AAT AAC GGA 1163
Ile Leu Leu Tyr Asn Asn Ala Asp Ile Ser Ile Lys Asp Asn Asn Gly
275 280 285

CGC AAT CCT ATC GAT ACG GCG TTT AAG TAT ATT AAC AGA GAT AGC GTT 1211
Arg Asn Pro Ile Asp Thr Ala Phe Lys Tyr Ile Asn Arg Asp Ser Val
290 295 300

ATA AAA GAA CTT CTC CGA AAC GCC GTG TTA ATT AAC GAG GTC GGT AAA 1259
Ile Lys Glu Leu Leu Arg Asn Ala Val Leu Ile Asn Glu Val Gly Lys
305 310 315 320

TTA AAA GAT ACT ACT ATC TTA GAA CAC AAA GAA ATA AAA GAC AAT ACC 1307
Leu Lys Asp Thr Thr Ile Leu Glu His Lys Glu Ile Lys Asp Asn Thr
325 330 335

GTG TTT TCA AAC TTT GTG TAC GAA TGT AAT GAA GAA ATT AAA AAA ATG 1355
Val Phe Ser Asn Phe Val Tyr Glu Cys Asn Glu Glu Ile Lys Lys Met
340 345 350

AAG AAA ACT AAA TGT GTC GGT GAC TAT AGT ATG TTT GAC GTA TAC ATG 1403
Lys Lys Thr Lys Cys Val Gly Asp Tyr Ser Met Phe Asp Val Tyr Met
355 360 365

ATA AGG TAT AAA CAC AAA TAT GAC GGT AAT AAG GAT AGT ATT AAA GAC 1451
Ile Arg Tyr Lys His Lys Tyr Asp Gly Asn Lys Asp Ser Ile Lys Asp
370 375 380

TAT TTG CGT TGT CTT GAT GAT AAT AGT ACT CGT ATG TTA AAA ACT ATA 1499
Tyr Leu Arg Cys Leu Asp Asp Asn Ser Thr Arg Met Leu Lys Thr Ile
385 390 395 400

GAT ATT AAT GAA TTT CCT ATA TAT TCT ATG TAT CTC GTA AGA TGC CTA 1547
Asp Ile Asn Glu Phe Pro Ile Tyr Ser Met Tyr Leu Val Arg Cys Leu
405 410 415

TAT GAT ATG GTA ATA TAT TAAAAGAAAT GGGCTCTTGC ATACATAATC 1595
Tyr Asp Met Val Ile Tyr
420

GGTATAAAAA ATAACGAAAT TATTAGCGGT TACATATCTT ACGGCGGCCG CGGCCCTCGA 1655

GGCCAGTAGC TCAGTATTTC CTATAAACTC TAATATTGAG AGTTTGATAT CCGGAGAAGT 1715

TTAGACCAAC CGCTAGAATC TAATATTTCA TCTAATTTTG ATCTACTTTT TTCTAATATT 1775

TTATGTCTAT TACTGGCTAA GGATATGGAA GTTTTAAGAC GATCTCCGTA ATTATAGAAA 1835

TAGTAAGTAT TAATTTCCTT TATTATAGGA TTATTTACTA AGTGATGTAA CAGGTTCATG 1895

TTTTTACTAA TAACGAATAT ATCTAAAGAG TAAAACATAT TAATACGAAT TTTAGATATA 1955

TCTTTTAGTT CTTCCTTACA ACTCAACCAA ATACTTTTAA ACGTATCATC GCTTTGAATA 2015

ATTTCTCTCA AGGGGTTTAC TTCACTTCTG ATATCGTGAC GTATAAAATC TTGTATACAT 2075

ATATGTGCTA TGATATATCT AAAAGAAAAC ATATTACTGT TAAGGCTCTT ATCGATGACC 2135

CTACTATCTC TAAGTTCAGC ACCATAATGT AATAATATAT TTACTATACC ATGATATTCT 2195

AATGCTATTA ATAAAGGATA TTGATTCCTT ATGTTAATAG CATTTACATC CGCTCCGTTA 2255

TCTAATAACA TTTTTATAAC TTCTGGTTTA CAATTCTTTT TACACGCATA ATGCAACGGA 2315

GTAGATAAGT ATTTGTTTTT AGAATTAACA TTAGCTCCTC TATCTATGAG CGTTTTTACA 2375

CTCATATACG GATTTGTTCC ATATAAGGCA AAATGTAAAA CCGTTCCTAT CTTCTGCGAT 2435

AACGCTTCTA TATCGGCCCC GTAATCTAAA AGAGTGTTTA TGATAACTAC ATTGTTTCTT 2495

ACAGCGGCAT AATGAATAGG CGTCTTGTCA CAATAATCTC TAGCATTTAC GTTCGCTCCC 2555

AATTCTAACA ACGTTATAAC TGTATCTTTA TATCTATCTA GAGTAGAGGC TTGATGTAAT 2615

GGAGTGATAT ACAGACTATC AGCGGCGTTA ACATCTGCAC CCCGCATTAT TAAAGTTCTA 2675

ATGTTTTCTG TATCGTATCC ATTCTTAGCC ATGAGATACA GAGGAGTTTC TCCTTTAATG 2735

TTTTTAGCGT TAACATCTAT TCCTCTTTCC AATAACTTGG GTACTAGTCT ACTTAACGAA 2795

GGTGCTTGTA CCGTGTAATG CAAAGGAGTA TTCTTATAAA CATCTATAGA ATTC 2849

(2) INFORMATION FOR SEQ ID NO:6:

(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:6:

Met Ser Val Asp Trp Arg Thr Glu Ile Tyr Ser Gly Asp Ile Ser Leu
1 5 10 15

Val Glu Lys Leu Ile Lys Asn Lys Gly Asn Cys Ile Asn Ile Ser Val
20 25 30

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

Lys Ile Val Glu Leu Phe Ile Lys His Gly Ala Gln Val Asn His Val
50 55 60

Asn Thr Lys Ile Pro Asn Pro Leu Leu Thr Ala Ile Lys Ile Gly Ser
65 70 75 80

His Asp Ile Val Lys Leu Leu Leu Ile Asn Gly Val Asp Thr Ser Ile
85 90 95

Leu Pro Val Pro Cys Ile Asn Lys Glu Met Ile Lys Thr Ile Leu Asp
100 105 110

Ser Gly Val Lys Val Asn Thr Lys Asn Ala Lys Ser Lys Thr Phe Leu
115 120 125

His Tyr Ala Ile Lys Asn Asn Asp Leu Glu Val Ile Lys Met Leu Phe
130 135 140

Glu Tyr Gly Ala Asp Val Asn Ile Lys Asp Asp Asn Ile Cys Tyr Ser
145 150 155 160

Ile His Ile Ala Thr Arg Ser Asn Ser Tyr Glu Ile Ile Lys Leu Leu
165 170 175

Leu Glu Lys Gly Ala Tyr Ala Asn Val Lys Asp Asn Tyr Gly Asn Ser
180 185 190

Pro Leu His Asn Ala Ala Lys Tyr Gly Asp Tyr Ala Cys Ile Lys Leu
195 200 205

Val Leu Asp His Thr Asn Asn Ile Ser Asn Lys Cys Asn Asn Gly Val
210 215 220

Thr Pro Leu His Asn Ala Ile Leu Tyr Asn Arg Ser Ala Val Glu Leu
225 230 235 240

Leu Ile Asn Asn Arg Ser Ile Asn Asp Thr Asp Val Asp Gly Tyr Thr
245 250 255

Pro Leu His Tyr Ala Leu Gln Pro Pro Cys Ser Ile Asp Ile Ile Asp
260 265 270

Ile Leu Leu Tyr Asn Asn Ala Asp Ile Ser Ile Lys Asp Asn Asn Gly
275 280 285

Arg Asn Pro Ile Asp Thr Ala Phe Lys Tyr Ile Asn Arg Asp Ser Val
290 295 300

Ile Lys Glu Leu Leu Arg Asn Ala Val Leu Ile Asn Glu Val Gly Lys
305 310 315 320

Leu Lys Asp Thr Thr Ile Leu Glu His Lys Glu Ile Lys Asp Asn Thr
325 330 335

Val Phe Ser Asn Phe Val Tyr Glu Cys Asn Glu Glu Ile Lys Lys Met
340 345 350

Lys Lys Thr Lys Cys Val Gly Asp Tyr Ser Met Phe Asp Val Tyr Met
355 360 365

Ile Arg Tyr Lys His Lys Tyr Asp Gly Asn Lys Asp Ser Ile Lys Asp
370 375 380

Tyr Leu Arg Cys Leu Asp Asp Asn Ser Thr Arg Met Leu Lys Thr Ile
385 390 395 400

Asp Ile Asn Glu Phe Pro Ile Tyr Ser Met Tyr Leu Val Arg Cys Leu
405 410 415

Tyr Asp Met Val Ile Tyr
420

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Asn Ser Ile Asp Val Tyr Lys Asn Thr Pro Leu His Tyr Thr Val Gln
1 5 10 15

Ala Pro Ser Leu Ser Arg Leu Val Pro Lys Leu Leu Glu Arg Gly Ile
20 25 30

Asp Val Asn Ala Lys Asn Ile Lys Gly Glu Thr Pro Leu Tyr Leu Met
35 40 45

Ala Lys Asn Gly Tyr Asp Thr Glu Asn Ile Arg Thr Leu Ile Met Arg
50 55 60

Gly Ala Asp Val Asn Ala Ala Asp Ser Leu Tyr Ile Thr Pro Leu His
65 70 75 80

Gln Ala Ser Thr Leu Asp Arg Tyr Lys Asp Thr Val Ile Thr Leu Leu
85 90 95

Glu Leu Gly Ala Asn Val Asn Ala Arg Asp Tyr Cys Asp Lys Thr Pro
100 105 110

Ile His Tyr Ala Ala Val Arg Asn Asn Val Val Ile Ile Asn Thr Leu
115 120 125

Leu Asp Tyr Gly Ala Asp Ile Glu Ala Leu Ser Gln Lys Ile Gly Thr
130 135 140

Val Leu His Phe Ala Leu Tyr Gly Thr Asn Pro Tyr Met Ser Val Lys
145 150 155 160

Thr Leu Ile Asp Arg Gly Ala Asn Val Asn Ser Lys Asn Lys Tyr Leu
165 170 175

Ser Thr Pro Leu His Tyr Ala Cys Lys Lys Asn Cys Lys Pro Glu Val
180 185 190

Ile Lys Met Leu Leu Asp Asn Gly Ala Asp Val Asn Ala Ile Asn Ile
195 200 205

Arg Asn Gln Tyr Pro Leu Leu Ile Ala Leu Glu Tyr His Gly Ile Val
210 215 220

Asn Ile Leu Leu His Tyr Gly Ala Glu Leu Arg Asp Ser Arg Val Ile
225 230 235 240

Asp Lys Ser Leu Asn Ser Asn Met Phe Ser Phe Arg Tyr Ile Ile Ala
245 250 255

His Ile Cys Ile Gln Asp Phe Ile Arg His Asp Ile Arg Ser Glu Val
260 265 270

Asn Pro Leu Arg Glu Ile Ile Gln Ser Asp Asp Thr Phe Lys Ser Ile
275 280 285

Trp Leu Ser Cys Lys Glu Glu Leu Lys Asp Ile Ser Lys Ile Arg Ile
290 295 300

Asn Met Phe Tyr Ser Leu Asp Ile Phe Val Ile Ser Lys Asn Met Asn
305 310 315 320

Leu Leu His His Leu Val Asn Asn Pro Ile Ile Lys Glu Ile Asn Thr
325 330 335

Tyr Tyr Phe Tyr Asn Tyr Gly Asp Arg Leu Lys Thr Ser Ile Ser Leu
340 345 350

Ala Ser Asn Arg His Lys Ile Leu Glu Lys Ser Arg Ser Lys Leu Asp
355 360 365

Glu Ile Leu Asp Ser Ser Gly Trp Ser Lys Leu Leu Arg Ile Ser Asn
370 375 380

Ser Gln Tyr *
385

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 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:8:

AAAAATTGAA AAACTATTCT AATTTATTGC ACGGAGATCT 40

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 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:9:

AATTTCATTT TGTTTTTTTC TATGCTATAA AT 32

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 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:10:

GTATCCTAAA ATTGAATTGT AATTATCGAT AATAAAT 37

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 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:11:

TTTTTTTTTT TTTTTTTTTT GGCATATAAA TGAATTCGGA TC 42

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4177 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: 115..1860

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2095..3756

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

CATACTGGCC TCGAGGGCCG CGGCCGCCTG CAGGTCGACT CTAGAAAAAA TTGAAAAACT 60

ATTCTAATTT ATTGCACGGA GATCTTTTTT TTTTTTTTTT TTTTTGGCAT ATAA ATG 117
Met
1

AAT TCG GAT CCG GAC CGC GCC GTT AGC CAA GTT GCG TTA GAG AAT GAT 165
Asn Ser Asp Pro Asp Arg Ala Val Ser Gln Val Ala Leu Glu Asn Asp
5 10 15

GAA AGA GAG GCA AAA AAT ACA TGG CGC TTG ATA TTC CGG ATT GCA ATC 213
Glu Arg Glu Ala Lys Asn Thr Trp Arg Leu Ile Phe Arg Ile Ala Ile
20 25 30

TTA TTC TTA ACA GTA GTG ACC TTG GCT ATA TCT GTA GCC TCC CTT TTA 261
Leu Phe Leu Thr Val Val Thr Leu Ala Ile Ser Val Ala Ser Leu Leu
35 40 45

TAT AGC ATG GGG GCT AGC ACA CCT AGC GAT CTT GTA GGC ATA CCG ACT 309
Tyr Ser Met Gly Ala Ser Thr Pro Ser Asp Leu Val Gly Ile Pro Thr
50 55 60 65

AGG ATT TCC AGG GCA GAA GAA AAG ATT ACA TCT ACA CTT GGT TCC AAT 357
Arg Ile Ser Arg Ala Glu Glu Lys Ile Thr Ser Thr Leu Gly Ser Asn
70 75 80

CAA GAT GTA GTA GAT AGG ATA TAT AAG CAA GTG GCC CTT GAG TCT CCA 405
Gln Asp Val Val Asp Arg Ile Tyr Lys Gln Val Ala Leu Glu Ser Pro
85 90 95

TTG GCA TTG TTA AAT ACT GAG ACC ACA ATT ATG AAC GCA ATA ACA TCT 453
Leu Ala Leu Leu Asn Thr Glu Thr Thr Ile Met Asn Ala Ile Thr Ser
100 105 110

CTC TCT TAT CAG ATT AAT GGA GCT GCA AAC AAC AGC GGG TGG GGG GCA 501
Leu Ser Tyr Gln Ile Asn Gly Ala Ala Asn Asn Ser Gly Trp Gly Ala
115 120 125

CCT ATT CAT GAC CCA GAT TAT ATA GGG GGG ATA GGC AAA GAA CTC ATT 549
Pro Ile His Asp Pro Asp Tyr Ile Gly Gly Ile Gly Lys Glu Leu Ile
130 135 140 145

GTA GAT GAT GCT AGT GAT GTC ACA TCA TTC TAT CCC TCT GCA TTT CAA 597
Val Asp Asp Ala Ser Asp Val Thr Ser Phe Tyr Pro Ser Ala Phe Gln
150 155 160

GAA CAT CTG AAT TTT ATC CCG GCG CCT ACT ACA GGA TCA GGT TGC ACT 645
Glu His Leu Asn Phe Ile Pro Ala Pro Thr Thr Gly Ser Gly Cys Thr
165 170 175

CGA ATA CCC TCA TTT GAC ATG AGT GCT ACC CAT TAC TGC TAC ACC CAT 693
Arg Ile Pro Ser Phe Asp Met Ser Ala Thr His Tyr Cys Tyr Thr His
180 185 190

AAT GTA ATA TTG TCT GGA TGC AGA GAT CAC TCA CAC TCA CAT CAG TAT 741
Asn Val Ile Leu Ser Gly Cys Arg Asp His Ser His Ser His Gln Tyr
195 200 205

TTA GCA CTT GGT GTG CTC CGG ACA TCT GCA ACA GGG AGG GTA TTC TTT 789
Leu Ala Leu Gly Val Leu Arg Thr Ser Ala Thr Gly Arg Val Phe Phe
210 215 220 225

TCT ACT CTG CGT TCC ATC AAC CTG GAC GAC ACC CAA AAT CGG AAG TCT 837
Ser Thr Leu Arg Ser Ile Asn Leu Asp Asp Thr Gln Asn Arg Lys Ser
230 235 240

TGC AGT GTG AGT GCA ACT CCC CTG GGT TGT GAT ATG CTG TGC TCG AAA 885
Cys Ser Val Ser Ala Thr Pro Leu Gly Cys Asp Met Leu Cys Ser Lys
245 250 255

GCC ACG GAG ACA GAG GAA GAA GAT TAT AAC TCA GCT GTC CCT ACG CGG 933
Ala Thr Glu Thr Glu Glu Glu Asp Tyr Asn Ser Ala Val Pro Thr Arg
260 265 270

ATG GTA CAT GGG AGG TTA GGG TTC GAC GGC CAA TAT CAC GAA AAG GAC 981
Met Val His Gly Arg Leu Gly Phe Asp Gly Gln Tyr His Glu Lys Asp
275 280 285

CTA GAT GTC ACA ACA TTA TTC GGG GAC TGG GTG GCC AAC TAC CCA GGA 1029
Leu Asp Val Thr Thr Leu Phe Gly Asp Trp Val Ala Asn Tyr Pro Gly
290 295 300 305

GTA GGG GGT GGA TCT TTT ATT GAC AGC CGC GTG TGG TTC TCA GTC TAC 1077
Val Gly Gly Gly Ser Phe Ile Asp Ser Arg Val Trp Phe Ser Val Tyr
310 315 320

GGA GGG TTA AAA CCC AAT ACA CCC AGT GAC ACT GTA CAG GAA GGG AAA 1125
Gly Gly Leu Lys Pro Asn Thr Pro Ser Asp Thr Val Gln Glu Gly Lys
325 330 335

TAT GTG ATA TAC AAG CGA TAC AAT GAC ACA TGC CCA GAT GAG CAA GAC 1173
Tyr Val Ile Tyr Lys Arg Tyr Asn Asp Thr Cys Pro Asp Glu Gln Asp
340 345 350

TAC CAG ATT CGA ATG GCC AAG TCT TCG TAT AAG CCT GGA CGG TTT GGT 1221
Tyr Gln Ile Arg Met Ala Lys Ser Ser Tyr Lys Pro Gly Arg Phe Gly
355 360 365

GGG AAA CGC ATA CAG CAG GCT ATC TTA TCT ATC AAA GTG TCA ACA TCC 1269
Gly Lys Arg Ile Gln Gln Ala Ile Leu Ser Ile Lys Val Ser Thr Ser
370 375 380 385

TTA GGC GAA GAC CCG GTA CTG ACT GTA CCG CCC AAC ACA GTC ACA CTC 1317
Leu Gly Glu Asp Pro Val Leu Thr Val Pro Pro Asn Thr Val Thr Leu
390 395 400

ATG GGG GCC GAA GGC AGA ATT CTC ACA GTA GGG ACA TCC CAT TTC TTG 1365
Met Gly Ala Glu Gly Arg Ile Leu Thr Val Gly Thr Ser His Phe Leu
405 410 415

TAT CAG CGA GGG TCA TCA TAC TTC TCT CCC GCG TTA TTA TAT CCT ATG 1413
Tyr Gln Arg Gly Ser Ser Tyr Phe Ser Pro Ala Leu Leu Tyr Pro Met
420 425 430

ACA GTC AGC AAC AAA ACA GCC ACT CTT CAT AGT CCT TAT ACA TTC AAT 1461
Thr Val Ser Asn Lys Thr Ala Thr Leu His Ser Pro Tyr Thr Phe Asn
435 440 445

GCC TTC ACT CGG CCA GGT AGT ATC CCT TGC CAG GCT TCA GCA AGA TGC 1509
Ala Phe Thr Arg Pro Gly Ser Ile Pro Cys Gln Ala Ser Ala Arg Cys
450 455 460 465

CCC AAC TCA TGT GTT ACT GGA GTC TAT ACA GAT CCA TAT CCC CTA ATC 1557
Pro Asn Ser Cys Val Thr Gly Val Tyr Thr Asp Pro Tyr Pro Leu Ile
470 475 480

TTC TAT AGA AAC CAC ACC TTG CGA GGG GTA TTC GGG ACA ATG CTT GAT 1605
Phe Tyr Arg Asn His Thr Leu Arg Gly Val Phe Gly Thr Met Leu Asp
485 490 495

GGT GAA CAA GCA AGA CTT AAC CCT GCG TCT GCA GTA TTC GAT AGC ACA 1653
Gly Glu Gln Ala Arg Leu Asn Pro Ala Ser Ala Val Phe Asp Ser Thr
500 505 510

TCC CGC AGT CGC ATA ACT CGA GTG AGT TCA AGC AGC ATC AAA GCA GCA 1701
Ser Arg Ser Arg Ile Thr Arg Val Ser Ser Ser Ser Ile Lys Ala Ala
515 520 525

TAC ACA ACA TCA ACT TGT TTT AAA GTG GTC AAG ACC AAT AAG ACC TAT 1749
Tyr Thr Thr Ser Thr Cys Phe Lys Val Val Lys Thr Asn Lys Thr Tyr
530 535 540 545

TGT CTC AGC ATT GCT GAA ATA TCT AAT ACT CTC TTC GGA GAA TTC AGA 1797
Cys Leu Ser Ile Ala Glu Ile Ser Asn Thr Leu Phe Gly Glu Phe Arg
550 555 560

ATC GTC CCG TTA CTA GTT GAG ATC CTC AAA GAT GAC GGG GTT AGA GAA 1845
Ile Val Pro Leu Leu Val Glu Ile Leu Lys Asp Asp Gly Val Arg Glu
565 570 575

GCC AGG TCT GGC TAGTTGAGTC AACTATGAAA GAGTTGGAAA GATGGCATTG 1897
Ala Arg Ser Gly
580

TATCACCTAT CTTCTGCGAC ATCAAGAATC AAACCGAATG CCCGGATCCA TAATTAATTA 1957

ATTAATTTTT ATCCCTCGAC TCTAGAAAAA ATTGAAAAAC TATTCTAATT TATTGCACGG 2017

AGATCTTTTT TTTTTTTTTT TTTTTTGGCA TATAAATGAA TTCGGATCGA TCCCGGTTGG 2077

CGCCCTCCAG GTGCAGG ATG GGC TCC AGA CCT TCT ACC AAG AAC CCA GCA 2127
Met Gly Ser Arg Pro Ser Thr Lys Asn Pro Ala
1 5 10

CCT ATG ATG CTG ACT ATC CGG GTC GCG CTG GTA CTG AGT TGC ATC TGT 2175
Pro Met Met Leu Thr Ile Arg Val Ala Leu Val Leu Ser Cys Ile Cys
15 20 25

CCG GCA AAC TCC ATT GAT GGC AGG CCT CTT GCA GCT GCA GGA ATT GTG 2223
Pro Ala Asn Ser Ile Asp Gly Arg Pro Leu Ala Ala Ala Gly Ile Val
30 35 40

GTT ACA GGA GAC AAA GCA GTC AAC ATA TAC ACC TCA TCC CAG ACA GGA 2271
Val Thr Gly Asp Lys Ala Val Asn Ile Tyr Thr Ser Ser Gln Thr Gly
45 50 55

TCA ATC ATA GTT AAG CTC CTC CCG AAT CTG CCA AAG GAT AAG GAG GCA 2319
Ser Ile Ile Val Lys Leu Leu Pro Asn Leu Pro Lys Asp Lys Glu Ala
60 65 70 75

TGT GCG AAA GCC CCC TTG GAT GCA TAC AAC AGG ACA TTG ACC ACT TTG 2367
Cys Ala Lys Ala Pro Leu Asp Ala Tyr Asn Arg Thr Leu Thr Thr Leu
80 85 90

CTC ACC CCC CTT GGT GAC TCT ATC CGT AGG ATA CAA GAG TCT GTG ACT 2415
Leu Thr Pro Leu Gly Asp Ser Ile Arg Arg Ile Gln Glu Ser Val Thr
95 100 105

ACA TCT GGA GGG GGG AGA CAG GGG CGC CTT ATA GGC GCC ATT ATT GGC 2463
Thr Ser Gly Gly Gly Arg Gln Gly Arg Leu Ile Gly Ala Ile Ile Gly
110 115 120

GGT GTG GCT CTT GGG GTT GCA ACT GCC GCA CAA ATA ACA GCG GCC GCA 2511
Gly Val Ala Leu Gly Val Ala Thr Ala Ala Gln Ile Thr Ala Ala Ala
125 130 135

GCT CTG ATA CAA GCC AAA CAA AAT GCT GCC AAC ATC CTC CGA CTT AAA 2559
Ala Leu Ile Gln Ala Lys Gln Asn Ala Ala Asn Ile Leu Arg Leu Lys
140 145 150 155

GAG AGC ATT GCC GCA ACC AAT GAG GCT GTG CAT GAG GTC ACT GAC GGA 2607
Glu Ser Ile Ala Ala Thr Asn Glu Ala Val His Glu Val Thr Asp Gly
160 165 170

TTA TCG CAA CTA GCA GTG GCA GTT GGG AAG ATG CAG CAG TTC GTT AAT 2655
Leu Ser Gln Leu Ala Val Ala Val Gly Lys Met Gln Gln Phe Val Asn
175 180 185

GAC CAA TTT AAT AAA ACA GCT CAG GAA TTA GAC TGC ATC AAA ATT GCA 2703
Asp Gln Phe Asn Lys Thr Ala Gln Glu Leu Asp Cys Ile Lys Ile Ala
190 195 200

CAG CAA GTT GGT GTA GAG CTC AAC CTG TAC CTA ACC GAA TCG ACT ACA 2751
Gln Gln Val Gly Val Glu Leu Asn Leu Tyr Leu Thr Glu Ser Thr Thr
205 210 215

GTA TTC GGA CCA CAA ATC ACT TCA CCT GCC TTA AAC AAG CTG ACT ATT 2799
Val Phe Gly Pro Gln Ile Thr Ser Pro Ala Leu Asn Lys Leu Thr Ile
220 225 230 235

CAG GCA CTT TAC AAT CTA GCT GGT GGG AAT ATG GAT TAC TTA TTG ACT 2847
Gln Ala Leu Tyr Asn Leu Ala Gly Gly Asn Met Asp Tyr Leu Leu Thr
240 245 250

AAG TTA GGT ATA GGG AAC AAT CAA CTC AGC TCA TTA ATC GGT AGC GGC 2895
Lys Leu Gly Ile Gly Asn Asn Gln Leu Ser Ser Leu Ile Gly Ser Gly
255 260 265

TTA ATC ACC GGT AAC CCT ATT CTA TAC GAC TCA CAG ACT CAA CTC TTG 2943
Leu Ile Thr Gly Asn Pro Ile Leu Tyr Asp Ser Gln Thr Gln Leu Leu
270 275 280

GGT ATA CAG GTA ACT CTA CCT TCA GTC GGG AAC CTA AAT AAT ATG CGT 2991
Gly Ile Gln Val Thr Leu Pro Ser Val Gly Asn Leu Asn Asn Met Arg
285 290 295

GCC ACC TAC TTG GAA ACC TTA TCC GTA AGC ACA ACC AGG GGA TTT GCC 3039
Ala Thr Tyr Leu Glu Thr Leu Ser Val Ser Thr Thr Arg Gly Phe Ala
300 305 310 315

TCG GCA CTT GTC CCA AAA GTG GTG ACA CGG GTC GGT TCT GTG ATA GAA 3087
Ser Ala Leu Val Pro Lys Val Val Thr Arg Val Gly Ser Val Ile Glu
320 325 330

GAA CTT GAC ACC TCA TAC TGT ATA GAA ACT GAC TTA GAT TTA TAT TGT 3135
Glu Leu Asp Thr Ser Tyr Cys Ile Glu Thr Asp Leu Asp Leu Tyr Cys
335 340 345

ACA AGA ATA GTA ACG TTC CCT ATG TCC CCT GGT ATT TAC TCC TGC TTG 3183
Thr Arg Ile Val Thr Phe Pro Met Ser Pro Gly Ile Tyr Ser Cys Leu
350 355 360

AGC GGC AAT ACA TCG GCC TGT ATG TAC TCA AAG ACC GAA GGC GCA CTT 3231
Ser Gly Asn Thr Ser Ala Cys Met Tyr Ser Lys Thr Glu Gly Ala Leu
365 370 375

ACT ACA CCA TAT ATG ACT ATC AAA GGC TCA GTC ATC GCT AAC TGC AAG 3279
Thr Thr Pro Tyr Met Thr Ile Lys Gly Ser Val Ile Ala Asn Cys Lys
380 385 390 395

ATG ACA ACA TGT AGA TGT GTA AAC CCC CCG GGT ATC ATA TCG CAA AAC 3327
Met Thr Thr Cys Arg Cys Val Asn Pro Pro Gly Ile Ile Ser Gln Asn
400 405 410

TAT GGA GAA GCC GTG TCT CTA ATA GAT AAA CAA TCA TGC AAT GTT TTA 3375
Tyr Gly Glu Ala Val Ser Leu Ile Asp Lys Gln Ser Cys Asn Val Leu
415 420 425

TCC TTA GGC GGG ATA ACT TTA AGG CTC AGT GGG GAA TTC GAT GTA ACT 3423
Ser Leu Gly Gly Ile Thr Leu Arg Leu Ser Gly Glu Phe Asp Val Thr
430 435 440

TAT CAG AAG AAT ATC TCA ATA CAA GAT TCT CAA GTA ATA ATA ACA GGC 3471
Tyr Gln Lys Asn Ile Ser Ile Gln Asp Ser Gln Val Ile Ile Thr Gly
445 450 455

AAT CTT GAT ATC TCA ACT GAG CTT GGG AAT GTC AAC AAC TCG ATC AGT 3519
Asn Leu Asp Ile Ser Thr Glu Leu Gly Asn Val Asn Asn Ser Ile Ser
460 465 470 475

AAT GCC TTG AAT AAG TTA GAG GAA AGC AAC AGA AAA CTA GAC AAA GTC 3567
Asn Ala Leu Asn Lys Leu Glu Glu Ser Asn Arg Lys Leu Asp Lys Val
480 485 490

AAT GTC AAA CTG ACC AGC ACA TCT GCT CTC ATT ACC TAT ATC GTT TTG 3615
Asn Val Lys Leu Thr Ser Thr Ser Ala Leu Ile Thr Tyr Ile Val Leu
495 500 505

ACT ATC ATA TCT CTT GTT TTT GGT ATA CTT AGC CTG ATT CTA GCA TGC 3663
Thr Ile Ile Ser Leu Val Phe Gly Ile Leu Ser Leu Ile Leu Ala Cys
510 515 520

TAC CTA ATG TAC AAG CAA AAG GCG CAA CAA AAG ACC TTA TTA TGG CTT 3711
Tyr Leu Met Tyr Lys Gln Lys Ala Gln Gln Lys Thr Leu Leu Trp Leu
525 530 535

GGG AAT AAT ACC CTA GAT CAG ATG AGA GCC ACT ACA AAA ATG TGAACACAGA 3763
Gly Asn Asn Thr Leu Asp Gln Met Arg Ala Thr Thr Lys Met
540 545 550

TGAGGAACGA AGGTTTCCCT AATAGTAATT TGTGTGAAAG TTCTGGTAGT CTGTCAGTTC 3823

GGAGAGTTAA GAAAAAAAAA AAACCCCCCC CCCCCCCCCC CCCCCCCCCT GCAGGCATCG 3883

TGGTGTCACG CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA CGATCAAGGC 3943

GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG 4003

TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT 4063

CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC TGGTGAGTGA TCCATAATTA 4123

ATTAATTAAT TTTTATCCCG GGTCGACCTG CAGGCGGCCG CGGCCCTCGA GGCC 4177

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 581 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Asn Ser Asp Pro Asp Arg Ala Val Ser Gln Val Ala Leu Glu Asn
1 5 10 15

Asp Glu Arg Glu Ala Lys Asn Thr Trp Arg Leu Ile Phe Arg Ile Ala
20 25 30

Ile Leu Phe Leu Thr Val Val Thr Leu Ala Ile Ser Val Ala Ser Leu
35 40 45

Leu Tyr Ser Met Gly Ala Ser Thr Pro Ser Asp Leu Val Gly Ile Pro
50 55 60

Thr Arg Ile Ser Arg Ala Glu Glu Lys Ile Thr Ser Thr Leu Gly Ser
65 70 75 80

Asn Gln Asp Val Val Asp Arg Ile Tyr Lys Gln Val Ala Leu Glu Ser
85 90 95

Pro Leu Ala Leu Leu Asn Thr Glu Thr Thr Ile Met Asn Ala Ile Thr
100 105 110

Ser Leu Ser Tyr Gln Ile Asn Gly Ala Ala Asn Asn Ser Gly Trp Gly
115 120 125

Ala Pro Ile His Asp Pro Asp Tyr Ile Gly Gly Ile Gly Lys Glu Leu
130 135 140

Ile Val Asp Asp Ala Ser Asp Val Thr Ser Phe Tyr Pro Ser Ala Phe
145 150 155 160

Gln Glu His Leu Asn Phe Ile Pro Ala Pro Thr Thr Gly Ser Gly Cys
165 170 175

Thr Arg Ile Pro Ser Phe Asp Met Ser Ala Thr His Tyr Cys Tyr Thr
180 185 190

His Asn Val Ile Leu Ser Gly Cys Arg Asp His Ser His Ser His Gln
195 200 205

Tyr Leu Ala Leu Gly Val Leu Arg Thr Ser Ala Thr Gly Arg Val Phe
210 215 220

Phe Ser Thr Leu Arg Ser Ile Asn Leu Asp Asp Thr Gln Asn Arg Lys
225 230 235 240

Ser Cys Ser Val Ser Ala Thr Pro Leu Gly Cys Asp Met Leu Cys Ser
245 250 255

Lys Ala Thr Glu Thr Glu Glu Glu Asp Tyr Asn Ser Ala Val Pro Thr
260 265 270

Arg Met Val His Gly Arg Leu Gly Phe Asp Gly Gln Tyr His Glu Lys
275 280 285

Asp Leu Asp Val Thr Thr Leu Phe Gly Asp Trp Val Ala Asn Tyr Pro
290 295 300

Gly Val Gly Gly Gly Ser Phe Ile Asp Ser Arg Val Trp Phe Ser Val
305 310 315 320

Tyr Gly Gly Leu Lys Pro Asn Thr Pro Ser Asp Thr Val Gln Glu Gly
325 330 335

Lys Tyr Val Ile Tyr Lys Arg Tyr Asn Asp Thr Cys Pro Asp Glu Gln
340 345 350

Asp Tyr Gln Ile Arg Met Ala Lys Ser Ser Tyr Lys Pro Gly Arg Phe
355 360 365

Gly Gly Lys Arg Ile Gln Gln Ala Ile Leu Ser Ile Lys Val Ser Thr
370 375 380

Ser Leu Gly Glu Asp Pro Val Leu Thr Val Pro Pro Asn Thr Val Thr
385 390 395 400

Leu Met Gly Ala Glu Gly Arg Ile Leu Thr Val Gly Thr Ser His Phe
405 410 415

Leu Tyr Gln Arg Gly Ser Ser Tyr Phe Ser Pro Ala Leu Leu Tyr Pro
420 425 430

Met Thr Val Ser Asn Lys Thr Ala Thr Leu His Ser Pro Tyr Thr Phe
435 440 445

Asn Ala Phe Thr Arg Pro Gly Ser Ile Pro Cys Gln Ala Ser Ala Arg
450 455 460

Cys Pro Asn Ser Cys Val Thr Gly Val Tyr Thr Asp Pro Tyr Pro Leu
465 470 475 480

Ile Phe Tyr Arg Asn His Thr Leu Arg Gly Val Phe Gly Thr Met Leu
485 490 495

Asp Gly Glu Gln Ala Arg Leu Asn Pro Ala Ser Ala Val Phe Asp Ser
500 505 510

Thr Ser Arg Ser Arg Ile Thr Arg Val Ser Ser Ser Ser Ile Lys Ala
515 520 525

Ala Tyr Thr Thr Ser Thr Cys Phe Lys Val Val Lys Thr Asn Lys Thr
530 535 540

Tyr Cys Leu Ser Ile Ala Glu Ile Ser Asn Thr Leu Phe Gly Glu Phe
545 550 555 560

Arg Ile Val Pro Leu Leu Val Glu Ile Leu Lys Asp Asp Gly Val Arg
565 570 575

Glu Ala Arg Ser Gly
580

(2) INFORMATION FOR SEQ ID NO:14:

(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:14:

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

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

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:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182 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:15:

GGCCTCGAGG GCCGCGGCCG CCTGCAGGTC GACTCTAGAA AAAATTGAAA AACTATTCTA 60

ATTTATTGCA CGGAGATCTT TTTTTTTTTT TTTTTTTTTG GCATATAAAT GAATTCGGAT 120

CCGGACCGCG CCGTTAGCCA AGTTGCGTTA GAGAATGATG AAAGAGAGGC AAAAAATACA 180

TG 182

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 178 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:16:

ATCTTCTGCG ACATCAAGAA TCAAACCGAA TGCCCGGATC CATAATTAAT TAATTAATTT 60

TTATCCCTCG ACTCTAGAAA AAATTGAAAA ACTATTCTAA TTTATTGCAC GGAGATCTTT 120

TTTTTTTTTT TTTTTTTTGG CATATAAATG AATTCGGATC GATCCCGGTT GGCGCCCT 178

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 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:17:

AAAAACCCCC CCCCCCCCCC CCCCCCCCCC CTGCAGGCAT CGTGGTGTCA CGCTCGTCGT 60

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 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:

ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTTC TGTGACTGGT GAGTGATCCA 60

TAATTAATTA ATTAATTTTT ATCCCGGGTC GACCTGCAGG CGGCCGCGGC CCTCGAGGCC 120

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1305 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..1305

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

```
ATG CAC CGT CCT CAT CTC AGA CGG CAC TCG CGT TAC TAC GCG AAA GGA      48
Met His Arg Pro His Leu Arg Arg His Ser Arg Tyr Tyr Ala Lys Gly
 1               5                  10                  15

GAG GTG CTT AAC AAA CAC ATG GAT TGC GGT GGA AAA CGG TGC TGC TCA      96
Glu Val Leu Asn Lys His Met Asp Cys Gly Gly Lys Arg Cys Cys Ser
            20                  25                  30

GGC GCA GCT GTA TTC ACT CTT TTC TGG ACT TGT GTC AGG ATT ATG CGG     144
Gly Ala Ala Val Phe Thr Leu Phe Trp Thr Cys Val Arg Ile Met Arg
        35                  40                  45

GAG CAT ATC TGC TTT GTA CGC AAC GCT ATG GAC CGC CAT TTA TTT TTG     192
Glu His Ile Cys Phe Val Arg Asn Ala Met Asp Arg His Leu Phe Leu
    50                  55                  60

AGG AAT GCT TTT TGG ACT ATC GTA CTG CTT TCT TCC TTC GCT AGC CAG     240
Arg Asn Ala Phe Trp Thr Ile Val Leu Leu Ser Ser Phe Ala Ser Gln
65                  70                  75                  80

AGC ACC GCC GCC GTC ACG TAC GAC TAC ATT TTA GGC CGT CGC GCG CTC     288
Ser Thr Ala Ala Val Thr Tyr Asp Tyr Ile Leu Gly Arg Arg Ala Leu
                85                  90                  95

GAC GCG CTA ACC ATA CCG GCG GTT GGC CCG TAT AAC AGA TAC CTC ACT     336
Asp Ala Leu Thr Ile Pro Ala Val Gly Pro Tyr Asn Arg Tyr Leu Thr
            100                 105                 110

AGG GTA TCA AGA GGC TGC GAC GTT GTC GAG CTC AAC CCG ATT TCT AAC     384
Arg Val Ser Arg Gly Cys Asp Val Val Glu Leu Asn Pro Ile Ser Asn
        115                 120                 125

GTG GAC GAC ATG ATA TCG GCG GCC AAA GAA AAA GAG AAG GGG GGC CCT     432
Val Asp Asp Met Ile Ser Ala Ala Lys Glu Lys Glu Lys Gly Gly Pro
    130                 135                 140

TTC GAG GCC TCC GTC GTC TGG TTC TAC GTG ATT AAG GGC GAC GAC GGC     480
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Phe Glu Ala Ser Val Val Trp Phe Tyr Val Ile Lys Gly Asp Asp Gly
145                 150                 155                 160

GAG GAC AAG TAC TGT CCA ATC TAT AGA AAA GAG TAC AGG GAA TGT GGC      528
Glu Asp Lys Tyr Cys Pro Ile Tyr Arg Lys Glu Tyr Arg Glu Cys Gly
                165                 170                 175

GAC GTA CAA CTG CTA TCT GAA TGC GCC GTT CAA TCT GCA CAG ATG TGG      576
Asp Val Gln Leu Leu Ser Glu Cys Ala Val Gln Ser Ala Gln Met Trp
            180                 185                 190

GCA GTG GAC TAT GTT CCT AGC ACC CTT GTA TCG CGA AAT GGC GCG GGA      624
Ala Val Asp Tyr Val Pro Ser Thr Leu Val Ser Arg Asn Gly Ala Gly
        195                 200                 205

CTG ACT ATA TTC TCC CCC ACT GCT GCG CTC TCT GGC CAA TAC TTG CTG      672
Leu Thr Ile Phe Ser Pro Thr Ala Ala Leu Ser Gly Gln Tyr Leu Leu
    210                 215                 220

ACC CTG AAA ATC GGG AGA TTT GCG CAA ACA GCT CTC GTA ACT CTA GAA      720
Thr Leu Lys Ile Gly Arg Phe Ala Gln Thr Ala Leu Val Thr Leu Glu
225                 230                 235                 240

GTT AAC GAT CGC TGT TTA AAG ATC GGG TCG CAG CTT AAC TTT TTA CCG      768
Val Asn Asp Arg Cys Leu Lys Ile Gly Ser Gln Leu Asn Phe Leu Pro
                245                 250                 255

TCG AAA TGC TGG ACA ACA GAA CAG TAT CAG ACT GGA TTT CAA GGC GAA      816
Ser Lys Cys Trp Thr Thr Glu Gln Tyr Gln Thr Gly Phe Gln Gly Glu
            260                 265                 270

CAC CTT TAT CCG ATC GCA GAC ACC AAT ACA CGA CAC GCG GAC GAC GTA      864
His Leu Tyr Pro Ile Ala Asp Thr Asn Thr Arg His Ala Asp Asp Val
        275                 280                 285

TAT CGG GGA TAC GAA GAT ATT CTG CAG CGC TGG AAT AAT TTG CTG AGG      912
Tyr Arg Gly Tyr Glu Asp Ile Leu Gln Arg Trp Asn Asn Leu Leu Arg
    290                 295                 300

AAA AAG AAT CCT AGC GCG CCA GAC CCT CGT CCA GAT AGC GTC CCG CAA      960
Lys Lys Asn Pro Ser Ala Pro Asp Pro Arg Pro Asp Ser Val Pro Gln
305                 310                 315                 320

GAA ATT CCC GCT GTA ACC AAG AAA GCG GAA GGG CGC ACC CCG GAC GCA     1008
Glu Ile Pro Ala Val Thr Lys Lys Ala Glu Gly Arg Thr Pro Asp Ala
                325                 330                 335

GAA AGC AGC GAA AAG AAG GCC CCT CCA GAA GAC TCG GAG GAC GAC ATG     1056
Glu Ser Ser Glu Lys Lys Ala Pro Pro Glu Asp Ser Glu Asp Asp Met
            340                 345                 350

CAG GCA GAG GCT TCT GGA GAA AAT CCT GCC GCC CTC CCC GAA GAC GAC     1104
Gln Ala Glu Ala Ser Gly Glu Asn Pro Ala Ala Leu Pro Glu Asp Asp
        355                 360                 365

GAA GTC CCC GAG GAC ACC GAG CAC GAT GAT CCA AAC TCG GAT CCT GAC     1152
Glu Val Pro Glu Asp Thr Glu His Asp Asp Pro Asn Ser Asp Pro Asp
    370                 375                 380

TAT TAC AAT GAC ATG CCC GCC GTG ATC CCG GTG GAG GAG ACT ACT AAA     1200
Tyr Tyr Asn Asp Met Pro Ala Val Ile Pro Val Glu Glu Thr Thr Lys
385                 390                 395                 400

AGT TCT AAT GCC GTC TCC ATG CCC ATA TTC GCG GCG TTC GTA GCC TGC     1248
Ser Ser Asn Ala Val Ser Met Pro Ile Phe Ala Ala Phe Val Ala Cys
                405                 410                 415
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GCG GTC GCG CTC GTG GGG CTA CTG GTT TGG AGC ATC GTA AAA TGC GCG 1296
Ala Val Ala Leu Val Gly Leu Leu Val Trp Ser Ile Val Lys Cys Ala
420 425 430

CGT AGC TAA 1305
Arg Ser
435

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 434 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met His Arg Pro His Leu Arg Arg His Ser Arg Tyr Tyr Ala Lys Gly
1 5 10 15

Glu Val Leu Asn Lys His Met Asp Cys Gly Gly Lys Arg Cys Cys Ser
20 25 30

Gly Ala Ala Val Phe Thr Leu Phe Trp Thr Cys Val Arg Ile Met Arg
35 40 45

Glu His Ile Cys Phe Val Arg Asn Ala Met Asp Arg His Leu Phe Leu
50 55 60

Arg Asn Ala Phe Trp Thr Ile Val Leu Leu Ser Ser Phe Ala Ser Gln
65 70 75 80

Ser Thr Ala Ala Val Thr Tyr Asp Tyr Ile Leu Gly Arg Arg Ala Leu
85 90 95

Asp Ala Leu Thr Ile Pro Ala Val Gly Pro Tyr Asn Arg Tyr Leu Thr
100 105 110

Arg Val Ser Arg Gly Cys Asp Val Val Glu Leu Asn Pro Ile Ser Asn
115 120 125

Val Asp Asp Met Ile Ser Ala Ala Lys Glu Lys Glu Lys Gly Gly Pro
130 135 140

Phe Glu Ala Ser Val Val Trp Phe Tyr Val Ile Lys Gly Asp Asp Gly
145 150 155 160

Glu Asp Lys Tyr Cys Pro Ile Tyr Arg Lys Glu Tyr Arg Glu Cys Gly
165 170 175

Asp Val Gln Leu Leu Ser Glu Cys Ala Val Gln Ser Ala Gln Met Trp
180 185 190

Ala Val Asp Tyr Val Pro Ser Thr Leu Val Ser Arg Asn Gly Ala Gly
195 200 205

Leu Thr Ile Phe Ser Pro Thr Ala Ala Leu Ser Gly Gln Tyr Leu Leu
210 215 220

Thr Leu Lys Ile Gly Arg Phe Ala Gln Thr Ala Leu Val Thr Leu Glu
225 230 235 240

Val Asn Asp Arg Cys Leu Lys Ile Gly Ser Gln Leu Asn Phe Leu Pro
245 250 255

Ser Lys Cys Trp Thr Thr Glu Gln Tyr Gln Thr Gly Phe Gln Gly Glu
260 265 270

His Leu Tyr Pro Ile Ala Asp Thr Asn Thr Arg His Ala Asp Asp Val
275 280 285

Tyr Arg Gly Tyr Glu Asp Ile Leu Gln Arg Trp Asn Asn Leu Leu Arg
290 295 300

Lys Lys Asn Pro Ser Ala Pro Asp Pro Arg Pro Asp Ser Val Pro Gln
305 310 315 320

Glu Ile Pro Ala Val Thr Lys Lys Ala Glu Gly Arg Thr Pro Asp Ala
325 330 335

Glu Ser Ser Glu Lys Lys Ala Pro Pro Glu Asp Ser Glu Asp Asp Met
340 345 350

Gln Ala Glu Ala Ser Gly Glu Asn Pro Ala Ala Leu Pro Glu Asp Asp
355 360 365

Glu Val Pro Glu Asp Thr Glu His Asp Asp Pro Asn Ser Asp Pro Asp
370 375 380

Tyr Tyr Asn Asp Met Pro Ala Val Ile Pro Val Glu Glu Thr Thr Lys
385 390 395 400

Ser Ser Asn Ala Val Ser Met Pro Ile Phe Ala Ala Phe Val Ala Cys
405 410 415

Ala Val Ala Leu Val Gly Leu Leu Val Trp Ser Ile Val Lys Cys Ala
420 425 430

Arg Ser

What is claimed is:

1. A recombinant fowlpox virus comprising a foreign DNA sequence inserted into the fowlpox virus genomic DNA, wherein the foreign DNA sequence is inserted within a non-essential region of the fowlpox virus genomic DNA and is capable of being expressed in a fowlpox virus infected host cell.

2. The recombinant fowlpox virus of claim 1, wherein the foreign DNA sequence is inserted into an open reading frame within the non-essential region the fowlpox virus genomic DNA.

3. The recombinant fowlpox virus of claim 1, wherein the foreign DNA sequence encodes a polypeptide.

4. The recombinant fowlpox virus of claim 3, wherein the polypeptide is antigenic.

5. The recombinant fowlpox virus of claim 1, further comprising a foreign DNA sequence which encodes a detectable marker.

6. The recombinant fowlpox virus of claim 5, wherein the detectable marker is *E. coli beta-galactosidase*.

7. The recombinant fowlpox virus of claim 5, wherein the detectable marker is *E. coli beta-glucuronidase*.

8. The recombinant fowlpox virus of claim 1, wherein the foreign DNA sequence encodes a cytokine.

9. The recombinant fowlpox virus of claim 8, wherein the cytokine is chicken myelomonocytic growth

factor (cMGF) or chicken interferon (cIFN).

10. The recombinant fowlpox virus of claim 9, designated S-FPV-100.

11. The recombinant fowlpox virus of claim 9, designated S-FPV-101.

12. The recombinant fowlpox virus of claim 9, further comprising a newcastle disease virus hemagglutinin (NDV HN), or a newcastle disease virus fusion (NDV F).

13. The recombinant fowlpox virus of claim 12, designated S-FPV-099.

14. The recombinant fowlpox virus of claim 8, wherein the cytokine is selected from a group consisting of interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, and interleukin receptors.

15. The recombinant fowlpox virus of claim 4, wherein the antigenic polypeptide is derived from the group consisting of: human herpesvirus, herpes simplex 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.

16. The recombinant fowlpox virus of claim 4, wherein the antigenic polypeptide is hepatitis B virus core protein or hepatitis B virus surface protein.

17. The recombinant fowlpox virus of claim 4, wherein

the antigenic polypeptide is equine influenza virus neuraminidase or hemagglutinin.

18. The recombinant fowlpox virus of claim 17, wherein the antigenic polypeptide is selected from the group consisting of: equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Kentucky 92 neuraminidase, equine influenza virus type 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.

19. The recombinant fowlpox virus of claim 4, wherein the antigenic polypeptide is derived from the group consisting of: hog cholera virus gE1, hog cholera virus gE2, swine influenza virus hemagglutinin, neuraminidase, matrix and nucleoprotein, pseudorabies virus glycoprotein B, pseudorabies virus glycoprotein C and pseudorabies virus glycoprotein D, and PRRS virus ORF7.

20. The recombinant fowlpox virus of claim 4, wherein the antigenic polypeptide is selected from the group consisting of: infectious bovine rhinotracheitis virus gE, bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.

21. The recombinant fowlpox virus of claim 4, wherein

the antigenic polypeptide is bovine viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53.

22. The recombinant fowlpox virus of claim 4, wherein the foreign DNA sequence encodes an antigenic polypeptide which is derived or derivable from a group consisting of: infectious feline immunodeficiency virus gag, feline immunodeficiency virus env, infectious laryngotracheitis virus glycoprotein B, infectious laryngotracheitis virus gI, infectious laryngotracheitis virus gD, infectious bovine rhinotracheitis virus glycoprotein G, infectious bovine rhinotracheitis virus glycoprotein E, pseudorabies virus glycoprotein 50, pseudorabies virus II glycoprotein B, pseudorabies virus III glycoprotein C, pseudorabies virus glycoprotein E, pseudorabies virus glycoprotein H, marek's disease virus glycoprotein A, marek's disease virus glycoprotein B, marek's disease virus glycoprotein D, newcastle disease virus hemagglutinin or neuraminadase, newcastle disease virus fusion, infectious bursal disease virus VP2, infectious bursal disease virus VP3, infectious bursal disease virus VP4, infectious bursal disease virus polyprotein, infectious bronchitis virus spike, infectious bronchitis virus matrix, and chick anemia virus.

23. The recombinant fowlpox virus of claim 1, wherein the foreign DNA sequence is under control of a promoter.

24. The recombinant fowlpox virus of claim 23, wherein the foreign DNA sequence is under control of an endogenous upstream poxvirus promoter.

25. The recombinant fowlpox virus of claim 23, wherein the foreign DNA sequence is under control of a heterologous upstream promoter.

26. The recombinant fowlpox virus of claim 23, wherein the promoter is selected from: synthetic pox viral promoter, pox synthetic late promoter 1, pox synthetic late promoter 2 early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, and pox E10R promoter.

27. A homology vector for producing a recombinant fowlpox virus by inserting foreign DNA into the viral genome of a fowlpox virus which comprises a double-stranded DNA molecule consisting essentially of:

a) double stranded foreign DNA not usually present within the fowlpox virus viral genome;

b) at one end the foreign DNA, double-stranded fowlpox virus DNA homologous to the viral genome located at one side of the non-essential region of the coding region of the fowlpox virus viral genome; and

c) at the other end of the foreign DNA, double-stranded fowlpox virus DNA homologous to the viral genome located at the other side of the non-essential region of the coding region of the fowlpox virus viral genome.

28. The homology vector of claim 27, wherein the foreign DNA sequence encodes a cytokine.

29. The homology vector of claim 27, wherein the cytokine is chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN).

30. The homology vector of claim 27, wherein the foreign DNA sequence encodes a polypeptide.

31. A homology vector of claim 30, wherein the polypeptide is antigenic.

32. The homology vector of claim 27, wherein the foreign DNA sequence is under control of a promoter.

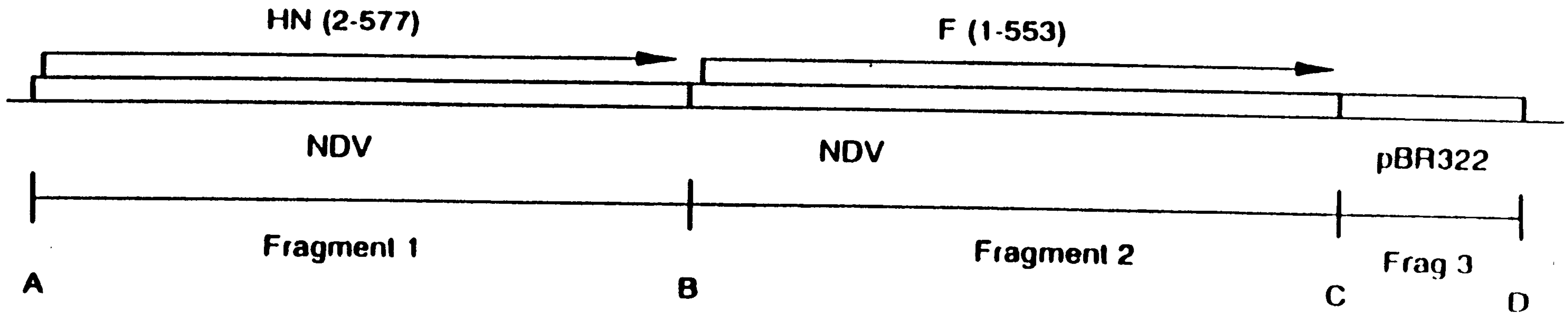
33. A vaccine for immunizing an animal against fowlpox virus which comprises an effective immunizing amount of the recombinant fowlpox virus of claims 1 and a suitable carrier.

34. A method of immunizing an animal against a human pathogen which comprises administering to the animal an effective immunizing dose of the vaccine of claim 32.

35. A method of immunizing an animal against an animal pathogen which comprises administering to the animal an effective immunizing dose of the vaccine of claim 32.

36. A method of enhancing an avian immune response which comprises administering to a person an effective dose of a recombinant fowlpox virus of claim 1 and a suitable carrier.

Figure 1A



SfiI

Junc. A GGCCTCGAGGGCCGCGGCCGCCTGCAGGTCGACTCTAGAAAAAATTGAAAAACTATTCTA

Linker EP1

Junc. A cont. ATTTATTGCACGGAGATCTTTTTTTTTTTTTTTTTTTTTTGGCATATAAATGAATTCGGATCC

EP1 LP2 LP2

FIGURE 1B

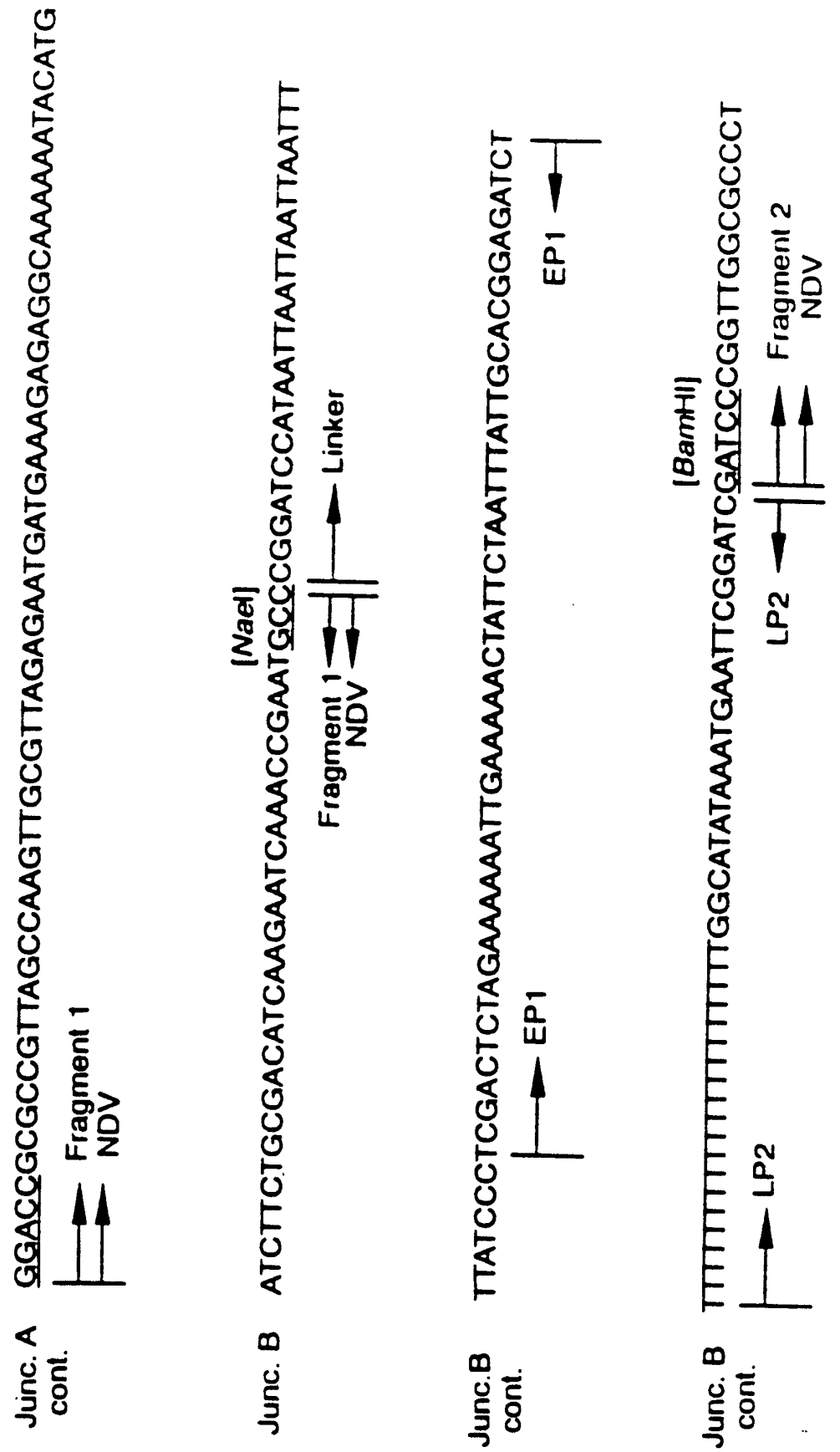

Junc. A cont.
AvaII
GGACCGCGCCGTTAGCCAAGTTGCGTTAGAGAATGATGAAAGAGAGGCAAAAAATACATG
Fragment 1 NDV
Junc. B
[NaeI]
ATCTTCTGCGACATCAAGAATCAAACCGAATGCCCGGATCCATAATTAATTAATTT
Fragment 1 NDV
Linker
Junc.B cont.
TTATCCCTCGACTCTAGAAAAAATTGAAAAACTATTCTAATTTATTGCACGGAGATCT
EP1
EP1
Junc. B cont.
[BamHI]
TTTTTTTTTTTTTTTTTTTTGGCATATAAATGAATTCGGATCGATCCCGGTTGGCGCCCT
LP2
LP2
Fragment 2 NDV

Figure 1C

PstI

Junc. C AAAAACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCTGCAGGCATCGTGGTGTCACGCTCGTCGT

Fragment 2 NDV ◄◄ ‖ ►► Fragment 3 pBR322

[*ScaI*]

Junc. D ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTGATCCA

Fragment 3 pBR322 ◄◄ ‖ ► Linker

SfiI

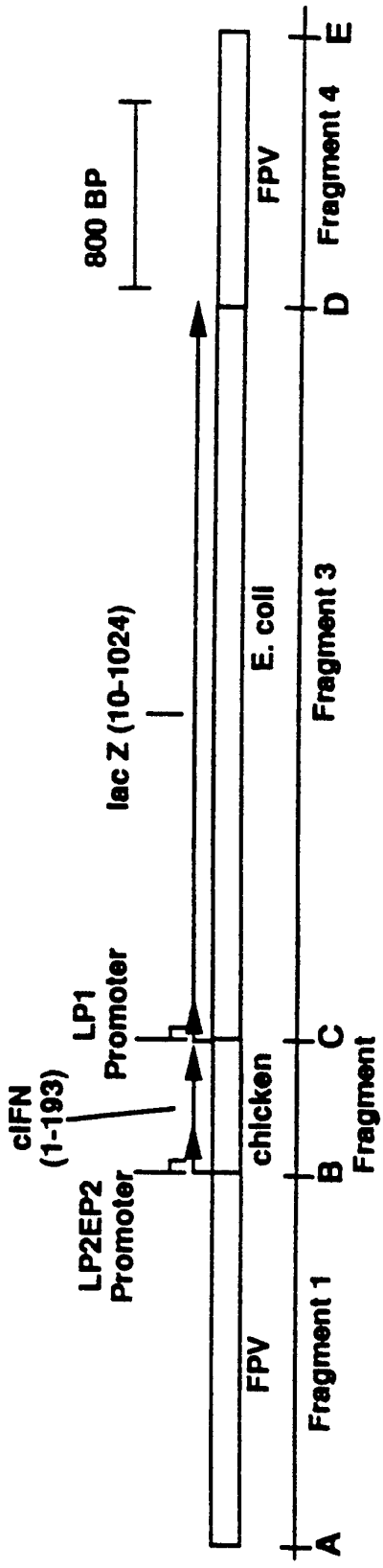
Junc. D cont. TAATTAATTAATTAATTTTTATCCCGGGTCGACCTGCAGGCGGCCGCGGCCCTCGAGGCC

Linker ◄ |

FIGURE 2A

DNA	Origin	Sites	Size
Vector	pSP64	EcoR I–EcoR I	~2999 BP
Fragment 1	FPV 2.8 kb EcoR I	EcoR I–SnaB I	~1626 BP
Fragment 2	chicken IFN	EcoR I†–Bgl II†	~577 BP
Fragment 3	pJF751	Bam HI–Pvu II	~3002 BP
Fragment 4	FPV 2.8 kb EcoR I	SnaB I–EcoR I	~1184 BP

†Restriction sites introduced by PCR cloning



751-07.D1; FPV-099, FPV-101

FIGURE 2B

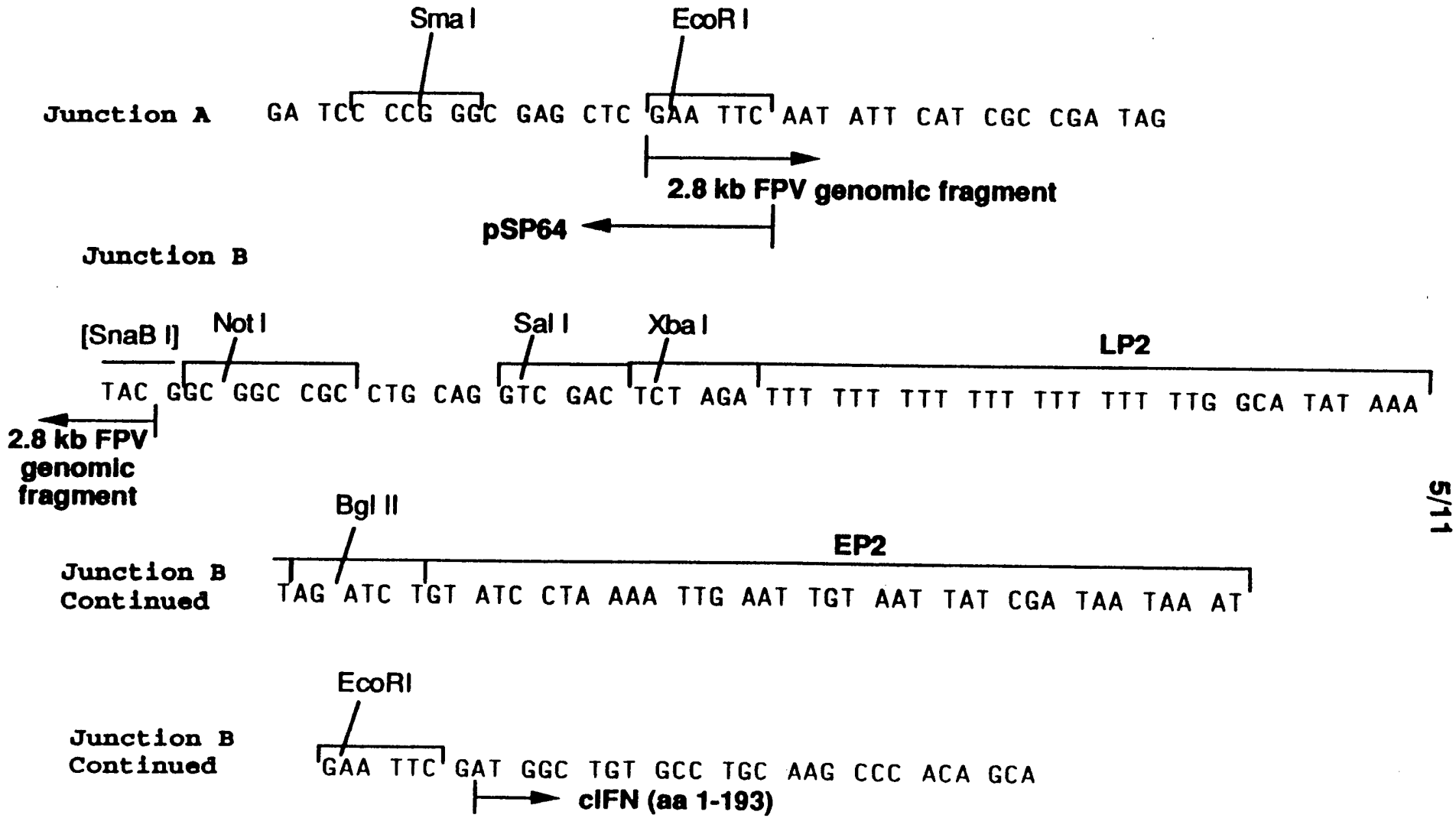
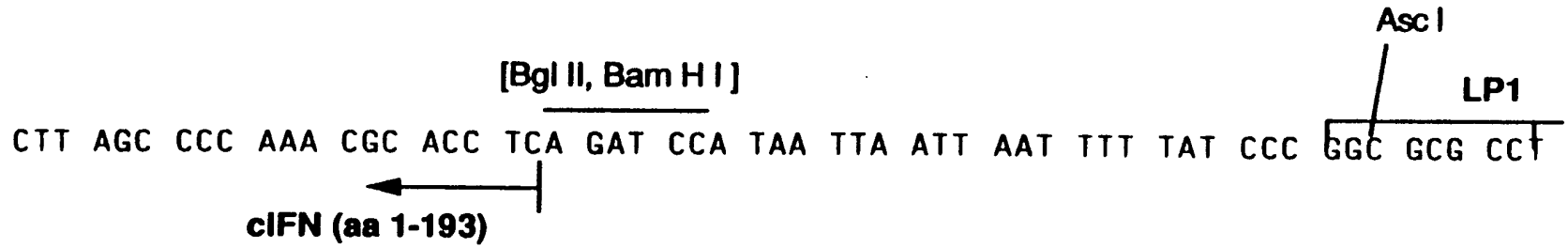


FIGURE 2C

Junction C



Junction C Continued

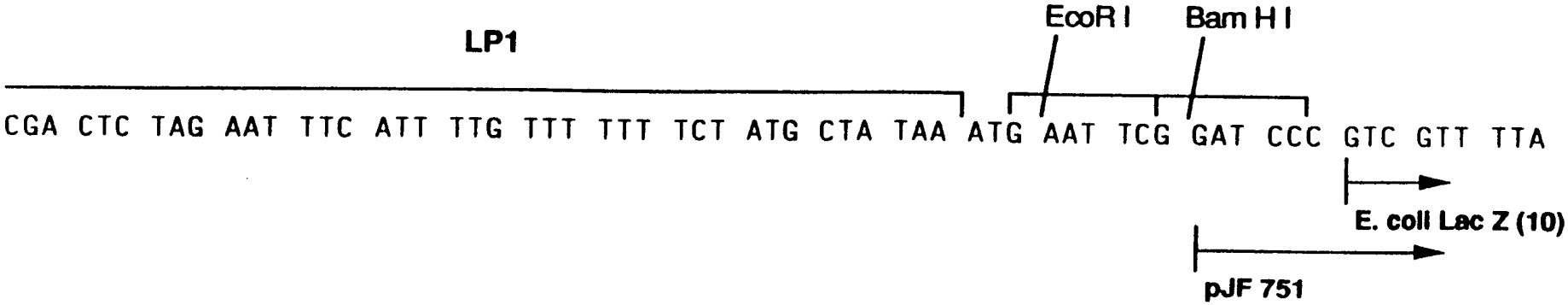


FIGURE 2D

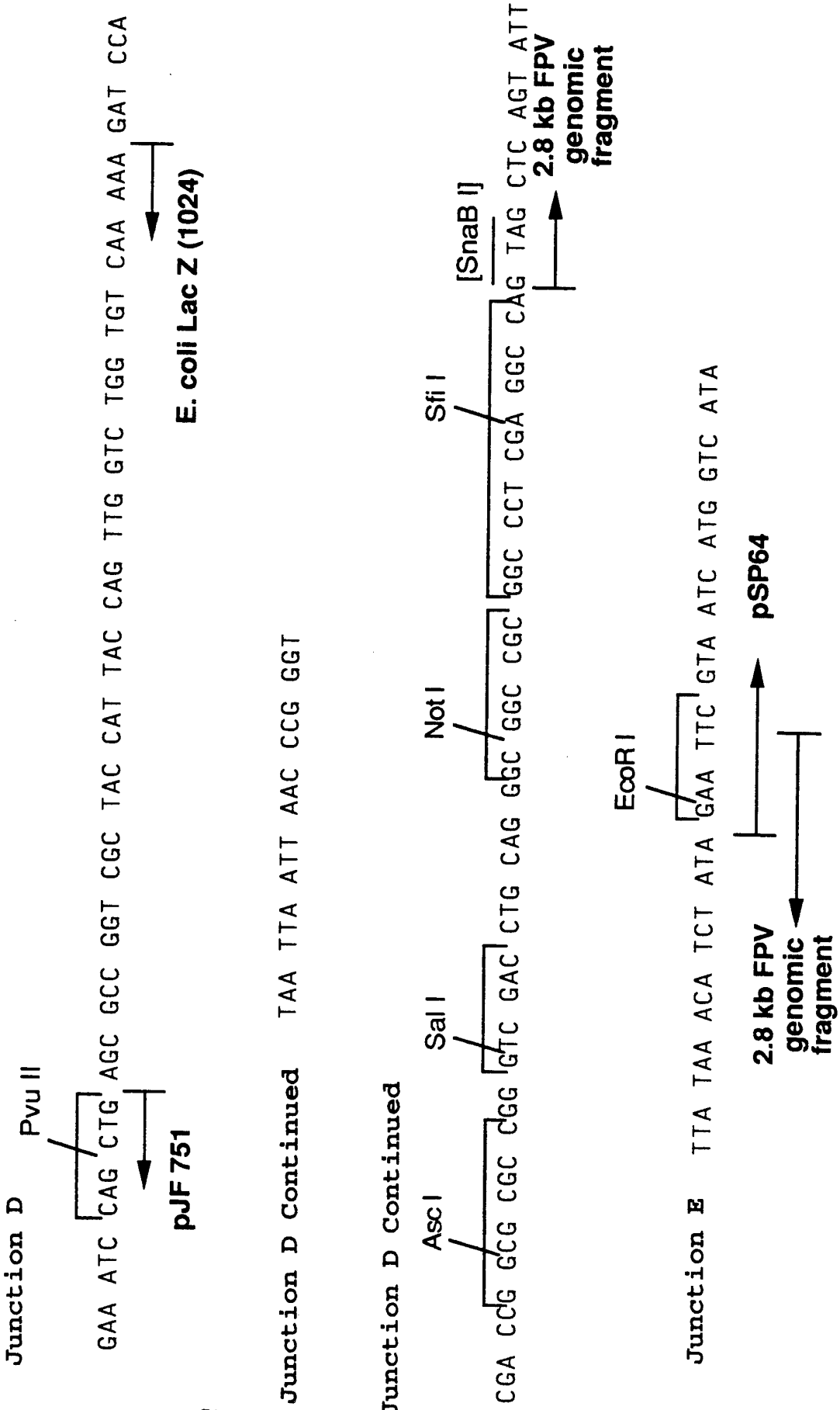
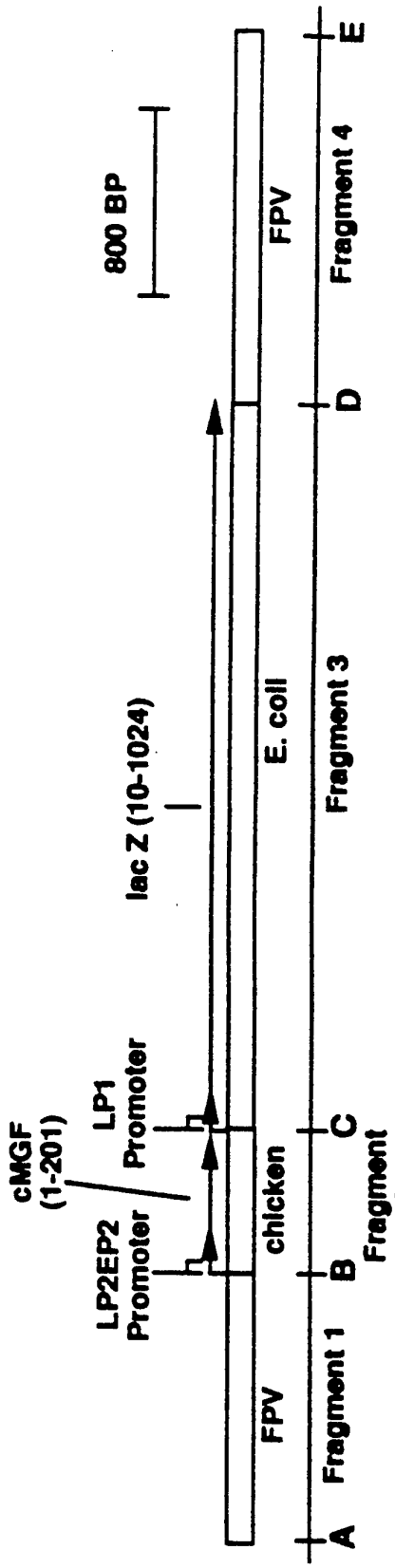

Junction D
Pvu II
GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT CCA
pJF 751
E. coli Lac Z (1024)
Junction D Continued
TAA TTA ATT AAC CCG GGT
Junction D Continued
Asc I
Sal I
Not I
Sfi I
[SnaB I]
CGA CCG GCG CGC CGG GTC GAC CTG CAG GGC GGC CGC GGC CCT CGA GGC CAG TAG CTC AGT ATT
2.8 kb FPV genomic fragment
EcoR I
Junction E
TTA TAA ACA TCT ATA GAA TTC GTA ATC ATG GTC ATA
pSP64
2.8 kb FPV genomic fragment

FIGURE 3A

DNA	Origin	Sites	Size
Vector	pSP64	EcoR I–EcoR I	~2999 BP
Fragment 1	FPV 2.8 kb EcoR I	EcoR I–SnaB I	~1184 BP
Fragment 2	chicken MGF	EcoR I†–BamH I†	~640 BP
Fragment 3	pJF751	Bam HI–Pvu II	~3002 BP
Fragment 4	FPV 2.8 kb EcoR I	SnaB I–EcoRI	~1626 BP

†Restriction sites introduced by PCR cloning



751-56.C1; FPV-100

FIGURE 3B

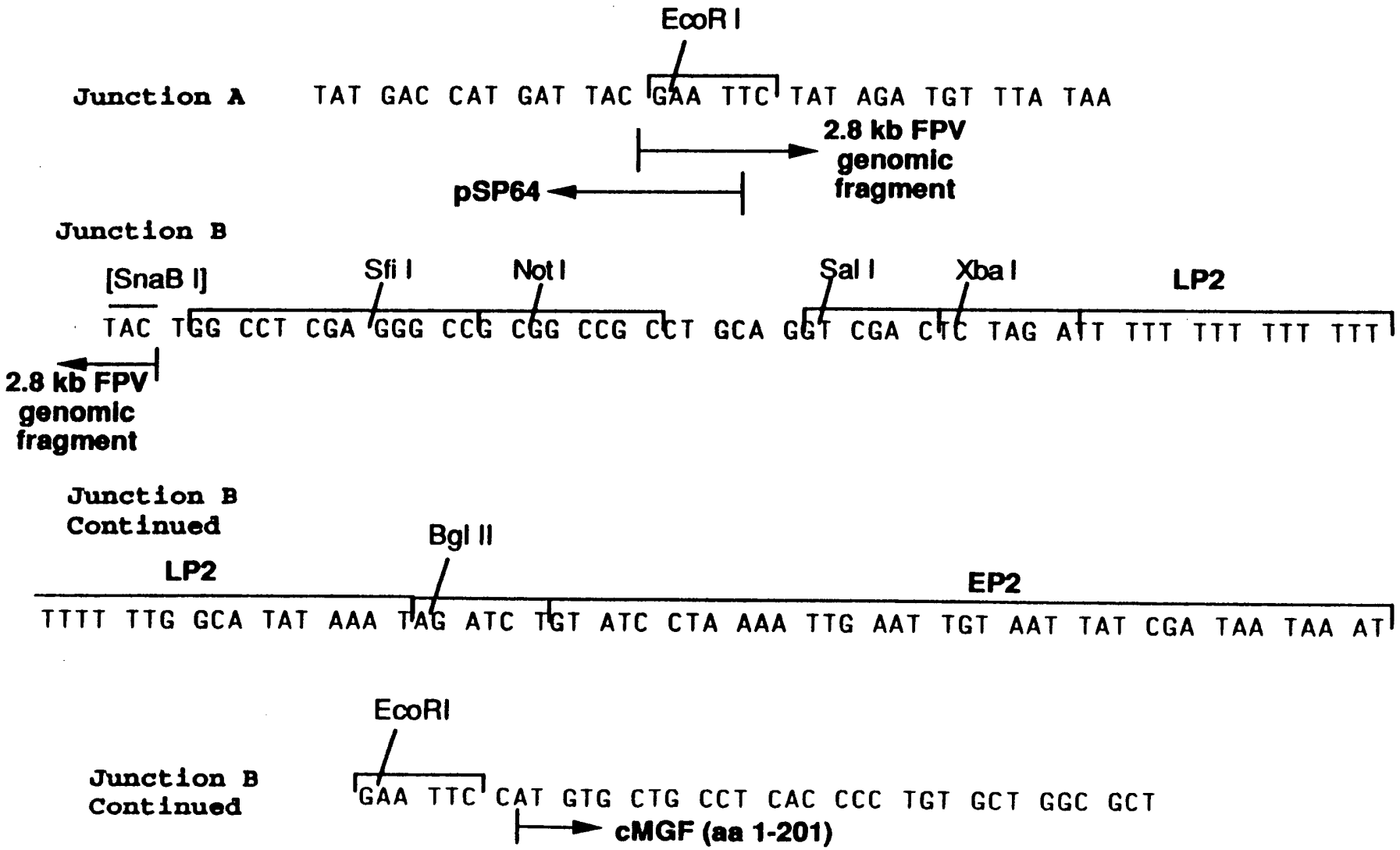


FIGURE 3C

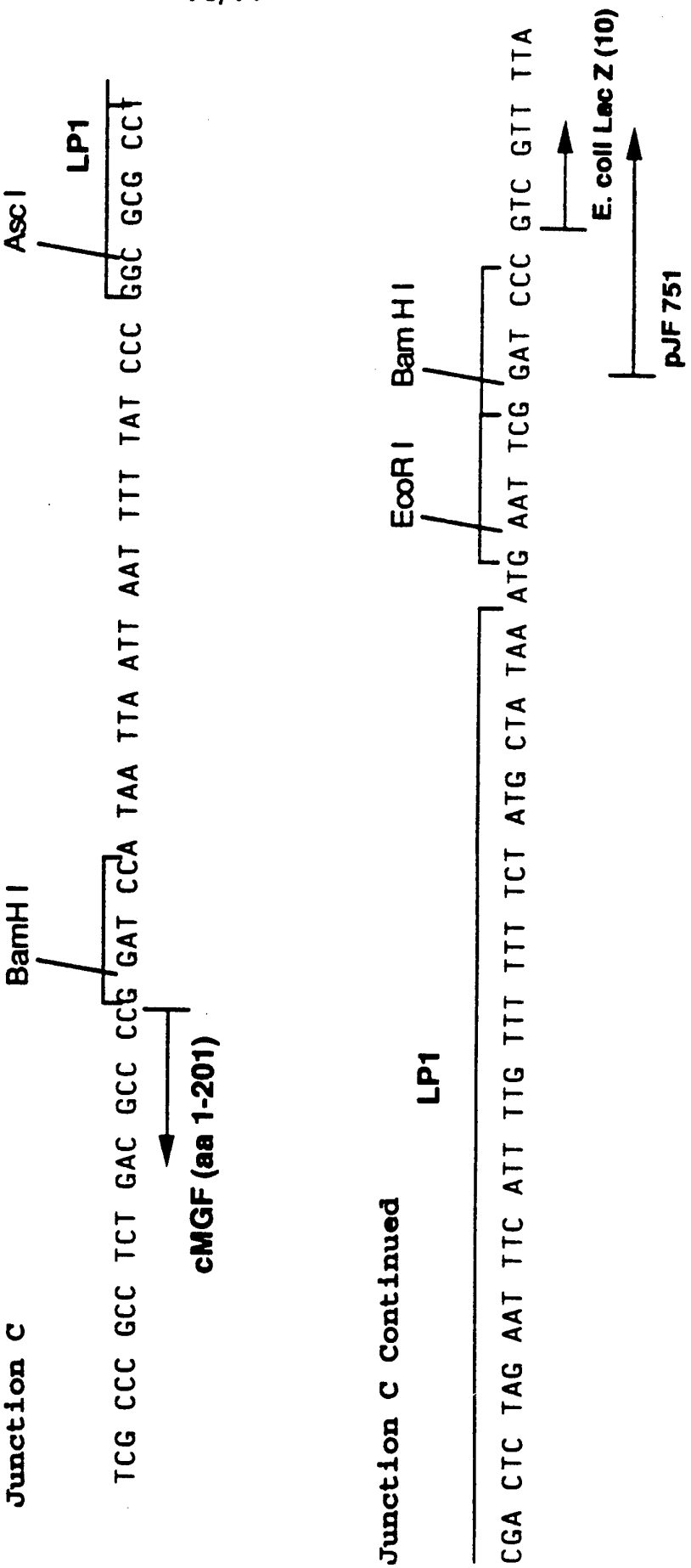
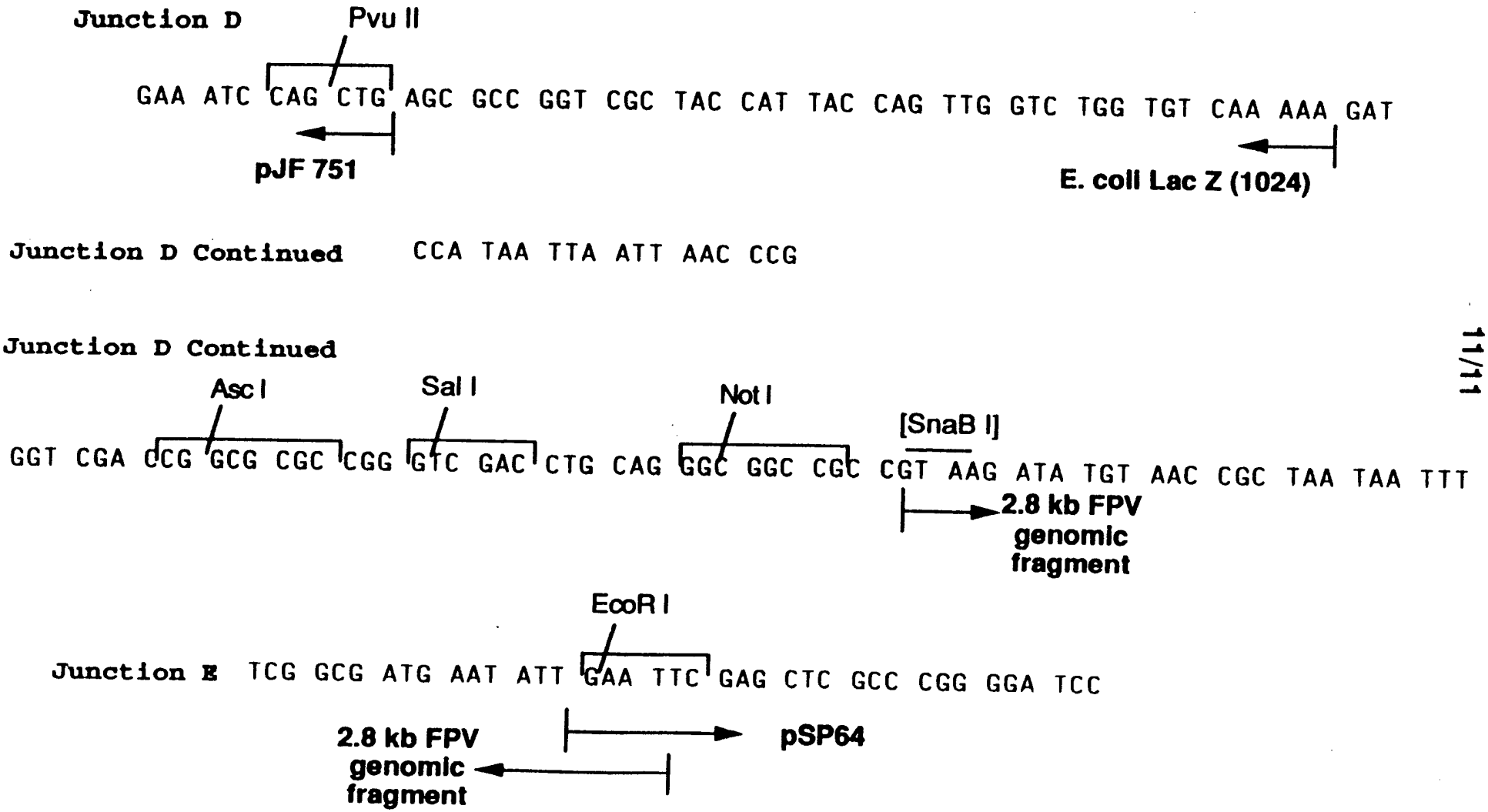

Junction C
BamH I
Asc I
LP1
TCG CCC GCC TCT GAC GCC CCG GAT CCA TAA TTA ATT AAT TTT TAT CCC GGC GCG CCT
cMGF (aa 1-201)
Junction C Continued
LP1
EcoR I
Bam H I
CGA CTC TAG AAT TTC ATT TTG TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA
E. coli Lac Z (10)
pJF 751

FIGURE 3D


Junction D
Pvu II
GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT
pJF 751
E. coli Lac Z (1024)
Junction D Continued
CCA TAA TTA ATT AAC CCG
Junction D Continued
Asc I
Sal I
Not I
[SnaB I]
GGT CGA CCG GCG CGC CGG GTC GAC CTG CAG GGC GGC CGC CGT AAG ATA TGT AAC CGC TAA TAA TTT
2.8 kb FPV genomic fragment
EcoR I
Junction E TCG GCG ATG AAT ATT GAA TTC GAG CTC GCC CGG GGA TCC
2.8 kb FPV genomic fragment
pSP64

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11187

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 7/01, 15/63, 15/00; A61K 39/12, 39/275, 45/00
US CL :424/199.1, 93.2; 435/235.1, 320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/199.1, 93.2; 435/69.1, 69.3, 172.3, 235.1, 320.1; 935/65

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

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

APS, MEDLINE, BIOSIS, CAPLUS, CAB
SEARCH TERMS: TURKEY, CHICKEN, FOWL, DELETION, MUTANTS, CYTOKINE, LYMPHOKINE, FOWLPOX, AVIPOX, PROMOTER, NEWCASTLE, NDV

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	YANAGIDA et al. Protective Immunity against Newcastle Disease Virus Induced by Fowl Poxvirus Recombinants. New York: Cold Spring Harbor Press. 1990, Vaccines90: pages 85-89, see entire document.	1-6, 23-26, 32-36 ---------- 1-11, 14-26, 28-29, 32-36,
X	WO 91/02072 A1 (NATIONAL RESEARCH DEVELOPMENT CORP) 21 February 1991 (21/02/91), see entire document, especially page 4.	27, 30-32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 SEPTEMBER 1996	25 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DANNY LEE Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11187

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEONG et al. Selective Induction of Immune Responses by Cytokines Coexpressed in Recombinant Fowlpox Virus. Journal of Virology. December 1994, Vol. 68, No. 12, pages 8125-8130, see entire document, especially pages 8126-8127.	8, 14
Y, P	US 5,505,941 A (PAOLETTI) 09 April 1996 (09/04/96), especially column 5, lines 4-24.	1-7, 10-11, 15-26, 32-36
Y	KLASING, Avian Leucocytic Cytokines. Poultry Sciences. July 1994, Vol. 73, No. 7, pages 1035-1043, see entire document.	8-9, 14, 28-29
Y	US 5,387,519 A (YANAGIDA) 07 February 1995 (07/02/95), especially figure 1J; column 8, lines 24-32; column 11, lines 19-29.	12-13
Y	US 5,286,639 A (YANAGIDA) 15 February 1994 (15/02/94), columns 1-8.	12-13
Y	RAMSHAW et al. Cytokine expression by recombinant viruses- a new vaccine strategy. TIBTECH. December 1992, Vol. 10, pages 424-426, see entire document, especially pages 424-425.	8, 14
A	SCHNITZLEIN et al. Genomic and antigenic characterization of avipoxviruses. Virus Research. 1988, Vol. 10, pages 65-76, see entire document.	1-36
A	PERKUS et al. Recombinant Vaccinia Virus: Immunization Against Multiple Pathogens. Science. December 1985, Vol. 229, pages 981-984, see entire document.	1-36
A	BOURSNELL et al. Insertion of the fusion gene from Newcastle disease virus into a non-essential region in the terminal repeats of fowlpox virus and demonstration of protective immunity induced by the recombinant. Journal of General Virology. 1990, Vol. 71, pages 621-628, see entire document.	1-36
A	US 5,180,675 A (DRILLIEN) 19 January 1993 (19/01/93), see entire document.	1-36