AUSTRALIA

PATENTS ACT 1952

B

APPLICATION BY ASSIGNEE OF INVENTOR

# DECLARATION IN SUPPORT OF AN APPLICATION FOR A PATENT

NAME OF APPLICANT

In support of an application made by:

Institute for Animal Health Limited

TITLE

for a patent for an invention entitled:

Viral Nucleotide Sequences

FULL NAME AND ADDRESS OF SIGNATORY

I, ~~Xxxxxxxxxxxxx~~ Arthur G. Campbell

of Institute for Animal Health Limited

Compton, Nr Newbury, Berkshire RG16 ONN, United Kingdom

do solemnly and sincerely declare as follows:

1. I am authorised by the above mentioned applicant for the patent to make this declaration on its behalf.

FULL NAME AND ADDRESS OF INVENTOR(S)

2. The name and address of each actual inventor of the invention is as follows:

Annette Mary Griffin of Institute for Animal Health Limited, Houghton Laboratory, Houghton, Huntingdon, Cambridgeshire PE17 2DA

~~Louis Joseph Norman Ross of Institute for Animal Health~~ Limited, Houghton Laboratory, Houghton, Huntingdon, Cambridgeshire PE17 2DA

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SEE NOTES OVER

3. The facts upon which the applicant is entitled to make this application are as follows:

By virtue of contract of employment, the applicant would be entitled to have assigned to it a patent granted to the actual inventors in respect of said invention.

DELETE PARAGRAPHS 3 AND 4 FOR NON-CONVENTION APPLICATION

4. The basic application(s) as defined by Section 141 of the Act was (were) made as follows:

Country Great Britain on 13 September 1988

in the name(s) Institute for Animal Health Limited

and in on

in the name(s)

PLACE AND DATE OF SIGNING

5. The basic application(s) referred to in the preceding paragraph was (were) the first application(s) made in a Convention country in respect of the invention the subject of this application.

Declared at Compton

this 6th day of March 199[illegible]

Signed [signature]

Position ~~Institute Secretary~~ Acting Secretary

GRIFFITH HACK & CO

PATENT AND TRADE MARK ATTORNEYS

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**VIRAL NUCLEOTIDE SEQUENCES**

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Various genes of herpes virus of turkeys (HVT), Marek's disease virus (MDV) and infectious laryngotracheitis virus (ILTV) have been identified as non-essential regions (and candidates for insertion sites for foreign genes) and/or as antigen-encoding regions. The former include the HVT homologue of the HSV (herpes simplex virus)gC gene, the TK (thymidine kinase) region of MDV or ILTV, ORF3 of ILTV (as defined herein), the ribonucleotide reductase (large subunit) gene of ILTV, MDV or HVT and the ribonucleotide reductase (small subunit) gene of MDV. The antigen-encoding regions include the HVT homologues of the HSV gB, gC and gH gene, the ILTV homologue of HSV gB, ORF2 of ILTV, and the HVT homologue of the HSV-1 immediate early genes IE-175 and IE-68. Manipulation of these genes allows vaccines to be prepared comprising attenuated virus or virus carrying heterologous antigen-encoding sequences.

The term "minor variations thereof" is intended to include changes in the nucleotide sequence which do not

.../2

affect its essential nature, for example minor substitutions of nucleo-tides for one another.

1. A nucleotide sequence substantially free of other sequences which would adjoint the sequence in the wild-type virus associated with the sequence, the sequence being selected from the group consisting of:

a) the HVT homologue of the HSV gB gene,

b) the HVT homologue of the HSV gC gene,

c) the HVT homologue of the HSV gH gene, or the 273-320 or 867-926 nucleotide region thereof,

d) the TK gene of ILTV,

e) the ILTV homologue of the HSV gB gene,

f) ORF2 of ILTV,

g) ORF3 of ILTV,

h) the ribonucleotide reductase gene (large subunit) of ILTV,

i) the ribonucleotide reductase (large subunit) gene of HVT,

(j) the ribonucleotide reductase (small subunit) gene of MDV,

(k) the ribonucleotide reductase (large subunit) gene of MDV,

(l) the HVT homologue of the immediate early gene IE-175 of HSV-I, and

(m) the HVT homologue of the immediate early gene IE-68 of HSV-I,

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(57) Abstract

Various genes of herpes virus of turkeys (HVT), Marek's disease virus (MDV) and infectious laryngotracheitis virus (ILTV) have been identified as non-essential regions (and candidates for insertion sites for foreign genes) and/or as antigen-encoding regions. The former include the HVT homologue of the HSV (herpes simplex virus) gC gene, the TK (thymidine kinase) region of MDV or ILTV, ORF3 of ILTV (as defined herein), the ribonucleotide reductase (large subunit) gene of ILTV, MDV or HVT and the ribonucleotide reductase (small subunit) gene of MDV. The antigen-encoding regions include the HVT homologues of the HSV gB, gC and gH genes, the ILTV homologue of HSV gB, ORF2 of ILTV, and the HVT homologue of the HSV-1 immediate early genes IE-175 and IE-68. Manipulation of these genes allows vaccines to be prepared comprising attenuated virus or virus carrying heterologous antigen-encoding sequences.

# VIRAL NUCLEOTIDE SEQUENCES

The present invention relates to viral nucleotide sequences which may be manipulated to provide vaccines against disease.

## Background and Description of prior art

Herpesviruses are large double stranded DNA viruses consisting of an icosahedral capsid surrounded by an envelope. The group has been classified as alpha, beta and gammaherpesviruses on the basis of genome structure and biological properties [Roizman, B et al (1981) Intervirology 16, 201-217]. Avian herpes viruses include Marek's Disease Virus (MDV) (a gammaherpesvirus) which causes a lymphomatous disease of considerable economic importance in chickens [reviewed in Payne, L.N. (ed) Marek's Disease (1985), Martinus Nijhoff Publishing, Boston] and Infectious Laryngotracheitis Virus (ILTV) (an alphaherpesvirus) which causes an acute upper respiratory tract infection in chickens resulting in mortality and loss of egg production.

A recent unexpected finding in out laboratory is that there is sufficient amino acid homology between MDV, ILTV and mammalian herpesviruses, particularly varicella zoster (VZV) and Herpes Simplex Virus (HSV) to allow

identification of numerous conserved genes. These include the MDV and Herpesvirus of Turkeys (HVT) homologues of glycoproteins gB, gC and gH of HSV; the ILTV, MDV and HVT homologues of TK and ribonucleotide reductase genes and the ILTV homologue of gB and genes 34 and 35 of VZV [Buckmaster, A et al, (1988) J. gen. Virol, 69, 2033-2042.

Strains of MDV have been classified into three serotypes. Type 1 comprises pathogenic strains and their attenuated derivatives. Type 2 are a group of naturally-occurring non-pathogenic strains and type 3 is HVT. For more than a decade, vaccination with HVT has been remarkably effective in controlling Marek's disease. However, in recent years, new strains of MDV have been isolated which cause disease despite vaccination with HVT. Losses due to these 'very virulent' strains have occurred in parts of the U.S.A., Europe and the Middle East. Although the degree of protection can be improved by using a mixture of HVT, type 2 MDV and attenuated derivatives of very virulent strains for vaccination, the results have been erratic. These observations and the fact that there are MDV type-specific epitopes that are not shared by HVT or type 2 MDV have led us to the conclusion that improved vaccines might be constructed which are antigenically more related to MDV than existing vaccines. [Reviewed by Ross and Biggs in Goldman J.M.

and Epstein M.A. (eds) Leukaemia and Lymphoma Research, Vaccine Intervention against Virus-Induced Tumour, p 13-31, Macmillan, 1986].

Infectious laryngotracheitis is also a worldwide problem. Sporadic outbreaks occur in which the severity of clinical symptoms varies considerably. Virus can persist in birds that have recovered and may be shed at intermittent intervals after recovery. An attenuated field strain is currently used as a vaccine. However, it has retained some degree of pathogenicity. Mortality due to the vaccine may reach 10% in young chicks.

A number of herpesvirus antigens have been shown to confer protective immunity when expressed in a recombinant vaccinia virus. These include the gB gene of HSV [Cantin E.M. et al (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 5908-5912], gD of HSV [Paoletti, E. et al (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 193-197] and gp50 of pseudorabies virus (PRV), a homologue of HSV gD [Marchioli, C.C. et al (1987) J. Virol. 61, 3977-3981]. Because of the absolute requirement of gB for virus penetration and infectivity and because it is conserved among herpes-viruses, gB and its homologues are important immunogens. Moreover, the presence of gB at the surface of infected cells has been shown to be an important target for humoral and cell-mediated immune responses

[Blacklaws, B.A. et al J.gen. Virol. 68, 1103-1114 (1987); McLaughlin-Taylor, E. et al (1988) J. gen. Virol. 69, 1731-1734]. The recently described glycoprotein gH of HSV is also essential for infectivity and may also be an important immunogen [Desai, P.J. et al (1988) J. gen. Virol. 69, 1147-1156]. It has also been shown that gIII of pseudorabies virus (PRV), a homologue of gC, is a major target for neutralizing antibody and for cytotoxic T cells although it is a non-essential protein. Also of interest is the unexpected participation of immediate early proteins in T cell mediated cytotoxic reactions in cells infected with cytomegalovirus (CMV) [Kozinowski U.H. et al (1987) J. Virol. 61, 2054-2058]. Similar antigens could play an important role in the rejection of latently infected and transformed lymphocytes in Marek's disease since immediate early RNA transcripts have been detected in lymphoblastoid cell lines established from Marek's disease tumours.

Although many recombinant vaccines have been constructed using the poxvirus vaccinia as a vector, there are also reports of the use of herpesviruses as vectors for the expression of foreign genes. Thus hepatitis antigen has been expressed in HSV [Shih, M.F. et al (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 5867-5870] and human tissue plasminogen activator has been expressed in PRV [Thomsen, D.R. et al (1987) Gene 57, 261-265. In

both cases, foreign genes were inserted in cloned fragments of non-essential herpes genes which were then introduced into the virus vector by homologous recombination. The hepatitis virus gene was fused to a herpesvirus promoter and the recombinant DNA was inserted within the TK gene of HSV. Homologous recombination following co-transfection of the recombinant DNA and wild-type HSV DNA resulted in TK$^-$ virus clones that expressed the hepatitis antigen.

In the case of PRV, the gX gene mapping in $U_s$ was used as the site for insertion of the foreign gene. The strategy used involved insertion of the TK gene of HSV in the gX gene of a PRV mutant that had a defect in its TK gene resulting in a TK positive virus. The human tissue plasminogen activator gene was then inserted within a cloned fragement of HSV TK and the recombinant was introduced into the PRV mutant by homologous recombination. TK$^-$ virus was selected which expressed the human gene (Thomsen et al as above). Similarly, VZV has been used as a vector [Lowe et al (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 3896-3900].

Several herpesvirus genes have also been shown to be associated with virulence and to be non-essential for growth in vitro. These include the TK genes of HSV [Jamieson, A.T. et al (1974) J. gen. Virol. 24, 465-480;

Field, H. and Wildy, P., (1987) J. Hygiene (Cambridge) 81, 267-277] and of PRV. Indeed it has long been known that PRV is readily attenuated by deletion of TK activity [Tatarov, G. (1968) Zentralbl. Vet. Med 15B, 848-853]. Furthermore, attenuation of the Bartha strain of PRV has been attributed to a defect in gI, a non-essential structural glycoprotein mapping in $U_s$ [Mettenleiter, T. et al (1987) J. Virol. 61, 4030-4032].

Genes of HSV mapping in the internal repeat region (TRS) flanking the long unique sequence have also been associated with pathogenicity [Rosen, A. et al (1986) Virus Research 5, 157-175; Thompson, R.L. et al (1983) Virology 131, 180-192]. Several additional genes of HSV have been shown to be non-essential for growth in vitro although it is not known whether they are associated with virulence. These include UL24 (Sanders, P.G., (1982), J. gen. Virol. 63, 277-295, large subunit of ribonucleotide reductase (Goldstein D.J. and Weller, S.K. (1988) J. Virol. 62, 196-205), gC (Draper K.G. et al (1984) J. Virol. 51, 578-585), dUTPase (Fisher, F.B. & Preston, V.G. (1986) Virology 148, 190-197), and $U_L55$ and $U_L$ 56 (MacLean, A.R. & Brown, S.M. (1987) J. gen. Virol. 68, 1339-1350).

Moreover there is evidence that several genes of HSV mapping in $U_s$ are also non-essential for growth in vitro

[Weber, P.C. et al (1987) Science 236, 576-579].

Summary of the invention

One aspect of the present invention provides a nucleotide sequence substantially free of ~~the~~ other sequences which would adjoin ~~it~~ the sequence in the wild-type virus associated with the sequence, the sequence being selected from the group consisting of:

(a) the HVT homologue of the HSV gB gene,

(b) the HVT homologue of the HSV gC gene,

(c) the HVT homologue of the HSV gH gene,

(d) the TK gene of ILTV,

(e) the ILTV homologue of the HSV gB gene,

(f) ORF2 of ILTV,

(g) ORF3 of ILTV,

(h) the ribonucleotide reductase (large subunit) gene of ILTV,

(i) the ribonucleotide reductase (large subunit) gene of HVT,

(j) the ribonucleotide reductase (large subunit) gene of MDV,

(k) the ribonucleotide reductase (large subunit) gene of MDV,

(l) the HVT homologue of the immediate early gene IE-175 of HSV-I, and

(m) the HVT homologue of the immediate early gene IE-68 of HSV-I,

and minor variations thereof.

Each of sequences (a) to (m) may be associated with further elements such as suitable stop and start signals and other 5' and 3' non-coding sequences, including promoters, enabling expression of the sequence. Such further elements may be those associated with the sequence in its naturally-occurring state or may be heterologous to that sequence.

In particular the promoter may be one associated with one of the sequences (l) and (m) above.

The term "minor variations thereof" is intended to include changes in the nucleotide sequence which do not affect its essential nature, for example minor substitutions of nucleo-tides for one another. In the case of sequences which are intended for insertion into a vector to encode an antigen, the "essential nature" of the sequence refers to the (glyco)protein encoded. Conservative changes in the nucleotide sequence which give rise to the same antigen will clearly be included, as will changes which cause conservative alterations in the amino acid sequence which do not affect adversely the antigenic nature of the antigen, in particular, antigenic portions of the antigen sequences may be used alone, for example the regions corresponding to nucleotides 273-320 or 867-926 of HVT gH and minor variations thereof. These sequences and the peptides encoded thereby form a further aspect of the invention. In the case of a sequence which is an insertion site, it is necessary only that the sequence should be non-essential for the infectivity and replication of the virus and have sufficient homology with the defined sequence to enable recombination to occur. Thus an insertion of one nucleotide into the sequence could completely change the reading frame from then on in a downstream direction. In the case of an

antigen-encoding sequence this would usually alter the amino acid sequence undesirably (depending on where the frameshift occurred), but in the case of an insertion site, the degree of homology would be almost the same, thereby allowing recombination to take place with almost the same ease.

Generally speaking, in an insertion site, if a nucleotide homology of at least 75% is present, the sequence is regarded as a "minor variation". Preferably, the sequence is at least 80, 85, 90, 95 or 99% homologous.

It will be appreciated that such degrees of homology relate to substantially the entire portion of each sequence (a) to (m) defined above. Shorter sequences may be used as probes in the identification or isolation of such longer sequences, but in this case the degree of homology will in general need to be greater in order to ensure accurate hybridisation.

Thus, a further aspect of the invention provides sub-sequences of at least 13 nucleotides having at least 90% (preferably 95%, 99% or 100%) homology with at least one portion of any of the said sequences (a) to (m) above.

In the above list, sequences (a) to (c), (e), (f), (l) and (m) are useful for expressing viral antigens. Sequences (b), (d) and (g) to (k) and, in addition, the TK region of MDV are useful as non-essential sites suitable for insertion of antigen-expressing genes. Thus, sequence (b) is useful for both functions.

The sequences may readily be isolated from naturally-occurring ILTV, HVT and MDV viruses, using the sequence information given herein and standard techniques, for example involving the preparation of oligonucleotide probles and use thereof to hybridise to the naturally-occurring DNA.

Antigenic ILTV and HVT sequences, i.e. sequences (a) to (c), (e), (f), (l) and (m) above, may be expressed in any suitable host and, in particular, in HVT or MDV. Suitable non-essential sites for insertion of one ILTV sequence include the MDV homologue of the HSV gC gene, the HVT homologue of the HSV gC gene, the TK gene of HVT or MDV, the ribonucleotide reductase (large subunit) gene of HVT or MDV and the ribonucleotide reductase (small subunit) gene of MDV.

A second aspect of the invention provides insertional or deletional mutants of MDV, HVT and ILTV as follows:

(i) for HVT, a mutation in the region homologous to the HSV gC gene or in the ribonucleotide reductase gene or the TK gene,

(ii) for MDV, a mutation in the region homologous to the HSV gC gene or in the ribonucleotide reductase (small subunit) gene or in the ribonucleotide reductase (large subunit) gene,

(iii) for ILTV, a mutation in the TK gene, ORF3 or the ribonucleotide reductase (large subunit) gene.

Each mutation may be in the coding or non-coding sequences of the regions identified.

Such mutant forms of HVT, MDV and ILTV may be used as, or created in the course of preparing, viral vectors for heterologous antigen-encoding sequences, or indeed as vectors for any other sequence which one wishes to express in a fowl in which the vector will replicate. Such sequences include, but are not limited to, (a) to (c), (e), (f), (l) and (m).

By "heterologous", we mean that the antigen-expressing sequence has not previously been found in the same place in relation to the remainder of the viral genome. For example, an antigen-expressing gene might be isolated from a virulent strain of ILTV and inserted into

the TK region of a less virulent strain of ILTV; this insertion would be regarded as "heterologous" if it did not result in a naturally-occurring virus.

The heterologous sequence may alternatively be one coding for an antigen associated with any one of the following diseases: avian encephalomyelitis (epidemic tremor), avian influenza (fowl plague), avian leukosis, avian paramyxoviruses other than Newcastle disease (PMV2 to PMV7), avian reovirus diseases (enteric disease, tenosynovitis), chicken anaemia (caused by chicken anaemia agent), coccidiosis (Eimeria is a pathogenic agent of coccidiosis), egg drop syndrome (EDS76), fowl pox, infectious bronchitis (IBV is used to signify Infectious Bronchitus Virus), infectious bursal disease (Gumboro) (IBD is used to signify Infectious Bursal Disease), inclusion body hepatitis (adenovirus), lymphoproliferative disease of turkeys, Newcastle disease, reticuloendotheliosis in chickens, reticuloendotheliosis in turkeys, rotavirus enteritis, turkey haemorrhagic enteritis and turkey rhinotracheitis. The sequence may alternatively encode paramyosin (a muscle protein common to all invertebrate parasites) or an antigenic part thereof, somatostatin or a growth-promoting part thereof or an immune regulator.

The vector in accordance with the invention may provide multivalent vaccine protection. For example, a vaccine comprising ILTV carrying an MDV antigen coding sequence would be expected to protect against ILT and

Marek's Disease.

Furthermore, the mutant ILTV viruses themselves are potentially useful in vaccines as attenuated viruses, without necessarily having a heterologous sequence inserted.

A convenient process for preparing the deletional or insertional mutants of the second aspect of the invention comprises simply introducing into a suitable cell, for example by co-transfection, a deletional or insertional mutant version of the appropriate region (for example, the TK region) and either whole viral DNA or a whole virus (for example the wild-type virus). The naked DNA of such viruses has been found to be infectious, provided that it has not been sheared. A calcium phosphate precipitate of the DNA is generally advantageous. Suitable cells include chicken embryo fibroblasts, chicken kidney cells and duck embryo fibroblasts, all preferably grown in sub-confluent monolayers in Petri dishes.

The transfected DNA and the whole viral DNA will then recombine with one another in the infected cells by homologous recombination and the desired recombinants can be screened for, for example by the detection of hybridisation to suitable probes or by an immunoassay

using suitable antibodies to the gene product of the region in question.

For homologous recombination to take place, the viral DNA must replicate. At present, no cell-free replication system for MDV, HVT or ILTV is known. However, if such a system becomes available, then the process of the invention could be operated therein. The environment in which the replication and recombination occur is not critical.

The ILTV and HVT regions which were identified above as being responsible for encoding immunologically useful viral antigens can be inserted into suitable vectors, for example into HVT or into other vectors such as fowlpoxvirus, bacteria or fungi. In the case of viral vectors, especially herpesvirus vectors and poxvirus vectors, such insertion can be achieved by recombination betwen the antigen-encoding sequence, flanked by suitable non-essential sequences, and the vector's genome in a suitable host cell as described above. A promoter which is endogenous to the host will usually be used to control expression of the heterologous (viral antigen-encoding) sequence. In the case of bacteria and fungi, the antigen-encoding sequence may be inserted using known or yet-to-be-discovered techniques of DNA manipulation. A non-pathogenic strain of Salmonella may be used as such a

host. The heterologous sequence may be inserted into the host's genome or be carried on an independently-replicating plasmid.

The flanking sequences which are used may comprise all, virtually all or less of the region into which the heterologous sequence is to be inserted. If all the region is employed, then the sequence of that region will clearly still be present in the resulting virus, but the function of that region will have been deleted. If less than the whole region is used as flanking sequences, then the result will be a structural as well as functional deletion. Either approach may be used.

Thus, the construction of deletional or insertional mutants of ILTV can yield improved vaccines. Alternatively, the expression of ILTV glycoproteins or other ILTV proteins engineered into HVT, fowl pox or other vectors can constitute effective vaccines.

To prepare a vaccine in which HVT, MDV or ILTV is the virus or vector, the virus is grown in suitable cells such as chick embryo fibroblasts in a standard culture medium which as 199 medium (Wellcome or Flow Laboratories) for 3 to 4 days at about 37°C. The cells are harvested by scraping from the surface of the culture or by trypsinisation and suspended in medium containing

1mM EDTA or 10% dimethyl sulphoxide and in either case 4% calf serum before storage in liquid nitrogen in sealed ampoules.

For vaccination, typically, day-old chicks are injected intramuscularly with about 1,000 plaque-forming units. Immunity follows within a few days.

It should be noted that MDV and HVT are cell-associated viruses and are infectious only when present in cells. Thus, a vaccine based on such viruses will always include suitable infected cells.

The vaccines of the invention may be used to protect any fowl susceptible to ILTV or HTV, including commercially-reared poultry such as chickens, turkeys, ducks and quail.

Preferred aspects of the invention will now be described by way of example and with reference to the accompanying drawings, in which:

Figure 1 is a map of the MDV genome showing in part the BamH1 site distribution and the location of the gB and TK genes;

Figure 2 (on 18 sheets) shows the nucleotide

sequence of the gB gene of the RB1B strain of MDV, with the numbering referring to the MDV nucleotides. the sequence of part of the HVT gB gene shown under the line, homologies indicated by vertical bars, and amino acid differences between MDV gB and HVT gB shown above the line;

Figure 3 is a map of the HVT genome showing the positions of the gH (hatched), TK (solid black) and major capsid protein (MCP, dotted) genes, with HindIII sites shown as "H";

Figure 4 (on 8 sheets) shows the nucleotide sequence of most of the HVT gH gene, with the corresponding amino acid sequence shown above the line;

Figure 5 (on 10 sheets) shows the nucleotide sequence of the HVT TK gene, with the numbering referring to the HVT nucleotides. the sequence of part of the MDV TK gene shown under the line, homologies indicated by vertical bars and amino acid differences between MDV TK and HVT TK shown above the line;

Figure 6 (on 6 sheets) shows the nucleotide sequence of the gC gene of the RBIB strain of MDV, with corresponding amino acids shown above the line;

Figure 7 (on 11 sheets) shows the nucleotide and predicted amino acid sequence of a 5400 base pair region of the ILTV genome containing the TK gene cluster. Amino acid sequences predicted for the products of the major open reading frames (ORFs) are indicated in the single letter code below the sequence for the strand and above the sequence for the complementary strand. The locations of potential 'TATA' boxes are underlined. ORF 4 is the ILT TK gene sequence:

Figure 8 is a representation of the gene organisation in the TK-containing part of the ILTV genome. Overlapping pUC 13 plasmid clones containing the EcoR1 (pILEc1) and BglII (pILBg2) generated fragments of ILTV DNA are indicated. Open reading frames (ORFs) are depicted as open boxes with the direction of transcription indicated by the arrow;

Figure 9 shows part of the nucleotide sequence of the ILTV gB gene;

Figure 10 shows part of the nucleotide sequence of the ILTV ribonucleotide reductase (large subunit);

Figure 11 shows part of the nucleotide sequence of the HVT homologue of the VZV62/HSV-1 IE 175 gene:

Figure 12 shows part of the nucleotide sequence of the HVT ribonucleotide reductase (large subunit) gene;

Figure 13 (on 2 sheets) shows part of the nucleotide sequence of the MDV ribonucleotide reductase (large subunit) gene;

Figure 14 shows part of the nucleotide sequence of the MDV ribonucleotide reductase (small subunit) gene;

Figure 15 shows part of the nucleotide sequence of the MDV homologue of the HSV-1 IE-175 gene;

Figure 16 shows part of the MDV homologue of the HSV-1 IE-68 gene;

Figure 17 is a schematic representation of homologous recombination at a non-essential region of a viral genome and a homologous region of DNA cloned within a plasmid vector; and

Figure 18 is a map of plasmid pILBg2, showing restriction sites and the locations of the TK gene and ORFs 3 and 5.

EXAMPLES: General Approaches

Selected short sequences of the avian herpesviruses cloned in the bacteriophage vector M13 were used as probes to identify longer fragments that might contain the entire genes of interest. This was achieved by

Southern blot hybridization of restriction fragments. Full details are given below.

Virus Strains. The 'highly oncogenic' strain RB1B of MDV [Schat, K.A. et al (1982) Avian Pathol. 11, 593-605] was obtained from Professor B. Calnek, Cornell University, Ithaca, U.S.A. The virus received has been plaque purified in chicken kidney vells in tissue culture. It was passaged twice in SPF RIR chickens and 4 times in chick embryo fibroblasts (CEF). Its 'highly oncogenic' nature was demonstrated by a high incidence of gross tumours when inoculated in genetically resistant N-line chickens.

The FC126 strain of HVT [Witter, R.L. et al (1970) Am. J. Vet. Res. 31, 525-538], obtained from the Wellcome Research Laboratories, Beckenham, Kent, had been passaged 14 times in CEF. It was subsequently grown in duck embryo fibroblasts (DEF) and CEF in our laboratory. It was then plaque-purified and grown further in CEF. Viral DNA used for cloning in the present work was extracted from virus that had been passed 29 times since the original isolation.

The Thorne strain of ILTV was passaged twice in eggs, once in chicken kidney cells (CKC) and plaque-purified three times in CKC.

Tissue culture. CEF were grown in roller bottles in 199 medium (Wellcome), supplemented with penicillin, streptomycin, Fungizone (Regd. T.M.) and calf serum as described previously [Ross, L.J.N. et al (1975) J. gen. Virol. 28, 37-47].

CKC were grown in 10 cm Petri dishes [Churchill, A.E. and Biggs P.M., (1967) Nature, 215, 528-530].

Isolation of MDV DNA. Cell associated RB1B was inoculated onto confluent monolayers of CEF in roller bottles at a multiplicity of infection of approximately 0.001 plaque-forming units (pfu) per cell, and the cultures were incubated at 37°C. After 3 days, the medium was discarded and replaced with fresh 199 medium containing 2% calf serum. Cells were harvested for virus purification after 2 to 3 days when cytopathic effect was extensive. Virus was obtained by rate zonal centrifugation of the cytoplasmic fraction of infected cells [Lee, Y.S. et al (1980) J. gen. Virol. 51, 245-253]. Viral DNA was extracted by treating purified virus with sarcosyl, proteinase K and Tris buffer pH 9 overnight at 37°C and purified by rate zonal centrifugation in glycerol gradients as described previously (Lee et al, 1980). High molecular weight viral DNA was precipitated with ethanol and resuspended in 10 mM Tris pH 7.5 im 1mM EDTA (TE).

Isolation of ILTV DNA. (a) Infected CKC were harvested 2-3 days after inoculation, washed in PBS, and resuspended in ice-cold TE by vortexing. Cells were lysed by addition of the non-ionic detergent NP40 (final 1%) vortexing and incubation on ice for 15 min. After treatment with RNAse, the preparation was centrifuged at 2000 rpm for 5 min in a bench top centrifuge (Centaur). The supernatant was collected and incubated at 37°C for 30 min in the presence of SDS (final 1%) and proteinase K (final 0.5 mg/ml). The mixture was extracted twice with phenol-chloroform and once with chloroform and the DNA was then precipitated with ethanol and 1/10 vol of 3M sodium acetate.

(b) Viral DNA was also isolated from the media of virally infected cells in the following way. The media of infected cells were harvested at 2-3 days post infection and centrifuged at 3000 for 5 mins at 4°C rpm in a bench centrifuge. The supernatant was collected and centrifuged at 19K rpm in an ultracentrifuge (Sorvall) for 1 hr at 4°C. The viral pellet was resuspended in TE, digested with RNAse A, then disrupted with SDS and proteinase K as described above. Finally, DNA was extracted from the disrupted virus as described above.

Cloning of MDV DNA. One *fg* of MDV DNA was cut with the restriction enzyme BamH1 and ligated to BamH1-cut, dephosphorylated pUC13 DNA (Pharmacia). Competent E.coli

strain TG1 cells were transformed according to standard procedures [Hanahan, D. (1983) J. Mol. Biol. 166, 557-580] and were grown in the presence of ampicillin and X-gal. White colonies were picked and tested for the presence or MDV inserts by hybridization to nick-translated MDV DNA [Grunstein M. and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3961]. Positive colonies were cultured in small volume and plasmid DNA isolated by the procedure of Holmes, D.S. and Quigley, M. [(1981) Anal. Biochem. 114, 193-297]. The size of the inserts was determined by electrophoresis of BamH1 digests of the recombinant DNA in agarose gels. Plasmids containing MDV inserts ranging from less than 1 to 18 Kbp were obtained.

Cloning of ILTV DNA. EcoR1 and Bg1II libraries of ILTV DNA were obtained by cloning digests of viral DNA in pUC13 as described above.

Random sequencing of viral DNA. Sonicated fragments of viral DNA were cloned into SmaI-cut, dephosphorylated M13.mp10 (Amersham International PLC) and plaques containing MDV inserts were identified by hybridization to MDV DNA. The sequence was determined by the dideoxy method [Sanger, F. et al (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5463-5467] using $^{35}$S dATP).

The same procedure was used to sequence cloned fragments of MDV, HVT and ILTV DNA except that plaques were identified by hybridization to labelled insert so as to avoid colonies containing pUC13 fragments.

EXAMPLE 1: gB gene of MDV

An M13 clone of HVT homologous to the gB gene of VZV and HSV hybridized to BamH1 fragment I3 of MDV (see Figure 1). Sequencing of this fragment obtained from a BamH1 library of the RB1B strain of MDV showed that two thirds of the gene, starting with the $NH_2$ terminus, was contained within I3. The remainder of the gene was identified in the adjacent restriction fragment K3. Figure 1 shows the map position of the gene which is 2.6Kbp long. Its mRNA has been estimated to be approximately 2.8 Kb. The translated protein is 865 amino acids long (Figure 2). This includes approximately 20 amino acids which may be part of a signal sequence domain. The primary translated sequence of MDV gB has a few features in common with gB of other herpes viruses such as the alignment of cysteine residues and the presence of hydrophobic sequences which are presumably capable of spanning a lipid bilayer [Pellet, P.E. et al (1985), J. Virol. 53, 243-253]. However, MDV gB has only 48% amino acid similarity with gB of HSV and has many unique features such as the insertion of 23 amino acids

(residues 1851-1920, Figure 2) and the presence of extra sites with glycosylation potential. Comparison of the sequence of MDV gB with limited sequence data (702 bases) available for HVT gB (Figure 2) has shown 76.9% nucleic acid similarity and 87.1% amino acid similarity between these two glycoproteins. Amino acid substitutions in HVT gB compared to MDV gB were particularly marked in a region (residues 1323 - 1433) equivalent to a domain of HSV gB associated with virus neutralization [Pellet P.E. et al (1985) as above]. Amino acid substitutions between MDV and HVT gB were also noted in other regions of unknown function.

EXAMPLE 2: gH gene of HVT and gH gene of MDV

An M13 clone of HVT containing sequences homologous to HSV gH was isolated during our earlier work on gene identification and mapping (Buckmaster et al (1988) as above). This clone, when used as a probe, hybridized to a 6Kbp HindIII fragment of HVT (Figure 3). Sequencing revealed that this fragment contained approximately one quarter of the gH gene including the carboxy terminus. The adjacent HindIII fragment (3.2 Kbp) containing the remainder of the gH gene was identified by hybridization using a cloned HpaI fragment of HVT which overlapped the HindIII site. Figure 4 shows the sequence of the coding region of the gH gene of HVT (2.3 Kbp) and flanking

sequences. The % amino acid identity between the gH gene of HVT and its homologue in HSV1, VZV and EBV was only 20, 24 and 20 respectively (estimated from maximised amino acid overlaps of 630, 644 and 153 respectively).

EXAMPLE 3: TK gene of HVT and TK gene of MDV

The whole coding region of the TK gene of HVT (1053 bp) was contained within the 3.2 Kbp HindIII fragment described above (Figure 3). The sequence of the entire gene and flanking regions is shown in Figure 5. Similarly the whole of the MDV TK gene is contained within the 3.6 Kbp BamH1 K2 fragment of MDV (Figure 1). The sequence of MDV TK gene determined so far is shown in Figure 5. Comparison of the MDV and HVT TK sequences indicates that the two genes have approximately 60% amino acid identity (estimated from 276 amino acid overlap). By contrast, the % amino acid identities between the TK gene of HVT and the TK genes of HSV 1, VZV and EBV are only 30, 27 and 24 respectively (estimated from amino acid overlaps of 320, 332 and 193 respectively). The predicted amino acid sequences of HVT and MDV TK show characteristic ATP and/or CTP binding site motifs described for a number of virus and eukaryotic proteins that are associated with phosphorylation (Gentry, G.A. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 6815-6819). These conserved sequences are examples of useful sites for insertion and expression of

foreign genes and for producing TK- deletion mutants.

EXAMPLE 4: A antigen gene of MDV (gP57-65) (gC homologue)

The A antigen gene is of interest in vaccine development both as an immunogen (it encodes a major glycopolypeptide product) and also because we have identified it as the homologue of HSV gC, a potential non-essential region. The A antigen gene was mapped within the BamH1 B fragment of MDV (Isfort et al 1987), and the nucleotide sequence determined for the GA strain of MDV (Coussens and Velicer, Abstract OP18.51, VII International Congress of Virology, 9-14 August, (1987) Edmonton, Canada; J. Virol. 62, 2373-2379). During the random sequencing studies described earlier (Buckmaster et al 1988), we identified an M13 clone (No. 130) which came from the A antigen gene. This clone was then used to identify a 2.3 Kbp EcoR1/PvuII fragment from the RB1B strain of MDV containing the A antigen. This fragment was cloned into a SmaI/EcoR1 cleaved pUC13 vector by standard protocols. One plasmid (pMB419) was sequenced by the M13 dideoxynucleotide method. The sequence of the MDV RB1B A antigen and the predicted amino acid sequence of the protein are presented in Figure 6. The A antigen regions of MDV and HTV are non-essential genes and they can therefore be used as sites in MDV and HVT into which other genes can be inserted into the virus by homologous

recombination. Several lines of evidence support this as outlined below.

1) During our study we isolated and sequenced another RB1B A antigen clone. This had one extra T residue in the string of T's 45 bases 3' to the A antigen ATG codon. This extra T would cause a frameshift which would make it impossible for the gene to encode functional A antigen. As it is probable that this gene was cloned from a replicating MDV, the results suggest that the A antigen is non-essential to the virus.

2) On conducting a similarity search it became clear that the MDV A antigen gene is the homologue of HSV gC and PRV gpIII glycoproteins. Both of these homologous genes are known to be non-essential [for the HSV homologue, see Rosenthal et al (1987) J. Virol. 61, 2438 - 2447].

3) Strains of MDV lacking A antigen as judged by agar gel diffusion tests [Churchill, A.E. et al (1969) J. gen. Virol. 4, 557-564] or producing low levels using the more sensitive 2D radio-immunoprecipitation (van Zaane, D. et al (1982) Virology 121, 133-146] have been reported.

Furthermore, in view of the fact that the A antigen is a major secreted glycoprotein, it may be a

particularly suitable location for the presentation of foreign epitopes within the A antigen as soluble, secreted proteins. This may be achieved by cloning oligonucleotides encoding these epitopes in frame within the A antigen gene.

STRATEGIES FOR INTRODUCING GENES INTO HVT AND ILTV VECTORS

Two possibilities can be envisaged. 1) Insertion into non-essential genes of the vector. 2) Substitution of foreign gene for corresponding gene of the vector. This would be possible only in regions which already have substantial homology as may be the case between some genes of MDV and HVT.

EXAMPLE 5: Insertion into non-essential genes of HVT, ILTV or MDV

(a) Insertion at the TK locus of the vector.

1) HVT, ILTV or MDV may be used as vectors for insertion and expression of avian herpesvirus genes. In particular gB, gD, gH or gC of RB1B MDV may be inserted into ILTV. Also gB and BS-17 of ILTV may be inserted into HVT or MDV. One may use the promoter associated with the inserted gene or use heterologous promoters,

including those of a different class of genes (for example the immediate early promoter to optimise expression of gB).

2) ILTV may be used as a general vector for the insertion and expression of genes unrelated to avian herpes viruses and likely to require manipulation of promoters for optimal expression.

The procedure to be used for gene insertion is substantially as described previously for the insertion of hepatitis antigen in HSV [Shih, M F et al (1984) Proc. Natl. Acad. Sci. USA, 81, 5867-5870].

MDV and HVT DNA obtained as described above is infectious provided that precautions are taken not to shear the DNA during extraction. Calcium phosphate precipitates of viral DNA prepared as described by Stow and Wilkie [(1976) J. gen. Virol. 33, 477] were added to sub-confluent monolayers of CEF. After absorption for 1h at 37°C, culture medium was added and cultures were incubated for 1 or 2 days until confluent. Monolayers were then trypsinised, replated (1:1 or 1:2) in 199 medium (Wellcome) containing 2 to 4% calf serum and incubated at 37°C until plaques developed, usually after 4 to 5 days. Approximately 200 plaques may be obtained per μg of HVT DNA and approximately 50 per μg of MDV DNA.

Restriction enzyme sites than could be used for the insertion of foreign antigens into the TK of HVT strain Fc-126 include: BanII, Bsp1286, DraIII, EcoRI, HincII, HpaI, NheI and NspbII.

Some of these enzymes also have sites in the plasmid vector into which the virus DNA fragments are cloned. Thus in order to linearize the clone DNA without also cutting within the vector, partial digests may be carried out.

None of the above enzymes should cause any disruption to flanking gene, HSV-1 homologues of which are known to play an important role in virus multiplication.

For homologous recombination and isolation of recombinant virus, genes of interest are inserted within non-essential genes such as TK or gC and co-transfected with wild-type viral DNA at molar ratios ranging from 10:1 to 2:1 as described above. Alternatively, intact wild-type virus may be used for co-infection.

Virus recombination may be detected by 'plaque lifts' which involve transfer of infected cells and released virus which have adhered to the agar overlay to nitrocellulose and hybridization of the denatured DNA

released from the cells and virus to suitable probes as described by Villareal, L. et al (1977) Science 196, 183-185. Virus which hybridizes to the probe may be recovered from the monolayer.

A similar procedure may be used to isolate recombinant virus which expressed epitopes of interest. In this instance the nitrocellulose "plaque lifts" are treated with antibody and the presence of bound antibody revealed using a suitable detection system such as labelled protein A or phosphatase conjugated antiglobulin antibody.

The gene of interest with appropriate promoters is first inserted within the cloned TK gene (Figure 7). The recombinant DNA is then co-transfected with infectious DNA of the vector in chick embryo fibroblasts or chicken kidney cells and TK- virus may be selected by growth in medium containing acyclovir [Ross, N. (1985), Development in Veterinary Virology, Martinus Nijhoff Publishing, Boston/Dordrecht/Lancaster Ed. L N Payne, pp 113-150 or FMAU [Schat, K A et al (1984) Antiviral Research 4, 159-270]. Alternatively, or in addition, plaques are screened for the presence of the gene of interest using 'plaque lifts' on nitrocellulose and hybridisation to any relevant labelled probe. Plaques are also screened for expression of the epitopes of interest using monoclonal antibodies or antipeptide antibodies.

The main advantage of this strategy is that the selection procedure increases the chances of obtaining virus recombinants containing the gene of interest. It also offers the opportunity of using different promoters for optimum expression. Thus the use of an immediate early promoter may allow expression in latently infected cells.

(b) Insertion at the gC locus of the vector.

Since the A antigen (HVT and MDV homologues of HSV gC) is not essential for virus growth in vivo and in vitro (see section on gC above) it is a potentially useful site for the insertion and expression of foreign genes. Moreover, since it is one of the most abundant antigens and is excreted, it may be particularly useful for enhancing the immunogenic properties of foreign proteins. The isolation of virus recombinants at this locus may be achieved by first inserting at least part of the gene of interest in frame within the gC gene and then co-transfecting with infectious viral DNA. Screening of virus plaques with sequence specific probes or with specific antibody allows the isolation of recombinants.

EXAMPLE 6: Substitution of ILTV genes for their homologues in HVT

Substitution may be achieved by co-transfection of cloned ILTV sequences and infectious HVT DNA as described in Example 5. Substitution of genes derived from ILTV for their counterparts in HVT may be effected.

Recombinants expressing ILTV sequences and epitopes may be detected using ILTV-specific monoclonal antibodies or anti-peptide antibodies raised against unique ILTV sequences as described above.

The advantage of this procedure is that it is relatively simple and does not require manipulation of promoters. However, it may be limited to genes which share substantial homology.

EXAMPLE 7: Strategies for obtaining TK- mutants of ILTV

Deletion mutants. Deletions may be introduced within any suitable part of the gene, for example the domains of the gene that are required for its function as a phosphorylating enzyme such as ATP and CTP binding sites. This may be achieved by restriction enzyme digestion, for example with SnaB1 or BclI, and religation of appropriate fragments followed by co-transfection with

infectious viral DNA or transfection into virally-infected cells. Reference may be made to Figures 7 and 8, and to the map of plasmid pILBg2 (Figure 18), in choosing restriction enzymes and so on. TK- virus may be selected in the presence of acyclovir [Ross, N. (1985) as above] or FMAU [Schat, K.A. et al (1984) as above]. Plaque-purified clones may then be tested for the absence of the deleted portion of the TK gene by hybridization.

The deletion mutants of ILTV may be used themselves as attenuated viruses for vaccine preparation, or may have sequences for heterologous antigens inserted.

Insertional mutants. A functional ß-galactosidase gene under the control of a herpesvirus promoter or any other suitable sequence or a single base is first introduced in a domain of the TK gene which is essential for TK activity. The recombinant DNA is then co-transfected with infectious viral DNA or transfected into virally-infected cells to allow homologous recombination to occur. Selection in the presence of acylovir or FMAU will yield TK- insertional mutants. If a ß-galactosidase gene is introduced, mutants can be detected by the production of blue plaques in the presence of X-gal.

The TK gene and surrounding sequences may be subcloned

into another suitable vector if necessary.

EXAMPLE 8: Insertion of MDV RB1B gB gene into HVT

(Not within the scope of the invention, but illustrates an analogous technique).

The HVT TK gene is cloned in the plasmid vector pUC13 to generate a plasmid, which may be termed pTK1B. This plasmid is linearised with, for example, the restriction endonuclease Rsr II which cleaves the plasmid only within the TK gene (nucleotide position 197 in Figure 5, enzyme recognition sequence CGGACCG). The "sticky" ends thus generated are end repaired by standard techniques (see "Molecular Cloning: a Laboratory Manual", ed. Maniatis T., Fritsch E.F., and Sambrook J. Cold Spring Harbor Laboratory 1982).

The RB1B gB was originally cloned on two plasmids which were termed RB1B-BamH1-$I_3$ and RB1B-BamH1-$K_3$. (Note $I_3$ had lost one BamH1 site during cloning.) To generate a complete gB copy on one plasmid, both plasmids were cleaved with BamH1 and the fragments ligated. However, the complete gB gene was later obtained independently on an EcoRI/SalI fragment. Ross et al, J. gen. Virol (1989) 70, 1789-1804 provides further information regarding the manipulation of viral genes. Recombinants containing the

desired configuration can be identified by restriction enzyme analysis of plasmid DNA's.

The recombinant plasmid is then cleaved with EcoR1, the ends are repaired and the plasmid is cloned into PTK1B prepared as above. The recombinant plasmid is then introduced into cells containing HVT virus (viral DNA) and homologous recombination will introduce the gB gene into the TK gene. HVT viral recombinants can be selected with acyclovir or FMAU or alternatively detected with labelled gB probes.

EXAMPLE 9: RB1B gC (A antigen) gene into HVT

Blunt ended PTK1B is prepared as in Example 8. The RB1B gC is cleaved from the plasmid pMB419 (Example 4) with the restriction endonucleases EcoR1 and HindIII (site within the pUC13 polylinker). The sticky ends generated are again end-repaired by standard protocols. The end-repaired gC fragment is then cloned into the linearized end-repaired pTK1B as in Example 8. (The cloning can be verified by analysis of the resulting clones with restriction enzymes, probing with radioactively labelled fragments, or DNA sequencing, or any combination of these).

The resulting plasmid with the RB1B gC gene cloned

into the HVT TK gene can then be introduced into the HVT genome by transfecting the plasmid into HVT-infected cells using calcium phosphate precipitation or electroporation. Homologous recombination, involving cross-overs either side of the gC gene, between the HVT virus and the flanking sequences of the HVT TK plasmid will carry the RB1B gC gene into the HVT viral genome. Viral recombinants can be selected for (as they are TK$^-$) or identified (eg by probing) as described above.

In analogous ways, the sequence information given above and in the Figures can be used to design cloning strategies for the insertion of these genes and others into the non-essential genes of the ILTV described here or to generate combinations of antigen genes into ILTV.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A nucleotide sequence substantially free of other sequences which would adjoint the sequence in the wild-type virus associated with the sequence, the sequence being selected from the group consisting of:

a) the HVT homologue of the HSV gB gene,

b) the HVT homologue of the HSV gC gene,

c) the HVT homologue of the HSV gH gene, or the 273-320 or 867-926 nucleotide region thereof,

d) the TK gene of ILTV,

e) the ILTV homologue of the HSV gB gene,

f) ORF2 of ILTV,

g) ORF3 of ILTV,

h) the ribonucleotide reductase gene (large subunit) of ILTV,

i) the ribonucleotide reductase (large subunit) gene of HVT,

(j) the ribonucleotide reductase (small subunit) gene of MDV,

(k) the ribonucleotide reductase (large subunit) gene of MDV,

(l) the HVT homologue of the immediate early gene IE-175 of HSV-I, and

(m) the HVT homologue of the immediate early gene IE-68 of HSV-I,

and minor variations thereof.

2. A sequence according to Claim 1 comprising the coding portion of the said sequence and at least part of the 5' and/or 3' non-coding portions thereof.

3. A plasmid vector comprising a sequence according to Claim 1 or 2 and a DNA portion allowing replication in a suitable host.

4. A plasmid vector according to Claim 3 which is suitable for transfection of an MDV-, HVT- or ILTV-susceptible cell.

5. An insertional or deletional mutant of MDV, HVT or ILT as follows:

(i) for HVT, a mutation in the region homologous to the HSV gC gene or in the ribonucleotide reductase (large subunit) gene or the TK gene,

(ii) for MDV, a mutation in the region homologous to the HSV gC gene or in the ribonucleotide reductase (small subunit) gene or in the ribonucleotide reductase (large subunit) gene,

(iii) for ILTV a mutation in the TK gene, ORF2, ORF3 or the ribonucleotide reductase (large subunit) gene.

6. A mutant virus according to Claim 5 wherein a heterologous gene is inserted into the said region of mutation.

7. A mutant virus according to Claim 6 wherein the heterologous gene codes for an antigen or part thereof associated with HVT, MDV, ILTV, IBV, IBD, Newcastle Disease or Eimeria.

8. A peptide encoded by any one of the said portions of HVT gH in Claim 1.

9. A process for preparing a mutant virus according to claim 5 comprising the steps of (i) allowing homologous recombination between (a) a deletional or insertional mutant copy of the said region and (b) either whole viral DNA from MDV, HVT or ILTV (as appropriate) or whole HVT, MDV or ILTV virus and (ii) isolating recombinant viruses.

10. A process according to claim 11 wherein, in step (i) the said mutant copy and either the whole viral DNA or the whole virus are introduced into a suitable cell such that the said recombination occurs during replication of the DNA therein.

11. A vaccine comprising ILTV-susceptible cells and a mutant ILT virus according to claim 5 such that the virus is attenuated, at least partially as a result of such mutation.

12. A vaccine effective against MDV, HVT or ILTV comprising susceptible cells and a mutant virus according to claim 5, 6 or 7.

13. A vaccination vector effective against ILT, HVT or MD, comprising a suitable microorganism which will replicate in a fowl, the microorganism carrying an ILTV or HVT vector comprising heterologous replicable DNA according to one or more sequences selected from the group consisting of:

a) the HVT homologue of the HSV gB gene,

b) the HVT homologue of the HSV gC gene,

c) the HVT homologue of the HSV gH gene, or the 273-320 or 867-926 nucleotide region thereof,

d) the ILTV homologue of the HSV gB gene,

e) ORF2 of ILTV,

f) the HVT homologue of the immediate early gene IE-175 of HSV-1,

g) the HVT homologue of the immediate early gene IE-68 of HSV-1,

14. A vaccine comprising a carrier medium and a vector according to claim 13.

15. A vaccine according to claim 14 wherein the carrier medium comprises ILTV-, HVT- or MDV-susceptible cells, and the vector is ILTV, HVT or MDV, as appropriate.

16. A method of vaccinating a fowl against a disease of the fowl comprising administering to the fowl a non-toxic immunity-conferring amount of vaccine according to any one of claims 11, 12, 14 and 15.

17. A fowl when vaccinated by a method according to claim 16.

18. A nucleotide sequence essentially free of other sequences which would adjoin the sequence in the wild type virus associated with the sequence, substantially as hereinbefore described with reference to the examples and the accompanying drawings.

19. An insertional or deletional mutant virus of MDV, HVT or ILT substantially as hereinbefore described with reference to the examples and the accompanying drawings.

20. A process for preparing a mutant virus substantially as hereinbefore described with reference to the examples and the accompanying drawings.

21. A vaccine effective against MDV, HVT or ILTV substantially as hereinbefore described with reference to the examples and the accompanying drawings.

22. A vaccination vector effective against ILT, HVT or MD substantially as hereinbefore described with reference to the examples and the accompanying figures.

23. A method of vaccinating a fowl against a disease of the fowl substantially as hereinbefore described with reference to the examples and the accompanying figures.

DATED this 28th day of October 1992

INSTITUTE FOR ANIMAL HEALTH LIMITED
By their Patent Attorneys
GRIFFITH HACK & CO

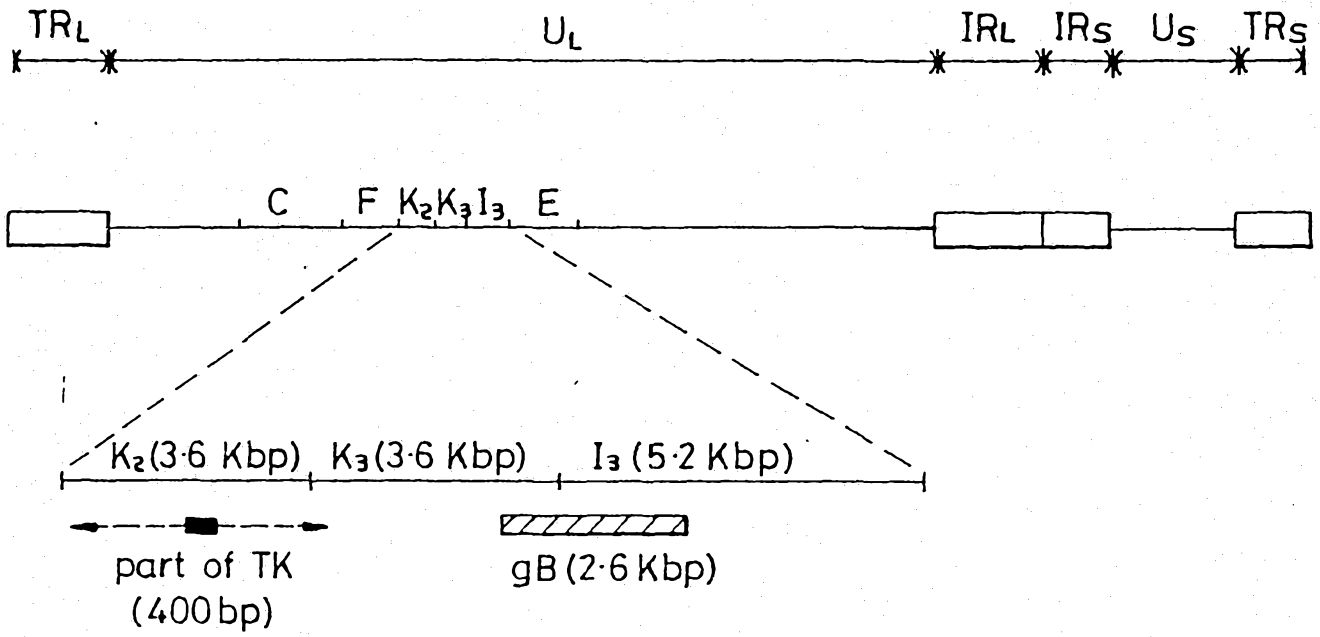

TRL
UL
IRL
IRS
US
TRS
C
F
K2K3I3
E
K2(3·6 Kbp)
K3(3·6 Kbp)
I3 (5·2 Kbp)
part of TK
(400 bp)
gB (2·6 Kbp)

Fig. 1

FIG 2A

TCGAGCTCGCCGGGGATGTTTAGTCACGATAGACATCGGT
10 20 30 40

TCGCCCAGCCGTCGAATACAGCATTATATTTTAGTGTTG
50 60 70 80

AAAATGTAGGGCTGCTTCCTCACTTAAAGGAGGAAATGGCT
90 100 110 120

CGATTCATGTTTCATAGCAGTAGAAAAACAGATTGGACCG
130 140 150 160

TCAGTAAGTTTAGAGGGTTTTATGACTTTAGCACTATAGA
170 180 190 200

TAATGTAACTGCGGCCCATCGCATGGCTTGGAAATATATC
210 220 230 240

AAAGAACTGATTTTTGCAACAGCTTTATTTTCTTCTGTAT
250 260 270 280

TTAAATGTGGCGAATTGCACATCTGTCGTGCCGACAGTTT
290 300 310 320

GCAGATCAACAGCAATGGAGACTATGTATGGAAAAATGGA
330 340 350 360

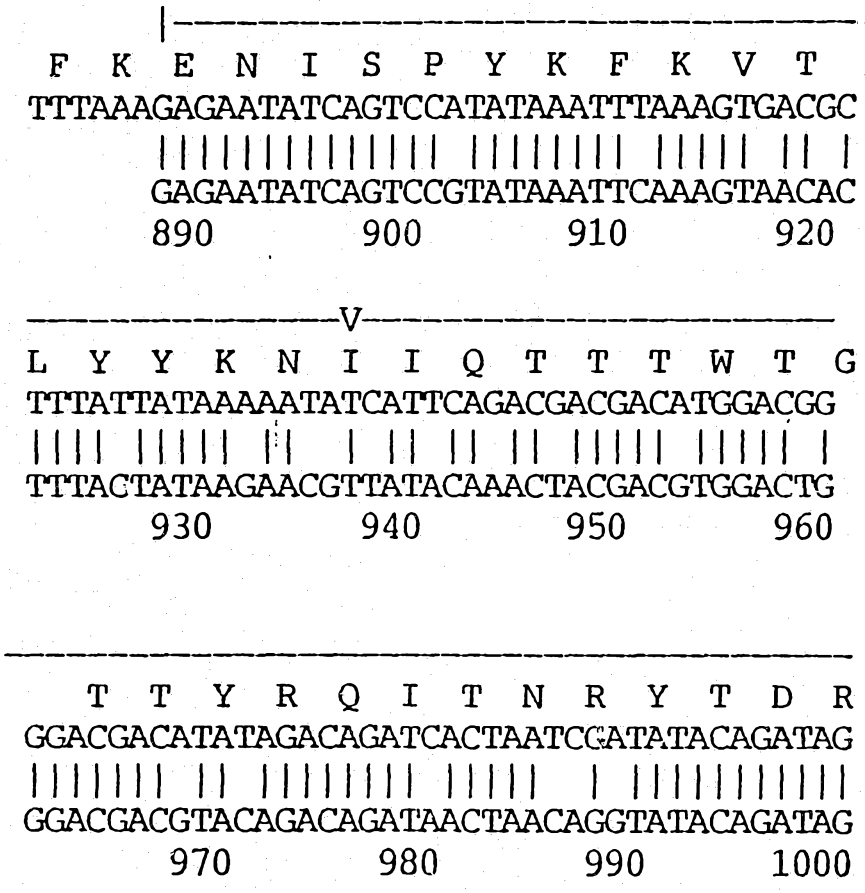
FIG 2B

```
ATATATATAACATATGAAACCGAATATCCACTTATAATGA
      370        380        390        400

TTCTGGGGTCAGAATCAAGCACTTCAGAAACGCAAAATAT
      410        420        430        440

GACTGCAATTATTGATACAGATGTTTTTTCGTTGCTTTAT
      450        460        470        480

TCTATTTTGCAGTATATGGCCCCCGTTACGGCAGATCAGG
      490        500        510        520

TGCGAGTAGAACAGATTACCAACAGCCACGCCCCCATCTG
      530        540        550        560

ACCCGTCCAATATTCTTGTGTCCCTGCATTTTATCTCACA
      570        580        590        600

                                       M  H
CAATTTATGAACAGCATCATTAAGATCATCTCACTATGCA
      610        620        630        640

  Y  F  R  R  N  C  I  F  F  L  I  V  I
CTATTTTAGGCGGAATTGCATTTTTTTCCTTATAGTTATT
      650        660        670        680
```

FIG 2C

```
L  Y  G  T  N  S  S  P  S  T  Q  N  V  T
CTATATGGTACGAACTCATCTCCGAGTACCCAAAATGTGA
      690        700        710        720

   S  R  E  V  V  S  S  V  Q  L  S  E  E
CATCAAGAGAAGTTGTTTCGAGCGTCCAGTTGTCTGAGGA
      730        740        750        760

   E  S  T  F  Y  L  C  P  P  P  V  G  S
AGAGTCTACGTTTTATCTTTGTCCCCCACCAGTGGGTTCA
      770        780        790        800

 T  V  I  R  L  E  P  P  R  K  C  P  E  P
ACCGTGATCCGTCTAGAACCGCCGCGAAAATGTCCCGAAC
      810        820        830        840

   R  K  A  T  E  W  G  E  G  I  A  I  L
CTAGAAAAGCCACCGAGTGGGGTGAAGGAATCGCGATATTA
      850        860        870        880
```

FIG 2D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GAGAATATCAGTCCGTATAAATTCAAAGTAACAC
890 900 910 920
V
L Y Y K N I I Q T T T W T G
TTTATTATAAAAATATCATTCAGACGACGACATGGACGG
TTTACTATAAGAACGTTATACAAACTACGACGTGGACTG
930 940 950 960
T T Y R Q I T N R Y T D R
GGACGACATATAGACAGATCACTAATCGATATACAGATAG
GGACGACGTACAGACAGATAACTAACAGGTATACAGATAG
970 980 990 1000

FIG 2E

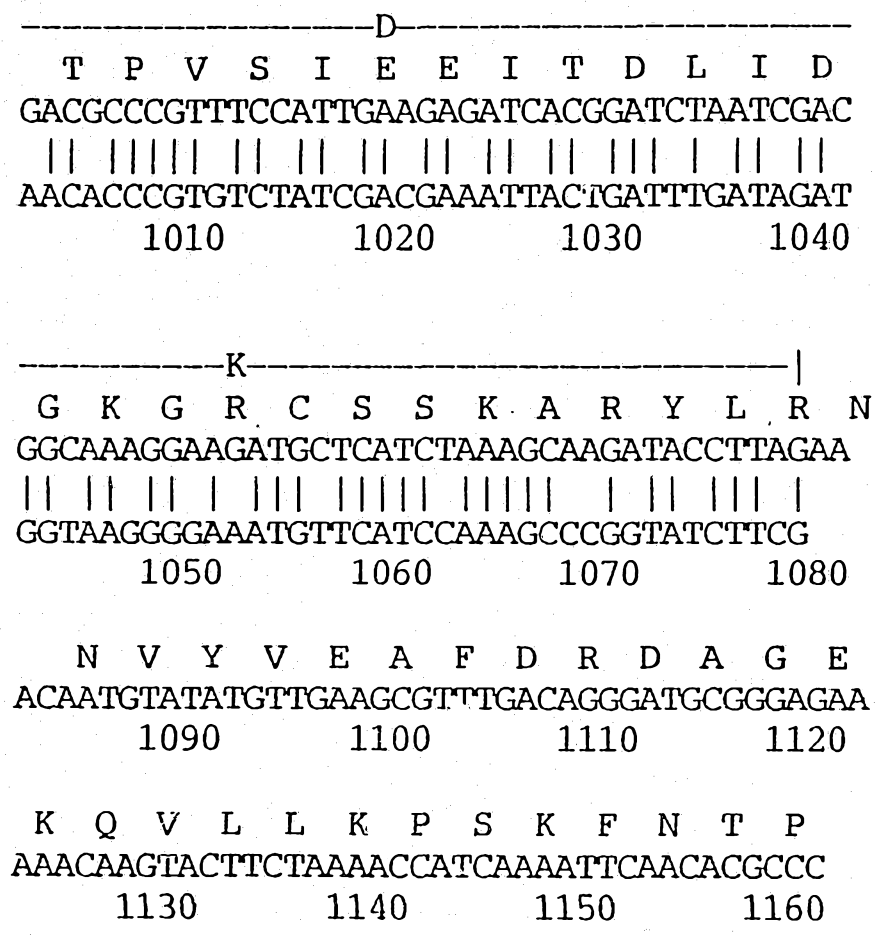

D
T P V S I E E I T D L I D
GACGCCCGTTTCCATTGAAGAGATCACGGATCTAATCGAC
AACACCCGTGTCTATCGACGAAATTACTGATTTGATAGAT
1010 1020 1030 1040
K
G K G R C S S K A R Y L R N
GGCAAAGGAAGATGCTCATCTAAAGCAAGATACCTTAGAA
GGTAAGGGGAAATGTTCATCCAAAGCCCGGTATCTTCG
1050 1060 1070 1080
N V Y V E A F D R D A G E
ACAATGTATATGTTGAAGCGTTTGACAGGGATGCGGGAGAA
1090 1100 1110 1120
K Q V L L K P S K F N T P
AAACAAGTACTTCTAAAACCATCAAAATTCAACACGCCC
1130 1140 1150 1160

FIG 2F

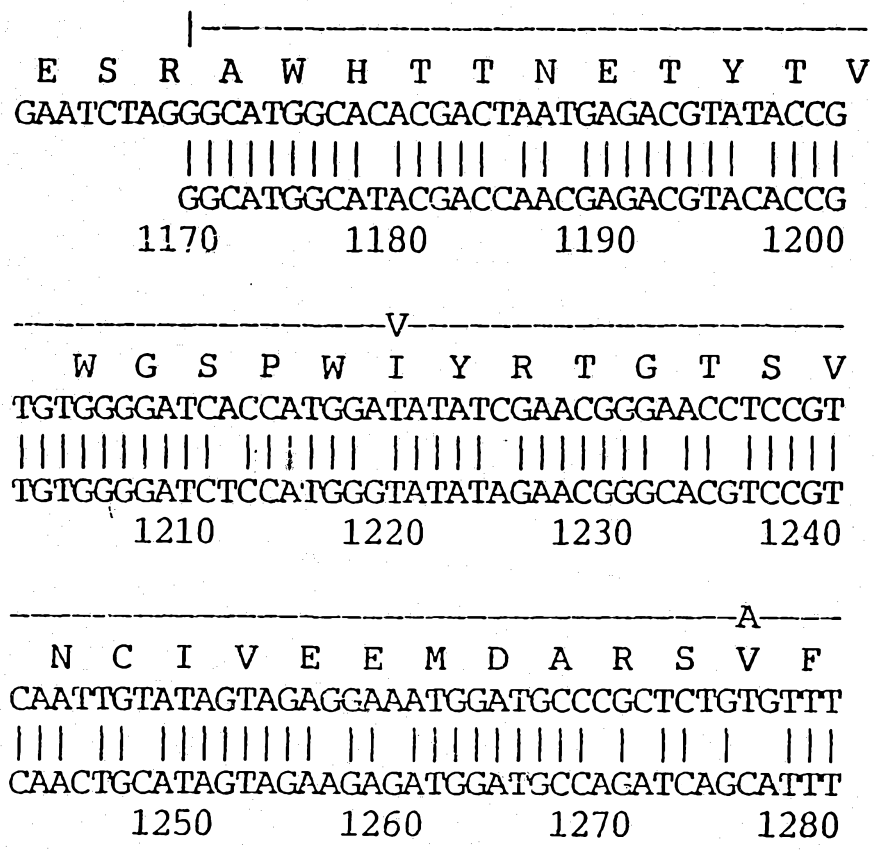

E S R A W H T T N E T Y T V
GAATCTAGGGCATGGCACACGACTAATGAGACGTATACCG
GGCATGGCATACGACCAACGAGACGTACACCG
1170 1180 1190 1200
V
W G S P W I Y R T G T S V
TGTGGGGATCACCATGGATATATCGAACGGGAACCTCCGT
TGTGGGGATCTCCATGGGTATATAGAACGGGCACGTCCGT
1210 1220 1230 1240
A
N C I V E E M D A R S V F
CAATTGTATAGTAGAGGAAATGGATGCCCGCTCTGTGTTT
CAACTGCATAGTAGAAGAGATGGATGCCAGATCAGCATTT
1250 1260 1270 1280

FIG 2G

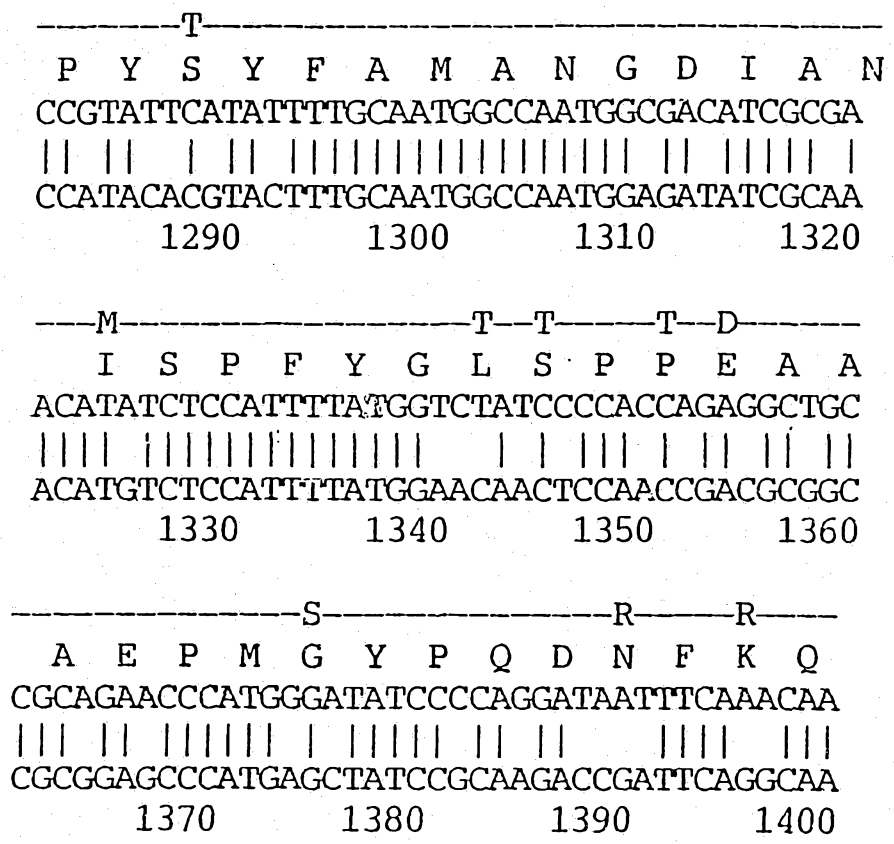

T
P Y S Y F A M A N G D I A N
CCGTATTCATATTTTGCAATGGCCAATGGCGACATCGCGA
CCATACACGTACTTTGCAATGGCCAATGGAGATATCGCAA
1290 1300 1310 1320
M T T T D
I S P F Y G L S P P E A A
ACATATCTCCATTTTATGGTCTATCCCCACCAGAGGCTGC
ACATGTCTCCATTTTATGGAACAACTCCAACCGACGCGGC
1330 1340 1350 1360
S R R
A E P M G Y P Q D N F K Q
CGCAGAACCCATGGGATATCCCCAGGATAATTTCAAACAA
CGCGGAGCCCATGAGCTATCCGCAAGACCGATTCAGGCAA
1370 1380 1390 1400

FIG 2H

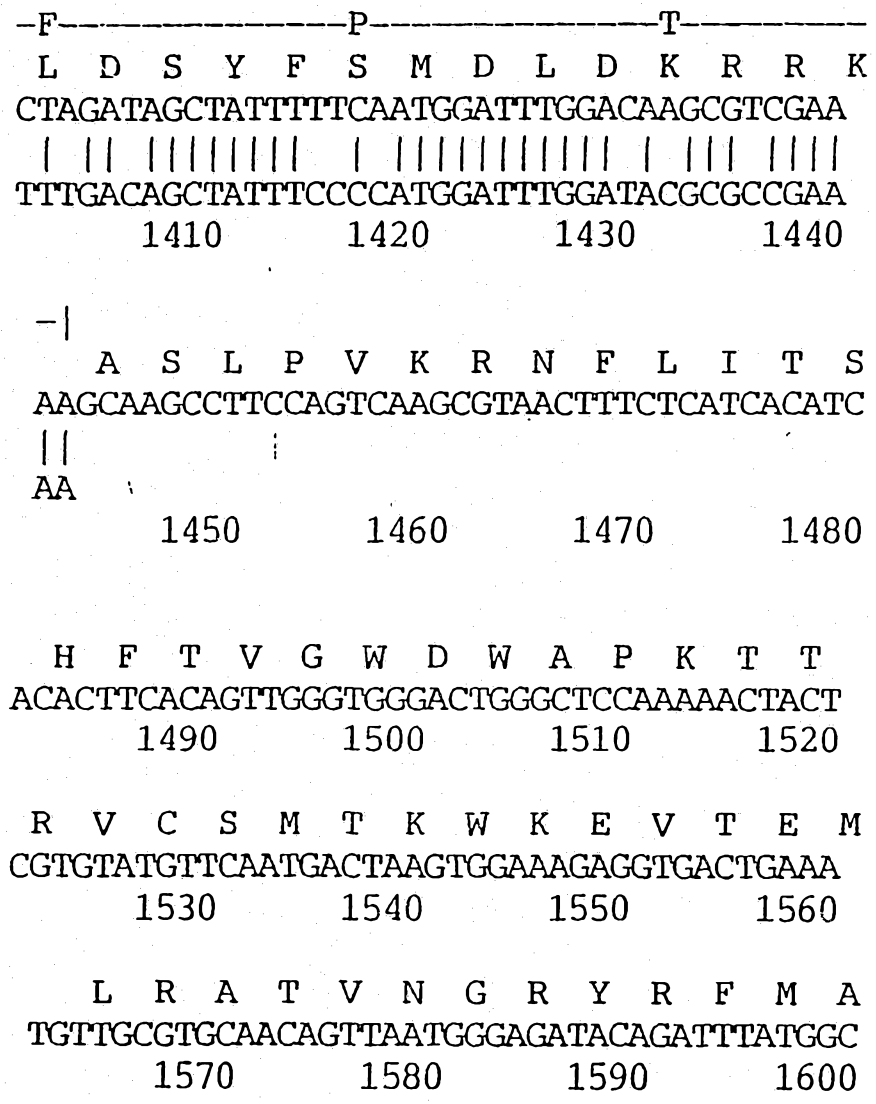

F
P
T
L D S Y F S M D L D K R R K
CTAGATAGCTATTTTTCAATGGATTTGGACAAGCGTCGAA
TTTGACAGCTATTTCCCCATGGATTTGGATACGCGCCGAA
1410 1420 1430 1440
A S L P V K R N F L I T S
AAGCAAGCCTTCCAGTCAAGCGTAACTTTCTCATCACATC
AA
1450 1460 1470 1480
H F T V G W D W A P K T T
ACACTTCACAGTTGGGTGGGACTGGGCTCCAAAAACTACT
1490 1500 1510 1520
R V C S M T K W K E V T E M
CGTGTATGTTCAATGACTAAGTGGAAAGAGGTGACTGAAA
1530 1540 1550 1560
L R A T V N G R Y R F M A
TGTTGCGTGCAACAGTTAATGGGAGATACAGATTTATGGC
1570 1580 1590 1600

FIG 2I

```
  R  E  L  S  A  T  F  I  S  N  T  T  E
CCGTGAACTTTCGGCAACGTTTATCAGTAATACGACTGAG
      1610       1620       1630       1640

  F  D  P  N  R  I  I  L  G  Q  C  I  K  R
TTTGATCCAAATCGCATCATATTAGGACAATGTATTAAAC
      1650       1660       1670       1680

     E  A  E  A  A  I  E  Q  I  F  R  T  K
 GCGAGGCAGAAGCAGCAATCGAGCAGATATTTAGGACAAA
      1690       1700       1710       1720

   Y  N  D  S  H  V  K  V  G  H  V  Q  Y
 ATATAATGACAGTCACGTCAAGGTTGGACATGTACAATA
      1730       1740       1750       1760

  F  L  A  L  G  G  F  I  V  A  Y  Q  P  V
TTTCTTGGCTCTCGGGGGATTTATTGTAGCATATCAGCCTG
      1770       1780       1790       1800

     L  S  K  S  L  A  H  M  Y  L  R  E  L
 TTCTATCCAAATCCCTGGCTCATATGTACCTCAGAGAATT
      1810       1820       1830       1840
```

FIG 2J

```
  M  R  D  N  R  T  D  E  M  L  D  L  V
GATGAGAGACAACAGGACCGATGAGATGCTCGACCTGGTA
      1850       1860       1870       1880

 N  N  K  H  A  I  Y  K  K  N  A  T  S  L
AACAATAAGCATGCAATTTATAAGAAAAATGCTACCTCAT
      1890       1900       1910       1920


    S  R  L  R  R  D  I  R  N  A  P  N  R
 TGTCACGATTGCGGCGAGATATTCGAAATGCACCAAATAG
       1930       1940       1950       1960
  K  I  T  L  D  D  T  T  A  I  K  S  T
AAAAATAACATTAGACGACACCACAGCTATTAAATCGACA
      1970       1980       1990       2000

 S  S  V  Q  F  A  M  L  Q  F  L  Y  D  H
TCGTCTGTTCAATTCGCCATGCTCCAATTTCTTTATGATC
      2010       2020       2030       2040

    I  Q  T  H  I  N  D  M  F  S  R  I  A
 ATATACAAACCCATATTAATGATATGTTTAGTAGGATTGC
       2050       2060       2070       2080
```

FIG 2K

```
  T  A  W  C  E  L  Q  N  R  E  L  V  L
CACAGCTTGGTGCGAATTGCAGAATAGAGAACTTGTTTTA
      2090       2100       2110       2120

 W  H  E  G  I  K  I  N  P  S  A  T  A  S
TGGCACGAAGGGATAAAGATTAATCCTAGCGCTACAGCGA
      2130       2140       2150       2160

                                  |------------
     A  T  L  G  R  R  V  A  A  K  M  L  G
  GTGCAACATTAGGAAGGAGAGTGGCTGCAAAGATGTTGGG
                                  || || ||||||||
                                  GCCAAAATGTTGGG
          2170       2180       2190       2200

------D----------------------I--E--T------S-
  D  V  A  A  V  S  S  C  T  A  I  D  A
GGATGTCGCTGCTGTATCGAGCTGCACTGCTATAGATGCG
 ||  |  ||  ||  |||||     ||  |  ||  |  |||  |
TGACGATGCCGCCGTATCATCATGTATTGAGACTGATTCA
      2210       2220       2230       2240
```

FIG 2L

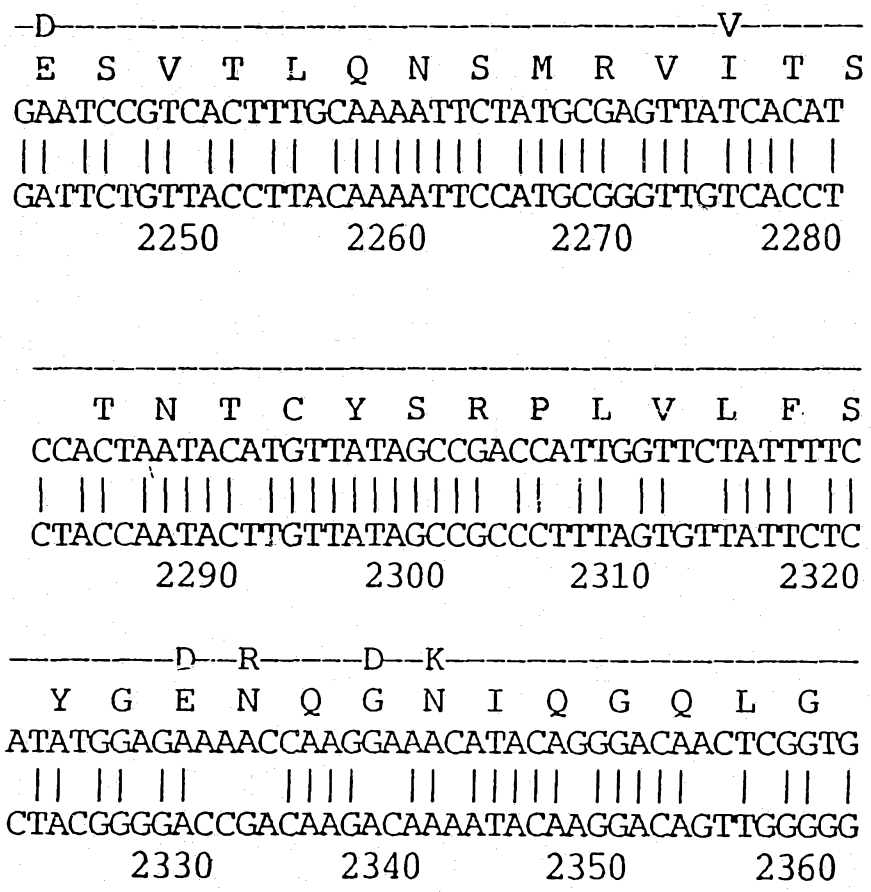

D
V
E S V T L Q N S M R V I T S
GAATCCGTCACTTTGCAAAATTCTATGCGAGTTATCACAT
GATTCTGTTACCTTACAAAATTCCATGCGGGTTGTCACCT
2250 2260 2270 2280
T N T C Y S R P L V L F S
CCACTAATACATGTTATAGCCGACCATTGGTTCTATTTTC
CTACCAATACTTGTTATAGCCGCCCTTTAGTGTTATTCTC
2290 2300 2310 2320
D R D K
Y G E N Q G N I Q G Q L G
ATATGGAGAAAACCAAGGAAACATACAGGGACAACTCGGTG
CTACGGGGACCGACAAGACAAAATACAAGGACAGTTGGGGG
2330 2340 2350 2360

FIG 2M

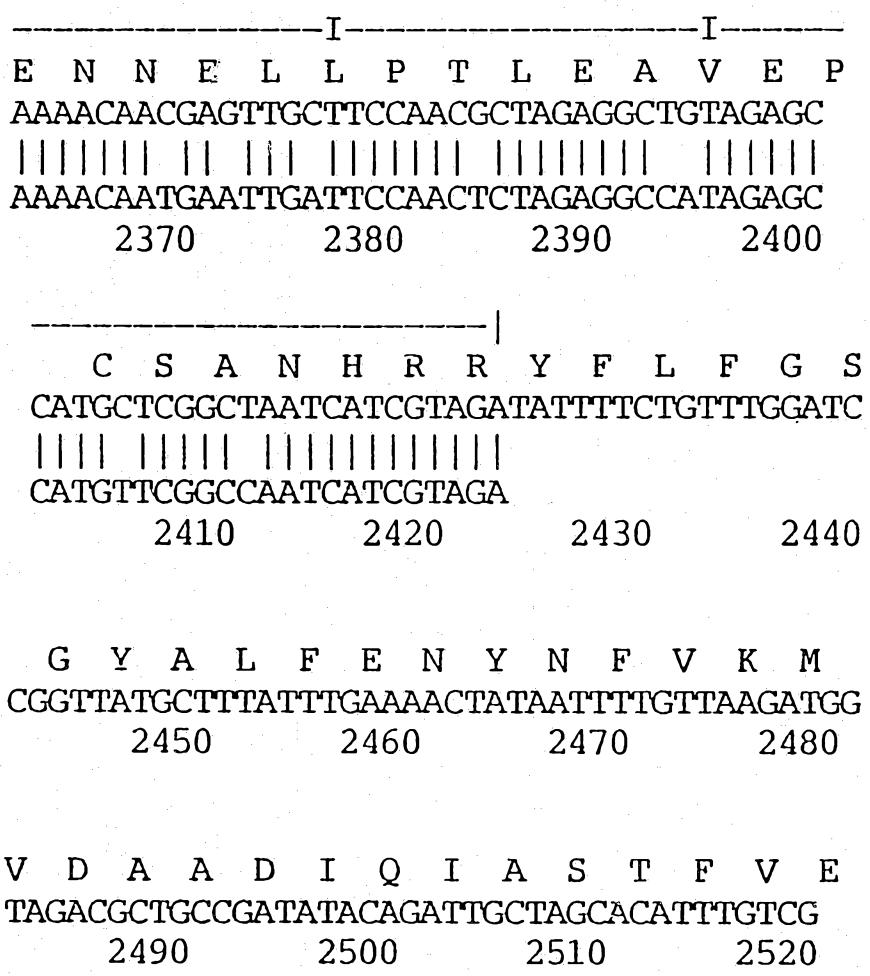

E N N E L L P T L E A V E P
AAAACAACGAGTTGCTTCCAACGCTAGAGGCTGTAGAGC
AAAACAATGAATTGATTCCAACTCTAGAGGCCATAGAGC
2370 2380 2390 2400
C S A N H R R Y F L F G S
CATGCTCGGCTAATCATCGTAGATATTTTCTGTTTGGATC
CATGTTCGGCCAATCATCGTAGA
2410 2420 2430 2440
G Y A L F E N Y N F V K M
CGGTTATGCTTTATTTGAAAACTATAATTTTGTTAAGATGG
2450 2460 2470 2480
V D A A D I Q I A S T F V E
TAGACGCTGCCGATATACAGATTGCTAGCACATTTGTCG
2490 2500 2510 2520

FIG 2N

```
   L  N  L  T  L  L  E  D  R  E  I  L  P
AGCTTAATCTAACCCTGCTAGAAGATCGGGAAATTTTGCC
      2530       2540       2550       2560

  L  S  V  Y  T  K  E  E  L  R  D  V  G
TTTATCCGTTTACACAAAAGAAGAGTTGCGTGATGTTGGT
      2570       2580       2590       2600

 V  L  D  Y  A  E  V  A  R  R  N  Q  L  H
GTATTGGATTATGCAGAAGTAGCTCGCCGCAATCAACTAC
      2610       2620       2630       2640

   E  L  K  F  Y  D  I  N  K  V  I  E  V
ATGAACTTAAATTTTATGACATAAACAAAGTAATAGAAGT
      2650       2660       2670       2680

  D  T  N  Y  A  F  M  N  G  L  A  E  L
GGATACAAATTACGCGTTTATGAACGGTTTGGCCGAATTG
      2690       2700       2710       2720

 F  N  G  M  G  Q  V  G  Q  A  I  G  K  V
TTTAACGGTATGGGTCAGGTAGGGCAAGCTATAGGCAAAG
      2730       2740       2750       2760
```

FIG 2Ø

```
   V  V  G  A  A  G  A  I  V  S  T  I  S
TTGTAGTAGGGGCTGCCGGTGCAATCGTATCTACCATATC
      2770       2780       2790       2800

  G  V  S  A  F  M  S  I  P  L  G  L  S
TGGTGTCTCTGCTTTCATGTCAATCCCTTTGGGGCTTTCG
      2810       2820       2830       2840

 A  I  G  L  I  I  I  A  G  L  V  A  A  F
GCAATCGGTTTAATCATTATAGCAGGACTCGTGGCTGCAT
      2850       2860       2870       2880

   L  A  Y  R  Y  V  N  K  L  K  S  N  P
TTTTAGCATATCGTTATGTAAACAAGCTTAAAAGCAATCC
      2890       2900       2910       2920

  M  K  A  L  Y  P  M  T  T  E  V  L  K
AATGAAAGCCCTTTATCCTATGACAACAGAAGTGCTTAAG
      2930       2940       2950       2960

 A  Q  A  T  R  E  L  H  G  E  E  S  D  D
GCACAGGCAACGCGTGAGTTGCATGGCGAGGAATCAGATG
      2970       2980       2990       3000
```

FIG 2P

```
   L  E  R  T  S  I  D  E  R  K  L  E  E
ATTTGGAACGAACATCTATTGATGAAAGAAAATTAGAAGA
      3010       3020       3030       3040

  A  R  E  M  I  K  Y  M  A  L  V  S  A
AGCTAGAGAAATGATAAAATATATGGCGTTAGTCTCCGCG
      3050       3060       3070       3080

 E  E  R  H  E  K  K  L  R  R  K  R  R  G
GAAGAACGCCACGAGAAAAAACTGCGGAGAAAGAGGCGAG
      3090       3100       3110       3120
   T  T  A  V  L  S  D  H  L  A  K  M  R
 GCACTACCGCCGTTCTATCGGACCACCTGGCAAAAATGAG
      3130       3140       3150       3160

  I  K  N  S  N  P  K  Y  D  K  L  P  T
GATTAAAAATAGTAACCCTAAATATGATAAGTTACCTACT
      3170       3180       3190       3200

 T  Y  S  D  S  E  D  D  A  V  *
ACATATTCAGACTCAGAAGATGATGCTGTGTAAGTGGGCA
      3210       3220       3230       3240

 CTATTATATTTGAACTGAATAAAACGCATAGAGCATGATA
      3250       3260       3270       3280
```

FIG 2Q

TGGTTTACTCATTTATTGCGAGATATAAAGCATATTCAAT
3290 3300 3310 3320

ACGATATATTGCGAACGTGATGCTAAAAACATAGCTCCCT
3330 3340 3350 3360

GTATTATTGATGCGCCATCATTTGATTAATAAATACATCG
3370 3380 3390 3400

ACGCCGGCATCACTGGTGCGGTGTATACCAGCTACGGCGC
3410 3420 3430 3440

TAGCATTCATGGTATCCCGTGATTGCTCGATGCTTTCCTT
3450 3460 3470 3480

CTGAATTCCGTCGGAACGCTCCTGAGAGATGGTCGCAGTT
3490 3500 3510 3520

ATTGGTACATTTCGACCAGCCTCCGGATCTGAAACTGGCA
3530 3540 3550 3560

CAGGAATGCACCGTGGAATTGGTAGAAGTTTTTCCTTCCG
3570 3580 3590 3600

FIG 2R

TGGAAGGCATAGGGCGTTCGACTCCCATGGGCCATGAAACTGTGGGATGT

3610 3620 3630 3640 3650

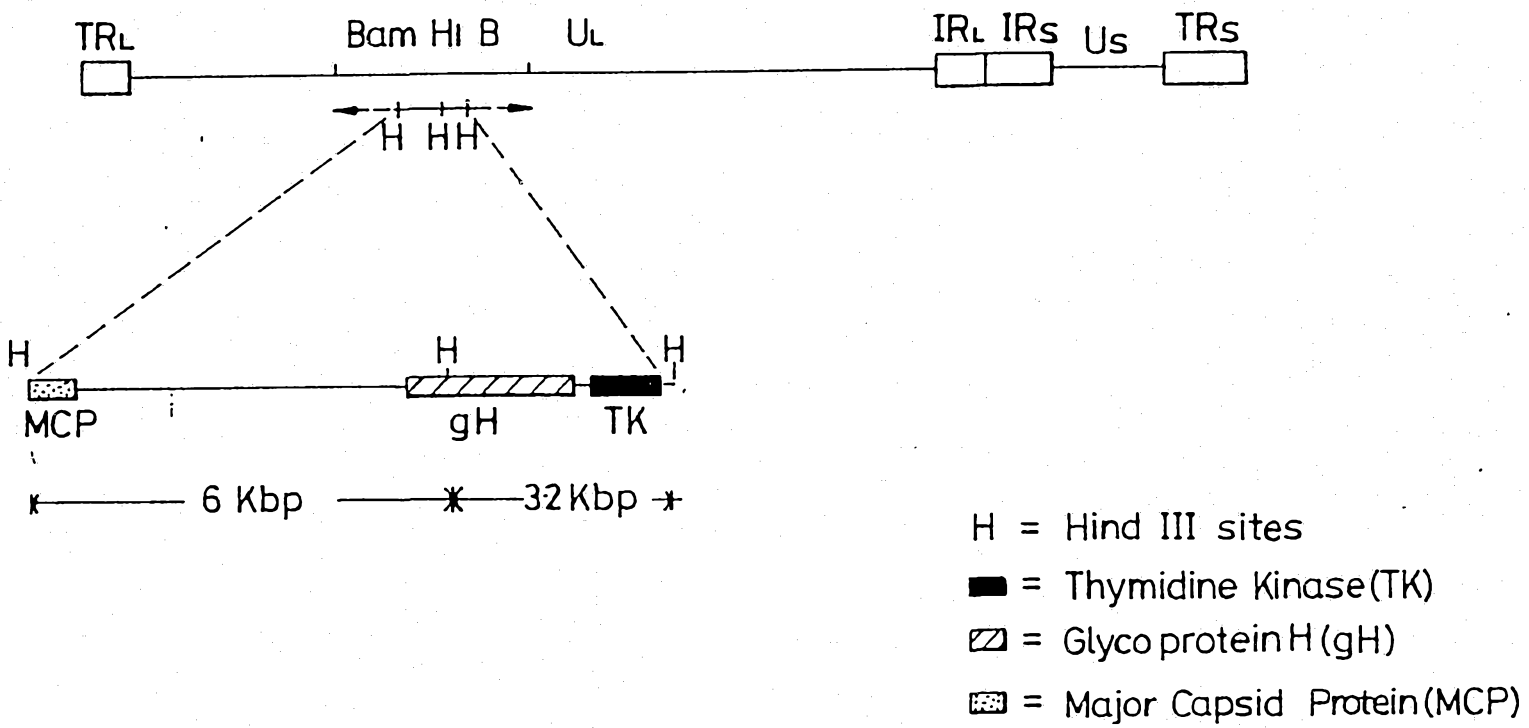

TRL
Bam HI B
UL
IRL IRS
Us
TRs
H HH
H
H
H
MCP
gH
TK
6 Kbp
32 Kbp
H = Hind III sites
= Thymidine Kinase (TK)
= Glyco protein H (gH)
= Major Capsid Protein (MCP)

Fig. 3

FIG 4A

```
 TATTATTGGTCCATGCTAGAATAGTCATACGCTACGATCT
         10         20         30         40

GTTGCTATATATGACTATCGCCAAACTGTTAAACCCGCGA
         50         60         70         80

AGAATATATTTCATATAAACCTAAGGGCCCCTCAGTCTGA
         90        100        110        120

                                M  K  F  Y  C  L
 TTTTTTGTGAAAACGTGTATACCATGAAGTTTTACTGCCT
        130        140        150        160

  I  R  F  M  I  I  A  N  L  Y  S  S  Y
AATCCGTTTCATGATCATAGCGAATCTTTATTCATCTTAC
        170        180        190        200

 Q  I  S  L  P  G  T  Y  P  S  Q  I  L  L
CAAATATCGCTTCCAGGCACATATCCATCGCAAATATTGC
        210        220        230        240

    D  M  K  N  S  P  L  V  R  F  N  I  S
 TTGACATGAAGAACTCGCCGCTCGTACGCTTTAATATATC
        250        260        270        280
```

FIG 4B

```
  T   R   D   Y   K   D   E   T   L   W   I   R   K
GACGCGTGATTATAAAGACGAGACACTCTGGATACGGAAA
       290         300         310         320

  N   S   T   F   V   Y   I   D   T   A   V   T   T   A
AATTCGACATTTGTTTATATCGATACGGCTGTGACGACAG
       330         340         350         360

    N   V   I   F   Y   L   P   I   G   Q   V   R   Q
 CGAACGTTATCTTTTATCTGCCGATCGGTCAGGTACGACA
       370         380         390         400

  M   V   F   F   K   R   P   I   S   R   L   L   T
AATGGTTTTTTTCAAGCGTCCAATATCCAGGCTACTAACG
       410         420         430         440

  S   N   N   L   V   K   F   I   N   T   G   S   Y   A
TCCAATAACCTGGTTAAATTTATTAATACCGGTTCATACG
       450         460         470         480

    N   H   T   F   K   T   E   L   S   P   Y   L   S
 CCAATCATACATTCAAGACAGAACTTTCACCCTATTTGTC
       490         500         510         520
```

FIG 4C

```
  K   T   N   T   P   L   K   K   Y   E   I   V   V.
GAAAACCAATACACCGTTGAAGAAATATGAAATTGTTGTC
      530       540       550       560

  D   Q   P   T   G   E   N   P   P   A   G   F   G   S
GATCAACCTACTGGAGAAAACCCTCCGGCAGGGTTCGGAA
      570       580       590       600

    L   K   P   A   D   F   L   N   P   G   Y   K   F
 GTTTAAAACCGGCAGACTTTCTCAACCCCGGATACAAGTT
      610       620       630       640

  V   L   T   S   E   L   V   G   A   Y   T   K   R
CGTTCTCACAAGCGAGTTGGTAGGAGCCTACACAAAACGA
      650       660       670       680

  S   C   F   V   D   P   M   D   S   L   V   P   I   D
TCTTGTTTTGTCGATCCGATGGATTCTCTCGTCCCGATAG
      690       700       710       720

    Y   D   H   V   R   T   I   I   F   G   S   A   G
 ATTATGATCATGTACGAACCATTATATTCGGATCTGCTGG
      730       740       750       760
```

FIG 4D

```
  M  E  I  L  M  K  M  G  I  T  L  A  S
GATGGAGATTTTAATGAAGATGGGAATTACTTTGGCATCT
       770        780        790        800

  M  T  I  S  T  K  Y  N  P  P  I  E  L  I
ATGACCATTTCGACGAAATATAATCCTCCTATTGAACTGA
       810        820        830        840

     I  S  A  K  Y  R  N  L  S  L  L  W  P
 TAATATCTGCAAAGTACCGAAATTTATCACTGTTGTGGCC
       850        860        870        880

  P  R  Q  Q  Y  E  P  V  N  K  G  T  G
ACCCCGACAACAATATGAACCTGTAAATAAAGGGACTGGA
       890        900        910        920

 R  P  H  W  I  Y  L  L  G  V  Y  R  N  V
CGCCCCCATTGGATCTACCTATTAGGTGTGTATAGAAACG
       930        940        950        960

    S  D  S  E  R  D  S  Y  M  N  M  I  K
 TTTCGGACTCCGAGCGTGACTCATACATGAATATGATTAA
       970        980        990       1000
```

FIG 4E

```
  S  L  G  D  S  M  D  Y  H  F  L  I  S
GAGTCTGGGCGATTCTATGGATTATCACTTCCTAATTAGC
      1010       1020       1030       1040

  R  A  H  A  Q  M  L  I  L  A  A  E  D  R
AGAGCGCATGCCCAGATGCTGATACTGGCAGCAGAGGACC
      1050       1060       1070       1080

     L  V  D  E  M  H  S  F  R  N  V  I  A
 GGCTCGTGGATGAAATGCATAGTTTCAGGAACGTTATTGC
      1090       1100       1110       1120

  R  L  F  V  S  L  F  A  F  I  R  N  A
GCGTTTATTTGTATCGTTGTTCGCATTCATACGTAACGCA
      1130       1140       1150       1160

  F  Q  S  G  Y  T  S  L  N  D  I  I  E  I
TTTCAGTCTGGCTACACCTCTCTTAATGACATAATTGAAA
      1170       1180       1190       1200

     E  A  D  L  R  L  I  V  E  G  I  S  S
 TCGAAGCCGATTTGAGGTTAATTGTAGAAGGCATTTCTTC
      1210       1220       1230       1240
```

FIG 4F

```
  A  A  F  R  K  D  A  S  T  H  F  L  I.
TGCTGCATTTCGTAAAGACGCTAGTACACACTTTCTTATA
      1250       1260       1270       1280

 S  G  T  P  I  K  D  S  K  A  D  L  I  K
TCGGGAACGCCCATAAAAGATAGCAAAGCGGATTTAATTA
      1290       1300       1310       1320

    S  L  L  S  K  V  I  R  P  I  S  G  H
 AATCGTTGTTGTCTAAAGTCATTCGACCAATTTCCGGACA
      1330       1340       1350       1360

  T  R  P  L  S  A  I  Q  H  L  F  L  L
TACACGTCCCTTATCTGCGATACAACATCTATTCCTTTTG
      1370       1380       1390       1400

 R  S  A  Y  A  L  D  I  P  R  Q  N  G  S
AGATCCGCTTATGCATTGGATATACCCCGTCAAAACGGAT
      1410       1420       1430       1440

    L  S  E  Q  V  S  T  V  A  L  S  F  I
 CTTTGAGCGAACAGGTATCTACAGTGGCACTGTCGTTCAT
      1450       1460       1470       1480
```

FIG 4G

```
  E  N  I  H  S  E  A  M  R  D  I  L  S.
TGAAAATATTCACAGCGAGGCCATGAGGGACATTCTGTCA
      1490       1500       1510       1520

 W  N  T  T  T  K  H  A  L  Y  Y  A  F  A
TGGAACACTACAACAAAGCATGCGTTGTATTATGCATTCG
      1530       1540       1550       1560

    S  I  L  Q  R  P  L  T  E  W  G  A  S
 CGAGTATTTTGCAACGGCCACTGACCGAATGGGGCGCCTC
      1570       1580       1590       1600

  R  N  A  R  R  A  I  L  L  A  S  S  M
AAGAAATGCACGGAGGGCAATACTATTAGCATCATCGATG
      1610       1620       1630       1640

 C  T  E  E  H  V  I  A  T  E  L  A  I  Q
TGTACAGAAGAGCATGTTATCGCAACTGAGTTGGCTATTC
      1650       1660       1670       1680

    E  L  Y  V  K  I  R  S  N  A  D  P  I
 AAGAACTGTATGTCAAAATCAGAAGTAATGCCGACCCAAT
      1690       1700       1710       1720
```

FIG 4H

```
  H   L   L   D   V   Y   T   P   C   L   S   S   L
ACACCTTCTAGACGTATATACACCATGTCTTTCTTCACTA
      1730        1740        1750        1760

  R   L   D   L   S   E   H   H   R   I   Y   A   M   A
CGATTGGACCTTTCCGAACACCATCGGATATACGCAATGG
      1770        1780        1790        1800

    D   V   V   F   Y   P   D   I   Q   Q   Y   L   K
 CAGATGTAGTTTTCTATCCAGACATTCAGCAGTATTTGAA
      1810        1820        1830        1840

  K   K   S   H   E   G   N   M   K   E   D   D   L
AAAAAAATCCCATGAGGGTAATATGAAGGAAGATGATCTC
      1850        1860        1870        1880

  E   T   K   A   E   Y   I   L   T   K   L
GAAACAAAGGCGGAATACATCCTCACCAAGCTT
      1890        1900        1910
```

FIG 5A

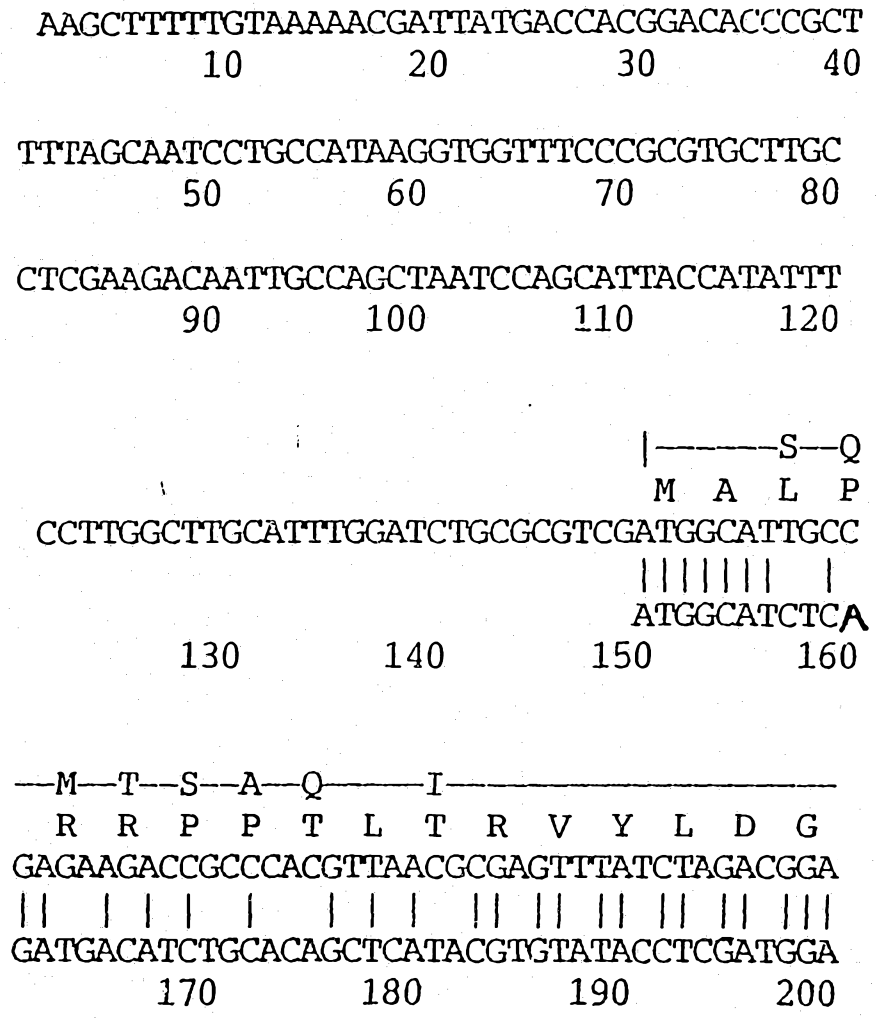

AAGCTTTTTGTAAAAACGATTATGACCACGGACACCCGCT
10 20 30 40
TTTAGCAATCCTGCCATAAGGTGGTTTCCCGCGTGCTTGC
50 60 70 80
CTCGAAGACAATTGCCAGCTAATCCAGCATTACCATATTT
90 100 110 120
|------S--Q
M A L P
CCTTGGCTTGCATTTGGATCTGCGCGTCGATGGCATTGCC
ATGGCATCTCA
130 140 150 160
--M--T--S--A--Q------I----------
R R P P T L T R V Y L D G
GAGAAGACCGCCCACGTTAACGCGAGTTTATCTAGACGGA
GATGACATCTGCACAGCTCATACGTGTATACCTCGATGGA
170 180 190 200

FIG 5B

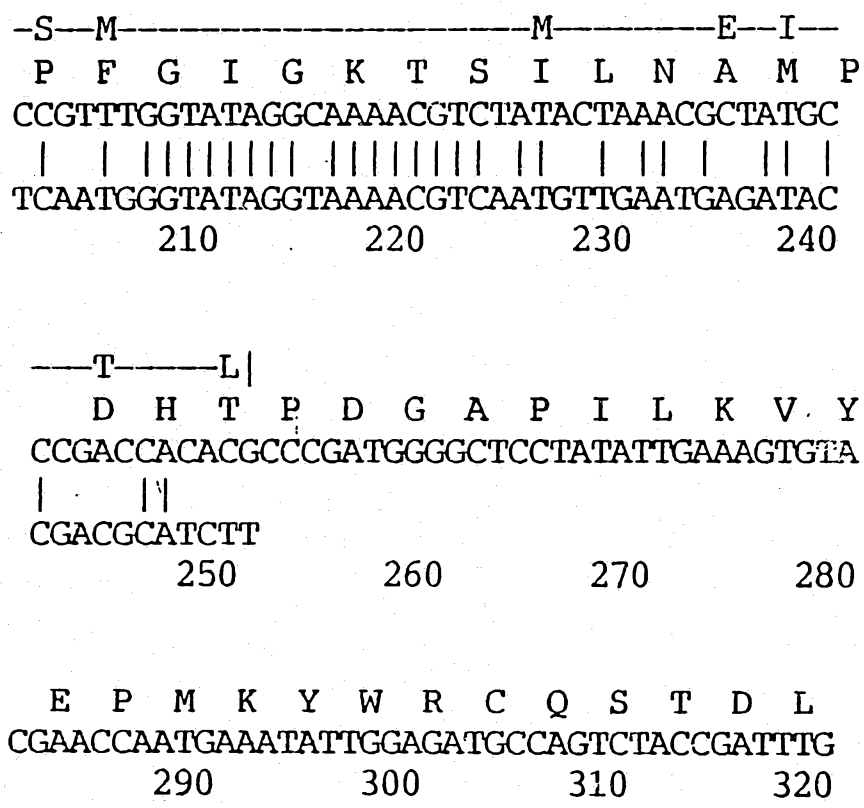

-S--M----------M--------E--I--
P F G I G K T S I L N A M P
CCGTTTGGTATAGGCAAAACGTCTATACTAAACGCTATGC
TCAATGGGTATAGGTAAAACGTCAATGTTGAATGAGATAC
210 220 230 240
---T-----L|
D H T P D G A P I L K V Y
CCGACCACACGCCCGATGGGGCTCCTATATTGAAAGTGTA
CGACGCATCTT
250 260 270 280
E P M K Y W R C Q S T D L
CGAACCAATGAAATATTGGAGATGCCAGTCTACCGATTTG
290 300 310 320

FIG 5C

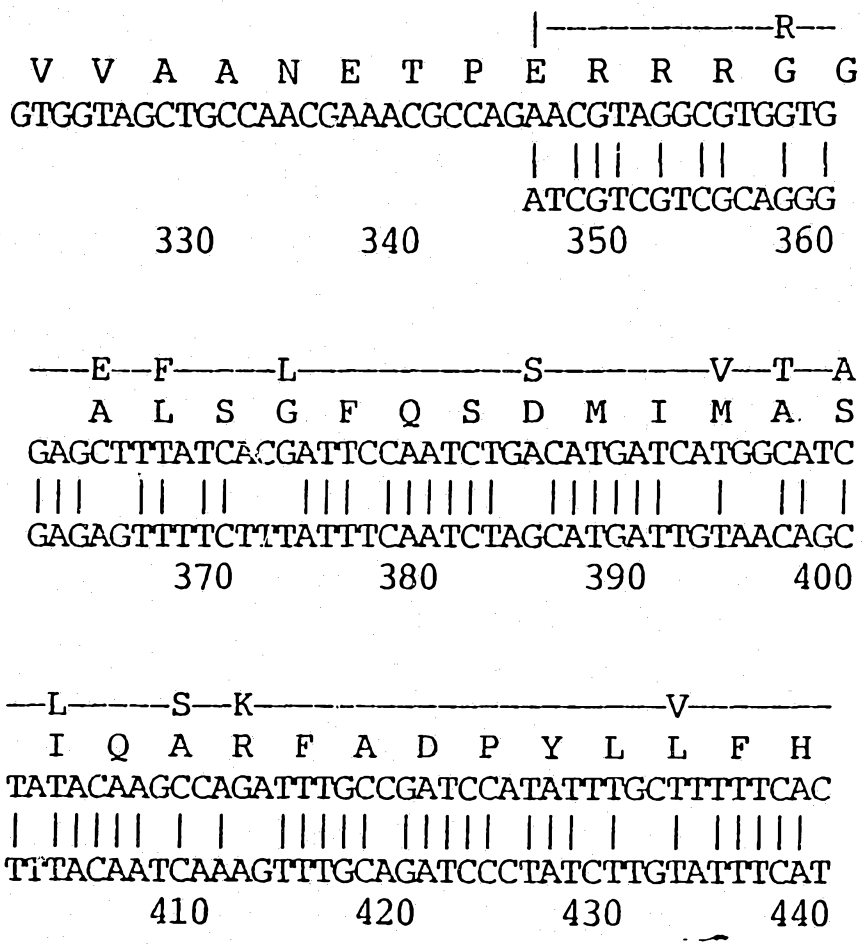

R
V V A A N E T P E R R R G G
GTGGTAGCTGCCAACGAAACGCCAGAACGTAGGCGTGGTG
ATCGTCGTCGCAGGG
330 340 350 360
E F L S V T A
A L S G F Q S D M I M A S
GAGCTTTATCACGATTCCAATCTGACATGATCATGGCATC
GAGAGTTTTCTTTATTTCAATCTAGCATGATTGTAACAGC
370 380 390 400
L S K V
I Q A R F A D P Y L L F H
TATACAAGCCAGATTTGCCGATCCATATTTGCTTTTTCAC
TTTACAATCAAAGTTTGCAGATCCCTATCTTGTATTTCAT
410 420 430 440

FIG 50

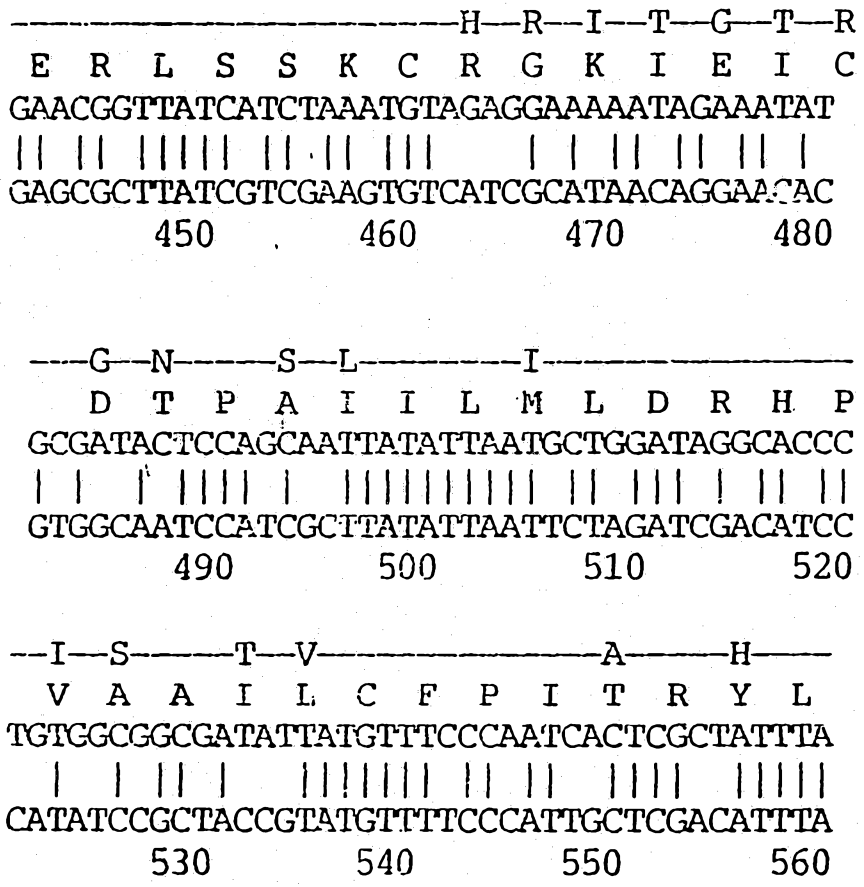

H R I T G T R
E R L S S K C R G K I E I C
GAACGGTTATCATCTAAATGTAGAGGAAAAATAGAAATAT
GAGCGCTTATCGTCGAAGTGTCATCGCATAACAGGAACAC
450 460 470 480
G N S L I
D T P A I I L M L D R H P
GCGATACTCCAGCAATTATATTAATGCTGGATAGGCACCC
GTGGCAATCCATCGCTTATATTAATTCTAGATCGACATCC
490 500 510 520
I S T V A H
V A A I L C F P I T R Y L
TGTGGCGGCGATATTATGTTTCCCAATCACTCGCTATTTA
CATATCCGCTACCGTATGTTTTCCCATTGCTCGACATTTA
530 540 550 560

FIG 5E

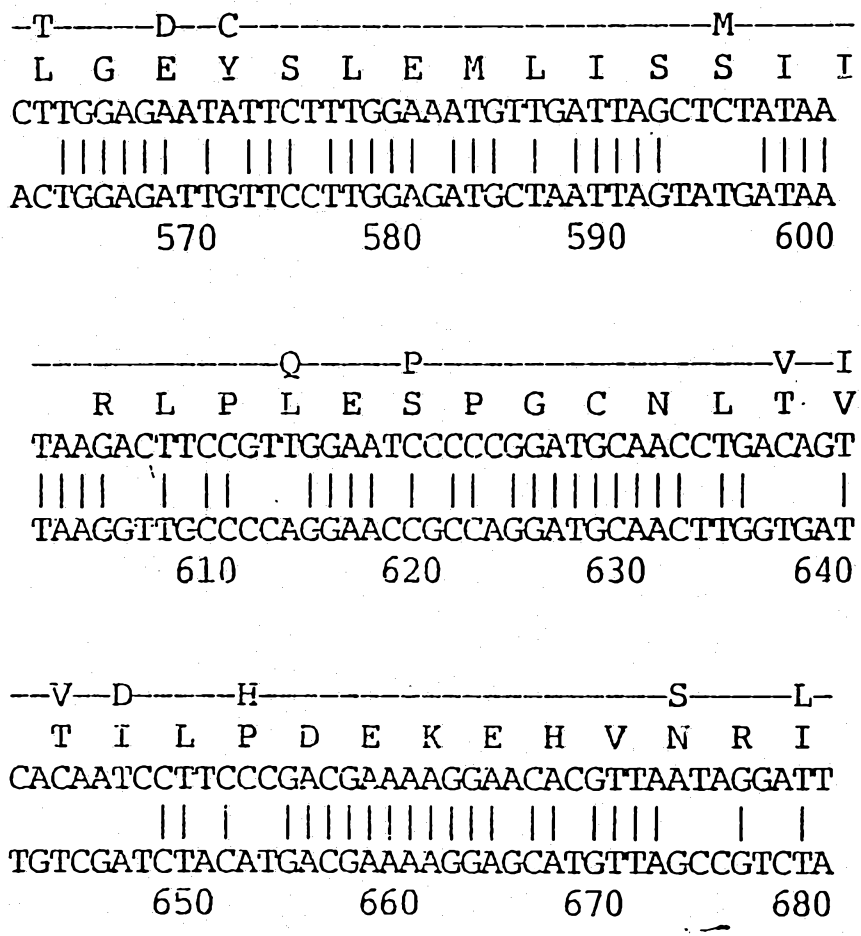

-T------D--C----------M------
L G E Y S L E M L I S S I I
CTTGGAGAATATTCTTTGGAAATGTTGATTAGCTCTATAA
ACTGGAGATTGTTCCTTGGAGATGCTAATTAGTATGATAA
570 580 590 600
----------Q------P----------V--I
R L P L E S P G C N L T V
TAAGACTTCCGTTGGAATCCCCCGGATGCAACCTGACAGT
TAAGGTTGCCCCAGGAACCGCCAGGATGCAACTTGGTGAT
610 620 630 640
--V--D------H----------S------L-
T I L P D E K E H V N R I
CACAATCCTTCCCGACGAAAAGGAACACGTTAATAGGATT
TGTCGATCTACATGACGAAAAGGAGCATGTTAGCCGTCTA
650 660 670 680

FIG 5F

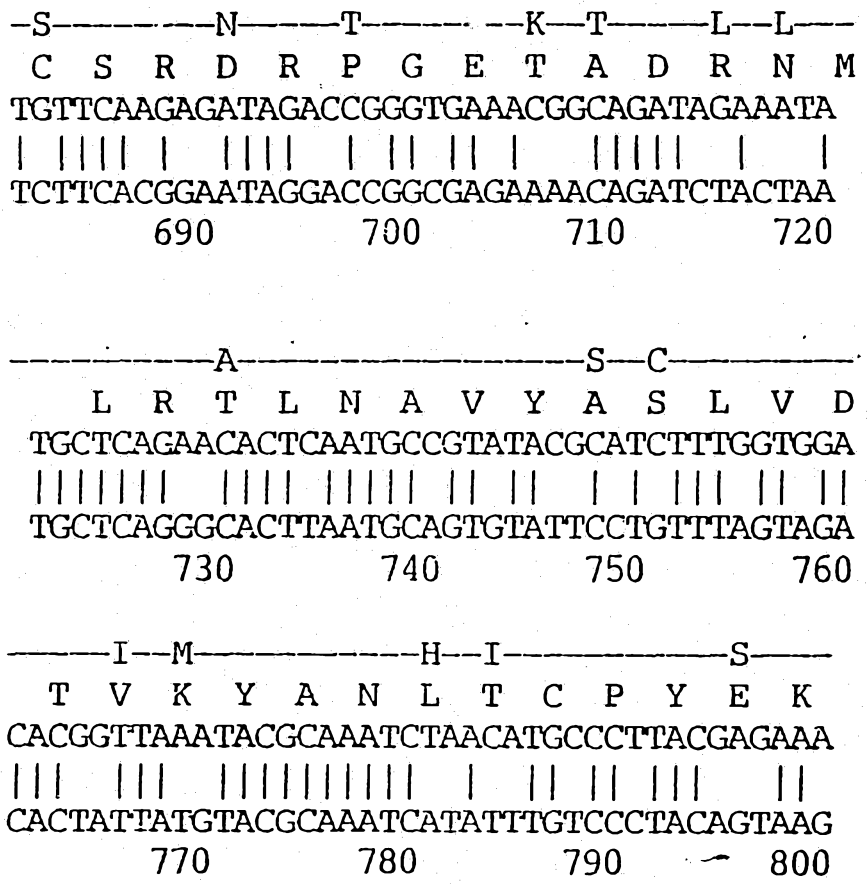

S N T K T L L
C S R D R P G E T A D R N M
TGTTCAAGAGATAGACCGGGTGAAACGGCAGATAGAAATA
TCTTCACGGAATAGGACCGGCGAGAAAACAGATCTACTAA
690 700 710 720
A S C
L R T L N A V Y A S L V D
TGCTCAGAACACTCAATGCCGTATACGCATCTTTGGTGGA
TGCTCAGGGCACTTAATGCAGTGTATTCCTGTTTAGTAGA
730 740 750 760
I M H I S
T V K Y A N L T C P Y E K
CACGGTTAAATACGCAAATCTAACATGCCCTTACGAGAAA
CACTATTATGTACGCAAATCATATTTGTCCCTACAGTAAG
770 780 790 800

FIG 5G

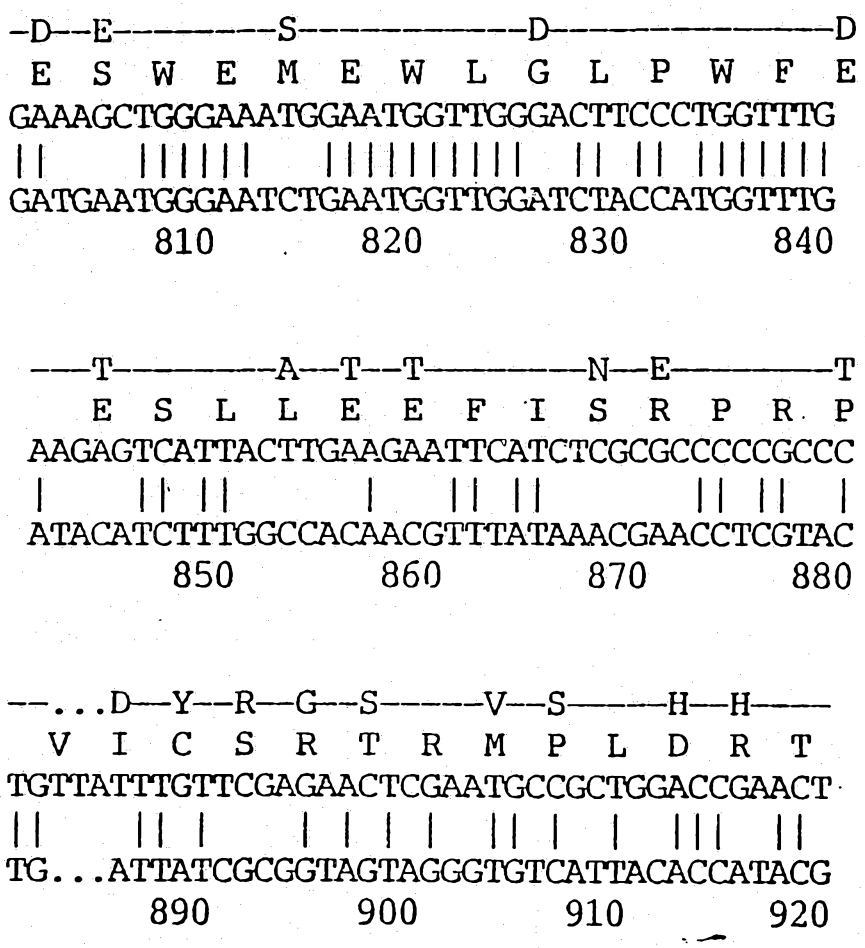

-D--E--------S----------D----------D
E S W E M E W L G L P W F E
GAAAGCTGGGAAATGGAATGGTTGGGACTTCCCTGGTTTG
GATGAATGGGAATCTGAATGGTTGGATCTACCATGGTTTG
810 820 830 840
---T--------A--T--T--------N--E--------T
E S L L E E F I S R P R P
AAGAGTCATTACTTGAAGAATTCATCTCGCGCCCCCGCCC
ATACATCTTTGGCCACAACGTTTATAAACGAACCTCGTAC
850 860 870 880
--...D--Y--R--G--S-----V--S-----H--H----
V I C S R T R M P L D R T
TGTTATTTGTTCGAGAACTCGAATGCCGCTGGACCGAACT
TG...ATTATCGCGGTAGTAGGGTGTCATTACACCATACG
890 900 910 920

FIG 5H

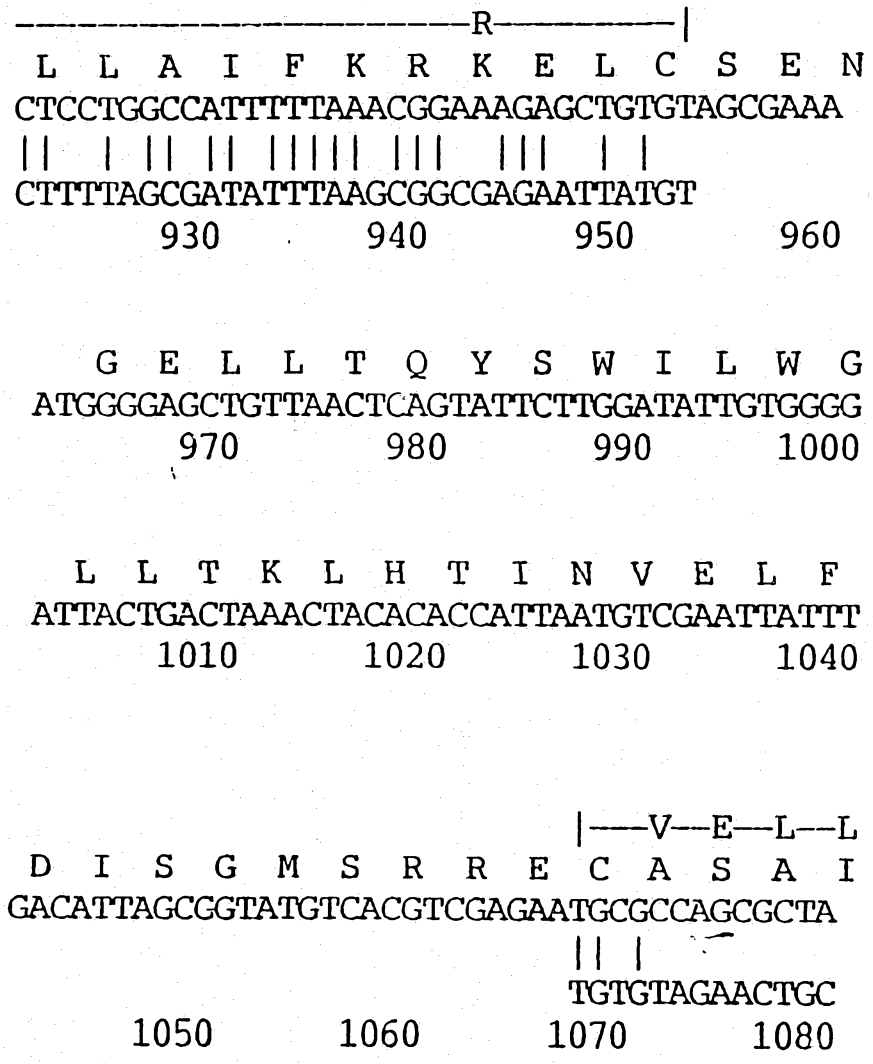

R
L L A I F K R K E L C S E N
CTCCTGGCCATTTTTAAACGGAAAGAGCTGTGTAGCGAAA
CTTTTAGCGATATTTAAGCGGCGAGAATTATGT
930 940 950 960
G E L L T Q Y S W I L W G
ATGGGGAGCTGTTAACTCAGTATTCTTGGATATTGTGGGG
970 980 990 1000
L L T K L H T I N V E L F
ATTACTGACTAAACTACACACCATTAATGTCGAATTATTT
1010 1020 1030 1040
V E L L
D I S G M S R R E C A S A I
GACATTAGCGGTATGTCACGTCGAGAATGCGCCAGCGCTA
TGTGTAGAACTGC
1050 1060 1070 1080

FIG 5I

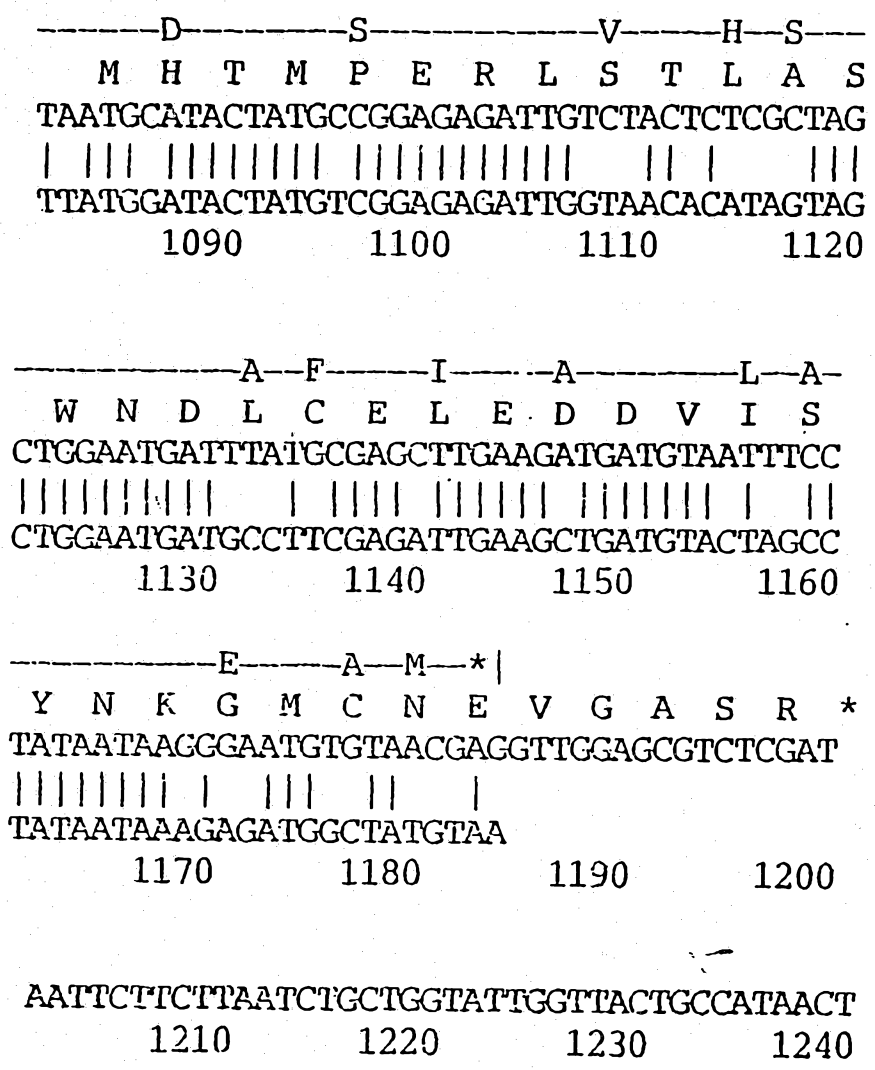

D S V H S
M H T M P E R L S T L A S
TAATGCATACTATGCCGGAGAGATTGTCTACTCTCGCTAG
TTATGGATACTATGTCGGAGAGATTGGTAACACATAGTAG
1090 1100 1110 1120
A F I A L A
W N D L C E L E D D V I S
CTGGAATGATTTATGCGAGCTTGAAGATGATGTAATTTCC
CTGGAATGATGCCTTCGAGATTGAAGCTGATGTACTAGCC
1130 1140 1150 1160
E A M *
Y N K G M C N E V G A S R *
TATAATAAGGGAATGTGTAACGAGGTTGGAGCGTCTCGAT
TATAATAAAGAGATGGCTATGTAA
1170 1180 1190 1200
AATTCTTCTTAATCTGCTGGTATTGGTTACTGCCATAACT
1210 1220 1230 1240

FIG 5J

TATTATTGGTCCATGCTAGAATAGTCATACGCTACGATCT
1250 1260 1270 1280

GTTGCTATATATGACTATCGCCAAACTGTTAAACCCGCGA
1290 1300 1310 1320

AGAATATATTTCATATAAACCTAAGGGCCCCTCAGTCTGA
1330 1340 1350 1360

TTTTTTGTGAAAACGTGTATACCA
1370 1380

FIG 6A

```
      1 CAGCTGCCTATGTAGTGAAATCTATACTGGGATTT

ATCATAACTAGTTTACTTGTTTGTATATTAGTAGCGCTATCT

TGACCAAATCGTTGTTCACATCTTGGCCATATACGTATTGATC

    121 GTTGTTTCGAACCGCGAATAAAACTTTCATACATAC

TAAACGATGGAGTTGTGTTTTATGAGCGTTGAAAACAAAGGT

ACCATCGGTTTAAAACTAAGTTGCATATCGTAATCCACAAAA

    241 ATCATTTTATACATCATCCCGAAGAGACACCAAACG

                            M  L  T  P  R  V
TAACCCTCTACATATCTTCCCTCATGCTCACGCCGCGTGTGT

L  R  A  L  G  W  T  G  L  F  F  L  L  L  S
TACGAGCTTTGGGGTGGACTGGACTCTTTTTTTTGCTTTTAT

            P  S  N  V  L  G  A  S  L  S  R
    361 CTCCGAGCAACGTCCTAGGAGCCAGCCTTAGCCGG

 D  L  E  T  P  P  F  L  S  F  D  P  S
GATCTCGAAACACCCCCATTTCTATCCTTTGATCCATCCA
```

FIG 6B

```
N  I  S  I  N  G  A  P  L  T  E  V  P  H  A  P
ACATTTCAATTAACGGCGCGCCTTTAACTGAGGTACCTCATGCAC

         S  T  E  S  V  S  T  N  S  E  S  T
  481 CTTCCACAGAAAGTGTGTCAACAAATTCGGAAAGTACC

 N  E  H  T  I  T  E  T  T  G  K  N  A  Y
AATGAACATACCATAACAGAAACGACGGGCAAGAACGCATACA

I  H  N  N  A  S  T  D  K  Q  N  A  N  D
TCCACAACAATGCGTCTACGGACAAGCAAAATGCGAACG

         T  H  K  T  P  N  I  L  C  D  T  E
  601 ACACTCATAAAACGCCCAATATACTCTGCGATACGGA

  E  V  F  V  F  L  N  E  T  G  R  F  V  C
AGAAGTTTTTGTTTTCCTTAACGAAACGGGAAGATTTGTTTGT

 T  L  K  V  D  P  P  S  D  S  E  W  S  N
ACTCTCAAAGTCGACCCCCCCTCGGATAGTGAATGGTCCA

         F  V  L  D  L  I  F  N  P  I  E  Y
  721 ACTTTGTTCTAGATCTGATCTTTAACCCAATTGAATA

  H  A  N  E  K  N  V  E  A  A  R  I  A  G
CCACGCCAACGAAAAGAATGTGGAAGCGGCGCGTATCGCTGGT
```

FIG 6C

```
 L  Y  G  V  P  G  S  D  Y  A  Y  P  R  Q
CTCTATGGAGTCCCCGGATCAGACTATGCATACCCACGTC

        S  E  L  I  S  S  I  R  R  D  P
  841 AATCTGAATTAATTTCTTCGATTCGACGAGATCCCC

Q  G  T  F  W  T  S  P  S  P  H  G  N  K
AGGGCACATTTTGGACGAGCCCATCACCTCATGGAAACAA

  Y  F  I  W  I  N  K  T  T  N  T  M  G  V  E
GTACTTCATATGGATAAACAAAACAACCAATACGATGGGCGTGG

        I  R  N  V  D  Y  A  D  N  G  Y
  961 AAATTAGAAATGTAGATTATGCTGATAATGGCTAC

 M  Q  V  I  M  R  D  H  F  N  R  P  L
ATGCAAGTCATTATGCGTGACCATTTTAATCGGCCTTTAA
I  D  K  H  I  Y  I  R  V  C  Q  R  P  A  S  V

TAGATAAACATATTTACATACGTGTGTGTCAACGACCTGCATCAG

        D  V  L  A  P  P  V  L  S  G  E  N
 1081 TGGATGTACTGGCCCCTCCAGTCCTCAGCGGAGAAAA

  Y  K  A  S  C  I  V  R  H  F  Y  P  P  G
TTACAAGGCATCTTGTATCGTTAGACACTTTTATCCCCCTGGA
```

FIG 6D

```
 S  V  Y  V  S  W  R  Q  N  G  N  I  A  T
TCTGTCTATGTATCTTGGAGACAGAATGGAAACATTGCAA

          P  R  K  D  R  D  G  S  F  W  W  F
1201 CTCCTCGGAAAGATCGCGATGGAAGTTTTTGGTGGTT

 E  S  G  R  G  A  T  L  V  S  T  I  T  L
CGAATCTGGTAGAGGAGCTACGTTGGTTTCTACAATAACATTG

 G  N  S  G  I  D  F  P  P  K  I  S  C  L
GGAAATTCAGGAATTGATTTCCCCCCCAAAATATCTTGTC

          V  A  W  K  Q  G  D  M  I  S  T  T
1321 TGGTTGCCTGGAAGCAGGGTGATATGATCAGCACGAC

 N  A  T  A  I  P  T  V  Y  H  H  P  R  L
GAATGCCACAGCTATCCCGACGGTATATCATCATCCCCGTTTA

 S  L  A  F  K  D  G  Y  A  I  C  T  I  E
TCCCTGGCTTTTAAAGATGGGTATGCAATATGTACTATAG

          C  V  P  S  E  I  T  V  R  W  L  V
1441 AATGTGTCCCCTCTGAGATTACTGTACGGTGGTTAGT

 H  D  E  A  Q  P  N  T  T  Y  N  T  V  V
ACATGATGAAGCGCAGCCTAACACAACTTATAATACTGTGGTT
```

FIG 6E

```
 T  G  L  C  R  T  I  D  R  H  R  N  L  L
ACAGGTCTCTGCCGGACCATCGATCGCCATAGAAATCTCC

          S  R  I  P  V  W  D  N  W  T  K  T
1561 TCAGCCGCATTCCAGTATGGGACAATTGGACGAAAAC

  K  Y  T  C  R  L  I  G  Y  P  F  D  E  D
AAAATATACGTGCAGACTCATAGGCTACCCCTTCGATGAAGAT

  K  F  Q  D  S  E  Y  Y  D  A  T  P  S  A
AAATTTCAAGATTCGGAATATTACGATGCAACTCCATCTG

          R  G  T  P  M  V  I  T  V  T  A  V
1681 CAAGAGGAACACCCATGGTTATTACGGTTACGGCAGT

  L  G  L  A  V  I  L  G  M  G  I  I  M  T
TTTGGGATTGGCTGTAATTTTAGGGATGGGGATAATCATGACT

  A  L  C  L  Y  N  S  T  R  K  N  I  R  L
GCCCTATGTTTATACAACTCCACACGAAAAAATATTCGAT

           *
1801 TATAATCTCATTGTTATGTAGTTGTGATTTATTAAAC

ATATTTTTTATAACTCTAGTATTCTCCGAGTACTTATATATT
```

FIG 6F

TATTTGTCAGACAATAATGCAATAGTGGAGAAACGTGAGG

1921 GGAGTCTGTAAACAGAATACGTATAATCATCTATTTG

AATAAAAGATTGTGGTATAAATGAAGATAGCGCAAGTCATTC

CAAGCTCTCCATTCTATTTAAACAATGTACAGTTTAAAGT

FIG 7A

```
  1 GAATTCTTGTAAAATATCATTAATTCCGCTACCAACGGGTTCCTTTTTTTCACATAGCTG
     F  E  Q  L  I  D  N  I  G  S  G  V  P  E  K  K  E  C  L  Q

 61 CTGCTCCGTAGTTATCTTTTTTGCTGGAACCATTTCCCCAGGGAAGTACGGGTCATTCTC
     Q  E  T  T  I  K  K  A  P  V  M  E  G  P  F  Y  P  D  N  E

121 AGAAATGGTCCTAGCGGATCTCTTCCGAGGGTTATTCTCCGAGTATTCGCTATCCCGTTT
     S  I  T  R  A  S  R  K  R  P  N  N  E  S  Y  E  S  D  R  K

181 ATTATTTTTCTCAAGAGCCGAAATCAAGGCATCTAAGCGTGAAACAACAGTCTGCTCATG
     N  N  K  E  L  A  S  I  L  A  D  L  R  S  V  V  T  Q  E  H

241 TAATGATGGATACTGTATAGGATACGTTCCAGCCCCAGGAAACTGGCCGAAGTTCGAACT
     L  S  P  Y  Q  I  P  Y  T  G  A  G  P  F  Q  G  F  N  S  S

301 ATAATAATTTGGATGCACGGGGTAAGTAGATGCGGGGCTTTGATAAGGAATATATGGGAA
     Y  Y  N  P  H  V  P  Y  T  S  A  P  S  Q  Y  P  I  Y  P  F

361 ACGCCCGCAGAAATTTGGGTCTTCGTAAAGAGCCGGTCTGTAAGTGTGGTTCATCCCCGG
     R  G  C  F  N  P  D  E  Y  L  A  P  R  Y  T  H  N  M  G  P
```

FIG 7B

```
421 ACCCATAAAAGATTCTAGGCGAGGGTGTCGTTCGAATCCACGCGGCCAAATATCACGTCC
      G   M   F   S   E   L   R   P   H   R   E   F   G   R   P   W   I   D   R   G

481 TGGTGAACATTCGTGAGTAGAGTCCCGCGACCTACGGGAAAACTCTCGTGATGGCGACAC
      P   S   C   E   H   T   S   D   R   S   R   R   S   F   E   R   S   P   S   V

541 GGGTCTCCGGTCGTTATCTCTGCGGGCTGATGCCGCAAGAAGACTCGCATACTTCTCGAA
      P   R   R   D   N   D   R   R   A   S   A   A   L   L   S   A   Y   K   E   F

601 TGGGACATAGACCATATCTTCACGAGATGCGGACGGTGAAGCTCCGTTTTTTGTTTGCAA
      P   V   Y   V   M   D   E   R   S   A   S   P   S   A   G   N   K   T   Q   L

661 GAACTGGCCGGAGCATGTCGCCGACTCCATGGCTGGAACGTGTGTTTGCCCGGACGGTTG
      F   Q   G   S   C   T   A   S   E   M   A   P   V   H   T   Q   G   S   P   Q

721 CTGACTCTCGCTTATATCGAAGCTTGGGGCCGTTTGCGTAGAAACTGAACGGTCTTTAAT
      Q   S   E   S   I   D   F   S   P   A   T   Q   T   S   V   S   R   D   K   I

781 AGCATTCTCAGGAGTCTCTTCGGAGTGTTCCTTAATCCCTATGGTTATTACCTCATTCGC
      A   N   E   P   T   E   E   S   H   E   K   I   G   I   T   I   V   E   N   A

841 TTGTAAATATTTCGGGTTGTAAACAGCTGCCATCTCGCGTCGCGTTTTCAAACAGGTACC
      Q   L   Y   K   P   N   Y   V   A   A   M   E   R   R   T   K   L   C   T   G

901 CCTATCCATCAAAAACGCCCCGTGAATAAATTTCTTCATCAGGACTCGCGTAATTTCTGG
      R   D   M   L   F   A   G   H   I   F   K   K   M   L   V   R   T   I   E   P
```

FIG 7C

```
961  TCGCGACAACTCTCTAGGGACTTCTGCACATTTCTCACGTGTAGCTATTTCATACAGCTG
       R  S  L  E  R  P  V  E  A  C  K  E  R  T  A  I  E  Y  L  Q

1021 TTCTTTAATTGGTGCGCTCAAATCATCAAATGCTCCAATAGCTTCCGAAGCGGTGGCCCC
       E  K  I  P  A  S  L  D  D  F  A  G  I  A  E  S  A  T  A  G

1081 GTAAATTGCTACCGTGCCTTCACGTCTACCCAACTCACAGAGCGCCACGTGTGCAAAAAA
       Y  I  A  V  T  G  E  R  R  G  L  E  C  L  A  V  H  A  F  F

1141 ATCTTTTCCGGGCACTTCGTTCTTTTCAAGCCGCCTTGAAGAGAGCGATAGAGAAGGTAG
       D  K  G  P  V  E  N  K  E  L  R  R  S  S  L  S  L  S  P  L

1201 AATGTTGCTGAGAAGATAGAGAAATTTCTCTGTCTCCGTCAAAACCATCCCCTCTTCGGC
       I  N  S  L  L  Y  L  F  K  E  T  E  T  L  V  M  G  E  E  A

1261 ATTCGCAAAAAGAGCATCATCTTGCACGTAGCTTAAAAAAATAGGTGCAAGAGCAGTTGA
       N  A  F  L  A  D  D  Q  V  Y  S  L  F  I  P  A  L  A  T  S

1321 CACGACACCCAAACAAAACAGTCCTCTCGGCAGGTCTAAGATCGTCAGCACTGTACCTAC
       V  V  G  L  C  F  L  G  R  P  L  D  L  I  T  L  V  T  G  V

1381 CACGCAGGATGACTCGTGGTCAATGTTTATCGGAATTGTTCCAGGCAAAACTGGTAAAGC
       V  C  S  S  E  H  D  T  N  I  P  I  T  G  P  L  V  P  L  A

1441 CGATTTGCTTTGCTCGCGAGTAAGCTCATATTCTCGCCCGGCACTTTCTTGGTGGTCATA
       S  K  S  Q  E  R  T  L  E  Y  E  R  G  A  S  E  Q  H  D  Y
```

FIG 7D

```
1501 TACTACCAAATAGCCGGCAACGAAGATATATTTGATGTCGACGTTCTCCGACATAGCGAG
      V  V  L  Y  G  A  V  F  I  Y  K  I  D  V  N  E  S  M  <<ORF1

1561 ACCGACTCGACCCGCAAAGTATCAACACACTGACAAAACAGACGGACTGATCAGAAAGAT

1621 ATAACCCTTTTATTGTCTAAACAGAGACGCGATCGCGAAAATACTAAGCATTATCCATAT

1681 GTCACGTGATGTGGCAAGCATCCAAGACACATAAAATAGATCAGGTCAGAATCAGACTCC
                                                      *  V  G

1741 ACGTTGAATGTCCTCAATATTCCTTTCAAATGCTTTTTTTGCATCAAATACCTCAAGTAA
      R  Q  I  D  E  I  N  R  E  F  A  K  K  A  D  F  V  E  L  L

1801 CCTGGACACTCCCTCTTCAACGTCACCTGTCAATGAATCGTGTACCGCCAAAACAGCAGC
      R  S  V  G  E  E  V  D  G  T  L  S  D  H  V  A  L  V  A  A

1861 TGCCCCGCTACCCACATGTGACGTTTTTCTGAGATCAAGCTCAATTAGATTACAGAGGGA
      A  G  S  G  V  H  S  T  K  R  L  D  L  E  I  L  N  C  L  S

1921 GGAATAGTACTCCCCCAACCGTACCGCTGTCGGTATTCCTTTAACGCGCCCCGTTATGCA
      S  Y  Y  E  G  L  R  V  A  T  P  I  G  K  V  R  G  T  I  C

1981 GAGTGCAGCTAAACCAGGAAAGAAACCAGTAACTTCACATCTGTTGTCATATCTATACAT
      L  A  A  L  G  P  F  F  G  T  V  E  C  R  N  D  Y  R  Y  M
```

FIG 7E

```
2041 AGGTACAACATATTTCTCGAATAAAAAGAACAAGTTGTTGTCGCGACTGGCCATATCTTG
       P  V  V  Y  K  E  F  L  F  F  L  N  N  D  R  S  A  M  D  Q

2101 TCCATTGTCATCAAATGTGCTTGCGGTGGCTTGAGGTCTTGACCCAGGGGCGACGGGCCA
       G  N  D  D  F  T  S  A  T  A  Q  P  R  S  G  P  A  V  P  W

2161 TTTAGCTGCCTCCTGTTCCGATTGGGTTCCAAGTGGCAATTCAAAGAACGCAGATGGCTG
       K  A  A  E  Q  E  S  Q  T  G  L  P  L  E  F  F  A  S  P  Q

2221 AAATCGATTGAGATGTGTGCTCGTATAGACATTACTATTAAACATTAGCTTTTGCAAGAC
       F  R  N  L  H  T  S  T  Y  V  N  S  N  F  M  L  K  Q  L  V

2281 TAGGAGCAGAGATATCGAGTCTATAACCGTTCGCACAAGCGGATCATCTTCATGAAGAAT
       L  L  L  S  I  S  D  I  V  T  R  V  L  P  D  D  E  H  L  I

2341 TAACGGCGTTCGTCTCGAAGGGAAAAATGAAATGGAGAGAGCAGCAATGGGGTCATCAAA
       L  P  T  R  R  S  P  F  F  S  I  S  L  A  A  I  P  D  D  F

2401 ACGAACAAAATCTGGATCTATTGGAAGCAGAGTTTCTTTCGTTATATCTCGTACTGTGGT
       R  V  F  D  P  D  I  P  L  L  T  E  K  T  I  D  R  V  T  T

2461 GGAAAGGGTCCCAGTACGCTTTAGCAATTGATACACTATGTGAGGATCGAACACTCCATT
       S  L  T  G  T  R  K  L  L  Q  Y  V  I  H  P  D  F  V  G  N

2521 CGAATATTTTCTGCCGTCAATTGGGGAAAAAAAGTTTACGGGGAGTTTTGACCGGTCAAA
       S  Y  K  R  G  D  I  P  S  F  F  N  V  P  L  K  S  R  D  F
```

FIG 7F

```
2581 TGAAAATGTAACTGAGCCCCACTCGGCCCGAATATCTTCCAGCATATAATTTAAATACGT
      S  F  T  V  S  G  W  E  A  R  I  D  E  L  M  Y  N  L  Y  T

2641 TGGAACATGTCTTAATGCATCTTCAAATATAGAAACATCAAGACCATGTCTAATGTTTGA
      P  V  H  R  L  A  D  E  F  I  S  V  D  L  G  H  R  I  N  S

2701 AGCAGATTGTCTAGACCGCGAGTAGTGCAGTAAGCATAAAACAGTAGCCTCGAATCCGCT
      A  S  Q  R  S  R  S  Y  H  L  L  C  L  V  T  A  E  F  G  S

2761 ATATTGTCGAGTCCCAGCATAAATTTCCATCGCCGATCGCAGCGCTCGAATAGCCGTTTT
      Y  Q  R  T  G  A  Y  I  E  M  A  S  R  L  A  R  I  A  T  K

2821 CATAAACCGCCCCGAGATGCGCGTGTCTCCCATTACATTCAAAACCCTAGCGATAGCTTT
      M  F  R  G  S  I  R  T  D  G  M  V  N  L  V  R  A  I  A  K

2881 ATTGTTATCTAGGAGCTGAGTCTGTAGCGCGCGAAACCAAGACCCGAATCCCAGCCGGCC
      N  N  D  L  L  Q  T  Q  L  A  R  F  W  S  G  F  G  L  R  G

2941 ATTGCCAGCAATAGCAAACGAAGTTGTCAGAAAATCTACTTGAAAATCTGTATTAAAAGT
      N  G  A  I  A  F  S  T  T  L  F  D  V  Q  F  D  T  N  F  T

3001 TAATGGTTCTCCATTCTTAACTATCCAAATTACGTTCGCAGGGACATCCTCGCCAGGCGC
      L  P  E  G  N  K  V  T  W  I  V  N  A  P  V  D  E  G  P  A

3061 ACGGATGTCTAGTGTTTTATAAGACCCAACAATGTCCTTCTCCTGCACGGCCCGCTGGGC
      R  I  D  L  T  K  Y  S  G  V  I  D  K  E  Q  V  A  R  Q  A
```

FIG 7G

```
3121 TAAATCATCCAAGACATCAATTACAGTCTCTAGGGGATATTCAATAGTTTGCAATGCCGA
      L  D  D  L  V  D  I  V  T  E  L  P  Y  E  I  T  Q  L  A  S

3181 TGAGACATGTACGGGAACAGTAGCGCTCATATTTTCCAGTTGCTCTAAAGCTGCCTTGGC
      S  V  H  V  P  V  T  A  S  M  N  E  L  Q  E  L  A  A  K  A

3241 AGCCCTGTTCCGCCTTTCCAGATCTTCAATCCTTGCACGATTGGTCATGATTTCTGCGTC
      A  R  N  R  R  E  L  D  E  I  R  A  R  N  T  M  I  E  A  D

3301 TATTAGAGTTCCTTCGAAAAGCTTAGATGCATATTTACTCGAGCCTTCCGCGCAGGAGAT
      I  L  T  G  E  F  L  K  S  A  Y  K  S  S  G  E  A  C  S  I

3361 AAAGTTTAAACGATCGGCTAAAACCATCAAGGTAGACGGCTTGTTGCTGTCCTTTGGTAA
      F  N  L  R  D  A  L  V  M  L  T  S  P  K  N  S  D  K  P  L

3421 ATTCATAGGTTCGAATCTGGGTCGAAACATGGTTCGGACAAGTTTTTGGACTCCTCCGTA
      N  M  P  E  F  R  P  R  F  M <<ORF 2
         *  L  N  S  D  P  D  F  C  P  E  S  L  N  K  S  E  E  T  S

3481 GAAGTGGAATAGCTCGCGGGTTTGGTTGCGGACTCTTTTCCCACGCGCGACGAAAACAAT
         T  S  Y  S  A  P  K  T  A  S  E  K  G  V  R  S  S  F  L  A

3541 GCAGCTACGGTTTGTAAGGCTGAAGGGGCGGGAGACGCTAGATCCGCATTCTTTGCACTT
         A  V  T  Q  L  A  S  P  A  P  S  A  L  D  A  N  K  A  S  E
```

FIG 7H

```
3601 TCTTGTTGATACTTACTGCGCGCATGCGACACGCGTGGCTCTAATTTCGCGCAGAAAGTC
        Q  Q  Y  K  S  R  A  H  S  V  R  P  E  L  K  A  C  F  T  K

3661 TTTAAATACTGGCTTCTGGTAGCTAGAGTGACAAAGAAAAGCTCAGACGATACATTTATA
        L  Y  Q  S  R  T  A  L  T  V  F  F  L  E  S  S  V  N  I  V

3721 ACCGTTTCCCCAACCGTTTTTACCGCCAAGATGTTCATCCCCCGTTGAGCAACAAATACC
        T  E  G  V  T  K  V  A  L  I  N  M  G  R  Q  A  V  F  V  L

3781 AGTAGAGAAAGAATCTTTACTTTCTCAGTGCCAGGAACAGCCAGATTCTCCAACAATCGT
        L  S  L  I  K  V  K  E  T  G  P  V  A  L  N  E  L  L  R  A

3841 GCAGAATCGCGAAGTTGGAGAGTGCCAGTATATCCTTGTCTTAATTTACTTTCAGTCATC
        S  D  R  L  Q  L  T  G  T  Y  G  Q  R  L  K  S  E  T  M  L

3901 AAAGATTTGGAAAAACGACATGTTTTCAGTTCAATCACAATACATTTCATTTCATGTTGT
        S  K  S  F  R  C  T  K  L  E  I  V  I  C  K  M  E  H  Q  T

3961 GTCTCCAGCAAACAAATGCAATCAGGTTTCCGCAACCCTAGGTTCACTTCAAACATGACT
        E  L  L  C  I  C  D  P  K  R  L  G  L  N  V  E  F  M  V  V

4021 ACAATTTTGCCCCCGGCAGGTTTGCATTGGGGAATTATCGTATAGGCCAGCCTTCCGTCT
        I  K  G  G  A  P  K  C  Q  P  I  I  T  Y  A  L  R  G  D  G

4081 CCACCCCCTTCAAAGACTTCCTCCAGTGATCTGACGAGAGCTCGGTAAAAGCGATTATGG
        G  G  E  F  V  E  E  L  S  R  V  L  A  R  Y  F  R  N  H  C
```

FIG 7I

```
                                                ORF 4 >> M  A  V  A  G  A  V  K  T
4141 CAACGGATTCCGGCATTTAGTCTAGCCCGCAGAGATGGCCGTAGCTGGCGCCGTGAAAAC
       R  I  G  A  N  L  R  A  R  L  S  P  R  L  Q  R  R  S  F  K

       S  G  G  V  Q  F  C  S  E  F  E  N  D  D  S  D  F  R  R  V
4201 TTCCGGTGGTGTGCAGTTTTGCTCCGAGTTCGAGAACGATGACTCCGACTTTCGCCGCGT
       R  H  H  A  T  K  S  R  T  R  S  R  H  S  R  S  E  G  R  Q

       V  L  L  Y  V  D  G  P  F  G  V  G  K  T  V  T  A  K  T  L
4261 TGTACTTCTTTACGTCGACGGGCCATTCGGAGTCGGTAAAACAGTCACTGCAAAGACGTT
       V  E  K  R  R  R  A  M <<ORF 3

       M  Q  M  P  N  W  R  G  C  R  L  Y  L  A  E  P  M  Q  A  W
4321 GATGCAAATGCCAAATTGGAGAGGTTGCCGTCTATACTTAGCGGAACCTATGCAAGCATG

       R  Q  W  F  G  G  A  D  M  I  K  E  I  N  E  I  Q  T  L  K
4381 GCGCCAATGGTTTGGCGGAGCGGATATGATCAAAGAAATTAATGAAATACAAACCCTAAA

       A  S  G  K  L  E  C  R  E  A  S  P  V  A  V  A  E  V  Q  M
4441 GGCTTCCGGAAAACTTGAATGTCGGGAGGCGTCTCCGGTTGCCGTAGCGGAAGTTCAGAT

       T  I  A  A  P  L  R  I  M  N  H  V  I  Y  N  Y  L  G  S  E
4501 GACTATTGCTGCCCCACTAAGAATAATGAACCACGTCATTTATAATTATTTGGGATCTGA

       R  C  Y  S  A  A  A  S  G  P  D  D  V  L  F  L  V  D  R  H
4561 ACGCTGCTACAGCGCAGCTGCATCCGGACCAGATGATGTCTTATTCCTCGTAGATAGGCA
```

FIG 7J

```
      P  L  A  A  C  L  C  F  P  V  A  Q  Y  L  S  G  A  L  E  F
4621  CCCACTCGCGGCATGTTTGTGTTTCCCTGTTGCACAATATCTAAGCGGAGCGCTCGAATT

      G  D  L  I  T  L  L  S  G  I  P  D  I  P  T  H  S  N  I  V
4681  TGGAGATTTAATAACTTTATTGTCAGGAATTCCTGACATTCCAACACACTCCAACATTGT

      L  M  D  L  D  I  C  E  Q  A  R  R  I  I  Q  R  G  R  P  G
4741  TTTAATGGATTTGGATATTTGCGAACAGGCACGGCGTATAATACAAAGGGGGCGCCCAGG

      E  T  V  D  W  T  Y  L  C  A  L  R  N  S  Y  I  C  L  M  N
4801  GGAAACGGTCGACTGGACGTATTTGTGTGCATTACGTAACTCGTACATCTGCCTCATGAA

      T  T  T  Y  L  Q  R  T  S  Y  P  A  L  L  K  E  Q  E  A  L
4861  TACTACCACCTACCTCCAACGTACATCTTATCCAGCATTGTTGAAGGAGCAAGAAGCCTT

      T  S  A  T  L  L  K  F  K  R  E  C  L  E  T  A  T  V  P  E
4921  AACAAGTGCCACGCTCTTAAAATTCAAGAGAGAGTGCTTAGAAACTGCTACTGTTCCAGA

      I  N  P  S  I  D  Q  T  L  F  A  I  L  A  F  D  Q  Q  N  V
4981  AATCAATCCTTCAATCGACCAGACGCTATTTGCAATATTAGCTTTTGATCAGCAAAATGT

      H  G  E  R  L  K  T  V  L  S  F  V  V  Q  K  L  A  T  V  L
5041  TCACGGGGAAAGATTAAAAACTGTACTTTCATTTGTGGTTCAAAAACTCGCGACGGTATT

      K  N  L  C  I  F  Y  L  P  A  H  G  L  T  P  E  A  C  A  L
5101  GAAAAACTTGTGCATTTTTTACTTACCAGCACATGGCCTCACCCCGGAGGCATGTGCACT
```

FIG 7K

```
      K  C  L  E  F  A  E  T  A  S  S  L  T  T  K  R  A  A  I  A
5161  GAAATGTTTAGAGTTTGCCGAGACGGCAAGTTCTCTTACAACCAAACGAGCGGCGATCGC

      S  L  I  D  A  V  E  R  Y  N  A  D  M  G  S  *
5221  GAGCTTAATTGACGCAGTAGAGCGCTACAATGCTGATATGGGTTCGTAATGTTCCGCTTC

                              M  S  F  T  H  F  L  A  L  Y  S  F  L
5281  CATAATCCTTCACAATAAGAGTATGTCCTTTACTCATTTCCTTGCTTTGTACTCATTCTT

      L  E  R  A  W  L  H  Q  Q  P  A  P  M  G  H  A  R  E  I  F
5341  ACTCGAGAGAGCGTGGCTTCACCAGCAACCCGCGCCGATGGGACACGCGAGAGAAATATT
```

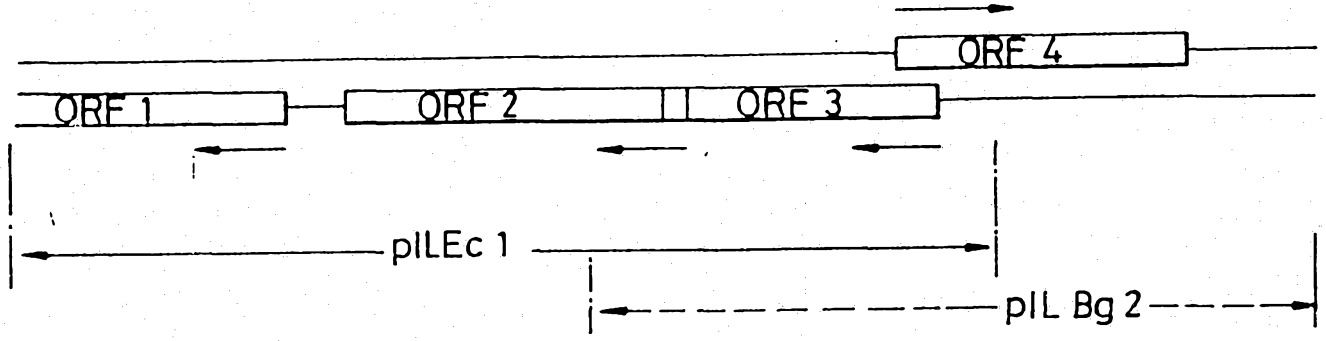



Fig. 8

Figure 9 ILTV gB

```
 V  L  I  S  N  W  R  Q  C  C  R  R  A  Q  P  R  H  C  H  R
CGTGCTAATCTCCAATTGGCGCCAGTGTTGCAGGCGGGCCCAGCCACGACATTGTCACCG
        10         20         30         40         50         60

 H  A  E  S  T  N  M  T  E  G  R  A  V  V  F  K  Q  N  I  A
ACATGCCGAGTCGACTAACATGACTGAAGGAAGGGCCGTAGTCTTCAAGCAAAACATTGC
        70         80         90        100        110        120

 P  Y  V  F  N  V  T  L  Y  Y  K  H  I  T  T  V  S
CCCGTACGTCTTTAATGTGACTCTATACTATAAACATATAACCACAGTTAG
       130        140        150        160        170
```

Figure 10 ILTV Ribonucleotide reductase.

```
  Q  R  R  R  K  P  T  G  L  C  V  Y  F  E  P  W  H  C  D  T
GCAACGGCGCCGCAAACCAACAGGGCTTTGTGTTTATTTCGAGCCATGGCATTGCGACAC
        10        20        30        40        50        60

  L  K  I  L  K  I  R  G  S  S  A  G  N  E  E  F  R  C  D  N
ATTAAAGATTCTGAAAATTCGTGGCAGCTCGGCCGGGAATGAAGAGTTCAGATGCGATAA
        70        80        90       100       110       120

  I  F  T  A  I  W  M  P  D  L  F  M  K  R  L  Q  Q  P  G  S
CATTTTTACTGCCATTTGGATGCCTGATCTTTTTATGAAAAGACTGCAGCAGCCAGGAAG
       130       140       150       160       170       180

  K  W  T
TAAATGGACA
       190
```

Fig 11

HVT HOMOLOGUES OF VZV62/ HSV-1 IE 175

```
  S  N  V  V  R  Y  M  C  G  N  T  V  L
 TCGAATGTGGTGCGATACATGTGCGGGAACACGGTACTCC
        10        20        30        40

P  P  D  P  H  G  T  P  V  V  I  N  V  P
CCCCGGATCCCCATGGCACCCCCGTGGTGATCAACGTTCC
        50        60        70        80

  E  G  T  S  E  T  M  A  E  L  T  V  A
CGAGGGAACGTCCGAAACTATGGCGGAGCTTACTGTTGCT
        90       100       110       120

  H  V  G  R  K  S  I  G  W  P  T  S  E
 CACGTTGGGCGCAAGTCTATTGGGTGGCCGACCTCCGAGT
        130       140       150       160

W  H  S  A  T  I  L  Q  K  D  N  D  S  R  L  V  I  I  R
GGCATTCCGCTACAATCCTGCAGAAAGATAATGATAGTCGGCTGGTAATTATACGCC
        170       180       190       200       210
```

FIG 12

HVT HOMOLOGUE OF RIBONUCLEOTIDE REDUCTASE (LARGE SUBUNIT)

```
  Q  V  T  E  V  S  E  G  F  A  P  L  F
 CAAGTGACCGAGGTTAGCGAAGGATTTGCCCCTTTGTTCA
          10         20         30         40

S  N  M  F  S  K  V  T  S  A  G  E  L  L
GTAACATGTTCAGCAAGGTGACAAGTGCCGGGGAACTGCT
          50         60         70         80

  R  P  N  S  Q  L  M  R  E  L  R  Q  I
TAGACCCAACAGTCAATTAATGCGGGAGCTGAGACAAATA
       90         100        110        120

  Y  P  D  N
 TATCCCGATAAT
          130
```

FIG 13A

MDV HOMOLOGUE OF RIBONUCLEOTIDE REDUCTASE ( LARGE SUB-UNIT )

```
   G  I  M  E  G  S  D  V  P  T  E  K  S
 GGGGATAATGGAAGGAAGTGATGTACCGACGGAAAAATCT
         10         20         30         40

 H  S  G  R  E  R  N  R  S  M  G  I  G
CATTCTGGCCGAGAACGTAACAGATCGATGGGCATCGGCG
         50         60         70         80

V  Q  G  F  H  T  A  F  L  S  M  G  L  D
TGCAGGGCTTTCATACAGCTTTTCTATCTATGGGTCTTGA
         90        100        110        120

   L  C  D  E  R  A  R  S  L  N  K  L  I
 TTTATGCGATGAACGCGCTAGATCCCTCAACAAGCTAATT
        130        140        150        160

 F  E  F  M  L  L  E  A  M  T  V  S  C
TTTGAATTCATGTTATTGGAGGCGATGACAGTTAGTTGCG
        170        180        190        200

E  F  C  E  R  G  L  P  P  F  A  D  F  S
AATTCTGCGAACGAGGCCTGCCGCCGTTTGCTGATTTCTC
        210        220        230        240
```

FIG 13B

```
  N  S  Y  Y  A  R  G  R  L  H  F  D  G
TAACAGTTATTATGCACGAGGACGTCTGCATTTCGATGGG
        250        260        270        280

 W  A  N  V  E  L  A  A  V  E  E  W  N
TGGGCTAATGTAGAATTGGCTGCAGTGGAAGAGTGGAATA
        290        300        310        320
```

FIG 14

MDV HOMOLOGUE OF RIBONUCLEOTIDE REDUCTASE ( SMALL SUB-UNIT )

```
   L  D  V  E  A  I  L  C  Y  V  R  Y  S
TATTGGATGTTGAAGCAATATTATGTTACGTACGTTACAG
        10        20        30        40

  R  G  Q  T  T  E  R  I  D  M  P  P  I
CCGCGGACAGACTACTGAAAGAATAGATATGCCACCTATT
        50        60        70        80

 Y  N  E  P  K  P  T  A  D  F  P  H  A  L
TACAACGAACCTAAACCTACAGCTGATTTTCCGCATGCAC
     90       100       110       120

   T  A  S  N  N  T  N  F  F  E  R  R  N
TGACAGCTTCAAATAATACCAACTTCTTTGAGAGAAGAAA
        130       140       150       160

  T  A  Y  S  G  S  V  S  N  D  L  *
TACTGCATACTCTGGAAGCGTGTCAAACGATCTTTAA
        170       180       190
```

FIG 15

MDV HOMOLOGUE OF HSV-1 IE-175

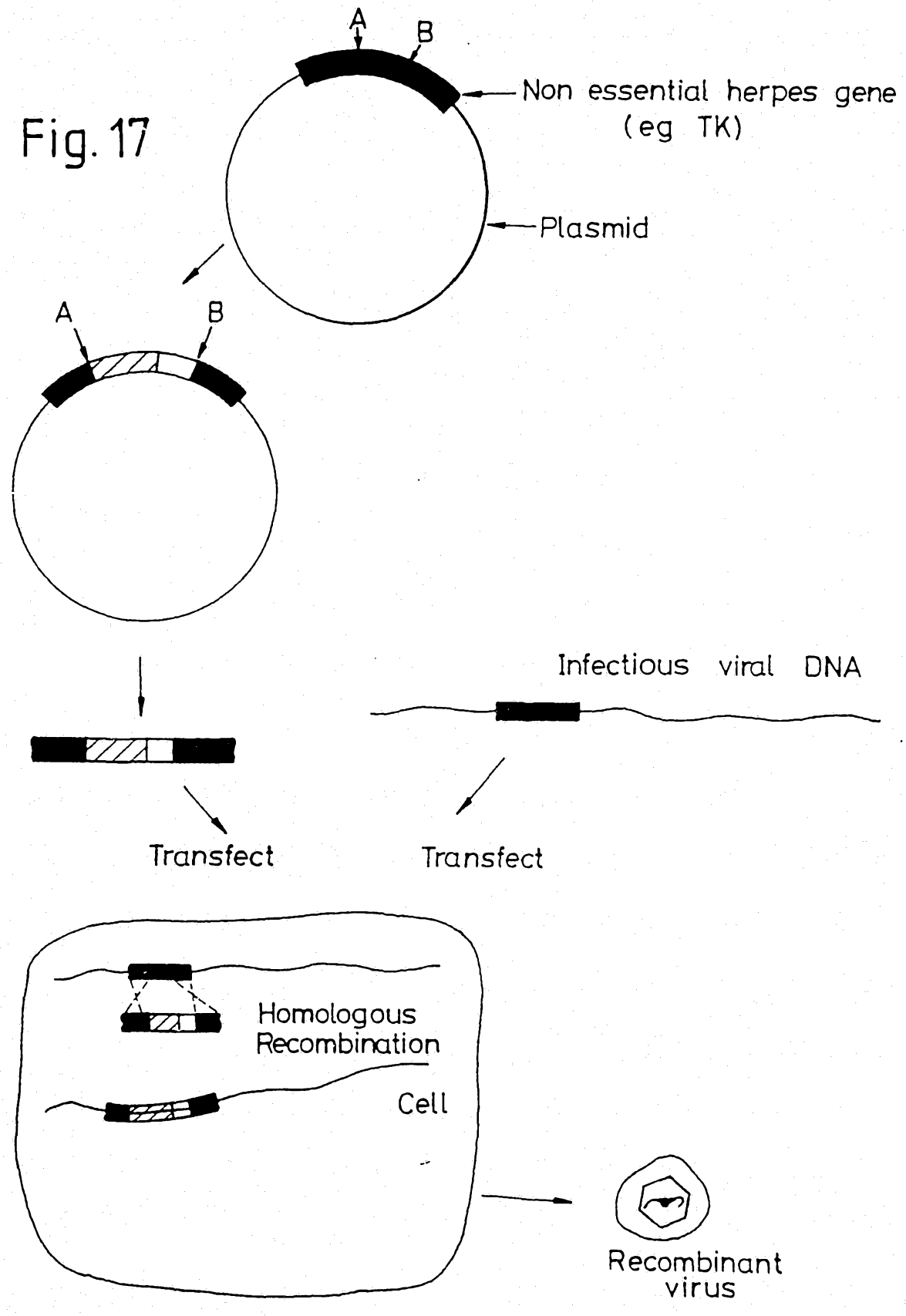
```
  P   I   P   V   Y   V   E   E   M   K   D   Y   A
CCCATTCCCGTCTATGTAGAGGAAATGAAAGATTATGCCA
        10          20          30          40

K   Q   Y   D   A   L   V   N   S   L   F   H   K   S
AACAATACGACGCTCTCGTAAACTCTTTGTTTCACAAAAG
        50          60          70          80

  M   K   V   N   P   L   N   W   M   H   H   G   K
CATGAAAGTAAATCCTCTGAACTGGATGCACCACGGGAAG
        90          100         110         120

  L   S   T   A   D   A   A   L   N   H   I   Y   V
CTGTCTACCGCCGATGCTGCCCTAAACCACATATATGTTC
        130         140         150         160

Q   K   F   Q   S   S   Y   D   S   P   G   A   A   V
AGAAATTCCAGAGTTCATACGATTCGCCCGGAGCGGCTGT
        170         180         190         200

  T   G   T   V   N
AACTGGCACAGTTAACA
        210
```

FIG 16

MDV HOMOLOGUE OF HSV-1 IE-68

```
    S  D  Q  D  F  E  L  N  N  V  G  K  F
 CGTCCGATCAAGACTTTGAACTTAATAATGTGGGCAAATT
         10        20        30        40

   C  P  L  P  W  K  P  D  V  A  R  L  C
TTGTCCTCTACCATGGAAACCCGATGTCGCTCGGTTATGT
         50        60        70        80

 A  D  T  N  K  L  F  R  C  F  I  R  C  R
GCGGATACAAACAAACTATTTCGATGTTTTATTCGATGTC
         90        100       110       120

    L  N  S  G  P  F  H  D  A  L  R  R  A
 GACTAAATAGCGGTCCGTTCCACGATGCTCTTCGGAGAGC
         130       140       150       160

  L  F  D  I  H  M  I  G  R  M  G  Y  R  L  N
ACTATTCGATATTCATATGATTGGTCGAATGGGATATCGACTAAA
         170       180       190       200
```

Fig. 17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(eg TK)
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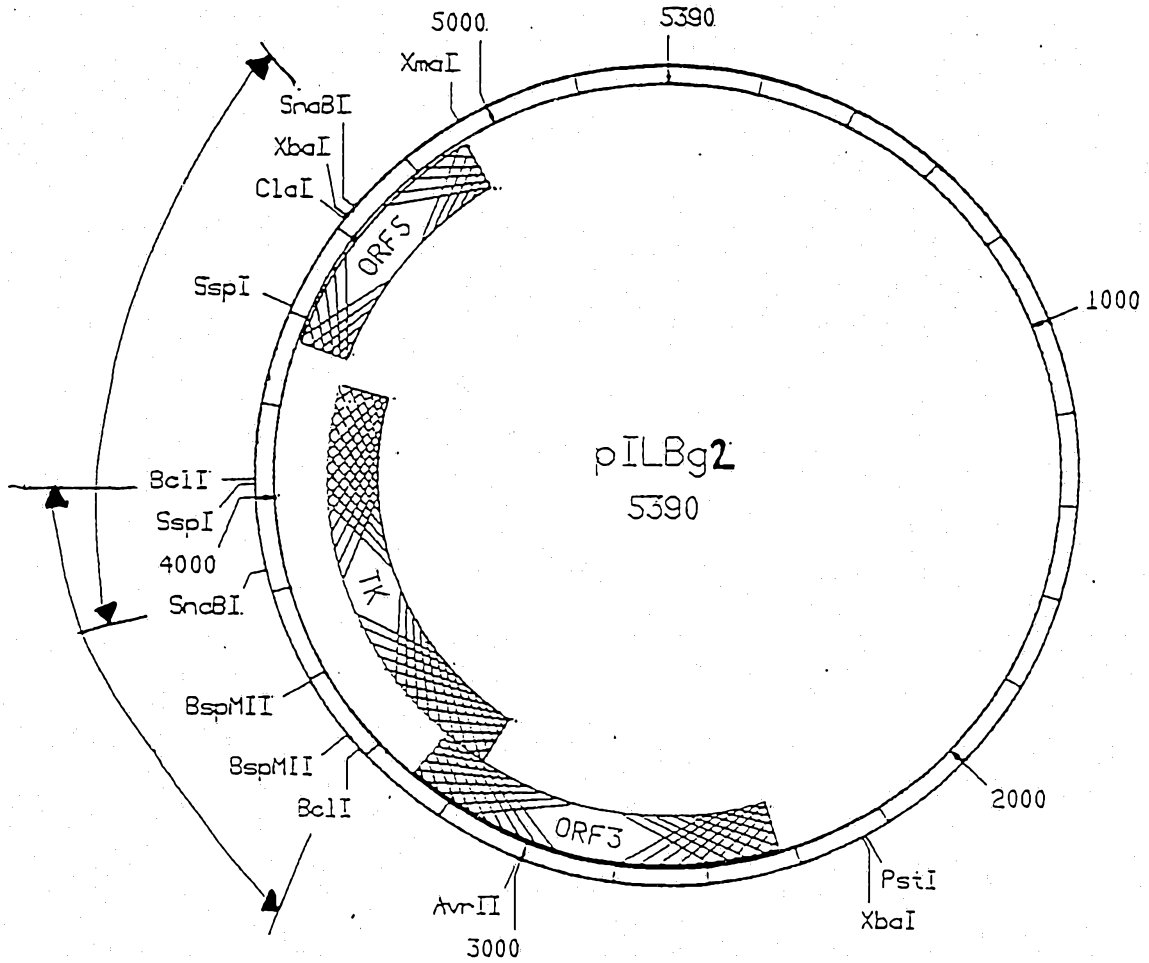
FIG.18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# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/01075

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 12 N 15/38, C 12 N 15/86, A 61 K 39/245, A 61 K 39/255

## II. FIELDS SEARCHED

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| IPC[5] | C 12 N, A 61 K |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category [10] | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| X | J. Gen. Virol., volume 69, 1988, SGM, (GB), A.E. Buckmaster et al.: "Gene sequence and mapping data from Marek's Disease virus and herpesvirus of turkeys: implications for herpesvirus classification", pages 2033-2042 see figure 3; page 2039, lines 6-35 cited in the application | 1-4 |
| Y | -- | 13-15 |
| Y | WO, A, 87/04463 (SYNTRO CORP.) 30 July 1987 see page 116 and figure 19 | 13-15 |
| A | -- Chemical Abstracts, volume 110, no. 17, 24 April 1989, (Columbus, Ohio, US), see page 231, abstract 149170c, & US, A, 128836 (UNITED STATES DEPT. OF AGRICULTURE) 15 July 1988 | |

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* Special categories of cited documents: [10]

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 12th December 1989 | 23. 04. 90 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | M. Peis M. PEIS |

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 16-17, because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1 (iv) - PCT:
Method of treatment of the human or animal body by surgery or therapy as wel as diagnostic methods.

Claim 17: Plant or anima varieties or essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.(Pls. see Rule 39.1 (ii) - PCT

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

**VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]**

This International Searching Authority found multiple inventions in this international application as follows:

For further information pls. see Form PCT/ISA/206 mailed to you on the 12.02.1990

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-4 partially; 13-15 partially as they relate to sequence a.

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8901075 SA 31083

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 11/04/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO-A- 8704463 | 30-07-87 | AU-A- 7026687 | 14-08-87 |
| | | EP-A- 0256092 | 24-02-88 |
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