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⑤④ Virsraksts: Kompozīcija, antivielu noteikšanas paņēmieni, diagnostikas reaģentu komplekss

⑤⑦ Kopsavilkums: Vīrusu hepatīta C (HCV) antigēnu kombinācija, kurai ir plašāks imunoloģiskās aktivitātes diapazons nekā jebkuram atsevišķam HCV antigēnam, sastāv no antigēna no HCV D domēna un vēl vismaz viena papildus HCV antigēna no domēniem NS3, NS4, NS5 un domēna S hibrīdas olbaltumvielas veidā. Kompozīcija ir vienkārša fiziska maisījuma veidā vai atsevišķu, ar kopēju cietu matrici saistītu, antigēnu veidā.

* pieteikums pieņemts ievērojot LR Ministru Padomes 1992.gada 28.februāra lēmumu Nr.72(PSRS ir norādītā valsts PCT pieteikumā)

Izgudrojuma formula

1. *Kompozīcija* no hepatīta C vīrusa (HCV) epitopa secībām vienā vai vairākos polipeptīdos, kas iegūti ar ķīmisko sintēzi vai rekombinantā ekspresijā, imobilizēti uz cietas nesējvielas un paredzēti HCV noteikšanai ar imūnanalīzi, kas *atšķiras* ar to, ka tā satur:

- a) pirmo epitopa secību no HCV poliproteīna C domēna;
 - b) otro epitopa secību no HCV poliproteīna otra domēna, kurš var būt -
 - I) HCV poliproteīna NS3 domēns;
 - II) HCV poliproteīna NS4 domēns, vai
 - III) HCV poliproteīna NS5 domēns;
 - c) trešo epitopa secību no HCV poliproteīna trešā domēna, kurš var būt -
 - I) HCV poliproteīna NS3 domēns;
 - II) HCV poliproteīna NS4 domēns, vai
 - III) HCV poliproteīna NS5 domēns;
- pie kam trešais domēns atšķiras no otrā domēna.

2. *Kompozīcija* pēc 1. punkta, kas *atšķiras* ar to, ka otrais domēns ir NS3.

3. *Kompozīcija* pēc 1. punkta, kas *atšķiras* ar to, ka otrais domēns ir NS4.

4. *Kompozīcija* pēc 1. punkta, kas *atšķiras* ar to, ka tā satur:

- a) pirmo epitopa secību no HCV poliproteīna C domēna;
- b) otro epitopa secību no HCV poliproteīna NS3 domēna;
- c) trešo epitopa secību no HCV poliproteīna NS4 domēna.

5. *Kompozīcija* pēc jebkura no iepriekšējiem punktiem, kas *atšķiras* ar to, ka cietā nesējviela ir mikrotitrēšanas plates šūnas virsma, lodītes virsma vai indikatora nūjiņas virsma.

6. *Kompozīcija* pēc jebkura no iepriekšējiem punktiem, kas *atšķiras* ar to, ka pirmā, otrā un trešā epitopa secība ir ietvertas attiecīgi pirmajā, otrajā un trešajā polipeptīdā, kas katrs atsevišķi piesaistīts cietās nesējvielas virsmai.

7. *Kompozīcija* pēc 6. punkta, kas *atšķiras* ar to, ka cietā nesējviela ir indikatora nūjiņa un polipeptīdi ir uzklāti uz tās atsevišķi viens no otra tādā veidā, ka pirmā, otrā vai trešā polipeptīda saistīšanos var novērot atsevišķi.

8. *Kompozīcija* pēc jebkura no 1.-5. punktiem, kas *atšķiras* ar to, ka kompozīcija ir savienota polipeptīda formā.

9. *Paņēmiens* hepatīta C vīrusa (HCV) antivielu noteikšanai tādās zīdītāja organisma sastāvdaļās, kuras varētu saturēt minētās antivielas, kas *atšķiras* ar to, ka minēto organisma sastāvdaļu kontaktē ar kompozīciju pēc jebkura no iepriekšējiem punktiem tādus apstākļos, kas dod iespēju notikt antivielas reakcijai ar antigēnu, un nosaka minēto antivielu un minēto polipeptīdu epitopa secību imūno kompleksu veidošanos.

10. *Diagnosticas reaģentu komplekts* hepatīta C vīrusa (HCV) antivielu noteikšanai tādās zīdītāja organisma sastāvdaļās, kuras varētu saturēt minētās antivielas, kas iesaiņotā veidā satur:

- a) HCV epitopa secību kompozīciju pēc jebkura no iepriekšējiem punktiem;
- b) parastos analītiskos reaģentus un
- c) instrukcijas analīzes veikšanai.

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COMBINATIONS OF HEPATITIS C VIRUS (HCV) ANTIGENS
FOR USE IN IMMUNOASSAYS FOR ANTI-HCV ANTIBODIES

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Description

Technical Field

The present invention is in the field of
15 immunoassays for HCV (previously called Non-A, Non-B
hepatitis virus). More particularly, it concerns combina-
tions of HCV antigens that permit broad range immunoassays
for anti-HCV antibodies.

20 Background

The disease known previously as Non-A, Non-B
hepatitis (NANBH) was considered to be a transmissible
disease or family of diseases that were believed to be
viral-induced, and that were distinguishable from other
25 forms of viral-associated liver diseases, including that
caused by the known hepatitis viruses, i.e., hepatitis A
virus (HAV), hepatitis B virus (HBV), and delta hepatitis
virus (HDV), as well as the hepatitis induced by
cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH
30 was first identified in transfused individuals. Transmis-
sion from man to chimpanzee and serial passage in
chimpanzees provided evidence that NANBH was due to a
transmissible infectious agent or agents. Epidemiologic
evidence suggested that there may be three types of NANBH:
35 a water-borne epidemic type; a blood-borne or parenterally
transmitted type; and a sporadically occurring (community

acquired) type. However, until recently, no transmissible agent responsible for NANBH had been identified, and clinical diagnosis and identification of NANBH had been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBH antigens and antibodies were agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

In 1987, scientists at Chiron Corporation (the owner of the present application) identified the first nucleic acid definitively linked to blood-borne NANBH: See, e.g., EPO Pub. No. 318,216; Houghton et al., Science 244:359 (1989). These publications describe the cloning of an isolate from a new viral class, hepatitis C virus (HCV), the prototype isolate described therein being named "HCV1." HCV is a Flavi-like virus, with an RNA genome.

U.S. Patent Application Serial No. 456,637 (Houghton et al.), incorporated herein by reference, describes the preparation of various recombinant HCV polypeptides by expressing HCV cDNA and the screening of those polypeptides for immunological reactivity with sera from HCV patients. That limited screening showed that at least five of the polypeptides tested were very immunogenic; specifically, those identified as 5-1-1, C100, C33c, CA279a, and CA290a. Of these five polypeptides, 5-1-1 is located in the putative NS4 domain; C100 spans the putative NS3 and NS4 domains; C33c is located within the putative NS3 domain and CA279a and CA290a are located within the putative C domain. The screening also showed

Figure 2 is a schematic of the amino acid sequence of Figure 1 showing the putative domains of the HCV polypeptide.

5 Modes for Carrying Out the Invention

Definitions

"HCV antigen" intends a polypeptide of at least about 5 amino acids, more usually at least about 8 to 10 amino acids that defines an epitope found in an isolate of HCV. Preferably, the epitope is unique to HCV. When an antigen is designated by an alphanumeric code, the epitope is from the HCV domain specified by the alphanumeric.

"Synthetic" as used to characterize an HCV antigen intends that the HCV antigen has either been isolated from native sources or man-made such as by chemical or recombinant synthesis.

"Domains" intends those segments of the HCV polyprotein shown in Figure 2 which generally correspond to the putative structural and nonstructural proteins of HCV. Domain designations generally follow the convention used to name Flaviviral proteins. The locations of the domains shown in Figure 2 are only approximate. The designations "NS" denotes "nonstructural" domains, while "S" denotes the envelope domain, and "C" denotes the nucleocapsid or core domain.

"Fusion polypeptide" intends a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

"Common solid matrix" intends a solid body to which the individual HCV antigens or the fusion polypeptide comprised of HCV antigens are bound covalently or by noncovalent means such as hydrophobic adsorption.

5 "Mammalian body component" intends a fluid or tissue of a mammalian individual (e.g., a human) that commonly contains antibodies produced by the individual. Such components are known in the art and include, without limitation, blood, plasma, serum, spinal fluid, lymph
10 fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

"Immunologically reactive" means that the antigen in question will react specifically with anti-HCV
15 antibody commonly present in a significant proportion of sera from individuals infected with HCV.

"Immune complex" intends the combination or aggregate formed when an antibody binds to an epitope on an antigen.

20

Combinations of HCV Antigens

Figure 2 shows the putative domains of the HCV polyprotein. The domains from which the antigens used in the combinations derive are: C, S (or E), NS3, NS4, and
25 NS5. The C domain is believed to define the nucleocapsid protein of HCV. It extends from the N-terminal of the polyprotein to approximately amino acid 120 of Figure 1. The S domain is believed to define the virion envelope protein, and possibly the matrix (M) protein, and is
30 believed to extend from approximately amino acid 120 to amino acid 400 of Figure 1. The NS3 domain extends from approximately amino acid 1050 to amino acid 1640 and is believed to constitute the viral protease. The NS4 domain extends from the terminus of NS3 to approximately amino
35 acid 2000. The function of the NS4 protein is not known at this time. Finally, the NS5 domain extends from about

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35 acid 2000. The function of the NS4 protein is not known at this time. Finally, the NS5 domain extends from about

When produced by recombinant techniques, standard procedures for constructing DNA encoding the antigen, cloning that DNA into expression vectors, transforming host cells such as bacteria, yeast, insect, or mammalian cells, and expressing such DNA to produce the antigen may be employed. As indicated previously, it may be desirable to express the antigen as a fusion protein to enhance expression, facilitate purification, or enhance solubility. Examples of specific procedures for producing representative HCV antigens are described in the Examples, infra, and in parent application Serial No. 456,637.

Formulation of Antigens for Use in Immunoassay

The HCV antigens may be combined by producing them in the form of a fusion protein composed of two or more of the antigens, by immobilizing them individually on a common solid matrix, or by physically mixing them. Fusion proteins of the antigen may also be immobilized on (bound to) a solid matrix. Methods and means for covalently or noncovalently binding proteins to solid matrices are known in the art. The nature of the solid surface will vary depending upon the assay format. For assays carried out in microtiter wells, the solid surface will be the wall of the well or cup. For assays using beads, the solid surface will be the surface of the bead. In assays using a dipstick (i.e., a solid body made from a porous or fibrous material such as fabric or paper) the surface will be the surface of the material from which the dipstick is made. In agglutination assays the solid surface may be the surface of latex or gelatin particles. When individual antigens are bound to the matrix they may be distributed homogeneously on the surface or distributed thereon in a pattern, such as bands so that a pattern of antigen binding may be discerned.

Simple mixtures of the antigens comprise the antigens in any suitable solvent or dispersing medium.

Assay Formats Using Combinations of Antigens

The HCV antigens may be employed in virtually any assay format that employs a known antigen to detect
5 antibodies. A common feature of all of these assays is that the antigen is contacted with the body component suspected of containing HCV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically
10 be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen.

Design of the immunoassays is subject to a great
15 deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radio-
20 active, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

25 The immunoassay may be, without limitation, in a heterogenous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the
30 polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as
35 Immulon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech

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complished by inoculating 1500 ml of Luria broth containing ampicillin (100 micrograms/ml) with 15 ml of an overnight culture of the transformants. The cells were grown to an O.D. of 0.3, IPTG was added to yield a final concentration of 2 mM, and growth continued until the
5 cells attained a density of 1 O.D., at which time they were harvested by centrifugation at 3,000 x g at 4°C for 20 minutes. The packed cells can be stored at -80°C for several months.

10 In order to purify the SOD-C33c polypeptide the bacterial cells in which the polypeptide was expressed were subjected to osmotic shock and mechanical disruption, the insoluble fraction containing SOD-C33c was isolated and subjected to differential extraction with an alkaline-
15 NaCl solution, and the fusion polypeptide in the extract purified by chromatography on columns of S-Sepharose and Q-Sepharose.

The crude extract resulting from osmotic shock and mechanical disruption was prepared by the following
20 procedure. One gram of the packed cells were suspended in 10 ml of a solution containing 0.02 M Tris HCl, pH 7.5, 10 mM EDTA, 20% sucrose, and incubated for 10 minutes on ice. The cells were then pelleted by centrifugation at 4,000 x g for 15 min at 4°C. After the supernatant was removed,
25 the cell pellets were resuspended in 10 ml of Buffer A1 (0.01M Tris HCl, pH 7.5, 1 mM EDTA, 14 mM beta-mercaptoethanol [BME]), and incubated on ice for 10 minutes. The cells were again pelleted at 4,000 x g for 15 minutes at 4°C. After removal of the clear supernatant
30 (periplasmic fraction I), the cell pellets were resuspended in Buffer A1, incubated on ice for 10 minutes, and again centrifuged at 4,000 x g for 15 minutes at 4°C. The clear supernatant (periplasmic fraction II) was removed, and the cell pellet resuspended in 5 ml of Buffer
35 A2 (0.02 M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA, 1 mM PMSF). In order to disrupt the cells, the suspension (5

ml) and 7.5 ml of Dyno-mill lead-free acid washed glass beads (0.10-0.15 mm diameter) (obtained from Glen-Mills, Inc.) were placed in a Falcon tube, and vortexed at top speed for two minutes, followed by cooling for at least 2 min on ice; the vortexing-cooling procedure was repeated another four times. After vortexing, the slurry was filtered through a scintered glass funnel using low suction; the glass beads were washed two times with Buffer A2, and the filtrate and washes combined.

10 The insoluble fraction of the crude extract was collected by centrifugation at 20,000 x g for 15 min at 4°C, washed twice with 10 ml Buffer A2, and resuspended in 5 ml of MILLI-Q water.

15 A fraction containing SOD-C33c was isolated from the insoluble material by adding to the suspension NaOH (2 M) and NaCl (2 M) to yield a final concentration of 20 mM each, vortexing the mixture for 1 minute, centrifuging it 20,000 x g for 20 min at 4°C, and retaining the supernatant.

20 In order to purify SOD-C33c on S-Sepharose, the supernatant fraction was adjusted to a final concentration of 6M urea, 0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA. This fraction was then applied to a column of S-Sepharose Fast Flow (1.5 x 10 cm) which had been equilibrated with Buffer B (0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA).
25 After application, the column was washed with two column volumes of Buffer B. The flow through and wash fractions were collected. The flow rate of application and wash, was 1 ml/min; and collected fractions were 1 ml. In order
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35 Fractions containing SOD-C33c were pooled.

recombinants, competent HB101 cells were transformed with the recombinant vectors, and after cloning, the desired recombinants were identified on the basis of the predicted size of HindIII-SalI fragments excised from the clones.

- 5 One of the clones which contained the a HindIII-SalI fragment of the correct size was named pBR322/C100⁻d. Confirmation that this clone contained amplified C100 was by direct sequence analysis of the HindIII-SalI fragment.

- The expression vector containing C100 was
10 constructed by ligating the HindIII-SalI fragment from pBR322/C100⁻d to a 13.1 kb BamHI-SalI fragment of pBS24.1, and a 1369 bp BamHI-HindIII fragment containing the ADH2/GAP promoter. (The latter fragment is described in EPO 164,556). The pBS24.1 vector is described in commonly
15 owned U.S.S.N. 382,805 filed 19 July 1989. The ADH2/GAP promoter fragment was obtained by digestion of the vector pPGAP/AG/HindIII with HindIII and BamHI, followed by purification of the 1369 bp fragment on a gel.

- Competent HB101 cells were transformed with the
20 recombinant vectors; and correct recombinants were identified by the generation of a 2464 bp fragment and a 13.1 kb fragment generated by BamHI and SalI digestion of the cloned vectors. One of the cloned correct recombinant vectors was named pC100⁻d#3.

- 25 In order to express C100, competent cells of Saccharomyces cerevisiae strain AB122 (MATa leu2 ura3-53 prb 1-1122 pep4-3 prc1-407[cir-0]) were transformed with the expression vector pC100⁻d#3. The transformed cells were plated on URA-sorbitol, and individual transformants
30 were then streaked on Leu⁻ plates.

- Individual clones were cultured in Leu⁻, ura⁻
- medium with 2% glucose at 30°C for 24-36 hours. One liter of Yeast Extract Peptone Medium (YEP) containing 2% glucose was inoculated with 10 ml of the overnight
35 culture, and the resulting culture was grown at 30°C at an agitation rate of 400 rpm and an aeration rate of 1 L of

air per 1 L of medium per minute (i.e., 1vvm) for 48 hours. The pH of the medium was not controlled. The culture was grown in a BioFlo II fermentor manufactured by New Brunswick Science Corp. Following fermentation, the
5 cells were isolated and analyzed for C100 expression.

Analysis for expressed C100 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude
10 extracts were analyzed by electrophoresis on SDS polyacrylamide gels, and by Western blots. The Western blots were probed with rabbit polyclonal antibodies directed against the SOD-C100 polypeptide expressed in yeast. The expected size of the C100 polypeptide is 364
15 amino acids. By gel analysis the expressed polypeptide has a MW_r of 39.9K.

Both analytical methods demonstrated that the expressed C100 polypeptide was present in total cell lysates, but was absent from crude extracts. These
20 results suggest that the expressed C100 polypeptide may be insoluble.

Example 3: Expression of HCV Antigen S2

HCV antigen S2 contains a sequence from the
25 hydrophobic N-terminus of the S domain. It includes amino acids 199-328 of Figure 1.

The protocol for the construction of the expression vector encoding the S2 polypeptide and for its expression in yeast was analogous to that used for the
30 expression of the C100 polypeptide, described in Example 2.

The template for the PCR reaction was the vector pBR322/Pil4a, which had been linearized by digestion with HindIII. Pil4a is a cDNA clone that encodes amino acids
35 199-328.

The oligonucleotides used as primers for the amplification by PCR of the S2 encoding sequence were the following.

5 For the 5'-region of the S2 sequence:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG GGC TCT
ACC ACG TCA CCA ATG ATT GCC CTA AC 3';

and

10

for the 3'-region of the S2 sequence:

5'GAG TGC TCG TCG ACT CAT TAA GGG GAC CAG TTC
ATC ATC ATA TCC CAT GCC AT 3'.

15 The primer for the 5'-region introduces a HindIII site and an ATG start codon into the amplified product. The primer for the 3'-region introduces translation stop codons and a SalI site into the amplified product.

The PCR conditions were 29 cycles of 94°C for a
20 minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The main product of the PCR reaction was a 413 bp fragment, which was gel purified. The purified fragment was ligated to the large fragment obtained from
25 pBR322 digested with HindIII and SalI fragment, yielding the plasmid pBR322/S2d.

Ligation of the 413 bp HindIII-SalI S2 fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment
30 of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.77 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing
35 the sequence encoding S2 fused directly to the ADH2/GAP promoter is identified as pS2d#9.

Example 4: Synthesis of HCV C Antigen

HCV antigen C22 is from the C domain. It comprises amino acids 1-122 of Figure 1.

5 The protocol for the construction of the expression vector encoding the C polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described supra, except for the following.

10 The template for the PCR reaction was pBR322/Ag30a which had been linearized with HindIII. Ag30 is a cDNA clone that encodes amino acids 1-122. The oligonucleotides used as primers for the amplification by PCR of the C encoding sequence were the following.

15

For the 5'-region of the C sequence:

5' GAG TGC AGC TTC AAA ACA AAA TGA GCA CGA
ATC CTA AAC CTC AAA AAA AAA AC 3',

20

and

for the 3'-region of the C sequence:

5' GAG TGC TCG TCG ACT CAT TAA CCC AAA TTG CGC
GAC CTA CGC CGG GGG TCT GT 3'.

25

The primer for the 5'-region introduces a HindIII site into the amplified product, and the primer for the 3'-region introduces translation stop codons and a SalI site. The PCR was run for 29 cycles of 94°C for a minute, 37°C
30 for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The major product of PCR amplification is a 381 bp polynucleotide. Ligation of this fragment with the SalI-HindIII large SalI-HindIII fragment of pBR322 yielded
35 the plasmid pBR322/C2.

Ligation of the 381 bp HindIII-SalI C coding fragment excised from pBR322/C2 with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.74 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding C fused directly to the ADH2/GAP promoter is identified as pC22.

Analysis for expressed C polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The C polypeptide is expected to have 122 amino acids and by gel analysis the expressed polypeptide has a MW_r of approximately 13.6 Kd.

20 Example 5: Synthesis of NS5 Polypeptide

This polypeptide contains sequence from the N-terminus of the NS5 domain. Specifically it includes amino acids 2054 to 2464 of Figure 1. The protocol for the construction of the expression vector encoding the NS5 polypeptide and for its expression were analogous to that used for the expression of C33c (see Example 1).

Example 6: Radioimmunoassay (RIA) for Antibodies to HCV

The HCV antigens of Examples 1-5 were tested in an RIA format for their ability to detect antibodies to HCV in the serum of individuals clinically diagnosed as having HCV (Non-A, Non-B) and in serum from blood given by paid blood donors.

The RIA was based upon the procedure of Tsu and Herzenberg (1980) in SELECTED METHODS IN CELLULAR IMMUNOLOGY (W.H. Freeman & Co.), pp. 373-391. Generally,

microtiter plates (Immulon 2, Removawell strips) are coated with purified HCV antigen. The coated plates are incubated with the serum samples or appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with ^{125}I -labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

Specifically, one hundred microliter aliquots containing 0.1 to 0.5 micrograms of the HCV antigen in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the antigen solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent non-specific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The antigens in the coated wells were reacted with serum by adding 100 microliters of serum samples diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for 1 hr at 37°C . After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Antibody bound to the antigen was determined by the binding of ^{125}I -labeled $\text{F}'(\text{ab})_2$ sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were

added to each well, and the plates were incubated at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

Table 1 below presents the results of the tests on the serum from individuals diagnosed as having HCV.

Table 1

	<u>INDIVIDUAL</u>	<u>ANTIGEN</u>				
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
	CVH IVDA	P	P	P(+++)	P	P
	CVH IVDA	P	P	P(+)	P	P
15	CVH IVDA	P	P	P(+)	P	P
	CVH NOS	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
	AVH NOS HS	P	N	N	N	N
	AVH NOS HS	P	N	N	N	N
20	AVH NOS HS	P/N	N	N	N	N
	AVH PTVH	N	N	N	P/N	N
	AVH NOS HS	N	N	N	N	N
	AVH NOS	N	N	N	N	P
	AVH PTVH	N	N	N	N	N
25	AVH IVDA	N	P	N	P	P
	AVH PTVH	P	P/N	N	N	P
	AVH NOS	N	P	N	N	N
	AVH IVDA	N	P	N	P	P
	AVH NOS HS	P/N	N	N	N	N
30	AVH PTVH	N	N	N	N	N
	CVH IVDA	P	P	P	P	P
	CVH IVDA	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
	CVH PTVH	P	P	N	N	N
35	AVH PTVH	P	N	P(+)	P(+++)	N
	CVH PTVH	N	P	P	P	P
	CVH NOS HS	P	P	P	P	N
	CVH NOS	N	P	F/N	P	P

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	CVH IVDA	N	N	N	P N
	AVH IVDA	P	P	P	P P
5	AVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P F
	AVH IVDA	P/N	P	N	P P
	AVH IVDA	N	P	P	P N
	CVH PTVH	P	P/N	N	N N
10	CVH NOS	N	N	N	N N
	CVH NOS	N	N	N	N N
	CVH IVDA	P	P	P	P P
	AVH IVDA	P	P	P	P P
	CVH PTVH	P	P	P	P P
15	AVH PTVH?	N	P	P	P P
	AVH IVDA	N	P	N	P N
	AVH NOS	N	N	N	N N
	AVH NOS	N	N	N	N N
	CVH NOS	N	P	N	N P
20	CVH NOS	P	P	N	N N
	CVH NOS HS	P	P	P	P P
	CVH PTVH	P	P	N	P P
	AVH nurse	P	P	N	N N
	AVH IVDA	P	P	P	P N
25	AVH IVDA	N	P	P(+)	P(+++) N
	AVH nurse	P/N	P	N	N N
	AVH PTVH	P/N	P	P	N P
	AVH NOS	N	P/N	N	N P
	AVH NOS	N	P	N	P N
30	AVH PTVH	P	P/N	N	N N
	AVH PTVH	N	P	N	P P
	AVH PTVH	P	P	P	P P
	AVH PTVH	N	P	N	N P
	CVH PTVH	P/N	P	P(+)	P(+++) N
35	AVH PTVH	N	P/N	P(+)	P(+++) P

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	P	(?)	P	N P
	CVH PTVH	N	P	N	P P
5	CVH PTVH	N	P	P	P P
	CVH PTVH	N	N	N	N N
	AVH NOS	N	N	N	N N
	AVH nurse	P	P	N	N N
	CVH PTVH	N	P	N	N P
10	AVH IVDA	N	P	N	P/N N
	CVH PTVH	P	P	P(+)	P(+++) P
	AVH NOS	P	P	N	N N
	AVH NOS	P/N	P	N	N P
	AVH PTVH	P/N	P	P	P P
15	AVH NOS	N	P	P	P P/N
	AVH IVDA	N	P	N	N P
	AVH NOS	N	P/N	N	N N
	AVH NOS	P	P	N	N P
	AVH PTVH	N	P	P	P P
20	crypto	P	P	P	F P
	CVH NOS	N	P	P	P P
	CVH NOS	N	N	N	N N
	AVH PTVH	N	P	P(+)	P(++) N
	AVH PTVH	N	P/N	P(+)	P(++) P
25	AVH PTVH	N	P/N	P(+)	P(++) P
	CVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P P
	CVH IVDA	P	P	P	P P
30	AVH NOS	N	P	N	N N
	CVH IVDA	P	P	P	P P/N
	AVH IVDA	P	P	P	P N
	AVH NOS	P	P	N	N N
	AVH NOS	P	P	N	N N
35	CVH PTVH	P	P	N	N P/N

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	N	P	N	P P
	AVH NOS	N	N	N	N N
5	AVH NOS	N	P	N	N N
	AVH NOS	P	N	N	N N
	CVH NOS	N	N	N	N N
	AVH NOS	N	P/N	N	N N
	AVH IVDA	N	P	P	P P
10	crypto	N	P	N	N P/N
	crypto	P	P	P/N	P P
	AVH IVDA	N	N	P	P N
	AVH IVDA	N	P	P	P N
	AVH NOS	N	N	N	N N
15	AVH NOS	N	N	N	N N
	CVH IVDA	P	P	P	P P
	CVH PTVH	N	N	N	N N
	CVH PTVH	P	P	P(+)	P(+++) P
	CVH PTVH	P	P	P(+)	P(+++) P
20	CVH NOS	P/N	N	N	N N
	CVH NOS	N	N	N	N N
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
25	AVH IVDA	N	P	P	P P
	CVH NOS	N	N	N	N N
	CVH NOS	N	N	N	N N
	CVH PTVH	P	P	P	P P
	AVH NOS	P	P	N	N P/N
30	AVH NOS	N	P/N	N	N N
	CVH PTVH	P	P	N	N P
	CVH NOS	N	P/N	N	N N
	AVH NOS	N	P	N	N N
	AVH NOS	N	P	N	N N
35	CVH PTVH	N	P	N	N N

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	
				<u>C33c</u>	<u>NS5</u>
	AVH IVDA	N	P	N	P
	AVH NOS	P	N	N	N
5	CVH NOS	N	N	N	N
	CVH NOS	N	N	N	N
	CVH IVDA	P	P	P	P
	CVH IVDA	P/N	P	P	P
	CVH IVDA	P	P	P	P
10	CVH IVDA	N	P	P	P
	AVH NOS	N	P	N	N
	CVH IVDA	N	P	N	P
	CVH IVDA	N	P	N	P
	AVH PTVH	P	P	N	P
15	AVH PTVH	P	P	N	P
	CVH NOS	N	P/N	N	P/N
	CVH NOS	N	P	N	N
	CVH NOS	N	N	N	N
	CVH PTVH	P	P	P	P
20	CVH PTVH	P	P	P	P
	CVH PTVH	P	P	P	P
	AVH IVDA	N	P	N	P
	AVH IVDA	N	P	P(++)	P(+)
	CVH PTVH	P	P	P	P
25	AVH PTVH	N	P	P	P
	CVH PTVH?	N	P	P	P
	CVH PTVH?	P/N	P	P	P
	CVH NOS HS	P	P	N	N
	CVH IVDA	P	P	P	N
30	CVH PTVH	N	P	P	P
	CVH PTVH	P	P	P	P/N
	CVH NOS	P	P	P	P
	CVH IVDA	P	P	P	P
	CVH PTVH	P	P	P	N
35	CVH PTVH	P	P	P	P

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	CVH NOS	N	N	N	N P/N
	CVH NOS	N	P/N	N	N P/N
5	CVH PTVH	P	P	P	P P
	CVH NOS	N	P	N	N N
	CVH NOS	N	N	N	N N
	CVH NOS	P	P	N	N P/N
	CVH NOS	N	N	N	N N
10	CVH NOS HS	P	P	P	P P
	CVH NOS HS	P	P	P	P P
	CVH PTVH	N	N	N	N N
	AVH PTVH	N	P	P	P P
	AVH NOS			-	-
15	CVH PTVH	N	P	P/N	P(+++) N
	crypto	P	P	P	P P
	crypto	P	P	P	P P
	crypto	N	P	N	N N
	crypto	N	P	P	P P
20	CVH PTVH	P	P	P	P P
	crypto	N	N	N	N N
	crypto	N	P	N	N P/N
	crypto	N	P	N	N P
	crypto	P	P	P	P P
25	crypto	N	P	N	P N
	crypto			-	-
	crypto			-	-
	CVH NOS			-	-
	AVH-IVDA	N	P	N	P(+) P
30					
35					

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INDIVIDUALANTIGEN

	<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
AVH-IVDA	N	P/N	N	P(++)	N

5

AVH = acute viral hepatitis

CVH = chronic viral hepatitis

PTVH = post-transfusion viral hepatitis

IVDA = intravenous drug abuser

10

crypto = cryptogenic hepatitis

NOS = non-obvious source

P = positive

N = negative

15

Per these results, no single antigen reacted with all sera. C22 and C33c were the most reactive and S2 reacted with some sera from some putative acute HCV cases with which no other antigen reacted. Based on these results, the combination of two antigens that would provide the greatest range of detection is C22 and C33c. If one wished to detect a maximum of acute infections, S2 would be included in the combination.

20

Table 2 below presents the results of the testing on the paid blood donors.

25

Table 2

		<u>Antigens</u>				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
30	1	N	N	N	N	N
	2	N	N	N	N	N
	3	P	P	P	P	P
	4	N	N	N	N	N
	5	N	N	N	N	N
35	6	N	N	N	N	N
	7	N	N	N	N	N
	8	N	N	N	N	N

Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	9	N	N	N	N	N
	10	N	N	N	N	N
5	11	N	N	N	N	N
	12	N	N	N	N	N
	13	N	N	N	N	N
	14	N	N	N	N	N
	15	N	N	N	N	N
10	16	N	N	N	N	N
	17	N	N	N	N	N
	18	P	P	P	P	P
	19	P	P	N	P	P
	20	P	P	N	P	P
15	21	N	N	N	N	N
	22	N	P	P	N	P
	23	P	P	P	P	P
	24	N	N	N	N	N
	25	N	N	N	N	N
20	26	N	N	N	N	N
	27	N	N	N	N	N
	28	N	N	N	N	N
	29	N	N	N	N	N
	30	N	N	N	N	N
25	31	P	P	P	N	P
	32	N	N	N	N	N
	33	N	N	N	N	N
	34	N	N	N	N	P
	35	N	N	P	N	P
30	36	N	N	N	N	N
	37	N	N	N	N	N
	38	N	N	N	N	N
	39	N	N	N	N	N
	40	N	N	N	N	N
35	41	N	N	N	N	P
	42	N	N	N	N	N

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Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	43	N	N	N	N	N
	44	N	N	N	N	N
	45	N	N	N	N	N
	46	N	N	N	N	N
	47	P	P	N	N	P
10	48	N	N	N	N	N
	49	N	N	N	N	N
	50	N	N	N	N	N
	51	N	P	P	N	P
	52	N	N	N	N	N
15	53	N	P	P	N	P
	54	P	P	P	P	N
	55	N	N	N	N	N
	56	N	N	N	N	N
	57	N	N	N	N	N
20	58	N	N	N	N	N
	59	N	N	N	N	N
	60	N	N	N	N	N
	61	N	N	N	N	N
	62	N	N	N	N	N
25	63	N	N	N	N	N
	64	N	N	N	N	N
	65	N	N	N	N	N
	66	N	N	N	N	N
	67	N	N	N	N	N
30	68	N	N	N	N	N
	69	N	N	N	N	N
	70	P	P	P	P	P
	71	N	N	N	N	N
	72	N	N	N	N	N
35	73	P	P	P	P	N
	74	N	N	N	N	N
	75	N	N	N	N	N
	76	N	N	N	N	P

Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	77	N	N	N	N	N
	78	N	N	N	N	N
5	79	N	N	N	N	N
	80	N	N	N	N	N
	81	N	N	N	N	N
	82	N	N	N	N	N
	83	P	P	N	N	N
10	84	N	N	P	N	N
	85	N	N	N	N	N
	86	P	P	P	P	N
	87	N	N	N	N	N
	88	N	N	N	N	N
15	89	P	P	P	P	P
	90	P	P	P	P	N
	91	N	N	N	N	P
	92	P	P	P	N	N
	93	N	N	N	N	N
20	94	N	N	N	N	N
	95	N	N	N	N	N
	96	N	N	N	N	N
	97	N	N	N	N	N
	98	N	P	P	P	P
25	99	P	P	P	P	P
	100	N	N	N	N	N
	101	P	P	P	P	P
	102	N	N	N	N	N
	103	N	N	N	N	N
30	104		N	N	N	N
	105	P	P	P	P	N
	106	N	N	N	N	N
	107	N	N	N	N	N
	108	N	N	N	N	N
35	109	P	P	P	P	P
	110	P	P	P	N	P

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	111	P	P	P	N	P
	112	N	N	N	N	N
5	113	P	P	P	P	P
	114	N	N	N	N	N
	115	N	N	N	N	N
	116	P	P	P	P	P
	117	N	N	N	N	N
10	118	N	N	N	N	N
	119	N	N	N	N	N
	120	P	P	P	P	P
	121	N	N	N	N	N
	122	N	P	P	N	P
15	123	N	N	N	N	N
	124	N	N	N	N	N
	125	N	N	N	N	N
	126	P	N	N	N	N
	127	N	N	N	N	N
20	128	N	N	N	N	N
	129	N	N	N	N	N
	130	P	P	P	P	N
	131	N	N	N	N	P
	132	N	N	N	N	N
25	133	N	N	N	N	N
	134	N	N	N	N	N
	135	N	N	N	N	N
	136	N	N	N	N	N
	137	N	N	N	N	N
30	138	N	N	N	N	N
	139	N	N	N	N	N
	140	P	N	N	N	N
	141	P	N	P	P	P
	142	N	N	N	N	N
35	143	N	N	N	N	N
	144	N	N	N	N	N

Antigens						
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	145	N	N	N	N	N
	146	N	N	N	N	N
5	147	N	N	N	N	N
	148	N	N	N	N	N
	149	N	N	N	N	N
	150	N	N	N	N	N
	151	N	N	N	N	N
10	152	N	N	N	N	N
	153	N	N	N	N	N
	154	P	P	P	P	P
	155	N	N	N	N	N
	156	N	N	N	N	N
15	157	N	N	N	N	N
	158	N	N	N	N	N
	159	N	N	N	N	N
	160	N	N	N	N	N
	161	P	P	P	P	P
20	162	N	N	N	N	N
	163	N	N	N	N	N
	164	P	P	P	N	P
	165	N	N	N	N	N
	166	P	P	P	N	P
25	167	N	N	N	N	N
	168	N	N	N	N	N
	169	N	N	N	N	N
	170	N	N	N	N	N
	171	N	N	N	N	N
30	172	N	N	N	N	N
	173	N	N	N	N	N
	174	N	N	N	N	N
	175	N	N	N	N	N
	176	N	N	N	N	N
35	177	N	N	N	N	P
	178	N	N	N	N	N

-35-

		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
	179	N	N	N	N	N
	180	N	N	N	N	N
5	181	N	N	N	N	N
	182	N	N	N	N	N
	183	P	P	P	P	P
	184	N	N	N	N	N
	185	N	N	N	N	N
10	186	N	N	N	N	N
	187	N	N	N	N	N
	188	N	P	P	N	N
	189	N	N	N	N	N
	190	N	N	N	N	N
15	191	N	N	N	N	N
	192	N	N	N	N	N
	193	N	N	N	N	N
	194	N	N	N	N	N
	195	N	N	N	N	N
20	196	N	N	N	N	N
	197	N	N	N	N	P
	198	P	P	P	N	N
	199	N	N	N	N	P
	200	P	P	P	P	N
25						

) The results on the paid donors generally confirms the results from the sera of infected individuals.

30 Example 7: ELISA Determinations of HCV Antibodies Using Combination of HCV Antigens

Plates coated with a combination of C22 and C33c antigens are prepared as follows. A solution containing coating buffer (50mM Na Borate, pH 9.0), 21 ml/plate, BSA
 35 (25 micrograms/ml), C22 and C33c (2.50 micrograms/ml each) is prepared just prior to addition to the Removeawell

Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2ml/well of the solution is added to the plates, they are covered and incubated for 2 hours at 37°C, after which the solution is removed by aspiration.

5 The wells are washed once with 400 microliters wash buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100, 0.01% (W/V) Thimerosal). After removal of the wash solution, 200 microliters/well of Postcoat solution (10 mM sodium

10 phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein, 3% sucrose and 2 mM phenylmethanesulfonylfluoride (PMSF)) is added, the plates are loosely covered to prevent evaporation, and are allowed to stand at room temperature for 30 minutes. The wells are then aspirated

15 to remove the solution, and lyophilized dry overnight, without shelf heating. The prepared plates may be stored at 2-8°C in sealed aluminum pouches with dessicant (3 g Sorb-it™ packs).

In order to perform the ELISA determination, 20 microliters of serum sample or control sample is added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.01% (W/V) Thimerosal, 1% (W/V) Triton X-100, 100 micrograms/ml yeast extract). The

25 plates are sealed, and are incubated at 37°C for two hours, after which the solution is removed by aspiration, and the wells are washed three times with 400 microliters of wash buffer (phosphate buffered saline (PBS) containing 0.05% Tween 20). The washed wells are treated with 200

30 microliters of mouse anti-human IgG-horse radish peroxidase (HRP) conjugate contained in a solution of Ortho conjugate diluent (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50% (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM $K_3Fe(CN)_6$, 0.05% (W/V) Tween 20, 0.02% (W/V) Thimerosal). Treatment is for 1

35 hour at 37°C, the solution is removed by aspiration, and

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the wells are washed three times with 400 ml wash buffer, which is also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine
5 dihydrochloride per 5 ml of Developer solution) is added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30% H_2O_2 . The plates containing the substrate solution are incubated in the dark for 30 minutes at room
10 temperature, the reactions are stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

In a similar manner, ELISAs using fusion
proteins of C22 and C33c, and C22, C33c, and S2 and
15 combinations of C22 and C100, C22 and S2, C22 and an NS5 antigen, C22, C33c, and S2, and C22, C100, and S2 may be carried out.

Modifications of the above-described modes for
20 carrying out the invention that are obvious to those of skill in the fields of molecular biology, immunology, and related fields are intended to be within the scope of the following claims.

25

30

35

FIG. 1A

-341 GCCAGCCCCCTGATGGGGCGA
CGGTGGGGGACTACCCCCGCT

-319 CACTCCACCATGAATCACTCCCCCTGTGAGAACTACTGTCTTCACGCAGAAAGCGTCTAG
GTGAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAAGTGCGTCTTTCCGCAGATC

-259 CCATGGCGTTAGTATGAGTGTGTCAGCCCTCCAGGACCCCCCTCCCGGAGAGCCATA
GGTACCGCAATCATACTACAGCACGTCGGAGGTCCTGGGGGGAGGCCCTCTCGGTAT

-199 GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGA
CACCAGACGCCCTTGGCCACTCATGTGGCCCTTAACGGTCTGCTGGCCAGGAAGAACCT

-139 TCAACCCGCTCAATGCCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGT
AGTTGGGCGAGTTACGGACCTCTAAACCCGACGGGGCGTTCTGACGATCGGCTCATCA

- 79 GTGGGTGCGGAAAGGCCCTTGTGGTACTGCCCTGATAGGGTGCTTGCGAGTGCCCCGGGAG
CAACCCAGCGCTTTCGGGAACACCATGACGGAATATCCACGAACGCTCACGGGGCCCTC

- 19 GTCTCGTAGACCGTGCACC
CAGAGCATCTGGCACGTGG

Arg Thr

MetSerThrAsnProLysProGlnLysLysAsnLysArgAsnThrAsnArgArgProGln
1 ATGAGCACGGAATCCTAAACCTCAAAAAAACAACGTAACACCAACCGTCGCCACAG
TACTCGTGCTTAGGATTGGAGTTTTTTTTTTTGTTCATTTGTTGGCAGCGGGTGTGTC

AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg
61 GACGTCAAGTTCCTCCGGGTGGCGGTACAGATCGTTGGTGGAGTTTACTTGTGCGCGCAGG
CTGCAGTTCAAGGGCCACCGCCAGTCTAGCAACCACTCAAATGAACAACGGCGCGTCC

121 GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly
 GGGCCCTAGATTGGGTGTGGCGCGACGAGAAAGACTTCCGAGCGGTCCGAACCTCGAGGT
 CCGGGATCTAACCACACACGCGCGCTCTTCTGAAGCTCGCCAGCGTTGGAGCTCCA

 181 ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly
 AGACGTACGCCCTATCCCCAAGGCTCGTCGGCCCCGAGGGCAGGACCTGGGCTCAGCCCCGG
 TCTGCAGTCGGATAGGGGTTCCGAGCAGCCGGGCTCCCTCTGGACCCGAGTCGGGCCC

 241 TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro
 TACCCCTTGCCCCCTCTATGGCAATGAGGGCTGCGGGTGGCGGATGGCTCCTGCTCTCCC
 ATGGGAACCGGGGAGATACCGTTACTCCCGACGCCACCCGCCCTACCGAGGACAGAGG

 301 ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly
 CGTGGCTCTCGGCTAGCTGGGCCCCCACAGACCCCCCGGTAGGTCCGCGCAATTGGGT
 GCACCGAGAGCCGGATCGACCCCGGGTGTCTGGGGCCCGCATCCAGCGCGTTAAACCCA

 361 LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal
 AAGGTCATCGATACCCCTTACGTGGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTC
 TTCCAGTAGCTATGGGAATGCACGCCGGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAG

 421 GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp
 GCGGCCCTCTTGGAGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGAC
 CCGCGGGGAGAACCTCCGGGACGGTCCCCGGGACCGCGTACCGCAGGCCCAAGACCTTCTG

FIG. 1B

FIG. 1C

Thr

GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla
GGCGTGAACATAACAAGGAAACCTTCCTGGTGGCTCTTCTCTATCTCTCTGGCC
CCGCACTTGATACGTTGTCCCTTGAAGGACCAACGAGAAAGAGATAGAAAGAACCCGG

481

LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu
CTGCTCTCTTGTGACTGTGCCCGCTTCGGCCTACCAAGTGCGCAACTCCACGGGCTT
GACGAGAGAACGAACTGACACGGGGCGAAGCCGGATGGTTCACGCGTTGAGTGCCCCCGAA

541

TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle
TACCACGTCAACCAATGATTGCCCTAACTCAGATAATTGTGTACGAGGCGGCCGATGCCATC
ATGGTGCAGTGGTACTAACGGGATTGAGCTCATAACACATGCTCCGCCGGCTACGGTAG

601

LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal
CTGCACACTCCGGGTGCGTCCCTTGGCTTCGTGAGGGCAACGCCCTCGAGGTGTGGGTG
GACGTGTAGGGCCCCACGCAGGGAACGCAAGCACTCCCGTTGCGGAGCTCCACAACCCAC

661

AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg
GCGATGACCCCTACGGTGGCCACCAGGATGGCAAACTCCCCGCGACGCGAGCTTCGACGT
CGTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGCGCTGCGTCCGAAGCTGCA

721

HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu
CACATCGATCTGCTTGTCTGGGAGCGCCACCCCTCTGTTCGGCCCTCTACGTGGGGACCTA
GTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCCGGAGATGCACCCCTGGAT

781

CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr
TGCGGGTCTGTCTTCTTGTCTGGCCAACTGTTCACCTTCTCTCCAGGCGCCACTGGACG
ACGCCCCAGACAGAAAGAACAGCCGGTTGACAAAGTGGAGAGGGTCCGCGGTGACCTGC

841

ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp
 901 ACGCAAGTTGCAATTGCTCTATCTATCCCGCCATATAACGGGTACCCGATGGCATGG
 TCGGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCCAAGTGGCGTACCGTACC

 AspMetMetMetAsnTrpSerProThrAlaLeuValMetAlaGlnLeuLeuArgIle
 961 GATATGATGATGAACCTGGTCCCTACGACGGCGTGTGTAATGGCTCAGCTGCTCCGGATC
 CTATACTACTGTGACCAAGGGATGCTGCCGCAACCATTAACGAGTCGACGAGGCCTAG

 ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla
 1021 CCACAAGCCATCTTGACACATGATCGGTGGTGTCTCACTGGGAGTCCCTGGCGGCATAGCG
 GGTGTTCGGTAGAACCTGTACTAGCGACCAAGTAGTACCCCTCAGGACCGCCCGTATCGC

 TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuPheAlaGly
 1081 TATTCTCCATGGTGGGAACTGGCGAAGTCCCTGGTAGTGTCTGTCTATTGCGCGC
 ATAAAGAGGTACCAACCCCTTGACCCGCTTCCAGGACCATCACGACGATAAACGGCCG

 ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal
 1141 GTCGACGCGGAACCCACGTACCGGGGAAGTCCCGCCACACTGTGTCTGGATTGTGT
 CAGCTGCGCCCTTGGGTGCAGTGGCCCCCTTCACGGCCGGTGTGACACAGACCTAAACAA

 SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp
 1201 AGCCTCCTCGCACCGCGGCCAAGCAGAACGTCCAGCTGATCAACACCAACGGCAGTTGG
 TCGGAGGAGCGTGGTCCCGCGGTTCGTCTTGACAGTCCGACTAGTTGTGGTTGCCGTC AAC

FIG. 1D

FIG. 1E

1261	HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly CACCTCAATAGCACGGCCCTGAAGTCAATGATAGCCTCAACACCGGCTGGTGGCAGGG GTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTGTGCGCGACCAACCGTCCC
1321	LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg CTTTTCTATCACCAAGTTCAACTCTTCAGGCTGTCCCTGAGAGGCTAGCCAGCTGCCGA GAAAAGATAGTGTGTCAAGTTGAGAACTCCGACAGGACTCTCCGATCGGTGCGACGGCT
1381	ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro CCCCTTACCGATTTTGACCAAGGCTGGGGCCCTATCAGTTATGATGCCAACGGAAGCGCCCC GGGGAATGGCTAAAACTGTGCCGACCCCGGATAGTCAATACGGTTGCCCTTCGCCCGGGG
1441	AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys GACCAAGCGCCCTACTGCTGGCACTACCCCCCAAACCTTGCGGTATTGTGCCCGCGAAG CTGGTCGGGGGATGACGACCGGTATGGGGGTTTGTGGAACGCCATAACACGGGGCGCTTC
1501	SerValCysGlyProValTyrCysPheThrProSerProValValGlyThrThrAsp AGTGTGTGTGTCGGTATATTGCTTCACTCCAGCCCGTGGTGGTGGGAACGACCGAC TCACACACACGAGGCATATAACGAAGTGAGGGTCGGGGCACCAACCCCTTGCTGGCTG
1561	ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn AGGTCGGCGCGCCACCTACAGCTGGGGTGAAATGATACGACGCTCTTCGTCCCTTAAC TCCAGCCCGCGGGTGATGTGACCCCACTTTTACTATATGCCTGCAGAACGAGGAATTG
1621	AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe AATACCAGGCCACCGCTGGGCAATTGGTTCCGGTTGTACCTGGATGAACCTCAACTGGATT TTATGTCCTCCGGTGCGGACCCGTTAACCAAGCCAACATGGACCTACTTGAGTTGACCTAAG

1681 ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis
ACCAAAGTGTGGGAGCGCCTCCTTGTGTCACTCGGAGGGGGCAACAACACCCCTGCAC
TGGTTTCACACCGCCTCGCGGAGGAACACAGTAGCCTCCCCCGCTTGTGTGGGACGTG

1741 CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly
TGCCCCACTGATTGCTTCCGCAAGCATCCGACCGCCACATACCTCTCGGTGCGGTCCGGT
ACGGGGTGAATAACGAAGGCGTTCGTAGGCCCTGCGGTGTATGAGAGCCACGCCGAGGCCA

Ile

1801 ProTrpLeuThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys
CCCTGGATCACACCCAGGTGCTGGTCGACTACCCGTATAGGCTTTGGCATATATCCTTGT
GGACCTAGTGTGGTCCACGGACCAGCTGATGGGCATATCCGAACCGTAATAGGAACA

1861 ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu
ACCATCAACTACACCATATTTAAATCAGGATGTACGTGGAGGGGTCCGAACACAGGCTG
TGGTAGTTGATGTGGTATAAATTTTAGTCCTACATGCACCCCTCCCGCTTGTGTCCGAC

1921 GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer
GAAGCTGCCCTGCAACTGGACGGGGCGAACGTTGCGATCTGGAAGACAGGACAGGTCC
CTTCGACGGACGTTGACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGG

1981 GluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThr
GAGCTCAGCCCCGTTACTGCTGACCACTACACAGTGGCAGGTCTCCCGTGTCTCTCACA
CTCGAGTCGGGCAATGACGACTGGTGATGTGTACCGTCCAGGAGGGGCACAAGGAAGTGT

FIG. 1F

FIG. 1G

2041 ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln
ACCCTACCAGCCTTGTCCACCGGCTCATCCACCTCCACCAGAACATTTGTGGACGTGCAG
TGGGATGCTCGGAACAGGTGGCCGAGTAGTGGAGGTGCTTGTAAACACCTGCACGTC

2101 TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal
TACTTGTACGGGTGGGTCAAGCATCGCGTCTCTGGCCATTAAAGTGGAGTACGTCTGTT
ATGAACATGCCCCACCCAGTTCTGTAGCGCAGGACCCGGTAATTACCCCTCATGCAGCAA

2161 LeuLeuPheLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu
CTCCTGTTCTCTGCTTGCAGACGCGCGTCTGCTCCTGCTTGTGGATGATGCTACTC
GAGGACAAGGAAGACGAACTCTGCGCGCAGACGAGGACGAACACCTACTACGATGAG

2221 IleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla
ATATCCCAAGCGGAGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCC
TATAGGGTTCCGCTCCGCCGAAACCTCTTGGAGCATTAAGAATTACGTCGTAGGGACCGG

2281 GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly
GGGACGCACGGTCTTGTATCCTTCCTCGTGTCTTCTCTGCTTGCATGGTATTTGAAGGT
CCCTGCGTGCCAGAACATAGGAAGGAGCACAAAGACGAACGTACCATAAACTTCCCA

2341 LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeu
AAGTGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTCCTCTG
TTCACCCACGGGCTCGCCAGATGTGGAAGATGCCCTACACCGGAGGAGGACGAGGAC

2401 LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly
TTGGCGTTGCCCCAGCGGCGTACGCGCTGGACACGAGGTGGCCGCTCGTGTGGCGGT
AACCGCAACGGGTGCGCCCGCATGCGCGCACCTGTGCTCCACCGCGGCAGCACACCGCCA

2461 ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer
 GTTGTCTCGTCGGTTGATGGCGTGA CTCTGTCA CCATATTACAAGCGCTATATCAGC
 CAACAAGAGCAGCCCAACTACCGGACTGAGACAGTGGTATAATGTTCCGATATAGTCG

2521 TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp
 TGGTGCTTGTTGGTTCAGTATTTCTGAC CAGAGTGGAAAGCGCAACTGCACGTGTGG
 ACCACGAACACACCGAAGTCATAAAAGACTGTCTCACCTTCGCGTTGACGTGCACACC

2581 IleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal
 ATTCCCCCCTCAACGTCCGAGGGGGCGG CACCGCTCATCTTACTCATGTGTGCTGTA
 TAAGGGGGGAGTTGCAGGCTCCCCCGCTG CCGCAGTAGAATGAGTACACACGACAT

2641 HisProThrLeuValPheAspIleThrLysLeuLeuAlaValPheGlyProLeuTrp
 CACCCGACTCTGGTATTGTGACATCACCA AATTGCTGTGGCGTCTTCGGACCCCTTTGG
 GTGGCTGAGACCATAAACTGTAGTGGTTT AACGACGACCGCGCAGAGCCTGGGGAACCC

2701 IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg
 ATTCTTCAAGCCAGTTTGCTTAAGTACCCTA CTTTGTGCGCGTCCAAAGCCCTTCTCCGG
 TAAGAAGTTCGGTCAACCGAATTTTCATGGGATGAAACACGCGCAGGTTCCGGAGAGGCC

2761 PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys
 TTCTGCGCGTTAGCGGGAAGATGATCGGAGG CCAATTACGTGCAAAATGGTCATCATTAAG
 AAGACGCGCAATCGCGCCTTCTACTAGCCTCC GGTAAATGCACGTTTACCAGTAGTAATTC

FIG. 1H

FIG. 11

2821 LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla
TTAGGGCGGCTTACTGCGACCTATGTTTATAAACCATCTCACTCTCTTCGGGACTGGGCG
AATCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAACCCCTGACCCCGC

2881 HisAsnGlyLeuArgAspLeuAlaValAlaGluProValValPheSerGlnMetGlu
CACAAAGGCTTGGAGATCTGGCCGTGGCTGTAGAGCCAGTCTCTCTCCCAAATGGAG
GTGTTGCCGAACGCTCTAGACCGGACCGACATCTCGGTCAAGCAGAGGGTTTACCTC

2941 ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu
ACCAAGCTCATCAGTGGGGGCGAGATACCGCCGCGTGGGTGACATCATCAACGGCTTG
TGGTTCGAGTAGTGCAACCCCGCTCTATGGCGGCGCACGCTGTAGTAGTTGCCGAAC

3001 ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer
CCTGTTTCCGCGCGAGGGCGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCC
GGACAAAGGCGGCGTCCCCCGCTCTATGACGAGCCCGGTCTACCTTACCAGAGG

3061 LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu
AAGGGTGAGGTTGCTGGCGCCCATCACGGCGTACGCCCAGCAGACAAAGGGCTCCTA
TTCCCCACCTCCAACGACCGGGGTAGTGCCGCGCATGCGGTCTGTTCCTCCCGGAGGAT

3121 GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln
GGGTGCATAATCACAGCCCTAACTGGCCGGGACAAACCAAGTGGAGGGTGAGGTCCAG
CCCACGTATTAGTGGTCCGATTGACCGGCCCTGTTTGGTTTCACTCCCACTCCAGGTC

3181 IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr
ATTGTGTCAACTGCTGCCCAACCTTCTGGCAACGTGCATCAATGGGGTGTGCTGGACT
TAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCAACGACCTGA

ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet
GTCTACCA CGGGCCGGAACGAGGACCATCGCTCACCCAAAGGTCCTGTCATCCAGATG
CAGATGGTGCCCCGGCCTGCTCCTGGTAGCGCAGTGCGTTCCAGGACAGTAGGCTCTAC

3241

Tyr Thr Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu
TATAACCAATGTAGACCAAGACCTTGTTGGCTGGCCCTCCGCAAGGTAGCCGCTCATTC
ATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGCGTTCATCGGCGAGTAAC

3301

ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle
ACACCCCTGCACCTTGCAGCCTTACCTGGTACGAGGCACGCCGATGTCATT
TGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCACTGCTCCGTCCGGCTACAGTAA

3361

ProValArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr
CCCGTGCGCCGCGGGTGATAGCAGGGCAGCTGTGCGCCCGCCCATTTCTAC
GGGCACGGCGCCGCCACTATCGTCCCCGTGGACGACAGCGGGCGGGTAAAGGATG

3421

LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe
TTTGAAGGCTCCTCGGGGTCGCTGTGTGCCCGGGGCACGCCGTGGGCATATTT
AACTTTCGAGGAGCCCCCAGCGACACACGGGGCGCCCGTGGCGCACCCGTATAAA

3481

ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn
AGGGCCGGGTGCACCCGTGGAGTGGCTAAGCGGTGACTTATCCCTGTGGAGAAC
TCCCGGGCCACACGTGGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTG

3541

FIG. 1J

FIG. 1K

3601 LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro
CTAGAGACAACCATGAGTCCCGGTGTTACACGATAACTCCTCTCCACCAAGTAGTGCCC
GATCTGTGTACTCCAGGGGCCACAAGTGCCCTATTGAGGAGAGGTGTCATCACGGG

3661 GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal
CAGAGCTTCCAGGTGGCTCACCTCCATGCTCCACAGGCAGCGCAAAAGCAAGGTC
GTCTCGAAGTCCACCGAGTGAGGTACGAGGTGCCGTCCGTCGCGTTCGTGTTCCAG

3721 ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla
CCGGCTGCATATGCAGCTCAGGGCTATAAGTGCTAGTACTCAACCCCTCTGTGCTGCA
GGCCGACGTATACGTCGAGTCCCGATATTCCACGATCATGAGTTGGGGAGACAACGACGT

Leu

3781 ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr
ACACTGGGCTTTGGTGCTTACATGTCCAAAGGCTCATGGGATCGATCCTAACATCAGGACC
TGTGACCCGAAACCAACGAATGTACAGGTTCCGAGTACCCCTAGCTAGGATTGTAGTCCCTGG

3841 GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu
GGGGTGAGAAACAATTACCCTGGCAGCCCCCATCAGTACTCCACCTACGGCAAGTTCCTT
CCCCACTCTTGTTAATGGTGACCCGTGCGGGTAGTGTCATGAGGTGGATGCCGTTCAAGGAA

3901 AlaaspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer
GCCGACGGCGGTGCTCGGGGGCGCTATAGACATAATAATTGTGACGAGTGCCACTCC
CGGCTGCCGCCACGAGCCCCCGGAATACTGTATTATTAAACACTGCTCACGGTGAGG

(Val)

ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly
 3961 ACGGATGCCACATCCATCTTGGCATCGGCATCTCTTGACCAAGCAGAGACTGCGGG
 TGCCACGGTGTAGTAGAACCCGTAGCCGTGACAGGAAGTGTCTCTGACGCCCC

AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro
 4021 GCGAGACTGTTGTCTCGCCACCGCCACCCCTCCGGCTCCGTCACTGTGCCCATCCC
 CGCTCTGACCAACACGAGCGGTGGGTGGGAGGCCGAGGCAGTGACACGGGGTAGGG

AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle
 4081 AACATCGAGAGGTTGCTCTGTCCACCCAGGAGAGATCCCTTTTACGGCAAGCTATC
 TTGTAGCTCCTCCAACGAGACAGGTGGTGGCTCTCTAGGGAATAATGCCGTTCCGATAG

ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysCys
 4141 CCCCTCGAAGTAATCAAGGGGGGAGACATCTCATCTTCTGTCTCATTTCAAAGAAAGTGC
 GGGAGCTTCATTAGTTCCCTCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTCAGG

AspGluLeuAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly
 4201 GACGAACTCGCCGCAAGCTGGTCGATTTGGCATCAATGCCGTGGCTACTACCGCGGT
 CTGCTTGAGCGGGTTCGACCAAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCA

LeuAspValSerValIleProThrSerGlyAspValValValAlaThrAspAlaLeu
 4261 CTTGACGTGTCCTCATCCCGACCGCGGATGTGTCTGTCTGTGGCAACCGATGCCCTC
 GAACTGCACAGGAGTAGGGCTGTGTCGCCCTACACACAGCACCGTGGCTACGGGAG

Tyr

MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
 4321 ATGACCGGCTATACCGGCACTTCGACTCGGTGATAGACTGCAATACGTGTGTCAACCCAG
 TACTGGCCGATATGGCCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGTC

FIG. 1L

FIG. 1M

(Ser)

4381 ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
ACAGTCGATTTCAGCCTTGACCCCTACCTTCACCATTTAGACAAATCACGCTCCCCCAGGAT
TGTCAGCTAAAGTCGGAACCTGGGATGGAACTGTTAACTCTGTAGTGCAGGGGGTCCCTA

4441 AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
GCTGTCTCCCGCACTCAACGTCCGGGCAGGACTGGCAGGGGAAGCCAGGCATCTACAGA
CGACAGAGGGCGTGAGTTGCAGCCCCCGTCCCTGACCGTCCCCCTTCGGTCCGTAGATGTCT

4501 PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys
TTTGTGGCACCGGGAGCGCCCTCCGGCATGTTTCGACTCGTCCGTCTCTGTGAGTGC
AAACACCGTGGCCCCCTCGCGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCACC

4561 TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg
TATGACGCAGGCTGTGCTTGATGAGCTACGCCCGCCGAGACTACAGTTAGGCTACGA
ATACTGCGTCCGACACGAACCATACTCGAGTGGCGGGCTCTGATGTCAATCCGATGCT

4621 AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly
GCGTACATGAACACCCCGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTGGGAGGGC
CGCATGTACTTGTGGGGCCCCGAAGGGCACACGGTCCCTGGTAGAACTTAAACCCCTCCCG

4681 ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly
GTCTTTACAGGCCCTCACTCATATAGATGCCCACTTTCTATCCCGAGACAAAGCAGAGTGGG
CAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTCGTCTCACCC

4741 GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro
GAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCT
CTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACGCGATCCCCGAGTTCGGGGA

4801 ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly
CCCCATCGTGGACCAAGTGTGGAGTGTGATTGCGCTCAAGCCACCCCTCCATGGG
GGGGTAGCACCCCTGGTCTACACCTTCACAACTAAGCGGAGTTCGGGTGGGAGGTACCC

4861 ProThrProLeuLeuTyArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro
CCAACACCCCTGCTATACAGACTGGGCGCTGTTCAAGAAATCACCCCTGACGCACCCA
GGTGTGGGACGATATGTCTGACCCCGGACAAAGTCTTACTTTAGTGGGACTGCGTGGGT

4921 ValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValThrSerThrTrp
GTCACCAAAATACATCATGATGTCATGTGCGCGACCTGGAGTTCGTACGAGCACCTGG
CAGTGGTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTCTCGTGGACC

4981 ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysVal
GTGCTCGTTGGCGGCTCCTGGCTGCTTTGGCCGCGTATTGCTGTCAACAGGCTGCGTG
CACGAGCAACCGCCGACGACCGAAACCGCGGCATAAACGGACAGTTGTCCGACGCAC

5041 ValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAspArgGluVal
GTCATAGTGGCAGGTCGTCTTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTC
CAGTATCACCCCGTCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAG

5101 LeuTyArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGln
CTCTACCGAGAGTTCGATGAGATGGAAGAGTGTCTCTCAGCACTTACCGTACATCGAGCAA
GAGATGGCTCTCAAGCTACTCTACCTTCTCAGCAGAGTCGTGAATGGCATGTAGCTCGTT

FIG. 1N

FIG. 10

5161	GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSer GGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGCCCTCGGCCCTCCTGCAGACCGCTCC CCCTACTACGAGCGGCTCGTCAAGTTCGTCTCCGGGAGCCGAGGACGTCTGGCGCAGG
5221	ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe CGTCAGGCAGAGGTTATCGCCCTGCTGTCCAGACCAACTGGCAAAACTCGAGACCTTC GCAGTCCGCTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTTGTGAGCTCTGGAAG
5281	TrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr TGGGCGAAGCATATGTGGAACCTTCATCAGTGGGATACATACTTGGCGGGCTTGTCAACG ACCCGCTTCGTATACACCTTGAAGTAGTCACCCCTATGTATGAACCGCCCAACAGTTGC
5341	LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro CTGCCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTCAACGCCCA GACGGACCATTGGGGCGGTAAACGAAGTAACTACCGAAATGTCTGACGACAGTGGTGGGT
5401	LeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu CTAACCACTAGCCAAACCCCTCCTCTTCAACATATTTGGGGGGTGGTGGCTGCCCAGCTC GATTGGTGATCGGTTTGGGAGGAGAAAGTTGTATAACCCCCCACCACCGGGTCCGAG
5461	AlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGly GCCGCCCGCGTGCCTACTGCCCTTGTGGCGCTGGCTTAGCTGGCGCCCATCGGC CGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAATCGACCGGGCGGTAGCCG
5521	SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla AGTGTTGGACTGGGGAAGTCCCTCATAGACATCCTTGCAGGGTATGGCGGGCGGTGGCG TCACAAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCCG

(Gly)

5581 GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal
GGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGTCCCTCCACGGAGACCTGGTC
CCTCGAGAACACCGTAAGTTCTAGTACTGCCCACTCCAGGGGAGGTGCCCTCCTGGACCCAG

5541 AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla
AATCTACTGCCCGCCATCCTCTCGCCCGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCA
TTAGATGACGGCGGTAGGAGAGCGGCCCTCGGGAGCATCAGCCGCACCAACACGTCGT

5701 IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle
ATACTGCCCGCCGACGTTGGCCCGGCGAGGGGAGTGAGTGATGAACCGGCTGATA
TATGACGGCGCGGTGCAACCGGGCCGCTCCCCGTCACGTACCTACTTGGCCGACTAT

5761 AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla
GCCTTCGCCCTCCGGGGAAACCATGTTTCCCCCACCACCTACGTGCCGGAGAGCGATGCA
CGAAGCGGAGGGCCCCCTTGGTACAAAGGGGTGCGTGATGCACGGCCTCTCGCTACGT

(HisCys)

5821 AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu
GCTGCCCGCGTCACTGCCATACTCAGCAGCCCTCACTGTAAACCAGCTCCTGAGGCGACTG
CGACGGCGCAGTGACGGTATGAGTCGTGCGAGTGACATTGGGTCGAGGACTCCGCTGAC

5381 HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle
CACCAAGTGGATAAGCTCGGAGTGTACCACCTCCATGCTCCGGTTCCTGGCTAAGGGACATC
GTGGTCACCTATTTCGAGCCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAG

FIG. 1P

FIG. 1Q

5941 TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet
TGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAGCTAAGCTCATG
ACCCTGACCTATACGCTCCCACTCGCTGAAATCTCTGGACCGATTTCGATTTCGAGTAC

6001 ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg
CCACAGCTGCCCTGGATCCCTTTGTGTCTCTGCCAGCGGGGTATAAAGGGGTCTGGCGGA
GGTGTGACGGACCCCTAGGGGAAACACAGGACGGTCCGCCCCCATATTCCCCCAGACCGCT

(Val)
6061 GlyAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys
GTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAA
CACCTGCCGTAGTACGTGTGAGCGGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTT

6121 AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe
AACGGACGATGAGGATCGTCGGTCCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTC
TTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCTTGTAACCTCACCCCTGGAAG

6181 ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe
CCCATTAAATGCCTACACACGGGCCCTGTACCCCTTCTGCGCCGAACTACACGTTTC
GGGTAATTACGGATGTGGTCCCCGGGACATGGGGGGAAGGACCGGGCTTGATGTGCAAG

6241 AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis
GCGCTATGGAGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGACTTCCAC
CGCGATACCTCCACAGACGTCTCCTTATACACCTCTATTCCGTCCACCCCTGAAGGTG

6301 TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu
TACGTGACGGGTATGACTACTGACAAATCTCAAATGCCCGTGCCAGGTCCCATCGCCCCGAA
ATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGGCACGGTCCAGGGTAGCGGGCTT

6361 PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu
 TTTTTCACAGAAATTGGACGGGTGCGCCTACATAGGTTTGCGCCCCCTGCAAGCCCTTG
 AAAAGTGCTCTTAACCTGCCCCACGGGATGTATCCAAACGGGGGACGTTCCGGGAAC

 6421 LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu
 CTGCGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGTCGCAATTA
 GACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTATGGGCCATCCCAGCGTTAAT

 6481 ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis
 CCTTGCGAGCCCGAACCGGACGTGGCCGTGTGACGTCCTCATGCTACTCCCTCCCAT
 GGAACGCTCGGGCTTGGCCCTGCACCGGCACAACTGCAGGTACGAGTACTAGGGAGGGTA

 6541 IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer
 ATAACAGCAGAGCGCGCGCGAAGGTTGGCGAGGGGATCACCCCCCTCTGTGGCCAGC
 TATTGTCTCTCCGCGCGCGCTTCCAAACCGCTCCCTAGTGGGGGAGACACCGGTCG

 6601 SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp
 TCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCGCTAACCATGAC
 AGGAGCCGATCGGTCTGATAGGCGAGGTAGAGATTCCGTTGAACCTGGCGATTGGTACTG

 6661 SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn
 TCCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGAGATGGCGGCAAC
 AGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCCTCTACCCCGCGTTG

FIG. 1R

FIG. 1S

6721	IleThrArgValGluSerGluAsnLysValIleLeuAspSerPheAspProLeuVal ATCACAGGGTTGAGTCAGAAACAAGTGGTGAATTCTGGACTCCTTCGATCCGCTTGTG TAGTGGTCCCAACTCAGTCTTTTGTTCACCACTAAGACCTGAGGAAGCTAGGCGAACAC
6781	AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg GCGAGGAGGACGAGCGGAGATCTCCGTACCCGAGAAATCCTGCGGAAGTCTCGGAGA CGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCCTTCAGAGCCTCT
6841	PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr TTCGCCAGGCCCTGCCGTTTGGCGCGCGGACTATAACCCCCGCTAGTGGAGACG AAGCGGTCCGGACGGGCAAAACCCGCCGCGCTGATATTGGGGGCGGATCACCTCTGC
6901	TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProLys TGGAAAAGCCCGACTACGAACCACTGTGTCCATGGCTGTCCGCTTCCACCTCCAAAG ACCTTTTTCGGGCTGATGCTTGGTGACACCAAGTACCGACAGGCGAAGGTGGAGGTTC
6961	SerProProValProProArgLysLysArgThrValValLeuThrGluSerThrLeu TCCCCCTCCTGTGCCCTCCGCTCGGAAGAACGGACGGTGGTCCCTCACTGAATCAACCCTA AGGGAGGACACGGAGCGGAGCCTTCTTCGCCCTGCCACCCAGGAGTACTAGTTGGGAT
7021	(Ser) SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle TCTACTGCCCTTGGCCGAGCTCGCCACCAAGAGCTTTGGCAGCTCCTCAACTTCCGGCAT AGATGACGGAAACCGGCTCGAGCGGTGCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAA
7081	ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer ACGGCGGACAATACGACAACATCCTCTGAGCCCCGCCCTTCTGGCTGCCCCCGGACTCC TGCCCCGTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGCTGAGG

(PheAla)

7141 AspAlaGluSerTyrSerMetProProLeuGluGlyGluProGlyAspProAspLeu
 GACGCTGAGTCCTATTCCCTCCATGCCCCCCTGGAGGGAGCCTGGGATCCGGATCTT
 CTGCCACTCAGGATAAGGAGGTACGGGGGGACCTCCCCCTCGGACCCCTAGGCCCTAGAA

7201 SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys
 AGCGACGGGTCAATGTCACGGTCAGTAGTGAGGCCAACGCGGAGGATGTCGTGTGCTGC
 TCGCTGCCCAGTACCAGTTGCCAGTCACTCCGGTTGCGCCTCCTACGACACAGACG

7261 SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys
 TCAATGTCTTACTCTTGACACAGCGCACTCGTCAACCCCGTGCGCCCGGAAAGAACAGAAA
 AGTTACAGAAATGAGAACCTGTCCGCTGAGCAGTGGGCGCACGCGCGCTTCTTGTCTTT

7321 LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisAsnLeuValTyrSerThr
 CTGCCCATCAATGCACATAAGCAACTCGTGTCTACGTCAACCAATTTGGTGTATTCCACC
 GACGGGTAGTTACGTGATTTCGTTGAGCAACGATGCAGTGGTGTAAACCATAGGTGG

7381 ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu
 ACCTCAGCAGTGCTTGCCAAAGGCAGAAAGTCAATTTGACAGACTGCCAAGTTCTG
 TGGAGTGCCTCACGAAACGGTTTCCGTCTTCTTTCAGTGTAAACTGTCTGACGTTCAAGAC

7441 AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaSerLysValLysAla
 GACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCGCGTCAAAAGTGAAAGGCT
 CTGTCGGTAATGGTCCCTGCATGAGTTCCCTCCAAATTCGTGCGCCGAGTTTTCACCTCCGA

FIG. 1T

FIG. 1U

7501	(Phe) AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys AACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCCACACTCAGCCAAATCCAAG TTGAACGATAGGCATCTCCTTCGAACGTCGGAAGTGGGGGTGAGTCGGTTTAGGTTT
7561	PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn TTTGGTTATGGGCAAAAGACGTCCTGTCATGCCAGAAAGCCGTAAACCCACATCAAC AAACCAATACCCCGTTTCTGCAGGCAACGGTACGGTCTTTCCGGCATTTGGGTAGTTG
7621	SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla TCCGTGTGGAAGACCTTCTGGAAGACAAATGTAAACACCAATAGACACTACCATCATGGCT AGGCACACCTTTCTGGAAAGACCTTCTGTACATTGTGGTTATCTGTGATGGTAGTACCGA
7681	LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle AAGAACCAGAGGTTTCTGCGTTCAGCCTGAGAAAGGGGGTCTGTAAGCCAGCTCGTCTCATC TTCTTGCTCCAAAGACGCAAGTCGGAAGTCTTCTCCCGGATTCGGTCCGAGCAGAGTAG
7741	ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr GTGTTCCCGCATCTGGCGTGGCGTGTGCGAAAGATGGCTTTGTACGACGTGGTTACA CACAGGGGCTAGACCCCGCACGCGCACACGCTTTTCTACCGAAACATGTCTGCACCAATGT
7801	LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg AAGCTCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCAATACTACCCAGGACAGCGG TTCCAGGGGAACCGGCACTACCCCTTCGAGGATGCCCTAAGGTTATGAGTGGTCTGTGCCC
7861	ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp GTTGAATTCCCTCGTGCAAGCGTGAAGTCCAGAAACCCCAATGGGGTTCTCGTATGAT CAACTAAGGAGCACGTTTCGCACTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTA

7921 ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr
 ACCGGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGCAATCTAC
 TGGGCGACGAACTGAGGTGTCACTGACTCTCGCTGTAGGCATGCCCTCCTCCGTTAGATG

 7981 GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu
 CAATGTTGTGACCTCGACCCCCAAGCCCGCTGGCCCATCAAGTCCCTCACCGAGAGGCTT
 GTACAACACTGGAGCTGGGGTTCTGGGCGCACCGGTAGTTCAGGGAGTGGCTCTCCGAA

 8041 TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg
 TATGTTGGGGCCCTCTTACCAATTCAAGGGGAGAACTGCGGCTATCGCAGGTGCCGC
 ATACAACCCCCGGGAGAAATGGTTAAGTTCCTCCCTCTTGACGCCGATAGCGTCCACGGCG
 (Gly)

 8101 AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg
 GCGAGCGCGTACTGACAACTAGCTGTGGTAACACCCCTCACTGTCTACATCAAGGCCCGG
 CGCTCGCCGCATGACTGTTGATCGACACCATTTGTGGGAGTGAAACGATGTATTCGGGCC

 8161 AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu
 GCAGCCTGTCGAGCCGCGAGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA
 CGTCGGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGAGCACACACCGCTGCTGAAT

 8221 ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr
 GTCGTTATCTGTGAAGCGGGGGTCCAGGAGGACCGCGGAGCCTGAGAGCCTTCACG
 CAGCAATAGACACTTTCGCGCCCCCAGGTCTCTCGCGCCGCTCGGACTCTCGGAAGTGC

FIG. 1V

8281	GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu GAGGCTATGACCAAGTACTCCGCCCCCTGGGACCCCCACAAACCAGAAATACGACTTG CTCCGATACTGGTCCATGAGCGGGGGGACCCCTGGGGGTGTGGTCTTATGCTGAAC
8341	GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg GAGCTCATAACATCATGCTCCTCCAACGTGTCACTCGCCACGACGGCGCTGGAAGAGG CTCGAGTATTGTAGTACGAGGAGGTGCACAGTCAGCGGTGCTGCCGCGACCTTCTCTCC
8401	ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla GTCTACTACCTCACCCGTGACCTACAAACCCCTCGCGAGAGCTGCGTGGGAGACAGCA CAGATGATGGAGTGGCACTGGGATGTTGGGGGAGCGCTCTCGACGCACCCCTCTGTCTCGT
8461	ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp AGACACACTCCAGTCAATTCTCTGGCTAGGCAACATAATCATGTTTGCCCCCACACTGTGG TCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAAACGGGGTGTGACACC
8521	AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu GCGAGGATGATAGTATGATACCCATTTCTTTAGCGTCTTATAGCCAGGACCACTTGAA CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCTGAACTT
8581	GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro CAGGCCCTCGATTGCGAGATCTACGGGGCTGCTACTCCATAGAACCACTTGATCTACCT GTCCGGGAGCTAACGCTCTAGATGCCCGGACGATGAGGTATCTTGGTGAACTAGATGGA
8641	ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACTCCACAGTACTCTCCAGGT GGTAGTAAGTTTCTGAGGTACCGGAGTCCGCGTAAAGTGAGGTGTCAATGAGAGGTCCA

8701 GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp
 GAAATTAATAGGTGGCGCATGCCTCAGAAACTTGGGGTACCGCCCTTGCGAGCTTGG
 CTTAATTATCCACCGGGTACGGAGTCTTTGAACCCCATGGCGGAACGCTCGAACC

 Gly
 8761 ArgHisArgAlaArgSerValArgAlaArgLeuAlaArgGlyGlyArgAlaAlaIle
 AGACACGGGCGGAGCGTCCGCGCTAGGCTTCTGGCCAGAGGAGGCGCTGCCATA
 TCTGTGGCCCCGGCCTCGCAGGCGGATCCGAAGACCGGTCTCCTCCGACGGTAT

 8821 CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLysLeuThrProIleAla
 TGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAGCTCAAACTCACTCCAATAGCG
 ACACCGTTTCATGGAGAAAGTTGACCCCGTCATTCTTGTTCGAGTTTGAGTGAGGTATCGC

 8881 AlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyrSerGlyGlyAspIle
 GCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTCACGGCTGGCTACAGCGGGGAGACATT
 CGCGACCGGTCTGACCTGAACAGGCCGACCAAGTGCCGACCGATGTGCCCCCTCTGTAA

 8941 TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCysLeuLeuLeuAla
 TATCACAGCGTGTCTCATGCCCCGGCCCCGCTGGATCTGGTTTTCCTACTCCTGCTGCT
 ATAGTGTCCACAGAGTACGGGGCGGGCGACCTAGACCAAAACGGATGAGGACGAACGA
 (Pro)

FIG. 1X

FIG. 1Y

AlaGlyValGlyIleTyrLeuLeuProAsnArgOP
9001 GCAGGGGTAGGCATCTACCTCCTCCCCAACCCGATGAAGGTTGGGGTAAACACTCCGGCCT
CGTCCCCCATCCGTAGATGGAGGAGGGGTTGGCTACTTCCAAACCCCATTTGTGAGGCCGGA

() = Heterogeneity due possibly to 5' or 3'-
terminal cloning artefact

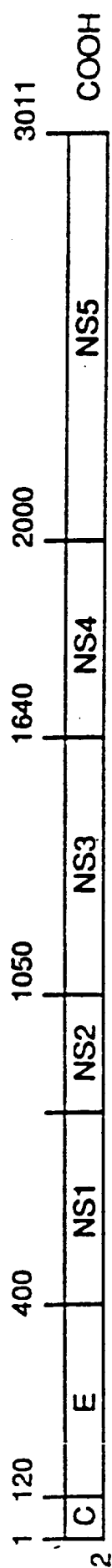


FIG. 2

CLAIMS

1. A combination of hepatitis C virus (HCV) epitope sequences in one or more polypeptides made by chemical synthesis or recombinant expression, immobilized on the surface of a solid matrix suitable for detection of HCV by immunoassay, comprising:

(a) a first epitope sequence from the C domain of the HCV polyprotein;

(b) a second epitope sequence from a second domain of the HCV polyprotein which domain is:

- (i) the NS3 domain of the HCV polyprotein;
- (ii) the NS4 domain of the HCV polyprotein; or
- (iii) the NS5 domain of the HCV polyprotein; and

(c) a third epitope sequence from a third domain of the HCV polyprotein which domain is;

- (i) the NS3 domain of the HCV polyprotein;
- (ii) the NS4 domain of the HCV polyprotein; or
- (iii) the NS5 domain of the HCV polyprotein;

wherein the third domain is different from the second domain.

2. A combination according to claim 1 wherein the second domain is NS3.
3. A combination according to claim 1 wherein the second domain is NS4.
4. A combination according to claim 1 which comprises:
 - (a) a first epitope sequence from the C domain of the HCV polyprotein;
 - (b) a second epitope sequence from the NS3 domain of the HCV polyprotein; and
 - (c) a third epitope sequence from the NS4 domain of the HCV polyprotein;
5. A combination according to any one claims 1 to 4 wherein the solid matrix is the surface of a microtiter plate well, a bead or dipstick.
6. A combination according to any one claims 1 to 5 wherein the first, second and third epitope sequences are contained in first, second and third polypeptides respectively individually bound to the solid matrix.
7. A combination according to claim 6 wherein the solid matrix is a dipstick and the polypeptides are distributed individually in a pattern such that binding to the first, second and third polypeptides may be discerned.
8. The combination to any one of claims 1 to 5 wherein the combination is in the form of a fusion polypeptide.

9. A method for detecting antibodies to hepatitis C virus (HIV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the combination of any one of claims 1 to 8 under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said polypeptide epitope sequences.

10. A kit for carrying out an assay for detecting antibodies to hepatitis C antigen (HCV) in a mammalian body component suspected of containing said antibodies comprising in packaged combination:

- (a) the combination of HCV epitope sequences of any of claims 1 to 9;
- (b) standard control reagents; and
- (c) instructions for carrying out the assay.

COMBINATIONS OF HEPATITIS C VIRUS (HCV) ANTIGENS
FOR USE IN IMMUNOASSAYS FOR ANTI-HCV ANTIBODIES

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Abstract of the Disclosure

Combinations of HCV antigens that have a broader
range of immunological reactivity than any single HCV
antigen. The combinations consist of an antigen from the
10 C domain of the HCV polyprotein, and at least one ad-
ditional HCV antigen from either the NS3 domain, the NS4
domain, the S domain, or the NS5 domain, and are in the
form of a fusion protein, a simple physical mixture, or
the individual antigens commonly bound to a solid matrix.

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