

RECOMBINANT AVIAN PARAMYXOVIRUS VACCINE AND METHOD FOR MAKING AND USING THEREOF

INCORPORATION BY REFERENCE

[0001] This application claims benefit of US provisional application Serial No. 61/235,912 filed August 21, 2009.

FIELD OF THE INVENTION

[0002] The invention relates to avian paramyxoviruses (APMV) and APMV sequences. The invention relates to viral vectors for the insertion and expression of foreign genes for use as safe immunization vehicles to protect against a variety of pathogens. It relates to vector vaccine in a reverse genetics system for the production of live attenuated vaccines. It also relates to polynucleotides which can be used for the production of subunits in an *in vitro* expression vector or as sequences to be integrated into a viral or plasmid type *in vivo* expression vector.

[0003] The present invention relates to unmodified and modified APMV virus, to methods of making and using the same, and to certain DNA and protein sequences. More in particular, the invention relates to APMV virus in which the naturally occurring genome of the virus has been altered ("APMV mutants" or "recombinant APMV") and to methods of making and using such APMV mutants or recombinant APMV.

BACKGROUND OF THE INVENTION

[0004] Viral vector vaccines represent one of the most rapidly growing areas in vaccine development. Many vaccines in clinical development for major global infectious diseases, HIV, tuberculosis and malaria, are based on viral vectors. Viral vector vaccines for animals are already on the market, e.g. avipox vector vaccines for companion animals and poultry, avian herpesviruses vectored vaccines for poultry, and vaccinia virus vectored vaccines for wildlife. But other livestock vector vaccines are in development. The advantage of viral vector vaccines is that they can be administered safely due to the use of a vector backbone which is strongly attenuated and does not cause disease in the animal itself. The disadvantage of currently used viral vectors is the existence of maternally derived or antibodies acquired due to a past infection. These antibodies will

neutralize the vector virus and thus diminish the success of the vector vaccine. One major impetus for the development of vector vaccines was the occurrence of highly pathogenic influenza virus H5N1 occurring first in Asia and later in Europe and Africa. Several vector vaccine candidates have been developed including fowl poxvirus (Taylor et al, 1988), vaccinia virus (Chambers et al., 1988), Rous sarcoma virus (Hunt et al, 1988), adenoviruses (Tang et al., 2002 , Gao et al, 2006), Venezuelan equine encephalitis virus (Schultz-Cherry et al, 2000), Newcastle disease virus (US6,719,979, Veits et al., 2006, Swayne et al, 2002, Park et al, 2006), herpesvirus of infectious laryngotracheitis (Veits et al. 2003), herpesvirus of turkey (Darteil et al., 1995), and adenovirus based vector vaccines (Hoelscher et al, 2008, Toro et al, 2007). The efficacy of these vector vaccines have been tested in naive birds, but so far no reports have been published on the efficacy of these vector vaccines in birds with a preexisting immunity to the viral vector and/or to the protein coded by the insert.

[0005] The virus family *Paramyxoviridae* includes both human (measles, mumps, parainfluenza and respiratory syncytial virus) and animal pathogens (Newcastle disease virus and rinderpest virus) that cause significant impact on public health as well as the global economy (Lamb et al., 2007). Members of this virus family are defined by having a monopartite, negative sense, single-stranded RNA genome. The *Paramyxoviridae* family consists of two subfamilies namely *Paramyxovirinae* and *Pneumovirinae*. Owing to recent reclassification, the subfamily *Paramyxovirinae* includes five genera, i.e. *Morbillivirus*, *Henipavirus*, *Rubulavirus*, *Respirovirus* and *Avulavirus* while *Pneumovirinae* includes *Pneumovirus* and *Metapneumovirus* (Mayo, 2002). Avian paramyxoviruses (APMV) are classified in the genus *Avulavirus* and comprise nine antigenically distinct serotypes that have been defined using hemagglutination inhibition (HI) tests (Alexander, 1988). Of the nine serotypes, isolates belonging to the APMV-1 subtype can cause a devastating disease in commercial poultry and are classified as velogenic Newcastle disease virus (NDV). Milder forms of NDV are designated as mesogenic and lentogenic isolates, wherein the latter form is mostly asymptomatic in domestic poultry. Isolates belonging to the APMV-2, 3, 6 and 7 have also been associated with disease in domestic poultry. Specifically, infections by isolates of APMV-2 and 3 can cause mild respiratory disease and problems in egg quality and quantity (Bankowski

et al., 1981; Redmann et al., 1991; Tumova et al., 1979; Zhang et al., 2007). Isolates of APMV-6 and 7 have been known to infect turkeys, ducks and migratory birds and can induce respiratory disease that may be complicated by secondary infection (Saif et al., 1997; Shortridge et al., 1980). On the other hand, isolates of APMV-4, 5, 8 and 9 have been isolated from ducks, waterfowl and other wild birds but the birds rarely show clinical signs after viral infection (Alexander et al., 1983; Capua et al., 2004; Gough et al., 1984; Maldonado et al., 1995; Shortridge et al., 1980).

[0006] The complete genomic sequences of several NDV isolates have been established and used to elucidate the various determinants of NDV virulence (de Leeuw et al., 1999; Krishnamurthy et al., 1998; Zou et al., 2005). In the recent two years several APMV sequences other than APMV1 have been published, such as GenBank accession number EU338414 for APMV-2, EU403085 for APMV-3, FJ177514 for APMV-4, EU622637 for APMV-6, FJ231524 for APMV-7, FJ215863, FJ215864 and FJ619036 for APMV-8, EU910942 for APMV-9. Besides the sequence information, not much is known about virulence factors. Isolates of APMV 2-9 have been mostly isolated from migratory birds. Interestingly, there are very few reports of experimental infection of chickens with such isolates (Saif et al., 1997). Since these APMV circulate widely in wild birds and in certain cases have been isolated from commercial flocks (Zhang et al., 2007) that sometimes cause disease in them (Saif et al., 1997; Shihmanter et al., 1998; Shihmanter et al., 1998), knowledge about their virulence in poultry is needed.

[0007] Most of the APMV isolates cause a relatively mild disease that may be exacerbated in the presence of concomitant bacterial or viral infections which might lead to economic impact. In particular, APMV-2 was first isolated as a secondary pathogen in 1956 from chickens affected by acute laryngotracheitis in Southern California (Bankowski et al., 1960). Since then numerous strains of this serotype have been isolated from several avian species signifying that APMV-2 is widely disseminated worldwide (Andral et al., 1984; Bradshaw et al., 1979; Fleury et al., 1979; Goodman et al., 1988; Lang et al., 1975; Lipkind et al., 1982; Lipkind et al., 1979; Zhang et al., 2006). Bankowski *et. al.* reported that natural as well as artificial exposure of laying turkey hens to APMV-2 caused a pronounced decline in hatchability and poultry yield (Bankowski et al., 1981). Initial examples of APMV-4 isolation were from hunter-killed feral ducks on

the Mississippi flyway in the United States (Webster et al., 1976) and from chickens, ducks and geese in Hong Kong during influenza surveillance programs of poultry (Alexander et al., 1979). Apart from an isolate from a ringed teal suffering from hemorrhagic enteritis (Gough et al., 1984), all other isolates were seemingly non-pathogenic in poultry and found to have wide distribution among waterfowl throughout the world (Stanislawek et al., 2002; Tumova et al., 1989; Yamane et al., 1982). Gough et al. reported that no clinical signs and very low HI titers (1:8 or less) were obtained after the intranasal inoculation of one-week old ducklings and two-week old chickens with the isolate from a ringed teal (Gough et al., 1984). Similarly, the first isolates of APMV-6 were also from domestic poultry in Hong Kong as a result of an influenza surveillance program and were reported to be non-pathogenic in chickens based on low HI titers from experimentally infected chickens (Shortridge et al., 1980). However, there have been reports of APMV-6 infection of turkeys leading to mild respiratory disease and egg production problems (Alexander, 2003).

[0008] APMV-8 (Goose/Delaware/1053/76) was first isolated in the USA from a hunter-killed Canada goose (*Branta canadensis*) (Rosenberger et al., 1974). A serological survey (from 1990 to 1992) of wildfowl in southern Spain showed a notable prevalence of APMV-8 antibodies in up to 43% of the tested sera (Maldonado et al., 1995). Another serological study to determine the status of live, healthy mallard ducks in New Zealand for APMV infection revealed the presence of APMV-8 antibodies in 56% of the tested sera (Stanislawek et al., 2002). Warke et al (2008) described that between 16% to 31% of investigated chicken sera might have had APMV-8 antibodies. But due to existing high titers against APMV1 the probability of a false positive HI test is possible since the sera do not react very specifically in the HI assay. With the exception of a few waterfowl isolates of APMV-8 isolated while the populations were being surveyed for avian influenza viruses (Stallknecht et al., 1991), there has been a dearth of information about the prevalence and pathogenicity of this virus.

[0009] The development of reverse genetics systems for the negative stranded RNA genome of NDV has made it possible to insert foreign gene sequences into the genome, thus making it possible to create recombinant NDV vectors for vaccination and gene therapy (Krishnamurthy et al., 2000; Peeters et al., 1999; Roemer-Oberdoerfer et al.,

1999). Recombinant NDV vectors expressing foreign viral proteins such as the HA protein of the H1 subtype of influenza A virus (Nakaya et al., 2001), VP2 protein of infectious bursal disease virus (IBDV) (Huang et al., 2004), avian influenza virus hemagglutinin of subtype H5 (Veits et al., 2006; Ge et al., 2007) and subtype H7 (Park et al., 2006) have been reported. However the efficacy of most of such vaccines has been demonstrated only in SPF birds. NDV causes a devastating disease in poultry leading to serious economic losses in the poultry industry. Commercial chickens therefore are routinely vaccinated against NDV in most countries of the world. Due to this, chickens from immunized parent flocks have a high level of maternally derived antibodies. Conventional live NDV vaccines provide protection even in the presence of these antibodies. However recombinant NDV vaccines (with foreign gene insertions) are generally more attenuated as compared to live NDV vaccines and their efficacy may be impaired in presence of NDV maternal antibodies. Therefore, there is a need for a vector vaccine platform which can provide the basis for safe vaccines for the expression of heterologous antigens. Ideally, the recombinant vaccine can induce a strong humoral immune response, can be applicable by mass administration, and is inexpensive.

SUMMARY OF THE INVENTION

[0010] The present invention relates to a vaccine or composition comprising (i) a recombinant APMV and (ii) a pharmaceutically or veterinarily acceptable carrier. The present invention encompasses methods for modifying the genome of APMV to produce recombinant APMV virus or APMV rival vector; modified APMV prepared by such methods; DNA and protein sequences; and methods for infecting cells and host animals with such recombinant APMV to provoke the amplification of exogenous DNA and proteins encoded by the exogenous DNA, including antigenic proteins, by said cells and host animals.

[0011] One aspect of the invention relates to APMV virus, DNA and protein sequences involved in making modified or recombinant virus. One embodiment of the invention relates to the genomic and protein sequence of APMV-2, 4, 6, or 8.

[0012] Another aspect of the invention relates to a modified recombinant APMV virus, which viruses have enhanced safety, strong humoral immune response, and a method of making such recombinant viruses.

[0013] Another aspect of the invention relates to a recombinant APMV virus vaccine or composition having an increased level of safety compared to known APMV or other recombinant vaccines.

[0014] In another aspect, the present invention provides unmodified and modified APMV viral vector for expressing a gene product in a host.

[0015] Another aspect of the invention is directed to a recombinant APMV virus modified by the insertion therein of DNA from any source into the intergenic region or the nonessential region of the APMV genome. Synthetically modified APMV virus recombinants carrying heterologous genes coding for and expressing an antigen, are used according to the invention to create novel compositions or vaccines.

[0016] Another aspect of the invention relates to an APMV viral vector which provides a reverse genetics system, wherein the vector can be used as a backbone for recombinant vaccines or compositions in different host animals.

[0017] In one aspect, the present invention relates to a pharmaceutical composition or vaccine for inducing an immunological response in a host animal inoculated with the composition or vaccine, the composition or vaccine including a pharmaceutical acceptable carrier and a modified APMV recombinant virus or viral vector. In yet another aspect of the invention, the recombinant APMV virus or viral vector includes, within a non-essential region of the virus genome, a heterologous DNA which encodes an antigenic protein derived from a pathogen wherein the composition or vaccine when administered to a host, is capable of inducing an immunological response specific to the protein encoded by the pathogen.

[0018] Another aspect of the invention relates to a method for inducing an immunological response in an animal to an antigen, which method comprises inoculating the animal with a vaccine or a pharmaceutical composition containing modified recombinant APMV virus or viral vector which comprises and expresses the antigenic determinant of a pathogen for said animal. Yet another aspect of the invention relates to a

method for inducing an immunological response in an animal to an antigen in a prime-boost regime.

[0019] Another aspect of the invention relates to a method of expressing a gene product in a cell culture in vitro by introducing into the cell a modified recombinant APMV virus, wherein the gene may be an antigenic protein derived from a pathogen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The following detailed description, given by way of example, and which is not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, in which:

[0021] Figure 1 is a table showing virus isolation from several organs of chickens after experimental infection with APMV-2, 4, 6 in embryonated chicken eggs.

[0022] Figure 2 is a table showing the histology results of several organs of chickens after experimental infection with APMV-2, 4, 6.

[0023] Figure 3 depicts HI antibody titers in SPF chickens experimentally inoculated with APMV-2, 4 or 6. Chicken sera samples collected at day 2, 4, 7, 14 and 28 post infection were subject to the HI test to analyze the presence of HI antibodies.

[0024] Figure 4 shows HI antibody titers in SPF chickens and ducks experimentally infected with APMV-8. Chickens and ducks were oro-nasal infected with a dose of 10^6 EID₅₀ of APMV-8. Sera samples were collected at day 2, 4, 7, 14, and 28 p. i. and analyzed by the HI test with the APMV-8 antigen. The HI serum titers (in log₂) are shown on the left axis

[0025] Figure 5 shows the development of HI antibody titers in SPF chickens during a prime/boost vaccination scheme with APMV-8. One-day old SPF chickens were infected at day 1 (prime) and day 14 (boost) with a dose of 10^6 EID₅₀ of APMV-8. Serum samples were obtained at day 7 and 14 p.i. after the first infection and day 7 and 14 p.i. after the second infection. The sera were subjected to the HI test. The HI serum titers (in log₂) are shown on the left axis.

[0026] Figure 6 shows the analysis of RT-PCR products by agarose gel electrophoresis. Tracheal tissues were taken at day 2 p.i. from non-infected ducks (C1-

C5) and APMV-8 infected ducks (I1-I5). The tissues were homogenized and RNA was prepared for RT-PCR. A water control (W) was prepared in parallel. The reaction products were separated on a 1.5 % agarose gel. The size of the fragment was controlled by using the 100 bp ladder (New England Biolabs). The sizes of the DNA fragments are shown at the right.

[0027] Figure 7 is a table showing virus isolation from chicken and ducks experimentally infected with APMV-8. The virus isolation from chicken tissue was performed in embryonated chicken eggs and the detection of viral RNA from duck tissue by RT-PCR.

[0028] Figure 8 is a table showing the results of the histological examination of several organs after infection of chicken and Pekin ducks with APMV-8.

[0029] Figure 9 shows the development of HI antibody titers in SPF chickens after infection with different doses of APMV-8. One-day old SPF chickens were infected at day 1 with different infectious doses (ID) of APMV-8 or mock-infected with virus transport medium (VTM). Blood was taken at day 7 and 14 p.i. and the obtained serum samples were analyzed by the HI test. The HI serum titers (in log₂) are shown on the left axis. The geometric mean titer (GMT) of the serum samples are shown in the lowest row.

[0030] Figure 10 shows the development of HI antibody titers in Pekin ducks after infection with different doses of APMV-8. One-day old SPF Pekin ducks were infected at day 1 with different infectious doses (ID) of APMV-8 or mock-infected with virus transport medium (VTM). Blood was taken at day 7 and 14 p.i. and the obtained serum samples were analyzed by the HI test. The HI serum titers (in log₂) are shown on the left axis. The geometric mean titer (GMT) of the serum samples are shown in the lowest row.

[0031] Figure 11 is a table showing the SEQ ID NOs of the corresponding DNA and protein sequences.

[0032] Figure 12 depicts the full length genome sequence of APMV-8 strain (APMV-8: SCWDS ID: MA-7, isolated from a mallard) and a genetic map of the full length APMV-8 genome.

[0033] Figure 12 depicts the DNA sequence (SEQ ID NO:2) encoding APMV-8 Nucleoprotein (NP) and the NP protein sequence (SEQ ID NO:3).

- [0034] Figure 13 depicts the DNA sequence (SEQ ID NO:4) encoding APMV-8 Phospho Protein (P) and the P protein sequence (SEQ ID NO:5).
- [0035] Figure 14 depicts the DNA sequence (SEQ ID NO:6) encoding APMV-8 Matrixprotein (M) and the M protein sequence (SEQ ID NO:7).
- [0036] Figure 15 depicts the DNA sequence (SEQ ID NO:8) encoding APMV-8 Fusionprotein (F) and the F protein sequence (SEQ ID NO:9).
- [0037] Figure 16 depicts the DNA sequence (SEQ ID NO:10) encoding APMV-8 Hemagglutinin/ neuraminidase (HN) and the HN protein sequence (SEQ ID NO:11).
- [0038] Figure 17 depicts the DNA sequence (SEQ ID NO:12) encoding APMV-8 Polymerase (L) and the L protein sequence (SEQ ID NO:13). This APMV-8 L(1) protein is translated from the ATG codon located at positions 8273-8275 of SEQ ID NO:1.
- [0039] Figure 18 depicts the protein sequence (2) of APMV-8 Polymerase (L) (SEQ ID NO:14). This APMV-8 L(2) protein is translated from the ATG codon located at positions 8297-8299 of SEQ ID NO:1. SEQ ID NO:14 does not contain the first 8 amino acids of SEQ ID NO:13.
- [0040] Figure 19A is a flow diagram of the APMV-8 reverse genetics system. Figure 19B depicts the result of replication of APMV-8 virus in MDCK cells.
- [0041] Figure 20 depicts the HI test result of commercial broiler chickens 2 weeks after APMV-8 vaccination.
- [0042] Figure 21 depicts the HI test result of commercial broiler chickens 4 weeks after APMV-8 vaccination.
- [0043] Figure 22 shows the HI test results after the *in ovo* vaccination at day 18 (study 1).
- [0044] Figure 23 depicts the HI test results after the *in ovo* vaccination at day 19 (study 2).
- [0045] Figure 24 shows the HI test results after the *in ovo* vaccination at day 18 (study 3).
- [0046] Figure 25 depicts 5'-full length genome (5'-FLG) and 3'-full length genome (3'-FLG) sequences, including the flanking sequences.
- [0047] Figure 26 depicts plasmid maps of pcNDA-NP, pcNDA-P, pcDNA-L, and pcDNA3-T7.

- [0048] Figure 27 depicts plasmid maps of pUC18-MG-APMV-8 and pCITE4A-EGFP.
- [0049] Figure 28 depicts plasmid map of pUC57-FL-APMV-8.
- [0050] Figure 29 depicts minigenome APMV-8 sequence.
- [0051] Figure 30 shows the NP protein sequence alignment and the sequence identity at the DNA and protein levels.
- [0052] Figure 31 shows the P protein sequence alignment and the sequence identity at the DNA and protein levels.
- [0053] Figure 32 shows the M protein sequence alignment and the sequence identity at the DNA and protein levels.
- [0054] Figure 33 shows the F protein sequence alignment and the sequence identity at the DNA and protein levels.
- [0055] Figure 34 shows the HN protein sequence alignment and the sequence identity at the DNA and protein levels.
- [0056] Figure 35 shows the L protein sequence alignment and the sequence identity at the DNA and protein levels.

DETAILED DESCRIPTION OF THE INVENTION

[0057] It is noted that in this disclosure and particularly in the claims, terms such as “comprises”, “comprised”, “comprising” and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean “includes”, “included”, “including”, and the like; and that terms such as “consisting essentially of” and “consists essentially of” have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0058] Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular*

Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

[0059] The singular terms “a”, “an”, and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicate otherwise. The word “or” means any one member of a particular list and also includes any combination of members of that list.

[0060] It is noted in this disclosure and the appended claims and/or paragraphs, the term “Avian Paramyxoviruses” or “APMV” is used interchangeably, and refers to and includes APMV-1, APMV-2, APMV-3, APMV-4, APMV-5, APMV-6, APMV-7, APMV-8, and APMV-9.

[0061] The term “animal” is used herein to include all mammals, birds and fish. The animal as used herein may be selected from the group consisting of equine (e.g., horse), canine (e.g., dogs, wolves, foxes, coyotes, jackals), feline (e.g., lions, tigers, domestic cats, wild cats, other big cats, and other felines including cheetahs and lynx), bovine (e.g., cattle), porcine (e.g., pig), ovine (e.g., sheep, goats, lambs, bison), avian (e.g., chicken, duck, goose, turkey, quail, pheasant, parrot, finches, hawk, crow, ostrich, emu and cassowary), primate (e.g., prosimian, tarsier, monkey, gibbon, ape), humans, and fish. The term “animal” also includes an individual animal in all stages of development, including embryonic and fetal stages.

[0062] The terms “polypeptide” and “protein” are used interchangeably herein to refer to a polymer of consecutive amino acid residues.

[0063] The terms “nucleic acid”, “nucleotide”, and “polynucleotide” are used interchangeably and refer to RNA, DNA, cDNA (complementary DNA), or crRNA (complementary RNA) and derivatives thereof, such as those containing modified backbones. It should be appreciated that the invention provides polynucleotides comprising sequences complementary to those described herein. The “polynucleotide” contemplated in the present invention includes both the forward strand (5' to 3') and reverse complementary strand (3' to 5'). Polynucleotides according to the invention can be prepared in different ways (e.g. by chemical synthesis, by gene cloning etc.) and can take various forms (e.g. linear or branched, single or double stranded, or a hybrid thereof, primers, probes etc.).

[0064] The term “genomic DNA” or “genome” is used interchangeably and refers to the heritable genetic information of a host organism. The genomic DNA comprises the DNA of the nucleus (also referred to as chromosomal DNA) but also the DNA of the plastids (e.g., chloroplasts) and other cellular organelles (e.g., mitochondria). The genomic DNA or genome contemplated in the present invention also refers to the RNA of a virus. The RNA may be a positive strand or a negative strand RNA. The term “genomic DNA” contemplated in the present invention includes the genomic DNA containing sequences complementary to those described herein. The term “genomic DNA” also refers to messenger RNA (mRNA), complementary DNA (cDNA), and complementary RNA (cRNA). The term “genomic RA (nucleic acid)” as used herein includes RNA, mRNA, cRNA, DNA and cDNA.

[0065] The term “gene” is used broadly to refer to any segment of polynucleotide associated with a biological function. Thus, genes or polynucleotides include introns and exons as in genomic sequence, or just the coding sequences as in cDNAs, such as an open reading frame (ORF), starting from the start codon (methionine codon) and ending with a termination signal (stop codon). Genes and polynucleotides can also include regions that regulate their expression, such as transcription initiation, translation and transcription termination. Thus, also included are promoters and ribosome binding regions (in general these regulatory elements lie approximately between 60 and 250 nucleotides upstream of the start codon of the coding sequence or gene; Doree S M *et al.*; Pandher K *et al.*; Chung J Y *et al.*), transcription terminators (in general the terminator is located within approximately 50 nucleotides downstream of the stop codon of the coding sequence or gene; Ward C K *et al.*). Gene or polynucleotide also refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

[0066] The term “heterologous DNA” as used herein refers to the DNA derived from a different organism, such as a different cell type or a different species from the recipient. The term also refers a DNA or fragment thereof on the same genome of the host DNA wherein the heterologous DNA is inserted into a region of the genome which is different from its original location.

[0067] As used herein, the term “antigen” or “immunogen” means a substance that induces a specific immune response in a host animal. The antigen may comprise a whole organism, killed, attenuated or live; a subunit or portion of an organism; a recombinant vector containing an insert with immunogenic properties; a piece or fragment of DNA capable of inducing an immune response upon presentation to a host animal; a polypeptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

[0068] The term “immunogenic protein or peptide” as used herein includes polypeptides that are immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral and/or cellular type directed against the protein. Preferably the protein fragment is such that it has substantially the same immunological activity as the total protein. Thus, a protein fragment according to the invention comprises or consists essentially of or consists of at least one epitope or antigenic determinant. An “immunogenic” protein or polypeptide, as used herein, includes the full-length sequence of the protein, analogs thereof, or immunogenic fragments thereof. By “immunogenic fragment” is meant a fragment of a protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques well known in the art. See, e.g., *Epitope Mapping Protocols in Methods in Molecular Biology*, Vol. 66 (Glenn E. Morris, Ed., 1996). For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Pat. No. 4,708,871; Geysen et al., 1984; Geysen et al., 1986. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols*, supra.

[0069] The term “immunogenic protein or peptide” further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. The term “conservative variation”

denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change or is another biologically similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, or the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like; or a similar conservative replacement of an amino acid with a structurally related amino acid that will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the definition of the reference polypeptide. All of the polypeptides produced by these modifications are included herein. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

[0070] A "host cell" denotes a prokaryotic or eukaryotic cell that has been genetically altered, or is capable of being genetically altered by administration of an exogenous polynucleotide, such as a recombinant plasmid or vector. When referring to genetically altered cells, the term refers both to the originally altered cell and to the progeny thereof. Polynucleotides comprising a desired sequence can be inserted into a suitable cloning or expression vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification. Polynucleotides can be introduced into host cells by any means known in the art. The vectors containing the polynucleotides of interest can be

introduced into the host cell by any of a number of appropriate means, including direct uptake, endocytosis, transfection, f-mating, electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is infectious, for instance, a retroviral vector). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0071] An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, and/or cytotoxic T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered viral titer in the infected host.

[0072] One embodiment of the invention provides the genomic DNA sequence and encoded protein sequences of APMV-8. The complementary genomic DNA(cDNA) sequence of APMV-8 strain of the present invention has a polynucleotide sequence as set forth in SEQ ID NO:1. The APMV-8 genomic cDNA sequence (SEQ ID NO:1) has 48% sequence identity to APMV-1 genomic DNA (SEQ ID NO:15), 61% sequence identity to APMV-2 genomic DNA (SEQ ID NO:16), 47.2% sequence identity to APMV-3 genomic DNA (SEQ ID NO:17), 47.6 % sequence identity to APMV-4 genomic DNA (SEQ ID NO:18), 52% sequence identity to APMV-6 genomic DNA (SEQ ID NO:19), 53% sequence identity to APMV-7 genomic DNA (SEQ ID NO:20), 99.1% sequence identity to APMV-8 genomic DNA (SEQ ID NO:37), 96.5% sequence identity to APMV-8 genomic DNA (SEQ ID NO:38), 96.4% sequence identity to APMV-8 genomic DNA (SEQ ID NO:39), 48% sequence identity to APMV-9 genomic DNA (SEQ ID NO:40). In another embodiment, the invention provides a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 4, 6, 8, 10 or 12, and variant or fragment thereof. The invention further comprises a complementary strand to a polynucleotide

described herein. In yet another embodiment, the invention provides a polypeptide having a sequence as set forth in SEQ ID NO:3, 5, 7, 9, 11, 13 or 14, and variant or fragment thereof.

[0073] Moreover, homologs of polynucleotides or polypeptides from APMV, for example APMV-8, APMV-2, APMV-4, APMV-6 strains are intended to be within the scope of the present invention. As used herein, the term “homologs” includes orthologs, analogs and paralogs. The term “analogs” refers to two polynucleotides or polypeptides that have the same or similar function, but that have evolved separately in unrelated organisms. The term “orthologs” refers to two polynucleotides or polypeptides from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides having the same or similar functions. The term “paralogs” refers to two polynucleotides or polypeptides that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related. Analogs, orthologs, and paralogs of a wild-type APMV polypeptide can differ from the wild-type APMV polypeptide by post-translational modifications, by amino acid sequence differences, or by both. In particular, homologs of the invention will generally exhibit at least 80-85%, 85-90%, 90-95%, or 95%, 96%, 97%, 98% , 99% sequence identity with all or part of the polynucleotide or polypeptide sequences of APMV-8, and will exhibit a similar function.

[0074] In another aspect, the present invention provides a genomic cDNA of APMV-8 having the sequence as set forth in SEQ ID NO:1. In yet another embodiment, the polynucleotide is a reverse complementary strand of the polynucleotide having the sequence as set forth in SEQ ID NO:1. In yet another embodiment, the polynucleotide or a reverse complementary strand of a polynucleotide of the present invention has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:1.

[0075] In one embodiment, the present invention provides a fragment of polynucleotide encoding an AMPV-8 polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 5, 7, 9, 11, 13 or 14. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide

having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 5, 7, 9, 11, 13 or 14, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides.

[0076] In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1, 2, 4, 6, 8, 10, or 12, or a variant thereof. In yet another embodiment, the polynucleotide is a reverse complementary strand of the polynucleotide having the sequence as set forth in SEQ ID NO:1. In yet another aspect, the present invention provides a polynucleotide or a reverse complementary strand of a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to one of a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 4, 6, 8, 10, or 12, or a variant thereof.

[0077] In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 5, 7, 9, 11, 13 or 14. In yet another aspect, the present invention provides fragments and variants of the APMV polypeptides identified above (SEQ ID NO: 3, 5, 7, 9, 11, 13 or 14) which may readily be prepared by one of skill in the art using well-known molecular biology techniques.

[0078] Variants are homologous polypeptides having an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 3, 5, 7, 9, 11, 13 or 14.

[0079] Variants include allelic variants. The term "allelic variant" refers to a polynucleotide or a polypeptide containing polymorphisms that lead to changes in the amino acid sequences of a protein and that exist within a natural population (e.g., a virus species or variety). Such natural allelic variations can typically result in 1- 5% variance in a polynucleotide or a polypeptide. Allelic variants can be identified by sequencing the nucleic acid sequence of interest in a number of different species, which can be readily

carried out by using hybridization probes to identify the same genetic locus in those species. Any and all such nucleic acid variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity of gene of interest, are intended to be within the scope of the invention.

[0080] The term “identity” with respect to sequences can refer to, for example, the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman). The sequence identity or sequence similarity of two amino acid sequences, or the sequence identity between two nucleotide sequences can be determined using Vector NTI software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

[0081] In one aspect, the present invention relates to a pharmaceutical composition or vaccine for inducing an immunological response in a host animal inoculated with the vaccine or composition, the vaccine or composition including a pharmaceutical acceptable carrier and a modified APMV recombinant virus or viral vector. In yet another aspect of the invention, the recombinant APMV virus or viral vector includes, within a non-essential region of the virus genome, a heterologous DNA sequence which encodes an antigenic protein derived from a pathogen wherein the composition or vaccine when administered to a host, is capable of inducing an immunological response specific to the protein encoded by the pathogen.

[0082] A “vector” refers to a recombinant DNA or RNA plasmid, bacteriophage, or virus that comprises a heterologous polynucleotide to be delivered to a target cell, either *in vitro* or *in vivo*. The heterologous polynucleotide may comprise a sequence of interest for purposes of prevention or therapy, and may optionally be in the form of an expression

cassette. As used herein, a vector needs not be capable of replication in the ultimate target cell or subject. The term includes vectors for cloning as well as viral vectors.

[0083] The term “engineered” or “recombinant” means a polynucleotide of semisynthetic, or synthetic origin that either does not occur in nature or is linked to another polynucleotide in an arrangement not found in nature.

[0084] The term “nonessential region” refers to a region of a virus genome which is not essential for replication and propagation of the virus in tissue culture and whose deletion or inactivation may reduce virulence in a variety of animal systems. Any nonessential region or portion thereof can be deleted from the APMV genome or a foreign sequence can be inserted in it, and the viability and stability of the recombinant APMV resulting from the deletion or insertion can be used to ascertain whether a deleted region or portion thereof is indeed nonessential. In one embodiment, the nonessential region of the APMV genome is any region on the APMV-2, 4, 6, or 8 genome that does not encode Polymerase (L). In yet another embodiment, the nonessential region comprises an open reading frame encoding a nonessential protein. In this aspect, the open reading frame is selected from the group consisting of nucleoprotein (NP), phosphoprotein (P), matrixprotein (M), fusionprotein (F), and hemagglutinin/neuraminidase (HN). In one embodiment, the nonessential region is located upstream of the NP gene. In another embodiment, the nonessential region is located downstream of the L gene. In yet another embodiment, the nonessential region is a non-coding or intergenic region. In this aspect, the non-coding or intergenic region may be a region between NP and P genes, between P and M genes, between M and F genes, or between F and HN genes on the APMV-2, 4, 6, or 8 genome. In yet another embodiment, the nonessential region may be in the region of nucleotide positions 1 - 140, 1526 - 1692, 2910 - 3085, 4195 - 4498, 6130 - 6382, 8116 - 8272, 8116 - 8289, or 15013 - 15342 of SEQ ID NO:1.

[0085] In another aspect, the invention includes APMV chimeras in which one part or the full gene or several parts or full genes of the APMV vector are replaced by similar genes from other viruses, in particular those belonging to the paramyxoviridae family.

[0086] In one embodiment of the invention, the vaccine or pharmaceutical composition comprises an antigen selected from the group of avian pathogens including,

but not limited to, *Salmonella typhimurium*, *Salmonella enteritidis*, Infectious Bronchitis virus (IBV), Newcastle Disease virus (NDV), egg drop syndrome virus (EDS), or Infectious Bursal Disease virus (IBDV), Infectious Laryngotracheitis virus (ILTV), avian adenoviruses, Marek's disease virus (MDV), fowlpox virus, duck enteritis virus (DEV), duck parvoviruses, avian influenza virus, APMV, such as APMV-1, and the like, and combinations thereof.

[0087] In another embodiment, the vaccine or pharmaceutical composition comprises an antigen selected from a feline pathogen such as, but not limited to, feline herpesvirus (FHV), feline calicivirus (FCV), feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline parvovirus (FPV), feline infectious peritonitis virus (FIPV), rabies virus, and the like, and combinations thereof.

[0088] In yet another embodiment, the vaccine or pharmaceutical composition of the present invention comprises an antigen selected from a canine pathogen including, but not limited to, rabies virus, canine herpesvirus (CHV), canine parvovirus (CPV), canine distemper virus (CDV), canine parainfluenza 2 (CPI2), canine coronavirus, *Leptospira canicola*, *Leptospira icterohaemorrhagiae*, *Leptospira grippotyphosa*, *Borrelia burgdorferi*, *Bordetella bronchiseptica* and the like, and combinations thereof.

[0089] In yet another embodiment, the vaccine or pharmaceutical composition comprises an antigen selected from an equine pathogen, such as equine herpesvirus (type 1 or type 4), equine influenza virus, tetanus, west nile virus, equine arterivirus and the like or combinations thereof.

[0090] In yet another embodiment, the vaccine or pharmaceutical composition comprises an antigen selected from a bovine, ovine or caprine pathogen, such as rabies virus, bovine rotavirus, bovine parainfluenza virus type 3 (bPIV-3), bovine coronavirus, bovine viral diarrhea virus (BVDV), foot and mouth disease virus (FMDV), Rinderpest virus (RPV), Peste des Petits Ruminants virus (PPRV), malignant catarrhal fever viruses, bovine respiratory syncytial virus (BRSV), Infectious Bovine Rhinotracheitis virus (IBR), *Escherichia coli*, *Pasteurella multocida*, *Pasteurella haemolytica* and the like, and combinations thereof.

[0091] In still another embodiment, the vaccine or pharmaceutical composition comprises an antigen selected from a porcine pathogen such as, but not limited to, swine

influenza virus (SIV), porcine circovirus type 2 (PCV-2), porcine reproductive respiratory syndrome virus (PRRS), pseudorabies virus (PRV), porcine parvovirus (PPV), FMDV, *Mycoplasma hyopneumoniae*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Escherichia coli* and the like, and combinations thereof.

[0092] Construction of recombinant virus is well known in the art as described in, e.g., US patent Nos. 4,769,330, 4,722,848, 4,603, 112, 5,174, 993, and 5,756,103, 6,719,979. Specifically, a recombinant APMV virus may be constructed in two steps. First, the gene of interest to be inserted into the virus, such as an open reading frame of an antigen from an APMV-1 (NDV) or avian influenza virus or other organism, is placed into an *E.coli* plasmid construct into which the cDNA homologous to a section of cDNA of the APMV is inserted. Separately, the cDNA gene sequence to be inserted is preceded by a promoter region (gene start region) and followed by a gene end region which is specific for the APMV vector. The gene start/foreign antigen/gene end DNA fragment is flanked by cDNA fragment homologous of APMV-8 cDNA containing unique restriction enzyme cleavage sites. The resulting plasmid construct is then amplified by growing in *E.coli* bacteria and isolated. Next the recombinant plasmid is used in a restriction enzyme digest to cut out the gene start/foreign antigen/gene end DNA fragment that is flanked by cDNA homologous of APMV-8 cDNA and this fragment is ligated into the appropriately cleaved full length construct of APMV-8.

[0093] The full length construct containing the gene of interest is transfected into cells along with plasmids containing polynucleotides for expression of the APMV nucleoprotein (NP), the APMV phosphoprotein (P) and the APMV RNA polymerase (L) as well as the T7 RNA polymerase. All APMV cDNA constructs are under the control of the T7 polymerase promoter. The rescue of infectious virus is performed as described in Römer-Oberdörfer et al., 1999 and as shown in Figure 19A. The expression of the T7 RNA polymerase in transfected cells can be obtained by different means including transfection of plasmid DNA containing an expression cassette of the T7 RNA polymerase, recombinant virus (such as fowlpox or canarypox virus) expressing the T7 RNA polymerase or in cells that express the T7 RNA polymerase. In another aspect, the polynucleotides for expression of the APMV nucleoprotein (NP), the APMV

phosphoprotein (P) and the APMV RNA polymerase (L) and the full length viral cRNA are under the control of the immediate early promoter of the human cytomegalovirus. The rescue of the virus is performed as described in Inoue K, et al., 2003.

[0094] Successful expression of the inserted cDNA of interest (foreign cDNA or heterologous cDNA) by the modified infectious virus requires two conditions. First, the insertion must be introduced into a region of the genome of the virus in order that the modified virus remains viable. The second condition for expression of inserted cDNA is the presence of a regulatory sequences allowing expression of the gene in the viral background (for instance: gene start, gene stop, promoter, enhancer, polyadenylation signals, intergenic and untranslated regions).

[0095] In general, it is advantageous to employ a strong promoter functional in eukaryotic cells. In one embodiment, the promoter used for the transcription of viral mRNA by the viral RNA polymerase is the “gene start sequence”. The “gene start sequence” is the binding site for the L protein to bind and to transcribe the downstream located viral RNA into viral mRNA.

[0096] In one embodiment, the invention provides for the administration of a therapeutically effective amount of an APMV vaccine for the delivery and expression of an antigen, epitope or immunogen in a target cell. Determination of the therapeutically effective amount is routine experimentation for one of ordinary skill in the art. In one embodiment, the APMV vaccine formulation comprises an expression vector comprising a polynucleotide that encodes an antigen, epitope or immunogen and a pharmaceutically or veterinarily acceptable carrier, vehicle or excipient. In another embodiment, the pharmaceutically or veterinarily acceptable carrier, vehicle or excipient facilitates transfection and/or improves preservation of the vector or protein.

[0097] The pharmaceutically or veterinarily acceptable carriers or vehicles or excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarily acceptable carrier or vehicle or excipient can be a 0.9% NaCl (e.g., saline) solution or a phosphate buffer. Other pharmaceutically or veterinarily acceptable carrier or vehicle or excipients that can be used for methods of this invention include, but are not limited to, poly-(L-glutamate) or polyvinylpyrrolidone. The pharmaceutically or veterinarily acceptable carrier or vehicle or excipients may be any compound or

combination of compounds facilitating the administration of the vector (or protein expressed from an inventive vector *in vitro*), or facilitating transfection and/or improve preservation of the vector (or protein). Doses and dose volumes are herein discussed in the general description and can also be determined by the skilled artisan from this disclosure read in conjunction with the knowledge in the art, without any undue experimentation.

[0098] In another embodiment, pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle may be a water-in-oil emulsion. Examples of suitable water-in-oil emulsions include oil-based water-in-oil vaccinal emulsions which are stable and fluid at 4°C containing: from 6 to 50 v/v % of an antigen-containing aqueous phase, from 12 to 25 v/v %, from 50 to 94 v/v % of an oil phase containing in total or in part a non-metabolizable oil (e.g., mineral oil such as paraffin oil) and/or metabolizable oil (e.g., vegetable oil, or fatty acid, polyol or alcohol esters), from 0.2 to 20 p/v % of surfactants, from 3 to 8 p/v %, the latter being in total or in part, or in a mixture either polyglycerol esters, said polyglycerol esters being polyglycerol (poly)ricinoleates, or polyoxyethylene ricin oils or else hydrogenated polyoxyethylene ricin oils. Examples of surfactants that may be used in a water-in-oil emulsion include ethoxylated sorbitan esters (e.g., polyoxyethylene (20) sorbitan monooleate (TWEEN 80®), available from AppliChem, Inc., Cheshire, CT) and sorbitan esters (e.g., sorbitan monooleate (SPAN 80®), available from Sigma Aldrich, St. Louis, MO). In addition, with respect to a water-in-oil emulsion, see also US Patent No. 6,919,084. In some embodiments, the antigen-containing aqueous phase comprises a saline solution comprising one or more buffering agents. An example of a suitable buffering solution is phosphate buffered saline. In one embodiment, the water-in-oil emulsion may be a water/oil/water (W/O/W) triple emulsion (see, e.g., U.S. Patent No. 6,358,500). Examples of other suitable emulsions are described in U.S. Patent No. 7,371,395.

[0099] The pharmaceutical compositions and vaccines according to the invention may comprise or consist essentially of one or more adjuvants. Suitable adjuvants for use in the practice of the present invention are (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), such as oligodeoxyribonucleotide sequences having one or more non-methylated

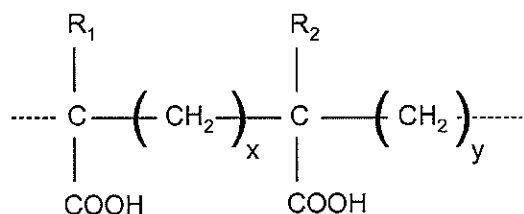
CpG units (Klinman et al., 1996; WO98/16247), (3) an oil in water emulsion, such as the SPT emulsion described on p 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, and the emulsion MF59 described on p 183 of the same work, (4) cation lipids containing a quaternary ammonium salt, e.g., DDA (5) cytokines, (6) aluminum hydroxide or aluminum phosphate, (7) saponin or (8) other adjuvants discussed in any document cited and incorporated by reference into the instant application, or (9) any combinations or mixtures thereof.

[0100] The oil in water emulsion (3), which is especially appropriate for viral vectors, can be based on: light liquid paraffin oil (European pharmacopoeia type), isoprenoid oil such as squalane, squalene, oil resulting from the oligomerization of alkenes, e.g. isobutene or decene, esters of acids or alcohols having a straight-chain alkyl group, such as vegetable oils, ethyl oleate, propylene glycol, di(caprylate/caprato), glycerol tri(caprylate/caprato) and propylene glycol dioleate, or esters of branched, fatty alcohols or acids, especially isostearic acid esters. The oil is used in combination with emulsifiers to form an emulsion. The emulsifiers may be nonionic surfactants, such as: esters of on the one hand sorbitan, mannide (e.g. anhydromannitol oleate), glycerol, polyglycerol or propylene glycol and on the other hand oleic, isostearic, ricinoleic or hydroxystearic acids, said esters being optionally ethoxylated, or polyoxypropylene-polyoxyethylene copolymer blocks, such as Pluronic, e.g., L121. Among the type (1) adjuvant polymers, preference is given to polymers of crosslinked acrylic or methacrylic acid, especially crosslinked by polyalkenyl ethers of sugars or polyalcohols. These compounds are known under the name carbomer (Pharmeuropa, vol. 8, no. 2, June 1996). One skilled in the art can also refer to U.S. Patent No. 2,909,462, which provides such acrylic polymers crosslinked by a polyhydroxyl compound having at least three hydroxyl groups, preferably no more than eight such groups, the hydrogen atoms of at least three hydroxyl groups being replaced by unsaturated, aliphatic radicals having at least two carbon atoms. The preferred radicals are those containing 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals can also contain other substituents, such as methyl. Products sold under the name Carbopol (BF Goodrich, Ohio, USA) are especially suitable. They are crosslinked by allyl

saccharose or by allyl pentaerythritol. Among them, reference is made to Carbopol 974P, 934P and 971P.

[0101] As to the maleic anhydride-alkenyl derivative copolymers, preference is given to EMA (Monsanto), which are straight-chain or crosslinked ethylene-maleic anhydride copolymers and they are, for example, crosslinked by divinyl ether. Reference is also made to J. Fields et al., 1960.

[0102] With regard to structure, the acrylic or methacrylic acid polymers and EMA are preferably formed by basic units having the following formula:



in which:

-R1 and R2, which can be the same or different, represent H or CH3

-x = 0 or 1, preferably x = 1

-y = 1 or 2, with x + y = 2.

For EMA, x = 0 and y = 2 and for carbomers x = y = 1.

[0103] These polymers are soluble in water or physiological salt solution (20 g/l NaCl) and the pH can be adjusted to 7.3 to 7.4, e.g., by soda (NaOH), to provide the adjuvant solution in which the expression vector(s) can be incorporated. The polymer concentration in the final immunological or vaccine composition can range between 0.01 and 1.5% w/v, between 0.05 and 1% w/v, and between 0.1 and 0.4% w/v.

[0104] Another aspect of the invention relates to a method for inducing an immunological response in an animal to an antigen, which method comprises inoculating the animal with a vaccine or a pharmaceutical composition including modified recombinant APMV virus which comprises and encodes the antigen of a pathogen for said animal. Yet another aspect of the invention relates to a method for inducing an immunological response in an animal to an antigen in a prime-boost administration regimen, which is comprised of at least one primary administration and at least one booster administration using at least one common polypeptide, antigen, epitope or immunogen. The immunological composition or vaccine used in primary administration

may be same, may be different in nature from those used as a booster. In one aspect of the prime-boost protocol of the invention, a composition or vaccine comprising the recombinant APMV virus (viral vector) of the present invention is administered followed by the administration of an inactivated viral vaccine or composition comprising an antigen, or a vaccine or composition comprising a subunit (protein, antigen), or a DNA plasmid vaccine or composition that contains or expresses an antigen. Likewise, a prime-boost protocol may comprise the administration of an inactivated viral vaccine or composition comprising an antigen, or a vaccine or composition comprising a subunit (protein, antigen), or a DNA plasmid vaccine or composition that contains or expresses an antigen, followed by the administration of a composition or vaccine comprising the recombinant APMV virus (viral vector) of the present invention. It is further noted that both the primary and the secondary administrations may comprise the composition or vaccine comprising the recombinant APMV virus (viral vector) of the present invention.

[0105] The primary administration may comprise one or more administrations of the same viral vector-based immunological compositions of vaccines. Similarly, the booster administration may comprise one or more administrations of the same viral vector-based or immunological composition of vaccine. The administration route of the prime and the boost may be the same or different. Similarly, the origin of the protective gene present in the prime and the boost may be the same or different (e.g. different strain).

[0106] The various administrations are preferably carried out 1 to 6 weeks apart, and more particularly about 3 weeks apart. According to a preferred mode, an annual booster, preferably using the viral vector-based immunological composition of vaccine, is also envisaged. The animals are preferably at least one day old at the time of the first administration.

[0107] A variety of administration routes may be used such as subcutaneously or intramuscularly, intradermally, transdermally, spray, drinking water, eye drop, intranasal, oral, oral baits, *in ovo* or a combination (e.g. oculonasal, oronasal).

[0108] The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Example 1 APMV-2, APMV-4, and APMV-6

A. Viruses and Birds

[0109] One-day-old SPF (specific pathogen free) chickens (Merial, Gainesville, GA) were housed in positive pressure Horsfall-Baur isolation units. Feed and water were provided *ad libitum* and the birds were examined twice daily. The viruses (APMV-2, 4, and 6) used for experimental studies were isolated from wild birds and classified by the National Veterinary Service Laboratory (NVSL, Ames Iowa, USA). Viruses were propagated in 9-day-old embryonated SPF chicken eggs (SunRise Farms, Catskill, NY, USA) by inoculation via the allantoic route. The allantoic fluid was harvested at day 3 after inoculation, aliquoted, and stored at -80°C. The APMV subtype was confirmed by the HI test using standard sera (NVSL, Ames, Iowa, USA). The 50% egg infectious dose (EID₅₀) for each isolate was determined by inoculating 10-fold serial dilutions of allantoic fluid in embryonated SPF eggs. The titer was calculated following the method as described by Reed and Muench (Reed, LJ et al., 1938, Am. J. Epidemiol. 27:493-497).

B. Experimental infection

[0110] Twenty-five one-day-old SPF chickens per group were infected with 10⁶ EID₅₀ per chicken by the ocular-nasal route. Chickens of the control group were mock inoculated with PBS (phosphate buffered saline). At days 2, 4, 7, 14, and 28 p. i. (post infection) five birds from each group were bled via the wing vein to collect serum samples, euthanized with CO₂, and necropsied. Tissue specimens of the trachea, lung, pancreas and gut were collected. For each organ, a fresh pair of sterile scissors and forceps was used. Half of the tissue sample was placed in a Lysing Matrix D tube (MP Biomedicals, Solon, OH) containing viral transport medium (1X minimal essential medium, 7.5% sodium bicarbonate, 15 mM HEPES, 1% fetal bovine serum, 4,000 U/ml penicillin, 400 µg/ml gentamycin, 8 µg/ml amphotericin B, 4,000 µg/ml streptomycin, 1000 µg/ml kanamycin sulfate). The second half of the tissue sample was fixed in 10% buffered formalin and embedded in paraffin wax. Sections of the paraffin-embedded tissues were stained with Mayer's hematoxylin and eosin (H&E).

[0111] The results showed that mild diarrhea was observed at day four and seven p. i. in birds infected with APMV-2 or APMV 4. During necropsy, birds infected with

APMV-2 showed slightly enlarged pancreas at day two and four after infection. No other gross lesions were observed in any group.

C. Serology

[0112] The hemagglutination (HA) and hemagglutination-inhibition (HI) tests were used to detect virus in allantoic fluid and analyze the presence of HI antibodies in collected serum samples, respectively. The tests were conducted by standard procedures using 0.8% chicken red blood cells resuspended in PBS. The HI test was performed by the diluted-serum constant-antigen method. Eight HA units of viral antigen was used for each serum dilution.

[0113] The HI antibody titers were investigated at day 2, 4, 7, 14, and 28 p.i. Positive HI titers ($\geq 1:16$) were observed in serum samples of APMV-2 infected birds at day 7 (1/5), day 14 (5/5), and day 28 p.i. (5/5). Interestingly, only one chicken infected with APMV-4 developed an HI titer which has been considered as positive during the course of the experiment at day 14 p.i. Similarly for APMV-6, two chickens out of five developed a HI titer of 1:16, only on day 28 p.i.. Mock inoculated birds remained negative for HI antibodies to all three APMV used in this experiment. In addition, to exclude cross contamination all sera were tested for HI antibodies against the other two antigens and remained negative.

D. Virus Isolation

[0114] The tissue samples collected in the Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA) were homogenized twice using the Fastprep[®]-24 (MP Biomedicals, Solon, OH) at a setting of 4.0 M/S for 20 seconds. Following incubation for 15 min at room temperature, the homogenized samples were centrifuged for 20 min at 2000 g at 4°C. Sterility was tested after inoculation of 50 µl of the obtained supernatant in 2 ml tryptose phosphate broth (TPB) (DIFCO, Becton Dickinson, Sparks, MD, USA) supplemented with 10% hydrolactalbumin by incubation at 37°C in an orbital shaker overnight. Non-sterile samples were filtered with 0.45 µm syringe filters (Whatman Inc., Florham Park, NJ, USA). Samples were stored at -80°C. Virus isolation was performed by inoculation of 0.1 ml into the allantoic cavity of 9-day-old embryonated SPF chicken eggs. After incubation for three days at 37.5°C, the allantoic fluid was harvested and tested for the presence of haemagglutinating activity by HA.

[0115] To analyze sites of virus replication, several organs (trachea, lung, gut, pancreas) were analyzed for infectious virus by virus isolation in embryonated eggs (Figure 1). Overall, replicating virus was only detected in a few chickens. Briefly, APMV-4 was recovered on day 2 from trachea, lung and pancreas while APMV-6 was isolated from lung and pancreas. At day 4, APMV-2 was isolated from trachea and lung while APMV-6 was isolated from all tested organs except the gut. At day 7, APMV-2 was isolated from a single gut sample, APMV-4 was isolated from the pancreas while APMV-6 was isolated from lung and pancreas samples. Surprisingly, at day 14 p.i. no virus was isolated whereas at day 28 p.i. APMV-2, 4 and 6 could be isolated from the pancreas. No virus was isolated from mock inoculated birds. The identity of the back isolated virus was confirmed by HI test using standard sera as provided by NVSL.

[0116] To evaluate the pathological potential of the investigated viruses, microscopical lesions in the obtained organs were analyzed (Figure 2). At day 2 p.i., a catarrhal tracheitis in addition to ciliary loss on the respiratory epithelium and mild enteritis was observed in all infected chickens. At day 4 p.i., APMV-2 infected chickens showed an increased number of hypertrophic mucous glands in the trachea and focal ulcerations of the respiratory epithelium. APMV-4 infected birds showed changes highly suggestive of a respiratory infection such as mild tracheitis, mild to moderate multifocal lymphocytic pancreatitis and also a focal BALT (Bronchus-Associated Lymphoid Tissue) hyperplasia at day 4 p.i. The investigation on organs of APMV-6 infected chickens revealed tracheal changes such as catarrhal and ulcerative tracheitis and a focal pancreatitis that are consistent with a viral stimulation. At day 7 p.i., APMV-2 infected chickens showed focal tracheal attenuation or replacement of respiratory epithelium as indicative of healing. Birds infected with APMV-4 showed mild BALT hyperplasia while APMV-6 infected birds showed cystic enteropathy, focal enteritis and lymphocytic infiltrates in the pancreas. In addition to mild lymphocytic enteritis and mild GALT hyperplasia, APMV-2 infected birds also showed healing changes such as tracheal attenuation at day 14 p.i. Organ samples from chicken infected with APMV-4 or 6 showed changes suggestive of viral infection such as mild interstitial pneumonia, catarrhal tracheitis and BALT or GALT hyperplasia at day 14 p.i. All investigated samples obtained from infected chicken showed lesions such as GALT hyperplasia,

lymphocytic pancreatitis and lymphocytic bronchitis at day 28 p.i. At day 2, birds of the control group showed a mild catarrhal tracheitis which could be attributed to environmental factors.

[0117] Figure 3 shows low HI titers (up to 1:32 at day 14) from SPF chickens infected with APMV-4 or APMV-6 indicating that only APMV-2 infection elicited an HI response that could be characterized as seropositive. Nevertheless, all three viruses were recovered from the infected birds' trachea, lungs, gut and pancreas up to day 7 p.i. and from the pancreas up to day 28 p.i.. Infection with APMV-2, 4, or 6 showed characteristic histopathological lesions (summarized in figure 2) in all the infected birds, indicative of stimulation with a viral antigen. The viral isolation and histological profiles of the infected birds clearly depicted the tropism of the viruses in the infected birds (trachea and lungs from day 2 to day 7 and gut, lung and pancreas from day 7 onwards). All isolates were detected in the pancreas up to 28 days p.i. but virus isolation was not possible at day 14 p.i.. This indicated that the investigated APMV can probably persist and later become reactivated. Thus virus carriers can be present in infected flocks. Only APMV-2 induced HI antibodies while HI antibodies of chickens infected with APMV-4 and 6 were not detected.

Example 2 APMV-8

A. Viruses and Birds

[0118] One-day-old SPF chickens (Merial, Gainesville, GA, USA) and Pekin ducks (Metzer Farms, Gonzales, CA, USA) were housed in positive pressure Horsfall Baur isolation units. Feed and water were provided *ad libitum*, and the birds were examined twice daily. The APMV-8 virus (APMV-8: SCWDS ID: MA-7) used for experimental studies was isolated from a mallard and classified by the National Veterinary Service Laboratory (NVSL, Ames Iowa, USA). The virus was propagated in 9-day-old embryonated SPF chicken eggs by inoculation via the allantoic route. Allantoic fluids were harvested at day 3 after inoculation, pooled, aliquoted and stored at -80 °C. The APMV-8 subtype was confirmed by HI testing using standard sera as provided by the National Veterinary Service Laboratory (Ames, IA, USA). The EID₅₀ was determined by inoculating 10-fold serial dilutions of allantoic fluid in embryonated SPF eggs. The titer

was calculated following the method as described by Reed and Muench (Reed & Muench, 1938).

B. Experimental infection

[0119] Twenty five one-day-old SPF chickens or Pekin ducks per group were infected via the oculonasal route with 10^6 EID₅₀ per bird diluted in PBS. Birds of the control chicken or duck group were mock inoculated with PBS. Five birds from each group were bled via the brachial vein to collect serum samples, humanely euthanized with CO₂, and necropsy was performed at two, four, seven, fourteen and twenty eight days post infection (d p.i). Tissue specimens of the trachea, lung, pancreas, and gut (duodenum) were collected. For each organ, a fresh pair of sterile scissors and forceps was used. Half of the tissue sample was placed in a Lysing Matrix D tube (MP Biomedicals, Solon, OH, USA) containing viral transport medium (VTM, 1X minimal essential medium, 7.5% sodium bicarbonate, 15 mM HEPES, 1% fetal bovine serum, 4,000 U/ml penicillin, 400 µg/ml gentamycin, 8 µg/ml amphotericin B, 4,000 µg/ml streptomycin, 1000 µg/ml kanamycin sulfate). The second half of the tissue sample was fixed in 10% buffered formalin and routinely processed, embedded, sectioned and stained with hematoxylin and eosin (H&E).

C. Virus Isolation

[0120] The tissue samples collected in the Lysing Matrix D tubes were homogenized twice using the FastPrep-24 (MP Biomedicals) at a setting of 4.0 M/S for 20 seconds. The homogenized samples were incubated for 15 min at room temperature and then centrifuged for 20 min at 2000xg at 4°C. 50 µl of the obtained supernatant was inoculated in 2 ml sterile TFB supplemented with 10% hydrolytalbumin followed by incubation at 37°C in an orbital shaker overnight to test for sterility. Non-sterile samples were filtered with 0.45 µm syringe filters (Whatman Inc.). Samples were stored at -80°C. Virus isolation was performed by inoculation of 0.1 ml into the allantoic cavity of 9-day-old embryonated SPF chicken eggs. After incubation for three days at 37.5°C, the allantoic fluid was harvested and tested for the presence of hemagglutinating activity by HA.

D. Serology

[0121] The hemagglutination (HA) and hemagglutination-inhibition (HI) tests were used to detect virus in allantoic fluid and to analyze the presence of HI antibodies in

collected serum samples, respectively. The tests were conducted by standard procedures using 0.8% chicken red blood cells resuspended in PBS. The HI test was performed by the diluted-serum constant-antigen method. Eight HA units of viral antigen were used for each serum dilution. The geometric mean titer was determined as described previously (Brugh, 1978).

[0122] Figure 4 shows HI antibody titers in SPF chickens and ducks infected with APMV-8. Chickens and ducks were oro-nasal infected with a dose of 10^6 EID₅₀ of APMV-8. Sera samples were collected at day 2, 4, 7, 14, and 28 p.i. and analyzed by the HI test with the APMV-8 antigen. The HI serum titers (in log₂) are shown on the left axis.

E. Pathogenicity indices of APMV-8 in chicken

[0123] To assess the virulence of APMV-8, the intracerebral pathogenicity index (ICPI) was determined following the World Organization for Animal Health (OIE, 2008) procedures for Newcastle disease virus. The mean dead time (MDT) in chicken embryos was determined as described previously (Swayne et al., 1998) using a serial dilution of APMV-8 from 10^{-1} through 10^{-8} .

[0124] The determination of the mean dead time (MDT) in embryonated eggs as well as the assessment of the intracerebral pathogenicity index (ICPI) is an important measurement for the pathogenicity of the virus. With respect to the MDT in embryonated eggs, none of the embryos of the inoculated eggs died after the 7 day period and thus the APMV-8 isolate can be classified as lentogenic. The presence of virus was confirmed by HA test using the allantoic fluid of eggs inoculated with the 10^{-6} dilution. All the chickens intracerebrally inoculated showed no clinical signs over the observation time resulting in an ICPI value of zero which results in a lentogenic phenotype.

F. APMV-8 booster vaccination

[0125] Ten one-day-old SPF chickens per group were infected via the oculo-nasal route with 10^6 EID₅₀ per bird. Birds of the control chicken group were mock inoculated with PBS. The birds were infected again with the same dose 14 days after the first infection. The presence of infectious virus was monitored at day 2, 4, 7 and 14 after the first infection and day 2 and 4 after the second infection by virus isolation from tracheal

swabs using 9-day-old embryonated SPF eggs. Antibody response was monitored by HI titer of serum samples collected at 0, 7, and 14 days after each vaccination.

[0126] In order to investigate if a second immunization would allow developing a more sustainable HI titer, a prime/boost scheme experiment was performed (Figure 5). On day 7 after the first infection, all ten birds showed an HI ranging from 64 to 1024 (GMT 207). The titer declined at 14 d p.i. after the first infection (GMT 84) but increased after the boost infection at day 14 after the initial infection. On day 7 after the boost vaccination, the GMT increased to 137 and decreased again to a GMT of 73 at day 14 after the second infection. Infectious virus could be isolated from tracheal swabs at day 2 (5/10 birds) and day 4 (4/10) birds after the first infection. After the second infection no virus was isolated from the swabs taken at day 2 and 4 p.i..

G. Detection of viral RNA by RT-PCR

[0127] The detection of viral RNA from tissue samples was performed after homogenization of tissue samples followed by isolation of RNA by using the High Pure RNA isolation kit (Roche, Mannheim, Germany). A primer pair (8NPf1, 8NPr, see table 1) was used in an RT-PCR using the Superscript III One Step RT-PCR kit with Platinum Taq (Invitrogen, Carlsbad, CA, USA) following the instructions of the manufacturer. The obtained reaction products were analyzed on a 1% agarose gel (Figure 6). Tracheal tissues were taken at day 2 p.i. from non-infected ducks (C1-C5) and APMV-8 infected ducks (I1-I5). The tissues were homogenized and RNA was prepared for RT-PCR. A water control (W) was prepared in parallel. The reaction products were separated on a 1.5% agarose gel. The size of the fragment was controlled by using the 100 bp ladder (New England Biolabs, Boston, MA, USA). The sizes of the DNA fragments are shown at the right.

Table 1 Oligonucleotides used for RT-PCR for the detection of
viral RNA in tissue samples

Name	Sequence	orientation	Location ^A	SEQ ID NO
APMV- PolyT	TTTTTTTTTTTTTTTTTACCAAACAR RGAA	sense	1-14	21
8NPf1	CAGGAGACCTGATGTTGCCTCAAC	sense	200-223	22
8NPr	GCAGGCGATCTATAGTCTCTGATAG	antisense	618-642	23

H. Determination of the minimal infectious dose in chicken and ducks

[0128] In order to determine which viral titer would be sufficient in chickens to detect a seroconversion, one-day-old SPF chickens were infected with different doses of APMV-8. The birds were housed as described above. Ten chickens per group were infected each with 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , or 10^6 EID₅₀. The virus was diluted in VTM. One group was inoculated with VTM and served as control. The birds were bled at day 7 and 14 after infection via the brachial vein. Based on the results obtained during the chicken experiment three-day-old Pekin ducks were infected with different amount of virus. The infection dose was chosen 10^3 , 10^4 , 10^5 , or 10^6 EID₅₀ per duck. One group was mock-infected with VTM. The birds were bled at day 7 and 14 after infection via the leg vein. The serum sample was analyzed for the presence of virus specific antibodies by HI as described above.

I. Determination of the pathogenicity in ducks and chickens

[0129] During the experiments, no clinical signs were observed in chickens and ducks. During necropsy, three infected chickens showed slightly enlarged pancreas and inflamed duodenum at day two and four p.i.. No other gross lesions were observed in any group.

[0130] The serological response was examined at 2, 4, 7, 14, and 28 d p.i. by investigation of the HI titers in the serum (Figure 4). Serum samples of APMV-8 infected chickens showed positive HI titers (≥ 16) starting with 7 d p.i. (5/5, GMT: 111), 14 d p.i. (5/5, GMT: 48), and 28 d p.i. (5/5, GMT: 48). Serum samples of APMV-8 infected ducks also showed positive HI titers (≥ 16) at 7 (5/5, GMT 21), 14 (5/5, GMT 28), and 28 (4/5, GMT 14) d.p.i. The HI titers ranged from 32 to 256 for chickens, while for ducks the range was from 16 to 64. Sham inoculated birds remained negative for HI antibodies to APMV-8 at all time points investigated in both species.

[0131] To determine the sites of virus replication in chickens and ducks, several organs (trachea, lung, duodenum, and pancreas) were analyzed for infectious virus by virus isolation in embryonated eggs (Figure 7). In chickens, APMV-8 was recovered at 2 d p.i. from trachea, lung, and duodenum. At 4 d p.i., APMV-8 was isolated from all the analyzed organs; while at 7 d p.i., APMV-8 was isolated only from the pancreas. At 14

and 28 d p.i. no virus was isolated from any organ. No virus was isolated from sham inoculated birds. The identity of the back isolated virus was confirmed by HI test using standard sera as provided by NVSL. No virus was isolated from any of the collected duck tissues at any time point even after two subpassages in 9-day-old embryonated SPF chicken eggs. Therefore, RT-PCR primers were designed based on the APMV-8 sequence information available to detect the presence of viral RNA in the collected tissue samples (Figure 7). At 2 d p.i viral RNA was detected in the trachea (Figure 6), gut and pancreas while at 4 d p.i viral RNA was detected in all the organs analyzed. At 7, 14, and 28 d p.i, viral RNA was detected only in the trachea and lung. RT-PCR using RNA obtained from organs of mock inoculated birds did not result in the amplification of an RT-PCR fragment which indicates the absence of APMV-8 in these birds.

[0132] To evaluate the pathological potential of the investigated virus, the organs were analyzed for the presence of microscopic lesions (Figure 8). At 2 d p.i., a mild multifocal proliferative tracheitis was observed in all infected chickens. The remaining organs showed no difference to the control group. APMV-8 infected chickens showed focal attenuation or regeneration of respiratory epithelium in the trachea at 4 d p.i. as indicative of healing. Additionally, the birds also showed mild multifocal lymphocytic pancreatitis which indicated a viral infection. Infected chicken showed changes in the lung at 7 d p.i. such as moderate to severe BALT, tracheal changes such as catarrhal tracheitis and multifocal lymphocytic pancreatitis. These findings are consistent with an antigenic stimulation. At 14 d p.i, tracheal changes consistent with healing and pancreatic changes such as lymphocytic pancreatitis suggestive of viral infection were observed in infected chickens. At 28 d p.i., only a mild catarrhal tracheitis and mild enteritis were observed in some of the infected chickens. In infected ducks, multifocal mild lymphocytic tracheitis, lung changes (interstitial pneumonia) and intestinal changes (lymphocytic enteritis) were observed at 2 d p.i while tracheal changes consistent with respiratory infection were seen at 4 d.p.i.. At 7 d p.i., infected ducks showed lymphocytic tracheitis and pancreatitis consistent with viral infection whereas the observed catarrhal tracheitis was a suggestive of healing at 14 d p.i.. In addition, infected ducks showed a lymphocytic pancreatitis at 14 d p.i.. Later, at 28 d p.i., in the lung of infected ducks a BALT hyperplasia and also mild multifocal heterophilic tracheitis were noticed. Both

pathological microscopic lesions were indicative of a viral infection. In the non infected controls no changes in the examined organs were observed.

J. Determination of the minimal dose needed for induction of an immune response

[0133] In order to examine the minimal infectious dose which is necessary to induce a seroconversion in chicken, tenfold dilutions of APMV-8 were used to infect ten one-day-old SPF chickens (Figure 9). For the HI test, 4 HA units were used which results in a threshold of 16 to be considered as positive. The serum samples taken at day 14 p.i. showed that an EID₅₀ of 10³ was sufficient to induce an immune response in 4/10 chickens which was considered as positive (GMT 11). At day 14 after infection with an EID₅₀ of 10⁴ nine of ten birds showed a titer ≥ 16 (GMT 34). The infection with an EID₅₀ of 10⁵ and 10⁶ induced an HI titer of ≥ 16 at day 14 p.i. in all birds with a GMT of 73 and 137, respectively.

[0134] Based on this result, 8 ducks each were infected with APMV-8 starting with a dose EID₅₀ of 10³ per bird up to a dose of EID₅₀ of 10⁶ per bird (Figure 10). Six out of eight ducks developed significant titers (≥ 16) 14 days after infection with a GMT of 14 after infection with an EID₅₀ of 10⁴/bird. At day 14 p.i. 6/8 ducks infected with an EID₅₀ of 10⁵/bird and 7/8 ducks infected with an EID₅₀ of 10⁶/birds developed significant titers (≥ 16) with a GMT of 17 and 23, respectively.

Example 3 Determination of the full length sequence of APMV-8

[0135] For the determination of the full length sequence of APMV-8, viral RNA sequence information is initially needed. To this end, the 3'-end of the viral genome was cloned by using a primer (APMV-polyT, see table 1) which contained a degenerated sequence based on available 3'- sequences of APMV1 (Genbank accession No. AF077761), APMV-2 (Genbank accession No. EU338414), and APMV-6 (Genbank accession No. EF569970). Viral RNA was purified from allantoic fluid using the High Pure RNA isolation kit (Roche, Mannheim, Germany). The sequence was amplified using the 5' RACE System for Rapid Amplification of cDNA Ends Version 2.0 (Invitrogen) following the manufacturer's instructions. Several fragments were obtained, gel eluted

and cloned into the Topo TA cloning vector (Invitrogen) and positive selected clones were sequenced. The obtained nucleotide sequences were analyzed in an nblast search against the NCBI database which resulted in no similarities. A tblastx search against the NCBI database showed similarities to the nucleoprotein of 83% similarity of an Avian paramyxovirus 2 (APMV-2/Chicken/California/Yucaipa/56, Genbank accession No. EU338414) and of 56% similarity of an Avian paramyxovirus 6 strain (APMV-6/Goose/FarEast/4440/2003, Genbank accession No. EF569970). Using this primer, the primer walking method was employed using the 5' RACE System for Rapid Amplification of cDNA Ends Version 2.0 (Invitrogen). The 5'-RACE produced an approximately 800 bp fragment. Using this technique, new sequence information was obtained based on sequence information from the previous sequence which has been used for the delineation of new oligonucleotides. The 5'-end of the viral genome was also determined by the 5'-RACE method. The 3'-end of the viral genome was obtained after ligation of the RNA with T4 RNA ligase1 (New England Biolabs). The ligation reaction was purified again with High Pure RNA isolation kit (Roche) and an RT-PCR was performed using Superscript III One Step RT-PCR kit with Platinum Taq (Invitrogen). The obtained cDNA fragment was cloned into the pCR2.1 vector (Invitrogen) and sequenced. Three plasmids from each cloned fragment were sequenced in both directions, thus resulting in sequence 6x-coverage of per nucleotide.

[0136] The full length genome sequence of the analyzed APMV-8 strain is 15342 nucleotides, this is in accordance to the rule of six (Calain, P. & Roux, L., 1993) for *Paramyxovirinae*. Six open reading frames (ORF) have been detected and are encoding for proteins. The order of the proteins was determined as 3'-NP-P-M-F-HN-L-5' (the genome sequence SEQ ID NO:1 is in the 5' to 3' orientation antigenomic) using similarities of the protein sequence to proteins of other avian paramyxoviruses. The putative start and stop codons of the ORF's and the theoretical molecular weight (Swiss Institute of Bioinformatics ExPASy website) of the proteins are shown in table 2.

Table 2 Parameter of the proteins encoded by the APMV-8 sequence

Protein	Start codon	Stop codon	Theoretical MW (kD)
Nucleoprotein	141-143	1524-1526	51.2

(NP)			
Phospho protein (P)	1693-1695	2908-2910	43.5
Matrixprotein (M)	3076-3078	4193-4195	40.6
Fusioprotein (F)	4499-4501	6128-6130	58.5
Hemagglutinin/neuraminidase	6383-6385	8114-8116	63.5
(HN)			
Polymerase (L)	8273-8275 or	15011-15013	254.6
	8297-8299		253.6

[0137] The putative genomic leader and trailer sequences were determined by determination of the putative gene start sequence of the NP gene (leader) and putative gene end sequence of the L protein (trailer). The leader sequence is located from nucleotide 1 to nucleotide 55. The putative gene start sequence (nt 56-63) of the NP gene ends the leader sequence. The trailer sequence is localized behind the last gene end sequence in the viral genome. Due to the presence of two putative gene end sequences for the RNA polymerase gene (nt 15161-15171 or 15288 -15297) two putative trailer sequences have been identified (nt 15172-15342 or nt 15289-15342). The location of the putative gene start sequence (poly G containing sequences) and gene end sequences (signal sequence for a polyadenylation) and the intergenic sequences were summarized in table 3.

Table 3 Sequence and location of putative gene start, intergenic, and gene end sequence of APMV-8

Gene	Gene start	Gene end	intergenic
Nucleoprotein	56-63	1615-1625	1626-1627
Phosphoprotein	1628-1635	2991-3001	3002-3031
Matrixprotein	3032-3039	4404-4416	4417-4441
Fusionprotein	4442-4449	6260-6271	6272-6278
Hemagglutinin/neuraminidase	6279-6287	8261-8273	8274-8275
RNA polymerase	8275-8283	15161-15171	
		or	
		15288-15297	

Table 4 SEQ ID NO v. DNA and protein sequences.

SEQ ID NO	Gene name	Type
1	APMV-8 genome sequence	DNA or RNA
2	APMV-8 Nucleoprotien (NP)	DNA or RNA
3	APMV-8 Nucleoprotien (NP)	Protein
4	APMV-8 phospho protein (P)	DNA or RNA
5	APMV-8 phospho protein (P)	Protein
6	APMV-8 Matrixprotein (M)	DNA or RNA
7	APMV-8 Matrixprotein (M)	Protein
8	APMV-8 Fusioprotein (F)	DNA or RNA
9	APMV-8 Fusioprotein (F)	Protein
10	APMV-8 Hemagglutinin/neuraminidase (HN)	DNA or RNA
11	APMV-8 Hemagglutinin/neuraminidase (HN)	Protein
12	APMV-8 Polymerase (L) DNA	DNA or RNA
13	APMV-8 Polymerase (L) protein 1	Protein
14	APMV-8 Polymerase (L) protein 2	Protein

[0138] The putative gene start sequences for APMV-8 were conserved containing a poly (C)₅ sequence followed by a 3'-GCU-5' sequence. The only exception is the putative gene start sequence for the viral RNA polymerase (3'-CUCCCCGCU-5'). The putative gene end sequences were also conserved and contain a poly (U)₆ sequence at the genomic viral 5' sequence (Table 5).

Table 5 Gene Start and Gene End sequences of APMV-8

	Gene Name	Sequences (5-3' antigenomic orientation)	SEQ ID NO
Gene start	NP gene	CCCCCGCUUCUGUCA	24
	P gene	CCCCCGCUGGAGUUA	25
	M gene	CCCCCGCUUCUGUGC	26
	F gene	CCCCCGCUUUAGAAC	27

	HN gene	CCCCCGCUGGGUAAA	28
	L	CUCCCGCUGGAGAUG	29
Gene end	NP gene	AACUAAAUUCUUUUUU	30
	P gene	UAACUAAUUCUUUUUU	31
	M gene	AGGAUUAUAUUUUUU	32
	F gene	CUAUAAAUAUUUUUU	33
	HN gene	UACUAAAUUCUUUUUU	34
	L gene (1)	ACUAAAUAUCUUUUUU	35
	L gene (2)	UUAUUGAUUUUUUUUU	36

[0139] These sequences were predicted based on sequences which were described for other paramyxoviruses of the genus *Avulavirus* (Chang et al., 2001, Nayak et al, 2008, Jeon et al., 2008). There are two possible start codons for the ORF of the RNA polymerase. The first start codon (nt 8273-8275) is localized in the gene end –intergenic region- gene start region between the HN ORF and the viral RNA polymerase ORF. This makes this start codon unlikely but not impossible. The second start codon (8297 -8299) is down stream of the gene end –intergenic region- gene start region and may act as initiation codon for the start of the translation of the RNA polymerase of APMV-8.

[0140] The genome of APMV-8 is 15342 nt long. This is larger than APMV-1 (SQ ID NO:15, 15186 nt, de Leeuw & Peeters, 1999), APMV-2 (SEQ ID NO:16, 14,904 nt, Subbiah et al., 2008), and APMV-4 (SEQ ID NO:18, 15054 nt, Nayak et al., 2008), and smaller than APMV-3 (SEQ ID NO:17, 16,272 nt, Kumar et al., 2008) and APMV-9 (SEQ ID NO:20, 15,438 nt, Samuel et al., 2009). The length of 55 nt of the leader sequence seemed to be conserved between all APMV (Krishnamurthy & Samal, 1998, de Leeuw & Peeters, 1999, Subbiah et al., 2008, Nayak et al., 2008, Kumar et al., 2008, Samuel et al., 2009) whereas the trailer sequence seemed to be variable in length. The gene start and gene end sequences of the viral genes were also highly conserved for APMV-8 (as shown in Table 5). This has been also described for sequences of APMV-2 (Subbiah et al, 2008), APMV-3 (Kumar et al, 2008), APMV-4 (Jeon et al., 2008, Nayak et al, 2008), APMV-6 (Chang et al, 2001), and recently for APMV-9 (Samuel et al., 2009). The number of the nucleotides of the full length sequence is a multiple of six

which is agreement with the role of six for the genomes of paramyxoviruses (Kolakofsky et al., 1998).

[0141] The sequence identity between APMV-1, 2, 3, 4, 6, 8, and 9 genome sequences are shown in Table 6.

Table 6 sequence identity percentage between the genome of
APMV-1, 2, 3, 4, 6, 7, 8, and 9

APMV		1	2	3	4	6	7	8	8	8	8	9
	SEQ ID NO	15	16	17	18	19	20	1	37	38	39	40
8	1	48	61	47.2	47.6	52	53	100	99.1	96.5	96.4	48

[0142] The percent sequence identity between two nucleic acid or polypeptide sequences is determined using Vector NTI 11.0 (PC) software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA). A gap opening penalty of 15 and a gap extension penalty of 6.66 are used for determining the percent identity of two nucleic acids. A gap opening penalty of 10 and a gap extension penalty of 0.1 are used for determining the percent identity of two polypeptides. The percent identity was calculated based on the shorter sequence.

Example 4 Vaccination of one-day-old broiler chickens with APMV-8 strain

[0143] Twenty one-day-old broiler chickens were separated into two groups according to table 7 shown below.

[0144] At day 1, the one-day-old chickens were bled to determine the antibody status directed against Newcastle disease virus (NDV) and APMV-8 using the hemagglutination inhibition assay (HI test). The test was performed using allantoic fluid from either NDV strain Lasota or APMV-8 infected SPF eggs. Four HA units of NDV strain Lasota or APMV-8 and 1% chicken red blood cells were used for the HI test. The resulting HI titers

show that the serum of the chickens contained HI antibodies directed against NDV but no detectable antibodies against APMV-8 virus.

Table 7. Infection/vaccination of one-day-old broiler chickens

Treatment	Group 1 (ten one-day-old chickens)	Group 2 (control) (ten one-day-old chickens)
Day 1: vaccination with APMV-8 strain	Yes	No
Day 14: HI test	Yes	Yes
Day 14: second vaccination with APMV-8 strain (boost)	5 chickens (group 1-1): 2nd vaccination/ 1 st vaccination. 5 chickens (group 1-2): no 2 nd vaccination/ 1 st vaccination	5 chickens (group 2-1): 2nd vaccination 5 chickens (group 2-2): not vaccinated
Day 28: HI test	Yes	Yes

[0145] At day 1, group 1 of 10 chickens were infected with via the nasal route with 10^6 EID₅₀ of APMV-8 strain, group 2 of 10 chickens were not infected serving as control.

[0146] Fourteen days after infection (day 14), the chickens were bled and the obtained serum samples were analyzed again for the presence of HI antibodies directed against NDV and APMV-8 (Figure 20). The result showed that the APMV-8 vaccinated chickens showed HI titers using APMV-8 as antigen. The APMV-8 specific HI titers were between 128 and 2048. The HI titers against NDV declined to a HI titer below 16, thus they are not considered as NDV positive. The result showed that chickens maternal derived antibodies directed against NDV did not omit the infection with APMV-8, thus interference of such antibodies with APMV-8 vaccination is unlikely.

[0147] Fourteen days after infection (day 14), the chickens in group 1 and group 2 were split. Five chickens in each of group 1 (group 1-1) and group 2 (group 2-1) were again infected with 10^6 EID₅₀ of APMV-8 strain (table 7), the remaining five chickens in each group (group 1-2 and group 2-2) were not infected. This experiment is designed to investigate where a later infection of chickens would have an effect on the infection and whether a second infection (boost vaccination) would increase the antibody titer.

Fourteen days later (day 28), all chickens were bled again and the serum was investigated for the presence of APMV-8 and NDV antibodies. The serum titers (Figure 21) showed that a first vaccination at day 14 (group 2-1) did induce APMV-8 specific antibody titers ranging from 32 to 512. In chickens vaccinated only at day 1 (group 1-2), the antibody titers declined to titers in a range from 128 to 512. In chickens which have been vaccinated at day 1 and day 14 (group 1-1), the APMV-8 specific antibody titer did not increase, suggesting that the virus used for the second infection was neutralized by APMV-8 specific antibodies induced by the first infection. The serum of the non-vaccinated controls (group 2-2) did not contain APMV-8 specific HI antibodies. At day 28, the NDV antibodies declined further, only 11 chickens out of the 20 chickens showed any HI titers with the NDV antigen whereas at day 14 fourteen chickens showed low antibody titers against NDV.

Example 5 *In ovo* vaccination of embryonated SPF eggs

[0148] This study was performed to test if an *in ovo* vaccination with APMV-8 would result in an antibody response in chickens and if *in ovo* vaccination would interfere with hatchability and livability.

[0149] In study 1, 108 SPF eggs were *in ovo* vaccinated with APMV-8 virus strain using the INOVOJECT (Pfizer Animal Health, NY, USA) at day 18 of incubation. The virus was diluted in 0.9% sterile NaCl saline. The back titration of the diluted virus revealed a titer of $10^{5.5}$ EID₅₀/100µl. As control, 108 eggs were inoculated with 0.9% sterile NaCl saline. The volume for the inoculation was 100µl per egg. Eighty chickens hatched from the control group and forty-five chickens hatched from the APMV-8 vaccinated group. Ten chickens from each group were transferred to one Horsefall-Bauer unit. In addition, the chickens of the APMV-8 vaccinated group and five chickens of the control group were co-mingled in a Horsefall-Bauer unit to test transmission of APMV-8 after vaccination. Water and feed were provided *ad libitum*. Fourteen and twenty-eight days after hatch, blood samples were taken and tested for the presence of HI antibodies directed against APMV-8 using 4 HA units and 1% of chicken red blood cells. The results (Figure 22) showed that *in ovo* vaccination at day 18 of incubation resulted in an immune response as indicated by the presence of HI titers in the tested serum samples.

Fourteen days after hatch, an HI titer from 256 to 4048 was observed in the *in ovo* vaccinated group. In the serum of the contact chickens, an HI titer from 256 to 2048 was observed 14 days after contact with chickens from the vaccinated group, indicating shedding of the virus used for vaccination. The control group did not show any HI titer specific for APMV-8. Fourteen days later, the chickens were bled again and showed titers from 256 to 4096 in the APMV-8 vaccinated group and 256 to 1024 in the APMV-8 contact group. The control group showed no presence of APMV-8 HI antibodies.

[0150] In study 2, 88 SPF (specific pathogen free) eggs were *in ovo* vaccinated using the INOVOJECT at day 19 of incubation. The APMV-8 virus strain was diluted in 0.9% sterile NaCl saline. The titer of the virus was $10^{5.75}$ EID₅₀/100 μ l as observed after back titration of the diluted virus. As control, 88 SPF eggs were inoculated with 0.9% sterile NaCl saline. The volume for the inoculation was 100 μ l per egg. Seventy-four chickens hatched from the control group (NaCl) and seventy-six chickens hatched from the APMV-8 vaccinated group. Ten chickens from each group were transferred to one Horsfall-Bauer unit. Water and feed were provided *ad libitum*. Fourteen days after hatch, blood samples were taken and tested for the presence of HI antibodies directed against APMV-8 using 4 HA units and 1% of chicken red blood cells. The results (Figure 23) showed that *in ovo* vaccination at day 19 of incubation resulted in an immune response with HI titers specific for APMV-8. Fourteen days after hatch, an HI titer from 256 to 4048 was observed in the APMV-8 *in ovo* vaccinated group. The control group did not show any HI titer specific for APMV-8. The chickens were bled again at day 28 after hatch. The HI titers for the APMV-8 vaccinated group ranged from 512 to 4096 (Figure 23) whereas the sera of the control chickens were still APMV-8 negative.

[0151] In a third study, 108 SPF eggs in group 1 and group 2 were *in ovo* vaccinated using the INOVOJECT at day 18 of incubation with $10^{3.5}$ EID₅₀ and $10^{4.5}$ EID₅₀, respectively. The APMV-8 virus strain was diluted in 0.9% sterile NaCl saline. In a third group 108 SPF eggs were inoculated with 0.9% sterile NaCl saline as control. Eighty-three chickens hatched from group 1, seventy-nine chickens hatched from group 2, and eighty-eight chickens hatched from group 3 (see table 8). After hatch, ten chickens from each group were transferred to one Horsfall-Bauer unit. Water and feed were provided *ad libitum*. After hatch, ten chickens from group 3 were vaccinated subcutaneously in the

neck region with a volume of 100 μ l 10^6 EID₅₀ APMV-8 virus. Fourteen days after hatch, blood samples were taken and tested for the presence of HI antibodies directed against APMV-8 using 4 HA units and 1% of chicken red blood cells (CRBS). The results (Figure 24) showed that *in ovo* vaccination at day 18 of incubation resulted in an immune response with HI titers specific for APMV-8. Fourteen days after hatch, an HI titer from 4096 to 16384 was observed in the APMV-8 *in ovo* vaccinated groups. The control group did not show any HI titer specific for APMV-8. The group subcutaneously vaccinated with APMV-8 showed a seroconversion with a titer ranging from 64 to 4096. The chickens were bled again at day 28 after hatch and tested for the presence of APMV-8 specific HI antibodies. The HI titer at four weeks after hatch decreased and ranged between 512 and 4096. The control group did not show any HI titer specific for APMV-8. The HI titer in the group which was subcutaneously vaccinated showed HI titers ranging between 32 and 256.

Table 8

	Group 1	Group 2	Group 3 (control)
SPF eggs	108	108	108
Hatched chicken	83	79	88
Vaccination with APMV-8 at day 18 of incubation	$10^{3.5}$ EID ₅₀ 100 μ l per egg	$10^{4.5}$ EID ₅₀ 100 μ l per egg	0.9% sterile NaCl saline

Example 6 Development of reverse genetics of the APMV-8 strain and generation of APMV-8 mutants expressing heterologous genes

Construction of the expression plasmids containing the NP, P, and L genes of APMV-8

[0152] For the establishment of a reverse genetics system for paramyxoviruses, the establishment of plasmids expressing the proteins involved in viral RNA replication is essential. The open reading frames (ORF) of three APMV-8 proteins (nucleoprotein NP, phosphoprotein P, RNA dependent RNA polymerase protein or protein L) were cloned into the eukaryotic expressions vector pcDNA3 (Invitrogen, California, USA). To this end, the RNA of allantoic fluid containing APMV-8 was purified using the High Pure

RNA Isolation Kit (Roche, Basel, Switzerland). The purified RNA was used for reverse transcription polymerase chain reaction (RT-PCR) using the Titan One Tube RT-PCR Kit (Roche). The ORFs of the proteins were amplified using the appropriate primer pairs: [NP (NP-FP, NP-RP), P (P-FP, P-RP), L (L-FP, L-RP), see table 9]. The reactions products were separated on a 0.7% agarose gel and eluted from the gel using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) following the protocol provided by the manufacturer. The RT-PCR fragments were incubated with the appropriate restriction enzymes (NP and P with Eco RI/NotI; L with Kpn I/NotI), gel eluted again and ligated into the eukaryotic expression vector pcDNA3 cleaved with the appropriate restriction enzymes. The ligation reactions were transformed into Top10F cells (Invitrogen) and plasmid DNA harvested from the Top10F cells was digested with the appropriate restriction enzymes (see above). Plasmids containing DNA fragments (pcDNA-NP, pcDNA-P, pcDNA-L) with the appropriate size were sequenced.

Table 9. Primer for amplification of the genes for the proteins of the RNP complexes

Primer name	Primer sequence ^a	Orientation ^b	Position ^c	SEQ ID NO:
NP-FP-pc3	Eco RI <u>ccGAATTC ATGTCATCTGTGTTCAATGAGTATCAGG</u>	sense	141-168	41
NP-RP-pc3	Not I <u>ccGCGGCCGC TTACCATTCTAGCCCGTTCTCGTATG</u>	antisense	1501-1526	42
P-FP-pc3	Eco RI <u>ccGAATTCATGGATTTCGCCAATGATGAAG</u>	sense	1693-1714	43
P-RP-pc3	Not I <u>ccGCGGCCGC TTACGCATTATATATTGCCTGCTTGACTCG</u>	antisense	2881-2910	44
L-FP-pc3	Kpn I <u>ccGGTACCATGGATATAAAACAAGTTGACCTG</u>	sense	8292-8320	45
L-RP-pc3	Not I <u>ccGCGGCCGC TTATTCAACTTGATGATTGCACCG</u>	antisense	14989-15013	46

[0153] ^a The primer sequence contains the restriction enzyme cleavage sites used for cloning. The restriction sites are bold and identified. The start and stop codons are highlighted by italics. Virus specific sequences are underlined.

[0154] ^b The orientation of the primer sequence in accordance with the viral messenger RNA.

[0155] ^c The position are the virus specific sequences in the full length genome as shown.

Construction of a plasmid containing the full length genome of APMV-8

[0156] For the generation of a plasmid containing the full length APMV-8 genome, the cDNA genome of the virus was synthesized (Genscript, New York, USA) based on the generated consensus sequence in two different parts (5'-FLG, 3'-FLG, see Figure 25). The 5'-part (5'-FLG, SEQ ID NO:47) of the viral sequence was synthesized from nucleotide 1-5564. Preceding the APMV-8 sequence is a sequence cassette consisting of the CMV-IE promoter, followed by a restriction enzyme cleavage sequence for XmaI (for possible subsequent cloning procedures) and the hammerhead ribozyme sequence. At the 5'-end and the 3'-end of the sequence, restriction enzyme cleavage sites for Not I and SacII were added, respectively. The synthesized 3'-part (3'-FLG, SEQ ID NO:48) of the sequence (nucleotide 5503-15342) is followed by the hepatitis delta ribozyme sequence and poly-A signal sequence of the bovine growth hormone. For cloning purpose, the sequence for the Not I restriction enzyme was added at the 5'- end of 3'-FLG and the sequence for the Sac II restriction enzyme was added at the 3'-end of the sequence. Within the overlapping parts of 5'-FLG and 3'-FLG (nucleotide 5503-5564), the sequence for a unique restriction enzyme (Bmt I) was located which cleaves at nucleotide 5541 of the full length sequence. Both parts of the DNA (5'-FLG, 3'-FLG) were ligated separately into the plasmid pUC57 (Genscript) resulting in the plasmids pUC57/5'-FLG and pUC57/3'-FLG. To clone both fragments together, pUC57/5'-FLG was cleaved with BmtI and Sac II and the 5'-FLG-containing plasmid was gel eluted. In parallel, pUC57/3'-FLG was cleaved with the same enzymes and the fragment 3'-FLG was eluted. The 3'-FLG was subsequently ligated into 5'-FLG-containing plasmid to obtain a plasmid which contains the full length cDNA sequence of the APMV-8 genome under the control of the CMV-IE promoter (pUC57-FL-APMV-8).

Construction of the plasmid containing the minigenome of APMV-8

[0157] The plasmid containing all functional elements of a minigenome for APMV-8 (pMG-APMV-8) was constructed using the method described by Conzelmann, *et al.* (J Virol. 68:713-719, 1994). Plasmid pMG-APMV-8 contains the trailer and leader region of the APMV-8 genome which is flanked by the T7 promoter and the antigenome

hepatitis delta virus ribozyme sequence (Collins, et al., PNAS USA 88:9663-9667, 1991). The antigenome hepatitis delta virus ribozyme sequence is followed by a T7 transcription terminator sequence. Between the trailer and leader region the coding sequence of the enhanced green fluorescent protein in antisense orientation is located. Preceding the trailer sequence and immediate after the T7 promoter three additional G residues are located. The insert is flanked by the restriction enzyme cleavage sites Eco RI and Not I and was cloned blunt end into the plasmid pUC57. This construct was subcloned into the plasmid pUC18. To this end, plasmid pMG-APMV-8 then was cleaved with Eco RI and Hind III and the appropriate fragment was gel eluted and ligated into the appropriately cleaved plasmid pUC18 to obtain pUC18-MG-APMV-8. The presence of the insert was confirmed by sequencing.

Generation of an expression plasmid allowing the expression of the T7 polymerase

[0158] For the generation of a plasmid encoding the T7 DNA dependent RNA polymerase (T7 polymerase) the coding sequence (GenBank accession number AY264778) was synthesized by Genscript. The T7 polymerase sequence (SEQ ID NO:49) was modified for optimization of the codon usage for expression in a eukaryotic system and to remove possible splice donor/acceptor sites in the sequence. The T7 polymerase encoding sequence was flanked by an EcoRI (5') and NotI (3') site. The synthesized fragment was cloned blunt end into the vector pUC57 (pUC57-T7). This plasmid was cleaved with EcoRI/NotI and the T7 polymerase-encoding fragment was gel eluted. The fragment was then cloned into the eukaryotic expressions vector pcDNA3 (Invitrogen) to obtain pcDNA3-T7. The presence of the fragment in the vector pcDNA3-T7 was verified by sequencing.

Generation of a plasmid for the expression of the enhanced green fluorescent protein with the use of an internal ribosomal entry site under the control of a T7 promoter.

[0159] To test the functionality of the T7 polymerase, the open reading frame of the enhanced green fluorescent protein (EGFP) was amplified by PCR using the plasmid pEGFP-N1 (Clontech, California, USA) and the primer pair: EGFP-FP (CCGGATCCATGGTGAGCAAGGGCGAGGAGCTG) SEQ ID NO:50 and

EGFP-RP (CCGCGGCCGCTTACTTGTACAGCTCGTCCATGCCG) SEQ ID NO:51

[0160] The obtained PCR fragment was gel eluted and incubated with the restriction enzymes BamHI and NotI. The reaction product was gel eluted and ligated into the appropriately cleaved vector pCITE 4A (Novagen). The obtained plasmid (pCITE4A-EGFP) was used for subsequent experiments. Plasmids pCITE4A-EGFP and pcDNA3-T7 were transfected alone or in combination into the chicken cell line DF1 grown in 24-well-plates using Lipofectin 2000 (Invitrogen). Twenty four hours after transfection, the medium was removed and sterile phosphate buffered saline (PBS) was added. The cells were evaluated using the inverted fluorescence microscope Axiovert 40 CFL (Zeiss, Jena, Germany). Green fluorescence was only observed in wells of the tissue culture plate which was co-transfected with both plasmids. This result indicates that both plasmids, pCITE4A-EGFP and pcDNA3-T7, were functional.

Validation of the functionality of the expressed viral proteins NP, P, and L using the minigenome plasmid

[0161] DF1 cells were co-transfected with pcDNA3-T7, pUC18-MG-APMV-8, pcDNA-NP, pcDNA-P, and pcDNA-L to validate the functionality of the expressed NP, P and L proteins. Twenty four hours after transfection, the medium was removed and sterile phosphate buffered saline (PBS) was added. The cells were evaluated using the inverted fluorescence microscope Axiovert 40 CFL (Zeiss, Jena, Germany). Green fluorescence was only observed in wells of the tissue culture plate which was co-transfected with the 5 plasmids. This result indicates that the expressed viral proteins NP, P and L were functional to transcribe the APMV-8 minigenome into mRNA and express the EGFP protein encoded by pUC18-MG-APMV-8.

Rescue of APMV8 virus from plasmid containing the full length sequence of APMV-8

[0162] DF1 cells were co-transfected with pUC57-FL-APMV-8, pcDNA-NP, pcDNA-P, and pcDNA-L. After 48 to 96 hours, the supernatants of the DF1 cells were inoculated in 10-day-old embryonated eggs to propagate the virus. After 3 to 5 days, the allantoic fluid was harvested and tested for hemagglutination activity (HA) using 1% chicken red blood cells. Allantoic fluid tested positive for HA activity was used for three

procedures. 1) DF1 cells were infected with the allantoic fluid and tested 36 hours after infection with an APMV-8 specific antiserum for the presence of APMV-8 protein expression in an indirect immunofluorescence assay. 2) The allantoic fluid was tested for APMV-8 specificity using an APMV-8 specific chicken serum (provided by the National Central Veterinary Laboratory, Ames, Iowa, USA) by an hemagglutination inhibition assay. 3) The rescue virus was identified by RT-PCR using APMV-8 specific oligonucleotides. The absence of viral cDNA was verified by omitting the RT step during the reaction. Samples tested positive in all three assays were further propagated in embryonated SPF chicken eggs.

Propagation of APMV-8 in cells other than chicken origin

[0163] Cells from different species [hamster (Baby hamster kidney cells, BHK-21 cells), monkey (Vero cells, cell line with the origin of the kidney of an African green monkey), and canine (Madin-Darby canine kidney cells, MDCK), and quail (Quail muscle cell line QM7)] were grown in 24 well tissue culture plates and infected with a multiplicity of infection of 0.01. The cells were fixed with ice cold ethanol 24 hours after infection and analyzed for the presence of APMV-8 specific proteins by indirect immunofluorescence using an APMV-8 specific antiserum from an APMV-8 infected SPF chicken. The binding of the antibodies was visualized by using a goat anti-chicken IgY specific FITC conjugate. Non-infected cells were used as negative control. Only in the APMV-8 infected cells was green fluorescence observed. This indicated that APMV-8 was able to infect cells from species other than chicken.

[0164] The replication of APMV-8 was increased in presence of trypsin. MDCK cells were infected with APMV-8 as described above. After infection the cells were rinsed with serum-free medium and either overlaid with trypsin-containing serum-free medium in a concentration of 1ug/ml or with serum-free medium only. Twenty four, forty eight, and ninety six hours after infection the cell supernatants were removed and the TCID₅₀ was determined on DF1 cells using indirect immunofluorescence as described above. The obtained data indicated that in presence of trypsin APMV-8 replicated to a higher titer than in absence of this enzyme (Figure 19B).

[0165] The results of this example showed that, like AMPV-1, APMV-8 is able to penetrate into cells of different species and to initiate its replication cycle. It is therefore a suitable vector for multiple species.

Production of recombinant APMV-8 virus expressing foreign genes using the reverse genetics system

[0166] For the generation of a recombinant APMV-8 virus (viral vector) expressing the hemagglutinin (HA) gene of a highly pathogenic avian influenza (HPAI), the coding sequence for the HA gene of HPAI of the H5 or H7 subtype virus is inserted in the non-essential regions, for example, between the M and F genes or between the P and M genes of the APMV-8 genome in the plasmid containing the full length APMV-8 genome. To this end the coding sequence of the hemagglutinin open reading frame is flanked by all the necessary regulatory sequences of the F gene which includes the gene start sequence, the 5' non-coding sequence, the 3' non-coding sequence and the gene stop sequence. The construct is synthesized in a way that the restriction enzyme cleavage sites Bsu 36I and Nhe I are used for the ligation of the appropriate fragment into the existing plasmid containing full length APMV-8 genome due to their uniqueness in the plasmid construct. The resulting plasmid is designated transcription plasmid which contains the hemagglutinin gene in the nonessential region of the full length APMV-8 genome. Using this approach the coding sequences of a variety of viral and bacterial antigen can be cloned into the backbone of the APMV-8 sequence. Other possible antigens which could be inserted into the APMV-8 genome are the fusion protein of the Newcastle disease virus, the S protein of avian bronchitis virus, other hemagglutinin genes from non-H5 and non-H7 avian influenza virus, the chicken anemia virus structural protein gene VP1, glycoprotein genes from infectious laryngotracheitis virus.

Example 7 Vaccination of animals

[0167] The animals are vaccinated with one, two administrations or a prime-boost regime of the composition or vaccine containing the recombinant APMV-8 virus (viral vector) as described in example 6. For chickens/avian, various administrations are

performed, for example, *in ovo* administration at D18 or D19, subcutaneous (SC) at one-day-old, or mucosal administration (spray, drinking water, eye drop) at different ages. The dose is between 3 and 7 log₁₀ (preferably 4-6 log₁₀ EID₅₀). For mammals, mucosal route (intra-nasal, intra-ocular, oral) or parenteral (IM, SC, needle-free, trans-dermal or intra-dermal) is used. The dose ranges from 5 to 9 log (preferably 6-8 log). Two administrations are usually performed at 3-4 weeks interval. Heterologous prime-boost (for instance, boost with proteins) would be also advantageous.

[0168] The protective efficacy induced by the composition or vaccine is evaluated against the specific pathogen challenge in the animals. The protective effect is evaluated by clinical observations and/or viral load of the specific pathogen in tissues, blood or mucosal swabs. The blood samples from the vaccinated animals are taken at various stages and tested for serology. The results show that the composition or vaccine of the present invention is immunogenic and provides protection in the vaccinated animals.

[0169] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

[0170] All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

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CLAIMS

What we claim is:

1. A composition or vaccine comprising (i) a recombinant APMV viral vector and (ii) a pharmaceutically or veterinarily acceptable carrier.
2. The composition or vaccine of claim 1, wherein the APMV is APMV-8, APMV-2, APMV-4 or APMV-6.
3. The composition or vaccine of claim 1 or 2, wherein the APMV viral vector comprises a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1 or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1.
4. The composition or vaccine of any one of claims 1-3, wherein the APMV viral vector further comprises an isolated polynucleotide encoding an antigen, and wherein the polynucleotide is inserted in a nonessential region of the APMV genome.
5. The composition or vaccine of claim 3, wherein the nonessential region is selected from the regions consisting of untranslated region located upstream of the NP open reading frame, intergenic regions between two open reading frames of the APMV genome, and untranslated region located downstream of the L open reading frame.
6. A method for producing recombinant APMV viral vector, wherein the method comprises the introduction into the APMV genome an isolated polynucleotide in a nonessential region of the APMV genome.
7. The method of claim 6, wherein the APMV is APMV-8, APMV-2, APMV-4 or APMV-6.
8. The method of claim 6 or 7, wherein the APMV comprises a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1 or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1.
9. The method of any one of claims 6-8, wherein the nonessential region is selected from the regions consisting of untranslated region located upstream of the NP open

- reading frame, intergenic regions between two open reading frames of the APMV genome, and untranslated region located downstream of the L open reading frame.
10. The method of any one of claims 6-9, wherein the method comprises the steps of:
- a) preparing expression plasmids expressing NP, P and L genes;
 - b) preparing a transcription plasmid comprising an isolated polynucleotide in a nonessential region of the full length APMV genome;
 - c) transfection of the expression plasmids and transcription plasmid into a host cell;
 - d) rescuing/recovering the infectious APMV virus from the host cell.
11. The method of any one of claims 6-9, wherein the method comprises the steps of:
- a) preparing expression plasmids expressing NP, P and L genes;
 - b) preparing a transcription plasmid comprising an isolated polynucleotide in a nonessential region of the full length APMV genome;
 - c) preparing an expression plasmid expressing T7 polymerase
 - d) transfection of the expression plasmids and transcription plasmid into a host cell;
 - e) rescuing/recovering the infectious APMV virus from the host cell.
12. A method for inducing an immunological response in an animal to an antigen comprising inoculating the animal with a composition or vaccine comprising a recombinant APMV viral vector, wherein the recombinant APMV viral vector comprises and expresses the antigen of a pathogen for said animal.
13. The method of claim 12, wherein the immunological response in an animal to the antigen is induced in a prime-boost regime.
14. The method of claim 12, wherein the animal is avian, equine, canine, feline, porcine, bovine, ovine, or human.
15. An unmodified or modified APMV virus comprising a polynucleotide selected from the group consisting of:
- a) a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1 or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1;
 - b) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:3, a polynucleotide

having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:2, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:2;

- c) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:5, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:4, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:4;
- d) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:7, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:6, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:6;
- e) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:9, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:8, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:8;
- f) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:11, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:10, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:10; and
- g) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:13, a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide

having the sequence as set forth in SEQ ID NO:14, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:12, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:12.

16. The modified APMV virus of claim 15 further comprising an isolated polynucleotide inserted in a nonessential region of APMV genome.

17. An isolated polynucleotide selected from the group consisting of:

- a) a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1 or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1;
- b) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:3, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:2, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:2;
- c) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:5, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:4, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:4;
- d) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:7, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:6, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:6;

- e) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:9, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:8, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:8;
- f) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:11, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:10, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:10; and
- g) a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:13, a polynucleotide having at least 90% sequence identity to a polynucleotide encoding a polypeptide having the sequence as set forth in SEQ ID NO:14, a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:12, or a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:12.

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Trp Ser Leu Ala Cys Leu Asn Ile Arg Trp Leu Ala Ser Asp Ser Ser
50 55 60

Thr Thr Pro Met Lys Gln Gly Ala Ile Leu Ser Leu Leu Ser Leu His
65 70 75 80

Ser Asp Asn Met Arg Ala His Ala Thr Leu Ala Ala Arg Ser Ala Asp
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Ala Ser Leu Thr Ile Leu Glu Val Asp Glu Val Asp Ile Ser Asn Ser
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His Pro Phe Leu Asp Thr Asp Ile Glu Thr Arg Asp Pro Leu Asp Leu
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Ser Lys Arg Leu Ala Lys Tyr Gln Gln Gln Gly Arg Leu Val Lys Gln
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Val Leu Leu His Ser Val Val Arg Thr Glu Phe Met Arg Val Ile Arg
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Ile Lys Ser Glu Ser Thr Asp Gly His Ile Arg Gly Thr Val Asn Gln
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Ser Glu Gln Val Pro Glu Gln Asn Gln Ser Arg Ser Ser Pro Gly Asp
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Sequence

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 Asp Ile Ala Lys Val Val Asn Thr Thr Pro Leu Ile Arg Asn Asp Ile
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 Asn Gln Leu Lys Ala Thr Thr Ala Leu Met Ser Asn Gln Ile Ala Ser
 275 280 285
 Ile Gln Ile Leu Asp Pro Gly Asn Ala Gly Val Arg Ser Leu Ser Glu
 290 295 300
 Met Lys Ser Val Thr Lys Lys Ala Ala Val Val Ile Ala Gly Phe Gly
 305 310 315 320
 Asp Asp Pro Thr Gln Ile Ile Glu Glu Gly Ile Met Ala Lys Asp Ala
 325 330 335
 Leu Gly Lys Pro Val Pro Pro Thr Ser Val Ile Ser Ala Lys Ala Gln
 340 345 350
 Thr Ser Ser Gly Val Ser Lys Gly Glu Ile Glu Gly Leu Ile Ala Leu
 355 360 365
 Val Glu Thr Leu Val Asp Asn Asp Lys Lys Ala Ala Lys Leu Ile Lys
 370 375 380

Sequence

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Ala Ile Tyr Asn Ala
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gatgacttca aacctgagac agtcactgca ggactggtag tggtaggtgc gatccgaatg 300
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<211> 369
<212> PRT
<213> artificial sequence

<220>
<223> APMV8 M protein

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20 25 30

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35 40 45

Gln Asn Ala Lys Glu Ser Ser Ile Phe Val Thr Leu Tyr Gly Phe Ile
50 55 60

Gln Ala Ile Lys Glu Ser Ser Asp Arg Ser Lys Phe Phe His Pro Lys
65 70 75 80

Asp Asp Phe Lys Pro Glu Thr Val Thr Ala Gly Leu Val Val Val Gly
85 90 95

Ala Ile Arg Met Met Ala Asp Val Asn Thr Ile Ser Asn Asp Ala Leu
100 105 110

Ala Leu Glu Ile Thr Val Lys Lys Ser Ala Thr Ser Gln Glu Lys Met
115 120 125

Thr Val Met Phe His Asn Ser Pro Pro Ser Leu Arg Thr Ala Ile Thr
130 135 140

Ile Arg Ala Gly Gly Phe Ile Ser Asn Ala Asp Glu Asn Ile Lys Cys
145 150 155 160

Ala Ser Lys Leu Thr Ala Gly Val Gln Tyr Ile Phe Arg Pro Met Phe
165 170 175

Val Ser Ile Thr Lys Leu His Asn Gly Lys Leu Tyr Arg Val Pro Lys
180 185 190

Ser Ile His Ser Ile Ser Ser Thr Leu Leu Tyr Ser Val Met Leu Glu
195 200 205

Val Gly Phe Lys Val Asp Ile Gly Lys Asp His Pro Gln Ala Lys Met
210 215 220

Leu Lys Lys Val Thr Ile Gly Asp Ala Asp Thr Tyr Trp Gly Phe Ala
225 230 235 240

Trp Phe His Leu Cys Asn Phe Lys Lys Thr Ser Ser Lys Gly Lys Pro
245 250 255

Arg Thr Leu Asp Glu Leu Lys Thr Lys Val Lys Asn Met Gly Leu Lys
260 265 270

Sequence

Leu Glu Leu His Asp Leu Trp Gly Pro Thr Ile Val Val Gln Ile Thr
 275 280 285

Gly Lys Ser Ser Lys Tyr Ala Gln Gly Phe Phe Ser Ser Asn Gly Thr
 290 295 300

Cys Cys Leu Pro Ile Ser Arg Ser Ala Pro Glu Leu Gly Lys Leu Leu
 305 310 315 320

Trp Ser Cys Ser Ala Thr Ile Gly Asp Ala Thr Val Val Ile Gln Ser
 325 330 335

Ser Glu Lys Gly Glu Leu Leu Arg Ser Asp Asp Leu Glu Ile Arg Gly
 340 345 350

Ala Val Ala Ser Lys Lys Gly Arg Leu Gly Ser Phe His Pro Phe Lys
 355 360 365

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Sequence

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Val Tyr Pro Val Asn Ser Ile Asp Asn Thr Leu Ile Ala Pro Ile Gly
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Val Ala Ser Ala Asn Glu Trp Gln Leu Ala Ala Tyr Thr Thr Ser Leu
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Ser Gly Thr Ile Ala Val Arg Phe Leu Pro Val Leu Pro Asp Asn Met
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Thr Thr Cys Leu Lys Glu Thr Ile Thr Thr Tyr Asn Asn Thr Val Asn
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Asn Ile Leu Gly Pro Leu Lys Ser Asn Leu Asp Ala Leu Leu Ser Ser
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Glu Thr Tyr Pro Gln Thr Arg Leu Ile Gly Ala Val Ile Gly Ser Ile
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Ala Leu Gly Val Ala Thr Ser Ala Gln Ile Thr Ala Ala Val Ala Leu
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Sequence

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Gln Thr Ala Ile Ala Leu Gly Lys Ile Gln Ser Phe Val Asn Glu Glu
165 170 175

Ile Leu Pro Ser Ile Asn Gln Leu Ser Cys Glu Val Thr Ala Asn Lys
180 185 190

Leu Gly Val Tyr Leu Ser Leu Tyr Leu Thr Glu Leu Thr Thr Ile Phe
195 200 205

Gly Ala Gln Leu Thr Asn Pro Ala Leu Thr Ser Leu Ser Tyr Gln Ala
210 215 220

Leu Tyr Asn Leu Cys Gly Gly Asn Met Ala Met Leu Thr Gln Lys Ile
225 230 235 240

Gly Ile Lys Gln Gln Asp Val Asn Ser Leu Tyr Glu Ala Gly Leu Ile
245 250 255

Thr Gly Gln Val Ile Gly Tyr Asp Ser His Tyr Gln Leu Leu Val Ile
260 265 270

Gln Val Asn Tyr Pro Ser Ile Ser Glu Val Thr Gly Val Arg Ala Thr
275 280 285

Glu Leu Val Thr Val Ser Val Thr Thr Asp Lys Gly Glu Gly Lys Ala
290 295 300

Ile Val Pro Gln Phe Val Ala Glu Ser Arg Val Thr Ile Glu Glu Leu
305 310 315 320

Asp Val Ala Ser Cys Lys Phe Ser Ser Thr Thr Leu Tyr Cys Arg Gln
325 330 335

Val Asn Thr Arg Ala Leu Pro Pro Leu Val Ala Ser Cys Leu Arg Gly
340 345 350

Asn Tyr Asp Asp Cys Gln Tyr Thr Thr Glu Ile Gly Ala Leu Ser Ser
355 360 365

Arg Tyr Ile Thr Leu Asp Gly Gly Val Leu Val Asn Cys Lys Ser Ile
370 375 380

Val Cys Arg Cys Leu Asn Pro Ser Lys Ile Ile Ser Gln Asn Thr Asn
385 390 395 400

Sequence

Ala Ala Val Thr Tyr Val Asp Ala Thr Ile Cys Lys Thr Ile Gln Leu
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Asp Asp Ile Gln Leu Gln Leu Glu Gly Ser Leu Ser Ser Val Tyr Ala
420 425 430

Arg Asn Ile Ser Ile Glu Ile Ser Gln Val Thr Thr Ser Gly Ser Leu
435 440 445

Asp Ile Ser Ser Glu Ile Gly Asn Ile Asn Asn Thr Val Asn Arg Val
450 455 460

Glu Asp Leu Ile His Gln Ser Glu Glu Trp Leu Ala Lys Val Asn Pro
465 470 475 480

His Ile Val Asn Asn Thr Thr Leu Ile Val Leu Cys Val Leu Ser Ala
485 490 495

Leu Ala Val Ile Trp Leu Ala Val Leu Thr Ala Ile Ile Ile Tyr Leu
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Arg Thr Lys Leu Lys Thr Ile Ser Ala Leu Ala Val Thr Asn Thr Ile
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gccagtgcac ccagtatgta tatctcaatg ggagttctcc atgtgtcctc atctggcact 660
cccatttttc ttactactgc aagtgagctg atagacgatg gagttaaccg taagtcatgc 720

Sequence

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Thr Thr Gly Cys Leu Ala Leu Ser Ile Val Ser Ile Val Gln Ile Gly
 35 40 45

Asn Leu Lys Ile Pro Ser Val Gly Asp Leu Ala Asp Glu Val Val Thr
 50 55 60

Pro Leu Lys Thr Thr Leu Ser Asp Thr Leu Arg Asn Pro Ile Asn Gln
 65 70 75 80

Ile Asn Asp Ile Phe Arg Ile Val Ala Leu Asp Ile Pro Leu Gln Val
 85 90 95

Sequence

Thr Ser Ile Gln Lys Asp Leu Ala Ser Gln Phe Asn Met Leu Ile Asp
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 Ser Leu Asn Ala Ile Lys Leu Gly Asn Gly Thr Asn Leu Ile Ile Pro
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 Val Asp Ala Gly Gly Ser Ile Gly Phe Lys Gln Phe Ser Leu Ile Glu
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 His Pro Ser Phe Ile Ala Gly Pro Thr Thr Thr Arg Gly Cys Thr Arg
 165 170 175
 Ile Pro Thr Phe His Met Ser Glu Ser His Trp Cys Tyr Ser His Asn
 180 185 190
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 195 200 205
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 210 215 220
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 225 230 235 240
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 245 250 255
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 260 265 270
 Arg His Gly Arg Phe Ser Phe Asn Gly Ser Phe Val Glu Ala Glu Leu
 275 280 285
 Pro Val Ser Ser Met Phe Ser Ser Phe Ser Ala Asn Tyr Pro Ala Val
 290 295 300
 Gly Ser Gly Glu Ile Val Lys Asp Arg Ile Leu Phe Pro Ile Tyr Gly
 305 310 315 320
 Gly Ile Lys Gln Thr Ser Pro Glu Phe Thr Glu Leu Val Lys Tyr Gly
 325 330 335
 Leu Phe Val Ser Thr Pro Thr Thr Val Cys Gln Ser Ser Trp Thr Tyr
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 Asp Gln Val Lys Ala Ala Tyr Arg Pro Asp Tyr Ile Ser Gly Arg Phe
 355 360 365

Sequence

Trp Ala Gln Val Ile Leu Ser Cys Ala Leu Asp Ala Val Asp Leu Ser
370 375 380

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

Glu Gly Arg Ile Met Lys Ile Gly Ile Asp Tyr Phe Tyr Tyr Gln Arg
405 410 415

Ser Ser Ser Trp Trp Pro Leu Ala Phe Val Thr Lys Leu Asp Pro Gln
420 425 430

Glu Leu Ala Asp Thr Asn Ser Ile Trp Leu Thr Asn Ser Ile Pro Ile
435 440 445

Pro Gln Ser Lys Phe Pro Arg Pro Ser Tyr Ser Glu Asn Tyr Cys Thr
450 455 460

Lys Pro Ala Val Cys Pro Ala Thr Cys Val Thr Gly Val Tyr Ser Asp
465 470 475 480

Ile Trp Pro Leu Thr Ser Ser Ser Ser Leu Pro Ser Ile Ile Trp Ile
485 490 495

Gly Gln Tyr Leu Asp Ala Pro Val Gly Arg Thr Tyr Pro Arg Phe Gly
500 505 510

Ile Ala Asn Gln Ser His Trp Tyr Leu Gln Glu Asp Ile Leu Pro Thr
515 520 525

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

Arg Asn Arg Val Phe Cys Val Thr Ile Ala Glu Phe Ala Asp Gly Leu
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Page 22

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Sequence

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Thr His Tyr Cys Gln Tyr Phe Asn Ile Asn Leu Ala Asn Ile Asp Pro
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Ile Thr His Pro Lys Ser Leu Tyr Trp Leu Ser Arg Leu Thr Ile Ala
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Ser Ala Gly Thr Phe Arg His Met Lys Asp Arg Ile Leu Cys Thr Val
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Gly Ser Glu Phe Gly His Lys Ile Gln Asp Leu Phe Ser Leu Leu Ser
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Ser Trp Leu His Ile Lys Gln Thr Met Arg Tyr Leu Ile Lys Gln Ser
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Arg Thr Thr Asn Ser Ala His Leu Thr Glu Ile Ile Lys Glu Glu Trp
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Ala Val Leu Glu Ser Leu Ser Tyr Gly Ser Val Gln Leu His Asp Ala
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Asp Asn Thr Leu Ser Lys Arg Val Asp Pro Lys His Lys Asn Thr Ile
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Gln Trp Asp Val Ile Phe Arg Lys Ser Leu Arg Arg Ser His Thr Gln
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Leu Asp Ser Ala Glu Phe Asp Pro Gly Lys Glu Phe Ala Tyr Val Thr
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Tyr Ser Pro Pro Glu Asp Pro Thr Leu Glu Asp Ile Asp Lys Ala Pro
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Cys Gln Lys Met Trp Thr Met Ile Ser Ile Ser Ala Ile His Cys Val
755 760 765

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Gln Val Ile Ala Ile Thr Lys Glu Leu Phe Arg Gly Glu Lys Ala Cys
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835 840 845

Glu Gly Arg Leu Leu Ser Gln Val Leu Lys Asn Ala Ala Lys Leu Cys
850 855 860

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885 890 895

Thr Ala Phe Val Leu Asn Leu Val Tyr Ile Met Thr Gln Ile Leu Phe
900 905 910

Asp Glu His Tyr Ser Ile Val Cys Asp His His Ser Val Lys Ser Leu
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Ile Gly Ser Lys Asn His Arg Asn Leu Leu Tyr Ser Ser Leu Ile Pro
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Gly Gln Leu Gly Gly Phe Asn Phe Leu Asn Ile Ser Arg Leu Phe Thr
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 Tyr Thr Leu Asn Ile Pro Tyr Thr Gln Leu Pro Thr Thr Tyr Leu
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 Ser Leu Val Asn Leu Pro Val Ser Arg Lys Lys Cys Glu Lys Ile
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Met Asp Ile Lys Gln Val Asp Leu Ile Ile Gln Pro Glu Val His Leu
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Asp Ser Pro Ile Ile Leu Asn Lys Leu Ala Leu Leu Trp Arg Leu Ser
20 25 30

Gly Leu Pro Met Pro Ala Asp Leu Arg Gln Lys Ser Val Val Met His
35 40 45

Ile Pro Asp His Ile Leu Glu Lys Ser Glu Tyr Arg Ile Lys His Arg
50 55 60

Leu Gly Lys Ile Lys Ser Asp Ile Thr His Tyr Cys Gln Tyr Phe Asn
65 70 75 80

Ile Asn Leu Ala Asn Ile Asp Pro Ile Thr His Pro Lys Ser Leu Tyr
85 90 95

Trp Leu Ser Arg Leu Thr Ile Ala Ser Ala Gly Thr Phe Arg His Met
100 105 110

Lys Asp Arg Ile Leu Cys Thr Val Gly Ser Glu Phe Gly His Lys Ile
115 120 125

Gln Asp Leu Phe Ser Leu Leu Ser His Lys Leu Val Gly Asn Gly Asp
130 135 140

Leu Phe Asn Gln Ser Leu Ser Gly Thr Arg Leu Thr Ala Ser Pro Leu
145 150 155 160

Ser Pro Leu Cys Asn Gln Phe Val Ser Asp Ile Lys Ser Ala Val Thr
165 170 175

Thr Pro Trp Ser Glu Ala Arg Trp Ser Trp Leu His Ile Lys Gln Thr
180 185 190

Met Arg Tyr Leu Ile Lys Gln Ser Arg Thr Thr Asn Ser Ala His Leu
195 200 205

Thr Glu Ile Ile Lys Glu Glu Trp Gly Leu Val Gly Ile Thr Pro Asp
210 215 220

Sequence

Leu Val Ile Leu Phe Asp Arg Val Asn Asn Ser Leu Thr Ala Leu Thr
225 230 235 240

Phe Glu Met Val Leu Met Tyr Ser Asp Val Leu Glu Ser Arg Asp Asn
245 250 255

Ile Val Leu Val Gly Arg Leu Ser Thr Phe Leu Gln Pro Val Val Ser
260 265 270

Arg Leu Glu Val Leu Phe Asp Leu Val Asp Ser Leu Ala Lys Ile Leu
275 280 285

Gly Asp Thr Ile Tyr Glu Ile Ile Ala Val Leu Glu Ser Leu Ser Tyr
290 295 300

Gly Ser Val Gln Leu His Asp Ala Ser His Ser His Ala Gly Ser Phe
305 310 315 320

Phe Ser Phe Asn Met Asn Glu Leu Asp Asn Thr Leu Ser Lys Arg Val
325 330 335

Asp Pro Lys His Lys Asn Thr Ile Met Ser Ile Ile Arg Gln Cys Phe
340 345 350

Ser Asn Leu Asp Val Asp Gln Ala Ala Glu Met Leu Cys Leu Met Arg
355 360 365

Leu Phe Gly His Pro Met Leu Thr Ala Pro Asp Ala Ala Ala Lys Val
370 375 380

Arg Lys Ala Met Cys Ala Pro Lys Leu Val Glu His Asp Thr Ile Leu
385 390 395 400

Gln Thr Leu Ser Phe Phe Lys Gly Ile Ile Ile Asn Gly Tyr Arg Arg
405 410 415

Ser His Ser Gly Leu Trp Pro Asn Val Glu Pro Ser Ser Ile Tyr Asp
420 425 430

Asp Asp Leu Arg Gln Leu Tyr Leu Glu Ser Ala Glu Ile Ser His His
435 440 445

Phe Met Leu Lys Asn Tyr Lys Ser Leu Ser Met Ile Glu Phe Lys Lys
450 455 460

Ser Ile Asp Tyr Asp Leu His Asp Asp Leu Ser Thr Phe Leu Lys Asp
465 470 475 480

Arg Ala Ile Cys Arg Pro Lys Ser Gln Trp Asp Val Ile Phe Arg Lys
485 490 495

Sequence

Ser Leu Arg Arg Ser His Thr Gln Ser Gln Tyr Leu Asp Glu Ile Lys
500 505 510

Ser Asn Arg Leu Leu Ile Asp Phe Leu Asp Ser Ala Glu Phe Asp Pro
515 520 525

Gly Lys Glu Phe Ala Tyr Val Thr Thr Met Asp Tyr Leu His Asp Asn
530 535 540

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

Gly Arg Ile Phe Ala Lys Met Thr Arg Asn Met Arg Ser Cys Gln Val
565 570 575

Ile Leu Glu Ser Leu Leu Ser Lys His Ile Cys Lys Phe Phe Lys Glu
580 585 590

Asn Gly Val Ser Met Glu Gln Leu Ser Leu Thr Lys Ser Leu Leu Ala
595 600 605

Met Ser Gln Leu Ser Pro Lys Val Ser Thr Leu Gln Asp Thr Ala Ser
610 615 620

Arg His Val Gly Asn Ser Lys Ser Gln Ile Ala Thr Ser Asn Pro Ser
625 630 635 640

Arg His His Ser Thr Pro Asn Gln Met Ser Leu Ser Asn Arg Lys Thr
645 650 655

Val Val Ala Thr Phe Leu Thr Thr Asp Leu Glu Lys Tyr Cys Leu Gln
660 665 670

Trp Arg Tyr Ser Thr Ile Lys Leu Phe Ala Gln Ala Leu Asn Gln Leu
675 680 685

Phe Gly Ile Asp His Gly Phe Glu Trp Ile His Leu Arg Leu Met Asn
690 695 700

Ser Thr Leu Phe Val Gly Asp Pro Tyr Ser Pro Pro Glu Asp Pro Thr
705 710 715 720

Leu Glu Asp Ile Asp Lys Ala Pro Asn Asp Asp Ile Phe Ile Val Ser
725 730 735

Pro Arg Gly Gly Ile Glu Gly Leu Cys Gln Lys Met Trp Thr Met Ile
740 745 750

Ser Ile Ser Ala Ile His Cys Val Ala Glu Lys Ile Gly Ala Arg Val
755 760 765

Sequence

Ala Ala Met Val Gln Gly Asp Asn Gln Val Ile Ala Ile Thr Lys Glu
770 775 780

Leu Phe Arg Gly Glu Lys Ala Cys Asp Val Arg Asp Glu Leu Asp Glu
785 790 795 800

Leu Gly Gln Val Phe Phe Asp Glu Phe Lys Arg His Asn Tyr Ala Ile
805 810 815

Gly His Asn Leu Lys Leu Asn Glu Thr Ile Gln Ser Gln Ser Phe Phe
820 825 830

Val Tyr Ser Lys Arg Ile Phe Phe Glu Gly Arg Leu Leu Ser Gln Val
835 840 845

Leu Lys Asn Ala Ala Lys Leu Cys Met Val Ala Asp His Leu Gly Glu
850 855 860

Asn Thr Val Ser Ser Cys Ser Asn Leu Ser Ser Thr Ile Ala Arg Leu
865 870 875 880

Val Glu Asn Gly Phe Glu Lys Asp Thr Ala Phe Val Leu Asn Leu Val
885 890 895

Tyr Ile Met Thr Gln Ile Leu Phe Asp Glu His Tyr Ser Ile Val Cys
900 905 910

Asp His His Ser Val Lys Ser Leu Ile Gly Ser Lys Asn His Arg Asn
915 920 925

Leu Leu Tyr Ser Ser Leu Ile Pro Gly Gln Leu Gly Gly Phe Asn Phe
930 935 940

Leu Asn Ile Ser Arg Leu Phe Thr Arg Asn Ile Gly Asp Pro Val Thr
945 950 955 960

Cys Ser Leu Ser Asp Leu Lys Cys Phe Ile Ala Ala Gly Leu Leu Pro
965 970 975

Pro Tyr Val Leu Lys Asn Val Val Leu Arg Glu Pro Gly Pro Gly Thr
980 985 990

Trp Leu Thr Leu Cys Ser Asp Pro Tyr Thr Leu Asn Ile Pro Tyr Thr
995 1000 1005

Gln Leu Pro Thr Thr Tyr Leu Lys Lys His Thr Gln Arg Ser Leu
1010 1015 1020

Leu Ser Arg Ala Val Asn Pro Leu Leu Ala Gly Val Gln Val Pro
1025 1030 1035

Sequence

Asn	Gln	His	Glu	Glu	Glu	Glu	Met	Leu	Ala	Arg	Phe	Leu	Leu	Asp
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Arg	Glu	Tyr	Val	Met	Pro	Arg	Val	Ala	His	Val	Ile	Leu	Glu	Ser
1055						1060					1065			
Ser	Val	Leu	Gly	Lys	Arg	Lys	Gln	Ile	Gln	Gly	Leu	Ile	Asp	Thr
1070						1075					1080			
Thr	Pro	Thr	Ile	Ile	Arg	Thr	Ser	Leu	Val	Asn	Leu	Pro	Val	Ser
1085						1090					1095			
Arg	Lys	Lys	Cys	Glu	Lys	Ile	Ile	Asn	Tyr	Ser	Leu	Asn	Tyr	Ile
1100						1105					1110			
Ala	Glu	Cys	His	Asp	Ser	Leu	Leu	Ser	Gln	Val	Cys	Phe	Ser	Asp
1115						1120					1125			
Asn	Lys	Glu	Tyr	Leu	Trp	Ser	Thr	Ser	Leu	Ile	Ser	Val	Glu	Thr
1130						1135					1140			
Cys	Ser	Val	Thr	Ile	Ala	Asp	Tyr	Leu	Arg	Ala	Val	Ser	Trp	Ser
1145						1150					1155			
Asn	Ile	Leu	Gly	Gly	Arg	Asn	Ile	Ser	Gly	Val	Thr	Thr	Pro	Asp
1160						1165					1170			
Thr	Ile	Glu	Leu	Ile	Gln	Gly	Cys	Leu	Ile	Gly	Glu	Asn	Ser	Ser
1175						1180					1185			
Cys	Thr	Leu	Cys	Glu	Ser	His	Asp	Asp	Ala	Phe	Thr	Trp	Met	His
1190						1195					1200			
Leu	Pro	Gly	Pro	Leu	Tyr	Ile	Pro	Glu	Pro	Ser	Val	Thr	Asn	Ser
1205						1210					1215			
Lys	Met	Arg	Val	Pro	Tyr	Leu	Gly	Ser	Lys	Thr	Glu	Glu	Arg	Lys
1220						1225					1230			
Thr	Ala	Ser	Met	Ala	Ala	Ile	Lys	Gly	Met	Ser	His	His	Leu	Arg
1235						1240					1245			
Ala	Val	Leu	Arg	Gly	Thr	Ser	Val	Phe	Ile	Trp	Ala	Phe	Gly	Asp
1250						1255					1260			
Thr	Asp	Ile	Asn	Trp	Asp	Asn	Ala	Leu	Gln	Ile	Ala	Gln	Ser	Arg
1265						1270					1275			
Cys	Asn	Ile	Thr	Leu	Asp	Gln	Met	Arg	Leu	Leu	Thr	Pro	Ile	Pro
1280						1285					1290			

Sequence

Ser Ser Ser Asn Ile Gln His Arg Leu Asp Asp Gly Ile Ser Thr
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 Gln Lys Phe Thr Pro Ala Ser Leu Ala Arg Ile Thr Ser Phe Val
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 His Ile Cys Asn Asp Ser Gln Arg Leu Glu Lys Asp Gly Ser Ser
 1325 1330 1335
 Val Asp Ser Asn Leu Ile Tyr Gln Gln Ile Met Leu Leu Gly Leu
 1340 1345 1350
 Ser Ile Phe Glu Thr Met Tyr Ser Met Asp Gln Lys Trp Val Phe
 1355 1360 1365
 Asn Asn His Thr Leu His Leu His Thr Gly His Ser Cys Cys Pro
 1370 1375 1380
 Arg Glu Leu Asp Ile Ser Leu Val Asn Pro Pro Arg His Gln Thr
 1385 1390 1395
 Pro Glu Leu Thr Ser Thr Thr Thr Asn Pro Phe Leu Tyr Asp Gln
 1400 1405 1410
 Leu Pro Leu Asn Gln Glu Asn Leu Thr Thr Leu Glu Ile Lys Thr
 1415 1420 1425
 Phe Lys Phe Asn Glu Leu Asn Ile Asp Gly Leu Asp Phe Gly Glu
 1430 1435 1440
 Gly Ile Gln Leu Leu Ser Arg Cys Thr Ala Arg Leu Met Ala Glu
 1445 1450 1455
 Cys Ile Leu Glu Glu Gly Ile Gly Ser Ser Val Lys Asn Glu Ala
 1460 1465 1470
 Ile Val Asn Phe Asp Asn Ser Val Asn Trp Ile Ser Glu Cys Leu
 1475 1480 1485
 Met Cys Asp Ile Arg Ser Leu Cys Val Asn Leu Gly Gln Glu Ile
 1490 1495 1500
 Leu Cys Ser Leu Ala Tyr Gln Met Tyr Tyr Leu Arg Ile Arg Gly
 1505 1510 1515
 Arg Arg Ala Ile Leu Asn Tyr Leu Asp Thr Thr Leu Gln Arg Ile
 1520 1525 1530
 Pro Val Ile Gln Leu Ala Asn Ile Ala Leu Thr Ile Ser His Pro
 1535 1540 1545

Sequence

Glu Ile Phe Arg Arg Ile Val Asn Thr Gly Ile His Asn Gln Ile
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 Lys Gly Pro Tyr Val Ala Thr Thr Asp Phe Ile Ala Ala Ser Arg
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 Asp Ile Ile Leu Ser Gly Ala Arg Glu Tyr Leu Ser Tyr Leu Ser
 1580 1585 1590
 Ser Gly Gln Glu Asp Cys Tyr Thr Phe Phe Asn Cys Gln Asp Gly
 1595 1600 1605
 Asp Leu Thr Pro Lys Met Glu Gln Tyr Leu Ala Arg Arg Ala Cys
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 Leu Leu Thr Leu Leu Tyr Asn Thr Gly His Gln Ile Pro Val Ile
 1625 1630 1635
 Arg Ser Leu Thr Pro Ile Glu Lys Cys Lys Val Leu Thr Glu Tyr
 1640 1645 1650
 Asn Gln Gln Ile Glu Tyr Ala Asp Gln Glu Phe Ser Ser Val Leu
 1655 1660 1665
 Lys Val Val Asn Ala Leu Leu Gln Asn Pro Lys Ile Asp Ala Leu
 1670 1675 1680
 Val Ser Asn Leu Tyr Phe Thr Thr Arg Arg Val Leu Ser Asn Leu
 1685 1690 1695
 Arg Ser Cys Asp Lys Ala Arg Ser Tyr Ile Glu Tyr Leu Tyr Thr
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 Glu Asp Phe Gly Glu Lys Glu Asp Thr Val Gln Tyr Asp Ile Met
 1715 1720 1725
 Thr Thr Asn Asp Ile Ile Leu Thr His Gly Leu Phe Thr Gln Ile
 1730 1735 1740
 Glu Ile Ser Tyr Gln Gly Asn Ser Leu His Lys Phe Leu Thr Pro
 1745 1750 1755
 Asp Asn Ala Pro Gly Ser Leu Ile Pro Phe Ser Ile Ser Pro Asn
 1760 1765 1770
 Ser Leu Ala Cys Asp Pro Leu His His Leu Leu Lys Ser Val Gly
 1775 1780 1785
 Thr Ser Ser Thr Ser Trp Tyr Lys Tyr Ala Ile Ala Tyr Ala Val
 1790 1795 1800

Sequence

Ser Glu Lys Arg Ser Ala Arg Leu Gly Gly Ser Leu Tyr Ile Gly
 1805 1810 1815
 Glu Gly Ser Gly Ser Val Met Thr Leu Leu Glu Tyr Leu Glu Pro
 1820 1825 1830
 Ser Val Asp Ile Phe Tyr Asn Ser Leu Phe Ser Asn Gly Met Asn
 1835 1840 1845
 Pro Pro Gln Arg Asn Tyr Gly Leu Met Pro Leu Gln Phe Val Asn
 1850 1855 1860
 Ser Val Val Tyr Lys Asn Leu Thr Ala Lys Ser Glu Cys Lys Leu
 1865 1870 1875
 Gly Phe Val Gln Gln Phe Lys Pro Leu Trp Arg Asp Ile Asp Ile
 1880 1885 1890
 Glu Thr Asn Val Thr Asp Pro Ser Phe Ile Asn Phe Ala Leu Asn
 1895 1900 1905
 Glu Ile Pro Met Gln Ser Leu Lys Arg Val Asn Cys Asp Val Glu
 1910 1915 1920
 Phe Asp Arg Gly Met Pro Ile Glu Arg Val Ile Gln Gly Tyr Thr
 1925 1930 1935
 His Ile Leu Leu Val Ala Thr Tyr Gly Leu Gln Gln Asp Ser Ile
 1940 1945 1950
 Leu Trp Val Lys Val Tyr Arg Thr Ser Glu Lys Val Phe Gln Phe
 1955 1960 1965
 Leu Leu Ser Ala Met Ile Met Ile Phe Gly Tyr Val Lys Ile His
 1970 1975 1980
 Arg Asn Gly Tyr Met Ser Thr Lys Asp Glu Glu Tyr Ile Leu Met
 1985 1990 1995
 Ser Asp Cys Lys Glu Pro Val Asn Tyr Thr Ala Val Pro Asn Ile
 2000 2005 2010
 Leu Thr Arg Val Ser Asp Leu Val Ser Lys Asn Leu Ser Leu Ile
 2015 2020 2025
 His Pro Glu Asp Leu Arg Lys Val Arg Cys Glu Thr Asp Ser Leu
 2030 2035 2040
 Asn Leu Lys Cys Asn His Ile Tyr Glu Lys Ile Ile Ala Arg Lys
 2045 2050 2055

Sequence

Ile Pro Leu Gln Val Ser Ser Thr Asp Ser Leu Leu Leu Gln Leu
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Gly Gly Val Ile Asn Ser Val Gly Ser Thr Asp Pro Arg Glu Val
2075 2080 2085

Ala Thr Leu Ser Ser Ile Glu Cys Met Asp Tyr Val Val Ser Ser
2090 2095 2100

Ile Asp Leu Ala Ile Leu Glu Ala Asn Ile Val Ile Ser Glu Ser
2105 2110 2115

Ala Gly Leu Asp Leu Ala Leu Met Leu Gly Pro Phe Asn Leu Asn
2120 2125 2130

Lys Leu Lys Lys Ile Asp Thr Ile Leu Lys Ser Ser Thr Tyr Gln
2135 2140 2145

Leu Ile Pro Tyr Trp Leu Arg Tyr Glu Tyr Ser Ile Asn Pro Arg
2150 2155 2160

Ser Leu Ser Phe Leu Ile Thr Lys Leu Gln Gln Cys Arg Ile Ser
2165 2170 2175

Trp Ser Asp Met Ile Thr Ile Ser Glu Phe Arg Lys Lys Ser Lys
2180 2185 2190

Arg Pro Ile Phe Ile Lys Arg Val Ile Gly Asn Gln Gln Leu Lys
2195 2200 2205

Ser Phe Phe Asn Glu Ser Ser Ser Ile Val Leu Thr Arg Ala Glu
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Val Lys Val Cys Ile Lys Phe Leu Gly Ala Ile Ile Lys Leu Lys
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catgtcttcc gtatttgatg agtacgaaca gtcctcgcg gctcagactc gcccgaatgg 180
agctcatgga gggggagaaa aaggaggtag cttaaaagta gacgtcccgg tattcactct 240
taacagtgat gaccagaag atagatggag ctttgtggtt ttctgcctcc ggattgctgt 300

Sequence

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Sequence

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Sequence

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Sequence

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Sequence

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Sequence

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Sequence

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Sequence

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 Ser Lys Arg Leu Ala Lys Tyr Gln Gln Gln Gly Arg Leu Val Lys Gln
 195 200 205
 Val Leu Leu His Ser Val Val Arg Thr Glu Phe Met Arg Val Ile Arg
 210 215 220
 Gly Ser Leu Val Leu Arg Gln Phe Met Val Ser Glu Cys Lys Arg Ala
 225 230 235 240
 Ser Ala Met Gly Gly Asp Thr Ser Arg Tyr Tyr Ala Met Val Gly Asp
 245 250 255
 Ile Ser Leu Tyr Ile Lys Asn Ala Gly Leu Thr Ala Phe Phe Leu Thr
 260 265 270

Sequence

Leu Lys Phe Gly Val Gly Thr Gln Tyr Pro Thr Leu Ala Met Ser Val
275 285

Phe Ser Ser Asp Leu Lys Arg Leu Ala Ala Leu Ile Arg Leu Tyr Lys
290 295 300

Thr Lys Gly Asp Asn Ala Pro Tyr Met Ala Phe Leu Glu Asp Ser Asp
305 310 315 320

Met Gly Asn Phe Ala Pro Ala Asn Tyr Ser Thr Met Tyr Ser Tyr Ala
325 330 335

Met Gly Ile Gly Thr Ile Leu Glu Ala Ser Val Ser Arg Tyr Gln Tyr
340 345 350

Ala Arg Asp Phe Thr Ser Glu Asn Tyr Phe Arg Leu Gly Val Glu Thr
355 360 365

Ala Gln Ser Gln Gln Gly Ala Phe Asp Glu Arg Thr Ala Arg Glu Met
370 375 380

Gly Leu Thr Glu Glu Ser Lys Gln Gln Val Arg Ser Leu Leu Met Ser
385 390 395 400

Val Asp Met Gly Pro Ser Ser Val His Glu Pro Ser Arg Pro Ala Phe
405 410 415

Ile Ser Gln Glu Glu Asn Arg Gln Pro Ala Gln Asn Ser Ser Asp Thr
420 425 430

Gln Gly Gln Thr Lys Pro Val Pro Lys Gln Pro Ala Pro Arg Ala Asp
435 440 445

Ser Asp Asp Ile Asp Pro Tyr Glu Asn Gly Leu Glu Trp
450 455 460

<210> 55
<211> 1218
<212> DNA
<213> artificial sequence

<220>
<223> P gene of FJ215863

<400> 55
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aaggagattc agaaatccga actcaagcct ccccaaacca ccggacgacc acctgtcagt 120
caagggaaca caagaaatct aactgatcta tgggaaaaag agactgcaag tcagaccaag 180
acaccggccc aatctccaca aaccacacaa gttcagtctg atgaaaatga ggagggagaa 240
atcaagtccg agtcaattga tggccacatc agaggaactg ttaatcaatc agagcaagtc 300

Sequence

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ccagaacaaa accagagcag atcttcacca ggtgatgata tcgacagagc tctcaacaag      360
cttgaaggga gaatcaattc aatcagctca atggacaaag aaattaaaaa gggccctcgc      420
atccagaatc tccctgggtc ccaggcggca actcaacagg cgacccaccc attggcaggg      480
gacaccccca acatgcaagc acagacaaaa gccctggcga agccacatca agaggcaatc      540
aatcctggca accaggacac aggagagagt attcatttac caccttccat ggcaccacca      600
gagtcattag ttggtgcaat ccgcaatgca cccaatttcg tgccagacca atctatgacg      660
aatgtagatg cggggagtggt ccaactacat gcatcatgtg cagagatgat aagtagaatg      720
tttgtagaag ttatatccaa gcttgataaa ctcgagtcga gactgaatga tatagcaaaa      780
gttgtgaaca ccacccccct tattaggaat gatattaacc aacttaaggc cacaaccgca      840
ctgatgtcca accaaattgc ttccatacaa attcttgacc caggggaatgc aggggtgagg      900
tccctctctg aaatgaaatc tgtgacgaag aaagctgctg ttgtaattgc aggggttgga      960
gacgacccaa ctcaaattat tgaagaaggc attatggcca aagatgctct tggaaaacct     1020
gtgcctccaa catctgttat ctcagctaaa gctcagactt cttccggtgt gagtaagggt     1080
gaaatagaag gattgattgc attggtggaa acattagttg acaatgacaa gaaggcagca     1140
aaactgatta aaatgattga tcaagttaaa tcccacgccg attacgcccg agtcaagcag     1200
gcaatatata atcgctaa                                     1218

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<210> 56
 <211> 405
 <212> PRT
 <213> artificial sequence

<220>
 <223> P protein AC048298

<400> 56

Met Asp Phe Ala Asn Asp Glu Glu Ile Ala Glu Leu Leu Asn Leu Ser
 1 5 10 15

Thr Asn Val Ile Lys Glu Ile Gln Lys Ser Glu Leu Lys Pro Pro Gln
 20 25 30

Thr Thr Gly Arg Pro Pro Val Ser Gln Gly Asn Thr Arg Asn Leu Thr
 35 40 45

Asp Leu Trp Glu Lys Glu Thr Ala Ser Gln Thr Lys Thr Pro Ala Gln
 50 55 60

Ser Pro Gln Thr Thr Gln Val Gln Ser Asp Glu Asn Glu Glu Gly Glu
 65 70 75 80

Ile Lys Ser Glu Ser Ile Asp Gly His Ile Arg Gly Thr Val Asn Gln
 85 90 95

Sequence

Ser Glu Gln Val Pro Glu Gln Asn Gln Ser Arg Ser Ser Pro Gly Asp
100 105 110

Asp Leu Asp Arg Ala Leu Asn Lys Leu Glu Gly Arg Ile Asn Ser Ile
115 120 125

Ser Ser Met Asp Lys Glu Ile Lys Lys Gly Pro Arg Ile Gln Asn Leu
130 135 140

Pro Gly Ser Gln Ala Ala Thr Gln Gln Ala Thr His Pro Leu Ala Gly
145 150 155 160

Asp Thr Pro Asn Met Gln Ala Gln Thr Lys Ala Leu Ala Lys Pro His
165 170 175

Gln Glu Ala Ile Asn Pro Gly Asn Gln Asp Thr Gly Glu Ser Ile His
180 185 190

Leu Pro Pro Ser Met Ala Pro Pro Glu Ser Leu Val Gly Ala Ile Arg
195 200 205

Asn Ala Pro Gln Phe Val Pro Asp Gln Ser Met Thr Asn Val Asp Ala
210 215 220

Gly Ser Val Gln Leu His Ala Ser Cys Ala Glu Met Ile Ser Arg Met
225 230 235 240

Phe Val Glu Val Ile Ser Lys Leu Asp Lys Leu Glu Ser Arg Leu Asn
245 250 255

Asp Ile Ala Lys Val Val Asn Thr Thr Pro Leu Ile Arg Asn Asp Ile
260 265 270

Asn Gln Leu Lys Ala Thr Thr Ala Leu Met Ser Asn Gln Ile Ala Ser
275 280 285

Ile Gln Ile Leu Asp Pro Gly Asn Ala Gly Val Arg Ser Leu Ser Glu
290 295 300

Met Lys Ser Val Thr Lys Lys Ala Ala Val Val Ile Ala Gly Phe Gly
305 310 315 320

Asp Asp Pro Thr Gln Ile Ile Glu Glu Gly Ile Met Ala Lys Asp Ala
325 330 335

Leu Gly Lys Pro Val Pro Pro Thr Ser Val Ile Ser Ala Lys Ala Gln
340 345 350

Thr Ser Ser Gly Val Ser Lys Gly Glu Ile Glu Gly Leu Ile Ala Leu
355 360 365

Sequence

Val Glu Thr Leu Val Asp Asn Asp Lys Lys Ala Ala Lys Leu Ile Lys
 370 375 380

Met Ile Asp Gln Val Lys Ser His Ala Asp Tyr Ala Arg Val Lys Gln
 385 390 395 400

Ala Ile Tyr Asn Ala
 405

<210> 57
 <211> 1110
 <212> DNA
 <213> artificial sequence

<220>
 <223> M gene of FJ215863

<400> 57
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 agggtagatt caattggcga tgtgcagaac gccaaagagt cctcgatatt cgtgactcta 180
 tatggtttca tccaagcaat taaggagagt acagatcgat cgaaattctt ccatccaaaa 240
 gatgacttca aacctgagac agtcactgca ggactggtag tggtaggtgc gatccgaatg 300
 atggctgatg ttaataccat ctctaatac gactagcgc tggagatcac tgtaagaaa 360
 tctgcaactt ctcaagagaa aatgacggtg atgttcacac atagcccccc ttcattgaga 420
 actgcaataa ctatccgagc aggagggttc atctcgaatg cagacgagaa tataaaatgt 480
 gccagcaagt tgactgcagg agtgcagtac atattccgcc caatgtttgt ttcaatcact 540
 aaattacaca atggcaaact atataggggtg cccaaaagca tccacagcat ctctgccact 600
 ctactgtata gtgtgatgtt ggaggtagga ttcaaagtgg atattgggaa ggatcatccc 660
 caggcaaaga tgctgaagaa ggtcacaatc ggcgatgcag acacatactg ggggtttgca 720
 tggttccacc tgtgcaattt caaaaagaca tcctctaagg gaaagccaag aacgctagac 780
 gaactaaaga caaaagtcaa aaacatgggg ttgaaattgg agttacatga cttgtggggg 840
 ccgactattg tggtcctaat cactggcaag agcagcaaata atgctcaagg atttttttcc 900
 tccaatggta cttgttgctt cccaatcagc agatctgcac cagggtcttg gaagcttctg 960
 tggctctgtt cagcaactat cgggtgacgc acagttgtta tccagtcaag cgagaaaggg 1020
 gaactcctaa ggtctgatga cctcgagata cgagggtgctg tggcctccaa gaaaggtaga 1080
 ctgagctcat ttcacccctt caagaaatga 1110

<210> 58
 <211> 369
 <212> PRT
 <213> artificial sequence

<220>
 <223> M protein of AC048299

Sequence

<400> 58

Met Ala Tyr Thr Thr Leu Lys Leu Trp Val Asp Glu Gly Asp Met Ser
 1 5 10 15

Ser Ser Leu Leu Ser Phe Pro Leu Val Leu Lys Glu Thr Asp Arg Gly
 20 25 30

Thr Lys Glu Leu Gln Pro Gln Val Arg Val Asp Ser Ile Gly Asp Val
 35 40 45

Gln Asn Ala Lys Glu Ser Ser Ile Phe Val Thr Leu Tyr Gly Phe Ile
 50 55 60

Gln Ala Ile Lys Glu Ser Thr Asp Arg Ser Lys Phe Phe His Pro Lys
 65 70 75 80

Asp Asp Phe Lys Pro Glu Thr Val Thr Ala Gly Leu Val Val Val Gly
 85 90 95

Ala Ile Arg Met Met Ala Asp Val Asn Thr Ile Ser Asn Asp Ala Leu
 100 105 110

Ala Leu Glu Ile Thr Val Lys Lys Ser Ala Thr Ser Gln Glu Lys Met
 115 120 125

Thr Val Met Phe His Asn Ser Pro Pro Ser Leu Arg Thr Ala Ile Thr
 130 135 140

Ile Arg Ala Gly Gly Phe Ile Ser Asn Ala Asp Glu Asn Ile Lys Cys
 145 150 155 160

Ala Ser Lys Leu Thr Ala Gly Val Gln Tyr Ile Phe Arg Pro Met Phe
 165 170 175

Val Ser Ile Thr Lys Leu His Asn Gly Lys Leu Tyr Arg Val Pro Lys
 180 185 190

Ser Ile His Ser Ile Ser Ser Thr Leu Leu Tyr Ser Val Met Leu Glu
 195 200 205

Val Gly Phe Lys Val Asp Ile Gly Lys Asp His Pro Gln Ala Lys Met
 210 215 220

Leu Lys Lys Val Thr Ile Gly Asp Ala Asp Thr Tyr Trp Gly Phe Ala
 225 230 235 240

Trp Phe His Leu Cys Asn Phe Lys Lys Thr Ser Ser Lys Gly Lys Pro
 245 250 255

Arg Thr Leu Asp Glu Leu Lys Thr Lys Val Lys Asn Met Gly Leu Lys
 260 265 270

Sequence

Leu Glu Leu His Asp Leu Trp Gly Pro Thr Ile Val Val Gln Ile Thr
275 280 285

Gly Lys Ser Ser Lys Tyr Ala Gln Gly Phe Phe Ser Ser Asn Gly Thr
290 295 300

Cys Cys Leu Pro Ile Ser Arg Ser Ala Pro Gly Leu Gly Lys Leu Leu
305 310 315 320

Trp Ser Cys Ser Ala Thr Ile Gly Asp Ala Thr Val Val Ile Gln Ser
325 330 335

Ser Glu Lys Gly Glu Leu Leu Arg Ser Asp Asp Leu Glu Ile Arg Gly
340 345 350

Ala Val Ala Ser Lys Lys Gly Arg Leu Ser Ser Phe His Pro Phe Lys
355 360 365

Lys

<210> 59
<211> 1632
<212> DNA
<213> artificial sequence

<220>
<223> F gene of FJ215863

<400> 59
atgggtcaaa tatcagtata tctaattaat agcgtgctat tattgctggt atatcctgtg 60
aattcgattg acaatacact cattgcccc aatcgaggtg ccagcgcaaa tgaatggcag 120
cttgctgcat atacaacatc actttcaggg acaattgccg tgcgattcct acctgtgctc 180
ccggataata tgactacctg tcttagagaa acaatcacta catataataa tactgtcaac 240
aacatcttag gccactcaa atccaatctg gatgcactgc tctcatctga gacttatccc 300
cagacaagat taattggggc agttataggt tcaattgctc tcggtgttgc aacatcggct 360
caaatcactg ctgcagttgc tctcaagcaa gcgcaagaca atgcaaggaa catactagca 420
ctcaaagaag cactgtccaa aaccaatgag gcggtcaagg agcttagtag tgggttacia 480
caaacagcta ttgcacttgg taagatacag agttttgtga atgaggaaat tctgccatct 540
atcaaccaac tgagctgcga ggtgacagcc aataaacttg ggggtgtattt atctctgtat 600
ctcacagaac tgaccacat attcgggtgca cagctgacta accctgcatt gacttcatta 660
tcatatcaag cgctgtacia cctgtgtggt ggcaacatgg caatgcttac tcagaagatt 720
ggaattaaac aacaagacgt taattcgcta tatgaagccg gactaatcac aggacaagtc 780
attggttatg actctcagta ccagctgctg gtcattccagg tcaattatcc aagcatttct 840
gaggtcactg gtgtacgtgc aacagaatta gtcactgtta gtgtaacaac agacaagggg 900

Sequence

```

gaagggaaag caattgtacc ccaatttgta gctgaaagtc gggtgactat tgaagagctt      960
gatgtagcat cttgtaaatt cagcagcacg accctatatt gcaggcaggt caacacaagg      1020
gcacttcccc cgctagtagc tagctgtctt cgaggtaact atgatgattg tcaatataacc      1080
acagagattg gagcattatc atccccggtat ataacactag atgggggggt cttagttaat      1140
tgtaagtcaa ttgtttgtag gtgccttaat ccaagtaaga tcattctctca aaatacaaac      1200
gctgcagtaa catatgttga tgccacaatc tgcaaaacaa ttcaattgga tgatatacaa      1260
ctccagctgg aagggtcact atcatcagtt tatgcaagaa acatctcaat tgagatcagt      1320
caggtgacca cctccgggtc tttagatatc agcagtgaga taggaaacat caataatacg      1380
gtgaatcgtg tggaggattt aattcaccaa tcagaggaat ggctggcaaa ggttaaccga      1440
cacattgtta ataatacaac actaatcgta ctctgtgtgt taagtgcgct tgctgtgatc      1500
tggtctggcag tattaacggc tattataata tacttgagaa caaagttgaa gactatatcg      1560
gcattagctg taaccaatac aatacagtct aaccctatg ttaaccaaac gaaacgtgaa      1620
tctaagtttt ga      1632

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<210> 60
 <211> 543
 <212> PRT
 <213> artificial sequence

<220>
 <223> F protein of AC048300
 <400> 60

Met Gly Gln Ile Ser Val Tyr Leu Ile Asn Ser Val Leu Leu Leu Leu
 1 5 10 15

Val Tyr Pro Val Asn Ser Ile Asp Asn Thr Leu Ile Ala Pro Ile Gly
 20 25 30

Val Ala Ser Ala Asn Glu Trp Gln Leu Ala Ala Tyr Thr Thr Ser Leu
 35 40 45

Ser Gly Thr Ile Ala Val Arg Phe Leu Pro Val Leu Pro Asp Asn Met
 50 55 60

Thr Thr Cys Leu Arg Glu Thr Ile Thr Thr Tyr Asn Asn Thr Val Asn
 65 70 75 80

Asn Ile Leu Gly Pro Leu Lys Ser Asn Leu Asp Ala Leu Leu Ser Ser
 85 90 95

Glu Thr Tyr Pro Gln Thr Arg Leu Ile Gly Ala Val Ile Gly Ser Ile
 100 105 110

Ala Leu Gly Val Ala Thr Ser Ala Gln Ile Thr Ala Ala Val Ala Leu
 115 120 125

Sequence

Lys Gln Ala Gln Asp Asn Ala Arg Asn Ile Leu Ala Leu Lys Glu Ala
 130 135 140
 Leu Ser Lys Thr Asn Glu Ala Val Lys Glu Leu Ser Ser Gly Leu Gln
 145 150 155 160
 Gln Thr Ala Ile Ala Leu Gly Lys Ile Gln Ser Phe Val Asn Glu Glu
 165 170 175
 Ile Leu Pro Ser Ile Asn Gln Leu Ser Cys Glu Val Thr Ala Asn Lys
 180 185 190
 Leu Gly Val Tyr Leu Ser Leu Tyr Leu Thr Glu Leu Thr Thr Ile Phe
 195 200 205
 Gly Ala Gln Leu Thr Asn Pro Ala Leu Thr Ser Leu Ser Tyr Gln Ala
 210 215 220
 Leu Tyr Asn Leu Cys Gly Gly Asn Met Ala Met Leu Thr Gln Lys Ile
 225 230 235 240
 Gly Ile Lys Gln Gln Asp Val Asn Ser Leu Tyr Glu Ala Gly Leu Ile
 245 250 255
 Thr Gly Gln Val Ile Gly Tyr Asp Ser Gln Tyr Gln Leu Leu Val Ile
 260 265 270
 Gln Val Asn Tyr Pro Ser Ile Ser Glu Val Thr Gly Val Arg Ala Thr
 275 280 285
 Glu Leu Val Thr Val Ser Val Thr Thr Asp Lys Gly Glu Gly Lys Ala
 290 295 300
 Ile Val Pro Gln Phe Val Ala Glu Ser Arg Val Thr Ile Glu Glu Leu
 305 310 315 320
 Asp Val Ala Ser Cys Lys Phe Ser Ser Thr Thr Leu Tyr Cys Arg Gln
 325 330 335
 Val Asn Thr Arg Ala Leu Pro Pro Leu Val Ala Ser Cys Leu Arg Gly
 340 345 350
 Asn Tyr Asp Asp Cys Gln Tyr Thr Thr Glu Ile Gly Ala Leu Ser Ser
 355 360 365
 Arg Tyr Ile Thr Leu Asp Gly Gly Val Leu Val Asn Cys Lys Ser Ile
 370 375 380
 Val Cys Arg Cys Leu Asn Pro Ser Lys Ile Ile Ser Gln Asn Thr Asn
 385 390 395 400

Sequence

Ala Ala Val Thr Tyr Val Asp Ala Thr Ile Cys Lys Thr Ile Gln Leu
405 410 415

Asp Asp Ile Gln Leu Gln Leu Glu Gly Ser Leu Ser Ser Val Tyr Ala
420 425 430

Arg Asn Ile Ser Ile Glu Ile Ser Gln Val Thr Thr Ser Gly Ser Leu
435 440 445

Asp Ile Ser Ser Glu Ile Gly Asn Ile Asn Asn Thr Val Asn Arg Val
450 455 460

Glu Asp Leu Ile His Gln Ser Glu Glu Trp Leu Ala Lys Val Asn Pro
465 470 475 480

His Ile Val Asn Asn Thr Thr Leu Ile Val Leu Cys Val Leu Ser Ala
485 490 495

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

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

Gln Ser Asn Pro Tyr Val Asn Gln Thr Lys Arg Glu Ser Lys Phe
530 535 540

<210> 61
<211> 1734
<212> DNA
<213> artificial sequence

<220>
<223> HN gene of FJ215863

<400> 61
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attattagca tagttcaaat tggaaatttg aaaattcctt ctgtagggga tctggctgat 180
gaagtgggtga cacccttgaa aaccactctg tctgatacac tcaggaatcc aattaaccag 240
ataaatgaca tatttaggat tgttgccctt gatattccat tgcaagtgac caatatccaa 300
aaagaccttg caagtcaatt taacatgttg atagatagtt taaatgctat caaattaggc 360
aacgggacca acctatcat acctacatca gacaaggagt atgcaggagg aattggaaac 420
cctgtattta ctgtcgatgc tggaggttct ataggattca aacagtttag cttaatagaa 480
catccgagct ttattgctgg acctacaacg acccgaggct gtacaagaat acccactttt 540
cacatgtcag aaagtcattg gtgctactca cacaacatca tcgctgctgg ctgtcaagat 600
gccagtgcac ccagtatgta tatctcaatg ggagttctcc atgtgtcctc atctggcact 660

Sequence

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cccatTTTTtC ttactactgc aagtGagctg atagacgatg gagttaaccg taagtcatgc      720
agcattgtag caacccgatt tggctgtgac attttgtgca gtattgtcac agagaaggag      780
ggagatgatt actggtctga tactccgact ccaatgcgcc acggccgttt ttcattcaat      840
ggtagttttg tagaagccga actaccagtg tccagtatgt tctcgtcatt ctctgccaac      900
taccctgctg tgggatcagg cgaaattgta aaagatagaa tattattccc aatttacgga      960
ggtataaagc agacttcacc agagtttacc gaattagtga aatacggact ctttgtatca     1020
acacctacaa ctgtgtgcca gagtagctgg acttatgacc aggtaaaagc agcgtatagg     1080
ccagattaca tatcaggccg gttctgggca caagtgatac tcagctgcgc tcttgatgca     1140
gtcgacttat caagttgtat tgtaaagatt atgaatagca gcacagtgat gatggcagcg     1200
gaaggaagga taatgaagat agggattgat tacttttact atcagcggtc atcttcttgg     1260
tggccattgg catttggttac aaaactagac ccgcaagaat tggcagacac aaactcaata     1320
tggctgacca attccatacc aatcccgcaa tcaaagttcc ctcggccttc atattcagaa     1380
aattattgca caaagccagc agtttgcctt gctacttgtg tcaactgggtgt gtactctgat     1440
atttggcccc tgacctcatc ttcatcactc ccgagcataa tttggatcgg ccagtacctt     1500
gatgctcctg ttagaaggac ttatcccaga tttggaattg caaatcagtc aactgggtac     1560
ctccaagaag atattctacc cacttcacc gcaagtgcgt attcaaccac tacatgtttt     1620
aagaatactg ccaggaatag agtgttctgc gtcaccattg ccgaatttgc agatggggtg     1680
tttggagagt acaggataac acctcagttg tacgaattag tgagaaataa ttga           1734

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<210> 62
 <211> 577
 <212> PRT
 <213> artificial sequence

<220>
 <223> HN protein of AC048301

<400> 62

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Arg Lys Thr Thr Trp Arg Ala Ile Phe Arg Trp Ser Val Leu Leu Ile
 20 25 30

Thr Thr Gly Cys Leu Ala Leu Ser Ile Ile Ser Ile Val Gln Ile Gly
 35 40 45

Asn Leu Lys Ile Pro Ser Val Gly Asp Leu Ala Asp Glu Val Val Thr
 50 55 60

Pro Leu Lys Thr Thr Leu Ser Asp Thr Leu Arg Asn Pro Ile Asn Gln
 65 70 75 80

Sequence

Ile Asn Asp Ile Phe Arg Ile Val Ala Leu Asp Ile Pro Leu Gln Val
85 90 95

Thr Asn Ile Gln Lys Asp Leu Ala Ser Gln Phe Asn Met Leu Ile Asp
100 105 110

Ser Leu Asn Ala Ile Lys Leu Gly Asn Gly Thr Asn Leu Ile Ile Pro
115 120 125

Thr Ser Asp Lys Glu Tyr Ala Gly Gly Ile Gly Asn Pro Val Phe Thr
130 135 140

Val Asp Ala Gly Gly Ser Ile Gly Phe Lys Gln Phe Ser Leu Ile Glu
145 150 155 160

His Pro Ser Phe Ile Ala Gly Pro Thr Thr Thr Arg Gly Cys Thr Arg
165 170 175

Ile Pro Thr Phe His Met Ser Glu Ser His Trp Cys Tyr Ser His Asn
180 185 190

Ile Ile Ala Ala Gly Cys Gln Asp Ala Ser Ala Ser Ser Met Tyr Ile
195 200 205

Ser Met Gly Val Leu His Val Ser Ser Ser Gly Thr Pro Ile Phe Leu
210 215 220

Thr Thr Ala Ser Glu Leu Ile Asp Asp Gly Val Asn Arg Lys Ser Cys
225 230 235 240

Ser Ile Val Ala Thr Arg Phe Gly Cys Asp Ile Leu Cys Ser Ile Val
245 250 255

Thr Glu Lys Glu Gly Asp Asp Tyr Trp Ser Asp Thr Pro Thr Pro Met
260 265 270

Arg His Gly Arg Phe Ser Phe Asn Gly Ser Phe Val Glu Ala Glu Leu
275 280 285

Pro Val Ser Ser Met Phe Ser Ser Phe Ser Ala Asn Tyr Pro Ala Val
290 295 300

Gly Ser Gly Glu Ile Val Lys Asp Arg Ile Leu Phe Pro Ile Tyr Gly
305 310 315 320

Gly Ile Lys Gln Thr Ser Pro Glu Phe Thr Glu Leu Val Lys Tyr Gly
325 330 335

Leu Phe Val Ser Thr Pro Thr Thr Val Cys Gln Ser Ser Trp Thr Tyr
340 345 350

Sequence

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

Trp Ala Gln Val Ile Leu Ser Cys Ala Leu Asp Ala Val Asp Leu Ser
370 375 380

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

Glu Gly Arg Ile Met Lys Ile Gly Ile Asp Tyr Phe Tyr Tyr Gln Arg
405 410 415

Ser Ser Ser Trp Trp Pro Leu Ala Phe Val Thr Lys Leu Asp Pro Gln
420 425 430

Glu Leu Ala Asp Thr Asn Ser Ile Trp Leu Thr Asn Ser Ile Pro Ile
435 440 445

Pro Gln Ser Lys Phe Pro Arg Pro Ser Tyr Ser Glu Asn Tyr Cys Thr
450 455 460

Lys Pro Ala Val Cys Pro Ala Thr Cys Val Thr Gly Val Tyr Ser Asp
465 470 475 480

Ile Trp Pro Leu Thr Ser Ser Ser Ser Leu Pro Ser Ile Ile Trp Ile
485 490 495

Gly Gln Tyr Leu Asp Ala Pro Val Arg Arg Thr Tyr Pro Arg Phe Gly
500 505 510

Ile Ala Asn Gln Ser His Trp Tyr Leu Gln Glu Asp Ile Leu Pro Thr
515 520 525

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

Arg Asn Arg Val Phe Cys Val Thr Ile Ala Glu Phe Ala Asp Gly Leu
545 550 555 560

Phe Gly Glu Tyr Arg Ile Thr Pro Gln Leu Tyr Glu Leu Val Arg Asn
565 570 575

Asn

<210> 63
<211> 6717
<212> DNA
<213> artificial sequence

<220>
<223> L gene of FJ215863

Sequence

<400> 63
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cgacaaaaat ccgtagtgat gcacatcccg gaccacatct tagaaaaatc agaatatcgg 180
atcaagcacc gtctagggaa aatcaagagt gacataacac attactgcca gtattttaat 240
attaatttgg caaatcttga tccgataacc caccctaaaa gtttgtattg gttatccaga 300
ctaacaatag ctagtgctgg aacctttaga catatgaaag atagaatctt gtgtacagtt 360
ggctctgaat tcggacacaa aattcaagat ttattttcac tgttgagcca taaactagta 420
ggtaacggtg atttatttaa tcaaagtctc tcaggtacac gtttgactgc gagtccgtta 480
tcccctttat gcgatcaatt tgtctctgac atcaagtctg cagtcacgac accctgggtca 540
gaagctcgtt ggtcttggct tcatatcaaa caaacaatga gatattctaat aaaacaatca 600
tgcactacaa attcggctca ttttaacagaa atcataaaag aggaatgggg tttagtaggt 660
attactccag atcttgtcat tctttttgac agagtcaata atagtctgac tgcattaaca 720
tttgagatgg ttctaattga ttcagatgta ttagaatccc gtgacaatat tgtgttagtg 780
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Sequence

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Sequence

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Sequence

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<212> PRT
<213> artificial sequence

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<220>
<223> L protein of AC048302
<400> 64

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```

Asp Ser Pro Ile Ile Leu Asn Lys Leu Ala Leu Leu Trp Arg Leu Ser
20          25          30

```

```

Gly Leu Pro Met Pro Ala Asp Leu Arg Gln Lys Ser Val Val Met His
35          40          45

```

```

Ile Pro Asp His Ile Leu Glu Lys Ser Glu Tyr Arg Ile Lys His Arg
50          55          60

```

```

Leu Gly Lys Ile Lys Ser Asp Ile Thr His Tyr Cys Gln Tyr Phe Asn
65          70          75          80

```

```

Ile Asn Leu Ala Asn Leu Asp Pro Ile Thr His Pro Lys Ser Leu Tyr
85          90          95

```

```

Trp Leu Ser Arg Leu Thr Ile Ala Ser Ala Gly Thr Phe Arg His Met
100          105          110

```

```

Lys Asp Arg Ile Leu Cys Thr Val Gly Ser Glu Phe Gly His Lys Ile
115          120          125

```

```

Gln Asp Leu Phe Ser Leu Leu Ser His Lys Leu Val Gly Asn Gly Asp
130          135          140

```

Sequence

Leu Phe Asn Gln Ser Leu Ser Gly Thr Arg Leu Thr Ala Ser Pro Leu
 145 150 155 160
 Ser Pro Leu Cys Asp Gln Phe Val Ser Asp Ile Lys Ser Ala Val Thr
 165 170 175
 Thr Pro Trp Ser Glu Ala Arg Trp Ser Trp Leu His Ile Lys Gln Thr
 180 185 190
 Met Arg Tyr Leu Ile Lys Gln Ser Cys Thr Thr Asn Ser Ala His Leu
 195 200 205
 Thr Glu Ile Ile Lys Glu Glu Trp Gly Leu Val Gly Ile Thr Pro Asp
 210 215 220
 Leu Val Ile Leu Phe Asp Arg Val Asn Asn Ser Leu Thr Ala Leu Thr
 225 230 235 240
 Phe Glu Met Val Leu Met Tyr Ser Asp Val Leu Glu Ser Arg Asp Asn
 245 250 255
 Ile Val Leu Val Gly Arg Leu Ser Thr Phe Leu Gln Pro Val Val Ser
 260 265 270
 Arg Leu Glu Val Leu Phe Asp Leu Val Asp Ser Leu Ala Lys Ile Leu
 275 280 285
 Gly Asp Thr Ile Tyr Glu Ile Ile Ala Val Leu Glu Ser Leu Ser Tyr
 290 295 300
 Gly Ser Val Gln Leu His Asp Ala Ser His Ser His Ala Gly Ser Phe
 305 310 315 320
 Phe Ser Phe Asn Met Asn Glu Leu Asp Asn Thr Leu Ser Lys Arg Val
 325 330 335
 Asp Pro Lys His Lys Asn Thr Ile Met Ser Ile Ile Arg Gln Cys Phe
 340 345 350
 Ser Asn Leu Asp Val Asp Gln Ala Ala Glu Met Leu Cys Leu Met Arg
 355 360 365
 Leu Phe Gly His Pro Met Leu Thr Ala Pro Asp Ala Ala Ala Lys Val
 370 375 380
 Arg Lys Ala Met Cys Ala Pro Lys Leu Val Glu His Asp Thr Ile Leu
 385 390 395 400
 Gln Thr Leu Ser Phe Phe Lys Gly Ile Ile Ile Asn Gly Tyr Arg Arg
 405 410 415

Sequence

Ser His Ser Gly Leu Trp Pro Asn Val Glu Pro Ser Ser Ile Tyr Asp
420 425 430

Asp Asp Leu Arg Gln Leu Tyr Leu Glu Ser Ala Glu Ile Ser His His
435 440 445

Phe Met Leu Lys Asn Tyr Lys Ser Leu Ser Met Ile Glu Phe Lys Lys
450 455 460

Ser Ile Asp Tyr Asp Leu His Asp Asp Leu Ser Thr Phe Leu Lys Asp
465 470 475 480

Arg Ala Ile Cys Arg Pro Lys Ser Gln Trp Asp Val Ile Phe Arg Lys
485 490 495

Ser Leu Arg Arg Ser His Thr Gln Ser Gln Tyr Leu Asp Glu Ile Lys
500 505 510

Ser Asn Arg Leu Leu Ile Asp Phe Leu Asp Ser Ala Glu Phe Asp Pro
515 520 525

Glu Lys Glu Phe Ala Tyr Val Thr Thr Met Asp Tyr Leu His Asp Asn
530 535 540

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

Gly Arg Ile Phe Ala Lys Met Thr Arg Asn Met Arg Ser Cys Gln Val
565 570 575

Ile Leu Glu Ser Leu Leu Ser Lys His Ile Cys Lys Phe Phe Lys Glu
580 585 590

Asn Gly Val Ser Met Glu Gln Leu Ser Leu Thr Lys Ser Leu Leu Ala
595 600 605

Met Ser Gln Leu Ser Pro Lys Val Ser Thr Leu Gln Asp Thr Ala Ser
610 615 620

Arg His Val Gly Asn Ser Lys Ser Gln Ile Ala Thr Ser Asn Pro Ser
625 630 635 640

Arg His His Ser Thr Ala Asn Gln Met Ser Leu Ser Asn Arg Lys Thr
645 650 655

Val Val Ala Thr Phe Leu Thr Thr Asp Leu Glu Lys Tyr Cys Leu Gln
660 665 670

Trp Arg Tyr Ser Thr Ile Lys Leu Phe Ala Gln Ala Leu Asn Gln Leu
675 680 685

Sequence

Phe Gly Ile Asp His Gly Phe Glu Trp Ile His Leu Arg Leu Met Asn
 690 695 700

Ser Thr Leu Phe Val Gly Asp Pro Tyr Ser Pro Pro Glu Asp Pro Thr
 705 710 715 720

Leu Glu Asp Ile Asp Lys Ala Pro Asn Asp Asp Ile Phe Ile Val Ser
 725 730 735

Pro Arg Gly Gly Ile Glu Gly Leu Cys Gln Lys Met Trp Thr Met Ile
 740 745 750

Ser Ile Ser Ala Ile His Cys Val Ala Glu Lys Ile Gly Ala Arg Val
 755 760 765

Ala Ala Met Val Gln Gly Asp Asn Gln Val Ile Ala Ile Thr Lys Glu
 770 775 780

Leu Phe Arg Gly Glu Lys Ala Cys Asp Val Arg Asp Glu Leu Asp Glu
 785 790 795 800

Leu Gly Gln Val Phe Phe Asp Glu Phe Lys Arg His Asn Tyr Ala Ile
 805 810 815

Gly His Asn Leu Lys Leu Asn Glu Thr Ile Gln Ser Gln Ser Phe Phe
 820 825 830

Val Tyr Ser Lys Arg Ile Phe Phe Glu Gly Arg Leu Leu Ser Gln Val
 835 840 845

Leu Lys Asn Ala Ala Lys Leu Cys Met Val Ala Asp His Leu Gly Glu
 850 855 860

Asn Thr Val Ser Ser Cys Ser Asn Leu Ser Ser Thr Ile Ala Arg Leu
 865 870 875 880

Val Glu Asn Gly Phe Glu Lys Asp Thr Ala Phe Val Leu Asn Leu Val
 885 890 895

Tyr Ile Met Thr Gln Ile Leu Phe Asp Glu His Tyr Ser Ile Val Cys
 900 905 910

Asp His His Ser Val Lys Ser Leu Ile Gly Ser Lys Asn His Arg Asn
 915 920 925

Leu Leu Tyr Ser Ser Leu Ile Pro Gly Gln Leu Gly Gly Phe Asn Phe
 930 935 940

Leu Asn Ile Ser Arg Leu Phe Thr Arg Asn Ile Gly Asp Pro Val Thr
 945 950 955 960

Sequence

Cys Ser Leu Ser Asp Leu Lys Cys Phe Ile Ala Ala Gly Leu Leu Pro
965 970 975

Pro Tyr Val Leu Lys Asn Val Val Leu Arg Glu Pro Gly Pro Gly Thr
980 985 990

Trp Leu Thr Leu Cys Ser Asp Pro Tyr Thr Leu Asn Ile Pro Tyr Thr
995 1000 1005

Gln Leu Pro Thr Thr Tyr Leu Lys Lys His Thr Gln Arg Ser Leu
1010 1015 1020

Leu Ser Arg Ala Val Asn Pro Leu Leu Ala Gly Val Gln Val Pro
1025 1030 1035

Asn Gln His Glu Glu Glu Glu Val Leu Ala Arg Phe Leu Leu Asp
1040 1045 1050

Arg Glu Tyr Val Met Pro Arg Val Ala His Val Ile Leu Glu Thr
1055 1060 1065

Ser Val Leu Gly Lys Arg Lys Gln Ile Gln Gly Leu Ile Asp Thr
1070 1075 1080

Thr Pro Thr Ile Ile Arg Thr Ser Leu Val Asn Leu Pro Val Ser
1085 1090 1095

Arg Lys Lys Cys Glu Lys Ile Ile Asn Tyr Ser Leu Asn Tyr Ile
1100 1105 1110

Ala Glu Cys His Asp Ser Leu Leu Ser Gln Val Cys Phe Ser Asp
1115 1120 1125

Asn Lys Glu Tyr Leu Trp Ser Thr Ser Leu Ile Ser Val Glu Thr
1130 1135 1140

Cys Ser Val Thr Ile Ala Asp Tyr Leu Arg Ala Val Ser Trp Ser
1145 1150 1155

Asn Ile Leu Gly Gly Arg Asn Ile Ser Gly Val Thr Thr Pro Asp
1160 1165 1170

Thr Ile Glu Leu Ile Gln Gly Cys Leu Ile Gly Glu Asn Ser Ser
1175 1180 1185

Cys Thr Leu Cys Glu Ser His Asp Asp Ala Phe Thr Trp Met His
1190 1195 1200

Leu Pro Gly Pro Leu Tyr Ile Pro Glu Pro Ser Val Thr Asn Ser
1205 1210 1215

Sequence												
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Ala	Val	Leu	Arg	Gly	Thr	Ser	Val	Phe	Ile	Trp	Ala	Phe Gly Asp
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Cys	Asn	Ile	Thr	Leu	Asp	Gln	Met	Arg	Leu	Leu	Thr	Pro Ile Pro
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Ser	Ser	Ser	Asn	Ile	Gln	His	Arg	Leu	Asp	Asp	Gly	Ile Ser Thr
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Gln	Lys	Phe	Thr	Pro	Ala	Ser	Leu	Ala	Arg	Ile	Thr	Ser Phe Val
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His	Ile	Cys	Asn	Asp	Ser	Gln	Arg	Leu	Glu	Lys	Asp	Gly Ser Ser
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Val	Asp	Ser	Asn	Leu	Ile	Tyr	Gln	Gln	Ile	Met	Leu	Leu Gly Leu
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Ser	Ile	Phe	Glu	Thr	Met	Tyr	Ser	Met	Asp	Gln	Lys	Trp Val Phe
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Asn	Asn	His	Thr	Leu	His	Leu	His	Thr	Gly	His	Ser	Cys Cys Pro
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Arg	Glu	Leu	Asp	Ile	Ser	Leu	Val	Asn	Pro	Pro	Arg	His Gln Thr
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Pro	Glu	Leu	Thr	Ser	Thr	Thr	Thr	Asn	Pro	Phe	Leu	Tyr Asp Gln
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Leu	Pro	Leu	Asn	Gln	Glu	Asn	Leu	Thr	Thr	Leu	Glu	Ile Lys Thr
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Phe	Lys	Phe	Asn	Glu	Leu	Asn	Ile	Asp	Gly	Leu	Asp	Phe Gly Glu
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Gly	Ile	Gln	Leu	Leu	Ser	Arg	Cys	Thr	Ala	Arg	Leu	Met Ala Glu
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Cys	Ile	Leu	Glu	Glu	Gly	Ile	Gly	Ser	Ser	Val	Lys	Asn Glu Ala
	1460					1465					1470	

Sequence														
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Pro	Val	Ile	Gln	Leu	Ala	Asn	Ile	Ala	Leu	Thr	Ile	Ser	His	Pro
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Glu	Ile	Phe	Arg	Arg	Ile	Val	Asn	Thr	Gly	Ile	His	Asn	Gln	Ile
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Lys	Gly	Pro	Tyr	Val	Ala	Thr	Thr	Asp	Phe	Ile	Ala	Ala	Ser	Arg
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Sequence

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 Asp Asn Ala Pro Gly Ser Leu Ile Pro Phe Ser Ile Ser Pro Asn
 1760 1765 1770
 Ser Leu Ala Cys Asp Pro Leu His His Leu Leu Lys Ser Val Gly
 1775 1780 1785
 Thr Ser Ser Thr Ser Trp Tyr Lys Tyr Ala Ile Ala Tyr Ala Val
 1790 1795 1800
 Ser Glu Lys Arg Ser Ala Arg Leu Gly Gly Ser Leu Tyr Ile Gly
 1805 1810 1815
 Glu Gly Ser Gly Ser Val Met Thr Leu Leu Glu Tyr Leu Glu Pro
 1820 1825 1830
 Ser Val Asp Ile Phe Tyr Asn Ser Leu Phe Ser Asn Gly Met Asn
 1835 1840 1845
 Pro Pro Gln Arg Asn Tyr Gly Leu Met Pro Leu Gln Phe Val Asn
 1850 1855 1860
 Ser Val Val Tyr Lys Asn Leu Thr Ala Lys Ser Glu Cys Lys Leu
 1865 1870 1875
 Gly Phe Val Gln Gln Phe Lys Pro Leu Trp Arg Asp Ile Asp Ile
 1880 1885 1890
 Glu Thr Asn Val Thr Asp Pro Ser Phe Val Asn Phe Ala Leu Asn
 1895 1900 1905
 Glu Ile Pro Met Gln Ser Leu Lys Arg Val Asn Cys Asp Val Glu
 1910 1915 1920
 Phe Asp Arg Gly Met Pro Ile Glu Arg Val Ile Gln Gly Tyr Thr
 1925 1930 1935
 His Ile Leu Leu Val Ala Thr Tyr Gly Leu Gln Gln Asp Ser Ile
 1940 1945 1950
 Leu Trp Val Lys Val Tyr Arg Thr Ser Glu Lys Val Phe Gln Phe
 1955 1960 1965
 Leu Leu Ser Ala Met Ile Met Ile Phe Gly Tyr Val Lys Ile His
 1970 1975 1980

Sequence														
Arg	Asn	Gly	Tyr	Met	Ser	Thr	Lys	Asp	Glu	Glu	Tyr	Ile	Leu	Met
1985						1990					1995			
Ser	Asp	Cys	Lys	Glu	Pro	Val	Asn	Tyr	Thr	Ala	Val	Pro	Asn	Ile
2000						2005					2010			
Leu	Thr	Arg	Val	Ser	Asp	Leu	Val	Ser	Lys	Asn	Leu	Ser	Leu	Ile
2015						2020					2025			
His	Pro	Glu	Asp	Leu	Arg	Lys	Val	Arg	Cys	Glu	Thr	Asp	Ser	Leu
2030						2035					2040			
Asn	Leu	Lys	Cys	Asn	His	Ile	Tyr	Glu	Lys	Ile	Ile	Ala	Arg	Lys
2045						2050					2055			
Ile	Pro	Leu	Gln	Val	Ser	Ser	Thr	Asp	Ser	Leu	Leu	Leu	Gln	Leu
2060						2065					2070			
Gly	Gly	Val	Ile	Asn	Ser	Val	Gly	Ser	Thr	Asp	Pro	Arg	Glu	Val
2075						2080					2085			
Ala	Thr	Leu	Ser	Ser	Ile	Glu	Cys	Met	Asp	Tyr	Val	Val	Ser	Ser
2090						2095					2100			
Ile	Asp	Leu	Ala	Ile	Leu	Glu	Ala	Asn	Ile	Val	Ile	Ser	Glu	Ser
2105						2110					2115			
Ala	Gly	Leu	Asp	Leu	Ala	Leu	Met	Leu	Gly	Pro	Phe	Asn	Leu	Asn
2120						2125					2130			
Lys	Leu	Lys	Lys	Ile	Asp	Thr	Ile	Leu	Lys	Ser	Ser	Thr	Tyr	Gln
2135						2140					2145			
Leu	Ile	Pro	Tyr	Trp	Leu	Arg	Tyr	Glu	Tyr	Ser	Ile	Asn	Pro	Arg
2150						2155					2160			
Ser	Leu	Ser	Phe	Leu	Ile	Thr	Lys	Leu	Gln	Gln	Cys	Arg	Ile	Ser
2165						2170					2175			
Trp	Ser	Asp	Met	Ile	Thr	Ile	Ser	Glu	Phe	Arg	Lys	Lys	Ser	Lys
2180						2185					2190			
Arg	Pro	Ile	Phe	Ile	Lys	Arg	Val	Ile	Gly	Asn	Gln	Gln	Leu	Lys
2195						2200					2205			
Ser	Phe	Phe	Asn	Glu	Ser	Ser	Ser	Ile	Val	Leu	Thr	Arg	Ala	Glu
2210						2215					2220			
Val	Lys	Val	Cys	Ile	Lys	Phe	Leu	Gly	Ala	Ile	Ile	Lys	Leu	Lys
2225						2230					2235			

Sequence

<210> 65
 <211> 1386
 <212> DNA
 <213> artificial sequence

<220>
 <223> NP gene of FJ215864

<400> 65
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 aggagacctg atgttgacctc aacagggttta ctcagggcgg aaataacctgt ctgtgttaca 120
 ttgtctcaag accccgggtga gagatggagc cttgcttgcc ttaatatccg atggcttgtg 180
 agtgattcat caaccacacc aatgaagcag ggagcaatat tgtcactgct gagtctacat 240
 tcagacaata tgcgagctca cgcaacatta gcagcaagggt ctgcagatgc ttcactcacc 300
 atacttgagg tagatgaagt agatattggc aactccctaa tcaaattcaa cgctagaagt 360
 ggtgtatctg ataaacgatc aaatcaattg cttgcaattg cggatgacat ccccaaaagt 420
 tgcagtaatg ggcattccatt tcttgacaca gacattgaga ccagagaccc gctcgatcta 480
 tcagagacca tagaccgcct gcaggggtatt gcagctcaga tatgggtgtc agccataaag 540
 agcatgacag cgcctgacac cgcattcagag tcagaaagta agaggctggc caaataccaa 600
 caacaaggcc gactgggttaa gcaagtactt ttgcattctg tagtcaggac agaatttatg 660
 agagttattc ggggcagctt ggtactgctc cagtttatgg ttagcgagtg caagagggct 720
 tcagccatgg gcggagacac atctaggtac tatgctatgg tgggtgacat cagtctgtac 780
 atcaagaatg caggattgac tgcatttttc ctcaccctga agttcggggg tggtagccag 840
 tatccaacct tagcaatgag tgttttctcc agtgacctta aaagacttgc tgcactcatc 900
 aggctgtaca aaaccaaggg agacaatgca ccatacatgg cattcctgga ggactccgat 960
 atgggaaatt ttgctccagc aaattatagc acaatgtact cttatgccat gggcattggg 1020
 acgattctgg aagcatctgt atctcgatac cagtatgcta gagactttac cagtgagaat 1080
 tatttccgtc ttggagttga gacagcccaa agccagcagg gagcgtttga cgagagaaca 1140
 gcccagagaga tgggcttgac tgaggaatcc aaacagcagg ttagatcact gctaattgtca 1200
 gtagacatgg gtcccagttc agttcgcgag ccatcccgcc ctgcattcat cagtcaagaa 1260
 gaaaataggc agcctgcca gaattcttca gatactcagg gtcagaccaa gccagtcccg 1320
 aatcaaccgg caccaagggc cgaccagat gacattgatc catacgagaa cgggctagaa 1380
 tggtaa 1386

<210> 66
 <211> 461
 <212> PRT
 <213> artificial sequence

<220>
 <223> NP protein of ACO48303

<400> 66

Sequence

Met Ser Ser Val Phe Asn Glu Tyr Gln Ala Leu Gln Glu Gln Leu Val
1 5 10 15

Lys Pro Ala Val Arg Arg Pro Asp Val Ala Ser Thr Gly Leu Leu Arg
20 25 30

Ala Glu Ile Pro Val Cys Val Thr Leu Ser Gln Asp Pro Gly Glu Arg
35 40 45

Trp Ser Leu Ala Cys Leu Asn Ile Arg Trp Leu Val Ser Asp Ser Ser
50 55 60

Thr Thr Pro Met Lys Gln Gly Ala Ile Leu Ser Leu Leu Ser Leu His
65 70 75 80

Ser Asp Asn Met Arg Ala His Ala Thr Leu Ala Ala Arg Ser Ala Asp
85 90 95

Ala Ser Leu Thr Ile Leu Glu Val Asp Glu Val Asp Ile Gly Asn Ser
100 105 110

Leu Ile Lys Phe Asn Ala Arg Ser Gly Val Ser Asp Lys Arg Ser Asn
115 120 125

Gln Leu Leu Ala Ile Ala Asp Asp Ile Pro Lys Ser Cys Ser Asn Gly
130 135 140

His Pro Phe Leu Asp Thr Asp Ile Glu Thr Arg Asp Pro Leu Asp Leu
145 150 155 160

Ser Glu Thr Ile Asp Arg Leu Gln Gly Ile Ala Ala Gln Ile Trp Val
165 170 175

Ser Ala Ile Lys Ser Met Thr Ala Pro Asp Thr Ala Ser Glu Ser Glu
180 185 190

Ser Lys Arg Leu Ala Lys Tyr Gln Gln Gln Gly Arg Leu Val Lys Gln
195 200 205

Val Leu Leu His Ser Val Val Arg Thr Glu Phe Met Arg Val Ile Arg
210 215 220

Gly Ser Leu Val Leu Arg Gln Phe Met Val Ser Glu Cys Lys Arg Ala
225 230 235 240

Ser Ala Met Gly Gly Asp Thr Ser Arg Tyr Tyr Ala Met Val Gly Asp
245 250 255

Ile Ser Leu Tyr Ile Lys Asn Ala Gly Leu Thr Ala Phe Phe Leu Thr
260 265 270

Sequence

Leu Lys Phe Gly Val Gly Thr Gln Tyr Pro Thr Leu Ala Met Ser Val
275 280 285

Phe Ser Ser Asp Leu Lys Arg Leu Ala Ala Leu Ile Arg Leu Tyr Lys
290 295 300

Thr Lys Gly Asp Asn Ala Pro Tyr Met Ala Phe Leu Glu Asp Ser Asp
305 310 315 320

Met Gly Asn Phe Ala Pro Ala Asn Tyr Ser Thr Met Tyr Ser Tyr Ala
325 330 335

Met Gly Ile Gly Thr Ile Leu Glu Ala Ser Val Ser Arg Tyr Gln Tyr
340 345 350

Ala Arg Asp Phe Thr Ser Glu Asn Tyr Phe Arg Leu Gly Val Glu Thr
355 360 365

Ala Gln Ser Gln Gln Gly Ala Phe Asp Glu Arg Thr Ala Arg Glu Met
370 375 380

Gly Leu Thr Glu Glu Ser Lys Gln Gln Val Arg Ser Leu Leu Met Ser
385 390 395 400

Val Asp Met Gly Pro Ser Ser Val Arg Glu Pro Ser Arg Pro Ala Phe
405 410 415

Ile Ser Gln Glu Glu Asn Arg Gln Pro Ala Gln Asn Ser Ser Asp Thr
420 425 430

Gln Gly Gln Thr Lys Pro Val Pro Asn Gln Pro Ala Pro Arg Ala Asp
435 440 445

Pro Asp Asp Ile Asp Pro Tyr Glu Asn Gly Leu Glu Trp
450 455 460

<210> 67
<211> 1218
<212> DNA
<213> artificial sequence

<220>
<223> P gene of FJ215864

<400> 67
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aaggagattc agaaatctga actcaagcct ccccaaacca ctgggcgacc acctgtcagt 120
caagggaaca caagaaatct aactgatcta tgggaaaagg agactgcaag tcagaacaag 180
acatcggtc aatctccaca aaccacacaa gttcagtctg atggaaatga ggaggaagaa 240
atcaaatcag agtcaattga tggccacatc agtggaactg ttaatcaatt agagcaagtc 300
ccagaacaaa accagagcag atcttcacca ggtgatgatc tcgacagagc tctcaacaag 360

Sequence

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cttgaagggga gaatcaactc aatcagctca atggataaag aaattaaaaa gggccctcgc      420
atccagaatc tccctggggtc ccaagcagca actcaacagg cgacccaccc attggcaggg      480
gacaccccgga acatgcaagc acggacaaaa cccctgacca agccacatca agaggcaatc      540
aatcctggca accaggacac aggagagaat attcatttac caccttccat ggcaccacca      600
gagtcattag ttggtgcaat ccgcaatgta cccaattcg tgccagacca atctatgacg      660
aatgtagatg cggggagtgt ccaactacat gcatcatgtg cagagatgat aagtagaatg      720
tttgtagaag ttatatctaa gcttgataaa ctcgagtcga gactgaatga tatagcaaaa      780
gttgtaaaca ccacccccct tatcaggaat gatattaacc aacttaaggc cacaactgca      840
ctgatgtcca accaaattgc ttccatacaa attcttgacc cagggaatgc aggggtgagg      900
tccctctctg aaatgagatc tgtgacgaag aaagctgctg ttgtaattgc aggatttgga      960
gacgacccaa ctcaaattat tgaagaaggc atcatggcca aagatgctct tggaaaacct     1020
gtgcctccaa catctgttat cgcagccaaa gctcagactt cttccggtgt gagtaagggc     1080
gaaatagaag gattgattgc attggtggaa acattagttg acaatgacaa gaaggcagcg     1140
aaactgatta aaatgattga tcaagttaaa tcccacgccg attacgcccg agtcaagcag     1200
gcaatatata atgcataa                                     1218

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<210> 68
 <211> 405
 <212> PRT
 <213> artificial sequence

<220>
 <223> P protein of AC048304
 <400> 68

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Met Asp Phe Ala Asn Asp Glu Glu Ile Ala Glu Leu Leu Asn Leu Ser
1          5          10          15

Thr Thr Val Ile Lys Glu Ile Gln Lys Ser Glu Leu Lys Pro Pro Gln
20          25          30

Thr Thr Gly Arg Pro Pro Val Ser Gln Gly Asn Thr Arg Asn Leu Thr
35          40          45

Asp Leu Trp Glu Lys Glu Thr Ala Ser Gln Asn Lys Thr Ser Ala Gln
50          55          60

Ser Pro Gln Thr Thr Gln Val Gln Ser Asp Gly Asn Glu Glu Glu Glu
65          70          75          80

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

Leu Glu Gln Val Pro Glu Gln Asn Gln Ser Arg Ser Ser Pro Gly Asp
100         105         110

```

Sequence

Asp Leu Asp Arg Ala Leu Asn Lys Leu Glu Gly Arg Ile Asn Ser Ile
 115 120 125
 Ser Ser Met Asp Lys Glu Ile Lys Lys Gly Pro Arg Ile Gln Asn Leu
 130 135 140
 Pro Gly Ser Gln Ala Ala Thr Gln Gln Ala Thr His Pro Leu Ala Gly
 145 150 155 160
 Asp Thr Pro Asn Met Gln Ala Arg Thr Lys Pro Leu Thr Lys Pro His
 165 170 175
 Gln Glu Ala Ile Asn Pro Gly Asn Gln Asp Thr Gly Glu Asn Ile His
 180 185 190
 Leu Pro Pro Ser Met Ala Pro Pro Glu Ser Leu Val Gly Ala Ile Arg
 195 200 205
 Asn Val Pro Gln Phe Val Pro Asp Gln Ser Met Thr Asn Val Asp Ala
 210 215 220
 Gly Ser Val Gln Leu His Ala Ser Cys Ala Glu Met Ile Ser Arg Met
 225 230 235 240
 Phe Val Glu Val Ile Ser Lys Leu Asp Lys Leu Glu Ser Arg Leu Asn
 245 250 255
 Asp Ile Ala Lys Val Val Asn Thr Thr Pro Leu Ile Arg Asn Asp Ile
 260 265 270
 Asn Gln Leu Lys Ala Thr Thr Ala Leu Met Ser Asn Gln Ile Ala Ser
 275 280 285
 Ile Gln Ile Leu Asp Pro Gly Asn Ala Gly Val Arg Ser Leu Ser Glu
 290 295 300
 Met Arg Ser Val Thr Lys Lys Ala Ala Val Val Ile Ala Gly Phe Gly
 305 310 315 320
 Asp Asp Pro Thr Gln Ile Ile Glu Glu Gly Ile Met Ala Lys Asp Ala
 325 330 335
 Leu Gly Lys Pro Val Pro Pro Thr Ser Val Ile Ala Ala Lys Ala Gln
 340 345 350
 Thr Ser Ser Gly Val Ser Lys Gly Glu Ile Glu Gly Leu Ile Ala Leu
 355 360 365
 Val Glu Thr Leu Val Asp Asn Asp Lys Lys Ala Ala Lys Leu Ile Lys
 370 375 380

Sequence

Met Ile Asp Gln Val Lys Ser His Ala Asp Tyr Ala Arg Val Lys Gln
385 390 395 400

Ala Ile Tyr Asn Ala
405

<210> 69
<211> 1110
<212> DNA
<213> artificial sequence

<220>
<223> M gene of FJ215864

<400> 69
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tcattcccgt tgggtactaaa agagacagac agaggcacia agaagcttca accacaggta 120
agggtagatt caattggcga tgtgcagaat gccaaagagt cctcgatatt cgtgactcta 180
tatggtttca tccaagcaat taaggagaat tcagatcgat cgaaattctt ccatccaaaa 240
gatgacttca aacctgagac agtcactgca ggactggtag tagtgggtgc aatccgaatg 300
atggctgatg tcaataccat ctctaatagat gactagcgc tggagatcac tgtaagaaa 360
tctgcaactt ctcaagagaa aatgacgggtg atgttccaca atagcccccc ttcattgaga 420
actgcaataa ctatccgagc agggaggtttc atctcgaatg cagacgaaaa tataaaatgt 480
gccagcaagt tgactgcagg agtgcagtag atattccgtc caatgtttgt ttcaatcact 540
aaattacaca atggcaaaact atataggggtg cccaaaagta tccacagcat ctcgtctacc 600
ctactgtata gtgtgatgtt ggaggtagga ttcaaagtgg acatcgggaa ggatcatccc 660
caggcaaaaa tgctgaagag ggtcacaatt ggcgatgcag acacatactg gggatttgca 720
tggttccacc tgtgcaattt caaaaagaca tcctctaagg gaaagccgag aacgctagac 780
gaactgaaga caaaagtcaa aaatatgggg ttgaaattgg agttacatga cctatgggggt 840
ccgactattg tgggtccaaat cactggcaag agcagcaaat atgctcaagg atttttttct 900
tccaatggta cttgttgctt cccaatcagc agatctgcac cagagcttgg gaagcttctg 960
tggtcctgct cagcaactat tggtgacgca acagttgtta tccaatcaag cgagaagggg 1020
gaactcctaa ggtctgatga tctcgagata cgagggtgctg tggcctcaa gaaaggtaga 1080
ctgagctcat ttcacccctt caaaaaatga 1110

<210> 70
<211> 369
<212> PRT
<213> artificial sequence

<220>
<223> M protein of AC048305

<400> 70

Sequence

Met Ala Tyr Thr Thr Leu Lys Leu Trp Val Asp Glu Gly Asp Met Ser
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Ser Ser Leu Leu Ser Phe Pro Leu Val Leu Lys Glu Thr Asp Arg Gly
20 25 30

Thr Lys Lys Leu Gln Pro Gln Val Arg Val Asp Ser Ile Gly Asp Val
35 40 45

Gln Asn Ala Lys Glu Ser Ser Ile Phe Val Thr Leu Tyr Gly Phe Ile
50 55 60

Gln Ala Ile Lys Glu Asn Ser Asp Arg Ser Lys Phe Phe His Pro Lys
65 70 75 80

Asp Asp Phe Lys Pro Glu Thr Val Thr Ala Gly Leu Val Val Val Gly
85 90 95

Ala Ile Arg Met Met Ala Asp Val Asn Thr Ile Ser Asn Asp Ala Leu
100 105 110

Ala Leu Glu Ile Thr Val Lys Lys Ser Ala Thr Ser Gln Glu Lys Met
115 120 125

Thr Val Met Phe His Asn Ser Pro Pro Ser Leu Arg Thr Ala Ile Thr
130 135 140

Ile Arg Ala Gly Gly Phe Ile Ser Asn Ala Asp Glu Asn Ile Lys Cys
145 150 155 160

Ala Ser Lys Leu Thr Ala Gly Val Gln Tyr Ile Phe Arg Pro Met Phe
165 170 175

Val Ser Ile Thr Lys Leu His Asn Gly Lys Leu Tyr Arg Val Pro Lys
180 185 190

Ser Ile His Ser Ile Ser Ser Thr Leu Leu Tyr Ser Val Met Leu Glu
195 200 205

Val Gly Phe Lys Val Asp Ile Gly Lys Asp His Pro Gln Ala Lys Met
210 215 220

Leu Lys Arg Val Thr Ile Gly Asp Ala Asp Thr Tyr Trp Gly Phe Ala
225 230 235 240

Trp Phe His Leu Cys Asn Phe Lys Lys Thr Ser Ser Lys Gly Lys Pro
245 250 255

Arg Thr Leu Asp Glu Leu Lys Thr Lys Val Lys Asn Met Gly Leu Lys
260 265 270

Sequence

Leu Glu Leu His Asp Leu Trp Gly Pro Thr Ile Val Val Gln Ile Thr
275 280 285

Gly Lys Ser Ser Lys Tyr Ala Gln Gly Phe Phe Ser Ser Asn Gly Thr
290 295 300

Cys Cys Leu Pro Ile Ser Arg Ser Ala Pro Glu Leu Gly Lys Leu Leu
305 310 315 320

Trp Ser Cys Ser Ala Thr Ile Gly Asp Ala Thr Val Val Ile Gln Ser
325 330 335

Ser Glu Lys Gly Glu Leu Leu Arg Ser Asp Asp Leu Glu Ile Arg Gly
340 345 350

Ala Val Ala Ser Lys Lys Gly Arg Leu Ser Ser Phe His Pro Phe Lys
355 360 365

Lys

<210> 71
<211> 1632
<212> DNA
<213> artificial sequence

<220>
<223> F gene of FJ215864

<400> 71
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aattcgattg acaatacact cgttgcccc aatcgagtcg ccagcgcaaa tgaatggcag 120
cttgctgcat atacaacatc actttcaggg acaattgccg tgcgattcct acctgtgctc 180
ccggataata tgactacctg tcttagagaa acaataacta catataataa tactgtcaac 240
aacatcttag gccactcaa atccaatctg gatgcactgc tctcatctga gacttatccc 300
cagacaagat taattggggc agttataggt tcaattgctc ttggtgttgc aacatcggct 360
caaatcactg ctgcagtcgc tctcaagcaa gcacaagata atgcaagaaa catactggca 420
ctcaaagagg cactgtccaa aactaatgag gcggtcaagg agcttagcag tggattgcaa 480
caaacagcta ttgcacttgg taagatacag agctttgtga atgaggaaat tctgccatct 540
atcaaccaac tgagctgcga ggtgacagcc aataaacttg ggggtgtattt atctctgtat 600
ctcacagaac tgaccactat attcggtgca cagttgacta accctgcatt gacttcatta 660
tcatatcaag cgctgtacaa cctgtgtggt ggcaacatgg caatgcttac tcagaagatt 720
ggaattaaac agcaagacgt taattcgcta tatgaagccg gactaatcac aggacaagtc 780
attggttatg actctcagta ccagctgctg gtcattccagg tcaattatcc aagcatttct 840
gaggtaactg gtgtgctgac gacagaatta gtcactgtta gtgtaacaac agacaagggg 900
gaagggaaag caattgtacc ccaatttcta gctgaaagtc ggggtgactat tgaggagctt 960

Sequence

```

gatgtagcat cttgtaaatt cagcagcaca accctatact gcaggcaggt caacacaagg      1020
gcacttcccc cgctagtggc tagctgtctc cgaggtaact atgatgattg tcaatatacc      1080
acagagattg gagcattatc atcccggtat ataacactag atggaggggt cttagtcaat      1140
tgtaagtcaa ttgtttgtag gtgccttaat ccaagtaaga tcatctctca aaatacaaat      1200
gctgcagtaa catatgttga tgctacaata tgcaaaacaa ttcaattgga tgacatacaa      1260
ctccagttgg aagggtcact atcatcagtt tatgcaagga acatctcaat tgagatcagt      1320
caggtgacta cctccggttc tttggatatac agcagtgaga tagggaacat caataatacg      1380
gtgaatcgtg tggaggattt aatccaccaa tcggaggaat ggctggcaaa agttaacca      1440
cacattgtta ataatactac actaattgta ctctgtgtgt taagtgcgct tgctgtgatc      1500
tggctggcag tattaacggc tattataata tacttgagaa caaagttgaa gactatatcg      1560
gcattggctg taaccaatac aatacagtct aatccctatg ttaaccaaac gaaacgtgaa      1620
tctaagtttt ga                                                              1632

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<210> 72
<211> 543
<212> PRT
<213> artificial sequence

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<220>
<223> F protein of AC048306

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<400> 72

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Met Gly Lys Ile Ser Ile Tyr Leu Ile Asn Ser Val Leu Leu Leu Leu
1           5           10           15

```

```

Val Tyr Pro Val Asn Ser Ile Asp Asn Thr Leu Val Ala Pro Ile Gly
20           25           30

```

```

Val Ala Ser Ala Asn Glu Trp Gln Leu Ala Ala Tyr Thr Thr Ser Leu
35           40           45

```

```

Ser Gly Thr Ile Ala Val Arg Phe Leu Pro Val Leu Pro Asp Asn Met
50           55           60

```

```

Thr Thr Cys Leu Arg Glu Thr Ile Thr Thr Tyr Asn Asn Thr Val Asn
65           70           75           80

```

```

Asn Ile Leu Gly Pro Leu Lys Ser Asn Leu Asp Ala Leu Leu Ser Ser
85           90           95

```

```

Glu Thr Tyr Pro Gln Thr Arg Leu Ile Gly Ala Val Ile Gly Ser Ile
100          105          110

```

```

Ala Leu Gly Val Ala Thr Ser Ala Gln Ile Thr Ala Ala Val Ala Leu
115          120          125

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Sequence

Lys Gln Ala Gln Asp Asn Ala Arg Asn Ile Leu Ala Leu Lys Glu Ala
130 135 140

Leu Ser Lys Thr Asn Glu Ala Val Lys Glu Leu Ser Ser Gly Leu Gln
145 150 155 160

Gln Thr Ala Ile Ala Leu Gly Lys Ile Gln Ser Phe Val Asn Glu Glu
165 170 175

Ile Leu Pro Ser Ile Asn Gln Leu Ser Cys Glu Val Thr Ala Asn Lys
180 185 190

Leu Gly Val Tyr Leu Ser Leu Tyr Leu Thr Glu Leu Thr Thr Ile Phe
195 200 205

Gly Ala Gln Leu Thr Asn Pro Ala Leu Thr Ser Leu Ser Tyr Gln Ala
210 215 220

Leu Tyr Asn Leu Cys Gly Gly Asn Met Ala Met Leu Thr Gln Lys Ile
225 230 235 240

Gly Ile Lys Gln Gln Asp Val Asn Ser Leu Tyr Glu Ala Gly Leu Ile
245 250 255

Thr Gly Gln Val Ile Gly Tyr Asp Ser Gln Tyr Gln Leu Leu Val Ile
260 265 270

Gln Val Asn Tyr Pro Ser Ile Ser Glu Val Thr Gly Val Arg Ala Thr
275 280 285

Glu Leu Val Thr Val Ser Val Thr Thr Asp Lys Gly Glu Gly Lys Ala
290 295 300

Ile Val Pro Gln Phe Val Ala Glu Ser Arg Val Thr Ile Glu Glu Leu
305 310 315 320

Asp Val Ala Ser Cys Lys Phe Ser Ser Thr Thr Leu Tyr Cys Arg Gln
325 330 335

Val Asn Thr Arg Ala Leu Pro Pro Leu Val Ala Ser Cys Leu Arg Gly
340 345 350

Asn Tyr Asp Asp Cys Gln Tyr Thr Thr Glu Ile Gly Ala Leu Ser Ser
355 360 365

Arg Tyr Ile Thr Leu Asp Gly Gly Val Leu Val Asn Cys Lys Ser Ile
370 375 380

Val Cys Arg Cys Leu Asn Pro Ser Lys Ile Ile Ser Gln Asn Thr Asn
385 390 395 400

Sequence

Ala Ala Val Thr Tyr Val Asp Ala Thr Ile Cys Lys Thr Ile Gln Leu
405 410 415

Asp Asp Ile Gln Leu Gln Leu Glu Gly Ser Leu Ser Ser Val Tyr Ala
420 425 430

Arg Asn Ile Ser Ile Glu Ile Ser Gln Val Thr Thr Ser Gly Ser Leu
435 440 445

Asp Ile Ser Ser Glu Ile Gly Asn Ile Asn Asn Thr Val Asn Arg Val
450 455 460

Glu Asp Leu Ile His Gln Ser Glu Glu Trp Leu Ala Lys Val Asn Pro
465 470 475 480

His Ile Val Asn Asn Thr Thr Leu Ile Val Leu Cys Val Leu Ser Ala
485 490 495

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

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

Gln Ser Asn Pro Tyr Val Asn Gln Thr Lys Arg Glu Ser Lys Phe
530 535 540

<210> 73
<211> 1734
<212> DNA
<213> artificial sequence

<220>
<223> HN gene of FJ215864

<400> 73
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Sequence

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Thr Thr Gly Cys Leu Ala Leu Ser Ile Val Ser Ile Val Gln Ile Gly
35 40 45

Asn Leu Lys Ile Pro Ser Val Gly Asp Leu Ala Asp Glu Val Val Thr
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Pro Leu Lys Thr Thr Leu Ser Asp Thr Leu Arg Asn Pro Ile Asn Gln
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Ile Asn Asp Ile Phe Arg Ile Val Ala Leu Asp Ile Pro Leu Gln Val
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Sequence

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Sequence

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Page 175

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Sequence

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Sequence

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Sequence

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Sequence

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 Cys Ser Leu Ser Asp Leu Lys Cys Phe Ile Ala Ala Gly Leu Leu Pro
 965 970 975

Sequence

Pro Tyr Val Leu Lys Asn Val Val Leu Arg Glu Pro Gly Pro Gly Thr
980 985 990

Trp Leu Thr Leu Cys Ser Asp Pro Tyr Thr Leu Asn Ile Pro Tyr Thr
995 1000 1005

Gln Leu Pro Thr Thr Tyr Leu Lys Lys His Thr Gln Arg Ser Leu
1010 1015 1020

Leu Ser Arg Ala Val Asn Pro Leu Leu Ala Gly Val Gln Val Pro
1025 1030 1035

Asn Gln His Glu Glu Glu Glu Met Leu Ala Arg Phe Leu Leu Asp
1040 1045 1050

Arg Glu Tyr Val Met Pro Arg Val Ala His Val Ile Leu Glu Thr
1055 1060 1065

Ser Val Leu Gly Lys Arg Lys Gln Ile Gln Gly Leu Ile Asp Thr
1070 1075 1080

Thr Pro Thr Ile Ile Arg Thr Ser Leu Val Asn Leu Pro Val Ser
1085 1090 1095

Arg Lys Lys Cys Glu Lys Ile Ile Asn Tyr Ser Leu Asn Tyr Ile
1100 1105 1110

Ala Glu Cys His Asp Ser Leu Leu Ser Gln Ile Cys Phe Ser Asp
1115 1120 1125

Asn Lys Glu Tyr Leu Trp Ser Thr Ser Leu Ile Ser Val Glu Thr
1130 1135 1140

Cys Ser Val Thr Ile Ala Asp Tyr Leu Arg Ala Val Ser Trp Ser
1145 1150 1155

Asn Ile Leu Gly Gly Arg Asn Ile Ser Gly Val Thr Thr Pro Asp
1160 1165 1170

Thr Ile Glu Leu Ile Gln Gly Cys Leu Ile Gly Glu Asn Ser Ser
1175 1180 1185

Cys Thr Leu Cys Glu Ser His Asp Asp Ala Phe Thr Trp Met His
1190 1195 1200

Leu Pro Gly Pro Leu Tyr Ile Pro Glu Pro Ser Val Thr Asn Ser
1205 1210 1215

Lys Met Arg Val Pro Tyr Leu Gly Ser Lys Thr Glu Glu Arg Lys
1220 1225 1230

Sequence

Thr	Ala	Ser	Met	Ala	Ala	Ile	Lys	Gly	Met	Ser	His	His	Leu	Arg
	1235					1240					1245			
Ala	Val	Leu	Arg	Gly	Thr	Ser	Val	Phe	Ile	Trp	Ala	Phe	Gly	Asp
	1250					1255					1260			
Thr	Asp	Ile	Asn	Trp	Asp	Asn	Ala	Leu	Gln	Ile	Ala	Gln	Ser	Arg
	1265					1270					1275			
Cys	Asn	Ile	Thr	Leu	Asp	Gln	Met	Arg	Leu	Leu	Thr	Pro	Ile	Pro
	1280					1285					1290			
Ser	Ser	Ser	Asn	Ile	Gln	His	Arg	Leu	Asp	Asp	Gly	Ile	Ser	Thr
	1295					1300					1305			
Gln	Lys	Phe	Thr	Pro	Ala	Ser	Leu	Ala	Arg	Ile	Thr	Ser	Phe	Val
	1310					1315					1320			
His	Ile	Cys	Asn	Asp	Ser	Gln	Arg	Leu	Glu	Lys	Asp	Gly	Ser	Ser
	1325					1330					1335			
Val	Asp	Ser	Asn	Leu	Ile	Tyr	Gln	Gln	Ile	Met	Leu	Leu	Gly	Leu
	1340					1345					1350			
Ser	Ile	Phe	Glu	Thr	Met	Tyr	Ser	Met	Asp	Gln	Lys	Trp	Val	Phe
	1355					1360					1365			
Asn	Asn	His	Thr	Leu	His	Leu	His	Thr	Gly	His	Ser	Cys	Cys	Pro
	1370					1375					1380			
Arg	Glu	Leu	Asp	Ile	Ser	Leu	Val	Asn	Pro	Pro	Arg	His	Gln	Thr
	1385					1390					1395			
Pro	Glu	Leu	Thr	Ser	Thr	Thr	Thr	Asn	Pro	Phe	Leu	Tyr	Asp	Gln
	1400					1405					1410			
Leu	Pro	Leu	Asn	Gln	Asp	Asn	Leu	Thr	Thr	Leu	Glu	Ile	Lys	Thr
	1415					1420					1425			
Phe	Lys	Phe	Asn	Glu	Leu	Asn	Ile	Asp	Gly	Leu	Asp	Phe	Gly	Glu
	1430					1435					1440			
Gly	Ile	Gln	Leu	Leu	Ser	Arg	Cys	Thr	Ala	Arg	Leu	Met	Ala	Glu
	1445					1450					1455			
Cys	Ile	Leu	Glu	Glu	Gly	Ile	Gly	Ser	Ser	Val	Lys	Asn	Glu	Ala
	1460					1465					1470			
Ile	Val	Asn	Phe	Asp	Asn	Ser	Val	Asn	Trp	Ile	Ser	Glu	Cys	Leu
	1475					1480					1485			

Sequence

Met	Cys	Asp	Ile	Arg	Ser	Leu	Cys	Val	Asn	Leu	Gly	Gln	Glu	Ile
	1490					1495					1500			
Leu	Cys	Ser	Leu	Ala	Tyr	Gln	Met	Tyr	Tyr	Leu	Arg	Ile	Arg	Gly
	1505					1510					1515			
Arg	Arg	Ala	Ile	Leu	Asn	Tyr	Leu	Asp	Thr	Thr	Leu	Gln	Arg	Ile
	1520					1525					1530			
Pro	Val	Ile	Gln	Leu	Ala	Asn	Ile	Ala	Leu	Thr	Ile	Ser	His	Pro
	1535					1540					1545			
Glu	Ile	Phe	Arg	Arg	Ile	Val	Asn	Thr	Gly	Ile	His	Asn	Gln	Ile
	1550					1555					1560			
Lys	Gly	Pro	Tyr	Val	Ala	Thr	Thr	Asp	Phe	Ile	Ala	Ala	Ser	Arg
	1565					1570					1575			
Asp	Ile	Ile	Leu	Ser	Gly	Ala	Arg	Glu	Tyr	Leu	Ser	Tyr	Leu	Ser
	1580					1585					1590			
Ser	Gly	Gln	Glu	Asp	Cys	Tyr	Thr	Phe	Phe	Asn	Cys	Gln	Asp	Gly
	1595					1600					1605			
Asp	Leu	Thr	Pro	Lys	Met	Glu	Gln	Tyr	Leu	Ala	Arg	Arg	Ala	Cys
	1610					1615					1620			
Leu	Leu	Thr	Leu	Leu	Tyr	Asn	Thr	Gly	His	Gln	Ile	Pro	Ile	Ile
	1625					1630					1635			
Arg	Ser	Leu	Thr	Pro	Ile	Glu	Lys	Cys	Lys	Val	Leu	Thr	Glu	Tyr
	1640					1645					1650			
Asn	Gln	Gln	Ile	Glu	Tyr	Ala	Asp	Gln	Glu	Phe	Ser	Ser	Val	Leu
	1655					1660					1665			
Lys	Val	Val	Asn	Ala	Leu	Leu	Gln	Asn	Pro	Asn	Ile	Asp	Ala	Leu
	1670					1675					1680			
Val	Ser	Asn	Leu	Tyr	Phe	Thr	Thr	Arg	Arg	Val	Leu	Ser	Asn	Leu
	1685					1690					1695			
Arg	Ser	Cys	Asp	Lys	Ala	Ile	Ser	Tyr	Ile	Glu	Tyr	Leu	Tyr	Thr
	1700					1705					1710			
Glu	Asp	Phe	Gly	Glu	Lys	Glu	Asp	Thr	Val	Gln	Tyr	Asp	Ile	Met
	1715					1720					1725			
Thr	Thr	Asn	Asp	Ile	Ile	Leu	Thr	His	Gly	Leu	Phe	Thr	Gln	Ile
	1730					1735					1740			

Sequence

Glu	Ile	Ser	Tyr	Gln	Gly	Ser	Ser	Leu	His	Lys	Phe	Leu	Thr	Pro
	1745					1750					1755			
Asp	Asn	Ala	Pro	Gly	Ser	Leu	Ile	Pro	Phe	Ser	Ile	Ser	Pro	Asn
	1760					1765					1770			
Ser	Leu	Ala	Cys	Asp	Pro	Leu	His	His	Leu	Leu	Lys	Ser	Val	Gly
	1775					1780					1785			
Thr	Ser	Ser	Thr	Ser	Trp	Tyr	Lys	Tyr	Ala	Ile	Ala	Tyr	Ala	Val
	1790					1795					1800			
Ser	Glu	Lys	Arg	Ser	Ala	Arg	Leu	Gly	Gly	Ser	Leu	Tyr	Ile	Gly
	1805					1810					1815			
Glu	Gly	Ser	Gly	Ser	Val	Met	Thr	Leu	Leu	Glu	Tyr	Leu	Glu	Pro
	1820					1825					1830			
Ser	Val	Asp	Ile	Phe	Tyr	Asn	Ser	Leu	Phe	Ser	Asn	Gly	Met	Asn
	1835					1840					1845			
Pro	Pro	Gln	Arg	Asn	Tyr	Gly	Leu	Met	Pro	Leu	Gln	Phe	Val	Asn
	1850					1855					1860			
Ser	Val	Val	Tyr	Lys	Asn	Leu	Thr	Ala	Lys	Ser	Glu	Cys	Lys	Leu
	1865					1870					1875			
Gly	Phe	Val	Gln	Gln	Phe	Lys	Pro	Leu	Trp	Arg	Asp	Ile	Asp	Ile
	1880					1885					1890			
Glu	Thr	Asn	Val	Thr	Asp	Pro	Ser	Phe	Val	Asn	Phe	Ala	Leu	Asn
	1895					1900					1905			
Glu	Ile	Pro	Met	Gln	Ser	Leu	Lys	Arg	Val	Asn	Cys	Asp	Val	Glu
	1910					1915					1920			
Phe	Asp	Arg	Gly	Met	Pro	Ile	Glu	Arg	Val	Ile	Gln	Gly	Tyr	Thr
	1925					1930					1935			
His	Ile	Leu	Leu	Val	Ala	Thr	Tyr	Gly	Leu	Gln	Gln	Asp	Ser	Ile
	1940					1945					1950			
Leu	Trp	Val	Lys	Val	Tyr	Arg	Thr	Ser	Glu	Lys	Val	Phe	Gln	Phe
	1955					1960					1965			
Leu	Leu	Ser	Ala	Met	Ile	Met	Ile	Phe	Gly	Tyr	Val	Lys	Ile	His
	1970					1975					1980			
Arg	Asn	Gly	Tyr	Met	Ser	Ala	Lys	Asp	Glu	Glu	Tyr	Ile	Leu	Met
	1985					1990					1995			

Sequence

Ser Asp Cys Lys Glu Pro Val Asn Tyr Thr Ala Val Pro Asn Ile
2000 2005 2010

Leu Thr Arg Val Ser Asp Leu Val Ser Lys Asn Leu Ser Leu Ile
2015 2020 2025

His Pro Glu Asp Leu Arg Lys Val Arg Cys Glu Thr Asp Ser Leu
2030 2035 2040

Asn Leu Lys Cys Asn His Ile Tyr Glu Lys Ile Ile Ala Arg Lys
2045 2050 2055

Ile Pro Leu Gln Val Ser Ser Thr Asp Ser Leu Leu Leu Gln Leu
2060 2065 2070

Gly Gly Val Ile Asn Ser Val Gly Ser Thr Asp Pro Arg Glu Val
2075 2080 2085

Ala Thr Leu Ser Ser Ile Glu Cys Met Asp Tyr Val Val Ser Ser
2090 2095 2100

Ile Asp Leu Ala Ile Leu Glu Ala Asn Ile Val Ile Ser Glu Ser
2105 2110 2115

Ala Gly Leu Asp Leu Ala Leu Met Leu Gly Pro Phe Asn Leu Asn
2120 2125 2130

Lys Leu Lys Lys Ile Asp Thr Ile Leu Lys Ser Ser Thr Tyr Gln
2135 2140 2145

Leu Ile Pro Tyr Trp Leu Arg Tyr Glu Tyr Ser Ile Asn Pro Arg
2150 2155 2160

Ser Leu Ser Phe Leu Ile Thr Lys Leu Gln Gln Cys Arg Ile Ser
2165 2170 2175

Trp Ser Asp Met Ile Thr Ile Ser Glu Phe Cys Lys Lys Ser Lys
2180 2185 2190

Arg Pro Ile Phe Ile Lys Arg Val Ile Gly Asn Gln Gln Leu Lys
2195 2200 2205

Ser Phe Phe Asn Glu Ser Ser Ser Ile Val Leu Thr Arg Ala Glu
2210 2215 2220

Val Lys Val Cys Ile Lys Phe Leu Gly Ala Ile Ile Lys Leu Lys
2225 2230 2235

<210> 77
<211> 1570

Sequence

<212> DNA
<213> artificial sequence

<220>
<223> NP gene of FJ619036

<400> 77
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 agaacaactt gtaaagccgg ctgtcaggag acctgatgtt gcctcaacag gtttactcag 180
 ggcggaaata cctgtctgtg ttacattgtc tcaagacccc ggtgagagat ggagccttgc 240
 ttgccttaat atccgatggc ttgtgagtga ttcacaaacc acaccaatga agcagggagc 300
 aatattgtca ctgctgagtc tacattcaga caatatgcga gctcacgcaa cattagcagc 360
 aaggtctgca gatgcttcac tcaccatact tgaggtagat gaagtagata ttggcaactc 420
 cctaataaaa ttcaacgcta gaagtgggtg atctgataaa cgatcaaata aattgcttgc 480
 aattgcggtg gacatcccca aaagttagcag taatgggcat ccatttcttg acacagacat 540
 tgagaccaga gaccgctcg atctatcaga gaccatagac cgcctgcagg gtattgcagc 600
 tcagatatgg gtgtcagcca taaagagcat gacagcgctt gacaccgcat cagagtcaga 660
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 ttctgtagtc aggacagaat ttatgagagt tattcggggc agcttggtac tgcgccagtt 780
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 tatggtgggt gacatcagtc tgtacatcaa gaatgcagga ttgactgcat ttttcctcac 900
 cctgaagttc ggggttggtg cccagtatcc aaccttagca atgagtgttt tctccagtga 960
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 gtactcttat gccatgggca ttgggacgat tctggaagca tctgtatctc gataccagta 1140
 tgctagagac ttaccagtg agaattatct ccgtcttgga gttgagacag cccaaagcca 1200
 gcagggagcg ttgacgaga gaacagcccc agagatgggc ttgactgagg aatccaaaca 1260
 gcaggttaga tcaactgcta tgtcagtaga catgggtccc agttcagttc gcgagccatc 1320
 ccgccctgca ttcacagtc aagaagaaaa taggcagcct gccagaatt cttcagatac 1380
 tcagggtcag accaagccag tcccgaatca acccgacca agggccgacc cagatgacat 1440
 tgatccatac gagaacgggc tagaatggta attcaatcac ctgcacacat ccacctatac 1500
 accaattctg tgacatatta acctaatcaa acatttcata aactatagta gtcattgatt 1560
 taagaaaaaa 1570

<210> 78
<211> 461
<212> PRT
<213> artificial sequence

<220>

Sequence

<223> NP protein of ACN88139

<400> 78

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 20 25 30

Ala Glu Ile Pro Val Cys Val Thr Leu Ser Gln Asp Pro Gly Glu Arg
 35 40 45

Trp Ser Leu Ala Cys Leu Asn Ile Arg Trp Leu Val Ser Asp Ser Ser
 50 55 60

Thr Thr Pro Met Lys Gln Gly Ala Ile Leu Ser Leu Leu Ser Leu His
 65 70 75 80

Ser Asp Asn Met Arg Ala His Ala Thr Leu Ala Ala Arg Ser Ala Asp
 85 90 95

Ala Ser Leu Thr Ile Leu Glu Val Asp Glu Val Asp Ile Gly Asn Ser
 100 105 110

Leu Ile Lys Phe Asn Ala Arg Ser Gly Val Ser Asp Lys Arg Ser Asn
 115 120 125

Gln Leu Leu Ala Ile Ala Asp Asp Ile Pro Lys Ser Cys Ser Asn Gly
 130 135 140

His Pro Phe Leu Asp Thr Asp Ile Glu Thr Arg Asp Pro Leu Asp Leu
 145 150 155 160

Ser Glu Thr Ile Asp Arg Leu Gln Gly Ile Ala Ala Gln Ile Trp Val
 165 170 175

Ser Ala Ile Lys Ser Met Thr Ala Pro Asp Thr Ala Ser Glu Ser Glu
 180 185 190

Ser Lys Arg Leu Ala Lys Tyr Gln Gln Gln Gly Arg Leu Val Lys Gln
 195 200 205

Val Leu Leu His Ser Val Val Arg Thr Glu Phe Met Arg Val Ile Arg
 210 215 220

Gly Ser Leu Val Leu Arg Gln Phe Met Val Ser Glu Cys Lys Arg Ala
 225 230 235 240

Ser Ala Met Gly Gly Asp Thr Ser Arg Tyr Tyr Ala Met Val Gly Asp
 245 250 255

Sequence

Ile Ser Leu Tyr Ile Lys Asn Ala Gly Leu Thr Ala Phe Phe Leu Thr
260 265 270

Leu Lys Phe Gly Val Gly Thr Gln Tyr Pro Thr Leu Ala Met Ser Val
275 280 285

Phe Ser Ser Asp Leu Lys Arg Leu Ala Ala Leu Ile Arg Leu Tyr Lys
290 295 300

Thr Lys Gly Asp Asn Ala Pro Tyr Met Ala Phe Leu Glu Asp Ser Asp
305 310 315 320

Met Gly Asn Phe Ala Pro Ala Asn Tyr Ser Thr Met Tyr Ser Tyr Ala
325 330 335

Met Gly Ile Gly Thr Ile Leu Glu Ala Ser Val Ser Arg Tyr Gln Tyr
340 345 350

Ala Arg Asp Phe Thr Ser Glu Asn Tyr Phe Arg Leu Gly Val Glu Thr
355 360 365

Ala Gln Ser Gln Gln Gly Ala Phe Asp Glu Arg Thr Ala Arg Glu Met
370 375 380

Gly Leu Thr Glu Glu Ser Lys Gln Gln Val Arg Ser Leu Leu Met Ser
385 390 395 400

Val Asp Met Gly Pro Ser Ser Val Arg Glu Pro Ser Arg Pro Ala Phe
405 410 415

Ile Ser Gln Glu Glu Asn Arg Gln Pro Ala Gln Asn Ser Ser Asp Thr
420 425 430

Gln Gly Gln Thr Lys Pro Val Pro Asn Gln Pro Ala Pro Arg Ala Asp
435 440 445

Pro Asp Asp Ile Asp Pro Tyr Glu Asn Gly Leu Glu Trp
450 455 460

<210> 79
<211> 1374
<212> DNA
<213> artificial sequence

<220>
<223> P gene of FJ619036

<400> 79
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acaagatgga cttcgccaat gatgaagaaa ttgcagaact tctgaacctc agcaccactg 120
taatcaagga gattcagaaa tctgaactca agcctcccca aaccactggg cgaccacctg 180
tcagtcaagg gaacacaaga aatctaactg atctatggga aaaggagact gcaagtcaga 240

Sequence

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acaagacatc ggctcaatct ccacaaacca cacaagttca gtctgatgga aatgaggagg      300
aagaaatcaa atcagagtca attgatggcc acatcagtgg aactgttaat caattagagc      360
aagtcccaga acaaaaccag agcagatctt caccagggtga tgatctcgac agagctctca      420
acaagcttga agggagaatc aactcaatca gctcaatgga taaagaaatt aaaaagggcc      480
ctcgcatcca gaatctccct ggggtcccaag cagcaactca acaggcgacc caccattgg      540
cagggggacac cccgaacatg caggcacgga caaaacccct gaccaagcca catcaagagg      600
caatcaatcc tggcaaccag gacacaggag agaatattca tttaccacct tccatggcac      660
caccagagtc attagttggg gcaatccgca atgtacccca attcgtgcca gaccaatcta      720
tgacgaatgt agatgcgggg agtgtccaac tacatgcatc atgtgcagag atgataagta      780
gaatgcttgt agaagttata tctaagcttg ataaactcga gtcgagactg aatgatatag      840
caaaagttgt aaacaccacc ccccttatca ggaatgatat taaccaactt aaggccacaa      900
ctgcactgat gtccaaccaa attgcttcca tacaaattct tgacccaggg aatgcagggg      960
tgagggtccct ctctgaaatg agatctgtga cgaagaaagc tgctgttgta attgcaggat     1020
ttggagacga cccaactcaa attattgaag aaggatcat ggccaaagat gctcttgaa     1080
aacctgtgcc tccaacatct gttatcgag ccaaagctca gacttcttcc ggtgtgagta     1140
agggtgaaat agaaggattg attgcattgg tggaaacatt agttgacaat gacaagaagg     1200
cagcgaaaact gattaaaatg attgatcaag ttaaattcca cgccgattac gcccgagtca     1260
agcaggcaat atataatgca taatattgta attatacaaa caatcaatac tgctgtcggg     1320
tgcaccacc ttagcaaatc aataatcttt taaaattgat tgattaagaa aaaa           1374

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<210> 80
 <211> 405
 <212> PRT
 <213> artificial sequence

<220>
 <223> P protein of ACN88140
 <400> 80

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20          25          30
Thr Thr Gly Arg Pro Pro Val Ser Gln Gly Asn Thr Arg Asn Leu Thr
35          40          45
Asp Leu Trp Glu Lys Glu Thr Ala Ser Gln Asn Lys Thr Ser Ala Gln
50          55          60
Ser Pro Gln Thr Thr Gln Val Gln Ser Asp Gly Asn Glu Glu Glu Glu
65          70          75          80

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Sequence

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

Leu Glu Gln Val Pro Glu Gln Asn Gln Ser Arg Ser Ser Pro Gly Asp
100 105 110

Asp Leu Asp Arg Ala Leu Asn Lys Leu Glu Gly Arg Ile Asn Ser Ile
115 120 125

Ser Ser Met Asp Lys Glu Ile Lys Lys Gly Pro Arg Ile Gln Asn Leu
130 135 140

Pro Gly Ser Gln Ala Ala Thr Gln Gln Ala Thr His Pro Leu Ala Gly
145 150 155 160

Asp Thr Pro Asn Met Gln Ala Arg Thr Lys Pro Leu Thr Lys Pro His
165 170 175

Gln Glu Ala Ile Asn Pro Gly Asn Gln Asp Thr Gly Glu Asn Ile His
180 185 190

Leu Pro Pro Ser Met Ala Pro Pro Glu Ser Leu Val Gly Ala Ile Arg
195 200 205

Asn Val Pro Gln Phe Val Pro Asp Gln Ser Met Thr Asn Val Asp Ala
210 215 220

Gly Ser Val Gln Leu His Ala Ser Cys Ala Glu Met Ile Ser Arg Met
225 230 235 240

Leu Val Glu Val Ile Ser Lys Leu Asp Lys Leu Glu Ser Arg Leu Asn
245 250 255

Asp Ile Ala Lys Val Val Asn Thr Thr Pro Leu Ile Arg Asn Asp Ile
260 265 270

Asn Gln Leu Lys Ala Thr Thr Ala Leu Met Ser Asn Gln Ile Ala Ser
275 280 285

Ile Gln Ile Leu Asp Pro Gly Asn Ala Gly Val Arg Ser Leu Ser Glu
290 295 300

Met Arg Ser Val Thr Lys Lys Ala Ala Val Val Ile Ala Gly Phe Gly
305 310 315 320

Asp Asp Pro Thr Gln Ile Ile Glu Glu Gly Ile Met Ala Lys Asp Ala
325 330 335

Leu Gly Lys Pro Val Pro Pro Thr Ser Val Ile Ala Ala Lys Ala Gln
340 345 350

Sequence

Thr Ser Ser Gly Val Ser Lys Gly Glu Ile Glu Gly Leu Ile Ala Leu
355 360 365

Val Glu Thr Leu Val Asp Asn Asp Lys Lys Ala Ala Lys Leu Ile Lys
370 375 380

Met Ile Asp Gln Val Lys Ser His Ala Asp Tyr Ala Arg Val Lys Gln
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Ala Ile Tyr Asn Ala
405

<210> 81
<211> 1110
<212> DNA
<213> artificial sequence

<220>
<223> M gene of FJ619036

<400> 81
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agggtagatt caattggcga tgtgcagaat gccaaagagt cctcgatatt cgtgactcta 180
tatggtttca tccaagcaat taaggagaat tcagatcgat cgaaattctt ccatccaaaa 240
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gaactcctaa ggtctgatga tctcgagata cgagggtgctg tggcctccaa gaaaggtaga 1080
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<210> 82
<211> 369

Sequence

<212> PRT
<213> artificial sequence

<220>
<223> M protein of ACN88143

<400> 82

Met Ala Tyr Thr Thr Leu Lys Leu Trp Val Asp Glu Gly Asp Met Ser
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Ser Ser Leu Leu Ser Phe Pro Leu Val Leu Lys Glu Thr Asp Arg Gly
20 25 30

Thr Lys Lys Leu Gln Pro Gln Val Arg Val Asp Ser Ile Gly Asp Val
35 40 45

Gln Asn Ala Lys Glu Ser Ser Ile Phe Val Thr Leu Tyr Gly Phe Ile
50 55 60

Gln Ala Ile Lys Glu Asn Ser Asp Arg Ser Lys Phe Phe His Pro Lys
65 70 75 80

Asp Asp Phe Lys Pro Glu Thr Val Thr Ala Gly Leu Val Val Val Gly
85 90 95

Ala Ile Arg Met Met Ala Asp Val Asn Thr Ile Ser Asn Asp Ala Leu
100 105 110

Ala Leu Glu Ile Thr Val Lys Lys Ser Ala Thr Ser Gln Glu Lys Met
115 120 125

Thr Val Met Phe His Asn Ser Pro Pro Ser Leu Arg Thr Ala Ile Thr
130 135 140

Ile Arg Ala Gly Gly Phe Ile Ser Asn Ala Asp Glu Asn Ile Lys Cys
145 150 155 160

Ala Ser Lys Leu Thr Ala Gly Val Gln Tyr Ile Phe Arg Pro Met Phe
165 170 175

Val Ser Ile Thr Lys Leu His Asn Gly Lys Leu Tyr Arg Val Pro Lys
180 185 190

Ser Ile His Ser Ile Ser Ser Thr Leu Leu Tyr Ser Val Met Leu Glu
195 200 205

Val Gly Phe Lys Val Asp Ile Gly Lys Asp His Pro Gln Ala Lys Met
210 215 220

Leu Lys Arg Val Thr Ile Gly Asp Ala Asp Thr Tyr Trp Gly Phe Ala
225 230 235 240

Trp Phe His Leu Cys Asn Phe Lys Lys Thr Ser Ser Lys Gly Lys Pro
245 250 255

Leu Glu Leu His Asp Leu Trp Gly Pro Thr Ile Val Val Gln Ile Thr
275 280 285

Gly Lys Ser Ser Lys Tyr Ala Gln Gly Phe Phe Ser Ser Asn Gly Thr
290 295 300

Cys Cys Leu Pro Ile Ser Arg Ser Ala Pro Glu Leu Gly Lys Leu Leu
305 310 315 320

Trp Ser Cys Ser Ala Thr Ile Gly Asp Ala Thr Val Val Ile Gln Ser
325 330 335

Ser Glu Lys Gly Glu Leu Leu Arg Ser Asp Asp Leu Glu Ile Arg Gly
340 345 350

Ala Val Ala Ser Lys Lys Gly Arg Leu Ser Ser Phe His Pro Phe Lys
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Sequence

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Val Tyr Pro Val Asn Ser Ile Asp Asn Thr Leu Val Ala Pro Ile Gly
          20           25           30

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Val Ala Ser Ala Asn Glu Trp Gln Leu Ala Ala Tyr Thr Thr Ser Leu
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Ser Gly Thr Ile Ala Val Arg Phe Leu Pro Val Leu Pro Asp Asn Met
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```

Thr Thr Cys Leu Arg Glu Thr Ile Thr Thr Tyr Asn Asn Thr Val Asn
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Asn Ile Leu Gly Pro Leu Lys Ser Asn Leu Asp Ala Leu Leu Ser Ser
          85           90           95

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Sequence

Glu Thr Tyr Pro Gln Thr Arg Leu Ile Gly Ala Val Ile Gly Ser Ile
100 105 110

Ala Leu Gly Val Ala Thr Ser Ala Gln Ile Thr Ala Ala Val Ala Leu
115 120 125

Lys Gln Ala Gln Asp Asn Ala Arg Asn Ile Leu Ala Leu Lys Glu Ala
130 135 140

Leu Ser Lys Thr Asn Glu Ala Val Lys Glu Leu Ser Ser Gly Leu Gln
145 150 155 160

Gln Thr Ala Ile Ala Leu Gly Lys Ile Gln Ser Phe Val Asn Glu Glu
165 170 175

Ile Leu Pro Ser Ile Asn Gln Leu Ser Cys Glu Val Thr Ala Asn Lys
180 185 190

Leu Gly Val Tyr Leu Ser Leu Tyr Leu Thr Glu Leu Thr Thr Ile Phe
195 200 205

Gly Ala Gln Leu Thr Asn Pro Ala Leu Thr Ser Leu Ser Tyr Gln Ala
210 215 220

Leu Tyr Asn Leu Cys Gly Gly Asn Met Ala Met Leu Thr Gln Lys Ile
225 230 235 240

Gly Ile Lys Gln Gln Asp Val Asn Ser Leu Tyr Glu Ala Gly Leu Ile
245 250 255

Thr Gly Gln Val Ile Gly Tyr Asp Ser Gln Tyr Gln Leu Leu Val Ile
260 265 270

Gln Val Asn Tyr Pro Ser Ile Ser Glu Val Thr Gly Val Arg Ala Thr
275 280 285

Glu Leu Val Thr Val Ser Val Thr Thr Asp Lys Gly Glu Gly Lys Ala
290 295 300

Ile Val Pro Gln Phe Val Ala Glu Ser Arg Val Thr Ile Glu Glu Leu
305 310 315 320

Asp Val Ala Ser Cys Lys Phe Ser Ser Thr Thr Leu Tyr Cys Arg Gln
325 330 335

Val Asn Thr Arg Ala Leu Pro Pro Leu Val Ala Ser Cys Leu Arg Gly
340 345 350

Asn Tyr Asp Asp Cys Gln Tyr Thr Thr Glu Ile Gly Ala Leu Ser Ser
355 360 365

Sequence

Arg Tyr Ile Thr Leu Asp Gly Gly Val Leu Val Asn Cys Lys Ser Ile
370 375 380

Val Cys Arg Cys Leu Asn Pro Ser Lys Ile Ile Ser Gln Asn Thr Asn
385 390 395 400

Ala Ala Val Thr Tyr Val Asp Ala Thr Ile Cys Lys Thr Ile Gln Leu
405 410 415

Asp Asp Ile Gln Leu Gln Leu Glu Gly Ser Leu Ser Ser Val Tyr Ala
420 425 430

Arg Asn Ile Ser Ile Glu Ile Ser Gln Val Thr Thr Ser Gly Ser Leu
435 440 445

Asp Ile Ser Ser Glu Ile Gly Asn Ile Asn Asn Thr Val Asn Arg Val
450 455 460

Glu Asp Leu Ile His Gln Ser Glu Glu Trp Leu Ala Lys Val Asn Pro
465 470 475 480

His Ile Val Asn Asn Thr Thr Leu Ile Val Leu Cys Val Leu Ser Ala
485 490 495

Leu Ala Val Ile Trp Leu Ala Val Leu Thr Ala Ile Ile Ile Tyr Leu
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Arg Thr Lys Leu Lys Thr Ile Ser Ala Leu Ala Val Thr Asn Thr Ile
515 520 525

Gln Ser Asn Pro Tyr Val Asn Gln Thr Lys Arg Glu Ser Lys Phe
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aacgggacca accttatcat acctacatca gataaggagt atgcaggagg aattggaaac 420
cctgtcttta ctgtcgaatg tggaggttct ataggattca agcaatttag cttaatagaa 480

Sequence

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Thr Thr Gly Cys Leu Ala Leu Ser Ile Val Ser Ile Val Gln Ile Gly
 35 40 45

Asn Leu Lys Ile Pro Ser Val Gly Asp Leu Ala Asp Glu Val Val Thr
 50 55 60

Sequence

Pro Leu Lys Thr Thr Leu Ser Asp Thr Leu Arg Asn Pro Ile Asn Gln
65 70 75 80

Ile Asn Asp Ile Phe Arg Ile Val Ala Leu Asp Ile Pro Leu Gln Val
85 90 95

Thr Ser Ile Gln Lys Asp Leu Ala Ser Gln Phe Ser Met Leu Ile Asp
100 105 110

Ser Leu Asn Ala Ile Lys Leu Gly Asn Gly Thr Asn Leu Ile Ile Pro
115 120 125

Thr Ser Asp Lys Glu Tyr Ala Gly Gly Ile Gly Asn Pro Val Phe Thr
130 135 140

Val Asp Ala Gly Gly Ser Ile Gly Phe Lys Gln Phe Ser Leu Ile Glu
145 150 155 160

His Pro Ser Phe Ile Ala Gly Pro Thr Thr Thr Arg Gly Cys Thr Arg
165 170 175

Ile Pro Thr Phe His Met Ser Glu Ser His Trp Cys Tyr Ser His Asn
180 185 190

Ile Ile Ala Ala Gly Cys Gln Asp Ala Ser Ala Ser Ser Met Tyr Ile
195 200 205

Ser Met Gly Val Leu His Val Ser Ser Ser Gly Thr Pro Ile Phe Leu
210 215 220

Thr Thr Ala Ser Glu Leu Ile Asp Asp Gly Val Asn Arg Lys Ser Cys
225 230 235 240

Ser Ile Val Ala Thr Gln Phe Gly Cys Asp Ile Leu Cys Ser Ile Val
245 250 255

Ile Glu Lys Glu Gly Asp Asp Tyr Trp Ser Asp Thr Pro Thr Pro Met
260 265 270

Arg His Gly Arg Phe Ser Phe Asn Gly Ser Phe Val Glu Thr Glu Leu
275 280 285

Pro Val Ser Ser Met Phe Ser Ser Phe Ser Ala Asn Tyr Pro Ala Val
290 295 300

Gly Ser Gly Glu Ile Val Lys Asp Arg Ile Leu Phe Pro Ile Tyr Gly
305 310 315 320

Gly Ile Lys Gln Thr Ser Pro Glu Phe Thr Glu Leu Val Lys Tyr Gly
325 330 335

Sequence

Leu Phe Val Ser Thr Pro Thr Thr Val Cys Gln Ser Ser Trp Thr Tyr
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 Asp Gln Val Lys Ala Ala Tyr Arg Pro Asp Tyr Ile Ser Gly Arg Phe
 355 360 365
 Trp Ala Gln Val Ile Leu Ser Cys Ala Leu Asp Ala Val Asp Leu Ser
 370 375 380
 Ser Cys Ile Val Lys Ile Met Asn Ser Ser Thr Val Met Met Ala Ala
 385 390 395 400
 Glu Gly Arg Ile Ile Lys Ile Gly Ile Asp Tyr Phe Tyr Tyr Gln Arg
 405 410 415
 Ser Ser Ser Trp Trp Pro Leu Ala Phe Val Thr Lys Leu Asp Pro Gln
 420 425 430
 Glu Leu Ala Asp Thr Asn Ser Ile Trp Leu Thr Asn Ser Ile Pro Ile
 435 440 445
 Pro Gln Ser Lys Phe Pro Arg Pro Ser Tyr Ser Glu Asn Tyr Cys Thr
 450 455 460
 Lys Pro Ala Val Cys Pro Ala Thr Cys Val Thr Gly Val Tyr Ser Asp
 465 470 475 480
 Ile Trp Pro Leu Thr Ser Ser Ser Ser Leu Pro Ser Ile Ile Trp Ile
 485 490 495
 Gly Gln Tyr Leu Asp Ala Pro Val Gly Arg Thr Tyr Pro Arg Phe Gly
 500 505 510
 Ile Ala Asn Gln Ser His Trp Tyr Leu Gln Glu Asp Ile Leu Pro Thr
 515 520 525
 Ser Thr Ala Ser Ala Tyr Ser Thr Thr Thr Cys Phe Lys Asn Thr Ala
 530 535 540
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Asn

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Sequence

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Sequence

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Sequence

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ttacttgttg	ctacttacgg	attgcagcaa	gattcaatac	tgtgggtgaa	agtatatagg	5880
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Sequence

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gtgtcgaaga atctgagtct tatccatcca gaagacctca gaaaggtaag gtgtgaaaca      6120
gattccctga atttgaagtg caatcatatt tatgagaaaa taattgctag aaaaattcca      6180
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gatatgataa caatctctga attttgcaag aaatccaagc ggcctatatt tattaacga      6600
gtaatagggg atcaacggct gaaatcattc tttaatgaaa gctcaagtat tgttttgacc      6660
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Asp Ser Pro Ile Ile Leu Asn Lys Leu Ala Leu Leu Trp Arg Leu Ser
           20          25          30

Gly Leu Pro Met Pro Ala Asp Leu Arg Gln Lys Ser Val Val Met His
           35          40          45

Ile Pro Asp His Ile Leu Glu Lys Ser Glu Tyr Arg Ile Lys His Arg
           50          55          60

Leu Gly Lys Ile Lys Ser Asp Ile Ala His Tyr Cys Gln Tyr Phe Asn
65          70          75          80

Ile Asn Leu Ala Asn Leu Asp Pro Ile Thr His Pro Lys Ser Leu Tyr
           85          90          95

Trp Leu Ser Arg Leu Thr Ile Ala Ser Ala Gly Thr Phe Arg His Met
           100         105         110

Lys Asp Arg Ile Leu Cys Thr Val Gly Ser Glu Phe Gly His Lys Ile
           115         120         125

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Sequence

Gln Asp Leu Phe Ser Leu Leu Ser His Lys Leu Val Gly Asn Gly Asp
130 135 140

Leu Phe Asn Gln Ser Leu Ser Gly Thr Arg Leu Thr Ala Ser Pro Leu
145 150 155 160

Ser Pro Leu Cys Asn Gln Phe Val Ser Asp Ile Lys Ser Ala Val Thr
165 170 175

Thr Pro Trp Ser Glu Ala Arg Trp Ser Trp Leu His Ile Lys Gln Thr
180 185 190

Met Arg Tyr Leu Ile Lys Gln Ser Arg Thr Thr Asn Ser Ala His Leu
195 200 205

Thr Glu Ile Ile Lys Glu Glu Trp Gly Leu Val Gly Ile Thr Pro Asp
210 215 220

Leu Val Ile Leu Phe Asp Arg Val Asn Asn Ser Leu Thr Ala Leu Thr
225 230 235 240

Phe Glu Met Val Leu Met Tyr Ser Asp Val Leu Glu Ser Arg Asp Asn
245 250 255

Ile Val Leu Val Gly Arg Leu Ser Thr Phe Leu Gln Pro Val Val Ser
260 265 270

Arg Leu Glu Val Leu Phe Asp Leu Val Asp Ser Leu Ala Lys Thr Leu
275 280 285

Gly Asp Thr Ile Tyr Glu Ile Ile Ala Val Leu Glu Ser Leu Ser Tyr
290 295 300

Gly Ser Val Gln Leu His Asp Ala Ser His Ser His Ala Gly Ser Phe
305 310 315 320

Phe Ser Phe Asn Met Asn Glu Leu Asp Asn Thr Leu Ser Lys Arg Val
325 330 335

Asp Pro Lys His Lys Asn Thr Ile Met Ser Ile Ile Arg Gln Cys Phe
340 345 350

Ser Asn Leu Asp Val Asp Gln Ala Ala Glu Met Leu Cys Leu Met Arg
355 360 365

Leu Phe Gly His Pro Met Leu Thr Ala Pro Asp Ala Ala Ala Lys Val
370 375 380

Arg Lys Ala Met Cys Ala Pro Lys Leu Val Glu His Asp Thr Ile Leu
385 390 395 400

Sequence

Gln Thr Leu Ser Phe Phe Lys Gly Ile Ile Ile Asn Gly Tyr Arg Arg
 405 410 415
 Ser His Ser Gly Leu Trp Pro Asn Val Glu Pro Ser Ser Ile Tyr Asp
 420 425 430
 Asp Asp Leu Arg Gln Leu Tyr Leu Glu Ser Ala Glu Ile Ser His His
 435 440 445
 Phe Met Leu Lys Asn Tyr Lys Ser Leu Ser Met Ile Glu Phe Lys Lys
 450 455 460
 Ser Ile Asp Tyr Asp Leu His Asp Asp Leu Ser Thr Phe Leu Lys Asp
 465 470 475 480
 Arg Ala Ile Cys Arg Pro Lys Ser Gln Trp Asp Val Ile Phe Arg Lys
 485 490 495
 Ser Leu Arg Arg Ser His Thr Arg Ser Gln Tyr Met Asp Glu Ile Lys
 500 505 510
 Ser Asn Arg Leu Leu Ile Asp Phe Leu Asp Ser Ala Asp Phe Asp Pro
 515 520 525
 Glu Lys Glu Phe Ala Tyr Val Thr Thr Met Asp Tyr Leu His Asp Asn
 530 535 540
 Glu Phe Cys Ala Ser Tyr Ser Leu Lys Glu Lys Glu Ile Lys Thr Thr
 545 550 555 560
 Gly Arg Ile Phe Ala Lys Met Thr Arg Asn Met Arg Ser Cys Gln Val
 565 570 575
 Ile Leu Glu Ser Leu Leu Ser Lys His Ile Cys Lys Phe Phe Lys Glu
 580 585 590
 Asn Gly Val Ser Met Glu Gln Leu Ser Leu Thr Lys Ser Leu Leu Ala
 595 600 605
 Met Ser Gln Leu Ser Pro Lys Val Ser Thr Leu Gln Asp Thr Ala Ser
 610 615 620
 Arg His Val Gly Asn Ser Lys Ser Gln Ile Ala Thr Ser Asn Pro Ser
 625 630 635 640
 Arg His His Ser Thr Thr Asn Gln Met Ser Leu Ser Asn Arg Lys Thr
 645 650 655
 Val Val Ala Thr Phe Leu Thr Thr Asp Leu Glu Lys Tyr Cys Leu Gln
 660 665 670

Sequence

Trp Arg Tyr Ser Thr Ile Lys Leu Phe Ala Gln Ala Leu Asn Gln Leu
 675 680 685
 Phe Gly Ile Asp His Gly Phe Glu Trp Ile His Leu Arg Leu Met Asn
 690 695 700
 Ser Thr Leu Phe Val Gly Asp Pro Tyr Ser Pro Pro Glu Asp Pro Thr
 705 710 715 720
 Leu Glu Asp Ile Asp Lys Ala Pro Asn Asp Asp Ile Phe Ile Val Ser
 725 730 735
 Pro Arg Gly Gly Ile Glu Gly Leu Cys Gln Lys Met Trp Thr Met Ile
 740 745 750
 Ser Ile Ser Ala Ile His Cys Val Ala Glu Lys Ile Gly Ala Arg Val
 755 760 765
 Ala Ala Met Val Gln Gly Asp Asn Gln Val Ile Ala Ile Thr Lys Glu
 770 775 780
 Leu Phe Arg Gly Glu Lys Ala Cys Asp Val Arg Asp Glu Leu Asp Glu
 785 790 795 800
 Leu Gly Gln Val Phe Phe Asp Glu Phe Lys Arg His Asn Tyr Ala Ile
 805 810 815
 Gly His Asn Leu Lys Leu Asn Glu Thr Ile Gln Ser Gln Ser Phe Phe
 820 825 830
 Val Tyr Ser Lys Arg Ile Phe Phe Glu Gly Arg Leu Leu Ser Gln Val
 835 840 845
 Leu Lys Asn Ala Ala Lys Leu Cys Met Val Ala Asp His Leu Gly Glu
 850 855 860
 Asn Thr Val Ser Ser Cys Ser Asn Leu Ser Ser Thr Ile Ala Arg Leu
 865 870 875 880
 Val Glu Asn Gly Phe Glu Lys Asp Thr Ala Phe Val Leu Asn Leu Val
 885 890 895
 Tyr Ile Met Thr Gln Ile Leu Phe Asp Glu His Tyr Ser Ile Val Cys
 900 905 910
 Asp His Asn Ser Val Lys Ser Leu Ile Gly Ser Lys Asn Tyr Arg Asn
 915 920 925
 Leu Leu Tyr Ser Ser Leu Ile Pro Gly Gln Leu Gly Gly Phe Asn Phe
 930 935 940

Sequence

Leu Asn Ile Ser Arg Leu Phe Thr Arg Asn Ile Gly Asp Pro Val Thr
 945 950 955 960
 Cys Ser Leu Ser Asp Leu Lys Cys Phe Ile Ala Ala Gly Leu Leu Pro
 965 970 975
 Pro Tyr Val Leu Lys Asn Val Val Leu Arg Glu Pro Gly Pro Gly Thr
 980 985 990
 Trp Leu Thr Leu Cys Ser Asp Pro Tyr Thr Leu Asn Ile Pro Tyr Thr
 995 1000 1005
 Gln Leu Pro Thr Thr Tyr Leu Lys Lys His Thr Gln Arg Ser Leu
 1010 1015 1020
 Leu Ser Arg Ala Val Asn Pro Leu Leu Ala Gly Val Gln Val Pro
 1025 1030 1035
 Asn Gln His Glu Glu Glu Glu Met Leu Ala Arg Phe Leu Leu Asp
 1040 1045 1050
 Arg Glu Tyr Val Met Pro Arg Val Ala His Val Thr Leu Glu Thr
 1055 1060 1065
 Ser Val Leu Gly Lys Arg Lys Gln Ile Gln Gly Leu Ile Asp Thr
 1070 1075 1080
 Thr Pro Thr Ile Ile Arg Thr Ser Leu Val Asn Leu Pro Val Ser
 1085 1090 1095
 Arg Lys Lys Cys Glu Lys Ile Ile Asn Tyr Ser Leu Asn Tyr Ile
 1100 1105 1110
 Ala Glu Cys His Asp Ser Leu Leu Ser Gln Ile Cys Phe Ser Asp
 1115 1120 1125
 Asn Lys Glu Tyr Leu Trp Ser Thr Ser Leu Ile Ser Val Glu Thr
 1130 1135 1140
 Cys Ser Val Thr Ile Ala Asp Tyr Leu Arg Ala Val Ser Trp Ser
 1145 1150 1155
 Asn Ile Leu Gly Gly Arg Ser Ile Ser Gly Val Thr Thr Pro Asp
 1160 1165 1170
 Thr Ile Glu Leu Ile Gln Gly Cys Leu Ile Gly Glu Asn Ser Ser
 1175 1180 1185
 Cys Thr Leu Cys Glu Ser His Asp Asp Ala Phe Thr Trp Met His
 1190 1195 1200

Sequence

Leu	Pro	Gly	Pro	Leu	Tyr	Ile	Pro	Glu	Pro	Ser	Val	Thr	Asn	Ser
1205						1210					1215			
Lys	Met	Arg	Val	Pro	Tyr	Leu	Gly	Ser	Lys	Thr	Glu	Glu	Arg	Lys
1220						1225					1230			
Thr	Ala	Ser	Met	Ala	Ala	Ile	Lys	Gly	Met	Ser	His	His	Leu	Arg
1235						1240					1245			
Ala	Val	Leu	Arg	Gly	Thr	Ser	Val	Phe	Ile	Trp	Ala	Ser	Gly	Asp
1250						1255					1260			
Thr	Asp	Ile	Asn	Trp	Asp	Asn	Ala	Leu	Gln	Ile	Ala	Gln	Ser	Arg
1265						1270					1275			
Cys	Asn	Ile	Thr	Leu	Asp	Gln	Met	Arg	Leu	Leu	Thr	Pro	Ile	Pro
1280						1285					1290			
Ser	Ser	Ser	Asn	Ile	Gln	Arg	Arg	Leu	Asp	Asp	Gly	Ile	Ser	Thr
1295						1300					1305			
Gln	Lys	Phe	Thr	Pro	Ala	Ser	Leu	Ala	Arg	Ile	Thr	Ser	Ser	Val
1310						1315					1320			
His	Ile	Cys	Asn	Asp	Ser	Gln	Arg	Leu	Glu	Lys	Asp	Gly	Ser	Ser
1325						1330					1335			
Val	Asp	Ser	Asn	Leu	Ile	Tyr	Gln	Gln	Ile	Met	Leu	Leu	Gly	Leu
1340						1345					1350			
Ser	Ile	Phe	Glu	Thr	Met	Tyr	Ser	Met	Asp	Gln	Lys	Trp	Val	Phe
1355						1360					1365			
Asn	Asn	His	Thr	Leu	His	Leu	His	Thr	Gly	His	Ser	Cys	Cys	Pro
1370						1375					1380			
Arg	Glu	Leu	Asp	Ile	Ser	Leu	Val	Asn	Pro	Pro	Arg	His	Gln	Thr
1385						1390					1395			
Pro	Glu	Leu	Thr	Ser	Thr	Thr	Thr	Asn	Pro	Phe	Leu	Tyr	Asp	Gln
1400						1405					1410			
Leu	Pro	Leu	Asn	Gln	Asp	Asn	Leu	Thr	Thr	Leu	Glu	Ile	Lys	Thr
1415						1420					1425			
Phe	Lys	Phe	Asn	Glu	Leu	Asn	Ile	Asp	Gly	Leu	Asp	Phe	Gly	Glu
1430						1435					1440			
Gly	Ile	Gln	Leu	Leu	Ser	Arg	Cys	Thr	Ala	Arg	Leu	Met	Ala	Glu
1445						1450					1455			

Sequence

Cys Ile Leu Glu Glu Gly Ile Gly Ser Ser Val Lys Asn Glu Ala
 1460 1465 1470
 Ile Val Asn Phe Asp Asn Ser Val Asn Trp Ile Ser Glu Cys Leu
 1475 1480 1485
 Met Cys Asp Ile Arg Ser Leu Cys Val Asn Leu Gly Gln Glu Ile
 1490 1495 1500
 Leu Cys Ser Leu Ala Tyr Gln Met Tyr Tyr Leu Arg Ile Arg Gly
 1505 1510 1515
 Arg Arg Ala Ile Leu Asn Tyr Leu Asp Thr Thr Leu Gln Arg Ile
 1520 1525 1530
 Pro Val Ile Gln Leu Ala Asn Ile Ala Leu Thr Ile Ser His Pro
 1535 1540 1545
 Glu Ile Phe Arg Arg Ile Val Asn Thr Gly Ile His Asn Gln Ile
 1550 1555 1560
 Lys Gly Pro Tyr Val Ala Thr Thr Asp Phe Ile Ala Ala Ser Arg
 1565 1570 1575
 Asp Ile Ile Leu Ser Gly Ala Arg Glu Tyr Leu Ser Tyr Leu Ser
 1580 1585 1590
 Ser Gly Gln Glu Asp Cys Tyr Thr Phe Phe Asn Cys Gln Asp Gly
 1595 1600 1605
 Asp Leu Thr Pro Lys Met Glu Gln Tyr Leu Ala Arg Arg Ala Cys
 1610 1615 1620
 Leu Leu Thr Leu Leu Tyr Asn Thr Gly His Gln Ile Pro Ile Ile
 1625 1630 1635
 Arg Ser Leu Thr Pro Ile Glu Lys Cys Lys Val Leu Thr Glu Tyr
 1640 1645 1650
 Asn Gln Gln Ile Glu Tyr Ala Asp Gln Glu Phe Ser Ser Val Leu
 1655 1660 1665
 Lys Val Val Asn Ala Leu Leu Gln Asn Pro Asn Ile Asp Ala Leu
 1670 1675 1680
 Val Ser Asn Leu Tyr Phe Thr Thr Arg Arg Val Leu Ser Asn Leu
 1685 1690 1695
 Arg Ser Cys Asp Lys Ala Ile Ser Tyr Ile Glu Tyr Leu Tyr Thr
 1700 1705 1710

Sequence

Glu Asp Phe Gly Glu Lys Glu Asp Thr Val Gln Tyr Asp Ile Met
 1715 1720 1725
 Thr Thr Asn Asp Ile Ile Leu Thr His Gly Leu Phe Thr Gln Ile
 1730 1735 1740
 Glu Ile Ser Tyr Gln Gly Ser Ser Leu His Lys Phe Leu Thr Pro
 1745 1750 1755
 Asp Asn Ala Pro Gly Ser Leu Ile Pro Phe Ser Ile Ser Pro Asn
 1760 1765 1770
 Ser Leu Ala Cys Asp Pro Leu His His Leu Leu Lys Ser Val Gly
 1775 1780 1785
 Thr Ser Ser Thr Ser Trp Tyr Lys Tyr Ala Ile Ala Tyr Ala Val
 1790 1795 1800
 Ser Glu Lys Arg Ser Ala Arg Leu Gly Gly Ser Leu Tyr Ile Gly
 1805 1810 1815
 Glu Gly Ser Gly Ser Val Met Thr Leu Leu Glu Tyr Leu Glu Pro
 1820 1825 1830
 Ser Val Asp Ile Phe Tyr Asn Ser Leu Phe Ser Asn Gly Met Asn
 1835 1840 1845
 Pro Pro Gln Arg Asn Tyr Gly Leu Met Pro Leu Gln Phe Val Asn
 1850 1855 1860
 Ser Val Val Tyr Lys Asn Leu Thr Ala Lys Ser Glu Cys Lys Leu
 1865 1870 1875
 Gly Phe Val Gln Gln Phe Lys Pro Leu Trp Arg Asp Ile Asp Ile
 1880 1885 1890
 Glu Thr Asn Val Thr Asp Pro Ser Phe Val Asn Phe Ala Leu Asn
 1895 1900 1905
 Glu Ile Pro Met Gln Ser Leu Lys Arg Val Asn Cys Asp Val Glu
 1910 1915 1920
 Phe Asp Arg Gly Met Pro Ile Glu Arg Val Ile Gln Gly Tyr Thr
 1925 1930 1935
 His Ile Leu Leu Val Ala Thr Tyr Gly Leu Gln Gln Asp Ser Ile
 1940 1945 1950
 Leu Trp Val Lys Val Tyr Arg Thr Ser Glu Lys Val Phe Gln Phe
 1955 1960 1965

Sequence

Leu Leu Ser Ala Met Ile Met Ile Phe Gly Tyr Val Lys Ile His
 1970 1975 1980
 Arg Asn Gly Tyr Met Ser Ala Lys Asp Glu Glu Tyr Ile Leu Met
 1985 1990 1995
 Ser Asp Cys Lys Glu Pro Val Asn Tyr Thr Ala Val Pro Asn Ile
 2000 2005 2010
 Leu Thr Arg Val Ser Asp Leu Val Ser Lys Asn Leu Ser Leu Ile
 2015 2020 2025
 His Pro Glu Asp Leu Arg Lys Val Arg Cys Glu Thr Asp Ser Leu
 2030 2035 2040
 Asn Leu Lys Cys Asn His Ile Tyr Glu Lys Ile Ile Ala Arg Lys
 2045 2050 2055
 Ile Pro Leu Gln Val Ser Ser Thr Asp Ser Leu Leu Leu Gln Leu
 2060 2065 2070
 Gly Gly Val Ile Asn Ser Val Gly Ser Thr Asp Pro Arg Glu Val
 2075 2080 2085
 Ala Thr Leu Ser Ser Ile Glu Cys Met Asp Tyr Val Val Ser Ser
 2090 2095 2100
 Ile Asp Leu Ala Ile Leu Glu Ala Asn Ile Val Ile Ser Glu Ser
 2105 2110 2115
 Ala Asp Leu Asp Leu Ala Leu Met Leu Gly Pro Phe Asn Leu Asn
 2120 2125 2130
 Lys Leu Lys Lys Ile Asp Thr Ile Leu Lys Ser Ser Thr Tyr Gln
 2135 2140 2145
 Leu Ile Pro Tyr Trp Leu Arg Tyr Glu Tyr Ser Ile Asn Pro Arg
 2150 2155 2160
 Ser Leu Ser Phe Leu Ile Thr Lys Leu Gln Gln Cys Arg Ile Ser
 2165 2170 2175
 Trp Ser Asp Met Ile Thr Ile Ser Glu Phe Cys Lys Lys Ser Lys
 2180 2185 2190
 Arg Pro Ile Phe Ile Lys Arg Val Ile Gly Asn Gln Arg Leu Lys
 2195 2200 2205
 Ser Phe Phe Asn Glu Ser Ser Ser Ile Val Leu Thr Arg Ala Glu
 2210 2215 2220

Sequence

Val Lys Val Cys Ile Lys Phe Leu Gly Ala Ile Ile Lys Leu Lys
2225 2230 2235