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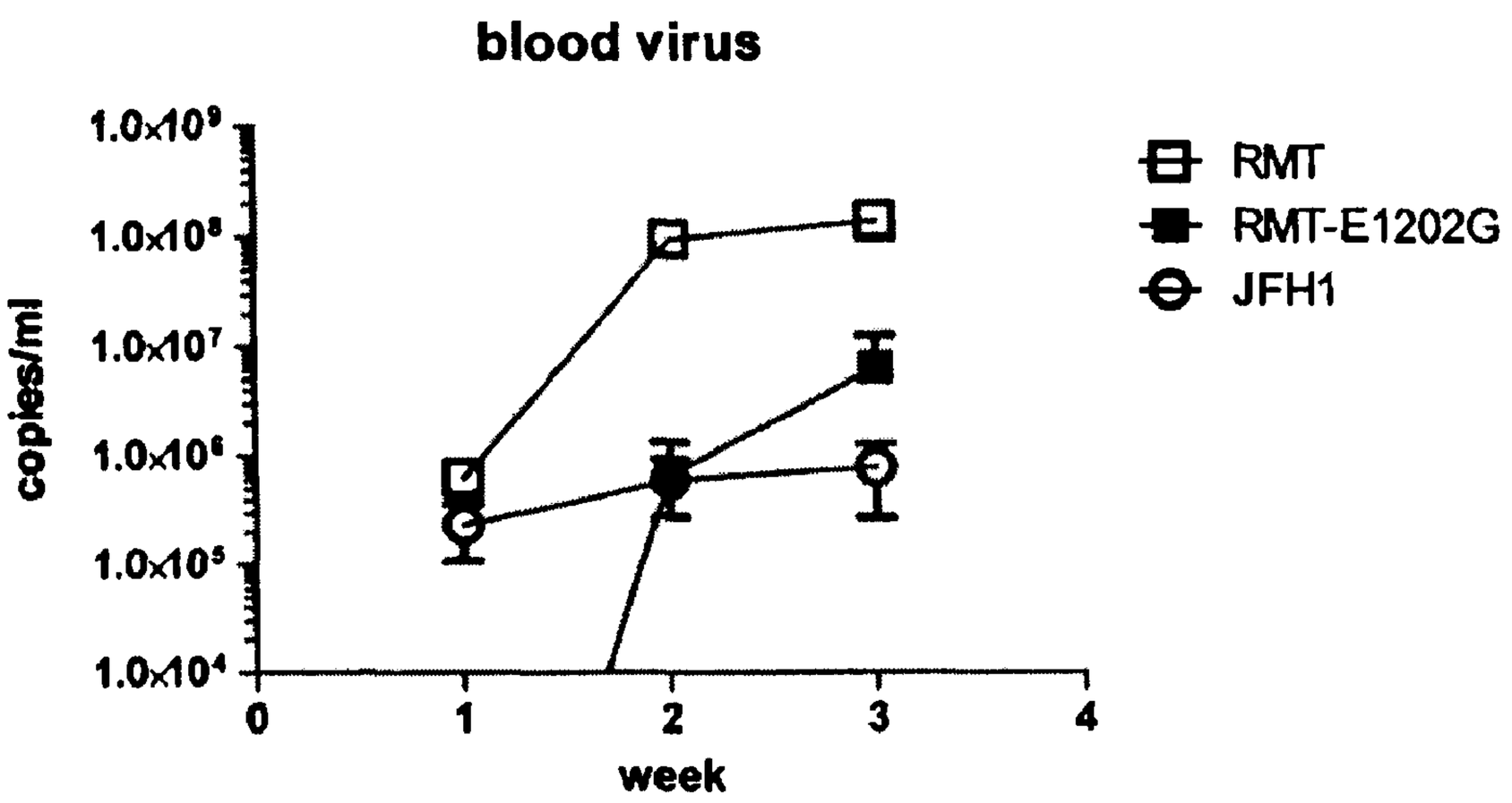
(54) **Titre : POLYNUCLEOTIDE ISSU D'UNE NOUVELLE SOUCHE DE VIRUS DE L'HEPATITE C ET SON UTILISATION**
(54) **Title: POLYNUCLEOTIDE DERIVED FROM NOVEL HEPATITIS C VIRUS STRAIN AND USE THEREOF**



(57) **Abrégé/Abstract:**
A polynucleotide encoding the amino acid shown in SEQ ID NO:2 or SEQ ID NO: 5, or encoding an amino acid sequence having not less than 98% identity thereto; preferably a polynucleotide comprising replacement of the amino acid corresponding to glutamic

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(57) **Abrégé(suite)/Abstract(continued):**
acid at position 1202 of SEQ ID NO:2 (position 177 of SEQ ID NO:5) with glycine, replacement of the amino acid corresponding to glutamic acid at position 1056 (position 31 of SEQ ID NO:5) with valine, and replacement of the amino acid corresponding to alanine at position 2199 (position 1174 of SEQ ID NO:5) with threonine.

ABSTRACT

A polynucleotide encoding the amino acid shown in SEQ ID NO:2 or SEQ ID NO: 5, or encoding an amino acid sequence having not less than 98% identity thereto; preferably a polynucleotide comprising replacement of the amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2 (position 177 of SEQ ID NO:5) with glycine, replacement of the amino acid corresponding to glutamic acid at position 1056 (position 31 of SEQ ID NO:5) with valine, and replacement of the amino acid corresponding to alanine at position 2199 (position 1174 of SEQ ID NO:5) with threonine.

DESCRIPTION

POLYNUCLEOTIDE DERIVED FROM NOVEL HEPATITIS C VIRUS STRAIN AND USE THEREOF

TECHNICAL FIELD

[0001]

The present invention relates to a polynucleotide derived from a novel strain of hepatitis C virus, a cell and a non-human mammal to which the polynucleotide has been introduced, and a replicon RNA prepared using the polynucleotide.

BACKGROUND ART

[0002]

Hepatitis C virus (hereinafter referred to as HCV) is a virus which belongs to Flaviviridae and has a genome of a single-stranded (+)-strand sense RNA, and known to cause hepatitis C. Recent studies have revealed that HCV can be divided into many types based on the genotype or the serotype. According to a phylogenetic analysis by Simmonds et al. using nucleotide sequences of HCV strains, HCV can be divided into 6 types, that is, the genotype 1a, genotype 1b, genotype 2a, genotype 2b, genotype 3a and genotype 3b, each of which can be further divided into several subtypes (Non-patent Document 1).

[0003]

At present, therapy for hepatitis C is carried out mainly with interferon-α or interferon-β, or by a combination therapy using interferon-α and a purine nucleoside-derivative ribavirin. However, even in cases where these therapies are carried out, a therapeutic effect can be observed in only about 60% of the overall patients, and, even in cases where the effect was observed in patients, recurrence occurs in more than half of the patients if the therapy was discontinued. The therapeutic effect of

interferon is known to be correlated with the genotype of HCV, and it is said that the effect is low against the genotype 1b while the effect is high against the genotype 2a (Non-patent Document 2).

Therefore, in order to evaluate the effect of an agent, it is important to establish a system which allows infection with, and growth of, each type of the virus, and to thereby investigate the effect of the agent against each type of the virus.

[0004]

Until recently, growing of HCV in a cell culture system and infection of cultured cells with HCV had been difficult, and the only animal which can be infected with HCV and can be used in experiments was chimpanzee, so that research on the replication mechanism of HCV and on its infection mechanism had been difficult. However, recently, HCV subgenomic RNA replicons were prepared as HCV-derived RNAs having self-replication capacity (Patent Document 1; Non-patent Document 3; Non-patent Document 4; Non-patent Document 5), and analysis of the replication mechanism of HCV using cultured cells became possible. These HCV subgenomic RNA replicons were prepared by replacing structural proteins existing in the downstream of HCV IRES in the 5'-untranslated region of the HCV genomic RNA of the clone called Con1, which belongs to the genotype 1b, with a neomycin resistance gene and EMCV-IRES linked to the downstream thereof. It has been demonstrated that introduction of the RNA replicons into Huh7 human liver cancer cells followed by culturing of the cells in the presence of neomycin allows self-replication of the replicons in the Huh7 cells. It is considered that the systems for evaluation of replication of HCV using this RNA replicon system can be a powerful tool for development of anti-HCV drugs

[0005]

However, since encoded viral proteins are different among HCVs having different genotypes, sufficient elucidation of the replication mechanism of HCV is

difficult by only analyzing a subgenomic RNA replicon derived from HCV of the genotype 1b. Therefore, it is thought that preparation of HCV RNA replicons of many genotypes is necessary for research on the replication mechanism of HCV and anti-HCV drugs.

[0006]

In Patent Document 2, identification of the virus and the genomic sequence are disclosed for the JFH-1 strain, which belongs to the 2a type. Further, in Patent Document 3, the JFH 2.1 strain and 2.2 strain, which similarly belong to the 2a type, and preparation of replicon RNAs using them are disclosed. On the other hand, in terms of the 1a type, there is a report on the H77 strain (Non-patent Document 6), but a recombinant virus obtained from this strain does not have a sufficient replication efficiency. Further, although a replicon was successfully prepared by introduction of 5 types of mutations which were predicted based on the 1b type, this replicon could be replicated only in a special cell.

PRIOR ART DOCUMENTS

Patent Documents

[0007]

[Patent Document 1] JP 2001-17187 A

[Patent Document 2] JP 2002-171978 A

[Patent Document 3] WO 2005/028652

Non-patent Documents

[0008]

[Non-patent Document 1] DUSHEIKO et al., "Hepatitis C Virus Genotypes: an Investigation of Type-specific Differences in Geographic Origin and Disease", Hepatology, 1994, PP. 13-18

[Non-patent Document 2] A new type of hepatitis C virus in patients in Thailand, Mori S et al., Biochem. Biophis. Res. Commun., (1992) 183, 334-342

[Non- patent Document 3] Replication of Subgenomic Hepatitis C Virus RNAs in a Hepatoma Cell Line, Lohmann V et al., Science, (1999) 285, 110-113

[Non-patent Document 4] Efficient Initiation of HCV RNA Replication in Cell Culture, Blight KJ et al., Science, (2000) 290, 1972-1974

[Non-patent Document 5] Selectable Subgenomic and Genome-Length Dicistronic RNAs Derived from an Infectious Molecular Clone of the HCV-N Strain of Hepatitis C Virus Replicate Efficiently in Cultured Huh7 Cells, Ikeda M et al., J. Virol., (2002) 76 2997-3006

[Non- patent Document 6] Efficient Replication of Hepatitis C Virus Genotype 1a RNAs in Cell Culture, Blight KJ et al., J. Virol. (2003) 77 3181-3190

DISCLOSURE OF THE INVENTION

[0009]

The present invention aims to provide a polynucleotide derived from a novel strain of HCV, and to use this for providing an HCV replicon RNA having high replication efficiency and HCV replicon cells with which continuous mass production of an HCV protein is possible.

[0010]

The inventors of the present invention intensively studied to solve the above problems, and, as a result, a novel RMT strain, which is grouped into the 1a type of HCV, was isolated from the serum of a hepatitis C patient; the nucleotide sequence of the strain was determined; and it was discovered that the virus has high infection efficiency. The inventors of the present invention then succeeded in preparing replicon RNAs having high replication efficiency using this virus, thereby completed the present invention. That is, the present invention provides the followings.

[0011]

(1) A polynucleotide which encodes the amino acid sequence shown in SEQ ID NO:2 or an amino acid sequence having not less than 98% identity thereto, wherein said polynucleotide can express nonstructural proteins and structural proteins of hepatitis C virus (HCV).

(2) The polynucleotide according to (1), wherein said amino acid sequence shown in SEQ ID NO:2 or an amino acid sequence having not less than 98% identity thereto comprises replacement of the amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2 with glycine.

(3) The polynucleotide according to (1), wherein said amino acid sequence shown in SEQ ID NO:2 or an amino acid sequence having not less than 98% identity thereto

comprises replacement of the amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2 with glycine, replacement of the amino acid corresponding to glutamic acid at position 1056 with valine, and replacement of the amino acid corresponding to alanine at position 2199 with threonine.

(4) The polynucleotide according to (2) or (3), wherein said amino acid sequence shown in SEQ ID NO:2 or an amino acid sequence having not less than 98% identity thereto further comprises replacement of the amino acid corresponding to serine at position 2321 of SEQ ID NO:2 with proline and/or replacement of the amino acid corresponding to leucine at position 2889 with phenylalanine

(5) The polynucleotide according to any one of (1) to (4), wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:1 or a nucleotide sequence having not less than 95% homology thereto, and wherein, in cases where the polynucleotide is RNA, the nucleotide "t" in SEQ ID NO:1 is read as "u".

(6) A hepatitis C virus particle comprising the polynucleotide according to any one of (1) to (5).

(7) A non-human mammal to which the polynucleotide according to any one of (1) to (5) has been introduced and which produces a recombinant HCV.

(8) The non-human mammal according to (7), wherein said non-human mammal is a human hepatocyte chimeric mouse.

(9) A cell to which the polynucleotide according to any one of (1) to (5) has been introduced and which produces a recombinant HCV.

(10) A method of screening or evaluating a drug candidate substance which inhibits replication or protein translation of HCV, said method comprising administering or adding said drug candidate substance to the non-human mammal according to (7) or (8) or to the cell according to (9) and evaluating replication capacity of HCV or capacity of translation of HCV protein.

(11) A polynucleotide which encodes the amino acid sequence shown in SEQ ID NO:5 or an amino acid sequence having not less than 98% identity thereto, and wherein said polynucleotide can express nonstructural proteins of HCV.

(12) The polynucleotide according to (11), wherein said amino acid sequence shown in SEQ ID NO: 5 or an amino acid sequence having not less than 98% identity thereto comprises replacement of the amino acid corresponding to glutamic acid at position 177 of SEQ ID NO:5 with glycine.

(13) The polynucleotide according to (11), wherein said amino acid sequence shown in SEQ ID NO: 5 or an amino acid sequence having not less than 98% identity thereto comprises replacement of the amino acid corresponding to glutamic acid at position 177 of SEQ ID NO:5 with glycine, replacement of the amino acid corresponding to glutamic acid at position 31 with valine, and replacement of the amino acid corresponding to alanine at position 1174 with threonine.

(14) The polynucleotide according to (12) or (13), wherein said amino acid sequence shown in SEQ ID NO:5 or an amino acid sequence having not less than 98% identity thereto further comprises replacement of the amino acid corresponding to serine at position 1296 of SEQ ID NO:5 with proline and/or replacement of the amino acid corresponding to leucine at position 1864 with phenylalanine.

(15) The polynucleotide according to any one of (11) to (14), further comprising an IRES sequence.

(16) The polynucleotide according to any one of (11) to (15), further comprising a selection marker gene or a reporter gene.

(17) The polynucleotide according to any one of (11) to (16), wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:3 or a polynucleotide which is able to hybridize with the complementary sequence of SEQ ID NO:3 under stringent conditions, and wherein, in cases where the polynucleotide is RNA, the nucleotide "t" in SEQ ID NO:3 is read as "u".

(18) The polynucleotide according to (17), wherein said polynucleotide is a replicon RNA.

(19) A replicon-replicating cell prepared by introducing the replicon RNA according to (18).

(20) The replicon-replicating cell according to (19), wherein the cell is a human liver-derived cell, human uterine cervix-derived cell or human fetal kidney-derived cell.

(21) The replicon-replicating cell according to (19) or (20), wherein the cell is a Huh7 cell, HepG2 cell, IMY-N9 cell, HeLa cell or 293 cell.

(22) A method of screening or evaluating a drug candidate substance which inhibits replication or protein translation of HCV, said method comprising adding a drug candidate substance to the replicon-replicating cell according to any one of (19) to (21) and evaluating replication capacity or protein translation of said replicon RNA.

[0011a]

The present invention further provides the following:

- a polynucleotide which encodes an amino acid sequence having not less than 98% identity to the full length of the amino acid sequence shown in SEQ ID NO:2, in which the amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2 is replaced with glycine and wherein said polynucleotide can express nonstructural proteins and structural proteins of hepatitis C virus (HCV);

- a recombinant hepatitis C virus particle comprising the polynucleotide as described herein;

- a cell to which the polynucleotide as described herein has been introduced and which produces a recombinant HCV;

- a method of screening or evaluating a drug candidate substance which inhibits replication or protein translation of HCV, said method comprising administering or

adding said drug candidate substance to the cell as described herein and evaluating replication capacity of HCV or capacity of translation of HCV protein

- a polynucleotide which encodes an amino acid sequence having not less than 98% identity to the full length of the amino acid sequence shown in SEQ ID NO:5, in which the amino acid corresponding to glutamic acid at position 177 of SEQ ID NO:5 is replaced with glycine and wherein said polynucleotide can express nonstructural proteins of HCV;

- a replicon-replicating cell prepared by introducing the replicon RNA as described herein; and

- a method of screening or evaluating a drug candidate substance which inhibits replication or protein translation of HCV, said method comprising adding a drug candidate substance to the replicon-replicating cell as described herein and evaluating replication capacity or protein translation of said replicon RNA.

[0012]

By using a polynucleotide of the novel HCV strain of the present invention, HCV-1a-type-infected model animals and cells can be efficiently obtained.

Further, by using a polynucleotide of the novel HCV strain of the present invention, replicon RNAs and replicon-replicating cells having high replication efficiency can be obtained at a high probability. These replicon-replicating cells can be used as culture systems for continuous production of HCV-derived RNAs and HCV proteins. Further, the replicon-replicating cells can also be used as test systems for screening of various substances that influence replication of HCV or translation of HCV proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013]

Fig. 1 is a diagram showing results of an experiment on infection of human hepatocyte chimeric mice with the serum derived from a patient suffering from 1a-type hepatitis C.

Fig. 2 is a diagram showing the positions of primers for analysis of the sequence of the RMT strain, which primers were designed based on the sequence of the H77 strain.

Fig. 3 is a diagram showing the structure of an RMT recombinant virus expression vector.

Fig. 4 is a diagram showing results of comparison of the efficiency of infection to human hepatocyte chimeric mice between the RMT strain and the JFH1 strain.

Fig. 5 is a diagram showing the structure of an expression vector for a replicon RNA derived from the RMT strain.

Fig. 6 is a diagram (photographs) showing the colony formation capacities of replicon RNAs having various mutations, in the presence of an agent.

Fig. 7 is a diagram showing the replication efficiencies of RMT recombinant viruses in Huh7 cells.

Fig. 8 is a diagram showing results of investigation of the effects of antiviral agents in Huh7 cells, using an RMT recombinant virus (to which a triple mutation was introduced).

Fig. 9 is a diagram (micrographs) showing results of fluorescent antibody staining of the HCV-core protein in Huh7 cells infected with culture supernatant (A) or concentrated culture supernatant (B) of #11 cells. The arrowheads indicate stained positions.

DESCRIPTION OF THE EMBODIMENTS

[0014]

The present invention will be described below in detail.

In the present specification, a "polynucleotide" means either RNA or DNA. That is, a "polynucleotide which can express a viral protein" may be either RNA which can be translated into the protein, or DNA which can be transcribed into RNA,

followed by being translated into the protein.

Further, the polynucleotide may be either single-stranded or double-stranded, and may be either naturally occurred or artificially synthesized. Further, the polynucleotide may be partially modified and may be a derivative. It should be noted that each nucleotide sequence in SEQUENCE LISTING is represented as DNA for convenience, but, in cases where the polynucleotide is RNA, the nucleotide symbol "t" is read as "u".

[0015]

The polynucleotide of the first embodiment of the present invention is a polynucleotide encoding structural proteins and nonstructural proteins of HCV.

Examples of such a polynucleotide include the one encoding the amino acid sequence shown in SEQ ID NO:2.

The amino acid sequence shown in SEQ ID NO:2 is composed of a region comprising structural proteins (Core, E1 and E2) and a region comprising nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).

[0016]

Such structural proteins (Core, E1 and E2) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) of HCV are translated as a continuous polyprotein from the translated region and then released by limited hydrolysis by protease. Among these structural proteins and nonstructural proteins (that is, viral proteins of HCV), Core is a core protein, E1 and E2 are envelope proteins, and the nonstructural proteins are proteins involved in replication of the virus itself. Further, it is known that NS2 has a metalloprotease activity and NS3 has a serine protease activity (one third from N-terminus of the whole protein) and a helicase activity (two thirds from C-terminus of the whole protein). Further, it has been reported that NS4A is a cofactor for the protease activity of NS3, and that NS5B has an RNA-dependent RNA polymerase activity.

[0017]

In the amino acid sequence shown in SEQ ID NO:2, amino acid positions 1 to 191 correspond to the Core protein; amino acid positions 192 to 383 correspond to the E1 protein; amino acid positions 384 to 809 correspond to the E2 protein; amino acid positions 810 to 1026 correspond to the NS2 protein; amino acid positions 1027 to 1657 correspond to the NS3 protein; amino acid positions 1658 to 1711 correspond to the NS4A protein; amino acid positions 1712 to 1972 correspond to the NS4B protein; amino acid positions 1973 to 2420 correspond to the NS5A protein; and amino acid positions 2421 to 3011 correspond to the NS5B protein.

[0018]

The polynucleotide of the present invention is derived from the RMT strain, and may be derived either from the RMT strain itself or from a derivative of the RMT strain.

That is, in the sequence of the polynucleotide of the first embodiment of the present invention, a substitution(s), deletion(s), addition(s), insertion(s) and/or the like may exist(s) as long as the polynucleotide encodes the respective viral proteins described above which are functional. More particularly, the polynucleotide of the first embodiment of the present invention may be a polynucleotide encoding an amino acid sequence having not less than 98% identity, preferably not less than 99% identity, to the entire amino acid sequence shown in SEQ ID NO:2

[0019]

Further, the polynucleotide of the first embodiment of the present invention may be a polynucleotide encoding an amino acid sequence which is the same as the amino acid sequence shown in SEQ ID NO:2 except that one or more amino acids, particularly 1 to 50, preferably 1 to 30, more preferably 1 to 10, still more preferably 1 to 5 amino acids are substituted, deleted, added and/or inserted, as long as the polynucleotide encodes the respective viral proteins described above which are

functional.

[0020]

In order to maintain high replication capacity in cultured cells, preferably, the amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2 is replaced with glycine, and, more preferably, the amino acid corresponding to glutamic acid at position 1202 is replaced with glycine; the amino acid corresponding to glutamic acid at position 1056 is replaced with valine; and the amino acid corresponding to alanine at position 2199 is replaced with threonine. Further, in addition to the above replacements, the polynucleotide may have replacement of the amino acid corresponding to serine at position 2321 of SEQ ID NO:2 with proline and/or replacement of the amino acid corresponding to leucine at position 2889 with phenylalanine.

The "amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2" herein means the amino acid existing at the position of glutamic acid at position 1202 of SEQ ID NO:2 based on comparison of sequences, and, for example, in cases where position 1202 becomes position 1201 due to deletion of an amino acid in the upstream of position 1202, the amino acid at position 1201 is referred to as the "amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2".

[0021]

The polynucleotide of the first embodiment of the present invention encodes the structural proteins and the nonstructural proteins of HCV, and, preferably, the polynucleotide comprises the 5'-untranslated region and the 3'-untranslated region in the upstream and the downstream, respectively, of the proteins, and can produce an HCV having the capacities of infection and self-replication. Examples of such a polynucleotide include viral genomic RNAs, and DNAs that express the RNAs.

[0022]

Particular examples of such a polynucleotide include the polynucleotide

having the nucleotide sequence shown in SEQ ID NO:1.

In the nucleotide sequence shown in SEQ ID NO:1, nucleotide positions 1 to 341 correspond to the "5'-untranslated region (5'-UTR)"; nucleotide positions 342 to 914 correspond to the Core protein-coding region, nucleotide positions 915 to 1490 correspond to the E1 protein-coding region; nucleotide positions 1491 to 2768 correspond to the E2 protein-coding region; nucleotide positions 2769 to 3419 correspond to the NS2 protein-coding region; nucleotide positions 3420 to 5312 correspond to the NS3 protein-coding region; nucleotide positions 5313 to 5474 correspond to the NS4A protein-coding region; nucleotide positions 5475 to 6257 correspond to the NS4B-coding region; nucleotide positions 6258 to 7601 correspond to the NS5A protein-coding region; nucleotide positions 7602 to 9374 correspond to the NS5B protein-coding region; and nucleotide positions 9375 to 9598 correspond to the "3'-untranslated region (3'-UTR)".

[0023]

The polynucleotide of the first embodiment of the present invention is not restricted to the polynucleotide having the nucleotide sequence of SEQ ID NO:1, and a polynucleotide which is able to hybridize with the complementary strand of SEQ ID NO:1 under stringent conditions is also included in the polynucleotide of the present invention as long as the polynucleotide encodes the respective desired proteins described above. The "stringent conditions" herein means conditions under which the so-called specific hybrid is formed while a non-specific hybrid is not formed, and examples of the stringent conditions include: conditions under which DNAs having a high homology, for example, a not less than 95% homology, to each other hybridize with each other, while DNAs having a lower homology to each other do not hybridize with each other; and conditions under which one time, more preferably 2 or 3 times, of washing is carried out at a salt concentration and a temperature corresponding to those in normal washing conditions for Southern

hybridization, such as at 60°C in 0.1×SSC, 0.1% SDS, more preferably at 68°C in 0.1×SSC, 0.1% SDS.

[0024]

By introducing the polynucleotide of the first embodiment of the present invention into a non-human mammal, a 1a-type-HCV-infected model animal can be prepared. Examples of the non-human mammal include mouse, rat, rabbit, dog and chimpanzee, and the non-human mammal is preferably a human hepatocyte chimeric mouse.

A uPA/SCID mouse was prepared by crossing a mouse prepared by gene transfer of the urokinase plasminogen activator (uPA) gene linked to an enhancer and a promoter of albumin (uPA-Tg mouse) with a SCID mouse, and human hepatocytes were transplanted to the resulting mouse, to prepare a human hepatocyte chimeric mouse wherein not less than 70% of the mouse liver was replaced with human hepatocytes. By inoculating HCV to the thus prepared human hepatocyte chimeric mouse, an HCV-persistently-infected chimeric mouse can be prepared.

Such a 1a-type-HCV-infected model animal can be used for research on the control mechanisms of infection and replication of 1a-type HCV, and for evaluation and screening of drug candidate substances.

[0025]

Further, by introducing the polynucleotide of the first embodiment of the present invention into cultured cells, 1a-type-HCV-producing cells can be obtained. The type of the cells is not restricted, and the cells are preferably mammalian-derived cells, more preferably human liver-derived cells, human uterine cervix-derived cells or human fetal kidney-derived cells. Particular examples of the cells include Huh7 cells, HepG2 cells, IMY-N9 cells, HeLa cells and 293 cells. Such 1a-type-HCV-producing cells can be used for research on the control mechanisms of infection and replication of 1a-type HCV, and for evaluation and screening of drug candidate

substances.

[0026]

The polynucleotide of the second embodiment of the present invention is a polynucleotide encoding nonstructural proteins of HCV.

Examples of such a polynucleotide include the one encoding the amino acid sequence shown in SEQ ID NO:5.

The amino acid sequence shown in SEQ ID NO:5 comprises nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).

[0027]

In the amino acid sequence shown in SEQ ID NO:5, amino acid positions 2 to 632 correspond to the NS3 protein; amino acid positions 633 to 686 correspond to the NS4A protein; amino acid positions 687 to 947 correspond to the NS4B protein; amino acid positions 948 to 1395 correspond to the NS5A protein; and amino acid positions 1396 to 1986 correspond to the NS5B protein

[0028]

In the sequence of the polynucleotide of the second embodiment of the present invention, a substitution(s), deletion(s), addition(s), insertion(s) and/or the like may exist as long as the polynucleotide encodes the respective viral proteins described above which are functional. More particularly, the polynucleotide of the second embodiment of the present invention may be a polynucleotide encoding an amino acid sequence having not less than 98% identity, preferably not less than 99% identity, to the entire amino acid sequence shown in SEQ ID NO:5.

[0029]

In the sequence the polynucleotide of the second embodiment of the present invention, a substitution(s), deletion(s), addition(s), insertion(s) and/or the like may exist(s) as long as the polynucleotide encodes the respective viral proteins described above which are functional. More particularly, the polynucleotide of the second

embodiment of the present invention may be a polynucleotide encoding an amino acid sequence which is the same as the amino acid sequence shown in SEQ ID NO:5 except that one or more amino acids, particularly 1 to 30, preferably 1 to 10, more preferably 1 to 5 amino acids are substituted, deleted, added and/or inserted.

[0030]

The polynucleotide of the second embodiment of the present invention is preferably an RNA having the 5'-untranslated region and the 3'-untranslated region in the upstream and downstream, respectively, of the region encoding the nonstructural proteins, and having the capacity of self-replication in a host cell; or a DNA which can produce the RNA. In the present specification, the RNA having the capacity of self-replication in a host cell is referred to as a "replicon RNA" or "RNA replicon".

[0031]

One embodiment of the HCV replicon RNA of the present invention is a replicon RNA composed of a nucleotide sequence comprising at least the 5'-untranslated region; a sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein; and the 3'-untranslated region, in the genomic RNA of the RMT strain.

The HCV replicon RNA, or the DNA expressing it, of the present invention preferably comprises a mutation(s) which enable(s) its efficient replication in a host cell.

Examples of such a mutation(s) include replacement of the amino acid corresponding to glutamic acid at position 177 of SEQ ID NO:5 (position 1202 of SEQ ID NO:2) with glycine; and the triple mutation by replacement of the amino acid corresponding to glutamic acid at position 177 of SEQ ID NO:5 (position 1202 of SEQ ID NO:2) with glycine, replacement of the amino acid corresponding to glutamic acid at position 31 of SEQ ID NO:5 (position 1056 of SEQ ID NO:2) with

valine, and replacement of the amino acid corresponding to alanine at position 1174 of SEQ ID NO:5 (position 2199 of SEQ ID NO:2) with threonine is more preferred. Further, in addition to the above replacements, replacement of the amino acid corresponding to serine at position 1296 of SEQ ID NO:5 with proline and/or replacement of the amino acid corresponding to leucine at position 1864 with phenylalanine may be contained.

[0032]

The HCV replicon RNA of the present invention may further comprise an IRES sequence, and may still further comprise a selection marker gene or reporter gene.

The selection marker gene or reporter gene may be linked to the upstream of the IRES sequence, or to the upstream or the downstream of the "sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein"; or may be inserted into the "sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein".

[0033]

A preferred embodiment of the HCV replicon RNA of the present invention is a replicon RNA composed of a polynucleotide comprising the 5'-untranslated region; at least one selection marker gene or reporter gene; at least one IRES sequence; a nucleotide sequence in the genomic RNA of HCV, encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein; and the 3'-untranslated region.

[0034]

The replicon RNA of the present invention more preferably has the 5'-untranslated region of the genomic RNA of HCV; has a selection marker gene or reporter gene, an IRES sequence, and the "sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein", in this order, in the

downstream of the 5'-untranslated region; and has the 3'-untranslated region of the genomic RNA of HCV, in the 3'-end.

[0035]

A more preferred embodiment of the HCV replicon RNA of the present invention is a replicon RNA composed of RNA having the nucleotide sequence shown in SEQ ID NO:3. A nucleic acid which is able to hybridize with the complementary strand of SEQ ID NO:3 under stringent conditions is also included therein. The meaning of the "stringent conditions" is as mentioned above.

In the nucleotide sequence shown in SEQ ID NO:3, nucleotide positions 1 to 341 correspond to the "5'-UTR"; nucleotide positions 342 to 1181 correspond to a neomycin resistance gene; nucleotide positions 1185 to 1764 correspond to EMCV IRES; nucleotide positions 1765 to 7722 correspond to the "nonstructural protein (NS3, NS4A, NS4B, NS5A and NS5B proteins)-coding region"; and nucleotide positions 7723 to 7946 correspond to the "3'-UTR".

Although replicon RNAs were explained above, DNAs that can express the replicon RNAs are also, of course, included in the polynucleotide of the present invention.

[0036]

In the present invention, "having the capacity of self-replication" means that, in cases where a replicon RNA is introduced to a cell, the replicon RNA can replicate the replicon RNA itself in the cell. The capacity of autonomous replication can be confirmed by, for example, transfection of the replicon RNA into a Huh7 cell and culturing of the Huh7 cell, followed by subjecting RNA extracted from cells in the obtained culture to Northern blot hybridization or RT-PCR using a probe or primer with which the introduced replicon RNA can be specifically detected, to detect the presence of the replicon RNA. The particular operation to confirm the capacity of autonomous replication can be carried out according to descriptions in Examples of

the present description, about measurement of the colony forming capacity, confirmation of expression of an HCV protein, detection of a replicon RNA, and the like.

[0037]

The replicon RNA of the present invention may comprise, in addition to the above-described sequences, an RNA having an arbitrary foreign gene which is to be expressed in the cell to which the replicon RNA is to be introduced. The foreign gene may be linked to the downstream of the 5'-untranslated region; linked to the upstream or the downstream of the selection marker gene or reporter gene; linked to the upstream or the downstream of the "sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein"; or inserted into the "sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein". The replicon RNA comprising a foreign gene can express the protein encoded by the foreign gene when the replicon RNA is translated in the cell to which the replicon RNA is introduced. Thus, the replicon RNA comprising a foreign gene can be suitably used also in cases where a specific gene product is produced in the cell, such as the cases of gene therapy.

[0038]

In the present invention, a "selection marker gene" means a gene which can add selectivity to cells such that only cells wherein the gene is expressed can be selected. Common examples of the selection marker gene include antibiotic resistance genes. Examples of selection marker genes suitable in the present invention include the neomycin resistance gene, thymidine kinase gene, kanamycin resistance gene, pyrithiamin resistance gene, adenyltransferase gene, zeocin resistance gene and puromycin resistance gene, among which the neomycin resistance gene and thymidine kinase gene are preferred, and the neomycin resistance gene is more preferred.

[0039]

In the present invention, a "reporter gene" means a marker gene encoding a gene product which can be used as an index of expression of the gene. Common examples of the reporter gene include structural genes of enzymes that catalyze a luminous reaction or a color reaction. Examples of reporter gene suitable in the present invention include the chloramphenicol acetyltransferase gene, β-galactosidase gene, luciferase gene, green fluorescent protein gene, aequorin gene derived from jerry fish and secreted placental alkaline phosphatase (SEAP) gene.

Either only one or both of the above selection marker gene and reporter gene may be contained in the replicon RNA.

[0040]

The "IRES sequence" in the present invention means an internal ribosome binding site which can allow a ribosome to bind to the inside of RNA to initiate translation. Suitable examples of the IRES sequence in the present invention include, but are not limited to, EMCV IRES (internal ribosome binding site of encephalomyocarditis virus), FMDV IRES and HCV IRES, among which EMCV IRES and HCV IRES are more preferred, and EMCV IRES is most preferred.

[0041]

The replicon RNA of the present invention may further comprise a ribozyme. The ribozyme is inserted such that it links the selection marker gene, reporter gene or foreign gene in the 5'-side replicon RNA to the IRES sequence and the "sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein" in the 3'-side, to allow their cleavage and separation by the self-cleavage activity of the ribozyme.

[0042]

In the replicon RNA of the present invention, the above-mentioned selection marker gene; reporter gene; sequence encoding virus proteins in the genomic RNA of

hepatitis C virus derived from a patient suffering from fulminant hepatitis; foreign gene, and the like are linked together such that translation occurs in the replicon RNA in the correct reading frame. Among these sequences, sequences encoding proteins may be linked to each other via protease cleavage sites or the like such that these sequences are expressed as a fusion protein with the polyprotein translated from the "sequence encoding the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein" of hepatitis C virus and then separated into the respective proteins by protease.

[0043]

The HCV replicon RNA of the present invention can be prepared using arbitrary genetic engineering techniques which are known to those skilled in the art. For example, the HCV replicon RNA can be prepared by the following method.

First, the NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein, and DNA corresponding to the RNA in the 3'-untranslated region are inserted into a cloning vector by a conventional method to prepare a DNA clone. On the other hand, the 5'-untranslated region is inserted in the downstream of an RNA promoter, to prepare a DNA clone. The "DNA corresponding to the RNA" means DNA having the nucleotide sequence wherein u (uracil) in the nucleotide sequence of the RNA is replaced with t (thymine). The RNA promoter is preferably one contained in a plasmid clone. Preferred examples of the RNA promoter include, but are not limited to, a T7 RNA promoter, SP6 RNA promoter and SP3 RNA promoter, among which a T7 RNA promoter is especially preferred.

[0044]

Subsequently, for example, in the prepared DNA clone of the 5'-untranslated region, a selection marker gene or a reporter gene is inserted into the downstream of the 5'-untranslated region, and an IRES sequence is inserted into the further downstream. Thereafter, by linking the both clones to each other, a DNA for

expressing the HCV replicon RNA of the present invention can be obtained.

[0045]

Subsequently, RNA is synthesized by RNA polymerase using the thus prepared DNA clone as a template. The RNA synthesis can be initiated from the 5'-untranslated region and the IRES sequence by a conventional method. In cases where the template DNA is a plasmid clone, the above-described DNA region linked to the downstream of the RNA promoter may be cleaved out using a restriction enzyme, to synthesize RNA using the DNA fragment as a template. Thus, the replicon RNA of the present invention can be obtained.

[0046]

By introducing the thus prepared replicon RNA into a cell wherein the replicon RNA is to be replicated, a cell wherein the replicon RNA is continuously replicating can be obtained. In the present specification, the cell wherein the replicon RNA is continuously replicating is referred to as a "replicon-replicating cell".

[0047]

As the cell to which the replicon RNA is to be introduced, an arbitrary cell can be used as long as the cell can be subcultured, and the cell is preferably a eukaryotic cell, more preferably a human liver-derived cell, human uterine cervix-derived cell or human fetal kidney-derived cell, still more preferably a Huh7 cell, HepG2 cell, IMY-N9 cell, HeLa cell or 293 cell. These cells may be those obtained from a commercial source; may be obtained from a cell depository; or may be those established from an arbitrary cell (e.g., from a cancer cell or stem cell).

[0048]

The introduction of the replicon RNA into cells may be carried out using an arbitrary technique known to those skilled in the art. Examples of the introduction method include electroporation, the particle gun method, lipofection, the calcium phosphate method, microinjection and the DEAE sepharose method, among which

electroporation is especially preferred.

The amount of the replicon RNA to be used for the introduction into cells may be determined depending on the type of the introduction method, and is preferably 1 picogram to 100 micrograms, more preferably 10 picograms to 10 micrograms.

[0049]

In cases where a replicon RNA comprising a selection marker gene or a reporter gene is used for the introduction into cells, cells to which the replicon RNA was introduced wherein the replicon RNA is continuously replicating can be selected using expression of the selection marker gene or the reporter gene. More particularly, for example, cells subjected to treatment for introduction of such a replicon RNA into the cells may be cultured in a medium wherein selection is possible based on expression of the selection marker gene or the reporter gene.

[0050]

For example, in cases where a neomycin resistance gene is contained as a selection marker gene in the replicon RNA, cells subjected to treatment for introduction of the replicon RNA into the cells are plated on a culture dish, and the cells are cultured for 16 to 24 hours, followed by addition of G418 (neomycin) to the culture dish at a concentration of 0.05 milligram/milliliter to 3.0 milligrams/milliliter. Thereafter, with replacement of the culture medium twice per week, the culture is continued, and viable cells are stained with crystal violet after preferably 10 days to 40 days, more preferably 14 days to 28 days of culture after the plating. By this, cells to which the replicon RNA was introduced wherein the replicon RNA is replicating continuously can be selected as colonies.

From the formed colonies, cells can be cloned by a conventional method. The thus obtained cell clone wherein the replicon RNA of interest is continuously replicating is referred to as a "replicon-replicating cell clone" in the present

specification.

[0051]

Whether the replicon RNA of interest is actually continuously replicating in the established cell clone is preferably confirmed by detection of the replicon RNA replicated from the introduced replicon RNA in the cell clone, confirmation of incorporation of the selection marker gene or the reporter gene in the introduced replicon RNA into the host genomic DNA, and confirmation of expression of an HCV protein.

The replicon RNA replicated from the introduced replicon RNA in the cell clone (which is referred to as a "replicated replicon RNA" in the present specification, for convenience) may be detected by an arbitrary RNA detection method known to those skilled in the art. For example, the replicated replicon RNA can be detected by subjecting total RNA extracted from the cell clone to Northern hybridization or RT-PCR using a DNA fragment specific to the introduced replicon RNA, as a probe or a primer.

[0052]

Incorporation of the selection marker gene or the reporter gene in the introduced replicon RNA into the host genomic DNA can be confirmed by, for example, performing PCR to amplify at least a part of the selection marker gene or the reporter gene in the host genomic DNA extracted from the cell clone, thereby confirming the presence/absence of the amplified product.

[0053]

Expression of an HCV protein can be confirmed by, for example, allowing an antibody against the HCV protein, which should be expressed from the introduced replicon RNA, to react with protein extracted from the cell clone. This method can be carried out by an arbitrary protein detection method known to those skilled in the art, and, more particularly, for example, it can be carried out by blotting a protein

sample extracted from the cell clone onto a nitrocellulose membrane and reacting an anti-HCV-protein antibody (e.g., an anti-NS3 specific antibody, or an antiserum collected from a patient suffering from hepatitis C) therewith, followed by detecting the anti-HCV-protein antibody. If the HCV protein is detected in the protein extracted from the cell clone, it can be judged that, in the cell clone, the replicon RNA derived from HCV is continuously replicating and expressing the HCV protein.

[0054]

Thus, a cell clone (replicon-replicating cell clone) wherein the replicon RNA of interest has been confirmed to be continuously replicating can be obtained.

[0055]

The replicon-replicating cell of the present invention can be suitably used also for producing an HCV protein. Production of an HCV protein from replicon-replicating cells can be carried out according to a conventional method by those skilled in the art. More particularly, for example, replicon-replicating cells are cultured, and, from the obtained culture (which contains cultured cells and a culture medium), the protein can be obtained by a conventional method.

[0056]

The replicon RNA-replicating cell of the present invention can be used also as a test system for screening or evaluation of substances that suppress replication of HCV or translation of HCV proteins. More particularly, for example, by culturing replicon-replicating cells in the presence of a drug candidate substance and detecting replication of a replicon RNA in the obtained culture, followed by judging whether the substance suppresses replication of the replicon RNA, a substance that suppresses replication of HCV can be screened. In such a case, the detection of replication of the replicon RNA in the obtained culture may be either detection of the amount or the presence/absence of the replicon RNA in RNA extracted from the replicon RNA-replicating cells, or detection of the amount or the presence/absence of an HCV

protein contained in protein in the culture or in the replicon RNA-replicating cells contained in the culture.

Further, by culturing replicon-replicating cells in the presence of a drug candidate substance and detecting a protein derived from the replicon RNA in the obtained culture, followed by judging whether the substance suppresses production of the protein, a substance that suppresses translation of the protein of HCV can be screened.

The drug candidate substance is not restricted, and may be, for example, a low-molecular synthetic compound or a compound contained in a natural product. Further, the drug candidate substance may be a peptide or a nucleic acid. Test substances may be used individually in the screening, or a compound library containing these substances may be used.

EXAMPLES

[0057]

The present invention will now be described more particularly by way of Examples. However, the scope of the claims should not be limited by the Examples.

(Example 1) Cloning of Hepatitis C Virus

1. Origin of Virus

Using a 27-G disposable injection needle, 100 μl of the serum of a patient infected with the genotype 1a (G-52998-035: International Reagents Co., Ltd.) was intravenously inoculated to a human hepatocyte chimeric mouse (Tateno et al. American Journal Pathology 2004, 165:901-912) prepared by PhoenixBio Co., Ltd., at the orbital venous plexus. From the following week of the inoculation, 10 μL of blood was collected once per week under diethyl ether anesthesia at the orbital venous plexus using Intramedic™ Polyethylene Tubing (0.58×0.965 mm, Becton-Dickinson Japan, Tokyo). Immediately after the blood collection, the blood was transferred to a 500- μL safe-lock microtest tube containing a serum separation agent

(Eppendorf Co., Ltd., Tokyo). The blood was left to stand for 5 minutes at room temperature and then centrifuged at 13200×g for 3 minutes to obtain serum, which was then stored at -80°C. From 1 μL of the collected serum, RNA was extracted using SepaGene RV-R (Sanko Junyaku Co., Ltd., Tokyo), and the RNA was dissolved in 10 μL of Nuclease-free water (Ambion, Inc./Applied Biosystems Japan, Tokyo) containing 1 mM DTT (Promega KK, Tokyo) and 4 U/mL ribonuclease inhibitor (TAKARA BIO INC., Shiga). The dissolved RNA was stored at -80±10°C until quantification of the serum HCV RNA.

When the serum HCV RNA was quantified, HCV RNA synthesized in vitro was used as a copy standard. The quantification limit of the serum HCV RNA level was from 4.0×10^4 copies/mL to 1.0×10^9 copies/mL in the serum.

In the PCR reaction solution, 2.5 μL of the stock solution of the dissolved RNA or diluted RNA was used, and PCR was carried out using TaqMan* EZ RT-PCR Core Reagents (Applied Biosystems Japan, Tokyo). The PCR reaction was performed at 50°C for 2 minutes → at 60°C for 30 minutes → at 95°C for 5 minutes → (at 95°C for 20 seconds → at 62°C for 1 minute) ×50 cycles, and the reaction and analysis were carried out using ABI Prism 7700 (Applied Biosystems Japan). The serum HCV RNA level was calculated by averaging the levels measured in 2 wells.

As primers and a probe, the following sequences were used.

Forward primer: 5'-CGGGAGAGCCATAGTGG-3' (SEQ ID NO:6)

Reverse primer: 5'-AGTACCACAAGGCCTTTCG-3' (SEQ ID NO:7)

Probe: 5'-CTGCGGAACCGGTGAGTACAC-3' (SEQ ID NO:8) (5'-end: FAM; 3'-end: TAMRA)

The results indicate that infection of HCV was established and the blood virus level was maintained at a high level. The process, and timing of collection of the serum are shown in Fig. 1.

* Trademark

As a result, it was revealed that this virus maintains a high infection/replication efficiency.

[0058]

2. Preparation of Total RNA of Virus and Synthesis of cDNA

From 10 μl of the chimeric mouse serum obtained in the above-described 1, total RNA was extracted by the AGPC (acid-guanidinium-isothiocyanate-phenol-chloroform) method using ISOGEN-LS (manufactured by Nippon Gene Co., Ltd.), and the extracted total RNA was dissolved in RNase-free water. Using the thus obtained total RNA, reverse transcription was performed with a primer (the antisense sequence from the 9569th nucleotide having a length of 21 nucleotides) prepared based on the sequence of the HCV-H77 strain (accession No: AF011751), using LongRange Reverse Transcriptase (manufactured by Qiagen), at 25°C for 10minutes and then at 42°C for 90 minutes, followed by treatment for inactivation of the reverse transcriptase at 85°C for 5 minutes, addition of RNase H (manufactured by Invitrogen) and incubation at 37°C for 20 minutes to digest viral RNA so that only cDNA exists.

[0059]

3. Determination of Sequence

Nested PCR was performed using the synthesized cDNA as a template for PCR. The reaction was carried out by adding the above-described reaction solution at a ratio of 1/10 with respect to the total volume, and using Phusion DNA polymerase (manufactured by Fynnzymes) as the enzyme. PCR primers were designed based on the sequence of HCV-H77 (Fig. 2). Three fragments, that is, those corresponding to nucleotide positions 9 to 4054, 2952 to 7057, and 5963 to 9569, are expected to be amplified in the first PCR reaction. In the second PCR, 3 fragments, that is, those corresponding to nucleotide positions 23-4033, 2967-7035, and 5981-9554, were amplified using the respective reaction solutions as templates.

In the first PCR and the second PCR, 20 cycles and 30 cycles, respectively, of the reaction under the conditions of: denaturation at 98°C for 10 seconds; annealing at 60°C for 30 seconds; and then at 72°C for 2.5 minutes were performed. These DNA fragments were separated by agarose electrophoresis and purified using Wizard SV gel and PCR clean-up system (manufactured by Promega), followed by being cloned using TOPO cloning kit (manufactured by Invitrogen). Appeared colonies were picked up and allowed to grow, followed by purification of plasmid DNAs and determination of the sequences of not less than 15 clones for each fragment using Big Dye Terminator Mix and an automated DNA sequencer model 3100 (manufactured by Applied Biosystems). From the obtained sequence information, the most frequent nucleotide at each nucleotide position was determined to obtain a consensus sequence. At each of all the nucleotide positions, not less than 11 clones out of 15 clones shared the same nucleotide.

Subsequently, using the 5'-RACE method and the 3'-RACE method, the nucleotide sequences of the 5'-end and the 3'-end of the viral RNA were determined. For determination of the 5'-end sequence, 5'-end cDNA was synthesized using the 5'-RACE system (manufactured by Invitrogen, Version 2.0) and a primer (the antisense sequence from the 261st nucleotide having a length of 21 nucleotides), and a poly-C sequence was added to the 5'-end of the synthesized cDNA by terminal deoxynucleotidyl transferase. Thereafter, the 5'-end was amplified by nested-PCR.

Further, in order to determine the 3'-end sequence, a poly-A sequence was added to the extracted viral RNA using poly(A) polymerase (manufactured by Takara Shuzo Co., Ltd.), and cDNA was prepared using an oligo d(T) primer, followed by PCR amplification using a specific primer (the sense sequence from the 9385th nucleotide having a length of 24 nucleotides) and the oligo d(T) primer. These DNA fragments were similarly subjected to TOPO cloning, and the sequences of not less than 10 clones were determined, to obtain a consensus sequence. The positions

in the HCV-H77 sequence and the lengths of the primers used for the cloning are shown in Fig. 2. Among these, the primers corresponding to the regions 2952 to 2972 and 5981 to 6000 were prepared according to the RMT sequence based on the sequence information preliminarily obtained.

Thus, a consensus sequence of the total viral genome was obtained, and the strain was designated the HCV-RMT strain. The obtained nucleotide sequence, which has a length of 9598 nucleotides, is shown in SEQ ID NO:1. The nucleotide sequence had a long translated region between position 342 and position 9374, which encodes 3011 amino acid residues. The amino acid sequence of the translated region is described in SEQ ID NO:2.

The genomic nucleotide sequence of the RMT strain had a homology of 92.8% in terms of the nucleotide sequence, and 95.1% in terms of the amino acid sequence, to the H77 strain, which similarly belongs to the 1a type

[0060]

(Example 2) Confirmation of Infectivity to Chimeric Mouse

1. Construction of Viral RNA Expression Vector

An expression vector was prepared using the HCV-RMT strain sequence obtained in Example 1. A promoter sequence for T7 RNA polymerase was linked to the 5'-end, and a restriction enzyme *Xba*I-cleavage site was linked to the 3'-end. The resulting fragment was inserted into pBR322. Further, T at nucleotide position 3941 was converted to C, to destroy the *Xba*I cleavage site in the HCV-RMT sequence, without changing the amino acid sequence. For introduction of the mutation, QuikChangeII Site-Directed Mutagenesis kit (manufactured by Stratagene) was used. Thus, the expression vector was made such that, by digesting the vector with *Xba*I, removing single-stranded portions with mung bean nuclease and using the resulting DNA fragment as a template for T7 polymerase, RNA having the length of the HCV genome can be synthesized. The prepared expression vector was

designated pRMTx, and its structure is shown in Fig. 3.

[0061]

2. Preparation HCV-RMT Strain RNA and Injection Thereof to Liver of Human Hepatocyte Chimeric Mouse

pRMTx was sufficiently digested with *Xba*I, and single-stranded portions after the *Xba*I digestion were removed with Mung bean nuclease. Subsequently, using this DNA fragment, RNA was synthesized using RiboMAX (manufactured by Promega KK). After reaction at 37°C for 90 minutes, DNase treatment was carried out at 37°C for 30 minutes, and the synthesized RNA was purified through a microspin-G25 column (manufactured by GE HealthCare), followed by being subjected to phenol extraction and ethanol precipitation. A part of the thus obtained RNA was subjected to agarose electrophoresis to confirm that RNA having the desired size was produced.

A human hepatocyte chimeric mouse of 10 to 14 weeks old which had human albumin in the mouse blood at a concentration of not less than 6 mg/ml was subjected to celiotomy, and 400 μL/75 μg of this RNA was injected to the liver directly using an injector. From the following week of the inoculation, 10 μL of blood was collected once per week under diethyl ether anesthesia at the orbital venous plexus using Intramedic™ Polyethylene Tubing, and the copy number of the HCV-RNA in the serum was measured in the same manner as described above. Further, pRMTx-E1202G, which was prepared by introducing E1202G (a mutation replacing glutamic acid at position 1202 of SEQ ID NO:2 with glycine) to pRMTx, was also subjected to a similar experiment.

As a result, as shown in Fig. 4, in the case of the HCV-RMT strain, a higher copy number of the HCV RNA was detected in the chimeric mouse serum, compared to the JFH1 strain used as a control. Thus, it was revealed that the HCV-RMT strain has a high replication capacity in the chimeric mouse. Further, also in the

case of the HCV-RMT-E1202G strain, a higher HCV RNA copy number was detected 3 weeks later, compared to the JFH1 strain.

[0062]

(Example 3) Confirmation of Replicon-replicating Capacity and Identification of Acclimation Mutations

1. Construction of Replicon RNA Expression Vector

According to a conventional method (Lohman et al., Science, 1999), nucleotide positions 391 to 3419 of the HCV-RMT sequence in the pRMTx vector were deleted, and, instead, a sequence for a neomycin resistance protein and, subsequently, an EMCV-IRES sequence and an initiation codon were inserted. The prepared expression vector was designated pRMTx-neo, and its structure is shown in Fig. 5; the nucleotide sequence of the replicon RNA prepared from the expression vector is shown in SEQ ID NO:3; and the amino acid sequences of the proteins produce by translation are shown in SEQ ID NOs: 4 (neomycin resistance protein) and 5 (nonstructural protein).

[0063]

2. Preparation of Replicon RNA

pRMTx-neo was sufficiently digested with *Xba*I, and single-stranded portions after the *Xba*I digestion were removed with Mung bean nuclease. Subsequently, using this DNA fragment, RNA was synthesized using RiboMAX (manufactured by Promega KK). After reaction at 37°C for 90 minutes, DNase treatment was carried out at 37°C for 30 minutes, and the synthesized RNA was purified through a microspin-G25 column (manufactured by GE HealthCare), followed by being subjected to phenol extraction and ethanol precipitation. A part of the thus obtained RNA was subjected to agarose electrophoresis to confirm that RNA having the desired size was produced.

[0064]

3. Establishment of Replicon-replicating Cell Clone and Identification of Replicon Sequence Mutations

To Huh7 cells, 30 μg of the synthesized replicon RNA prepared in the above 2 was introduced by electroporation. The Huh7 cell to which the RNA was introduced was plated on a culture dish, and, after 16 hours of culture, G418 (neomycin) was added to the culture dish at a concentration of 300 μg/ml. Thereafter, the culture was continued, with the culture medium being replaced 3 times per week. As a result, 2 colonies were formed 21 days after the plating. These colonies were cloned and the culture was continued, after which one strain of a replicon-replicating clone maintaining stable replication was successfully established. From the established clone, total RNA was prepared, and PCR amplification was carried out to determine a consensus sequence. As a result, 3 mutations, that is, change from glutamic acid to valine at amino acid position 1056 (E1056V), change from glutamic acid to glycine at amino acid position 1202 (E1202G) and change from alanine to threonine at amino acid position 2199 (A2199T) were confirmed.

[0065]

4. Elucidation of Usefulness of Replicon Sequence Mutations

The 3 mutations confirmed in the replicon-replicating clone were introduced to pRMTx-neo individually or in combination. From the vectors to which the mutations were introduced, replicon RNAs were prepared, and 5 μg each of these replicon RNAs were introduced to Huh7 cells by electroporation in the same manner as in the above-described 3. After 21 days of G418 selection, viable cells were stained with crystal violet, and, as a result, colony formation was confirmed as shown in Fig. 6.

Based on these results obtained by comparison among the 3 mutations, the degree of enhancement of the growth capacity in the Huh7 cells was highest in E1202G, followed by E1056V and A2199T in this order. Further, it was revealed

that the clone to which all the 3 mutations were introduced has a higher growth capacity (replication capacity) than any of the clones to which the mutations were introduced alone.

[0066]

(Example 4) Confirmation of Growth Capacity of Acclimation Mutation-introduced HCV-RMT Strain in Cultured Cells

E1202G or the 3 acclimation mutations (E1202G, E1056V and A2199T) was/were introduced to pRMTx, and the obtained vectors were designated pRMTx-E1202G and pRMTx-tri, respectively. From pRMTx, pRMTx-E1202G and pRMTx-tri, viral genomic RNAs were prepared (RMT, RMT-E1202G and RMT-tri, respectively), and 30 μg each of the prepared genomic RNAs were introduced to Huh7 cells by electroporation. The cells were plated on a culture dish and subcultured 3 to 4 times per week, during which the cell were sampled. From the sampled cells, total RNA was purified by the AGPC method, and the copy number of the HCV genome per 1 μg of the total RNA was quantified according to a conventional method (Takeuchi et al., Gastroenterology, 1999) by reverse transcription real-time PCR using QuantiTect Multiplex PCR kit (manufactured by Qiagen). Daily changes in the copy number of the HCV genome in the cells to which RMT-RNA, RMT-E1202G-RNA and RMT-tri-RNA were respectively introduced are shown in Fig. 7. It was revealed that, while RMT-RNA, which has no acclimation mutation, quickly disappears, RMT-tri-RNA maintains a viral genomic amount of about 1×10^6 copies/μg for not less than 1 month. Further, it was revealed that RMT-E1202G-RNA also maintains a viral genomic amount of not less than 1×10^5 copies/μg even on Day 12.

[0067]

Further, 10 days after the introduction of the genome, the cells in the state where the HCV genome was maintained were plated on a 96-well plate, and an agent

was added thereto 16 hours later, followed by purification of total RNA 48 hours later from each well using ABI prizm 6100 and a purification kit (manufactured by Applied Biosystems). The HCV amount in the RNA, which was obtained by the above real-time PCR, and the total RNA amount quantified using an absorption spectrophotometer are shown in Fig. 8. As anti-HCV agents, an HCV polymerase inhibitor (MK0608), HCV protease inhibitor (BILN2061), IFN-α and cyclophilin inhibitor (cyclosporin A) were used. For all the anti-HCV agents, growth inhibition capacities against HCV, whose levels were almost the same as those described in documents were observed, and it was thereby confirmed that the cells wherein the HCV-RMT strain is continuously growing are useful as an evaluation system for anti-HCV agents.

[0068]

(Example 5) Confirmation of Production of Infectious Virus from Cells Wherein Acclimation Mutation-introduced HCV-RMT Strain is Continuously Growing

The RMT-tri-introduced cells, wherein the growth was confirmed in Example 4, single cell culture was carried out by the limiting dilution method. Selection was carried out among clones using as an index the amount of the intracellular virus, and the #11 clone, wherein expression of the HCV-core protein could be confirmed in all the cells by the fluorescent antibody method and the intracellular HCV genomic amount was 1×10^8 copies/μg, was obtained. By treating uninfected Huh7 cells with the culture supernatant of the clone after filtration through a 0.22 μm-filter, infected cells were confirmed by the fluorescent antibody method applied to the core protein (Fig. 9). By reducing the total volume of the culture supernatant to about one seventieth using an ultrafiltration membrane, the HCV copies were 40-fold concentrated. The concentrated culture supernatant showed a 45 times higher infectivity titer compared to the culture supernatant before the concentration.

The HCV sequence in the #11 cells was determined and, as a result, it was

revealed that, compared to the RMT strain, all the 10 clones sequenced had the mutation at amino acid position 2321 from serine to proline (S2321P) and the mutation at amino acid position 2889 from leucine to phenylalanine (L2889F). These mutations were suggested to be contributing to the infectivity and the growth capacity of the RMT strain.

INDUSTRIAL APPLICABILITY

[0069]

By using the polynucleotide of the present invention derived from the HCV-RMT strain, which belongs to the genotype 1a, and the identified acclimation mutations, an HCV replicon RNA and the HCV genomic RNA of the genotype 1 showing good replication efficiency can be prepared at a high probability. Replicon-replicating cells to which the replicon RNA was introduced, and HCV-growing cells to which the HCV genomic RNA was introduced can be used as culture systems for continuous production of an RNA derived from HCV, or an HCV protein. Further, the replicon-replicating cells and the HCV-growing cells can be used as test systems for screening various substances that influence replication of HCV and/or translation of HCV proteins.

[0070]

[Description of Sequence Listing]

1. Nucleotide sequence of entire genome of HCV-RMT strain

2. Amino acid sequence encoded by HCV-RMT strain

3. Nucleotide sequence of replicon derived from HCV-RMT strain

4. Amino acid sequence of neomycin resistance protein

5. Amino acid sequence of nonstructural protein encoded by HCV-RMT strain

6. Nucleotide sequence of Forward Primer

7. Nucleotide sequence of Reverse Primer

8. Nucleotide sequence of probe

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 72689-208 Seq 06-02-12 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> TOKYO METROPOLITAN ORGANIZATION FOR MEDICAL RESEARCH

<120> Polynucleotide from a novel strain of Hepatitis C virus and use thereof

<130> OP-10162-PCT

<140> PCT/JP2010/064417
<141> 2010-08-25

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<170> PatentIn version 3.3

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gtgcttgcga gtgccccggg aggtctcgta gaccgtgcac c atg agc acg aat cct 356
Met Ser Thr Asn Pro
1 5

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Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp
10 15 20

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Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu
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ccg cgc agg ggc cct aga ttg ggt gtg cgc gcg acg agg aag act tcc      500
Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser
        40                  45                  50

gag cgg tcg caa cct cga ggt aga cgt cag cct atc ccc aag gcg cgt      548
Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg
    55                  60                  65

cgg ccc gag ggc agg acc tgg gct cag ccc ggg tac cct tgg ccc ctc      596
Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu
70                  75                  80                  85

tat ggc aat gag ggc tgc ggg tgg gcg gga tgg ctc ctg tcc ccc cgt      644
Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg
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Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg
            105                 110                 115

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Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu
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Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg
    135                 140                 145

gcc ctg gcg cat ggc gtc cgg gtt ctg gaa gac ggc gtg aac tat gca      836
Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala
150                 155                 160                 165

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Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu
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ctc tct tgc ctg act gtg ccc gca tca gcc tac caa gta cgc aac tcc      932
Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Gln Val Arg Asn Ser
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Ser Gly Leu Tyr His Val Thr Asn Asp Cys Pro Asn Ser Ser Ile Val
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Tyr Glu Thr Ala Asp Thr Ile Leu His Ser Pro Gly Cys Val Pro Cys
    215                 220                 225

gtt cgc gag gat aac gcc tcg aga tgt tgg gtg ccg gtg gcc ccc aca     1076
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230                 235                 240                 245
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atc gat ctg ctt gtc ggg agc gcc acc ctc tgc tcg gcc ctc tat gta     1172
Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu Tyr Val
            265                 270                 275

ggg gac ttg tgc ggg tcc gtc ttt ctt gtc agc caa ctg ttc act ttt     1220
Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Phe
        280                 285                 290

tcc ccc agg cgc cac tgg aca acg caa gac tgc aac tgt tct atc tac     1268
Ser Pro Arg Arg His Trp Thr Thr Gln Asp Cys Asn Cys Ser Ile Tyr
    295                 300                 305

ccc ggc cat ata acg ggt cac cgc atg gcg tgg gat atg atg atg aac     1316
Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn
310                 315                 320                 325

tgg tcc cct acg gtc gcg ctg gta atg gct cag ctg ctc agg gtc cca     1364
Trp Ser Pro Thr Val Ala Leu Val Met Ala Gln Leu Leu Arg Val Pro
                330                 335                 340

caa gcc atc ttg gac atg atc gct ggt gct cac tgg gga gtc cta gcg     1412
Gln Ala Ile Leu Asp Met Ile Ala Gly Ala His Trp Gly Val Leu Ala
            345                 350                 355

ggc ata gcg tat ttc tcc atg gtg ggg aac tgg gcg aag gtt ctg gtg     1460
Gly Ile Ala Tyr Phe Ser Met Val Gly Asn Trp Ala Lys Val Leu Val
        360                 365                 370

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Val Leu Leu Leu Phe Ala Gly Val Asp Ala Gln Thr Tyr Val Thr Gly
    375                 380                 385

ggg agt gcc gct cgg ggc gcg tcc gga ctc gcc aac ctt ttt aca ccg     1556
Gly Ser Ala Ala Arg Gly Ala Ser Gly Leu Ala Asn Leu Phe Thr Pro
390                 395                 400                 405

ggc gcc aag cag gat atc cag ctg atc aac acc aat ggc agt tgg cac     1604
Gly Ala Lys Gln Asp Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp His
                410                 415                 420

atc aac cgt acg gcc ttg aac tgt aat gcg agc ctt gac act ggt tgg     1652
Ile Asn Arg Thr Ala Leu Asn Cys Asn Ala Ser Leu Asp Thr Gly Trp
            425                 430                 435

gtc gca ggg ctc ttt tat tac cat aaa ttc aac tct tca ggt tgc cct     1700
Val Ala Gly Leu Phe Tyr Tyr His Lys Phe Asn Ser Ser Gly Cys Pro
        440                 445                 450

gag agg atg gcc agc tgc aaa ccc ctt gcc gat ttc gac cag ggc tgg     1748
Glu Arg Met Ala Ser Cys Lys Pro Leu Ala Asp Phe Asp Gln Gly Trp
    455                 460                 465
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ggc cct att cgc cac gcc aac gga agc ggc ccc gaa cat cga ccc tac     1796
Gly Pro Ile Arg His Ala Asn Gly Ser Gly Pro Glu His Arg Pro Tyr
470                 475                 480                 485

tgc tgg cac tac ccc cca aaa cct tgt ggt atc gtg tca gca cag act     1844
Cys Trp His Tyr Pro Pro Lys Pro Cys Gly Ile Val Ser Ala Gln Thr
                490                 495                 500

gta tgt ggc cca gtg tat tgc ttc acc ccc agc ccc gta gtg gtg gga     1892
Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val Gly
            505                 510                 515

acg acc aac cgg ttg ggc gtg ccc acc tac agt tgg ggc acc aat gat     1940
Thr Thr Asn Arg Leu Gly Val Pro Thr Tyr Ser Trp Gly Thr Asn Asp
        520                 525                 530

acg gac gtt ttc gtc ctt aat aac acc agg cca ccg ttg ggc aat tgg     1988
Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro Pro Leu Gly Asn Trp
    535                 540                 545

ttc ggt tgc act tgg atg aac tca tct gga ttt acc aaa gtg tgc gga     2036
Phe Gly Cys Thr Trp Met Asn Ser Ser Gly Phe Thr Lys Val Cys Gly
550                 555                 560                 565

gcg cct cct tgt gtc atc gga gga gtg ggc aac aac acc ctg cac tgc     2084
Ala Pro Pro Cys Val Ile Gly Gly Val Gly Asn Asn Thr Leu His Cys
                570                 575                 580

ccc act gac tgt ttc cgc aag cat ccg gaa gcc acg tac tct cgg tgt     2132
Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr Ser Arg Cys
            585                 590                 595

ggc tcc ggt ccc tgg atc acg ccc agg tgc ctg gtc cac tat cct tat     2180
Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys Leu Val His Tyr Pro Tyr
        600                 605                 610

agg ctt tgg cat tat cct tgc acc gtc aac tac acc ctg ttc aaa gtc     2228
Arg Leu Trp His Tyr Pro Cys Thr Val Asn Tyr Thr Leu Phe Lys Val
    615                 620                 625

agg atg tac gtg gga ggg gtc gag cac agg ctg gaa gtt gct tgc aac     2276
Arg Met Tyr Val Gly Gly Val Glu His Arg Leu Glu Val Ala Cys Asn
630                 635                 640                 645

tgg acg cgg ggc gag cgt tgt gat ctg gac gac agg gac agg tcc gag     2324
Trp Thr Arg Gly Glu Arg Cys Asp Leu Asp Asp Arg Asp Arg Ser Glu
                650                 655                 660

ctc agc ccg ctg ctg ctg tcc acc aca cag tgg cag gtc ctc ccg tgt     2372
Leu Ser Pro Leu Leu Leu Ser Thr Thr Gln Trp Gln Val Leu Pro Cys
            665                 670                 675

tct ttc acg acc ttg cca gcc ttg act acc ggc ctc atc cac ctc cac     2420
Ser Phe Thr Thr Leu Pro Ala Leu Thr Thr Gly Leu Ile His Leu His
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Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly Val Gly Ser Ser Ile
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gtg tcc tgg gcc atc aag tgg gaa tac gtc atc ctt ctg ttc ctc ctg     2516
Val Ser Trp Ala Ile Lys Trp Glu Tyr Val Ile Leu Leu Phe Leu Leu
710                 715                 720                 725

ctt gca gac gcg cgc atc tgc tcc tgc ttg tgg atg atg cta ctc ata     2564
Leu Ala Asp Ala Arg Ile Cys Ser Cys Leu Trp Met Met Leu Leu Ile
                730                 735                 740

tcc caa gcg gag gcg gct ttg gag aac ctt gtg tta ctc aat gca gcg     2612
Ser Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Leu Leu Asn Ala Ala
            745                 750                 755

tct ctg gcc ggg acg cac ggt ctt gcg tcc ttc ctc gtg ttc ttc tgc     2660
Ser Leu Ala Gly Thr His Gly Leu Ala Ser Phe Leu Val Phe Phe Cys
        760                 765                 770

ttt gca tgg tat ctg aag ggt agg tgg gtg ccc gga gtg gcc tac gcc     2708
Phe Ala Trp Tyr Leu Lys Gly Arg Trp Val Pro Gly Val Ala Tyr Ala
    775                 780                 785

ttc tac ggg atg tgg cct ctc ctc ctg ctc ctg ttg gcg ttg ccc cag     2756
Phe Tyr Gly Met Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Gln
790                 795                 800                 805

cgg gca tac gcg ctg gac acg gag atg gcc gcg tcg tgt ggc ggc gtt     2804
Arg Ala Tyr Ala Leu Asp Thr Glu Met Ala Ala Ser Cys Gly Gly Val
                810                 815                 820

gtt ctt gtc ggg ttg atg gcg cta act ctg tca ccg tac tac aag agc     2852
Val Leu Val Gly Leu Met Ala Leu Thr Leu Ser Pro Tyr Tyr Lys Ser
            825                 830                 835

tat atc agc tgg tgc tta tgg tgg ctt cag tac ttc cta act agg ata     2900
Tyr Ile Ser Trp Cys Leu Trp Trp Leu Gln Tyr Phe Leu Thr Arg Ile
        840                 845                 850

gaa gcg cta ctg cac gtg tgg gtc ccc ccc ctc aat gtc cga gga ggg     2948
Glu Ala Leu Leu His Val Trp Val Pro Pro Leu Asn Val Arg Gly Gly
    855                 860                 865

cgc gac gct gtc atc ctg ctc acg tgt gtt gta cac ccg acc ttg gta     2996
Arg Asp Ala Val Ile Leu Leu Thr Cys Val Val His Pro Thr Leu Val
870                 875                 880                 885

ttc gac atc acc aag cta cta ctg gcc gtc ttc gga ccc ctt tgg att     3044
Phe Asp Ile Thr Lys Leu Leu Leu Ala Val Phe Gly Pro Leu Trp Ile
                890                 895                 900

ctc caa acc agt ctg ctc aag gtg ccc tac ttc gtg cgc gtc caa ggc     3092
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gtg cag atg gtc atc atc aag ttg ggg gcg ctt act ggc acc tac atc     3188
Val Gln Met Val Ile Ile Lys Leu Gly Ala Leu Thr Gly Thr Tyr Ile
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tac aac cat ctc act cct ctc cgg gac tgg gcg cac aac ggc cta cga     3236
Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His Asn Gly Leu Arg
950                 955                 960                 965

gat ctg gcc gtg gct gta gag cca gtc gtc ttt tcc cag atg gag acc     3284
Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser Gln Met Glu Thr
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aag ctc atc acg tgg ggg gcg gac acc gcc gcg tgc ggt gac atc atc     3332
Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp Ile Ile
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aac ggc ctg  cct gtc tct gcc cgt  agg ggc caa gag ata  ttg ctc     3377
Asn Gly Leu  Pro Val Ser Ala Arg  Arg Gly Gln Glu Ile  Leu Leu
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gga ccg gcc  gat gga atg gtc tct  aag ggg tgg agg ttg  ctg gcg     3422
Gly Pro Ala  Asp Gly Met Val Ser  Lys Gly Trp Arg Leu  Leu Ala
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ccc atc acg  gcg tat gcc cag cag  aca agg ggc ctc ctg  gga tgc     3467
Pro Ile Thr  Ala Tyr Ala Gln Gln  Thr Arg Gly Leu Leu  Gly Cys
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ata att act  agc ctg acc ggc cgg  gac aaa aac cag gtg  gag ggt     3512
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gag gtc cag  att gtg tca act gct  gcc cag acc ttc ctg  gca acc     3557
Glu Val Gln  Ile Val Ser Thr Ala  Ala Gln Thr Phe Leu  Ala Thr
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tgc atc aac  gga gtg tgc tgg act  gtc tac cac ggg gcc  gga aca     3602
Cys Ile Asn  Gly Val Cys Trp Thr  Val Tyr His Gly Ala  Gly Thr
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agg acc atc  gcg tca ccc aaa ggt  ccc gtc atc cag atg  tat act     3647
Arg Thr Ile  Ala Ser Pro Lys Gly  Pro Val Ile Gln Met  Tyr Thr
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aat gta gac  caa gac ctt gta ggc  tgg ccc gct ccc caa  ggt gcc     3692
Asn Val Asp  Gln Asp Leu Val Gly  Trp Pro Ala Pro Gln  Gly Ala
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cgc tca ttg  aca ccc tgc act tgc  ggc tcc tcg gac ctt  tac ttg     3737
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agc agg ggc  agc ctg ctc tcg ccc  cgg cct atc tct tac  ttg aaa      3827
Ser Arg Gly  Ser Leu Leu Ser Pro  Arg Pro Ile Ser Tyr  Leu Lys
        1150                 1155                 1160

ggc tct tcg  ggg ggc cca ttg ctg  tgc ccc gcg gga cac  gcc gta      3872
Gly Ser Ser  Gly Gly Pro Leu Leu  Cys Pro Ala Gly His  Ala Val
        1165                 1170                 1175

ggc ata ttc  agg gcc gcg gtg tgc  acc cgt gga gtg gct  aag gcg      3917
Gly Ile Phe  Arg Ala Ala Val Cys  Thr Arg Gly Val Ala  Lys Ala
        1180                 1185                 1190

gtg gac ttt  atc ccc gta gag agt  cta gag aca acc atg  agg tcc      3962
Val Asp Phe  Ile Pro Val Glu Ser  Leu Glu Thr Thr Met  Arg Ser
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ccg gtg ttc  aca gac aac tcc tcc  cca cca gca gtg ccc  cag agc      4007
Pro Val Phe  Thr Asp Asn Ser Ser  Pro Pro Ala Val Pro  Gln Ser
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ttc cag gtg  gcc cac ctg cat gct  ccc acc ggc agc ggt  aag agt      4052
Phe Gln Val  Ala His Leu His Ala  Pro Thr Gly Ser Gly  Lys Ser
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acc aag gtc  ccg gcc gca tac gcg  gct cag ggc tac aag  gtg ctg      4097
Thr Lys Val  Pro Ala Ala Tyr Ala  Ala Gln Gly Tyr Lys  Val Leu
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gtg ctc aac  ccc tcc gtt gct gca  aca ctg ggc ttt ggt  gct tat      4142
Val Leu Asn  Pro Ser Val Ala Ala  Thr Leu Gly Phe Gly  Ala Tyr
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atg tcc aag  gcc cat ggg att gat  cct aac atc agg act  ggg gtg      4187
Met Ser Lys  Ala His Gly Ile Asp  Pro Asn Ile Arg Thr  Gly Val
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agg aca atc  act act ggc agc ccc  atc acg tac tcc acc  tac ggc      4232
Arg Thr Ile  Thr Thr Gly Ser Pro  Ile Thr Tyr Ser Thr  Tyr Gly
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aag ttc ctt  gct gat ggc ggg tgc  tcg ggg ggt gct tat  gac ata      4277
Lys Phe Leu  Ala Asp Gly Gly Cys  Ser Gly Gly Ala Tyr  Asp Ile
        1300                 1305                 1310

ata att tgt  gac gag tgc cac tct  acg gat gca aca tcc  gtc ttg      4322
Ile Ile Cys  Asp Glu Cys His Ser  Thr Asp Ala Thr Ser  Val Leu
        1315                 1320                 1325

ggc atc ggc  act gtc ctt gac caa  gca gag act gcg ggg  gcg aga      4367
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ccc cat cct  aac atc gag gag gtt  gct ctg tcc acc acc  gga gag      4457
Pro His Pro  Asn Ile Glu Glu Val  Ala Leu Ser Thr Thr  Gly Glu
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atc ccc ttt  tac ggc aag gct atc  ccc ctt gag gca atc  aag ggg      4502
Ile Pro Phe  Tyr Gly Lys Ala Ile  Pro Leu Glu Ala Ile  Lys Gly
        1375                 1380                 1385

ggg aga cat  ctc atc ttc tgc cat  tca aaa aag aag tgc  gac gag      4547
Gly Arg His  Leu Ile Phe Cys His  Ser Lys Lys Lys Cys  Asp Glu
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ctc gct gca  aag ctg gtc gcg atg  ggc gtc aat gcc gtg  gct tac      4592
Leu Ala Ala  Lys Leu Val Ala Met  Gly Val Asn Ala Val  Ala Tyr
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tac cgc ggc  ctc gac gtg tcc gtc  atc cca acc agt ggc  gat gtt      4637
Tyr Arg Gly  Leu Asp Val Ser Val  Ile Pro Thr Ser Gly  Asp Val
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gtc gtc gtg  gca act gat gcc ctc  atg acc ggc tat acc  ggc gac      4682
Val Val Val  Ala Thr Asp Ala Leu  Met Thr Gly Tyr Thr  Gly Asp
        1435                 1440                 1445

ttt gac tcg  gtg ata gac tgc aac  acg tgt gtc acc cag  aca gtc      4727
Phe Asp Ser  Val Ile Asp Cys Asn  Thr Cys Val Thr Gln  Thr Val
        1450                 1455                 1460

gac ttc agc  ctt gac cct acc ttc  acc att gag aca acc  acg ctc      4772
Asp Phe Ser  Leu Asp Pro Thr Phe  Thr Ile Glu Thr Thr  Thr Leu
        1465                 1470                 1475

ccc cag gac  gct gtc tcc cgc act  caa cgt cgg ggc agg  act ggc      4817
Pro Gln Asp  Ala Val Ser Arg Thr  Gln Arg Arg Gly Arg  Thr Gly
        1480                 1485                 1490

agg ggg aag  cca ggc atc tac aga  ttt gtg gca ccg ggg  gag cgc      4862
Arg Gly Lys  Pro Gly Ile Tyr Arg  Phe Val Ala Pro Gly  Glu Arg
        1495                 1500                 1505

ccc tcc ggc  atg ttt gac tcg tcc  gtc ctc tgt gag tgc  tat gac      4907
Pro Ser Gly  Met Phe Asp Ser Ser  Val Leu Cys Glu Cys  Tyr Asp
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gcg ggc tgt  gct tgg tat gag ctc  aca ccc gcc gag acc  aca gtt      4952
Ala Gly Cys  Ala Trp Tyr Glu Leu  Thr Pro Ala Glu Thr  Thr Val
        1525                 1530                 1535

agg cta cga  gca tat atg aac acc  ccg ggg ctc ccc gtg  tgc caa      4997
Arg Leu Arg  Ala Tyr Met Asn Thr  Pro Gly Leu Pro Val  Cys Gln
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ata gac gcc  cat ttc cta tcc cag  aca aag cag agt ggg  gaa aac      5087
Ile Asp Ala  His Phe Leu Ser Gln  Thr Lys Gln Ser Gly  Glu Asn
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ctt cct tac  ctg gta gcg tac caa  gcc acc gtg tgc gct  agg gct      5132
Leu Pro Tyr  Leu Val Ala Tyr Gln  Ala Thr Val Cys Ala  Arg Ala
        1585                 1590                 1595

cag gcc ccc  ccc cca tcg tgg gac  cag atg tgg aag tgc  ttg atc      5177
Gln Ala Pro  Pro Pro Ser Trp Asp  Gln Met Trp Lys Cys  Leu Ile
        1600                 1605                 1610

cgc ctc aag  ccc acc ctt cat ggg  cca aca cct ctg cta  tac aga      5222
Arg Leu Lys  Pro Thr Leu His Gly  Pro Thr Pro Leu Leu  Tyr Arg
        1615                 1620                 1625

ctg ggc gct  gtt cag aat gaa gtc  acc ctg acg cac cca  atc acc      5267
Leu Gly Ala  Val Gln Asn Glu Val  Thr Leu Thr His Pro  Ile Thr
        1630                 1635                 1640

aag tac atc  atg aca tgc atg tcg  gct gac ctg gag gtc  gtc acg      5312
Lys Tyr Ile  Met Thr Cys Met Ser  Ala Asp Leu Glu Val  Val Thr
        1645                 1650                 1655

agt acc tgg  gtg ctc gtc ggc ggc  gtc ctg gct gct ttg  gcc gcg      5357
Ser Thr Trp  Val Leu Val Gly Gly  Val Leu Ala Ala Leu  Ala Ala
        1660                 1665                 1670

tat tgc cta  tcc aca ggc tgc gtg  gtc ata gta ggc agg  att gtc      5402
Tyr Cys Leu  Ser Thr Gly Cys Val  Val Ile Val Gly Arg  Ile Val
        1675                 1680                 1685

ttg tcc ggg  aag ccg gct atc ata  cct gac agg gaa gtc  ctc tac      5447
Leu Ser Gly  Lys Pro Ala Ile Ile  Pro Asp Arg Glu Val  Leu Tyr
        1690                 1695                 1700

cgg gag ttc  gat gag atg gaa gag  tgc tct cag cac ttg  ccg tac      5492
Arg Glu Phe  Asp Glu Met Glu Glu  Cys Ser Gln His Leu  Pro Tyr
        1705                 1710                 1715

atc gag cag  ggg atg atg ctc gcc  gag cag ttc aag cag  aag gcc      5537
Ile Glu Gln  Gly Met Met Leu Ala  Glu Gln Phe Lys Gln  Lys Ala
        1720                 1725                 1730

ctc ggc ctc  ctg cag acc gcg tcc  cgc cag gca gag gtc  atc gcc      5582
Leu Gly Leu  Leu Gln Thr Ala Ser  Arg Gln Ala Glu Val  Ile Ala
        1735                 1740                 1745

cct acc gtc  caa acc aac tgg cag  aga ctc gag gcc ttc  tgg gcg      5627
Pro Thr Val  Gln Thr Asn Trp Gln  Arg Leu Glu Ala Phe  Trp Ala
        1750                 1755                 1760
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aag cat atg  tgg aac ttc atc agt  ggg ata caa tat ctg  gcg ggc      5672
Lys His Met  Trp Asn Phe Ile Ser  Gly Ile Gln Tyr Leu  Ala Gly
       1765                 1770                 1775

ctg tca acg  ttg cct ggt aat ccc  gcc att gca tca ttg  atg gct      5717
Leu Ser Thr  Leu Pro Gly Asn Pro  Ala Ile Ala Ser Leu  Met Ala
       1780                 1785                 1790

ttt aca gct  gcc gtc acc agc cca  cta acc acc ggc caa  act ctc      5762
Phe Thr Ala  Ala Val Thr Ser Pro  Leu Thr Thr Gly Gln  Thr Leu
       1795                 1800                 1805

ctc ttc aac  att ttg ggg ggg tgg  gtg gct gcc cag ctc  gca gcc      5807
Leu Phe Asn  Ile Leu Gly Gly Trp  Val Ala Ala Gln Leu  Ala Ala
       1810                 1815                 1820

ccc ggt gcc  gct acc gcc ttt gtg  ggc gct ggc tta gcc  ggc gcc      5852
Pro Gly Ala  Ala Thr Ala Phe Val  Gly Ala Gly Leu Ala  Gly Ala
       1825                 1830                 1835

gcc atc ggc  agt gtt gga ctg ggg  aag gtc ctc gtg gac  atc ctt      5897
Ala Ile Gly  Ser Val Gly Leu Gly  Lys Val Leu Val Asp  Ile Leu
       1840                 1845                 1850

gca ggg tat  ggc gcg ggc gtg gcg  gga gct ctt gta gca  ttt aag      5942
Ala Gly Tyr  Gly Ala Gly Val Ala  Gly Ala Leu Val Ala  Phe Lys
       1855                 1860                 1865

atc atg agc  ggt gag gtt ccc tcc  aca gag gac ctg gtc  aat cta      5987
Ile Met Ser  Gly Glu Val Pro Ser  Thr Glu Asp Leu Val  Asn Leu
       1870                 1875                 1880

ctg cct gcc  atc ctt tcg ccc gga  gcc ctt gta gtc ggt  gtg gtc      6032
Leu Pro Ala  Ile Leu Ser Pro Gly  Ala Leu Val Val Gly  Val Val
       1885                 1890                 1895

tgc gca gca  ata cta cgc cgg cac  gtt ggc ccg ggc gag  gga gca      6077
Cys Ala Ala  Ile Leu Arg Arg His  Val Gly Pro Gly Glu  Gly Ala
       1900                 1905                 1910

gtg cag tgg  atg aac cgg ttg ata  gcc ttc gcc tcc cgg  ggg aac      6122
Val Gln Trp  Met Asn Arg Leu Ile  Ala Phe Ala Ser Arg  Gly Asn
       1915                 1920                 1925

cac gtt tcc  ccc acg cac tac gtg  ccg gag agc gat gca  gct gcc      6167
His Val Ser  Pro Thr His Tyr Val  Pro Glu Ser Asp Ala  Ala Ala
       1930                 1935                 1940

cgc gtc act  gcc ata ctc agc agc  ctc act gtg acc cag  ctc ctg      6212
Arg Val Thr  Ala Ile Leu Ser Ser  Leu Thr Val Thr Gln  Leu Leu
       1945                 1950                 1955

agg cga cta  cac cag tgg cta agc  tcg gag tgt acc act  cca tgc      6257
Arg Arg Leu  His Gln Trp Leu Ser  Ser Glu Cys Thr Thr  Pro Cys
       1960                 1965                 1970
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tcc ggt tcc  tgg cta agg gac atc  tgg gac tgg ata tgc  gag gtg      6302
Ser Gly Ser  Trp Leu Arg Asp Ile  Trp Asp Trp Ile Cys  Glu Val
        1975                 1980                 1985

ctg agc gat  ttt aag acc tgg ctg  aag gcc aag ctc atg  cca caa      6347
Leu Ser Asp  Phe Lys Thr Trp Leu  Lys Ala Lys Leu Met  Pro Gln
        1990                 1995                 2000

ctg cct ggg  att ccc ttt gtg tcc  tgc caa cgc ggg tac  agg ggg      6392
Leu Pro Gly  Ile Pro Phe Val Ser  Cys Gln Arg Gly Tyr  Arg Gly
        2005                 2010                 2015

gtc tgg cga  gga gat ggc att atg  cac act cgc tgc ccc  tgt gga      6437
Val Trp Arg  Gly Asp Gly Ile Met  His Thr Arg Cys Pro  Cys Gly
        2020                 2025                 2030

gct gag atc  gcc gga cat gtc aag  aac ggg acg atg agg  atc gtc      6482
Ala Glu Ile  Ala Gly His Val Lys  Asn Gly Thr Met Arg  Ile Val
        2035                 2040                 2045

ggt cct aag  acc tgc aga aac acg  tgg agt ggg acc ttc  ccc atc      6527
Gly Pro Lys  Thr Cys Arg Asn Thr  Trp Ser Gly Thr Phe  Pro Ile
        2050                 2055                 2060

aac gcc tac  acc acg ggc ccc tgt  acc ccc ctt cct gcg  ccg aac      6572
Asn Ala Tyr  Thr Thr Gly Pro Cys  Thr Pro Leu Pro Ala  Pro Asn
        2065                 2070                 2075

tat acg ttc  gcg ctg tgg agg gtg  tct gcg gag gaa tac  gtg gaa      6617
Tyr Thr Phe  Ala Leu Trp Arg Val  Ser Ala Glu Glu Tyr  Val Glu
        2080                 2085                 2090

ata agg cag  gtg ggg gac ttc cac  tac gtg acg ggc atg  act gct      6662
Ile Arg Gln  Val Gly Asp Phe His  Tyr Val Thr Gly Met  Thr Ala
        2095                 2100                 2105

gac aac ctt  aag tgc cca tgc cag  gtc cca tcg ccc gaa  ttt ttc      6707
Asp Asn Leu  Lys Cys Pro Cys Gln  Val Pro Ser Pro Glu  Phe Phe
        2110                 2115                 2120

aca gaa ctg  gat ggg gtg cgc ctg  cat agg ttt gcg ccc  cct tgc      6752
Thr Glu Leu  Asp Gly Val Arg Leu  His Arg Phe Ala Pro  Pro Cys
        2125                 2130                 2135

aag ccc ttg  cta cga gat gag gtg  tcg ttc aga gta gga  cta cac      6797
Lys Pro Leu  Leu Arg Asp Glu Val  Ser Phe Arg Val Gly  Leu His
        2140                 2145                 2150

gac tac ccg  gtg ggg tcg cag tta  cct tgc gag cct gaa  ccg gat      6842
Asp Tyr Pro  Val Gly Ser Gln Leu  Pro Cys Glu Pro Glu  Pro Asp
        2155                 2160                 2165

gtg gcc gta  ctg acg tcc atg ctc  acc gat ccc tcc cat  ata acg      6887
Val Ala Val  Leu Thr Ser Met Leu  Thr Asp Pro Ser His  Ile Thr
        2170                 2175                 2180
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gca gag gcg  gct ggg agg agg tta  gca agg gga tcg ccc  cct tct      6932
Ala Glu Ala  Ala Gly Arg Arg Leu  Ala Arg Gly Ser Pro  Pro Ser
        2185                 2190                 2195

ctg gcc agc  tcc tcg gcc agc cag  ctg tcc gct cca tct  ctc aaa      6977
Leu Ala Ser  Ser Ser Ala Ser Gln  Leu Ser Ala Pro Ser  Leu Lys
        2200                 2205                 2210

gca act tgc  acc acc aac cac gac  tcc cct gac gcc gag  ctc ata      7022
Ala Thr Cys  Thr Thr Asn His Asp  Ser Pro Asp Ala Glu  Leu Ile
        2215                 2220                 2225

gag gct aac  ctc ctg tgg agg cag  gag atg ggc ggc aac  atc acc      7067
Glu Ala Asn  Leu Leu Trp Arg Gln  Glu Met Gly Gly Asn  Ile Thr
        2230                 2235                 2240

agg gtt gag  tca gag aac aaa gtg  gta gtc ctg gac tcc  ttc gat      7112
Arg Val Glu  Ser Glu Asn Lys Val  Val Val Leu Asp Ser  Phe Asp
        2245                 2250                 2255

ccg ctt gtg  gca gaa gag gac gaa  cgg gag atc tcc gtg  gcc gca      7157
Pro Leu Val  Ala Glu Glu Asp Glu  Arg Glu Ile Ser Val  Ala Ala
        2260                 2265                 2270

gag atc ctg  cgg aag tct cgg aga  ttc gct ccg gcc ctg  ccc att      7202
Glu Ile Leu  Arg Lys Ser Arg Arg  Phe Ala Pro Ala Leu  Pro Ile
        2275                 2280                 2285

tgg gca cgg  ccg gac tac aac ccc  ccg tta ctg gag acg  tgg aaa      7247
Trp Ala Arg  Pro Asp Tyr Asn Pro  Pro Leu Leu Glu Thr  Trp Lys
        2290                 2295                 2300

aag ccg gac  tac gag cca cct gtg  gtc cat ggc tgc ccg  ctt cca      7292
Lys Pro Asp  Tyr Glu Pro Pro Val  Val His Gly Cys Pro  Leu Pro
        2305                 2310                 2315

cct cca aag  tcc cct cct gtg cct  ccg ccc cgg aag aag  cgg acg      7337
Pro Pro Lys  Ser Pro Pro Val Pro  Pro Pro Arg Lys Lys  Arg Thr
        2320                 2325                 2330

gtg gtc ctc  act gaa tca act gta  tcc act gcc ttg gct  gag ctt      7382
Val Val Leu  Thr Glu Ser Thr Val  Ser Thr Ala Leu Ala  Glu Leu
        2335                 2340                 2345

gct acc aag  agc ttt ggc agc tct  tca act tcc ggt ata  acg ggc      7427
Ala Thr Lys  Ser Phe Gly Ser Ser  Ser Thr Ser Gly Ile  Thr Gly
        2350                 2355                 2360

gac aac acg  aca gcg tcc tct gag  ccc gcc ccc tct gtc  tgc cct      7472
Asp Asn Thr  Thr Ala Ser Ser Glu  Pro Ala Pro Ser Val  Cys Pro
        2365                 2370                 2375

cca gac tcc  gac gct gag tcc tat  tct tcc atg ccc ccc  ctg gag      7517
Pro Asp Ser  Asp Ala Glu Ser Tyr  Ser Ser Met Pro Pro  Leu Glu
        2380                 2385                 2390
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ggg gag cct  ggg gat ccg gat ctc  agc gac ggg tca tgg  tcg acg      7562
Gly Glu Pro  Gly Asp Pro Asp Leu  Ser Asp Gly Ser Trp  Ser Thr
        2395                 2400                 2405

gtc agt agt  ggg gct ggc acg gag  gat gtc gtg tgt tgc  tca atg      7607
Val Ser Ser  Gly Ala Gly Thr Glu  Asp Val Val Cys Cys  Ser Met
        2410                 2415                 2420

tcc tat tcc  tgg aca ggc gca ctc  atc acc ccg tgt gcc  gcg gaa      7652
Ser Tyr Ser  Trp Thr Gly Ala Leu  Ile Thr Pro Cys Ala  Ala Glu
        2425                 2430                 2435

gaa caa aaa  ttg cct atc aac gca  ctg agc aac tca tta  ctg cgt      7697
Glu Gln Lys  Leu Pro Ile Asn Ala  Leu Ser Asn Ser Leu  Leu Arg
        2440                 2445                 2450

cac cac aac  ctc gtg tat tcc acc  acc tca cgc agt gct  tgc caa      7742
His His Asn  Leu Val Tyr Ser Thr  Thr Ser Arg Ser Ala  Cys Gln
        2455                 2460                 2465

agg cag aag  aaa gtc aca ttt gac  aga ctg caa gtt ctg  gac aac      7787
Arg Gln Lys  Lys Val Thr Phe Asp  Arg Leu Gln Val Leu  Asp Asn
        2470                 2475                 2480

cac tac cag  gac gtg ctc aag gag  gtt aag gcg gcg gcg  tca aaa      7832
His Tyr Gln  Asp Val Leu Lys Glu  Val Lys Ala Ala Ala  Ser Lys
        2485                 2490                 2495

gtg aag gct  aac ttg cta tcc gta  gag gaa gct tgc agc  ctg acg      7877
Val Lys Ala  Asn Leu Leu Ser Val  Glu Glu Ala Cys Ser  Leu Thr
        2500                 2505                 2510

ccc cca cat  tca gcc aga tca aaa  ttt ggc tat ggg gca  aaa gac      7922
Pro Pro His  Ser Ala Arg Ser Lys  Phe Gly Tyr Gly Ala  Lys Asp
        2515                 2520                 2525

gtc cgt tgc  cat gcc aga aag gcc  gta aac cac atc aac  tcc gtg      7967
Val Arg Cys  His Ala Arg Lys Ala  Val Asn His Ile Asn  Ser Val
        2530                 2535                 2540

tgg aaa gac  ctt ctg gaa gac agt  gtt aca cca ata gac  aca acc      8012
Trp Lys Asp  Leu Leu Glu Asp Ser  Val Thr Pro Ile Asp  Thr Thr
        2545                 2550                 2555

atc atg gct  aag aac gaa gtt ttc  tgc gtt cag cct gag  aag ggg      8057
Ile Met Ala  Lys Asn Glu Val Phe  Cys Val Gln Pro Glu  Lys Gly
        2560                 2565                 2570

ggt cgt aag  cca gct cgt ctc atc  gtg tac cct gac ctg  ggt gtg      8102
Gly Arg Lys  Pro Ala Arg Leu Ile  Val Tyr Pro Asp Leu  Gly Val
        2575                 2580                 2585

cgc gtg tgc  gag aaa atg gcc ctg  tac gac gtg gta aaa  aaa ctc      8147
Arg Val Cys  Glu Lys Met Ala Leu  Tyr Asp Val Val Lys  Lys Leu
        2590                 2595                 2600
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ccc ctg gcc  gtg atg gga agc tcc  tac gga ttc cag tac  tca cca      8192
Pro Leu Ala  Val Met Gly Ser Ser  Tyr Gly Phe Gln Tyr  Ser Pro
        2605                 2610                 2615

gga cag cgg  gtt gaa ttc ctc gtg  caa gcg tgg aag tcc  aag ggg      8237
Gly Gln Arg  Val Glu Phe Leu Val  Gln Ala Trp Lys Ser  Lys Gly
        2620                 2625                 2630

acc cca atg  ggg ttc tcg tat gat  acc cgc tgc ttt gac  tct aca      8282
Thr Pro Met  Gly Phe Ser Tyr Asp  Thr Arg Cys Phe Asp  Ser Thr
        2635                 2640                 2645

gtc act gag  agc gat atc cgt acg  gag gag gca atc tac  cag tgt      8327
Val Thr Glu  Ser Asp Ile Arg Thr  Glu Glu Ala Ile Tyr  Gln Cys
        2650                 2655                 2660

tgt gac ctg  gac ccc caa gcc cgc  gtg gcc atc aag tcc  ctc acc      8372
Cys Asp Leu  Asp Pro Gln Ala Arg  Val Ala Ile Lys Ser  Leu Thr
        2665                 2670                 2675

gag agg ctt  tat gtc ggg ggt cct  ctt acc aat tca agg  ggg gaa      8417
Glu Arg Leu  Tyr Val Gly Gly Pro  Leu Thr Asn Ser Arg  Gly Glu
        2680                 2685                 2690

aac tgc ggc  tat cgc agg tgc cgc  gca agc ggc gta ctg  aca act      8462
Asn Cys Gly  Tyr Arg Arg Cys Arg  Ala Ser Gly Val Leu  Thr Thr
        2695                 2700                 2705

agc tgt ggt  aac acc ctc act tgc  tac atc aag gcc cga  gca gcc      8507
Ser Cys Gly  Asn Thr Leu Thr Cys  Tyr Ile Lys Ala Arg  Ala Ala
        2710                 2715                 2720

tgt cga gcc  gca ggg ctc cgg gac  tgc acc atg ctc gtg  tgt ggc      8552
Cys Arg Ala  Ala Gly Leu Arg Asp  Cys Thr Met Leu Val  Cys Gly
        2725                 2730                 2735

gac gac tta  gtc gtt atc tgt gaa  agc cag ggg gtc caa  gag gat      8597
Asp Asp Leu  Val Val Ile Cys Glu  Ser Gln Gly Val Gln  Glu Asp
        2740                 2745                 2750

aca gcg agc  ctg aga gcc ttc acg  gag gct atg acc agg  tac tcc      8642
Thr Ala Ser  Leu Arg Ala Phe Thr  Glu Ala Met Thr Arg  Tyr Ser
        2755                 2760                 2765

gct ccc ccc  ggg gac ccc ccc caa  cca gaa tac gac ttg  gag ctc      8687
Ala Pro Pro  Gly Asp Pro Pro Gln  Pro Glu Tyr Asp Leu  Glu Leu
        2770                 2775                 2780

ata aca tcg  tgc tcc tct aac gtg  tca gtc gcc cac gac  gac act      8732
Ile Thr Ser  Cys Ser Ser Asn Val  Ser Val Ala His Asp  Asp Thr
        2785                 2790                 2795

gga aag agg  gtc tat tac ctt acc  cgt gac cct aca act  ccc ctc      8777
Gly Lys Arg  Val Tyr Tyr Leu Thr  Arg Asp Pro Thr Thr  Pro Leu
        2800                 2805                 2810
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gcg aga gcc  gcg tgg gag aca gca  aga cac act cca gtc  aat tcc      8822
Ala Arg Ala  Ala Trp Glu Thr Ala  Arg His Thr Pro Val  Asn Ser
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tgg cta ggc  aac ata atc atg ttt  gcc ccc aca ttg tgg  gtg aga      8867
Trp Leu Gly  Asn Ile Ile Met Phe  Ala Pro Thr Leu Trp  Val Arg
        2830                 2835                 2840

atg ata ctg  ctg ccc cac ttc ttc  agt gtc ctc atg gcc  agg gac      8912
Met Ile Leu  Leu Pro His Phe Phe  Ser Val Leu Met Ala  Arg Asp
        2845                 2850                 2855

caa ctt gaa  cag gcc ctt gat tgc  gaa atc tac gga gcc  tgc tac      8957
Gln Leu Glu  Gln Ala Leu Asp Cys  Glu Ile Tyr Gly Ala  Cys Tyr
        2860                 2865                 2870

tcc ata gaa  cca ctg gac cta cct  cca atc att caa aga  ctc cat      9002
Ser Ile Glu  Pro Leu Asp Leu Pro  Pro Ile Ile Gln Arg  Leu His
        2875                 2880                 2885

ggc ctt agc  gca ttt tca ctc cac  agt tac tct cca ggt  gaa atc      9047
Gly Leu Ser  Ala Phe Ser Leu His  Ser Tyr Ser Pro Gly  Glu Ile
        2890                 2895                 2900

aat agg gtg  gcc gca tgc ctc aga  aaa ctt ggg gtc ccg  ccc ttg      9092
Asn Arg Val  Ala Ala Cys Leu Arg  Lys Leu Gly Val Pro  Pro Leu
        2905                 2910                 2915

cga gct tgg  aga cac cgg gcc cgg  agc atc cgc gct aag  ctt ctg      9137
Arg Ala Trp  Arg His Arg Ala Arg  Ser Ile Arg Ala Lys  Leu Leu
        2920                 2925                 2930

tcc aga gga  ggc agg gct gcc acg  tgt ggc aag tac ctc  ttc aat      9182
Ser Arg Gly  Gly Arg Ala Ala Thr  Cys Gly Lys Tyr Leu  Phe Asn
        2935                 2940                 2945

tgg gca gta  aga aca aag ctc aaa  ctc act cca ata gcg  gcc gct      9227
Trp Ala Val  Arg Thr Lys Leu Lys  Leu Thr Pro Ile Ala  Ala Ala
        2950                 2955                 2960

agc cag ctg  gac ttg tcc ggt tgg  ttc acg gct ggc tac  agc ggg      9272
Ser Gln Leu  Asp Leu Ser Gly Trp  Phe Thr Ala Gly Tyr  Ser Gly
        2965                 2970                 2975

gga gac att  tat cac agc gtg tct  cgt gcc cgg ccc cgc  tgg ttc      9317
Gly Asp Ile  Tyr His Ser Val Ser  Arg Ala Arg Pro Arg  Trp Phe
        2980                 2985                 2990

tgg ttt tgc  cta ctc ctg ctt gct  gca ggg gta ggc atc  tac ctc      9362
Trp Phe Cys  Leu Leu Leu Leu Ala  Ala Gly Val Gly Ile  Tyr Leu
        2995                 3000                 3005

ctc ccc aac  cga tgaaggttgg ggtaaacact ccggcctctt aggccattcc      9414
Leu Pro Asn  Arg
        3010
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tgtttttttt ttttttttct tttgtttttt ttgttttttt ttttttttct cctttctttt   9474
ttttttttct tcctttcttc tttaatggtg gctccatctt agccctagtc acggctagct   9534
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atgt                                                                9598
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<210> 2
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            20                  25                  30
Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala
        35                  40                  45
Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
    50                  55                  60
Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly
65                  70                  75                  80
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp
                85                  90                  95
Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
            100                 105                 110
Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
        115                 120                 125
Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu
    130                 135                 140
Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
145                 150                 155                 160
Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
                165                 170                 175
Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr
            180                 185                 190
Gln Val Arg Asn Ser Ser Gly Leu Tyr His Val Thr Asn Asp Cys Pro
        195                 200                 205
Asn Ser Ser Ile Val Tyr Glu Thr Ala Asp Thr Ile Leu His Ser Pro
    210                 215                 220
Gly Cys Val Pro Cys Val Arg Glu Asp Asn Ala Ser Arg Cys Trp Val
225                 230                 235                 240
Pro Val Ala Pro Thr Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr
                245                 250                 255
Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys
            260                 265                 270
Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser
        275                 280                 285
Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr Gln Asp Cys
    290                 295                 300
Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp
305                 310                 315                 320
Asp Met Met Met Asn Trp Ser Pro Thr Val Ala Leu Val Met Ala Gln
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Leu Leu Arg Val Pro Gln Ala Ile Leu Asp Met Ile Ala Gly Ala His
            340                 345                 350
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Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met Val Gly Asn Trp
355 360 365
Ala Lys Val Leu Val Val Leu Leu Leu Phe Ala Gly Val Asp Ala Gln
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Thr Tyr Val Thr Gly Gly Ser Ala Ala Arg Gly Ala Ser Gly Leu Ala
385 390 395 400
Asn Leu Phe Thr Pro Gly Ala Lys Gln Asp Ile Gln Leu Ile Asn Thr
405 410 415
Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Ala Ser
420 425 430
Leu Asp Thr Gly Trp Val Ala Gly Leu Phe Tyr Tyr His Lys Phe Asn
435 440 445
Ser Ser Gly Cys Pro Glu Arg Met Ala Ser Cys Lys Pro Leu Ala Asp
450 455 460
Phe Asp Gln Gly Trp Gly Pro Ile Arg His Ala Asn Gly Ser Gly Pro
465 470 475 480
Glu His Arg Pro Tyr Cys Trp His Tyr Pro Pro Lys Pro Cys Gly Ile
485 490 495
Val Ser Ala Gln Thr Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser
500 505 510
Pro Val Val Val Gly Thr Thr Asn Arg Leu Gly Val Pro Thr Tyr Ser
515 520 525
Trp Gly Thr Asn Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro
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Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Ser Gly Phe
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Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly Gly Val Gly Asn
565 570 575
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580 585 590
Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys Leu
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Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Tyr
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675 680 685
Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly
690 695 700
Val Gly Ser Ser Ile Val Ser Trp Ala Ile Lys Trp Glu Tyr Val Ile
705 710 715 720
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Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn Leu Val
740 745 750
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755 760 765
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770 775 780
Gly Val Ala Tyr Ala Phe Tyr Gly Met Trp Pro Leu Leu Leu Leu Leu
785 790 795 800

Leu Ala Leu Pro Gln Arg Ala Tyr Ala Leu Asp Thr Glu Met Ala Ala
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Ser Cys Gly Gly Val Val Leu Val Gly Leu Met Ala Leu Thr Leu Ser
820 825 830
Pro Tyr Tyr Lys Ser Tyr Ile Ser Trp Cys Leu Trp Trp Leu Gln Tyr
835 840 845
Phe Leu Thr Arg Ile Glu Ala Leu Leu His Val Trp Val Pro Pro Leu
850 855 860
Asn Val Arg Gly Gly Arg Asp Ala Val Ile Leu Leu Thr Cys Val Val
865 870 875 880
His Pro Thr Leu Val Phe Asp Ile Thr Lys Leu Leu Leu Ala Val Phe
885 890 895
Gly Pro Leu Trp Ile Leu Gln Thr Ser Leu Leu Lys Val Pro Tyr Phe
900 905 910
Val Arg Val Gln Gly Leu Leu Arg Ala Cys Ala Leu Ala Arg Lys Val
915 920 925
Ala Gly Gly His Tyr Val Gln Met Val Ile Ile Lys Leu Gly Ala Leu
930 935 940
Thr Gly Thr Tyr Ile Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala
945 950 955 960
His Asn Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe
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Ser Gln Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala
980 985 990
Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly Gln
995 1000 1005
Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp
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Arg Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly
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Leu Leu Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn
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Gln Val Glu Gly Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr
1055 1060 1065
Phe Leu Ala Thr Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His
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Gly Ala Gly Thr Arg Thr Ile Ala Ser Pro Lys Gly Pro Val Ile
1085 1090 1095
Gln Met Tyr Thr Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala
1100 1105 1110
Pro Gln Gly Ala Arg Ser Leu Thr Pro Cys Thr Cys Gly Ser Ser
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Asp Leu Tyr Leu Val Thr Arg His Ala Asp Val Ile Pro Val Arg
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Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu Ser Pro Arg Pro Ile
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Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu Leu Cys Pro Ala
1160 1165 1170
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Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu Glu Thr
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Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Ala
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Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly
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Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly
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Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly
1250 1255 1260
Phe Gly Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile
1265 1270 1275
Arg Thr Gly Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr
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Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly
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Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ala
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Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala Thr Pro Pro Gly
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Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val Ala Leu Ser
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1370 1375 1380
Ala Ile Lys Gly Gly Arg His Leu Ile Phe Cys His Ser Lys Lys
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Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala Met Gly Val Asn
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Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly
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Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val
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Pro Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys
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Glu Cys Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala
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Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr
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Gly Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr Lys Gln
1565 1570 1575
Ser Gly Glu Asn Leu Pro Tyr Leu Val Ala Tyr Gln Ala Thr Val
1580 1585 1590
Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp Asp Gln Met Trp
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Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly Pro Thr Pro
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Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val Thr Leu Thr
1625 1630 1635
His Pro Ile Thr Lys Tyr Ile Met Thr Cys Met Ser Ala Asp Leu
1640 1645 1650

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Glu Val  Val Thr Ser Thr Trp  Val Leu Val Gly Gly  Val Leu Ala
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Ala Leu  Ala Ala Tyr Cys Leu  Ser Thr Gly Cys Val  Val Ile Val
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Gly Arg  Ile Val Leu Ser Gly  Lys Pro Ala Ile Ile  Pro Asp Arg
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Glu Val  Leu Tyr Arg Glu Phe  Asp Glu Met Glu Glu  Cys Ser Gln
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His Leu  Pro Tyr Ile Glu Gln  Gly Met Met Leu Ala  Glu Gln Phe
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Lys Gln  Lys Ala Leu Gly Leu  Leu Gln Thr Ala Ser  Arg Gln Ala
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Glu Val  Ile Ala Pro Thr Val  Gln Thr Asn Trp Gln  Arg Leu Glu
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Ala Phe  Trp Ala Lys His Met  Trp Asn Phe Ile Ser  Gly Ile Gln
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Tyr Leu  Ala Gly Leu Ser Thr  Leu Pro Gly Asn Pro  Ala Ile Ala
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Ser Leu  Met Ala Phe Thr Ala  Ala Val Thr Ser Pro  Leu Thr Thr
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Gly Gln  Thr Leu Leu Phe Asn  Ile Leu Gly Gly Trp  Val Ala Ala
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Gln Leu  Ala Ala Pro Gly Ala  Ala Thr Ala Phe Val  Gly Ala Gly
    1820                 1825                 1830
Leu Ala  Gly Ala Ala Ile Gly  Ser Val Gly Leu Gly  Lys Val Leu
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Val Asp  Ile Leu Ala Gly Tyr  Gly Ala Gly Val Ala  Gly Ala Leu
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Val Ala  Phe Lys Ile Met Ser  Gly Glu Val Pro Ser  Thr Glu Asp
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Leu Val  Asn Leu Leu Pro Ala  Ile Leu Ser Pro Gly  Ala Leu Val
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Val Gly  Val Val Cys Ala Ala  Ile Leu Arg Arg His  Val Gly Pro
    1895                 1900                 1905
Gly Glu  Gly Ala Val Gln Trp  Met Asn Arg Leu Ile  Ala Phe Ala
    1910                 1915                 1920
Ser Arg  Gly Asn His Val Ser  Pro Thr His Tyr Val  Pro Glu Ser
    1925                 1930                 1935
Asp Ala  Ala Ala Arg Val Thr  Ala Ile Leu Ser Ser  Leu Thr Val
    1940                 1945                 1950
Thr Gln  Leu Leu Arg Arg Leu  His Gln Trp Leu Ser  Ser Glu Cys
    1955                 1960                 1965
Thr Thr  Pro Cys Ser Gly Ser  Trp Leu Arg Asp Ile  Trp Asp Trp
    1970                 1975                 1980
Ile Cys  Glu Val Leu Ser Asp  Phe Lys Thr Trp Leu  Lys Ala Lys
    1985                 1990                 1995
Leu Met  Pro Gln Leu Pro Gly  Ile Pro Phe Val Ser  Cys Gln Arg
    2000                 2005                 2010
Gly Tyr  Arg Gly Val Trp Arg  Gly Asp Gly Ile Met  His Thr Arg
    2015                 2020                 2025
Cys Pro  Cys Gly Ala Glu Ile  Ala Gly His Val Lys  Asn Gly Thr
    2030                 2035                 2040
Met Arg  Ile Val Gly Pro Lys  Thr Cys Arg Asn Thr  Trp Ser Gly
    2045                 2050                 2055
Thr Phe  Pro Ile Asn Ala Tyr  Thr Thr Gly Pro Cys  Thr Pro Leu
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Pro Glu Phe Phe Thr Glu Leu Asp Gly Val Arg Leu His Arg Phe
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Ala Pro Pro Cys Lys Pro Leu Leu Arg Asp Glu Val Ser Phe Arg
2135 2140 2145
Val Gly Leu His Asp Tyr Pro Val Gly Ser Gln Leu Pro Cys Glu
2150 2155 2160
Pro Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro
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Ser His Ile Thr Ala Glu Ala Ala Gly Arg Arg Leu Ala Arg Gly
2180 2185 2190
Ser Pro Pro Ser Leu Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala
2195 2200 2205
Pro Ser Leu Lys Ala Thr Cys Thr Thr Asn His Asp Ser Pro Asp
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Gly Asn Ile Thr Arg Val Glu Ser Glu Asn Lys Val Val Val Leu
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Asp Ser Phe Asp Pro Leu Val Ala Glu Glu Asp Glu Arg Glu Ile
2255 2260 2265
Ser Val Ala Ala Glu Ile Leu Arg Lys Ser Arg Arg Phe Ala Pro
2270 2275 2280
Ala Leu Pro Ile Trp Ala Arg Pro Asp Tyr Asn Pro Pro Leu Leu
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Glu Thr Trp Lys Lys Pro Asp Tyr Glu Pro Pro Val Val His Gly
2300 2305 2310
Cys Pro Leu Pro Pro Pro Lys Ser Pro Pro Val Pro Pro Pro Arg
2315 2320 2325
Lys Lys Arg Thr Val Val Leu Thr Glu Ser Thr Val Ser Thr Ala
2330 2335 2340
Leu Ala Glu Leu Ala Thr Lys Ser Phe Gly Ser Ser Ser Thr Ser
2345 2350 2355
Gly Ile Thr Gly Asp Asn Thr Thr Ala Ser Ser Glu Pro Ala Pro
2360 2365 2370
Ser Val Cys Pro Pro Asp Ser Asp Ala Glu Ser Tyr Ser Ser Met
2375 2380 2385
Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly
2390 2395 2400
Ser Trp Ser Thr Val Ser Ser Gly Ala Gly Thr Glu Asp Val Val
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Cys Cys Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Ile Thr Pro
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Cys Ala Ala Glu Glu Gln Lys Leu Pro Ile Asn Ala Leu Ser Asn
2435 2440 2445
Ser Leu Leu Arg His His Asn Leu Val Tyr Ser Thr Thr Ser Arg
2450 2455 2460
Ser Ala Cys Gln Arg Gln Lys Lys Val Thr Phe Asp Arg Leu Gln
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Val Leu Asp Asn His Tyr Gln Asp Val Leu Lys Glu Val Lys Ala
2480 2485 2490

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Cys Ser  Leu Thr Pro Pro His  Ser Ala Arg Ser Lys  Phe Gly Tyr
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Gly Ala  Lys Asp Val Arg Cys  His Ala Arg Lys Ala  Val Asn His
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Ile Asn  Ser Val Trp Lys Asp  Leu Leu Glu Asp Ser  Val Thr Pro
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Ile Asp  Thr Thr Ile Met Ala  Lys Asn Glu Val Phe  Cys Val Gln
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Pro Glu  Lys Gly Gly Arg Lys  Pro Ala Arg Leu Ile  Val Tyr Pro
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Asp Leu  Gly Val Arg Val Cys  Glu Lys Met Ala Leu  Tyr Asp Val
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Val Lys  Lys Leu Pro Leu Ala  Val Met Gly Ser Ser  Tyr Gly Phe
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Gln Tyr  Ser Pro Gly Gln Arg  Val Glu Phe Leu Val  Gln Ala Trp
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Lys Ser  Lys Gly Thr Pro Met  Gly Phe Ser Tyr Asp  Thr Arg Cys
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Phe Asp  Ser Thr Val Thr Glu  Ser Asp Ile Arg Thr  Glu Glu Ala
    2645                 2650                 2655
Ile Tyr  Gln Cys Cys Asp Leu  Asp Pro Gln Ala Arg  Val Ala Ile
    2660                 2665                 2670
Lys Ser  Leu Thr Glu Arg Leu  Tyr Val Gly Gly Pro  Leu Thr Asn
    2675                 2680                 2685
Ser Arg  Gly Glu Asn Cys Gly  Tyr Arg Arg Cys Arg  Ala Ser Gly
    2690                 2695                 2700
Val Leu  Thr Thr Ser Cys Gly  Asn Thr Leu Thr Cys  Tyr Ile Lys
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Ala Arg  Ala Ala Cys Arg Ala  Ala Gly Leu Arg Asp  Cys Thr Met
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Leu Val  Cys Gly Asp Asp Leu  Val Val Ile Cys Glu  Ser Gln Gly
    2735                 2740                 2745
Val Gln  Glu Asp Thr Ala Ser  Leu Arg Ala Phe Thr  Glu Ala Met
    2750                 2755                 2760
Thr Arg  Tyr Ser Ala Pro Pro  Gly Asp Pro Pro Gln  Pro Glu Tyr
    2765                 2770                 2775
Asp Leu  Glu Leu Ile Thr Ser  Cys Ser Ser Asn Val  Ser Val Ala
    2780                 2785                 2790
His Asp  Asp Thr Gly Lys Arg  Val Tyr Tyr Leu Thr  Arg Asp Pro
    2795                 2800                 2805
Thr Thr  Pro Leu Ala Arg Ala  Ala Trp Glu Thr Ala  Arg His Thr
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Pro Val  Asn Ser Trp Leu Gly  Asn Ile Ile Met Phe  Ala Pro Thr
    2825                 2830                 2835
Leu Trp  Val Arg Met Ile Leu  Leu Pro His Phe Phe  Ser Val Leu
    2840                 2845                 2850
Met Ala  Arg Asp Gln Leu Glu  Gln Ala Leu Asp Cys  Glu Ile Tyr
    2855                 2860                 2865
Gly Ala  Cys Tyr Ser Ile Glu  Pro Leu Asp Leu Pro  Pro Ile Ile
    2870                 2875                 2880
Gln Arg  Leu His Gly Leu Ser  Ala Phe Ser Leu His  Ser Tyr Ser
    2885                 2890                 2895
Pro Gly  Glu Ile Asn Arg Val  Ala Ala Cys Leu Arg  Lys Leu Gly
    2900                 2905                 2910
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Val Pro  Pro Leu Arg Ala Trp  Arg His Arg Ala Arg  Ser Ile Arg
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Ala Lys  Leu Leu Ser Arg Gly  Gly Arg Ala Ala Thr  Cys Gly Lys
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Tyr Leu  Phe Asn Trp Ala Val  Arg Thr Lys Leu Lys  Leu Thr Pro
    2945                 2950                 2955
Ile Ala  Ala Ala Ser Gln Leu  Asp Leu Ser Gly Trp  Phe Thr Ala
    2960                 2965                 2970
Gly Tyr  Ser Gly Gly Asp Ile  Tyr His Ser Val Ser  Arg Ala Arg
    2975                 2980                 2985
Pro Arg  Trp Phe Trp Phe Cys  Leu Leu Leu Leu Ala  Ala Gly Val
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Gly Ile  Tyr Leu Leu Pro Asn  Arg
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ccccccctccc gggagagcca tagtggtctg cggaaccggt gagtacaccg gaattgccag    180
gacgaccggg tcctttcttg gatcaacccg ctcaatgcct ggagatttgg gcgtgccccc    240
gcaagactgc tagccgagta gtgttgggtc gcgaaaggcc ttgtggtact gcctgatagg    300

gtgcttgcga gtgccccggg aggtctcgta gaccgtgcac c atg agc acg aat cct    356
                                              Met Ser Thr Asn Pro
                                              1               5

aaa cct caa aga aaa acc aaa cgt aac acc aac atg atc gaa caa gat      404
Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Met Ile Glu Gln Asp
                10                  15                  20

gga ttg cac gca ggt tct ccg gcc gct tgg gtg gag agg cta ttc ggc      452
Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val Glu Arg Leu Phe Gly
            25                  30                  35

tat gac tgg gca caa cag aca atc ggc tgc tct gat gcc gcc gtg ttc      500
Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser Asp Ala Ala Val Phe
        40                  45                  50
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cgg ctg tca gcg cag ggg cgc ccg gtt ctt ttt gtc aag acc gac ctg      548
Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe Val Lys Thr Asp Leu
    55                  60                  65

tcc ggt gcc ctg aat gaa ctg cag gac gag gca gcg cgg cta tcg tgg      596
Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala Ala Arg Leu Ser Trp
70                  75                  80                  85

ctg gcc acg acg ggc gtt cct tgc gca gct gtg ctc gac gtt gtc act      644
Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val Leu Asp Val Val Thr
                90                  95                  100

gaa gcg gga agg gac tgg ctg cta ttg ggc gaa gtg ccg ggg cag gat      692
Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu Val Pro Gly Gln Asp
            105                 110                 115

ctc ctg tca tct cac ctt gct cct gcc gag aaa gta tcc atc atg gct      740
Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys Val Ser Ile Met Ala
        120                 125                 130

gat gca atg cgg cgg ctg cat acg ctt gat ccg gct acc tgc cca ttc      788
Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro Ala Thr Cys Pro Phe
    135                 140                 145

gac cac caa gcg aaa cat cgc atc gag cga gca cgt act cgg atg gaa      836
Asp His Gln Ala Lys His Arg Ile Glu Arg Ala Arg Thr Arg Met Glu
150                 155                 160                 165

gcc ggt ctt gtc gat cag gat gat ctg gac gaa gag cat cag ggg ctc      884
Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu Glu His Gln Gly Leu
                170                 175                 180

gcg cca gcc gaa ctg ttc gcc agg ctc aag gcg cgc atg ccc gac ggc      932
Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala Arg Met Pro Asp Gly
            185                 190                 195

gag gat ctc gtc gtg acc cat ggc gat gcc tgc ttg ccg aat atc atg      980
Glu Asp Leu Val Val Thr His Gly Asp Ala Cys Leu Pro Asn Ile Met
        200                 205                 210

gtg gaa aat ggc cgc ttt tct gga ttc atc gac tgt ggc cgg ctg ggt     1028
Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp Cys Gly Arg Leu Gly
    215                 220                 225

gtg gcg gac cgc tat cag gac ata gcg ttg gct acc cgt gat att gct     1076
Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala Thr Arg Asp Ile Ala
230                 235                 240                 245

gaa gag ctt ggc ggc gaa tgg gct gac cgc ttc ctc gtg ctt tac ggt     1124
Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe Leu Val Leu Tyr Gly
                250                 255                 260

atc gcc gct ccc gat tcg cag cgc atc gcc ttc tat cgc ctt ctt gac     1172
Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe Tyr Arg Leu Leu Asp
            265                 270                 275
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gag ttc ttc tgagtttaaa ccctctccct ccccccccccc taacgttact              1221
Glu Phe Phe
        280

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ttgccgtctt ttggcaatgt gagggcccgg aaacctggcc ctgtcttctt gacgagcatt   1341
cctaggggtc tttcccctct cgccaaagga atgcaaggtc tgttgaatgt cgtgaaggaa   1401
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tgtatgggat ctgatctggg gcctcggtgc acatgcttta catgtgttta gtcgaggtta   1701
aaaaacgtct aggccccccg aaccacgggg acgtggtttt cctttgaaaa acacgataat   1761

acc atg gcg ccc atc acg gcg tat gcc cag cag aca agg ggc ctc ctg      1809
    Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu
                    285                 290                 295

gga tgc ata att act agc ctg acc ggc cgg gac aaa aac cag gtg gag      1857
Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu
                300                 305                 310

ggt gag gtc cag att gtg tca act gct gcc cag acc ttc ctg gca acc      1905
Gly Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr
            315                 320                 325

tgc atc aac gga gtg tgc tgg act gtc tac cac ggg gcc gga aca agg      1953
Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg
        330                 335                 340

acc atc gcg tca ccc aaa ggt ccc gtc atc cag atg tat act aat gta      2001
Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val
    345                 350                 355

gac caa gac ctt gta ggc tgg ccc gct ccc caa ggt gcc cgc tca ttg      2049
Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu
360                 365                 370                 375

aca ccc tgc act tgc ggc tcc tcg gac ctt tac ttg gtc acg agg cac      2097
Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His
                380                 385                 390

gcc gat gtc att ccc gtg cgc cgg cgg ggt gat agc agg ggc agc ctg      2145
Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu
            395                 400                 405

ctc tcg ccc cgg cct atc tct tac ttg aaa ggc tct tcg ggg ggc cca      2193
Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro
        410                 415                 420

ttg ctg tgc ccc gcg gga cac gcc gta ggc ata ttc agg gcc gcg gtg      2241
Leu Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val
    425                 430                 435
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tgc acc cgt gga gtg gct aag gcg gtg gac ttt atc ccc gta gag agc      2289
Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser
440                 445                 450                 455

cta gag aca acc atg agg tcc ccg gtg ttc aca gac aac tcc tcc cca      2337
Leu Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro
                460                 465                 470

cca gca gtg ccc cag agc ttc cag gtg gcc cac ctg cat gct ccc acc      2385
Pro Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr
            475                 480                 485

ggc agc ggt aag agt acc aag gtc ccg gcc gca tac gcg gct cag ggc      2433
Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly
        490                 495                 500

tac aag gtg ctg gtg ctc aac ccc tcc gtt gct gca aca ctg ggc ttt      2481
Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe
    505                 510                 515

ggt gct tat atg tcc aag gcc cat ggg att gat cct aac atc agg act      2529
Gly Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr
520                 525                 530                 535

ggg gtg agg aca atc act act ggc agc ccc atc acg tac tcc acc tac      2577
Gly Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr
                540                 545                 550

ggc aag ttc ctt gct gat ggc ggg tgc tcg ggg ggt gct tat gac ata      2625
Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile
            555                 560                 565

ata att tgt gac gag tgc cac tct acg gat gca aca tcc gtc ttg ggc      2673
Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Val Leu Gly
        570                 575                 580

atc ggc act gtc ctt gac caa gca gag act gcg ggg gcg aga ctg gtc      2721
Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val
    585                 590                 595

gtg ctc gcc acc gct acc cct ccg ggc tct gtc act gtg ccc cat cct      2769
Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro
600                 605                 610                 615

aac atc gag gag gtt gct ctg tcc acc acc gga gag atc ccc ttt tac      2817
Asn Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr
                620                 625                 630

ggc aag gct atc ccc ctt gag gca atc aag ggg ggg aga cat ctc atc      2865
Gly Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile
            635                 640                 645

ttc tgc cat tca aaa aag aag tgc gac gag ctc gct gca aag ctg gtc      2913
Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val
        650                 655                 660
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gcg atg ggc gtc aat gcc gtg gct tac tac cgc ggc ctc gac gtg tcc     2961
Ala Met Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser
    665                 670                 675

gtc atc cca acc agt ggc gat gtt gtc gtc gtg gca act gat gcc ctc     3009
Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu
680                 685                 690                 695

atg acc ggc tat acc ggc gac ttt gac tcg gtg ata gac tgc aac acg     3057
Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr
                700                 705                 710

tgt gtc acc cag aca gtc gac ttc agc ctt gac cct acc ttc acc att     3105
Cys Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile
            715                 720                 725

gag aca acc acg ctc ccc cag gac gct gtc tcc cgc act caa cgt cgg     3153
Glu Thr Thr Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg
        730                 735                 740

ggc agg act ggc agg ggg aag cca ggc atc tac aga ttt gtg gca ccg     3201
Gly Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro
    745                 750                 755

ggg gag cgc ccc tcc ggc atg ttt gac tcg tcc gtc ctc tgt gag tgc     3249
Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys
760                 765                 770                 775

tat gac gcg ggc tgt gct tgg tat gag ctc aca ccc gcc gag acc aca     3297
Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr
                780                 785                 790

gtt agg cta cga gca tat atg aac acc ccg ggg ctc ccc gtg tgc caa     3345
Val Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln
            795                 800                 805

gac cat ctt gaa ttt tgg gag ggc gtc ttc acg ggt ctc acc cat ata     3393
Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile
        810                 815                 820

gac gcc cat ttc cta tcc cag aca aag cag agt ggg gaa aac ctt cct     3441
Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro
    825                 830                 835

tac ctg gta gcg tac caa gcc acc gtg tgc gct agg gct cag gcc ccc     3489
Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro
840                 845                 850                 855

ccc cca tcg tgg gac cag atg tgg aag tgc ttg atc cgc ctc aag ccc     3537
Pro Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro
                860                 865                 870

acc ctt cat ggg cca aca cct ctg cta tac aga ctg ggc gct gtt cag     3585
Thr Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln
            875                 880                 885
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aat gaa gtc acc ctg acg cac cca atc acc aag tac atc atg aca tgc     3633
Asn Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys
        890                 895                 900

atg tcg gct gac ctg gag gtc gtc acg agt acc tgg gtg ctc gtc ggc     3681
Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly
    905                 910                 915

ggc gtc ctg gct gct ttg gcc gcg tat tgc cta tcc aca ggc tgc gtg     3729
Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val
920                 925                 930                 935

gtc ata gta ggc agg att gtc ttg tcc ggg aag ccg gct atc ata cct     3777
Val Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro
                940                 945                 950

gac agg gaa gtc ctc tac cgg gag ttc gat gag atg gaa gag tgc tct     3825
Asp Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ser
            955                 960                 965

cag cac ttg ccg tac atc gag cag ggg atg atg ctc gcc gag cag ttc     3873
Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe
        970                 975                 980

aag cag aag gcc ctc ggc ctc ctg cag acc gcg tcc cgc cag gca gag     3921
Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg Gln Ala Glu
    985                 990                 995

gtc  atc gcc cct acc gtc  caa acc aac tgg cag  aga ctc gag gcc      3966
Val  Ile Ala Pro Thr Val  Gln Thr Asn Trp Gln  Arg Leu Glu Ala
1000                1005                1010

ttc  tgg gcg aag cat atg  tgg aac ttc atc agt  ggg ata caa tat      4011
Phe  Trp Ala Lys His Met  Trp Asn Phe Ile Ser  Gly Ile Gln Tyr
1015                1020                1025

ctg  gcg ggc ctg tca acg  ttg cct ggt aat ccc  gcc att gca tca      4056
Leu  Ala Gly Leu Ser Thr  Leu Pro Gly Asn Pro  Ala Ile Ala Ser
1030                1035                1040

ttg  atg gct ttt aca gct  gcc gtc acc agc cca  cta acc acc ggc      4101
Leu  Met Ala Phe Thr Ala  Ala Val Thr Ser Pro  Leu Thr Thr Gly
1045                1050                1055

caa  act ctc ctc ttc aac  att ttg ggg ggg tgg  gtg gct gcc cag      4146
Gln  Thr Leu Leu Phe Asn  Ile Leu Gly Gly Trp  Val Ala Ala Gln
1060                1065                1070

ctc  gca gcc ccc ggt gcc  gct acc gcc ttt gtg  ggc gct ggc tta      4191
Leu  Ala Ala Pro Gly Ala  Ala Thr Ala Phe Val  Gly Ala Gly Leu
1075                1080                1085

gcc  ggc gcc gcc atc ggc  agt gtt gga ctg ggg  aag gtc ctc gtg      4236
Ala  Gly Ala Ala Ile Gly  Ser Val Gly Leu Gly  Lys Val Leu Val
1090                1095                1100
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gac  atc ctt gca ggg tat  ggc gcg ggc gtg gcg  gga gct ctt gta      4281
Asp  Ile Leu Ala Gly Tyr  Gly Ala Gly Val Ala  Gly Ala Leu Val
1105                 1110                 1115

gca  ttt aag atc atg agc  ggt gag gtt ccc tcc  aca gag gac ctg      4326
Ala  Phe Lys Ile Met Ser  Gly Glu Val Pro Ser  Thr Glu Asp Leu
1120                 1125                 1130

gtc  aat cta ctg cct gcc  atc ctt tcg ccc gga  gcc ctt gta gtc      4371
Val  Asn Leu Leu Pro Ala  Ile Leu Ser Pro Gly  Ala Leu Val Val
1135                 1140                 1145

ggt  gtg gtc tgc gca gca  ata cta cgc cgg cac  gtt ggc ccg ggc      4416
Gly  Val Val Cys Ala Ala  Ile Leu Arg Arg His  Val Gly Pro Gly
1150                 1155                 1160

gag  gga gca gtg cag tgg  atg aac cgg ttg ata  gcc ttc gcc tcc      4461
Glu  Gly Ala Val Gln Trp  Met Asn Arg Leu Ile  Ala Phe Ala Ser
1165                 1170                 1175

cgg  ggg aac cac gtt tcc  ccc acg cac tac gtg  ccg gag agc gat      4506
Arg  Gly Asn His Val Ser  Pro Thr His Tyr Val  Pro Glu Ser Asp
1180                 1185                 1190

gca  gct gcc cgc gtc act  gcc ata ctc agc agc  ctc act gtg acc      4551
Ala  Ala Ala Arg Val Thr  Ala Ile Leu Ser Ser  Leu Thr Val Thr
1195                 1200                 1205

cag  ctc ctg agg cga cta  cac cag tgg cta agc  tcg gag tgt acc      4596
Gln  Leu Leu Arg Arg Leu  His Gln Trp Leu Ser  Ser Glu Cys Thr
1210                 1215                 1220

act  cca tgc tcc ggt tcc  tgg cta agg gac atc  tgg gac tgg ata      4641
Thr  Pro Cys Ser Gly Ser  Trp Leu Arg Asp Ile  Trp Asp Trp Ile
1225                 1230                 1235

tgc  gag gtg ctg agc gat  ttt aag acc tgg ctg  aag gcc aag ctc      4686
Cys  Glu Val Leu Ser Asp  Phe Lys Thr Trp Leu  Lys Ala Lys Leu
1240                 1245                 1250

atg  cca caa ctg cct ggg  att ccc ttt gtg tcc  tgc caa cgc ggg      4731
Met  Pro Gln Leu Pro Gly  Ile Pro Phe Val Ser  Cys Gln Arg Gly
1255                 1260                 1265

tac  agg ggg gtc tgg cga  gga gat ggc att atg  cac act cgc tgc      4776
Tyr  Arg Gly Val Trp Arg  Gly Asp Gly Ile Met  His Thr Arg Cys
1270                 1275                 1280

ccc  tgt gga gct gag atc  gcc gga cat gtc aag  aac ggg acg atg      4821
Pro  Cys Gly Ala Glu Ile  Ala Gly His Val Lys  Asn Gly Thr Met
1285                 1290                 1295

agg  atc gtc ggt cct aag  acc tgc aga aac acg  tgg agt ggg acc      4866
Arg  Ile Val Gly Pro Lys  Thr Cys Arg Asn Thr  Trp Ser Gly Thr
1300                 1305                 1310
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ttc  ccc atc aac gcc tac  acc acg ggc ccc tgt  acc ccc ctt cct      4911
Phe  Pro Ile Asn Ala Tyr  Thr Thr Gly Pro Cys  Thr Pro Leu Pro
1315                 1320                 1325

gcg  ccg aac tat acg ttc  gcg ctg tgg agg gtg  tct gcg gag gaa      4956
Ala  Pro Asn Tyr Thr Phe  Ala Leu Trp Arg Val  Ser Ala Glu Glu
1330                 1335                 1340

tac  gtg gaa ata agg cag  gtg ggg gac ttc cac  tac gtg acg ggc      5001
Tyr  Val Glu Ile Arg Gln  Val Gly Asp Phe His  Tyr Val Thr Gly
1345                 1350                 1355

atg  act gct gac aac ctt  aag tgc cca tgc cag  gtc cca tcg ccc      5046
Met  Thr Ala Asp Asn Leu  Lys Cys Pro Cys Gln  Val Pro Ser Pro
1360                 1365                 1370

gaa  ttt ttc aca gaa ctg  gat ggg gtg cgc ctg  cat agg ttt gcg      5091
Glu  Phe Phe Thr Glu Leu  Asp Gly Val Arg Leu  His Arg Phe Ala
1375                 1380                 1385

ccc  cct tgc aag ccc ttg  cta cga gat gag gtg  tcg ttc aga gta      5136
Pro  Pro Cys Lys Pro Leu  Leu Arg Asp Glu Val  Ser Phe Arg Val
1390                 1395                 1400

gga  cta cac gac tac ccg  gtg ggg tcg cag tta  cct tgc gag cct      5181
Gly  Leu His Asp Tyr Pro  Val Gly Ser Gln Leu  Pro Cys Glu Pro
1405                 1410                 1415

gaa  ccg gat gtg gcc gta  ctg acg tcc atg ctc  acc gat ccc tcc      5226
Glu  Pro Asp Val Ala Val  Leu Thr Ser Met Leu  Thr Asp Pro Ser
1420                 1425                 1430

cat  ata acg gca gag gcg  gct ggg agg agg tta  gca agg gga tcg      5271
His  Ile Thr Ala Glu Ala  Ala Gly Arg Arg Leu  Ala Arg Gly Ser
1435                 1440                 1445

ccc  cct tct ctg gcc agc  tcc tcg gcc agc cag  ctg tcc gct cca      5316
Pro  Pro Ser Leu Ala Ser  Ser Ser Ala Ser Gln  Leu Ser Ala Pro
1450                 1455                 1460

tct  ctc aaa gca act tgc  acc acc aac cac gac  tcc cct gac gcc      5361
Ser  Leu Lys Ala Thr Cys  Thr Thr Asn His Asp  Ser Pro Asp Ala
1465                 1470                 1475

gag  ctc ata gag gct aac  ctc ctg tgg agg cag  gag atg ggc ggc      5406
Glu  Leu Ile Glu Ala Asn  Leu Leu Trp Arg Gln  Glu Met Gly Gly
1480                 1485                 1490

aac  atc acc agg gtt gag  tca gag aac aaa gtg  gta gtc ctg gac      5451
Asn  Ile Thr Arg Val Glu  Ser Glu Asn Lys Val  Val Val Leu Asp
1495                 1500                 1505

tcc  ttc gat ccg ctt gtg  gca gaa gag gac gaa  cgg gag atc tcc      5496
Ser  Phe Asp Pro Leu Val  Ala Glu Glu Asp Glu  Arg Glu Ile Ser
1510                 1515                 1520
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gtg  gcc gca gag atc ctg  cgg aag tct cgg aga  ttc gct ccg gcc      5541
Val  Ala Ala Glu Ile Leu  Arg Lys Ser Arg Arg  Phe Ala Pro Ala
1525                 1530                 1535

ctg  ccc att tgg gca cgg  ccg gac tac aac ccc  ccg tta ctg gag      5586
Leu  Pro Ile Trp Ala Arg  Pro Asp Tyr Asn Pro  Pro Leu Leu Glu
1540                 1545                 1550

acg  tgg aaa aag ccg gac  tac gag cca cct gtg  gtc cat ggc tgc      5631
Thr  Trp Lys Lys Pro Asp  Tyr Glu Pro Pro Val  Val His Gly Cys
1555                 1560                 1565

ccg  ctt cca cct cca aag  tcc cct cct gtg cct  ccg ccc cgg aag      5676
Pro  Leu Pro Pro Pro Lys  Ser Pro Pro Val Pro  Pro Pro Arg Lys
1570                 1575                 1580

aag  cgg acg gtg gtc ctc  act gaa tca act gta  tcc act gcc ttg      5721
Lys  Arg Thr Val Val Leu  Thr Glu Ser Thr Val  Ser Thr Ala Leu
1585                 1590                 1595

gct  gag ctt gct acc aag  agc ttt ggc agc tct  tca act tcc ggt      5766
Ala  Glu Leu Ala Thr Lys  Ser Phe Gly Ser Ser  Ser Thr Ser Gly
1600                 1605                 1610

ata  acg ggc gac aac acg  aca gcg tcc tct gag  ccc gcc ccc tct      5811
Ile  Thr Gly Asp Asn Thr  Thr Ala Ser Ser Glu  Pro Ala Pro Ser
1615                 1620                 1625

gtc  tgc cct cca gac tcc  gac gct gag tcc tat  tct tcc atg ccc      5856
Val  Cys Pro Pro Asp Ser  Asp Ala Glu Ser Tyr  Ser Ser Met Pro
1630                 1635                 1640

ccc  ctg gag ggg gag cct  ggg gat ccg gat ctc  agc gac ggg tca      5901
Pro  Leu Glu Gly Glu Pro  Gly Asp Pro Asp Leu  Ser Asp Gly Ser
1645                 1650                 1655

tgg  tcg acg gtc agt agt  ggg gct ggc acg gag  gat gtc gtg tgt      5946
Trp  Ser Thr Val Ser Ser  Gly Ala Gly Thr Glu  Asp Val Val Cys
1660                 1665                 1670

tgc  tca atg tcc tat tcc  tgg aca ggc gca ctc  atc acc ccg tgt      5991
Cys  Ser Met Ser Tyr Ser  Trp Thr Gly Ala Leu  Ile Thr Pro Cys
1675                 1680                 1685

gcc  gcg gaa gaa caa aaa  ttg cct atc aac gca  ctg agc aac tca      6036
Ala  Ala Glu Glu Gln Lys  Leu Pro Ile Asn Ala  Leu Ser Asn Ser
1690                 1695                 1700

tta  ctg cgt cac cac aac  ctc gtg tat tcc acc  acc tca cgc agt      6081
Leu  Leu Arg His His Asn  Leu Val Tyr Ser Thr  Thr Ser Arg Ser
1705                 1710                 1715

gct  tgc caa agg cag aag  aaa gtc aca ttt gac  aga ctg caa gtt      6126
Ala  Cys Gln Arg Gln Lys  Lys Val Thr Phe Asp  Arg Leu Gln Val
1720                 1725                 1730
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ctg  gac aac cac tac cag  gac gtg ctc aag gag  gtt aag gcg gcg      6171
Leu  Asp Asn His Tyr Gln  Asp Val Leu Lys Glu  Val Lys Ala Ala
1735                 1740                 1745

gcg  tca aaa gtg aag gct  aac ttg cta tcc gta  gag gaa gct tgc      6216
Ala  Ser Lys Val Lys Ala  Asn Leu Leu Ser Val  Glu Glu Ala Cys
1750                 1755                 1760

agc  ctg acg ccc cca cat  tca gcc aga tca aaa  ttt ggc tat ggg      6261
Ser  Leu Thr Pro Pro His  Ser Ala Arg Ser Lys  Phe Gly Tyr Gly
1765                 1770                 1775

gca  aaa gac gtc cgt tgc  cat gcc aga aag gcc  gta aac cac atc      6306
Ala  Lys Asp Val Arg Cys  His Ala Arg Lys Ala  Val Asn His Ile
1780                 1785                 1790

aac  tcc gtg tgg aaa gac  ctt ctg gaa gac agt  gtt aca cca ata      6351
Asn  Ser Val Trp Lys Asp  Leu Leu Glu Asp Ser  Val Thr Pro Ile
1795                 1800                 1805

gac  aca acc atc atg gct  aag aac gaa gtt ttc  tgc gtt cag cct      6396
Asp  Thr Thr Ile Met Ala  Lys Asn Glu Val Phe  Cys Val Gln Pro
1810                 1815                 1820

gag  aag ggg ggt cgt aag  cca gct cgt ctc atc  gtg tac cct gac      6441
Glu  Lys Gly Gly Arg Lys  Pro Ala Arg Leu Ile  Val Tyr Pro Asp
1825                 1830                 1835

ctg  ggt gtg cgc gtg tgc  gag aaa atg gcc ctg  tac gac gtg gta      6486
Leu  Gly Val Arg Val Cys  Glu Lys Met Ala Leu  Tyr Asp Val Val
1840                 1845                 1850

aaa  aaa ctc ccc ctg gcc  gtg atg gga agc tcc  tac gga ttc cag      6531
Lys  Lys Leu Pro Leu Ala  Val Met Gly Ser Ser  Tyr Gly Phe Gln
1855                 1860                 1865

tac  tca cca gga cag cgg  gtt gaa ttc ctc gtg  caa gcg tgg aag      6576
Tyr  Ser Pro Gly Gln Arg  Val Glu Phe Leu Val  Gln Ala Trp Lys
1870                 1875                 1880

tcc  aag ggg acc cca atg  ggg ttc tcg tat gat  acc cgc tgc ttt      6621
Ser  Lys Gly Thr Pro Met  Gly Phe Ser Tyr Asp  Thr Arg Cys Phe
1885                 1890                 1895

gac  tct aca gtc act gag  agc gat atc cgt acg  gag gag gca atc      6666
Asp  Ser Thr Val Thr Glu  Ser Asp Ile Arg Thr  Glu Glu Ala Ile
1900                 1905                 1910

tac  cag tgt tgt gac ctg  gac ccc caa gcc cgc  gtg gcc atc aag      6711
Tyr  Gln Cys Cys Asp Leu  Asp Pro Gln Ala Arg  Val Ala Ile Lys
1915                 1920                 1925

tcc  ctc acc gag agg ctt  tat gtc ggg ggt cct  ctt acc aat tca      6756
Ser  Leu Thr Glu Arg Leu  Tyr Val Gly Gly Pro  Leu Thr Asn Ser
1930                 1935                 1940
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agg  ggg gaa aac tgc ggc  tat cgc agg tgc cgc  gca agc ggc gta      6801
Arg  Gly Glu Asn Cys Gly  Tyr Arg Arg Cys Arg  Ala Ser Gly Val
1945                 1950                 1955

ctg  aca act agc tgt ggt  aac acc ctc act tgc  tac atc aag gcc      6846
Leu  Thr Thr Ser Cys Gly  Asn Thr Leu Thr Cys  Tyr Ile Lys Ala
1960                 1965                 1970

cga  gca gcc tgt cga gcc  gca ggg ctc cgg gac  tgc acc atg ctc      6891
Arg  Ala Ala Cys Arg Ala  Ala Gly Leu Arg Asp  Cys Thr Met Leu
1975                 1980                 1985

gtg  tgt ggc gac gac tta  gtc gtt atc tgt gaa  agc cag ggg gtc      6936
Val  Cys Gly Asp Asp Leu  Val Val Ile Cys Glu  Ser Gln Gly Val
1990                 1995                 2000

caa  gag gat aca gcg agc  ctg aga gcc ttc acg  gag gct atg acc      6981
Gln  Glu Asp Thr Ala Ser  Leu Arg Ala Phe Thr  Glu Ala Met Thr
2005                 2010                 2015

agg  tac tcc gct ccc ccc  ggg gac ccc ccc caa  cca gaa tac gac      7026
Arg  Tyr Ser Ala Pro Pro  Gly Asp Pro Pro Gln  Pro Glu Tyr Asp
2020                 2025                 2030

ttg  gag ctc ata aca tcg  tgc tcc tct aac gtg  tca gtc gcc cac      7071
Leu  Glu Leu Ile Thr Ser  Cys Ser Ser Asn Val  Ser Val Ala His
2035                 2040                 2045

gac  gac act gga aag agg  gtc tat tac ctt acc  cgt gac cct aca      7116
Asp  Asp Thr Gly Lys Arg  Val Tyr Tyr Leu Thr  Arg Asp Pro Thr
2050                 2055                 2060

act  ccc ctc gcg aga gcc  gcg tgg gag aca gca  aga cac act cca      7161
Thr  Pro Leu Ala Arg Ala  Ala Trp Glu Thr Ala  Arg His Thr Pro
2065                 2070                 2075

gtc  aat tcc tgg cta ggc  aac ata atc atg ttt  gcc ccc aca ttg      7206
Val  Asn Ser Trp Leu Gly  Asn Ile Ile Met Phe  Ala Pro Thr Leu
2080                 2085                 2090

tgg  gtg aga atg ata ctg  ctg ccc cac ttc ttc  agt gtc ctc atg      7251
Trp  Val Arg Met Ile Leu  Leu Pro His Phe Phe  Ser Val Leu Met
2095                 2100                 2105

gcc  agg gac caa ctt gaa  cag gcc ctt gat tgc  gaa atc tac gga      7296
Ala  Arg Asp Gln Leu Glu  Gln Ala Leu Asp Cys  Glu Ile Tyr Gly
2110                 2115                 2120

gcc  tgc tac tcc ata gaa  cca ctg gac cta cct  cca atc att caa      7341
Ala  Cys Tyr Ser Ile Glu  Pro Leu Asp Leu Pro  Pro Ile Ile Gln
2125                 2130                 2135

aga  ctc cat ggc ctt agc  gca ttt tca ctc cac  agt tac tct cca      7386
Arg  Leu His Gly Leu Ser  Ala Phe Ser Leu His  Ser Tyr Ser Pro
2140                 2145                 2150
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ggt  gaa atc aat agg gtg  gcc gca tgc ctc aga  aaa ctt ggg gtc      7431
Gly  Glu Ile Asn Arg Val  Ala Ala Cys Leu Arg  Lys Leu Gly Val
2155                 2160                 2165

ccg  ccc ttg cga gct tgg  aga cac cgg gcc cgg  agc atc cgc gct      7476
Pro  Pro Leu Arg Ala Trp  Arg His Arg Ala Arg  Ser Ile Arg Ala
2170                 2175                 2180

aag  ctt ctg tcc aga gga  ggc agg gct gcc acg  tgt ggc aag tac      7521
Lys  Leu Leu Ser Arg Gly  Gly Arg Ala Ala Thr  Cys Gly Lys Tyr
2185                 2190                 2195

ctc  ttc aat tgg gca gta  aga aca aag ctc aaa  ctc act cca ata      7566
Leu  Phe Asn Trp Ala Val  Arg Thr Lys Leu Lys  Leu Thr Pro Ile
2200                 2205                 2210

gcg  gcc gct agc cag ctg  gac ttg tcc ggt tgg  ttc acg gct ggc      7611
Ala  Ala Ala Ser Gln Leu  Asp Leu Ser Gly Trp  Phe Thr Ala Gly
2215                 2220                 2225

tac  agc ggg gga gac att  tat cac agc gtg tct  cgt gcc cgg ccc      7656
Tyr  Ser Gly Gly Asp Ile  Tyr His Ser Val Ser  Arg Ala Arg Pro
2230                 2235                 2240

cgc  tgg ttc tgg ttt tgc  cta ctc ctg ctt gct  gca ggg gta ggc      7701
Arg  Trp Phe Trp Phe Cys  Leu Leu Leu Leu Ala  Ala Gly Val Gly
2245                 2250                 2255

atc  tac ctc ctc ccc aac  cga tgaaggttgg ggtaaacact ccggcctctt      7752
Ile  Tyr Leu Leu Pro Asn  Arg
2260                 2265

aggccattcc tgttttttt ttttttttct tttgtttttt ttgttttttt ttttttttt    7812
cctttctttt ttttttttt tcctttcttc tttaatggtg gctccatctt agccctagtc    7872
acggctagct gtgaaaggtc cgtgagccgc atgactgcag agagtgctga tactggcctc    7932
tctgcagatc atgt                                                     7946
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<213> Artificial sequence

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<223> Synthetic Construct

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

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

Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
130 135 140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145 150 155 160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
165 170 175
Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro
180 185 190
Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly
195 200 205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
210 215 220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
225 230 235 240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
245 250 255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
260 265 270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
275 280 285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Val Leu Gly Ile
290 295 300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val
305 310 315 320
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
325 330 335
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
340 345 350
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
355 360 365
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
370 375 380
Met Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
385 390 395 400
Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
405 410 415
Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
420 425 430
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
435 440 445
Thr Thr Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
450 455 460
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
465 470 475 480
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
485 490 495
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
500 505 510
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
515 520 525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
530 535 540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545 550 555 560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
565 570 575

Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
580 585 590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
595 600 605
Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys Met
610 615 620
Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
625 630 635 640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
645 650 655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
660 665 670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ser Gln
675 680 685
His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe Lys
690 695 700
Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg Gln Ala Glu Val
705 710 715 720
Ile Ala Pro Thr Val Gln Thr Asn Trp Gln Arg Leu Glu Ala Phe Trp
725 730 735
Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
740 745 750
Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
755 760 765
Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Gly Gln Thr Leu Leu Phe
770 775 780
Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro Gly Ala
785 790 795 800
Ala Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Ile Gly Ser
805 810 815
Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
820 825 830
Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu Val
835 840 845
Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser Pro
850 855 860
Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg His
865 870 875 880
Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile Ala
885 890 895
Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro Glu
900 905 910
Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu Thr Val
915 920 925
Thr Gln Leu Leu Arg Arg Leu His Gln Trp Leu Ser Ser Glu Cys Thr
930 935 940
Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile Cys
945 950 955 960
Glu Val Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met Pro
965 970 975
Gln Leu Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg Gly
980 985 990
Val Trp Arg Gly Asp Gly Ile Met His Thr Arg Cys Pro Cys Gly Ala
995 1000 1005
Glu Ile Ala Gly His Val Lys Asn Gly Thr Met Arg Ile Val Gly
1010 1015 1020

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Pro Lys  Thr Cys Arg Asn Thr  Trp Ser Gly Thr Phe  Pro Ile Asn
    1025                 1030                 1035
Ala Tyr  Thr Thr Gly Pro Cys  Thr Pro Leu Pro Ala  Pro Asn Tyr
    1040                 1045                 1050
Thr Phe  Ala Leu Trp Arg Val  Ser Ala Glu Glu Tyr  Val Glu Ile
    1055                 1060                 1065
Arg Gln  Val Gly Asp Phe His  Tyr Val Thr Gly Met  Thr Ala Asp
    1070                 1075                 1080
Asn Leu  Lys Cys Pro Cys Gln  Val Pro Ser Pro Glu  Phe Phe Thr
    1085                 1090                 1095
Glu Leu  Asp Gly Val Arg Leu  His Arg Phe Ala Pro  Pro Cys Lys
    1100                 1105                 1110
Pro Leu  Leu Arg Asp Glu Val  Ser Phe Arg Val Gly  Leu His Asp
    1115                 1120                 1125
Tyr Pro  Val Gly Ser Gln Leu  Pro Cys Glu Pro Glu  Pro Asp Val
    1130                 1135                 1140
Ala Val  Leu Thr Ser Met Leu  Thr Asp Pro Ser His  Ile Thr Ala
    1145                 1150                 1155
Glu Ala  Ala Gly Arg Arg Leu  Ala Arg Gly Ser Pro  Pro Ser Leu
    1160                 1165                 1170
Ala Ser  Ser Ser Ala Ser Gln  Leu Ser Ala Pro Ser  Leu Lys Ala
    1175                 1180                 1185
Thr Cys  Thr Thr Asn His Asp  Ser Pro Asp Ala Glu  Leu Ile Glu
    1190                 1195                 1200
Ala Asn  Leu Leu Trp Arg Gln  Glu Met Gly Gly Asn  Ile Thr Arg
    1205                 1210                 1215
Val Glu  Ser Glu Asn Lys Val  Val Val Leu Asp Ser  Phe Asp Pro
    1220                 1225                 1230
Leu Val  Ala Glu Glu Asp Glu  Arg Glu Ile Ser Val  Ala Ala Glu
    1235                 1240                 1245
Ile Leu  Arg Lys Ser Arg Arg  Phe Ala Pro Ala Leu  Pro Ile Trp
    1250                 1255                 1260
Ala Arg  Pro Asp Tyr Asn Pro  Pro Leu Leu Glu Thr  Trp Lys Lys
    1265                 1270                 1275
Pro Asp  Tyr Glu Pro Pro Val  Val His Gly Cys Pro  Leu Pro Pro
    1280                 1285                 1290
Pro Lys  Ser Pro Pro Val Pro  Pro Pro Arg Lys Lys  Arg Thr Val
    1295                 1300                 1305
Val Leu  Thr Glu Ser Thr Val  Ser Thr Ala Leu Ala  Glu Leu Ala
    1310                 1315                 1320
Thr Lys  Ser Phe Gly Ser Ser  Ser Thr Ser Gly Ile  Thr Gly Asp
    1325                 1330                 1335
Asn Thr  Thr Ala Ser Ser Glu  Pro Ala Pro Ser Val  Cys Pro Pro
    1340                 1345                 1350
Asp Ser  Asp Ala Glu Ser Tyr  Ser Ser Met Pro Pro  Leu Glu Gly
    1355                 1360                 1365
Glu Pro  Gly Asp Pro Asp Leu  Ser Asp Gly Ser Trp  Ser Thr Val
    1370                 1375                 1380
Ser Ser  Gly Ala Gly Thr Glu  Asp Val Val Cys Cys  Ser Met Ser
    1385                 1390                 1395
Tyr Ser  Trp Thr Gly Ala Leu  Ile Thr Pro Cys Ala  Ala Glu Glu
    1400                 1405                 1410
Gln Lys  Leu Pro Ile Asn Ala  Leu Ser Asn Ser Leu  Leu Arg His
    1415                 1420                 1425
His Asn  Leu Val Tyr Ser Thr  Thr Ser Arg Ser Ala  Cys Gln Arg
    1430                 1435                 1440
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Gln Lys  Lys Val Thr Phe Asp  Arg Leu Gln Val Leu  Asp Asn His
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Tyr Gln  Asp Val Leu Lys Glu  Val Lys Ala Ala Ala  Ser Lys Val
    1460                 1465                 1470
Lys Ala  Asn Leu Leu Ser Val  Glu Glu Ala Cys Ser  Leu Thr Pro
    1475                 1480                 1485
Pro His  Ser Ala Arg Ser Lys  Phe Gly Tyr Gly Ala  Lys Asp Val
    1490                 1495                 1500
Arg Cys  His Ala Arg Lys Ala  Val Asn His Ile Asn  Ser Val Trp
    1505                 1510                 1515
Lys Asp  Leu Leu Glu Asp Ser  Val Thr Pro Ile Asp  Thr Thr Ile
    1520                 1525                 1530
Met Ala  Lys Asn Glu Val Phe  Cys Val Gln Pro Glu  Lys Gly Gly
    1535                 1540                 1545
Arg Lys  Pro Ala Arg Leu Ile  Val Tyr Pro Asp Leu  Gly Val Arg
    1550                 1555                 1560
Val Cys  Glu Lys Met Ala Leu  Tyr Asp Val Val Lys  Lys Leu Pro
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Leu Ala  Val Met Gly Ser Ser  Tyr Gly Phe Gln Tyr  Ser Pro Gly
    1580                 1585                 1590
Gln Arg  Val Glu Phe Leu Val  Gln Ala Trp Lys Ser  Lys Gly Thr
    1595                 1600                 1605
Pro Met  Gly Phe Ser Tyr Asp  Thr Arg Cys Phe Asp  Ser Thr Val
    1610                 1615                 1620
Thr Glu  Ser Asp Ile Arg Thr  Glu Glu Ala Ile Tyr  Gln Cys Cys
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Asp Leu  Asp Pro Gln Ala Arg  Val Ala Ile Lys Ser  Leu Thr Glu
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Arg Leu  Tyr Val Gly Gly Pro  Leu Thr Asn Ser Arg  Gly Glu Asn
    1655                 1660                 1665
Cys Gly  Tyr Arg Arg Cys Arg  Ala Ser Gly Val Leu  Thr Thr Ser
    1670                 1675                 1680
Cys Gly  Asn Thr Leu Thr Cys  Tyr Ile Lys Ala Arg  Ala Ala Cys
    1685                 1690                 1695
Arg Ala  Ala Gly Leu Arg Asp  Cys Thr Met Leu Val  Cys Gly Asp
    1700                 1705                 1710
Asp Leu  Val Val Ile Cys Glu  Ser Gln Gly Val Gln  Glu Asp Thr
    1715                 1720                 1725
Ala Ser  Leu Arg Ala Phe Thr  Glu Ala Met Thr Arg  Tyr Ser Ala
    1730                 1735                 1740
Pro Pro  Gly Asp Pro Pro Gln  Pro Glu Tyr Asp Leu  Glu Leu Ile
    1745                 1750                 1755
Thr Ser  Cys Ser Ser Asn Val  Ser Val Ala His Asp  Asp Thr Gly
    1760                 1765                 1770
Lys Arg  Val Tyr Tyr Leu Thr  Arg Asp Pro Thr Thr  Pro Leu Ala
    1775                 1780                 1785
Arg Ala  Ala Trp Glu Thr Ala  Arg His Thr Pro Val  Asn Ser Trp
    1790                 1795                 1800
Leu Gly  Asn Ile Ile Met Phe  Ala Pro Thr Leu Trp  Val Arg Met
    1805                 1810                 1815
Ile Leu  Leu Pro His Phe Phe  Ser Val Leu Met Ala  Arg Asp Gln
    1820                 1825                 1830
Leu Glu  Gln Ala Leu Asp Cys  Glu Ile Tyr Gly Ala  Cys Tyr Ser
    1835                 1840                 1845
Ile Glu  Pro Leu Asp Leu Pro  Pro Ile Ile Gln Arg  Leu His Gly
    1850                 1855                 1860
```

Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro Gly Glu Ile Asn
1865 1870 1875
Arg Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro Pro Leu Arg
1880 1885 1890
Ala Trp Arg His Arg Ala Arg Ser Ile Arg Ala Lys Leu Leu Ser
1895 1900 1905
Arg Gly Gly Arg Ala Ala Thr Cys Gly Lys Tyr Leu Phe Asn Trp
1910 1915 1920
Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Ala Ala Ala Ser
1925 1930 1935
Gln Leu Asp Leu Ser Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly
1940 1945 1950
Asp Ile Tyr His Ser Val Ser Arg Ala Arg Pro Arg Trp Phe Trp
1955 1960 1965
Phe Cys Leu Leu Leu Leu Ala Ala Gly Val Gly Ile Tyr Leu Leu
1970 1975 1980
Pro Asn Arg
1985

<210> 6
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> primer

<400> 6
cgggagagcc atagtgg 17

<210> 7
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> primer

<400> 7
agtaccacaa ggcctttcg 19

<210> 8
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> probe

<400> 8
ctgcggaacc ggtgagtaca c 21

CLAIMS:

1. A polynucleotide which encodes an amino acid sequence having not less than 98% identity to the full length of the amino acid sequence shown in SEQ ID NO:2, in which the amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2 is replaced with glycine and wherein said polynucleotide can express nonstructural proteins and structural proteins of hepatitis C virus (HCV).

2. The polynucleotide according to claim 1, in which the amino acid corresponding to glutamic acid at position 1202 of SEQ ID NO:2 is replaced with glycine, the amino acid corresponding to glutamic acid at position 1056 is replaced with valine, and the amino acid corresponding to alanine at position 2199 is replaced with threonine.

3. The polynucleotide according to claim 1 or 2, in which the amino acid corresponding to serine at position 2321 of SEQ ID NO:2 is replaced with proline and/or the amino acid corresponding to leucine at position 2889 is replaced with phenylalanine.

4. The polynucleotide according to any one of claims 1 to 3 wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:1 or a nucleotide sequence having not less than 95% identity to the full length of the nucleotide sequence shown in SEQ ID NO:1, and wherein, in cases where the polynucleotide is RNA, the nucleotide "t" in SEQ ID NO:1 is read as "u".

5. A recombinant hepatitis C virus particle comprising the polynucleotide according to any one of claims 1 to 4.

6. A cell to which the polynucleotide according to any one of claims 1 to 4 has been introduced and which produces a recombinant HCV.

7. A method of screening or evaluating a drug candidate substance which inhibits replication or protein translation of HCV, said method comprising administering or adding said drug candidate substance to the cell according to claim 6 and evaluating replication capacity of HCV or capacity of translation of HCV protein.

8. A polynucleotide which encodes an amino acid sequence having not less than 98% identity to the full length of the amino acid sequence shown in SEQ ID NO:5, in which the amino acid corresponding to glutamic acid at position 177 of SEQ ID NO:5 is replaced with glycine and wherein said polynucleotide can express nonstructural proteins of HCV.

9. The polynucleotide according to claim 8, in which the amino acid corresponding to glutamic acid at position 177 of SEQ ID NO:5 is replaced with glycine, the amino acid corresponding to glutamic acid at position 31 is replaced with valine, and the amino acid corresponding to alanine at position 1174 is replaced with threonine.

10. The polynucleotide according to claim 8 or 9, in which the amino acid corresponding to serine at position 1296 of SEQ ID NO:5 is replaced with proline and/or the amino acid corresponding to leucine at position 1864 is replaced with phenylalanine.

11. The polynucleotide according to any one of claims 8 to 10, further comprising an IRES sequence.

12. The polynucleotide according to any one of claims 8 to 11, further comprising a selection marker gene or a reporter gene.

13. The polynucleotide according to any one of claims 8 to 12, wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:3 or a polynucleotide which is able to hybridize with the complementary sequence of SEQ ID NO:3 under stringent conditions comprising washing at 68°C in 0.1×SSC, 0.1% SDS, and wherein, in cases where the polynucleotide is RNA, the nucleotide "t" in SEQ ID NO:3 is read as "u".

14. The polynucleotide according to any one of claims 8 to 13, wherein said polynucleotide is a replicon RNA.

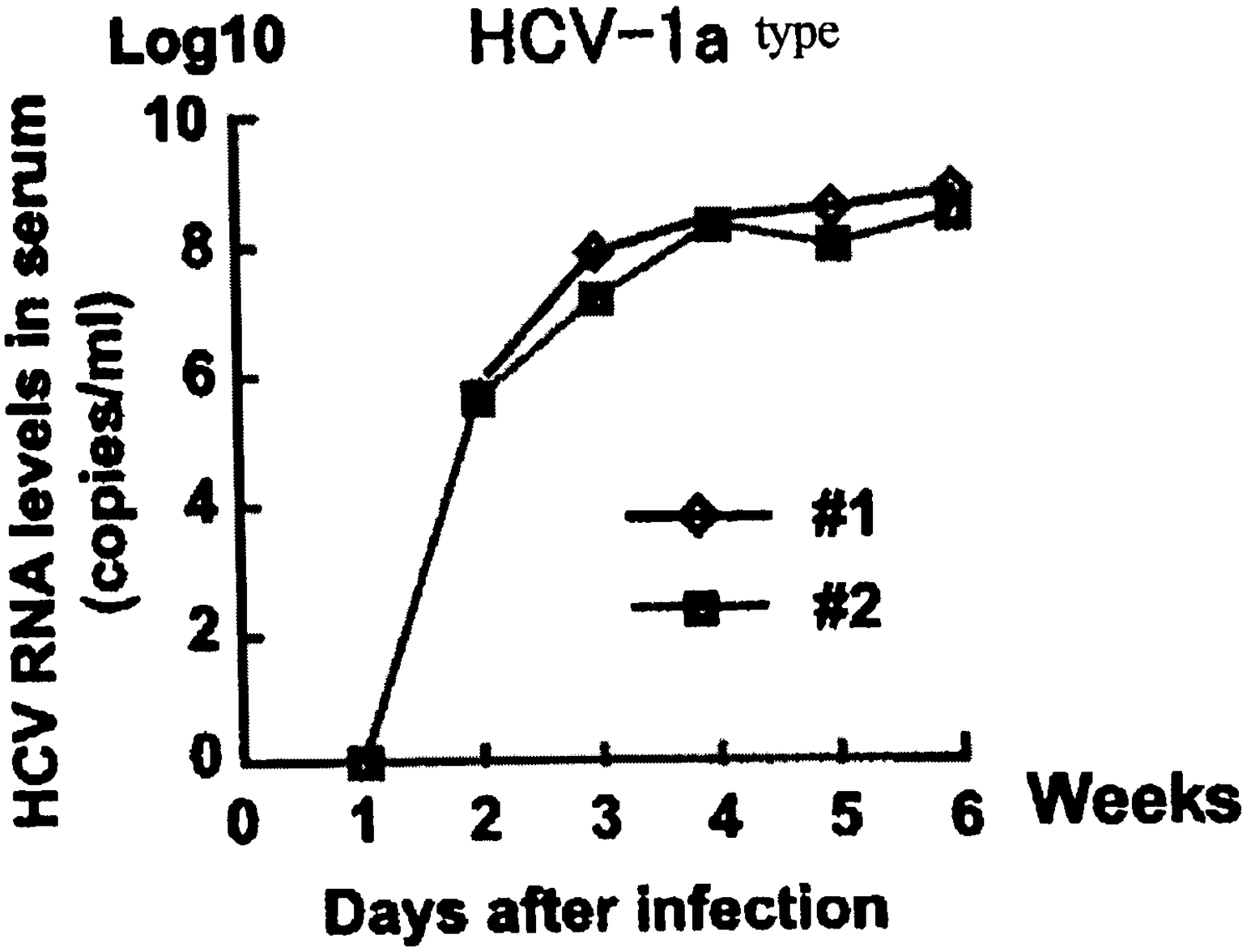
15. A replicon-replicating cell prepared by introducing the replicon RNA according to claim 14.

16. The replicon-replicating cell according to claim 15, wherein the cell is a human liver-derived cell, human uterine cervix-derived cell or human fetal kidney-derived cell.

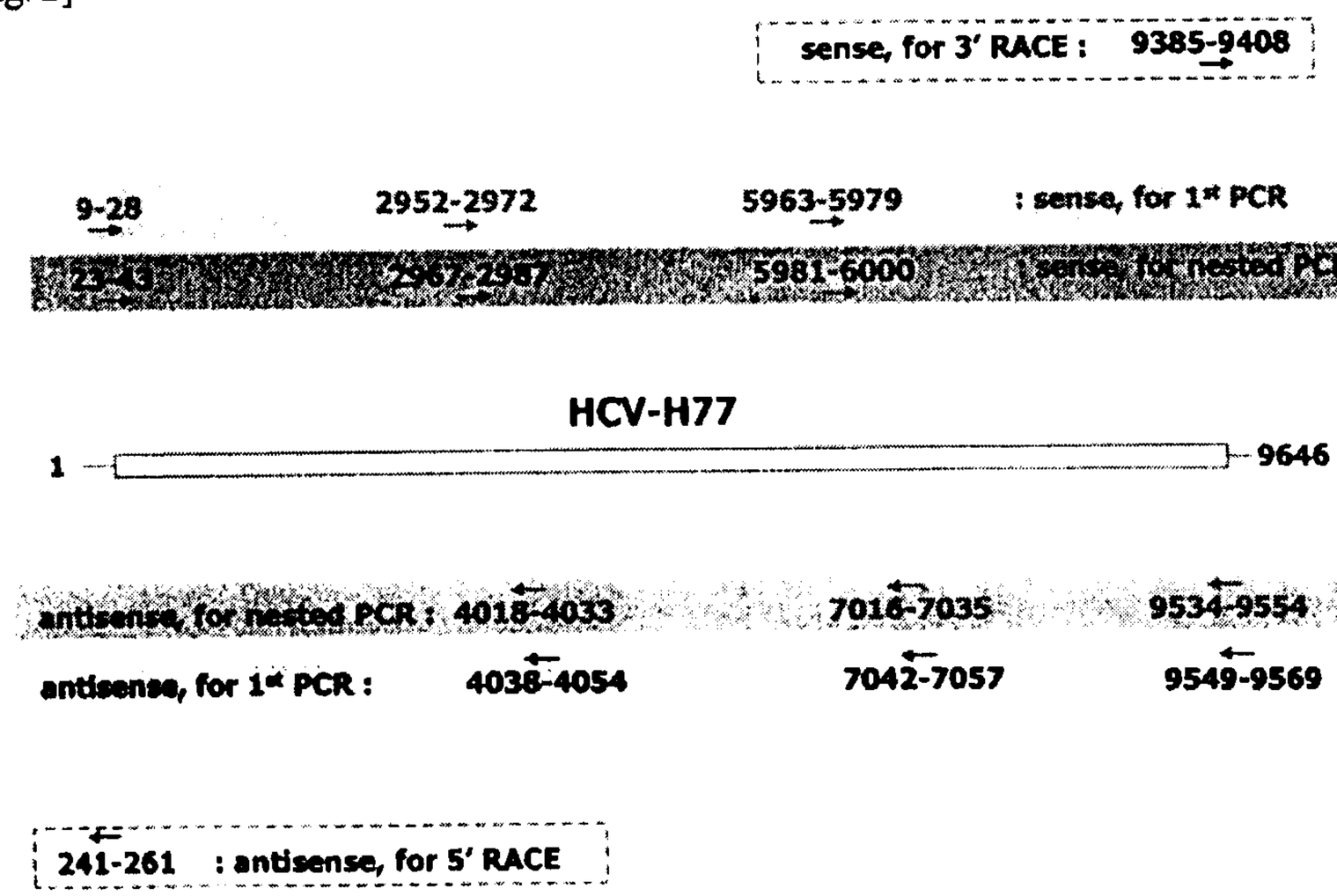
17. The replicon-replicating cell according to claim 15 or 16, wherein the cell is a Huh7 cell, HepG2 cell, IMY-N9 cell, HeLa cell or 293 cell.

18. A method of screening or evaluating a drug candidate substance which inhibits replication or protein translation of HCV, said method comprising adding a drug candidate substance to the replicon-replicating cell according to any one of claims 15 to 17 and evaluating replication capacity or protein translation of said replicon RNA.

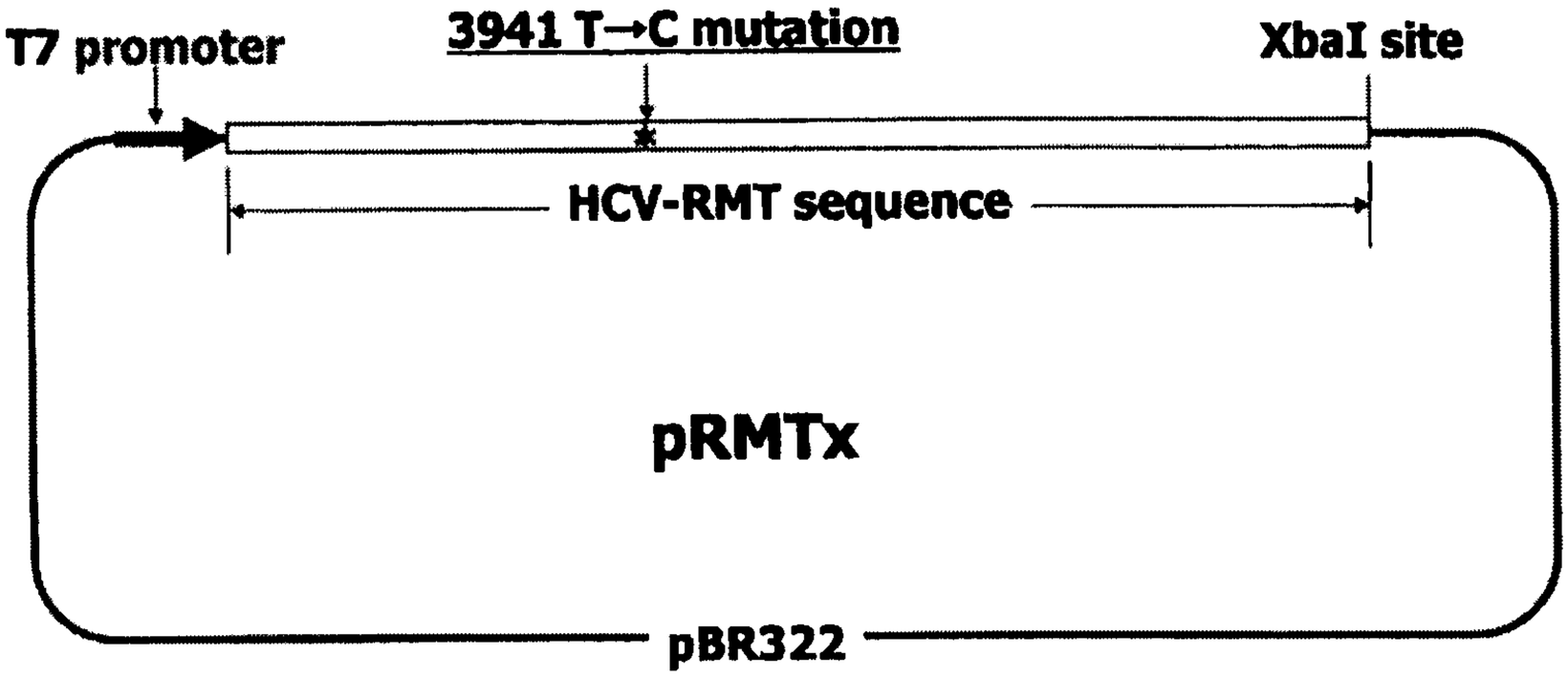
[Fig. 1]


HCV-1a type
Log10
HCV RNA levels in serum (copies/ml)
10
8
6
4
2
0
0 1 2 3 4 5 6 Weeks
Days after infection
#1
#2

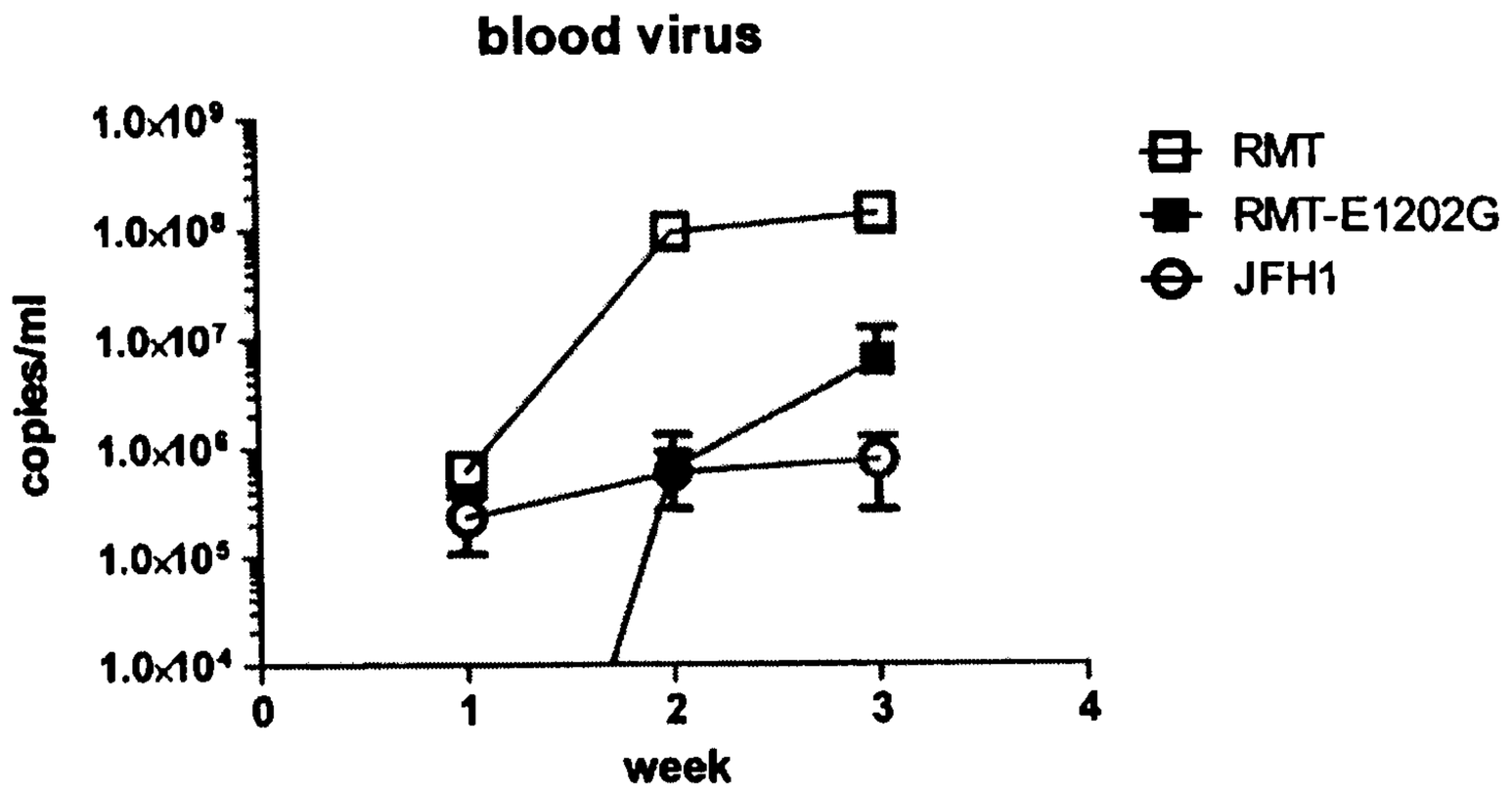
[Fig. 2]


sense, for 3' RACE : 9385-9408
9-28
2952-2972
5963-5979
: sense, for 1st PCR
23-43
2967-2987
5981-6000
: sense, for nested PCR
HCV-H77
1
9646
antisense, for nested PCR : 4018-4033
7016-7035
9534-9554
antisense, for 1st PCR : 4038-4054
7042-7057
9549-9569
241-261 : antisense, for 5' RACE

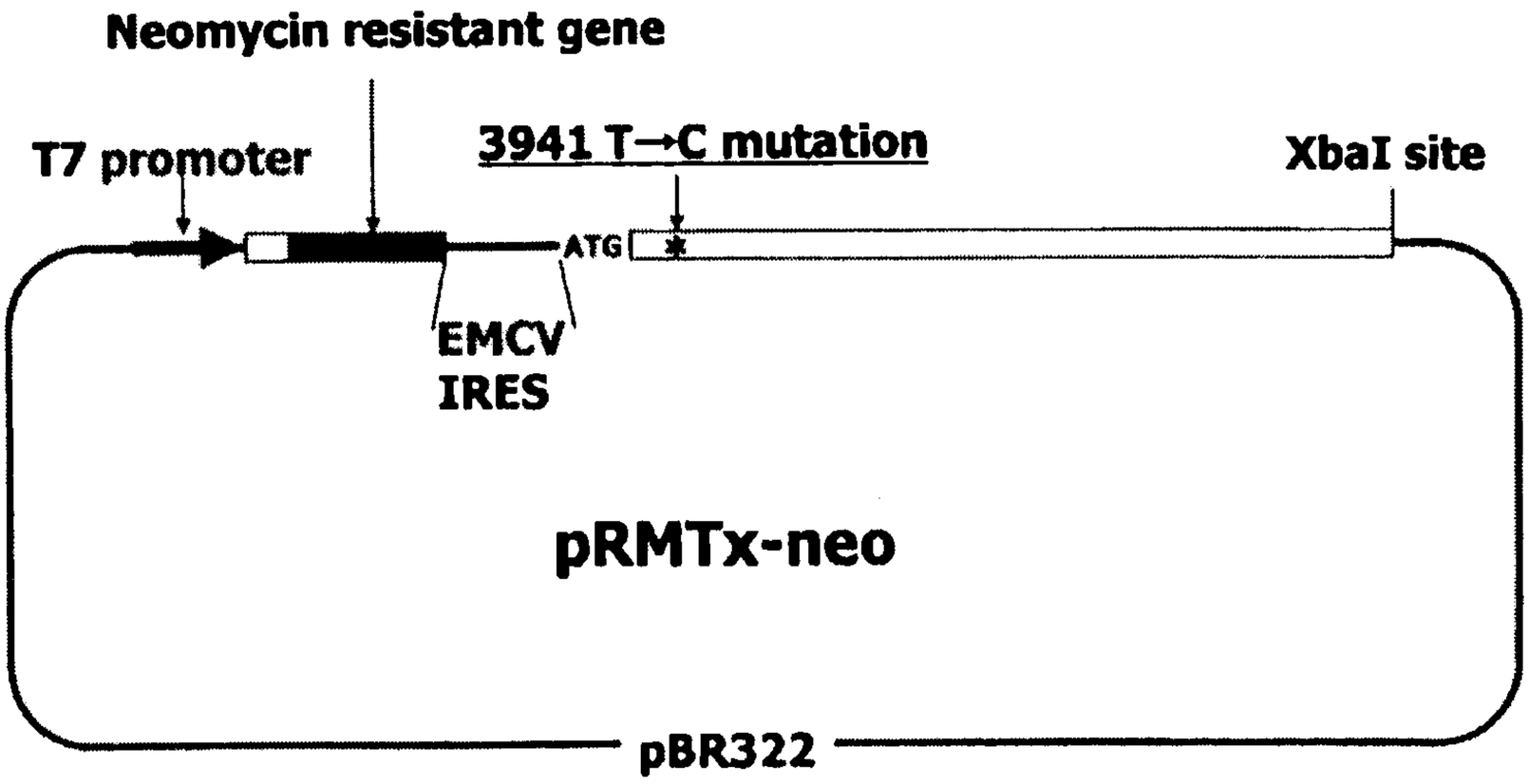
[Fig. 3]



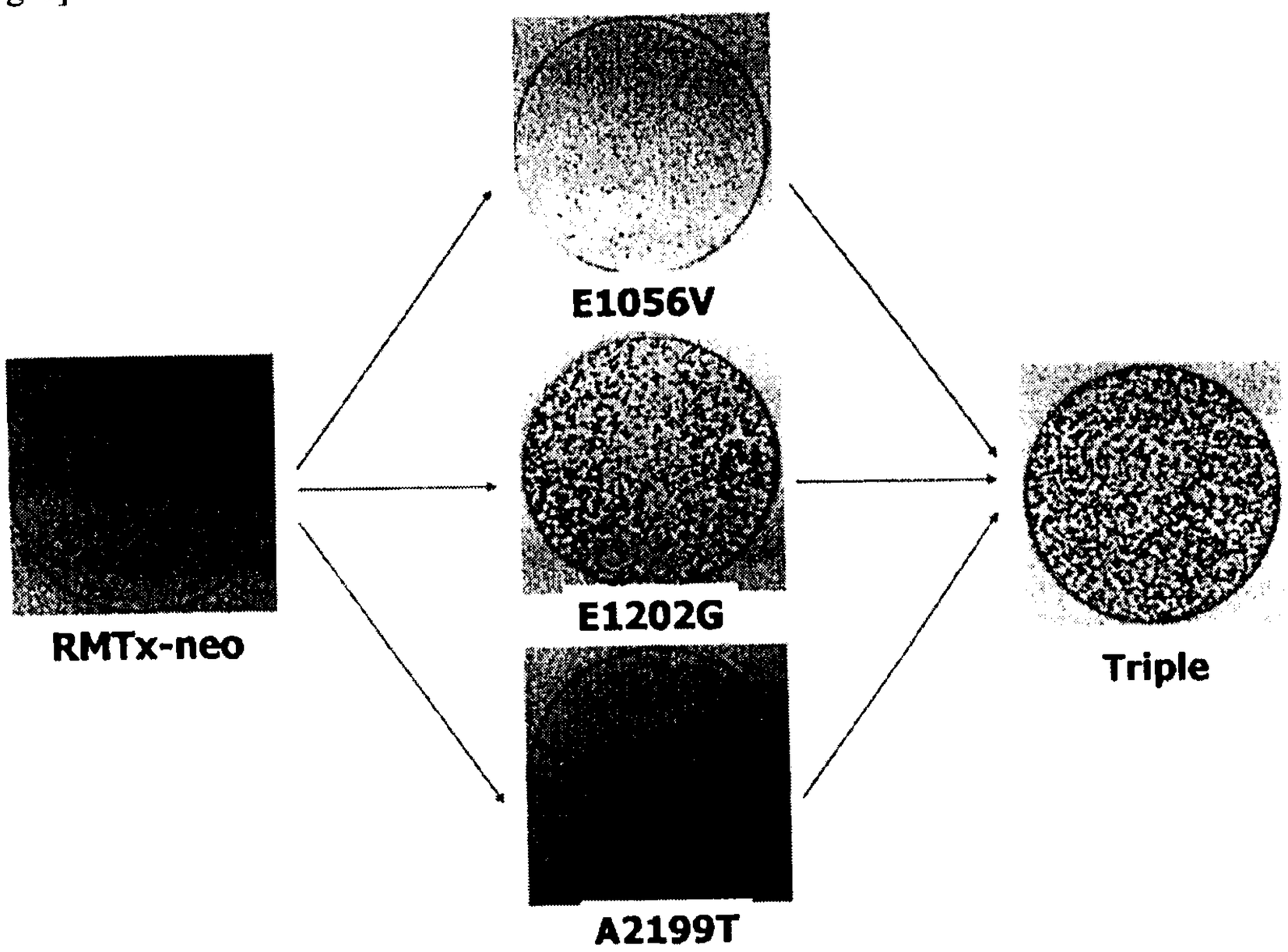
[Fig. 4]



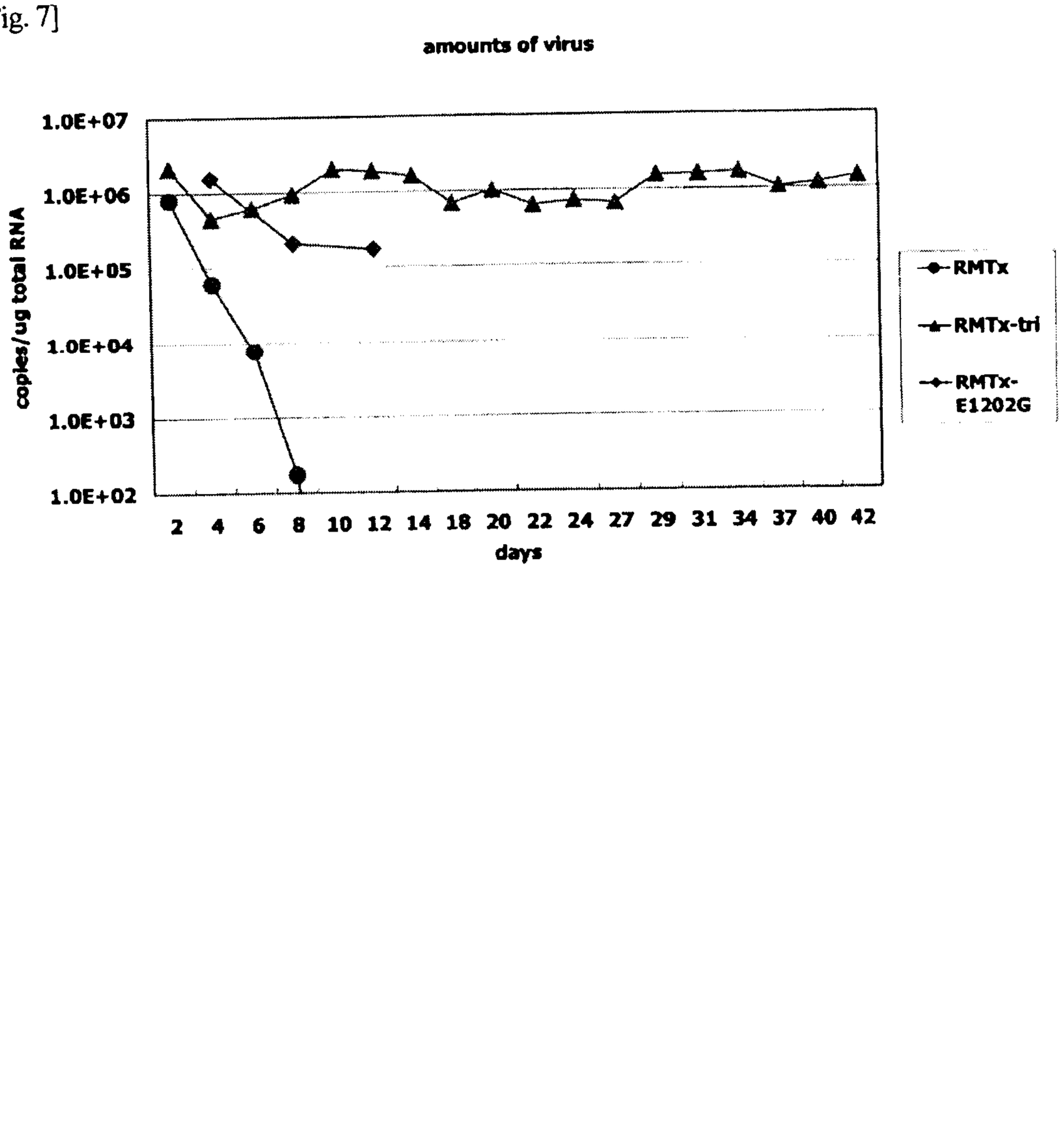
[Fig. 5]



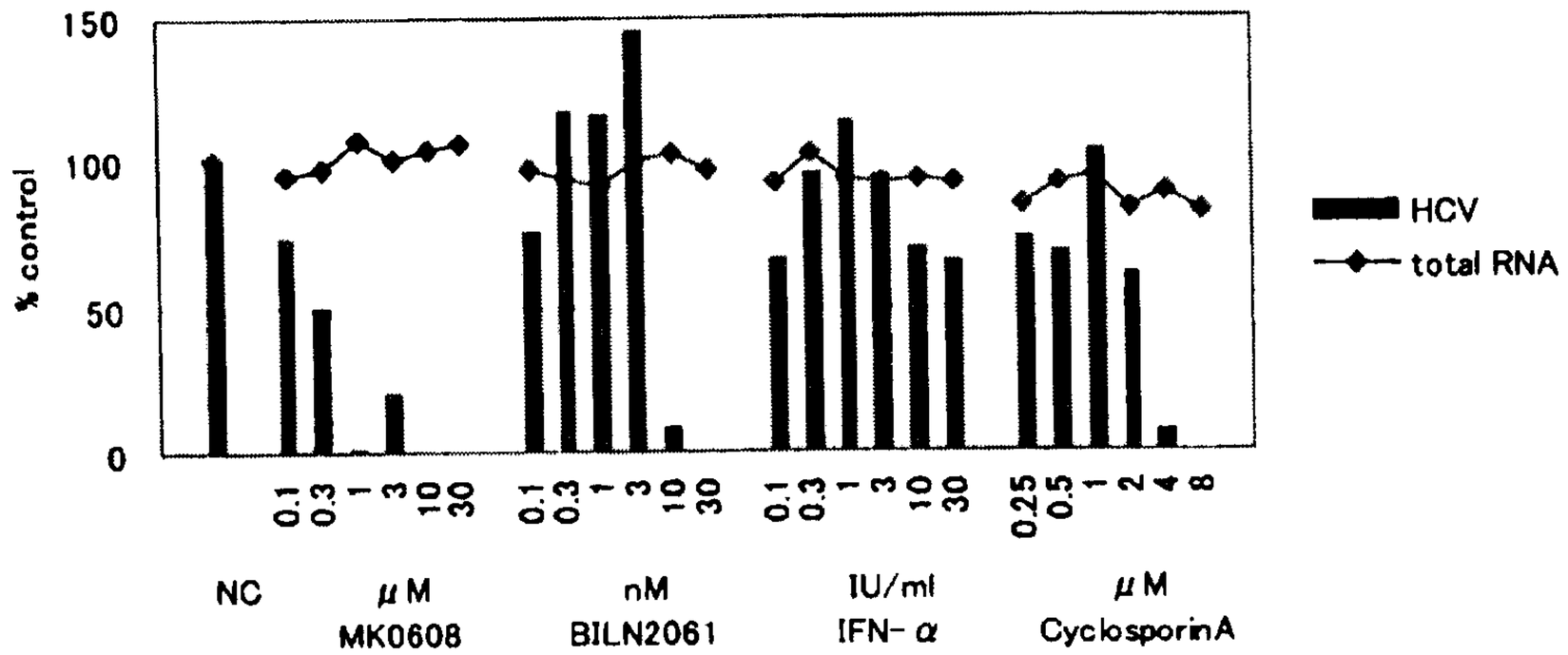
[Fig. 6]


E1056V
RMTx-neo
E1202G
Triple
A2199T

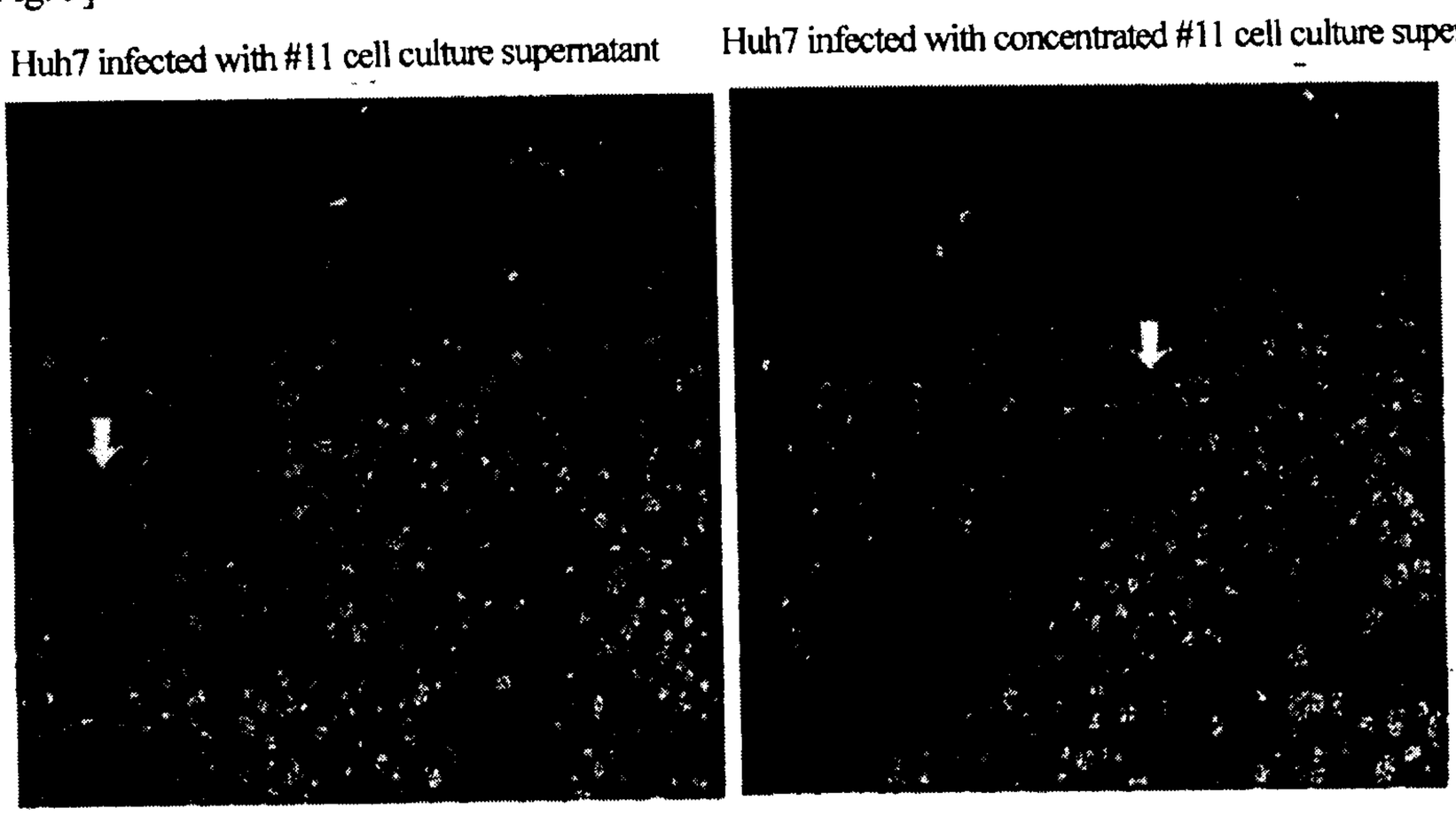
[Fig. 7]

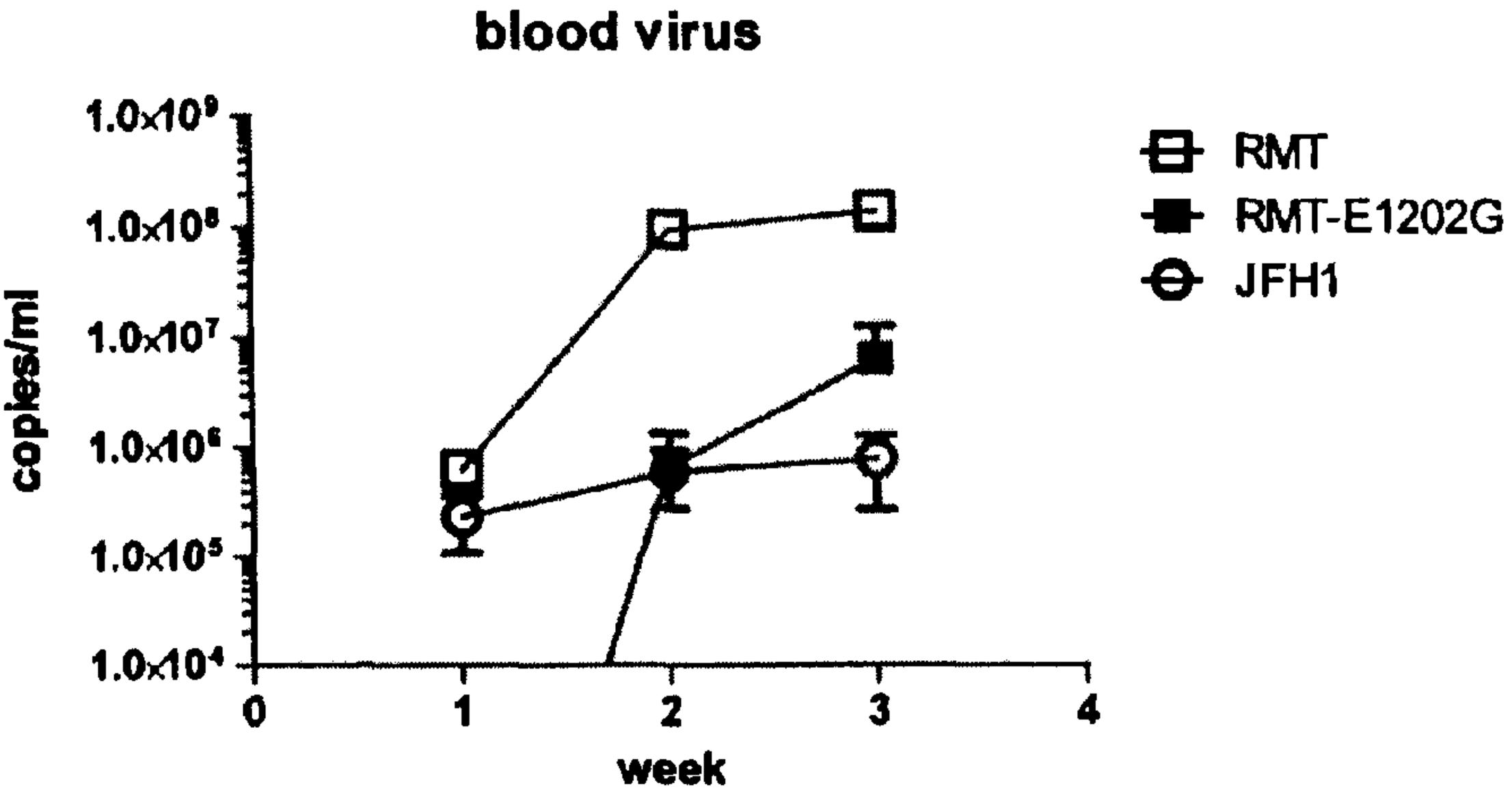

amounts of virus
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
copies/ug total RNA
2 4 6 8 10 12 14 18 20 22 24 27 29 31 34 37 40 42
days
RMTx
RMTx-tri
RMTx-E1202G

[Fig. 8]


150
100
50
0
% control
HCV
total RNA
0.1
0.3
1
3
10
30
0.1
0.3
1
3
10
30
0.1
0.3
1
3
10
30
0.25
0.5
1
2
4
8
NC
μM
MK0608
nM
BILN2061
IU/ml
IFN-α
μM
CyclosporinA

[Fig. 9]


Huh7 infected with #11 cell culture supernatant
Huh7 infected with concentrated #11 cell culture supernatant


blood virus
1.0×10⁹
1.0×10⁸
1.0×10⁷
1.0×10⁶
1.0×10⁵
1.0×10⁴
copies/ml
0
1
2
3
4
week
RMT
RMT-E1202G
JFH1