



Office de la propriété
intellectuelle
du Canada

un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An Agency of
Industry Canada

(11) © 1,341,307
(21) 611,209
(22) 1989/09/13
(45) 2001/10/23
(52) 195-1.22
C.L. CR 167-100
195-1.23
195-1.28
530-13.00
530-15.12

(51) Int.Cl. ⁷ C12N 15/38; C12N 15/63; C12N 15/86; A61K 39/245; C07K 14/03

(19) (CA) **CANADIAN PATENT** (12)

(54) Viral Nucleotide Sequences

(72) Griffin, Annette Mary, U.K.
Ross, Louis Joseph Norman, U.K.
Scott, Simon David, U.K.
Binns, Matthew McKinley, U.K.

(73) MERIAL SOCIETE PAR ACTIONS SIMPLIFIEE, U.K.

(30) (GB) U.K. 8821441.6 1988/09/13

(57) 33 Claims

Canada

<http://lopi.gc.ca> • Ottawa-Hull K1A 0C9 • <http://cipo.gc.ca>
OPIC • CIPQ 191

OPIC



CIPQ

611,209

134 1307

ABSTRACT

VIRAL NUCLEOTIDE SEQUENCES

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 ~~our~~⁵ 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 fragment 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

According to the present invention there is provided a DNA fragment having a nucleotide sequence which corresponds to the nucleotide sequence of a gene of HVT, MDV or ILTV and which comprises a coding portion of a nucleotide sequence selected from the group consisting of:

- (a) the HVT nucleotide sequence indicated in Figure 2 between nucleotide positions 890-1081, 1172-1445 and 2190-2426, of the HVT gB gene,
- (b) the HVT nucleotide sequence shown in Figure 4 of the HVT gH gene,
- (c) the HVT nucleotide sequence shown in Figure 12 of the HVT ribonucleotide reductase (large subunit) gene,
- (d) the HVT nucleotide sequence shown in Figure 11 of the HVT immediate early gene IE-175,
- (e) the MDV nucleotide sequence shown in Figure 14 of the MDV ribonucleotide reductase (small subunit) gene,
- (f) the MDV nucleotide sequence shown in Figure 13 of the MDV ribonucleotide reductase (large subunit) gene,
- (g) the ILTV nucleotide sequence of the ILTV TK gene shown in Figure 7,
- (h) the ILTV nucleotide sequence shown in Figure 9 of the ILTV gB gene,

- (i) the ILTV nucleotide sequence of ILTV ORF2 shown in Figure 7,
- (j) the ILTV nucleotide sequence of ILTV ORF3 shown in Figure 7,
- (k) the ILTV nucleotide sequence shown in Figure 10, of the ILTV ribonucleotide reductase (large subunit) gene,
- (l) the HVT nucleotide sequence shown in Figure 11, of the HVT IE-175 gene,

and variations thereof which do not affect the essential nature of said DNA fragments or the protein encoded thereby.

According to another aspect of the invention there is provided a DNA fragment comprising an antigenic portion of a nucleotide sequence selected from the group consisting of:

- (a) the sequence from HTV gH gene indicated in Figure 2 between positions 890-1081, 1172-1445 and/or 2190-2426,
- (b) the sequence from HVT gH gene shown in Figure 4,
- (c) the sequence from HVT ribonucleotide reductase (large subunit) gene shown in Figure 12,
- (d) the sequence from HVT IE-175 gene shown in Figure 11,
- (e) the sequence from MDV ribonucleotide reductase (small subunit) gene shown in Figure 14,
- (f) the sequence from MDV ribonucleotide reductase (large subunit) gene shown in Figure 13,
- (g) the sequence of ILTV TK gene shown in Figure 7,
- (h) the sequence from ILTV gB gene shown in Figure 9,
- (i) the sequence of ILTV ORF2 gene shown in Figure 7,
- (j) the sequence of ILTV ORF3 gene shown in Figure 7,

1341307

8a

- (k) the sequence from ILTV ribonucleotide reductase (large subunit) gene shown in Figure 10,

- (1) the sequence from HVT IE-175 gene shown in Figure 11, and variations thereof which do not affect the essential nature of said nucleotide fragments or the protein encoded thereby.

The invention also provides a plasmid vector comprising a sequence described above and a DNA portion allowing replication in a host.

According to another aspect of the invention, there is provided an insertional or deletional mutant of MDV, HVT or ILTV 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.

The invention also provides a process for preparing such a mutant virus 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 or whole HVT, MDV or ILTV virus and (ii) isolating recombinant viruses.

The invention also provides a vaccine comprising ILTV susceptible cells and a mutant ILTV virus according to the

9(a)

invention such that the virus is attenuated as a result of such mutation.

The invention further provides a vaccine effective against MDV, HVT or ILTV comprising susceptible cells and a mutant virus according to the invention.

In another aspect, the invention provides a vaccination vector effective against ILTV, HVT or MDV, comprising a microorganism which will replicate in a fowl, the microorganism expressing a DNA fragment comprising a gene chosen among the group consisting of the HVT gB gene, the HVT gH gene, the ILTV gB gene, the ILTV ORF2, the HVT IE-175 gene, and an antigenic portion thereof.

The invention also relates to a vaccine comprising a vaccination vector of the invention.

The invention further provides a recombinant ILTV modified by the presence, in a non-essential region of the ILTV genome, of DNA not naturally occurring in ILTV.

The invention also provides a recombinant ILTV modified by a mutation in an ILTV gene such that the recombinant ILTV is attenuated as a result of said mutation.

The invention further provides a vaccine comprising a recombinant ILTV of the invention.

94b

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 nucleotides 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 probes 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.

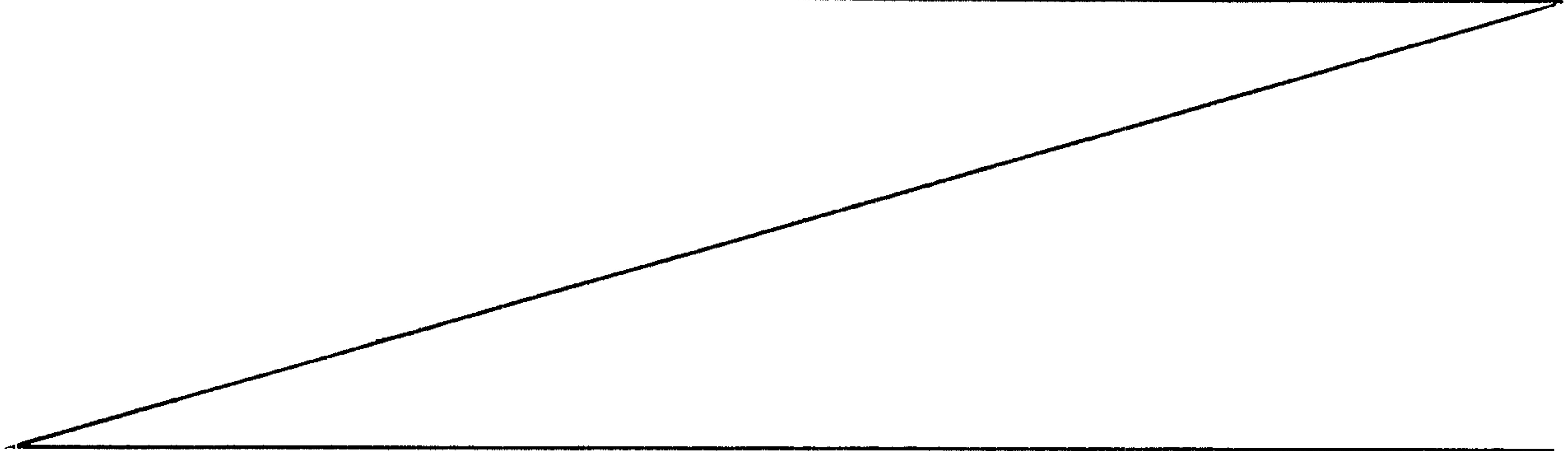
A second aspect of the invention provides insertional or deletional mutants of MDV, HVT and ILTV as follows:

- (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, egg drop syndrome (EDS76), fowl pox, infectious bronchitis, infectious bursal disease (Gumboro), 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 vectors 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 between 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 RB1B 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 (one 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 MDV homologue of 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 in 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 BglII 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 ³⁵S 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 NH₂ 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 BamHI 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 et al, 1984 as above].

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 μ g of HVT DNA and approximately 50 per μ g 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 anti-globulin 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) as above] 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 hybridization 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 β -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 acyclovir or FMAU will yield TK⁻ insertional mutants. If a β -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-I₃ and RB1B-BamH1-K₃. (Note I₃ 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 EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS.

1. A DNA fragment having a nucleotide sequence which corresponds to the nucleotide sequence of a gene of HVT, MDV or ILTV and which comprises a coding portion of a nucleotide sequence selected from the group consisting of:

- (a) the HVT nucleotide sequence indicated in Figure 2 between nucleotide positions 890-1081, 1172-1445 and 2190-2426, of the HVT gB gene,
- (b) the HVT nucleotide sequence shown in Figure 4 of the HVT gH gene,
- (c) the HVT nucleotide sequence shown in Figure 12 of the HVT ribonucleotide reductase (large subunit) gene,
- (d) the HVT nucleotide sequence shown in Figure 11 of the HVT immediate early gene IE-175,
- (e) the MDV nucleotide sequence shown in Figure 14 of the MDV ribonucleotide reductase (small subunit) gene,
- (f) the MDV nucleotide sequence shown in Figure 13 of the MDV ribonucleotide reductase (large subunit) gene,
- (g) the ILTV nucleotide sequence of the ILTV TK gene shown in Figure 7,
- (h) the ILTV nucleotide sequence shown in Figure 9 of the ILTV gB gene,
- (i) the ILTV nucleotide sequence of ILTV ORF2 shown in Figure 7,
- (j) the ILTV nucleotide sequence of ILTV ORF3 shown in Figure 7,

- (k) the ILTV nucleotide sequence shown in Figure 10, of the ILTV ribonucleotide reductase (large subunit) gene,
 - (l) the HVT nucleotide sequence shown in Figure 11, of the HVT IE-175 gene,
- and variations thereof which do not affect the essential nature of said DNA fragments or the protein encoded thereby.
2. A DNA fragment comprising an antigenic portion of a nucleotide sequence selected from the group consisting of:

- (a) the sequence from HTV gB gene indicated in Figure 2 between positions 890-1081, 1172-1445 and/or 2190-2426,
- (b) the sequence from HVT gH gene shown in Figure 4,
- (c) the sequence from HVT ribonucleotide reductase (large subunit) gene shown in Figure 12,
- (d) the sequence from HVT IE-175 gene shown in Figure 11,
- (e) the sequence from MDV ribonucleotide reductase (small subunit) gene shown in Figure 14,
- (f) the sequence from MDV ribonucleotide reductase (large subunit) gene shown in Figure 13,
- (g) the sequence of ILTV TK gene shown in Figure 7,
- (h) the sequence from ILTV gB gene shown in Figure 9,
- (i) the sequence of ILTV ORF2 gene shown in Figure 7,
- (j) the sequence of ILTV ORF3 gene shown in Figure 7,
- (k) the sequence from ILTV ribonucleotide reductase (large subunit) gene shown in Figure 10,
- (l) the sequence from HVT IE-175 gene shown in Figure 11, and variations thereof which do not affect the

essential nature of said nucleotide fragments or the protein encoded thereby.

3. A sequence according to claim 1 further comprising a 5' and/or 3' non-coding portion of the sequence.

4. A plasmid vector comprising a sequence according to claim 1 or 2 and a DNA portion allowing replication in a host.

5. A plasmid vector according to claim 4 which is designed to transfect an MDV-, HVT- or ILTV susceptible cell.

6. An insertional or deletional mutant of MDV, HVT or ILTV 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.

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

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

9. A process for preparing a mutant virus according to claim 6 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 or whole HVT, MDV or ILTV virus and (ii) isolating recombinant viruses.

10. A process according to claim 9 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 ILTV virus according to claim 6 such that the virus is attenuated as a result of such mutation.

12. A vaccine effective against MDV, HVT or ILTV comprising susceptible cells and a mutant virus according to claims 6, 7 or 8.

13. A vaccination vector effective against ILTV, HVT or MDV, comprising a microorganism which will replicate in a fowl, the microorganism expressing a DNA fragment comprising a gene chosen among the group consisting of:

the HVT gB gene,

the HVT gH gene,

the ILTV gB gene,

the ILTV ORF2,

the HVT IE-175 gene,

and an antigenic portion thereof.

14. The vaccination vector according to claim 13, which comprises the HVT nucleotide sequence indicated in Figure 2 between nucleotide positions 890-1081, 1172-1445 and 2190-2426, including with variations which do not affect the essential nature of said sequence.

15. The vaccination vector according to claim 13, which comprises the HVT nucleotide sequence indicated in Figure 4, including with variations which do not affect the essential nature of said sequence.

16. The vaccination vector according to claim 13, which comprises the ILT nucleotide sequence indicated in Figure 9, including with variations which do not affect the essential nature of said sequence.

17. The vaccination vector according to claim 13, which comprises the ILT ORF2 nucleotide sequence indicated in Figure 7, including with variations which do not affect the essential nature of said sequence.

18. The vaccination vector according to any one of claims 13-17, wherein the microorganism is a viral vector.

19. The vaccination vector according to claim 18, wherein the viral vector is a poxvirus.

20. The vaccination vector according to claim 19, wherein the poxvirus is a fowlpox virus.

21. The vaccination vector according to claim 18, wherein the viral vector is an herpesvirus.

22. The vaccination vector according to claim 18, wherein the viral vector is MDV or HVT.

23. The vaccination vector according to claim 18, wherein the viral vector is ILTV.

24. A vaccine comprising a vaccination vector according to any one of claims 13-23.

25. The vaccine according to claim 24, wherein it comprises a HVT or a MDV vector and HVT- or MDV-susceptible cells.

26. A recombinant ILTV modified by the presence, in a non-essential region of the ILTV genome, of DNA not naturally occurring in ILTV.

27. The ILTV of claim 26, wherein the DNA is expressed in a host by the production of a protein or an antigen.

28. The ILTV of claim 27, wherein the DNA codes for an antigen or an antigenic part of the antigen, and comes from HVT, MDV, IBV, IBDV, Eimeria, avian paramyxoviruses, fowlpox, or pathogens responsible for Newcastle Disease, avian encephalomyelitis, avian influenza, avian leukosis, avian reovirus diseases, chicken anaemia, coccidiosis, egg drop syndrome, inclusion body hepatitis, lymphoproliferative disease of turkey, reticuloendotheliosis in chickens, reticuloendotheliosis in turkeys, rotavirus enteritis, turkey hemorrhagic enteritis or turkey rhinotracheitis, or for paramyosin, somatostatin or growth-promoting part thereof, and immune regulator.

29. The ILTV of claim 28, wherein the antigen is selected from the group consisting of the genes gB, gC, gD, gH of MDV and gB, gH or HVT.

30. A recombinant ILTV modified by a mutation in an ILTV gene such that the recombinant ILTV is attenuated as a result of said mutation.

31. The ILTV of claim 30, wherein the mutation is in the TK, ORF3 or ribonucleotide reductase, large subunit, gene.

32. The ILTV of claim 30, modified by the presence, in a non-essential region of the ILTV genome, of DNA not naturally occurring in ILTV.

33. A vaccine comprising a recombinant ILTV according to any one of claims 26-32.

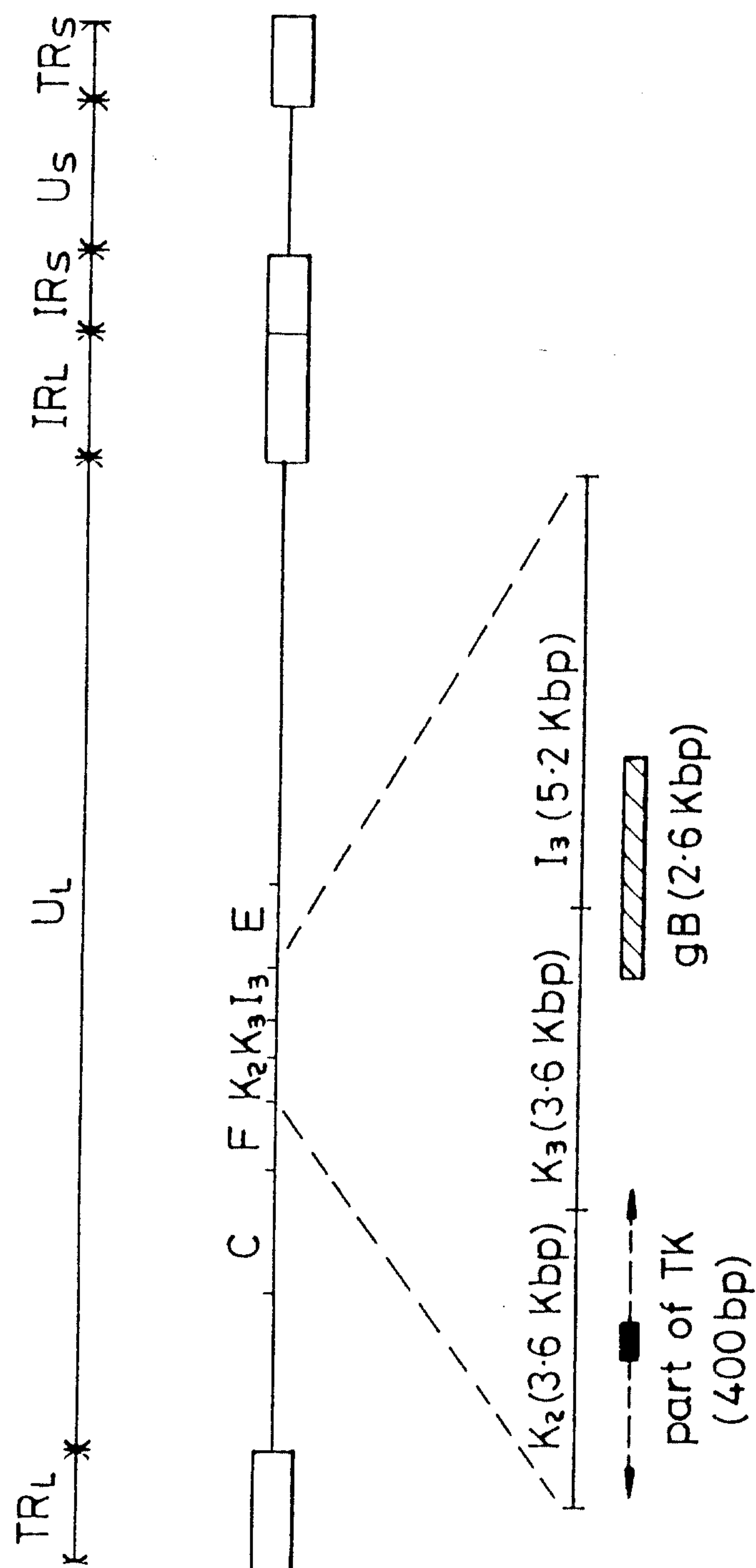


Fig. 1

FIG 2A

TCGAGCTCGCCGGGATGTTTAGTCACGATAGACATCGGT
10 20 30 40

TCGCCCAGCCGTCGAATACAGCATTTATATTTTAGTGTG
50 60 70 80

AAAATGTAGGGCTGCTTCCTCAGTTAAAGGAGGAAATGGCT
90 100 110 120

CGATTCAATGTTTCATAGCAGTAGAATAACAGATTGGACCG
130 140 150 160

TCAGTAAGTTTAGAGGGTTTATGACTTTAGCACTATAGA
170 180 190 200

TAAATGTAAC TGCGGCCCATCGCATGGCTTGGAATATATC
210 220 230 240

AAAGAACTGATTTTGTGCAACAGCTTTATTTCTTCTGTAT
250 260 270 280

TTAAATGTGGCGAATTGCACATCTGTCGTGCCGACAGTTT
290 300 310 320

GCAGATCAACAGCAATGGAGACTATGTATGGAAATGGA
330 340 350 360

1 3 4 1 3 0 7

FIG 2B

ATATATATAACATATGAAACCGAATATCCACTTATAATGA
370 380 390 400

TTCTGGGTCAGAATCAAGCACTTCAGAAACGCAAAATAT
410 420 430 440

GACTGCAATTATGATACAGATGTTTTTTCGTTGCTTTAT
450 460 470 480

TCTATTTTGCAGTATATGGCCCCCGTTACGGCAGATCAGG
490 500 510 520

TGCGAGTAGAACAGATTACCAACAGCCACGCCCATCTG
530 540 550 560

ACCCGTCCAATATTCTTGTGTCCCTGCATTTTATCTCACA
570 580 590 600

CAATTATGAACAGCATCATTAAGATCATCTCACTATGCA
610 620 630 640

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

M H

1 3 4 1 3 0 7

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
CATCAAGAGAAGTTGTTCGAGCGTCCAGTTGTCTGAGGA
730 740 750 760

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

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

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

1 3 4 1 3 0 7

FIG 2D

F K E N I S P Y K F K V T
TTTAAAGAGAATATCAGTCCATATAAAATTTAAAGTGACGC
|||||
GAGAATATCAGTCCGTATAAAATTCAAAGTAACAC
890 900 910 920

-----V-----
L Y Y K N I I Q T T T W T G
TTTATTATAAAATATCATTCAGACGACGACATCGACGG
|||||
TTTACTATAAGAACGTTATACAAACTACGACGTGGACTG
930 940 950 960

T T Y R Q I T N R Y T D R
GGACGACATATAGACAGATCACTAATCGATATACAGATAG
|||||
GGACGACGTACAGACAGATACTAACAGGTATACAGATAG
970 980 990 1000

1 3 4 1 3 0 7

FIG 2E

-----D-----

T P V S I E E I T D L I D
GACGCCGTTTCCATTGAAGAGATCACGGATCTAATCGAC
AACACCCGTGTCATCGACGAAATTACTGATTTGATAGAT
1010 1020 1030 1040

-----K-----													
G	K	G	R	C	S	S	K	A	R	Y	L	R	N
GGCAAGGAAGATGCTCATCTAAAGCAAGATACCTTAGAA													
GGTAAGGGGAAATGTTTCATCCAAAGCCGGTATCTTCG													
1050				1060				1070				1080	

N V Y V E A F D R D A G E
ACAATGTATATGTTGAAGCGTTTGACACGGATGCGGAGAA
1090 1100 1110 1120

K Q V L L K P S K F N T P
AAACAAGTACTTCTAAACCATCAAAATTCAACACGCCC
1130 1140 1150 1160

FIG 2F

|-----
E S R A W H T T N E T Y T V
GAATCTAGGCGCATGGCACACGACTAATGAGACGTATACCG
|||||
GGCATGGCATAACGACCAACGAGACGTACACCG
1170 1180 1190 1200

-----V-----
W G S P W I Y R T G T S V
TGTGGGATCACCATGGATATATCGAACGGAACTCCGT
|||||
TGTGGGATCTCCATGGGTATATAGAACGGACGTCCGT
1210 1220 1230 1240

-----A-----
N C I V E E M D A R S V F
CAATGTATAGTAGAGGAATGGATGCCCGCTCTGTGTTT
|||||
CAACTGCATAGTAGAAGAGATGGATGCCAGATCAGCATTT
1250 1260 1270 1280

-----T-----													
P	Y	S	Y	F	A	M	A	N	G	D	I	A	N
CCGTATTTCATATTTTGCAATGGCCAATGGGACATCGCGA													
CCATACACGTACTTTTGCAATGGCCAATGGAGATATCGCAA													
1290				1300				1310				1320	

	M	T	T	T	D							
I	S	P	F	Y	G	L	S	P	P	E	A	A
ACATATCTCCATTTTATGGTCTATCCCAAGGCTGC												
ACATGTCTCCATTTTATCGAACAACCTCCAACGCGGC												
1330				1340					1350			1360

-----S-----R-----R-----												
A	E	P	M	G	Y	P	Q	D	N	F	K	Q
CGCAGAACCATGGGATATCCCAGGATATTTCAAACAA												
CGCGGAGCCCATGAGCTATCCGCAAGACCGATTTCAGGCAA												
1370				1380				1390				1400

FIG 2H

-F-----P-----T-----
L D S Y F S M D L D K R R K
CTAGATAGCTATTTTCAATGGATTGGACAAGCGTCGAA
|
TTTGACAGCTATTTCCCCATGGATTGGATACGCGCCGAA
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
ACACTTCACAGTTGGTGGGACTGGGCTCCAAAACTACT
1490 1500 1510 1520

R V C S M T K W K E V T E M
CGTGATGTTCAATGACTAAGTGGAAGAGGTGACTGAA
1530 1540 1550 1560

L R A T V N G R Y R F M A
TGTTGCGTGAACAGTTAATGGAGATACAGATTTATGCG
1570 1580 1590 1600

1 3 4 1 3 0 7

9/66

FIG 2I

R E L S A T F I S N T T E
CCGTGAACCTTTCGGCAACGTTTATCAGTAATACGACTGAG
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
GCGAGGCAGAGCAGCAATCGAGCAGATATTAGGACAAA
1690 1700 1710 1720

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

F L A L G G F I V A Y Q P V
TTTCTTGGCTCTCGGGGATTTATTTGTAGCATATCAGCCTG
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
GATGAGAGACAACAGACCGATGAGATGCTCGACCTGGTA
1850 1860 1870 1880

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

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

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

I Q T H I N D M F S R I A
ATATACAAACCCATATTAAATGATATGTTTAGTAGGATTGC
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
TGGCACGAGGATAAAGATTAAATCCTAGCGCTACAGCGA
2130 2140 2150 2160

A T L G R R V A A K M L G
GTGCAACATTAGGAAGGAGAGTGGCTGCAAGATGTGGG
||| ||| ||| ||| |||
GCCAAAATGTGGG
2170 2180 2190 2200

-----D-----I---E---T---S---
D V A A V S S C T A I D A
GGATGTCGCTGTATCGAGCTGCACTGCTATAGATGCG
||| ||| ||| ||| ||| ||| |||
TGACGATGCCCGGTATCATCATGTATTGAGACTGATTCA
2210 2220 2230 2240

FIG 2L

-D-----V-----
E S V T L Q N S M R V I T S
GAATCCGTCACCTTTGCAAAATTCATGCGAGTTATCACAT
|||||
GATTCTGTACCTTACAAAATTCATGCGGTTGTTCACCT
2250 2260 2270 2280

T N T C Y S R P L V L F S
CCACTAATACATGTTATAGCCGACCATTTGGTCTATTTC
|||||
CTACCAATACTTGTATTATAGCCGCCCTTTAGTGTATTCTC
2290 2300 2310 2320

-----D--R-----D--K-----
Y G E N Q G N I Q G Q L G
ATATGGAGAAACCAAGGAAACATACAGGGACAACCTCGGTG
|||||
CTACGGGACCGACAAGACAAAATACAAGGACAGTTGGGGG
2330 2340 2350 2360

1 3 4 1 3 0 7

FIG 2M

-----I-----
E N N E L L P T L E A V E P
AAACAACGAGTTGCTTCCAACGCTAGAGGCTGTAGAGC
||||| ||| ||||| ||||| ||||| |||||
AAACAATGAATTGATTCCAACCTCTAGAGGCCATAGAGC
2370 2380 2390 2400

-----|-----
C S A N H R R Y F L F G S
CATGCTCGGCTAATCATCGTAGATATTTTCTGTTTGATC
||||| ||||| ||||| ||||| |||||
CATGTTCCGGCCAATCATCGTAGA
2410 2420 2430 2440

G Y A L F E N Y N F V K M
CGGTTATGCTTTATTTGAAAACTATAATTTTGTAAAGATGG
2450 2460 2470 2480

V D A A D I Q I A S T F V E
TAGACGCTGCCGATATACAGATTGCTAGCACATTTGTCTG
2490 2500 2510 2520

FIG 2N

L N L T L L E D R E I L P
AGCTTAATCTAACCCCTGCTAGAAGATCGGAAATTTTGCC
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
ATGAACCTTAAATTTTATGACATAAACAAGTAATAGAAGT
2650 2660 2670 2680

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

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

FIG 2ø

V V G A A G A I V S T I S
TTGTAGTAGGGCTGCCGGTGCAATCGTATCTACCATATC
2770 2780 2790 2800

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

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

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

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

A Q A T R E L H G E E S D D
GCACAGGCAACGCGTGAGTTGCATGCCGAGGAATCAGATG
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
AGCTAGAGAAATGATAAAATATATGCGTTAGTCTCCGCG
3050 3060 3070 3080

E E R H E K K L R R K R R G
GAAGAACGCCACGAGAAAAACTCCGGAGAAAGAGCGGAG
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
GATTAAAAATAGTAACCCCTAAATATGATAAGTTACCTACT
3170 3180 3190 3200

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

CTATTATATTGAACTGAATAAAACGCATAGCATGATA
3250 3260 3270 3280

FIG 2Q

TGGTTTACTCATTTATTGCGAGATATAAAGCATATTCAAT
3290 3300 3310 3320

ACGATATATTGCGAACGTGATGCTAAAAACATAGCTCCCT
3330 3340 3350 3360

GTATTATTGATGCGCCATCATTTTGATTAAATAACATCG
3370 3380 3390 3400

ACGCCGGCATCACTGGTGCGGTGTATACCAGCTACGGCGC
3410 3420 3430 3440

TAGCATTCATGGTATCCCGTGATTGCTCGATGCTTTCCTT
3450 3460 3470 3480

CTGAATTCCGTCCGGAACGCTCCTGAGAGATGGTCGCAGTT
3490 3500 3510 3520

ATTGGTACATTTTCGACCAGCCTCCGGATCTGAAACTGGCA
3530 3540 3550 3560

CAGGAATGCACCGTGGAATTGGTAGAAGTTTTCCTTCCG
3570 3580 3590 3600

FIG 2R

TCGAAGGCATAGGCGTTTCGACTCCCATGGGCCATGAAACTGTGGGATGT
3610 3620 3630 3640 3650

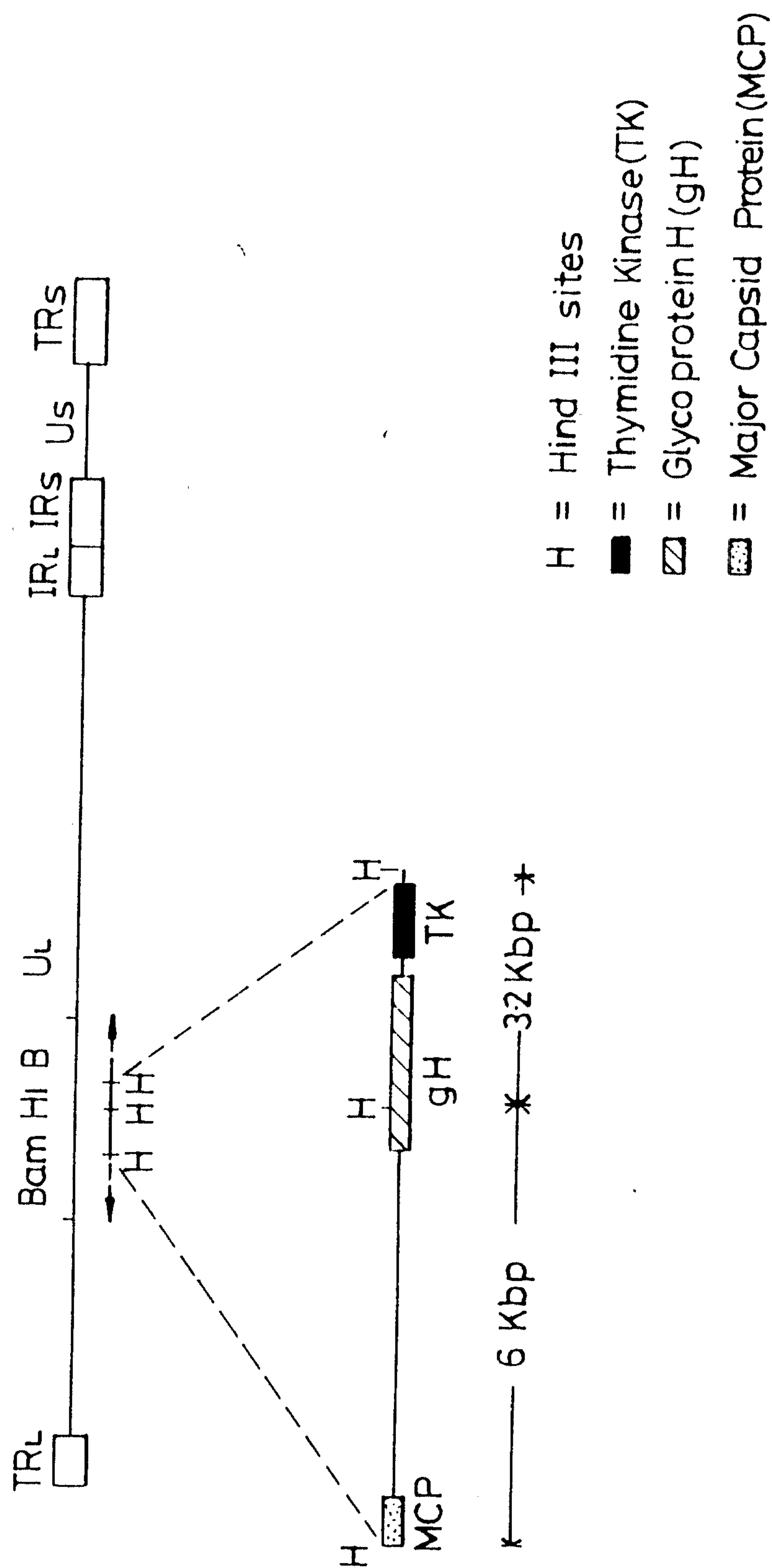


Fig. 3

FIG 4A

TATTATTGGTCCATGCTAGAAATAGTCATACGCTACGATCT
10 20 30 40

GTTGCTATATGACTATCGCCAAACTGTTAAACCCGCGA
50 60 70 80

AGAAATATATTTCATATAAAACCTAAGGGCCCTCAGTCTGA
90 100 110 120

TTTTTTGTGAAAACGTGTATACCATGAAGTTTACTGCCT
130 140 150 160

I R F M I I A N L Y S S Y
AATCCGTTTCATGATCATAGCGAATCTTTATTCTTCTTAC
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
AATGGTTTTTCAAGCGTGCCAAATATCCAGGCTACTAACG
410 420 430 440

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

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

FIG 4C

K T N T P L K K Y E I V V
GAAACCAATACACCGTTGAAGAAATATGAAATTGTTGTC
530 540 550 560

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

L K P A D F L N P G Y K F
GTTTAAACCGGCAGACTTCTCAACCCCGGATACAAAGTT
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
TCTTGTTTGTGATCCGATGGATTCTCTCGTCCCGATAG
690 700 710 720

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

FIG 4D

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

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

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

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

R P H W I Y L L G V Y R N V
CGCCCCATTGGATCTACCTATTAGGTGTGTATAGAAACG
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
GAGTCTGGGCGATTCTATGGATTATCACTTCCCTAATTAGC
1010 1020 1030 1040

R A H A Q M L I L A A E D R
AGAGCGCATGCCCGAGATGCTGATACTGGCAGCAGAGGACC
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
GCGTTTATTGTATCGTTGTTCGCATTTCATACGTAACGCA
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
TCGAAGCCGATTGAGGTTAATTGTAGAAGGCATTTCTTC
1210 1220 1230 1240

FIG 4F

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

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

S L L S K V I R P I S G H
AATCGTTGTTGCTAAAGTCATTTCGACCAATTTCGGGACA
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
CTTGAGCGAACAGGTATCTACAGTGGCACTGTCGTTTCAT
1450 1460 1470 1480

FIG 4G

E N I H S E A M R D I L S
TGAAAAATATTCACAGCGAGGCCCATGAGGGACATTCTGTCA
1490 1500 1510 1520

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

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

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

C T E E H V I A T E L A I Q
TGACAGAAGAGCATGTTATCGCAACTGAGTTGGCTATTC
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 L
ACACCTTCTAGACGTATATACACCATGTCTTTCTTCACTA
1730 1740 1750 1760

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

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

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

E T K A E Y I L T K L
GAAACAAAGCGGAATACATCCTCACCAGCTT
1890 1900 1910

AAGCTTTTGTAAACGATTATGACCGACACCCGCT
10 20 30 40

TTAGCAATCCTGCCATAAGGTGGTTTCCCGCGTGTTC

CTCGAAGACAATTGCCAGCTAATCCAGCATTACCATATTT
90 100 110 120

|-----S---Q
 M A L P
 CCTTGGCTTGCATTGGATCTGCCGCTCGATGGCATTGCC
 |||||
 ATGGCATCTCA
 130 140 150 160

```

M-T-S-A-Q-I-
R R P P T L T R V Y L D G
GAGAAGACCGCCACGTTAACGCGAGTTTATCTAGACGGA
||| ||| | | | | | | | | | | | | | | | |
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
CCGTTGGTATAGGCAAAACGTCCTATACTAAACGCTATGC
| | | | | | | | | | | | | | | |
TCAATGGGTATAGGTAAACGTCATGTGTAATGAGATAC
210 220 230 240

---T---L|
D H T P D G A P I L K V Y
CCGACCACACGCCGATGGGGCTCTATATTGAAAGTGTA
| |
CGACGCATCTT
250 260 270 280

E P M K Y W R C Q S T D L
CGAACCAATGAAATATTGGAGATGCCAGTCTACCGATTG
290 300 310 320

FIG 5C

V V A A N E T P E R R R G G
GTGGTAGCTGCCAACGAAACGCCAGAACGTAGCGTGCTG
|-----R--
| | | | | | | |
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
GAGCTTTATCACGATTCCAATCTGACATGATCATGCGCATC
| | | | | | | | | | | | | | | |
GAGAGTTTCTTTTATTCAATCTAGCATGATTGTAACAGC 370 380 390 400

---L---S--K---V---
I Q A R F A D P Y L L F H
TATACAAGCCAGATTGCGCATCCATATTGCTTTTTCAC
| | | | | | | | | | | | | | | |
TTTACAATCAAAGTTTGCAGATCCCTATCTTGTATTTCAT 410 420 430 440

FIG 5D

-----H-R-I-T-G-T-R
E R L S S K C R G K I E I C
GAACGGTTATCATCTAAATGTAGAGGAAATAAGAAATAT
|||||
GAGCGCTTATCGTCGAAGTGTATCGCATACAGGAACAC
450 460 470 480

-----G-N-S-L-I-----
D T P A I I L M L D R H P
GCCGATACTCCAGCAATTATATTAATGCTGGATAGCACCC
|||||
GTGGCAATCCATCGCTTATATTAATTCTAGATCGACATCC
490 500 510 520

-----I-S-T-V-----A-H-----
V A A I L C F P I T R Y L
TGTCGGCGGATATTATGTTTCCCAATCACTCGCTATTTA
| | | | |
CATATCCGCTACCGTATGTTTCCCATGTGCTCGACATTTA
530 540 550 560

FIG 5E

-T---D-C-----M-
L G E Y S L E M L I S S I I
CTTGGAGAAATATTCTTTGGAAATGTTGATTAGCTCTATAA
||||| | | | | | | | | | | | | | | |
ACTGGAGATTGTTCTTGGAGATGCTAATTAGTATGATAA
570 580 590 600

-----Q-P-----V-I
R L P L E S P G C N L T V
TAAGACTTCCGTGGAAATCCCCGGATGCAACCTGACAGT
|||| | | | | | | | | | | | | | | |
TAAGGTTCCCCCAGGAACCGCCAGGATGCAACTTGGTGAT
610 620 630 640

--V-D---H-----S---L-
T I L P D E K E H V N R I
CACAATCCTTCCCGACGAAAGAACACACGTTAATAGGATT
|| | | | | | | | | | | | | | | |
TGTCGATCTACATGACGAAAGAGCATGTTAGCCGTCTA
650 660 670 680

FIG 5F

-S-----N-----T-----K-----L-----L-----
C S R D R P G E T A D R N M
TGTTCAAGAGATAGACCGGTTGAACGGCAGATAGAAATA
| | | | | | | | | | | | | | | |
TCTTCACGGAATAGGACCGCGGAGAAACAGATCTACTAA
690 700 710 720

-----A-----S-----C-----
L R T L N A V Y A S L V D
TGCTCAGAACACTCAATGCCGTATACGCATCTTTGGTGGA
| | | | | | | | | | | | | | | |
TGCTCAGGCGCACTTAATGCAGTGTATTCCTGTTTAGTAGA
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

G E L L T Q Y S W I L W G
ATGGGAGCTGTTAACTCAGTATTCCTTGGATATTGTGGG
970 980 990 1000

L L T K L H T I N V E L F
ATTACTGACTAAACTACACACCATTAATGTCTGAATTATT
1010 1020 1030 1040

D	I	S	G	M	S	R	R	E	C	A	S	A	I		
GACATTAGCGGTATGTCACGTCGAGAATGCCCCAGCGCTA															
TGTTGTAAGAACTGC															
1050									1060					1070	1080

FIG 5I

-----D-----S-----V-----H-----S-----
M H T M P E R L S T L A S
TAATGCATACTATGCCGAGAGATTGTCTACTCTCGCTAG
| | | | | | | | | | | | | | | | | |
TTATGGATACTATGTCCGAGAGATTGGTAACACATAGTAG
1090 1100 1110 1120

-----A-----F-----I-----A-----L-----A-----
W N D L C E L E D D V I S
CTGGAATGATTTATGCCAGCTTGAAGATGATGTAATTCC
| | | | | | | | | | | | | | | | | |
CTGGAATGATGCCCTTCGAGATTGAAGCTGATGTACTAGCC
1130 1140 1150 1160

-----E-----A-----M-----*|
Y N K G M C N E V G A S R *
TATAATAAGCGAATGTGTAAACGAGGTTGGAGCGTCTCGAT
| | | | | | | | | | | | | | | | | |
TATAATAAAGAGATGGCTATGTAA
1170 1180 1190 1200

AATTCTTCTTAAATCTGCTGGTATGTGTTACTGCCATAACT
1210 1220 1230 1240

FIG 5J

TATTATTGGTCCATGCTAGATAAGTACATACGCTACGATCT
1250 1260 1270 1280

GTTGCTATATGACTATCGCCAAACTGTTAAACCCGCCGA
1290 1300 1310 1320

AGAATATATTTTCATATAAACCTAAGGGCCCCCTCAGTCTGA
1330 1340 1350 1360

TTTTTTGTGAAACGTGTATACCA
1370 1380

FIG 6B

N I S I N G A P L T E V P H A P
ACATTTCATTAAACGGCGGCCCTTTAACTGAGGTACCTCATGCAC

S T E S V S T N S E S T
481 CTTCCACAGAAAGTGTGTCAACAAATTTCGGAAGTACC

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
TCCACAACAATGCGTCTACGGACAAGCAAAATGCCGAACG

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

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

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

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

H A N E K N V E A A R I A G
CCACGCCAACGAAAGAATGTGGAAGCGCGGTATCGCTGGT

FIG 6C

L Y G V P G S D Y A Y P R Q
CTCTATGGAGTCCCGGATCAGACTATGCATACCCACGTC

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

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

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

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
ATGCAAGTCATTTATGCGTGACCATTTTAATCGGCCTTAA
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 TGGATGTACTGGCCCCCTCCAGTCTCAGCGGAGAAAA

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

FIG 6A

```
1 CAGCTGCCCTATGTAGTGAATCTATACTGGGATT  
ATCATAACTAGTTTACTTGTGTTGTATATTAGTAGCGCTATCT  
TGACCAAATCGTTGTTACATCTTGGCCATATACGTATTGATC  
121 GTTGTTCGAACCGCGAATAAACTTTCATACATAC  
TAAACGATCGAGTTGTGTTTATGAGCGTTGAAAACAAGGT  
ACCATCGGTTTAAAACTAAGTTGCATATCGTAATCCACAAA  
241 ATCATTTTATACATCATCCCGAAGAGACACCAAACG  
M L T P R V  
TAACCCCTCTACATATCTTCCCTCATGCTCACGCCGCGTGTGT  
L R A L G W T G L F F L L L S  
TAGGAGCTTTGGGGTGGACTGGACTCTTTTGTGCTTTTAT  
P S N V L G A S L S R  
361 CTCCGAGCAACGTCTTAGGAGCCAGCCTTAGCCCG  
D L E T P P F L S F D P S  
GATCTGAAACACCCCCATTCTATCCTTTGATCCATCCA
```

FIG 6D

S V Y V S W R Q N G N I A T
TCTGTCTATGTATCTTGGAGACAGAATGGAACATTCGCAA

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

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
GGAATTCAGGAATTGATTTCCCCCCCCAAATATCTTGTC

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

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

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

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

H D E A Q P N T T Y N T V V
ACATGATGAAGCGCAGCCTAACACAACCTATAATACTGTGGTT

FIG 6E

T G L C R T I D R H R N L L
ACAGGTCTCTGCCGACCATCGATCGCCATAGAAATCTCC

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

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

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

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
TTTGGATTGGCTGTAATTTAGGGATGGGATAATCATGACT

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

*

1801 TATAATCTCATTTGTTATGTAGTTGTGATTTATTAAAC

ATATTTTTTATAACTCTAGTATTCTCCGAGTACTTATATATT

FIG 6F

TATTTGTGAGACAATAATGCAATAGTCGAGAAACGTGAGG

1921 GGAGTCTGTAAACAGAAATACGTATAATCATCTATTG

AATAAAAGATTGTGGTATAAATGAAGATAGCCGAAGTCATT

CAAGCTCTCCATTCTATTTTAAACAATGTACAGTTTAAAGT

FIG 7A

1 GAATTCTTGTAATAATCATTAATTCCGCTACCAACGGGTTCCCTTTTTCACATAGCTG
F E Q L I D N I G S G V P E K K E C L Q
61 CTGCTCCGTAGTTATCTTTTGTGCTGGAACCATTTCCCGAGGAAGTACGGGTCAATCTC
Q E T I K K A P V M E G P F Y P D N E
121 AGAAATGGTCCCTAGCGGATCTCTTCCGAGGGTTATTCTCCGAGTATTCGCTATCCCGTTT
S I T R A S R K R P N N E S Y E S D R K
181 ATATTTTCTCAAGAGCCGAAATCAAGGCATCTAAGCGTGAAACAACAGTCTGCTCATG
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 ATAATAATTGGATGCACGGGGTAAGTAGATGCGGGGCTTTGATAAGGAATATATGGGAA
Y Y N P H V P Y T S A P S Q Y P I Y P F
361 ACGCCCGCAGAAATTTGGGTCTTCGTAAAGAGCCGGTCTGTAAAGTGTGGTTCATCCCCGG
R G C F N P D E Y L A P R Y T H N M G P

FIG. 7B

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

481 TGGTGAACATTCTGTAGTAGAGTCCCGGACCTACGGGAAACTCTCGTGATGGCGACAC
P S C E H T S D R S 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 TGGGACATAGACCATATCTTCACGAGATGCGGACGGTGAAGCTCCGTTTGTGTCGAA
P V Y V M D E R S A S P S A G N K T Q L

661 GAACTGGCCGGAGCATGTGCGCCGACTCCATGGCTGGAACGTGTGTTGCCCGGACGGTTG
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 TTGTAAATATTTCGGGTGTGTAACAGCTGCCATCTCGCGTCGCGTTTCAACAGGTACC
Q L Y K P N Y ~~V~~ A A M E R R T K L C T G

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

FIG 7C

961 TCGCGACAACTCTAGGGACTTCTGCACATTCTCACGTGTAGCTATTTCATACAGCTG
 R S L E R P V E A C K E R T A I E Y L Q
 1021 TTCTTTAATTGGTGGCTCAAAATCATCAAAATGCTCCAATAGCTTCCGAAGCGGTGGCCCC
 E K I P A S L D D F A G I A E S A T A G
 1081 GTAAATTGCTACCGTGCCCTTCACGTCTACCCAACTCACAGAGCGCCACGTGTGCAAAAAA
 Y I A V T G E R R G L E C L A V H A F F
 1141 ATCTTTCCGGGCACTTCGTTCTTTCAAGCCGCCCTTGAAGAGAGCGGATAGAGAAGGTAG
 D K G P V E N K E L R R S S L S L S P L
 1201 AATGTTGCTGAGAAGATAGAGAAATTCTCTGTCTCCGTCAAAACCATCCCCCTCTTCGGC
 I N S L L Y L F K E T E T L V M G E E A
 1261 ATTCGCAAAAAGAGCATCATCTTGACCGTAGCTTAAAAAAATAGTGCAAGAGCAGTTGA
 N A F L A D D Q V Y S L F I P A L A T S
 1321 CACGACACCCAAACAAACAGTCCTCTCGGCAGGTCTAAGATCGTCAGCACTGTACCTAC
 V V G L C F L G R P L D L I T L V T G V
 1381 CACGCAGGATGACTCGTGGTCAATGTTTATCGGAATTGTCCAGGCAAACTGGTAAAGC
 V C S S E H D T N I P I T G P L V P L A
 1441 CGATTGCTTGTCTCGCGAGTAAGCTCATATTCTCGCCCGGCACTTCTTGGTGGTCATA
 S K S Q E R T L E Y E R G A S E Q H D Y

FIG 7D

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

1561 ACCGACTCGACCCGCAAGTATCAACACACTGACAAACAGACGGACTGATCAGAAAGAT

1621 ATAACCCCTTTTATTGTCTAAACAGAGACGCGATCGCGAAAATACTAAGCATATCCATAT

1681 GTCACGTGATGTGGCAAGCATCCAAGACACATAAAATAGATCAGGTCAGAAATCAGACTCC
* V G

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

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

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

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

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

FIG 7E

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

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

2161 TTAGCTGCCCTCCTGTTCCGATTGGGTTCCAAGTGGCAATTCAAGAACGCAGATGGCTG
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 TAGGAGCAGAGATATCGAGTCTATAACCGTTCGCACAAGCGGATCATCTTCAATGAAGAAT
L L L S I S D I V T R V L P D D E H L I

2341 TAACGGCGTTCGTCTCGAAGGGAATAATGAAATGGAGAGCAGCAATGGGGTCATCAAA
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 GGAAGGGTCCCAGTACGCTTTAGCAATTGATACACTATGTGAGGATCGAACACTCCATT
S L T G T R K L Q Y V I H P D F V G N

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

FIG 7F

2581 TGAAAAATGTAAGCCCACTCGGCCGAATATCTCCAGCATATAATTAAATACGT
 S F T V S G W E A R I D E L M Y N L Y T
 2641 TGGAACATGTCTTAATGCATCTTCAAATATAGAAACATCAAGACCATGTCTAATGTTGA
 P V H R L A D E F I S V D L G H R I N S
 2701 AGCAGATTGTCTAGACCGGAGTAGTGCAGTAAGCATAAACAGTAGCCTCGAATCCGCT
 A S Q R S R S Y H L L C L V T A E F G S
 2761 ATATTGTCGAGTCCCAGCATAAATTCCATCGCCGATCGCAGCGCTCGAATAGCCGTTT
 Y Q R T G A Y I E M A S R L A R I A T K
 2821 CATAAACCGCCCGAGATGCGCGTGTCTCCCATTAACATTCAAAACCCCTAGCGATAGCTTT
 M F R G S I R T D G M V N L V R A I A K
 2881 ATTGTTATCTAGGAGCTGAGTCTGTAGCGCGGAACCAAGACCCGAATCCAGCCGGCC
 N N D L L Q T Q L A R F W S G F G L R G
 2941 ATTGCCAGCAATAGCAACGAAGTTGTCAGAAAATCTACTTGAAAATCTGTATTAAGT
 N G A I A F S T T L F D V Q F D T N F T
 3001 TAATGGTCTCCTTAACCTATCCAAATTACGTTCCGAGGGACATCCTCGCCAGCGGC
 L P E G N K V I W I V N A P V D E G P A
 3061 ACGGATGTCTAGTGTTTATAAGACCCAACAAATGTCCTTCTCCTGCACGGCCGCTGGGC
 R I D L T K Y S G V I D K E Q V A R Q A

FIG 7G

3121 TAAATCATCCAAGACATCAATTACAGTCTCTAGGGATATTCAATAGTTTGCAATGCCGA
 L D D L V D I V T E L P Y E I T Q L A S
 3181 TGAGACATGTACGGGAACAGTAGCGCTCATATTTCCAGTTGCTCTAAAGCTGCCCTTGGC
 S V H V P V T A S M N E L Q E L A A K A
 3241 AGCCCTGTCCGCCCTTCCAGATCTTCAATCCTTGCACGATTGGTCATGATTTCTGCGTC
 A R N 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 AAAGTTTAAACGATCGGCTAAACCATCAAGGTAGACGGCTTGTTGCTGTCTTGGTAA
 F N L R D A L V M L T S P K N S D K P L
 3421 ATTCATAGGTTTGAATCTGGGTCGAAACATGGTTCGGACAAGTTTGTGGACTCCTCCGTA
 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 GAAGTGGAATAGCTCGCGGGTTTGTTGCGGACTCTTTTCCACGCGCAGAAACAAT
 T S Y S A P K T A S E K G V R S S F L A
 3541 GCAGCTACGGTTTGTAAAGGCTGAAGGGGCGGAGACGCTAGATCCGCATTTTGCACTT
 A V T Q L A S P A P S A L D A N K A S E

FIG 7H

3601 TCTTGTGATACTTACTGCGCGCATGCGACGCGTGGCTCTAATTTCGCGCAGAAAGTC
 Q Q Y K S R A H S V R P E L K A C F T K
 3661 TTTAAATACTGGCTTCTGGTAGCTAGAGTGACAAAGAAAGCTCAGACGATACATTATATA
 L Y Q S R T A L T V F F L E S S V N I V
 3721 ACCGTTTCCCAACCGTTTTACCGCCAAGATGTTCAATCCCCGTTGAGCAACAATACC
 T E G V T K V A L I N M G R Q A V F V L
 3781 AGTAGAGAAAGAAATCTTTACTTTCTCAGTGCCAGGAACAGCCAGATTCTCCAACAATCGT
 L S L I K V K E T G P V A L N E L L R A
 3841 GCAGAAATCGGAAGTTGGAGAGTGCCAGTATATCCTTGCTTAATTACTTTCAGTCATC
 S D R L Q L T G T Y G Q R L K S E T M L
 3901 AAAGATTTGGAAAAACGACATGTTTTCAGTTCAATCACAATACATTTTCATTTGTTGT
 S K S F R C T K L E I V I C K M E H Q T
 3961 GTCTCCAGCAAAATGCAATCAGGTTTCCGCAACCCTAGGTTCACTTCAAACATGACT
 E L L C I C D P K R L G L N V E F M V V
 4021 ACAATTTGCCCCCGCAGGTTTGCAATTGGGGAATTATCGTATAGGCCAGCCTTCCGTCT
 I K G G A P K C Q P I I T Y A L R G D G
 4081 CCACCCCTTCAAAGACTTCTCCAGTGATCTGACGAGAGCTCGGTAAAGCGATTATGG
 G G E F V E L S R V L A R Y F R N H C

FIG 7I

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

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

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

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

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

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

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

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

FIG 7J

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

 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 TTTAATGGATTGGATATTGCGAATTCGGAACAGGCACGGCGTATAATACAAAGGGCGGCCAGG

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

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

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

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

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

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

FIG 7K

K C L E F A E T A S S L T T K R A A I A
5161 GAAATGTTTAGAGTTTGCCGAGACGGCAAGTTCTCTTACAACCAACGAGCGGCGATCGC
S L I D A V E R Y N A D M G S *
5221 GAGCTTAATTGACGCAGTAGAGCGCTACAATGCTGATATGGGTTCCGTAATGTTCCGCTTC
M S F T H F L A L Y S F L
5281 CATAATCCTTCACAAATAAGAGTATGTCCTTTACTCATTTCCCTTGCTTGTACTCATTTCTT
L E R A W L H Q Q P A P M G H A R E I F
5341 ACTCGAGAGCGGTGGCTTCACCAAGCAACCCGCCGATGGGACACGCGAGAGAAATATT

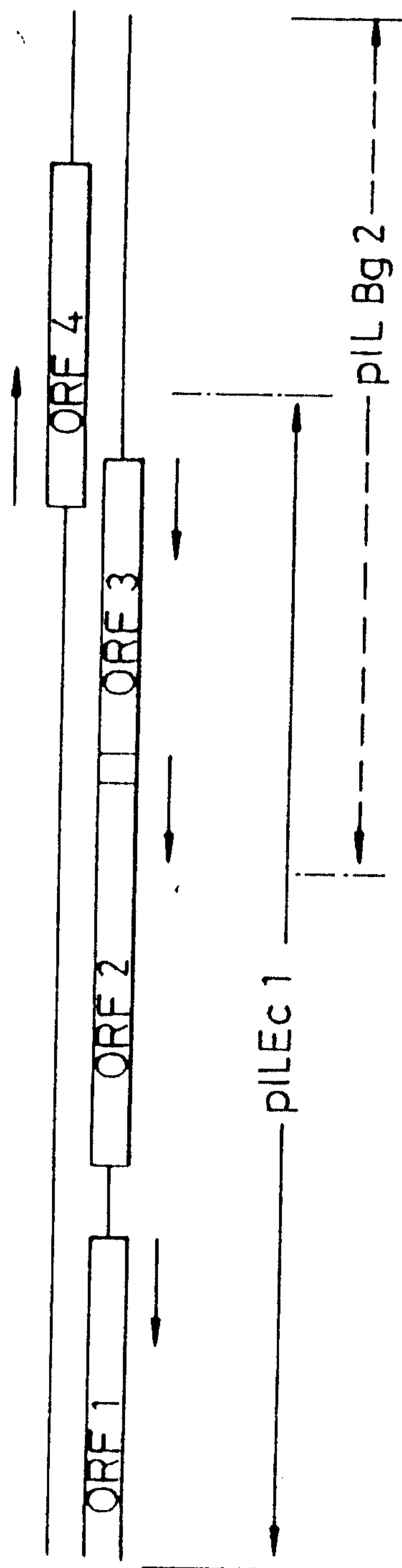


Fig. 8

Figure 9 ILTV gB

V L I S N W R Q C C R R A Q P R H C H R
CGTGCTAATCTCCAATTGGCGCCAGTGTGACGGCGGCCAGCCACGACATTGTCACCCG
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
ACATGCCGAGTCGACTAACATGACTGAAGGAAGGCCGTAGTCTTCAAGCAAAACATTGC
70 80 90 100 110 120

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

1 3 4 1 3 0 7

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
GCAACGGCGCCGCAACCAACAGGGCTTGTGTTTATTCGAGCCCATGGCATTCGACAC
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
ATTAAAGATTCTGAAAATTCTGGCAGCTCGGCCGGAATGAAGAGTTCAGATGCGATAA
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
CATTTTACTGCCATTGGATGCCCTGATCTTTTATGAAAAGACTGCAGCAGCCAGGAAG
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
TCGAATGTGGTGGGATACATGTGCGGGAACACGGTACTCC
10 20 30 40

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

E G T S E T M A E L T V A
CGAGGGAACGTCCGAACTATGGCGGAGCTTACTGTGCT
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
GGCATTCGCTACAATCCTGCAGAAAGATAATGATAGTCGGCTGGTAATTATACGCC
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
CAAGTGACCGAGGTTAGCGAAGGATTGCCCCCTTTGTTCA
10 20 30 40

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

R P N S Q L M R E L R Q I
TAGACCAACAGTCAATTAAATGCCGGAGCTGAGACAAATA
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
CATCTGGCCGAGAACGTAACAGATCGATGGGCATCGGCG
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
TTTGAATTCATGTTATTGGAGGCGGATGACAGTTAGTTGCG
170 180 190 200

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

FIG 13B

N S Y Y A R G R L H F D G
TAACAGTTATTATGCACGAGGACGCTCTGCATTTCGATGGG
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
CCGCGACAGACTACTGAAAGAAATAGATATGCCACCTATT
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
TGACAGCTTCAAAATAATACCAACTTCTTTGAGAGAAGAAA
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
CCCATTCCTCGTCTATGTAGAGGAAATGAAAGATTATGCCA
10 20 30 40

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

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

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

Q K F Q S S Y D S P G A A V
AGAAATTCAGAGTTTCATACGATTCCCGGAGCGGCTGT
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
TTGTCCCTCTACCATGGAAACCCGATGTCGCTCGGTTATGT
50 60 70 80

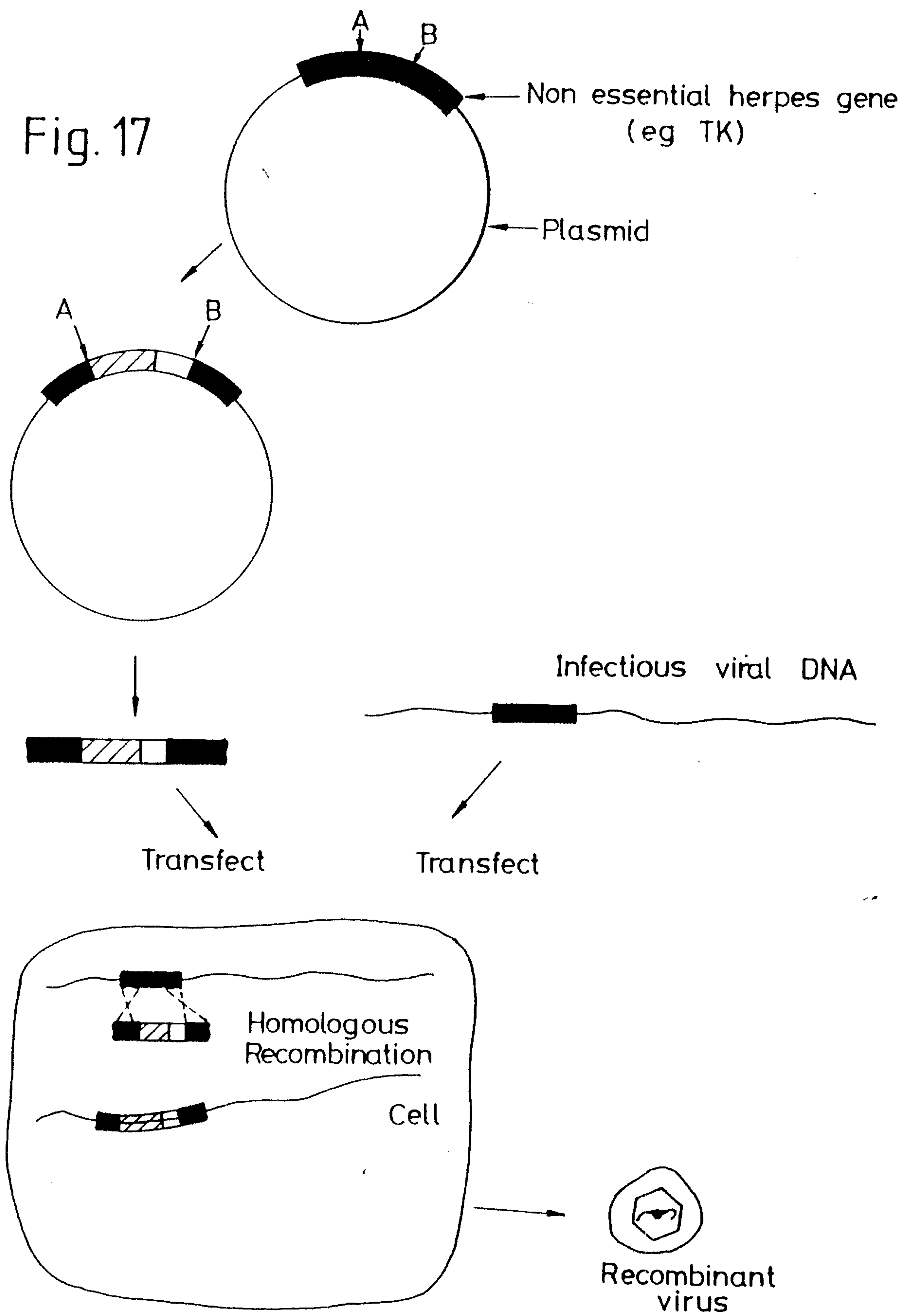
A D T N K L F R C F I R C R
GCGGATACAAACAACACTATTTCGATGTTTATTCGATGTC
90 100 110 120

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
ACTATTCGATATTCAATATGATTTGGTCGAATGGGATATCGACTAAA
170 180 190 200

66/66

Fig. 17



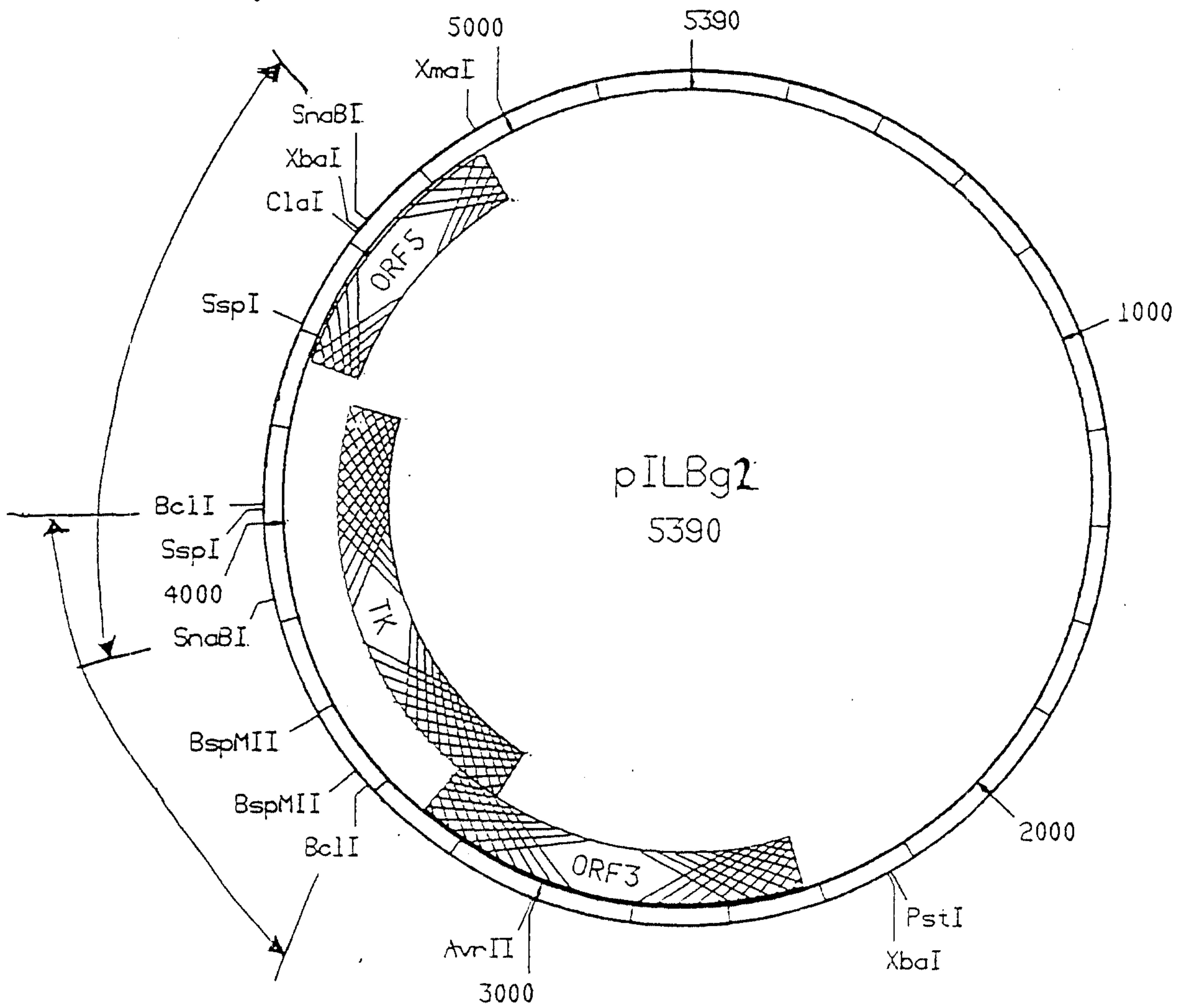


Fig. 18