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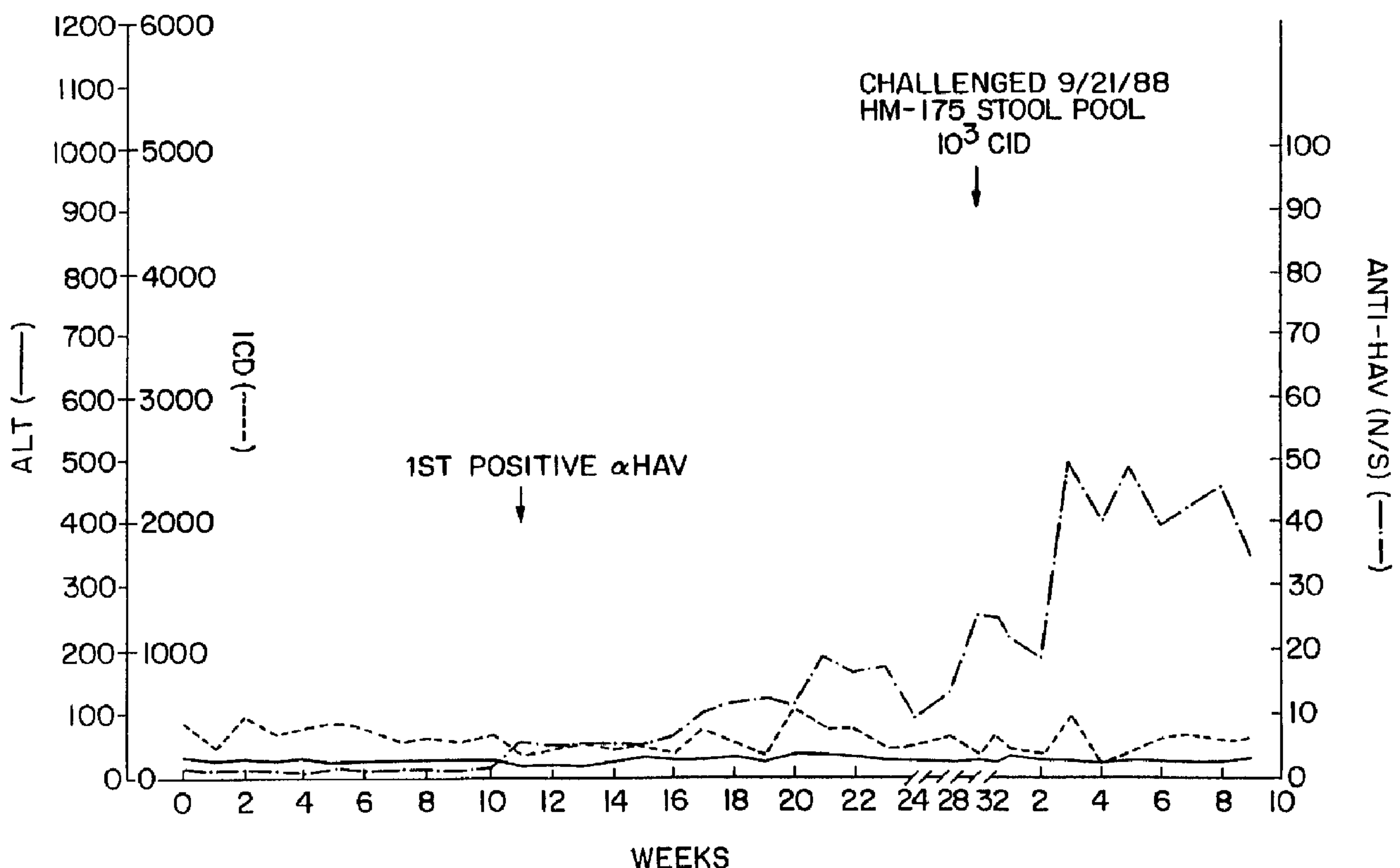
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(57) Abrégé/Abstract:

A live hepatitis A virus adapted to growth in MRC-5 cells, which HAV is preferably characterized by suitable attenuation for effective vaccine administration to humans and animals without inactivation, methods for adapting HAV to growth in MRC-5, vaccine compositions and method of vaccinating humans against HAV infection.

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HEPATITIS A VIRUS VACCINES

ABSTRACT

A live hepatitis A virus adapted to growth in MRC-5 cells, which HAV is preferably characterized by suitable attenuation for effective vaccine administration to humans and animals without inactivation, methods for adapting HAV to growth in MRC-5, vaccine compositions and method of vaccinating humans against HAV infection.

HEPATITIS A VIRUS VACCINES

Field of the Invention

The present invention relates generally to the field of vaccinal compositions useful in the prophylaxis of hepatitis A.

5 More specifically, the invention provides a novel live hepatitis A virus (HAV) and recombinant and chimeric HAVs, the genomes of which are modified from that of their parental strain HM-175 to provide them with the ability to propagate in MRC-5 cells and retain appropriate attenuation for use as live vaccines in humans
10 and other primates.

Background of the Invention

In the United States, hepatitis A virus is the cause of approximately 25% of all clinical hepatitis cases, accounting for approximately 150,000 such cases. Populations at high risk of
15 acquiring hepatitis A in industrialized countries include the socially disadvantaged, medical personnel, military personnel, staff and adult contacts of children in day-care centers, male homosexuals, drug addicts and travellers to endemic areas.

In developing countries, virtually the entire population
20 is infected with hepatitis A virus at an early age. Much of this infection results in subclinical and inapparent infection, but, as countries improve their hygienic conditions, infection with hepatitis A virus

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occurs at progressively older ages, resulting in a higher proportion of clinical disease. Thus, there is a paradoxical increase in clinical hepatitis A as the overall rate of infection diminishes. To successfully immunize against hepatitis A in the United States and in other industrialized countries as well as in developing countries, it will be necessary to vaccinate the entire pediatric population. There will be an increasing need for hepatitis A vaccines in such countries for the foreseeable future.

Research in HAV vaccines has focused on inactivated, or killed, viruses. However, in vaccine therapy there are several advantages to a live vaccine, rather than an inactivated, vaccine. With a live vaccine, one can use a lower dosage and smaller number of doses, because a live vaccine replicates in the vaccinee to produce more antigen and can stimulate the immune system of the vaccinee to make both IgA and IgM. Inactivated vaccines, such as the Salk polio vaccine, which stimulates production of IgG only in vaccinees, do not protect against infection by ingested virus, only against disease.

A major obstacle to the development of live, attenuated vaccine has been the difficulty in adapting HAV to a cell line that supports rapid viral growth and is licensed for vaccine production. Wild type hepatitis A virus (HAV) grows poorly in cell culture.

U. S. Patent Nos. 4,532,215 and 4,636,469 describe, respectively, a strain of HAV designated HM-175 initially isolated from human feces of a patient in Melbourne, Australia, and adapted to passage in vitro in African green monkey kidney (AGMK) culture cells and methods for obtaining same by serial passaging. U. S. Patent No. 4,620,978 describes a vaccine employing the

HAV HM-175, triply cloned in AGMK cell culture and attenuated. U.S. Patent No. 4,894,228 describes HM-175 Pass 35, which differs from wild-type HM-175 by nucleotide changes in the genome, is attenuated for chimpanzees, elicits serum neutralizing antibodies and is suitable for use as an attenuated HAV vaccine. It discloses the complete nucleotide sequence of HAV, strain HM-175/7. See, also, B.C. Ross et al, J. Gen. Virol., 70:2805 - 2810 (1989); R.W. Jansen et al, Virol., 163:299 - 307 (1988) and Tedeschi et al, J. Med. Virol., (in press). The disclosures of these patents and articles may be referred to for further details.

N. Fineschi et al, J. Hepatol., 13(4):S146 - S151 (1991) describes an HAV isolate, LSH/S, which is a candidate for an inactivated vaccine. It was adapted to grow in human diploid MRC-5 cells, a preferred licensed cell for vaccine development. This document compares only a small part of its nucleotide sequence to that of wild-type HM-175.

Provost et al, J. Med. Virol., 20:165 - 175 (1986) described the F and F' variants of the CR326 hepatitis A virus strain. While it is reported to be immunogenic in volunteer vaccinees, the F variant also caused abnormal serum ALT levels in a substantial proportion of individuals.

Another recent publication from this group of investigators has described further work with the F' variant [K. Midthun et al, J. Infect. Dis., 163:735 - 739 (1991)]. They observed that the immunogenicity of the F' vaccine product is dose dependent, i.e. a $10^{7.3}$ TCID₅₀ evoked an antibody response in 100% of volunteers within 9 weeks after immunization whereas lower doses were immunogenic in a smaller percentage of volunteers and anti-HAV was observed 4 to 6 months after immunization.

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Chinese investigators have recently described studies of a potential live attenuated hepatitis A vaccine prepared from the H2 strain of HAV [J. S. Mao et al, J. Infect. Dis., 159:621-624 (1989)]. Twelve volunteers received the vaccine by the subcutaneous route.

A live attenuated hepatitis A vaccine could have a significant impact on the eradication of the disease. It could be anticipated that a live attenuated vaccine which requires minimal purification and no adjuvant would be less costly than presently available inactivated hepatitis A vaccines.

There is a need in the art for methods and compositions for effective vaccination of humans and animals against hepatitis A.

Summary of the Invention

In one aspect, the present invention provides a live hepatitis A virus adapted to growth in MRC-5 cells. This virus is preferably characterized by attenuation. The attenuated virus may be recombinant or chimeric. More preferably, the HAV is characterized by suitable attenuation for effective vaccine administration to primates, preferably humans, without inactivation. The HAV may be characterized by containing one or more of fourteen specific nucleotides which differ from nucleotides in the same position in the genome of HAV HM-175, Pass 35 [SEQ ID NO:1].

In another aspect, the invention provides a vaccine useful for protecting humans or other primates against hepatitis A which vaccine contains at least one above-described HAV adapted to growth in MRC-5 cells. Preferably, the vaccine is effective in inducing a protective antibody response without adjuvant.

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In still another aspect, the invention provides a method for protecting humans against hepatitis A virus infection which comprises administering to the human patient an effective amount of a vaccine composition of this invention.

In a further aspect, the invention provides a method for preparing a live HAV adapted to growth in MRC-5 cells by incorporating into a selected area of the genome of an HAV one or more of fourteen specific nucleotides. The HAV genome so modified is preferably HAV HM-175, Pass 35 or a related cell culture-adapted mutant.

In another embodiment, the HAV may be constructed using another HAV cDNA clone and inserting appropriate nucleotides into its genome. According to this method an attenuated, MRC-5-adapted HAV is provided without requiring further passaging in MRC-5 or other primate cell lines.

In still another aspect, the invention provides polynucleotide sequences encoding the recombinant or chimeric HAVs described above. Preferably these sequences are cDNAs useful as master seeds for vaccine preparation.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. These results were obtained from a chimpanzee that was infected with the attenuated HAV at time 0 and challenged with virulent virus at week 28.

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Fig. 2 is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. The conditions were the same as for Fig. 1.

Fig. 3 is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. The conditions were the same as for Fig. 1.

Fig. 4 is a graph plotting anti-HAV antibody production vs. time (weeks) vs. ALT and ICD levels for the chimpanzee studies with Virus 4380 and subsequent challenge with wild-type HAV, as described in Example 1. These results were obtained from the chimpanzee that was not infected with the attenuated HAV, and therefore developed hepatitis following challenge with the virulent virus. The conditions were the same as for Fig. 1.

Fig. 5 is a bar graph of endpoint dilutions of several of the chimeric viruses, Viruses #2, 3, 4, 5, and 6, listed in Table VI.

Detailed Description of the Invention

The present invention provides hepatitis A virus (HAV) adapted to growth in the human fibroblast-like cell line, MRC-5, a cell substrate suitable for commercial production and licensing of inactivated and live, attenuated hepatitis A vaccines. In addition to such adapted HAVs, the invention provides a method for adapting a selected HAV to growth in that human cell line and preparing an MRC-5-adapted, attenuated HAV without

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passaging in other primate cells. The HAV of this invention and the preparative method also preferably provides the HAV with sufficient attenuation to enable its efficacy as a vaccine for humans and animals.

5 Although the prior art discloses other candidate vaccine strains of hepatitis A virus which have been adapted to growth in human diploid fibroblasts, the genetic changes in the virus genome necessary and
10 sufficient for such adaptation have not been characterized. Thus, these strains cannot be manipulated *in vitro* to assure a reproducible and fully-characterized vaccine product.

 The present invention is based on the wild-type HAV, strain HM-175, which is described in detail in the
15 above-cited and incorporated art [Cohen et al., J. Virol., 61:50-59 (1987); SEQ ID NO:1 and 2]. Briefly described, the wild type, infectious HAV HM-175 virus was previously adapted to growth in primary African green monkey kidney (AGMK) cells at 37°C. After 26 passages in
20 AGMK, the virus was cloned three times in AGMK cells by serial dilution, then passaged three more times to provide passage 32 (P-32). P-32 was found to be attenuated as described in R. A. Karron et al, J. Infec. Dis., 157:338-345 (1988).

25 The P-32 virus described above was passaged three more times in AGMK, and molecularly cloned. The virus that was cloned was called P-35 and the full-length clone was referred to as pHAV/7. pHAV/7 is an infectious cDNA clone of the virus that can be maintained in a
30 monoclonal state and amplified at will with diminished risk of spontaneous mutations. The resulting P-35 virus grew well in fetal rhesus monkey kidney (FRhK) cells and minimally in human fibroblastoid lung cells (MRC-5).

U.S. Patent 4,894,228 and Cohen et al, Proc. Natl. Acad. Sci., USA, 84:2497 - 2501 (1987) provide the HAV nucleotide sequences of wild-type HAV strain HM-175 (see Fig. 1 of the patent; SEQ ID NO:1 and 2) and the nucleotide differences between HAV HM-175, Pass 35, clone pHAV/7 and the wild-type sequence [SEQ ID NO:1]. Thus, these documents, which may be reviewed for further details, provide the sequence of pHAV/7, P-35 [SEQ ID NO:1 and 2]. The nucleotide numbers used herein to which the mutations of this invention correspond (Tables I and VI below) are the nucleotide numbers assigned to positions of the wild-type sequence of Fig. 1 [SEQ ID NO:1 and 2] from U.S. Patent 4,894,228 containing the mutations for P-35. Note that the nucleotides deleted in P-35 are assigned the nucleotide position of the wild-type sequence [SEQ ID NO:1]. Thus, for example, nucleotide position 131 represents a nucleotide that was deleted between wild-type and P-35. The P-35 cDNA, i.e. HAV/HM-175/7, is on deposit at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland under Accession No. 67495, deposited August 7, 1987. One of skill in the art can readily construct the nucleotide and amino acid sequences of P-35 by use of the above-cited art.

Thereafter the P-32 AGMK cell-adapted and attenuated virus was further manipulated to enable it to be adapted for growth in MRC-5 cells, so that it is available for large scale vaccine production. Passage 32 was double plaque cloned in MRC-5 to form Passage 37. Passage 37 was passaged once in MRC-5 of a selected clone 25-4-21. The resulting Passage 38 was passaged three times in MRC-5 cells, resulting in Passage 41, the master seed, designated 87J19. This master seed virus stock was also called virus 4380 and is referred to throughout this disclosure by the latter name.

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Live attenuated virus HAV 4380, was deposited on April 4, 1990 at the Collection Nationale de Cultures de Microorganismes, Institut Pasteur, 25, rue du Docteur Roux, 75724, Paris CEDEX 15 under Accession No. I-936.

5 This deposit was made under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms and has not been publicly disseminated. HAV 4380 is a cell culture-adapted and attenuated strain of hepatitis A virus strain HM-175, adapted to growth in
10 a human fibroblast cell line (MRC-5) suitable for vaccine development by incubation at a reduced temperature of 32-35°C. Growth of the virus is determined by detection of viral antigen in a serological assay. The adapted virus is purified by plaque-purification, using an accepted
15 method (radioimmunofocus assay).

After a total of nine passages in MRC-5 cells at reduced temperature, the resultant virus was characterized for its biological characteristics in cell culture and in two primate species that are considered to
20 be surrogates for man, i.e., marmosets and chimpanzees. See, e.g., Example 1 below. The HAV 4380 virus was found to be temperature-sensitive (i.e., only grew at reduced temperatures) in MRC-5 cells but was still capable of growing at 37°C in primary African green monkey kidney
25 cells. The virus was further attenuated in virulence, compared to the parent virus HM-175, P-32, when tested in chimpanzees and marmoset monkeys, in which species the virus replicated poorly or not at all. This reduced capacity for replication in primates was further
30 confirmed in human volunteers, as described in Example 2.

A candidate inactivated hepatitis A vaccine was prepared from the HAV 4380 and demonstrated to be safe (i.e., it does not produce hepatitis or other serious adverse effects) and immunogenic in humans. It was also
35 found to induce antibody production without adjuvant.

HAV 4380, as it currently exists, grows well in a cell substrate suitable for commercial vaccine production. It also does not infect human beings when administered by the oral or intravenous route at doses of up to 10^7 tissue culture infectious doses, even when not inactivated. HAV 4380 is suitable for use as a live HAV vaccine in humans. However, as indicated in Example 2, vaccine 4380 is believed to be somewhat over-attenuated, because it is not infectious, which characteristic reduces its efficiency when used as an attenuated vaccine.

In order to produce other vaccine candidates which are maximized for desirable levels of attenuation and good growth in MRC-5 cells, the inventors discovered genetic changes that occurred in the genome of the MRC-5-adapted HAV 4380 virus that altered its growth characteristics and made it more suitable for vaccine production than the related AGMK-adapted virus HM-175, Passage 35. The discovery of the following mutations in the nucleotide sequences in 4380, when compared to HM-175 Pass 35 [Cohen et al, cited above; and U. S. Patent No. 4,894,228, Fig. 1; SEQ ID NO:1 and 2], permit the manipulation of the HAV genome by genetic engineering techniques.

Thus, knowledge of the genomic differences between the AGMK-adapted passages of HM-175 and the more attenuated 4380 permit the construction of chimeric viruses having the improved growth characteristics, i.e., rapid and efficient growth in MRC-5 cell culture, but with a level of attenuation of virulence for primate species, including man, that will permit the virus to replicate efficiently without producing hepatitis or other untoward effects. This invention permits the design of a chimeric HAV that can achieve the optimum characteristics for a candidate live-attenuated hepatitis

A vaccine. Such a virus will also permit the design of preferred inactivated vaccine candidates, if desired. The present invention identifies the mutations that are believed to have occurred during adaptation to growth of the HM-175 HAV, passage 32, strain in MRC-5 cells. One or a combination of these mutations are responsible for MRC-5 cell adaptation and overattenuation in HAV 4380. The nucleotide sequence of the MRC-5 cell-adapted virus HAV 4380 was compared with that of the AGMK-adapted, HM-175 virus, passage 35, clone 7. Nucleotide consensus sequences were determined directly from polymerase chain reaction products.

The inventors have discovered that there are at least fourteen unique nucleotide differences between the pass-35 HM-175/7 virus [SEQ ID NO:1] and the MRC-5-adapted virus 4380, at least twelve of which mutations apparently occurred during the period of adaptation of the Pass 32 hepatitis A virus (HAV), strain HM-175 [SEQ ID NO:1] to growth in MRC-5 cells. Table I lists these twelve mutations by nucleotide differences and resulting amino acid (AA) differences, if any, acquired by the MRC-5-adapted virus HAV 4380. The other two mutations appear to be back-mutations to wild-type sequence, or more likely, to represent mutations that occurred between passages 32 and 35 in AGMK cells, and that therefore represent differences between the biologically characterized P-32 virus and the molecularly cloned P-35 virus. Note that the partial sequence of LSH/S HAV of Fineschi et al, cited above, overlaps with only the mutation observed at position 5145.

In the Table, A represents adenine, G represents guanine, C represents cytosine, and U represents uridine or thymine; Leu represents leucine, Phe represents phenylalanine, Ile represents isoleucine, Val represents valine and Ser represents serine.

 TABLE I

Nucleotide Changes Occurring During
 Adaptation of HM-175 in MRC-5 Cells:
Comparison with sequence of HM-175/7 (P-35)

	<u>Nucleotide Change</u>	<u>Region of Genome</u>	<u>AA Change</u>
	591 A to G	5' nc	NA
	646 G to A	5' nc	NA
10	669 C to U	5' nc	NA
	687 U to G	5' nc	NA
	2864 U to A	VP1	No change
	3934 A to G	2B	No change
	4418 A to U	2C	Leu to Phe
15	4643 A to U	2C	No Change
	5145 A to G	3A	Ile to Val
	5745 A to U	3C	Thr to Ser
	6216 U to C	3D	No change
	6908 U to C	3D	No change

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 New HAV vaccine candidates are designed by introducing one or more of these nucleotides into an HAV at a nucleotide position homologous to the nucleotide position in the genomic sequence of the AGMK-adapted virus HM-175, Pass 35 [SEQ ID NO:1]. These nucleotides identified in Table I may be introduced at analogous and/or homologous nucleotide positions to those of P-35 in the genomic sequences of other HAV strains and variants to produce a recombinant or chimeric HAV of this invention. By the phrase "analogous or homologous nucleotide position" is meant a nucleotide in an HAV other than HAV HM-175, Pass 35 [SEQ ID NO:1] which is present in the same viral region, e.g., 2C, 3D and the like, at a position in that region similar to that of the nucleotide of Table I. In other words, the nucleotide position may differ in position number due to deletions in other regions of the virus; but one of skill in the art can readily determine its functional similarity to the nucleotide position in HM-175, Pass 35 [SEQ ID NO:1].

While such nucleotide positions may not have the identical nucleotide position numbers corresponding to the wild-type HM-175 [SEQ ID NO:1], it is anticipated that these analogous and/or homologous positions can be readily identified to enable HAVs other than strain HM-175 derivatives to be modified to create novel HAVs according to this invention.

Similarly, the inventors are able to manipulate the genome of a progenitor or intermediate of HAV 4380 with resort to this knowledge and can thereby 'reverse' certain mutations in 4380 to create new chimeric HAV viruses. One or more of these nucleotides, or varying combinations thereof, can be incorporated, by chimera formation or oligonucleotide-directed mutagenesis, into an HAV strain, most readily the cDNA clone HAV/HM-175/7, to produce new viable virus which has acquired the ability to grow in MRC-5 cells. Other HM-175 HAV derivatives are available from the American Type Culture Collection under ATCC designation numbers VR 2089, VR 2090, VR 2091, VR 2092, VR 2093, VR 2097, VR 2098, and VR 2099. These and other HAVs may be employed to derive desired HAVs of this invention. Since there are indications that the MRC-5-adapted virus 4380 may be over-attenuated for humans, it is important to be able to remove or introduce selected mutations into HM-175. The construction of nine exemplary chimeric viruses containing one or more such mutations is described in detail in Example 3 below.

The mutagenic and genetic engineering techniques employed to construct chimeric or recombinant HAVs which incorporate one or more of these mutations are conventional and known to those of skill in the art [see, for example, Sambrook et al., Molecular Cloning. A Laboratory Manual, 2d edition, Cold Spring Harbor

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Laboratory, Cold Spring Harbor, New York, (1989)]. Other conventional techniques, including polymerase chain reactions and chemical synthetic techniques may also be used to design HAVs of this invention. Similarly, it is anticipated that homologous mutations may be made using other HM-175 passages. It also may be possible to adapt similar changes to HAV strains other than HM-175 by introducing these nucleotides into homologous regions.

Chimeric and recombinant viruses of this invention may be designed by application of similar techniques and selecting one or more different combinations of the nucleotides (mutations) appearing in Tables I and VI. For example, data from growth analyses of the chimeric viruses of Example 3 demonstrate that one or more of the four MRC-5 specific mutations in the 5' non-coding region (mutations at nucleotide positions 591, 646, 669, and 687 of HM-175/7) and one or both of the MRC-5 specific mutations in the 2C region (mutations at nucleotide positions 4418 and 4643 of HM-175/7) may be desirable for optimal growth of the virus in MRC-5 cells. Other mutations may also be involved. Specific exemplary chimeric HAVs of this invention are characterized by the mutations in the genome of HAV HM-175/7 that appear in viruses designated #2 through #10 in Table VI of Example 3 below.

HAVs of this invention may be characterized by the presence of one or more of these nucleotides of Tables I or VI in analogous genomic positions of HAV HM-175 derivatives or other HAV strains. HAVs of this invention may also be characterized by two or more such nucleotides, where one nucleotide in the HAV parent strain is a guanine (G) at position 5145 of pHAV/7 or the analogous position of another HAV strain.

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It is further anticipated that additional mutations may appear in a few regions of HAV that have yet to be sequenced. The mutations appearing in Table I may be incorporated in any combination, and/or with other mutations yet to be identified to construct a number of chimeric or recombinant HAVs with desired characteristics for use as live HAV vaccines.

Additional chimeras and recombinant viruses constructed by oligonucleotide-directed mutagenesis may be designed and evaluated for assessment of the individual effects of the mutations and combinations thereof on viral growth in MRC-5 cells and on adaptation to growth in selected cell culture. The attenuation phenotype of these chimeric viruses may be evaluated in marmosets or chimpanzees by techniques such as described below in Example 1 for HAV 4380.

Also provided by this invention are the polynucleotide sequences encoding the HAVs of this invention. Such polynucleotide sequences are preferably cDNA sequences, which can form a master seed for the HAV vaccine. A cDNA sequence of this invention comprises a DNA sequence encoding a selected HAV genome characterized by the presence of one or more of the nucleotides identified as the twelve mutations in Table I in any desired combination which imparts desired characteristics to the novel HAV. Such cDNAs may be obtained by conventional techniques known to those of skill in the art. See, e.g., Sambrook et al, cited above, and U. S. Patent No. 4, 894, 228.

Thus, the present invention provides a live vaccine composition useful in protecting against HAV infection and a prophylactic method entailing administering to a primate, preferably a human, an

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effective amount of such a composition. This vaccine composition may contain one or more of the HAVs of the invention, including HAV 4380, as well as the chimeric and recombinant HAVs described herein. The vaccine
5 composition may also contain mixtures of two or more of the HAVs, if desired.

A vaccinal composition may be formulated to contain a carrier or diluent and one or more of the HAVs of the invention. Suitable pharmaceutically acceptable
10 carriers facilitate administration of the viruses but are physiologically inert and/or nonharmful. Carriers may be selected by one of skill in the art. Exemplary carriers include sterile saline, lactose, sucrose, calcium
phosphate, gelatin, dextrin, agar, pectin, peanut oil,
15 olive oil, sesame oil, and water. Additionally, the carrier or diluent may include a time delay material, such as glycerol monostearate or glycerol distearate alone or with a wax. In addition, slow release polymer formulations can be used.

20 Optionally, the vaccine composition may further contain preservatives, chemical stabilizers, other antigenic proteins, and conventional pharmaceutical ingredients. Suitable ingredients which may be used in a vaccinal composition in conjunction with the viruses
25 include, for example, casamino acids, sucrose, gelatin, phenol red, N-Z amine, monopotassium diphosphate, lactose, lactalbumin hydrolysate, and dried milk. Typically, stabilizers, adjuvants, and preservatives are optimized to determine the best formulation for efficacy
30 in the target human or animal. Suitable preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, parachlorophenol.

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A vaccine composition of this invention is most preferably produced without an adjuvant. However, where necessary, one or more of the above described vaccine components may be admixed or adsorbed with a conventional adjuvant. The adjuvant is used as a non-specific irritant to attract leukocytes or enhance an immune response. Such adjuvants include, among others, mineral oil and water, aluminum hydroxide, Amphigen, Avridine, L121/squalene, D-lactide-poly lactide/glycoside, pluronic plyois, muramyl dipeptide, killed *Bordetella*, saponins, and Quil A.

Alternatively, or in addition to the HAV of the invention, other agents useful in treating HAV infection, e.g., immunostimulatory agents, are expected to be useful in reducing and eliminating disease symptoms. The development of vaccine or therapeutic compositions containing these agents is within the skill of one of skill in the art in view of the teaching of this invention.

According to the method of the invention, a human or an animal may be vaccinated against HAV infection by administering an effective amount of a vaccine composition described above. An effective amount is defined as that amount of HAV vaccine capable of inducing protection in the vaccinee against HAV infection and/or against hepatitis. The vaccine may be administered by any suitable route. Such a composition may be administered parenterally, preferably intramuscularly or subcutaneously. However, it may also be formulated to be administered by any other suitable route, including orally.

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Suitable effective amounts of the HAVs of this invention can be determined by one of skill in the art based upon the level of immune response desired. Such a composition may be administered once, and/or a booster
5 may also be administered. However, suitable dosage adjustments may be made by the attending physician or veterinarian depending upon the age, sex, weight and general health of the human or animal patient.

Similarly, suitable doses of the vaccine
10 composition of the invention can be readily determined by one of skill in the art. The dosage can be adjusted depending upon the human patient or the animal species being treated, i.e. its weight, age, and general health.

The following examples illustrate the preferred
15 methods for obtaining HAVs of the invention and using them as vaccine compositions. These examples are not intended to limit the scope thereof.

Example 1: Test of MRC-5-Adapted HAV 4380 Vaccine in
Marmosets and Chimpanzees

20 The attenuation of hepatitis A virus (HAV), strain HM-175, by serial passage in cell culture has previously been demonstrated. Following 32 passages in primary AGMK cells, the virus was completely attenuated for chimpanzees and almost completely attenuated for
25 marmosets. Subsequently, according to this invention, the virus was adapted to growth in MRC-5 cells and recloned by plaque purification.

HAV 4380 was prepared from Volunteer lot 87J19, passage level 41 of strain HM-175 HAV that was derived
30 from previously characterized passage levels of the virus that have also been prepared as volunteer pools. Two such earlier passage pools were shown to be attenuated

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for chimpanzees and marmosets. However, neither was administered to volunteers because it was recognized that primary African green monkey kidney cells, the substrate for those volunteer pools, would not be available in sufficient quantities to produce an economically viable vaccine. Therefore, the virus was adapted to MRC-5 cells and further passaged to prepare volunteer lot 87J19 or HAV 4380.

The purpose of this experiment is to test the level of attenuation of this virus for marmosets and chimpanzees, prior to phase I trials in volunteers. Lot 87J19 was tested for safety and immunogenicity in four chimpanzees and four Saguinus mystax marmosets. Two additional marmosets served as uninoculated controls. The chimpanzees used in this study were bred and raised in captivity; the marmosets were wild-caught animals. An inoculum of 10^4 TCID₅₀ of candidate vaccine lot 87J19 was administered intravenously to each animal. Residual inoculum was frozen and the titer reconfirmed subsequently in two different laboratories.

According to the experimental protocol, marmosets identified by the arbitrary ID numbers 570, 572, 566, and 575 and chimpanzees identified by the arbitrary ID numbers 1300, 1333, 1309 and 1313 received an inoculum of HAV 4380 from Clone 25-4-21, Lot 87 J 19575, 17/11/87 at a dose and route of administration of 10^3 dilution/1ml/I.V. Marmosets No. 541 and 578 received a diluent at a dose and route of 1ml/I.V.

A. Infection: Three of four chimpanzees and one of four marmosets were infected, as determined by development of anti-HAV detectable by commercial radioimmunoassay (HAVAB, Abbott Laboratories, Chicago, IL). The chimpanzees seroconverted ten to eleven weeks following inoculation; the single marmoset seroconversion

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occurred eight weeks following inoculation. This marmoset subsequently died on week eleven of the study and another, noninfected, marmoset subsequently died on week fourteen, but neither death was attributable to the inoculum.

All three chimpanzees that seroconverted also developed IgM anti-HAV. Two of these, Chimp 1309 and Chimp 1313, developed IgM anti-HAV on weeks ten and thirteen, respectively, when tested by the standard HAVAB-M (Abbott Laboratories, Chicago, IL) at a final serum dilution of 1:4,000. When sera were tested at a dilution of 1:40, Chimp 1313 and Chimp 1333 seroconverted at weeks nine and five, respectively. The HAVAB-M test is a capture assay utilizing anti-human IgM and has not been standardized for use with sera from primates less closely related to man than to the chimpanzee. For this reason, the marmosets were not tested for IgM anti-HAV.

B. Biochemistry: Biochemical evidence of hepatitis was monitored by weekly determinations of serum alanine amino transferase (ALT) and isocitric dehydrogenase (ICD). The former is the most reliable indirect means of diagnosing hepatitis in the chimpanzee and the latter is comparably sensitive for evaluating marmosets. None of the chimpanzees or marmosets had elevation of liver enzymes attributable to the inoculum. All values for chimpanzees were within normal limits. The only infected marmoset, number 582, had normal liver enzymes up to the time of its death. Marmosets 566, 570, and 578 each had one or more abnormal liver enzyme values, but the first two of these animals were not infected by the inoculum, as judged by failure to seroconvert, and the third was an uninoculated negative control.

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5 Marmosets often have less stable liver enzyme values than chimpanzees, in part because they are, by nature, relatively fragile animals and because they are jungle-caught and therefore usually infected with a variety of endo- and ecto-parasites, including microfilaria.

10 C. Histology: Histologic sections prepared from serial weekly liver biopsies obtained from the chimpanzees and marmosets were evaluated under code for histopathologic changes. Although some animals had a high base-line of histopathologic changes, none of the animals had evidence of histopathologic changes more severe than those seen in preinoculation biopsies. Equally important, there were no histologic changes that were temporally related to seroconversion in infected animals. The two marmosets that died were subjected to more extensive evaluation. Both animals had evidence of systemic disease that was probably etiologically related to their deaths, but histologic changes in the liver were diagnostic of chronic, not acute, disease and therefore not related to the inoculum.

20 A comparison of histopathologic changes observed in chimpanzees and marmosets with these various volunteer pools and wild-type virus was performed. See
25 Tables II and III below.

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Table II
HISTOPATHOLOGY: CHIMPANZEES

5	<u>Inoculum</u> [*]	<u># Inoc.</u>	<u># Infected</u>	<u>Liver Histopathology</u>	
				<u>#</u>	<u>Range of Severity</u> ^{**}
	W.T.	4	4	4	± - 3+
	P-21	6	6	1	0 - 3+
	P-32	6	6	0	0
10	MRC-5	4	3	0	0

^{*} Dose: 10^3 - 10^5 ID₅₀ IV
^{**} Scale of 0-3+

15

Table III
HISTOPATHOLOGY: MARMOSETS

20	<u>Inoculum</u> [*]	<u># Inoc.</u>	<u># Infected</u>	<u>Liver Histopathology</u>	
				<u>#</u>	<u>Range of Severity</u> ^{**}
	W.T.	4	4	4	1+ - 2+
	P-21	8	8	8	1+ - 3+
	P-32	5	5	3	0 - 2+
	MRC-5	4	1	0	0

25

^{*} Dose: 10^3 - 10^5 ID₅₀ IV
^{**} Scale of 0-3+

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Lot 87J19 appears to be more attenuated than the other volunteer pools or wild-type virus, based upon infectivity and severity of histopathologic changes.

5 D. Immunofluorescence: Serial snap-frozen
liver biopsies obtained from infected animals were
evaluated for expression of viral antigen by
immunofluorescence. Only one animal, Chimp 1313, was
definitely but weakly positive for intrahepatic viral
antigen. This animal was positive for only one week.
10 These results were compared with those obtained in the
previous study of other volunteer pools and wild-type
virus. As seen in Tables IV and V, intrahepatic
replication was further diminished in both chimpanzees
and marmosets when compared with the AGMK-grown virus and
15 wild-type virus.

Table IV

VIRAL REPLICATION IN THE LIVER:
MARMOSETS (IMMUNOFLUORESCENCE)

20	<u>Inoculum</u>		<u># Inoc.</u>		<u># Infected</u>		<u>Mean</u>	<u>Mean</u>
							<u>Peak</u>	<u>Duration (wks)</u>
	W.T.		4		4		2.5+	12.2
	P-21		8		8		1+	3.5
	P-32		5		5		<1+	2.4
25	MRC-5		4		1		0	0

* Dose: 10^3 - 10^5 ID₅₀ I.V.

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Table V

VIRAL REPLICATION IN THE LIVER:
CHIMPANZEES (IMMUNOFLUORESCENCE)

5				Mean Peak	Mean Duration (wks)
	<u>Inoculum*</u>	<u># Inoc.</u>	<u># Infected</u>		
	W.T.	4	4	1+	1.8
	P-21	6	6	<1+	0.5
	P-32	6	6	<1+	0.6
10	MRC-5	4	3	<1+	0.3

* Dose: 10^5 - 10^5 ID₅₀ I.V.

15 E. Protection: Although the single infected marmoset on study died, all four chimpanzees were available for challenge with wild-type parent HAV to determine if the levels of anti-HAV present in infected animals were protective. Consequently, the three infected and one uninfected animal were challenged with approximately 10^3 chimpanzee infectious doses of wild-type HM-175 strain HAV (human stool suspension), administered intravenously (Fig. 1 through 4).

20 All three previously infected chimpanzees were protected against type A hepatitis, as measured by persistently normal serum enzyme values (Figs. 1 through 3). All three protected animals had an anamnestic antibody response to the challenge virus, suggesting that there was limited replication. In contrast, the previously uninfected chimpanzee developed high enzyme values diagnostic of hepatitis following challenge with

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wild-type virus (Fig. 4). Thus, volunteer pool 87J19 produced an inapparent infection in chimpanzees that stimulated protection against subsequent challenge with virulent wild-type virus.

5 The results of these portions of the experiment demonstrate that volunteer pool 87J19 of HAV 4380, strain HM-175 (adapted to MRC-5 cells) was significantly more attenuated for chimpanzees and marmosets than its parent, HAV, strain HM-175 (AGMK, Pass-32). It is clear from
10 these studies that HAV 4380, strain HM-175 volunteer pool 87J19, is highly attenuated for chimpanzees and marmosets which are accepted surrogates for man in the study of hepatitis A viruses.

Example 2: Clinical study of volunteers.

15 In this clinical trial, volunteers received increasing titers of the live attenuated hepatitis A vaccine 4380, volunteer pool 87J19, which was previously tested in chimpanzees and marmosets as described in
20 Example 1. These pre-clinical studies demonstrated that the vaccine was safe, immunogenic, and efficacious in experimental animal models.

 Volunteers were admitted to a closed clinical ward at the United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. Eight
25 volunteers received the live attenuated hepatitis A vaccine (1ml) by the oral route in the following manner: two received a 10^4 TCID₅₀ dose, two a 10^5 TCID₅₀ dose, two a 10^6 TCID₅₀ dose, and two a 10^7 TCID₅₀ dose. Six volunteers received the vaccine by the intramuscular route in the
30 deltoid area in the following manner: 2 received a 10^5 TCID₅₀ dose, 2 a 10^6 TCID₅₀ dose and 2 a 10^7 TCID₅₀ dose.

Each volunteer remained on the ward for three days after immunization. Local or systemic side effects were monitored during the admission period and for 12 weeks following the immunization. Volunteers were asked
5 to return at 6 and 12 months for serological follow-up.

Sera were obtained prior to immunization and once a week for the next 12 weeks. In volunteers who completed the appropriate follow-up time, sera were also obtained at 6 and 12 months after initial administration
10 of vaccine. Serum specimens were tested for alanine aminotransferase (ALT) and antibody to hepatitis A. ALT was tested with a Kodak EKTATM Chem 700XR analyzer (Rochester, NY); normal values were 0 to 50 IU/ml. Antibody to hepatitis A was tested by four different
15 methods, including a commercial radioimmunoassay (HAVAB, Abbott Laboratories, N. Chicago, IL). Second, an enzyme-linked immunoassay developed by SmithKline Beecham (SKB-ELISA), which was more sensitive than the standard HAVAB, in which a level of ≥ 20 milli-International Units (mIU)
20 was considered positive. Selected sera were tested by the RIFIT (radioimmunofocus) assay for neutralizing antibody to hepatitis A. With this test, a serum titer of $\geq 1:10$ was considered positive [S. M. Lemon et al, J. Infect. Dis., 148:1033-1039 (1983)]. Finally sera were
25 tested for IgM anti-HAV by commercial radioimmunoassay (HAVAB-M, Abbott Laboratories, N. Chicago, IL).

Stools were collected from the volunteers two to three times per week for the first 12 weeks and were tested for the presence of hepatitis A virus by
30 radioimmunoassay [R. H. Purcell et al, J. Immunol., 116:349-356 (1976)] and molecular biology techniques [J. Ticehurst et al, J. Clin. Microbiol., 25:1822-1829 (1987)].

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All volunteers remained healthy during the follow-up period (14 weeks to one year). No systemic complaints were present immediately after immunization or during long-term follow-up. Serum alanine aminotransferase levels remained normal in all 14 individuals during the period of observation.

Antibody to hepatitis A was not observed in any of the eight volunteers who received the vaccine by the oral route or in the two volunteers who received the 10^5 TCID₅₀ dose by the intramuscular route. The four volunteers who received higher doses of vaccine (10^6 TCID₅₀ or 10^7 TCID₅₀) all had detectable antibody by the SKB ELISA as early as 3 weeks after immunization. Detectable levels persisted for the 12 weeks of observation.

Selected sera tested for neutralizing antibody had titers ranging from 1:10 to 1:40 in a volunteer who received a 10^6 dose and 1:40 to 1:2560 in a volunteer who received a 10^7 TCID₅₀ dose. The commercial HAVAB assay detected anti-HAV in only one of the volunteers, who received the 10^7 dose. IgM anti-HAV was not detected in any of the volunteers who received the vaccine orally. Sera from volunteers who received 10^7 TCID₅₀ I.M. had detectable IgM anti-HAV.

Stools from all volunteers who received the oral vaccine were negative for hepatitis A virus, while those from volunteers who had received the vaccine by intramuscular route are in the process of being tested.

Although only a small number of volunteers received the vaccine orally, it appeared that the vaccine is not immunogenic by this route. This is likely due to over-attenuation of the virus, although other causes, such as inactivation in the gastrointestinal tract or too small an inoculum, should be considered. The vaccine was safe and immunogenic by the intramuscular route at doses

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of 10^6 and 10^7 TCID₅₀. The antibody response was prompt: anti-HAV was observed within 3 weeks of immunization, persisted during the period of observation, and did not diminish in titer. Such a response to one single
5 inoculation of a preparation which lacked an adjuvant, is remarkable. If indeed, anti-HAV persists for a long time after one dose, the logistics of administration of this product would be much simpler and more successful than with present hepatitis A vaccines. The presence of IgM
10 anti-HAV in volunteers who received 10^7 TCID₅₀ without evidence of hepatitis is suggestive of asymptomatic replication of the virus.

Example 3: Construction of Chimeric Viruses

Several exemplary chimeric viruses were
15 generated to evaluate the effect of several of the mutations of Table I on host range and/or attenuation in primates. The sequence of the MRC-5-adapted virus 4380 was obtained using reverse-transcriptase:polymerase chain reaction (RT:PCR) to amplify regions of the virus as cDNA
20 prior to sequencing (hence T instead of U in Table VI below). Numbers 2-10 in Table VI designate chimeric viruses made by inserting mutations found in the MRC-5-adapted virus 4380 into the cDNA clone of pHAV/7 encoding the attenuated HM-175 virus, Pass 35, of Cohen et al., J. Virol., 61:3035-3039 (1987) [SEQ ID NO:1 and 2].
25 Mutations introduced by "chimera" means a portion of the 4380 virus genome was amplified by RT:PCR, digested with specific restriction enzymes and the fragment used to replace the homologous fragment in the cDNA clone pHAV/7.
30 Mutations introduced by mutagenesis were inserted by oligonucleotide-directed mutagenesis of the cDNA clone pHAV/7 using the Amersham mutagenesis protocol.

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The chimeric cDNAs were transcribed into RNA *in vitro* and the nucleic acids (both RNA and DNA) transfected into FRhK-4 cells to generate chimeric viruses. Quantification of chimeric virus growth for the exemplary chimeras was performed by slot-blot assay.

Table VI reports the results of the construction and testing of nine chimeric viruses. As used in Table VI, the following terms are defined: Cell culture refers to virus containing indicated mutations selected by growth in MRC-5 cells. Mutagenesis refers to oligonucleotide-directed mutagenesis of P-35 or HM-175 cDNA clones. A chimeric viral genome refers to the construction of a chimeric viral genome using portions of P-35 cDNA clone and PCR-generated fragments of the MRC-5 cell-adapted virus 4380. ND means that this study has not yet been performed. The + symbol refers to virus that has some growth in that cell type. The - symbol refers to virus that has little or no growth in that cell type. The two cell types employed to test the growth of the chimeric viruses are the human lung fibroblast-like cell line MRC-5 and fetal rhesus monkey kidney epithelial-like cell line, FRhK-4. Note that Virus #1 in the Table refers to MRC-5-adapted HAV 4380. Viruses # 2 through 10 are chimeric viruses of this invention.

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TABLE VI

Differences in Nucleotide Sequence of
MRC-5-Adapted hepatitis A Virus:
Comparison with P-35 HM-175 Virus

5	Nucleotide Differences from P-35 HM-175	Method Mutation Introduced	Growth of Mutated Virus in Cell Cultures	
			<u>FRhK-4</u>	<u>MRC-5</u>
10	Virus #1 (MRC-5-adapted)	Cell Culture	+	+
	591 A to G			
	646 G to A			
	669 C to T			
15	687 T to G			
	2864 T to A			
	3196 G to A			
	3934 A to G			
	4418 A to T			
20	4643 A to T			
	5145 A to G			
	5745 A to T			
	6216 T to C			
	6908 T to C			
25	7032 C to T			
	Virus #2	Chimera	+	+
	591 A to G			
	646 G to A			
	669 C to T			
30	687 T to G			
	Virus #3	Chimera	+	+
	124 C to T			
	131 d to T			
	132 d to T			
35	133 d to T			
	134 d to G			
	152 G to A			
	203-207 d to T			
	591 A to G			
40	646 G to A			
	669 C to T			
	687 T to G			

45	d = Base at this position deleted in P-35 compared to wild-type			

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TABLE VI (cont)

Differences in Nucleotide Sequence of
MRC-5-Adapted hepatitis A Virus
Comparison with P-35 HM-175 Virus

5	Nucleotide Differences from P-35 HM-175	Method Mutation Introduced	Growth of Mutated Virus in Cell Culture	
			<u>FRhK-4</u>	<u>MRC-5</u>
	Virus #4	Chimera	+	+
10	591 A to G 646 G to A 669 C to T 687 T to G 4418 A to T			
15	4643 A to T			
	Virus #5	Chimera	+	+
	124 C to T 131 d to T 132 d to T 133 d to T 134 d to T 152 G to A 203-207 d to T			
20	591 A to G 646 G to A 669 C to T 687 T to G 4418 A to T 4643 A to T			
25				
30	Virus #6	Chimera	+	-
	4418 A to T 4643 A to T			
	Virus #7	Chimera Mutagenesis	+	ND
35	591 A to G 4418 A to T 4643 A to T			
40	----- d = Base at this position deleted in P-35 compared to wild-type			

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TABLE VI (cont)

Differences in Nucleotide Sequence of
MRC-5-Adapted hepatitis A Virus
Comparison with P-35 HM-175 Virus

	Nucleotide Differences from P-35 HM-175	Method Mutation Introduced	Growth of Mutated Virus in Cell Culture	
			<u>FRhK-4</u>	<u>MRC-5</u>
5				
10	Virus #8 646 G to A 4418 A to T 4643 A to T	Chimera Mutagenesis	+	ND
15	Virus #9 669 C to T 4418 A to T 4643 A to T	Chimera Mutagenesis	+	ND
20	Virus #10 687 T to G 4418 A to T 4643 A to T	Chimera Mutagenesis	+	ND

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Introduction of four mutations found in the 5' noncoding region, at nucleotide positions 591, 646, 669, and 687 of the P-35 genome, appear to be important for HAV host range in cell culture. They allow some growth of the transfected virus in MRC-5 cells, but do not account entirely for MRC-5 cell culture adaptation. Introduction of two mutations, at nucleotides 4418 and 4643 in the 2C region of the MRC-5-adapted virus, with the 5' mutations allow full growth in MRC-5 cells. Thus the four mutations in the 5' noncoding region and the two mutations in the 2C region of the genome of the MRC-5 cell-adapted virus appear to act synergistically in the adaptation of this virus to efficient growth in MRC-5 cells. Introduction of only the two mutations in the 2C region into the P-35 AGMK genome does not permit discernible growth of the transfected virus in MRC-5 cells.

Example 4: Comparison of End Point Dilution of Chimeric Viruses in FRhK-4 Versus MRC-5 Cells

Chimeric viruses with the composition described in Table VI were serially diluted in ten-fold increments, and an equal aliquot of each dilution was plated onto FRhK-4 and MRC-5 cells. After 21 days incubation at 34.5°C to permit virus growth, the cells were lysed by the addition of a buffer solution containing sodium dodecyl sulfate. The viral RNA was extracted with phenol and quantified by slot blot hybridization using a [32p]-labeled riboprobe specific for Hepatitis A virus. A radioautograph of the slot blot obtained from the FRhK-4 cells and from the MRC-5 cells illustrates that the endpoint dilution of the MRC-5-adapted virus was the same

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in both cell lines, indicating that this virus can grow in either cell line. In contrast, the P35 HM-175 virus had an endpoint dilution of 10^{-5} on FRhK-4 cells and $< 10^{-1}$ on MRC-5 cells, demonstrating that this virus is unable to grow successfully on MRC-5 cells. As Fig. 5 illustrates, virus #6 was most like the pass 35 virus while virus #4 was most like the MRC-5 adapted virus and viruses #2, 3, and 5 were intermediate. These results show that certain mutations from the MRC-5-adapted virus can be introduced into the pHAV/7 cDNA clone to generate new viruses which also have acquired the ability to grow in MRC-5 cells.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7493 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 735..7415

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCAAGAGGG GTCTCCGGGA ATTTCCGGAG TCCCTCTTGG AAGTCCATGG	50
TGAGGGGACT TGATACCTCA CCGCCGTTTG CCTAGGCTAT AGGCTAAATT	100
TTCCCTTTCC CTTTTCCTT TCCTATTCCC TTTGTTTTGC TTGTAAATAT	150
TAATTCCTGC AGG TTCAGG TTCTTAAATC TGTTTCTCTA TAAGAACACT	200
CATTTTTCAC GCTTTCTGTC TTCTTTCTTC CAGGGCTCTC CCCTTGCCCT	250
AGGCTCTGGC CGTTGCGCCC GGCGGGGTCA ACTCCATGAT TAGCATGGAG	300
CTGTAGGAGT CTAAATTGGG GACACAGATG TTTGGAACGT CACCTTGCAG	350
TGTTAACTTG GCTTTCATGA ATCTCTTTGA TCTTCCACAA GGGGTAGGCT	400
ACGGGTGAAA CCTCTTAGGC TAATACTTCT ATGAAGAGAT GCCTTGGATA	450
GGGTAACAGC GGCGGATATT GGTGAGTTGT TAAGACAAAA ACCATTCAAC	500
GCCGGAGGAC TGACTCTCAT CCAGTGGATG CATTGAGTGG ATTGACTGTC	550
AGGGCTGTCT TTAGGCTTAA TTCCAGACCT CTCTGTGCTT AGGGCAAACA	600
TCATTTGGCC TTAAATGGGA TTCTGTGAGA GGGGATCCCT CCATTGACAG	650
CTGGACTGTT CTTTGGGGCC TTATGTGGTG TTTGCCTCTG AGGTACTCAG	700
GGGCATTTAG GTTTTTCCTC ATTCTTAAAT AATA ATG AAC ATG TCT AGA	749
	Met Asn Met Ser Arg
	1 5
CAA GGT ATT TTC CAG ACT GTT GGG AGT GGT CTT GAC CAC ATC	791
Gln Gly Ile Phe Gln Thr Val Gly Ser Gly Leu Asp His Ile	
10 15	

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CTG Leu 20	TCT Ser	TTG Leu	GCA Ala	GAC Asp	ATT Ile 25	GAG Glu	GAA Glu	GAG Glu	CAA Gln	ATG Met 30	ATT Ile	CAA Gln	TCA Ser	833
GTT Val	GAT Asp 35	AGG Arg	ACT Thr	GCA Ala	GTG Val	ACT Thr 40	GGT Gly	GCT Ala	TCT Ser	TAT Tyr	TTT Phe 45	ACT Thr	TCT Ser	875
GTG Val	GAT Asp	CAA Gln 50	TCT Ser	TCA Ser	GTT Val	CAT His 55	ACA Thr	GCT Ala	GAG Glu	GTT Val	GGA Gly 60	TCA Ser	CAC His	917
CAG Gln	GTT Val	GAA Glu	CCT Pro 65	TTG Leu	AGA Arg	ACC Thr	TCT Ser	GTT Val 70	GAT Asp	AAA Lys	CCC Pro	GGT Gly	TCA Ser 75	959
AAG Lys	AAG Lys	ACT Thr	CAG Gln	GGA Gly 80	GAG Glu	AAA Lys	TTT Phe	TTC Phe	TTG Leu 85	ATT Ile	CAT His	TCT Ser	GCA Ala	1001
GAT Asp 90	TGG Trp	CTT Leu	ACT Thr	ACA Thr	CAT His 95	GCT Ala	CTT Leu	TTC Phe	CAT His	GAA Glu 100	GTT Val	GCA Ala	AAA Lys	1043
TTG Leu 105	GAT Asp	GTG Val	GTG Val	AAA Lys	TTA Leu 110	TTA Leu	TAC Tyr	AAT Asn	GAG Glu	CAG Gln	TTT Phe 115	GCT Ala	GTT Val	1085
CAA Gln 120	GGG Gly	TTG Leu	TTG Leu	AGA Arg	TAC Tyr	CAT His	ACA Thr 125	TAT Tyr	GCA Ala	AGA Arg	TTT Phe	GGC Gly 130	ATT Ile	1127
GAA Glu	ATT Ile	CAA Gln 135	GTT Val	CAG Gln	ATA Ile	AAC Asn	CCT Pro	ACA Thr 140	CCT Pro	TTC Phe	CAA Gln	CAG Gln	GGG Gly 145	1169
GGA Gly	TTG Leu	ATC Ile	TGT Cys	GCT Ala 150	ATG Met	GTT Val	CCT Pro	GGT Gly	GAC Asp 155	CAG Gln	AGC Ser	TAT Tyr	GGT Gly	1211
TCT Ser 160	ATA Ile	GCA Ala	TCA Ser	TTG Leu	ACT Thr 165	GTT Val	TAT Tyr	CCT Pro	CAT His	GGT Gly 170	TTG Leu	TTA Leu	AAT Asn	1253
TGC Cys 175	AAT Asn	ATT Ile	AAC Asn	AAT Asn	GTG Val	GTT Val 180	AGA Arg	ATA Ile	AAG Lys	GTT Val	CCA Pro 185	TTT Phe	ATT Ile	1295
TAC Tyr	ACA Thr 190	AGA Arg	GGT Gly	GCT Ala	TAC Tyr	CAC His	TTT Phe 195	AAA Lys	GAT Asp	CCA Pro	CAA Gln	TAC Tyr 200	CCA Pro	1337

GTT	TGG	GAA	TTG	ACA	ATT	AGA	GTT	TGG	TCA	GAA	TTA	AAT	ATT	1379
Val	Trp	Glu	Leu	Thr	Ile	Arg	Val	Trp	Ser	Glu	Leu	Asn	Ile	
			205					210					215	
GGG	ACA	GGA	ACT	TCA	GCT	TAT	ACT	TCA	CTC	AAT	GTT	TTA	GCT	1421
Gly	Thr	Gly	Thr	Ser	Ala	Tyr	Thr	Ser	Leu	Asn	Val	Leu	Ala	
				220					225					
AGA	TTT	ACA	GAT	TTG	GAG	TTG	CAT	GGA	TTA	ACT	CCT	CTT	TCT	1463
Arg	Phe	Thr	Asp	Leu	Glu	Leu	His	Gly	Leu	Thr	Pro	Leu	Ser	
230					235					240				
ACA	CAA	ATG	ATG	AGA	AAT	GAA	TTT	AGG	GTC	AGT	ACT	ACT	GAG	1505
Thr	Gln	Met	Met	Arg	Asn	Glu	Phe	Arg	Val	Ser	Thr	Thr	Glu	
	245					250					255			
AAT	GTG	GTG	AAT	CTG	TCA	AAT	TAT	GAA	GAT	GCA	AGA	GCA	AAG	1547
Asn	Val	Val	Asn	Leu	Ser	Asn	Tyr	Glu	Asp	Ala	Arg	Ala	Lys	
	260						265					270		
ATG	TCT	TTT	GCT	TTG	GAT	CAG	GAA	GAT	TGG	AAA	TCT	GAT	CCG	1589
Met	Ser	Phe	Ala	Leu	Asp	Gln	Glu	Asp	Trp	Lys	Ser	Asp	Pro	
			275					280					285	
TCC	CAG	GGT	GGT	GGG	ATC	AAA	ATT	ACT	CAT	TTT	ACT	ACT	TGG	1631
Ser	Gln	Gly	Gly	Gly	Ile	Lys	Ile	Thr	His	Phe	Thr	Thr	Trp	
				290					295					
ACA	TCT	ATT	CCA	ACT	TTG	GCT	GCT	CAG	TTT	CCA	TTT	AAT	GCT	1673
Thr	Ser	Ile	Pro	Thr	Leu	Ala	Ala	Gln	Phe	Pro	Phe	Asn	Ala	
300					305					310				
TCA	GAC	TCA	GTT	GGT	CAA	CAA	ATT	AAA	GTT	ATT	CCA	GTT	GAC	1715
Ser	Asp	Ser	Val	Gly	Gln	Gln	Ile	Lys	Val	Ile	Pro	Val	Asp	
	315					320					325			
CCA	TAT	TTT	TTC	CAA	ATG	ACA	AAT	ACG	AAT	CCT	GAC	CAA	AAA	1757
Pro	Tyr	Phe	Phe	Gln	Met	Thr	Asn	Thr	Asn	Pro	Asp	Gln	Lys	
		330					335					340		
TGT	ATA	ACT	GCT	TTG	GCT	TCT	ATT	TGT	CAG	ATG	TTT	TGT	TTT	1799
Cys	Ile	Thr	Ala	Leu	Ala	Ser	Ile	Cys	Gln	Met	Phe	Cys	Phe	
			345					350					355	
TGG	AGA	GGA	GAT	CTT	GTC	TTT	GAT	TTT	CAA	GTT	TTT	CCC	ACC	1841
Trp	Arg	Gly	Asp	Leu	Val	Phe	Asp	Phe	Gln	Val	Phe	Pro	Thr	
				360					365					
AAA	TAT	CAT	TCA	GGT	AGA	TTA	CTG	TTT	TGT	TTT	GTT	CCT	GGC	1883
Lys	Tyr	His	Ser	Gly	Arg	Leu	Leu	Phe	Cys	Phe	Val	Pro	Gly	
370					375					380				

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AAT Asn 385	GAG Glu	CTA Leu	ATA Ile	GAT Asp	GTT Val	TCT Ser	GGA Gly	ATC Ile	ACA Thr	TTA Leu	AAG Lys	CAA Gln	GCA Ala	1925
ACT Thr	ACT Thr	GCT Ala 400	CCT Pro	TGT Cys	GCA Ala	GTA Val	ATG Met 405	GAT Asp	ATT Ile	ACA Thr	GGA Gly	GTG Val 410	CAG Gln	1967
TCA Ser	ACT Thr	TTG Leu	AGA Arg 415	TTT Phe	CGT Arg	GTT Val	CCC Pro	TGG Trp 420	ATT Ile	TCT Ser	GAC Asp	ACT Thr	CCT Pro 425	2009
TAC Tyr	AGA Arg	GTG Val	AAC Asn	AGG Arg 430	TAT Tyr	ACA Thr	AAG Lys	TCA Ser	GCA Ala	CAT His	CAG Gln	AAA Lys	GGT Gly	2051
GAG Glu 440	TAC Tyr	ACT Thr	GCC Ala	ATT Ile	GGG Gly 445	AAG Lys	CTT Leu	ATT Ile	GTG Val	TAT Tyr 450	TGT Cys	TAT Tyr	AAC Asn	2093
AGA Arg 455	TTG Leu	ACC Thr	TCT Ser	CCT Pro	TCT Ser	AAC Asn 460	GTT Val	GCT Ala	TCC Ser	CAT His	GTC Val 465	AGA Arg	GTG Val	2135
AAT Asn	GTT Val 470	TAT Tyr	CTT Leu	TCA Ser	GCA Ala	ATT Ile	AAC Asn 475	TTG Leu	GAA Glu	TGT Cys	TTT Phe	GCT Ala 480	CCT Pro	2177
CTT Leu	TAT Tyr	CAT His	GCT Ala 485	ATG Met	GAT Asp	GTT Val	ACT Thr	ACA Thr 490	CAA Gln	GTT Val	GGA Gly	GAT Asp	GAT Asp 495	2219
TCT Ser	GGA Gly	GGT Gly	TTT Phe	TCA Ser 500	ACA Thr	ACA Thr	GTT Val	TCT Ser	ACA Thr 505	GAA Glu	CAG Gln	AAT Asn	GTT Val	2261
CCA Pro 510	GAT Asp	CCC Pro	CAA Gln	GTT Val	GGT Gly 515	ATA Ile	ACA Thr	ACC Thr	ATG Met	AAA Lys 520	GAT Asp	TTG Leu	AAA Lys	2303
GGA Gly 525	AAA Lys	GCT Ala	AAC Asn	AGA Arg	GGG Gly 530	AAA Lys	ATG Met	GAT Asp	GTT Val	TCA Ser	GGA Gly 535	GTA Val	CAA Gln	2345
GCA Ala	CCT Pro 540	GTG Val	GGA Gly	GCT Ala	ATC Ile	ACA Thr	ACA Thr 545	ATT Ile	GAG Glu	GAT Asp	CCA Pro	GTT Val 550	TTA Leu	2387
GCA Ala 555	AAG Lys	AAA Lys	GTA Val	CCT Pro	GAG Glu	ACA Thr	TTT Phe	CCT Pro 560	GAA Glu	TTG Leu	AAA Lys	CCT Pro	GGA Gly 565	2429

GAA	TCC	AGA	CAT	ACA	TCA	GAT	CAT	ATG	TCC	ATC	TAC	AAG	TTT	2471
Glu	Ser	Arg	His	Thr	Ser	Asp	His	Met	Ser	Ile	Tyr	Lys	Phe	
				570					575					
ATG	GGA	AGG	TCT	CAT	TTC	TTG	TGC	ACT	TTT	ACA	TTC	AAT	TCA	2513
Met	Gly	Arg	Ser	His	Phe	Leu	Cys	Thr	Phe	Thr	Phe	Asn	Ser	
580					585					590				
AAT	AAT	AAA	GAG	TAC	ACA	TTT	CCT	ATA	ACC	TTG	TCT	TCA	ACC	2555
Asn	Asn	Lys	Glu	Tyr	Thr	Phe	Pro	Ile	Thr	Leu	Ser	Ser	Thr	
595						600					605			
TCT	AAT	CCT	CCT	CAT	GGT	TTG	CCA	TCA	ACA	CTG	AGG	TGG	TTT	2597
Ser	Asn	Pro	Pro	His	Gly	Leu	Pro	Ser	Thr	Leu	Arg	Trp	Phe	
		610					615					620		
TTC	AAC	TTG	TTT	CAG	TTG	TAT	AGA	GGG	CCT	TTA	GAT	CTG	ACA	2639
Phe	Asn	Leu	Phe	Gln	Leu	Tyr	Arg	Gly	Pro	Leu	Asp	Leu	Thr	
			625					630					635	
ATT	ATT	ATT	ACA	GGA	GCA	ACT	GAT	GTA	GAT	GGC	ATG	GCC	TGG	2681
Ile	Ile	Ile	Thr	Gly	Ala	Thr	Asp	Val	Asp	Gly	Met	Ala	Trp	
				640					645					
TTC	ACT	CCA	GTA	GGT	CTT	GCC	GTT	GAT	ACT	CCT	TGG	GTA	GAG	2723
Phe	Thr	Pro	Val	Gly	Leu	Ala	Val	Asp	Thr	Pro	Trp	Val	Glu	
650					655					660				
AAG	GAG	TCA	GCT	TTG	TCT	ATT	GAC	TAC	AAA	ACT	GCT	CTT	GGA	2765
Lys	Glu	Ser	Ala	Leu	Ser	Ile	Asp	Tyr	Lys	Thr	Ala	Leu	Gly	
	665					670					675			
GCT	GTC	AGA	TTT	AAC	ACA	AGG	AGA	ACA	GGG	AAC	ATT	CAG	ATT	2807
Ala	Val	Arg	Phe	Asn	Thr	Arg	Arg	Thr	Gly	Asn	Ile	Gln	Ile	
		680					685					690		
AGA	TTA	CCA	TGG	TAT	TCT	TAT	TTA	TAT	GCT	GTG	TCT	GGA	GCA	2849
Arg	Leu	Pro	Trp	Tyr	Ser	Tyr	Leu	Tyr	Ala	Val	Ser	Gly	Ala	
			695				700						705	
CTG	GAT	GGT	TTG	GGT	GAC	AAG	ACA	GAT	TCT	ACA	TTT	GGA	TTG	2891
Leu	Asp	Gly	Leu	Gly	Asp	Lys	Thr	Asp	Ser	Thr	Phe	Gly	Leu	
				710					715					
GTT	TCT	ATT	CAG	ATT	GCA	AAT	TAC	AAT	CAT	TCT	GAT	GAA	TAC	2933
Val	Ser	Ile	Gln	Ile	Ala	Asn	Tyr	Asn	His	Ser	Asp	Glu	Tyr	
720					725					730				
TTG	TCT	TTT	AGT	TGT	TAT	TTG	TCT	GTC	ACA	GAA	CAA	TCA	GAG	2975
Leu	Ser	Phe	Ser	Cys	Tyr	Leu	Ser	Val	Thr	Glu	Gln	Ser	Glu	
	735					740					745			

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TTT	TAT	TTT	CCC	AGA	GCT	CCA	TTG	AAC	TCA	AAT	GCC	ATG	TTA	3017
Phe	Tyr	Phe	Pro	Arg	Ala	Pro	Leu	Asn	Ser	Asn	Ala	Met	Leu	
		750					755					760		
TCC	ACT	GAA	TCA	ATG	ATG	AGC	AGA	ATT	GCA	GCT	GGA	GAC	TTG	3059
Ser	Thr	Glu	Ser	Met	Met	Ser	Arg	Ile	Ala	Ala	Gly	Asp	Leu	
			765					770					775	
GAG	TCA	TCA	GTG	GAT	GAT	CCT	AGA	TCA	GAG	GAA	GAT	AAA	AGA	3101
Glu	Ser	Ser	Val	Asp	Asp	Pro	Arg	Ser	Glu	Glu	Asp	Lys	Arg	
				780					785					
TTT	GAG	AGT	CAT	ATA	GAA	TGC	AGG	AAG	CCA	TAT	AAA	GAA	CTG	3143
Phe	Glu	Ser	His	Ile	Glu	Cys	Arg	Lys	Pro	Tyr	Lys	Glu	Leu	
790					795					800				
AGA	TTA	GAA	GTT	GGG	AAA	CAA	AGA	CTC	AAG	TAT	GCT	CAG	GAA	3185
Arg	Leu	Glu	Val	Gly	Lys	Gln	Arg	Leu	Lys	Tyr	Ala	Gln	Glu	
	805					810					815			
GAA	TTG	TCA	AAT	GAA	GTA	CTT	CCA	CCC	CCT	AGG	AAA	ATG	AAG	3227
Glu	Leu	Ser	Asn	Glu	Val	Leu	Pro	Pro	Pro	Arg	Lys	Met	Lys	
		820					825					830		
GGA	CTG	TTT	TCA	CAA	GCC	AAA	ATT	TCT	CTT	TTT	TAT	ACT	GAG	3269
Gly	Leu	Phe	Ser	Gln	Ala	Lys	Ile	Ser	Leu	Phe	Tyr	Thr	Glu	
			835					840					845	
GAG	CAT	GAA	ATA	ATG	AAG	TTT	TCC	TGG	AGA	GGT	GTG	ACT	GCT	3311
Glu	His	Glu	Ile	Met	Lys	Phe	Ser	Trp	Arg	Gly	Val	Thr	Ala	
				850					855					
GAT	ACT	AGA	GCT	TTA	AGG	AGG	TTT	GGA	TTC	TCT	TTG	GCC	GCA	3353
Asp	Thr	Arg	Ala	Leu	Arg	Arg	Phe	Gly	Phe	Ser	Leu	Ala	Ala	
860					865					870				
GGC	AGA	AGT	GTG	TGG	ACT	CTT	GAA	ATG	GAT	GCT	GGG	GTT	CTT	3395
Gly	Arg	Ser	Val	Trp	Thr	Leu	Glu	Met	Asp	Ala	Gly	Val	Leu	
	875					880					885			
ACT	GGG	AGA	CTG	ATT	AGA	TTG	AAT	GAT	GAG	AAA	TGG	ACA	GAA	3437
Thr	Gly	Arg	Leu	Ile	Arg	Leu	Asn	Asp	Glu	Lys	Trp	Thr	Glu	
		890					895					900		
ATG	AAG	GAT	GAC	AAG	ATT	GTT	TCA	TTG	ATT	GAA	AAG	TTT	ACA	3479
Met	Lys	Asp	Asp	Lys	Ile	Val	Ser	Leu	Ile	Glu	Lys	Phe	Thr	
			905					910					915	
AGT	AAC	AAA	TAT	TGG	TCC	AAA	GTG	AAT	TTC	CCA	CAT	GGG	ATG	3521
Ser	Asn	Lys	Tyr	Trp	Ser	Lys	Val	Asn	Phe	Pro	His	Gly	Met	
				920					925					

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TTG	GAT	CTT	GAA	GAA	ATT	GCT	GCC	AAT	TCT	AAG	GAT	TTT	CCT	3563
Leu	Asp	Leu	Glu	Glu	Ile	Ala	Ala	Asn	Ser	Lys	Asp	Phe	Pro	
930					935					940				
AAC	ATG	TCT	GAA	ACG	GAT	TTG	TGT	TTC	TTG	CTG	CAT	TGG	TTA	3605
Asn	Met	Ser	Glu	Thr	Asp	Leu	Cys	Phe	Leu	Leu	His	Trp	Leu	
945						950					955			
AAT	CCA	AAG	AAA	ATT	AAT	TTA	GCA	GAT	AGA	ATG	CTT	GGA	TTG	3647
Asn	Pro	Lys	Lys	Ile	Asn	Leu	Ala	Asp	Arg	Met	Leu	Gly	Leu	
		960					965					970		
TCT	GGA	GTT	CAG	GAA	ATT	AAA	GAA	CAA	GGT	GTT	GGA	TTA	ATA	3689
Ser	Gly	Val	Gln	Glu	Ile	Lys	Glu	Gln	Gly	Val	Gly	Leu	Ile	
			975					980					985	
GCA	GAG	TGT	AGA	ACT	TTC	TTA	GAT	TCT	ATT	GCT	GGA	ACT	TTA	3731
Ala	Glu	Cys	Arg	Thr	Phe	Leu	Asp	Ser	Ile	Ala	Gly	Thr	Leu	
				990					995					
AAA	TCT	ATG	ATG	TTT	GGA	TTT	CAT	CAT	TCT	GTG	ACT	GTT	GAA	3773
Lys	Ser	Met	Met	Phe	Gly	Phe	His	His	Ser	Val	Thr	Val	Glu	
1000					1005					1010				
ATT	ATA	AAC	ACT	GTG	CTC	TGT	TTT	GTT	AAG	AGT	GGA	ATT	TTG	3815
Ile	Ile	Asn	Thr	Val	Leu	Cys	Phe	Val	Lys	Ser	Gly	Ile	Leu	
	1015				1020						1025			
CTT	TAT	GTA	ATA	CAA	CAA	TTG	AAT	CAG	GAT	GAA	CAT	TCT	CAC	3857
Leu	Tyr	Val	Ile	Gln	Gln	Leu	Asn	Gln	Asp	Glu	His	Ser	His	
		1030					1035					1040		
ATA	ATT	GGT	TTG	TTG	AGA	GTC	ATG	AAT	TAT	GCA	GAT	ATT	GGT	3899
Ile	Ile	Gly	Leu	Leu	Arg	Val	Met	Asn	Tyr	Ala	Asp	Ile	Gly	
			1045					1050					1055	
TGT	TCA	GTT	ATT	TCA	TGT	GGC	AAA	GTT	TTT	TCC	AAA	ATG	CTG	3941
Cys	Ser	Val	Ile	Ser	Cys	Gly	Lys	Val	Phe	Ser	Lys	Met	Leu	
				1060					1065					
GAA	ACA	GTC	TTT	AAT	TGG	CAA	ATG	GAC	TCC	AGA	ATG	ATG	GAG	3983
Glu	Thr	Val	Phe	Asn	Trp	Gln	Met	Asp	Ser	Arg	Met	Met	Glu	
1070					1075					1080				
TTA	AGG	ACT	CAG	AGT	TTT	TCC	AAC	TGG	TTA	AGA	GAT	ATT	TGT	4025
Leu	Arg	Thr	Gln	Ser	Phe	Ser	Asn	Trp	Leu	Arg	Asp	Ile	Cys	
	1085					1090					1095			
TCT	GGG	ATC	ACC	ATT	TTT	AAA	AAC	TTC	AAG	GAT	GCA	ATT	TAT	4067
Ser	Gly	Ile	Thr	Ile	Phe	Lys	Asn	Phe	Lys	Asp	Ala	Ile	Tyr	
		1100				1105						1110		

TGG	CTT	TAT	ACA	AAA	TTA	AAG	GAC	TTT	TAT	GAA	GTG	AAT	TAT	4109
Trp	Leu	Tyr	Thr	Lys	Leu	Lys	Asp	Phe	Tyr	Glu	Val	Asn	Tyr	
			1115					1120					1125	
GGC	AAG	AAG	AAG	GAC	ATT	TTA	AAT	ATT	CTT	AAA	GAT	AAC	CAA	4151
Gly	Lys	Lys	Lys	Asp	Ile	Leu	Asn	Ile	Leu	Lys	Asp	Asn	Gln	
				1130					1135					
CAA	AAA	ATA	GAG	AAA	GCC	ATT	GAG	GAA	GCC	GAT	GAA	TTT	TGC	4193
Gln	Lys	Ile	Glu	Lys	Ala	Ile	Glu	Glu	Ala	Asp	Glu	Phe	Cys	
1140					1145					1150				
ATT	TTG	CAA	ATC	CAA	GAT	GTG	GAA	AAA	TTT	GAA	CAG	TAT	CAG	4235
Ile	Leu	Gln	Ile	Gln	Asp	Val	Glu	Lys	Phe	Glu	Gln	Tyr	Gln	
	1155					1160					1165			
AAA	GGG	GTT	GAC	TTG	ATA	CAA	AAA	TTG	AGA	ACT	GTT	CAT	TCA	4277
Lys	Gly	Val	Asp	Leu	Ile	Gln	Lys	Leu	Arg	Thr	Val	His	Ser	
	1170						1175					1180		
ATG	GCT	CAG	GTT	GAT	CCA	AAT	TTA	ATG	GTT	CAT	TTG	TCA	CCT	4319
Met	Ala	Gln	Val	Asp	Pro	Asn	Leu	Met	Val	His	Leu	Ser	Pro	
			1185					1190					1195	
TTG	AGA	GAT	TGT	ATA	GCA	AGA	GTT	CAT	CAG	AAA	CTT	AAA	AAC	4361
Leu	Arg	Asp	Cys	Ile	Ala	Arg	Val	His	Gln	Lys	Leu	Lys	Asn	
				1200					1205					
CTT	GGA	TCT	ATA	AAT	CAG	GCA	ATG	GTA	ACG	AGA	TGT	GAG	CCA	4403
Leu	Gly	Ser	Ile	Asn	Gln	Ala	Met	Val	Thr	Arg	Cys	Glu	Pro	
1210					1215					1220				
GTT	GTT	TGT	TAT	TTA	TAT	GGC	AAA	AGA	GGG	GGA	GGA	AAG	AGC	4445
Val	Val	Cys	Tyr	Leu	Tyr	Gly	Lys	Arg	Gly	Gly	Gly	Lys	Ser	
	1225					1230					1235			
TTA	ACA	TCA	ATT	GCA	TTG	GCA	ACC	AAA	ATT	TGT	AAA	CAT	TAT	4487
Leu	Thr	Ser	Ile	Ala	Leu	Ala	Thr	Lys	Ile	Cys	Lys	His	Tyr	
			1240				1245					1250		
GGT	GTT	GAG	CCT	GAA	AAG	AAT	ATC	TAT	ACT	AAA	CCT	GTG	GCT	4529
Gly	Val	Glu	Pro	Glu	Lys	Asn	Ile	Tyr	Thr	Lys	Pro	Val	Ala	
			1255					1260					1265	
TCA	GAT	TAC	TGG	GAT	GGA	TAT	AGT	GGA	CAA	TTA	GTT	TGC	ATC	4571
Ser	Asp	Tyr	Trp	Asp	Gly	Tyr	Ser	Gly	Gln	Leu	Val	Cys	Ile	
				1270				1275						
ATT	GAT	GAT	ATT	GGC	CAA	AAC	ACA	ACA	GAT	GAG	GAT	TGG	TCA	4613
Ile	Asp	Asp	Ile	Gly	Gln	Asn	Thr	Thr	Asp	Glu	Asp	Trp	Ser	
1280					1285					1290				

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GAT TTT TGT CAG TTA GTG TCA GGA TGT CCA ATG AGA TTA AAC	4655
Asp Phe Cys Gln Leu Val Ser Gly Cys Pro Met Arg Leu Asn	
1295 1300 1305	
ATG GCC TCT CTT GAG GAG AAG GGT AGG CAT TTT TCT TCT CCT	4697
Met Ala Ser Leu Glu Glu Lys Gly Arg His Phe Ser Ser Pro	
1310 1315 1320	
TTT ATA ATA GCA ACT TCA AAT TGG TCA AAT CCA AGT CCA AAA	4739
Phe Ile Ile Ala Thr Ser Asn Trp Ser Asn Pro Ser Pro Lys	
1325 1330 1335	
ACA GTT TAT GTT AAG GAA GCA ATT GAC CGC AGA CTC CAT TTC	4781
Thr Val Tyr Val Lys Glu Ala Ile Asp Arg Arg Leu His Phe	
1340 1345	
AAG GTT GAA GTT AAA CCT GCT TCA TTT TTC AAA AAT CCT CAC	4823
Lys Val Glu Val Lys Pro Ala Ser Phe Phe Lys Asn Pro His	
1350 1355 1360	
AAT GAT ATG TTG AAT GTT AAT TTA GCT AAA ACA AAT GAT GCA	4865
Asn Asp Met Leu Asn Val Asn Leu Ala Lys Thr Asn Asp Ala	
1365 1370 1375	
ATC AAA GAT ATG TCT TGT GTT GAT TTG ATA ATG GAT GGA CAT	4907
Ile Lys Asp Met Ser Cys Val Asp Leu Ile Met Asp Gly His	
1380 1385 1390	
AAT GTT TCA TTG ATG GAT TTG CTC AGT TCT TTA GTC ATG ACA	4949
Asn Val Ser Leu Met Asp Leu Leu Ser Ser Leu Val Met	
1395 1400 1405	
GTT GAA ATT AGA AAA CAA AAC ATG ACT GAA TTC ATG GAG TTG	4991
Val Glu Ile Arg Lys Gln Asn Met Thr Glu Phe Met Glu Leu	
1410 1415	
TGG TCT CAG GGA ATT TCA GAT GAT GAT AAT GAT AGT GCA GTA	5033
Trp Ser Gln Gly Ile Ser Asp Asp Asp Asn Asp Ser Ala Val	
1420 1425 1430	
GCT GAG TTT TTC CAG TCT TTT CCA TCT GGT GAA CCA TCG AAC	5075
Ala Glu Phe Phe Gln Ser Phe Pro Ser Gly Glu Pro Ser Asn	
1435 1440 1445	
TCT AAA TTA TCT GGC TTT TTC CAA TCT GTT ACT AAT CAC AAG	5117
Ser Lys Leu Ser Gly Phe Phe Gln Ser Val Thr Asn His Lys	
1450 1455 1460	
TGG GTT GCT GTG GGA GCT GCA GTT GGC ATT CTT GGA GTG CTC	5159
Trp Val Ala Val Gly Ala Ala Val Gly Ile Leu Gly Val Leu	
1465 1470 1475	

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GTT	GGA	GGA	TGG	TTT	GTG	TAT	AAG	CAT	TTC	TCC	CGC	AAA	GAG	5201
Val	Gly	Gly	Trp	Phe	Val	Tyr	Lys	His	Phe	Ser	Arg	Lys	Glu	
				1480					1485					
GAG	GAA	CCA	ATC	CCA	GCT	GAA	GGG	GTA	TAT	CAT	GGT	GTA	ACT	5243
Glu	Glu	Pro	Ile	Pro	Ala	Glu	Gly	Val	Tyr	His	Gly	Val	Thr	
1490					1495					1500				
AAG	CCC	AAG	CAA	GTG	ATT	AAA	TTA	GAT	GCA	GAT	CCA	GTA	GAA	5285
Lys	Pro	Lys	Gln	Val	Ile	Lys	Leu	Asp	Ala	Asp	Pro	Val	Glu	
	1505					1510					1515			
TCT	CAG	TCA	ACT	TTG	GAA	ATA	GCA	GGA	CTG	GTT	AGG	AAG	AAC	5327
Ser	Gln	Ser	Thr	Leu	Glu	Ile	Ala	Gly	Leu	Val	Arg	Lys	Asn	
		1520					1525					1530		
TTG	GTT	CAG	TTT	GGA	GTT	GGA	GAG	AAG	AAT	GGA	TGT	GTG	AGA	5369
Leu	Val	Gln	Phe	Gly	Val	Gly	Glu	Lys	Asn	Gly	Cys	Val	Arg	
			1535					1540					1545	
TGG	GTT	ATG	AAT	GCC	TTG	GGA	GTG	AAA	GAT	GAT	TGG	CTG	CTT	5411
Trp	Val	Met	Asn	Ala	Leu	Gly	Val	Lys	Asp	Asp	Trp	Leu	Leu	
				1550					1555					
GTG	CCT	TCC	CAT	GCT	TAT	AAA	TTT	GAG	AAA	GAT	TAT	GAA	ATG	5453
Val	Pro	Ser	His	Ala	Tyr	Lys	Phe	Glu	Lys	Asp	Tyr	Glu	Met	
1560					1565					1570				
ATG	GAG	TTT	TAT	TTT	AAT	AGA	GGT	GGA	ACT	TAC	TAT	TCA	ATT	5495
Met	Glu	Phe	Tyr	Phe	Asn	Arg	Gly	Gly	Thr	Tyr	Tyr	Ser	Ile	
	1575					1580					1585			
TCA	GCT	GGT	AAT	GTT	GTT	ATT	CAA	TCT	TTG	GAT	GTG	GGA	TTC	5537
Ser	Ala	Gly	Asn	Val	Val	Ile	Gln	Ser	Leu	Asp	Val	Gly	Phe	
		1590					1595					1600		
CAG	GAT	GTT	GTT	CTG	ATG	AAG	GTT	CCT	ACA	ATT	CCT	AAG	TTT	5579
Gln	Asp	Val	Val	Leu	Met	Lys	Val	Pro	Thr	Ile	Pro	Lys	Phe	
			1605					1610					1615	
AGA	GAT	ATT	ACT	CAG	CAT	TTT	ATT	AAG	AAA	GGG	GAT	GTG	CCT	5621
Arg	Asp	Ile	Thr	Gln	His	Phe	Ile	Lys	Lys	Gly	Asp	Val	Pro	
				1620					1625					
AGA	GCT	TTG	AAT	CGC	CTG	GCA	ACA	TTA	GTG	ACA	ACT	GTA	AAT	5663
Arg	Ala	Leu	Asn	Arg	Leu	Ala	Thr	Leu	Val	Thr	Thr	Val	Asn	
1630					1635					1640				
GGA	ACC	CCT	ATG	TTA	ATT	TCT	GAG	GGC	CCA	CTA	AAG	ATG	GAA	5705
Gly	Thr	Pro	Met	Leu	Ile	Ser	Glu	Gly	Pro	Leu	Lys	Met	Glu	
	1645					1650					1655			

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GAG AAA GCT ACT TAT GTT CAT AAG AAA AAT GAT GGT ACA ACA	5747
Glu Lys Ala Thr Tyr Val His Lys Lys Asn Asp Gly Thr Thr	
1660 1665 1670	
GTT GAT TTA ACT GTG GAT CAG GCA TGG AGA GGA AAA GGC GAA	5789
Val Asp Leu Thr Val Asp Gln Ala Trp Arg Gly Lys Gly Glu	
1675 1680 1685	
GGT CTT CCT GGA ATG TGT GGT GGG GCC TTG GTT TCA TCG AAT	5831
Gly Leu Pro Gly Met Cys Gly Gly Ala Leu Val Ser Ser Asn	
1690 1695	
CAA TCT ATA CAG AAT GCA ATC TTG GGC ATC CAT GTT GCT GGA	5873
Gln Ser Ile Gln Asn Ala Ile Leu Gly Ile His Val Ala Gly	
1700 1705 1710	
GGA AAT TCA ATT CTT GTT GCA AAA TTG GTT ACT CAA GAA ATG	5915
Gly Asn Ser Ile Leu Val Ala Lys Leu Val Thr Gln Glu Met	
1715 1720 1725	
TTC CAA AAT ATT GAT AAG AAA ATT GAA AGT CAG AGA ATT ATG	5957
Phe Gln Asn Ile Asp Lys Lys Ile Glu Ser Gln Arg Ile Met	
1730 1735 1740	
AAA GTG GAG TTT ACT CAG TGT TCA ATG AAT GTG GTC TCC AAA	5999
Lys Val Glu Phe Thr Gln Cys Ser Met Asn Val Val Ser Lys	
1745 1750 1755	
ACG CTT TTT AGA AAG AGT CCC ATT TAT CAT CAC ATT GAT AAA	6041
Thr Leu Phe Arg Lys Ser Pro Ile Tyr His His Ile Asp Lys	
1760 1765	
ACC ATG ATT AAT TTT CCT GCA GCT ATG CCC TTT TCT AAA GCT	6083
Thr Met Ile Asn Phe Pro Ala Ala Met Pro Phe Ser Lys Ala	
1770 1775 1780	
GAA ATT GAT CCA ATG GCT GTG ATG TTA TCT AAG TAT TCA TTA	6125
Glu Ile Asp Pro Met Ala Val Met Leu Ser Lys Tyr Ser Leu	
1785 1790 1795	
CCT ATT GTA GAA GAA CCA GAG GAT TAT AAA GAG GCT TCA ATT	6167
Pro Ile Val Glu Glu Pro Glu Asp Tyr Lys Glu Ala Ser Ile	
1800 1805 1810	
TTT TAT CAA AAT AAA ATA GTG GGT AAG ACT CAG TTA GTT GAT	6209
Phe Tyr Gln Asn Lys Ile Val Gly Lys Thr Gln Leu Val Asp	
1815 1820 1825	
GAT TTT TTA GAT CTT GAT ATG GCC ATT ACA GGG GCC CCA GGA	6251
Asp Phe Leu Asp Leu Asp Met Ala Ile Thr Gly Ala Pro Gly	
1830 1835	

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ATT Ile 1840	GAT Asp	GCT Ala	ATC Ile	AAC Asn	ATG Met 1845	GAT Asp	TCA Ser	TCT Ser	CCT Pro	GGA Gly 1850	TTT Phe	CCT Pro	TAT Tyr	6293
GTC Val 1855	CAG Gln	GAG Glu	AAG Lys	TTG Leu	ACC Thr	AAA Lys 1860	AGA Arg	GAT Asp	TTA Leu	ATT Ile	TGG Trp 1865	TTG Leu	GAT Asp	6335
GAA Glu 1870	AAT Asn	GGT Gly	TTA Leu	TTG Leu	CTG Leu	GGA Gly 1875	GTT Val	CAT His	CCA Pro	AGA Arg	TTG Leu	GCT Ala 1880	CAG Gln	6377
AGA Arg 1885	ATC Ile	TTA Leu	TTC Phe	AAT Asn	ACT Thr	GTC Val	ATG Met	ATG Met 1890	GAA Glu	AAT Asn	TGT Cys	TCT Ser	GAT Asp 1895	6419
TTG Leu	GAT Asp	GTT Val	GTT Val	TTT Phe 1900	ACA Thr	ACC Thr	TGT Cys	CCA Pro	AAA Lys 1905	GAT Asp	GAA Glu	TTG Leu	AGA Arg	6461
CCA Pro 1910	TTA Leu	GAG Glu	AAA Lys	GTG Val	TTG Leu 1915	GAA Glu	TCA Ser	AAA Lys	ACA Thr	AGA Arg 1920	GCT Ala	ATT Ile	GAT Asp	6503
GCT Ala 1925	TGT Cys	CCT Pro	CTG Leu	GAT Asp	TAC Tyr	TCA Ser 1930	ATT Ile	TTG Leu	TGC Cys	CGA Arg	ATG Met 1935	TAT Tyr	TGG Trp	6545
GGT Gly 1940	CCA Pro	GCT Ala	ATT Ile	AGT Ser	TAT Tyr	TTT Phe 1945	CAT His	TTG Leu	AAT Asn	CCA Pro	GGT Gly	TTC Phe 1950	CAT His	6587
ACA Thr	GGT Gly	GTT Val	GCT Ala 1955	ATT Ile	GGC Gly	ATA Ile	GAT Asp	CCT Pro 1960	GAT Asp	AGA Arg	CAG Gln	TGG Trp 1965	GAT Asp	6629
GAA Glu	TTA Leu	TTT Phe	AAA Lys	ACA Thr 1970	ATG Met	ATA Ile	AGA Arg	TTC Phe 1975	GGA Gly	GAT Asp	GTT Val	GGT Gly	CTT Leu	6671
GAT Asp 1980	TTA Leu	GAT Asp	TTC Phe	TCT Ser	GCT Ala 1985	TTT Phe	GAT Asp	GCT Ala	AGT Ser	CTT Leu 1990	AGT Ser	CCA Pro	TTT Phe	6713
ATG Met 1995	ATT Ile	AGA Arg	GAA Glu	GCA Ala	GGT Gly 2000	AGA Arg	ATC Ile	ATG Met	AGT Ser	GAA Glu 2005	CTA Leu	TCT Ser	GGA Gly	6755
ACT Thr 2010	CCA Pro	TCC Ser	CAT His	TTT Phe	GGC Gly	ACA Thr 2015	GCT Ala	CTT Leu	ATC Ile	AAT Asn	ACT Thr 2020	ATC Ile	ATT Ile	6797

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TAT	TCC	AAG	CAT	TTG	CTG	TAT	AAC	TGT	TGT	TAC	CAT	GTC	TGT	6839
Tyr	Ser	Lys	His	Leu	Leu	Tyr	Asn	Cys	Cys	Tyr	His	Val	Cys	
			2025					2030					2035	
GGT	TCA	ATG	CCC	TCT	GGG	TCT	CCT	TGT	ACA	GCT	TTG	CTA	AAT	6881
Gly	Ser	Met	Pro	Ser	Gly	Ser	Pro	Cys	Thr	Ala	Leu	Leu	Asn	
			2040					2045						
TCA	ATT	ATT	AAT	AAT	GTC	AAT	TTG	TAT	TAT	GTG	TTT	TCC	AAG	6923
Ser	Ile	Ile	Asn	Asn	Val	Asn	Leu	Tyr	Tyr	Val	Phe	Ser	Lys	
2050					2055					2060				
ATA	TTT	GGA	AAG	TCT	CCA	GTT	TTC	TTT	TGT	CAG	GCT	TTG	AAG	6965
Ile	Phe	Gly	Lys	Ser	Pro	Val	Phe	Phe	Cys	Gln	Ala	Leu	Lys	
2065					2070					2075				
ATT	CTC	TGT	TAT	GGA	GAT	GAT	GTT	TTA	ATA	GTT	TTC	TCT	CGA	7007
Ile	Leu	Cys	Tyr	Gly	Asp	Asp	Val	Leu	Ile	Val	Phe	Ser	Arg	
	2080						2085					2090		
GAT	GTT	CAG	ATT	GAT	AAT	CTT	GAT	TTG	ATT	GGA	CAA	AAA	ATT	7049
Asp	Val	Gln	Ile	Asp	Asn	Leu	Asp	Leu	Ile	Gly	Gln	Lys	Ile	
			2095					2100					2105	
GTA	GAT	GAG	TTT	AAG	AAA	CTT	GGC	ATG	ACA	GCT	ACT	TCT	GCT	7091
Val	Asp	Glu	Phe	Lys	Lys	Leu	Gly	Met	Thr	Ala	Thr	Ser	Ala	
			2110					2115						
GAC	AAG	AAT	GTA	CCT	CAG	CTG	AAA	CCA	GTT	TCG	GAA	TTG	ACT	7133
Asp	Lys	Asn	Val	Pro	Gln	Leu	Lys	Pro	Val	Ser	Glu	Leu	Thr	
2120					2125					2130				
TTT	CTC	AAA	AGA	TCT	TTC	AAT	TTG	GTA	GAG	GAT	AGA	ATT	AGA	7175
Phe	Leu	Lys	Arg	Ser	Phe	Asn	Leu	Val	Glu	Asp	Arg	Ile	Arg	
	2135					2140					2145			
CCT	GCA	ATT	TCG	GAA	AAA	ACA	ATT	TGG	TCT	TTA	ATA	GCA	TGG	7217
Pro	Ala	Ile	Ser	Glu	Lys	Thr	Ile	Trp	Ser	Leu	Ile	Ala	Trp	
		2150					2155					2160		
CAG	AGA	AGT	AAC	GCT	GAG	TTT	GAG	CAG	AAT	TTA	GAA	AAT	GCT	7259
Gln	Arg	Ser	Asn	Ala	Glu	Phe	Glu	Gln	Asn	Leu	Glu	Asn	Ala	
			2165				2170						2175	
CAG	TGG	TTT	GCT	TTT	ATG	CAT	GGC	TAT	GAG	TTT	TAT	CAG	AAA	7301
Gln	Trp	Phe	Ala	Phe	Met	His	Gly	Tyr	Glu	Phe	Tyr	Gln	Lys	
			2180					2185						
TTT	TAT	TAT	TTT	GTT	CAG	TCC	TGT	TTG	GAG	AAA	GAG	ATG	ATA	7343
Phe	Tyr	Tyr	Phe	Val	Gln	Ser	Cys	Leu	Glu	Lys	Glu	Met	Ile	
2190					2195					2200				

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GAA TAC AGA CTT AAA TCT TAT GAT TGG TGG AGA ATG AGA TTT 7385
 Glu Tyr Arg Leu Lys Ser Tyr Asp Trp Trp Arg Met Arg Phe
 2205 2210 2215

TAT GAC CAG TGT TTC ATT TGT GAC CTT TCA TGATTTGTTT 7425
 Tyr Asp Gln Cys Phe Ile Cys Asp Leu Ser
 2220 2225

AAACAAATTT TCTTAAAATT TCTGAGGTTT GTTTATTTCT TTTATCAGTA 7475

AATAAAAAAA AAAAAAAA 7493

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2227 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asn Met Ser Arg Gln Gly Ile Phe Gln Thr Val Gly Ser Gly Leu
 1 5 10 15
 Asp His Ile Leu Ser Leu Ala Asp Ile Glu Glu Glu Gln Met Ile Gln
 20 25 30
 Ser Val Asp Arg Thr Ala Val Thr Gly Ala Ser Tyr Phe Thr Ser Val
 35 40 45
 Asp Gln Ser Ser Val His Thr Ala Glu Val Gly Ser His Gln Val Glu
 50 55 60
 Pro Leu Arg Thr Ser Val Asp Lys Pro Gly Ser Lys Lys Thr Gln Gly
 65 70 75 80
 Glu Lys Phe Phe Leu Ile His Ser Ala Asp Trp Leu Thr Thr His Ala
 85 90 95
 Leu Phe His Glu Val Ala Lys Leu Asp Val Val Lys Leu Leu Tyr Asn
 100 105 110
 Glu Gln Phe Ala Val Gln Gly Leu Leu Arg Tyr His Thr Tyr Ala Arg
 115 120 125
 Phe Gly Ile Glu Ile Gln Val Gln Ile Asn Pro Thr Pro Phe Gln Gln
 130 135 140

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

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Arg	Val	Pro	Trp	Ile	Ser	Asp	Thr	Pro	Tyr	Arg	Val	Asn	Arg	Tyr	Thr
			420					425					430		
Lys	Ser	Ala	His	Gln	Lys	Gly	Glu	Tyr	Thr	Ala	Ile	Gly	Lys	Leu	Ile
		435					440					445			
Val	Tyr	Cys	Tyr	Asn	Arg	Leu	Thr	Ser	Pro	Ser	Asn	Val	Ala	Ser	His
	450					455					460				
Val	Arg	Val	Asn	Val	Tyr	Leu	Ser	Ala	Ile	Asn	Leu	Glu	Cys	Phe	Ala
465					470					475					480
Pro	Leu	Tyr	His	Ala	Met	Asp	Val	Thr	Thr	Gln	Val	Gly	Asp	Asp	Ser
				485					490					495	
Gly	Gly	Phe	Ser	Thr	Thr	Val	Ser	Thr	Glu	Gln	Asn	Val	Pro	Asp	Pro
			500					505					510		
Gln	Val	Gly	Ile	Thr	Thr	Met	Lys	Asp	Leu	Lys	Gly	Lys	Ala	Asn	Arg
		515					520					525			
Gly	Lys	Met	Asp	Val	Ser	Gly	Val	Gln	Ala	Pro	Val	Gly	Ala	Ile	Thr
	530					535					540				
Thr	Ile	Glu	Asp	Pro	Val	Leu	Ala	Lys	Lys	Val	Pro	Glu	Thr	Phe	Pro
545					550					555					560
Glu	Leu	Lys	Pro	Gly	Glu	Ser	Arg	His	Thr	Ser	Asp	His	Met	Ser	Ile
				565					570					575	
Tyr	Lys	Phe	Met	Gly	Arg	Ser	His	Phe	Leu	Cys	Thr	Phe	Thr	Phe	Asn
			580					585					590		
Ser	Asn	Asn	Lys	Glu	Tyr	Thr	Phe	Pro	Ile	Thr	Leu	Ser	Ser	Thr	Ser
		595					600					605			
Asn	Pro	Pro	His	Gly	Leu	Pro	Ser	Thr	Leu	Arg	Trp	Phe	Phe	Asn	Leu
	610					615					620				
Phe	Gln	Leu	Tyr	Arg	Gly	Pro	Leu	Asp	Leu	Thr	Ile	Ile	Ile	Thr	Gly
625					630					635					640
Ala	Thr	Asp	Val	Asp	Gly	Met	Ala	Trp	Phe	Thr	Pro	Val	Gly	Leu	Ala
				645					650					655	
Val	Asp	Thr	Pro	Trp	Val	Glu	Lys	Glu	Ser	Ala	Leu	Ser	Ile	Asp	Tyr
			660					665					670		
Lys	Thr	Ala	Leu	Gly	Ala	Val	Arg	Phe	Asn	Thr	Arg	Arg	Thr	Gly	Asn
		675					680					685			

Ile	Gln	Ile	Arg	Leu	Pro	Trp	Tyr	Ser	Tyr	Leu	Tyr	Ala	Val	Ser	Gly
690						695					700				
Ala	Leu	Asp	Gly	Leu	Gly	Asp	Lys	Thr	Asp	Ser	Thr	Phe	Gly	Leu	Val
705					710					715					720
Ser	Ile	Gln	Ile	Ala	Asn	Tyr	Asn	His	Ser	Asp	Glu	Tyr	Leu	Ser	Phe
				725					730					735	
Ser	Cys	Tyr	Leu	Ser	Val	Thr	Glu	Gln	Ser	Glu	Phe	Tyr	Phe	Pro	Arg
			740					745					750		
Ala	Pro	Leu	Asn	Ser	Asn	Ala	Met	Leu	Ser	Thr	Glu	Ser	Met	Met	Ser
		755					760					765			
Arg	Ile	Ala	Ala	Gly	Asp	Leu	Glu	Ser	Ser	Val	Asp	Asp	Pro	Arg	Ser
	770					775					780				
Glu	Glu	Asp	Lys	Arg	Phe	Glu	Ser	His	Ile	Glu	Cys	Arg	Lys	Pro	Tyr
785					790					795					800
Lys	Glu	Leu	Arg	Leu	Glu	Val	Gly	Lys	Gln	Arg	Leu	Lys	Tyr	Ala	Gln
				805					810					815	
Glu	Glu	Leu	Ser	Asn	Glu	Val	Leu	Pro	Pro	Pro	Arg	Lys	Met	Lys	Gly
			820					825					830		
Leu	Phe	Ser	Gln	Ala	Lys	Ile	Ser	Leu	Phe	Tyr	Thr	Glu	Glu	His	Glu
		835					840					845			
Ile	Met	Lys	Phe	Ser	Trp	Arg	Gly	Val	Thr	Ala	Asp	Thr	Arg	Ala	Leu
	850					855					860				
Arg	Arg	Phe	Gly	Phe	Ser	Leu	Ala	Ala	Gly	Arg	Ser	Val	Trp	Thr	Leu
865					870					875					880
Glu	Met	Asp	Ala	Gly	Val	Leu	Thr	Gly	Arg	Leu	Ile	Arg	Leu	Asn	Asp
				885					890					895	
Glu	Lys	Trp	Thr	Glu	Met	Lys	Asp	Asp	Lys	Ile	Val	Ser	Leu	Ile	Glu
			900					905					910		
Lys	Phe	Thr	Ser	Asn	Lys	Tyr	Trp	Ser	Lys	Val	Asn	Phe	Pro	His	Gly
		915					920					925			
Met	Leu	Asp	Leu	Glu	Glu	Ile	Ala	Ala	Asn	Ser	Lys	Asp	Phe	Pro	Asn
	930					935					940				
Met	Ser	Glu	Thr	Asp	Leu	Cys	Phe	Leu	Leu	His	Trp	Leu	Asn	Pro	Lys
945					950					955					960

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Lys Ile Asn Leu Ala Asp Arg Met Leu Gly Leu Ser Gly Val Gln Glu
965 970 975

Ile Lys Glu Gln Gly Val Gly Leu Ile Ala Glu Cys Arg Thr Phe Leu
980 985 990

Asp Ser Ile Ala Gly Thr Leu Lys Ser Met Met Phe Gly Phe His His
995 1000 1005

Ser Val Thr Val Glu Ile Ile Asn Thr Val Leu Cys Phe Val Lys Ser
1010 1015 1020

Gly Ile Leu Leu Tyr Val Ile Gln Gln Leu Asn Gln Asp Glu His Ser
1025 1030 1035 1040

His Ile Ile Gly Leu Leu Arg Val Met Asn Tyr Ala Asp Ile Gly Cys
1045 1050 1055

Ser Val Ile Ser Cys Gly Lys Val Phe Ser Lys Met Leu Glu Thr Val
1060 1065 1070

Phe Asn Trp Gln Met Asp Ser Arg Met Met Glu Leu Arg Thr Gln Ser
1075 1080 1085

Phe Ser Asn Trp Leu Arg Asp Ile Cys Ser Gly Ile Thr Ile Phe Lys
1090 1095 1100

Asn Phe Lys Asp Ala Ile Tyr Trp Leu Tyr Thr Lys Leu Lys Asp Phe
1105 1110 1115 1120

Tyr Glu Val Asn Tyr Gly Lys Lys Lys Asp Ile Leu Asn Ile Leu Lys
1125 1130 1135

Asp Asn Gln Gln Lys Ile Glu Lys Ala Ile Glu Glu Ala Asp Glu Phe
1140 1145 1150

Cys Ile Leu Gln Ile Gln Asp Val Glu Lys Phe Glu Gln Tyr Gln Lys
1155 1160 1165

Gly Val Asp Leu Ile Gln Lys Leu Arg Thr Val His Ser Met Ala Gln
1170 1175 1180

Val Asp Pro Asn Leu Met Val His Leu Ser Pro Leu Arg Asp Cys Ile
1185 1190 1195 1200

Ala Arg Val His Gln Lys Leu Lys Asn Leu Gly Ser Ile Asn Gln Ala
1205 1210 1215

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

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Gly Gly Gly Lys Ser Leu Thr Ser Ile Ala Leu Ala Thr Lys Ile Cys
1235 1240 1245

Lys His Tyr Gly Val Glu Pro Glu Lys Asn Ile Tyr Thr Lys Pro Val
1250 1255 1260

Ala Ser Asp Tyr Trp Asp Gly Tyr Ser Gly Gln Leu Val Cys Ile Ile
1265 1270 1275 1280

Asp Asp Ile Gly Gln Asn Thr Thr Asp Glu Asp Trp Ser Asp Phe Cys
1285 1290 1295

Gln Leu Val Ser Gly Cys Pro Met Arg Leu Asn Met Ala Ser Leu Glu
1300 1305 1310

Glu Lys Gly Arg His Phe Ser Ser Pro Phe Ile Ile Ala Thr Ser Asn
1315 1320 1325

Trp Ser Asn Pro Ser Pro Lys Thr Val Tyr Val Lys Glu Ala Ile Asp
1330 1335 1340

Arg Arg Leu His Phe Lys Val Glu Val Lys Pro Ala Ser Phe Phe Lys
1345 1350 1355 1360

Asn Pro His Asn Asp Met Leu Asn Val Asn Leu Ala Lys Thr Asn Asp
1365 1370 1375

Ala Ile Lys Asp Met Ser Cys Val Asp Leu Ile Met Asp Gly His Asn
1380 1385 1390

Val Ser Leu Met Asp Leu Leu Ser Ser Leu Val Met Thr Val Glu Ile
1395 1400 1405

Arg Lys Gln Asn Met Thr Glu Phe Met Glu Leu Trp Ser Gln Gly Ile
1410 1415 1420

Ser Asp Asp Asp Asn Asp Ser Ala Val Ala Glu Phe Phe Gln Ser Phe
1425 1430 1435 1440

Pro Ser Gly Glu Pro Ser Asn Ser Lys Leu Ser Gly Phe Phe Gln Ser
1445 1450 1455

Val Thr Asn His Lys Trp Val Ala Val Gly Ala Ala Val Gly Ile Leu
1460 1465 1470

Gly Val Leu Val Gly Gly Trp Phe Val Tyr Lys His Phe Ser Arg Lys
1475 1480 1485

Glu Glu Glu Pro Ile Pro Ala Glu Gly Val Tyr His Gly Val Thr Lys
1490 1495 1500

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Pro Lys Gln Val Ile Lys Leu Asp Ala Asp Pro Val Glu Ser Gln Ser
1505 1510 1515 1520

Thr Leu Glu Ile Ala Gly Leu Val Arg Lys Asn Leu Val Gln Phe Gly
1525 1530 1535

Val Gly Glu Lys Asn Gly Cys Val Arg Trp Val Met Asn Ala Leu Gly
1540 1545 1550

Val Lys Asp Asp Trp Leu Leu Val Pro Ser His Ala Tyr Lys Phe Glu
1555 1560 1565

Lys Asp Tyr Glu Met Met Glu Phe Tyr Phe Asn Arg Gly Gly Thr Tyr
1570 1575 1580

Tyr Ser Ile Ser Ala Gly Asn Val Val Ile Gln Ser Leu Asp Val Gly
1585 1590 1595 1600

Phe Gln Asp Val Val Leu Met Lys Val Pro Thr Ile Pro Lys Phe Arg
1605 1610 1615

Asp Ile Thr Gln His Phe Ile Lys Lys Gly Asp Val Pro Arg Ala Leu
1620 1625 1630

Asn Arg Leu Ala Thr Leu Val Thr Thr Val Asn Gly Thr Pro Met Leu
1635 1640 1645

Ile Ser Glu Gly Pro Leu Lys Met Glu Glu Lys Ala Thr Tyr Val His
1650 1655 1660

Lys Lys Asn Asp Gly Thr Thr Val Asp Leu Thr Val Asp Gln Ala Trp
1665 1670 1675 1680

Arg Gly Lys Gly Glu Gly Leu Pro Gly Met Cys Gly Gly Ala Leu Val
1685 1690 1695

Ser Ser Asn Gln Ser Ile Gln Asn Ala Ile Leu Gly Ile His Val Ala
1700 1705 1710

Gly Gly Asn Ser Ile Leu Val Ala Lys Leu Val Thr Gln Glu Met Phe
1715 1720 1725

Gln Asn Ile Asp Lys Lys Ile Glu Ser Gln Arg Ile Met Lys Val Glu
1730 1735 1740

Phe Thr Gln Cys Ser Met Asn Val Val Ser Lys Thr Leu Phe Arg Lys
1745 1750 1755 1760

Ser Pro Ile Tyr His His Ile Asp Lys Thr Met Ile Asn Phe Pro Ala
1765 1770 1775

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Ala Met Pro Phe Ser Lys Ala Glu Ile Asp Pro Met Ala Val Met Leu	1780	1785	1790
Ser Lys Tyr Ser Leu Pro Ile Val Glu Glu Pro Glu Asp Tyr Lys Glu	1795	1800	1805
Ala Ser Ile Phe Tyr Gln Asn Lys Ile Val Gly Lys Thr Gln Leu Val	1810	1815	1820
Asp Asp Phe Leu Asp Leu Asp Met Ala Ile Thr Gly Ala Pro Gly Ile	1825	1830	1835
Asp Ala Ile Asn Met Asp Ser Ser Pro Gly Phe Pro Tyr Val Gln Glu	1845	1850	1855
Lys Leu Thr Lys Arg Asp Leu Ile Trp Leu Asp Glu Asn Gly Leu Leu	1860	1865	1870
Leu Gly Val His Pro Arg Leu Ala Gln Arg Ile Leu Phe Asn Thr Val	1875	1880	1885
Met Met Glu Asn Cys Ser Asp Leu Asp Val Val Phe Thr Thr Cys Pro	1890	1895	1900
Lys Asp Glu Leu Arg Pro Leu Glu Lys Val Leu Glu Ser Lys Thr Arg	1905	1910	1915
Ala Ile Asp Ala Cys Pro Leu Asp Tyr Ser Ile Leu Cys Arg Met Tyr	1925	1930	1935
Trp Gly Pro Ala Ile Ser Tyr Phe His Leu Asn Pro Gly Phe His Thr	1940	1945	1950
Gly Val Ala Ile Gly Ile Asp Pro Asp Arg Gln Trp Asp Glu Leu Phe	1955	1960	1965
Lys Thr Met Ile Arg Phe Gly Asp Val Gly Leu Asp Leu Asp Phe Ser	1970	1975	1980
Ala Phe Asp Ala Ser Leu Ser Pro Phe Met Ile Arg Glu Ala Gly Arg	1985	1990	1995
Ile Met Ser Glu Leu Ser Gly Thr Pro Ser His Phe Gly Thr Ala Leu	2005	2010	2015
Ile Asn Thr Ile Ile Tyr Ser Lys His Leu Leu Tyr Asn Cys Cys Tyr	2020	2025	2030
His Val Cys Gly Ser Met Pro Ser Gly Ser Pro Cys Thr Ala Leu Leu	2035	2040	2045

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Asn Ser Ile Ile Asn Asn Val Asn Leu Tyr Tyr Val Phe Ser Lys Ile
2050 2055 2060

Phe Gly Lys Ser Pro Val Phe Phe Cys Gln Ala Leu Lys Ile Leu Cys
2065 2070 2075 2080

Tyr Gly Asp Asp Val Leu Ile Val Phe Ser Arg Asp Val Gln Ile Asp
2085 2090 2095

Asn Leu Asp Leu Ile Gly Gln Lys Ile Val Asp Glu Phe Lys Lys Leu
2100 2105 2110

Gly Met Thr Ala Thr Ser Ala Asp Lys Asn Val Pro Gln Leu Lys Pro
2115 2120 2125

Val Ser Glu Leu Thr Phe Leu Lys Arg Ser Phe Asn Leu Val Glu Asp
2130 2135 2140

Arg Ile Arg Pro Ala Ile Ser Glu Lys Thr Ile Trp Ser Leu Ile Ala
2145 2150 2155 2160

Trp Gln Arg Ser Asn Ala Glu Phe Glu Gln Asn Leu Glu Asn Ala Gln
2165 2170 2175

Trp Phe Ala Phe Met His Gly Tyr Glu Phe Tyr Gln Lys Phe Tyr Tyr
2180 2185 2190

Phe Val Gln Ser Cys Leu Glu Lys Glu Met Ile Glu Tyr Arg Leu Lys
2195 2200 2205

Ser Tyr Asp Trp Trp Arg Met Arg Phe Tyr Asp Gln Cys Phe Ile Cys
2210 2215 2220

Asp Leu Ser
2225

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A mutant of hepatitis A virus strain HM-175, having the DNA SEQ ID NO. 1, wherein at least nucleotide positions 591 and 687 are changed to guanine, 646 is changed to adenine and 669 is changed to thymine.
2. The mutant of claim 1, wherein additionally nucleotides at positions 4418 and 4643 are changed to thymine.
3. The mutant of claim 1 or 2, wherein at least nucleotide positions 3934 and 5145 are changed to guanine, 5745 is changed to thymine and 6908 is changed to cytosine.
4. The mutant of any one of claims 1 to 3 having the identifying characteristics of strain HAV 4380, deposited at CNCM under Accession No. 1-936.
5. The HAV 4380 strain of claim 4 adapted to grow in the human fibroblast cell line MRC-5 by incubation at 32°C to 35°C and capable of growing at 37°C in primary African green monkey kidney cells (AGMK cells).
6. A hepatitis A virus vaccine, comprising a mutant according to any one of claims 1 to 5.
7. A method for preparing a mutant of hepatitis A virus strain HM-175 having the DNA SEQ ID NO. 1, comprising the step of modifying said hepatitis A virus strain wherein at least nucleotide positions 591 and 687 in DNA SEQ ID NO. 1 are changed to guanine, 646 is changed to adenine and 669 is changed to thymine.
8. A method according to claim 7, wherein additionally

nucleotides at positions 4418 and 4643 are changed to thymine.

9. A method according to claim 7 or 8, wherein at least nucleotide positions 3934 and 5145 are changed to guanine, 5745 is changed to thymine and 6908 is changed to cytosine.

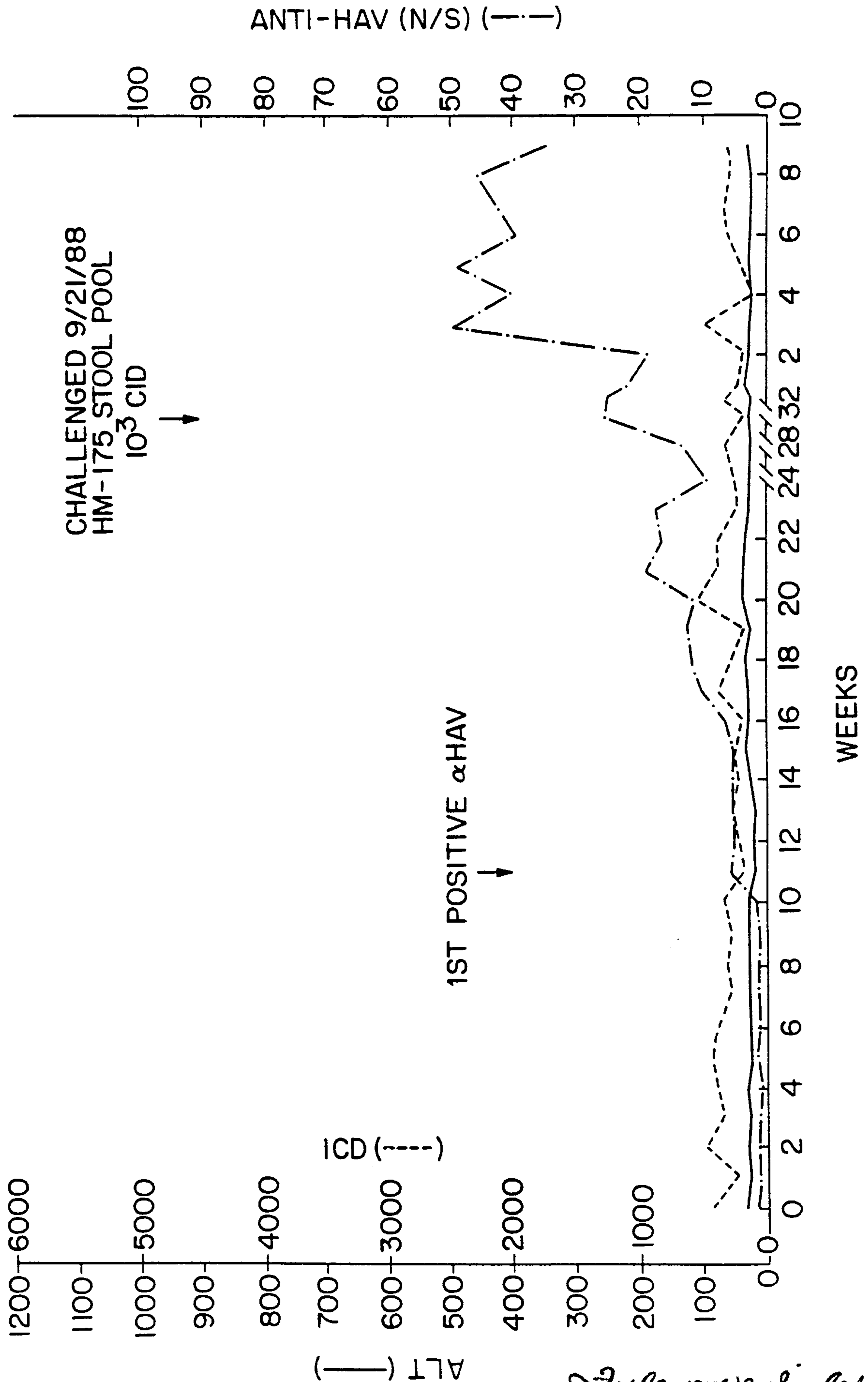
10. The use of a mutant of HAV virus according to any one of claims 1 to 5 for the preparation of a vaccine for immunizing a human or primate against hepatitis A.

11. A nucleic acid molecule which encodes the mutant of any one of claims 1 to 5.

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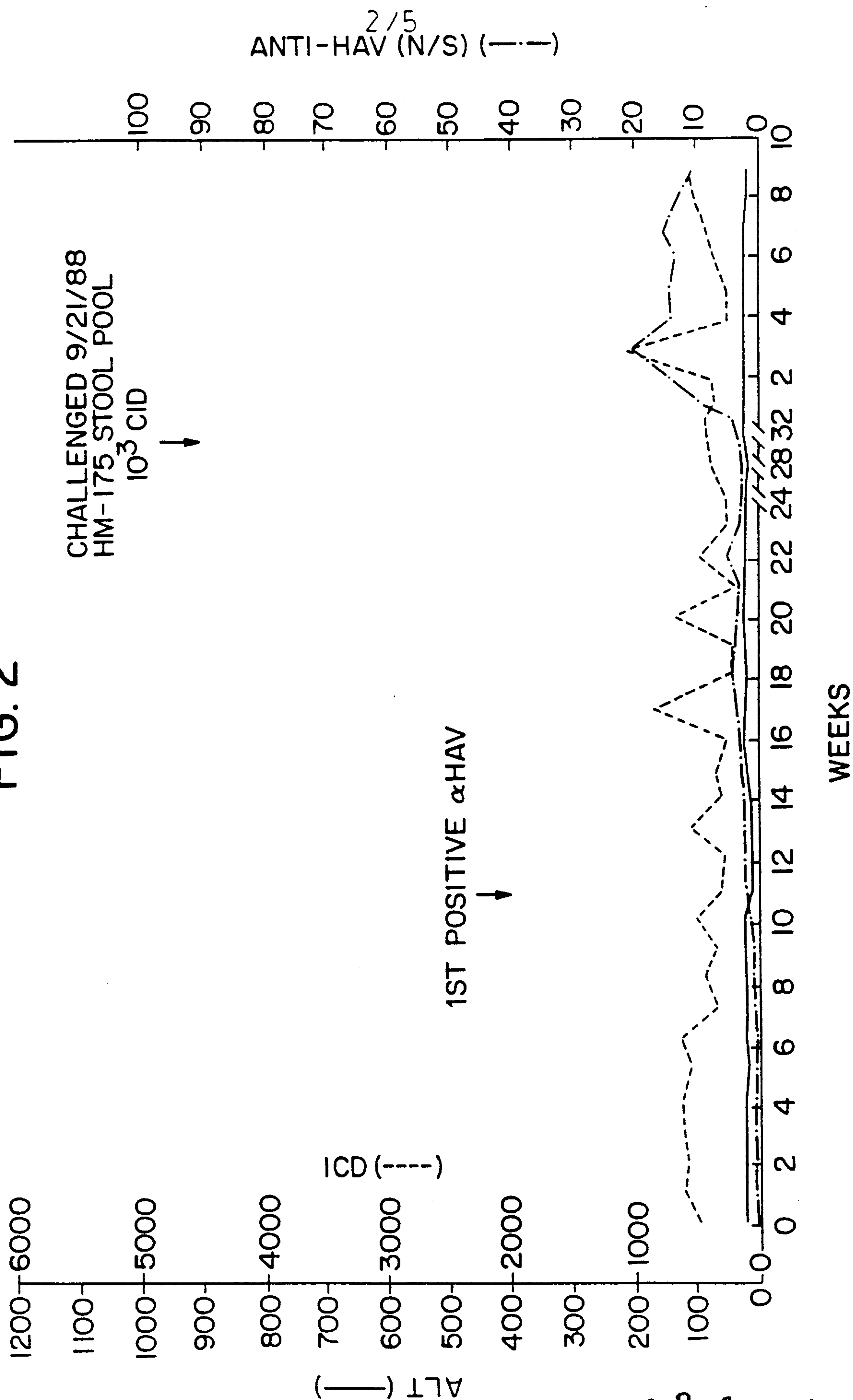
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FIG. 1



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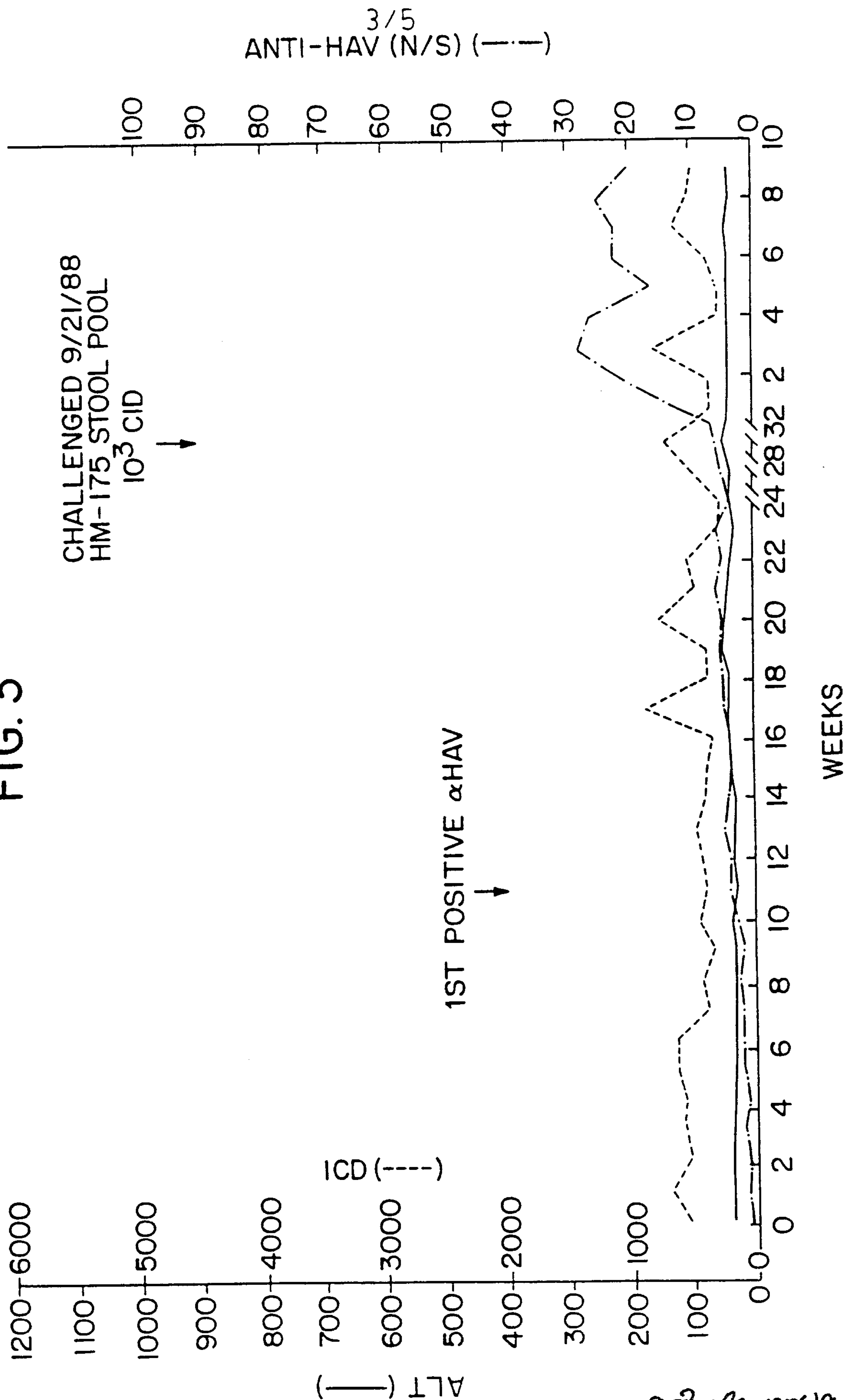
FIG. 2



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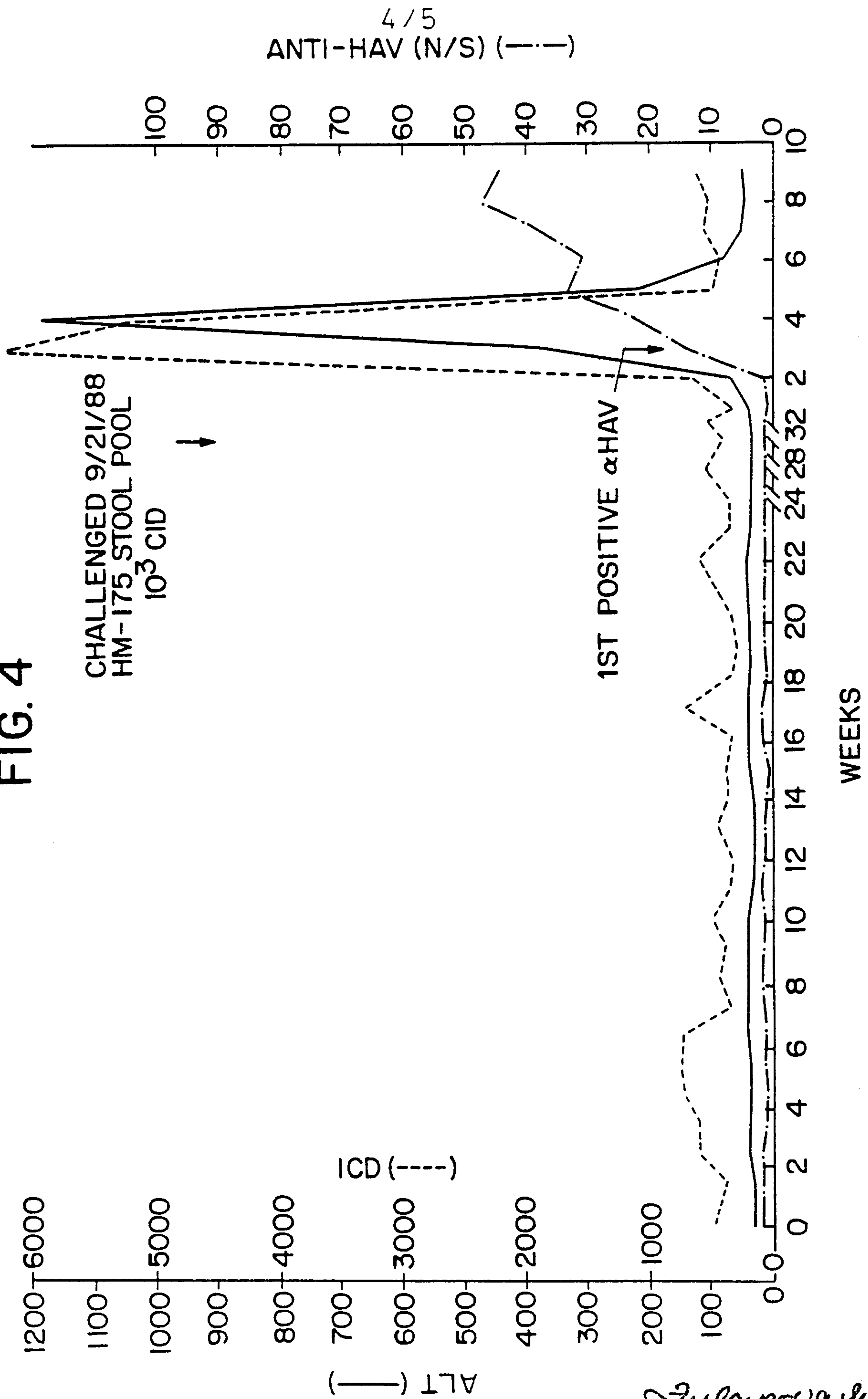
FIG. 3



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FIG. 4

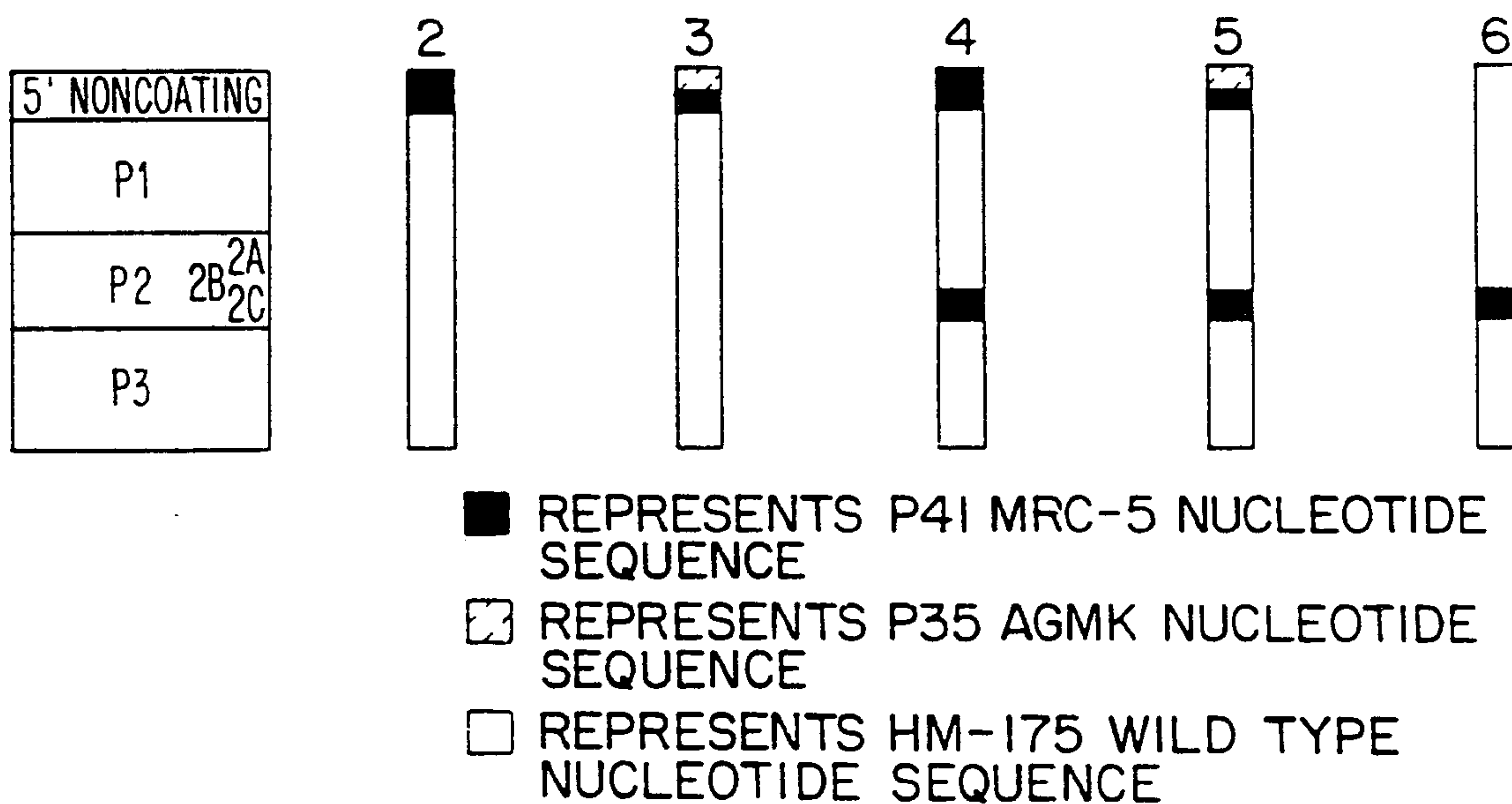
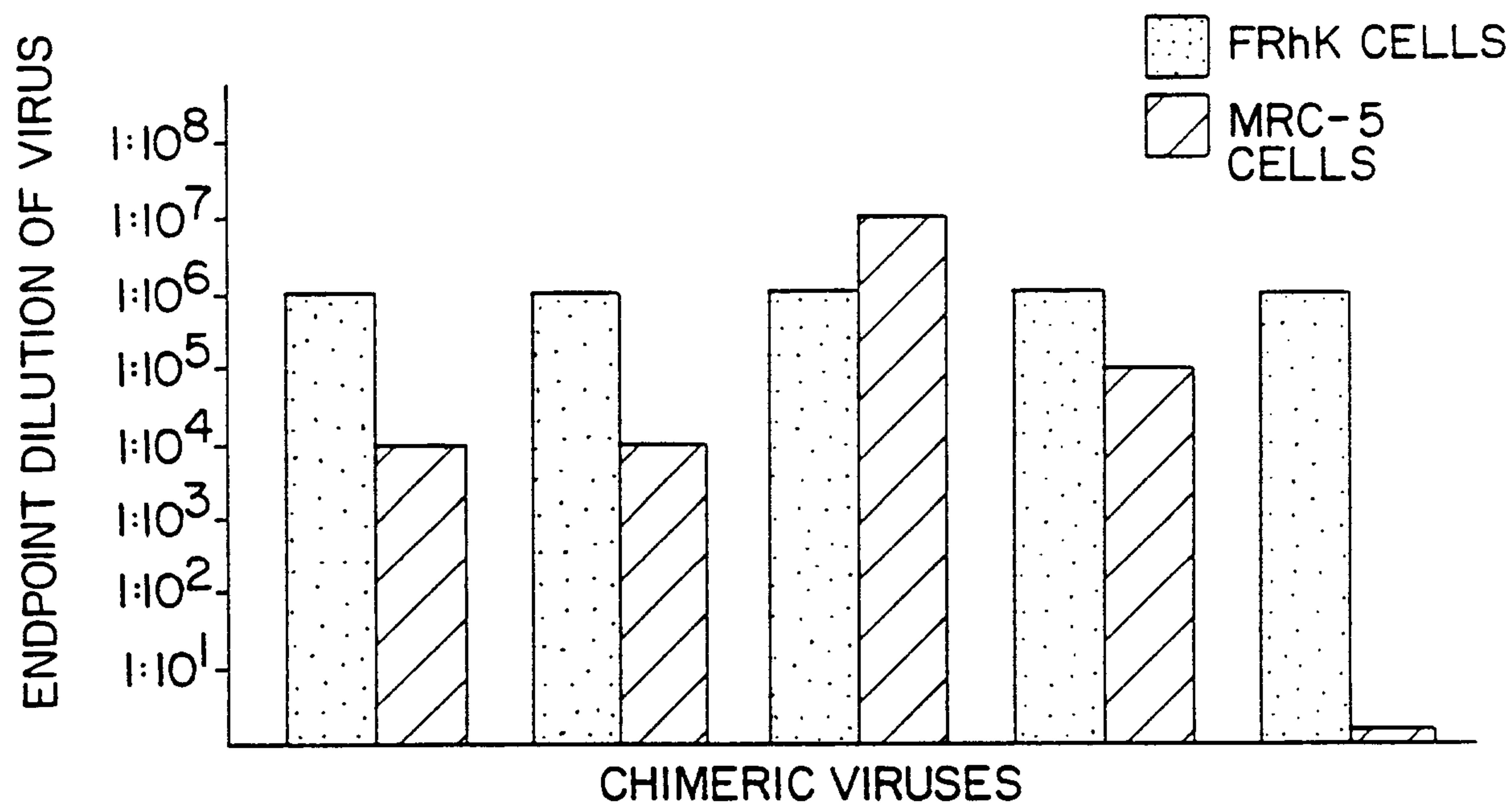


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FIG. 5



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