



IMMUNOREACTIVE ANTIGENS OF HEPATITIS E VIRUS

The present invention relates generally to molecules such as peptides, polypeptides and proteins which carry epitopes and in particular B cell epitopes from antigenic proteins encoded by Hepatitis E Virus. These molecules are preferentially immunoreactive to convalescent or acute phase circulating antibodies to the Hepatitis E Virus and are useful in the development of diagnostic, therapeutic and prophylactic agents for Hepatitis E Virus.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Viral hepatitis results from infection with one of at least five very different viral agents. Available serological tests allow the diagnosis of acute hepatitis due to infection with Hepatitis A Virus (HAV) and Hepatitis B Virus (HBV). HBV is required for propagation of the delta agent, or Hepatitis D Virus (HDV); this co-infection results in a high proportion of cases progressing to chronic active hepatitis. The clinical and diagnostic exclusion of HAV and HBV has led to the recognition of other hepatitides that were formerly grouped together as non-A, non-B hepatitis [(NANBH)] (Prince *et al.*, 1974; Feinstone *et al.*, 1975; Tabor, 1985). NANBH is caused by more than one viral agent and can be transmitted by either parenteral or fecal/oral routes (Bradley, 1990a; Reyes and Baroudy, 1991).

The cloning of a blood-borne agent, termed Hepatitis C Virus (HCV), led to the development of a specific assay for circulating antibody to HCV (Choo *et al.*, 1989; Kuo



et al., 1989; Kubo *et al.*, 1989, Maeno *et al.*, 1990). This assay predominantly detects infections at the chronic stage, but has facilitated the identification of HCV as the cause of up to 90% of parenterally transmitted NANBH. A second epidemiologically distinct form of NANBH has been shown to occur in both epidemic and sporadic patterns in developing countries and is referred to as enterically transmitted non-A, non-B hepatitis (ET-NANBH) due to its water-borne mode of virus transmission and presumed enteric route of infection (Khuroo, 1980; Wong *et al.*, 1980). ET-NANBH has been documented in India, Pakistan, Burma, USSR, Costa Rica, Mexico and countries in Africa where epidemic outbreaks can generally be traced to fecal contamination of drinking water (Bradley and Maynard, 1986; Bradley, 1990b). The causative viral agent was previously shown to passage successfully in cynomolgus macaques (cyno) and tamarins with typical liver enzyme elevations and recovery of morphologically similar 27 to 34 nm virus like particles from the feces of clinical specimens and experimental animals (Balayan *et al.*, 1983; Andiaparidze *et al.*, 1986; Bradley *et al.*, 1987; Arankalle *et al.*, 1988).

Reyes *et al.* (1990) recently reported the isolation of a partial cDNA clone from the virus responsible for ET-NANBH, and termed the newly identified agent the Hepatitis E Virus (HEV). The expressions "enterically transmitted non-A, non-B hepatitis", "ET-NANBH", "Hepatitis E Virus" and "HEV" are used interchangeably herein to refer to a virus or type of virus which is capable of causing infectious hepatitis from contaminated water, which is transmissible and capable of passage in cynomolgus macaques and tamarins, which is serologically distinct from HAV, HBV, HCV and HDV and which comprises a genomic nucleotide sequence, a portion of which, at least is homologous to or substantially similar to or capable of hybridizing under low stringency conditions to the all or part of the nucleotide sequences set forth in Figure 1 and/or Figure 2. Preferred homologies and a definition of stringency conditions are set forth below.

The HEV clone was from a Burma isolate of the virus and hybridised with cDNA made from five other distinct geographic isolates. These molecular epidemiological findings are consistent with the available serologic data based on the use of immune electron microscopy and immunofluorescence blocking studies that indicate a single major agent is responsible for the majority of ET-NANBH seen worldwide (Purcell and

Ticehurst, 1988; Bradley *et al.* 1988a; Krawczynski and Bradley, 1989). Tam *et al.* (1991) subsequently reported on the molecular cloning of the complete HEV (Burma; B) viral clone together with the deduced amino acid sequences.

HEV has a single stranded genome of polyadenylated RNA of positive polarity which encodes three open reading frames (ORF's) designated ORF1, ORF2 and ORF3. Open reading frame 2 and ORF3 are partially overlapping in different reading frames and are thought to encode structural proteins of the virus. Open reading frame 1, on the other hand, encodes replicative proteins and overlaps ORF3 by one nucleotide.

Despite the availability of immunoassays for the detection of antibodies to some strains of HEV, it has been observed that IgM and IgG antibody titres wane rapidly following infection. Consequently, it has proven difficult to interpret serological surveys from both endemic and non-endemic areas. Furthermore, the relationship between antibody interactivity and immunity to reinfection remains unclear. These and other factors have delayed the development of suitable diagnostic and therapeutic protocols for the HEV, for which a need clearly exists. In particular, it would be most beneficial to develop an assay which could distinguish between acute phase antibodies (contemporary antibodies) generated in response to HEV infection and convalescent phase antibodies, which remain in the circulatory and/or secretory system after infection and may contribute to immunity to reinfection.

In work leading up to the present invention, the inventors sought to identify epitopes, and in particular B cell epitopes, on peptides, polypeptides and proteins encoded within the HEV genome in order to improve upon current diagnostic procedures and to further develop therapeutic and prophylactic compositions for HEV. In accordance with the present invention, peptides, polypeptides and proteins, were recombinantly expressed from nucleic acid molecules derived from ORFs in the HEV genome. The epitopic and in particular B cell epitopic regions within these molecules were identified on the basis of interactivity to antibodies specific to HEV, thereby forming the basis for a new range of diagnostic, therapeutic and prophylactic procedures for HEV. Interestingly, the inventors have determined that one portion of a molecule encoded by an ORF may inhibit

forming the basis for a new range of diagnostic, therapeutic and prophylactic procedures for HEV. Interestingly, the inventors have determined that one portion of a molecule encoded by an ORF may inhibit or otherwise reduce the immunointeractivity of another portion of the same molecule. They have further determined that the inhibitory effect may be overcome by using non-full length molecules or reducing the inhibitory effect by physical or chemical processes.

Accordingly, one aspect of the present invention provides a recombinant molecule encoded by a sequence of nucleotides selected from the list consisting of open reading frame (ORF) 2 and ORF 3 of Hepatitis E Virus (HEV) or a mutant or derivative of said ORF2 or ORF3. More particularly, the present invention provides a recombinant molecule encoded by a sequence of nucleotides comprising "ORF3" or a part of "ORF2" and which molecule is preferentially immunologically interactive in either convalescent phase or acute phase antibodies to HEV are conveniently described respectively as those regions of the HEV genome beginning at nucleotide 5106 extending 369 bases and terminating at nucleotide 5474 (ORF3) and that region beginning at nucleotide 5147 extending 1980 bases and terminating 68 bases upstream of the poly(A) tail (ORF2) using the numbering system of Tam *et al.* (1991).

In a particularly preferred embodiment, the present invention is directed to a recombinant polypeptide encoded by a nucleotide sequence selected from the list consisting of clone 2.0 (SEQ ID NO:15), clone 2.1 (SEQ ID NO:17), clone 2.2 (SEQ ID NO:19), clone 2.3 (SEQ ID NO:21) and clone 2.4 (SEQ ID NO:23) of Hepatitis E Virus (HEV) as depicted in Figure 5 or encoded by a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to a nucleotide sequence selected from clone 2.0, clone 2.1, clone 2.2, clone 2.3 and clone 2.4 of HEV as depicted in Figure 5.

The term "recombinant molecule" in this context includes a peptide, polypeptide, protein or a chemical equivalent thereof. The recombinant molecule may or may not be glycosylated. A recombinant molecule also extends to a fusion between an HEV derived peptide, polypeptide or protein and a peptide, polypeptide or protein of non-HEV origin such as but not limited to glutathione-S-transferase (GST) or its derivatives, polylysine, polyhistidine, thioredoxin or any other molecule capable of bioaffinity chromatography.

In accordance with the present invention, it has been surprisingly discovered that recombinant molecules encoded by ORF2 and in particular the 3' end of ORF2 and ORF3 are capable of interacting with convalescent phase antibodies to HEV whereas recombinant molecules encoded by the 5' end of ORF2 are interactive more preferentially with acute phase antibodies generated to the virus particle *per se*. According to this aspect of the present invention, there is provided a recombinant peptide or polypeptide which is preferentially immunologically reactive with convalescent phase antibodies generated during HEV infection. In a related aspect of the present invention, there is provided a recombinant peptide or polypeptide which is preferentially immunologically interactive with acute phase antibodies generated during HEV infection.

As used herein, the 3' end of an ORF (e.g. ORF2) is considered the carboxy (C)-terminal end portion of the molecule encoded by the ORF and the 5' end is considered the amino (N)-terminal end portion.

More particularly, it has further been surprisingly discovered that the 5' (i.e. the N-terminal) end portion of ORF2 exhibits greater immunointeractivity to acute phase antibodies to HEV and the 3' (i.e. C-terminal) end portion of ORF2 exhibits greater immunointeractivity to convalescent phase antibodies to HEV, when present on the same molecule, however, the N-terminal end portion appears to inhibit or reduce the immunointeractivity of the C-terminal end portion.

Accordingly, another aspect of the present invention contemplates a recombinant molecule encoded by a sequence of nucleotides comprising ORF3 or a non-full length ORF2 derived from the 3' end of ORF2 of HEV and wherein said recombinant molecule is preferentially interactive with convalescent phase antibodies to HEV. In a related aspect there is provided a recombinant molecule encoded by a sequence of nucleotides comprising a non-full length ORF2 derived from the 5' end of ORF2 of HEV and wherein said recombinant molecule is preferentially interactive with acute phase antibodies to HEV.

In one embodiment the sequence of nucleotides is from the 5' end of ORF2 and encodes

a molecule more interactive with acute phase antibodies relative to convalescent phase antibodies. In another embodiment, the nucleotide sequence is from ORF3 or the 3' end of ORF2 and encodes a molecule more interactive with convalescent phase antibodies relative to acute phase antibodies. The preferred nucleotide sequence of the

treatments to expose epitopes, for example by denaturing followed by renaturing of the molecule or the use of antagonists to prevent the inhibition of, for example, the N-terminal end portion over the C-terminal end portion binding to HEV antibodies.

"Acute and "convalescent" phases are used in their broadest sense to mean respectively the period during infection (acute) and the period beginning from after the peak of infection extending through a convalescent period to a period post infection. The acute phase of infection can conveniently be considered, for example, to be approximately 3 to 6 months following exposure to HEV and the convalescent phase is generally after this period.

Yet another aspect of the present invention is directed to a recombinant polypeptide comprising first and second amino acid sequences wherein said first amino acid sequence is encoded by a nucleotide sequence selected from or within ORF2 and ORF 3 of HEV and wherein said second amino acid sequence is a non-HEV encoded peptide, polypeptide or protein such as but not exclusively GST. Preferably, the first amino acid sequence is encoded by ORF3 or the 3' end of ORF2 and is interactive with convalescent phase antibodies to HEV. Alternatively, the first amino acid sequence is from the 5' end portion of ORF2 and is preferably interactive with acute phase antibodies to HEV.

The presence of selectively or preferentially immunologically reactive antigens provides a means of distinguishing between past (convalescent) and acute (contemporary) phase infection with HEV. Accordingly, the present invention also extends to a method for distinguishing between convalescent and acute phase infection with HEV in an individual which method measures the reactivity of serum to immune interactive recombinant molecule encoded by ORF2 and/or ORF3 or more preferably parts thereof. Most preferably, these parts include the 3' end portion of ORF2 or the 5' end portion of ORF2 or is a chemically modified form of a full length molecule such as to expose epitopes on both the N- and C- termini.

The present invention also provides an isolated nucleic acid molecule such as DNA or cDNA molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a peptide, polypeptide or protein interactive with acute phase antibodies to HEV. In an alternative embodiment, the peptide, polypeptide or protein is interactive with both convalescent and/or acute phase circulating antibodies to HEV. Accordingly, this aspect of the present invention provides a recombinant molecule carrying a B cell epitope of a polypeptide or protein encoded by ORF2 or ORF3 or more preferably a part thereof. More particularly, the sequence of nucleotides is selected from a sequence comprising or within nucleotides 6326 to 7129 (ORF2) and 5106 to 5474 (ORF3) in the HEV genome using the nucleotide numbering system of Tam *et al* (1991) or a nucleotide region substantially equivalent thereto. Preferably, the nucleotide sequence is operably linked to an expression control sequence such as in the form of an expression vector.

The recombinant molecule according to this aspect of the present invention is generally an isolated nucleic acid molecule comprising DNA (eg cDNA or genomic DNA) or mRNA. The recombinant molecule may be isolated directly from the HEV genome or may be generated *in vitro* by, for example, the stepwise addition of nucleotides or groups of nucleotides. The present invention further extends to a host cell such as bacterium, yeast, mammalian or insect cell transformed with such a recombinant molecule. A preferred mammalian cell is the Chinese Hamster Ovary (CHO) cell line. A preferred bacterium is *E. coli*. Preferably, the nucleic acid molecules are DNA, at least parts of which have a nucleotide sequence substantially corresponding to all or part of the nucleotide sequence shown in Figures 1 (SEQ ID NO: 1) or 2 (SEQ ID NO: 3) or a part, fragment, derivative, homologue or analogue thereof or one or more sequences complementary thereto. The present invention, however also extends to any single or multiple nucleotide substitutions, deletions and/or additions to the sequence shown in Figures 1 or 2 and which still encode an epitopic region of the HEV genome or fragment or derivative thereof having the requisite antigenic profile and interactive with antibodies to HEV. Most preferably, the nucleotide sequence encode either the 3' end of ORF2 or the 5' end of ORF2 such that the nucleotide sequence encodes a peptide or polypeptide which interacts preferably with convalescent phase antibodies or acute phase antibodies,

respectively.

Furthermore, when the nucleic acid molecule is RNA, the ribonucleotide sequence will, in a preferred embodiment, be substantially complementary to one or more of the nucleotide sequences shown in Figures 1 or 2 a part, fragment, derivative, homologue or analogue thereof. Another aspect of this invention is directed to a synthetic (e.g. recombinant) peptide, polypeptide or protein which is immunologically interactive with convalescent phase antibodies generating during HEV infection. Furthermore, the invention is further directed to a synthetic (eg: recombinant) peptide, polypeptide or protein which is immunologically interactive with acute and convalescent circulating antibodies generated during HEV infection.

Such synthetic peptides, polypeptides or proteins may, for example, be prepared by recombinant means such as by the expression of a host cell transformed with the recombinant molecules described above. The peptide, polypeptide or protein may be fused to another peptide, polypeptide or protein. Alternatively, it may be prepared by chemical synthesis, such as by the Merrifield solid-phase synthesis procedure. Furthermore, although synthetic or fragments thereof represent a preferred embodiment, the present invention also extends to biologically pure preparations of the naturally occurring epitopes or their fragments. By "biologically pure" is meant a preparation of at least 60%, preferably at least 70%, more preferably at least 80% and still more preferably at least 90% by weight peptide, polypeptide or protein.

Another aspect of the present invention contemplates a peptide, polypeptide or protein carrying a B cell epitope interactive with antibodies to HEV, said peptide, polypeptide or protein being encoded by a sequence of nucleotides capable of hybridising under low stringency conditions to one or more regions of the nucleotide sequence set forth in Figure 1 (SEQ ID NO: 1) or 2 (SEQ ID NO: 3).

More particularly, one aspect of this embodiment is directed to a nucleic acid molecule comprising a sequence of nucleotides which:

- (i) encodes a peptide, polypeptide or protein interactive with acute and/or convalescent phase antibodies to HEV; and
- (ii) is capable of hybridizing under low stringency conditions to all or part of the nucleotide sequence set forth in Figure 1 (SEQ ID NO: 1) under low stringency conditions.

In a related aspect, the nucleotide sequence encodes a peptide, polypeptide or protein interactive with acute phase antibodies and is capable of hybridizing under low stringency conditions to all or part of the nucleotide sequence set forth in Figure 1 or Figure 2.

In a preferred embodiment, the nucleotide sequence is capable of hybridizing to the 5' end of ORF2 and encodes a molecule capable of preferential interaction with acute phase antibodies to HEV. Most preferably, the nucleotide sequence is sequence 2.3 or 2.4 of Figure 5.

Another aspect of this embodiment of the present invention provides a recombinant peptide, polypeptide or protein encoded by a nucleotide sequence capable of hybridizing under low stringency conditions to all or part of the nucleotide sequence set forth in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3). This aspect of the present invention includes recombinant or synthetic forms of naturally occurring proteins encoded by the aforementioned nucleotide sequences as well as peptide, polypeptide or protein derivatives thereof which are encoded by modified nucleotide sequences still capable of hybridizing to the nucleotide sequence in Figure 1 (SEQ ID NO: 1) or 2 (SEQ ID NO: 3) and which derivatives are interactive with acute and/or convalescent phase antibodies to HEV.

For the purposes of defining the level of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which is herein incorporated by reference where the washing step disclosed is considered high stringency. A low stringency is defined herein as being in 4-6X SSC/0.1-0.5% w/v SDS at 37-45°C for 2-3

hours. Depending on the source and concentration of nucleic acid involved in the hybridization, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 1-4X SSC/0.25-0.5% w/v SDS at $\geq 45^{\circ}\text{C}$ for 2-3 hours or high stringency conditions which are considered herein to be 0.1-1X SSC/0.1% w/v SDS at $\geq 60^{\circ}\text{C}$ for 1-3 hours.

In yet a further aspect, the present invention contemplates a peptide, polypeptide or protein carrying a B cell epitope interactive with antibodies to HEV, said peptide, polypeptide or protein having a substantially similar amino acid sequence to one or more regions of the amino acid sequences set forth in Figure 1 (SEQ ID NO: 2) or 2 (SEQ ID NO: 4). By "substantially similar" is meant a sequence having at least 50%, preferably at least 60%, more preferably at least 75% and still more preferably at least 90% homology (i.e. similarity) with the sequences set forth in Figure 1 (SEQ ID NO: 2) or 2 (SEQ ID NO: 4) at the amino acid level. Preferably, the peptide, polypeptide or protein comprise, the N-terminal encoding region of ORF2 which is preferentially interactive with acute phase antibodies to HEV. Alternatively, the peptide, polypeptide or protein comprises the C-terminal encoding region of ORF2 which is preferentially interactive with convalescent phase antibodies to HEV. In a further alternative, the peptide, polypeptide or protein is a full length ORF encoded molecule but chemically or physically modified or disrupted to expose both the N- and C- terminal epitopes.

The present invention also extends to a method for maintaining interactive conformation of synthetic (eg recombinant) peptides or polypeptides wherein the synthetic peptides or polypeptides are further purified after undergoing a denaturation procedure and subsequently renatured. By way of example the synthetic peptide may be denatured by adding denaturing concentrations of buffers including but not limited to, sodium dodecyl sulphate (SDS), 2-mercaptoethanol, or urea. Purification of denatured protein can be undertaken by procedures well known to the skilled artisan including but not limited to procedures for electrophoretic resolution on polyacrylamide gels or size exclusion chromatography. The denaturing buffers are removed by any number of means including but not limited to dilution, dialysis, chromatography or by binding to a solid support, for example membrane support or microtitre plate.

Accordingly, another aspect of the present invention contemplates a method of enhancing the immunointeractivity of a polypeptide or protein encoded by ORF 2 of HEV, said method comprising chemically or physically modifying the polypeptide or protein to enable both convalescent and acute phase antibodies to HEV to bind to said polypeptide or protein without substantial N-terminal inhibition of C-terminal binding to convalescent phase antibodies to HEV.

In a most preferred embodiment, the present invention extends to naturally occurring or synthetic (eg recombinant) peptide, polypeptide or protein corresponding to an approximately 30KDa protein from the ORF2 region and/or an approximately 14KDa protein from the ORF3 region and to nucleotide sequences coding for same as well as to fragments, derivatives, homologues or immunological relatives thereof. By "derivatives" is meant to include any single or multiple amino acid substitution, deletion and/or addition relative to the naturally occurring sequence or to the sequences as shown in Figures 1 (SEQ ID NO: 2) and 2 (SEQ ID NO: 4) and including any single or multiple substitution, deletion and/or addition to other molecules associated with the peptide or polypeptide including carbohydrate, lipid and/or other proteinacious moieties. Such derivatives, therefore, include glycosylated or non-glycosylated forms or molecules with altered glycosylation patterns.

The present invention is also directed to a method for distinguishing past (i.e. convalescent phase) or contemporary (i.e. acute phase) infection with HEV whether or not by natural infection or following vaccination, whereby the antisera from infected individuals are preferentially immunologically distinguished by interactive peptides of ORF3 or the N-terminus of ORF2. Alternatively, such antisera are preferably immunologically distinguished by interactive peptides of the C-terminal end portion of ORF2.

The present invention also contemplates a method for the detection of antibodies associated with HEV which method comprises contacting a peptide or polypeptide corresponding to all, or an antigenic portion of the epitopic regions of the HEV genome or a derivative thereof with a biological sample from a patient to be tested for a time and

under conditions sufficient for a complex to form between the peptide or polypeptide and an antibody reactive to HEV and then detecting the complex. Preferably, the biological sample is serum, a blood derived component such as whole blood, serum or plasma or a secretory component such as saliva. Even more preferably, the peptide or polypeptide is immobilised onto a solid support before, during or after contact with the serum. Methods of detection are well known and include colorimetric, fluorometric and radioactive procedures. Other detection means can also be used such as involving immunoblotting. An example of such immunoblotting techniques includes the well known Western Blot technique. This assay can be varied in any number of ways without departing from the scope of the present invention. Another assay contemplated herein to be useful is the AGEN red cell agglutination assay as described by Wilson *et al.* (1991).

The present invention also extends to the use of peptides or polypeptide corresponding to the epitopic regions of the HEV genome or antigenic fragments thereof, as antigens in a diagnostic test for HEV, or for screening asymptomatic individuals by detection or determination of the titre of antibodies in a patient's serum, for example using enzyme-linked immunoassay or radio immunoassay (RIA) technology or an agglutination assay using antigen-coated beads or the like.

This aspect of the present invention may conveniently be carried out by the detection and/or determination of the titre of antibodies in a biological sample (e.g. whole blood, plasma, serum or saliva) from a human subject, said method comprising contacting said sample with peptides or polypeptides corresponding to epitopic regions of the HEV genome or a fragment or derivative thereof for a time and under conditions sufficient for a complex to form between the peptide or polypeptide and an antibody reactive to HEV and then detecting the complex and/or amount of peptide or polypeptide which has been bound in the complex. Preferably, the peptide or polypeptide is immobilised onto a solid support before, during or after contact with the sample and the peptides or polypeptides are as hereinbefore defined.

Alternatively, HEV infection may be detected or at least a negative result re-confirmed by screening for HEV associated immune complexes. It is possible, for

example, that a negative antibody result could have been caused by antibodies forming complexes with HEV thereby not being available for binding in the aforementioned assay. To conveniently detect HEV immune complexes, serum or other biological fluid is contacted with an anti-HEV antibody (e.g. a monoclonal antibody) for a time and under conditions sufficient for a HEV-antibody immune complex to bind. Preferably, the anti-HEV antibody is first immobilised onto a solid support. An anti-immunoglobulin antibody, generally with a label or other reporter molecule attached, is then used to screen for the antibody component of the HEV complex.

One skilled in the art will immediately recognise that the assays as contemplated herein may be modified without departing from the scope of the present invention. All such modifications and variations of these assays are encompassed by the present invention.

The presence of HEV convalescent and/or acute phase antibodies in a subject's biological fluid (such as whole blood, plasma, serum or saliva) can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. This includes both single-site and two-site, or "sandwich", assays of the non-competitive types, as well as in the traditional competitive binding assays. Sandwich assays are particularly useful and include ELISA dip-stick assays and Immunofluorometric assays (IFA). Competitive binding assays include radioimmunoassays (RIA). Western blot assays may also be used. The choice of assay will depend on the sensitivity required, materials available and the biological sample employed.

A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in one assay, an unlabelled peptide, polypeptide or protein (hereinafter abbreviated to "polypeptide") capable of preferably binding to acute or convalescent phase antibodies to HEV is immobilised onto a solid substrate and the biological sample to be tested for HEV antibodies brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of a polypeptide-antibody primary complex, an

immunoglobulin specific antibody, labelled with a reporter molecule capable of producing a detectable signal, is then added and incubated, allowing time sufficient for the formation of a secondary complex of polypeptide-antibody-labelled antibody. Any unreacted material is washed away, and the presence of the VPF is determined by observation of a signal produced by the reporter molecule on the second antibody. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing with a control sample containing known amounts of HEV antibody. Variations of this assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound polypeptide, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and then added simultaneously to the bound polypeptide. These techniques are well known to those skilled in the art, and the possibility of variations will be readily apparent. The antibodies used above may be monoclonal or polyclonal.

In this assay, the HEV derived polypeptide or antigenic parts thereof is either covalently or passively bound to a solid surface. The solid substrate is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microtitre plates, or any other surface suitable for conducting an immunoassay such as a dipstick. The binding processes are well-known in the art and generally consist of cross-linking, covalently binding or physically adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of an HEV polypeptide-antibody complex. The most commonly used reporter molecules in this type of assay include enzymes, fluorophores and radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the antimmunoglobulin antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase

and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change.

Alternatively, fluorescent compounds, such as fluorescein, anthranide such as europium and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in an enzyme immunoassay (EIA), the fluorescent labelled antibody is allowed to bind to the polypeptide in the polypeptide-antibody complex. After washing off the unbound reagent, the remaining complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent molecules or bioluminescent molecules may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

Accordingly, one aspect of the present invention contemplates a method of detecting convalescent and/or acute phase antibodies in HEV in a biological sample, said method comprising the steps of contacting said biological sample with an immobilised polypeptide preferably interactive with acute phase or convalescent phase antibodies to HEV for a time and under conditions sufficient for a polypeptide-antibody complex to form and subjecting said complex to a detecting means. The latter complex may be detected by, for example, the addition of an antimmunoglobulin antibody labelled with a reporter molecule.

Alternatively, a competitive immunoassay may be used. The most convenient assay of this type is a radioimmunoassay (RIA). In another assay, the AGEN red cell agglutination test may be employed (see Wilson *et al.* 1991).

The invention also extends to use of the peptides and/or polypeptides, or fragments, or derivatives of the present invention in the treatment or prophylaxis of patients.

As contemplated herein prophylaxis may be achieved in a patient by immunising a patient with a vaccine containing antigens corresponding to the epitopic regions of the HEV genome and a suitable adjuvant sufficient to elicit an antibody response in a patient. Methods for production of vaccines are well known to those skilled in the art and by way of example a convenient reference is Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pennsylvania, USA. Preferred HEV antigens include the N-terminal region of ORF2 or the C-terminal region of ORF2.

Due to the nature of the epitopic region(s) of the HEV antigen it should therefore be possible to discriminate between naturally infected individuals and vaccinated individuals.

The present invention encompasses other forms of kits and diagnostic assays including a kit comprising a container adapted to contain a synthetic peptide or polypeptide corresponding to the epitopic region of the HEV genome or its fragments, derivatives, homologues and/or immunological relatives. Preferred antigens are the N-terminal region of ORF2 and the C-terminal region of ORF2 of HEV. The kit may contain a second container adapted to contain or receive a sample to be tested. A third container may be present adapted to contain reagents for detecting HEV-antibody complexes. Alternatively, where the kit is to detect HEV immune complexes, the kit may comprise one or more containers (e.g. wells) adapted to contain a HEV specific antibody (e.g. a monoclonal antibody). Additional containers with the kit may then contain receptacles for receiving fluid samples and a labelled antibody.

In further accordance with the present invention, expression of the cDNA insert encoding the epitopic regions of HEV described herein or fragments thereof, may be achieved in a number of different ways.

As an example, successful expression of the antigens encoding the epitopic regions of HEV as a fusion protein can be achieved using the pGEX vectors which give expression of glutathione S-transferase fusion proteins, using *E. coli* as the host cells. Expression could also be achieved, by way of example, using pEV vectors or the polyhistidine expression vectors again using *E. coli* as the host cells. Any other suitable fusion protein capable of use in a bioaffinity chromatography may also be employed such as polylysine, FLAG and thioredoxin. Alternatively, the epitopic region of HEV may be expressed as a non-fused polypeptide, by using appropriate vector and host cell combinations. Other vector and host cell combinations which can be used in accordance with the present invention including a number of well described yeast shuttle vectors for use in yeast cells, or eukaryotic vectors useful in continuous cell lines, (eg CHO cells) yeast cells and/or insect cells or transgenic animals.

The present invention is applicable to any strain of HEV which has a genomic organisation comprising open reading frames equivalent to ORF2 and ORF3 as defined by Tam *et al* (1991). Of the strains isolated to date, any strain of HEV is useful in the practice of the present invention.

The present invention will now be further described with reference to the following non-limiting Figures and Examples.

In the Figures:

Figure 1 shows the nucleotide and deduced amino acid sequence of the 76kDa protein encoded by Open Reading Frame 2, of the strain of hepatitis E virus isolated from an epidemic in Xinjiang autonomous region of China.

Figure 2 shows the nucleotide deduced amino acid sequences of the 14kDa protein encoded by Open Reading Frame 3 of the HEV genome, of the strain of hepatitis E virus isolated from an epidemic in Xinjiang autonomous region of China.

Figure 3 shows a Western blot of sera taken at differing time points from an

infection of a rhesus monkey (*Macaca mulatta*) with the HEV virus using the 56kDa and/or 39kDa fusions proteins or GST as antigen.

Figure 4 shows a comparison of the results obtained using the Western blot of the present invention to screen sera samples from an Australian Aboriginal population versus results obtained with a commercially available EIA for HEV (Genelabs Diagnostics, Singapore).

Figure 5 shows the cloning strategy for the recombinant HEV-GST fusion proteins herein described. The cDNA fragments of 1658, 830 and 380 bp were generated after reverse transcription and PCR of HEV RNA extracted from the bile of an experimentally infected monkey. All DNA fragments were purified by agarose gel electrophoresis and Prep-A-Gene at each step.

Figure 6 shows the cloning strategies for (A): HEV cDNA clones; (B): recombinant HEV-GST fusion proteins ORF3, ORF2.1 and ORF2.2; and (C): the recombinant HEV-GST fusion proteins ORF2, ORF2.3 and ORF2.4. The recombinant HEV-GST fusion proteins herein described. The cDNA fragments of 1658, 830 and 380 bp were generated after reverse transcription and PCR of HEV RNA extracted from the bile of an experimentally infected monkey, while other PCR products were generated from the clones pGEX1-AC3 and pGEX1-AC2.2. All DNA fragments were purified by agarose gel electrophoresis and Prep-A-Gene at each step.

Figure 7 shows a Western blot of human acute hepatitis sera against recombinant GST-ORF2.1 and GST-ORF3 proteins. Approximately equal masses of each recombinant protein and unfused GST were mixed and electrophoresed on a 10% w/v acrylamide gel, proteins transferred to nitrocellulose, and the membrane blocked with casein. The membrane was then incubated with a 1:500 dilution of patient sera or 1:10,000 dilution of HRPO-streptavidin (marker lane), and immune complexes detected using HRPO-conjugated anti-human IgG and enhanced chemiluminescence. Lanes: MW, biotinylated molecular weight markers (sizes indicated at left); 1-10, acute hepatitis A; 11-18, acute hepatitis B; 19-23, acute hepatitis E. The positions of reactive degradation products of

GST-ORF2.1 and GST-ORF3 (closed and open circles, respectively), GST and each fusion protein are indicated at right.

Figure 8 shows a Western blot of GST-ORF2.1 and GST-ORF3 with sera from three rhesus macaques experimentally infected with HEV and subsequently rechallenged with aliquots of the original inoculum. (A), (B), (C) monkeys M31, M32 and M33, respectively. The days of rechallenge are indicated above each figure, and the days of serum collection below each figure. Lane MW, biotinylated molecular weight markers (sizes indicated at left). Note that reactivity to GST-ORF2.1 remains at high levels and is boosted following rechallenge, whereas reactivity to GST-ORF3 declines with time.

Figure 9 is a Western blot of GST-ORF2.1 and GST-ORF2.2 with sera from infected macaques. GST and fusion proteins GST-ORF2.1 and GST-ORF2.2 were mixed in equal proportions, electrophoresed and transferred essentially as in Figure 3. Serum samples are from the animals shown in Figure 8. (A), (B), (C) monkeys M31, M32 and M33, respectively. Lane MW, biotinylated molecular weight markers (sizes indicated at left) Note that reactivity to GST-ORF2.1 is weaker than that to GST-ORF2.2 during the acute phase of infection, but is boosted after rechallenge and subsequently remains at higher levels than that to GST-ORF2.2.

Figure 10 is a Western blot of (a) GST-ORF2, GST-ORF2.1, (b) GST-ORF 2.3 and GST-ORF2.4, with sera from infected macaques. GST and fusion proteins were mixed in equal proportions, electrophoresed and transferred essentially as in Figure 3. Serum samples are from the animals shown in Figure 8: (A), (B), (C), monkeys M31, M32 and M33. Lane MW, biotinylated molecular weight markers. Note that the reactivity of GST-ORF2 appears to lie in the N-terminal sequences overlapping with GST-ORF2.3 and GST-ORF2.4: breakdown products of GST-ORF2 as well as the shorter GST-ORF2.3 and GST-ORF2.4 fusion proteins are strongly reactive with acute phase sera.

EXAMPLE 1

HEPATITIS E VIRUS AND ANTIGENS THEREFROM

The strain of HEV used in these studies was isolated from infected individuals from the Xinjiang autonomous region of China during an epidemic between September 1986 and April 1988.

Aye TT *et al* (1992) reported that an HEV strain isolated from infected individuals from the Xinjiang autonomous region of China had 93.9% nucleotide sequence homology with the Burmese strain (Tam *et al.*, 1989).

Virus was cultured by infecting rhesus monkeys with fecal samples from infected individuals, using known methods (Cao, X-Y *et al*, 1991). The monkeys were acutely infected, with the HEV being excreted into the bile and faeces of the monkey.

The viral antigens were isolated as follows using reverse transcriptase PCR.:

- (a) **Extraction of RNA from bile.** Bile specimens were collected directly from the gall bladder of an infected rhesus monkey at day 7-14 after inoculation. An aliquot of 10 μ l of bile was mixed with 25 μ l nuclease free water and 465 μ l GIT (4M guanidine thiocyanate, 25mM sodium citrate, 0.5% v/v sarkosyl, 0.1M 2-mercaptoethanol). An aliquot of 50 μ l of 3M sodium acetate (pH5.2) was added to the mixture above followed by extraction with 450 μ l phenol/chloroform (5:1). After separation by centrifugation, the upper phase was transferred to a fresh tube containing 2 μ l of 10 mg/ml tRNA. The RNA was precipitated in 750 μ l of ice-cold isopropanol at -20°C for a minimum of 2 hours, then pelleted at 13,000 rpm for 15 minutes, washed with 80% v/v ethanol and repelleted as above. The dried pellet was resuspended in 10 μ l of nuclease free water and incubated at 65°C for 10 minutes, then stored at 4°C ready for RT-PCR. A similar method may be used on faeces.

- (b) Primers. Two pairs of primers were designed to isolate the whole fragment of ORF3 and one third of the 3' end of ORF2 (ORF2.1), respectively. These primers were modified to contain *Eco*R1 restriction sites to facilitate cloning. The nucleotide sequences of the primers, with the HEV-specific sequences underlined, are:

For ORF3: 5'-GTGAATTCATGAATAACATGTCTTTTGC-3'(forward)
(SEQ ID NO: 5)

5'-GTGAATTCTTAGCGGCGCGGCCCCAGCT-3'(reverse)
(SEQ ID NO: 6)

For ORF2: 5'-GTGAATTCCAGCTGTTCTACTCCCGTCC-3'(forward)
(SEQ ID NO: 7)

5'-GTGAATTCGGCACTTCGTTTATTGATGTT-3'(reverse)
(SEQ ID NO: 8)

A third primer pair was designed to amplify approximately two thirds of ORF2, terminating 27 nucleotides from the carboxy terminus (ORF2.0) This PCR product did not contain modified restriction enzyme sites, and instead was cloned using the 3' A overhang.

ORF2.0: 5'-TCGTAGACCTACCACAGCTG-3' (forward)
(SEQ ID NO: 9)

5'-TCTTAAGGCGCTGAAGCTCA-3' (reverse)
(SEQ ID NO: 10)

These primer sets were designed on the basis of published sequences for HEV.

To extend the collection of cDNA clones (Figure 6C), the first primer pair was designed to amplify the amino-terminal 330bp of ORF2 (ORF2.3). These primers were modified to contain BamH or EcoR 1 restriction sites to facilitate cloning. The nucleotide sequences of primers, with the HEV-specific sequences underlined, are:

P4740: 5'-GTGGATCCATGCGCCCTCGGCCTATTTTGT-3' (forward)

(SEQ ID NO: 11)

P3566: 5'-GTGAATTCTTAGCGGCGCGGCCCCAGCT-3' (reverse)

(SEQ ID NO: 12)

The second primer pair was designed to amplify 440bp fragment of ORF2 which overlaps AC2.3.

P2: 5'-TCGTAGACCTACCACAGCTG-3' (forward)

(SEQ ID NO: 13)

P5019: 5'-CACAAGCTCAGAGGCTATGCCG-3' (reverse)

(SEQ ID NO: 14)

- (c) RT-PCR. Prior to PCR amplification, the RNA was reverse transcribed into cDNA. To each RNA sample, 10µl of RT Master Mixture (4µl 25mM MgCl₂, 2.0µl 10XRT buffer, 2.0µl 10mM dNTP mixture, 1.0µl 200ng/µl reverse primer, 0.5µl rRNAsin, 0.5 µl AWW RT) was added. After a 60 minute incubation at 42°C, the reaction was boiled for 5 minutes and quenched in chip ice for 5 minutes.

For the PCR reaction, 30µl of PCR Master Mixture (19.1µl nuclease free water, 3.6µl 25mM MgCl₂, 3.0µl 10XTaq Buffer (500 mM KCl, 100mM Tris pH 9.0, 1% v/v Triton X-100), 3.0 µl 2mM dNTP, 1.0µl forward primer, 0.3µl AmpliTaq DNA polymerase (Promega) was mixed with the cDNA reaction and overlayed with 50 µl mineral oil. The amplifications were carried out in a thermocycler (DNA Thermal Cycler 480, PERKIN ELMER CETUS) with the following cycling program: 94°C denaturation for 1 minute, 55°C annealing for 1 minute and 72°C extension for 2 minutes. A total of 40 cycles were processed followed by a final extension at 72°C for 7 minutes.

EXAMPLE 2

CONSTRUCTION OF EXPRESSION VECTORS

The plasmids and cloning strategy used in this study are shown in Figures 5 and 6. PCR products were separated on a 1.0% v/v agarose gel. Fragments of about 380 bp (ORF3), 830 bp (ORF2.1) and 1658 bp (ORF2.0) were recovered by cutting the bands out of the gel. The DNA was purified using the Pre-A-Gene kit (Bio-Rad, USA). Fragments ORF3 and ORF2.0 were digested with *Eco*R1 and the restriction fragments (370bp or 820 bp) were separated on a gel and purified as above. Approximately 6ng of ORF3 or 16 ng of the ORF 2.1 DNA were ligated with 10 ng of pGEX1 vector *Eco*R1 cut, dephosphorylated, (Pharmacia Biotech, USA) to give the plasmids pGEX1-AC3 and pGEX1-AC2.1, respectively, transformed into *E. coli* strain NM522 (Pharmacia Biotech, USA) using the Gene Pulser (Bio-Rad, USA), and plated on LB-ampicillin. Plasmids were prepared from transformants by minipreparation and following *Eco*R1 digestion were examined for inserts of the expected sizes. Fragment ORF2.0 (20 ng) was ligated with 50 ng of dephosphorylated vector pCR (Invitrogen, San Diego, Ca) utilizing the T overhangs on plasmid and A overhangs on PCR product to give the plasmid pCR-AC2.0. After transformation and selection of recombinants, purified plasmids were subjected to partial *Eco*R1 digestion and the resulting fragment of 1670 bp purified and ligated into pGEX1 as above to give plasmid pGEX1-AC2.0. This plasmid and pGEX1-AC2.1 were digested to completion with *Kpn*I and *Bam*H1. The restriction fragment of 1390 bp from pGEX1-AC2.0 was then ligated with the fragment from pGEX1-AC2.0 containing the pGEX 1 sequence together with the ORF2 sequences downstream of the *Kpn*I site, to generate plasmid pGEX1-AC2.2.

The plasmids and cloning strategy used in this study are shown in Figures 5 and 6. Three clones, pGEX1-AC3, pGEX1-AC2.1 and pGEX1-AC2.2, were constructed in this laboratory earlier. DNA from plasmid pGEX1-AC3 and pGEX1-AC2.2 were extracted by minipreparation and used as templates for PCR to amplify two overlapped fragments. A PCR product from pGEX1-AC3 (ORF2.3), which contains 330bp nucleotides of 5' end of ORF2, was digested with *Bam*H1 and *Eco*R1

and ligated with pGEX1, to generate plasmid pGEX1-AC2.3. Another PCR product from pGEX1-AC2.2 was digested with Pvu 2 and EcoR1(360bp) and ligated with the restriction fragment of 312bp from ORF2.3 after BamH1 and Pvu2 digestion, as well as pGEX1. The resulting plasmid(pGEX1-AC2.4) contains first 672bp nucleotides of ORF2. pGEX1-AC2.4 was digested with EcoR1 then ligated with the EcoR1 fragment of 1328bp from pGEX2.2 to give plasmid pGEX-AC2, which contains full length of ORF2.

Selected colonies from each transformant were then induced with isopropylthio- β -D-galactoside (IPTG) and examined for expression of fusion proteins of the expected sizes.

EXAMPLE 3

PRODUCTION OF GST FUSION PROTEINS

The transformants of pGEX1-AC3, pGEX1-AC2.1 and -2.2, and pGEX1 (as a control) were grown for 5 h in L-Broth medium (LB) containing 50 μ g/ml of ampicillin. An aliquot of 0.5 μ l of the overnight culture was inoculated into 200 ml of LB plus ampicillin and incubated for 6 hr. IPTG (200 μ l of 100 mM stock) was added and the culture was grown overnight. Cells were pelleted at 4000 rpm for 15 min in a bench centrifuge. The pellet was washed in 10mM Tris (pH 7.5) and spun as before. For each gram of packed cells, 3 μ l of lysis buffer (50 mM Tris pH 8.0, 1 mM EDTA, 100 mM NaCl), 4 μ l of 100 mM phenylmethylsulfonylfluoride (PMSF) and 80 μ l of 10 mg/ml lysozyme were added. The suspension was incubated at room temperature for 30 min then 37° for an additional 30 min. The solution was sonicated for 3 min. and centrifuged at 4000 rpm for 15 min. The pellet (containing most of the fusion proteins) was washed with ice-cold 1M NaCl, 10 mM Tris pH 7.5. The protein pellet was resuspended in 3 ml of 10mM Tris (pH 7.5), and stored in aliquots at -20°.

EXAMPLE 4

SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

Each of the fusion proteins (GST-ORF3, GST-ORF2.1, GST-ORF2.2, GST-ORF2.3, GST-ORF2.4) was thoroughly resuspended in 50 mM Tris (pH 6.8), 2% v/v SDS, 5% v/v 2-mercaptoethanol, 8% v/v glycerol, 0.01% v/v bromophenol blue. In each experiment, unfused GST (prepared as above) was mixed with the fusion proteins before electrophoresis. The protein mixture was heated at 100° for 5 min and loaded on SDS-PAGE gels in a mini-gel apparatus (Bio-Rad, USA) with a separating gel of 10% w/v acrylamide, 0.267% w/v bis-acrylamide, using the discontinuous buffer system of Laemmli (2% w/v SDS, 5% v/v 2-mercaptoethanol, 8% v/v glycerol, 50 mM Tris[pH 6.8], 0.01% w/v bromophenol blue). The gels were electrophoresed at 75V for 20 min then 150V until the dye reached the bottom of the gel. The proteins were transferred to nitrocellulose membranes (Hybond-C extra, Amersham) on a Semi-Dry Transfer Cell (Bio-Rad, USA) in transfer buffer (0.15M glycine, 0.025M Tris [pH 8.5], 20% v/v methanol). Biotinylated marker proteins (Bio-Rad) were run in reference lanes.

EXAMPLE 5

WESTERN BLOT

The nitrocellulose membranes were blocked with 0.1M Tris (pH7.5), 0.1M NaCl, 0.3% v/v tween-20 (TBST) containing 3% w/v casein and 0.01% w/v sodium azide at room temperature for 1 hr. Test sera were diluted 1:500 in TBST plus 1% w/v casein and preabsorbed with 1% w/v) GST lysate suspension at 37° for 1.5 hr. The diluted sera were incubated at 35° with agitation for 1 hr with the membrane, using the Mini-Proean Multi Screen apparatus (Bio-Rad, USA). The membranes were washed three times for 5 min in TBST, incubated with horseradish peroxidase (HRPO)-conjugated rabbit anti-human IgG (diluted 1:5000; DAKO Co. Denmark) at 35° for 1 hr, then washed as before. Biotinylated markers were detected using HRPO-conjugated streptavidin (diluted 1:10,000; Amersham). Enzyme complexes were detected by enhanced chemiluminescence (ECL; Amersham). The membranes were

soaked in ECL reagents for 1 min, then placed between acetate sheets and exposed to Hyperfilm ECL (Amersham, USA) for 30 sec to 2 min at room temperature.

EXAMPLE 6

TEST SERA FOR WESTERN BLOT

Human acute hepatitis sera

Sera from other viral hepatitis cases in Australia were used as negative controls: 10 acute hepatitis A (positive for anti-HAV IgM, Abbot Diagnostics), 18 acute hepatitis B (positive for anti-HBc IgM, Abbot Diagnostics) and 58 hepatitis C (positive in second generation HCV EIA, Abbot Diagnostics). Sixty-six sera were collected from acute phase patients in Xinjiang, China during the Hepatitis E epidemic of 1988.

Serial sera from infected monkeys

Serum specimens were collected approximately twice weekly from 7 rhesus monkeys (M27, M31, M32, M33, M34, M35, M36) experimentally infected with HEV. Samples were stored at -20°.

EXAMPLE 7

EXPRESSION OF HEV-GST FUSION PROTEINS

Clones of putative HEV structural proteins were constructed in the highly efficient pGEX expression system, giving rise to proteins fused with GST (normal molecular mass of 26 kDa). The clones studied represent the complete sequence of ORF3 (GST-ORF3), and partially overlapping sequences within ORF2 (GST-ORF2.1, GST-ORF2.2, GST-ORF2.3, GST-ORF2.4) as shown in Figures 5 and 6. After induction with IPTG, bacterial lysates were found to contain unique proteins of about 37, 39.5, 49, 56, 86 and 97 kDa, consistent with the predicted molecular masses of these translated sequences. It should be noted that pGEX1-AC2, pGEX1-AC2.2 and pGEX1-AC2.1 encode proteins having common C-termini (representing the extreme

C-terminus of ORF2). pGEX1-AC2.1 encodes 273 amino acids, that is the most C-terminal one-third of ORF2, while pGEX1-AC2.2 has a N-terminal extension of 294 amino acids, and pGEX1-AC2 has a further extension of 93 amino acids and therefore represents 660 amino acids or the full-length ORF2. It should be further noted that pGEX1-AC2, pGEX1-AC2.3 and pGEX1-AC2.4 encode proteins having common N-termini (representing the extreme N-terminus of ORF2). pGEX1-AC2.3 encodes 1509 amino acids, while pGEX1-AC2.4 has a C-terminal extension of 110 amino acids and pGEX1-AC2 has a further extension of 400 amino acids or the full-length ORF2, as above.

EXAMPLE 8

DETECTION OF ANTI-HEV BY WESTERN BLOT

To select conditions for a sensitive diagnostic assay and to examine the dynamics of antibody responses to each fusion protein, western blots were conducted to detect anti-HEV IgG in sera from acute-phase hepatitis patients and from experimentally infected monkeys.

Detection of anti-HEV in human acute hepatitis sera

Sera from well-documented cases of acute hepatitis A, B, C and E were assayed by western blot (Figure 6, Table 1). The results show that none of 10 Hepatitis A and 18 Hepatitis B sera contained IgG against GST-ORF3 or GST-ORF2.1, but 3 of 58 hepatitis C were weakly reactive to GST-ORF2.1 and one of these was also reactive to GST-ORF3. Sixty-one of 66 (92%) acute HEV sera from Xinjiang contained anti-HEV IgG that bound specifically to the GST-ORF2.1 band but not to the negative control (GST). Of these, 55 were also positive for IgG to GST-ORF3, and one serum was reactive with GST-ORF3 but not with GST-ORF2.1. Nine of nineteen sera collected between 3 and 9 months after suspected HEV were reactive against GST-ORF2.1, but only 3 of these were active with GST-ORF3. When tested using a commercial EIA based on ORF3 of the Burmese and Mexican strains, only one of the convalescent sera was reactive and this serum was reactive against both GST-ORF2.1 and GST-ORF3.

Both fusion proteins were, therefore, immunoreactive by Western blot and resulted in a high detection rate of anti-HEV IgG in epidemic cases of HEV in China, with a high level of specificity indicated by the low reactive rate in other forms of viral hepatitis. Some HEV samples strongly positive for antibody to GST-ORF2.1 or 3 were also reactive with degradation products of the respective fusion proteins (indicated with closed and open circles respectively in Figure 6 and subsequent figures), but no sera showed reactivity with the control GST protein band.

Detection of anti-HEV in serial sera from rhesus monkeys

Rhesus monkeys were inoculated intravenously with a Chinese strain of HEV, and displayed an increase in ALT at 7-26 days indicating acute hepatitis. Serial blood samples were tested for anti-HEV IgG by Western blot, and the results for one monkey (M27) are shown in Figure 7. In this monkey, antibodies to both GST-ORF2.1 and GST-ORF3 were detected at day 16, with anti-GST-ORF2.1 continuing to increase throughout the study period whereas anti-GST-ORF3 reached a peak at 71 days and then declined. Degradation products of both fusion proteins were sometimes detected in Western blots, as indicated with open circles (GST-ORF3) or closed circles (GST-ORF2.1) in Figure 7 and subsequent figures.

All 7 monkeys seroconverted since antibody to GST-ORF2.1 was detected at day 14-21, immediately after ALT elevation. Antibody to GST-ORF3 was first detected at the same time or 7 days after first detection of GST-ORF2.1 in four monkeys, but was weakly detectable in only one serum sample from each of 2 monkeys and in no samples from the remaining monkey. Antibody to GST-ORF2.1, therefore, appears to be a more consistent marker of infection in macaques, as was also observed with samples from man (Table 1).

Two monkeys were rechallenged at days 53, 107 and 316, and a third monkey at days 107 and 316 after initial infection. All monkeys have shown biochemical evidence of hepatitis after primary inoculation and less severe hepatitis after the first rechallenge. Only one (M32) of two monkeys appeared to be infected after the second rechallenge, suggesting the acquisition of partial protective immunity after each

infection. Coincident with this partial immunity, we detected antibody to GST-ORF2.1 in each monkey (Figure 8), remaining at high levels throughout the study and sometimes being boosted after rechallenge. The boosted reactivity may result from limited viral replication or at least partly from viral antigen present in the inocula. It should be noted, however, that in both M31 and M32 no further increase in reactivity was detected following a third rechallenge, which suggests that the increased reactivity observed previously was due to virus replication.

Antibody to GST-ORF3 was detected at high levels in M31 and M32 between days 17 and approximately 60, but waned thereafter. Interestingly, antibody to GST-ORF3 was boosted after the second rechallenge in monkey 32, and in monkey 33 the only sample positive for this antibody was after rechallenge. The reactivity with GST-ORF3 closely reflects that observed using a commercial HEV EIA based on ORF3 synthetic proteins, in which the day 151 sample was also the only reactive sample from M33.

Comparison of GST-ORF2.1 and 2.2

The fusion protein GST-ORF2.1 contains only the carboxy-terminal one-third of the coding sequences from HEV ORF2. In order to examine whether additional sequences from ORF2 would show greater reactivity, sera from monkeys M31, M32 and M33 were reacted with GST-ORF2.1 and GST-ORF2.2, having an N-terminal extension of 294 amino acids. In the acute phase of infection, the extended protein was indeed more strongly reactive, although no samples were positive only for this protein. However, interestingly, the peak reactivity of the extended protein was in fact much lower than that of GST-ORF2.1 in each of the animals. In addition, the antibody was found to decline with time and was only weakly boosted after each rechallenge.

These two proteins were also used to screen some of the sera from patients with acute hepatitis A, B or E as described above, with no difference observed in the proportion of reactive sera, although in most cases the signal for GST-ORF2.2 was stronger than that for GST-ORF2.1, as also seen during the acute phase in monkeys

(Fig. 9).

GST fusion proteins GST-ORF2.1, GST-ORF2.2 and GST-ORF2 are partially overlapping with common carboxy termini (Figure 5, Figure 6). As shown in Figures 3 and 7-10, the shortest of these, GST-ORF2.1, is the most reactive with convalescent hepatitis E sera and therefore is the best protein for expression of the epitopes which are involved in protection from reinfection.

GST fusion proteins GST-ORF2, GST-ORF2.3 and GST-ORF2.4 are partially overlapping with common amino term (Figure 5). As shown in Figure 10, the reactivity in sera from experimentally infected macaques against the full-length GST-ORF2 closely approximates that seen with the shorter GST-ORF2.3 and GST-ORF2.4 proteins: that is, they are most reactive with acute phase sera and poorly reactive with convalescent phase sera. The reactivity of full-length GST-ORF2 therefore lies within the region b5147 to 5477 in ORF2 according to Tam, and no reactivity is seen against the sequences b6326 to 7145 which overlap with GST-ORF2.1. The sequences b5147 to 5477 as in GST-ORF2.3 or GST-ORF2.4 are therefore suitable for the detection of acute phase antibodies to HEV, and on the basis of the greater reactivity in macaque M33 compared to that against GST-ORF3, we claim that these proteins will enhance the detection of acute hepatitis E virus infection.

Taken together with the lower reactivity of GST-ORF2.2 and GST-ORF2 compared with GST-ORF2.1 (Figure 9, Figure 10), this again suggests that the inclusion of amino terminal sequences of ORF2 inhibits the reactivity of carboxy terminal sequences which may be included in the same protein. It would be expected that they might also reduce the immunogenicity of similar proteins, and we therefore claim that proteins containing sequens between b5147 and 6325 are suitable for diagnostic reagents but not for vaccines unless they can be demonsrated not to interfere with the reactivity of the sequences 6326 to 7145.

It is further claimed that antibodies to GST-ORF3 and/or GST-ORF2.3 and/or GST-ORF2.4 could be used to detect clinical or subclinical infection with HEV in

vaccine recipients who have antibodies to GST-ORF2.1 as a result of active or passive immunisation.

EXAMPLE 9

Recent infection can be diagnosed on the basis of antibody to ORF3 and/or increasing titre of antibody to C-terminus ORF2 and/or antibody to the N-terminal part of ORF2, while past infection of HEV can be diagnosed by the presence of antibody to ORF2 with or without antibody to ORF3. Antibody to ORF3 is expected to be present only in a proportion of individuals infected with the virus, and not in individuals who are exposed to viral antigen in the form of an inactivated or live, attenuated vaccine. This is also likely to be true for antibodies to ORF1.

EXAMPLE 10

DEVELOPMENT OF VACCINES AGAINST HEV

Conventional approaches to recombinant vaccines generally rely on using either synthetic peptides which are small enough to be chemically synthesised, or recombinant or fusion proteins representing as large a portion of the antigen of interest as possible. Consequently, most work on HEV has previously focussed on full-length open reading frame (ORF) 2. However, a key feature of the present invention is the disparity between the length of recombinant HEV proteins and their reactivity. Recombinant protein, GST-ORF2.1, representing only the C-terminal 1/3rd of ORF2, is in fact far more reactive with convalescent sera than is a full length GST-ORF2 protein. Furthermore, the reactivity of the full-length protein is most likely attributable to the N-terminal sequences, as its reactivity with convalescent sera almost exactly mirrors that of such N-terminal proteins (Figure 10). Full-length ORF2, therefore, does not efficiently present the epitopes found in GST-ORF2.1, which are likely to be important for protective humoral immunity.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The

invention also includes all of the steps, features, compositions and compounds referred to our indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Table 1. Detection of anti-HEV IgG by Western Blotting

Infection	Number	Number positive (%)		Total (%) ^a
		GST-ORF2.1	GST-ORF3	
Hepatitis A	10	0	0	0
Hepatitis B	18	0	0	0
Hepatitis C	58	3 (5)	1 (2)	3 (5)
Hepatitis E (acute) ^b	66	61 (92)	56 (85)	62 (94)
Hepatitis E (convalescent) ^c	19	9 (47)	3 (16)	9 (47)

^a Positive for GST-ORF2.1 and/or GST-ORF3.^b Less than three months after onset of disease^c Three to nine months after onset of disease

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT (Other than U.S.): THE MACFARLANE BURNET CENTRE FOR
MEDICAL RESEARCH LIMITED

(U.S. only): ANDERSON, D.A.
TORRESI, J.
LOCARNINI, S.A.
LI, FAN
HUI, ZHUANG

(ii) TITLE OF INVENTION: IMMUNOREACTIVITY ANTIGENS OF HEPATITIS
E VIRUS

(iii) NUMBER OF SEQUENCES: 24

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE
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(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT *International*
(B) FILING DATE: 23-SEP-1994

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PM1423
(B) FILING DATE: 24-SEP-1993

(A) APPLICATION NUMBER: PM2964
(B) FILING DATE: 15-DEC-1993

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1984 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1980

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

ATG CGC CCT CGG CCT ATT TTG CTG TTG CTC CTC ATG TTT CTG CCT ATG	48
Met Arg Pro Arg Pro Ile Leu Leu Leu Leu Met Phe Leu Pro Met	
1 5 10 15	
CTG CCC GCG CCA CCG CCC GGT CAG CCG TCT GGC CGC CGT CGT GGG CGG	96
Leu Pro Ala Pro Pro Pro Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg	
20 25 30	
CGC AGC GGC GGT TCC GGC GGT GGT TTC TGG GGT GAC CGG GTT GAT TCT	144
Arg Ser Gly Gly Ser Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser	
35 40 45	
CAG CCC TTC GCA ATC CCC TAT ATT CAT CCA ACC AAC CCC TTC GCC CCC	192
Gln Pro Phe Ala Ile Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Pro	
50 55 60	
GAT GTC ACC GCT GCG GCC GGG GCT GGA CCT CGT GTT CGC CAA CCC GCC	240
Asp Val Thr Ala Ala Ala Gly Ala Gly Pro Arg Val Arg Gln Pro Ala	
65 70 75 80	
CGA CCA CTC GGC TCC GCT TGG CGT GAC CAG GCC CAG CGC CCC GCC GTT	288
Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ala Gln Arg Pro Ala Val	
85 90 95	
GCC TCA CGT CGT AGA CCT ACC ACA GCT GGG GCC GCG CCG CTA ACC GCG	336
Ala Ser Arg Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala	
100 105 110	
GTC GCT CCG GCC CAT GAC ACC CCG CCA GTG CCT GAT GTT GAC TCC CGC	384
Val Ala Pro Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg	
115 120 125	
GGC GCC ATC CTG CGC CGG CAG TAT AAC CTA TCA ACA TCT CCC CTT ACT	432
Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr	
130 135 140	

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TCT Ser 145	TCC Ser	GTG Val	GCC Ala	ACC Thr	GGT Gly 150	ACA Thr	AAC Asn	TTG Leu	GTT Val	CTA Leu 155	TAC Tyr	GCC Ala	GCT Ala	CCT Pro	CTT Leu 160	480
AGC Ser	CCA Pro	CTT Leu	CTA Leu	CCC Pro 165	CTC Leu	CAG Gln	GAC Asp	GGC Gly	ACC Thr 170	AAT Asn	ACT Thr	CAT His	ATA Ile	ATG Met	GCC Ala 175	528
ACA Thr	GAA Glu	GCT Ala	TCT Ser 180	AAT Asn	TAT Tyr	GCC Ala	CAG Gln	TAC Tyr 185	CGG Arg	GTT Val	GCT Ala	CGT Arg	GCC Ala 190	ACA Thr	ATT Ile	576
CGC Arg	TAC Tyr	CGC Arg 195	CCG Pro	CTG Leu	GTC Val	CCC Pro	AAC Asn 200	GCT Ala	GTT Val	GGT Gly	GGC Gly	TAC Tyr 205	GCC Ala	ATC Ile	TCC Ser	624
ATC Ile 210	TCG Ser	TTC Phe	TGG Trp	CCA Pro	CAG Gln	ACC Thr 215	ACC Thr	ACC Thr	ACC Thr	CCG Pro	ACG Thr 220	TCC Ser	GTT Val	GAC Asp	ATG Met	672
AAT Asn 225	TCA Ser	ATA Ile	ACC Thr	TCG Ser	ACG Thr 230	GAT Asp	GTT Val	CGT Arg	ATT Ile	TTA Leu 235	GTC Val	CAG Gln	CCC Pro	GGC Gly	ATA Ile 240	720
GCC Ala	TCC Ser	GAG Glu	CTT Leu	GTT Val 245	ATC Ile	CCA Pro	AGT Ser	GAG Glu	CGC Arg 250	CTA Leu	CAC His	TAC Tyr	CGT Arg	AAC Asn 255	CAA Gln	768
GGT Gly	TGG Trp	CGC Arg	TCT Ser 260	GTT Val	GAG Glu	ACC Thr	TCC Ser	GGG Gly 265	GTG Val	GCG Ala	GAG Glu	GAG Glu	GAG Glu	GCC Ala 270	ACC Thr	816
TCT Ser	GGT Gly 275	CTT Leu	GTT Val	ATG Met	CTC Leu	TGC Cys	ATA Ile 280	CAT His	GGC Gly	TCA Ser	CCT Pro	GTA Val 285	AAT Asn	TCT Ser	TAT Tyr	864
ACT Thr 290	AAT Asn	ACA Thr	CCT Pro	TAT Tyr	ACC Thr	GGT Gly 295	GCC Ala	CTC Leu	GGG Gly	CTG Leu	TTG Leu 300	GAC Asp	TTT Phe	GCC Ala	CTC Leu	912
GAA Glu 305	CTT Leu	GAG Glu	TTC Phe	CGC Arg	AAC Asn 310	CTC Leu	ACC Thr	CCC Pro	GGT Gly	AAT Asn 315	ACC Thr	AAC Asn	ACG Thr	CGG Arg	GTC Val 320	960
TCC Ser	CGT Arg	TAC Tyr	TCC Ser	AGC Ser 325	ACT Thr	GCC Ala	CGT Arg	CAC His	CGC Arg 330	CTT Leu	CGT Arg	CGC Arg	GGT Gly	GCA Ala 335	GAT Asp	1008
GGG Gly	ACT Thr	GCC Ala	GAG Glu 340	CTT Leu	ACC Thr	ACC Thr	ACG Thr	GCT Ala 345	GCT Ala	ACC Thr	CGC Arg	TTC Phe	ATG Met 350	AAG Lys	GAC Asp	1056

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CTC	TAT	TTT	ACT	AGT	ACT	AAT	GGT	GTC	GGT	GAG	ATC	GGC	CGT	GGG	ATA	1104
Leu	Tyr	Phe	Thr	Ser	Thr	Asn	Gly	Val	Gly	Glu	Ile	Gly	Arg	Gly	Ile	
		355					360					365				
GCG	CTT	ACC	CTG	TTT	AAC	CTT	GCT	GAC	ACC	CTG	CTT	GGC	GGT	CTA	CCG	1152
Ala	Leu	Thr	Leu	Phe	Asn	Leu	Ala	Asp	Thr	Leu	Leu	Gly	Gly	Leu	Pro	
	370					375					380					
ACA	GAA	TTG	ATT	TCG	TCG	GCT	GGT	GGC	CAG	CTG	TTC	TAC	TCT	CGT	CCC	1200
Thr	Glu	Leu	Ile	Ser	Ser	Ala	Gly	Gly	Gln	Leu	Phe	Tyr	Ser	Arg	Pro	
385					390					395					400	
GTC	GTC	TCA	GCC	AAT	GGC	GAG	CCG	ACT	GTT	AAG	CTT	TAT	ACA	TCT	GTA	1248
Val	Val	Ser	Ala	Asn	Gly	Glu	Pro	Thr	Val	Lys	Leu	Tyr	Thr	Ser	Val	
				405				410						415		
GAG	AAT	GCT	CAG	CAG	GAT	AAG	GGT	ATT	GCA	ATC	CCG	CAT	GAC	ATC	GAC	1296
Glu	Asn	Ala	Gln	Gln	Asp	Lys	Gly	Ile	Ala	Ile	Pro	His	Asp	Ile	Asp	
			420					425					430			
CTC	GGG	GAG	TCT	CGT	GTA	GTT	ATT	CAG	GAT	TAT	GAC	AAC	CAA	CAT	GAG	1344
Leu	Gly	Glu	Ser	Arg	Val	Val	Ile	Gln	Asp	Tyr	Asp	Asn	Gln	His	Glu	
		435					440					445				
CAG	GAC	CGA	CCG	ACA	CCT	TCC	CCA	GCC	CCA	TCG	CGC	CCT	TFT	TCT	GTC	1392
Gln	Asp	Arg	Pro	Thr	Pro	Ser	Pro	Ala	Pro	Ser	Arg	Pro	Phe	Ser	Val	
	450					455					460					
CTC	CGA	GCT	AAT	GAT	GTG	CTT	TGG	CTT	TCT	CTC	ACC	GCT	GCC	GAG	TAT	1440
Leu	Arg	Ala	Asn	Asp	Val	Leu	Trp	Leu	Ser	Leu	Thr	Ala	Ala	Glu	Tyr	
465					470					475				480		
GAC	CAG	TCC	ACT	TAC	GGC	TCT	TCG	ACC	GGC	CCA	GTC	TAT	GTC	TCT	GAC	1488
Asp	Gln	Ser	Thr	Tyr	Gly	Ser	Ser	Thr	Gly	Pro	Val	Tyr	Val	Ser	Asp	
				485				490						495		
TCT	GTG	ACC	TTG	GTT	AAT	GTT	GCG	ACC	GGC	GCG	CAG	GCC	GTT	GCC	CGG	1536
Ser	Val	Thr	Leu	Val	Asn	Val	Ala	Thr	Gly	Ala	Gln	Ala	Val	Ala	Arg	
			500				505						510			
TCA	CTC	GAC	TGG	ACC	AAG	GTC	ACA	CTT	GAT	GGT	CGC	CCC	CTT	TCC	ACC	1584
Ser	Leu	Asp	Trp	Thr	Lys	Val	Thr	Leu	Asp	Gly	Arg	Pro	Leu	Ser	Thr	
		515					520					525				
ATC	CAG	CAG	TAT	TCA	AAG	ACC	TTC	TTT	GTC	CTG	CCG	CTC	CGC	GGT	AAG	1632
Ile	Gln	Gln	Tyr	Ser	Lys	Thr	Phe	Phe	Val	Leu	Pro	Leu	Arg	Gly	Lys	
	530					535					540					
CTC	TCC	TTT	TGG	GAG	GCA	GGT	ACT	ACT	AAA	GCC	GGG	TAC	CCT	TAT	AAT	1680
Leu	Ser	Phe	Trp	Glu	Ala	Gly	Thr	Thr	Lys	Ala	Gly	Tyr	Pro	Tyr	Asn	
545					550					555					560	

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TAT AAC ACC ACT GCT AGT GAC CAA CTG CTC GTT GAG AAT GCC GCT GGG Tyr Asn Thr Thr Ala Ser Asp Gln Leu Leu Val Glu Asn Ala Ala Gly 565 570 575	1728
CAT CGG GTT GCT ATT TCC ACT TAC ACC ACT AGC CTG GGT GCT GGT CCC His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro 580 585 590	1776
GTC TCT ATT TCC GCG GTT GCT GTT TTA GCC CCC CAC TCC GCG CTA GCA Val Ser Ile Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala 595 600 605	1824
TTG CTT GAG GAT ACC ATG GAC TAC CCT GCC CGC GCC CAT ACT TTC GAT Leu Leu Glu Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp 610 615 620	1872
GAC TTC TGC CCG GAG TGC CGC CCC CTT GGC CTC CAG GGC TGT GCT TTT Asp Phe Cys Pro Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe 625 630 635 640	1920
CAG TCT ACT GTC GCT GAG CTT CAG CGC CTT AAG ATG AAG GTG GGT AAA Gln Ser Thr Val Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys 645 650 655	1968
ACT CGG GAG TTA TAGT Thr Arg Glu Leu 660	1984

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 660 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Arg Pro Arg Pro Ile Leu Leu Leu Leu Leu Met Phe Leu Pro Met 1 5 10 15
Leu Pro Ala Pro Pro Pro Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg 20 25 30
Arg Ser Gly Gly Ser Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser 35 40 45
Gln Pro Phe Ala Ile Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Pro 50 55 60
Asp Val Thr Ala Ala Ala Gly Ala Gly Pro Arg Val Arg Gln Pro Ala 65 70 75 80

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Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ala Gln Arg Pro Ala Val
85 90 95

Ala Ser Arg Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala
100 105 110

Val Ala Pro Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg
115 120 125

Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr
130 135 140

Ser Ser Val Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu
145 150 155 160

Ser Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala
165 170 175

Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile
180 185 190

Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser
195 200 205

Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met
210 215 220

Asn Ser Ile Thr Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile
225 230 235 240

Ala Ser Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln
245 250 255

Gly Trp Arg Ser Val Glu Thr Ser Gly Val Ala Glu Glu Ala Thr
260 265 270

Ser Gly Leu Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr
275 280 285

Thr Asn Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu
290 295 300

Glu Leu Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val
305 310 315 320

Ser Arg Tyr Ser Ser Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp
325 330 335

Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp
340 345 350

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

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Ala Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro
370 375 380

Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro
385 390 395 400

Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val
405 410 415

Glu Asn Ala Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp
420 425 430

Leu Gly Glu Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu
435 440 445

Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val
450 455 460

Leu Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr
465 470 475 480

Asp Gln Ser Thr Tyr Gly Ser Ser Thr Gly Pro Val Tyr Val Ser Asp
485 490 495

Ser Val Thr Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg
500 505 510

Ser Leu Asp Trp Thr Lys Val Thr Leu Asp Gly Arg Pro Leu Ser Thr
515 520 525

Ile Gln Gln Tyr Ser Lys Thr Phe Phe Val Leu Pro Leu Arg Gly Lys
530 535 540

Leu Ser Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn
545 550 555 560

Tyr Asn Thr Thr Ala Ser Asp Gln Leu Leu Val Glu Asn Ala Ala Gly
565 570 575

His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro
580 585 590

Val Ser Ile Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala
595 600 605

Leu Leu Glu Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp
610 615 620

Asp Phe Cys Pro Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe
625 630 635 640

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Gln Ser Thr Val Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys
645 650 655

Thr Arg Glu Leu
660

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 372 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..369

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG AAT AAC ATG TCT TTT GCT GCG CCC ATG GGT TCG CGA CCA TGC GCC	48
Met Asn Asn Met Ser Phe Ala Ala Pro Met Gly Ser Arg Pro Cys Ala	
1 5 10 15	
CTC GGC CTA TTT TGC TGT TGC TCC TCA TGT TTC TGC CTA TGC TGC CCG	96
Leu Gly Leu Phe Cys Cys Cys Ser Ser Cys Phe Cys Leu Cys Cys Pro	
20 25 30	
CGC CAC CGC CCG GTC AGC CGT CTG GCC GCC GTC GTG GGC GGC GCA GCG	144
Arg His Arg Pro Val Ser Arg Leu Ala Ala Val Val Gly Gly Ala Ala	
35 40 45	
GCG GTT CCG GCG GTG GTT TCT GGG GTG ACC GGG TTG ATT CTC AGC CCT	192
Ala Val Pro Ala Val Val Ser Gly Val Thr Gly Leu Ile Leu Ser Pro	
50 55 60	
TCG CAA TCC CCT ATA TTC ATC CAA CCA ACC CCT TCG CCC CCG ATG TCA	240
Ser Gln Ser Pro Ile Phe Ile Gln Pro Thr Pro Ser Pro Pro Met Ser	
65 70 75 80	
CCG CTG CGG CCG GGG CTG GAC CTC GTG TTC GCC AAC CCG CCC GAC CAC	288
Pro Leu Arg Pro Gly Leu Asp Leu Val Phe Ala Asn Pro Pro Asp His	
85 90 95	

-46-

TCG GCT CCG CTT GGC GTG ACC AGG CCC AGC GCC CCG CCG TTG CCT CAC	336
Ser Ala Pro Leu Gly Val Thr Arg Pro Ser Ala Pro Pro Leu Pro His	
100 105 110	

GTC GTA GAC CTA CCA CAG CTG GGG CCG CGC CGC TAA	372
Val Val Asp Leu Pro Gln Leu Gly Pro Arg Arg	
115 120	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GTGAATTCAT GAATAACATG TCTTTTGC 28

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
GTGAATTCTT AGCGGCGCGG CCCCAGCT 28

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GTGAATTCCA GCTGTTCTAC TCCCGTCC 28

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GTGAATTCGG CACTCCGTTT ATTTGATGTT 30

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCGTAGACCT ACCACAGCTG

20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCTTAAGGCG CTGAAGCTCA

20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTGGATCCAT GCGCCCTCGG CCTATTTTGT

30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTGAATTCTT AGCGGCGCGG CCCCAGCT

28

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCGTAGACCT ACCACAGCTG

20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CACAAGCTCA GAGGCTATGC CG

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2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1658 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..1658

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

T CGT AGA CCT ACC ACA GCT GGG GCC GCG CCG CTA ACC GCG GTC GCT	46
Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala Val Ala	
1 5 10 15	
CCG GCC CAT GAC ACC CCG CCA GTG CCT GAT GTT GAC TCC CGC GGC GCC	94
Pro Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg Gly Ala	
20 25 30	
ATC CTG CGC CGG CAG TAT AAC CTA TCA ACA TCT CCC CTT ACT TCT TCC	142
Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr Ser Ser	
35 40 45	
GTG GCC ACC GGT ACA AAC TTG GTT CTA TAC GCC GCT CCT CTT AGC CCA	190
Val Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Ser Pro	
50 55 60	
CTT CTA CCC CTC CAG GAC GGC ACC AAT ACT CAT ATA ATG GCC ACA GAA	238
Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr Glu	
65 70 75	
GCT TCT AAT TAT GCC CAG TAC CGG GTT GCT CGT GCC ACA ATT CGC TAC	286
Ala Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile Arg Tyr	
80 85 90 95	
CGC CCG CTG GTC CCC AAC GCT GTT GGT GGC TAC GCC ATC TCC ATC TCG	334
Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser Ile Ser	
100 105 110	
TTC TGG CCA CAG ACC ACC ACC ACC CCG ACG TCC GTT GAC ATG AAT TCA	382
Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn Ser	
115 120 125	
ATA ACC TCG ACG GAT GTT CGT ATT TTA GTC CAG CCC GGC ATA GCC TCC	430
Ile Thr Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala Ser	
130 135 140	
GAG CTT GTT ATC CCA AGT GAG CGC CTA CAC TAC CGT AAC CAA GGT TGG	478
Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly Trp	
145 150 155	
CGC TCT GTT GAG ACC TCC GGG GTG GCG GAG GAG GAG GCC ACC TCT GGT	526
Arg Ser Val Glu Thr Ser Gly Val Ala Glu Glu Glu Ala Thr Ser Gly	
160 165 170 175	
CTT GTT ATG CTC TGC ATA CAT GGC TCA CCT GTA AAT TCT TAT ACT AAT	574
Leu Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr Asn	
180 185 190	
ACA CCT TAT ACC GGT GCC CTC GGG CTG TTG GAC TTT GCC CTC GAA CTT	622
Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu Leu	
195 200 205	

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GAG	TTC	CGC	AAC	CTC	ACC	CCC	GGT	AAT	ACC	AAC	ACG	CGG	GTC	TCC	CGT	670
Glu	Phe	Arg	Asn	Leu	Thr	Pro	Gly	Asn	Thr	Asn	Thr	Arg	Val	Ser	Arg	
		210					215					220				
TAC	TCC	AGC	ACT	GCC	CGT	CAC	CGC	CTT	CGT	CGC	GGT	GCA	GAT	GGG	ACT	718
Tyr	Ser	Ser	Thr	Ala	Arg	His	Arg	Leu	Arg	Arg	Gly	Ala	Asp	Gly	Thr	
	225					230					235					
GCC	GAG	CTT	ACC	ACC	ACG	GCT	GCT	ACC	CGC	TTC	ATG	AAG	GAC	CTC	TAT	766
Ala	Glu	Leu	Thr	Thr	Thr	Ala	Ala	Thr	Arg	Phe	Met	Lys	Asp	Leu	Tyr	
240					245					250					255	
TTT	ACT	AGT	ACT	AAT	GGT	GTC	GGT	GAG	ATC	GGC	CGT	GGG	ATA	GCG	CTT	814
Phe	Thr	Ser	Thr	Asn	Gly	Val	Gly	Glu	Ile	Gly	Arg	Gly	Ile	Ala	Leu	
				260					265					270		
ACC	CTG	TTT	AAC	CTT	GCT	GAC	ACC	CTG	CTT	GGC	GGT	CTA	CCG	ACA	GAA	862
Thr	Leu	Phe	Asn	Leu	Ala	Asp	Thr	Leu	Leu	Gly	Gly	Leu	Pro	Thr	Glu	
			275					280					285			
TTG	ATT	TCG	TCG	GCT	GGT	GGC	CAG	CTG	TTC	TAC	TCT	CGT	CCC	GTC	GTC	910
Leu	Ile	Ser	Ser	Ala	Gly	Gly	Gln	Leu	Phe	Tyr	Ser	Arg	Pro	Val	Val	
		290					295					300				
TCA	GCC	AAT	GGC	GAG	CCG	ACT	GTT	AAG	CTT	TAT	ACA	TCT	GTA	GAG	AAT	958
Ser	Ala	Asn	Gly	Glu	Pro	Thr	Val	Lys	Leu	Tyr	Thr	Ser	Val	Glu	Asn	
	305					310					315					
GCT	CAG	CAG	GAT	AAG	GGT	ATT	GCA	ATC	CCG	CAT	GAC	ATC	GAC	CTC	GGG	1006
Ala	Gln	Gln	Asp	Lys	Gly	Ile	Ala	Ile	Pro	His	Asp	Ile	Asp	Leu	Gly	
320					325					330					335	
GAG	TCT	CGT	GTA	GTT	ATT	CAG	GAT	TAT	GAC	AAC	CAA	CAT	GAG	CAG	GAC	1054
Glu	Ser	Arg	Val	Val	Ile	Gln	Asp	Tyr	Asp	Asn	Gln	His	Glu	Gln	Asp	
			340						345					350		
CGA	CCG	ACA	CCT	TCC	CCA	GCC	CCA	TCG	CGC	CCT	TTT	TCT	GTC	CTC	CGA	1102
Arg	Pro	Thr	Pro	Ser	Pro	Ala	Pro	Ser	Arg	Pro	Phe	Ser	Val	Leu	Arg	
			355					360					365			
GCT	AAT	GAT	GTG	CTT	TGG	CTT	TCT	CTC	ACC	GCT	GCC	GAG	TAT	GAC	CAG	1150
Ala	Asn	Asp	Val	Leu	Trp	Leu	Ser	Leu	Thr	Ala	Ala	Glu	Tyr	Asp	Gln	
		370					375					380				
TCC	ACT	TAC	GGC	TCT	TCG	ACC	GGC	CCA	GTC	TAT	GTC	TCT	GAC	TCT	GTG	1198
Ser	Thr	Tyr	Gly	Ser	Ser	Thr	Gly	Pro	Val	Tyr	Val	Ser	Asp	Ser	Val	
	385					390					395					
ACC	TTG	GTT	AAT	GTT	GCG	ACC	GGC	GCG	CAG	GCC	GTT	GCC	CGG	TCA	CTC	1246
Thr	Leu	Val	Asn	Val	Ala	Thr	Gly	Ala	Gln	Ala	Val	Ala	Arg	Ser	Leu	
400					405				410						415	

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GAC TGG ACC AAG GTC ACA CTT GAT GGT CGC CCC CTT TCC ACC ATC CAG Asp Trp Thr Lys Val Thr Leu Asp Gly Arg Pro Leu Ser Thr Ile Gln 420 425 430	1294
CAG TAT TCA AAG ACC TTC TTT GTC CTG CCG CTC CGC GGT AAG CTC TCC Gln Tyr Ser Lys Thr Phe Phe Val Leu Pro Leu Arg Gly Lys Leu Ser 435 440 445	1342
TTT TGG GAG GCA GGT ACT ACT AAA GCC GGG TAC CCT TAT AAT TAT AAC Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn 450 455 460	1390
ACC ACT GCT AGT GAC CAA CTG CTC GTT GAG AAT GCC GCT GGG CAT CGG Thr Thr Ala Ser Asp Gln Leu Leu Val Glu Asn Ala Ala Gly His Arg 465 470 475	1438
GTT GCT ATT TCC ACT TAC ACC ACT AGC CTG GGT GCT GGT CCC GTC TCT Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Val Ser 480 485 490 495	1486
ATT TCC GCG GTT GCT GTT TTA GCC CCC CAC TCC GCG CTA GCA TTG CTT Ile Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala Leu Leu 500 505 510	1534
GAG GAT ACC ATG GAC TAC CCT GCC CGC GCC CAT ACT TTC GAT GAC TTC Glu Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe 515 520 525	1582
TGC CCG GAG TGC CGC CCC CTT GGC CTC CAG GGC TGT GCT TTT CAG TCT Cys Pro Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser 530 535 540	1630
ACT GTC GCT GAG CTT CAG CGC CTT AAG A Thr Val Ala Glu Leu Gln Arg Leu Lys 670 675	1658

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 552 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Arg	Arg	Pro	Thr	Thr	Ala	Gly	Ala	Ala	Pro	Leu	Thr	Ala	Val	Ala	Pro
1				5					10					15	
Ala	His	Asp	Thr	Pro	Pro	Val	Pro	Asp	Val	Asp	Ser	Arg	Gly	Ala	Ile
			20					25					30		

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

Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Ser Pro Leu
50 55 60

Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr Glu Ala
65 70 75 80

Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile Arg Tyr Arg
85 90 95

Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser Ile Ser Phe
100 105 110

Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn Ser Ile
115 120 125

Thr Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala Ser Glu
130 135 140

Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly Trp Arg
145 150 155 160

Ser Val Glu Thr Ser Gly Val Ala Glu Glu Glu Ala Thr Ser Gly Leu
165 170 175

Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr Asn Thr
180 185 190

Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu Leu Glu
195 200 205

Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val Ser Arg Tyr
210 215 220

Ser Ser Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp Gly Thr Ala
225 230 235 240

Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu Tyr Phe
245 250 255

Thr Ser Thr Asn Gly Val Gly Glu Ile Gly Arg Gly Ile Ala Leu Thr
260 265 270

Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr Glu Leu
275 280 285

Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val Val Ser
290 295 300

Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu Asn Ala
305 310 315 320

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Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp Leu Gly Glu
325 330 335

Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln Asp Arg
340 345 350

Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu Arg Ala
355 360 365

Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Asp Gln Ser
370 375 380

Thr Tyr Gly Ser Ser Thr Gly Pro Val Tyr Val Ser Asp Ser Val Thr
385 390 395 400

Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg Ser Leu Asp
405 410 415

Trp Thr Lys Val Thr Leu Asp Gly Arg Pro Leu Ser Thr Ile Gln Gln
420 425 430

Tyr Ser Lys Thr Phe Phe Val Leu Pro Leu Arg Gly Lys Leu Ser Phe
435 440 445

Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr
450 455 460

Thr Ala Ser Asp Gln Leu Leu Val Glu Asn Ala Ala Gly His Arg Val
465 470 475 480

Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Val Ser Ile
485 490 495

Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala Leu Leu Glu
500 505 510

Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys
515 520 525

Pro Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr
530 535 540

Val Ala Glu Leu Gln Arg Leu Lys
545 550

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 803 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..803

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CAG CTG TTC TAC TCT CGT CCC GTC GTC TCA GCC AAT GGC GAG CCG ACT	48
Gln Leu Phe Tyr Ser Arg Pro Val Val Ser Ala Asn Gly Glu Pro Thr	
5 10 15	
GTT AAG CTT TAT ACA TCT GTA GAG AAT GCT CAG CAG GAT AAG GGT ATT	96
Val Lys Leu Tyr Thr Ser Val Glu Asn Ala Gln Gln Asp Lys Gly Ile	
20 25 30	
GCA ATC CCG CAT GAC ATC GAC CTC GGG GAG TCT CGT GTA GTT ATT CAG	144
Ala Ile Pro His Asp Ile Asp Leu Gly Glu Ser Arg Val Val Ile Gln	
35 40 45	
AT TAT GAC AAC CAA CAT GAG CAG GAC CGA CCG ACA CCT TCC CCA GCC	192
Asp Tyr Asp Asn Gln His Glu Gln Asp Arg Pro Thr Pro Ser Pro Ala	
50 55 60	
CCA TCG CGC CCT TTT TCT GTC CTC CGA GCT AAT GAT GTG CTT TGG CTT	240
Pro Ser Arg Pro Phe Ser Val Leu Arg Ala Asn Asp Val Leu Trp Leu	
65 70 75 80	
TCT CTC ACC GCT GCC GAG TAT GAC CAG TCC ACT TAC GGC TCT TCG ACC	288
Ser Leu Thr Ala Ala Glu Tyr Asp Gln Ser Thr Tyr Gly Ser Ser Thr	
85 90 95	
GGC CCA GTC TAT GTC TCT GAC TCT GTG ACC TTG GTT AAT GTT GCG ACC	336
Gly Pro Val Tyr Val Ser Asp Ser Val Thr Leu Val Asn Val Ala Thr	
100 105 110	
GGC GCG CAG GCC GTT GCC CGG TCA CTC GAC TGG ACC AAG GTC ACA CTT	384
Gly Ala Gln Ala Val Ala Arg Ser Leu Asp Trp Thr Lys Val Thr Leu	
115 120 125	
GAT GGT CGC CCC CTT TCC ACC ATC CAG CAG TAT TCA AAG ACC TTC TTT	432
Asp Gly Arg Pro Leu Ser Thr Ile Gln Gln Tyr Ser Lys Thr Phe Phe	
130 135 140	
GTC CTG CCG CTC CGC GGT AAG CTC TCC TTT TGG GAG GCA GGT ACT ACT	480
Val Leu Pro Leu Arg Gly Lys Leu Ser Phe Trp Glu Ala Gly Thr Thr	
145 150 155 160	
AAA GCC GGG TAC CCT TAT AAT TAT AAC ACC ACT GCT AGT GAC CAA CTG	528
Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr Ala Ser Asp Gln Leu	
165 170 175	

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CTC GTT GAG AAT GCC GCT GGG CAT CGG GTT GCT ATT TCC ACT TAC ACC	576
Leu Val Glu Asn Ala Ala Gly His Arg Val Ala Ile Ser Thr Tyr Thr	
180 185 190	
ACT AGC CTG GGT GCT GGT CCC GTC TCT ATT TCC GCG GTT GCT GTT TTA	624
Thr Ser Leu Gly Ala Gly Pro Val Ser Ile Ser Ala Val Ala Val Leu	
195 200 205	
GCC CCC CAC TCC GCG CTA GCA TTG CTT GAG GAT ACC ATG GAC TAC CCT	672
Ala Pro His Ser Ala Leu Ala Leu Leu Glu Asp Thr Met Asp Tyr Pro	
210 215 220	
GCC CGC GCC CAT ACT TTC GAT GAC TTC TGC CCG GAG TGC CGC CCC CTT	720
Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro Glu Cys Arg Pro Leu	
225 230 235 240	
GGC CTC CAG GGC TGT GCT TTT CAG TCT ACT GTC GCT GAG CTT CAG CGC	768
Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Val Ala Glu Leu Gln Arg	
245 250 255	
CTT AAG ATG AAG GTG GGT AAA ACT CGG GAG TTA TA	803
Leu Lys Met Lys Val Gly Lys Thr Arg Glu Leu	
260 265	

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 267 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln Leu Phe Tyr Ser Arg Pro Val Val Ser Ala Asn Gly Glu Pro Thr	
1 5 10 15	
Val Lys Leu Tyr Thr Ser Val Glu Asn Ala Gln Gln Asp Lys Gly Ile	
20 25 30	
Ala Ile Pro His Asp Ile Asp Leu Gly Glu Ser Arg Val Val Ile Gln	
35 40 45	
Asp Tyr Asp Asn Gln His Glu Gln Asp Arg Pro Thr Pro Ser Pro Ala	
50 55 60	
Pro Ser Arg Pro Phe Ser Val Leu Arg Ala Asn Asp Val Leu Trp Leu	
65 70 75 80	
Ser Leu Thr Ala Ala Glu Tyr Asp Gln Ser Thr Tyr Gly Ser Ser Thr	
85 90 95	

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Gly Pro Val Tyr Val Ser Asp Ser Val Thr Leu Val Asn Val Ala Thr
100 105 110

Gly Ala Gln Ala Val Ala Arg Ser Leu Asp Trp Thr Lys Val Thr Leu
115 120 125

Asp Gly Arg Pro Leu Ser Thr Ile Gln Gln Tyr Ser Lys Thr Phe Phe
130 135 140

Val Leu Pro Leu Arg Gly Lys Leu Ser Phe Trp Glu Ala Gly Thr Thr
145 150 155 160

Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr Ala Ser Asp Gln Leu
165 170 175

Leu Val Glu Asn Ala Ala Gly His Arg Val Ala Ile Ser Thr Tyr Thr
180 185 190

Thr Ser Leu Gly Ala Gly Pro Val Ser Ile Ser Ala Val Ala Val Leu
195 200 205

Ala Pro His Ser Ala Leu Ala Leu Leu Glu Asp Thr Met Asp Tyr Pro
210 215 220

Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro Glu Cys Arg Pro Leu
225 230 235 240

Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Val Ala Glu Leu Gln Arg
245 250 255

Leu Lys Met Lys Val Gly Lys Thr Arg Glu Leu
260 265

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1686 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..1687

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

T CGT AGA CCT ACC ACA GCT GGG GCC GCG CCG CTA ACC GCG GTC GCT
Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala Val Ala
1 5 10 15

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CCG GCC CAT GAC ACC CCG CCA GTG CCT GAT GTT GAC TCC CGC GGC GCC	94
Pro Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg Gly Ala	
20 25 30	
ATC CTG CGC CGG CAG TAT AAC CTA TCA ACA TCT CCC CTT ACT TCT TCC	142
Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr Ser Ser	
35 40 45	
GTG GCC ACC GGT ACA AAC TTG GTT CTA TAC GCC GCT CCT CTT AGC CCA	190
Val Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Ser Pro	
50 55 60	
CTT CTA CCC CTC CAG GAC GGC ACC AAT ACT CAT ATA ATG GCC ACA GAA	238
Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr Glu	
65 70 75	
GCT TCT AAT TAT GCC CAG TAC CGG GTT GCT CGT GCC ACA ATT CGC TAC	286
Ala Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile Arg Tyr	
80 85 90 95	
CGC CCG CTG GTC CCC AAC GCT GTT GGT GGC TAC GCC ATC TCC ATC TCG	334
Arg Pro Leu Val Pro Asn Ala Val Gly Tyr Ala Ile Ser Ile Ser	
100 105 110	
TTC TGG CCA CAG ACC ACC ACC ACC CCG ACG TCC GTT GAC ATG AAT TCA	382
Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn Ser	
115 120 125	
ATA ACC TCG ACG GAT GTT CGT ATT TTA GTC CAG CCC GGC ATA GCC TCC	430
Ile Thr Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala Ser	
130 135 140	
GAG CTT GTT ATC CCA AGT GAG CGC CTA CAC TAC CGT AAC CAA GGT TGG	478
Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly Trp	
145 150 155	
CGC TCT GTT GAG ACC TCC GGG GTG GCG GAG GAG GAG GCC ACC TCT GGT	526
Arg Ser Val Glu Thr Ser Gly Val Ala Glu Glu Glu Ala Thr Ser Gly	
160 165 170 175	
CTT GTT ATG CTC TGC ATA CAT GGC TCA CCT GTA AAT TCT TAT ACT AAT	574
Leu Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr Asn	
180 185 190	
ACA CCT TAT ACC GGT GCC CTC GGG CTG TTG GAC TTT GCC CTC GAA CTT	622
Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu Leu	
195 200 205	
GAG TTC CGC AAC CTC ACC CCC GGT AAT ACC AAC ACG CGG GTC TCC CGT	670
Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val Ser Arg	
210 215 220	

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TAC	TCC	AGC	ACT	GCC	CGT	CAC	CGC	CTT	CGT	CGC	GGT	GCA	GAT	GGG	ACT	718
Tyr	Ser	Ser	Thr	Ala	Arg	His	Arg	Leu	Arg	Arg	Gly	Ala	Asp	Gly	Thr	
225						230					235					
GCC	GAG	CTT	ACC	ACC	ACG	GCT	GCT	ACC	CGC	TTC	ATG	AAG	GAC	CTC	TAT	766
Ala	Glu	Leu	Thr	Thr	Thr	Ala	Ala	Thr	Arg	Phe	Met	Lys	Asp	Leu	Tyr	
240					245				250						255	
TTT	ACT	AGT	ACT	AAT	GGT	GTC	GGT	GAG	ATC	GGC	CGT	GGG	ATA	GCG	CTT	814
Phe	Thr	Ser	Thr	Asn	Gly	Val	Gly	Glu	Ile	Gly	Arg	Gly	Ile	Ala	Leu	
				260				265						270		
ACC	CTG	TTT	AAC	CTT	GCT	GAC	ACC	CTG	CTT	GGC	GGT	CTA	CCG	ACA	GAA	862
Thr	Leu	Phe	Asn	Leu	Ala	Asp	Thr	Leu	Leu	Gly	Gly	Leu	Pro	Thr	Glu	
			275					280					285			
TTG	ATT	TCG	TCG	GCT	GGT	GGC	CAG	CTG	TTC	TAC	TCT	CGT	CCC	GTC	GTC	910
Leu	Ile	Ser	Ser	Ala	Gly	Gly	Gln	Leu	Phe	Tyr	Ser	Arg	Pro	Val	Val	
		290					295					300				
TCA	GCC	AAT	GGC	GAG	CCG	ACT	GTT	AAG	CTT	TAT	ACA	TCT	GTA	GAG	AAT	958
Ser	Ala	Asn	Gly	Glu	Pro	Thr	Val	Lys	Leu	Tyr	Thr	Ser	Val	Glu	Asn	
305						310					315					
GCT	CAG	CAG	GAT	AAG	GGT	ATT	GCA	ATC	CCG	CAT	GAC	ATC	GAC	CTC	GGG	1006
Ala	Gln	Gln	Asp	Lys	Gly	Ile	Ala	Ile	Pro	His	Asp	Ile	Asp	Leu	Gly	
320					325				330						335	
GAG	TCT	CGT	GTA	GTT	ATT	CAG	GAT	TAT	GAC	AAC	CAA	CAT	GAG	CAG	GAC	1054
Glu	Ser	Arg	Val	Val	Ile	Gln	Asp	Tyr	Asp	Asn	Gln	His	Glu	Gln	Asp	
			340					345						350		
CGA	CCG	ACA	CCT	TCC	CCA	GCC	CCA	TCG	CGC	CCT	TTT	TCT	GTC	CTC	CGA	1102
Arg	Pro	Thr	Pro	Ser	Pro	Ala	Pro	Ser	Arg	Pro	Phe	Ser	Val	Leu	Arg	
			355					360					365			
GCT	AAT	GAT	GTG	CTT	TGG	CTT	TCT	CTC	ACC	GCT	GCC	GAG	TAT	GAC	CAG	1150
Ala	Asn	Asp	Val	Leu	Trp	Leu	Ser	Leu	Thr	Ala	Ala	Glu	Tyr	Asp	Gln	
		370					375					380				
TCC	ACT	TAC	GGC	TCT	TCG	ACC	GGC	CCA	GTC	TAT	GTC	TCT	GAC	TCT	GTG	1198
Ser	Thr	Tyr	Gly	Ser	Ser	Thr	Gly	Pro	Val	Tyr	Val	Ser	Asp	Ser	Val	
	385					390					395					
ACC	TTG	GTT	AAT	GTT	GCG	ACC	GGC	GCG	CAG	GCC	GTT	GCC	CGG	TCA	CTC	1246
Thr	Leu	Val	Asn	Val	Ala	Thr	Gly	Ala	Gln	Ala	Val	Ala	Arg	Ser	Leu	
400					405					410					415	
GAC	TGG	ACC	AAG	GTC	ACA	CTT	GAT	GGT	CGC	CCC	CTT	TCC	ACC	ATC	CAG	1294
Asp	Trp	Thr	Lys	Val	Thr	Leu	Asp	Gly	Arg	Pro	Leu	Ser	Thr	Ile	Gln	
				420					425					430		

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CAG TAT TCA AAG ACC TTC TTT GTC CTG CCG CTC CGC GGT AAG CTC TCC Gln Tyr Ser Lys Thr Phe Phe Val Leu Pro Leu Arg Gly Lys Leu Ser 435 440 445	1342
TTT TGG GAG GCA GGT ACT ACT AAA GCC GGG TAC CCT TAT AAT TAT AAC Phe Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn 450 455 460	1390
ACC ACT GCT AGT GAC CAA CTG CTC GTT GAG AAT GCC GCT GGG CAT CGG Thr Thr Ala Ser Asp Gln Leu Leu Val Glu Asn Ala Ala Gly His Arg 465 470 475	1438
GTT GCT ATT TCC ACT TAC ACC ACT AGC CTG GGT GCT GGT CCC GTC TCT Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Val Ser 480 485 490 495	1486
ATT TCC GCG GTT GCT GTT TTA GCC CCC CAC TCC GCG CTA GCA TTG CTT Ile Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala Leu Leu 500 505 510	1534
GAG GAT ACC ATG GAC TAC CCT GCC CGC GCC CAT ACT TTC GAT GAC TTC Glu Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe 515 520 525	1582
TGC CCG GAG TGC CGC CCC CTT GGC CTC CAG GGC TGT GCT TTT CAG TCT Cys Pro Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser 530 535 540	1630
ACT GTC GCT GAG CTT CAG CGC CTT AAG ATG AAG GTG GGT AAA ACT CGG Thr Val Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg 545 550 555	1678
GAG TTA TA Glu Leu 560	1686

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 562 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala Val Ala Pro 1 5 10 15
Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile 20 25 30

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

Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Ser Pro Leu
50 55 60

Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr Glu Ala
65 70 75 80

Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile Arg Tyr Arg
85 90 95

Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser Ile Ser Phe
100 105 110

Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn Ser Ile
115 120 125

Thr Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala Ser Glu
130 135 140

Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly Trp Arg
145 150 155 160

Ser Val Glu Thr Ser Gly Val Ala Glu Glu Glu Ala Thr Ser Gly Leu
165 170 175

Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr Asn Thr
180 185 190

Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu Leu Glu
195 200 205

Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val Ser Arg Tyr
210 215 220

Ser Ser Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp Gly Thr Ala
225 230 235 240

Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu Tyr Phe
245 250 255

Thr Ser Thr Asn Gly Val Gly Glu Ile Gly Arg Gly Ile Ala Leu Thr
260 265 270

Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr Glu Leu
275 280 285

Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val Val Ser
290 295 300

Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu Asn Ala
305 310 315 320

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Gln Gln Asp Lys Gly Ile Ala Ile Pro His Asp Ile Asp Leu Gly Glu
325 330 335

Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln Asp Arg
340 345 350

Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu Arg Ala
355 360 365

Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Asp Gln Ser
370 375 380

Thr Tyr Gly Ser Ser Thr Gly Pro Val Tyr Val Ser Asp Ser Val Thr
385 390 395 400

Leu Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg Ser Leu Asp
405 410 415

Trp Thr Lys Val Thr Leu Asp Gly Arg Pro Leu Ser Thr Ile Gln Gln
420 425 430

Tyr Ser Lys Thr Phe Phe Val Leu Pro Leu Arg Gly Lys Leu Ser Phe
435 440 445

Trp Glu Ala Gly Thr Thr Lys Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr
450 455 460

Thr Ala Ser Asp Gln Leu Leu Val Glu Asn Ala Ala Gly His Arg Val
465 470 475 480

Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Val Ser Ile
485 490 495

Ser Ala Val Ala Val Leu Ala Pro His Ser Ala Leu Ala Leu Leu Glu
500 505 510

Asp Thr Met Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys
515 520 525

Pro Glu Cys Arg Pro Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr
530 535 540

Val Ala Glu Leu Gln Arg Leu Lys Met Lys Val Gly Lys Thr Arg Glu
545 550 555 560

Leu

(2) INFORMATION FOR SEQ ID NO:21:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 331 base pairs
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..331

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATG CGC CCT CGG CCT ATT TTG CTG TTG CTC CTC ATG TTT CTG CCT ATG	48
Met Arg Pro Arg Pro Ile Leu Leu Leu Leu Met Phe Leu Pro Met	
1 5 10 15	
CTG CCC GCG CCA CCG CCC GGT CAG CCG TCT GGC CGC CGT CGT GGG CGG	96
Leu Pro Ala Pro Pro Pro Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg	
20 25 30	
CGC AGC GGC GGT TCC GGC GGT GGT TTC TGG GGT GAC CGG GTT GAT TCT	144
Arg Ser Gly Gly Ser Gly Gly Phe Trp Gly Asp Arg Val Asp Ser	
35 40 45	
CAG CCC TTC GCA ATC CCC TAT ATT CAT CCA ACC AAC CCC TTC GCC CCC	192
Gln Pro Phe Ala Ile Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Pro	
50 55 60	
GAT GTC ACC GCT GCG GCC GGG GCT GGA CCT CGT GTT CGC CAA CCC GCC	240
Asp Val Thr Ala Ala Ala Gly Ala Gly Pro Arg Val Arg Gln Pro Ala	
65 70 75 80	
CGA CCA CTC GGC TCC GCT TGG CGT GAC CAG GCC CAG CGC CCC GCC GTT	288
Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ala Gln Arg Pro Ala Val	
85 90 100	
GCC TCA CGT CGT AGA CCT ACC ACA GCT GGG GCC GCG CCG CTA A	331
Ala Ser Arg Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu	
105 110 115	

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Arg Pro Arg Pro Ile Leu Leu Leu Leu Leu Met Phe Leu Pro Met
1 5 10 15

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

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 672 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..672

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATG CGC CCT CGG CCT ATT TTG CTG TTG CTC CTC ATG TTT CTG CCT ATG	48
Met Arg Pro Arg Pro Ile Leu Leu Leu Leu Leu Met Phe Leu Pro Met	
1 5 10 15	
CTG CCC GCG CCA CCG CCC GGT CAG CCG TCT GGC CGC CGT CGT GGG CGG	96
Leu Pro Ala Pro Pro Pro Gly Gln Pro Ser Gly Arg Arg Arg Gly Arg	
20 25 30	
CGC AGC GGC GGT TCC GGC GGT GGT TTC TGG GGT GAC CGG GTT GAT TCT	144
Arg Ser Gly Gly Ser Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser	
35 40 45	
CAG CCC TTC GCA ATC CCC TAT ATT CAT CCA ACC AAC CCC TTC GCC CCC	192
Gln Pro Phe Ala Ile Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Pro	
50 55 60	
GAT GTC ACC GCT GCG GCC GGG GCT GGA CCT CGT GTT CGC CAA CCC GCC	240
Asp Val Thr Ala Ala Ala Gly Ala Gly Pro Arg Val Arg Gln Pro Ala	
65 70 75 80	

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CGA CCA CTC GGC TCC GCT TGG CGT GAC CAG GCC CAG CGC CCC GCC GTT	288
Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ala Gln Arg Pro Ala Val	
85 90 95	
GCC TCA CGT CGT AGA CCT ACC ACA GCT GGG GCC GCG CCG CTA ACC GCG	336
Ala Ser Arg Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala	
100 105 110	
GTC GCT CCG GCC CAT GAC ACC CCG CCA GTG CCT GAT GTT GAC TCC CGC	384
Val Ala Pro Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg	
115 120 125	
GGC GCC ATC CTG CGC CGG CAG TAT AAC CTA TCA ACA TCT CCC CTT ACT	432
Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr	
130 135 140	
TCT TCC GTG GCC ACC GGT ACA AAC TTG GTT CTA TAC GCC GCT CCT CTT	480
Ser Ser Val Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu	
145 150 155 160	
AGC CCA CTT CTA CCC CTC CAG GAC GGC ACC AAT ACT CAT ATA ATG GCC	528
Ser Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala	
165 170 175	
ACA GAA GCT TCT AAT TAT GCC CAG TAC CGG GTT GCT CGT GCC ACA ATT	576
Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile	
180 185 190	
CGC TAC CGC CCG CTG GTC CCC AAC GCT GTT GGT GGC TAC GCC ATC TCC	624
Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser	
195 200 205	
ATC TCG TTC TGG CCA CAG ACC ACC ACC ACC CCG ACG TCC GTT GAC ATG	672
Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met	
210 215 220	

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 224 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met	Arg	Pro	Arg	Pro	Ile	Leu	Leu	Leu	Leu	Leu	Met	Phe	Leu	Pro	Met
1				5					10					15	
Leu	Pro	Ala	Pro	Pro	Pro	Gly	Gln	Pro	Ser	Gly	Arg	Arg	Arg	Gly	Arg
			20					25					30		

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Arg Ser Gly Gly Ser Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser
35 40 45

Gln Pro Phe Ala Ile Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Pro
50 55 60

Asp Val Thr Ala Ala Ala Gly Ala Gly Pro Arg Val Arg Gln Pro Ala
65 70 75 80

Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ala Gln Arg Pro Ala Val
85 90 95

Ala Ser Arg Arg Arg Pro Thr Thr Ala Gly Ala Ala Pro Leu Thr Ala
100 105 110

Val Ala Pro Ala His Asp Thr Pro Pro Val Pro Asp Val Asp Ser Arg
115 120 125

Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr
130 135 140

Ser Ser Val Ala Thr Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu
145 150 155 160

Ser Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala
165 170 175

Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Ala Arg Ala Thr Ile
180 185 190

Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser
195 200 205

Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met
210 215 220

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A recombinant polypeptide encoded by a nucleotide sequence selected from the list consisting of clone 2.1 (SEQ ID NO:17), clone 2.3 (SEQ ID NO:21) and clone 2.4 (SEQ ID NO:23) of Hepatitis E Virus (HEV) as set forth in Figure 5.
2. A recombinant polypeptide according to claim 1 wherein said recombinant molecule is interactive with convalescent phase antibodies to HEV.
3. A recombinant polypeptide according to claim 1 or 2 wherein said recombinant molecule is interactive with acute phase antibodies to HEV.
4. A recombinant polypeptide comprising first and second amino acid sequences wherein said first amino acid sequence is encoded by a nucleotide sequence selected from clone 2.1 (SEQ ID NO:17), clone 2.3 (SEQ ID NO:21) and clone 2.4 (SEQ ID NO:23) as set forth in Figure 5 and wherein said second amino acid sequence is a non-HEV encoded peptide, polypeptide or protein.
5. A recombinant polypeptide according to claim 4 wherein said first amino acid sequence is interactive with convalescent phase antibodies to HEV.
6. A recombinant polypeptide according to claim 4 or 5 wherein said first amino acid sequence is interactive with acute phase antibodies to HEV.
7. A recombinant polypeptide according to claim 4, 5 or 6 wherein said second amino acid sequence is glutathione-S-transferase (GST) or a part or derivative thereof.
8. An isolated nucleic acid molecule comprising a sequence of nucleotides selected from the list consisting of clone 2.1 (SEQ ID NO:17), clone 2.3 (SEQ ID NO:21) and clone 2.4 (SEQ ID NO:23) as set forth in Figure 5.

9. An isolated nucleic acid molecule according to claim 8 further comprising a second nucleotide sequence encoding or complementary to a sequence encoding a peptide, polypeptide or protein of non-HEV origin.
10. An isolated nucleic acid molecule according to claim 9 wherein the second nucleotide sequence encodes or is complementary to a sequence which encodes GST or a part or derivative thereof.
11. An expression vector having a nucleotide sequence according to claim 8 or 9.
12. A method for detecting HEV interactive antibodies in a biological sample said method comprising contacting a recombinant polypeptide according to any one of claims 1 to 7 with said biological sample for a time and under conditions sufficient to permit a complex to form with HEV antibodies if present in said biological sample and then detecting the complex wherein the presence of said complex is indicative of said HEV interactive antibodies.
13. A method according to claim 12 wherein said HEV interactive antibodies are HEV acute phase antibodies and said polypeptide comprises an ORF2 polypeptide of HEV produced by expression of a sequence of nucleotides as set forth in clone 2.1 (SEQ ID NO:17), 2.3 (SEQ ID NO:21) or 2.4 (SEQ ID NO:23) in Figure 5.
14. A method for detecting HEV acute phase antibodies in a biological sample said method comprising contacting said biological sample with a polypeptide for a time and under conditions sufficient to permit a complex to form with HEV antibodies if present in said biological sample and then detecting the complex wherein said polypeptide comprises an ORF2 polypeptide of HEV consisting essentially of amino acids 394-660 of ORF2 polypeptide of HEV.
15. A method for detecting HEV acute phase antibodies in a biological sample said method comprising contacting said biological sample with a polypeptide for a time

and under conditions sufficient to permit a complex to form with HEV antibodies if present in said biological sample and then detecting the complex wherein said polypeptide comprises an ORF2 polypeptide of HEV produced by expression of a 3' end portion of SEQ ID NO:1.

16. A method for detecting HEV convalescent phase antibodies in a biological sample said method comprising contacting said biological sample with a polypeptide for a time and under conditions sufficient to permit a complex to form with HEV antibodies if present in said biological sample and then detecting the complex wherein said polypeptide is produced by expression of a nucleic acid molecule comprising a 3' end portion of SEQ ID NO:1.
17. A method according to claim 12 wherein said HEV interactive antibodies are HEV convalescent phase antibodies and said polypeptide is produced by expression of nucleotides as set forth in clone 2.1 in Figure 5.
18. A method for detecting HEV convalescent phase antibodies in a biological sample said method comprising contacting said biological sample with a polypeptide for a time and under conditions sufficient to permit a complex to form with HEV antibodies if present in said biological sample and then detecting the complex wherein said polypeptide comprises an ORF2 polypeptide of HEV consisting essentially of amino acids 394-660 of ORF2 polypeptide of HEV.
19. A method according to claim 12 wherein said HEV interactive antibodies are acute phase antibodies and said polypeptide comprises an ORF2 polypeptide of HEV consisting essentially of amino acids as set forth in SEQ ID NO:18, SEQ ID NO:22 or SEQ ID NO:24.
20. A method according to any one of claims 12-19 wherein said biological sample is whole blood, plasma, serum or saliva.

21. A method according to any one of claims 12-20 wherein said recombinant polypeptide further comprises a sequence of amino acids from a non-HEV peptide, polypeptide or protein.
22. A method according to claim 21 wherein the further amino acid sequence is GST or a part or derivative thereof.
23. A vaccine to inhibit, reduce or ameliorate the effects of HEV infection in a human, said vaccine comprising a polypeptide according to any one of claims 1 to 7 and one or more carriers and/or diluents suitable for use in a vaccine.
24. Use of a recombinant polypeptide according to any one of claims 1 to 7 in the manufacture of a medicament for the treatment and/or prophylaxis of HEV.
25. Use of an ORF2 polypeptide of HEV consisting of amino acids 394-660 in the manufacture of a medicament for the treatment and/or prophylaxis of HEV.
26. Use of a polypeptide comprising an ORF2 polypeptide of HEV produced by expression of a 3' end nucleotide sequence of SEQ ID NO:1 in the manufacture of a medicament for the treatment and/or prophylaxis of HEV.
27. A kit for detecting antibodies to HEV, said kit comprising in compartmental form a first compartment comprising a recombinant polypeptide according to any one of claims 1 to 7 comprising one or more further compartments comprising agents suitable for detecting antibodies.
28. A kit for detecting antibodies to HEV, said kit comprising in compartmental form a first compartment comprising an ORF2 polypeptide of HEV consisting essentially of amino acids 394-660 of HEV and one or more further compartments comprising agents suitable for detecting antibodies.

29. The kit according to claim 27 wherein said polypeptide comprises one or more ORF2 polypeptides of HEV produced by expression of a sequence of nucleotides as set forth in clones 2.1 (SEQ ID NO:17), 2.3 (SEQ ID NO:21) or 2.4 (SEQ ID NO:23) in Figure 5.
30. A recombinant polypeptide according to any one of claims 1 to 7 or an isolated nucleic acid molecule according to any one of claims 8 to 10 or an expression vector according to claim 11 or a method according to any one of claims 12 to 22 or a use according to claims 24-26 or a vaccine according to claim 26 or a kit according to claims 27 to 29 substantially as hereinbefore described with reference to the Figures and/or Examples.