

DESCRIPTION

ATTENUATED CHIMERIC FLAVIVIRUS BEARING ATTENUATED JAPANESE ENCEPHALITIS VIRUS GENE AS BACKBONE

TECHNICAL FIELD

5 The present invention relates to an attenuated chimeric flavivirus having a gene of attenuated Japanese encephalitis virus as the backbone, which is useful as an attenuated live vaccine for preventing flavivirus infections.

BACKGROUND ART

10 Currently, more than about 60 kinds of viruses belonging to Flaviviridae (hereinafter abbreviated flaviviruses) are known, including Japanese encephalitis virus, West Nile virus, dengue 1-4 virus, yellow fever virus, St. Louis encephalitis virus, tick-borne encephalitis virus, Kunjin virus, Central
15 European encephalitis virus, Kyasanur Forest virus, Murray Valley encephalitis virus, Omsk hemorrhagic fever virus, Powassan virus, Russian spring-summer encephalitis virus, Yokose virus, Apoi virus, and Aroa virus.

 These flaviviruses have a genome of single-stranded (+)
20 RNA and are similar to each other in terms of gene structure. The open reading frame (ORF) of the flavivirus genome encodes three structural proteins (capsid (C) protein, pre-membrane (prM) protein, which is the precursor for membrane (M) protein, and envelope (E) protein) and subsequent seven non-structural
25 (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) from the 5' terminus thereof.

 These structural proteins and non-structural proteins of the flaviviruses are translated as single polyproteins; the polyproteins translated are then processed by the protease and
30 NS3 protein having protease activity of host cells and the virus, resulting in the formation of a mature virion comprising the above-described three kinds of structural proteins.

 It is known that many of the above-described flaviviruses infect to mammals including humans, and birds, via insects such

as mosquitoes and ticks, and cause encephalitis and/or febrile symptoms. Originally, each of these flavivirus species is indigenous to a particular region; therefore, the endemic area of the infection had been limited. However, in recent years,
5 due to development in traffics/distributions, climate change and the like, various flavivirus infections have expanded to places other than the original habitats of causal flaviviruses, posing an important problem with public health.

In preventing the expansion of viral infections,
10 prevention with vaccines is effective. However, despite the fact that a large number of viruses belonging to Flaviviridae have been recognized as described above, only attenuated live vaccines for yellow fever virus and Japanese encephalitis virus infections and inactivated vaccines for Japanese encephalitis
15 virus and tick-borne encephalitis virus infections are in practical application as vaccines for flavivirus infections. Particularly, attenuated live vaccines are useful as vaccines that are inexpensive and induce long-term immunity, but there are no approved live vaccines other than those described above.

20 In these circumstances, as a means of quickly developing novel attenuated live vaccines for various flavivirus infections, a strategy utilizing a chimeric flavivirus prepared by a gene engineering technique has recently been drawing attention.

25 For example, ChimeriVaxTM-JE is a chimeric flavivirus prepared by replacing the genes that encode two structural proteins (prM-E) of the yellow fever virus vaccine 17D strain with the corresponding genes of the Japanese encephalitis virus vaccine SA14-14-2 strain (see, for example, pamphlet for
30 International Patent Publication No. 98/37911 and pamphlet for International Patent Publication No. 01/39802). ChimeriVaxTM-JE is attenuated to the extent that allows its use as a vaccine as a result of a plurality of amino acid mutations from the wild type that are present in the virus polyprotein corresponding to

the E protein of the Japanese encephalitis virus vaccine SA14-14-2 strain (see, for example, Arroyo et al., J. Virol. 75:934-942, 2001).

Furthermore, on the basis of this technique for
5 ChimeriVaxTM-JE, chimeric flaviviruses with dengue 1-4 viruses (ChimeriVaxTM-DEN (1-4)) (see, for example, pamphlet for International Patent Publication No. 98/37911 and pamphlet for International Patent Publication No. 01/39802) and a chimeric flavivirus with West Nile virus (ChimeriVaxTM-West Nile) (see,
10 for example, pamphlet for International Patent Publication No. 2004/045529), which have a gene of the yellow fever virus vaccine 17D strain as the backbone, have also been developed.

These chimeric flaviviruses are also attenuated to the extent that allows their use as vaccines, with their
15 attenuation resulting mainly from amino acid substitutions from the wild type that are present in the virus polyprotein corresponding to the prM-E protein.

However, it has been reported that the attenuation of ChimeriVaxTM-DEN1 results mainly from amino acid mutations in
20 the E protein that occur during the passage of this chimeric flavivirus in cells for vaccine production (see, for example, Guirakhoo et al., J. Virol. 78:9998-10008, 2004). Furthermore, because re-infection with dengue viruses of different serotypes is likely as a cause of the onset of dengue hemorrhagic fever,
25 this vaccine has not found a practical application.

Also, ChimeriVaxTM-West Nile has the attenuation promoted by artificially introducing an amino acid mutation in the E protein derived from the wild-type highly virulent West Nile virus NY-99 strain (see, for example, pamphlet for
30 International Patent Publication No. 2004/045529).

As a chimeric flavivirus using a gene backbone other than yellow fever virus (YF-17D), a chimeric flavivirus prepared by replacing the gene that encodes the prM-E protein of dengue-4 virus with the corresponding gene of the West Nile virus NY99

strain (WN/DEN4 chimeric virus) has been reported (see, for example, Pletnev et al., Proc. Natl. Acad. Sci. USA 99:3036-3041, 2002).

Although the WN/DEN4 chimeric virus is attenuated
5 compared to the parent strains thereof, i.e., West Nile virus and dengue-4 virus, the mechanism of the attenuation has not yet been fully elucidated, and the virus has not found a practical application as a vaccine.

Disclosure of the Invention

10 When attenuated live chimeric flavivirus vaccines for various flavivirus infections is developed by conventional methods as described above, it is necessary to investigate the safety, that is, brain neurotoxicity, infectivity from peripheral to the central nervous system (brain nerve
15 invasiveness), infection-preventing effect, neutralizing antibody production and the like of the chimeric virus constructed for each combination of flavivirus species, and this may be a major cause of prolonging the period to the practical application of attenuated live vaccine.

20 Furthermore, live vaccines still pose the problem of reductions in antibody productivity as they are attenuated for the sake of safety. This is because attenuation of viruses by modifying the E protein as described above potentially reduces their immune induction potential (antibody productivity) as
25 vaccines because the antigenic determinant for inducing a neutralizing antibody is present in the E protein.

Accordingly, it is an object of the present invention to provide an attenuated chimeric flavivirus having an attenuating mutation in a portion other than the E protein.

30 The present inventors diligently investigated to solve the above-described problem, found that the Japanese encephalitis virus vaccine ML-17 strain for swine had a plurality of amino acid mutations intrinsic to the ML-17 strain in the prM and NS proteins other than the E protein, and

obtained the suggestion that these amino acid mutations might be involved in the attenuation of the ML-17 strain. Based on this finding, the present inventors were inspired to construct a chimeric flavivirus having the structural and non-structural
5 proteins other than the E protein (that is, C protein, prM protein, and NS protein) of a Japanese encephalitis virus comprising one or more of the amino acid mutations intrinsic to the ML-17 strain, conducted further investigations, and developed the present invention.

10 Accordingly, the present invention provides:

[1] A nucleic acid molecule comprising nucleotide sequences that encode the capsid protein, pre-membrane protein and non-structural protein of Japanese encephalitis virus, and a nucleotide sequence that encodes the envelope protein of a
15 second flavivirus,

wherein the nucleotide sequence(s) that encode(s) the pre-membrane protein and/or non-structural protein of Japanese encephalitis virus comprise(s) nucleotide mutations that produce one or more amino acid mutations that attenuate the
20 virus.

[2] The nucleic acid molecule described in [1], wherein the Japanese encephalitis virus is the ML-17 strain.

[3] The nucleic acid molecule described in [1] or [2], wherein the second flavivirus is selected from the group consisting of
25 West Nile virus, dengue 1-4 virus, yellow fever virus, St. Louis encephalitis virus, tick-borne encephalitis virus, Kunjin virus, Central European encephalitis virus, Kyasanur Forest virus, Murray Valley encephalitis virus, Omsk hemorrhagic fever virus, Powassan virus, Russian spring-summer
30 encephalitis virus, Yokose virus, Apoi virus, and Aroa virus.

[4] An attenuated chimeric flavivirus encoded by the nucleic acid molecule described in any one of [1] to [3].

[5] An attenuated live vaccine comprising the attenuated chimeric flavivirus described in [4].

- [6] A method of preparing the nucleic acid molecule described in [1], which comprises the following steps:
a step for replacing a nucleotide sequence that encodes the envelope protein in a nucleic acid molecule comprising a
5 nucleotide sequence that encodes Japanese encephalitis virus with the nucleotide sequence that encodes the envelope protein of the second flavivirus; and
a step for introducing nucleotide mutations that produce one or more amino acid mutations that attenuate the virus into the
10 nucleotide sequence(s) that encode(s) the pre-membrane protein and/or non-structural protein of Japanese encephalitis virus.
- [7] A method of preparing the nucleic acid molecule described in [1], which comprises the following step:
a step for replacing a nucleotide sequence that encodes the
15 envelope protein in a nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the pre-membrane protein and/or non-structural protein with the nucleotide sequence that encodes the envelope
20 protein of the second flavivirus.
- [8] A method of preparing an attenuated chimeric flavivirus, which comprises a step for expressing chimeric flavivirus proteins from the nucleic acid molecule described in any one of [1] to [3].
- 25 [9] A nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the pre-membrane protein and/or non-structural protein.
- [10] A vector comprising the nucleic acid molecule described
30 in [9].
- [11] An attenuated Japanese encephalitis virus encoded by the nucleic acid molecule described in [9].
- [12] A method of preparing the nucleic acid molecule described in [9], which comprises a step for introducing nucleotide

mutations that produce one or more amino acid mutations that attenuate the virus into nucleotide sequence(s) that encode(s) the pre-membrane protein and/or non-structural protein in a nucleic acid molecule comprising a nucleotide sequence that
5 encodes Japanese encephalitis virus.

[13] A method of preparing an attenuated Japanese encephalitis virus, which comprises a step for expressing Japanese encephalitis virus proteins from the nucleic acid molecule described in [9].

10 By the present invention, an attenuated chimeric flavivirus having attenuating mutation(s) in portion(s) other than the E protein is provided. Because the attenuation of the chimeric flavivirus of the present invention can be achieved without modifying the E protein, attenuated live vaccines for
15 various flavivirus infections can be brought into practical application in a short time, without reducing the immune induction potential.

Brief Description of the Drawings

Figure 1 shows the differences in the nucleotide sequence
20 of genomic cDNA and the amino acid sequence of polyprotein between the Japanese encephalitis virus L-17 strain and JaOH0566 strain. Nt represents nucleotide; AA represents amino acid. Amino acid positions are shown by number, as counted from the amino acid next to the starting methionine of the
25 polyprotein.

Figure 2 shows the results of a comparison of amino acids that have mutated from the parent strain (JaOH0566 strain) (indicated by bold letters) in the polyprotein of the Japanese encephalitis virus ML-17 strain, with the corresponding amino
30 acids in the polyproteins of the JaOH0566 strain, JaOArS982 strain, JaGAr01 strain, Nakayama strain, Beijing strain, SA14 strain, and SA14-14-2 strain. Amino acid positions are shown by number, as counted from the amino acid next to the starting methionine of the polyprotein. * represents that the amino

acid at the indicated position is the same as that of the ML-17 strain.

Figure 3 shows an outline of the method of preparing the full-length cDNA of the recombinant Japanese encephalitis virus MS-14 strain and MS-15 strain by the Long-PCR method.

Figure 4 shows an outline of the method of preparing the cDNA of the ML-17/WN (E gene) chimeric flavivirus by the Long-PCR method.

Best Mode for Embodiment of the Invention

The present invention provides a nucleic acid molecule comprising nucleotide sequences that encode the C protein, prM protein and NS protein of Japanese encephalitis virus (a first flavivirus), and a nucleotide sequence that encodes the E protein of a second flavivirus. Preferably, the present invention provides a nucleic acid molecule comprising a nucleotide sequence of the 5' untranslated region, the nucleotide sequences that encode the C protein, prM protein and NS protein, and a nucleotide sequence of the 3' untranslated region of Japanese encephalitis virus, and the nucleotide sequence that encodes the E protein of the second flavivirus. Furthermore, this nucleic acid molecule comprises nucleotide mutations that produce one or more amino acid mutations capable of attenuating the virus as described below, in the nucleotide sequence(s) that encode(s) the prM protein and/or NS protein of Japanese encephalitis virus.

The present invention also provides a chimeric flavivirus encoded by such a nucleic acid molecule.

In this specification, "a nucleic acid molecule" means a single-stranded or double-stranded DNA or RNA.

In this specification, "a nucleotide sequence" means a sequence of deoxyribonucleotide (shown by A, G, C, and T) or a sequence of ribonucleotide (shown by A, G, C, and U) unless otherwise specified.

In this specification, the left end represents the 5'

terminus and the right end represents the 3' terminus for a single-stranded nucleotide sequence; the left end represents the N terminus (amino terminus) and the right end represents the C terminus (carboxyl terminus) for an amino acid sequence
5 unless otherwise specified.

In this specification, amino acids are shown using 1-letter abbreviation or 3-letter abbreviation in the standard denotation system for amino acids unless otherwise specified.

In this specification, "attenuated" means that a virus is
10 low virulent (low toxic) such that the virus can be used safely as a vaccine for an animal subject to vaccination {for example, human and non-human mammals (for example, monkeys, horses, cattle, sheep, swine, dogs, cats, rabbits, rats, mice and the like), and birds and the like}.

15 In this specification, "the virus can be used (safely) as a vaccine" means that growth of the virus is observed at the site of inoculation of the vaccine but ends without manifesting serious symptoms, and that specific immunity is conferred which prevents the onset of a disease caused by a highly virulent
20 virus inoculated in a subsequent challenge test with the virus against an individual inoculated with the vaccine.

Japanese encephalitis virus used as a first flavivirus to prepare the chimeric flavivirus of the present invention is not subject to limitation, as long as the prM protein and/or NS
25 protein of the chimeric flavivirus of the present invention finally comprises one or more amino acid mutations that attenuate the virus as described below.

Therefore, any strain may be used out of the various strains of Japanese encephalitis virus (for example, ML-17
30 strain, JaOH0566 strain, JaOArS982 strain, JaGAr01 strain, Nakayama strain, Beijing strain, SA14 strain, SA14-14-2 strain and the like).

The genomes of various Japanese encephalitis virus strains have been cloned, and the complete or partial

nucleotide sequences thereof have been determined. See, for example, Sumiyoshi et al., Virology 161:497-510, 1987 for the JaOArS982 strain; McAda et al., Virology 158:348-360, 1987 for the Nakayama strain; Hashimoto et al., Virus Genes 1:305-317, 5 1988 for the Beijing strain; Nitayaphan et al., Virology 177:541-552, 1990 for the SA14 strain and SA14-14-2 strain.

Information on the genome sequences of various Japanese encephalitis viruses can also be obtained from publicly accessible gene databases such as GenBank. See, for example, 10 GenBank accession number: M18370 for the JaOArS982 strain; GenBank accession number: AF069076 for the JaGAR01 strain; GenBank accession number: L48961 for the Beijing-1 strain; GenBank accession number: M55506 for the SA14 strain; GenBank accession number: AF315119 for the SA14-14-2 strain.

15 To prepare a nucleic acid molecule comprising a nucleotide sequence that encodes the chimeric virus of the present invention, a nucleic acid molecule comprising a nucleotide sequence that encodes Japanese encephalitis virus is prepared, and can be used as the gene backbone for chimeric 20 flavivirus. As examples of the nucleic acid molecule comprising a nucleotide sequence that encodes Japanese encephalitis virus, genomic RNA, cDNA, synthetic RNA, synthetic DNA and the like can be mentioned.

Alternatively, to prepare a nucleic acid molecule 25 comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention, any fragment (for example, PCR-amplified DNA fragment and the like) of a nucleic acid molecule comprising a nucleotide sequence that encodes Japanese encephalitis virus can be used.

30 The genomic RNA of Japanese encephalitis virus can be prepared by a commonly known method from cells (for example, MMC-LK2 cells, HeLa cells, N2a cells, PS cells, BSC-1 cells, HL-CZ cells, LLC-MK2 cells, Vero cells, BHK cells, mosquito-derived C6/36 cells, mouse- or hamster-derived intracerebral

cells), growing chicken eggs and the like infected with Japanese encephalitis virus. Japanese encephalitis virus strain used to prepare a genomic RNA is not subject to limitation; for example, the strains as listed above can be
5 used.

A cDNA of Japanese encephalitis virus can be constructed from a genomic RNA according to a commonly known method (for example, the method described in Sumiyoshi et al., Virology 161:497-510, 1987).

10 Alternatively, a genomic RNA or cDNA of Japanese encephalitis virus or any fragment thereof can also be chemically synthesized on the basis of genome sequence information on Japanese encephalitis virus by a commonly known method.

15 The genomic RNA or cDNA of Japanese encephalitis virus or any fragment thereof can be amplified with a genomic RNA or cDNA as the template by Polymerase Chain Reaction (abbreviated as "PCR method"), Reverse Transcriptase-Polymerase Chain Reaction (abbreviated as "RT-PCR method"), Long Polymerase
20 Chain Reaction (abbreviated as "Long-PCR method"), and/or Long-Reverse Transcriptase-Polymerase Chain Reaction (abbreviated as "Long-RT-PCR method").

Alternatively, a genomic RNA or cDNA of Japanese encephalitis virus or any fragment thereof can also be inserted
25 to a vector and cloned.

As examples of the vector used, plasmids such as pBR322, pBR325, pBR327, pBR328, pUC7, pUC8, pUC9, pCU18, pUC19, pHSG298, pHSG299, pSC101, pGBM5, and pCRII can be mentioned. As the cloning vector, bacteriophage, cosmid, phagemid and the like
30 can also be used. These cloning vectors are commercially available from, for example, NIPPON GENE CO., LTD. and the like.

As the second flavivirus used in the present invention, a flavivirus other than Japanese encephalitis virus, for example, West Nile virus, dengue-4 virus, yellow fever virus, St. Louis

encephalitis virus, tick-borne encephalitis virus, Kunjin virus, Central European encephalitis virus, Kyasanur Forest virus, Murray Valley encephalitis virus, Omsk hemorrhagic fever virus, Powassan virus, Russian spring-summer encephalitis virus,
5 Yokose virus, Apoi virus, Aroa virus and the like can be mentioned. For these flavivirus species as well, any strain may be used out of the various mutant strains thereof.

Nucleotide sequences and amino acid sequences that encode the E proteins of various flaviviruses as described above are
10 commonly known, and furthermore, for many of these flavivirus species, the entire nucleotide sequence of the genome thereof has been reported. See, for example, the following: West Nile virus (for example, Wengler et al., Virology 147:264-274, 1985); dengue-1 virus (for example, Mason et al., Virology
15 161:262-267, 1987); dengue-2 virus (for example, Deubel et al., Virology 155:365-377, 1986; Gruenberg et al., J. Gen. Virol. 69:1391-1398, 1988; Hahn et al., Virology 162:167-180, 1988); dengue-3 virus (for example, Osatomi et al., Virus Genes 2:99-108, 1988); dengue-4 virus (for example, Mackow et al.,
20 Virology 159:217-228, 1987; Zhao et al., Virology 155:77-88, 1986); yellow fever virus (for example, Rice et al., Science 229:726-733, 1985); St. Louis encephalitis virus (for example, Trent et al., Virology 156:293-304, 1987); tick-borne encephalitis virus (for example, Mandl et al., Virology
25 166:197-205, 1988); Kunjin virus (for example, Coia et al., J. Gen. Virol. 69(Pt 1):1-21, 1988); Kyasanur Forest virus (for example, Venugopal et al., Journal of General Virology 75:227-232, 1994; Kuno et al., Journal of Virology 72:73-83, 1998); Murray Valley encephalitis virus (for example, Dalgarno et al.,
30 J. Mol. Biol. 187:309-323, 1986); Omsk hemorrhagic fever virus (for example, Lin et al., Virology 313:81-90, 2003; Li et al., Journal of General Virology 85:1619-1624, 2004; Gritsun et al., Journal of General Virology 74:287-291, 1993); Powassan virus (for example, Kuno et al., Am. J. Trop. Med. Hyg. 65:671-676,

2001; Mandl et al., Virology 194:173-184, 1993); Russian
spring-summer encephalitis virus (for example, Kuno et al.,
Journal of Virology 72:73-83, 1998); Apoi virus (for example,
Billoir et al., Journal of General Virology 81:781-790, 2000);
5 Aroa virus (for example, Gaunt et al., Journal of General
Virology 82:1867-1976, 2001).

Information on the nucleotide sequences of the genomes of
various flaviviruses can also be obtained from publicly
accessible gene databases such as GenBank. See, for example,
10 the following: West Nile virus (for example, GenBank accession
number: M12294; NC_001563); dengue-1 virus (for example,
GenBank accession number: M23027); dengue-2 virus (for example,
GenBank accession number: M19197; NC_001474); dengue-3 virus
(for example, GenBank accession number: M93130); dengue-4 virus
15 (for example, GenBank accession number: M14931); yellow fever
virus (for example, GenBank accession number: X03700;
NC_002031); St. Louis encephalitis virus (for example, GenBank
accession number: M16614); tick-borne encephalitis virus (for
example, GenBank accession number: U27495; NC_001672); Kunjin
20 virus (for example, GenBank accession number: AY274504;
AY274505); Kyasanur Forest virus (for example, GenBank
accession number: X74111); Murray Valley encephalitis virus
(for example, GenBank accession number: AF161266; NC_000943);
Omsk hemorrhagic fever virus (for example, GenBank accession
25 number: AY193805; AY438626; X66694; NC_005062); Powassan virus
(for example, GenBank accession number: AF310922; AF310920;
AF310912; L06436; NC_003687); Yokose virus (for example,
GenBank accession number: AB114858; NC_005039); Apoi virus (for
example, GenBank accession number: AF160193; NC_003676); Aroa
30 virus (for example, GenBank accession number: AF372413).

To prepare a nucleic acid molecule comprising a
nucleotide sequence that encodes the chimeric virus of the
present invention, a nucleic acid molecule comprising a
nucleotide sequence that encodes a second flavivirus is

prepared, and a region that encodes the E protein thereof can be used. As examples of the nucleic acid molecule comprising a nucleotide sequence that encodes a second flavivirus, genomic RNA, cDNA, synthetic RNA, synthetic DNA and the like can be
5 mentioned.

Alternatively, to prepare a nucleic acid molecule comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention, any fragment (for example, PCR-amplified DNA fragment and the like) of a nucleic acid
10 molecule comprising a nucleotide sequence that encodes a second flavivirus can be used.

The genomic RNA of a second flavivirus can be prepared by the same method as that for Japanese encephalitis virus.

A cDNA of a second flavivirus can also be constructed
15 from a genomic RNA by the same technique as that for Japanese encephalitis virus.

Alternatively, a genomic RNA or cDNA of a second flavivirus or any fragment thereof can also be chemically synthesized on the basis of commonly known genome sequence
20 information on the virus used as the second flavivirus, by a commonly known method.

The genomic RNA or cDNA of a second flavivirus or any fragment thereof can be amplified by the PCR method, RT-PCR method, Long-PCR method and/or Long-RT-PCR method, with a
25 genomic RNA or cDNA as the template, as in Japanese encephalitis virus.

Alternatively, a genomic RNA or cDNA of a second flavivirus or any fragment thereof can also be inserted to an appropriate vector as listed above and cloned, as in Japanese
30 encephalitis virus.

A nucleic acid molecule (DNA or RNA) comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention is prepared by replacing a nucleotide sequence that encodes the E protein in a nucleic acid molecule

comprising a nucleotide sequence that encodes Japanese encephalitis virus with a nucleotide sequence that encodes the envelope protein of a second flavivirus.

Replacement of the region that encodes the E protein in
5 a nucleic acid molecule comprising a nucleotide sequence that encodes Japanese encephalitis virus can be performed by a commonly known recombinant technology (for example, the method utilizing the Long-PCR method described in Morita et al., Virology 287:417-426, 2001, and the like).

10 Alternatively, a nucleic acid molecule (DNA or RNA) comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention can also be prepared by chemical synthesis by designing the entire nucleotide sequence that encodes the chimeric flavivirus of the present invention,
15 on the basis of genome sequence information on Japanese encephalitis virus and a second flavivirus.

When a nucleic acid molecule comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention is prepared as DNA, a promoter sequence for in vitro
20 transcription is introduced to the 5' terminus of the DNA. The DNA that encodes the chimeric flavivirus of the present invention is transcribed to RNA by an RNA polymerase corresponding to the promoter introduced, at any stage prior to expression of the chimeric flavivirus protein. As examples
25 of the promoter sequence used, the T7 RNA polymerase promoter, SP6 RNA polymerase promoter and the like can be mentioned.

The RNA that encodes the chimeric flavivirus of the present invention is introduced by a commonly known gene introduction technology in the art such as transfection,
30 electroporation, or microinjection, into cells suitable for protein expression (for example, C6/36 cells, Vero cells, BHK cells, MMC-LK2 cells, HeLa cells, N2a cells, PS cells and the like), and the chimeric flavivirus protein is expressed in these cells.

Alternatively, for the chimeric flavivirus of the present invention, the chimeric flavivirus protein can be obtained by utilizing a method of protein production not using a cell system, wherein a genetic information translation system for the organism is provided in a test tube by the addition of a substrate, enzyme and the like to cell homogenate or extract (also referred to as cell-free system expression; see, for example, US Patent No. 5,478,730; Madin et al., Proc. Natl. Acad. Sci. USA 97:559-564, 2000; Sawasaki et al., Proc. Natl. Acad. Sci. USA 99:14652-14657, 2002).

The nucleic acid molecule comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention comprises a nucleotide mutation that produces one or more amino acid mutations that attenuate the virus in the nucleotide sequence that encodes the prM protein and/or NS protein derived from Japanese encephalitis virus in this nucleic acid molecule.

In detail, when expressed with the amino acid sequences of the individual structural proteins and non-structural proteins of the Japanese encephalitis virus JaOArS982 strain (see H. Sumiyoshi et al., Virology 161:497-510, 1987) as a reference, the amino acid mutations that attenuate the virus are the following amino acid substitutions: substitution of the 1st methionine of the prM protein by isoleucine; substitution of the 148th asparagine of the prM protein (56th of M protein) by threonine; substitution of the 4th alanine of the NS2A protein by serine; substitution of the 51st asparagine of the NS4B protein by lysine; substitution of the 52nd valine of the NS4B protein by isoleucine; substitution of the 68th threonine of the NS4B protein by serine; substitution of the 126th leucine of the NS5 protein by methionine; and/or substitution of the 854th serine of the NS5 protein by asparagine.

Alternatively, the above-described amino acid substitutions can also be performed using conservative amino

acids for the amino acids introduced at the respective amino acid positions.

In this specification, "conservative amino acids" mean amino acids similar to each other in terms of physicochemical properties; examples thereof include amino acids classified under the same group, such as aromatic amino acids (Phe, Trp, Tyr), aliphatic amino acids (Ala, Leu, Ile, Val), polar amino acids (Gln, Asn), basic amino acids (Lys, Arg, His), acidic amino acids (Glu, Asp), amino acids having a hydroxyl group (Ser, Thr) and amino acids having a small side-chain (Gly, Ala, Ser, Thr, Met).

Preferably, the chimeric flavivirus of the present invention comprises at least substitution of the 1st methionine of the prM protein by isoleucine and/or substitution of the 148th asparagine of the prM protein (56th of M protein) by threonine, out of the above-described amino acid substitutions.

The above-described amino acid mutations can be introduced in any step for preparing a nucleic acid molecule comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention.

Introduction of the above-described amino acid mutations can be performed using, for example, the site-directed mutagenesis method utilizing the Long-PCR method, described in Morita et al., Virology 287:417-426, 2001, or by applying a commonly known method such as the Kunkel method or the gapped duplex method or a mutagenesis kit utilizing these methods (available from, for example, Takara Bio Inc.) and the like to a cDNA cloned to plasmid.

Alternatively, these mutations can be introduced to the chimeric flavivirus of the present invention by using a nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the prM and/or NS protein to prepare a nucleic acid molecule comprising a nucleotide

sequence that encodes the chimeric flavivirus of the present invention.

A nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the prM and/or NS protein can be prepared by artificially introducing these mutations to a genomic RNA or cDNA of a Japanese encephalitis virus strain not containing these mutations (for example, JaOH0566 strain, JaOArS982 strain, JaGAR01 strain, Nakayama strain, Beijing strain, SA14 strain, SA14-14-2 strain and the like), using the aforementioned commonly known method of site-directed mutagenesis.

A recombinant Japanese encephalitis virus can be prepared by expressing a virus protein from the nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the prM and/or NS protein, thus artificially prepared, by a commonly known method as described above.

By infecting this recombinant Japanese encephalitis virus to suitable cells as described above, and culturing the cells, a large amount of genomic RNA can be prepared from the cultured cells.

Furthermore, this recombinant Japanese encephalitis virus is an attenuated Japanese encephalitis virus per se, and can be a promising candidate for an attenuated live vaccine for Japanese encephalitis.

A nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the prM and/or NS protein can also be prepared by acquiring a genomic RNA or cDNA from a Japanese encephalitis virus mutant strain natively comprising one or more such amino acid mutations.

As examples of the Japanese encephalitis virus mutant

strain natively comprising one or more amino acid mutations that attenuate the virus in the prM and/or NS protein, the Japanese encephalitis virus ML-17 strain can be mentioned. The ML-17 strain is a Japanese encephalitis virus vaccine strain
5 for swine (see, for example, Yoshida et al., BIKEN JOURNAL 24:47-67, 1981), and is available from The Research Foundation for Microbial Diseases of Osaka University (based in Osaka University, 3-1, Yamadaoka, Suita-shi, Osaka).

Alternatively, from among mutant strains of Japanese
10 encephalitis virus resulting from passage in cells and the like, a strain having one or more amino acid mutations that attenuate the virus in the prM and/or NS protein may be selected by a commonly known method, and a genomic RNA or cDNA thereof can also be used to prepare the chimeric flavivirus of the present
15 invention.

The nucleic acid molecule (genomic RNA or cDNA) comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the prM and/or NS protein, thus prepared,
20 may be inserted to a cloning vector as described above after being fragmented as required. For example, utilizing such a recombinant vector, the chimeric flavivirus of the present invention can be prepared safely and conveniently.

Construction of the chimeric flavivirus of the present
25 invention can be confirmed by, for example, acquiring the gene from the chimeric virus prepared, determining all or a portion of the base sequence thereof or corresponding amino acid sequence (for example, the portion where the mutation has been introduced, the joint of the two viruses and the like), and
30 confirming that the sequence matches with the intended sequence.

The present invention also provides an attenuated live vaccine comprising the chimeric flavivirus of the present invention (hereinafter also referred to as the vaccine of the present invention). Because the E protein of virus contains an

antigenic determinant that induces a neutralizing antibody, inoculation of the chimeric flavivirus of the present invention as a vaccine results in the production of an antibody against the second flavivirus species used as the E protein.

5 To prevent various flavivirus infections, the vaccine of the present invention can be inoculated to, for example, animals such as humans and non-human mammals (for example, monkeys, horses, cattle, sheep, swine, dogs, cats, rabbits, rats, mice and the like), and birds.

10 The vaccine of the present invention can be produced in the form of a suspension or freeze-dried preparation. The vaccine of the present invention can comprise, in addition to the chimeric flavivirus of the present invention, a pharmaceutically acceptable stabilizer (for example, sucrose,
15 lactose, glucose, gelatin, gelatin hydrolysates, sodium L-glutamate, serum albumin and the like), soothing agent (for example, glucose and the like) and the like in common use in vaccine preparations.

 Upon vaccination, the vaccine of the present invention
20 can normally be dissolved or suspended in a pharmaceutically acceptable carrier. As examples of the carrier, liquid carriers such as water, saline (including physiological saline), and buffer solutions (for example, phosphate buffer solution) can be mentioned.

25 The vaccine of the present invention is representatively prescribed as a sterile aqueous solution containing 10^2 to 10^6 PFU of the chimeric flavivirus of the present invention per a dose of 0.1 to 1.0 ml, and can be inoculated, for example, subcutaneously, intradermally, intramuscularly and the like.

30 Alternatively, because some flaviviruses are known to infect via the mucosa, the vaccine of the present invention may be administered orally or transnasally according to the second flavivirus species chosen.

 Furthermore, a nucleic acid molecule (RNA or DNA)

comprising a nucleotide sequence that encodes the chimeric flavivirus of the present invention per se can also be used as a nucleic acid vaccine preparation.

Confirmation of the efficacy and safety of the chimeric virus constructed can be performed by, for example, evaluating brain neurotoxicity, infectivity from peripheral to the central nervous system (brain nerve invasiveness), infection-preventing effect, presence or absence of viremia, neutralizing antibody production and the like in animals (for example, mice, monkeys and the like) by a commonly known method of evaluation in the art.

The present invention is hereinafter described in more detail by means of the following Examples, which, however, are for illustrative purposes only and never limit the scope of the invention.

Examples

(Example 1)

(Determination of attenuating mutation sites of the Japanese encephalitis virus vaccine ML-17 strain)

To determine the attenuating mutation sites of the Japanese encephalitis virus vaccine ML-17 strain, first, the nucleotide sequence of full-length genomic cDNA and the amino acid sequence of polyprotein were compared between the ML-17 strain and its parent strain, i.e., the wild-type virulent Japanese encephalitis virus JaOH0566 strain.

The nucleotide sequence of full-length genomic cDNA of the ML-17 strain (obtained from The Research Foundation for Microbial Diseases of Osaka University) (SEQ ID NO:1) and the nucleotide sequence of full-length genomic cDNA of the JaOH0566 strain (SEQ ID NO:3) were determined according to the method disclosed in Sumiyoshi et al., Virology 161:497-510, 1987. For both strains, the cDNAs were constructed using the PCR primers shown in Table 1A, and the nucleotide sequences of the cDNAs constructed were determined using the primers for sequencing

shown in Table 1B. Furthermore, the amino acid sequence of the polyprotein of the ML-17 strain (SEQ ID NO:2) and the amino acid sequence of the polyprotein of the JaOH0566 strain (SEQ ID NO:4) were deduced from the nucleotide sequences of the
5 respective full-length genomic cDNAs.

(Table 1A)

Japanese encephalitis virus (JEV) PCR primers

Name of primer	Corresponding nucleotide position on the genome*	Nucleotide sequence
JEV 1-34F	1-34-F	AGAAGTTTATCTGTGTGAACCTCTTGGCTTAGTA (SEQ ID NO:5)
JEV 2851-2817R	2851-2817-R	GCAAAGAGAATGCTTTTTCCCATGCTTTCCAGCC (SEQ ID NO:6)
JEV 2707-2741F	2707-2741-F	TCCTGCTCAAAGAGAATGCAGTGGACCTCAGTGTG (SEQ ID NO:7)
JEV 5554-5520R	5554-5520-R	GTCATGAAGATGGCTGCTGCCTCCCCTAATTCCAC (SEQ ID NO:8)
JEV 5414-5449F	5414-5449-F	ACTGATGTCACCGAACAGAGTGCCCAACTACAACCT (SEQ ID NO:9)
JEV 8257-8223R	8257-8223-R	TTTTCTATAACCTTGGGCATGTAAGGGCAGAGAAC (SEQ ID NO:10)
JEV 8119-8153F	8119-8153-F	GGGAATCCTCTCCAAGTCCAGAAGTAGAAGAACAA (SEQ ID NO:11)
JEV 10976-10943R	10976-10943-R	AGATCCTGTGTTCTTCCTCACCACCAGCTACATA (SEQ ID NO:12)

10 (Table 1B)

Primers for Japanese encephalitis virus (JEV) sequencing

Name of primer	Corresponding nucleotide position on the genome*	Nucleotide sequence
JEV 1-20F	1-20-F	AGAAGTTTATCTGTGTGAAC (SEQ ID NO:13)
JEV 421-440F	421-440-F	AAGGCTCAATCATGTGGCTC (SEQ ID NO:14)
JEV 821-840F	821-840-F	GAAAGCCACACGGTATCTCA (SEQ ID NO:15)

JEV 1174-1193F	1174-1193-F	CTGACATCTCGACGGTGGCT (SEQ ID NO:16)
JEV 1559-1578F	1559-1578-F	TGGACTGAACACTGAAGCGT (SEQ ID NO:17)
JEV 1942-1961F	1942-1961-F	TTGTCATTGAACATATCCTAC (SEQ ID NO:18)
JEV 2326-2345F	2326-2345-F	GAACACTCTTTGGGGGAATG (SEQ ID NO:19)
JEV 2520-2539F	2520-2539-F	TGTGGAAGTGGCATCTTTGT (SEQ ID NO:20)
JEV 2746-2765F	2746-2765-F	TGAACAAGCCCGTGGGAAGA (SEQ ID NO:21)
JEV 3147-3166F	3147-3166-F	ACTTGGCCAGAGACACACAC (SEQ ID NO:22)
JEV 3538-3557F	3538-3557-F	ATGGTGAAATGGTTGACCCT (SEQ ID NO:23)
JEV 3935-3954F	3935-3954-F	AGCATGGATGATTGTCCGAG (SEQ ID NO:24)
JEV 4111-4130F	4111-4130-F	AGAAAGGAGCTGTACTCTTG (SEQ ID NO:25)
JEV 4339-4358F	4339-4358-F	CCTACGTGGTGTGAGGAAAA (SEQ ID NO:26)
JEV 4741-4760F	4741-4760-F	TTTTCCACACACTATGGCAC (SEQ ID NO:27)
JEV 5112-5131F	5112-5131-F	GACCGTCAGGAGGAACCAGT (SEQ ID NO:28)
JEV 5517-5535F	5517-5535-F	AAGGTGGAATTAGGGGAGG (SEQ ID NO:29)
JEV 5914-5932F	5914-5932-F	AAGAGGGAGAAGGCAGAGT (SEQ ID NO:30)
JEV 6319-6338F	6319-6338-F	ATGCCATACTGGAGGACAAC (SEQ ID NO:31)
JEV 6685-6704F	6685-6704-F	GAAAGGGTATAGGGAAGATG (SEQ ID NO:32)
JEV 7106-7125F	7106-7125-F	CACCACATCGCTAGCCTCAA (SEQ ID NO:33)
JEV 7508-7527F	7508-7527-F	GGTATTGGTGACGGCGGCTA (SEQ ID NO:34)
JEV 7920-7939F	7920-7939-F	TGTGGGCGTGGAGGATGGAG (SEQ ID NO:35)
JEV 8292-8311F	8292-8311-F	CTAGTGCGTCTCCCCCTGTC (SEQ ID NO:36)
JEV 8713-8732F	8713-8732-F	CTGACACCACCCCTTTTGA (SEQ ID NO:37)
JEV 9144-9162F	9144-9162-F	GCTTTGGGGTTCCTGAATG (SEQ ID NO:38)
JEV 9470-9488F	9470-9488-F	AGAAGATCAAAGGGGGAGT (SEQ ID NO:39)
JEV 9870-9889F	9870-9889-F	CCGTGCAGAGGACAGGATGA (SEQ ID NO:40)
JEV 10281-	10281-10298-F	TATGCGGCGATAAACCAG

10298F		(SEQ ID NO:41)
JEV 10662-10679F	10662-10679-F	GCGCAGCCCCAGGAGGAC (SEQ ID NO:42)
JEV 375-356R	375-356-R	CGTCAATGAGTGTTCCTCAAGT (SEQ ID NO:43)
JEV 3084-3065R	3084-3065-R	CAATCCAGTACGACAAGTCA (SEQ ID NO:44)
JEV 5757-5738R	5757-5738-R	TGACCTTTTTTCCCGCTCTT (SEQ ID NO:45)
JEV 8518-8499R	8518-8499-R	TTCTTGATTTTCTCCTGATT (SEQ ID NO:46)
JEV 10976-10955R	10976-10955-R	AGATCCTGTGTTCTTCCTCACC (SEQ ID NO:47)

*: F represents a forward primer; R represents a reverse primer.

As a result of a comparison of the nucleotide sequences of the genomic cDNAs, it was found, as shown in Figure 1, that the ML-17 strain had 25 nucleotide substitutions, with 10 amino acid substitutions produced due to 10 of these nucleotide substitutions, at the 127th, 274th, 1209th, 2462nd, 2463rd, 2479th, 2652nd, 2751st, 2896th, and 3380th amino acids, as counted from the amino acid next to the starting methionine of the polyprotein.

Furthermore, the above-described amino acid mutations in the polyprotein of the ML-17 strain were compared with the amino acid sequences of the corresponding sites of the polyproteins of the JaOArS982 strain, JaGAr01 strain, Nakayama strain, Beijing strain, and SA14 strain, which are other wild-type virulent Japanese encephalitis viruses, and the SA14-14-2 strain, which is another Japanese encephalitis virus vaccine.

The putative amino acid sequences of the polyproteins of the following strains were obtained from GenBank and used in this Example:

Amino acid sequence of the polyprotein of the JaOArS982 strain (GenBank accession number: M18370);

Amino acid sequence of the polyprotein of the JaGAr01 strain (GenBank accession number: AF069076);

Amino acid sequence of the polyprotein of the Beijing-1 strain

(GenBank accession number: L48961);

Amino acid sequence of the polyprotein of the SA14 strain

(GenBank accession number: M55506);

Amino acid sequence of the polyprotein of the SA14-14-2 strain

5 (GenBank accession number: AF315119).

For the amino acid sequence of the polyprotein of the Nakayama strain, the amino acid sequence of the region necessary for the comparison was obtained from the gene and/or amino acid partial sequence information described in GenBank
10 accession number: AF112297; McAda et al., Virology. 158(2):348-60, 1987 and the like, and used in this Example.

As shown in Figure 2, as a result of a comparison of the amino acid sequences of the polyproteins, a total of eight amino acid substitutions at the 127th, 274th, 1209th, 2462nd,
15 2463rd, 2479th, 2652nd, and 3380th amino acid positions, as counted from the amino acid next to the starting methionine of the polyprotein, out of the above-described 10 amino acid substitutions between the ML-17 strain and the JaOH0566 strain, were found to be amino acid mutations intrinsic to the ML-17
20 strain.

From a comparison with the positions in the individual structural proteins and non-structural proteins in the polyprotein of the Japanese encephalitis virus (JaOArS982 strain) described in Sumiyoshi et al., Virology 161:497-510,
25 1987, it was found that the positions of these eight amino acid substitutions in the polyprotein corresponded to the 1st and 148th amino acids of the prM protein (56th of M protein); the 4th amino acid of the NS2A protein; the 51st, 52nd, and 68th amino acids of the NS4B protein; and the 126th and 854th amino
30 acids of the NS5 protein, respectively.

(Example 2)

(Preparation of recombinant Japanese encephalitis virus MS-14 strain and MS-15 strain)

To confirm the effects of amino acid mutations intrinsic to the ML-17 strain on the attenuation of the virus, according to the site-directed mutagenesis method utilizing the Long-PCR method, described in Morita et al., Virology 287:417-426, 2001, 5 using the gene of the wild-type virulent Japanese encephalitis virus JaOArS982 strain as the backbone, MS-14 strain, which is a recombinant Japanese encephalitis virus incorporating a nucleotide mutation that produces substitution of the 1st methionine of the prM protein by isoleucine (substitution of G 10 by A at position 479 of the genome), and MS-15 strain, which is a recombinant Japanese encephalitis virus incorporating a nucleotide mutation that produces substitution of the 148th asparagine of the prM protein (56th of the M protein) by threonine (substitution of A by C at position 919 of the 15 genome), were prepared.

(Preparation of the MS-14 strain)

As shown in Figure 3, with the gene RNA of Japanese encephalitis virus (JaOArS982 strain) as the template, using a set of primer 1 and primer 2, a set of primer 3 and primer 4, 20 and a set of primer 5 and primer 6, by the Long-RT-PCR method, Japanese encephalitis virus gene fragments corresponding to the respective primer sets (fragments 1, 2, and 3) were prepared.

Furthermore, after each fragment was purified by agarose electrophoresis, first, with fragments 1 and 2 as the templates, 25 using a set of primers 1 and 4, again by the Long-PCR method, gene fragment 4 was prepared. This gene fragment was purified in the same manner; next, with gene fragments 3 and 4 as the templates, using a set of primers 1 and 6, the Long-PCR method was performed to prepare a full-length Japanese encephalitis 30 virus cDNA having the T7 promoter sequence at the 5' terminus thereof (fragment 5).

With this full-length Japanese encephalitis virus cDNA (fragment 5) as the template, an in vitro RNA synthesis reaction was performed to prepare an artificial full-length

Japanese encephalitis virus gene RNA. This RNA was introduced into mosquito cells (C6/36 cells) by the electroporation method and the cells were cultured for 5 days, after which the recombinant virus (MS-14 strain) emerging in the culture supernatant was recovered.

(Preparation of the MS-15 strain)

For the MS-15 strain, the recombinant virus was recovered according to the above-described method except that the recombinant virus was prepared using primer 7 instead of primer 2, and primer 8 instead of primer 3.

Furthermore, for control, a mutation-free virus was acquired using primer 9 instead of primer 2, and primer 10 instead of primer 3.

The primers used in this Example are shown in Table 2.

15

Name of primer	Corresponding nucleotide position on the genome*	Nucleotide sequence*
primer 1	T7 promoter-1-45-S	AAATTTAATACGACTCACTATAAGAAGTTTATCTGT GTGAACTTCTTGGCTTAGTATCGTTG (SEQ ID NO:48)
primer 9	972-927-R	AAGCCGGAGCGACCAACAGCAGGAGGATGGTAAATA CCACGCGTTG (SEQ ID NO:56)
primer 10	927-972-S	CAACGCGTGGTATTTACCATCCTCCTGCTGTTGGTC GCTCCGGCTT (SEQ ID NO:57)
primer 4	2670-2626-R	GCTCCAATCTAGTGACAGATCTGACTCCGCACACGC CTTCCTTGT (SEQ ID NO:51)
primer 5	2501-2547-S	CACAAGAAAAGAGATGAGATGTGGAAGTGGCATCTT TGTGCACAACG (SEQ ID NO:52)
primer 6	10976-10937-R	AGATCCTGTGTTCTTCCTCACCACCAGCTACATACT TCGG (SEQ ID NO:53)
primer 3	439-484/5-S (479G→A)	CGCAAGCTTGGCAGTTGTCATAGCTTACGCAGGAGC AATAAAGTTG (SEQ ID NO:50)
primer 2	471-515-R (479G→A)	CGTCATCAAAAGCTTCCCCTGGAAATTCGACAACCTT TATTGCTCC (SEQ ID NO:49)
primer 8	882-926-S (919A→C)	TTCCTGGCGCGACACTTGGCTGGATGCTTGGCAGT ACCAACGGT (SEQ ID NO:55)
primer 7	900-944-R (919A→C)	GGTAAATACCACGCGTTGACCGTTGGTACTGCCAAC CATCCAGCC (SEQ ID NO:54)

In the table above, S represents a sense sequence; R

represents a complementary sequence. The underline represents the T7 promoter sequence; the double underline represents a nucleotide mutation.

5 (Example 3)

(Evaluation of the growing potentials of the recombinant Japanese encephalitis virus MS-14 strain and MS-15 strain in BHK cells)

The growing potentials of the recombinant Japanese
10 encephalitis virus MS-14 strain and MS-15 strain prepared in Example 2 in BHK cells were evaluated. For control, the wild-type virulent Japanese encephalitis virus JaOArS982 strain was used.

Each of the MS-14 strain, MS-15 strain, and JaOArS982
15 strain was infected to BHK cells; 24, 48, and 72 hours after infection, cell culture supernatants were collected and checked for the emergence of virus. The virus contents in the culture supernatants at each time were measured by the plaque method. For all strains, growth in the BHK cells was confirmed.

20

(Example 4)

(Evaluation of the neurotoxicity of the recombinant Japanese encephalitis virus MS-14 strain and MS-15 strain in normal mature mice)

25 Using the recombinant Japanese encephalitis virus MS-14 strain and MS-15 strain prepared in Example 2, the neurotoxicity of each strain was evaluated by the intraperitoneal inoculation method in normal mature mice. For virulent control, the wild-type virulent Japanese encephalitis
30 virus JaOArS982 strain was used; for attenuated control, the Japanese encephalitis virus vaccine ML-17 strain was used.

Ten mice (4-week-old male and female ICR mice, 5 animals in each group) were given 10^1 to 10^6 plaque forming units of the MS-14 strain, MS-15 strain, ML-17 strain, or JaOArS982

strain by intracerebral inoculation, and observed daily for 3 weeks, and LD₅₀ was calculated by the Read-Muench method. The results of this test are shown in Table 3.

5 (Table 3)

Virus strain	Feature	LD ₅₀ (ffu)
JaOH0566	Wild type	6.3×10 ¹
ML-17	Vaccine strain	>1.0×10 ⁶
JaOArS982	Wild type	1.6×10 ¹
MS-14	Recombinant mutant: 127M→I (prM)	>1.0×10 ⁶
MS-15	Recombinant mutant: 274N→T (M)	1.0×10 ³
MS-14 rev*	Wild type (revertant)	3.2×10 ¹

*: MS-14 rev represents a revertant of MS-14, prepared from MS-14 using Long-PCR-based site-directed mutagenesis.

10 As shown in Table 3, the MS-14 strain and MS-15 strain were significantly attenuated compared to the virulent JaOArS982 strain. Particularly, the MS-14 strain had a toxicity similar to that of the ML-17 strain, which is a Japanese encephalitis virus vaccine strain.

15 This demonstrated that neurotoxicity in mice could be reduced by amino acid substitution in the prM protein.

From the results above, it was suggested that amino acid mutations introduced to the MS-14 strain and MS-15 strain, which are recombinant Japanese encephalitis viruses, might be
20 useful in preparing a Japanese encephalitis live vaccine. Furthermore, because MS-14 exhibits a low degree of pathogenicity in high-dose inoculation, it was suggested that a mutation on NS might also be necessary for attenuation.

25 (Example 5)

(Construction of cDNA of chimeric flavivirus of Japanese encephalitis virus and West Nile virus (ML-17/WN (E gene)))

To prepare ML-17/WN (E gene), which is a chimeric flavivirus wherein the E protein of the Japanese encephalitis virus vaccine ML-17 strain is replaced by the E protein of West Nile virus, a cDNA of ML-17/WN (E gene) was constructed in
5 accordance with the method of preparing a recombinant Japanese encephalitis virus utilizing the Long-PCR method, described in Morita et al., Virology 287:417-426,2001.

As shown in Figure 4, with the gene RNA of a Japanese encephalitis virus live vaccine strain (ML-17 strain) as the
10 template, using a set of primer 1A and primer 2A and a set of primer 5A and primer 6A, by the Long-RT-PCR method, Japanese encephalitis virus gene fragments corresponding to the respective primer sets (fragments 1A and 3A) were prepared.

Also, with the gene RNA of West Nile virus (NY99-35262-11
15 strain) as the template, using a set of primer 3A and primer 4A, by the Long-RT-PCR method, West Nile virus gene fragment 2A was prepared.

After each fragment was purified by agarose electrophoresis, first, with fragments 1A and 2A as the
20 templates, using a set of primers 1A and 4A, again by the Long-PCR method, gene fragment 4A was prepared.

This gene fragment was purified in the same manner; then, with gene fragments 3A and 4A as the templates, using a set of primers 1A and 6A, the Long-PCR method was performed to prepare
25 a chimeric virus cDNA (fragment 5A) of ML-17/WN (E gene), which has the T7 promoter sequence at the 5' terminus thereof and the West Nile virus E protein gene.

The primers used in this Example are shown in Table 4.

Name of primer	Corresponding nucleotide position on the genome*	Nucleotide sequence*
primer 5A	JE2478-2513 WN2431-N	GGAGGAGTTCTGCTCTTCCTCTCCGTGAACGTGCAC GCTGACACTGGATGTGCCATTGACATCACAAGAAAA GAG (SEQ ID NO:62)
primer 4A	JE2478-2513 WN2433-R	CTCTTTTCTTGTGATGTCAATGGCACATCCAGTGTC AGCGTGCACGTTACGGAGAGGAAGAGCAGAACTCC T (SEQ ID NO:61)
primer 6A	JE-NR	AGATCCTGTGTTCTTCCTCACCACCAGCTACATACT TCGG (SEQ ID NO:63)
primer 3A	ML17 941 WN 967 S	CACCATCCTCCTGCTGCTGGTCGCTCCGGCTTACAG TTTCAACTGCCTTGGAATGAGCAACAGAGACTTC (SEQ ID NO:60)
primer 2A	ML17 945 WN 967 R	CCAAGAAGTCTCTGTTGCTCATTCCAAGGCAGTTGA AACTGTAAGCCGGAGCGACCAGCAGCAGGAGGAT (SEQ ID NO:59)
primer 1A	<u>T7 promoter</u> JE1-40 N	AAATTTAATACGACTCACTATAAGAAGTTTATCTGT GTGAACTTCTTGGCTTAGTATCGTTG (SEQ ID NO:58)

*: JE represents the Japanese encephalitis virus JaOAr982 strain; ML17 represents the sequence of the Japanese encephalitis virus ML-17 strain; WN represents the sequence of West Nile virus. S or N represents a sense sequence; R represents a complementary sequence. The underline indicates the T7 promoter sequence.

10 (Example 6)

(Production of chimeric flavivirus from cDNA of ML-17/WN (E gene))

With the cDNA of the chimeric flavivirus of Japanese encephalitis virus and West Nile virus, constructed in Example 5 (fragment 5A in Figure 4), as the template, an in vitro RNA synthesis reaction was performed to prepare an artificial full-length chimeric virus gene RNA. This RNA was introduced into mosquito cells (C6/36 cells) by the electroporation method and the cells were cultured for 5 days, after which the chimeric virus emerging in the culture supernatant was recovered.

(Example 7)

(Evaluation of the growing potential of ML-17/WN (E gene) chimeric flavivirus)

5 The chimeric virus used was the ML-17/WN (E gene) as obtained in Example 6. ML-17/WN (E gene) and the ML-17 strain were grown in C6/36 cells and BHK cells, and the infected culture broths were dispensed and stored at -70°C. For both types of cells, an Eagle MEM culture broth supplemented with
10 non-essential amino acids was used, with fetal bovine serum added at a concentration as required (2 to 10%). The infectivity titers of the culture broths in the growth experiment are shown in Table 5. ML-17/WN (E gene) had a growing potential equivalent to that of ML-17.

15

Table 5 Growth of ML-17/WN (E gene) chimeric flavivirus and ML-17 in C6/36 cells and BHK cells

	Virus strain	Titer (ffu/ml)
C6/36	ML-17/WN E gene	2.1×10^7
	ML-17	2.0×10^7
BHK	ML-17/WN E gene	5.3×10^6
	ML-17	4.0×10^5

20 (Example 8)

(Evaluation of the neurotoxicity of ML-17/WN (E gene) chimeric virus)

Using the recombinant chimeric virus ML-17/WN (E gene) prepared in Example 6, the neurotoxicity was evaluated by the
25 intracerebral inoculation method in normal mature mice. Also examined by intraperitoneal administration was infection from peripheral to the central nervous system (brain nerve invasiveness). For control, the JaOH0566 strain, which is a wild-type highly virulent strain Japanese encephalitis viruses,
30 the West Nile virus NY99-3562-11 strain, and the Japanese

encephalitis virus vaccine ML-17 strain, which is an attenuated strain, were used.

C57BL/B6 mice at 4 weeks after birth were given 10^1 to 10^7 of each virus by intracerebral inoculation, and observed for 28 days. LD₅₀ was calculated by the Read-Muench method.

Table 6 shows the LD₅₀ of ML-17/WN (E gene) obtained from the experiment. ML-17/WN (E gene) was significantly attenuated compared to the highly virulent JaOH0566 strain. Particularly, ML-17/WN (E gene) had a toxicity similar to that of the ML-17 strain, which is a Japanese encephalitis virus vaccine strain.

Table 6 LD₅₀ of ML-17/WN (E gene) in C57BL/B6 mice

Virus strain	LD ₅₀ (ffu)
JaOH0566	6.0×10^1
NY99-35262-11	5.0×10^0
ML-17	$>1.0 \times 10^7$
ML-17/WN (E gene)	$>1.0 \times 10^7$

(Example 9)

(Property of the ML-17/WN (E gene) chimeric virus)

The infectivity of the ML-17/WN (E gene) chimeric virus to mosquitoes was investigated. *Culex tritaeniorhynchus* OK7 was allowed to suck a mixture of the virus in rabbit blood (antibody negative) by the transmembrane method. By evaluating the PFU (Plaque-Forming Unit) and the incidence rate in suckling mice, the infection rate was calculated.

After the mosquitoes allowed to suck the chimeric virus by the transmembrane method were reared for 10 days and 21 days, they were emulsified, and demonstration of the presence of the virus was attempted by intracerebral inoculation in suckling mice and PFU evaluation. Even when the ML-17/WN (E gene) virus was allowed to be sucked at not less than 10^2 PFU per mosquito, there was absolutely no evidence for the presence of the virus.

The ML-17/WN (E gene) chimeric virus did not exhibit

susceptibility to *Culex tritaeniorhynchus*.

(Example 10)

(Evaluation of the defensive potency of a live vaccine
5 comprising the ML-17/WN (E gene) chimeric virus)

An immunization experiment was performed using 2-week-old
C57BL/B6 and C3H/He mice.

An investigation was performed using the two inbred lines
of mice. The non-immunized control group was fatally infected
10 by an intracerebral challenge with West Nile virus (NY99-35262-
11 strain), whereas the group given ML-17/WN (E gene) by
intraperitoneal inoculation exhibited significant defense
against infection.

Industrial Applicability

15 According to the present invention, an attenuated
chimeric flavivirus having attenuating mutation(s) in
portion(s) other than the E protein is provided. Because the
attenuation of the chimeric flavivirus of the present invention
can be achieved without modifying the E protein, attenuated
20 live vaccines for various flavivirus infections can be brought
into practical application in a short time, without reducing
the immune induction potential.

This application is based on a patent application No.
2004-374630 filed in Japan, the contents of which are
25 entirely incorporated herein by reference.

Claims

1. A nucleic acid molecule comprising nucleotide sequences that encode the capsid protein, pre-membrane protein and non-structural protein of Japanese encephalitis virus, and a nucleotide sequence that encodes the envelope protein of a second flavivirus,
wherein the nucleotide sequence(s) that encode(s) the pre-membrane protein and/or non-structural protein of Japanese encephalitis virus comprise(s) nucleotide mutations that produce one or more amino acid mutations that attenuate the virus,
and wherein the nucleotide sequence that encodes the envelope protein of the second flavivirus comprises no modification.
2. A nucleic acid molecule of claim 1 wherein the second flavivirus is a virulent virus.
3. The nucleic acid molecule of claim 2, wherein the position of the amino acid mutation that attenuates said Japanese encephalitis virus is at least selected from a group consisting of the 127th, 274th, 1209th, 2462nd, 2463rd, 2479th, 2652nd, 2751st, 2896th and 3380th, counted from the amino acid next to a starting methionine of a polyprotein.
4. The nucleic acid molecule of claim 3, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is at least selected from a group consisting of the 127th isoleucine, 274th threonine, 1209th serine, 2462nd lysine, 2463rd isoleucine, 2479th serine, 2652nd methionine, 2751st alanine, 2896th alanine and 3380th asparagine, counted from the amino acid next to the starting methionine of the polyprotein.
5. The nucleic acid molecule of claim 4, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 127th isoleucine, 274th threonine, 1209th serine, 2462nd lysine, 2463rd isoleucine, 2479th serine, 2652nd methionine, 2751st alanine, 2896th alanine and 3380th asparagine, counted from the amino acid next to the starting methionine of the polyprotein.
6. The nucleic acid molecule of claim 4, wherein the amino acid mutation that

attenuates said Japanese encephalitis virus is the 127th isoleucine, counted from the amino acid next to the starting methionine of the polyprotein.

7. The nucleic acid molecule of claim 4, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 274th threonine, counted from the amino acid next to the starting methionine of the polyprotein.

8. The nucleic acid molecule of claim 5, wherein the Japanese encephalitis virus is a ML-17 strain.

9. The nucleic acid molecule of claim 6, wherein the Japanese encephalitis virus is a MS-14 strain.

10. The nucleic acid molecule of claim 7, wherein the Japanese encephalitis virus is the MS-15 strain.

11. The nucleic acid molecule of claim 5, said Japanese encephalitis virus is encoded by a nucleotide sequence of a full-length genomic cDNA of a ML-17 strain shown by SEQ ID NO:1.

12. The nucleic acid molecule of any one of claims 1 to 11, wherein the second flavivirus is selected from a group consisting of West Nile virus, dengue 1-4 virus, yellow fever virus, St. Louis encephalitis virus, tick-borne encephalitis virus, Kunjin virus, Central European encephalitis virus, Kyasanur Forest virus, Murray Valley encephalitis virus, Omsk hemorrhagic fever virus, Powassan virus, Russian spring-summer encephalitis virus, Yokose virus, Apoi virus, and Aroa virus.

13. The nucleic acid molecule of claim 12, wherein the second flavivirus is the West Nile virus.

14. The nucleic acid molecule of claim 1, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is at least selected from the group consisting of the 127th isoleucine, 274th threonine, 1209th serine, 2462nd lysine, 2463rd isoleucine, 2479th serine, 2652nd methionine, 2751st alanine, 2896th alanine and 3380th asparagine, counted from the amino acid next to the starting

methionine of the polyprotein, and the second flavivirus is the West Nile virus.

15. The nucleic acid molecule of claim 14, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 127th isoleucine, 274th threonine, 1209th serine, 2462nd lysine, 2463rd isoleucine, 2479th serine, 2652nd methionine, 2751st alanine, 2896th alanine and 3380th asparagine, counted from the amino acid next to the starting methionine of the polyprotein, and the second flavivirus is the West Nile virus.

16. The nucleic acid molecule of claim 14, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 127th isoleucine, counted from the amino acid next to the starting methionine of the polyprotein, and the second flavivirus is the West Nile virus.

17. The nucleic acid molecule of claim 14, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 274th threonine, counted from the amino acid next to the starting methionine of the polyprotein, and the second flavivirus is the West Nile virus.

18. The nucleic acid molecule of claim 15, wherein the Japanese encephalitis virus is a ML-17 strain, and the second flavivirus is the West Nile virus.

19. The nucleic acid molecule of claim 16, wherein the Japanese encephalitis virus is a MS-14 strain, and the second flavivirus is the West Nile virus.

20. The nucleic acid molecule of claim 17, wherein the Japanese encephalitis virus is a MS-15 strain, and the second flavivirus is the West Nile virus.

21. The nucleic acid molecule of claim 15, wherein said Japanese encephalitis virus is encoded by a nucleotide sequence of a full-length genomic cDNA of a ML-17 strain shown by SEQ ID NO:1, and the second flavivirus is the West Nile virus.

22. An attenuated chimeric flavivirus encoded by the nucleic acid molecule of any one of claims 1 to 21.

23. An attenuated live vaccine comprising the attenuated chimeric flavivirus of claim 22.

24. A method of preparing the nucleic acid molecule of any one of claims 1 to 21, which comprises the following steps:

a step for replacing a nucleotide sequence that encodes the envelope protein in a nucleic acid molecule comprising a nucleotide sequence that encodes Japanese encephalitis virus with the nucleotide sequence that encodes the envelope protein of a second flavivirus; and

a step for introducing nucleotide mutations that produce one or more amino acid mutations that attenuate the virus into the nucleotide sequence(s) that encode(s) the pre-membrane protein and/or non-structural protein of Japanese encephalitis virus, wherein the nucleotide sequence that encodes the envelope protein of the second flavivirus comprises no modification.

25. The method of claim 24 wherein the second flavivirus is a virulent virus.

26. A method of preparing the nucleic acid molecule of any one of claims 1 to 21, which comprises the following step:

a step for replacing a nucleotide sequence that encodes the envelope protein in a nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the pre-membrane protein and/or non-structural protein with the nucleotide sequence that encodes the envelope protein of the second flavivirus,

wherein the nucleotide sequence that encodes the envelope protein of the second flavivirus comprises no modification.

27. The method of claim 26 wherein the second flavivirus is a virulent virus.

28. A method of preparing an attenuated chimeric flavivirus, which comprises a step for expressing chimeric flavivirus proteins from the nucleic acid molecule of any one of claims 1 to 21.

29. A nucleic acid molecule comprising a nucleotide sequence that encodes a Japanese encephalitis virus having one or more amino acid mutations that attenuate the virus in the pre-membrane protein and/or non-structural protein,

wherein the position of the amino acid mutation that attenuates said Japanese encephalitis virus is at least selected from a group consisting of the 127th, 274th, 1209th, 2462nd, 2463rd, 2479th, 2652nd, 2751st, 2896th and 3380th, counted from the amino acid next to a starting methionine of a polyprotein.

30. The nucleic acid molecule of claim 29, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is at least selected from a group consisting of the 127th isoleucine, 274th threonine, 1209th serine, 2462nd lysine, 2463rd isoleucine, 2479th serine, 2652nd methionine, 2751st alanine, 2896th alanine and 3380th asparagine, counted from the amino acid next to the starting methionine of the polyprotein.

31. The nucleic acid molecule of claim 30, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 127th isoleucine, 274th threonine, 1209th serine, 2462nd lysine, 2463rd isoleucine, 2479th serine, 2652nd methionine, 2751st alanine, 2896th alanine and 3380th asparagine, counted from the amino acid next to the starting methionine of the polyprotein.

32. The nucleic acid molecule of claim 30, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 127th isoleucine, counted from the amino acid next to the starting methionine of the polyprotein.

33. The nucleic acid molecule of claim 30, wherein the amino acid mutation that attenuates said Japanese encephalitis virus is the 274th threonine, counted from the amino acid next to the starting methionine of the polyprotein.

34. The nucleic acid molecule of claim 31, which comprises the nucleotide sequence of a full-length genomic cDNA of a ML-17 strain shown by SEQ ID NO:1.

35. A vector comprising the nucleic acid molecule any one of claims 29-34.

36. An attenuated Japanese encephalitis virus encoded by the nucleic acid molecule of any one of claims 29-34.

37. An attenuated Japanese encephalitis virus that does not contain a ML-17 strain, is encoded by the nucleic acid molecule of any one of claims 29 to 33.

38. The attenuated Japanese encephalitis virus of claim 37, is a MS-14 strain.

39. The attenuated Japanese encephalitis virus of claim 37, which is a MS-15 strain.

40. A method of preparing the nucleic acid molecule of any one of claims 29-34, which comprises a step for introducing nucleotide mutations that produce one or more amino acid mutations that attenuate the virus into nucleotide sequence(s) that encode(s) the pre-membrane protein and/or non-structural protein in a nucleic acid molecule comprising a nucleotide sequence that encodes Japanese encephalitis virus.

41. A method of preparing an attenuated Japanese encephalitis virus, which comprises a step for expressing Japanese encephalitis virus proteins from the nucleic acid molecule of any one of claims 29-34.

SEQUENCE LISTING

<110> The Research Foundation for Microbial Diseases of Osaka
University

<120> ATTENUATED CHIMERIC FLAVIVIRUS HAVING GENE OF ATTENUATED JAPANESE
ENCEPHALITIS VIRUS AS BACKBONE

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<150> JP2004-374630

<151> 2004-12-24

<160> 63

<170> PatentIn version 3.3

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<211> 10976

<212> DNA

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1

5

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10

15

20

cgc gta ttc cca cta gtg gga gtg aag agg gta gta atg agc ttg ttg 209

Arg Val Phe Pro Leu Val Gly Val Lys Arg Val Val Met Ser Leu Leu

25

30

35

gac ggc aga ggg cca gta cgt ttc gtg ctg gct ctt atc acg ttc ttc 257

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40

45

50

aag ttt aca gca tta gcc ccg acc aag gcg ctt tta ggc cga tgg aaa 305

Lys Phe Thr Ala Leu Ala Pro Thr Lys Ala Leu Leu Gly Arg Trp Lys

55

60

65

70

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75

80

85

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 Glu Leu Gly Thr Leu Ile Asp Ala Val Asn Lys Arg Gly Arg Lys Gln

90

95

100

aac aaa aga gga gga aat gaa ggc tca atc atg tgg ctt gcg agc ttg 449
 Asn Lys Arg Gly Gly Asn Glu Gly Ser Ile Met Trp Leu Ala Ser Leu

105

110

115

gca gtt gtc ata gct tgt gca gga gcc ata aag ttg tca aat ttc cag 497
 Ala Val Val Ile Ala Cys Ala Gly Ala Ile Lys Leu Ser Asn Phe Gln

120

125

130

ggg aag ctt ttg atg acc att aac aac acg gac att gca gac gtt atc 545
 Gly Lys Leu Leu Met Thr Ile Asn Asn Thr Asp Ile Ala Asp Val Ile

135

140

145

150

gta att ccc acc tca aaa gga gag aac aga tgc tgg gtc cgg gca atc 593
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160

165

gac gtc ggc tac atg tgt gag gac act atc acg tac gaa tgt cct aag 641
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170

175

180

ctt gcc atg ggc aat gat cca gag gat gtg gac tgc tgg tgt gac aac 689
 Leu Ala Met Gly Asn Asp Pro Glu Asp Val Asp Cys Trp Cys Asp Asn

185

190

195

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 Gln Glu Val Tyr Val Gln Tyr Gly Arg Cys Thr Arg Thr Arg His Ser
 200 205 210

aag cga agc agg aga tcc gtg tcg gtc caa aca cat ggg gag agt tca 785
 Lys Arg Ser Arg Arg Ser Val Ser Val Gln Thr His Gly Glu Ser Ser
 215 220 225 230

cta gtg aat aaa aaa gag gct tgg ctg gat tca acg aaa gcc aca cga 833
 Leu Val Asn Lys Lys Glu Ala Trp Leu Asp Ser Thr Lys Ala Thr Arg
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 Tyr Leu Met Lys Thr Glu Asn Trp Ile Ile Arg Asn Pro Gly Tyr Ala
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 Arg Val Val Phe Thr Ile Leu Leu Leu Leu Val Ala Pro Ala Tyr Ser
 280 285 290

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Gly Ala Thr Trp Val Asp Leu Val Leu Glu Gly Asp Ser Cys Leu Thr				
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Ile Met Ala Asn Asp Lys Pro Thr Leu Asp Val Arg Met Ile Asn Ile				
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His Asn Glu Lys Arg Ala Asp Ser Ser Tyr Val Cys Lys Gln Gly Phe				
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Thr Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser				
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975

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Ala Leu Ile Ala Val Phe Lys	Ile Gln Pro Ala Phe	Leu Val Met	
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 Gly Trp Pro Ala Thr Glu Phe Leu Ser Ala Val Gly Leu Met Phe

1375 1380 1385

gcc atc gta ggt ggt ttg gcg gag ttg gat att gaa tcc atg tca 4304
 Ala Ile Val Gly Gly Leu Ala Glu Leu Asp Ile Glu Ser Met Ser

1390 1395 1400

ata ccc ttc atg ctg gca ggt ctc atg gca gtg tcc tac gtg gtg 4349
 Ile Pro Phe Met Leu Ala Gly Leu Met Ala Val Ser Tyr Val Val

1405 1410 1415

tca gga aaa gca aca gat atg tgg ctt gaa cgg gct gcc gac atc 4394
 Ser Gly Lys Ala Thr Asp Met Trp Leu Glu Arg Ala Ala Asp Ile

1420 1425 1430

agc tgg gag atg gat gct gca atc aca gga agc agt cgg agg ctg 4439
 Ser Trp Glu Met Asp Ala Ala Ile Thr Gly Ser Ser Arg Arg Leu

1435 1440 1445

gat gtg aag cta gat gat gac gga gat ttt cac ttg att gac gat 4484
 Asp Val Lys Leu Asp Asp Asp Gly Asp Phe His Leu Ile Asp Asp

1450 1455 1460

ccc ggt gtt cca tgg aag gtc tgg gtc ctg cgc atg tct tgc att 4529
 Pro Gly Val Pro Trp Lys Val Trp Val Leu Arg Met Ser Cys Ile
 1465 1470 1475

ggg tta gcc gcc ctc acg cct tgg gcc att gtt ccc gcc gct ttt 4574
 Gly Leu Ala Ala Leu Thr Pro Trp Ala Ile Val Pro Ala Ala Phe
 1480 1485 1490

ggt tat tgg ctc act tta aaa aca aca aaa aga ggg ggc gtg ttt 4619
 Gly Tyr Trp Leu Thr Leu Lys Thr Thr Lys Arg Gly Gly Val Phe
 1495 1500 1505

tgg gac acg cca tcc cca aaa cct tgc tca aaa gga gac acc act 4664
 Trp Asp Thr Pro Ser Pro Lys Pro Cys Ser Lys Gly Asp Thr Thr
 1510 1515 1520

aca gga gtt tac cgc att atg gct aga ggg att ctt ggc act tac 4709
 Thr Gly Val Tyr Arg Ile Met Ala Arg Gly Ile Leu Gly Thr Tyr
 1525 1530 1535

cag gcc ggc gtc gga gtc atg tac gag aat gtt ttc cac aca cta 4754
 Gln Ala Gly Val Gly Val Met Tyr Glu Asn Val Phe His Thr Leu
 1540 1545 1550

tgg cac aca act aga gga gca gct att atg agt gga gaa gga aaa 4799
 Trp His Thr Thr Arg Gly Ala Ala Ile Met Ser Gly Glu Gly Lys

1555	1560	1565	
ttg acg cca tac tgg ggt agt	gtg aaa gaa gac cgc	ata gct tac	4844
Leu Thr Pro Tyr Trp Gly Ser	Val Lys Glu Asp Arg	Ile Ala Tyr	
1570	1575	1580	
gga ggc cca tgg agg ttt gat	cga aaa tgg aat gga	act gat gac	4889
Gly Gly Pro Trp Arg Phe Asp	Arg Lys Trp Asn Gly	Thr Asp Asp	
1585	1590	1595	
gtg caa gtg atc gtg gta gaa	ccg ggg aag gct gca	gta aac atc	4934
Val Gln Val Ile Val Val Glu	Pro Gly Lys Ala Ala	Val Asn Ile	
1600	1605	1610	
cag aca aaa cca gga gtg ttt	cgg act ccc ttc ggg	gag gtt ggg	4979
Gln Thr Lys Pro Gly Val Phe	Arg Thr Pro Phe Gly	Glu Val Gly	
1615	1620	1625	
gct gtt agt ctg gat tat ccg	cga gga aca tcc ggc	tca ccc att	5024
Ala Val Ser Leu Asp Tyr Pro	Arg Gly Thr Ser Gly	Ser Pro Ile	
1630	1635	1640	
ctg gat tcc aat gga gac atc	ata ggc ctg tac ggc	aat gga gtt	5069
Leu Asp Ser Asn Gly Asp Ile	Ile Gly Leu Tyr Gly	Asn Gly Val	
1645	1650	1655	
gag ctt ggc gat ggc tca tac	gtc agc gcc atc gtg	cag ggt gac	5114

Glu Leu Gly Asp Gly Ser Tyr Val Ser Ala Ile Val Gln Gly Asp

1660

1665

1670

cgt cag gag gaa cca gtc cca gaa gct tac acc cca aac atg ttg

5159

Arg Gln Glu Glu Pro Val Pro Glu Ala Tyr Thr Pro Asn Met Leu

1675

1680

1685

aga aag aga cag atg acc gta cta gat ttg cac cct ggt tca ggg

5204

Arg Lys Arg Gln Met Thr Val Leu Asp Leu His Pro Gly Ser Gly

1690

1695

1700

aaa acc aag aaa att ctg cca caa ata att aag gac gct att cag

5249

Lys Thr Lys Lys Ile Leu Pro Gln Ile Ile Lys Asp Ala Ile Gln

1705

1710

1715

cag cgc cta aga aca gct gtg ttg gca ccg acg cgg gtg gta gca

5294

Gln Arg Leu Arg Thr Ala Val Leu Ala Pro Thr Arg Val Val Ala

1720

1725

1730

gca gaa atg gca gaa gct ttg aga ggg ctc cca gta cga tat caa

5339

Ala Glu Met Ala Glu Ala Leu Arg Gly Leu Pro Val Arg Tyr Gln

1735

1740

1745

act tca gca gtg cag aga gag cac caa ggg aat gaa ata gtg gat

5384

Thr Ser Ala Val Gln Arg Glu His Gln Gly Asn Glu Ile Val Asp

1750

1755

1760

gtg atg tgc cac gcc act ctg acc cat aga ctg atg tca ccg aac 5429
 Val Met Cys His Ala Thr Leu Thr His Arg Leu Met Ser Pro Asn

1765 1770 1775

aga gtg ccc aac tac aac cta ttt gtc atg gat gaa gct cat ttc 5474
 Arg Val Pro Asn Tyr Asn Leu Phe Val Met Asp Glu Ala His Phe

1780 1785 1790

acc gac cca gcc agt ata gcc gca cga gga tac att gct acc aag 5519
 Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly Tyr Ile Ala Thr Lys

1795 1800 1805

gtg gaa tta ggg gag gca gca gcc atc ttt atg aca gcg acc ccg 5564
 Val Glu Leu Gly Glu Ala Ala Ala Ile Phe Met Thr Ala Thr Pro

1810 1815 1820

cct gga acc acg gat cct ttt cct gac tca aat gcc cca atc cat 5609
 Pro Gly Thr Thr Asp Pro Phe Pro Asp Ser Asn Ala Pro Ile His

1825 1830 1835

gat ttg caa gat gag ata cca gac agg gcg tgg agc agt gga tac 5654
 Asp Leu Gln Asp Glu Ile Pro Asp Arg Ala Trp Ser Ser Gly Tyr

1840 1845 1850

gaa tgg atc aca gaa tat gcg gga aaa acc gtg tgg ttt gtg gca 5699
 Glu Trp Ile Thr Glu Tyr Ala Gly Lys Thr Val Trp Phe Val Ala

1855 1860 1865

agc gtg aaa atg ggg aac gag att gca atg tgc ctc caa aga gcg 5744
 Ser Val Lys Met Gly Asn Glu Ile Ala Met Cys Leu Gln Arg Ala
 1870 1875 1880

ggg aaa aag gtc atc caa ctc aac cgc aag tcc tat gac aca gaa 5789
 Gly Lys Lys Val Ile Gln Leu Asn Arg Lys Ser Tyr Asp Thr Glu
 1885 1890 1895

tac cca aaa tgt aag aat gga gac tgg gat ttt gtc atc acc act 5834
 Tyr Pro Lys Cys Lys Asn Gly Asp Trp Asp Phe Val Ile Thr Thr
 1900 1905 1910

gac att tct gaa atg ggg gcc aac ttc ggt gcg agc agg gtc atc 5879
 Asp Ile Ser Glu Met Gly Ala Asn Phe Gly Ala Ser Arg Val Ile
 1915 1920 1925

gac tgt aga aag agc gtg aag ccc acc atc tta gaa gag gga gaa 5924
 Asp Cys Arg Lys Ser Val Lys Pro Thr Ile Leu Glu Glu Gly Glu
 1930 1935 1940

ggc aga gtc atc ctc gga aac cca tcg ccc ata acc agt gca agc 5969
 Gly Arg Val Ile Leu Gly Asn Pro Ser Pro Ile Thr Ser Ala Ser
 1945 1950 1955

gca gct caa cgg agg ggc aga gta ggc aga aac cct aac cag gtt 6014
 Ala Ala Gln Arg Arg Gly Arg Val Gly Arg Asn Pro Asn Gln Val

1960	1965	1970	
gga gat	gaa tac cac tat ggg	ggg gcc acc agt gaa	gat gac agt 6059
Gly Asp	Glu Tyr His Tyr Gly	Gly Ala Thr Ser Glu	Asp Asp Ser
1975	1980	1985	
aac cta	gcc cat tgg aca gag	gca aag atc atg tta	gat aac ata 6104
Asn Leu	Ala His Trp Thr Glu	Ala Lys Ile Met Leu	Asp Asn Ile
1990	1995	2000	
cac atg	ccc aat gga ctg gtg	gcc cag ctc tat gga	cca gag agg 6149
His Met	Pro Asn Gly Leu Val	Ala Gln Leu Tyr Gly	Pro Glu Arg
2005	2010	2015	
gaa aag	gcc ttc aca atg gat	ggc gaa tac cgt ctc	aga ggt gaa 6194
Glu Lys	Ala Phe Thr Met Asp	Gly Glu Tyr Arg Leu	Arg Gly Glu
2020	2025	2030	
gaa aag	aaa aac ttc tta gag	ctg ctt agg acg gct	gac ctc ccg 6239
Glu Lys	Lys Asn Phe Leu Glu	Leu Leu Arg Thr Ala	Asp Leu Pro
2035	2040	2045	
gtg tgg	ctg gcc tac aag gtg	gcg tcc aat ggc atc	cag tac acc 6284
Val Trp	Leu Ala Tyr Lys Val	Ala Ser Asn Gly Ile	Gln Tyr Thr
2050	2055	2060	
gat aga	aag tgg tgt ttt gat	ggg ccg cgt acg aat	gcc ata ctg 6329

Asp Arg Lys Trp Cys Phe Asp Gly Pro Arg Thr Asn Ala Ile Leu

2065

2070

2075

gag gac aac acc gag gta gag ata gtc acc cgg atg ggt gag agg

6374

Glu Asp Asn Thr Glu Val Glu Ile Val Thr Arg Met Gly Glu Arg

2080

2085

2090

aaa atc ctc aag ccg aga tgg ctt gat gca aga gtt tat gca gat

6419

Lys Ile Leu Lys Pro Arg Trp Leu Asp Ala Arg Val Tyr Ala Asp

2095

2100

2105

cac caa gct ctc aag tgg ttc aaa gac ttc gca gca gga aag aga

6464

His Gln Ala Leu Lys Trp Phe Lys Asp Phe Ala Ala Gly Lys Arg

2110

2115

2120

tca gcc gtt agc ttc ata gag gtg ctc ggt cgt atg cct gag cat

6509

Ser Ala Val Ser Phe Ile Glu Val Leu Gly Arg Met Pro Glu His

2125

2130

2135

ttc atg gga aag acg cgg gaa gct tta gac acc atg tac ttg gtt

6554

Phe Met Gly Lys Thr Arg Glu Ala Leu Asp Thr Met Tyr Leu Val

2140

2145

2150

gca acg gct gag aaa ggt ggg aaa gca cac cga atg gct ctc gaa

6599

Ala Thr Ala Glu Lys Gly Gly Lys Ala His Arg Met Ala Leu Glu

2155

2160

2165

gag ctg cca gat gca ctg gaa acc att aca ctt att gtt gct atc 6644
 Glu Leu Pro Asp Ala Leu Glu Thr Ile Thr Leu Ile Val Ala Ile

2170

2175

2180

act gtg atg aca gga gga ttc ttt cta ctc atg atg cag cga aag 6689
 Thr Val Met Thr Gly Gly Phe Phe Leu Leu Met Met Gln Arg Lys

2185

2190

2195

ggt ata ggg aag atg ggt ctt gga gct cta gtg ctc acg cta gct 6734
 Gly Ile Gly Lys Met Gly Leu Gly Ala Leu Val Leu Thr Leu Ala

2200

2205

2210

acc ttc ttc ctg tgg gcg gca gag gtt ccc gga aca aaa ata gca 6779
 Thr Phe Phe Leu Trp Ala Ala Glu Val Pro Gly Thr Lys Ile Ala

2215

2220

2225

ggg acc ctg ctg atc gcc ctg ctg ctt atg gtg gtt ctc atc cca 6824
 Gly Thr Leu Leu Ile Ala Leu Leu Leu Met Val Val Leu Ile Pro

2230

2235

2240

gaa ccg gaa aag cag agg tca caa aca gat aat caa ctg gcg gtg 6869
 Glu Pro Glu Lys Gln Arg Ser Gln Thr Asp Asn Gln Leu Ala Val

2245

2250

2255

ttt ctc atc tgt gtc ttg acc gtg gtt gga gtg gtg gca gca aac 6914
 Phe Leu Ile Cys Val Leu Thr Val Val Gly Val Val Ala Ala Asn

2260

2265

2270

gag tac ggg atg cta gaa aaa acc aaa gca gac ctc aag agc atg 6959
 Glu Tyr Gly Met Leu Glu Lys Thr Lys Ala Asp Leu Lys Ser Met
 2275 2280 2285

ttt ggc gga aag acg cag gca tca gga ctg act gga tta cca agc 7004
 Phe Gly Gly Lys Thr Gln Ala Ser Gly Leu Thr Gly Leu Pro Ser
 2290 2295 2300

atg gca ctg gac ctg cgt cca gcc aca gct tgg gca ctg tat ggg 7049
 Met Ala Leu Asp Leu Arg Pro Ala Thr Ala Trp Ala Leu Tyr Gly
 2305 2310 2315

ggg agc aca gtc gtg cta acc cct ctt ctg aag cac ctg atc acg 7094
 Gly Ser Thr Val Val Leu Thr Pro Leu Leu Lys His Leu Ile Thr
 2320 2325 2330

tcg gaa tac gtc acc aca tcg cta gcc tca att aac tca caa gct 7139
 Ser Glu Tyr Val Thr Thr Ser Leu Ala Ser Ile Asn Ser Gln Ala
 2335 2340 2345

ggc tca tta ttt gtc ttg cca cga ggc gtg cct ttt acc gac cta 7184
 Gly Ser Leu Phe Val Leu Pro Arg Gly Val Pro Phe Thr Asp Leu
 2350 2355 2360

gac ttg acc gtt ggc ctc gtc ttc ctt ggc tgt tgg ggt caa atc 7229
 Asp Leu Thr Val Gly Leu Val Phe Leu Gly Cys Trp Gly Gln Ile

2365	2370	2375	
acc ctc	aca acg ttt ttg aca	gcc atg gtt ctg gcg	aca ctt cac 7274
Thr Leu	Thr Thr Phe Leu Thr	Ala Met Val Leu Ala	Thr Leu His
2380	2385	2390	
tat ggg	tac atg ctc cct gga	tgg caa gca gaa gca	ctc agg gct 7319
Tyr Gly	Tyr Met Leu Pro Gly	Trp Gln Ala Glu Ala	Leu Arg Ala
2395	2400	2405	
gcc cag	aga agg aca gcg gct	gga ata atg aag aat	gcc gtt gtt 7364
Ala Gln	Arg Arg Thr Ala Ala	Gly Ile Met Lys Asn	Ala Val Val
2410	2415	2420	
gac gga	atg gtc gcc act gat	gtg cct gaa ctg gaa	agg acc act 7409
Asp Gly	Met Val Ala Thr Asp	Val Pro Glu Leu Glu	Arg Thr Thr
2425	2430	2435	
cct ctg	atg caa aag aaa gtc	gga cag gtg ctc ctc	ata ggg gta 7454
Pro Leu	Met Gln Lys Lys Val	Gly Gln Val Leu Leu	Ile Gly Val
2440	2445	2450	
agc gtg	gca gcg ttc ctc gtc	aac ccc aaa atc acc	act gtg aga 7499
Ser Val	Ala Ala Phe Leu Val	Asn Pro Lys Ile Thr	Thr Val Arg
2455	2460	2465	
gaa gca	ggg gtg ttg gtg aca	gcg gct acg ctc tct	ttg tgg gac 7544

Glu Ala Gly Val Leu Val Thr Ala Ala Thr Leu Ser Leu Trp Asp

2470

2475

2480

aac gga gcc agt gcc gtt tgg aat tcc acc act gcc acg gga ctc

7589

Asn Gly Ala Ser Ala Val Trp Asn Ser Thr Thr Ala Thr Gly Leu

2485

2490

2495

tgc cat gta atg cga ggt agc tac ctg gct gga ggc tcc att gct

7634

Cys His Val Met Arg Gly Ser Tyr Leu Ala Gly Gly Ser Ile Ala

2500

2505

2510

tgg act ctc atc aag aac gct gac aag ccc tcc tta aaa agg gga

7679

Trp Thr Leu Ile Lys Asn Ala Asp Lys Pro Ser Leu Lys Arg Gly

2515

2520

2525

agg cct ggg ggc agg acg cta ggg gag cag tgg aag gaa aaa cta

7724

Arg Pro Gly Gly Arg Thr Leu Gly Glu Gln Trp Lys Glu Lys Leu

2530

2535

2540

aat gcc atg agc aga gaa gag ttt ttt aaa tac cgg aga gag gcc

7769

Asn Ala Met Ser Arg Glu Glu Phe Phe Lys Tyr Arg Arg Glu Ala

2545

2550

2555

ata atc gag gtg gac cgc act gaa gca cgc agg gct aga cgt gaa

7814

Ile Ile Glu Val Asp Arg Thr Glu Ala Arg Arg Ala Arg Arg Glu

2560

2565

2570

aat aac	ata gtg gga gga cat	ccg gtt tcg cga ggc	tca gca aaa	7859
Asn Asn	Ile Val Gly Gly His	Pro Val Ser Arg Gly	Ser Ala Lys	
2575	2580	2585		
ctc cgt	tgg ctc gta gag aaa	gga ttt gtc tcg cca	ata gga aaa	7904
Leu Arg	Trp Leu Val Glu Lys	Gly Phe Val Ser Pro	Ile Gly Lys	
2590	2595	2600		
gtc att	gat cta ggg tgt ggg	cgt gga gga tgg agc	tac tac gca	7949
Val Ile	Asp Leu Gly Cys Gly	Arg Gly Gly Trp Ser	Tyr Tyr Ala	
2605	2610	2615		
gca acc	ctg aag aag gtc cag	gaa gtc aga gga tac	acg aaa ggt	7994
Ala Thr	Leu Lys Lys Val Gln	Glu Val Arg Gly Tyr	Thr Lys Gly	
2620	2625	2630		
ggg gcg	gga cat gaa gaa ccg	atg ctc atg cag agc	tac ggc tgg	8039
Gly Ala	Gly His Glu Glu Pro	Met Leu Met Gln Ser	Tyr Gly Trp	
2635	2640	2645		
aac ctg	gtc tcc atg aag agt	gga gtg gac gtg ttt	tac aaa cct	8084
Asn Leu	Val Ser Met Lys Ser	Gly Val Asp Val Phe	Tyr Lys Pro	
2650	2655	2660		
tca gag	ccc agt gac act ctg	ttc tgc gac ata ggg	gaa tcc tcc	8129
Ser Glu	Pro Ser Asp Thr Leu	Phe Cys Asp Ile Gly	Glu Ser Ser	
2665	2670	2675		

cgc agt	cca gaa gta gaa gaa	caa cgc aca cta cgc	gtc cta gag	8174
Pro Ser	Pro Glu Val Glu Glu	Gln Arg Thr Leu Arg	Val Leu Glu	
2680	2685	2690		
atg aca	tct gac tgg ttg cac	cga gga cct aga gag	ttc tgt ata	8219
Met Thr	Ser Asp Trp Leu His	Arg Gly Pro Arg Glu	Phe Cys Ile	
2695	2700	2705		
aaa gtt	ctt tgc ccc tac atg	ccc aag gtt ata gaa	aaa atg gaa	8264
Lys Val	Leu Cys Pro Tyr Met	Pro Lys Val Ile Glu	Lys Met Glu	
2710	2715	2720		
gtc ctg	caa cgc cgc ttc gga	ggg ggg cta gtg cgt	ctt ccc ctg	8309
Val Leu	Gln Arg Arg Phe Gly	Gly Gly Leu Val Arg	Leu Pro Leu	
2725	2730	2735		
tcc cgc	aac tcc aat cac gag	atg tac tgg gtt agt	gga gcc gct	8354
Ser Arg	Asn Ser Asn His Glu	Met Tyr Trp Val Ser	Gly Ala Ala	
2740	2745	2750		
ggc aat	gtg gtg cac gct gtg	aac atg acc agc cag	gta cta ctg	8399
Gly Asn	Val Val His Ala Val	Asn Met Thr Ser Gln	Val Leu Leu	
2755	2760	2765		
ggg cga	atg gat cgc aca gtg	tgg aga ggg cca aag	tat gag gaa	8444
Gly Arg	Met Asp Arg Thr Val	Trp Arg Gly Pro Lys	Tyr Glu Glu	

2770	2775	2780	
gat gtc aac tta ggg agc gga aca aga gcc gtg gga aag gga gaa			8489
Asp Val Asn Leu Gly Ser Gly Thr Arg Ala Val Gly Lys Gly Glu			
2785	2790	2795	
gtc cat agc aat cag gag aaa atc aag aag aga atc cag aag ctt			8534
Val His Ser Asn Gln Glu Lys Ile Lys Lys Arg Ile Gln Lys Leu			
2800	2805	2810	
aaa gaa gaa ttc gcc aca acg tgg cac aaa gac cct gag cat cca			8579
Lys Glu Glu Phe Ala Thr Thr Trp His Lys Asp Pro Glu His Pro			
2815	2820	2825	
tac cgc act tgg aca tac cac gga agc tat gaa gtg aag gct act			8624
Tyr Arg Thr Trp Thr Tyr His Gly Ser Tyr Glu Val Lys Ala Thr			
2830	2835	2840	
ggc tca gct agt tct ctc gtc aac gga gtg gtg aag ctc atg agc			8669
Gly Ser Ala Ser Ser Leu Val Asn Gly Val Val Lys Leu Met Ser			
2845	2850	2855	
aaa cct tgg gac gcc att gcc aac gtc acc acc atg gcc atg act			8714
Lys Pro Trp Asp Ala Ile Ala Asn Val Thr Thr Met Ala Met Thr			
2860	2865	2870	
gac acc acc cct ttt gga cag caa aga gtt ttc aag gag aaa gtt			8759

Asp Thr Thr Pro Phe Gly Gln Gln Arg Val Phe Lys Glu Lys Val

2875

2880

2885

gac acg aag gct cct gag cca cca gct gga gct aag gaa gtg ctc

8804

Asp Thr Lys Ala Pro Glu Pro Pro Ala Gly Ala Lys Glu Val Leu

2890

2895

2900

aac gag acc acc aac tgg ctg tgg gcc tac ttg tca cgg gaa aaa

8849

Asn Glu Thr Thr Asn Trp Leu Trp Ala Tyr Leu Ser Arg Glu Lys

2905

2910

2915

aga ccc cgc ttg tgc acc aag gaa gaa ttc ata aag aaa gtc aat

8894

Arg Pro Arg Leu Cys Thr Lys Glu Glu Phe Ile Lys Lys Val Asn

2920

2925

2930

agc aac gcg gct ctt gga gca gtg ttc gct gaa cag aat caa tgg

8939

Ser Asn Ala Ala Leu Gly Ala Val Phe Ala Glu Gln Asn Gln Trp

2935

2940

2945

agc acg gcg cgt gag gct gtg gat gac ccg cgg ttt tgg gag atg

8984

Ser Thr Ala Arg Glu Ala Val Asp Asp Pro Arg Phe Trp Glu Met

2950

2955

2960

gtt gat gaa gag agg gaa aac cat ctg cga gga gag tgt cac aca

9029

Val Asp Glu Glu Arg Glu Asn His Leu Arg Gly Glu Cys His Thr

2965

2970

2975

tgt atc tat aac atg atg gga aaa aga gag aag aag cct gga gag 9074

Cys Ile Tyr Asn Met Met Gly Lys Arg Glu Lys Lys Pro Gly Glu

2980

2985

2990

ttt gga aaa gct aaa gga agc agg gcc att tgg ttc atg tgg ctt 9119

Phe Gly Lys Ala Lys Gly Ser Arg Ala Ile Trp Phe Met Trp Leu

2995

3000

3005

gga gca cgg tat cta gag ttt gaa gct ttg ggg ttc ctg aat gaa 9164

Gly Ala Arg Tyr Leu Glu Phe Glu Ala Leu Gly Phe Leu Asn Glu

3010

3015

3020

gat cat tgg ctg agc cga gag aat tca gga ggt gga gtg gaa ggc 9209

Asp His Trp Leu Ser Arg Glu Asn Ser Gly Gly Gly Val Glu Gly

3025

3030

3035

tca ggc gtc caa aag ctg gga tac atc ctc cgt gat ata gca gga 9254

Ser Gly Val Gln Lys Leu Gly Tyr Ile Leu Arg Asp Ile Ala Gly

3040

3045

3050

aag caa gga gga aaa atg tac gct gat gat acc gcc ggg tgg gac 9299

Lys Gln Gly Gly Lys Met Tyr Ala Asp Asp Thr Ala Gly Trp Asp

3055

3060

3065

act aga att acc aga act gat tta gaa aat gaa gcc aag gtg ctg 9344

Thr Arg Ile Thr Arg Thr Asp Leu Glu Asn Glu Ala Lys Val Leu

3070

3075

3080

gag ctt	cta gac ggt gaa cac	cgc atg ctc gcc cga	gcc ata att	9389
Glu Leu	Leu Asp Gly Glu His	Arg Met Leu Ala Arg	Ala Ile Ile	
3085	3090	3095		
gaa ttg	act tac agg cac aaa	gtg gtc aag gtc atg	aga cct gca	9434
Glu Leu	Thr Tyr Arg His Lys	Val Val Lys Val Met	Arg Pro Ala	
3100	3105	3110		
gca gaa	gga aag acc gtg atg	gac gtg ata tca agg	gag gat caa	9479
Ala Glu	Gly Lys Thr Val Met	Asp Val Ile Ser Arg	Glu Asp Gln	
3115	3120	3125		
agg ggg	agt gga cag gtg gtc	act tat gct ctt aac	act ttc acg	9524
Arg Gly	Ser Gly Gln Val Val	Thr Tyr Ala Leu Asn	Thr Phe Thr	
3130	3135	3140		
aac atc	gct gtc cag ctc gtc	agg ctg atg gag gct	gag ggg gtc	9569
Asn Ile	Ala Val Gln Leu Val	Arg Leu Met Glu Ala	Glu Gly Val	
3145	3150	3155		
att gga	cca caa cac ttg gaa	cag cta cct aga aaa	aac aag ata	9614
Ile Gly	Pro Gln His Leu Glu	Gln Leu Pro Arg Lys	Asn Lys Ile	
3160	3165	3170		
gct gtc	agg acc tgg ctc ttt	gag aat gga gag gag	aga gtg tcc	9659
Ala Val	Arg Thr Trp Leu Phe	Glu Asn Gly Glu Glu	Arg Val Ser	

3175	3180	3185	
agg atg gct atc agc gga gac	gac tgt gtc gtc aag	ccg ctg gac	9704
Arg Met Ala Ile Ser Gly Asp	Asp Cys Val Val Lys	Pro Leu Asp	
3190	3195	3200	
gac aga ttc gcc acg gcc ctc	cac ttc ctc aac gca	atg tca aag	9749
Asp Arg Phe Ala Thr Ala Leu	His Phe Leu Asn Ala	Met Ser Lys	
3205	3210	3215	
gtc aga aaa gac atc cag gaa	tgg aag cct tca cat	ggc tgg cac	9794
Val Arg Lys Asp Ile Gln Glu	Trp Lys Pro Ser His	Gly Trp His	
3220	3225	3230	
gat tgg cag caa gtt ccc ttc	tgc tct aac cat ttt	cag gag att	9839
Asp Trp Gln Gln Val Pro Phe	Cys Ser Asn His Phe	Gln Glu Ile	
3235	3240	3245	
gtg atg aaa gat gga agg agt	ata gtt gtc ccg tgc	aga gga cag	9884
Val Met Lys Asp Gly Arg Ser	Ile Val Val Pro Cys	Arg Gly Gln	
3250	3255	3260	
gat gag ctg ata ggc agg gct	cgc atc tcc cca gga	gct gga tgg	9929
Asp Glu Leu Ile Gly Arg Ala	Arg Ile Ser Pro Gly	Ala Gly Trp	
3265	3270	3275	
aat gtg aag gac aca gct tgt	ctg gcc aaa gca tat	gca cag atg	9974

Asn Val Lys Asp Thr Ala Cys Leu Ala Lys Ala Tyr Ala Gln Met

3280

3285

3290

tgg cta ctc cta tac ttc cat cgt agg gac ttg cgt ctc atg gca 10019

Trp Leu Leu Leu Tyr Phe His Arg Arg Asp Leu Arg Leu Met Ala

3295

3300

3305

aat gcg att tgc tca gca gtg cca gtg gat tgg gtg ccc acg ggc 10064

Asn Ala Ile Cys Ser Ala Val Pro Val Asp Trp Val Pro Thr Gly

3310

3315

3320

agg aca tcc tgg tcg ata cac tcg aaa gga gag tgg atg acc aca 10109

Arg Thr Ser Trp Ser Ile His Ser Lys Gly Glu Trp Met Thr Thr

3325

3330

3335

gaa gac atg ctg cag gtc tgg aac aga gtc tgg att gaa gaa aat 10154

Glu Asp Met Leu Gln Val Trp Asn Arg Val Trp Ile Glu Glu Asn

3340

3345

3350

gaa tgg atg gtg gac aag act cca ata aca agc tgg aca gac gtt 10199

Glu Trp Met Val Asp Lys Thr Pro Ile Thr Ser Trp Thr Asp Val

3355

3360

3365

ccg tat gtg gga aag cgg gag gac atc tgg tgt ggc aac ctc atc 10244

Pro Tyr Val Gly Lys Arg Glu Asp Ile Trp Cys Gly Asn Leu Ile

3370

3375

3380

gga acg cga tcc aga gca acc tgg gct gag aac atc tac gcg gcg 10289

Gly Thr Arg Ser Arg Ala Thr Trp Ala Glu Asn Ile Tyr Ala Ala

3385

3390

3395

ata aac cag gtt aga gct gtc att ggg aaa gaa aat tat gtt gac 10334

Ile Asn Gln Val Arg Ala Val Ile Gly Lys Glu Asn Tyr Val Asp

3400

3405

3410

tac atg acc tca ctc agg aga tac gaa gat gtc ttg atc cag gaa 10379

Tyr Met Thr Ser Leu Arg Arg Tyr Glu Asp Val Leu Ile Gln Glu

3415

3420

3425

gac agg gtc atc tagtgtgatt taagggtggaa aagcagatta tgtaaataat 10431

Asp Arg Val Ile

3430

gtaaatgaga aaatgcatgc atatggagtc aggccagcaa aagctgccac cggataactgg 10491

gtagacgggtg ctgtctgcgt cccagtccca ggaggactgg gttaacaaat ctgacaacag 10551

aaagtgagaa aacctcaga accgtctcgg aagcagggtcc ctgctcactg gaagttgaag 10611

gaccaacgtc aggccacaaa tttgtgccac tccgtgagg agtgcggcct gcgcagcccc 10671

aggaggactg ggttaccaa ggcgttgagc cccacggcc caagcctcgt ctaggatgca 10731

atagacgagg tgtaaggact agaggttaga ggagaccccg tggaacaac aacatgcggc 10791

ccaagccccc tccaagctgt agaggaggtg gaaggactag aggttagagg agaccccgca 10851

tttgcataaa acagcatatt gacacctggg aatagactgg gagatcttct gctctatctc 10911

aacatcagct actaggcaca gagcgccgaa gtatgtagct ggtggtgagg aagaacacag 10971

gatct 10976

<210> 2

<211> 3432

<212> PRT

<213> Japanese encephalitis virus

<400> 2

Met Thr Lys Lys Pro Gly Gly Pro Gly Lys Asn Arg Ala Ile Asn Met

1 5 10 15

Leu Lys Arg Gly Leu Pro Arg Val Phe Pro Leu Val Gly Val Lys Arg

20 25 30

Val Val Met Ser Leu Leu Asp Gly Arg Gly Pro Val Arg Phe Val Leu

35 40 45

Ala Leu Ile Thr Phe Phe Lys Phe Thr Ala Leu Ala Pro Thr Lys Ala

50

55

60

Leu Leu Gly Arg Trp Lys Ala Val Glu Lys Ser Val Ala Met Lys His

65

70

75

80

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

85

90

95

Lys Arg Gly Arg Lys Gln Asn Lys Arg Gly Gly Asn Glu Gly Ser Ile

100

105

110

Met Trp Leu Ala Ser Leu Ala Val Val Ile Ala Cys Ala Gly Ala Ile

115

120

125

Lys Leu Ser Asn Phe Gln Gly Lys Leu Leu Met Thr Ile Asn Asn Thr

130

135

140

Asp Ile Ala Asp Val Ile Val Ile Pro Thr Ser Lys Gly Glu Asn Arg

145 150 155 160

Cys Trp Val Arg Ala Ile Asp Val Gly Tyr Met Cys Glu Asp Thr Ile

165 170 175

Thr Tyr Glu Cys Pro Lys Leu Ala Met Gly Asn Asp Pro Glu Asp Val

180 185 190

Asp Cys Trp Cys Asp Asn Gln Glu Val Tyr Val Gln Tyr Gly Arg Cys

195 200 205

Thr Arg Thr Arg His Ser Lys Arg Ser Arg Arg Ser Val Ser Val Gln

210 215 220

Thr His Gly Glu Ser Ser Leu Val Asn Lys Lys Glu Ala Trp Leu Asp

225 230 235 240

Ser Thr Lys Ala Thr Arg Tyr Leu Met Lys Thr Glu Asn Trp Ile Ile

245 250 255

Arg Asn Pro Gly Tyr Ala Phe Leu Ala Ala Val Leu Gly Trp Met Leu
260 265 270

Gly Ser Thr Asn Gly Gln Arg Val Val Phe Thr Ile Leu Leu Leu Leu
275 280 285

Val Ala Pro Ala Tyr Ser Phe Asn Cys Leu Gly Met Gly Asn Arg Asp
290 295 300

Phe Ile Glu Gly Ala Ser Gly Ala Thr Trp Val Asp Leu Val Leu Glu
305 310 315 320

Gly Asp Ser Cys Leu Thr Ile Met Ala Asn Asp Lys Pro Thr Leu Asp
325 330 335

Val Arg Met Ile Asn Ile Glu Ala Ser Gln Leu Ala Glu Val Arg Ser
340 345 350

Tyr Cys Tyr His Ala Ser Val Thr Asp Ile Ser Thr Val Ala Arg Cys
355 360 365

Pro Thr Thr Gly Glu Ala His Asn Glu Lys Arg Ala Asp Ser Ser Tyr

370

375

380

Val Cys Lys Gln Gly Phe Thr Asp Arg Gly Trp Gly Asn Gly Cys Gly

385

390

395

400

Leu Phe Gly Lys Gly Ser Ile Asp Thr Cys Ala Lys Phe Ser Cys Thr

405

410

415

Ser Lys Ala Ile Gly Arg Thr Ile Gln Pro Glu Asn Ile Lys Tyr Lys

420

425

430

Val Gly Ile Phe Val His Gly Ala Thr Thr Ser Glu Asn His Gly Asn

435

440

445

Tyr Ser Ala Gln Val Gly Ala Ser Gln Ala Ala Lys Phe Thr Val Thr

450

455

460

Pro Asn Ala Pro Ser Ile Thr Leu Lys Leu Gly Asp Tyr Gly Glu Val

465

470

475

480

Thr Leu Asp Cys Glu Pro Arg Ser Gly Leu Asn Thr Glu Ala Phe Tyr
485 490 495

Val Met Thr Val Gly Ser Lys Ser Phe Leu Val His Arg Glu Trp Phe
500 505 510

His Asp Leu Ala Leu Pro Trp Thr Ser Pro Ser Ser Thr Ala Trp Arg
515 520 525

Asn Arg Glu Leu Leu Met Glu Phe Glu Glu Ala His Ala Thr Lys Gln
530 535 540

Ser Val Val Ala Leu Gly Ser Gln Glu Gly Gly Leu His Gln Ala Leu
545 550 555 560

Ala Gly Ala Ile Val Val Glu Tyr Ser Ser Ser Val Lys Leu Thr Ser
565 570 575

Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Ala Leu Lys Gly

580

585

590

Thr Thr Tyr Gly Met Cys Thr Glu Lys Phe Ser Phe Ala Lys Asn Pro

595

600

605

Ala Asp Thr Gly His Gly Thr Val Val Ile Glu Leu Ser Tyr Ser Gly

610

615

620

Ser Asp Gly Pro Cys Lys Ile Pro Ile Val Ser Val Ala Ser Leu Asn

625

630

635

640

Asp Met Thr Pro Val Gly Arg Leu Val Thr Val Asn Pro Phe Val Ala

645

650

655

Thr Ser Ser Ala Asn Ser Lys Val Leu Val Glu Met Glu Pro Pro Phe

660

665

670

Gly Asp Ser Tyr Ile Val Val Gly Arg Gly Asp Lys Gln Ile Asn His

675

680

685

His Trp His Lys Ala Gly Ser Thr Leu Gly Lys Ala Phe Ser Thr Thr
690 695 700

Leu Lys Gly Ala Gln Arg Leu Ala Ala Leu Gly Asp Thr Ala Trp Asp
705 710 715 720

Phe Gly Ser Ile Gly Gly Val Phe Asn Ser Ile Gly Lys Ala Val His
725 730 735

Gln Val Phe Gly Gly Ala Phe Arg Thr Leu Phe Gly Gly Met Ser Trp
740 745 750

Ile Thr Gln Gly Leu Met Gly Ala Leu Leu Leu Trp Met Gly Val Asn
755 760 765

Ala Arg Asp Arg Ser Ile Ala Leu Ala Phe Leu Ala Thr Gly Gly Val
770 775 780

Leu Val Phe Leu Ala Thr Asn Val His Ala Asp Thr Gly Cys Ala Ile
785 790 795 800

Asp Ile Thr Arg Lys Glu Met Arg Cys Gly Ser Gly Ile Phe Val His

805

810

815

Asn Asp Val Glu Ala Trp Val Asp Arg Tyr Lys Tyr Leu Pro Glu Thr

820

825

830

Pro Arg Ser Leu Ala Lys Ile Val His Lys Ala His Lys Glu Gly Val

835

840

845

Cys Gly Val Arg Ser Val Thr Arg Leu Glu His Gln Met Trp Glu Ala

850

855

860

Val Arg Asp Glu Leu Asn Val Leu Leu Lys Glu Asn Ala Val Asp Leu

865

870

875

880

Ser Val Val Val Asn Lys Pro Val Gly Arg Tyr Arg Ser Ala Pro Lys

885

890

895

Arg Leu Ser Met Thr Gln Glu Lys Phe Glu Met Gly Trp Lys Ala Trp

900

905

910

Gly Lys Ser Ile Leu Phe Ala Pro Glu Leu Ala Asn Ser Thr Phe Val
 915 920 925

Val Asp Gly Pro Glu Thr Lys Glu Cys Pro Asp Glu His Arg Ala Trp
 930 935 940

Asn Ser Met Gln Ile Glu Asp Phe Gly Phe Gly Ile Thr Ser Thr Arg
 945 950 955 960

Val Trp Leu Lys Ile Arg Glu Glu Ser Thr Asp Glu Cys Asp Gly Ala
 965 970 975

Ile Ile Gly Thr Ala Val Lys Gly His Val Ala Val His Ser Asp Leu
 980 985 990

Ser Tyr Trp Ile Glu Ser Arg Tyr Asn Asp Thr Trp Lys Leu Glu Arg
 995 1000 1005

Ala Val Phe Gly Glu Val Lys Ser Cys Thr Trp Pro Glu Thr His

1010	1015	1020
Thr Leu Trp Gly Asp Gly Val	Glu Glu Ser Glu Leu	Ile Ile Pro
1025	1030	1035
His Thr Ile Ala Gly Pro Lys	Ser Lys His Asn Arg	Arg Glu Gly
1040	1045	1050
Tyr Lys Thr Gln Asn Gln Gly	Pro Trp Asp Glu Asn	Gly Ile Val
1055	1060	1065
Leu Asp Phe Asp Tyr Cys Pro	Gly Thr Lys Val Thr	Ile Thr Glu
1070	1075	1080
Asp Cys Gly Lys Arg Gly Pro	Ser Val Arg Thr Thr	Thr Asp Ser
1085	1090	1095
Gly Lys Leu Ile Thr Asp Trp	Cys Cys Arg Ser Cys	Ser Leu Pro
1100	1105	1110

Pro	Leu	Arg	Phe	Arg	Thr	Glu	Asn	Gly	Cys	Trp	Tyr	Gly	Met	Glu
1115						1120						1125		

Ile	Arg	Pro	Val	Arg	His	Asp	Glu	Thr	Thr	Leu	Val	Arg	Ser	Gln
1130						1135						1140		

Val	Asp	Ala	Phe	Asn	Gly	Glu	Met	Val	Asp	Pro	Phe	Gln	Leu	Gly
1145						1150						1155		

Leu	Leu	Val	Met	Phe	Leu	Ala	Thr	Gln	Glu	Val	Leu	Arg	Lys	Arg
1160						1165						1170		

Trp	Thr	Ala	Arg	Leu	Thr	Ile	Pro	Ala	Val	Leu	Gly	Ala	Leu	Leu
1175						1180						1185		

Val	Leu	Met	Leu	Gly	Gly	Ile	Thr	Tyr	Thr	Asp	Leu	Ala	Arg	Tyr
1190						1195						1200		

Val	Val	Leu	Val	Ala	Ala	Ser	Phe	Ala	Glu	Ala	Asn	Ser	Gly	Gly
1205						1210						1215		

Asp Val Leu His Leu Ala Leu Ile Ala Val Phe Lys Ile Gln Pro
1220 1225 1230

Ala Phe Leu Val Met Asn Met Leu Ser Thr Arg Trp Thr Asn Gln
1235 1240 1245

Glu Asn Val Val Leu Val Leu Gly Ala Ala Phe Phe Gln Leu Ala
1250 1255 1260

Ser Val Asp Leu Gln Ile Gly Val His Gly Ile Leu Asn Ala Ala
1265 1270 1275

Ala Ile Ala Trp Met Ile Val Arg Ala Ile Thr Phe Pro Thr Thr
1280 1285 1290

Ser Ser Val Thr Met Pro Val Leu Ala Leu Leu Thr Pro Gly Met
1295 1300 1305

Arg Ala Leu Tyr Leu Asp Thr Tyr Arg Ile Ile Leu Leu Val Ile
1310 1315 1320

Gly Ile Cys Ser Leu Leu Gln Glu Arg Lys Lys Thr Met Ala Lys
1325 1330 1335

Lys Lys Gly Ala Val Leu Leu Gly Leu Ala Leu Thr Ser Thr Gly
1340 1345 1350

Trp Phe Ser Pro Thr Thr Ile Ala Ala Gly Leu Met Val Cys Asn
1355 1360 1365

Pro Asn Lys Lys Arg Gly Trp Pro Ala Thr Glu Phe Leu Ser Ala
1370 1375 1380

Val Gly Leu Met Phe Ala Ile Val Gly Gly Leu Ala Glu Leu Asp
1385 1390 1395

Ile Glu Ser Met Ser Ile Pro Phe Met Leu Ala Gly Leu Met Ala
1400 1405 1410

Val Ser Tyr Val Val Ser Gly Lys Ala Thr Asp Met Trp Leu Glu

1415

1420

1425

Arg Ala Ala Asp Ile Ser Trp Glu Met Asp Ala Ala Ile Thr Gly

1430

1435

1440

Ser Ser Arg Arg Leu Asp Val Lys Leu Asp Asp Asp Gly Asp Phe

1445

1450

1455

His Leu Ile Asp Asp Pro Gly Val Pro Trp Lys Val Trp Val Leu

1460

1465

1470

Arg Met Ser Cys Ile Gly Leu Ala Ala Leu Thr Pro Trp Ala Ile

1475

1480

1485

Val Pro Ala Ala Phe Gly Tyr Trp Leu Thr Leu Lys Thr Thr Lys

1490

1495

1500

Arg Gly Gly Val Phe Trp Asp Thr Pro Ser Pro Lys Pro Cys Ser

1505

1510

1515

Lys Gly Asp Thr Thr Thr Gly Val Tyr Arg Ile Met Ala Arg Gly
1520 1525 1530

Ile Leu Gly Thr Tyr Gln Ala Gly Val Gly Val Met Tyr Glu Asn
1535 1540 1545

Val Phe His Thr Leu Trp His Thr Thr Arg Gly Ala Ala Ile Met
1550 1555 1560

Ser Gly Glu Gly Lys Leu Thr Pro Tyr Trp Gly Ser Val Lys Glu
1565 1570 1575

Asp Arg Ile Ala Tyr Gly Gly Pro Trp Arg Phe Asp Arg Lys Trp
1580 1585 1590

Asn Gly Thr Asp Asp Val Gln Val Ile Val Val Glu Pro Gly Lys
1595 1600 1605

Ala Ala Val Asn Ile Gln Thr Lys Pro Gly Val Phe Arg Thr Pro
1610 1615 1620

Phe Gly Glu Val Gly Ala Val Ser Leu Asp Tyr Pro Arg Gly Thr
1625 1630 1635

Ser Gly Ser Pro Ile Leu Asp Ser Asn Gly Asp Ile Ile Gly Leu
1640 1645 1650

Tyr Gly Asn Gly Val Glu Leu Gly Asp Gly Ser Tyr Val Ser Ala
1655 1660 1665

Ile Val Gln Gly Asp Arg Gln Glu Glu Pro Val Pro Glu Ala Tyr
1670 1675 1680

Thr Pro Asn Met Leu Arg Lys Arg Gln Met Thr Val Leu Asp Leu
1685 1690 1695

His Pro Gly Ser Gly Lys Thr Lys Lys Ile Leu Pro Gln Ile Ile
1700 1705 1710

Lys Asp Ala Ile Gln Gln Arg Leu Arg Thr Ala Val Leu Ala Pro
1715 1720 1725

Thr Arg Val Val Ala Ala Glu Met Ala Glu Ala Leu Arg Gly Leu
1730 1735 1740

Pro Val Arg Tyr Gln Thr Ser Ala Val Gln Arg Glu His Gln Gly
1745 1750 1755

Asn Glu Ile Val Asp Val Met Cys His Ala Thr Leu Thr His Arg
1760 1765 1770

Leu Met Ser Pro Asn Arg Val Pro Asn Tyr Asn Leu Phe Val Met
1775 1780 1785

Asp Glu Ala His Phe Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly
1790 1795 1800

Tyr Ile Ala Thr Lys Val Glu Leu Gly Glu Ala Ala Ala Ile Phe
1805 1810 1815

Met Thr Ala Thr Pro Pro Gly Thr Thr Asp Pro Phe Pro Asp Ser

1820	1825	1830
Asn Ala Pro Ile His Asp Leu Gln Asp Glu Ile Pro Asp Arg Ala		
1835	1840	1845
Trp Ser Ser Gly Tyr Glu Trp Ile Thr Glu Tyr Ala Gly Lys Thr		
1850	1855	1860
Val Trp Phe Val Ala Ser Val Lys Met Gly Asn Glu Ile Ala Met		
1865	1870	1875
Cys Leu Gln Arg Ala Gly Lys Lys Val Ile Gln Leu Asn Arg Lys		
1880	1885	1890
Ser Tyr Asp Thr Glu Tyr Pro Lys Cys Lys Asn Gly Asp Trp Asp		
1895	1900	1905
Phe Val Ile Thr Thr Asp Ile Ser Glu Met Gly Ala Asn Phe Gly		
1910	1915	1920

Ala Ser	Arg Val Ile Asp Cys	Arg Lys Ser Val Lys	Pro Thr Ile
1925	1930	1935	

Leu Glu	Glu Gly Glu Gly Arg	Val Ile Leu Gly Asn	Pro Ser Pro
1940	1945	1950	

Ile Thr	Ser Ala Ser Ala Ala	Gln Arg Arg Gly Arg	Val Gly Arg
1955	1960	1965	

Asn Pro	Asn Gln Val Gly Asp	Glu Tyr His Tyr Gly	Gly Ala Thr
1970	1975	1980	

Ser Glu	Asp Asp Ser Asn Leu	Ala His Trp Thr Glu	Ala Lys Ile
1985	1990	1995	

Met Leu	Asp Asn Ile His Met	Pro Asn Gly Leu Val	Ala Gln Leu
2000	2005	2010	

Tyr Gly	Pro Glu Arg Glu Lys	Ala Phe Thr Met Asp	Gly Glu Tyr
2015	2020	2025	

Arg Leu Arg Gly Glu Glu Lys Lys Asn Phe Leu Glu Leu Leu Arg
2030 2035 2040

Thr Ala Asp Leu Pro Val Trp Leu Ala Tyr Lys Val Ala Ser Asn
2045 2050 2055

Gly Ile Gln Tyr Thr Asp Arg Lys Trp Cys Phe Asp Gly Pro Arg
2060 2065 2070

Thr Asn Ala Ile Leu Glu Asp Asn Thr Glu Val Glu Ile Val Thr
2075 2080 2085

Arg Met Gly Glu Arg Lys Ile Leu Lys Pro Arg Trp Leu Asp Ala
2090 2095 2100

Arg Val Tyr Ala Asp His Gln Ala Leu Lys Trp Phe Lys Asp Phe
2105 2110 2115

Ala Ala Gly Lys Arg Ser Ala Val Ser Phe Ile Glu Val Leu Gly
2120 2125 2130

Arg Met Pro Glu His Phe Met Gly Lys Thr Arg Glu Ala Leu Asp
2135 2140 2145

Thr Met Tyr Leu Val Ala Thr Ala Glu Lys Gly Gly Lys Ala His
2150 2155 2160

Arg Met Ala Leu Glu Glu Leu Pro Asp Ala Leu Glu Thr Ile Thr
2165 2170 2175

Leu Ile Val Ala Ile Thr Val Met Thr Gly Gly Phe Phe Leu Leu
2180 2185 2190

Met Met Gln Arg Lys Gly Ile Gly Lys Met Gly Leu Gly Ala Leu
2195 2200 2205

Val Leu Thr Leu Ala Thr Phe Phe Leu Trp Ala Ala Glu Val Pro
2210 2215 2220

Gly Thr Lys Ile Ala Gly Thr Leu Leu Ile Ala Leu Leu Leu Met

2225

2230

2235

Val Val Leu Ile Pro Glu Pro Glu Lys Gln Arg Ser Gln Thr Asp

2240

2245

2250

Asn Gln Leu Ala Val Phe Leu Ile Cys Val Leu Thr Val Val Gly

2255

2260

2265

Val Val Ala Ala Asn Glu Tyr Gly Met Leu Glu Lys Thr Lys Ala

2270

2275

2280

Asp Leu Lys Ser Met Phe Gly Gly Lys Thr Gln Ala Ser Gly Leu

2285

2290

2295

Thr Gly Leu Pro Ser Met Ala Leu Asp Leu Arg Pro Ala Thr Ala

2300

2305

2310

Trp Ala Leu Tyr Gly Gly Ser Thr Val Val Leu Thr Pro Leu Leu

2315

2320

2325

Lys His Leu Ile Thr Ser Glu Tyr Val Thr Thr Ser Leu Ala Ser
2330 2335 2340

Ile Asn Ser Gln Ala Gly Ser Leu Phe Val Leu Pro Arg Gly Val
2345 2350 2355

Pro Phe Thr Asp Leu Asp Leu Thr Val Gly Leu Val Phe Leu Gly
2360 2365 2370

Cys Trp Gly Gln Ile Thr Leu Thr Thr Phe Leu Thr Ala Met Val
2375 2380 2385

Leu Ala Thr Leu His Tyr Gly Tyr Met Leu Pro Gly Trp Gln Ala
2390 2395 2400

Glu Ala Leu Arg Ala Ala Gln Arg Arg Thr Ala Ala Gly Ile Met
2405 2410 2415

Lys Asn Ala Val Val Asp Gly Met Val Ala Thr Asp Val Pro Glu
2420 2425 2430

Leu Glu Arg Thr Thr Pro Leu Met Gln Lys Lys Val Gly Gln Val

2435

2440

2445

Leu Leu Ile Gly Val Ser Val Ala Ala Phe Leu Val Asn Pro Lys

2450

2455

2460

Ile Thr Thr Val Arg Glu Ala Gly Val Leu Val Thr Ala Ala Thr

2465

2470

2475

Leu Ser Leu Trp Asp Asn Gly Ala Ser Ala Val Trp Asn Ser Thr

2480

2485

2490

Thr Ala Thr Gly Leu Cys His Val Met Arg Gly Ser Tyr Leu Ala

2495

2500

2505

Gly Gly Ser Ile Ala Trp Thr Leu Ile Lys Asn Ala Asp Lys Pro

2510

2515

2520

Ser Leu Lys Arg Gly Arg Pro Gly Gly Arg Thr Leu Gly Glu Gln

2525

2530

2535

Trp Lys Glu Lys Leu Asn Ala Met Ser Arg Glu Glu Phe Phe Lys
2540 2545 2550

Tyr Arg Arg Glu Ala Ile Ile Glu Val Asp Arg Thr Glu Ala Arg
2555 2560 2565

Arg Ala Arg Arg Glu Asn Asn Ile Val Gly Gly His Pro Val Ser
2570 2575 2580

Arg Gly Ser Ala Lys Leu Arg Trp Leu Val Glu Lys Gly Phe Val
2585 2590 2595

Ser Pro Ile Gly Lys Val Ile Asp Leu Gly Cys Gly Arg Gly Gly
2600 2605 2610

Trp Ser Tyr Tyr Ala Ala Thr Leu Lys Lys Val Gln Glu Val Arg
2615 2620 2625

Gly Tyr Thr Lys Gly Gly Ala Gly His Glu Glu Pro Met Leu Met

2630

2635

2640

Gln Ser Tyr Gly Trp Asn Leu Val Ser Met Lys Ser Gly Val Asp

2645

2650

2655

Val Phe Tyr Lys Pro Ser Glu Pro Ser Asp Thr Leu Phe Cys Asp

2660

2665

2670

Ile Gly Glu Ser Ser Pro Ser Pro Glu Val Glu Glu Gln Arg Thr

2675

2680

2685

Leu Arg Val Leu Glu Met Thr Ser Asp Trp Leu His Arg Gly Pro

2690

2695

2700

Arg Glu Phe Cys Ile Lys Val Leu Cys Pro Tyr Met Pro Lys Val

2705

2710

2715

Ile Glu Lys Met Glu Val Leu Gln Arg Arg Phe Gly Gly Gly Leu

2720

2725

2730

Val Arg Leu Pro Leu Ser Arg Asn Ser Asn His Glu Met Tyr Trp

2735

2740

2745

Val Ser Gly Ala Ala Gly Asn Val Val His Ala Val Asn Met Thr

2750

2755

2760

Ser Gln Val Leu Leu Gly Arg Met Asp Arg Thr Val Trp Arg Gly

2765

2770

2775

Pro Lys Tyr Glu Glu Asp Val Asn Leu Gly Ser Gly Thr Arg Ala

2780

2785

2790

Val Gly Lys Gly Glu Val His Ser Asn Gln Glu Lys Ile Lys Lys

2795

2800

2805

Arg Ile Gln Lys Leu Lys Glu Glu Phe Ala Thr Thr Trp His Lys

2810

2815

2820

Asp Pro Glu His Pro Tyr Arg Thr Trp Thr Tyr His Gly Ser Tyr

2825

2830

2835

Glu Val Lys Ala Thr Gly Ser Ala Ser Ser Leu Val Asn Gly Val

2840

2845

2850

Val Lys Leu Met Ser Lys Pro Trp Asp Ala Ile Ala Asn Val Thr

2855

2860

2865

Thr Met Ala Met Thr Asp Thr Thr Pro Phe Gly Gln Gln Arg Val

2870

2875

2880

Phe Lys Glu Lys Val Asp Thr Lys Ala Pro Glu Pro Pro Ala Gly

2885

2890

2895

Ala Lys Glu Val Leu Asn Glu Thr Thr Asn Trp Leu Trp Ala Tyr

2900

2905

2910

Leu Ser Arg Glu Lys Arg Pro Arg Leu Cys Thr Lys Glu Glu Phe

2915

2920

2925

Ile Lys Lys Val Asn Ser Asn Ala Ala Leu Gly Ala Val Phe Ala

2930

2935

2940

Glu Gln Asn Gln Trp Ser Thr Ala Arg Glu Ala Val Asp Asp Pro
2945 2950 2955

Arg Phe Trp Glu Met Val Asp Glu Glu Arg Glu Asn His Leu Arg
2960 2965 2970

Gly Glu Cys His Thr Cys Ile Tyr Asn Met Met Gly Lys Arg Glu
2975 2980 2985

Lys Lys Pro Gly Glu Phe Gly Lys Ala Lys Gly Ser Arg Ala Ile
2990 2995 3000

Trp Phe Met Trp Leu Gly Ala Arg Tyr Leu Glu Phe Glu Ala Leu
3005 3010 3015

Gly Phe Leu Asn Glu Asp His Trp Leu Ser Arg Glu Asn Ser Gly
3020 3025 3030

Gly Gly Val Glu Gly Ser Gly Val Gln Lys Leu Gly Tyr Ile Leu

3035

3040

3045

Arg Asp Ile Ala Gly Lys Gln Gly Gly Lys Met Tyr Ala Asp Asp

3050

3055

3060

Thr Ala Gly Trp Asp Thr Arg Ile Thr Arg Thr Asp Leu Glu Asn

3065

3070

3075

Glu Ala Lys Val Leu Glu Leu Leu Asp Gly Glu His Arg Met Leu

3080

3085

3090

Ala Arg Ala Ile Ile Glu Leu Thr Tyr Arg His Lys Val Val Lys

3095

3100

3105

Val Met Arg Pro Ala Ala Glu Gly Lys Thr Val Met Asp Val Ile

3110

3115

3120

Ser Arg Glu Asp Gln Arg Gly Ser Gly Gln Val Val Thr Tyr Ala

3125

3130

3135

Leu Asn Thr Phe Thr Asn Ile Ala Val Gln Leu Val Arg Leu Met

3140

3145

3150

Glu Ala Glu Gly Val Ile Gly Pro Gln His Leu Glu Gln Leu Pro

3155

3160

3165

Arg Lys Asn Lys Ile Ala Val Arg Thr Trp Leu Phe Glu Asn Gly

3170

3175

3180

Glu Glu Arg Val Ser Arg Met Ala Ile Ser Gly Asp Asp Cys Val

3185

3190

3195

Val Lys Pro Leu Asp Asp Arg Phe Ala Thr Ala Leu His Phe Leu

3200

3205

3210

Asn Ala Met Ser Lys Val Arg Lys Asp Ile Gln Glu Trp Lys Pro

3215

3220

3225

Ser His Gly Trp His Asp Trp Gln Gln Val Pro Phe Cys Ser Asn

3230

3235

3240

His Phe Gln Glu Ile Val Met Lys Asp Gly Arg Ser Ile Val Val

3245

3250

3255

Pro Cys Arg Gly Gln Asp Glu Leu Ile Gly Arg Ala Arg Ile Ser

3260

3265

3270

Pro Gly Ala Gly Trp Asn Val Lys Asp Thr Ala Cys Leu Ala Lys

3275

3280

3285

Ala Tyr Ala Gln Met Trp Leu Leu Leu Tyr Phe His Arg Arg Asp

3290

3295

3300

Leu Arg Leu Met Ala Asn Ala Ile Cys Ser Ala Val Pro Val Asp

3305

3310

3315

Trp Val Pro Thr Gly Arg Thr Ser Trp Ser Ile His Ser Lys Gly

3320

3325

3330

Glu Trp Met Thr Thr Glu Asp Met Leu Gln Val Trp Asn Arg Val

3335

3340

3345

Trp Ile Glu Glu Asn Glu Trp Met Val Asp Lys Thr Pro Ile Thr
3350 3355 3360

Ser Trp Thr Asp Val Pro Tyr Val Gly Lys Arg Glu Asp Ile Trp
3365 3370 3375

Cys Gly Asn Leu Ile Gly Thr Arg Ser Arg Ala Thr Trp Ala Glu
3380 3385 3390

Asn Ile Tyr Ala Ala Ile Asn Gln Val Arg Ala Val Ile Gly Lys
3395 3400 3405

Glu Asn Tyr Val Asp Tyr Met Thr Ser Leu Arg Arg Tyr Glu Asp
3410 3415 3420

Val Leu Ile Gln Glu Asp Arg Val Ile
3425 3430

<211> 10976

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gcagttttaa cagtttttta gaacggaaga taacc atg act aaa aaa cca gga 113

Met Thr Lys Lys Pro Gly

1 5

ggg ccc ggt aaa aac cgg gct atc aat atg ctg aaa cgc ggc cta ccc 161

Gly Pro Gly Lys Asn Arg Ala Ile Asn Met Leu Lys Arg Gly Leu Pro

10

15

20

cgc gta ttc cca cta gtg gga gtg aag agg gta gta atg agc ttg ttg 209

Arg Val Phe Pro Leu Val Gly Val Lys Arg Val Val Met Ser Leu Leu

25

30

35

gac ggc aga ggg cca gta cgt ttc gtg ctg gct ctt atc acg ttc ttc 257

Asp Gly Arg Gly Pro Val Arg Phe Val Leu Ala Leu Ile Thr Phe Phe

40

45

50

aag ttt aca gca tta gcc ccg acc aag gcg ctt tta ggc cga tgg aaa 305
 Lys Phe Thr Ala Leu Ala Pro Thr Lys Ala Leu Leu Gly Arg Trp Lys
 55 60 65 70

gca gtg gaa aag agt gta gca atg aaa cat ctc act agt ttc aaa cga 353
 Ala Val Glu Lys Ser Val Ala Met Lys His Leu Thr Ser Phe Lys Arg
 75 80 85

gaa ctt gga aca ctc att gac gcc gtg aac aag cgg ggc aga aag caa 401
 Glu Leu Gly Thr Leu Ile Asp Ala Val Asn Lys Arg Gly Arg Lys Gln
 90 95 100

aac aaa aga gga gga aat gaa ggc tca atc atg tgg ctt gcg agc ttg 449
 Asn Lys Arg Gly Gly Asn Glu Gly Ser Ile Met Trp Leu Ala Ser Leu
 105 110 115

gca gtt gtc ata gct tgt gca gga gcc atg aag ttg tca aat ttc cag 497
 Ala Val Val Ile Ala Cys Ala Gly Ala Met Lys Leu Ser Asn Phe Gln
 120 125 130

ggg aag ctt ttg atg acc att aac aac acg gac att gca gac gtt atc 545
 Gly Lys Leu Leu Met Thr Ile Asn Asn Thr Asp Ile Ala Asp Val Ile
 135 140 145 150

gta att ccc acc tca aaa gga gag aac aga tgc tgg gtc cgg gca atc 593
 Val Ile Pro Thr Ser Lys Gly Glu Asn Arg Cys Trp Val Arg Ala Ile

155	160	165	
gac gtc ggc tac atg tgt gag gac act atc acg tac gaa tgt cct aag			641
Asp Val Gly Tyr Met Cys Glu Asp Thr Ile Thr Tyr Glu Cys Pro Lys			
170	175	180	
ctt gcc atg ggc aat gat cca gag gat gtg gac tgc tgg tgt gac aac			689
Leu Ala Met Gly Asn Asp Pro Glu Asp Val Asp Cys Trp Cys Asp Asn			
185	190	195	
caa gaa gtc tac gtc caa tat gga cgg tgc acg cgg acc agg cat tcc			737
Gln Glu Val Tyr Val Gln Tyr Gly Arg Cys Thr Arg Thr Arg His Ser			
200	205	210	
aag cga agc agg aga tcc gtg tgc gtc caa aca cat ggg gag agt tca			785
Lys Arg Ser Arg Arg Ser Val Ser Val Gln Thr His Gly Glu Ser Ser			
215	220	225	230
cta gtg aat aaa aaa gag gct tgg ctg gat tca acg aaa gcc aca cga			833
Leu Val Asn Lys Lys Glu Ala Trp Leu Asp Ser Thr Lys Ala Thr Arg			
235	240	245	
tat ctc atg aaa act gag aac tgg atc ata agg aat cct ggc tat gct			881
Tyr Leu Met Lys Thr Glu Asn Trp Ile Ile Arg Asn Pro Gly Tyr Ala			
250	255	260	
ttc ctg gcg gcg gta ctc ggc tgg atg ctt ggc agt aac aac ggt caa			929

Phe Leu Ala Ala Val Leu Gly Trp Met Leu Gly Ser Asn Asn Gly Gln

265

270

275

cgc gtg gta ttc acc atc ctc ctg ctg ctg gtc gct ccg gct tac agt 977

Arg Val Val Phe Thr Ile Leu Leu Leu Leu Val Ala Pro Ala Tyr Ser

280

285

290

ttt aat tgt ctg gga atg ggc aat cgt gac ttc ata gaa gga gcc agt 1025

Phe Asn Cys Leu Gly Met Gly Asn Arg Asp Phe Ile Glu Gly Ala Ser

295

300

305

310

gga gcc act tgg gtg gac ttg gtg cta gaa gga gat agc tgc ttg aca 1073

Gly Ala Thr Trp Val Asp Leu Val Leu Glu Gly Asp Ser Cys Leu Thr

315

320

325

att atg gca aac gac aaa cca aca ttg gac gtc cgc atg atc aac atc 1121

Ile Met Ala Asn Asp Lys Pro Thr Leu Asp Val Arg Met Ile Asn Ile

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340

gaa gct agc caa ctt gct gag gtt aga agt tac tgt tat cat gct tca 1169

Glu Ala Ser Gln Leu Ala Glu Val Arg Ser Tyr Cys Tyr His Ala Ser

345

350

355

gtc act gac atc tcg acg gtg gct cgg tgc ccc acg act gga gaa gcc 1217

Val Thr Asp Ile Ser Thr Val Ala Arg Cys Pro Thr Thr Gly Glu Ala

360

365

370

cac aac gag aag cga gct gat agt agc tat gtg tgc aaa caa ggc ttc 1265
 His Asn Glu Lys Arg Ala Asp Ser Ser Tyr Val Cys Lys Gln Gly Phe
 375 380 385 390

act gat cgt ggg tgg ggc aac gga tgt gga ctt ttc ggg aag gga agc 1313
Thr Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
395 400 405

att gac aca tgt gca aaa ttc tcc tgc acc agc aaa gcg att ggg aga 1361
Ile Asp Thr Cys Ala Lys Phe Ser Cys Thr Ser Lys Ala Ile Gly Arg
410 415 420

aca atc cag cca gaa aac atc aaa tac aaa gtt ggc att ttt gtg cat 1409
Thr Ile Gln Pro Glu Asn Ile Lys Tyr Lys Val Gly Ile Phe Val His
425 430 435

gga gcc act act tcg gaa aac cat ggg aat tat tca gcg caa gtt ggg 1457
Gly Ala Thr Thr Ser Glu Asn His Gly Asn Tyr Ser Ala Gln Val Gly
440 445 450

gcg tcc cag gcg gca aag ttc aca gta aca ccc aat gct cct tcg ata 1505
Ala Ser Gln Ala Ala Lys Phe Thr Val Thr Pro Asn Ala Pro Ser Ile
455 460 465 470

acc ctc aaa ctt ggt gac tac gga gaa gtc aca ctg gac tgt gag cca 1553
Thr Leu Lys Leu Gly Asp Tyr Gly Glu Val Thr Leu Asp Cys Glu Pro
475 480 485

agg agt gga ctg aac act gaa gcg ttt tac gtc atg acc gtg ggg tca 1601
 Arg Ser Gly Leu Asn Thr Glu Ala Phe Tyr Val Met Thr Val Gly Ser
 490 495 500

aag tca ttt ctg gtc cat agg gaa tgg ttt cat gac ctc gct ctc ccc 1649
 Lys Ser Phe Leu Val His Arg Glu Trp Phe His Asp Leu Ala Leu Pro
 505 510 515

tgg acg tcc cct tcg agc aca gcg tgg aga aac aga gaa ctc ctc atg 1697
 Trp Thr Ser Pro Ser Ser Thr Ala Trp Arg Asn Arg Glu Leu Leu Met
 520 525 530

gag ttt gaa gag gcg cac gcc aca aaa cag tcc gtt gtt gct ctt ggg 1745
 Glu Phe Glu Glu Ala His Ala Thr Lys Gln Ser Val Val Ala Leu Gly
 535 540 545 550

tca cag gaa gga ggc ctc cat cag gcg ttg gca gga gcc atc gtg gtg 1793
 Ser Gln Glu Gly Gly Leu His Gln Ala Leu Ala Gly Ala Ile Val Val
 555 560 565

gag tac tca agt tca gtg aag tta aca tca ggc cac ctg aaa tgt agg 1841
 Glu Tyr Ser Ser Ser Val Lys Leu Thr Ser Gly His Leu Lys Cys Arg
 570 575 580

ctg aaa atg gac aaa ctg gct ctg aaa ggc aca acc tat ggc atg tgc 1889
 Leu Lys Met Asp Lys Leu Ala Leu Lys Gly Thr Thr Tyr Gly Met Cys

585	590	595	
aca gaa aaa ttc tcc ttc gcg aaa aat ccg gcg gac act ggt cac ggg			1937
Thr Glu Lys Phe Ser Phe Ala Lys Asn Pro Ala Asp Thr Gly His Gly			
600	605	610	
aca gtt gtc att gaa ctc tcc tac tct ggg agt gat ggc ccc tgc aaa			1985
Thr Val Val Ile Glu Leu Ser Tyr Ser Gly Ser Asp Gly Pro Cys Lys			
615	620	625	630
att ccg att gtc tcc gtt gcg agc ctc aat gac atg acc ccc gtc ggg			2033
Ile Pro Ile Val Ser Val Ala Ser Leu Asn Asp Met Thr Pro Val Gly			
	635	640	645
cgg ctg gtg aca gtg aac ccc ttc gtc gcg act tcc agt gcc aat tca			2081
Arg Leu Val Thr Val Asn Pro Phe Val Ala Thr Ser Ser Ala Asn Ser			
650	655	660	
aag gtg ctg gtc gag atg gaa ccc ccc ttc gga gac tcc tac atc gta			2129
Lys Val Leu Val Glu Met Glu Pro Pro Phe Gly Asp Ser Tyr Ile Val			
665	670	675	
gtt gga cgg gga gac aag cag atc aac cac cat tgg cat aaa gct gga			2177
Val Gly Arg Gly Asp Lys Gln Ile Asn His His Trp His Lys Ala Gly			
680	685	690	
agc acg ctg ggc aaa gcc ttt tca aca act ttg aag gga gct cag aga			2225

Ser Thr Leu Gly Lys Ala Phe Ser Thr Thr Leu Lys Gly Ala Gln Arg
 695 700 705 710

ctg gca gcg ttg ggt gac aca gcc tgg gac ttt ggc tcc att gga ggg 2273
 Leu Ala Ala Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Ile Gly Gly
 715 720 725

gtc ttc aac tcc ata gga aaa gcc gtt cac caa gtg ttt ggt ggt gcc 2321
 Val Phe Asn Ser Ile Gly Lys Ala Val His Gln Val Phe Gly Gly Ala
 730 735 740

ttc aga aca ctc ttc ggg gga atg tct tgg atc aca caa ggg cta atg 2369
 Phe Arg Thr Leu Phe Gly Gly Met Ser Trp Ile Thr Gln Gly Leu Met
 745 750 755

ggt gcc cta cta ctc tgg atg ggc gtc aac gca cga gac cga tca att 2417
 Gly Ala Leu Leu Leu Trp Met Gly Val Asn Ala Arg Asp Arg Ser Ile
 760 765 770

gct ttg gcc ttc tta gcc aca gga ggt gtg ctc gtg ttc tta gcg acc 2465
 Ala Leu Ala Phe Leu Ala Thr Gly Gly Val Leu Val Phe Leu Ala Thr
 775 780 785 790

aat gtg cat gct gac act gga tgt gcc att gac atc aca aga aaa gag 2513
 Asn Val His Ala Asp Thr Gly Cys Ala Ile Asp Ile Thr Arg Lys Glu
 795 800 805

atg agg tgt gga agt ggc atc ttc gtg cac aac gac gtg gaa gcc tgg 2561
Met Arg Cys Gly Ser Gly Ile Phe Val His Asn Asp Val Glu Ala Trp

810

815

820

gtg gat agg tat aaa tat ttg cca gaa acg ccc aga tcc cta gca aag 2609
Val Asp Arg Tyr Lys Tyr Leu Pro Glu Thr Pro Arg Ser Leu Ala Lys

825

830

835

atc gtc cac aaa gcg cac aag gaa ggc gtg tgc gga gtc aga tct gtc 2657
Ile Val His Lys Ala His Lys Glu Gly Val Cys Gly Val Arg Ser Val

840

845

850

act aga ctg gag cat caa atg tgg gaa gcc gta cgg gat gaa ttg aac 2705
Thr Arg Leu Glu His Gln Met Trp Glu Ala Val Arg Asp Glu Leu Asn
855 860 865 870

gtc ctg ctc aaa gag aat gca gtg gac ctc agt gtg gtt gtg aac aag 2753
Val Leu Leu Lys Glu Asn Ala Val Asp Leu Ser Val Val Val Asn Lys

875

880

885

ccc gtg ggg aga tat cgc tca gcc cct aaa cgc ctg tcc atg acg caa 2801
Pro Val Gly Arg Tyr Arg Ser Ala Pro Lys Arg Leu Ser Met Thr Gln

890

895

900

gag aag ttt gaa atg ggc tgg aaa gca tgg gga aaa agc att ctc ttt 2849
Glu Lys Phe Glu Met Gly Trp Lys Ala Trp Gly Lys Ser Ile Leu Phe

905

910

915

gcc ccg gaa ttg gcc aac tcc aca ttt gtc gta gat gga cct gag aca 2897

Ala Pro Glu Leu Ala Asn Ser Thr Phe Val Val Asp Gly Pro Glu Thr

920

925

930

aag gaa tgc cct gat gag cac aga gct tgg aac agc atg caa atc gaa 2945

Lys Glu Cys Pro Asp Glu His Arg Ala Trp Asn Ser Met Gln Ile Glu

935

940

945

950

gac ttc ggc ttt ggc atc aca tca acc cgt gtg tgg ctg aag att aga 2993

Asp Phe Gly Phe Gly Ile Thr Ser Thr Arg Val Trp Leu Lys Ile Arg

955

960

965

gag gag agc act gac gag tgt gat gga gcg atc ata ggt acg gct gtc 3041

Glu Glu Ser Thr Asp Glu Cys Asp Gly Ala Ile Ile Gly Thr Ala Val

970

975

980

aaa gga cat gtg gca gtc cat agt gac ttg tcg tac tgg att gag agt 3089

Lys Gly His Val Ala Val His Ser Asp Leu Ser Tyr Trp Ile Glu Ser

985

990

995

cgc tac aac gac aca tgg aaa ctt gag agg gca gtc ttt gga gaa 3134

Arg Tyr Asn Asp Thr Trp Lys Leu Glu Arg Ala Val Phe Gly Glu

1000

1005

1010

gtt aaa tcc tgc act tgg cca gag aca cac acc cta tgg gga gat 3179

Val Lys Ser Cys Thr Trp Pro Glu Thr His Thr Leu Trp Gly Asp

1015	1020	1025	
ggt gtt gag gaa agt gaa ctc	atc atc ccg cac acc	ata gcc gga	3224
Gly Val Glu Glu Ser Glu Leu	Ile Ile Pro His Thr	Ile Ala Gly	
1030	1035	1040	
cca aaa agc aag cat aat cgg	agg gaa ggg tat aag	aca caa aac	3269
Pro Lys Ser Lys His Asn Arg	Arg Glu Gly Tyr Lys	Thr Gln Asn	
1045	1050	1055	
cag gga cct tgg gac gag aat	ggc ata gtc ttg gac	ttt gac tat	3314
Gln Gly Pro Trp Asp Glu Asn	Gly Ile Val Leu Asp	Phe Asp Tyr	
1060	1065	1070	
tgc cca ggg aca aaa gtc acc	att aca gag gat tgt	ggc aag aga	3359
Cys Pro Gly Thr Lys Val Thr	Ile Thr Glu Asp Cys	Gly Lys Arg	
1075	1080	1085	
ggc cct tcg gtc aga acc act	act gac agt gga aag	ttg atc act	3404
Gly Pro Ser Val Arg Thr Thr	Thr Asp Ser Gly Lys	Leu Ile Thr	
1090	1095	1100	
gac tgg tgc tgt cgc agt tgc	tcc ctt ccg ccc cta	cga ttc cgg	3449
Asp Trp Cys Cys Arg Ser Cys	Ser Leu Pro Pro Leu	Arg Phe Arg	
1105	1110	1115	
aca gaa aat ggc tgc tgg tac	gga atg gaa atc aga	cct gtc agg	3494

Thr Glu Asn Gly Cys Trp Tyr Gly Met Glu Ile Arg Pro Val Arg

1120

1125

1130

cat gat gaa aca aca ctc gtc aga tcg cag gtt gat gct ttt aat

3539

His Asp Glu Thr Thr Leu Val Arg Ser Gln Val Asp Ala Phe Asn

1135

1140

1145

ggt gaa atg gtt gac cct ttt cag ctg ggc ctt ctg gtg atg ttt

3584

Gly Glu Met Val Asp Pro Phe Gln Leu Gly Leu Leu Val Met Phe

1150

1155

1160

ctg gcc acc cag gag gtc ctt cgc aag agg tgg acg gcc aga ttg

3629

Leu Ala Thr Gln Glu Val Leu Arg Lys Arg Trp Thr Ala Arg Leu

1165

1170

1175

acc att cct gcg gtt ttg ggg gcc cta ctt gtg ctg atg ctt ggg

3674

Thr Ile Pro Ala Val Leu Gly Ala Leu Leu Val Leu Met Leu Gly

1180

1185

1190

ggc atc act tac act gat ttg gcg agg tat gtg gtg cta gtc gct

3719

Gly Ile Thr Tyr Thr Asp Leu Ala Arg Tyr Val Val Leu Val Ala

1195

1200

1205

gcc gct ttc gca gag gcc aac agt gga gga gat gtc ctg cac ctt

3764

Ala Ala Phe Ala Glu Ala Asn Ser Gly Gly Asp Val Leu His Leu

1210

1215

1220

gct ttg att gcc gtt ttc aag atc caa cca gca ttt tta gtg atg 3809
 Ala Leu Ile Ala Val Phe Lys Ile Gln Pro Ala Phe Leu Val Met

1225

1230

1235

aac atg ctt agc acg aga tgg acg aac caa gaa aat gtg gtt ctg 3854
 Asn Met Leu Ser Thr Arg Trp Thr Asn Gln Glu Asn Val Val Leu

1240

1245

1250

gtc cta ggg gct gcc ttt ttc caa ttg gcc tca gta gat ctg caa 3899
 Val Leu Gly Ala Ala Phe Phe Gln Leu Ala Ser Val Asp Leu Gln

1255

1260

1265

ata gga gtt cac gga atc ctg aat gcc gcc gct ata gca tgg atg 3944
 Ile Gly Val His Gly Ile Leu Asn Ala Ala Ala Ile Ala Trp Met

1270

1275

1280

att gtc cgg gcg atc acc ttc ccc aca acc tcc tcc gtc acc atg 3989
 Ile Val Arg Ala Ile Thr Phe Pro Thr Thr Ser Ser Val Thr Met

1285

1290

1295

cca gtc tta gcg ctt cta act ccg gga atg agg gct cta tac cta 4034
 Pro Val Leu Ala Leu Leu Thr Pro Gly Met Arg Ala Leu Tyr Leu

1300

1305

1310

gat act tac aga atc atc ctc ctc gtc ata ggg att tgc tct ctg 4079
 Asp Thr Tyr Arg Ile Ile Leu Leu Val Ile Gly Ile Cys Ser Leu

1315

1320

1325

ttg caa gag agg aaa aag acc atg gca aaa aag aaa gga gct gta 4124
 Leu Gln Glu Arg Lys Lys Thr Met Ala Lys Lys Lys Gly Ala Val
 1330 1335 1340

ctc ttg ggc tta gcg ctc aca tcc act gga tgg ttt tcg ccc acc 4169
 Leu Leu Gly Leu Ala Leu Thr Ser Thr Gly Trp Phe Ser Pro Thr
 1345 1350 1355

act ata gct gcc gga cta atg gtc tgc aac cca aac aag aag aga 4214
 Thr Ile Ala Ala Gly Leu Met Val Cys Asn Pro Asn Lys Lys Arg
 1360 1365 1370

ggg tgg cca gct act gag ttt ttg tcg gca gtt gga ttg atg ttt 4259
 Gly Trp Pro Ala Thr Glu Phe Leu Ser Ala Val Gly Leu Met Phe
 1375 1380 1385

gcc atc gta ggt ggt ttg gcg gag ttg gat att gaa tcc atg tca 4304
 Ala Ile Val Gly Gly Leu Ala Glu Leu Asp Ile Glu Ser Met Ser
 1390 1395 1400

ata ccc ttc atg ctg gca ggt ctc atg gca gtg tcc tac gtg gtg 4349
 Ile Pro Phe Met Leu Ala Gly Leu Met Ala Val Ser Tyr Val Val
 1405 1410 1415

tca gga aaa gca aca gat atg tgg ctt gaa cgg gct gcc gac atc 4394
 Ser Gly Lys Ala Thr Asp Met Trp Leu Glu Arg Ala Ala Asp Ile

1420	1425	1430	
agc tgg	gag atg gat gct gca	atc aca gga agc agt	cgg agg ctg 4439
Ser Trp	Glu Met Asp Ala Ala	Ile Thr Gly Ser Ser	Arg Arg Leu
1435	1440	1445	
gat gtg	aag cta gat gat gac	gga gat ttt cac ttg	att gac gat 4484
Asp Val	Lys Leu Asp Asp Asp	Gly Asp Phe His Leu	Ile Asp Asp
1450	1455	1460	
ccc ggt	gtt cca tgg aag gtc	tgg gtc ctg cgc atg	tct tgc att 4529
Pro Gly	Val Pro Trp Lys Val	Trp Val Leu Arg Met	Ser Cys Ile
1465	1470	1475	
ggg tta	gcc gcc ctc acg cct	tgg gcc att gtt ccc	gcc gct ttt 4574
Gly Leu	Ala Ala Leu Thr Pro	Trp Ala Ile Val Pro	Ala Ala Phe
1480	1485	1490	
ggt tat	tgg ctc act tta aaa	aca aca aaa aga ggg	ggc gtg ttt 4619
Gly Tyr	Trp Leu Thr Leu Lys	Thr Thr Lys Arg Gly	Gly Val Phe
1495	1500	1505	
tgg gac	acg cca tcc cca aaa	cct tgc tca aaa gga	gac acc act 4664
Trp Asp	Thr Pro Ser Pro Lys	Pro Cys Ser Lys Gly	Asp Thr Thr
1510	1515	1520	
aca gga	gtt tac cgc att atg	gct aga ggg att ctt	ggc act tac 4709

Thr Gly	Val Tyr Arg Ile Met	Ala Arg Gly Ile Leu	Gly Thr Tyr	
1525	1530	1535		
cag gcc	ggc gtc gga gtc atg	tac gag aat gtt ttc	cac aca cta	4754
Gln Ala	Gly Val Gly Val Met	Tyr Glu Asn Val Phe	His Thr Leu	
1540	1545	1550		
tgg cac	aca act aga gga gca	gct att atg agt gga	gaa gga aaa	4799
Trp His	Thr Thr Arg Gly Ala	Ala Ile Met Ser Gly	Glu Gly Lys	
1555	1560	1565		
ttg acg	cca tac tgg ggt agt	gtg aaa gaa gac cgc	ata gct tac	4844
Leu Thr	Pro Tyr Trp Gly Ser	Val Lys Glu Asp Arg	Ile Ala Tyr	
1570	1575	1580		
gga ggc	cca tgg agg ttt gat	cga aaa tgg aat gga	act gat gac	4889
Gly Gly	Pro Trp Arg Phe Asp	Arg Lys Trp Asn Gly	Thr Asp Asp	
1585	1590	1595		
gtg caa	gtg atc gtg gta gaa	ccg ggg aag gct gca	gta aac atc	4934
Val Gln	Val Ile Val Val Glu	Pro Gly Lys Ala Ala	Val Asn Ile	
1600	1605	1610		
cag aca	aaa cca gga gtg ttt	cgg act ccc ttc ggg	gag gtt ggg	4979
Gln Thr	Lys Pro Gly Val Phe	Arg Thr Pro Phe Gly	Glu Val Gly	
1615	1620	1625		

gct gtt agt ctg gat tac ccg cga gga aca tcc ggc tca ccc att 5024

Ala Val Ser Leu Asp Tyr Pro Arg Gly Thr Ser Gly Ser Pro Ile

1630

1635

1640

ctg gat tcc aat gga gac atc ata ggc ctg tac ggc aat gga gtt 5069

Leu Asp Ser Asn Gly Asp Ile Ile Gly Leu Tyr Gly Asn Gly Val

1645

1650

1655

gag ctt ggc gat ggc tca tac gtc agc gcc atc gtg cag ggt gac 5114

Glu Leu Gly Asp Gly Ser Tyr Val Ser Ala Ile Val Gln Gly Asp

1660

1665

1670

cgt cag gag gaa cca gtc cca gaa gct tac acc cca aac atg ttg 5159

Arg Gln Glu Glu Pro Val Pro Glu Ala Tyr Thr Pro Asn Met Leu

1675

1680

1685

aga aag aga cag atg acc gta cta gat ttg cac cct ggt tca ggg 5204

Arg Lys Arg Gln Met Thr Val Leu Asp Leu His Pro Gly Ser Gly

1690

1695

1700

aaa acc aag aaa att ctg cca caa ata att aag gac gct att cag 5249

Lys Thr Lys Lys Ile Leu Pro Gln Ile Ile Lys Asp Ala Ile Gln

1705

1710

1715

cag cgc cta aga aca gct gtg ttg gca ccg acg cgg gtg gta gca 5294

Gln Arg Leu Arg Thr Ala Val Leu Ala Pro Thr Arg Val Val Ala

1720

1725

1730

gca gaa	atg gca gaa gct ttg	aga ggg ctc cca gta	cga tat caa	5339
Ala Glu	Met Ala Glu Ala Leu	Arg Gly Leu Pro Val	Arg Tyr Gln	
1735	1740	1745		

act tca	gca gtg cag aga gag	cac caa ggg aat gaa	ata gtg gat	5384
Thr Ser	Ala Val Gln Arg Glu	His Gln Gly Asn Glu	Ile Val Asp	
1750	1755	1760		

gtg atg	tgc cac gcc act ctg	acc cat aga ctg atg	tca ccg aac	5429
Val Met	Cys His Ala Thr Leu	Thr His Arg Leu Met	Ser Pro Asn	
1765	1770	1775		

aga gtg	ccc aac tac aac cta	ttt gtc atg gat gaa	gct cat ttc	5474
Arg Val	Pro Asn Tyr Asn Leu	Phe Val Met Asp Glu	Ala His Phe	
1780	1785	1790		

acc gac	cca gcc agt ata gcc	gca cga gga tac att	gct acc aag	5519
Thr Asp	Pro Ala Ser Ile Ala	Ala Arg Gly Tyr Ile	Ala Thr Lys	
1795	1800	1805		

gtg gaa	tta ggg gag gca gca	gcc atc ttt atg aca	gcg acc ccg	5564
Val Glu	Leu Gly Glu Ala Ala	Ala Ile Phe Met Thr	Ala Thr Pro	
1810	1815	1820		

cct gga	acc acg gat cct ttt	cct gac tca aat gcc	cca atc cat	5609
Pro Gly	Thr Thr Asp Pro Phe	Pro Asp Ser Asn Ala	Pro Ile His	

1825	1830	1835	
gat ttg	caa gat gag ata cca	gac agg gcg tgg agc	agt gga tac 5654
Asp Leu	Gln Asp Glu Ile Pro	Asp Arg Ala Trp Ser	Ser Gly Tyr
1840	1845	1850	
gaa tgg	atc aca gaa tat gcg	gga aaa acc gtg tgg	ttt gtg gca 5699
Glu Trp	Ile Thr Glu Tyr Ala	Gly Lys Thr Val Trp	Phe Val Ala
1855	1860	1865	
agc gtg	aaa atg ggg aac gag	att gca atg tgc ctc	caa aga gcg 5744
Ser Val	Lys Met Gly Asn Glu	Ile Ala Met Cys Leu	Gln Arg Ala
1870	1875	1880	
ggg aaa	aag gtc atc caa ctc	aac cgc aag tcc tat	gac aca gaa 5789
Gly Lys	Lys Val Ile Gln Leu	Asn Arg Lys Ser Tyr	Asp Thr Glu
1885	1890	1895	
tac cca	aaa tgt aag aat gga	gac tgg gat ttt gtc	atc acc act 5834
Tyr Pro	Lys Cys Lys Asn Gly	Asp Trp Asp Phe Val	Ile Thr Thr
1900	1905	1910	
gac att	tct gaa atg ggg gcc	aac ttc ggt gcg agc	agg gtc atc 5879
Asp Ile	Ser Glu Met Gly Ala	Asn Phe Gly Ala Ser	Arg Val Ile
1915	1920	1925	
gac tgt	aga aag agc gtg aag	ccc acc atc tta gaa	gag gga gaa 5924

Asp Cys Arg Lys Ser Val Lys Pro Thr Ile Leu Glu Glu Gly Glu

1930

1935

1940

ggc aga gtc atc ctc gga aac cca tcg ccc ata acc agt gca agc

5969

Gly Arg Val Ile Leu Gly Asn Pro Ser Pro Ile Thr Ser Ala Ser

1945

1950

1955

gca gct caa cgg agg ggc aga gta ggc aga aac cct aac cag gtt

6014

Ala Ala Gln Arg Arg Gly Arg Val Gly Arg Asn Pro Asn Gln Val

1960

1965

1970

gga gat gaa tac cac tat ggg ggg gcc acc agt gaa gat gac agt

6059

Gly Asp Glu Tyr His Tyr Gly Gly Ala Thr Ser Glu Asp Asp Ser

1975

1980

1985

aac cta gcc cat tgg aca gag gca aag atc atg tta gat aac ata

6104

Asn Leu Ala His Trp Thr Glu Ala Lys Ile Met Leu Asp Asn Ile

1990

1995

2000

cac atg ccc aat gga ctg gtg gcc cag ctc tat gga cca gag agg

6149

His Met Pro Asn Gly Leu Val Ala Gln Leu Tyr Gly Pro Glu Arg

2005

2010

2015

gaa aag gcc ttc aca atg gat ggc gaa tac cgt ctc aga ggt gaa

6194

Glu Lys Ala Phe Thr Met Asp Gly Glu Tyr Arg Leu Arg Gly Glu

2020

2025

2030

gaa aag aaa aac ttc tta gag ctg ctt agg acg gct gac ctc ccg 6239

Glu Lys Lys Asn Phe Leu Glu Leu Leu Arg Thr Ala Asp Leu Pro

2035

2040

2045

gtg tgg ctg gcc tac aag gtg gcg tcc aat ggc atc cag tac acc 6284

Val Trp Leu Ala Tyr Lys Val Ala Ser Asn Gly Ile Gln Tyr Thr

2050

2055

2060

gat aga aag tgg tgt ttt gat ggg ccg cgt acg aat gcc ata ctg 6329

Asp Arg Lys Trp Cys Phe Asp Gly Pro Arg Thr Asn Ala Ile Leu

2065

2070

2075

gag gac aac acc gag gta gag ata gtc acc cgg atg ggt gag agg 6374

Glu Asp Asn Thr Glu Val Glu Ile Val Thr Arg Met Gly Glu Arg

2080

2085

2090

aaa atc ctc aag ccg aga tgg ctt gat gca aga gtt tat gca gat 6419

Lys Ile Leu Lys Pro Arg Trp Leu Asp Ala Arg Val Tyr Ala Asp

2095

2100

2105

cac caa gct ctc aag tgg ttc aaa gac ttc gca gca gga aag aga 6464

His Gln Ala Leu Lys Trp Phe Lys Asp Phe Ala Ala Gly Lys Arg

2110

2115

2120

tca gcc gtt agc ttc ata gag gtg ctc ggt cgt atg cct gag cat 6509

Ser Ala Val Ser Phe Ile Glu Val Leu Gly Arg Met Pro Glu His

2125

2130

2135

ttc atg gga aag acg cgg gaa gct tta gac acc atg tac ttg gtt 6554
Phe Met Gly Lys Thr Arg Glu Ala Leu Asp Thr Met Tyr Leu Val
2140 2145 2150

gca acg gct gag aaa ggt ggg aaa gca cac cga atg gct ctc gaa 6599
Ala Thr Ala Glu Lys Gly Gly Lys Ala His Arg Met Ala Leu Glu
2155 2160 2165

gag ctg cca gat gca ctg gaa acc att aca ctt att gtt gct atc 6644
Glu Leu Pro Asp Ala Leu Glu Thr Ile Thr Leu Ile Val Ala Ile
2170 2175 2180

act gtg atg aca gga gga ttc ttt cta ctc atg atg cag cga aag 6689
Thr Val Met Thr Gly Gly Phe Phe Leu Leu Met Met Gln Arg Lys
2185 2190 2195

ggt ata ggg aag atg ggt ctt gga gct cta gtg ctc acg cta gct 6734
Gly Ile Gly Lys Met Gly Leu Gly Ala Leu Val Leu Thr Leu Ala
2200 2205 2210

acc ttc ttc ctg tgg gcg gca gag gtt ccc gga aca aaa ata gca 6779
Thr Phe Phe Leu Trp Ala Ala Glu Val Pro Gly Thr Lys Ile Ala
2215 2220 2225

ggg acc ctg ctg atc gcc ctg ctg ctt atg gtg gtt ctc atc cca 6824
Gly Thr Leu Leu Ile Ala Leu Leu Leu Met Val Val Leu Ile Pro

2230	2235	2240	
gaa ccg gaa aag cag agg tca caa aca gat aat caa ctg gcg gtg			6869
Glu Pro Glu Lys Gln Arg Ser Gln Thr Asp Asn Gln Leu Ala Val			
2245	2250	2255	
ttt ctc atc tgt gtc ttg acc gtg gtt gga gtg gtg gca gca aac			6914
Phe Leu Ile Cys Val Leu Thr Val Val Gly Val Val Ala Ala Asn			
2260	2265	2270	
gag tac ggg atg cta gaa aaa acc aaa gca gac ctc aag agc atg			6959
Glu Tyr Gly Met Leu Glu Lys Thr Lys Ala Asp Leu Lys Ser Met			
2275	2280	2285	
ttt ggc gga aag acg cag gca tca gga ctg act gga tta cca agc			7004
Phe Gly Gly Lys Thr Gln Ala Ser Gly Leu Thr Gly Leu Pro Ser			
2290	2295	2300	
atg gca ctg gac ctg cgt cca gcc aca gct tgg gca ctg tat ggg			7049
Met Ala Leu Asp Leu Arg Pro Ala Thr Ala Trp Ala Leu Tyr Gly			
2305	2310	2315	
ggg agc aca gtc gtg cta acc cct ctt ctg aag cac ctg atc acg			7094
Gly Ser Thr Val Val Leu Thr Pro Leu Leu Lys His Leu Ile Thr			
2320	2325	2330	
tcg gaa tac gtc acc aca tcg cta gcc tca att aac tca caa gct			7139

Ser Glu Tyr Val Thr Thr Ser Leu Ala Ser Ile Asn Ser Gln Ala

2335

2340

2345

ggc tca tta ttt gtc ttg cca cga ggc gtg cct ttt acc gac cta

7184

Gly Ser Leu Phe Val Leu Pro Arg Gly Val Pro Phe Thr Asp Leu

2350

2355

2360

gac ttg acc gtt ggc ctc gtc ttc ctt ggc tgt tgg ggt caa atc

7229

Asp Leu Thr Val Gly Leu Val Phe Leu Gly Cys Trp Gly Gln Ile

2365

2370

2375

acc ctc aca acg ttt ttg aca gcc atg gtt ctg gcg aca ctt cac

7274

Thr Leu Thr Thr Phe Leu Thr Ala Met Val Leu Ala Thr Leu His

2380

2385

2390

tat ggg tac atg ctc cct gga tgg caa gca gaa gca ctc agg gca

7319

Tyr Gly Tyr Met Leu Pro Gly Trp Gln Ala Glu Ala Leu Arg Ala

2395

2400

2405

gcc cag aga agg aca gcg gct gga ata atg aag aat gcc gtt gtt

7364

Ala Gln Arg Arg Thr Ala Ala Gly Ile Met Lys Asn Ala Val Val

2410

2415

2420

gac gga atg gtc gcc act gat gtg cct gaa ctg gaa agg acc act

7409

Asp Gly Met Val Ala Thr Asp Val Pro Glu Leu Glu Arg Thr Thr

2425

2430

2435

cct ctg atg caa aag aaa gtc gga cag gtg ctc ctc ata ggg gta 7454
 Pro Leu Met Gln Lys Lys Val Gly Gln Val Leu Leu Ile Gly Val

2440

2445

2450

agc gtg gca gcg ttc ctc gtc aac cct aat gtc acc act gtg aga 7499
 Ser Val Ala Ala Phe Leu Val Asn Pro Asn Val Thr Thr Val Arg

2455

2460

2465

gaa gca ggg gtg ttg gtg aca gcg gct acg ctc act ttg tgg gac 7544
 Glu Ala Gly Val Leu Val Thr Ala Ala Thr Leu Thr Leu Trp Asp

2470

2475

2480

aac gga gcc agt gcc gtt tgg aat tcc acc act gcc acg gga ctc 7589
 Asn Gly Ala Ser Ala Val Trp Asn Ser Thr Thr Ala Thr Gly Leu

2485

2490

2495

tgc cat gta atg cga ggt agc tac ctg gct gga ggc tcc att gct 7634
 Cys His Val Met Arg Gly Ser Tyr Leu Ala Gly Gly Ser Ile Ala

2500

2505

2510

tgg act ctc atc aag aac gct gac aag ccc tcc tta aaa agg gga 7679
 Trp Thr Leu Ile Lys Asn Ala Asp Lys Pro Ser Leu Lys Arg Gly

2515

2520

2525

agg cct ggg ggc agg acg cta ggg gag cag tgg aag gaa aaa cta 7724
 Arg Pro Gly Gly Arg Thr Leu Gly Glu Gln Trp Lys Glu Lys Leu

2530

2535

2540

aat gcc atg agc aga gaa gag ttt ttt aaa tac cgg aga gag gcc 7769
 Asn Ala Met Ser Arg Glu Glu Phe Phe Lys Tyr Arg Arg Glu Ala
 2545 2550 2555

ata atc gag gtg gac cgc act gaa gca cgc agg gct aga cgt gaa 7814
 Ile Ile Glu Val Asp Arg Thr Glu Ala Arg Arg Ala Arg Arg Glu
 2560 2565 2570

aat aac ata gtg gga gga cat ccg gtt tcg cga ggc tca gca aaa 7859
 Asn Asn Ile Val Gly Gly His Pro Val Ser Arg Gly Ser Ala Lys
 2575 2580 2585

ctc cgt tgg ctc gta gag aaa gga ttt gtc tcg cca ata gga aaa 7904
 Leu Arg Trp Leu Val Glu Lys Gly Phe Val Ser Pro Ile Gly Lys
 2590 2595 2600

gtc att gat cta ggg tgt ggg cgt gga gga tgg agc tac tac gca 7949
 Val Ile Asp Leu Gly Cys Gly Arg Gly Gly Trp Ser Tyr Tyr Ala
 2605 2610 2615

gca acc ctg aag aag gtc cag gaa gtc aga gga tac acg aaa ggt 7994
 Ala Thr Leu Lys Lys Val Gln Glu Val Arg Gly Tyr Thr Lys Gly
 2620 2625 2630

ggg gcg gga cat gaa gaa ccg atg ctc atg cag agc tac ggc tgg 8039
 Gly Ala Gly His Glu Glu Pro Met Leu Met Gln Ser Tyr Gly Trp

2635	2640	2645	
aac ctg	gtc tcc ctg aag agt	gga gtg gac gtt ttt	tac aaa cct 8084
Asn Leu	Val Ser Leu Lys Ser	Gly Val Asp Val Phe	Tyr Lys Pro
2650	2655	2660	
tca gag	ccc agt gac act ctg	ttc tgc gac ata ggg	gaa tcc tcc 8129
Ser Glu	Pro Ser Asp Thr Leu	Phe Cys Asp Ile Gly	Glu Ser Ser
2665	2670	2675	
cca agt	cca gaa gta gaa gaa	caa cgc aca cta cgc	gtc cta gag 8174
Pro Ser	Pro Glu Val Glu Glu	Gln Arg Thr Leu Arg	Val Leu Glu
2680	2685	2690	
atg aca	tct gac tgg ttg cac	cga gga cct aga gag	ttc tgt ata 8219
Met Thr	Ser Asp Trp Leu His	Arg Gly Pro Arg Glu	Phe Cys Ile
2695	2700	2705	
aaa gtt	ctt tgc ccc tac atg	ccc aag gtt ata gaa	aaa atg gaa 8264
Lys Val	Leu Cys Pro Tyr Met	Pro Lys Val Ile Glu	Lys Met Glu
2710	2715	2720	
gtc ctg	cag cgc cgc ttc gga	ggt ggg cta gtg cgt	ctt ccc ctg 8309
Val Leu	Gln Arg Arg Phe Gly	Gly Gly Leu Val Arg	Leu Pro Leu
2725	2730	2735	
tcc cgc	aac tcc aat cac gag	atg tac tgg gtt agt	gga ccg gct 8354

Ser Arg Asn Ser Asn His Glu Met Tyr Trp Val Ser Gly Pro Ala

2740

2745

2750

ggc aat gtg gtg cac gct gtg aac atg acc agc cag gta cta ctg

8399

Gly Asn Val Val His Ala Val Asn Met Thr Ser Gln Val Leu Leu

2755

2760

2765

ggg cga atg gat cgc aca gtg tgg aga ggg cca aag tat gag gaa

8444

Gly Arg Met Asp Arg Thr Val Trp Arg Gly Pro Lys Tyr Glu Glu

2770

2775

2780

gat gtc aac tta ggg agc gga aca aga gcc gtg gga aag gga gaa

8489

Asp Val Asn Leu Gly Ser Gly Thr Arg Ala Val Gly Lys Gly Glu

2785

2790

2795

gtc cat agc aat cag gag aaa atc aag aag aga atc cag aag ctt

8534

Val His Ser Asn Gln Glu Lys Ile Lys Lys Arg Ile Gln Lys Leu

2800

2805

2810

aaa gaa gaa ttc gcc aca acg tgg cac aaa gac cct gag cat cca

8579

Lys Glu Glu Phe Ala Thr Thr Trp His Lys Asp Pro Glu His Pro

2815

2820

2825

tac cgc act tgg aca tac cac gga agc tat gaa gtg aag gct act

8624

Tyr Arg Thr Trp Thr Tyr His Gly Ser Tyr Glu Val Lys Ala Thr

2830

2835

2840

ggc tca gct agt tct ctc gtc aac gga gtg gtg aag ctc atg agc 8669
 Gly Ser Ala Ser Ser Leu Val Asn Gly Val Val Lys Leu Met Ser

2845

2850

2855

aaa cct tgg gac gcc att gcc aac gtc acc acc atg gcc atg act 8714
 Lys Pro Trp Asp Ala Ile Ala Asn Val Thr Thr Met Ala Met Thr

2860

2865

2870

gac acc acc cct ttt gga cag caa aga gtt ttc aag gag aaa gtt 8759
 Asp Thr Thr Pro Phe Gly Gln Gln Arg Val Phe Lys Glu Lys Val

2875

2880

2885

gac acg aag gct cct gag cca cca act gga gct aag gaa gtg ctc 8804
 Asp Thr Lys Ala Pro Glu Pro Pro Thr Gly Ala Lys Glu Val Leu

2890

2895

2900

aac gag acc acc aac tgg ctg tgg gcc tac ttg tca cgg gaa aaa 8849
 Asn Glu Thr Thr Asn Trp Leu Trp Ala Tyr Leu Ser Arg Glu Lys

2905

2910

2915

aga ccc cgc ttg tgc acc aag gaa gaa ttc ata aag aaa gtc aat 8894
 Arg Pro Arg Leu Cys Thr Lys Glu Glu Phe Ile Lys Lys Val Asn

2920

2925

2930

agc aac gcg gct ctt gga gca gtg ttc gct gaa cag aat caa tgg 8939
 Ser Asn Ala Ala Leu Gly Ala Val Phe Ala Glu Gln Asn Gln Trp

2935

2940

2945

agc acg gcg cgt gag gct gtg gat gac ccg cgg ttt tgg gag atg 8984
 Ser Thr Ala Arg Glu Ala Val Asp Asp Pro Arg Phe Trp Glu Met
 2950 2955 2960

gtt gat gaa gag agg gaa aac cat ctg cga gga gag tgt cac aca 9029
 Val Asp Glu Glu Arg Glu Asn His Leu Arg Gly Glu Cys His Thr
 2965 2970 2975

tgt atc tat aac atg atg gga aaa aga gag aag aag cct gga gag 9074
 Cys Ile Tyr Asn Met Met Gly Lys Arg Glu Lys Lys Pro Gly Glu
 2980 2985 2990

ttt gga aaa gct aaa gga agc agg gcc att tgg ttc atg tgg ctt 9119
 Phe Gly Lys Ala Lys Gly Ser Arg Ala Ile Trp Phe Met Trp Leu
 2995 3000 3005

gga gca cgg tat cta gag ttt gaa gct ttg ggg ttc ctg aat gaa 9164
 Gly Ala Arg Tyr Leu Glu Phe Glu Ala Leu Gly Phe Leu Asn Glu
 3010 3015 3020

gat cat tgg ctg agc cga gag aat tca gga ggt gga gtg gaa ggc 9209
 Asp His Trp Leu Ser Arg Glu Asn Ser Gly Gly Gly Val Glu Gly
 3025 3030 3035

tca ggc gtc caa aag ctg gga tac atc ctc cgt gat ata gca gga 9254
 Ser Gly Val Gln Lys Leu Gly Tyr Ile Leu Arg Asp Ile Ala Gly

3040	3045	3050	
aag caa gga gga aaa atg tac gct gat gat acc gcc ggg tgg gac			9299
Lys Gln Gly Gly Lys Met Tyr Ala Asp Asp Thr Ala Gly Trp Asp			
3055	3060	3065	
act aga att acc aga act gat tta gaa aat gaa gcc aag gtg ctg			9344
Thr Arg Ile Thr Arg Thr Asp Leu Glu Asn Glu Ala Lys Val Leu			
3070	3075	3080	
gag ctt cta gac ggt gaa cac cgc atg ctc gcc cga gcc ata att			9389
Glu Leu Leu Asp Gly Glu His Arg Met Leu Ala Arg Ala Ile Ile			
3085	3090	3095	
gaa ttg act tac agg cac aaa gtg gtc aag gtc atg aga cct gca			9434
Glu Leu Thr Tyr Arg His Lys Val Val Lys Val Met Arg Pro Ala			
3100	3105	3110	
gca gaa gga aag acc gtg atg gac gtg ata tca agg gag gat caa			9479
Ala Glu Gly Lys Thr Val Met Asp Val Ile Ser Arg Glu Asp Gln			
3115	3120	3125	
agg ggg agt gga cag gtg gtc act tat gct ctt aac act ttc acg			9524
Arg Gly Ser Gly Gln Val Val Thr Tyr Ala Leu Asn Thr Phe Thr			
3130	3135	3140	
aac atc gct gtc cag ctc gtc agg ctg atg gag gct gag ggg gtc			9569

Asn Ile Ala Val Gln Leu Val Arg Leu Met Glu Ala Glu Gly Val

3145

3150

3155

att gga cca caa cac ttg gaa cag cta cct aga aaa aac aag ata

9614

Ile Gly Pro Gln His Leu Glu Gln Leu Pro Arg Lys Asn Lys Ile

3160

3165

3170

gct gtc agg acc tgg ctc ttt gag aat gga gag gag aga gtg tcc

9659

Ala Val Arg Thr Trp Leu Phe Glu Asn Gly Glu Glu Arg Val Ser

3175

3180

3185

agg atg gct atc agc gga gac gac tgt gtc gtc aag ccg ctg gac

9704

Arg Met Ala Ile Ser Gly Asp Asp Cys Val Val Lys Pro Leu Asp

3190

3195

3200

gac aga ttc gcc acg gcc ctc cac ttc ctc aac gca atg tca aag

9749

Asp Arg Phe Ala Thr Ala Leu His Phe Leu Asn Ala Met Ser Lys

3205

3210

3215

gtc aga aaa gac atc cag gaa tgg aag cct tca cat ggc tgg cac

9794

Val Arg Lys Asp Ile Gln Glu Trp Lys Pro Ser His Gly Trp His

3220

3225

3230

gat tgg cag caa gtt ccc ttc tgc tct aac cat ttt cag gag att

9839

Asp Trp Gln Gln Val Pro Phe Cys Ser Asn His Phe Gln Glu Ile

3235

3240

3245

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3250

3255

3260

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3265

3270

3275

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3285

3290

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3295

3300

3305

aat gcg att tgc tca gca gtg cca gtg gat tgg gtg ccc acg ggc 10064
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3315

3320

agg aca tcc tgg tcg ata cac tcg aaa gga gag tgg atg acc aca 10109
 Arg Thr Ser Trp Ser Ile His Ser Lys Gly Glu Trp Met Thr Thr

3325

3330

3335

gaa gac atg ctg cag gtc tgg aac aga gtc tgg att gaa gaa aat 10154
 Glu Asp Met Leu Gln Val Trp Asn Arg Val Trp Ile Glu Glu Asn

3340

3345

3350

gaa tgg atg gtg gac aag act cca ata aca agc tgg aca gac gtt 10199
 Glu Trp Met Val Asp Lys Thr Pro Ile Thr Ser Trp Thr Asp Val
 3355 3360 3365

ccg tat gtg gga aag cgg gag gac atc tgg tgt ggc agc ctc atc 10244
 Pro Tyr Val Gly Lys Arg Glu Asp Ile Trp Cys Gly Ser Leu Ile
 3370 3375 3380

gga acg cga tcc aga gca acc tgg gct gag aac atc tac gcg gcg 10289
 Gly Thr Arg Ser Arg Ala Thr Trp Ala Glu Asn Ile Tyr Ala Ala
 3385 3390 3395

ata aac cag gtt aga gct gtc att ggg aaa gaa aat tat gtt gac 10334
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 3400 3405 3410

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 3415 3420 3425

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 Asp Arg Val Ile
 3430

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 gatct 10976

<210> 4

<211> 3432

<212> PRT

<213> Japanese encephalitis virus

<400> 4

Met Thr Lys Lys Pro Gly Gly Pro Gly Lys Asn Arg Ala Ile Asn Met

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Leu Lys Arg Gly Leu Pro Arg Val Phe Pro Leu Val Gly Val Lys Arg
 20 25 30

Val Val Met Ser Leu Leu Asp Gly Arg Gly Pro Val Arg Phe Val Leu
 35 40 45

Ala Leu Ile Thr Phe Phe Lys Phe Thr Ala Leu Ala Pro Thr Lys Ala
 50 55 60

Leu Leu Gly Arg Trp Lys Ala Val Glu Lys Ser Val Ala Met Lys His
65 70 75 80

Leu Thr Ser Phe Lys Arg Glu Leu Gly Thr Leu Ile Asp Ala Val Asn
 85 90 95

Lys Arg Gly Arg Lys Gln Asn Lys Arg Gly Gly Asn Glu Gly Ser Ile
 100 105 110

Met Trp Leu Ala Ser Leu Ala Val Val Ile Ala Cys Ala Gly Ala Met

115

120

125

Lys Leu Ser Asn Phe Gln Gly Lys Leu Leu Met Thr Ile Asn Asn Thr

130

135

140

Asp Ile Ala Asp Val Ile Val Ile Pro Thr Ser Lys Gly Glu Asn Arg

145

150

155

160

Cys Trp Val Arg Ala Ile Asp Val Gly Tyr Met Cys Glu Asp Thr Ile

165

170

175

Thr Tyr Glu Cys Pro Lys Leu Ala Met Gly Asn Asp Pro Glu Asp Val

180

185

190

Asp Cys Trp Cys Asp Asn Gln Glu Val Tyr Val Gln Tyr Gly Arg Cys

195

200

205

Thr Arg Thr Arg His Ser Lys Arg Ser Arg Arg Ser Val Ser Val Gln

210

215

220

Thr His Gly Glu Ser Ser Leu Val Asn Lys Lys Glu Ala Trp Leu Asp

225

230

235

240

Ser Thr Lys Ala Thr Arg Tyr Leu Met Lys Thr Glu Asn Trp Ile Ile

245

250

255

Arg Asn Pro Gly Tyr Ala Phe Leu Ala Ala Val Leu Gly Trp Met Leu

260

265

270

Gly Ser Asn Asn Gly Gln Arg Val Val Phe Thr Ile Leu Leu Leu Leu

275

280

285

Val Ala Pro Ala Tyr Ser Phe Asn Cys Leu Gly Met Gly Asn Arg Asp

290

295

300

Phe Ile Glu Gly Ala Ser Gly Ala Thr Trp Val Asp Leu Val Leu Glu

305

310

315

320

Gly Asp Ser Cys Leu Thr Ile Met Ala Asn Asp Lys Pro Thr Leu Asp

325

330

335

Val Arg Met Ile Asn Ile Glu Ala Ser Gln Leu Ala Glu Val Arg Ser

340

345

350

Tyr Cys Tyr His Ala Ser Val Thr Asp Ile Ser Thr Val Ala Arg Cys

355

360

365

Pro Thr Thr Gly Glu Ala His Asn Glu Lys Arg Ala Asp Ser Ser Tyr

370

375

380

Val Cys Lys Gln Gly Phe Thr Asp Arg Gly Trp Gly Asn Gly Cys Gly

385

390

395

400

Leu Phe Gly Lys Gly Ser Ile Asp Thr Cys Ala Lys Phe Ser Cys Thr

405

410

415

Ser Lys Ala Ile Gly Arg Thr Ile Gln Pro Glu Asn Ile Lys Tyr Lys

420

425

430

Val Gly Ile Phe Val His Gly Ala Thr Thr Ser Glu Asn His Gly Asn

435

440

445

Tyr Ser Ala Gln Val Gly Ala Ser Gln Ala Ala Lys Phe Thr Val Thr

450

455

460

Pro Asn Ala Pro Ser Ile Thr Leu Lys Leu Gly Asp Tyr Gly Glu Val

465

470

475

480

Thr Leu Asp Cys Glu Pro Arg Ser Gly Leu Asn Thr Glu Ala Phe Tyr

485

490

495

Val Met Thr Val Gly Ser Lys Ser Phe Leu Val His Arg Glu Trp Phe

500

505

510

His Asp Leu Ala Leu Pro Trp Thr Ser Pro Ser Ser Thr Ala Trp Arg

515

520

525

Asn Arg Glu Leu Leu Met Glu Phe Glu Glu Ala His Ala Thr Lys Gln

530

535

540

Ser Val Val Ala Leu Gly Ser Gln Glu Gly Gly Leu His Gln Ala Leu
545 550 555 560

Ala Gly Ala Ile Val Val Glu Tyr Ser Ser Ser Val Lys Leu Thr Ser
565 570 575

Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Ala Leu Lys Gly
580 585 590

Thr Thr Tyr Gly Met Cys Thr Glu Lys Phe Ser Phe Ala Lys Asn Pro
595 600 605

Ala Asp Thr Gly His Gly Thr Val Val Ile Glu Leu Ser Tyr Ser Gly
610 615 620

Ser Asp Gly Pro Cys Lys Ile Pro Ile Val Ser Val Ala Ser Leu Asn
625 630 635 640

Asp Met Thr Pro Val Gly Arg Leu Val Thr Val Asn Pro Phe Val Ala
645 650 655

Thr Ser Ser Ala Asn Ser Lys Val Leu Val Glu Met Glu Pro Pro Phe

660

665

670

Gly Asp Ser Tyr Ile Val Val Gly Arg Gly Asp Lys Gln Ile Asn His

675

680

685

His Trp His Lys Ala Gly Ser Thr Leu Gly Lys Ala Phe Ser Thr Thr

690

695

700

Leu Lys Gly Ala Gln Arg Leu Ala Ala Leu Gly Asp Thr Ala Trp Asp

705

710

715

720

Phe Gly Ser Ile Gly Gly Val Phe Asn Ser Ile Gly Lys Ala Val His

725

730

735

Gln Val Phe Gly Gly Ala Phe Arg Thr Leu Phe Gly Gly Met Ser Trp

740

745

750

Ile Thr Gln Gly Leu Met Gly Ala Leu Leu Leu Trp Met Gly Val Asn

755

760

765

Ala Arg Asp Arg Ser Ile Ala Leu Ala Phe Leu Ala Thr Gly Gly Val
770 775 780

Leu Val Phe Leu Ala Thr Asn Val His Ala Asp Thr Gly Cys Ala Ile
785 790 795 800

Asp Ile Thr Arg Lys Glu Met Arg Cys Gly Ser Gly Ile Phe Val His
805 810 815

Asn Asp Val Glu Ala Trp Val Asp Arg Tyr Lys Tyr Leu Pro Glu Thr
820 825 830

Pro Arg Ser Leu Ala Lys Ile Val His Lys Ala His Lys Glu Gly Val
835 840 845

Cys Gly Val Arg Ser Val Thr Arg Leu Glu His Gln Met Trp Glu Ala
850 855 860

Val Arg Asp Glu Leu Asn Val Leu Leu Lys Glu Asn Ala Val Asp Leu

865

870

875

880

Ser Val Val Val Asn Lys Pro Val Gly Arg Tyr Arg Ser Ala Pro Lys

885

890

895

Arg Leu Ser Met Thr Gln Glu Lys Phe Glu Met Gly Trp Lys Ala Trp

900

905

910

Gly Lys Ser Ile Leu Phe Ala Pro Glu Leu Ala Asn Ser Thr Phe Val

915

920

925

Val Asp Gly Pro Glu Thr Lys Glu Cys Pro Asp Glu His Arg Ala Trp

930

935

940

Asn Ser Met Gln Ile Glu Asp Phe Gly Phe Gly Ile Thr Ser Thr Arg

945

950

955

960

Val Trp Leu Lys Ile Arg Glu Glu Ser Thr Asp Glu Cys Asp Gly Ala

965

970

975

Ile Ile Gly Thr Ala Val Lys Gly His Val Ala Val His Ser Asp Leu

980

985

990

Ser Tyr Trp Ile Glu Ser Arg Tyr Asn Asp Thr Trp Lys Leu Glu Arg

995

1000

1005

Ala Val Phe Gly Glu Val Lys Ser Cys Thr Trp Pro Glu Thr His

1010

1015

1020

Thr Leu Trp Gly Asp Gly Val Glu Glu Ser Glu Leu Ile Ile Pro

1025

1030

1035

His Thr Ile Ala Gly Pro Lys Ser Lys His Asn Arg Arg Glu Gly

1040

1045

1050

Tyr Lys Thr Gln Asn Gln Gly Pro Trp Asp Glu Asn Gly Ile Val

1055

1060

1065

Leu Asp Phe Asp Tyr Cys Pro Gly Thr Lys Val Thr Ile Thr Glu

1070

1075

1080

Asp Cys Gly Lys Arg Gly Pro Ser Val Arg Thr Thr Thr Asp Ser

1085

1090

1095

Gly Lys Leu Ile Thr Asp Trp Cys Cys Arg Ser Cys Ser Leu Pro

1100

1105

1110

Pro Leu Arg Phe Arg Thr Glu Asn Gly Cys Trp Tyr Gly Met Glu

1115

1120

1125

Ile Arg Pro Val Arg His Asp Glu Thr Thr Leu Val Arg Ser Gln

1130

1135

1140

Val Asp Ala Phe Asn Gly Glu Met Val Asp Pro Phe Gln Leu Gly

1145

1150

1155

Leu Leu Val Met Phe Leu Ala Thr Gln Glu Val Leu Arg Lys Arg

1160

1165

1170

Trp Thr Ala Arg Leu Thr Ile Pro Ala Val Leu Gly Ala Leu Leu

1175

1180

1185

Val Leu Met Leu Gly Gly Ile Thr Tyr Thr Asp Leu Ala Arg Tyr
1190 1195 1200

Val Val Leu Val Ala Ala Ala Phe Ala Glu Ala Asn Ser Gly Gly
1205 1210 1215

Asp Val Leu His Leu Ala Leu Ile Ala Val Phe Lys Ile Gln Pro
1220 1225 1230

Ala Phe Leu Val Met Asn Met Leu Ser Thr Arg Trp Thr Asn Gln
1235 1240 1245

Glu Asn Val Val Leu Val Leu Gly Ala Ala Phe Phe Gln Leu Ala
1250 1255 1260

Ser Val Asp Leu Gln Ile Gly Val His Gly Ile Leu Asn Ala Ala
1265 1270 1275

Ala Ile Ala Trp Met Ile Val Arg Ala Ile Thr Phe Pro Thr Thr

1280	1285	1290
Ser Ser Val Thr Met Pro Val Leu Ala Leu Leu Thr Pro Gly Met		
1295	1300	1305
Arg Ala Leu Tyr Leu Asp Thr Tyr Arg Ile Ile Leu Leu Val Ile		
1310	1315	1320
Gly Ile Cys Ser Leu Leu Gln Glu Arg Lys Lys Thr Met Ala Lys		
1325	1330	1335
Lys Lys Gly Ala Val Leu Leu Gly Leu Ala Leu Thr Ser Thr Gly		
1340	1345	1350
Trp Phe Ser Pro Thr Thr Ile Ala Ala Gly Leu Met Val Cys Asn		
1355	1360	1365
Pro Asn Lys Lys Arg Gly Trp Pro Ala Thr Glu Phe Leu Ser Ala		
1370	1375	1380

Val Gly Leu Met Phe Ala Ile Val Gly Gly Leu Ala Glu Leu Asp

1385

1390

1395

Ile Glu Ser Met Ser Ile Pro Phe Met Leu Ala Gly Leu Met Ala

1400

1405

1410

Val Ser Tyr Val Val Ser Gly Lys Ala Thr Asp Met Trp Leu Glu

1415

1420

1425

Arg Ala Ala Asp Ile Ser Trp Glu Met Asp Ala Ala Ile Thr Gly

1430

1435

1440

Ser Ser Arg Arg Leu Asp Val Lys Leu Asp Asp Asp Gly Asp Phe

1445

1450

1455

His Leu Ile Asp Asp Pro Gly Val Pro Trp Lys Val Trp Val Leu

1460

1465

1470

Arg Met Ser Cys Ile Gly Leu Ala Ala Leu Thr Pro Trp Ala Ile

1475

1480

1485

Val Pro Ala Ala Phe Gly Tyr Trp Leu Thr Leu Lys Thr Thr Lys

1490

1495

1500

Arg Gly Gly Val Phe Trp Asp Thr Pro Ser Pro Lys Pro Cys Ser

1505

1510

1515

Lys Gly Asp Thr Thr Thr Gly Val Tyr Arg Ile Met Ala Arg Gly

1520

1525

1530

Ile Leu Gly Thr Tyr Gln Ala Gly Val Gly Val Met Tyr Glu Asn

1535

1540

1545

Val Phe His Thr Leu Trp His Thr Thr Arg Gly Ala Ala Ile Met

1550

1555

1560

Ser Gly Glu Gly Lys Leu Thr Pro Tyr Trp Gly Ser Val Lys Glu

1565

1570

1575

Asp Arg Ile Ala Tyr Gly Gly Pro Trp Arg Phe Asp Arg Lys Trp

1580

1585

1590

Asn Gly	Thr Asp Asp Val Gln	Val Ile Val Val Glu	Pro Gly Lys
1595	1600	1605	

Ala Ala	Val Asn Ile Gln Thr	Lys Pro Gly Val Phe	Arg Thr Pro
1610	1615	1620	

Phe Gly	Glu Val Gly Ala Val	Ser Leu Asp Tyr Pro	Arg Gly Thr
1625	1630	1635	

Ser Gly	Ser Pro Ile Leu Asp	Ser Asn Gly Asp Ile	Ile Gly Leu
1640	1645	1650	

Tyr Gly	Asn Gly Val Glu Leu	Gly Asp Gly Ser Tyr	Val Ser Ala
1655	1660	1665	

Ile Val	Gln Gly Asp Arg Gln	Glu Glu Pro Val Pro	Glu Ala Tyr
1670	1675	1680	

Thr Pro	Asn Met Leu Arg Lys	Arg Gln Met Thr Val	Leu Asp Leu
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1685

1690

1695

His Pro Gly Ser Gly Lys Thr Lys Lys Ile Leu Pro Gln Ile Ile

1700

1705

1710

Lys Asp Ala Ile Gln Gln Arg Leu Arg Thr Ala Val Leu Ala Pro

1715

1720

1725

Thr Arg Val Val Ala Ala Glu Met Ala Glu Ala Leu Arg Gly Leu

1730

1735

1740

Pro Val Arg Tyr Gln Thr Ser Ala Val Gln Arg Glu His Gln Gly

1745

1750

1755

Asn Glu Ile Val Asp Val Met Cys His Ala Thr Leu Thr His Arg

1760

1765

1770

Leu Met Ser Pro Asn Arg Val Pro Asn Tyr Asn Leu Phe Val Met

1775

1780

1785

Asp Glu Ala His Phe Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly
1790 1795 1800

Tyr Ile Ala Thr Lys Val Glu Leu Gly Glu Ala Ala Ala Ile Phe
1805 1810 1815

Met Thr Ala Thr Pro Pro Gly Thr Thr Asp Pro Phe Pro Asp Ser
1820 1825 1830

Asn Ala Pro Ile His Asp Leu Gln Asp Glu Ile Pro Asp Arg Ala
1835 1840 1845

Trp Ser Ser Gly Tyr Glu Trp Ile Thr Glu Tyr Ala Gly Lys Thr
1850 1855 1860

Val Trp Phe Val Ala Ser Val Lys Met Gly Asn Glu Ile Ala Met
1865 1870 1875

Cys Leu Gln Arg Ala Gly Lys Lys Val Ile Gln Leu Asn Arg Lys
1880 1885 1890

Ser	Tyr	Asp	Thr	Glu	Tyr	Pro	Lys	Cys	Lys	Asn	Gly	Asp	Trp	Asp
1895				1900				1905						

Phe	Val	Ile	Thr	Thr	Asp	Ile	Ser	Glu	Met	Gly	Ala	Asn	Phe	Gly
1910				1915				1920						

Ala	Ser	Arg	Val	Ile	Asp	Cys	Arg	Lys	Ser	Val	Lys	Pro	Thr	Ile
1925				1930				1935						

Leu	Glu	Glu	Gly	Glu	Gly	Arg	Val	Ile	Leu	Gly	Asn	Pro	Ser	Pro
1940				1945				1950						

Ile	Thr	Ser	Ala	Ser	Ala	Ala	Gln	Arg	Arg	Gly	Arg	Val	Gly	Arg
1955				1960				1965						

Asn	Pro	Asn	Gln	Val	Gly	Asp	Glu	Tyr	His	Tyr	Gly	Gly	Ala	Thr
1970				1975				1980						

Ser	Glu	Asp	Asp	Ser	Asn	Leu	Ala	His	Trp	Thr	Glu	Ala	Lys	Ile
1985				1990				1995						

Met Leu Asp Asn Ile His Met Pro Asn Gly Leu Val Ala Gln Leu
2000 2005 2010

Tyr Gly Pro Glu Arg Glu Lys Ala Phe Thr Met Asp Gly Glu Tyr
2015 2020 2025

Arg Leu Arg Gly Glu Glu Lys Lys Asn Phe Leu Glu Leu Leu Arg
2030 2035 2040

Thr Ala Asp Leu Pro Val Trp Leu Ala Tyr Lys Val Ala Ser Asn
2045 2050 2055

Gly Ile Gln Tyr Thr Asp Arg Lys Trp Cys Phe Asp Gly Pro Arg
2060 2065 2070

Thr Asn Ala Ile Leu Glu Asp Asn Thr Glu Val Glu Ile Val Thr
2075 2080 2085

Arg Met Gly Glu Arg Lys Ile Leu Lys Pro Arg Trp Leu Asp Ala

2090

2095

2100

Arg Val Tyr Ala Asp His Gln Ala Leu Lys Trp Phe Lys Asp Phe

2105

2110

2115

Ala Ala Gly Lys Arg Ser Ala Val Ser Phe Ile Glu Val Leu Gly

2120

2125

2130

Arg Met Pro Glu His Phe Met Gly Lys Thr Arg Glu Ala Leu Asp

2135

2140

2145

Thr Met Tyr Leu Val Ala Thr Ala Glu Lys Gly Gly Lys Ala His

2150

2155

2160

Arg Met Ala Leu Glu Glu Leu Pro Asp Ala Leu Glu Thr Ile Thr

2165

2170

2175

Leu Ile Val Ala Ile Thr Val Met Thr Gly Gly Phe Phe Leu Leu

2180

2185

2190

Met Met Gln Arg Lys Gly Ile Gly Lys Met Gly Leu Gly Ala Leu
2195 2200 2205

Val Leu Thr Leu Ala Thr Phe Phe Leu Trp Ala Ala Glu Val Pro
2210 2215 2220

Gly Thr Lys Ile Ala Gly Thr Leu Leu Ile Ala Leu Leu Leu Met
2225 2230 2235

Val Val Leu Ile Pro Glu Pro Glu Lys Gln Arg Ser Gln Thr Asp
2240 2245 2250

Asn Gln Leu Ala Val Phe Leu Ile Cys Val Leu Thr Val Val Gly
2255 2260 2265

Val Val Ala Ala Asn Glu Tyr Gly Met Leu Glu Lys Thr Lys Ala
2270 2275 2280

Asp Leu Lys Ser Met Phe Gly Gly Lys Thr Gln Ala Ser Gly Leu
2285 2290 2295

Thr Gly Leu Pro Ser Met Ala Leu Asp Leu Arg Pro Ala Thr Ala
2300 2305 2310

Trp Ala Leu Tyr Gly Gly Ser Thr Val Val Leu Thr Pro Leu Leu
2315 2320 2325

Lys His Leu Ile Thr Ser Glu Tyr Val Thr Thr Ser Leu Ala Ser
2330 2335 2340

Ile Asn Ser Gln Ala Gly Ser Leu Phe Val Leu Pro Arg Gly Val
2345 2350 2355

Pro Phe Thr Asp Leu Asp Leu Thr Val Gly Leu Val Phe Leu Gly
2360 2365 2370

Cys Trp Gly Gln Ile Thr Leu Thr Thr Phe Leu Thr Ala Met Val
2375 2380 2385

Leu Ala Thr Leu His Tyr Gly Tyr Met Leu Pro Gly Trp Gln Ala
2390 2395 2400

Glu Ala Leu Arg Ala Ala Gln Arg Arg Thr Ala Ala Gly Ile Met
2405 2410 2415

Lys Asn Ala Val Val Asp Gly Met Val Ala Thr Asp Val Pro Glu
2420 2425 2430

Leu Glu Arg Thr Thr Pro Leu Met Gln Lys Lys Val Gly Gln Val
2435 2440 2445

Leu Leu Ile Gly Val Ser Val Ala Ala Phe Leu Val Asn Pro Asn
2450 2455 2460

Val Thr Thr Val Arg Glu Ala Gly Val Leu Val Thr Ala Ala Thr
2465 2470 2475

Leu Thr Leu Trp Asp Asn Gly Ala Ser Ala Val Trp Asn Ser Thr
2480 2485 2490

Thr Ala Thr Gly Leu Cys His Val Met Arg Gly Ser Tyr Leu Ala

2495

2500

2505

Gly Gly Ser Ile Ala Trp Thr Leu Ile Lys Asn Ala Asp Lys Pro

2510

2515

2520

Ser Leu Lys Arg Gly Arg Pro Gly Gly Arg Thr Leu Gly Glu Gln

2525

2530

2535

Trp Lys Glu Lys Leu Asn Ala Met Ser Arg Glu Glu Phe Phe Lys

2540

2545

2550

Tyr Arg Arg Glu Ala Ile Ile Glu Val Asp Arg Thr Glu Ala Arg

2555

2560

2565

Arg Ala Arg Arg Glu Asn Asn Ile Val Gly Gly His Pro Val Ser

2570

2575

2580

Arg Gly Ser Ala Lys Leu Arg Trp Leu Val Glu Lys Gly Phe Val

2585

2590

2595

Ser Pro Ile Gly Lys Val Ile Asp Leu Gly Cys Gly Arg Gly Gly
2600 2605 2610

Trp Ser Tyr Tyr Ala Ala Thr Leu Lys Lys Val Gln Glu Val Arg
2615 2620 2625

Gly Tyr Thr Lys Gly Gly Ala Gly His Glu Glu Pro Met Leu Met
2630 2635 2640

Gln Ser Tyr Gly Trp Asn Leu Val Ser Leu Lys Ser Gly Val Asp
2645 2650 2655

Val Phe Tyr Lys Pro Ser Glu Pro Ser Asp Thr Leu Phe Cys Asp
2660 2665 2670

Ile Gly Glu Ser Ser Pro Ser Pro Glu Val Glu Glu Gln Arg Thr
2675 2680 2685

Leu Arg Val Leu Glu Met Thr Ser Asp Trp Leu His Arg Gly Pro
2690 2695 2700

Arg Glu Phe Cys Ile Lys Val Leu Cys Pro Tyr Met Pro Lys Val
2705 2710 2715

Ile Glu Lys Met Glu Val Leu Gln Arg Arg Phe Gly Gly Gly Leu
2720 2725 2730

Val Arg Leu Pro Leu Ser Arg Asn Ser Asn His Glu Met Tyr Trp
2735 2740 2745

Val Ser Gly Pro Ala Gly Asn Val Val His Ala Val Asn Met Thr
2750 2755 2760

Ser Gln Val Leu Leu Gly Arg Met Asp Arg Thr Val Trp Arg Gly
2765 2770 2775

Pro Lys Tyr Glu Glu Asp Val Asn Leu Gly Ser Gly Thr Arg Ala
2780 2785 2790

Val Gly Lys Gly Glu Val His Ser Asn Gln Glu Lys Ile Lys Lys
2795 2800 2805

Arg Ile Gln Lys Leu Lys Glu Glu Phe Ala Thr Thr Trp His Lys
 2810 2815 2820

Asp Pro Glu His Pro Tyr Arg Thr Trp Thr Tyr His Gly Ser Tyr
 2825 2830 2835

Glu Val Lys Ala Thr Gly Ser Ala Ser Ser Leu Val Asn Gly Val
 2840 2845 2850

Val Lys Leu Met Ser Lys Pro Trp Asp Ala Ile Ala Asn Val Thr
 2855 2860 2865

Thr Met Ala Met Thr Asp Thr Thr Pro Phe Gly Gln Gln Arg Val
 2870 2875 2880

Phe Lys Glu Lys Val Asp Thr Lys Ala Pro Glu Pro Pro Thr Gly
 2885 2890 2895

Ala Lys Glu Val Leu Asn Glu Thr Thr Asn Trp Leu Trp Ala Tyr

2900

2905

2910

Leu Ser Arg Glu Lys Arg Pro Arg Leu Cys Thr Lys Glu Glu Phe

2915

2920

2925

Ile Lys Lys Val Asn Ser Asn Ala Ala Leu Gly Ala Val Phe Ala

2930

2935

2940

Glu Gln Asn Gln Trp Ser Thr Ala Arg Glu Ala Val Asp Asp Pro

2945

2950

2955

Arg Phe Trp Glu Met Val Asp Glu Glu Arg Glu Asn His Leu Arg

2960

2965

2970

Gly Glu Cys His Thr Cys Ile Tyr Asn Met Met Gly Lys Arg Glu

2975

2980

2985

Lys Lys Pro Gly Glu Phe Gly Lys Ala Lys Gly Ser Arg Ala Ile

2990

2995

3000

Trp Phe	Met Trp Leu Gly Ala	Arg Tyr Leu Glu Phe	Glu Ala Leu
3005	3010	3015	

Gly Phe	Leu Asn Glu Asp His	Trp Leu Ser Arg Glu	Asn Ser Gly
3020	3025	3030	

Gly Gly	Val Glu Gly Ser Gly	Val Gln Lys Leu Gly	Tyr Ile Leu
3035	3040	3045	

Arg Asp	Ile Ala Gly Lys Gln	Gly Gly Lys Met Tyr	Ala Asp Asp
3050	3055	3060	

Thr Ala	Gly Trp Asp Thr Arg	Ile Thr Arg Thr Asp	Leu Glu Asn
3065	3070	3075	

Glu Ala	Lys Val Leu Glu Leu	Leu Asp Gly Glu His	Arg Met Leu
3080	3085	3090	

Ala Arg	Ala Ile Ile Glu Leu	Thr Tyr Arg His Lys	Val Val Lys
3095	3100	3105	

Val Met Arg Pro Ala Ala Glu Gly Lys Thr Val Met Asp Val Ile

3110

3115

3120

Ser Arg Glu Asp Gln Arg Gly Ser Gly Gln Val Val Thr Tyr Ala

3125

3130

3135

Leu Asn Thr Phe Thr Asn Ile Ala Val Gln Leu Val Arg Leu Met

3140

3145

3150

Glu Ala Glu Gly Val Ile Gly Pro Gln His Leu Glu Gln Leu Pro

3155

3160

3165

Arg Lys Asn Lys Ile Ala Val Arg Thr Trp Leu Phe Glu Asn Gly

3170

3175

3180

Glu Glu Arg Val Ser Arg Met Ala Ile Ser Gly Asp Asp Cys Val

3185

3190

3195

Val Lys Pro Leu Asp Asp Arg Phe Ala Thr Ala Leu His Phe Leu

3200

3205

3210

Asn Ala Met Ser Lys Val Arg Lys Asp Ile Gln Glu Trp Lys Pro
3215 3220 3225

Ser His Gly Trp His Asp Trp Gln Gln Val Pro Phe Cys Ser Asn
3230 3235 3240

His Phe Gln Glu Ile Val Met Lys Asp Gly Arg Ser Ile Val Val
3245 3250 3255

Pro Cys Arg Gly Gln Asp Glu Leu Ile Gly Arg Ala Arg Ile Ser
3260 3265 3270

Pro Gly Ala Gly Trp Asn Val Lys Asp Thr Ala Cys Leu Ala Lys
3275 3280 3285

Ala Tyr Ala Gln Met Trp Leu Leu Leu Tyr Phe His Arg Arg Asp
3290 3295 3300

Leu Arg Leu Met Ala Asn Ala Ile Cys Ser Ala Val Pro Val Asp

3305

3310

3315

Trp Val Pro Thr Gly Arg Thr Ser Trp Ser Ile His Ser Lys Gly

3320

3325

3330

Glu Trp Met Thr Thr Glu Asp Met Leu Gln Val Trp Asn Arg Val

3335

3340

3345

Trp Ile Glu Glu Asn Glu Trp Met Val Asp Lys Thr Pro Ile Thr

3350

3355

3360

Ser Trp Thr Asp Val Pro Tyr Val Gly Lys Arg Glu Asp Ile Trp

3365

3370

3375

Cys Gly Ser Leu Ile Gly Thr Arg Ser Arg Ala Thr Trp Ala Glu

3380

3385

3390

Asn Ile Tyr Ala Ala Ile Asn Gln Val Arg Ala Val Ile Gly Lys

3395

3400

3405

Glu Asn Tyr Val Asp Tyr Met Thr Ser Leu Arg Arg Tyr Glu Asp

3410

3415

3420

Val Leu Ile Gln Glu Asp Arg Val Ile

3425

3430

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20

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18

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caatccagta cgacaagtca

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