



(86) Date de dépôt PCT/PCT Filing Date: 1998/02/25

(87) Date publication PCT/PCT Publication Date: 1998/09/17

(45) Date de délivrance/Issue Date: 2010/11/16

(85) Entrée phase nationale/National Entry: 1999/09/09

(86) N° demande PCT/PCT Application No.: US 1998/003710

(87) N° publication PCT/PCT Publication No.: 1998/040500

(30) Priorité/Priority: 1997/03/12 (US08/815,809)

(51) Cl.Int./Int.Cl. *C12N 15/63* (2006.01),
A61K 39/12 (2006.01), *A61K 39/285* (2006.01),
C12N 15/67 (2006.01), *C12N 15/85* (2006.01)

(72) Inventeurs/Inventors:

TARTAGLIA, JAMES, US;
JACOBS, BERTRAM L., US;
GOEBEL, SCOTT J., US;
COX, WILLIAM I., US;
GETTIG, RUSSELL ROBERT, US;

...

(73) Propriétaires/Owners:

ARIZONA STATE UNIVERSITY, US;
CONNAUGHT TECHNOLOGY CORPORATION, US

(74) Agent: SMART & BIGGAR

(54) Titre : VECTEURS PRESENTANT UNE EXPRESSION AMELIOREE, PROCEDES DE REALISATION ET
UTILISATIONS DESDITS VECTEURS

(54) Title: VECTORS HAVING ENHANCED EXPRESSION AND METHODS OF MAKING AND USES THEREOF

(57) **Abrégé/Abstract:**

Disclosed and claimed are vectors having enhanced expression and methods for making and using them. Enhancement of expression is from substantially co-temporal expression of at least one first nucleic acid molecule and at least one second nucleic acid molecule. The second nucleic acid molecule encodes a translation factor. The contemporaneous expression can be from operably linking the first and second nucleic molecules to a single promoter, or from operably linking the first nucleic acid molecule to a first promoter and the second nucleic molecule to a second promoter wherein the first and second promoters function substantially contemporaneously. Thus, the first and second nucleic acid molecules can be at the same locus in the vector or at different loci. The second nucleic acid molecule can encode one translation factor or more than one translation factor. The translation factor can be a K3L open reading frame, an E3L open reading frame, a VAI RNA, and EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, or combinations thereof. The vector can be a poxvirus such as an attenuated poxvirus; for instance, a NYVAC vaccinia virus or an ALVAC canarypox virus.

(72) Inventeurs(suite)/Inventors(continued): PINCUS, STEVEN E., US; PAOLETTI, ENZO, US

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/63, A61K 39/12, 39/285	A1	(11) International Publication Number: WO 98/40500 (43) International Publication Date: 17 September 1998 (17.09.98)
(21) International Application Number: PCT/US98/03710 (22) International Filing Date: 25 February 1998 (25.02.98) (30) Priority Data: 08/815,809 12 March 1997 (12.03.97) US (71) Applicants: VIROGENETICS CORPORATION [US/US]; Rensselaer Technology Park, 465 Jordan Road, Troy, NY 12180 (US). ARIZONA STATE UNIVERSITY [US/US]; Dept. of Microbiology, P.O. Box 872701, Tempe, AZ 85287-2701 (US). (72) Inventors: TARTAGLIA, James; 7 Christina Drive, Schenec- tady, NY 12303 (US). JACOBS, Bertram, L.; 522 West Lynwood Street, Phoenix, AZ 85003 (US). GOEBEL, Scott, J.; 19 Glade Mallow Road, Ballston Spa, NY 12020 (US). COX, William, I.; 1379 Maple Trail, Sand Lake, NY 12153 (US). GETTIG, Russell, Robert; R.D. 2, Box 421C, Averill Park, NY 12018 (US). PINCUS, Steven, E.; 78 Troy Road, East Greenbush, NY 12061 (US). PAOLETTI, Enzo; 297 Murray Avenue, Delmar, NY 12054 (US). (74) Agents: FROMMER, William, S. et al.; 745 Fifth Avenue, New York, NY 10151 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: VECTORS HAVING ENHANCED EXPRESSION AND METHODS OF MAKING AND USES THEREOF (57) Abstract Disclosed and claimed are vectors having enhanced expression and methods for making and using them. Enhancement of expression is from substantially co-temporal expression of at least one first nucleic acid molecule and at least one second nucleic acid molecule. The second nucleic acid molecule encodes a translation factor. The contemporaneous expression can be from operably linking the first and second nucleic molecules to a single promoter, or from operably linking the first nucleic acid molecule to a first promoter and the second nucleic molecule to a second promoter wherein the first and second promoters function substantially contemporaneously. Thus, the first and second nucleic acid molecules can be at the same locus in the vector or at different loci. The second nucleic acid molecule can encode one translation factor or more than one translation factor. The translation factor can be a K3L open reading frame, an E3L open reading frame, a VAI RNA, and EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, or combinations thereof. The vector can be a poxvirus such as an attenuated poxvirus; for instance, a NYVAC vaccinia virus or an ALVAC canarypox virus.		

77396-24

1

TITLE OF THE INVENTION**VECTORS HAVING ENHANCED EXPRESSION
AND METHODS OF MAKING AND USES THEREOF****FIELD OF THE INVENTION**

5 The present invention relates to enhanced vectors, and methods for making and using them. The vectors can have enhanced translation and/or expression, e.g., translation and/or expression from a nucleotide sequence of interest.

10 Several publications are referenced in this application. Full citation to these publications is found where cited or at the end of the specification, immediately preceding the claims or where the publication is mentioned. These publications relate to the state of the art to which
15 the invention pertains; however, there is no admission that any of these publications is indeed prior art.

BACKGROUND OF THE INVENTION

DNA such as plasmids or naked DNA, and other vectors, such as viral vectors, e.g., vaccinia virus and
20 more recently other poxviruses, have been used for the insertion and expression from foreign genes. The basic technique of inserting foreign genes into live infectious poxvirus involves recombination between pox DNA sequences flanking a foreign genetic element in a donor plasmid and
25 homologous sequences present donor plasmid and homologous sequences present in the rescuing poxvirus (Piccini et

77396-24

2

al., 1987). Recombinant poxviruses are constructed in steps known as in or analogous to methods in U.S. Patent Nos. 4,769,330, 4,772,848, 4,603,112, 5,505,941, and 5,494,807. A desire in

5 vector development is attenuated vectors, e.g., for enhanced safety; for instance, so that the vector may be employed in an immunological or vaccine composition.

For instance, the NYVAC vector, derived by deletion of specific virulence and host-range genes from
10 the Copenhagen strain of vaccinia (Tartaglia et al., 1992) has proven useful as a recombinant vector in eliciting a protective immune response against an expressed foreign antigen. Likewise, the ALVAC vector, a vaccine strain of canarypox virus, has also proven
15 effective as a recombinant viral vaccine vector (Perkus et al., 1995). In non-avian hosts, both these vectors do not productively replicate (with some exceptions as to NYVAC). Since all poxviruses replicate in the cytoplasm and encode most, if not all of the proteins required for
20 viral transcription (Moss 1990), appropriately engineered foreign coding sequences under the control of poxvirus promoters are transcribed and translated in the absence of productive viral replication.

It would be an improvement over the state of
25 the art to provide enhanced vectors, e.g., vectors having enhanced transcription or transcription and translation and/or expression, for instance such vectors which are attenuated; especially since attenuation may raise issues of expression levels and/or persistence, and it would be
30 an advancement to address such issues.

OBJECTS AND SUMMARY OF THE INVENTION

Recent studies on vaccinia replication have revealed certain poxvirus-encoded functions which play a role in the regulation of viral transcription and
35 translation (reviewed in Moss, 1990; Moss, 1992). Some of these vaccinia encoded functions (e.g., K3L, E3L, and combinations thereof) have now surprisingly been utilized

to increase the levels and persistence of gene expression (e.g., foreign gene expression) in vectors (e.g., the ALVAC vectors); and, are exemplary of the inventive vectors and methods.

5 Objects of the present invention may include at least one of: providing a method for increasing translation and/or expression from at least one nucleotide sequence of interest by a vector, such as a coding nucleotide sequence by a vector; a vector having
10 enhanced translation; providing a method for preparing a vector having enhanced translation and/or expression; providing a method for enhancing translation and/or expression from a vector; providing an improved vector, such as poxvirus vectors, e.g., improved NYVAC, ALVAC or
15 TROVAC vectors; and, products therefrom.

The invention therefore provides a vector for enhanced expression of at least one first nucleic acid molecule in a cell, said vector comprising the first nucleic acid molecule operably linked to a first promoter and at least one second nucleic acid
20 molecule operably linked to a second promoter and encoding a translation factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by
25 enhancing translation. Preferably the vector is employed in a cell in which enhanced translation results from the translation factor.

The first nucleotide sequence can be operably linked to a first promoter and the second nucleotide sequence can be operably linked to a second promoter, and
30 the first and second promoters are preferably functional substantially co-temporally or contemporaneously. Thus, the first and second nucleotide sequences can be at different loci within the vector. The first and second nucleotide sequences also can be at the same locus within
35 the vector, using the first and second promoters; or, by the first nucleotide sequence and the second nucleotide

77396-24

4

sequence being operably linked to a promoter.

The translation factor can effect inhibition of eIF-2 α phosphorylation or inhibition of PKR phosphorylation or otherwise sequesters dsRNA leading to an increase of the effective concentration of dsRNA. The second nucleotide sequence can be from the group consisting of: a K3L open reading frame, an E3L open reading frame, a viral associated RNA I (VAI), an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, and combinations thereof.

The first nucleotide sequence can be selected from the group of sequences encoding an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene and a fusion protein.

The vector can be a recombinant virus, such as a poxvirus; for instance, an orthopoxvirus or an avipoxvirus, e.g., a vaccinia virus, a fowlpox virus, a canarypox virus; preferably an attenuated virus such as an attenuated poxvirus, e.g., NYVAC, ALVAC, or TROVAC.

The invention further provides a method for preparing a vector as defined herein comprising modifying a vector to comprise the at least one second nucleic acid molecule operably linked to a second promoter and the first nucleic acid molecule operably linked to a first promoter, so that there is co-temporal expression of the first and second nucleic acid molecules. Preferably the vector is so modified that there is substantially co-temporal or contemporaneous expression of the first and second nucleotide sequences; and, more preferably, the vector is also so modified that the translation factor is with respect to the cell in which the vector is to be employed.

The method can comprise operably linking the first nucleotide sequence to a first promoter and the second nucleotide sequence to a second promoter, wherein the first and second promoters are functional substantially co-temporally or contemporaneously. The

method can also comprise operably linking the first and second nucleotide sequences to a promoter.

The invention further provides an immunological, vaccine or therapeutic composition comprising at least one inventive vector and a pharmaceutically acceptable carrier or diluent.

The invention even still further provides a method for generating an immunological or therapeutic response in a host (animal, human, vertebrate, mammal, etc.) comprising administering to the host at least one inventive composition.

The invention additionally provides a method for increasing expression from at least one first nucleotide sequence by a vector comprising the first nucleotide sequence. The method comprises modifying the vector to comprise at least one second nucleotide sequence encoding a translation factor. There is preferably substantially co-temporal or contemporaneous expression of the first and second nucleotide sequences. Expression can be in a cell; and it is more preferred to have the translation factor expressed in a cell in which there is enhancement from expression of the particular translation factor. Expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing translation. The method can additionally comprise modifying the vector to comprise the first nucleotide sequence.

The invention in yet another embodiment provides a method for expressing at least one gene product in vitro comprising infecting, or transfecting, a suitable cell with at least one inventive vector. The products therefrom can be an epitope of interest, which can be useful in formulating therapeutic, immunological or vaccine compositions; or, for generating antibodies such as monoclonal antibodies; or, in assays, kits, tests and the like, such as diagnostic compositions, e.g., for detection of antibodies.

Thus, the invention can provide compositions and methods for *in vitro* translation and/or expression involving at least one inventive vector, e.g., methods for producing a gene product (which can be used as an antigen or epitope in a therapeutic, immunological or vaccine composition, or in a diagnostic or detection kit, assay or method, e.g., to ascertain the presence or absence of antibodies, or to generate antibodies, such as monoclonal antibodies, e.g., for use in a diagnostic or detection kit, assay or method), and/or for *ex vivo* translation and/or expression involving at least one inventive vector, e.g., methods for producing a gene product for stimulating cells for reinfusion into a host (e.g., animal, mammal, vertebrate, human).

Additionally, in a further embodiment the invention provides a method for expressing at least one nucleotide sequence (e.g., the at least one first nucleotide sequence) *in vivo* comprising administering at least one inventive vector to a host (human, animal, vertebrate, mammal, etc.). The nucleotide sequence can encode an epitope of interest. The method can obtain antibodies. From generating antibodies one can generate monoclonal antibodies; or, antibodies are useful in assays, kits, tests or diagnostic compositions, e.g., for detection of antigens.

The invention can thus provide methods and compositions for *in vivo* translation and/or expression involving the inventive vectors, e.g., administering at least one inventive vector or a composition comprising at least one inventive vector, for instance, therapeutic, immunological or vaccine compositions comprising at least one inventive vector and a suitable carrier or diluent (e.g., suitable for veterinary and human medicine).

77396-24

6a

The invention additionally provides use of the composition as defined herein for generating an immunological or therapeutic response in a host.

The invention additionally provides an *in vitro* method of increasing expression of at least one first nucleic acid molecule in a cell, by a vector comprising the first nucleic acid molecule operably linked to a first promoter, comprising modifying the vector to comprise at least one second nucleic acid molecule operably linked to a second promoter encoding a translation factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing translation.

The invention also provides use, for increasing expression of at least one first nucleic acid molecule in a cell, of a vector comprising the first nucleic acid molecule operably linked to a first promoter, and at least one second nucleic acid molecule operably linked to a second promoter encoding a translation factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing translation.

The invention also provides use in the preparation of a medicament for increasing expression of at least one first nucleic acid molecule in a cell, of a vector comprising the first nucleic acid molecule operably linked to a first promoter, and at least one second nucleic acid molecule operably linked to a second promoter encoding

77396-24

6b

a translation factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing translation.

The invention additionally provides a method of expressing a gene product *in vitro* comprising infecting, or transfecting, a suitable cell with a vector as defined herein, wherein the gene product is encoded by the first nucleic acid molecule.

The invention additionally provides use of the vector as defined herein in the manufacture of a medicament for generating an immunological or therapeutic response in a host.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

77396-24

7

BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, in which:

Figs. 1A and 1B show the nucleotide sequence of the ALVAC C6 insertion site containing the H6/K3L and E3L expression cassette (SEQ ID NO: 1);

Fig. 2 shows the DNA sequence of the coding region of FHV gB with modified T5NT motifs (SEQ ID NO: 2);

Figs. 3A to 3C show the DNA sequence of the H6 promoted FHV gB donor plasmid pC3H6FHVB (SEQ ID NO: 3);

Figs. 4A to 4C and 5A to 5G show DNA and amino acid sequences (SEQ ID NOs: 4, 5 and 6) of inserts in vCP1433 and vCP1452; and

Figs. 6A to 6H show the DNA sequence (SEQ ID NO: 7) of K3L E3L in vCP1452.

DETAILED DESCRIPTION

U.S. Patent No. 5,494,807, to Paoletti et al. relates to a modified recombinant virus having inactivated virus-encoded genetic functions so that the recombinant virus has attenuated virulence and enhanced safety. The viruses disclosed in Paoletti et al. can be poxviruses, e.g., a vaccinia virus or an avipox virus, such as fowlpox virus and canarypox virus, e.g., NYVAC, ALVAC and TROVAC. ALVAC was deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, USA, ATCC accession number VR-2547. TROVAC was likewise deposited under the terms of the Budapest Treaty with the ATCC, accession number 2553. And, vCP205, vCP1433, pMPC6H6K3E3 and pC3H6FHVB were also deposited with the ATCC under the terms of the Budapest Treaty, accession numbers VR-2557, VR-2556, 97912 and 97914, respectively, on March 6, 1997.

Like the Paoletti et al. issued U.S. Patent,

77396-24

8

Falkner et al., WO 95/30018, published November 9, 1995, relates to

poxviruses wherein loci for genetic functions associated with virulence (i.e., loci for "essential" functions) are
5 employed for insertion of exogenous DNA.

Further, recombinants can be made from early (DNA) and late defective mutants (see Condit and Niles, "Orthopoxvirus Genetics," pp 1-39, In: Poxviruses, Edited by R. W. Moyer and P. C. Turner (Springer-Verlag, 1990),
10 and documents cited therein), or from MVA which is said to be abortive late. Recombinants from defective mutants, abortive late viruses, viruses having essential genetic functions deleted or interrupted, or viruses having expression
15 without productive replication (e.g., ALVAC in mammalian systems) may be said to be attenuated. It would be useful to increase foreign gene expression, e.g., levels of foreign gene expression or persistence of expression, in vectors, especially attenuated vectors.

20 A means to increase foreign gene expression involves enhancing the overall efficiency of translation, e.g., mRNA translation, such as viral mRNA translation. Two vaccinia encoded functions (E3L and K3L) have recently been identified as playing a role in the
25 regulation of viral translation (Beattie et al., 1995a, 1995b, 1991; Chang et al., 1992; Davies et al., 1993). Both are capable of inhibiting the action of a cellular protein kinase (PKR) which, when activated by double stranded RNA (dsRNA), phosphorylates the translational
30 initiation factor eIF-2 α , leading to an inhibition of initiation of mRNA translation (reviewed in Jacobs and Langland, 1996). Vaccinia virus, which produces dsRNA during viral transcription, has thus evolved mechanisms to block the negative action of PKR on eIF-2 α and allow
35 for efficient translation of viral mRNA. (Asymmetric transcription gives rise to dsRNA; any viral or plasmid-

borne expression gives rise to it; dsRNA activates PKR and PKR becomes autophosphorylated, leading to phosphorylation of eIF-2 α .)

The vaccinia K3L ORF has been shown to have
5 significant amino acid homology to eIF-2 α (Goebel et al., 1990; Beattie et al., 1991; U.S. Patent No. 5,378,457; see also Beattie et al., 1995a, 1995b). This protein is believed to act as a pseudosubstrate for PKR and competes for the eIF-2 α binding site (Carroll et al., 1993; Davies
10 et al., 1992). The K3L gene product can bind to activated PKR and thus prevent phosphorylation of eIF-2 α with its resultant negative effect on translation initiation.

The vaccinia E3L gene codes for a protein which
15 is capable of specifically binding to dsRNA (Watson and Jacobs, 1991; Chang et al., 1992). This would tend to lower the amounts of dsRNA in the infected cell, and thus reduce the level of activated PKR. When E3L was deleted from vaccinia, the resulting virus lost this kinase
20 inhibitory function and further allowed activation of the 2' 5' oligoadenylate synthetase/RNase L pathway resulting in increased degradation of rRNA (Beattie et al., 1995a, 1995b). Thus, E3L appears to be critical for efficient mRNA translation in vaccinia infected cells at two
25 levels; mRNA stability and limiting eIF-2 α phosphorylation.

The ALVAC genome has been sequenced and searched for any homology to E3L/K3L or to any known dsRNA binding protein. Results have revealed no
30 significant homology of any ALVAC ORFs to these two vaccinia ORFs, nor the presence of any dsRNA binding motifs.

Thus, an approach to improving expression levels in recombinant ALVAC vectors was to express the
35 vaccinia E3L/K3L ORFs in ALVAC under the control of early vaccinia promoters. Through inhibition of PKR in the infected cells, the levels and persistence of foreign

gene expression could be enhanced.

Hence, ALVAC recombinants as discussed herein were generated in order to enhance foreign gene expression at the transcriptional or transcriptional and translational levels, as examples of the vectors and methods of the present invention.

Thus, exemplified herein is ALVAC recombinants having expression from the vaccinia E3L/K3L genes for enhancing or increasing the levels or persistence of an inserted foreign gene. The up-regulation of foreign gene expression can have a profound effect on the induction of a therapeutic or immunological response in a host administered or inoculated with recombinants derived from these new vectors, thereby leading to an enhanced immunological, e.g., protective, response, or an enhanced therapeutic response.

The scope of the invention, i.e., to manipulate expression from any of E3L and K3L to thereby enhance translational and/or expression efficiency, can be extended to other eukaryotic vector systems (i.e. DNA, viruses).

In fact, viruses in other families have also evolved mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation. In adenoviruses, the VAI RNA, transcribed by RNA pol III, has been well characterized and shown to bind directly to PKR, and thus, prevent its activation by dsRNA (Mathews and Shenk, 1991). Deletion of VAI from the adenovirus genome results in a mutant that replicates poorly and is deficient in levels of late gene expression (Thimmappaya et al., 1982). Similarly, Epstein-Barr virus, a herpesvirus, has an analogous RNA, called EBER, which also acts to prevent PKR activation by directly binding to the kinase (Clark et al., 1991; Sharp et al., 1993). The reovirus sigma 3 gene product has been shown to act in a similar manner as vaccinia E3L in binding dsRNA and thus preventing activation of PKR (Imani and

Jacobs, 1988; see also Beattie et al. 1995a). Indeed, one study has shown that the reovirus sigma 3 gene can partially compensate a vaccinia recombinant deleted of E3L (Beattie et al., 1995a). Further, a cellular protein
5 activated upon HIV infection (TRBP) has been shown to inhibit the activity of PKR (Park et al., 1994).

Thus, the present invention broadly relates to manipulation of expression, preferably by employing at least one one translation factor, e.g., a nucleotide
10 sequence encoding a product for overcoming the cellular anti-viral response of translational down-regulation through PKR activation in any eukaryotic vector system; for instance, to increase or enhance expression. And, the invention can pertain to any vector system,
15 including, plasmid or naked DNA vectors, viral vectors, such as poxvirus, adenovirus, herpesvirus, baculovirus, and the like. Thus, the nucleotide sequences can be RNA or DNA, for instance, as is suitable in view of the vector system.

20 Accordingly, the invention can relate to a vector modified to comprise at least one nucleotide sequence encoding at least one translation factor; a method for increasing translation and/or expression by a vector or for preparing an inventive vector, e.g., by
25 modifying the vector to comprise the at least one nucleotide sequence.

These methods can include substantially co-temporal expression from: (i) a first nucleotide sequence comprising at least one nucleotide sequence of interest,
30 and (ii) a second nucleotide sequence comprising at least one nucleotide sequence encoding a translation factor. The vector also can be modified to comprise the at least one nucleotide sequence of interest. The at least one nucleotide sequence of interest can be at least one
35 coding nucleotide sequence. The vector preferably has substantially co-temporal or contemporaneous expression of the first and second nucleotide sequences.

The substantially co-temporal expression can occur by employing promoters for the first and second nucleotide sequences which are functional at approximately the same time or stage of infection. Thus, 5 the nucleotide sequence of interest and the nucleotide sequences encoding the factor(s) can be positioned at different loci in the vector. Alternatively, substantially co-temporal expression can occur by positioning the first and second nucleotide sequences 10 within the same loci. Thus, substantially co-temporal expression can occur by operably linking to the nucleotide sequence of interest and/or to a promoter operably linked to the nucleotide sequence of interest, a nucleotide sequence encoding a translation factor.

15 The translation factor can be from any suitable system. Preferably the translation factor can effect inhibition of eIF-2 α phosphorylation or inhibition of PKR phosphorylation or otherwise decreases cellular dsRNA content which increases the effective concentration of 20 dsRNA. The translation factor can be selected from expression from the group consisting of: a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, a homolog thereof, and combinations 25 thereof. Thus, at least one nucleotide sequence encoding a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, or homologs thereof, or combinations thereof, can be used in the practice of the 30 invention. The term "effective" with respect to dsRNA concentration means the amount of dsRNA to activate PKR and/or eIF-2 α phosphorylation (the dsRNA being in a form therefor). With respect to RNA-based factors, the skilled artisan, without undue experimentation, can 35 obtain suitable DNA therefrom, for use in DNA-based vector systems; and, as to DNA-based factors, the skilled artisan can obtain RNA therefrom for use in RNA-based

vector systems.

The term "substantially co-temporal expression" or the term "substantially contemporaneous expression" means that the nucleotide sequence(s) encoding the translation factor(s) are expressed during approximately the same stage of infection as is the at least one nucleotide sequence of interest.

For instance, poxvirus genes are regulated in a temporal manner (Coupar, et al., Eur. J. Immunol., 1986, 16:1479-1487, at 1479). Thus, immediately after infection, a class of "early" genes is expressed (Id.). "Early genes" cease being expressed (i.e., early promoters cease functioning) at a time after infection prior to the "later" stage of infection (DNA replication commencement). The thymidine kinase ("TK") gene and TK promoter is an example of an immediate "early" gene and promoter (Hruby et al., J. Virol., 1982, 43(2):403-409, at 403). The TK gene is switched "off" about four hours after infection. "Late genes" are a class of genes not expressed until DNA replication has commenced (Coupar et al., *supra*). The PL11 promoter employed by Coupar et al. is an example of a "late" promoter. Thus, in Coupar et al., HA gene expression regulated by the PL11 promoter was not until after DNA replication, despite being in the TK region.

In contrast to canonical "early" genes and "late" genes the 7.5 kD gene and 7.5 kD promoter, is an example of an "early and late" gene and promoter. An "apparent exception to regulated transcription" (Davison and Moss, "Structure of Vaccinia Virus Early Promoters" J. Mol. Biol., 210-69, 249-69 (1989) at 749), the 7.5 kD promoter "contains regulatory signal for both early and late transcription" (Coupar et al., *supra*). Indeed, there are "independent early and late RNA start sites within the promoter region of the 7.5-kD gene" (Cochran et al., J. Virol., 59(1): 30-37 (April, 1985).

Coupar et al. observed "that temporal

regulation of HA expression by the promoters PF [early], P7.5 [early and late] and PL11 [late] was maintained when the promoters were transposed to interrupt the TK gene of [vaccinia virus]" (Id., at 1482). That is, Coupar et al. 5 observed that foreign gene expression under the control of an early vaccinia promoter occurred "early", foreign gene expression under control of a late vaccinia promoter occurred "late", and foreign gene expression under the control of the early and late vaccinia 7.5 kD promoter 10 occurred both early and late (See also id. at 1479: "[p]romoter sequences transposed to within the thymidine kinase (TK) gene continue to function in a temporally regulated manner" (citations omitted)).

Thus, the nucleotide sequence(s) encoding the 15 translation factor(s) can be under the control of a first type of promoter and the at least one nucleotide sequence of interest or the coding nucleotide sequence can be under the control of a second type of promoter, wherein the first and second promoters are both early, both late 20 (including intermediate), or both early and late; or, the first promoter can be early or late and the second promoter early and late; or the first promoter can be early and late and the second promoter early or late. The nucleotide sequence of interest and the nucleotide 25 sequence(s) encoding the translation factor(s) can be at the same locus or at different loci; or under the control of the same promoter.

Accordingly, the invention can relate to a method for preparing a vector having enhanced translation 30 and/or expression, or to a method for increasing or enhancing translation and/or expression in a vector comprising operably linking to at least one nucleotide sequence of interest, or to a promoter operably linked thereto, at least one nucleotide sequence for at least 35 one at least one translation factor. Preferably the translation factor effects an inhibition of eIF-2 α phosphorylation and/or effects an inhibition of

phosphorylation of PKR and/or a cellular kinase responsible for phosphorylation of eIF-2 α and/or effects a decrease in the effective concentration of dsRNA. The invention also can thus relate to vectors from such methods.

Alternatively, the inventive methods can comprise operably linking at least one nucleotide sequence of interest to a first type of promoter and operably linking at least one second nucleotide sequence encoding at least one translation factor to a second type of promoter, within a vector, wherein the first and second promoters are both functional at the same time or same stage of infection, e.g., the first and second promoters are both early, both late (including intermediate), or both early and late; or, the first promoter is early or late and the second promoter is early and late; or the first promoter is early and late and the second promoter is early or late. Of course, the first and second promoters can be the same promoter at two or more different loci, or the same promoter at one locus. And, the invention thus relates to vectors from such methods.

And, the term "nucleotide sequence" as used herein can mean nucleic acid molecule. Thus, a nucleotide sequence can be an isolated nucleic acid molecule, e.g., exogenous DNA.

Accordingly, the present invention can provide vectors modified to contain at least one exogenous nucleotide sequence, preferably encoding at least one epitope of interest, and at least one translation factor, wherein there is substantially temporal co-expression (or substantially co-temporal expression or substantially contemporaneous expression) of the exogenous nucleotide sequence and the factor(s); and, methods for making and using such vectors and products therefrom. Enhanced or improved expression is obtained by the vectors and methods of the invention; and, enhanced or improved

expression can mean enhanced levels and/or persistence of expression.

The invention can provide vectors, for instance, poxvirus vectors, e.g., NYVAC, ALVAC or TROVAC
5 recombinants, having expression from the vaccinia E3L and/or K3L (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus, plasmid or naked DNA, and the like, note discussion supra of viral
10 mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation) as a means for enhancing and/or increasing the levels and persistence of an inserted nucleotide sequence, e.g., a foreign gene.

15 As shown in the Examples below, ALVAC-HIV recombinant vCP1452 containing the K3L/E3L factors had enhanced expression on human cells in comparison to vCP1433 or vCP300. Indeed, enhanced expression is observed with the E3L/K3L translational factors in human
20 and canine cells.

Enhanced expression by translational factors such as E3L/K3L may be cell type dependent. For instance, while enhanced expression with E3L/K3L is observed in human and canine cells it is not observed in
25 murine and feline cells. From this disclosure and the knowledge in the art, the skilled artisan can select an appropriate translational factor for use with a particular cell type, without undue experimentation. For example, it should go without saying that the skilled
30 artisan knows the differences between cells. Thus it is preferred that the translational factor be expressed in a cell in which enhanced expression is observed, e.g., that the translational factor employed be with respect to the cell.

35 Further, preliminary immunogenicity studies in mice show no evidence of enhanced immunogenicity by the E3L/K3L translational factor. This corresponds to no

observed enhanced expression in murine cells. Accordingly, the skilled artisan from this disclosure and the knowledge in the art can select a translational factor which will provide enhanced immunogenicity in a
5 desired animal, without undue experimentation. If enhanced expression is observed *in vitro* in a particular cell line by a particular translational factor, e.g., E3L/K3L in human or canine cells, the skilled artisan can thus expect enhanced immunogenicity *in vivo* in the animal
10 (including human) from which the cells were derived by that particular translational factor, e.g., enhanced immunogenicity in humans and canines from the E3L/K3L translational factor.

In an abortive early system such as ALVAC or
15 NYVAC, one preferably expresses exogenous DNA and the translational factor(s) early; in an abortive late (including intermediate) system, one preferably expresses exogenous DNA and the translational factor late or early and late (as expression only early may may not
20 necessarily obtain optimal expression).

The selection of a suitable translation factor and time for its expression is within the ambit of the skilled artisan from this disclosure and knowledge in the art; for instance, the skilled artisan can select
25 expression of a translation factor based on the nature of the vector and of the promoter to be used with the factor; for example based on an abortive phenotype of the vector, e.g., MVA is said to be abortive late, and late (including intermediate) or early or early/late
30 expression from a translation factor may be employed with this vector; ALVAC is abortive early and early or early/late expression from a translation factor may be employed with this vector. The vector can also be a ts (temperature sensitive) mutant (with respect to early
35 (DNA) and late defective mutants which can be also used in the practice of this invention, reference is made to Condit and Niles, *supra*). Thus, the translation factor

77396-24

18

and the at least one nucleotide sequence of interest preferably are expressed early, late (including intermediate), or early/late, relative to the phenotype of the vector; and, preferably the translation factor
5 should be selected such that it indeed enhances translation and/or expression with respect to the cell type the vector is being employed in.

The methods for making a vector or recombinant can be by or analogous to the methods disclosed in U.S.
10 Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941, 5,338,683, 5,494,807, and 4,722,848, WO 95/30018, Paoletti, "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996, Moss, "Genetically engineered poxviruses for
15 recombinant gene expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996, Smith et al., U.S. Patent No. 4,745,051 (recombinant baculovirus), Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press
20 Inc.), Smith et al., "Production of Huma Beta Interferon in Insect Cells Infected with a Baculovirus Expression Vector," Molecular and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p. 2156-2165; Pennock et al., "Strong and Regulated Expression of *Escherichia coli* B-Galactosidase
25 in Infect Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573, EP Patent publication No. 265785, U.S. Patent No. 4,769,331 (recombinant herpesvirus), Roizman,
30 "The function of herpes simplex virus genes: A primer for genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996, Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA
35 93:11313-11318, October 1996, Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996, Frolov et al.,

77396-24

19

"Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996, Kitson et al., J. Virol. 65, 3068-3075, 1991; U.S. Patent Nos. 5,591,439, 5,552,143, Grunhaus et al., 1992,

5 "Adenovirus as cloning vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993, Ballay et al. EMBO Journal, vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April, 1990, Prevec et al., J. Gen Virol. 70, 429-434, PCT WO91/11525, Felgner et al. (1994), J. Biol. Chem. 269,

10 2550-2561, Science, 259:1745-49, 1993 and McClements et al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-

15 11420, October 1996, and U.S. Patents Nos 5,591,639, 5,589,466, and 5,580,859 relating to DNA expression vectors, *inter alia*.

20 As to the inserted nucleotide sequence in a vector of the invention, e.g., the foreign gene, the heterologous or exogenous nucleotide sequence, e.g., DNA, in vectors of the instant invention, preferably encodes an expression product comprising: an epitope of interest,

25 a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene or a fusion protein. With respect to these terms, reference is made to the following discussion, and generally to Kendrew, THE ENCYCLOPEDIA OF MOLECULAR BIOLOGY (Blackwell Science

30 Ltd., 1995) and Sambrook, Fritsch and Maniatis, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1982.

An epitope of interest is an immunologically relevant region of an antigen or immunogen or

35 immunologically active fragment thereof, e.g., from a pathogen or toxin of veterinary or human interest.

An epitope of interest can be prepared from an

antigen of a pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the pathogen, or from another antigen or toxin which elicits a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a canine distemper virus or measles or rinderpest antigen such as HA or F; a rabies glycoprotein, e.g., rabies glycoprotein G; an avian influenza antigen, e.g., turkey influenza HA, Chicken/Pennsylvania/1/83 influenza antigen such as a nudeoprotein (NP); a bovine leukemia virus antigen, e.g., gp51,30 envelope; a Newcastle Disease Virus (NDV) antigen, e.g., HN or F; a feline leukemia virus antigen (FeLV), e.g., FeLV envelope protein; RAV-1 env; matrix and/or preplomer of infectious bronchitis virus; a Herpesvirus glycoprotein, e.g., a glycoprotein from feline herpesvirus, equine herpesvirus, bovine herpesvirus, pseudorabies virus, canine herpesvirus, HSV, Marek's Disease Virus, or cytomegalovirus; a flavivirus antigen, e.g., a Japanese encephalitis virus (JEV) antigen, a Yellow Fever antigen, or a Dengue virus antigen; a malaria (*Plasmodium*) antigen, an immunodeficiency virus antigen, e.g., a feline immunodeficiency virus (FIV) antigen or a simian immunodeficiency virus (SIV) antigen or a human immunodeficiency virus antigen (HIV); a parvovirus antigen, e.g., canine parvovirus; an equine influenza antigen; an poxvirus antigen, e.g., an ectromelia antigen, a canarypox virus antigen or a fowlpox virus antigen; or an infectious bursal disease virus antigen, e.g., VP2, VP3, VP4.

An epitope of interest can be from an antigen of a human pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the pathogen, or from another antigen or toxin which elicits a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a measles virus antigen such as HA or F; a rabies glycoprotein, e.g.,

77396-24

21

rabies virus glycoprotein G; an influenza antigen, e.g., influenza virus HA or N; a Herpesvirus antigen, e.g., a glycoprotein of a herpes simplex virus (HSV), a human cytomegalovirus (HCMV), Epstein-Barr; a flavivirus antigen, a JEV, Yellow Fever virus or Dengue virus antigen; a Hepatitis virus antigen, e.g., HBsAg; an immunodeficiency virus antigen, e.g., an HIV antigen such as gp120, gp160; a Hantaan virus antigen; a *C. tetani* antigen; a mumps antigen; a pneumococcal antigen, e.g., PspA; a *Borrelia* antigen, e.g., OspA, OspB, OspC of *Borrelia* associated with Lyme disease such as *Borrelia burgdorferi*, *Borrelia afzelli* and *Borrelia garinii*; a chicken pox (varicella zoster) antigen; or a *Plasmodium* antigen.

Of course, the foregoing lists are intended as exemplary, as the epitope of interest can be derived from any antigen of any veterinary or human pathogen; and, to obtain an epitope of interest, one can express an antigen of any veterinary or human pathogen (such that the invention encompasses the exogenous or foreign nucleotide sequence(s) of interest encoding at least one antigen).

Since the heterologous DNA can be a growth factor or therapeutic gene, the inventive recombinants can be used in gene therapy. Gene therapy involves transferring genetic information; and, with respect to gene therapy and immunotherapy, reference is made to U.S. Patent No. 5,252,479, together with the documents cited in it and on its face, and to WO 94/16716, together with the documents cited therein. The growth factor or therapeutic gene, for example, can encode a disease-fighting protein, a molecule for treating cancer, a tumor suppressor, a cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, be selected from the group consisting

of a gene encoding alpha-globin, beta-globin, gamma-globin, granulocyte macrophage-colony stimulating factor, tumor necrosis factor, an interleukin, macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53, retinoblastoma, interferon, melanoma associated antigen or B7.

The invention further relates to an immunogenic, immunological or vaccine composition containing the inventive vector and an acceptable carrier or diluent (e.g., veterinary acceptable or pharmaceutically acceptable). An immunological composition containing the vector (or an expression product thereof) elicits an immunological response - local or systemic. The response can, but need not be protective. An immunogenic composition containing the inventive recombinants (or an expression product thereof) likewise elicits a local or systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms can be protective compositions).

The invention therefore also provides a method of inducing an immunological response in a host vertebrate comprising administering to the host an immunogenic, immunological or vaccine composition comprising the inventive recombinant virus or vector and an acceptable carrier or diluent. For purposes of this specification, "animal" includes all vertebrate species, except humans; and "vertebrate" includes all vertebrates, including animals (as "animal" is used herein) and humans. And, of course, a subset of "animal" is "mammal", which for purposes of this specification includes all mammals, except humans.

For human administration, the inventive

77396-24

23

recombinants or vectors, can provide the advantage of expression without productive replication. This thus provides the ability to use recombinants of the invention in immunocompromised individuals; and, provides a level
5 of safety to workers in contact with recombinants of the invention. Therefore, the invention comprehends methods for amplifying or expressing a protein by administering or inoculating a host with a recombinant virus or vector, whereby the host is not a natural host of the recombinant
10 virus or vector, and there is expression without productive replication.

The exogenous or heterologous DNA (or DNA foreign to vaccine virus) can be DNA encoding any of the aforementioned epitopes of interest, as listed above. In
15 this regard, with respect to *Borrelia* DNA, reference is made to U.S. Patent No. 5,523,089, WO93/08306, Molecular Microbiology (1989), 3(4), 479-486, and PCT publications WO 93/04175, and WO 96/06165.

20 With respect to pneumococcal epitopes of interest, reference is made to Briles et al. WO 92/14488; with respect to tumor viruses reference is made to Molecular Biology of Tumor Viruses, RNA TUMOR VIRUSES (Second Edition, Edited by
25 Weiss et al., Cold Spring Harbor Laboratory 1982) (e.g., page 44 et seq. - Taxonomy of Retroviruses).

With respect to DNA encoding epitopes of interest, attention is directed to documents cited
30 herein, see, e.g., documents cited *supra* and documents cited *infra*, for instance: U.S. Patents Nos. 5,174,993 and 5,505,941 (e.g., recombinant avipox virus, vaccinia virus; rabies glycoprotein (G), gene, turkey influenza hemagglutinin gene, gp51,30 envelope gene of bovine
35 leukemia virus, Newcastle Disease Virus (NDV) antigen, FeLV envelope gene, RAV-1 env gene, NP (nucleoprotein gene of Chicken/Pennsylvania/1/83 influenza virus), matrix and

77396-24

24

preplomer gene of infectious bronchitis virus; HSV gD),
U.S. Patent No. 5,338,683 (e.g., recombinant vaccinia
virus, avipox virus; DNA encoding Herpesvirus
glycoproteins, *inter alia*), U.S. Patent No. 5,494,807
5 (e.g., recombinant vaccinia, avipox; exogenous DNA
encoding antigens from rabies, Hepatitis B, JEV, YF,
Dengue, measles, pseudorabies, Epstein-Barr, HSV, HIV,
SIV, EHV, BHV, HCMV, canine parvovirus, equine influenza,
FeLV, FHV, Hantaan, C. tetani, avian influenza, mumps,
10 NDV, *inter alia*), U.S. Patent No. 5,503,834 (e.g.,
recombinant vaccinia, avipox, Morbillivirus, e.g.,
measles F, hemagglutinin, *inter alia*), U.S. Patent No.
4,722,848 (e.g., recombinant vaccinia virus; HSV tk, HSV
glycoproteins, e.g., gB, gD, influenza HA, Hepatitis B,
15 e.g., HBsAg, *inter alia*), U.K. Patent GB 2 269 820 B and
U.S. Patent No. 5,514,375 (recombinant poxvirus;
flavivirus structural proteins); WO 92/22641
(e.g., recombinant poxvirus; immunodeficiency virus, HTLV,
inter alia), WO 93/03145 (e.g., recombinant poxvirus; IBDV,
20 *inter alia*), WO 94/16716 (e.g., recombinant poxvirus;
cytokine and/or tumor associated antigens, *inter alia*),
U.S. Patent No. 5,843,456 (rabies combination
compositions), U.S. Patent No. 5,529,780 (canine
herpesvirus) and WO 96/3941 (cytomegalovirus).

25 As to antigens for use in vaccine or
immunological compositions, reference is made to the

documents and discussion set forth in the documents cited herein (see, e.g., documents cited *supra*); see also Stedman's Medical Dictionary (24th edition, 1982, e.g., definition of vaccine (for a list of antigens used in vaccine formulations; such antigens or epitopes of interest from those antigens can be used in the invention, as either an expression product of an inventive recombinant virus or vector, or in a multivalent composition containing an inventive recombinant virus or vector or an expression product therefrom).

As to epitopes of interest, one skilled in the art can determine an epitope or immunodominant region of a peptide or polypeptide and ergo the coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids (e.g., size, charge, etc.) and the codon dictionary, without undue experimentation.

A general method for determining which portions of a protein to use in an immunological composition focuses on the size and sequence of the antigen of interest. "In general, large proteins, because they have more potential determinants are better antigens than small ones. The more foreign an antigen, that is the less similar to self configurations which induce tolerance, the more effective it is in provoking an immune response." Ivan Roitt, Essential Immunology, 1988.

As to size: the skilled artisan can maximize the size of the protein encoded by the DNA sequence to be inserted into the viral vector (keeping in mind the packaging limitations of the vector). To minimize the DNA inserted while maximizing the size of the protein expressed, the DNA sequence can exclude introns (regions of a gene which are transcribed but which are subsequently excised from the primary RNA transcript).

At a minimum, the DNA sequence can code for a

peptide at least 8 or 9 amino acids long. This is the minimum length that a peptide needs to be in order to stimulate a CD8+ T cell response (which recognizes virus infected cells or cancerous cells). A minimum peptide
5 length of 13 to 25 amino acids is useful to stimulate a CD4+ T cell response (which recognizes special antigen presenting cells which have engulfed the pathogen). See Kendrew, *supra*. However, as these are minimum lengths, these peptides are likely to generate an immunological
10 response, i.e., an antibody or T cell response; but, for a protective response (as from a vaccine composition), a longer peptide is preferred.

With respect to the sequence, the DNA sequence preferably encodes at least regions of the peptide that
15 generate an antibody response or a T cell response. One method to determine T and B cell epitopes involves epitope mapping. The protein of interest "is fragmented into overlapping peptides with proteolytic enzymes. The individual peptides are then tested for their ability to
20 bind to an antibody elicited by the native protein or to induce T cell or B cell activation. This approach has been particularly useful in mapping T-cell epitopes since the T cell recognizes short linear peptides complexed with MHC molecules. The method is less effective for
25 determining B-cell epitopes" since B cell epitopes are often not linear amino acid sequence but rather result from the tertiary structure of the folded three dimensional protein. Janis Kuby, Immunology, (1992) pp. 79-80.

30 Another method for determining an epitope of interest is to choose the regions of the protein that are hydrophilic. Hydrophilic residues are often on the surface of the protein and are therefore often the regions of the protein which are accessible to the
35 antibody. Janis Kuby, Immunology, (1992) p. 81

Yet another method for determining an epitope of interest is to perform an X-ray crystallographic

analysis of the antigen (full length)-antibody complex.
Janis Kuby, Immunology, (1992) p. 80.

Still another method for choosing an epitope of interest which can generate a T cell response is to
5 identify from the protein sequence potential HLA anchor binding motifs which are peptide sequences which are known to be likely to bind to the MHC molecule.

The peptide which is a putative epitope of interest, to generate a T cell response, should be
10 presented in a MHC complex. The peptide preferably contains appropriate anchor motifs for binding to the MHC molecules, and should bind with high enough affinity to generate an immune response. Factors which can be considered are: the HLA type of the patient (vertebrate,
15 animal or human) expected to be immunized, the sequence of the protein, the presence of appropriate anchor motifs and the occurrence of the peptide sequence in other vital cells.

An immune response is generated, in general, as
20 follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatibility complex MHC" located on another cell's surface. There are two classes of MHC complexes - class I and class II, and each
25 class is made up of many different alleles. Different patients have different types of MHC complex alleles; they are said to have a 'different HLA type.'

Class I MHC complexes are found on virtually every cell and present peptides from proteins produced
30 inside the cell. Thus, Class I MHC complexes are useful for killing cells which when infected by viruses or which have become cancerous and as the result of expression of an oncogene. T cells which have a protein called CD8 on their surface, bind specifically to the MHC class
35 I/peptide complexes via the T cell receptor. This leads to cytolytic effector activities.

Class II MHC complexes are found only on

antigen- presenting cells and are used to present peptides from circulating pathogens which have been endocytosed by the antigen- presenting cells. T cells which have a protein called CD4 bind to the MHC class
5 II/peptide complexes via the T cell receptor. This leads to the synthesis of specific cytokines thich stimulate an immune response.

Some guidelines in determining whether a protein is an epitopes of interest which will stimulate a
10 T cell response, include: Peptide length - the peptide should be at least 8 or 9 amino acids long to fit into the MHC class I complex and at least 13-25 amino acids long to fit into a class II MHC complex. This length is a minimum for the peptide to bind to the MHC complex. It
15 is preferred for the peptides to be longer than these lengths because cells may cut the expressed peptides. The peptide should contain an appropriate anchor motif which will enable it to bind to the various class I or class II molecules with high enough specificity to
20 generate an immune response (See Bocchia, M. et al, Specific Binding of Leukemia Oncogene Fusion Protein Peptides to HLA Class I Molecules, Blood 85:2680-2684; Englehard, VH, Structure of peptides associated with class I and class II MHC molecules Ann. Rev. Immunol.
25 12:181 (1994)). This can be done, without undue experimentation, by comparing the sequence of the protein of interest with published structures of peptides associated with the MHC molecules. Protein epitopes recognized by T cell receptors are peptides generated by
30 enzymatic degradation of the protein molecule and are presented on the cell surface in association with class I or class II MHC molecules.

Further, the skilled artisan can ascertain an epitope of interest by comparing the protein sequence
35 with sequences listed in the protein data base.

Even further, another method is simply to generate or express portions of a protein of interest,

77396-24

29

generate monoclonal antibodies to those portions of the protein of interest, and then ascertain whether those antibodies inhibit growth in vitro of the pathogen from which the from which the protein was derived. The skilled artisan can use the other guidelines set forth in this disclosure and in the art for generating or expressing portions of a protein of interest for analysis as to whether antibodies thereto inhibit growth in vitro. For example, the skilled artisan can generate portions of a protein of interest by: selecting 8 to 9 or 13 to 25 amino acid length portions of the protein, selecting hydrophylic regions, selecting portions shown to bind from X-ray data of the antigen (full length)-antibody complex, selecting regions which differ in sequence from other proteins, selecting potential HLA anchor binding motifs, or any combination of these methods or other methods known in the art.

Epitopes recognized by antibodies are expressed on the surface of a protein. To determine the regions of a protein most likely to stimulate an antibody response one skilled in the art can preferably perform an epitope map, using the general methods described above, or other mapping methods known in the art.

As can be seen from the foregoing, without undue experimentation, from this disclosure and the knowledge in the art, the skilled artisan can ascertain the amino acid and corresponding DNA sequence of an epitope of interest for obtaining a T cell, B cell and/or antibody response. In addition, reference is made to Gefter et al., U.S. Patent No. 5,019,384, issued May 28, 1991, and the documents it cites (Note especially the "Relevant Literature" section of this patent, and column 13 of this patent which discloses that: "A large number of epitopes have been defined for a wide variety of organisms of interest. Of particular interest are those epitopes to which neutralizing antibodies are directed. Disclosures of

77396-24

30

such epitopes are in many of the references cited in the Relevant Literature section.")

With respect to expression of a biological response modulator, reference is made to Wohlstadter,
5 "Selection Methods," WO 93/19170, published 30 September 1993, and the documents cited therein.

For instance, a biological response modulator modulates biological activity; for instance, a biological
10 response modulator is a modulatory component such as a high molecular weight protein associated with non-NMDA excitatory amino acid receptors and which allosterically regulates affinity of AMPA binding (See Kendrew, *supra*). The recombinant of the present invention can express such
15 a high molecular weight protein.

More generally, nature has provided a number of precedents of biological response modulators. Modulation of activity may be carried out through mechanisms as complicated and intricate as allosteric induced
20 quaternary change to simple presence/absence, e.g., expression/degradation, systems. Indeed, the repression/activation of expression of many biological molecules is itself mediated by molecules whose activities are capable of being modulated through a
25 variety of mechanisms.

Table 2 of Neidhardt et al *Physiology of the Bacterial Cell* (Sinauer Associates Inc., Publishers, 1990), at page 73, lists chemical modifications to bacterial proteins. As is noted in that table, some
30 modifications are involved in proper assembly and other modifications are not, but in either case such modifications are capable of causing modulation of function. From that table, analogous chemical modulations for proteins of other cells can be
35 determined, without undue experimentation.

In some instances modulation of biological functions may be mediated simply through the

proper/improper localization of a molecule. Molecules may function to provide a growth advantage or disadvantage only if they are targeted to a particular location. For example, a molecule may be typically not
5 taken up or used by a cell, as a function of that molecule being first degraded by the cell by secretion of an enzyme for that degradation. Thus, production of the enzyme by a recombinant can regulate use or uptake of the molecule by a cell. Likewise, the recombinant can
10 express a molecule which binds to the enzyme necessary for uptake or use of a molecule, thereby similarly regulating its uptake or use.

Localization targeting of proteins carried out through cleavage of signal peptides another type of
15 modulation or regulation. In this case, a specific endoprotease catalytic activity can be expressed by the recombinant.

Other examples of mechanisms through which modulation of function may occur are RNA virus poly-
20 proteins, allosteric effects, and general covalent and non-covalent steric hindrance. HIV is a well studied example of an RNA virus which expresses non-functional poly-protein constructs. In HIV "the gag, pol, and env poly-proteins are processed to yield, respectively, the
25 viral structural proteins p17, p24, and p15--reverse transcriptase and integrase--and the two envelope proteins gp41 and gp120" (Kohl et al., PNAS USA 85:4686-90 (1988)). The proper cleavage of the poly-proteins is crucial for replication of the virus, and virions
30 carrying inactive mutant HIV protease are non-infectious (*Id.*). This is another example of the fusion of proteins down-modulating their activity. Thus, it is possible to construct recombinant viruses which express molecules which interfere with endoproteases, or which provide
35 endoproteases, for inhibiting or enhancing the natural expression of certain proteins (by interfering with or enhancing cleavage).

The functional usefulness of enzymes may also be modulated by altering their capability of catalyzing a reaction. Illustrative examples of modulated molecules are zymogens, formation/disassociation of multi-subunit
5 functional complexes, RNA virus poly-protein chains, allosteric interactions, general steric hindrance (covalent and non-covalent) and a variety of chemical modifications such as phosphorylation, methylation, acetylation, adenylation, and uridylation (see Table 1
10 of Neidhardt, *supra*, at page 315 and Table 2 at page 73).

Zymogens are examples of naturally occurring protein fusions which cause modulation of enzymatic activity. Zymogens are one class of proteins which are converted into their active state through limited
15 proteolysis. See Table 3 of Reich, *Proteases and Biological Control*, Vol. 2, (1975) at page 54). Nature has developed a mechanism of down-modulating the activity of certain enzymes, such as trypsin, by expressing these enzymes with additional "leader" peptide sequences at
20 their amino termini. With the extra peptide sequence the enzyme is in the inactive zymogen state. Upon cleavage of this sequence the zymogen is converted to its enzymatically active state. The overall reaction rates of the zymogen are "about 10^5 - 10^6 times lower than those
25 of the corresponding enzyme" (See Table 3 of Reich, *supra* at page 54).

It is therefore possible to down-modulate the function of certain enzymes simply by the addition of a peptide sequence to one of its termini. For example,
30 with knowledge of this property, a recombinant can express peptide sequences containing additional amino acids at one or both termini.

The formation or disassociation of multi-subunit enzymes is another way through which modulation
35 may occur. Different mechanisms may be responsible for the modulation of activity upon formation or disassociation of multi-subunit enzymes.

Therefore, sterically hindering the proper specific subunit interactions will down-modulate the catalytic activity. And accordingly, the recombinant of the invention can express a molecule which sterically
5 hinders a naturally occurring enzyme or enzyme complex, so as to modulate biological functions.

Certain enzyme inhibitors afford good examples of functional down-modulation through covalent steric hindrance or modification. Suicide substrates which
10 irreversibly bind to the active site of an enzyme at a catalytically important amino acid in the active site are examples of covalent modifications which sterically block the enzymatic active site. An example of a suicide substrate is TPCK for chymotrypsin (Fritsch, *Enzyme*
15 *Structure and Mechanism*, 2d ed; Freeman & Co. Publishers, 1984)). This type of modulation is possible by the recombinant expressing a suitable suicide substrate, to thereby modulate biological responses (e.g., by limiting enzyme activity).

20 There are also examples of non-covalent steric hindrance including many repressor molecules. The recombinant can express repressor molecules which are capable of sterically hindering and thus down-modulating the function of a DNA sequence by preventing particular
25 DNA-RNA polymerase interactions.

Allosteric effects are another way through which modulation is carried out in some biological systems. Aspartate transcarbamoylase is a well characterized allosteric enzyme. Interacting with the
30 catalytic subunits are regulatory domains. Upon binding to CTP or UTP the regulatory subunits are capable of inducing a quaternary structural change in the holoenzyme causing down-modulation of catalytic activity. In contrast, binding of ATP to the regulatory subunits is
35 capable of causing up-modulation of catalytic activity (Fritsch, *supra*). Using methods of the invention, molecules can be expressed which are capable of binding

77396-24

34

and causing modulatory quaternary or tertiary changes.

In addition, a variety of chemical modifications, e.g., phosphorylation, methylation, acetylation, adenylation, and uridylation may be carried out so as to modulate function. It is known that modifications such as these play important roles in the regulation of many important cellular components. Table 2 of Neidhardt, *supra*, at page 73, lists different bacterial enzymes which undergo such modifications. From that list, one skilled in the art can ascertain other enzymes of other systems which undergo the same or similar modifications, without undue experimentation. In addition, many proteins which are implicated in human disease also undergo such chemical modifications. For example, many oncogenes have been found to be modified by phosphorylation or to modify other proteins through phosphorylation or dephosphorylation. Therefore, the ability afforded by the invention to express modulators which can modify or alter function, e.g., phosphorylation, is of importance.

From the foregoing, the skilled artisan can use the present invention to express a biological response modulator, without any undue experimentation.

With respect to expression of fusion proteins by inventive recombinants, reference is made to Sambrook, Fritsch, Maniatis, *Molecular Cloning, A LABORATORY MANUAL* (2d Edition, Cold Spring Harbor Laboratory Press, 1989) (especially Volume 3), and Kendrew, *supra*. The teachings of Sambrook et al., can be suitably modified, without undue experimentation, from this disclosure, for the skilled artisan to generate recombinants or vectors expressing fusion proteins.

With regard to gene therapy and immunotherapy, reference is made to U.S. Patent Nos. 4,690,915 and 5,252,479, together with the documents cited therein and on their face, and to WO 94/16716,

77396-24

35

together with the documents cited therein.

A growth factor can be defined as multifunctional, locally acting intercellular signalling peptides which control both ontogeny and maintenance of tissue and function (see Kendrew, *supra*, especially at
5 page 455 et seq.).

The growth factor or therapeutic gene, for example, can encode a disease-fighting protein, a molecule for treating cancer, a tumor suppressor, a
10 cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, be selected from the group consisting of a gene encoding alpha-globin, beta-globin, gamma-globin, granulocyte macrophage-colony stimulating factor, tumor necrosis
15 factor, an interleukin (e.g., an interleukin selected from interleukins 1 to 14, or 1 to 11, or any combination thereof), macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53,
20 retinoblastoma, interferon, melanoma associated antigen or B7. U.S. Patent No. 5,252,479 provides a list of proteins which can be expressed in an adenovirus system for gene therapy, and the skilled artisan is directed to that disclosure. WO 94/16716 provides genes for cytokines
25 and tumor associated antigens and immunotherapy methods, including ex vivo methods, and the skilled artisan is directed to those disclosures.

Thus, one skilled in the art can create recombinants or vectors expressing a growth factor or
30 therapeutic gene and use the recombinants or vectors, from this disclosure and the knowledge in the art, without undue experimentation.

Moreover, from the foregoing and the knowledge

in the art, no undue experimentation is required for the skilled artisan to construct an inventive recombinant or vector which expresses an epitope of interest, a biological response modulator, a growth factor, a
5 recognition sequence, a therapeutic gene, or a fusion protein; or for the skilled artisan to use such a recombinant or vector.

As the recombinants or vectors of the invention can be used for expression of gene products *in vitro*,
10 techniques for protein purification can be employed in the practice of the invention, and such techniques, in general, include:

Briefly, the cells are disrupted and the protein of interest is released into an aqueous
15 "extract". There are many methods of cellular disintegration, which vary from relatively gentle to vigorous conditions, and the choice of one method over the other is dependent upon the source material. Animal tissues vary from the very easily broken erythrocytes to
20 tough collagenous material such as found in blood vessels and other smooth-muscle containing tissue. Bacteria vary from fairly fragile organisms that can be broken up by digestive enzymes or osmotic shock to more resilient species with thick cell walls, needing vigorous
25 mechanical treatment for disintegration.

Gentle techniques include cell lysis, enzymatic digestion, chemical solubilization, hand homogenization and mincing (or grinding); moderate techniques of cell disintegration include blade homogenization and grinding
30 with abrasive materials, i.e., sand or alumina; and vigorous techniques include french press, ultrasonication, bead mill or Manton-Gaulin homogenization. Each of the aforementioned techniques are art-recognized, and it is well within the scope of
35 knowledge of the skilled artisan to determine the appropriate method of cell disintegration based upon the starting material, and the teachings herein and in the

art.

Following cell disintegration, the extract is prepared by centrifuging off insoluble material. At this stage, one may proceed with the purification method, as an extract containing as much of the protein of interest as possible has been prepared, and, where appropriate, particulate and most nonprotein materials have been removed.

Standard techniques of protein purification may be employed to further purify the protein of interest, including: precipitation by taking advantage of the solubility of the protein of interest at varying salt concentrations, precipitation with organic solvents, polymers and other materials, affinity precipitation and selective denaturation; column chromatography, including high performance liquid chromatography (HPLC), ion-exchange, affinity, immuno affinity or dye-ligand chromatography; immunoprecipitation and the use of gel filtration, electrophoretic methods, ultrafiltration and isoelectric focusing. Each of the above-identified methods are well within the knowledge of the skilled artisan, and no undue experimentation is required to purify the proteins or epitopes of interest from expression of a recombinant or vector of the invention, using the standard methodologies outlined hereinabove, and in the literature, as well as the teachings in the Examples below.

As the expression products can provide an antigenic, immunological, or protective (vaccine) response, the invention further relates to products therefrom; namely, antibodies and uses thereof. More in particular, the expression products can elicit antibodies by administration of those products or of recombinants or vectors expressing the products. The antibodies can be monoclonal antibodies; and, the antibodies or expression products can be used in kits, assays, tests, and the like involving binding, so that the invention relates to these

uses too. Additionally, since the recombinants or vectors of the invention can be used to replicate DNA, the invention relates to the inventive recombinants as vectors and methods for replicating DNA by infecting or
5 transfecting cells with the recombinant and harvesting DNA therefrom. The resultant DNA can be used as probes or primers or for amplification.

The administration procedure for the inventive recombinants or vectors or expression products thereof,
10 compositions of the invention such as immunological, antigenic or vaccine compositions or therapeutic compositions can be via a parenteral route (intradermal, intramuscular or subcutaneous). Such an administration enables a systemic immune response. The administration
15 can be via a mucosal route, e.g., oral, nasal, genital, etc. Such an administration enables a local immune response.

More generally, the inventive antigenic, immunological or vaccine compositions or therapeutic
20 compositions can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical, medical or veterinary arts. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or
25 veterinary arts taking into consideration such factors as the breed or species, age, sex, weight, and condition of the particular patient, and the route of administration. The compositions can be administered alone, or can be co-administered or sequentially administered with other
30 compositions of the invention or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions can include purified native antigens or epitopes or antigens or epitopes from expression by an inventive recombinant or
35 vector or another vector system; and are administered taking into account the aforementioned factors.

Examples of compositions of the invention

77396-24

39

include liquid preparations for orifice, e.g., oral, nasal, anal, genital, e.g., vaginal, etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. In such compositions the recombinant or vector may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like.

Antigenic, immunological or vaccine compositions typically can contain an adjuvant and an amount of the recombinant or vector or expression product to elicit the desired response. In human applications, alum (aluminum phosphate or aluminum hydroxide) is a typical adjuvant. Saponin and its purified component Quil A, Freund's complete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. Chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff et al. J. Immunol. 147:410-415 (1991), encapsulation of the protein within a proteoliposome as described by Miller et al., J. Exp. Med. 176:1739-1744 (1992), and encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vesicular Systems, Inc., Nashua, NH) can also be used.

The composition may be packaged in a single dosage form for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or orifice administration, e.g., perlingual (i.e., oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and route of administration are determined by the nature of

the composition, by the nature of the expression product, by expression level if the recombinant is directly used, and by known factors, such as breed or species, age, sex, weight, condition and nature of host, as well as LD₅₀ and
5 other screening procedures which are known and do not require undue experimentation. Dosages of expressed product can range from a few to a few hundred micrograms, e.g., 5 to 500 µg. The inventive recombinant or vector can be administered in any suitable amount to achieve
10 expression at these dosage levels. The viral recombinants of the invention can be administered in an amount of about 10^{3.5} pfu; thus, the inventive viral recombinant is preferably administered in at least this amount; more preferably about 10⁴ pfu to about 10⁶ pfu;
15 however higher dosages such as about 10⁴ pfu to about 10¹⁰ pfu, e.g., about 10⁵ pfu to about 10⁹ pfu, for instance about 10⁶ pfu to about 10⁸ pfu can be employed. Suitable quantities of inventive plasmid or naked DNA in plasmid or naked DNA compositions can be 1 ug to 100 mg,
20 preferably 0.1 to 10 mg, but lower levels such as 0.1 to 2 mg or preferably 1-10 ug may be employed. Other suitable carriers or diluents can be water or a buffered saline, with or without a preservative. The expression product or recombinant or vector may be lyophilized for
25 resuspension at the time of administration or can be in solution.

The carrier may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a composition having
30 controlled release. An early example of this was the polymerization of methyl methacrylate into spheres having diameters less than one micron to form so-called nano particles, reported by Kreuter, J., Microcapsules and Nanoparticles in Medicine and Pharmacology, M. Donbrow
35 (Ed). CRC Press, p. 125-148.

Microencapsulation has been applied to the injection of microencapsulated pharmaceuticals to give a

controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are polycarbonates, polyesters, polyurethanes, polyorthoesters and polyamides, particularly those that are biodegradable.

A frequent choice of a carrier for pharmaceuticals and more recently for antigens is poly (d,l-lactide-co-glycolide) (PLGA). This is a biodegradable polyester that has a long history of medical use in erodible sutures, bone plates and other temporary prostheses where it has not exhibited any toxicity. A wide variety of pharmaceuticals including peptides and antigens have been formulated into PLGA microcapsules. A body of data has accumulated on the adaption of PLGA for the controlled release of antigen, for example, as reviewed by Eldridge, J.H., et al. Current Topics in Microbiology and Immunology, 1989, 146:59-66. The entrapment of antigens in PLGA microspheres of 1 to 10 microns in diameter has been shown to have a remarkable adjuvant effect when administered orally. The PLGA microencapsulation process uses a phase separation of a water-in-oil emulsion. The compound of interest is prepared as an aqueous solution and the PLGA is dissolved in a suitable organic solvents such as methylene chloride and ethyl acetate. These two immiscible solutions are co-emulsified by high-speed stirring. A non-solvent for the polymer is then added, causing precipitation of the polymer around the aqueous droplets to form embryonic microcapsules. The microcapsules are collected, and stabilized with one of

77396-24

42

an assortment of agents (polyvinyl alcohol (PVA), gelatin, alginates, polyvinylpyrrolidone (PVP), methyl cellulose) and the solvent removed by either drying in vacuo or solvent extraction.

5 Thus, solid, including solid-containing-liquid, liquid, and gel (including "gel caps") compositions are envisioned.

 Furthermore, the inventive vectors or recombinants can be used in any desired immunization or
10 administration regimen; e.g., as part of periodic vaccinations such as annual vaccinations as in the veterinary arts or as in periodic vaccinations as in the human medical arts, or as in a prime-boost regimen wherein an inventive vector or recombinant is
15 administered either before or after the administration of the same or of a different epitope of interest or recombinant or vector expressing such a same or different epitope of interest (including an inventive recombinant or vector expressing such a same or different epitope of
20 interest), see, e.g., documents cited herein.

 Additionally, the inventive vectors or recombinants and the expression products therefrom can stimulate an immune or antibody response in animals.
25 From those antibodies, by techniques well-known in the art, monoclonal antibodies can be prepared and, those monoclonal antibodies, can be employed in well known antibody binding assays, diagnostic kits or tests to determine the presence or absence of antigen(s) and
30 therefrom the presence or absence of the natural causative agent of the antigen or, to determine whether an immune response to that agent or to the antigen(s) has simply been stimulated.

 Monoclonal antibodies are immunoglobulin
35 produced by hybridoma cells. A monoclonal antibody reacts with a single antigenic determinant and provides greater specificity than a conventional, serum-derived

77396-24

43

antibody. Furthermore, screening a large number of monoclonal antibodies makes it possible to select an individual antibody with desired specificity, avidity and isotype. Hybridoma cell lines provide a constant,
5 inexpensive source of chemically identical antibodies and preparations of such antibodies can be easily standardized. Methods for producing monoclonal antibodies are well known to those of ordinary skill in the art, e.g., Koprowski, H. et al., U.S. Pat. No.
10 4,196,265, issued Apr. 1, 1989.

Uses of monoclonal antibodies are known. One such use is in diagnostic methods, e.g., David, G. and Greene, H., U.S. Pat. No. 4,376,110, issued Mar. 8, 1983.
15

Monoclonal antibodies have also been used to recover materials by immunoadsorption chromatography, e.g. Milstein, C., 1980, Scientific American 243:66, 70.

20 Furthermore, the inventive recombinants or vectors or expression products therefrom can be used to stimulate a response in cells *in vitro* or *ex vivo* for subsequent reinfusion into a patient. If the patient is seronegative, the reinfusion is to stimulate an immune
25 response, e.g., an immunological or antigenic response such as active immunization. In a seropositive individual, the reinfusion is to stimulate or boost the immune system against a pathogen.

The recombinants or vectors of the invention
30 are also useful for generating DNA for probes or for PCR primers which can be used to detect the presence or absence of hybridizable DNA or to amplify DNA, e.g., to detect a pathogen in a sample or for amplifying DNA.

Since viruses require translation of viral
35 mRNAs in order to generate viral proteins required for replication, it is evident that any function which blocks the action of PKR in the infected cell will have a

77396-24

44

positive effect on viral protein expression. Thus, co-expression, in some fashion, of the vaccinia E3L/K3L gene products, or a homolog of E3L and/or K3L, may provide a general mechanism for enhancing the expression levels of
5 heterologous gene products by vectors in general. The E3L/K3L or homologous functions may enhance or augment native anti-PKR mechanisms, and thus increase protein expression levels and/or persistence. This provides a useful element towards optimizing the efficiency of
10 eukaryotic virus systems as immunization vehicles. This approach could be further extended for improvement of DNA-based immunogens, e.g., naked DNA or plasmid DNA vector systems. Thus, increased or enhanced levels or persistence of expression can be obtained.

15 A better understanding of the present invention and of its many advantages will be had from the following non-limiting Examples, given by way of illustration.

EXAMPLES

Example 1 - ALVAC Recombinants

20 pMPC6H6K3E3 (ATCC No. 97912) was used as a donor plasmid in *in vivo* recombination (Piccini et al., 1987) with rescuing virus vCP205 (ATCC No. VR-2557; HIV expression cassette - vaccinia H6 promoter/HIV truncated env MN strain, I3L gag with
25 protease in ALVAC C3 insertion site); and the resulting recombinant virus was designated vCP1431A (vaccinia H6/K3L and E3L cassette in the C6 locus). With respect to the H6/K3L expression cassette and the vaccinia E3L gene with the endogenous promoter flanked by the ALVAC C6
30 insertion site sequences reference is made to Fig. 1 (SEQ ID NO: 1).

pC3H6FHVB (ATCC No. 97914; Fig. 3, SEQ ID NO: 3; H6 promoted FHV gB ORF with early transcriptional and translational stop signals at both 5' and 3' ends flanked
35 by the left and right arms of the ALVAC C3 locus) was used in *in vivo* recombination with the ALVAC (ATCC No.

77396-24

45

VR-2547) to generate vCP1459 (H6 promoted FHV gB expression cassette in deorfed C3 insertion locus). With respect to the FHV-1 gB coding region in which the two internal T₅NT motifs have been mutated, see Fig. 2 (SEQ ID NO: 2).

pC3H6FHVB was used in *in vivo* recombination with vCP1431A to generate vCP1460 (H6 promoted FHV gB expression cassette in the deorfed C3 insertion locus and vaccinia E3L/K3L genes in C6 locus).

10 pMPC5H6PN (HIV pol/nef "string of beads" cassette in the ALVAC C5 locus) was used in recombination with vCP205 to obtain vCP1433 (ATCC Deposit No. VR-2556). Thus, recombinant ALVAC-MN120TMGNPst (vCP1433) was generated by insertion of an expression cassette encoding
15 a synthetic polypeptide containing all of the known Pol CTL epitopes (Nixon and McMichael; 1991) and all of the known human Nef CTL epitopes into vCP205 at the insertion site known as C5.

pMPC6H6K3E3 (ATCC Deposit No. 97912; containing
20 vaccinia H6/K3L expression cassette and vaccinia E3L gene with endogenous promoter flanked by the ALVAC C6 insertion site sequences) was used in recombination with vCP1433 to obtain vCP1452. Figures 4 and 5 show the nucleotide and amino acid sequences of the vCP1433 and
25 vCP1452 inserts. Figure 6 shows the K3L E3L in C6 in vCP1452. vCP1452 contains the HIV type 1 gag and protease genes derived from the IIIB isolate, the gp120 envelope sequences derived from the MN isolate, and sequences encoding a polypeptide encompassing the known
30 human CTL epitopes from HIV-1 Nef and Pol (Nef1 and Nef2 CTL epitopes, and Pol1, Pol2 and Pol3 CTL epitopes). The expressed gp120 moiety is linked to the transmembrane (TM) anchor sequence (28 amino acids) of the envelope glycoprotein. In addition to the HIV coding sequences
35 vCP1452 contains the vaccinia virus E3L and K3L coding sequences inserted into the C6 site. The insertion sites and promoter linkages for this construct are shown in the

77396-24

46

Table below.

Table: Insertion sites and promoter linkages in vCP1452

	Insert	Insertion Site	Promoter
	HIV1 MN gp120 + TM	C3	H6
5	HIV1 IIIB gag (+ pro)	C3	I3L
	Pol3/Nef C term/Pol2/Nef central/Pol1	C5	H6
	Vaccinia virus E3L	C6	endogenous
	Vaccinia virus K3L	C6	H6

10 vCP300 is an ALVAC recombinant containing HIV gp120TM (MN), gag/pro (IIIB) (C3 locus), Nef (C6 locus), and Pol (C5 locus).

15 Plasmids for preparing these recombinants were prepared as follows:

K3L Expression Cassette

The K3L coding sequences were synthesized by PCR amplification using pSD407VC containing Copenhagen vaccinia HindIII K fragment as template, as described in
20 U.S. Patent No. 5,378,457. The oligonucleotides MPSYN 763 and MPSYN 764 (SEQ ID NOS: 8 and 9) were used as primers for the PCR reaction.

MPSYN 763

5' -

25 CCCTCTAGATCGCGATATCCGTTAAGTTTGTATCGTAATGCTTGCATTTTGTATTTC
GT-3'

MPSYN 764 5' - CCCGAATTCATAAAAATTATTGATGTCTACA-3'

The approximately 325bp PCR fragment was digested with XbaI and EcoRI yielding a 315bp fragment.
30 This 315bp fragment was purified by isolation from an agarose gel and ligated with XbaI and EcoRI digested pBSSK+ vector (from Stratagene LA Jolla, CA.). The nucleic acid sequence was confirmed directly from alkali denatured plasmid template as described in Hattori, M.
35 and Sakaki, Y., 1986, using the modified T7 polymerase

(Tabor, S. and Richardson, C.C. 1987) and Sequenase (from U.S. Biochemicals Cleveland, OH.). This plasmid was designated pBS 763/764. Digesting pBS 763/764 with *Nru*I and *Xho*I, a 340bp fragment was isolated for cloning into the plasmid vector pMM154 containing a cassette with the vaccinia H6 promoter controlling an irrelevant gene in the NYVAC *tk* insertion vector background, which was prepared by digestion with *Nru*I (partially) and *Xho*I, such that the 340bp fragment from pBS 763/764 containing the K3L gene could be directionally oriented next to the H6 promoter generating pMPTKH6K3L. The plasmid pMP42GPT containing the dominant selectable marker *Eco* gpt gene (Pratt D. and Subramani S. 1983) under the control of the Entomopox 42k promoter, was digested with *Sma*I and *Bam*HI to yield a 0.7 Kbp 42k-*Eco* gpt expression cassette. This 0.7 Kbp fragment was purified and ligated into *Sma*I and *Bam*HI cut pMPTKH6K3L generating the plasmid pMPTKH6K3Lgpt. This plasmid was digested with *Xho*I, generating a 1.2 Kbp fragment containing the H6/K3L and the 42k/*Eco*gpt expression cassette, which was then gel purified. The 1.2 Kbp *Xho*I fragment was inserted into the *Xho*I site of the ALVAC C6 insertion plasmid pC6L (described in U.S. Patent No. 5,494,807), generating pMPC6H6K3Lgpt.

25 E3L/K3L ALVAC Expression Cassette

The entire E3L gene is contained within a 2.3 Kbp *Eco*RI fragment isolated from pSD401VC, which contained a clone of the *Hind*III E fragment from Copenhagen vaccinia. The 2.3 Kbp *Eco*RI fragment was inserted into pMPC6H6K3Lgpt that had been partially digested with *Eco*RI, generating the plasmid pMPC6H6K3E3gpt. The plasmid pMPC6H6K3E3gpt was digested with *Xho*I and the resulting 6.8 Kbp vector fragment was purified and self-ligated, resulting in the plasmid pMPC6E3. The plasmid pMPTKH6K3L was digested with *Psp*AI and the resulting 560bp fragment containing the H6/K3L expression cassette was ligated into *Psp*AI digested

77396-24

48

pMPC6E3 resulting in the plasmid construct pMPC6H6K3E3.
Construction of the H6-promoted FHV gB donor plasmid

The entire coding region of the Feline Herpesvirus 1 glycoprotein gB (FHV-1 gB) was obtained by
 5 digestion of pJCA079 (FHV gB coding region in which 5' and 3' T₃NT sequences were mutated to change the early transcriptional stop signal without affecting amino acid sequences; the I3L vaccinia promoter has been coupled to the 5' end of the gB ORF; see Fig. 4, SEQ ID NOS: 4 and 5) with
 10 PstI and isolating a 3 Kbp fragment from an agarose gel. The purified PstI fragment was cloned into an ALVAC C3 insertion plasmid (pVQH6CP3LSA) also digested with PstI (the unique BamHI site in pVQH6CP3LSA was previously inactivated by digestion with BamHI, blunting the ends
 15 with Klenow polymerase and religation; pVQH6CP3LSA was obtained by digesting pVQH6CP3L, discussed in U.S. Patent No. 5,494,807, with NotI and NsiI, from which a 6623 bp fragment was isolated and ligated to annealed oligonucleotides CP34 (5'GGCCGCGTCGACATGCA3') and CP35
 20 (5'TGTCGACGC3') (SEQ ID NOS: 10 and 11). The resulting plasmid, pRAC5, was screened for proper orientation of the gB coding region with respect to the H6 promoter. To properly link the H6 promoter to the FHV gB initiation codon, an 800 bp PCR fragment was amplified from pJCA079
 25 using oligonucleotides RG789 (SEQ ID NO: 12) (5'-TTTCATTATCGCGATATC-CGTTAAGTTTGTATCGTAATGTCCACTCGTGGCGATC-3') and RG787 (SEQ ID NO:13) (5'-GGAGGGTTTCAGAGGCAG-3'). This purified fragment was digested with NruI/BamHI and ligated into pRAC5 also digested with NruI/BamHI. The
 30 resulting plasmid was the FHV gB donor plasmid, pC3H6FHVB.

"String of Beads" Cassette

The "string of beads" expression cassette for the nef and pol CTL epitopes (H6/Pol 3/Nef C term/Pol
 35 2/Nef central/Pol 1) was generated by PCR (polymerase chain reaction) as detailed below, using template pHXBD2 for pol epitopes and template 2-60-HIV.3 for Nef

77396-24

49

epitopes. Initial assembly was in two parts: (1) H6(partial promoter)/Pol 3/Nef C term(Nef 2); (2) Pol 2/Nef central (Nef 1)/Pol 1 in pBSSK. These were combined, then moved to pBSH6-11 for the assembly of the entire H6 promoter, then the H6/HIV cassette was moved to a C5 insertion plasmid.

(1) H6/Pol 3/Nef C term(Nef 2)

A 230 bp fragment (A) was derived by PCR to obtain the H6 linkage and Pol3 using synthetic oligonucleotides MPSYN783 and MPSYN784 and template pHXBD2. pHXBD2 was derived at the NIH/NCI (Dr. Nancy Miller) from a recombinant phage library of XbaI digested DNA from HTLV-III infected H9 cells cloned in lambda-J1 (Shaw et al., 1994). This plasmid contains the entire proviral DNA sequence of the HIV IIIB isolate.

A 110 bp fragment (B) was derived by PCR to obtain Nef2 using oligonucleotides MPSYN785/MPSYN786 and template p2-60-HIV.3.

PCR fragments A and B were combined in a PCR as template to obtain a 300bp fragment containing H6 linkage/Pol3/Nef2 using external primers MPSYN783/MPSYN786 (SEQ ID NOS: 14 and 17). The 300bp fragment was digested with XhoI/HindIII and a 290 bp fragment was isolated and ligated with similarly digested pBSSK to generate pBS783/786. The sequence was confirmed.

(2) Pol 2/Nef central (Nef 1)/Pol 1

A 210 bp fragment (C) containing Pol2 was derived by PCR using synthetic oligonucleotides MPSYN787/MPSYN788 (SEQ ID NOS: 18 and 19) and template pHXBD2.

A 270 bp fragment (D) containing Nef1 was derived by PCR using synthetic oligonucleotides MPSYN789/MPSYN790 (SEQ ID NOS: 20 and 21) and template p2-60-HIV.3.

35

A 170 bp fragment (E) containing Pol1 was derived by PCR using primers MPSYN791/MPSYN792 (SEQ ID

77396-24

50

NOS: 22 and 23) and template pHXBD2.

Fragments C and D were combined as template in a PCR for Pol 2/Nef 1 using external primers MPSYN787/MPSYN790 (SEQ ID NOS: 18 and 21) resulting in a 460 bp PCR product (C+D).

Fragments D and E were combined as template in a PCR for Nef 1/Pol 1 using external primers MPSYN789/MPSYN792 (SEQ ID NOS: 20 and 23), resulting in isolation of a 420 bp fragment (D+E).

Fragments (C+D) and (D+E) were combined as template in a PCR with external primers MPSYN787/MPSYN792 (SEQ ID NOS: 18 and 23) to obtain a 610 bp fragment containing Pol 2/Nef 1/Pol 1. This 610 bp fragment was digested with *HindIII*/*PstI*. The resulting 590 bp fragment was ligated with pBSSK cut with *HindIII*/*PstI* to generate pBS787/792. The sequence was confirmed.

MPSYN783: 5' CCC CTC GAG TCG CGA TAT CCG TTA AGT TTG TAT CGT AAT GCC ACT AAC AGA AGA AGC A 3' (58mer)

MPSYN784: 5' AAA TCT CCA CTC CAT CCT TGT TTT CAG ATT TTT AAA 3' (36 mer)

MPSYN785: 5' AAT CTG AAA ACA GGA ATG GAG TGG AGA TTT GAT TCT 3' (36 mer)

MPSYN786: 5' CCC AAG CTT ACA ATT TTT AAA ATA TTC AGG 3' (30 mer)

MPSYN787: 5' CCC AAG CTT ATG GCA ATA TTC CAA AGT AGC 3' (30 mer)

MPSYN788: 5' TGG AAA ACC TAC CAT GGT TGT AAG TCC CCA CCT CAA 3' (36 mer)

MPSYN789: 5' TGG GGA CTT ACA ACC ATG GTA GGT TTT CCA GTA ACA 3' (36 mer)

MPSYN790: 5' TAC AGT CTC AAT CAT TGG TAC TAG CTT GTA GCA CCA 3' (36 mer)

MPSYN791: 5' TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GTA 3' (36 mer)

MPSYN792: 5' CCC CCT GCA GAA AAA TTA AGG CCC AAT TTT TGA AAT 3' (36 mer)

(SEQ ID NOS: 14 to 23)

Assembly of entire cassette:

A 590 bp *HindIII*/*PstI* fragment was isolated from pBS787/792 and ligated with vector pBS783/786 cut with *HindIII*/*PstI* to generate pBS783/792. pBS783/792 was
5 cut with *EcoRV* and *PstI*, to generate an 880 bp fragment which was then ligated with similarly digested vector pBSH6-1 to generate pBSH6PN. Plasmid pBSH6PN was digested with *BamHI* and a 1060 bp fragment was isolated. pVQC5LSP1, a generic C5 donor plasmid, was digested with
10 *BamHI* and ligated with the 1060 bp fragment from pBSH6PN. The resulting plasmid, pMPC5H6PN, contains the HIV pol/nef "string of beads" cassette in the ALVAC C5 locus.

Example 2 - Expression studies

15 Dishes containing confluent monolayers of cells were infected at a multiplicity of infection (moi) of 2. After incubation for specified time periods, cells were incubated in labeling medium for 1 hour. At the end of the incubation, cells were harvested for
20 immunoprecipitation analysis as described (Harlow, E and Lane, D (1988) ; Langone, J. (1982)).

Cells were infected at an moi of 2 pfu/cell and incubated for specified time periods. At the appropriate time post-infection, cell lysates were prepared for RNA
25 analysis. The medium was aspirated and cells were harvested. RNA was isolated and prepared using the TRI-Reagent (Molecular Research Center Inc. Cincinnati, OH. 45212) as per manufacture instructions and analyzed by slot blot. Radiolabelled DNA probes were used to detect
30 specific RNA species.

ALVAC-HIV Recombinants

Immunoprecipitation (IP) was used to provide a semi-quantitative comparison of the temporal expression of the HIV-I cassette contained in the ALVAC recombinants
35 in MRC-5 infected cells. Heat inactivated sera from HIV patients was obtained and used for the IP as described in the methods. The antiserum will precipitate the 120 KDa

env protein and the various cleavage products from the gag protein precursor. In the analysis of the IP data it is apparent that the ALVAC recombinants such as vCP1431A containing the E3L/K3L cassette had a significant
5 increase in the level of expression at all times post infection when compared to the ALVAC recombinant vCP205 without the E3L/K3L cassette.

RNA slot blots were used to evaluate temporal transcriptional expression in MRC-5 cells infected with
10 the ALVAC recombinants vCP205 and vCP1431A. In this analysis comparisons were made to the levels of mRNA transcribed from the HIV-I cassette encoding the env and gag proteins. ALVAC recombinants containing the E3L/K3L cassette (vCP1431A) did not exhibit a significant
15 increase in the level of mRNA for the env and gag genes above that of the ALVAC recombinant vCP205.

The previously discussed role E3L/K3L plays in the down regulation of PKR in vaccinia infected cells thereby modulating translation seems to be operative in
20 the ALVAC recombinants containing the vaccinia E3L/K3L functions. The data has shown that translation is significantly enhanced in cells infected with ALVAC recombinants containing the E3L/K3L genes, while no significant increase in the level of transcription has
25 been detected. This exemplifies the impact of E3L/K3L expression on translation efficiency in poxvirus infected cells.

Immunoprecipitation analyses were also performed using radiolabeled lysates derived from CEF
30 cells infected with ALVAC parental virus, ALVAC-MN120TMG (vCP205), ALVAC-MN120TMGNPst (vCP1433), vCP1452 and vCP300, as described previously (Taylor et al., 1990), with human serum derived from HIV-seropositive individuals (anti-HIV). The analysis confirmed the
35 expression of the envelope sequences with a molecular weight of 120kDa and the Gag precursor protein with a molecular weight of 55kDa in the recombinants but not in

the parental virus. However, vCP300 exhibits diminished expression in comparison to vCP1452, i.e., vCP1452 surprisingly demonstrates enhanced expression due to expression of transcription and/or translation factors,
5 in accordance with the invention.

FAC scan analysis with the Human anti-HIV antibody demonstrated expression of gp120 on the surface of HeLa cells infected with ALVAC-MN120TMGNPst (vCP1433). No fluorescence was detected on cells infected with ALVAC
10 parental virus.

Appropriate expression of the inserted HIV genes was further confirmed by immunoprecipitation analysis (using polyclonal serum pool from HIV infected individuals) performed on a radiolabelled lysate of MRC5
15 cells infected with vCP1433 or vCP1452. The analysis confirmed the expression of the envelope sequences with a molecular weight of 120KDa and the Gag precursor protein with a molecular weight of 55 KDa in vCP1452.

vCP1452 had enhanced expression on human cells
20 in comparison to vCP1433 and vCP300. Indeed, enhanced expression was observed with the E3L/K3L translational factors in human and canine cells.

Preliminary immunogenicity studies in mice showed no evidence of enhanced immunogenicity by the
25 E3L/K3L translational factor. This corresponds to no observed enhanced expression in murine cells. Thus, the origin of the cell may be an important factor in in vitro or in vivo applications of the invention, as may be the nature of the vector, e.g., the phenotype of the vector
30 (e.g., abortive, and when abortive such as abortive early, abortive late); but, appropriate selection of a cell and vector phenotype and of time of expression of factor(s) and foreign and/or exogenous DNA are within the ambit of the skilled artisan, from this disclosure and
35 the knowledge in the art, without undue experimentation.

ALVAC-FHV gB Recombinants

Analysis of the expression for vCP1459 and

vCP1460 was accomplished by immunoprecipitation analysis using a sheep anti-FHV gB polyclonal sera. Human MRC-5 cells were inoculated at an moi =5 at time 0, and then pulsed for 1 hour with ³⁵S labelled methionine at times 3, 5 6, 24, 48 and 72 h p.i. The precipitated protein was separated on SDS-PAGE gels. Autoradiographs of these IPs were scanned using a densitometer. The methods used provide a semi-quantitative analysis of FHV gB expression at the specific time points.

10 Results show that all recombinants express the proper sized full-length, glycosylated FHV gB polypeptide (apparent MW of approximately 115 kDa). However, recombinant vCP1460 shows significant increase in the amount of gB protein (about 5 times) compared to vCP1459. 15 In addition, these expression levels persist even at 72 hr p.i. Thus, it appears that the expression of vaccinia E3L/K3L in ALVAC has a significant effect on the level and persistence of FHV gB expression.

Example 3 - Additional Vectors

20 Using the documents cited herein and the teaching herein, including in the foregoing Examples, plasmid and naked DNA vectors, and additional viral vectors, including poxvirus, e.g., NYVAC, TROVAC, ALVAC, MVA, ts (temperature sensitive) mutants, or early (DNA) 25 and late defective mutants, adenovirus, e.g., CAV such as CAV2, herpesvirus, e.g., Epstein Barr, are generated with enhanced translation, e.g., by using E3L, K3L, VAI, EBER, sigma 3, TRBP, or combinations thereof, to modify the vector to contain at least one translation factor; and 30 accordingly, enhanced expression, of exogenous coding nucleic acid molecules (such exogenous coding nucleic acid molecules including from documents cited herein or as otherwise known in the art, or from applying those teachings in conjunction with teachings herein) is 35 obtained.

Having thus described in detail preferred embodiments of the present invention, it is to be

WO 98/40500

PCT/US98/03710

55

understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from
5 the spirit or scope of the present invention.

References

- Ahn, B-Y. and Moss, B. 1992. RNA polymerase-associated transcription specificity factor encoded by vaccinia virus. Proc. Natl. Acad. Sci. 89: 3536-3540.
- 5
- Beattie, E., Denzler, K., Tartaglia, J., Paoletti, E. and Jacobs, B. L. 1995. Reversal of the interferon-sensitive phenotype of and E3L-minus vaccinia virus by expression of the reovirus S4 gene. J. Virol. 69: 499-505 ("Beattie et al. 1995a").
- 10
- Beattie, E., Paoletti, E., and Tartaglia, J. 1995. Distinct Patterns of IFN Sensitivity Observed in Cells Infected with Vaccinia K3L and E3L Mutant Viruses. Virology 210:254-263 ("Beattie et al 1995b")
- 15
- Beattie, E., Tartaglia, J. and Paoletti, E. 1991. Vaccinia virus-encoded eIF-2a homologue abrogates the antiviral effect of interferon. Virology 183: 419-422.
- 20
- Carroll, K., Elroy Stein, O., Moss, B. and Jagus, R. 1993. Recombinant vaccinia virus K3L gene product prevents activation of double-stranded RNA-dependent, initiation factor 2 alpha-specific protein kinase. J. Biol. Chem. 268: 12837-12842.
- 25
- Chang, H-W., Watson, J. and Jacobs, B. L. 1992. The vaccinia virus E3L gene encodes a double-stranded RNA-binding protein with inhibitory activity for the interferon-induced protein kinase. Proc. Natl. Acad. Sci. USA 89: 4825-4829.
- 30
- Clark, P. A., Schwemmle, M., Schickinger, J., Hilse, K., and Clemens, M. J. 1991. Binding of Epstein-Barr virus small RNA EBER-1 to double-stranded RNA-activated protein kinase DAI. Nucleic Acids Res. 19:243-248.
- 35

Davies, M. V., Chang, H. W. , Jacobs, B. L. and Kaufman, R. J. 1993. The E3L and K3L vaccinia virus gene products stimulate translation through inhibition of the double-stranded RNA-dependent protein kinase by different
5 mechanisms. J. Virol. 67: 1688-1692.

Davies, M. V., Elroy Stein, O., Jagus, R., Moss, B. and Kaufman, R. J. 1992. The vaccinia K3L gene product potentiates translation by inhibiting double-stranded-
10 RNA-activated protein kinase and phosphorylation of the alpha subunit of eukaryotic initiation factor 2. J. Virol. 66: 1943-1950.

Goebel, S. J., Johnson, G. P., Perkus, M. E., Davis, S. W., Winslow, J. P. and Paoletti, E. 1990. The complete
15 DNA sequence of vaccinia virus. Virology 179: 247-266.

Harlow, E. and Lane, D. (1988). Antibodies, A Laboratory Manual. Cold Spring Harbor Laboratory. 421-470.
20

Hattori, M., and Sakaki, Y. (1986). Dideoxy sequencing method using denatured plasmid templates. Anal. Biochem. 152, 232-237.

25 Imani, F. and Jacobs, B. L. 1988. Inhibitory activity for the interferon induced protein kinase is associated with the reovirus serotype 1 s3 protein. Proc. Natl. Acad. Sci. USA 85: 7887-7891.

30 Jacobs, B. L. and Langland, J. O. 1996. When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology 219: 339-349.

35 Langone, J. (1982). Applications of immobilized protein A in immunochemical techniques. J. Immunol. Methods. 55. 277-296.

- Mathews, M. B. and Shenk, T. 1991. Adenovirus virus-associated RNA and translation control. *J. Virol.* 65: 5657-5662.
- 5 Moss, B. 1990. Regulation of vaccinia virus transcription. *Annu. Rev. Biochem.* 59: 661-688.
- Moss, B. 1992. Molecular biology of poxviruses. In *Recombinant Poxviruses*. Binns M. M., Smith, G. L. (eds).
10 Boca Raton, FL: CRC Press; pg. 45-80.
- Park, H., Davies, M. V., Langland, L. O., Chang, H-W., Nam, Y. S., Tartaglia, J., Paoletti, E., Jacobs, B. L., Kaufman, R. J. and Venkatesan, S. 1994. A cellular
15 protein that binds several structured viral RNAs is an inhibitor of the interferon induced PKR protein kinase in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* 91: 4713-4717.
- 20 Perkus, M., Limbach, K., Paoletti, E. (1989). Cloning and expression of foreign genes in vaccinia virus, using a host range selection system. *J. Virology* 63. 3829-3836.
- Perkus, M. E., Tartaglia, J., and Paoletti, E. 1995.
25 Poxvirus-based vaccine candidates for cancer, AIDS and other infectious diseases. *J. of Leukocyte Biology* 58: 1-13.
- Sharp, T. V. , Schwemmle, M., Jeffrey, I., Laing, K.,
30 Mellor, H., Proud, C. G., Hilse, K. and Clemens, M. J. 1993. Comparative analysis of the regulation of the interferon-inducible protein kinase PKR by Epstein-Barr virus RNAs EBER-1 and EBER-2 and adenovirus VAI RNA. *Nucleic Acids Res.* 21: 4483-4490.
- 35 Tabor, S., and Richardson, C.C. (1987). DNA sequence analysis with a modified bacteriophage T7 polymerase.

Proc. Natl. Acad. Sci. USA 84, 4767-4771.

Tartaglia, J., Perkus, M. E., Taylor, J. et al. 1992.
NYVAC: A highly attenuated strain of vaccinia virus.

5 Virology 188: 217-32.

Thimmappaya, B. C., Weinberger, C., Schneider, R. J. and
Shenk, T. 1982. Adenovirus VAI RNA is required for
efficient translation of viral mRNAs at late times after
10 infection. Cell 31: 543-551.

Watson, J., Chang, H-W. and Jacobs, B. L. 1991.
Characterization of a vaccinia virus-induced dsRNA-
binding protein that may be the inhibitor of the dsRNA-
15 dependent protein kinase. Virology 185: 206-216.

Yuen, L, and Moss, B. (1987). Oligonucleotide sequence
signaling transcriptional termination of vaccinia virus
early genes. Proc. Natl. Acad. Sci. USA 84, 6417-6421.
20

Zhang, Y., Ahn, B-Y. and Moss, B. 1994. Targeting of a
multicomponent transcription apparatus into assembling
vaccinia virus particles requires RAP94, an RNA
polymerase-associated protein. J. Virol. 68: 1360-1370.
25

60
SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: VIROGENETICS CORPORATION -AND- ARIZONA STATE UNIVERSITY

(ii) TITLE OF INVENTION: VECTORS HAVING ENHANCED EXPRESSION AND METHODS
OF MAKING AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 23

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SMART & BIGGAR

(B) STREET: P.O. BOX 2999, STATION D

10 (C) CITY: OTTAWA

(D) STATE: ONT

(E) COUNTRY: CANADA

(F) ZIP: K1P 5Y6

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: ASCII (text)

(vi) CURRENT APPLICATION DATA:

20 (A) APPLICATION NUMBER: CA 2,283,462

(B) FILING DATE: 25-FEB-1998

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/815,809

(B) FILING DATE: 12-MAR-1997

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: SMART & BIGGAR

(B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER: 77354-45

30 (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (613)-232-2486

61

(B) TELEFAX: (613)-232-8440

(2) INFORMATION FOR SEQ ID NO.: 1:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 4434

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

GAGCTCGCGG CCGCCTATCA AAAGTCTTAA TGAGTTAGGT GTAGATAGTA TAGATATTAC 60
TACAAAGGTA TTCATATTTT CTATCAATTC TAAAGTAGAT GATATTAATA ACTCAAAGAT 120
GATGATAGTA GATAATAGAT ACGCTCATAT AATGACTGCA AATTTGGACG GTTCACATTT 180
TAATCATCAC GCGTTCATAA GTTTCAACTG CATAGATCAA AATCTCACTA AAAAGATAGC 240
CGATGTATTT GAGAGAGATT GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA 300
TACATAATGG ATTTTGTTAT CATCAGTTAT ATTTAACATA AGTACAATAA AAAGTATTAA 360
ATAAAAATAC TTACTIONTACGA AAAAATGACT AATTAGCTAT AAAAACCCAG ATCTCTCGAG 420
20 GTCGACGGTA TCGATAAGCT TGATATCGAA TTCATAAAAA TTATTGATGT CTACACATCC 480
TTTTGTAATT GACATCTATA TATCCTTTTG TATAATCAAC TCTAATCACT TTAACITTTTA 540
CAGTTTTCCC TACCAGTTTA TCCCTATATT CAACATATCT ATCCATATGC ATCTTAACAC 600
TCTCTGCCAA GATAGCTTCA GAGTGAGGAT AGTCAAAAAG ATAAATGTAT AGAGCATAAT 660
CCTTCTCGTA TACTCTGCCC TTTATTACAT CGCCCGCATT GGGCAACGAA TAACAAAATG 720
CAAGCATACG ATACAAACTT AACGGATATC GCGATAATGA AATAATTTAT GATTATTTCT 780
CGCTTTCAAT TTAACACAAC CCTCAAGAAC CTTTGTATTT ATTTTCACTT TTTAAGTATA 840
GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATTGTTT CGTTTTCCCC TTGGCGTATC 900
ACTAATTAAT TAACCCGGGC TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCAITTT 960
GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTAACG GGAACAGGGT 1020
30 TTGTTGATTC GCAAACITATT CTAATACATA ATTCTTCTGT TAATACGTCT TGCACGTAAT 1080
CTATTATAGA TGCCAAGATA TCTATATAAT TATTTTGTAAT GATGATGTTA ACTATGTGAT 1140

62

CTATATAAGT AGTGTAATAA TTCATGTATT TCGATATATG TTCCAACCTCT GTCTTTGTGA 1200
 TGTCTAGTTT CGTAATATCT ATAGCATCCT CAAAAAATAT ATTTCGCATAT ATTCCCAAGT 1260
 CTTCAGTTCT ATCTTCTAAA AAATCTTCAA CGTATGGAAT ATAATAATCT ATTTTACCTC 1320
 TTCTGATATC ATTAATGATA TAGTTTTTGA CACTATCTTC TGTCAATTGA TTCTTATTCA 1380
 CTATATCTAA GAAACGGATA GCGTCCCTAG GACGAACTAC TGCCATTAAT ATCTCTATTA 1440
 TAGCTTCTGG ACATAATTCA TCTATTATAC CAGAATTAAT GGGAACCTATT CCGTATCTAT 1500
 CTAACATAGT TTTAAGAAAG TCAGAATCTA AGACCTGATG TTCATATATT GGTTCATACA 1560
 TGAAATGATC TCTATTGATG ATAGTGACTA TTTCATTCTC TGAAAATTGG TAACTCATTC 1620
 TATATATGCT TTCCTTGTTG ATGAAGGATA GAATATACTC AATAGAATTT GTACCAACAA 1680
 10 ACTGTTCTCT TATGAATCGT ATATCATCAT CTGAAATAAT CATGTAAGGC ATACATTTAA 1740
 CAATTAGAGA CTTGTCTCCT GTTATCAATA TACTATTCTT GTGATAATTT ATGTGTGAGG 1800
 CAAATTTGTC CACGTTCTTT AATTTTGTTA TAGTAGATAT CAAATCCAAT GGAGCTACAG 1860
 TTCTTGGCTT AAACAGATAT AGTTTTTCTG GAACAAATTC TACAACATTA TTATAAAGGA 1920
 CTTTGGGTAG ATAAGTGGGA TGAAATCCTA TTTTAATTAA TGCTATCGCA TTGTCCTCGT 1980
 GCAAATATCC AAACGCTTTT GTGATAGTAT GGCATTCATT GTCTAGAAAC GCTCTACGAA 2040
 TATCTGTGAC AGATATCATC TTTAGAGAAT ATACTAGTCG CGTTAATAGT ACTACAATTT 2100
 GTATTTTTTA ATCTATCTCA ATAAAAAAT TAATATGTAT GATTCAATGT ATAATAAAC 2160
 TACTAACTGT TATTGATAAC TAGAATCAGA ATCTAATGAT GACGTAACCA AGAAGTTTAT 2220
 CTACTGCCAA TTTAGCTGCA TTATTTTGTAG CATCTCGTTT AGATTTTCCA TCTGCCTTAT 2280
 20 CGAATACTCT TCCGTCGATG TCTACACAGG CATAAAATGT AGGAGAGTTA CTAGGCCCAA 2340
 CTGATTCAAT ACGAAAAGAC CAATCTCTCT TAGTTATTTG GCAGTACTCA TTAATAATGG 2400
 TGACAGGGTT AGCATCTTTC CAATCAATAA TTTTTTTAGC CGGAATAACA TCATCAAAAG 2460
 ACTTATGATC CTCTCTCATT GATTTTTTCGC GGGATACATC ATCTATTATG ACGTCAGCCA 2520
 TAGCATCAGC ATCCGGCTTA TCCGCCTCCG TTGTCATAAA CCAACGAGGA GGAATATCGT 2580
 CGGAGCTGTA CACCATAGCA CTACGTTGAA GATCGTACAG AGCTTTATTA ACTTCTCGCT 2640
 TCTCCATATT AAGTTGTCTA GTTAGTTGTG CAGCAGTAGC TCCTTCGATT CCAATGTTTT 2700
 TAATAGCCGC ACACACAATC TCTGCGTCAG AACGCTCGTC AATATAGATC TTAGACATTT 2760
 TTAGAGAGAA CTAACACAAC CAGCAATAAA ACTGAACCTA CTTTATCATT TTTTATTCA 2820
 TCATCCTCTG GTGGTTCGTC GTTTCTATCG AATGTAGCTC TGATTAACCC GTCATCTATA 2880
 30 GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT 2940
 ATTCCTTGT TTTCATGTAT CGATTGCGTT GTAACATTAA GATTGCGAAA TGCTCTAAAT 3000

63

TTGGGAGGCT TAAAGTGTTG TTTGCAATCT CTACACGCGT GTCTAACTAG TGGAGGTTTCG 3060
TCAGCTGCTC TAGTTTGAAT CATCATCGGC GTAGTATTCC TACTTTTACA GTTAGGACAC 3120
GGTGTATTGT ATTTCTCGTC GAGAACGTTA AAATAATCGT TGTAACCTAC ATCCTTTATT 3180
TTATCTATAT TGTATTCTAC TCCTTTCTTA ATGCATTTTA TACCGAATAA GAGATAGCGA 3240
AGGAATTCTT TTTATTGATT AACTAGTCAA ATGAGTATAT ATAATTGAAA AAGTAAAATA 3300
TAAATCATAT AATAATGAAA CGAAATATCA GTAATAGACA GGAAGTGGCA GATTCTTCTT 3360
CTAATGAAGT AAGTACTGCT AAATCTCCAA AATTAGATAA AAATGATACA GCAAATACAG 3420
CTTCATTCAA CGAATTACCT TTTAATTTTT TCAGACACAC CTTATTACAA ACTAACTAAG 3480
TCAGATGATG AGAAAGTAAA TATAAATTTA ACTTATGGGT ATAATATAAT AAAGATTCAT 3540
10 GATATTAATA ATTTACTTAA CGATGTTAAT AGACTTATTC CATCAACCCC TTCAAACCTT 3600
TCTGGATATT ATAAAATACC AGTTAATGAT ATTAAAATAG ATTGTTTAAAG AGATGTAAAT 3660
AATTATTTGG AGGTAAAGGA TATAAAATTA GTCTATCTTT CACATGGAAA TGAATTACCT 3720
AATATTAATA ATTATGATAG GAATTTTTTA GGATTTACAG CTGTTATATG TATCAACAAT 3780
ACAGGCAGAT CTATGGTTAT GGTAACACAC TGTAACGGGA AGCAGCATTC TATGGTAACT 3840
GGCCTATGTT TAATAGCCAG ATCATTTTAC TCTATAAACA TTTTACCACA AATAATAGGA 3900
TCCTCTAGAT ATTTAATATT ATATCTAACA ACAACAAAAA AATTTAACGA TGTATGGCCA 3960
GAAGTATTTT CTACTAATAA AGATAAAGAT AGTCTATCTT ATCTACAAGA TATGAAAGAA 4020
GATAATCATT TAGTAGTAGC TACTAATATG GAAAGAAATG TATACAAAAA CGTGAAGCT 4080
TTTATATTAA ATAGCATATT ACTAGAAGAT TTAAATCTA GACTTAGTAT AACAAAACAG 4140
20 TTAAATGCCA ATATCGATTC TATATTTTAT CATAACAGTA GTACATTAAT CAGTGATATA 4200
CTGAAACGAT CTACAGACTC AACTATGCAA GGAATAAGCA ATATGCCAAT TATGTCTAAT 4260
ATTTTAACTT TAGAACTAAA ACGTTCTACC AATACTAAAA ATAGGATACG TGATAGGCTG 4320
TTAAAAGCTG CAATAAATAG TAAGGATGTA GAAGAAATAC TTTGTTCTAT ACCTTCGGAG 4380
GAAAGAACTT TAGAACAACT TAAGTTTAAAT CAAACTTGTA TTTATGAAGG TACC 4434

(2) INFORMATION FOR SEQ ID NO.: 2

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 2844

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS:

(D) TOPOLOGY:

64

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

ATGTCCACTC GTGGCGATCT TGGGAAGCGG CGACGAGGGA GTCGTTGGCA GGGACACAGT 60
GGCTATTTTC GACAGAGATG TTTTTTCCCT TCTCTACTCG GTATTGCAGC GACTGGCTCC 120
AGACATGGTA ACGGATCGTC GGGATTAACC AGACTAGCTA GATATGTTTC ATTTATCTGG 180
ATCGTACTAT TCTTAGTCGG TCCCCGTCCA GTAGAGGGTC AATCTGGAAG CACATCGGAA 240
CAACCCCGGC GGACTGTAGC TACCCCTGAG GTAGGGGGTA CACCACCAA ACCAACTACA 300
10 GATCCCACCG ATATGTCGGA TATGAGGGAA GCTCTCCGTG CGTCCCAAAT AGAGGCTAAC 360
GGACCATCGA CTTTCTATAT GTGTCCACCA CCTTCAGGAT CTACTGTCGT GCGTTTAGAG 420
CCACCACGGG CCTGTCCAGA TTATAAACTA GGGAAAATT TTACCGAGGG TATAGCTGTA 480
ATATTTAAAG AAAATATAGC GCCATATAAA TTCAAGGCAA ATATATACTA TAAAAACATT 540
ATTATGACAA CGGTATGGTC TGGGAGTTCC TATGCCGTTA CAACCAACCG ATATACAGAC 600
AGGGTTCCCG TGAAAGTTCA AGAGATTACA GATCTCATAG ATAGACGGGG TATGTGCCTC 660
TCGAAAGCTG ATTACGTTTC TAACAATTAT CAATTTACGG CCTTTGATCG AGACGAGGAT 720
CCCAGAGAAC TGCCTCTGAA ACCCTCCAAG TTCAACACTC CAGAGTCCCG TGGATGGCAC 780
ACCACCAATG AACATACAC AAAGATCGGT GCTGCTGGAT TTCACCACTC TGGGACCTCT 840
GTAAATTGCA TCGTAGAGGA AGTGGATGCA AGATCTGTAT ATCCATATGA CTCATTTGCT 900
20 ATCTCCACTG GTGACGTGAT TCACATGTCT CCATTCTTTG GGCTGAGGGA TGGAGCCCAT 960
GTAGAACATA CTAGTTATTC TTCAGACAGA TTTCAACAAA TCGAGGGATA CTATCCAATA 1020
GACTTGGATA CGCGATTACA ACTGGGGGCA CCAGTTTCTC GCAATTTTTT GGAAACTCCG 1080
CATGTGACAG TGGCCTGGAA CTGGACCCCA AAGTCTGGTC GGGTATGTAC CTTAGCCAAA 1140
TGGAGGGAAA TAGATGAAAT GCTACGCGAT GAATATCAGG GCTCCTATAG ATTTACAGCC 1200
AAGACCATAT CCGCTACTTT CATCTCCAAT ACTTCACAAT TTGAAATCAA TCGTATCCGT 1260
TTGGGGGACT GTGCCACCAA GGAGGCAGCC GAAGCCATAG ACCGGATTTA TAAGAGTAAA 1320
TATAGTAAAA CTCATATTCA GACTGGAACC CTGGAGACCT ACCTAGCCCG TGGGGGATTT 1380
CTAATAGCTT TCCGTCCCAT GATCAGCAAC GAACTAGCAA AGTTATATAT CAATGAATTA 1440
GCACGTTCCA ATCGCACGGT AGATCTCAGT GCACTCCTCA ATCCATCTGG GGAAACAGTA 1500
30 CAACGAACTA GAAGATCGGT CCCATCTAAT CAACATCATA GGTCGCGGCG CAGCACAATA 1560
GAGGGGGGTA TAGAAACCGT GAACAATGCA TCACTCCTCA AGACCACCTC ATCTGTGGAA 1620

65

TTCGCAATGC TACAATTTGC CTATGACTAC ATACAAGCCC ATGTAAATGA AATGTTGAGT 1680
CGGATAGCCA CTGCCTGGTG TACACTTCAG AACCGCGAAC ATGTGCTGTG GACAGAGACC 1740
CTAAAACTCA ATCCCGGTGG GGTGGTCTCG ATGGCCCTAG AACGTCGTGT ATCCGCGCGC 1800
CTACTTGGAG ATGCCGTCGC CGTAACACAA TGTGTTAACA TTTCTAGCGG ACATGTCTAT 1860
ATCCAAAATT CTATGCGGGT GACGGGTTCa TCAACGACAT GTTACAGCCG CCCTCTTGTT 1920
TCCTTCCGTG CCCTCAATGA CTCCGAATAC ATAGAAGGAC AACTAGGGGA AAACAATGAA 1980
CTTCTCGTGG AACGAAAACt AATTGAGCCT TGCACtGTCA ATAATAAGCG GTATTTTAAG 2040
TTTGGGGCAG ATTATGTATA TTTTGAGGAT TATGCGTATG TCCGTAAAGT CCCGCTATCG 2100
GAGATAGAAC TGATAAGTGC GTATGTGAAT TTAAATCTTA CTCTCCTAGA GGATCGTGAA 2160
10 TTTCTCCCAC TCGAAGTTTA TACACGAGCT GAGCTGGAAG ATACCGGCCT TTTGGACTAC 2220
AGCGAGATTC AACGCCGCAA CCAACTCCAC GCCTTAAAAT TTTATGATAT AGACAGCATA 2280
GTCAGAGTGG ATAATAATCT TGTCATCATG CGTGGTATGG CAAATTTCTT TCAGGGACTC 2340
GGGGATGTGG GGGCTGGTTT CGGCAAGGTG GTCTTAGGGG CTGCGAGTGC GGTAATCTCA 2400
ACAGTATCAG GCGTATCATC ATTTCTAAAC AACCCATTTG GAGCATTGGC CGTGGGACTG 2460
TTAATATTAG CTGGCATCGT CGCAGCATTC CTGGCATATC GCTATATATC TAGATTACGT 2520
GCAAATCCAA TGAAAGCCTT ATATCCTGTG ACGACTAGGA ATTTGAAACA GACGCTAAGA 2580
GCCCCGCTCAA CGGCTGGTGG GGATAGCGAC CCGGGAGTCG ATGACTTCGA TGAGGAAAAG 2640
CTAATGCAGG CAAGGGAGAT GATAAAATAT ATGTCCCTCG TATCGGCTAT GGAGCAACAA 2700
GAACATAAGG CGATGAAAAA GAATAAGGGC CCAGCGATCC TAACGAGTCA TCTCACTAAC 2760
20 ATGGCCCTCC GTCGCCGTGG ACCTAAATAC CAACGCCTCA ATAATCTTGA TAGCGGTGAT 2820
GATACTGAAA CAAATCTTGT CTAA 2844

(2) INFORMATION FOR SEQ ID NO.: 3

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6628

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

30 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

66

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

GCGGCCGCGT CGACATGCAT TGTTAGTTCT GTAGATCAGT AACGTATAGC ATACGAGTAT 60
 AATTATCGTA GGTAGTAGGT ATCCTAAAAT AAATCTGATA CAGATAATAA CTTTGTAAAT 120
 CAATTCAGCA ATTTCTCTAT TATCATGATA ATGATTAATA CACAGCGTGT CGTTATTTTT 180
 TGTTACGATA GTATTTCTAA AGTAAAGAGC AGGAATCCCT AGTATAATAG AAATAATCCA 240
 TATGAAAAAT ATAGTAATGT ACATATTTCT AATGTTAACA TATTTATAGG TAAATCCAGG 300
 AAGGGTAATT TTTACATATC TATATACGCT TATTACAGTT ATTAAAAATA TACTTGCAAA 360
 CATGTTAGAA GTAAAAAAGA AAGAACTAAT TTTACAAAGT GCTTTACCAA AATGCCAATG 420
 GAAATTACTT AGTATGTATA TAATGTATAA AGGTATGAAT ATCACAAACA GCAAATCGGC 480
 10 TATTCCCAAG TTGAGAAACG GTATAATAGA TATATTTCTA GATACCATTA ATAACCTTAT 540
 AAGCTTGACG TTTCTATAA TGCCTACTAA GAAACTAGA AGATACATAC ATACTAACGC 600
 CACACGAGAG TAACTACTCA TCGTATAACT ACTGTTGCTA ACAGTGACAC TGATGTTATA 660
 ACTCATCTTT GATGTGGTAT AAATGTATAA TAACTATATT AACTGGTAT TTTATTTTCAG 720
 TTATATACTA TATAGTATTA AAAATTATAT TTGTATAATT ATATTATTAT ATTCAGTGTA 780
 GAAAGTAAAA TACTATAAAT ATGTATCTCT TATTTATAAC TTATTAGTAA AGTATGTACT 840
 ATTCAGTTAT ATTGTTTTAT AAAAGCTAAA TGCTACTAGA TTGATATAAA TGAATATGTA 900
 ATAAATTAGT AATGTAGTAT ACTAATATTA ACTCACATTT GACTAATTAG CTATAAAAAC 960
 CCGGGCTGCA GCCCGGAAG CTTACAAAAA TTAGACAAGA TTTGTTTCAG TATCATCACC 1020
 GCTATCAAGA TTATTGAGGC GTTGGTATTT AGGTCCACGG CGACGGAGGG CCATGTTAGT 1080
 20 GAGATGACTC GTTAGGATCG CTGGGCCCTT ATTCTTTTTC ATCGCCTTAT GTTCTTGTTG 1140
 CTCCATAGCC GATACGAGGG ACATATATTT TATCATCTCC CTTGCCTGCA TTAGCTTTTC 1200
 CTCATCGAAG TCATCGACTC CCGGGTCGCT ATCCCCACCA GCCGTTGAGC GGGCTCTTAG 1260
 CGTCTGTTTC AAATTCCTAG TCGTCACAGG ATATAAGGCT TTCATTGGAT TTGCACGTAA 1320
 TCTAGATATA TAGCGATATG CCAGGAATGC TGCGACGATG CCAGCTAATA TTAACAGTCC 1380
 CACGGCCAAT GCTCCAAATG GGTGTTTGTAG AAATGATGAT ACGCCTGATA CTGTTGAGAT 1440
 TACCGCACTC GCAGCCCCTA AGACCACCTT GCCGAAACCA GCCCCACAT CCCCAGGTCC 1500
 CTGAAAGAAA TTTGCCATAC CACGCATGAT GACAAGATTA TTATCCACTC TGAATATGCT 1560
 GTCTATATCA TAAAATTTTA AGGCGTGGAG TTGGTTGCGG CGTTGAATCT CGCTGTAGTC 1620
 CAAAAGGCCG GTATCTTCCA GCTCAGCTCG TGTATAAACT TCGAGTGGGA GAAATTCACG 1680
 30 ATCCTCTAGG AGAGTAAGAT TTAAATTCAC ATACGCACTT ATCAGTTCTA TCTCCGATAG 1740
 CGGGACTTTA CGGACATACG CATAATCCTC AAAATATACA TAATCTGCCC CAAACTTAAA 1800

67

ATACCGCTTA TTATTGACAG TGCAAGGCTC AATTAGTTTT CGTTCCACGA GAAGTTCATT 1860
 GTTTTCCCCT AGTTGTCCTT CTATGTATTC GGAGTCATTG AGGGCACGGA AGGAAACAAG 1920
 AGGGCGGCTG TAACATGTCG TTGATGAACC CGTCACCCGC ATAGAATTTT GGATATAGAC 1980
 ATGTCCGCTA GAAATGTAA CACATTGTGT TACGGCGACG GCATCTCCAA GTAGGCGCGC 2040
 GGATACACGA CGTTCTAGGG CCATCGAGAC CACCCCACCG GGATTGAGTT TTAGGGTCTC 2100
 TGTCCACAGC ACATGTTTCG GGTTCCTGAAG TGTACACCAG GCAGTGGCTA TCCGACTCAA 2160
 CATTTCAATTT ACATGGGCTT GTATGTAGTC ATAGGCAAAT TGTAGCATTG CGAATTCCAC 2220
 AGATGAGGTG GTCTTGAGGA GTGATGCATT GTTCACGGTT TCTATACCCC CCTCTATTGT 2280
 GCTGCGCCGC GACCTATGAT GTTGATTAGA TGGGACCGAT CTTCTAGTTC GTTGTACTGT 2340
 10 TTCCCCAGAT GGATTGAGGA GTGCACTGAG ATCTACCGTG CGATTGGAAC GTGCTAATTC 2400
 ATTGATATAT AACTTTGCTA GTTCGTTGCT GATCATGGGA CGGAAAGCTA TTAGAAATCC 2460
 CCCACGGGCT AGGTAGGTCT CCAGGGTTCC AGTCTGAATA TGAGTTTTAC TATATTTACT 2520
 CTTATAAATC CGGTCTATGG CTTGCGCTGC CTCCTTGGTG GCACAGTCCC CCAAACGGAT 2580
 ACGATTGATT TCAAATTGTG AAGTATTGGA GATGAAAGTA GCGGATATGG TCTTGGCTGT 2640
 AAATCTATAG GAGCCCTGAT ATTCATCGCG TAGCATTTC TCTATTTCCC TCCATTTGGC 2700
 TAAGGTACAT ACCCGACCAG ACTTTGGGGT CCAGTTCCAG GCCACTGTCA CATGCGGAGT 2760
 TTCCAAAAAA TTGCGAGAAA CTGGTGCCCC CAGTTGTAAT CGCGTATCCA AGTCTATTGG 2820
 ATAGTATCCC TCGATTTGTT GAAATCTGTC TGAAGAATAA CTAGTATGTT CTACATGGGC 2880
 TCCATCCCTC AGCCCAAAGA ATGGAGACAT GTGAATCACG TCACCAGTGG AGATAGCAAA 2940
 20 TGAGTCATAT GGATATACAG ATCTTGCATC CACTTCCTCT ACGATGCAAT TTACAGAGGT 3000
 CCCAGAGTGG TGAAATCCAG CAGCACCGAT CTTTGTGTAT GTTTCATTGG TGGTGTGCCA 3060
 TCCACGGGAC TCTGGAGTGT TGAACCTGGA GGGTTTCAGA GGCAGTTCTC TGGGATCCTC 3120
 GTCTCGATCA AAGGCCGTAA ATTGATAATT GTTACGAACG TAATCAGCTT TCGAGAGGCA 3180
 CATACCCCGT CTATCTATGA GATCTGTAAT CTCTTGAAC TACACGGGAA CCCTGTCTGT 3240
 ATATCGGTTG GTTGTAACGG CATAGGAACT CCCAGACCAT ACCGTTGTCA TAATAATGTT 3300
 TTTATAGTAT ATATTTGCCT TGAATTTATA TGGCGCTATA TTTTCTTTAA ATATTACAGC 3360
 TATACCCTCG GTAAAATTTT TCCCTAGTTT ATAATCTGGA CAGGCCCGTG GTGGCTCTAA 3420
 ACGCACGACA GTAGATCCTG AAGGTGGTGG ACACATATAG AAAGTCGATG GTCCGTTAGC 3480
 CTCTATTTGG GACGCACGGA GAGCTTCCCT CATATCCGAC ATATCGGTGG GATCTGTAGT 3540
 30 TGGTTTTGGT GGTGTACCCC CTACCTCAGG GGTAGCTACA GTCCGCCGGG GTTGTTCGA 3600
 TGTGCTTCCA GATTGACCCT CTAATGGACG GGGACCGACT AAGAATAGTA CGATCCAGAT 3660

68

AAATGAAACA TATCTAGCTA GTCTGGTTAA TCCCGACGAT CCGTTACCAT GTCTGGAGCC 3720
 AGTCGCTGCA ATACCGAGTA GAGAAGGGAA AAAACATCTC TGTCGAAAAT AGCCACTGTG 3780
 TCCCTGCCAA CGACTCCCTC GTCGCCGCTT CCCAAGATCG CCACGAGTGG ACATTACGAT 3840
 ACAAACTTAA CGGATATCGC GATAATGAAA TAATTTATGA TTATTTCTCG CTTTCAATTT 3900
 AACACAACCC TCAAGAACCT TTGTATTTAT TTTCACTTTT TAAGTATAGA ATAAAGAAGC 3960
 TCTAATTAAT TAAGCTACAA ATAGTTTCGT TTTCACCTTG TCTAATAACT AATTAATTAA 4020
 CCCGGATCGA TCCCGATTTT TATGACTAGT TAATCAAATA AAAAGCATAC AAGCTATTGC 4080
 TTCGCTATCG TTACAAAATG GCAGGAATTT TGTGTAAACT AAGCCACATA CTTGCCAATG 4140
 AAAAAAATAG TAGAAAGGAT ACTATTTTAA TGGGATTAGA TGTTAAGGTT CCTTGGGATT 4200
 10 ATAGTAACTG GGCATCTGTT AACTTTTACG ACGTTAGGTT AGATACTGAT GTTACAGATT 4260
 ATAATAATGT TACAATAAAA TACATGACAG GATGTGATAT TTTTCCTCAT ATAACTCTTG 4320
 GAATAGCAAA TATGGATCAA TGTGATAGAT TTGAAAATTT CAAAAGCAA ATAAGTATC 4380
 AAGATTTACA GACTATTTCT ATAGTCTGTA AAGAAGAGAT GTGTTTTCTT CAGAGTAACG 4440
 CCTCTAAACA GTTGGGAGCG AAAGGATGCG CTGTAGTTAT GAAACTGGAG GTATCTGATG 4500
 AACTTAGAGC CCTAAGAAAT GTTCTGCTGA ATGCGGTACC CTGTTCGAAG GACGTGTTTG 4560
 GTGATATCAC AGTAGATAAT CCGTGGAATC CTCACATAAC AGTAGGATAT GTTAAGGAGG 4620
 ACGATGTCGA AAACAAGAAA CGCCTAATGG AGTGCATGTC CAAGTTTAGG GGGCAAGAAA 4680
 TACAAGTTCT AGGATGGTAT TAATAAGTAT CTAAGTATTT GGTATAATTT ATTAAATAGT 4740
 ATAATTATAA CAAATAATAA ATAACATGAT AACGGTTTTT ATTAGAATAA AATAGAGATA 4800
 20 ATATCATAAT GATATATAAT ACTTCATTAC CAGAAATGAG TAATGGAAGA CTTATAAATG 4860
 AACTGCATAA AGCTATAAGG TATAGAGATA TAAATTTAGT AAGGTATATA CTTAAAAAAT 4920
 GCAAATACAA TAACGTAAAT ATACTATCAA CGTCTTTGTA TTTAGCCGTA AGTATTTCTG 4980
 ATATAGAAAT GGTAATAATTA TTACTAGAAC ACGGTGCCGA TATTTTAAAA TGTAATAATC 5040
 CTCCTCTTCA TAAAGCTGCT AGTTTAGATA ATACAGAAAT TGCTAAACTA CTAATAGATT 5100
 CTGGCGCTGA CATAGAACAG ATACATTCTG GAAATAGTCC GTTATATATT TCTGTATATA 5160
 GAAACAATAA GTCATTAAC TATATTTTAT TAAAAAAGG TGTTAATTGT AATAGATTCT 5220
 TTCTAAATTA TTACGATGTA CTGTATGATA AGATATCTGA TGATATGTAT AAAATATTTA 5280
 TAGATTTTAA TATTGATCTT AATATACAAA CTAGAAATTT TGAAACTCCG TTACATTACG 5340
 CTATAAAGTA TAAGAATATA GATTTAATTA GGATATTGTT AGATAATAGT ATTAAAATAG 5400
 30 ATAAAAGTTT ATTTTTCAT AAACAGTATC TCATAAAGGC ACTTAAAAAT AATTGTAGTT 5460
 ACGATATAAT AGCGTTACTT ATAAATCACG GAGTGCCTAT AAACGAACAA GATGATTTAG 5520

69

GTAAAACCCC ATTACATCAT TCGGTAATTA ATAGAAGAAA AGATGTAACA GCACTTCTGT 5580
TAAATCTAGG AGCTGATATA AACGTAATAG ATGACTGTAT GGGCAGTCCC TTACATTACG 5640
CTGTTTCACG TAACGATATC GAAACAACAA AGACACTTTT AGAAAGAGGA TCTAATGTTA 5700
ATGTGGTTAA TAATCATATA GATACCGTTC TAAATATAGC TGTGTCATCT AAAAACAAAA 5760
CTATAGTAAA CTTATTACTG AAGTACGGTA CTGATACAAA GTTGGTAGGA TTAGATAAAC 5820
ATGTTATTCA CATAGCTATA GAAATGAAAG ATATTAATAT ACTGAATGCG ATCTTATTAT 5880
ATGGTTGCTA TGTAACGTC TATAATCATA AAGGTTTCAC TCCTCTATAC ATGGCAGTTA 5940
GTTCTATGAA AACAGAATTT GTTAACTCT TACTTGACCA CGGTGCTTAC GTAAATGCTA 6000
AAGCTAAGTT ATCTGGAAAT ACTCCTTTAC ATAAAGCTAT GTTATCTAAT AGTTTTAATA 6060
10 ATATAAAATT ACTTTTATCT TATAACGCCG ACTATAATTC TCTAAATAAT CACGGTAATA 6120
CGCCTCTAAC TTGTGTTAGC TTTTGTAGATG ACAAGATAGC TATTATGATA ATATCTAAAA 6180
TGATGTTAGA AATATCTAAA AATCCTGAAA TAGCTAATTC AGAAGGTTTT ATAGTAAACA 6240
TGGAACATAT AAACAGTAAT AAAAGACTAC TATCTATAAA AGAATCATGC GAAAAAGAAC 6300
TAGATGTTAT AACACATATA AAGTTAAATT CTATATATTC TTTTAATATC TTTCTTGACA 6360
ATAACATAGA TCTTATGGTA AAGTTCGTAA CTAATCCTAG AGTTAATAAG ATACCTGCAT 6420
GTATACGTAT ATATAGGGAA TTAATACGGA AAAATAAATC ATTAGCTTTT CATAGACATC 6480
AGCTAATAGT TAAAGCTGTA AAAGAGAGTA AGAATCTAGG AATAATAGGT AGGTTACCTA 6540
TAGATATCAA ACATATAATA ATGGAACTAT TAAGTAATAA TGATTTACAT TCTGTTATCA 6600
CCAGCTGTTG TAACCCAGTA GTATAAAG 6628

20

(2) INFORMATION FOR SEQ ID NO.: 4

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 2700

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

TTTTTTTCAT TATTTAGAAA TTATGCATTT TAGATCTTTA TAAGCGGCCG TGATTAACTA 60

70

GTCATAAAAA CCCGGGATCG ATTCTAGACT CGAGGGTACC GGATCTTAAT AAAAAAAGTA 120
 ATAAATCTTT AATACGTAAA ATCTAGAAAT ATTCGCCGGC ACTAATTGAT CAGTATTTTT 180
 GGGCCCTAGC TAAGATCTGA GCTCCCATGG CCTAGAATTA TAATTAGTCA TCAGGCAGGG 240
 CGAGAACGAG ACTATCTGCT CGTTAATTAA TTAGGTGAC GGATCCCCCA AAAAAACTA 300
 ATCAGCTATC GGGGTTAATT AATTAGTTAT ATTAATCAGT AGTCCGTCCC GCTCTTGCTC 360
 TGATAGACGA GCAATTAATT AATCCAGCTG CCTAGGGGGT TGTTTTTGAT TAGTCGATAG 420
 CCCCAATTAA TTAATCAATA TAGACAAGGT GAAAACGAAA CTATTTGTAG CTTAATTAAT 480
 TAGAGCTTCT TTATTCTATA CTTAAAAAGT GAAAATAAAT ACAAAGGTTC TTGAGGGTTG 540
 TGTAAATTG ATCTGTTCCA CTTTGTCTT GATAAACATC GAATTAATTA ATCTCGAAGA 600
 10 AATAAGATAT GAATTTTTCA CTTTATTTA TGTTCCTAAG AACTCCCAAC ACAATTTAAC 660
 AAAGCGAGAA ATAATCATAA ATTATTTTAT TATCGCGATA TCCGTTAAGT TTGTATCGTA 720
 ATGCCACTAA CAGAAGAAGC AGAGCTAGAA CTGGCAGAAA ACTTTCGCTC TTTATTAGTA 780
 TTTAATAAAG TAATAGCGCT ATAGGCAATT CAAACATAGC ATTACGGTGA TTGTCTTCTT 840
 CGTCTCGATC TTGACCGTCT TTTGAGAGAG ATTCTAAAAG AACCAGTACA TGGAGTGTAT 900
 TATGACCCAT CAAAAGACTT AATAGCAGAA ATACAGAAGC AGGGGCAAGG CCAATCTCTC 960
 TAAGATTTTC TTGGTCATGT ACCTCACATA ATACTGGGTA GTTTTCTGAA TTATCGTCTT 1020
 TATGTCTTCG TCCCCGTTCC GGTTTGGACA TATCAAATTT ATCAAGAGCC ATTTAAAAAT 1080
 CTGAAAACAG GAATGGAGTG GAGATTTGAT TCTAGATTAG CATTTTCATCA CGTAACCTGT 1140
 ATAGTTTAAA TAGTTCTCGG TAAATTTTAA GACTTTTGTC CTTACCTCAC CTCTAAACTA 1200
 20 AGATCTAATC GTAAAGTAGT GCATGCTAGA GAATTACATC CTGAATATTT TAAAAATTGT 1260
 AAGCTTATGG CAATATTCCA AAGTAGCATG AAAAAATCT TAGAGCCTTT TAGACGATCT 1320
 CTTAATGTAG GACTTATAAA ATTTTAAACA TTCGAATACC GTTATAAGGT TTCATCGTAC 1380
 TGTTTTTAGA ATCTCGGAAA ATCTAAACAA AATCCAGACA TAGTTATCTA TCAATACATG 1440
 GATGATTTGT ATGTAGGATC TGACTTAGAA ATAGGGCAGC ATAGAACAAA AATATTTGTT 1500
 TTAGGTCTGT ATCAATAGAT AGTTATGTAC CTAATAACA TACATCCTAG ACTGAATCTT 1560
 TATCCCGTCG TATCTTGTTT TTATGAGGAG CTGAGACAAC ATCTGTTGAG GTGGGGACTT 1620
 ACAACCATGG TAGGTTTTCC AGTAACACCT CAAGTACCTT TAAGACCAAT GACTCTCCTC 1680
 GACTCTGTTG TAGACAACTC CACCCCTGAA TGTTGGTACC ATCCAAAAGG TCATTGTGGA 1740
 GTTCATGGAA ATTCTGGTTA CTGATACAAA GCAGCTGTAG ATCTTTCTCA CTTTTTAAAA 1800
 30 GAAAAAGGAG GTTTAGAAGG GCTAATTCAT TCTCAACGAA GACAAGATAT TCTTATGTTT 1860
 CGTCGACATC TAGAAAGAGT GAAAAATTTT CTTTTTCCTC CAAATCTTCC CGATTAAGTA 1920

71

AGAGTTGCTT CTGTTCTATA AGAAGATTTG TGGATTTATC ATACACAAGG ATATTTTCCT 1980
 GATTGGCAGA ATTACACACC AGGACCAGGA GTCAGATACC CATTAACCTT TGGTCTAAAC 2040
 ACCTAAATAG TATGTGTTCC TATAAAAGGA CTAACCGTCT TAATGTGTGG TCCTGGTCCT 2100
 CAGTCTATGG GTAATTGGAA ACCATGGTGC TACAAGCTAG TACCAATGAT TGAGACTGTA 2160
 CCAGTAAAAT TAAAGCCAGG AATGGATGGC CCAAAGTTA AACAATGGCC ATTGACCACG 2220
 ATGTTGATC ATGGTTACTA ACTCTGACAT GGTCAATTTA ATTTTCGGTCC TTACCTACCG 2280
 GGTTTTCAAT TTGTTACCGG TAACACAGAA GAAAAATAA AAGCATTAGT AGAAATTTGT 2340
 ACAGAGATGG AAAAGGAAGG GAAAATTTCA AAAATTGGGC CTTAATTTTT CTTGTCTTCT 2400
 TTTTATTTT CGTAATCATC TTAAACATG TCTCTACCTT TTCCTTCCCT TTAAAGTTT 2460
 10 TTAACCCGGA ATTAAAAAGA GCAGCCCGGG GGATCCTTTT TATAGCTAAT TAGTCACGTA 2520
 CCTTTGAGAG TACCACTTCA GCTACCTCTT TTGTGTCTCA GAGTAACTTT CTTTAATCAA 2580
 TTCCAAAACA CGTCGGGCCC CTTAGGAAAA ATATCGATTA ATCAGTGCAT GGAAACTCTC 2640
 ATGGTGAAGT CGATGGAGAA AACACAGAGT CTCATTGAAA GAAATTAGTT AAGGTTTTGT 2700

(2) INFORMATION FOR SEQ ID NO.: 5

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 266

(B) TYPE: amino acid

(C) STRANDEDNESS:

20 (D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 5

Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser
 1 5 10 15

Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr
 20 25 30

30 Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Met Glu
 35 40 45

Trp Arg Phe Asp Ser Arg Leu Ala Phe His His Val Ala Arg Glu Leu
 50 55 60

His Pro Glu Tyr Phe Lys Asn Cys Lys Leu Met Ala Ile Phe Gln Ser
 65 70 75 80

10

20

30

40 (2) INFORMATION FOR SEQ ID NO.: 6

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 7616

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 6

50 TAATGTAGTA TACTAATATT AACTCACATT TGACTAATTA GCTATAAAAA CCCGGGATCG 60

73

ATTCTAGAAT AAAAATTATC CCTGCCTAAC TCTATTCACT ACAGAGAGTA CAGCAAAAAC 120
 ATTACATCAT ATGATTATAA TTGAGTGTA ACTGATTAAT CGATATTTTT GGGCCCTAGC 180
 TAAGATCTTA TTTTAAATAG GGACGGATTG AGATAAGTGA TGTCTCTCAT GTCGTTTTTG 240
 TATTCTTAAA CCTACCAAGC CTCCTACTAT CATTATGAAT AATCTTTTTT CTCTCTGCAC 300
 CACTCTTCTC TTTGCCTTGG TGGGTGCTAC TCCTAATGGT TCAATTGTTA CTACTTTATA 360
 ATAAGAATTT GGATGGTTCG GAGGATGATA GTAATACTTA TTAGAAAAAA GAGAGACGTG 420
 GTGAGAAGAG AAACGGAACC ACCCACGATG AGGATTACCA AGTTAACAAT GATGAAATAT 480
 TTTATATAAT TCACTTCTCC AATTGTCCCT CATATCTCCT CCTCCAGGTC TGAAGATCTC 540
 GGTGTCGTTT GTGTCCGTGT CCTTACCACC ATCTCTTGTT AATAGTAGCC CTGTAATATT 600
 10 AAATATATTA AGTGAAGAGG TTAACAGGGA GTATAGAGGA GGAGGTCCAG ACTTCTAGAG 660
 CCACAGCAAG CACAGGCACA GGAATGGTGG TAGAGAACAA TTATCATCGG GACATTATAA 720
 TGATGAACAT CTAATTTGTC CTTCAATGGG AGGGGCATAT ATTGCTTTTC CTACTTCCTG 780
 CCACATGTTT ATAATTTGTT TTATTTTGCA TTGAAGTGTG ATATTGTTAT TTGACCCTGT 840
 ACTACTTGTA GATTAAACAG GAAGTTACCC TCCCCGTATA TAACGAAAAG GATGAAGGAC 900
 GGTGTACAAA TATTAAACAA AATAAAACGT AACTTCACAC TATAACAATA AACTGGGACA 960
 AGTATTATTC CAAGTATTAT TACCATTCCA AGTACTATTA AACAGTGGTG ATGAATTACA 1020
 GTAGAAGAAT TCCCCTCCAC AATTAAACT GTGCATTACA ATTTCTGGGT CCCCTCCTGA 1080
 TCATAATAAG GTTCATAATA ATGGTAAGGT TCATGATAAT TTGTCACCAC TACTTAATGT 1140
 CATCTTCTTA AGGGGAGGTG TTAATTTTGA CACGTAATGT TAAAGACCCA GGGGAGGACT 1200
 20 GGATTGATTA AAGACTATTG TTTTATTCTT AAATTGTTCT TTTAATTTGC TAACTATCTG 1260
 TCTTAAAGTG TCATTCCATT TTGCTCTACT AATGTTACAA TGTGCTTGTC TTATAGTTCC 1320
 CCTAACTAAT TTCTGATAAC AAAATAAGAA TTTAACAAGA AAATTAAACG ATTGATAGAC 1380
 AGAATTTTAC AGTAAGGTAA AACGAGATGA TTACAATGTT ACACGAACAG AATATCAAGG 1440
 TATTATATTT TTTGTTGTAT AAAATGCTCT CCCTGGTCCT ATATGTATCC TTTTCTTTT 1500
 ATTGTAGTTG GGTCTTGTA AATTAATTTG TACAGATTCA TTCAGATGTA CTATGATGGT 1560
 ATAATATAAA AAACAACATA TTTTACGAGA GGGACCAGGA TATACATAGG AAAAAGAAAA 1620
 TAACATCAAC CCAGAACATG TTAATTAAAC ATGTCTAAGT AAGTCTACAT GATACTACCA 1680
 TTTAGCATTA TCATTGAAAT TCTCAGATCT AATTACTACC TCTTCTTCTG CTAGACTGCC 1740
 ATTTAACAGC AGTTGAGTTG ATACTACTGG CCTAATTCCA TGTGTACATT GACTGTGCT 1800
 30 AAATCGTAAT AGTAACTTTA AGAGTCTAGA TTAATGATGG AGAAGAAGAC GATCTGACGG 1860
 TAAATTGTCG TCAACTCAAC TATGATGACC GGATTAAGGT ACACATGTAA CATGACACGA 1920

GACATTTTTA CATGATCCTT TTCCACTGAA CTTTTTATCG TTACACTTTA GAATCGCAAA 1980
 ACCAGCCGGG GCACAATAGT GTATGGGAAT TGGCTCAAAG GATATCTTTG GACAAGCTTG 2040
 CTGTAAAAAT GTACTAGGAA AAGGTGACTT GAAAAATAGC AATGTGAAAT CTTAGCGTTT 2100
 TGGTCGGCCC CGTGTTATCA CATACCCTTA ACCGAGTTTC CTATAGAAAC CTGTTCGAAC 2160
 TGTAATGACT GAGGTATTAC AACTTATCAA CCTATAGCTG GTACTATCAT TATTTATTGA 2220
 TACTATATCA AGTTTATAAA GAAGTGCATA TTCTTTCTGC ATCTTATCTC TTATGCTTGT 2280
 ACATTACTGA CTCCATAATG TTGAATAGTT GGATATCGAC CATGATAGTA ATAAATAACT 2340
 ATGATATAGT TCAAATATTT CTTACGTAT AAGAAAGACG TAGAATAGAG AATACGAACA 2400
 GGTGATATTG AAAGAGCAGT TTTTCATTTT TCCTCCCTTT ATTGTTCCCT CGCTATTACT 2460
 10 ATTGTTATTA GCAGTACTAT TATTGGTATT AGTAGTATTC CTCAAATCAG TGCAATTTAA 2520
 CCACTATAAC TTTCTCGTCA AAAAGTAAAG AGGAGGGAAA TAACAAGGGA GCGATAATGA 2580
 TAACAATAAT CGTCATGATA ATAACCATAA TCATCATAAG GAGTTTAGTC ACGTTAAATT 2640
 AGTAACACAG AGTGGGGTTA ATTTTACACA TGGCTTTAGG CTTTGATCCC ATAAACTGAT 2700
 TATATCCTCA TGCATCTGTT CTACCATGTT ATTTTCCAC ATGTTAAAAT TTTCTGTCAC 2760
 TCATTGTGTC TCACCCCAAT TAAAATGTGT ACCGAAATCC GAAACTAGGG TATTTGACTA 2820
 ATATAGGAGT ACGTAGACAA GATGGTACAA TAAAAAGGTG TACAATTTTA AAAGACAGTG 2880
 ATTTACCAAT TCTACTTCTT GTGGGTTGGG GTCTGTGGGT ACACAGGCAT GTGTGGCCCA 2940
 AACATTATGT ACCTCTGTAT CATATGCTTT AGCATCTGAT GCACAAAATA GAGTGGTGGT 3000
 TAAATGGTTA AGATGAAGAA CACCCAACCC CAGACACCCA TGTGTCCGTA CACACCGGGT 3060
 20 TTGTAATACA TGGAGACATA GTATACGAAA TCGTAGACTA CGTGTTTTAT CTCACCACCA 3120
 TGCTTCTTTC CACACAGGTA CCCATAATA GACTGTGACC CACAATTTTT CTGTAGCACT 3180
 ACAGATCATC AACATCCCAA GGAGCATGGT GCCCCATCTC CACCCCATC TCCACAAGTG 3240
 ACGAAGAAAG GTGTGTCCAT GGGGTATTAT CTGACACTGG GTGTTAAAAA GACATCGTGA 3300
 TGTCTAGTAG TTGTAGGGTT CCTCGTACCA CGGGGTAGAG GTGGGGGTAG AGGTGTTTAC 3360
 CTGATATTTT TCCTTCACTC TCATTGCCAC TGTCTTCTGC TCTTTCATAT ACGATACAAA 3420
 CTTAACGCAT ATCGCGATAA TGAAATAATT TATGATTATT TCTCGCTTTC AATTTAACAC 3480
 GACTATAAAG AGGAAGTGAG AGTAACGGTG ACAGAAGACG AGAAAGTATA TGCTATGTTT 3540
 GAATTGCGTA TAGCGCTATT ACTTTATTAA ATACTAATAA AGAGCGAAAG TTAAATTGTG 3600
 AACCTCAAG AACCTTTGTA TTTATTTTCA CTTTTTAAGT ATAGAATAAA GAAGCTCTAA 3660
 30 TTAATTAAGC TACAAATAGT TTCGTTTTCA CCTTGTCTAA TAACTAATTA ATTAACCCGG 3720
 TTGGGAGTTC TTGGAAACAT AAATAAAAGT GAAAAATTCA TATCTTATTT CTTCGAGATT 3780

75

AATTAATTCG ATGTTTATCA AAGCAAAAGT GGAACAGATT ATTGATTAAT TAATTGGGCC 3840
 ATCTTGAGAT AAAGTGAAAA TATATATCAT TATATTACAA AGTACAATTA TTTAGGTTTA 3900
 ATCATGGGTG CGAGAGCGTC AGTATTAAGC GGGGGAGAAT TAGATCGATG GGAAAAAATT 3960
 TAGAACTCTA TTCACTTTT ATATATAGTA ATATAATGTT TCATGTTAAT AAATCCAAAT 4020
 TAGTACCCAC GCTCTCGCAG TCATAATTCG CCCCTCTTA ATCTAGCTAC CCTTTTTTAA 4080
 CGGTTAAGGC CAGGGGGAAA GAAAAAATAT AAATTAAAAC ATATAGTATG GGCAAGCAGG 4140
 GAGCTAGAAC GATTGCGAGT TAATCCTGGC CTGTTAGAAA CATCAGAAGG CTGTAGACAA 4200
 GCCAATTCCG GTCCCCCTTT CTTTTTTATA TTTAATTTTG TATATCATAC CCGTTCGTCC 4260
 CTCGATCTTG CTAAGCGTCA ATTAGGACCG GACAATCTTT GTAGTCTTCC GACATCTGTT 4320
 10 ATACTGGGAC AGCTACAACC ATCCCTTCAG ACAGGATCAG AAGAACTTAG ATCATTATAT 4380
 AATACAGTAG CAACCCTCTA TTGTGTGCAT CAAAGGATAG AGATAAAAGA CACCAAGGAA 4440
 TATGACCCTG TCGATGTTGG TAGGGAAGTC TGTCTAGTC TTCTTGAATC TAGTAATATA 4500
 TTATGTCATC GTTGGGAGAT AACACACGTA GTTTCCTATC TCTATTTTCT GTGGTTCCTT 4560
 GCTTTAGACA AGATAGAGGA AGAGCAAAAC AAAAGTAAGA AAAAAGCACA GCAAGCAGCA 4620
 GCTGACACAG GACACAGCAA TCAGGTCAGC CAAAATTACC CTATAGTGCA GAACATCCAG 4680
 CGAAATCTGT TCTATCTCCT TCTCGTTTTG TTTTCATTCT TTTTTCGTGT CGTTCGTCGT 4740
 CGACTGTGTC CTGTGTCGTT AGTCCAGTCG GTTTTAATGG GATATCACGT CTTGTAGGTC 4800
 GGGCAAATGG TACATCAGGC CATATCACCT AGAACTTTAA ATGCATGGGT AAAAGTAGTA 4860
 GAAGAGAAGG CTTTCAGCCC AGAAGTGATA CCCATGTTTT CAGCATTATC AGAAGGAGCC 4920
 20 CCCGTTTACC ATGTAGTCCG GTATAGTGGA TCTTGAAATT TACGTACCCA TTTTCATCAT 4980
 CTTCTCTTCC GAAAGTCGGG TCTTCACTAT GGGTACAAAA GTCGTAATAG TCTTCCTCGG 5040
 ACCCCACAAG ATTTAAACAC CATGCTAAAC ACAGTGGGGG GACATCAAGC AGCCATGCAA 5100
 ATGTTAAAAG AGACCATCAA TGAGGAAGCT GCAGAATGGG ATAGAGTGCA TCCAGTGCAT 5160
 TGGGGTGTTT TAAATTTGTG GTACGATTTG TGTCACCCCC CTGTAGTTCTG TCGGTACGTT 5220
 TACAATTTTC TCTGGTAGTT ACTCCTTCGA CGTCTTACCC TATCTCACGT AGGTCACGTA 5280
 GCAGGGCCTA TTGCACCAGG CCAGATGAGA GAACCAAGGG GAAGTGACAT AGCAGGAACT 5340
 ACTAGTACCC TTCAGGAACA AATAGGATGG ATGACAAATA ATCCACCTAT CCCAGTAGGA 5400
 CGTCCCGGAT AACGTGGTCC GGTCTACTCT CTTGGTTCCC CTTCACTGTA TCGTCCTTGA 5460
 TGATCATGGG AAGTCCTTGT TTATCCTACC TACTGTTTAT TAGGTGGATA GGGTCATCCT 5520
 30 GAAATTTATA AAAGATGGAT AATCCTGGGA TTAAATAAAA TAGTAAGAAT GTATAGCCCT 5580
 ACCAGCATTC TGGACATAAG ACAAGGACCA AAAGAACCCT TTAGAGACTA TGTAGACCGG 5640

76

CTTTAAATAT TTTCTACCTA TTAGGACCCT AATTTATTTT ATCATTCTTA CATATCGGGA 5700
 TGGTCGTAAG ACCTGTATTC TGTTCTGGT TTTCTTGGGA AATCTCTGAT ACATCTGGCC 5760
 TTCTATAAAA CTCTAAGAGC CGAGCAAGCT TCACAGGAGG TAAAAAATTG GATGACAGAA 5820
 ACCTTGTTGG TCCAAAATGC GAACCCAGAT TGTAAGACTA TTTTAAAAGC ATTGGGACCA 5880
 AAGATATTTT GAGATTCTCG GCTCGTTCGA AGTGTCTCC ATTTTTTAAC CTACTGTCTT 5940
 TGAACAACC AGGTTTTACG CTTGGGTCTA ACATTCTGAT AAAATTTTCG TAACCCTGGT 6000
 GCGGCTACAC TAGAAGAAAT GATGACAGCA TGTCAGGGAG TAGGAGGACC CGGCCATAAG 6060
 GCAAGAGTTT TGGCTGAAGC AATGAGCCAA GTAACAAATT CAGCTACCAT AATGATGCAG 6120
 CGCCGATGTG ATCTTCTTTA CTACTGTCGT ACAGTCCCTC ATCCTCCTGG GCCGGTATTC 6180
 10 CGTTCTCAAA ACCGACTTCG TTA CTCTCGGT CATTTGTTTAA GTCGATGGTA TTA CTACGTC 6240
 AGAGGCAATT TTAGGAACCA AAGAAAGATT GTTAAGTGTT TCAATTGTGG CAAAGAAGGG 6300
 CACACAGCCA GAAATTGCAG GGCCCTAGG AAAAAGGGCT GTTGGAATG TGGAAAGGAA 6360
 TCTCCGTTAA AATCCTTGGT TTCTTTCTAA CAATTCACAA AGTTAACACC GTTTCTTCCC 6420
 GTGTGTCGGT CTTTAACGTC CCGGGGATCC TTTTCCCGA CAACCTTTAC ACCTTTCCTT 6480
 GGACACCAAA TGAAAGATTG TACTGAGAGA CAGGCTAATT TTTTAGGGAA GATCTGGCCT 6540
 TCCTACAAGG GAAGGCCAGG GAATTTTCTT CAGAGCAGAC CAGAGCCAAC AGCCCCACCA 6600
 CCTGTGGTTT ACTTTCTAAC ATGACTCTCT GTCCGATTAA AAAATCCCTT CTAGACCGGA 6660
 AGGATGTTCC CTTCCGGTCC CTTAAAAGAA GTCTCGTCTG GTCTCGGTTG TCGGGGTGGT 6720
 GAAGAGAGCT TCAGGTCTGG GGTAGAGACA ACAACTCCCC CTCAGAAGCA GGAGCCGATA 6780
 20 GACAAGGAAC TGTATCCTTT AACTTCCCTC AGATCACTCT TTGGCAACGA CCCCTCGTCA 6840
 CTTCTCTCGA AGTCCAGACC CCATCTCTGT TGTTGAGGGG GAGTCTTCGT CCTCGGCTAT 6900
 CTGTTCTTG ACATAGGAAA TTGAAGGGAG TCTAGTGAGA AACCGTTGCT GGGGAGCAGT 6960
 CAATAAGAT AGGGGGGCAA CTAAAGGAAG CTCTATTAGA TACAGGAGCA GATGATACAG 7020
 TATTAGAAGA AATGAGTTTG CCAGGAAGAT GGAAACCAAA AATGATAGGG GGAATTGGAG 7080
 GTTATTTCTA TCCCCCGTT GATTTCTTC GAGATAATCT ATGTCCTCGT CTACTATGTC 7140
 ATAATCTTCT TTA CTCAAAC GGTCTTCTA CCTTTGGTTT TTA CTATCCC CCTTAACCTC 7200
 GTTTTATCAA AGTAAGACAG TATGATCAGA TACTCATAGA AATCTGTGGA CATAAAGCTA 7260
 TAGGTACAGT ATTAGTAGGA CCTACACCTG TCAACATAAT TGGAAGAAAT CTGTTGACTC 7320
 CAAAATAGTT TCATTCTGTC ATACTAGTCT ATGAGTATCT TTAGACACCT GTATTTTCGAT 7380
 30 ATCCATGTCA TAATCATCCT GGATGTGGAC AGTTGTATTA ACCTTCTTTA GACAACTGAG 7440
 AGATTGGTTG CACTTTAAAT TTTTAACCCG GGGGATCCCG ATTTTATGA CTAGTTAATC 7500

77

AAATAAAAAG CATAACAAGCT ATTGCTTCTC TAACCAACGT GAAATTTAAA AATTGGGCCC 7560
CCTAGGGCTA AAAATACTGA TCAATTAGTT TATTTTTCGT ATGTTTCGATA ACGAAG 7616

(2) INFORMATION FOR SEQ ID NO.: 7

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 8868

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

10 (ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 7

GAGCTCGCGG CCGCCTATCA AAAGTCTTAA TGAGTTAGGT GTAGATAGTA TAGATATTAC 60
TACAAAGGTA TTCATATTTT CTATCAATTC TAAAGTAGAT GATATTAATA CTCGAGCGCC 120
GGCGGATAGT TTTCAGAATT ACTCAATCCA CATCTATCAT ATCTATAATG ATGTTTCCAT 180
AAGTATAAAG GATAGTTAAG ATTTTCATCTA CTATAATTAT ACTCAAAGAT GATGATAGTA 240
GATAATAGAT ACGCTCATAT AATGACTGCA AATTTGGACG GTTCACATTT TAATCATCAC 300
GCGTTCATAA GTTTCAACTG CATAGATCAA TGAGTTTCTA CTACTATCAT CTATTATCTA 360
20 TGCGAGTATA TTAGTGACGT TTAAACCTGC CAAGTGTAAG ATTAGTAGTG CGCAAGTATT 420
CAAAGTTGAC GTATCTAGTT AATCTCACTA AAAAGATAGC CGATGTATTT GAGAGAGATT 480
GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA TACATAATGG ATTTTGTTAT 540
CATCAGTTAT TTAGAGTGAT TTTTCTATCG GCTACATAAA CTCTCTCTAA CCTGTAGATT 600
GATGCGATTT CTTTAATGTC AATATTTATT ATGTATTACC TAAAACAATA GTAGTCAATA 660
ATTTAACATA AGTACAATAA AAAGTATTAA ATAAAAATAC TTAGTTACGA AAAAATGACT 720
AATTAGCTAT AAAAACCAG ATCTCTCGAG GTCGACGGTA TCGATAAGCT TAAATTGTAT 780
TCATGTTATT TTTCATAATT TATTTTATG AATGAATGCT TTTTACTGA TTAATCGATA 840
TTTTTGGGTC TAGAGAGCTC CAGCTGCCAT AGCTATTCGA TGATATCGAA TTCATAAAAA 900
TTATTGATGT CTACACATCC TTTTGTAATT GACATCTATA TATCCTTTTG TATAATCAAC 960
30 TCTAATCACT TTAGTATAGC TTAAGTATTT TTAATAACTA CAGATGTGTA GGAAAACATT 1020
AACTGTAGAT ATATAGGAAA ACATATTAGT TGAGATTAGT GAAAACTTT TACAGTTTTC 1080

78

CCTACCAGTT TATCCCTATA TTCAACATAT CTATCCATAT GCATCTTAAC ACTCTCTGCC 1140
 AAGATAGCTT CAGATTGAAA ATGTCAAAAG GGATGGTCAA ATAGGGATAT AAGTTGTATA 1200
 GATAGGTATA CGTAGAATTG TGAGAGACGG TTCTATCGAA GTCTGTGAGG ATAGTCAAAA 1260
 AGATAAATGT ATAGAGCATA ATCCTTCTCG TATACTCTGC CCTTTATTAC ATCGCCCGCA 1320
 TTGGGCAACG AATACACTCC TATCAGTTTT TCTATTTACA TATCTCGTAT TAGGAAGAGC 1380
 ATATGAGACG GGAAATAATG TAGCGGGCGT AACCCGTTGC TTATACAAA TGCAAGCATA 1440
 CGATACAAAC TTAACGGATA TCGCGATAAT GAAATAATTT ATGATTATTT CTCGCTTTCA 1500
 ATTTAACACA ACCCTCAAGA ACTGTTTTAC GTTCGTATGC TATGTTTGAA TTGCCTATAG 1560
 CGCTATTACT TTATTAAATA CTAATAAAGA GCGAAAGTTA AATTGTGTTG GGAGTTCTTG 1620
 10 CTTTGTATTT ATTTTCACTT TTTAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA 1680
 CAGATTGTTT CGTTTTCCCC TTGGCGTATC ACTAATTAAT TAACCCGGGC GAAACATAAA 1740
 TAAAAGTGAA AAATTCATAT CTTATTTCTT TCGAGATTAA TTAATTACTT GTCTAACAAA 1800
 GCAAAAGGGG AACCGCATAG TGATTAATTA ATTGGGCCCCG TGCAGCTCGA GGAATTCAAC 1860
 TATATCGACA TATTTCAATTT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG 1920
 AGATGTAACG GGAACAGGGT TTGTTGATTC ACGTCGAGCT CCTTAAGTTG ATATAGCTGT 1980
 ATAAAGTAAA CATATGTGTA TTGGTAATGA TTGCATCTTA CATATCCTTC TCTACATTGC 2040
 CCTTGTCCCA AACAACTAAG GCAAACCTATT CTAATACATA ATTCTTCTGT TAATACGTCT 2100
 TGCACGTAAT CTATTATAGA TGCCAAGATA TCTATATAAT TATTTTGTA GATGATGTTA 2160
 ACTATGTGAT CGTTTGATAA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA 2220
 20 GATAATATCT ACGGTTCTAT AGATATATTA ATAAACATT CTACTACAAT TGATACACTA 2280
 CTATATAAGT AGTGTAATAA TTCATGTATT TCGATATATG TTCCAACCTCT GTCTTTGTGA 2340
 TGTCTAGTTT CGTAATATCT ATAGCATCCT CAAAAAATAT ATTCGCATAT GATATATTCA 2400
 TCACATTATT AAGTACATAA AGCTATATAC AAGGTTGAGA CAGAAACACT ACAGATCAAA 2460
 GCATTATAGA TATCGTAGGA GTTTTTTATA TAAGCGTATA ATTCCAAGT CTTCAGTTCT 2520
 ATCTTCTAAA AAATCTTCAA CGTATGGAAT ATAATAATCT ATTTTACCTC TTCTGATATC 2580
 ATTAATGATA TAGTTTTTGA CACTATCTTC TAAGGGTTCA GAAGTCAAGA TAGAAGATTT 2640
 TTTAGAAGTT GCATACCTTA TATTATTAGA TAAAATGGAG AAGACTATAG TAATTACTAT 2700
 ATCAAAAACCT GTGATAGAAG TGTCAATTGA TTCTTATTCA CTATATCTAA GAAACGGATA 2760
 GCGTCCCTAG GACGAACTAC TGCCATTAAT ATCTCTATTA TAGCTTCTGG ACATAATTCA 2820
 30 TCTATTATAC ACAGTTAACT AAGAATAAGT GATATAGATT CTTTGCCTAT CGCAGGGATC 2880
 CTGCTTGATG ACGGTAATTA TAGAGATAAT ATCGAAGACC TGTATTAAGT AGATAATATG 2940

CAGAATTAAT GGGAACTATT CCGTATCTAT CTAACATAGT TTTAAGAAAG TCAGAATCTA 3000
AGACCTGATG TTCATATATT GGTTCATACA TGAAATGATC TCTATTGATG GTCTTAATTA 3060
CCCTTGATAA GGCATAGATA GATTGTATCA AAATTCTTTC AGTCTTAGAT TCTGGACTAC 3120
AAGTATATAA CCAAGTATGT ACTTTACTAG AGATAACTAC ATAGTGAATA TTTCATTCTC 3180
TGAAAATTGG TAACTCATTC TATATATGCT TTCCTTGTTG ATGAAGGATA GAATATACTC 3240
AATAGAATTT GTACCAACAA ACTGTTCTCT TATCACTGAT AAAGTAAGAG ACTTTTAACC 3300
ATTGAGTAAG ATATATACGA AAGGAACAAC TACTTCCTAT CTTATATGAG TTATCTTAAA 3360
CATGGTTGTT TGACAAGAGA TATGAATCGT ATATCATCAT CTGAAATAAT CATGTAAGGC 3420
ATACATTTAA CAATTAGAGA CTTGTCTCCT GTTATCAATA TACTATTCTT GTGATAATTT 3480
10 ATGTGTGAGG ATACTTAGCA TATAGTAGTA GACTTTATTA GTACATTCCG TATGTAAATT 3540
GTTAATCTCT GAACAGAGGA CAATAGTTAT ATGATAAGAA CACTATTAAA TACACACTCC 3600
CAAATTTGTC CACGTTCTTT AATTTTGTTA TAGTAGATAT CAAATCCAAT GGAGCTACAG 3660
TTCTTGGCTT AAACAGATAT AGTTTTTCTG GAACAAATTC TACAACATTA GTTTAAACAG 3720
GTGCAAGAAA TTAAAACAAT ATCATCTATA GTTTAGGTTA CCTCGATGTC AAGAACCGAA 3780
TTTGTCTATA TCAAAAAGAC CTTGTTTAAG ATGTTGTAAT TTATAAAGGA CTTTGGGTAG 3840
ATAAGTGGGA TGAAATCCTA TTTTAATTAA TGCTATCGCA TTGTCCTCGT GCAAATATCC 3900
AAACGCTTTT GTGATAGTAT GGCATTCATT AATATTTCTT GAAACCCATC TATTCACCCT 3960
ACTTTAGGAT AAAATTAATT ACGATAGCGT AACAGGAGCA CGTTTATAGG TTTGCGAAAA 4020
CACTATCATA CCGTAAGTAA GTCTAGAAAC GCTCTACGAA TATCTGTGAC AGATATCATC 4080
20 TTTAGAGAAT ATACTAGTCG CGTTAATAGT ACTACAATTT GTATTTTTTA ATCTATCTCA 4140
ATAAAAAAAT CAGATCTTTG CGAGATGCTT ATAGACACTG TCTATAGTAG AAATCTCTTA 4200
TATGATCAGC GCAATTATCA TGATGTTAAA CATAAAAAAT TAGATAGAGT TATTTTTTTA 4260
TAATATGTAT GATTCAATGT ATAATAAAC TACTAACTGT TATTGATAAC TAGAATCAGA 4320
ATCTAATGAT GACGTAACCA AGAAGTTTAT CTACTGCCAA ATTATACATA CTAAGTTACA 4380
TATTGATTTG ATGATTGACA ATAATAATTG ATCTTAGTCT TAGATTACTA CTGCATTGGT 4440
TCTTCAAATA GATGACGGTT TTTAGCTGCA TTATTTTTAG CATCTCGTTT AGATTTTCCA 4500
TCTGCCTTAT CGAATACTCT TCCGTCGATG TCTACACAGG CATAAAATGT AAATCGACGT 4560
AATAAAAATC GTAGAGCAAA TCTAAAAGGT AGACGGAATA GCTTATGAGA AGGCAGCTAC 4620
AGATGTGTCC GTATTTTACA AGGAGAGTTA CTAGGCCCAA CTGATTCAAT ACGAAAAGAC 4680
30 CAATCTCTCT TAGTTATTTG GCAGTACTCA TTAATAATGG TGACAGGGTT TCCTCTCAAT 4740
GATCCGGGTT GACTAAGTTA TGCTTTTCTG GTTAGAGAGA ATCAATAAAC CGTCATGAGT 4800

80

AATTATTACC ACTGTCCCAA AGCATCTTTC CAATCAATAA TTTTTTTAGC CGGAATAACA 4860
 TCATCAAAAG ACTTATGATC CTCTCTCATT GATTTTTTCGC GGGATACATC TCGTAGAAAG 4920
 GTTAGTTATT AAAAAAATCG GCCTTATTGT AGTAGTTTTT TGAATACTAG GAGAGAGTAA 4980
 CTAAAAAGCG CCCTATGTAG ATCTATTATG ACGTCAGCCA TAGCATCAGC ATCCGGCTTA 5040
 TCCGCCTCCG TTGTCATAAA CCAACGAGGA GGAATATCGT CGGAGCTGTA TAGATAATAC 5100
 TGCAGTCGGT ATCGTAGTCG TAGGCCGAAT AGGCGGAGGC AACAGTATTT GGTTGCTCCT 5160
 CCTTATAGCA GCCTCGACAT CACCATAGCA CTACGTTGAA GATCGTACAG AGCTTTATTA 5220
 ACTTCTCGCT TCTCCATATT AAGTTGTCTA GTTAGTTGTG CAGCAGTAGC GTGGTATCGT 5280
 GATGCAACTT CTAGCATGTC TCGAAATAAT TGAAGAGCGA AGAGGTATAA TTCAACAGAT 5340
 10 CAATCAACAC GTCGTCATCG TCCTTCGATT CCAATGTTTT TAATAGCCGC ACACACAATC 5400
 TCTGCGTCAG AACGCTCGTC AATATAGATC TTAGACATTT TTAGAGAGAA AGGAAGCTAA 5460
 GGTTACAAAA ATTATCGGCG TGTGTGTTAG AGACGCAGTC TTGCGAGCAG TTATATCTAG 5520
 AATCTGTAAA AATCTCTCTT CTAACACAAC CAGCAATAAA ACTGAACCTA CTTTATCATT 5580
 TTTTATTCA TCATCCTCTG GTGGTTCGTC GTTCTATCG AATGTAGCTC TGATTAACCC 5640
 GTCATCTATA GATTGTGTTG GTCGTTATTT TGAATTGGAT GAAATAGTAA AAAAATAAGT 5700
 AGTAGGAGAC CACCAAGCAG CAAAGATAGC TTACATCGAG ACTAATTGGG CAGTAGATAT 5760
 GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT 5820
 ATTCCTTGT TTTCATGTAT CGATTGCGTT GTAACATTAA GATTGCGAAA CCACTACGAC 5880
 CAAGACCTCT AAGACCTCCT CTACCTAATA ATAGACCTTC TTAGAGACAA TAAAGGAACA 5940
 20 AAAGTACATA GCTAACGCAA CATTGTAATT CTAACGCTTT TGCTCTAAAT TTGGGAGGCT 6000
 TAAAGTGTTG TTTGCAATCT CTACACGCGT GTCTAACTAG TGGAGGTTTCG TCAGCTGCTC 6060
 TAGTTTGAAT CATCATCGGC GTAGTATTCC ACGAGATTTA AACCCTCCGA ATTTCACAAC 6120
 AAACGTTAGA GATGTGCGCA CAGATTGATC ACCTCCAAGC AGTCGACGAG ATCAAACCTTA 6180
 GTAGTAGCCG CATCATAAGG TACTTTTACA GTTAGGACAC GGTGTATTGT ATTTCTCGTC 6240
 GAGAACGTTA AAATAATCGT TGTAACCTAC ATCCTTTATT TTATCTATAT TGTATTCTAC 6300
 TCCTTTCTTA ATGAAAATGT CAATCCTGTG CCACATAACA TAAAGAGCAG CTCTTGCAAT 6360
 TTTATTAGCA ACATTGAGTG TAGGAAATAA AATAGATATA ACATAAGATG AGGAAAGAAT 6420
 ATGCATTTTA TACCGAATAA GAGATAGCGA AGGAATTCTT TTTATTGATT AACTAGTCAA 6480
 ATGAGTATAT ATAATTGAAA AAGTAAAATA TAAATCATAT AATAATGAAA TACGTAAAAT 6540
 30 ATGGCTTATT CTCTATCGCT TCCTTAAGAA AAATAACTAA TTGATCAGTT TACTCATATA 6600
 TATTAACCTT TTCATTTTAT ATTTAGTATA TTATTACTTT CGAAATATCA GTAATAGACA 6660

81

GGA ACTGGCA GATTCTTCTT CTAATGAAGT AAGTACTGCT AAATCTCCAA AATTAGATAA 6720
 AAATGATACA GCAAATACAG CTTCAATCAA GCTTTATAGT CATTATCTGT CCTTGACCGT 6780
 CTAAGAAGAA GATTACTTCA TTCATGACGA TTTAGAGGTT TTAATCTATT TTTACTATGT 6840
 CGTTTATGTC GAAGTAAGTT CGAATTACCT TTTAATTTTT TCAGACACAC CTTATTACAA 6900
 ACTAACTAAG TCAGATGATG AGAAAGTAAA TATAAATTTA ACTTATGGGT ATAATATAAT 6960
 AAAGATTCAT GCTTAATGGA AAATTAAAAA AGTCTGTGTG GAATAATGTT TGATTGATTC 7020
 AGTCTACTAC TCTTTCATTT ATATTTAAAT TGAATACCCA TATTATATTA TTTCTAAGTA 7080
 GATATTAATA ATTTACTTAA CGATGTTAAT AGACTTATTC CATCAACCCC TTCAAACCTT 7140
 TCTGGATATT ATAAAATACC AGTTAATGAT ATTAAAATAG ATTGTTTAAAG CTATAATTAT 7200
 10 TAAATGAATT GCTACAATTA TCTGAATAAG GTAGTTGGGG AAGTTTGGAA AGACCTATAA 7260
 TATTTTATGG TCAATTACTA TAATTTTATC TAACAAATTC AGATGTAAAT AATTATTTGG 7320
 AGGTAAAGGA TATAAAATTA GTCTATCTTT CACATGGAAA TGAATTACCT AATATTAATA 7380
 ATTATGATAG GAATTTTTTA GGATTTACAG TCTACATTTA TTAATAAACC TCCATTTCTT 7440
 ATATTTTAAT CAGATAGAAA GTGTACCTTT ACTTAATGGA TTATAATTAT TAATACTATC 7500
 CTTAAAAAAT CCTAAATGTC CTGTTATATG TATCAACAAT ACAGGCAGAT CTATGGTTAT 7560
 GGTAAACAC TGTAACGGGA AGCAGCATTC TATGGTAACT GGCCTATGTT TAATAGCCAG 7620
 ATCATTTTAC GACAATATAC ATAGTTGTTA TGTCCGTCTA GATACCAATA CCATTTTGTG 7680
 ACATTGCCCT TCGTCGTAAG ATACCATTGA CCGGATACAA ATTATCGGTC TAGTAAAATG 7740
 TCTATAACA TTTTACCACA AATAATAGGA TCCTCTAGAT ATTTAATATT ATATCTAACA 7800
 20 ACAACAAAAA AATTTAACGA TGTATGGCCA GAAGTATTTT CTACTAATAA AGATATTTGT 7860
 AAAATGGTGT TTATTATCCT AGGAGATCTA TAAATTATAA TATAGATTGT TGTTGTTTTT 7920
 TTAAATTGCT ACATACCGGT CTTCATAAAA GATGATTATT AGATAAAGAT AGTCTATCTT 7980
 ATCTACAAGA TATGAAAGAA GATAATCATT TAGTAGTAGC TACTAATATG GAAAGAAATG 8040
 TATACAAAAA CGTGGAAGCT TTTATATTAA TCTATTTCTA TCAGATAGAA TAGATGTTCT 8100
 ATACTTTCTT CTATTAGTAA ATCATCATCG ATGATTATAC CTTTCTTTAC ATATGTTTTT 8160
 GCACCTTCGA AAATATAATT ATAGCATATT ACTAGAAGAT TTAAAATCTA GACTTAGTAT 8220
 AACAAAACAG TTAAATGCCA ATATCGATTC TATATTTTAT CATAACAGTA GTACATTAAT 8280
 CAGTGATATA TATCGTATAA TGATCTTCTA AATTTTAGAT CTGAATCATA TTGTTTTGTC 8340
 AATTTACGGT TATAGCTAAG ATATAAAGTA GTATTGTCAT CATGTAATTA GTCACTATAT 8400
 30 CTGAAACGAT CTACAGACTC AACTATGCAA GGAATAAGCA ATATGCCAAT TATGTCTAAT 8460
 ATTTTAACTT TAGAACTAAA ACGTTCTACC AATACTAAAA ATAGGATACG GACTTTGCTA 8520

82

GATGTCTGAG TTGATACGTT CCTTATTCGT TATACGGTTA ATACAGATTA TAAAATTGAA 8580
ATCTTGATTT TGCAAGATGG TTATGATTTT TATCCTATGC TGATAGGCTG TTAAAAGCTG 8640
CAATAAATAG TAAGGATGTA GAAGAAATAC TTTGTTCTAT ACCTTCGGAG GAAAGAACTT 8700
TAGAACAACT TAAGTTTAAT CAAACTTGTA ACTATCCGAC AATTTTCGAC GTTATTTATC 8760
ATTCCTACAT CTTCTTTATG AAACAAGATA TGGAAGCCTC CTTTCTTGAA ATCTTGTTGA 8820
ATTCAAATTA GTTGAACAT TTTATGAAGG TACCAAATAC TTCCATGG 8868

(2) INFORMATION FOR SEQ ID NO.: 8

(i) SEQUENCE CHARACTERISTICS

10 (A) LENGTH: 59

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 8

CCCTCTAGAT CGCGATATCC GTTAAGTTTG TATCGTAATG CTTGCATTTT GTTATTCGT 59

20 (2) INFORMATION FOR SEQ ID NO.: 9

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 31

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

30 CCCGAATTCA TAAAATTAT TGATGTCTAC A

31

83

(2) INFORMATION FOR SEQ ID NO.: 10

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 17

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

GGCCGCGTCG ACATGCA

17

(2) INFORMATION FOR SEQ ID NO.: 11

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 9

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

20 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

TGTCGACGC

9

(2) INFORMATION FOR SEQ ID NO.: 12

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 55

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

30 (ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

84

(A) ORGANISM: Vaccinia virus

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

TTTCATTATC GCGATATCCG TTAAGTTTGT ATCGTAATGT CCACTCGTGG CGATC 55

(2) INFORMATION FOR SEQ ID NO.: 13

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 18

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

10 (D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

GGAGGGTTTC AGAGGCAG 18

(2) INFORMATION FOR SEQ ID NO.: 14

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 58

20 (B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

CCCCTCGAGT CGCGATATCC GTTAAGTTTG TATCGTAATG CCACTAACAG AAGAAGCA 58

(2) INFORMATION FOR SEQ ID NO.: 15

30 (i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 36

85

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

AAATCTCCAC TCCATCCTTG TTTTCAGATT TTTAAA

36

10 (2) INFORMATION FOR SEQ ID NO.: 16

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 36

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

20 AATCTGAAAA CAGGAATGGA GTGGAGATTT GATTCT

36

(2) INFORMATION FOR SEQ ID NO.: 17

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 30

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

30 (A) ORGANISM: Vaccinia virus

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

86

CCCAAGCTTA CAATTTTAA AATATTCAGG

30

(2) INFORMATION FOR SEQ ID NO.: 18

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 30

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

CCCAAGCTTA TGGCAATATT CCAAAGTAGC

30

(2) INFORMATION FOR SEQ ID NO.: 19

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 36

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

20 (D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

TGGAAAACCT ACCATGGTTG TAAGTCCCCA CCTCAA

36

(2) INFORMATION FOR SEQ ID NO.: 20

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 36

30 (B) TYPE: nucleic acid

(C) STRANDEDNESS:

87

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

TGGGGACTTA CAACCATGGT AGGTTTTCCA GTAACA

36

(2) INFORMATION FOR SEQ ID NO.: 21

(i) SEQUENCE CHARACTERISTICS

10 (A) LENGTH: 36

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

TACAGTCTCA ATCATTGGTA CTAGCTTGTA GCACCA

36

20 (2) INFORMATION FOR SEQ ID NO.: 22

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 36

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

30 TACAAGCTAG TACCAATGAT TGAGACTGTA CCAGTA

36

88

(2) INFORMATION FOR SEQ ID NO.: 23

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 36

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus

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

CCCCCTGCAG AAAAATTAAG GCCCAATTTT TGAAAT

36

77396-24

89

CLAIMS:

1. A vector for enhanced expression of at least one first nucleic acid molecule in a cell, said vector comprising the first nucleic acid molecule operably linked
5 to a first promoter and at least one second nucleic acid molecule operably linked to a second promoter and encoding a translation factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the
10 second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing translation.
2. The vector of claim 1, wherein the first and second nucleic acid molecules are at different loci within the vector.
- 15 3. The vector of claim 1, wherein the first and second nucleic acid molecules are at the same locus within the vector.
4. The vector of any one of claims 1 to 3, wherein the translation factor effects inhibition of eIF-2 α
20 phosphorylation or inhibition of PKR phosphorylation or otherwise sequesters dsRNA, increasing the effective concentration of dsRNA.
5. The vector of claim 4, wherein said at least one second molecule is a K3L open reading frame, an E3L open
25 reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, or any combination thereof.
6. The vector of any one of claims 1 to 5, wherein said first nucleic acid molecule encodes: an epitope of
30 interest, a biological response modulator, a growth factor,

77396-24

90

a recognition sequence, a therapeutic gene or a fusion protein.

7. The vector of any one of claims 1 to 6 which is a recombinant virus.

5 8. The vector of claim 7 which is a recombinant poxvirus.

9. A method for preparing a vector as claimed in any one of claims 1 to 8 comprising modifying a vector to comprise the at least one second nucleic acid molecule
10 operably linked to a second promoter and the first nucleic acid molecule operably linked to a first promoter, so that there is co-temporal expression of the first and second nucleic acid molecules.

10. An immunological, vaccine or therapeutic
15 composition comprising the vector of any one of claims 1 to 8 and a pharmaceutically acceptable carrier or diluent.

11. Use of the composition of claim 10 for generating an immunological or therapeutic response in a host.

12. An *in vitro* method of increasing expression of at
20 least one first nucleic acid molecule in a cell, by a vector comprising the first nucleic acid molecule operably linked to a first promoter, comprising modifying the vector to comprise at least one second nucleic acid molecule operably linked to a second promoter encoding a translation
25 factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing translation.

77396-24

91

13. Use, for increasing expression of at least one first nucleic acid molecule in a cell, of a vector comprising the first nucleic acid molecule operably linked to a first promoter, and at least one second nucleic acid molecule operably linked to a second promoter encoding a translation factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing translation.

14. Use in the preparation of a medicament for increasing expression of at least one first nucleic acid molecule in a cell, of a vector comprising the first nucleic acid molecule operably linked to a first promoter, and at least one second nucleic acid molecule operably linked to a second promoter encoding a translation factor, wherein the first and second promoters direct co-temporal expression of the first and second nucleic acid molecules, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing translation.

15. A method of expressing a gene product *in vitro* comprising infecting, or transfecting, a suitable cell with a vector as claimed in any one of claims 1 to 8, wherein the gene product is encoded by the first nucleic acid molecule.

16. Use of the vector of any one of claims 1 to 8 in the manufacture of a medicament for generating an immunological or therapeutic response in a host.

WO 98/40500

PCT/US98/03710

1/24
FIG. 1A

Nucleotide sequence of the ALVAC C6 insertion site containing the H6 / K3L and E3L expression cassette.

Characteristic

Position(s)

Left Arm

1-385

Right Arm

3273-4434

K3L orf

C727-464

H6 Promoter

C850-728

E3L

C2758-2188

1	GAGCTCGCGG	CCGCCCTATCA	AAAGTCTTAA	TGAGTTAGGT	GTAGATAGTA	TAGATATTAC
61	TACAAAGGTA	TTCATATTTT	CTATCAATTC	TAAAGTAGAT	GATATTAATA	ACTCAAAGAT
121	GATGATAGTA	GATAATAGAT	ACGCTCATAT	AATGACTGCA	AATTTGGACG	GTTTACATTT
181	TAATCATCAC	GCGTTTCATA	GTTTCAACTG	CATAGATCAA	AATCTCACTA	AAAAGATAGC
241	CGATGTATTT	GAGAGAGATT	GGACATCTAA	CTACGCTAAA	GAAATTACAG	TTATAAATAA
301	TACATAATGG	ATTTTGTTAT	CATCAGTTAT	ATTTAACATA	AGTACAATAA	AAAGTATTAA
361	ATAAAAATAC	TTACTTACGA	AAAAATGACT	AATTAGCTAT	AAAAACCCAG	ATCTCTCGAG
421	GTCGACGGTA	TGCATAAGCT	TGATATCGAA	TTCATAAAAA	TTATTGATGT	CTACACATCC
481	TTTTGTAAAT	GACATCTATA	TATCCTTTTG	TATAATCAAC	TCTAATCACT	TTAACTTTTA
541	CAGTTTTCCC	TACCAGTTTA	TCCCTATATT	CAACATATCT	ATCCATATGC	ATCTTAACAC
601	TCTCTGCCAA	GATAGCTTCA	GAGTGAGGAT	AGTCAAAAAG	ATAAATGTAT	AGAGCATAAT
661	CCTTCTCGTA	TACTCTGCCC	TTTATTACAT	CGCCCGCATT	GGGCAACGAA	TAACAAAATG
721	CAAGCATACG	ATACAAACTT	AACGGATATC	GCGATAATGA	AATAATTTAT	GATTATTTCT
781	CGCTTTCAAT	TTAACACAAC	CCTCAAGAAC	CTTTGTATTT	ATTTTCACTT	TTTAAGTATA
841	GAATAAAGAA	AGCTCTAATT	AATTAATGAA	CAGATTGTTT	CGTTTTCCCC	TTGGCGTATC
901	ACTAATTAAT	TAACCCGGGC	TGCAGCTCGA	GGAATTCAAC	TATATCGACA	TATTTTCATTT
961	GTATACACAT	AACCAATTACT	AACGTAGAAT	GTATAGGAAG	AGATGTAACG	GGAACAGGGT
1021	TTGTTGATTG	GCAAACATTT	CTAATACATA	ATTCTTCTGT	TAATACGTCT	TGCACGTAAT
1081	CTATTATAGA	TGCCAAGATA	TCTATATAAT	TATTTTGTAA	GATGATGTTA	ACTATGTGAT
1141	CTATATAAGT	AGTGTAATAA	TTCAATGTAT	TCGATATATG	TTCCAACCTCT	GTCTTTGTGA
1201	TGTCTAGTTT	CGTAATATCT	ATAGCATCCT	CAAAAAATAT	ATTGCGCATAT	ATTCCCAAGT
1261	CTTCAGTTCT	ATCTTCTAAA	AAATCTTCAA	CGTATGGAAT	ATAATAATCT	ATTTTACCTC
1321	TTCTGATATC	ATTAATGATA	TAGTTTTTGA	CACIATCTTC	TGTCAATTGA	TTCTTATTCA
1381	CTATATCTAA	GAAACGGATA	GCGTCCCTAG	GACGAACTAC	TGCCATTAAAT	ATCTCTATTA
1441	TAGCTTCTGG	ACATAATTCA	TCTATTATAC	CAGAATTAAT	GGGAACATAT	CCGTATCTAT
1501	CTAACATAGT	TTTAAGAAAG	TCAGAATCTA	AGACCTGATG	TTCATATATT	GGTTCATACA
1561	TGAAATGATC	TCTATTGATG	ATAGTGACTA	TTTCATTCTC	TGAAAATTGG	TAATCATTC
1621	TATATATGCT	TTCCCTTGTTG	ATGAAGGATA	GAATATACTC	AATAGAATTT	GTACCAACAA
1681	ACTGTTCTCT	TATGAATCGT	ATATCATCAT	CTGAAATAAT	CATGTAAGGC	ATACATTTAA
1741	CAATTAGAGA	CTTGTTCTCT	GTTATCAATA	TACTATTCTT	GTGATAATTT	ATGTGTGAGG
1801	CAAATTTGTC	CACGTTCTTT	AATTTTGTTA	TAGTAGATAT	CAAATCCAAT	GGAGCTACAG
1861	TTCTTGGCCT	AAACAGATAT	AGTTTTTCTG	GAACAAATTC	TACAACATTA	TTATAAAGGA
1921	CTTTGGGTAG	ATAAGTGGGA	TGAAATCCTA	TTTTAATTAA	TGCTATCGCA	TTGTCTCTGT
1981	GCAAATATCC	AAACGCTTTT	GTGATAGTAT	GGCATTCAAT	GTCTAGAAAC	GCTCTACGAA
2041	TATCTGTGAC	AGATATCATC	TTTAGAGAAT	ATACTAGTCG	CGTTAATAGT	ACTACAATTT
2101	GTATTTTTTA	ATCTATCTCA	ATAAAAAAT	TAATATGTAT	GATTCAATGT	ATAACTAAAC
2161	TACTAACTGT	TATTGATAAC	TAGAATCAGA	ATCTAATGAT	GACGTAACCA	AGAAGTTTAT
2221	CTACTGCCAA	TTTAGCTGCA	TTATTTTTAG	CATCTCGTTT	AGATTTTCCA	TCTGCCCTTAT
2281	CGAATACTCT	TCCGTGCGTG	TCTACACAGG	CATAAAATGT	AGGAGAGTTA	CTAGGCCCAA
2341	CTGATTCAAT	ACGAAAAGAC	CAATCTCTCT	TAGTTATTTG	GCAGTACTCA	TTAATAATGG
2401	TGACAGGGTT	AGCATCTTTC	CAATCAATAA	TTTTTTTTAGC	CGGAATAACA	TCATCAAAAG
2461	ACTTATGATC	CTCTCTCAT	GATTTTTCCG	GGGATACATC	ATCTATTATG	ACGTCAGCCA

2/24

FIG. 1B

```
2521 TAGCATCAGC ATCCGGGCHTA TCGGCCTCCG TTGTCATAAA CCAACGAGGA GGPATATCGT
2581 CGGAGCTGTA CACCATAGCA CTACGTTGAA GATCGTACAG AGCTTTATTA ACTTCCTCGCT
2641 TCTCCATATT AAGTTGTCTA GTTAGTTGTG CAGCAGTAGC TCCCTOGATT CCAATGTTTT
2701 TAATAGCOGC ACACACAATC TCTGCGTCAG AACGCTCGTC AATATAGATC TTAGACATTT
2761 TTAGAGAGAA CTAACACAAC CAGCAATAAA ACTGAACCTA CTTTATCATT TTTTATTTCA
2821 TCATCCTCTG GTGGTTGCTC GTTCTATCGG AATGTAGCTC TGATTAACCC GTCATCTATA
2881 GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT
2941 AHTTCCTTGT TTTTATGTAT CGATTGCGTT GTAACATTAA GATTGCGAAA TGCTCTAAAT
3001 TTGGGAGGCT TAAAGTGTTC TTTCGAATCT CTACACGCGT GTCTAACTAG TGGAGGTTCT
3061 TCAGCTGCTC TAGTTTGAAT CATCATCGGC GTAGTATTCC TACTTTTACA GTTAGGACAC
3121 GGTGTATTGT AHTTCTCGTC GAGAACGTTA AAATAATCGT TGTAACCTAC ATCCTTTATT
3181 TTATCTATAT TGTATTCTAC TCCTTTCTTA ATGCATTTTA TACCGAATAA GAGATAGCGA
3241 AGGAATTCTT TTTATTGATT AACTAGTCAA ATGAGTATAT ATAATTGAAA AAGTAAAATA
3301 TAAATCATAT AATAATGAAA CGAAATATCA GTAATAGACA GGAACCTGGCA GATTCTTCTT
3361 CTAATGAAGT AAGTACTGCT AAATCTCCAA AATTAGATAA AAATGATACA GCAAATACAG
3421 CTTCAATCAA CGAATTACCT TTTAATTTTT TCAGACACAC CTTATTACAA ACTAATAAG
3481 TCAGATGATG AGAAAGTAAA TATAAATTTA ACTTATGGGT ATAATATAAT AAAGATTTCAT
3541 GATATTAATA ATTTACTTTA CGATGTTAAT AGACTTATTC CATCAACCCC TTCAAACCTT
3601 TCTGGATATT ATAAAATACC AGTTAATGAT ATTAAAATAG ATTGTTTAAG AGATGTAAAT
3661 AATTATTTGG AGGTAAAGGA TATAAAATTA GTCTATCTTT CACATGGAAA TGAATTACCT
3721 AATATTAATA ATTATGATAG GAATTTTTTA GGATTTACAG CTGTTATATG TATCAACAAT
3781 ACAGGCAGAT CTATGGTTAT GGTAAAACAC TGTAACGGGA AGCAGCATTG TATGGTAACT
3841 GGCCTATGTT TAATAGCCAG ATCAATTTTAC TCTATAAACA TTTTACCACA AATAATAGGA
3901 TCCTCTAGAT AHTTAATATT ATATCTAACA ACAACAAAAA AATTTAACGA TGTATGGCCA
3961 GAAGTATTTT CTACTAATAA AGATAAAGAT AGTCTATCTT ATCTACAAGA TATGAAAGAA
4021 GATAATCATT TAGTAGTAGC TACTAATATG GAAAGAAATG TATACAAAAA CGTGGAAGCT
4081 TTTATATTAA ATAGCATATT ACTAGAAGAT TTAAAATCTA GACTTAGTAT AACAAAACAG
4141 TTAAATGCCA ATATCGATTG TATATTTTCA CATAACAGTA GTACATTAAAT CAGTGATATA
4201 CTGAAACGAT CTACAGACTC AACTATGCHA GGAATAAGCA ATATGCCAAT TATGTCTAAT
4261 AHTTTAACYT TGAACATAAA ACGTTCTACC AATACTAAAA ATAGGATACG TATAGGCTG
4321 TTAAAAGCTG CATAAATAG TAAGGATGTA GAAGAAATAC TTTGTTCTAT ACCTTCGGAG
4381 GAAAGAACYT TGAACAACT TAAGTTTAAT CAAACTTGTA TTTATGAAGG TACC
```

WO 98/40500

PCT/US98/03710

3/24

FIG. 2

DNA sequence of the coding region of FHV gB with modified T5NT motifs.

```

1  ATGTCCACTC  GTGGCGATCT  TGGGAAGCGG  CGACGAGGGA  GTCGTTGGCA  GGGACACAGT
61  GGCTATTTTC  GACAGAGATG  TTTTTTCCCT  TCTCTACTCG  GTATTGCAGC  GACTGGCTCC
121  AGACATGGTA  ACGGATCGTC  GGGATTAACC  AGACTAGCTA  GATATGTTTC  ATTTATCTGG
181  ATCGTACTAT  TCTTAGTCGG  TCCCCGTCCA  GTAGAGGGTC  AATCTGGAAG  CACATCGGAA
241  CAACCCCGGC  GGACTGTAGC  TACCCCTGAG  GTAGGGGGTA  CACCACCAA  ACCAACTACA
301  GATCCCACCG  ATATGTCGGA  TATGAGGGAA  GCTCTCCGTG  CGTCCCAAAT  AGAGGCTAAC
361  GGACCATCGA  CTTTCTATAT  GTGTCCACCA  CCTTCAGGAT  CTACTGTCGT  GCGTTTAGAG
421  CCACCACGGG  CCTGTCCAGA  TTATAAACTA  GGGAAAAATF  TTACCGAGGG  TATAGCTGTA
481  ATATTTAAAG  AAAATATAGC  GCCATATAAA  TTCAAGGCAA  ATATATACTA  TAAAAACATT
541  ATTATGACAA  CGGTATGGTC  TGGGAGTTCC  TATGCCGTTA  CAACCAACCG  ATATACAGAC
601  AGGGTTCCCC  TGAAAGTTCA  AGAGATTACA  GATCTCATAG  ATAGACGGGG  TATGTGCCTC
661  TCGAAAGCTG  ATTACGTTTC  TAACAATTAT  CAATTTACGG  CCTTTGATCG  AGACGAGGAT
721  CCCAGAGAAC  TGCCTCTGAA  ACCCTCCAAG  TTCAACACTC  CAGAGTCCCG  TGGATGGCAC
781  ACCACCAATG  AAACATACAC  AAAGATCGGT  GCTGCTGGAT  TTCACCACTC  TGGGACCTCT
841  GTAAATTGCA  TCGTAGAGGA  AGTGGATGCA  AGATCTGTAT  ATCCATATGA  CTCATTTGCT
901  ATCTCCACTG  GTGACGTGAT  TCACATGTCT  CCATTCTTTG  GGCTGAGGGA  TGGAGCCCAT
961  GTAGAACATA  CTAGTTATTC  TTCAGACAGA  TTTCAACAAA  TCGAGGGATA  CTATCCAATA
1021  GACTTGGATA  CCGGATTACA  ACTGGGGGCA  CCAGTTTCTC  GCAATTTTTT  GGAAACTCCG
1081  CATGTGACAG  TGGCCTGGAA  CTGGACCCCA  AAGTCTGGTC  GGGTATGTAC  CTTAGCCAAA
1141  TGGAGGGAAA  TAGATGAAAT  GCTACGCGAT  GAATATCAGG  GCTCCTATAG  ATTTACAGCC
1201  AAGACCATAT  CCGCTACTTT  CATCTCCAAT  ACTTCACAAT  TTGAAATCAA  TCGTATCCGT
1261  TTGGGGGACT  GTGCCACCAA  GGAGGCAGCC  GAAGCCATAG  ACCGGATTTA  TAAGAGTAAA
1321  TATAGTAAAA  CTCATATTCA  GACTGGAACC  CTGGAGACCT  ACCTAGCCCG  TGGGGGATTT
1381  CTAATAGCTT  TCCGTCCCAT  GATCAGCAAC  GAACTAGCAA  AGTTATATAT  CAATGAATTA
1441  GCACGTTCCA  ATCGCACGGT  AGATCTCAGT  GCACTCCTCA  ATCCATCTGG  GGAAACAGTA
1501  CAACGAACTA  GAAGATCGGT  CCCATCTAAT  CAACATCATA  GGTGCGGGCG  CAGCACAATA
1561  GAGGGGGGTA  TAGAAACCGT  GAACAATGCA  TCACTCCTCA  AGACCACCTC  ATCTGTGGAA
1621  TTCGCAATGC  TACAATTTGC  CTATGACTAC  ATACAAGCCC  ATGTAAATGA  AATGTTGAGT
1681  CGGATAGCCA  CTGCCTGGTG  TACACTTCAG  AACC GC GAAC  ATGTGCTGTG  GACAGAGACC
1741  CTAAAACTCA  ATCCCGGTGG  GGTGGTCTCG  ATGGCCCTAG  AACGTCGTGT  ATCCGCGCGC
1801  CTAATTGGAG  ATGCCGTCGC  CGTAACACAA  TGTGTTAACA  TTTCTAGCGG  ACATGTCTAT
1861  ATCCAAAATT  CTATGCGGGT  GACGGGTTCA  TCAACGACAT  GTTACAGCCG  CCCTCTTGTT
1921  TCCTTCCGTG  CCCTCAATGA  CTCCGAATAC  ATAGAAGGAC  AACTAGGGGA  AAACAATGAA
1981  CTTCTCGTGG  AACGAAAAC  AATTGAGCCT  TGCACGTGCA  ATAATAAGCG  GTATTTTAAG
2041  TTTGGGGCAG  ATTATGTATA  TTTTGAGGAT  TATGCGTATG  TCCGTAAAGT  CCCGCTATCG
2101  GAGATAGAAC  TGATAAGTGC  GTATGTGAAT  TTAAATCTTA  CTCTCCTAGA  GGATCGTGAA
2161  TTTCTCCAC  TCGAAGTTTA  TACACGAGCT  GAGCTGGAAG  ATACCGGCCT  TTTGGACTAC
2221  AGCGAGATTC  AACGCCGCAA  CCAACTCCAC  GCCTTAAAT  TTTATGATAT  AGACAGCATA
2281  GTCAGAGTGG  ATAATAATCT  TGTCATCATG  CGTGGTATGG  CAAATTTCTT  TCAGGGACTC
2341  GGGGATGTGG  GGGCTGGTTT  CGGCAAGGTG  GTCTTAGGGG  CTGCGAGTGC  GGTAATCTCA
2401  ACAGTATCAG  GCGTATCATC  ATTTCTAAAC  AACCCATTTG  GAGCATTGGC  CGTGGGACTG
2461  TTAATATTAG  CTGGCATCGT  CGCAGCATTC  CTGGCATATC  GCTATATATC  TAGATTACGT
2521  GCAAATCCAA  TGAAAGCCTT  ATATCCTGTG  ACGACTAGGA  ATTTGAAACA  GACGCTAAGA
2581  CCCCCTCAA  CCGCTGGTGG  GGATAGCGAC  CCGGGAGTCG  ATGACTTCGA  TGAGGAAAAG
2641  CTAATGCAGG  CAAGGGAGAT  GATAAAATAT  ATGTCCCTCG  TATCGGCTAT  GGAGCAACAA
2701  GAACATAAGG  CGATGAAAAA  GAATAAGGGC  CCAGCGATCC  TAACGAGTCA  TCTCACTAAC
2761  ATGGCCCTCC  GTCGCCGTGG  ACCTAAATAC  CAACGCCTCA  ATAATCTTGA  TAGCGGTGAT
2821  GATACTGAAA  CAAATCTTGT  CTAA

```

4 / 24

FIG. 3A

DNA sequence of the the H6 promoted FHV gB donor plasmid
pC3H6FHVB.

H6 promoter: 3958 - 3835

FHV gB coding region: 3834 - 991

C3 left arm: 15 - 939

C3 right arm: 4056 - 6628

```
1  GCGGCCGCGT  CGACATGCAT  TGTTAGTTCT  GTAGATCAGT  AACGTATAGC  ATACGAGTAT
61  AATTATCGTA  GGTAGTAGGT  ATCCTAAAT  AAATCTGATA  CAGATAATAA  CTTTGTAAT
121 CAATTCAGCA  ATTTCTCTAT  TATCATGATA  ATGATTAATA  CACAGCGTGT  CGTTATTTTT
181 TGTTACGATA  GTATTTCTAA  AGTAAAGAGC  AGGAATCCCT  AGTATAATAG  AAATAATCCA
241 TATGAAAAAT  ATAGTAATGT  ACATATTTCT  AATGTTAACA  TATTTATAGG  TAAATCCAGG
301 AAGGGTAATT  TTTACATATC  TATATACGCT  TATTACAGTT  ATTAAAAATA  TACTTGCAAA
361 CATGTTAGAA  GTAAAAAAGA  AAGAACTAAT  TTTACAAAGT  GCTTTACCAA  AATGCCAATG
421 GAAATTACTT  AGTATGTATA  TAATGTATAA  AGGTATGAAT  ATCACAACA  GCAAATCGGC
481 TATCCCAAG  TTGAGAAACG  GTATAATAGA  TATATTTCTA  GATACCATTA  ATAACCTTAT
541 AAGCTTGACG  TTTCTTATAA  TGCCTACTAA  GAAAACCTAGA  AGATACATAC  ATACTAACGC
601 CATACGAGAG  TAACTACTCA  TCGTATAACT  ACTGTTGCTA  ACAGTGACAC  TGATGTTATA
661 ACTCATCTTT  GATGTGGTAT  AAATGTATAA  TAACTATATT  ACACTGGTAT  TTTATTTTCAG
721 TTATATACTA  TATAGTATTA  AAAATTATAT  TTGTATAAAT  ATATTATTAT  ATTCAGTGTA
781 GAAAGTAAAA  TACTATAAAT  ATGTATCTCT  TATTTATAAC  TTATTAGTAA  AGTATGTACT
841 ATTCAGTTAT  ATTGTTTTAT  AAAAGCTAAA  TGCTACTAGA  TTGATATAAA  TGAATATGTA
901 ATAAATTAGT  AATGTAGTAT  ACTAATATTA  ACTCACATTT  GACTAATTAG  CTATAAAAC
961 CCGGGCTGCA  GCCCGGGAAG  CTTACAAAAA  TTAGACAAGA  TTTGTTTCAG  TATCATCACC
1021 GCTATCAAGA  TTATTGAGGC  GTTGGTATTT  AGGTCCACGG  CGACGGAGGG  CCATGTTAGT
1081 GAGATGACTC  GTTAGGATCG  CTGGGCCCTT  ATTCTTTTTC  ATCGCCTTAT  GTTCTTGTTG
1141 CTCCATAGCC  GATACGAGGG  ACATATATTT  TATCATCTCC  CTTGCCTGCA  TTAGCTTTTC
1201 CTCATCGAAG  TCATCGACTC  CCGGGTCGCT  ATCCCCACCA  GCCGTTGAGC  GGGCTCTTAG
1261 CGTCTGTTTC  AAATTCCTAG  TCGTCACAGG  ATATAAGGCT  TTCATTGGAT  TTGCACGTAA
1321 TCTAGATATA  TAGCGATATG  CCAGGAATGC  TGCGACGATG  CCAGCTAATA  TTAACAGTCC
1381 CACGGCCAAT  GCTCCAAATG  GGTGTGTTAG  AAATGATGAT  ACGCCTGATA  CTGTTGAGAT
1441 TACCGCACTC  GCAGCCCCTA  AGACCACCTT  GCCGAAACCA  GCCCCACAT  CCCCAGTCC
1501 CTGAAAGAAA  TTTGCCATAC  CACGCATGAT  GACAAGATTA  TTATCCACTC  TGAATATGCT
1561 GTCTATATCA  TAAAATTTTA  AGGCGTGGAG  TTGGTTGCGG  CGTTGAATCT  CGCTGTAGTC
1621 CAAAAGGCCG  GTATCTTCCA  GCTCAGCTCG  TGTATAAACT  TCGAGTGGGA  GAAATTCACG
1681 ATCCTCTAGG  AGAGTAAGAT  TTAAATTCAC  ATACGCACTT  ATCAGTTCTA  TCTCCGATAG
1741 CGGGACTTTA  CGGACATACG  CATAATCCTC  AAAATATACA  TAATCTGCCC  CAAACTTAAA
1801 ATACCGCTTA  TTATTGACAG  TGCAAGGCTC  AATTAGTTTT  CGTTCCACGA  GAAGTTCATT
1861 GTTTTCCCCT  AGTTGTCCTT  CTATGTATTC  GGAGTCATTG  AGGGCACGGA  AGGAAACAAG
1921 AGGGCGGCTG  TAACATGTCG  TTGATGAACC  CGTCACCCGC  ATAGAATTTT  GGATATAGAC
1981 ATGTCCGCTA  GAAATGTTAA  CACATTGTGT  TACGGCGACG  GCATCTCCAA  GTAGGCGCGC
2041 GGATACACGA  CGTTCTAGGG  CCATCGAGAC  CACCCACCG  GGATTGAGTT  TTAGGGTCTC
2101 TGTCCACAGC  ACATGTTTCG  GGTTCGTAAG  TGTACACCAG  GCAGTGGCTA  TCCGACTCAA
2161 CATTTTATTT  ACATGGGCTT  GTATGTAGTC  ATAGGCAAAT  TGTAGCATTG  CGAATTCCAC
2221 AGATGAGGTG  GTCTTGAGGA  GTGATGCATT  GTTCACGGTT  TCTATACCCC  CCTCTATTGT
```

5 / 24

FIG. 3B

```
2281 GCTGCGCCGC GACCTATGAT GTTGATTAGA TGGGACCGAT CTTCTAGTTC GTTGTACTGT
2341 TTCCCCAGAT GGATTGAGGA GTGCACTGAG ATCTACCGTG CGATTGGAAC GTGCTAATTC
2401 ATTGATATAT AACTTTGCTA GTTCGTTGCT GATCATGGGA CGGAAAGCTA TTAGAAATCC
2461 CCCACGGGCT AGGTAGGTCT CCAGGGTTCC AGTCTGAATA TGAGTTTTAC TATATTTACT
2521 CTTATAAATC CGGTCTATGG CTTCCGGCTGC CTCCTTGGTG GCACAGTCCC CCAAACGGAT
2581 ACGATTGATT TCAAATTGTG AAGTATTGGA GATGAAAGTA GCGGATATGG TCTTGGCTGT
2641 AAATCTATAG GAGCCCTGAT ATTCATCGCG TAGCATTTCA TCTATTTCCC TCCATTTGGC
2701 TAAGGTACAT ACCCGACCAG ACTTTGGGGT CCAGTTCCAG GCCACTGTCA CATGCGGAGT
2761 TTCCAAAAAA TTGCGAGAAA CTGGTGCCCC CAGTTGTAAT CGCGTATCCA AGTCTATTGG
2821 ATAGTATCCC TCGATTTGTT GAAATCTGTC TGAAGAATAA CTAGTATGTT CTACATGGGC
2881 TCCATCCCTC AGCCCAAAGA ATGGAGACAT GTGAATCACC TCACCAGTGG AGATAGCAAA
2941 TGAGTCATAT GGATATACAG ATCTTGCATC CACTTCCTCT ACGATGCAAT TTACAGAGGT
3001 CCCAGAGTGG TGAAATCCAG CAGCACCAGT CTTTGTGTAT GTTTCATTGG TGGTGTGCCA
3061 TCCACGGGAC TCTGGAGTGT TGAAC TTGGA GGGTTTCAGA GGCAGTTCTC TGGGATCCTC
3121 GTCTCGATCA AAGGCCGTAA ATTGATAATT GTTACGAACG TAATCAGCTT TCGAGAGGCA
3181 CATACCCCGT CTATCTATGA GATCTGTAAT CTCTTGAAC TACACGGGAA CCCTGTCTGT
3241 ATATCGGTTG GTTGTAACGG CATAGGAACT CCCAGACCAT ACCGTTGTCA TAATAATGTT
3301 TTTATAGTAT ATATTTGCCT TGAATTTATA TGGCGCTATA TTTTCTTTAA ATATTACAGC
3361 TATACCCTCG GTAAAATTTT TCCCTAGTTT ATAATCTGGA CAGGCCCGTG GTGGCTCTAA
3421 ACGCACGACA GTAGATCCTG AAGGTGGTGG ACACATATAG AAAGTCGATG GTCCGTTAGC
3481 CTCTATTTGG GACGCACGGA GAGCTTCCCT CATATCCGAC ATATCGGTGG GATCTGTAGT
3541 TGGTTTTGGT GGTGTACCCC CTACCTCAGG GGTAGCTACA GTCCGCCGGG GTTGTTCGGA
3601 TGTGCTTCCA GATTGACCCT CTACTGGACG GGGACCGACT AAGAATAGTA CGATCCAGAT
3661 AAATGAAACA TATCTAGCTA GTCTGGTTAA TCCCGACGAT CCGTTACCAT GTCTGGAGCC
3721 AGTCGCTGCA ATACCGAGTA GAGAAGGGAA AAAACATCTC TGTCGAAAAT AGCCACTGTG
3781 TCCCTGCCAA CGACTCCCTC GTCGCCGCTT CCCAAGATCG CCACGAGTGG ACATTACGAT
3841 ACAAACTTAA CGGATATCGC GATAATGAAA TAATTTATGA TTATTTCTCG CTTTCAATTT
3901 AACACAACCC TCAAGAACCT TTGTATTTAT TTTCACTTTT TAAGTATAGA ATAAAGAAGC
3961 TCTAATTAAT TAAGCTACAA ATAGTTTCGT TTTACCTTG TCTAATAACT AATTAATTAA
4021 CCCGGATCGA TCCCGATTTT TATGACTAGT TAATCAAATA AAAAGCATAA AAGCTATTGC
4081 TTCGCTATCG TTACAAAATG GCAGGAATTT TGTGTAAACT AAGCCACATA CTTGCCAATG
4141 AAAAAAATAG TAGAAAGGAT ACTATTTTAA TGGGATTAGA TGTAAAGGTT CCTTGGGATT
4201 ATAGTAACTG GGCATCTGTT AACTTTTACG ACGTTAGGTT AGATACTGAT GTTACAGATT
4261 ATAATAATGT TACAATAAAA TACATGACAG GATGTGATAT TTTTCCTCAT ATAACTCTTG
4321 GAATAGCAAA TATGGATCAA TGTGATAGAT TTGAAAATTT CAAAAGCAA ATAACTGATC
4381 AAGATTTACA GACTATTTCT ATAGTCTGTA AAGAAGAGAT GTGTTTTCTT CAGAGTAACG
4441 CCTCTAAACA GTTGGGAGCG AAAGGATGCG CTGTAGTTAT GAAACTGGAG GTATCTGATG
4501 AACTTAGAGC CCTAAGAAAT GTTCTGCTGA ATGCGGTACC CTGTTCGAAG GACGTGTTTG
4561 GTGATATCAC AGTAGATAAT CCSTGGAATC CTCACATAAC AGTAGGATAT GTTAAGGAGG
4621 ACGATGTGCA AAACAAGAAA CGCCTAATGG AGTGCATGTC CAAGTTTAGG GGGCAAGAAA
4681 TACAAGTTCT AGGATGGTAT TAATAAGTAT CTAAGTATTT GGTATAATTT ATTAAATAGT
4741 ATAATTATAA CAAATAATAA ATAACATGAT AACGGTTTTT ATTAGAATAA AATAGAGATA
4801 ATATCATAAT GATATATAAT ACTTCATTAC CAGAAATGAG TAATGGAAGA CTTATAAATG
4861 AACTGCATAA AGCTATAAGG TATAGAGATA TAAATTTAGT AAGGTATATA CTTAAAAAAT
4921 GCAAATACAA TAACGTAAAT ATACTATCAA CGTCTTTGTA TTTAGCCGTA AGTATTTCTG
4981 ATATAGAAAT GGTAAAATTA TTAGTAGAAC ACGGTGCCGA TATTTTAAAA TGTAAAAATC
5041 CTCCTCTTCA TAAAGCTGCT AGTTTAGATA ATACAGAAAT TGCTAAACTA CTAATAGATT
5101 CTGGCGCTGA CATAGAACAG ATACATTCTG GAAATAGTCC GTTATATATT TCTGTATATA
5161 GAAACAATAA GTCATTAACT AGATATTTAT TAAAAAAGG TGTTAATTGT AATAGATTCT
5221 TTCTAAATTA TTACGATGTA CTGTATGATA AGATATCTGA TGATATGTAT AAAATATTTA
5281 TAGATTTTAA TATTGATCTT AATATACAAA CTAGAAATTT TGAAACTCCG TTACATTACG
5341 CTATAAAGTA TAAGAATATA GATTTAATTA GGATATTGTT AGATAATAGT ATTAATAATG
5401 ATAAAAAGTTT ATTTTTGTCAT AACAGTATC TCATAAAGGC ACTTAAAAAT AATTGTAGTT
5461 ACGATATAAT AGCGTTACTT ATAAATCACC GAGTGCCTAT AAACGAACAA GATGATTTAG
```

6/24

FIG. 3C

```
5521 GTAAAACCCC ATTACATCAT TCGGTAATTA ATAGAAGAAA AGATGTAACA GCACTTCTGT
5581 TAAATCTAGG AGCTGATATA AACGTAATAG ATGACTGTAT GGGCAGTCCC TTACATTACG
5641 CTGTTTCACG TAACGATATC GAAACAACAA AGACACTTTT AGAAAGAGGA TCTAATGTTA
5701 ATGTGGTTAA TAATCATATA GATACCGTTC TAAATATAGC TGTTGCATCT AAAAACAAAA
5761 CTATAGTAAA CTTATTACTG AAGTACGGTA CTGATACAAA GTTGGTAGGA TTAGATAAAC
5821 ATGTTATTCA CATAGCTATA GAAATGAAAG ATATTAATAT ACTGAATGCG ATCTTATTAT
5881 ATGGTTGCTA TGTAACGTC TATAATCATA AAGGTTTCAC TCCTCTATAC ATGGCAGTTA
5941 GTTCTATGAA AACAGAATTT GTTAAACTCT TACTTGACCA CGGTGCTTAC GTAAATGCTA
6001 AAGCTAAGTT ATCTGGAAAT ACTCCTTTAC ATAAAGCTAT GTTATCTAAT AGTTTTAATA
6061 ATATAAAATT ACTTTTATCT TATAACGCCG ACTATAATTC TCTAAATAAT CACGGTAATA
6121 CGCCTCTAAC TTGTGTTAGC TTTTLAGATG ACAAGATAGC TATTATGATA ATATCTAAAA
6181 TGATGTTAGA AATATCTAAA AATCCTGAAA TAGCTAATTC AGAAGGTTTT ATAGTAAACA
6241 TGGAACATAT AAACAGTAAT AAAAGACTAC TATCTATAAA AGAATCATGC GAAAAAGAAC
6301 TAGATGTTAT AACACATATA AAGTTAAATT CTATATATTC TTTTAATATC TTTCTTGACA
6361 ATAACATAGA TCTTATGGTA AAGTTCGTAA CTAATCCTAG AGTTAATAAG ATACCTGCAT
6421 GTATACGTAT ATATAGGGAA TTAATACGGA AAAATAAATC ATTAGCTTTT CATAGACATC
6481 AGCTAATAGT TAAAGCTGTA AAAGAGAGTA AGAATCTAGG AATAATAGGT AGGTTACCTA
6541 TAGATATCAA ACATATAATA ATGGAACAT TAAGTAATAA TGATTTACAT TCTGTTATCA
6601 CCAGCTGTTG TAACCCAGTA GTATAAAG
```


FIG. 4B

	800	810	820	830	840	850	860	870	880																
*	*	*	*	*	*	*	*	*	*																
GAG	GAG	CTG	TIG	AGG	TGG	GGA	CTT	ACA	ACC	ATG	GIA	GGT	TTT	CCA	GTA	CCT	CAA	GTA	CCT	TIA	AGA	CCA	ATG	ACT	
CTC	CTC	GAC	AAC	TCC	ACC	CCT	GAA	TGT	TGG	TAC	CAI	CCA	AAA	GGT	CAT	TGT	GGA	GTT	CAT	GGA	AAT	TCT	GGT	TAC	TGA
Glu	Glu	Leu	Leu	Arg	Trp	Gly	Leu	Thr	Thr	Met	Val	Gly	Phe	Pro	Val	Thr	Pro	Gln	Val	Pro	Leu	Arg	Pro	Met	Thr

POL/NEF Epitopes.

FIG. 4C

890	900	910	920	930	940	950	960	970
★	★	★	★	★	★	★	★	★
TAC AAA GCA GCT GTA GAT CTT TCT CAC TTT TTA AAA GAA GGA GGT TTA GAA GGG CTA ATT CAT TCT CAA CGA AGA CAA GAT ATT CTT								
ATG TTT CGT CGA CAT CTA GAA AGA GTG AAA AAT TTT CTT TTT CCT CCA AAT CTT CCC GAT TAA GTA AGA GGT TCT TCT GGT CTA TAA GAA								
Tyr Lys Ala Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Ile His Ser Gln Arg Arg Gln Asp Ile Leu								
POL/NEF Epitopes								

980	990	1000	1010	1020	1030	1040	1050	1060
★	★	★	★	★	★	★	★	★
GAT TTG TGG ATT TAT CAT ACA CAA GGA TAT TTT CCT GAT TGG CAG AAT TAC ACA CCA GGA CCA GGC AGA TAC CCA TTA ACC TTT GGT								
CTA AAC ACC TAA ATA GTA TGT GTT CCT ATA AAA GGA CTA ACC GTC TTA ATG TGT GGT CCT GGT CAG TCT ATG GGT AAT TGG AAA CCA								
Asp Leu Trp Ile Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Val Arg Tyr Pro Leu Thr Phe Gly								
POL/NEF Epitopes								

1070	1080	1090	1100	1110	1120	1130	1140	1150
★	★	★	★	★	★	★	★	★
TGG TGC TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GGA GGA ATG GAT GGC CCA AAA GGT AAA CAA TGG CCA TTG								
ACC ACG ATG TTC GAT CAT GGT TAC TAA CTC TGA CAT GGT CAT TTT AAT TTC GGT CCT TAC CTA CCG GGT TTT CAA TTT GGT ACC GGT AAC								
Trp Cys Tyr Lys Leu Val Pro Met Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu								
POL/NEF Epitopes								

1160	1170	1180	1190	1200	1210	1220	1230	1240
★	★	★	★	★	★	★	★	★
ACA GAA GAA AAA ATA AAA GCA TTA GTA GAA ATT TGT ACA GAG ATG GAA AAG GAA GGG AAA ATT TCA AAA AAT GGG CCT TAA TTTTICT								
TGT CTT CTT TTT TAT TAT TTT CGT AAT CAT CTT TAA ACA TGT CTC TAC CTT TTC CTT CCC TTT TAA AGT TTT TAA CCC GGA ATT AAAAAGA								
Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro								
POL/NEF Epitopes								

1250	1260	1270	1280	1290	1300	1310	1320	1330	1340	1350
★	★	★	★	★	★	★	★	★	★	★
GCAGCCCGGG GGATCCCTTTT TATAGCTAAT TAGTCACGTA CCTTTGAGAG TACCACCTTCA GCTACCCTCTT TTGIGCTICA GAGTAACCTT CTTTAATCAA TTCCAAACA										
CGTCGGGCCC CCTAGGAAAT ATATCGATTA ATCAGTGCAT GGAACTCTC ATGGTGAAGT CGATGGAGAA AACACAGAGT CTCATIGAAA GAAATTAGTT AAGGTTTGT										

FIG. 5A

gag (+ pro) and gp120 (+ transmembrane)

FEATURES	From	To/Span	Description
frag	1	56	C3 flanking arm
frag	162	76 (C)	HIV1 (IIIB) env transmembrane region
frag	1728	163 (C)	HIV1 (MN) gp120 gene
frag	1853	1729 (C)	vaccinia H6 promoter
frag	1925	1983	vaccinia I3L promoter
frag	1984	3746	HIV1 (IIIB) gag/pro gene
frag	3753	3808	C3 flanking arm

1010/24

10120

10110

10100

10090

10080

10070

10060

10050

10040

10030

10020

10010

10000

99990

99980

99970

99960

99950

99940

99930

99920

99910

99900

99890

99880

99870

99860

99850

99840

99830

99820

99810

99800

99790

99780

99770

99760

99750

99740

99730

99720

99710

99700

99690

99680

99670

99660

99650

99640

99630

99620

99610

99600

99590

99580

99570

99560

99550

99540

99530

99520

99510

99500

99490

99480

99470

99460

99450

99440

99430

99420

99410

99400

99390

99380

99370

99360

99350

99340

99330

99320

99310

99300

99290

99280

99270

99260

99250

99240

99230

99220

99210

99200

99190

99180

99170

99160

99150

99140

99130

99120

99110

99100

99090

99080

99070

99060

99050

99040

99030

99020

99010

99000

98990

98980

98970

98960

98950

98940

98930

98920

98910

98900

98890

98880

98870

98860

98850

98840

98830

98820

98810

98800

98790

98780

98770

98760

98750

98740

98730

98720

98710

98700

98690

98680

98670

98660

98650

98640

98630

98620

98610

98600

98590

98580

98570

98560

98550

98540

98530

98520

98510

98500

98490

98480

98470

98460

98450

98440

98430

98420

98410

98400

98390

98380

98370

98360

98350

98340

98330

98320

98310

98300

98290

98280

98270

98260

98250

98240

98230

98220

98210

98200

98190

98180

98170

98160

98150

98140

98130

98120

98110

98100

98090

98080

98070

98060

98050

98040

98030

98020

98010

98000

97990

97980

97970

97960

97950

97940

97930

97920

97910

97900

97890

97880

97870

97860

97850

97840

97830

97820

97810

97800

97790

97780

97770

97760

97750

97740

97730

97720

97710

97700

97690

97680

97670

97660

97650

97640

97630

97620

97610

97600

97590

97580

97570

97560

97550

97540

97530

97520

97510

97500

97490

97480

97470

97460

97450

97440

97430

97420

97410

97400

97390

97380

97370

97360

97350

97340

97330

97320

97310

97300

97290

97280

97270

97260

97250

97240

97230

97220

97210

97200

97190

97180

97170

97160

97150

97140

97130

97120

97110

97100

97090

97080

97070

97060

97050

97040

97030

97020

97010

97000

96990

96980

96970

96960

96950

96940

96930

96920

96910

96900

96890

96880

96870

96860

96850

96840

96830

96820

96810

96800

96790

96780

96770

96760

96750

96740

96730

96720

96710

96700

96690

96680

96670

96660

96650

96640

96630

96620

96610

96600

96590

96580

96570

96560

96550

96540

96530

96520

96510

96500

96490

96480

96470

96460

96450

96440

96430

96420

96410

96400

96390

96380

96370

96360

96350

96340

96330

96320

96310

96300

96290

96280

96270

96260

96250

96240

96230

96220

96210

96200

96190

96180

96170

96160

96150

96140

96130

96120

96110

96100

96090

96080

96070

96060

96050

96040

96030

96020

96010

96000

95990

95980

95970

95960

95950

95940

95930

95920

95910

95900

95890

95880

95870

95860

95850

95840

95830

95820

95810

95800

95790

95780

95770

95760

95750

95740

95730

95720

95710

95700

95690

95680

95670

95660

95650

95640

95630

95620

95610

95600

95590

95580

95570

95560

95550

95540

95530

95520

95510

95500

95490

95480

95470

95460

95450

95440

95430

95420

95410

95400

95390

95380

95370

95360

95350

95340

95330

95320

95310

95300

95290

95280

95270

95260

95250

95240

95230

95220

95210

95200

95190

95180

95170

95160

95150

95140

95130

95120

95110

95100

95090

95080

95070

95060

95050

95040

95030

95020

95010

95000

94990

94980

94970

94960

94950

94940

94930

94920

94910

94900

94890

94880

94870

94860

94850

94840

94830

94820

94810

94800

94790

94780

94770

94760

94750

94740

94730

94720

94710

94700

94690

94680

94670

94660

94650

94640

94630

94620

94610

94600

94590

94580

94570

94560

94550

94540

94530

94520

94510

94500

94490

94480

94470

94460

94450

94440

94430

94420

94410

94400

94390

94380

94370

94360

94350

94340

94330

94320

94310

94300

94290

94280

94270

94260

94250

94240

94230

94220

94210

94200

94190

94180

94170

94160

94150

94140

94130

94120

94110

94100

94090

94080

94070

94060

94050

94040

94030

94020

94010

94000

93990

93980

93970

93960

93950

93940

93930

93920

93910

93900

93890

93880

93870

93860

93850

93840

93830

93820

93810

93800

93790

93780

93770

93760

93750

93740

93730

93720

93710

93700

93690

93680

93670

93660

93650

93640

93630

93620

93610

93600

93590

93580

93570

93560

93550

93540

93530

93520

93510

93500

93490

93480

93470

93460

93450

93440

93430

93420

93410

93400

93390

93380

93370

93360

93350

93340

93330

93320

93310

93300

93290

93280

93270

93260

93250

93240

93230

93220

93210

93200

93190

93180

93170

93160

93150

93140

93130

93120

93110

93100

93090

93080

93070

93060

93050

93040

93030

93020

93010

93000

92990

92980

92970

92960

92950

92940

92930

92920

92910

92900

92890

92880

92870

92860

92850

92840

92830

92820

92810

92800

92790

92780

92770

92760

92750

92740

92730

92720

92710

92700

92690

92680

92670

92660

92650

92640

92630

92620

92610

92600

92590

92580

92570

92560

92550

92540

92530

92520

92510

92500

92490

92480

92470

92460

92450

92440

92430

92420

92410

92400

92390

92380

92370

92360

92350

92340

92330

92320

92310

92300

92290

92280

92270

92260

92250

92240

92230

92220

92210

92200

92190

92180

92170

92160

92150

92140

92130

92120

92110

92100

92090

92080

92070

92060

92050

92040

92030

92020

92010

92000

91990

91980

91970

91960

91950

91940

91930

91920

91910

91900

91890

91880

91870

91860

91850

91840

91830

91820

91810

91800

91790

91780

91770

91760

91750

91740

91730

91720

91710

91700

91690

91680

91670

91660

91650

91640

91630

91620

91610

91600

91590

91580

91570

91560

91550

91540

91530

91520

91510

91500

91490

91480

91470

91460

91450

91440

91430

91420

91410

91400

91390

91380

91370

91360

91350

91340

91330

91320

91310

91300

91290

91280

91270

91260

91250

91240

91230

91220

91210

91200

91190

91180

91170

91160

91150

91140

91130

91120

91110

91100

91090

91080

91070

91060

91050

91040

91030

91020

91010

91000

90990

90980

90970

90960

90950

90940

90930

90920

90910

90900

90890

90880

90870

90860

90850

90840

90830

90820

90810

90800

90790

90780

90770

90760

90750

90740

90730

90720

90710

90700

90690

90680

90670

90660

90650

90640

90630

90620

90610

90600

90590

90580

90570

90560

90550

90540

90530

90520

90510

90500

90490

90480

90470

90460

90450

90440

90430

90420

90410

90400

90390

90380

90370

90360

90350

90340

90330

90320

90310

90300

90290

90280

90270

90260

90250

90240

90230

90220

90210

90200

90190

90180

90170

90160

90150

90140

90130

90120

90110

90100

90090

90080

90070

90060

90050

90040

90030

90020

90010

90000

89990

89980

89970

89960

89950

89940

89930

89920

89910

89900

89890

89880

89870

89860

89850

89840

89830

89820

89810

89800

89790

89780

89770

89760

89750

89740

89730

89720

89710

89700

89690

89680

89670

89660

89650

89640

89630

89620

89610

89600

89590

89580

89570

89560

89550

89540

89530

89520

89510

89500

89490

89480

89470

89460

89450

89440

89430

89420

89410

89400

89390

89380

89370

89360

89350

89340

89330

89320

89310

89300

89290

89280

89270

89260

89250

89240

89230

89220

89210

89200

89190

89180

89170

89160

89150

89140

89130

89120

89110

89100

89090

89080

89070

89060

89050

89040

89030

89020

89010

89000

88990

88980

88970

88960

88950

88940

88930

88920

88910

88900

88890

88880

88870

88860

88850

88840

88830

88820

88810

88800

88790

88780

88770

88760

88750

88740

88730

88720

88710

88700

88690

88680

88670

88660

88650

88640

88630

88620

88610

88600

88590

88580

88570

88560

88550

88540

88530

88520

88510

88500

88490

88480

88470

88460

88450

88440

88430

88420

88410

88400

88390

88380

88370

88360

88350

88340

88330

88320</

11 / 24

850 860 870 880 890 900 910 920 930 940 950 960

TTTAGCATTATCATTTGAAATTCACAGATCTAATTACTACCCTCTCTTCTCGCTAGACIGCCCAATTAAACAGGCAGTTGAGTGGATTAATCCATGTGATACATTTGCTACCTGTGCT
AAATCGTAAATAGTAACCTTTAAGAGCTCTAGATTAAATGATGGAGAAGAACGATCTGACCGGTAAATTTGCTCAACTCAACTATGATGACCGGATTAAAGGTACACATGTAAACATGACACCGA
K A N D N F N E S R I V V E E A L S G N L L Q T S V V P R I G H T C Q V T S

HIV1 (MN) GP120 GENE

[illegible]

	2180	2190	2200	2210	2220	2230	2240	2250	2260	2270	2280
--	------	------	------	------	------	------	------	------	------	------	------

FIG. 5E

ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATATACAGTAGCAACCCCTCTATTGTGTGCATGAAGGATAGAGATAAAGACACACCAAGGAA
TATGACCCCTGTCGATGTTGGTAGGGAAGCTGTCCTAGTCTTCTTGAATCTAGTAATATATTATGTCATCGTGGAGATAACACACAGTAGTTCCCTATCTCTATTTCTGTGGTTCCTT
I L G Q L Q P S L Q T G S E E L R S L Y N T V A T L Y C V H Q R I E I K D T K E
HIV1 (IIB) GAG/PRO GENE
>

2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
GGCTTTAGACAAGATAGAGGAAGCAAAACAAAGTAAGAAAGCACAGCAGCGCTGACACAGGACACAGCAATCAGGTGAGCCAAAATTACCTATAGTGCAGAACATCCAG
CGAAATCTGTTCTATCTCCTTCTCGTTTGTGTTTCATTCCTTTTTCGTGTCGTTTCGACTGTCCTGTCGTGCTAGTCCAGTCGGTTTTTAATGGGATATCACGCTCTTGTAGGTC
A L D K I E E E Q N K S K K A Q Q A A A D T G H S N Q V S Q N Y P I V Q N I Q
HIV1 (IIIB) GAG/PRO GENE

[illegible]

2530	2540	2550	2560	2570	2580	2590	2600	2610	2620	2630	2640
ACCCACAAAGATT	TAACACCATGCT	AAACACAGTGGG	GGGACATCAAG	CAGCCATGTTAA	AAGAGACCAT	CAATGAGGAAG	CTGCAGATGG	GATAGAGTGC	ATCCAGTGCAT		
TGGGGTGTTCTA	AAATTTGTGGT	ACGATTTGTGTC	ACCCCTGTAGT	TCGTCGGTACG	TTTCTCTGTA	GTACTCTTCG	ACGCTTACCC	TATCTCAGGT	ATCAGGTACGTA		
T P Q D L N T M L N T M L N T V G G H Q A A M Q M L K E T I N E E A A E W D R V H P V H											

HIV1 (IIIB) GAG/PRO GENE

[illegible]

FIG. 5F

2770	2780	2790	2800	2810	2820	2830	2840	2850	2860	2870	2880
GAAATTTATAAAGATGGATAATCCTGGGATTAAATAAATAGTAAGAAATGTATAGCCCTACAGCATTCCTGGACATAAGACACAAGGACCCAAAGAACCCCTTTAGAGACTATGTAGACCGG											
CTTTAAATATTTTCTACCTATTAGGACCCCTAATTTATTTTATCATCTTACATATCGGGATGGTCGTAGACACCTGTATTCCTGCTGTTTCTTGGGAAATCTCTGATACATCTGGCCC											
E I Y K R W I I L G L N K I V R M Y S P T S I L D I R Q G P K E P F R D Y V D R											
HIV1 (JIIIB) GAG/PRO GENE											
>											

2890	2900	2910	2920	2930	2940	2950	2960	2970	2980	2990	3000
TTCTATAAACTCTAAGAGCCGAGCAAGCTTCACAGGAGGTAA	AAATTCGATGACAGAAACCTTGGTTC	CAAAATGCGAACCCAGATTGTAAGACTAT	TTTAAAGCATTGGGACCA								
AAGATAATTTTGAGATTCTCGGCTCGTTCGAAGTGCTCCTCC	ATTTTAAACCTACTGCTCTTGGACAAACCAGGTTT	ACGCTTGGGCTCAACATTCTGATAAAATTTTCGTAACCCCTGGT									
F Y K T L R A E Q A S Q E V K N W M T E T L L V Q N A N P D C K T I L K A L G P											
HIV1 (111B) GAG/PRO GENE											
>											

3010	3020	3030	3040	3050	3060	3070	3080	3090	3100	3110	3120
GCGGCTACACTAGAAATGATGACAGCATGTCAGGGAGTAGGAGACCCGGCCATAAGGCAAGAGTTTTGGCTGAAGCAATGAGCCAAAGTAAACAATTCAGCTACCATATGATGCAG											5/24
CGCCGATGTGATCTTCTTTACTACTGTCGTACAGTCCCTCATCTCCTGGCCGGTATTCGGTTCTCAAAACCGACITCGTTACTCGGTTCAATGTTTAAAGTCGATGGTATTACTACGTC											
A A T L E E M M T A C Q G V G G P G H K A R V L A E A M S Q V T N S A T I M H Q											
HIV1 (JIIIB) GAG/PRO GENE											

3130	3140	3150	3160	3170	3180	3190	3200	3210	3220	3230	3240
AGAGGGCAATTTAGGAACCAAGAAAGATTGTTAAGTGTTTCAATTGIGGCAAGAGGGCACACAGCCAGAAATTCAGGGCCCTAGGAAATGTGGAAAGGAA											
TTCTCCGTTAAATCCTTGTTCTTTCTAACAAATTCACAAAGTTAACACCGTTTCTCCCGTGTCGGICTTTAACGTCCTCCGGGATCCCTTTTCCCGACAACCTTACACCTTTCCT											
R G N F R N Q R K I V K C F N C G K E G H T A R N C R A P R K K G C W K C G K E											
HIV1 (111B) GAG/PRO GENE											

3250	3260	3270	3280	3290	3300	3310	3320	3330	3340	3350	3360
GGACACCAATGAAAGATTGTACTGAGACAGGCTAATTTTTTAGGAAGATCTGGCCCTTCCCTACAAAGGGAAGCCAGGGAATTTCTTCAGAGCAGACCAGAGCCCAACAGCCCCACCA											
CCTGTGGTTTACTTTCTAACATGACTCTCTGTCGCGATTAAAAATCCCTTCTAGACCGGAAGGATGTTCCCTTCCGGTCCCTTAAAGAAGTCTCGTCTCGGTGTCGGGTGGT											
G H Q M K D C T E R Q A N F L G K I W P S Y K G R P G N F L Q S R P E P T A P P											
HIV1 (IIIB) GAG/PRO GENE											
>											

FIG. 5G

3370	3380	3390	3400	3410	3420	3430	3440	3450	3460	3470	3480	
GAAGAGAGCTTCAGGCTCTGGGGTAGAGACAACAACCTCCCTCAGAGCAGGAGCCGATAGACAAGGAACCTGTAATCCCTTAACCTCCCTCAGATCACICTTTGGCAACGACCCCTCGTCA	CTTCTCTCGAAGTCCAGACCCCACTCTCTGTGTGTGAGGGGAGCTCTCGTCCCTCGGCTATCTGTTCCCTTGACATAGGAAATTGAAGGGAGTCTAGTGAGAAACCGTTGCTGGGGAGCAGT	E E S F R S G V E T T T T P P Q K Q E P I D K E L Y P L T S L R S L F G N D P S S	P Q I T L W Q R P L V									
HIV1 (IIIB) GAG/PRO GENE												
3490	3500	3510	3520	3530	3540	3550	3560	3570	3580	3590	3600	
CAATAAGATAGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAGAAATGAGTTGCCAGGAAGATGGAACCAAAATGATAGGGGAATTGGAG	GTTATTCTATCCCCCGTIGATTTCCTTCGAGATAATCTATGCTCTACTATGTCATAATCTCTTACTCAACGGTCCCTTCTACCTTTGGTTTTTACTATCCCCCTTAACCTC	Q										
T I K I G G Q L K E A L L D T G A D D T V L E E M S L P G R W K P K M I G G I G	HIV1 (IIIB) GAG/PRO GENE											
3610	3620	3630	3640	3650	3660	3670	3680	3690	3700	3710	3720	
GTTTTATCAAAGTAAGACAGIATGATCAGATACATACATAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCACATAATTTGGAAGAAATCTGTGACTC	CAAAATAGTTTCATCTGTGICATCTAGTCTATGAGTATCTTTAGACACCTGTATTTTCGATATCCATGTCATAATCCTGATGTCGACAGTGTGTTAACCCTTCTTTAGACAACTGAG	G F I K V R Q Y D Q I L I E I C G H K A I G T V L V G P T P V N I I G R N L L T	HIV1 (IIIB) GAG/PRO GENE									
3730	3740	3750	3760	3770	3780	3790	3800					
AGATTGGTTGCACCTTAAATTTTAAACCCGGGGATCCCGATTTTATGACTAGTTAATCAATAAAAGCATAACAAGCTATTGCTTC	TCTAACCAACGTGAAATTTAAAAATGGGGCCCCIAGGGCTAAATAACIGATCAATTAGTTATTTTCGTATGTTCCGATAACGAAG	Q I G C T L N F	< C3 FLANKING ARM									
HIV1 (IIIB) GAG/PRO												

FIG. 6A

Q H R C M R K Y N V D I
-----K3L

WO 98/40500

PCT/US98/03710

FIG.6B⁵⁴⁰

550 560 570 580 590 600 610 620
* * * * *
AAC TTT TAC AGT TTT CCC TAC CAG TTT ATC CCT ATA TTC AAC ATA TCT ATC CAT ATG CAT CTT AAC ACT CTC TGC CAA GAT AGC TTC AGA
TTG AAA ATG TCA AAA GGG ATG GTC AAA TAG GGA TAT AAG TTG TAT AGA TAG GTA TAC GTA GAA TTG TGA GAG ACG GTT CTA TCG AAG TCT
<V K V T K G G V L K D R Y E V Y R D M H M K V S E A L I A E S
-----K3L-----

630 640 650 660 670 680 690 700 710
* * * * *
GIG AGG ATA GTC AAA AAG ATA AAT GIA TAG AGC ATA ATC CTT CTC GIA TAC TCT GCC CTT TAT TAC ATC GCC CGC ATT GGG CAA CGA AIA
CAC TCC TAT CAG TTT TTC TAT TTA CAT ATC TCG TAT TAG GAA GAG CAT ATG AGA CGG GAA ATA ATG TAG CGG GCG TAA CCC GTT GCT TAT
<H P Y D F L Y I Y L A Y D K E Y V R G K I V D G A N P L S Y
-----K3L-----

720 730 740 750 760 770 780 790 800 810
* * * * *
ACA AAA TGC AAG CAT ACG ATACAACTT AACGGATAIC GCGATAAIGA AATAATTAT GATTATTTCT CGCTTTCAT TTAACACAAC CCICAAGAAC
TGT TTT ACG TTC GTA TGC TATGTTIGAA TTGCTATAG CGCTATTACT TTATTAATA CTAATAAGA GCGAAAGTTA AATIGTGTTG GGAGTTCITG
<C F A L M
-----K3L-----

18/24

820 830 840 850 860 870 880 890 900 910 920
* * * * *
CTTTGATTT ATTTTCACCT TTTAAGTATA GAATAAAGAA AGCTCIAATT AATTAAAGAA CAGATTIGT CGTTTCCCC TTGGCGTAIC ACTAATTAAT TAACCCGGGCG
GAAACATAAA TAAAGTGAA AAATTCATAT CITATTTCT TCGAGATTAA TTAATTACTT GICTAACAAA GCAAAGGGG AACCGCATAG TGATTAAATTA ATTGGGCCCG

930 940 950 960 970 980 990 1000 1010 1020 1030
* * * * *
TGCAGCTCGA GGAATTC AAC TATATCGACA TATTTCATTT GIATACACAT AACCAATTACT AACGIAGAAT GIATAGGAAG AGATGTAACG GGAACAGGGT TTGTIGATTC
ACGTCGAGCT CCITTAAGTTG ATATAGCTGT ATAAAGTAAA CATATGIGTA TTGGTAAIGA TTGCATCTTA CATATCCTTC TCTACATTGC CCTTGICCCA AACCACTAAG

1040 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140
* * * * *
GCAAACATTT CIAATACATA ATTCTTCTGT TAATACGCT TGCACGTAAT CTATTATAGA TGCCAAGAIA TCTATATAAT TATTTIGTAA GATGATGTTA ACTATGTGAT
CGTTTGATAA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA GATAATATCT ACGTCTCTAT AGATATATTA ATAAACATT CTACTACAAT TGATACACTA

FIG. 6C

1150	1160	1170	1180	1190	1200	1210	1220	1230	1240	1250	
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	
CTATAAAGT	AGTGTAATAA	TTCATGTATT	TCGATAATG	TTCCAACICT	GICITTTGGA	IGICIAITCT	CGIAAIAICT	AIAGCAICCT	CAAAAAAIAI	ATTGCGCAIAI	
GATATAATCA	TCACATTATT	AAGTACATAA	AGCTATATAC	AAGGTTGAGA	CAGAAACACT	ACAGATCAAA	GCATTATAGA	TATCGTAGGA	GTTTTTTATA	TAAGCGGIATA	
1260	1270	1280	1290	1300	1310	1320	1330	1340	1350	1360	
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	
ATTCCCAAGT	CTTCAGITCT	ATCTTCTTAA	AAATCTTCAA	CGTATGGAAT	ATAATAAICT	ATTTTACCCTC	TTCIGATAIC	ATTAAGAIA	IAGTTTTIGA	CACIATCTTC	
TAAGGGTICA	GAAGTCAAGA	TAGAAGATT	TTTGAAGIT	GCATACCCTTA	TATTATTAGA	TAAAATGGAG	AAGACTATAG	TAATTACTAT	ATCAAAAAC	GTGATAGAAG	
1370	1380	1390	1400	1410	1420	1430	1440	1450	1460	1470	19/24
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	
IGTCAATIGA	TTCITATICA	CTATATICTAA	GAAACGGATA	GCGTCCCTAG	GACGAACIAC	TGCCATTAA	ATCTCTATTA	TAGCTTCTGG	ACATAAATICA	ICTATTTAIAC	
ACAGTTAACT	AAGAATAAGT	GATATAGATT	CTTTGCCAT	CGCAGGGATC	CTGCTTGATG	ACGGTAATTA	TAGAGATAAT	ATCGAAGACC	TGTATTAAAGT	AGATAATAIG	
1480	1490	1500	1510	1520	1530	1540	1550	1560	1570	1580	
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	
CAGAAATTAAT	GGGAACATAIT	CCGTATCTAT	CTAACATAGT	TTTAAGAAAG	TCAGAAICTA	AGACCTGATG	TTCATATATT	GGTTCATACA	TGAAATGAIC	ICTATTGAIG	
GTCTTAATTA	CCCTTGATAA	GGCATAGATA	GATTGTATCA	AAATTCCTTC	AGTCTTAGAT	TCCTGGACTAC	AAGTATATAA	CCAAGTATGT	ACTTTIAC	TAG AGATAACTAC	
1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690	
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	
ATAGTGACTA	TTTCATTCTC	TGAAAATTGG	TAACTCATTC	TATATAIGCT	TTCCTTGTTG	ATGAAGGATA	GAAIATACIC	AAIAGAATTT	GIACCAACAA	ACTGTTCTCT	
TATCACTGAT	AAAGTAAGAG	ACTTTTAACC	ATTGAGTAAG	ATATATACGA	AAGGAACAAC	TACTTCCCTAT	CTTATATGAG	TTAICTTAA	CATGGTTGTT	TGACAAGAGA	

WO 98/40500

PCT/US98/03710

FIG. 6D

1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
* * * * *
TATGAATCGT ATATCATCAT CTGAAATAAT CAITGAAGGC ATACATTTAA CAATTAGAGA CTGGCTCCCT GTTATCAATA TACTATTCCT GIGATAATTT AIGTIGAGG
ATACTTAGCA TATAGTAGTA GACTTTATTA GTACATTCCG TATGTAAATT GTTAATCTCT GAACAGAGCA CAATAGTTAT ATGATAAGAA CACTATTAAG CACTACACTCC

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910
* * * * *
CAAATTIGTC CACGTTCTTT AATTTTGTTA TAGTAGATAT CAAATCCCAAT GGAGCTACAG TTCTTGGCTT AAACAGATAT AGTTTTTCIG GAACAAATTC TACAACATTA
GTTTAAACAG GTGCAAGAAA TTAAACAAT ATCATCTATA GTTAGGTTA CCTCGATGTC AAGAACCAGAA TTIGTCTATA TCAAAAAGAC CTIGTTAAG AIGTTGTAAT

1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020
* * * * *
TTATAAAGGA CTTGGGTAG ATAAGTGGGA TGAATCCCTA TTTTAATTA TGTATCGCA TTGCTCCCTT GCAATAATCC AAACGCTTTT GIGATAGIAT GGCATTCAT
AATATTCCT GAAACCCATC TATTCACCCT ACTTAGGAT AAAATTAAT ACCATAGCGT AACAGGAGCA CGTTATAGG TTTCGAGAAA CACTATCATA CCGTAGTAA

20/24

2030 2040 2050 2060 2070 2080 2090 2100 2110 2120 2130
* * * * *
GCTAGAAC GCCTACGAA TATCTGIGAC AGATAATC TTTAGAGAAT ATACTAGTCG CGTTAATAGT ACTACAATTT GTATTTTTTA ATCTATCTCA ATAAAAAAT
CAGATCTTTG CGAGATGCTT ATAGACACTG TCTATAGTAG AAATCTCTTA TATGATCAGC GCAATTAICA TGATGTTAA CATAAAAAT TAGATAGAGT TATTTTTTA

2140 2150 2160 2170 2180 2190 2200 2210 2220 2230
* * * * *
TAATAIGIAT GATTCATGT ATAACATAAC TACTAACCTG TATIGATAAC TAGAATCA GAA TCT AAT GAT GAC GTA ACC AAG AAG TTT ATC TAC TGC CAA
ATTATACATA CTAAGTTACA TATTGATTG ATGATTGACA ATAACTATTG ATCTTAGT CTT AGA TTA CTA CTG CAT TGG TTC TTC AAA TAG ATG ACG GTT
<F R I I V Y G L L K D V A L

-----E3L-----

2240 2250 2260 2270 2280 2290 2300 2310 2320
* * * * *
TTT AGC TGC ATT ATT TTT AGC ATC TCG TTT AGA TTT TCC AIC TGC CTT ATC GAA TAC TCT ICC GTC GAT GTC ACA GGC ATA AAA TGT
AAA TCG ACG TAA TAA AAA TCG TAG AGC AAA TCT AAA AGG TAG ACG GAA TAG CTT ATG AGA AGG CAG CTA CAG ATG TGT CCG TAT TTT ACA
<K A A N N K A D R K S K G D A K D F V R G D I D V C A Y F T

-----E3L-----

2690	2700	2710	2720	2730	2740	2750	2760	2770																				
* *	* *	* *	* *	* *	* *	* *	* *	* *																				
ITCC	ITC	GAT	ICC	AAT	GTT	TTT	AAT	AGC	CGC	ACA	CAC	AAT	CYC	TGC	GTC	AGA	ACG	CIC	GTC	ACG	CAC	ATA	GAT	CIT	AGA	CAT	TT	TTAGAGAGAA
AGG	AAG	CTA	AGG	TTA	CAA	AAA	TTA	TCG	GCG	TGT	GTG	TTA	GAG	ACG	CAG	TCT	TGC	GAG	CAG	TIA	TAT	CIA	GAA	TCT	GTA	AA	AATCTCTCTT	
<G	E	I	G	I	N	K	I	A	A	C	V	I	E	A	D	S	R	E	D	I	Y	I	K	S	M			

FIG. 6F

2780	2790	2800	2810	2820	2830	2840	2850	2860	2870	2880
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
CTAACACAAC	CAGCAATAAA	ACTGAACCCTA	CITTAICATT	TTTTTATICA	TCATCCCTCIG	GTTGGTTCGIC	GTTTCTATCG	AAIGTAGCIC	TGATTAACCC	GTCACTCTATA
GATTGTGTTG	GTCGTTATTT	TGACTTGGAT	GAAATAGTAA	AAAAATAAGT	AGTAGGAGAC	CACCAAGCAG	CAAAGATAGC	TTACATCGAG	ACTAATTGGG	CAGTAGATAT
2890	2900	2910	2920	2930	2940	2950	2960	2970	2980	2990
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
GGTGATGCTG	GTICTGGAGA	TTCTGGAGGA	GAIGGATTAT	TATCIGGAAG	AATCTCIGTT	ATTICCTTGT	TTTCAIGTAT	CGATTGCGTT	GTAACATTAA	GATIGCGAAA
CCACTACGAC	CAAGACCTCT	AAGACCTCCT	CTACCTAATA	ATAGACCTTC	TTAGAGACAA	TAAAGGAACA	AAAGTACATA	GCTAACGCCAA	CATTGTAATT	CTAACGCITT
3000	3010	3020	3030	3040	3050	3060	3070	3080	3090	3100
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
TGCTCTAAAT	TTGGGAGGCT	TAAAGTGTG	TTTGCAATCT	CTACACCGGT	GICTAACIAG	TGGAGGTTCG	TCAGCIGCIC	TAGTTIGAAT	CAATCATCGGC	GTAGTATICC
ACGAGATTTA	AACCCCTCCGA	ATTTCACAAC	AAACGTTAGA	GATGIGCGCA	CAGATTGATC	ACCICCAAGC	AGTCGACGAG	ATCAAACCTTA	GTAGTAGCCG	CATCATAAGG
3110	3120	3130	3140	3150	3160	3170	3180	3190	3200	3210
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
TACITTTIACA	GTTAGGACAC	GGIGTATTGT	ATTICTCGIC	GAGAACGGTIA	AAATAATCGT	IGTAACCTCAC	ATCCTTTTAT	TTATCTATAT	IGIATTCIAC	TCCITTTCTIA
ATGAAAATGT	CAATCCCTGTG	CCACATAACA	TAAAGAGCAG	CYCTTGCAAT	TTTATTAGCA	ACATTGAGTG	TAGGAAATAA	AATAGATATA	ACATAAGATG	AGGAAAGAAT
3220	3230	3240	3250	3260	3270	3280	3290	3300	3310	3320
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
ATGCATTTTA	TACCGAATAA	GAGATAGCGA	AGGAATICTT	TTTTATTGAT	AACIAGICAA	ATGAGTATAT	ATAATTGAAA	AAGTAAATAA	TAAATCATAT	AATAATGAAA
TACGTAAAT	ATGGCTTATT	CTCTATCGCT	TCCITTAGAA	AAATAACTAA	TIGATCAGTT	TACICATATA	TATTAACCTT	TTCATTTTAT	ATTIAGIATA	TTATTACTTT

22/24

WO 98/40500

PCT/US98/03710

FIG. 6G

3330	3340	3350	3360	3370	3380	3390	3400	3410	3420	3430
* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *
CGAAATATCA	GTAATAGACA	GGAACIGGCA	GATTCCTCT	CTAATGAAGT	AAGTACIGCT	AAATCICCAA	AATTAGATAA	AAATGATACA	GCAAATACAG	CTTCATTCAA
GCCTTATAGT	CATTATCTGT	CCITGACCGT	CTAAGAAGAA	GATTACTTCA	TTCATGACGA	TTTAGAGGTT	TAAATCTATT	TTIACCTAAT	CGTTTATGTC	GAAGTAAAGTT
3440	3450	3460	3470	3480	3490	3500	3510	3520	3530	3540
* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *
CGAATTACCT	TTTAAATTTT	TCAGACACAC	CTTATTACAA	ACTAACAIAAG	TCAGATGAIG	AGAAAGTAA	TATAAATTTA	ACTTATGGGT	ATAATATAAT	AAAGATTTCAT
GCTTAAIGGA	AAATTAAAAA	AGCTGIGTIG	GAATAATGTT	TGATTGATTC	AGTCTACTAC	TCTTTCATTT	ATATTTAAT	TGAATACCCA	TATTATATTA	TTTCTAAGTA
3550	3560	3570	3580	3590	3600	3610	3620	3630	3640	3650
* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *
GATATTAAIA	ATTIACCTIAA	CGAIGTTAAT	AGACTTATTC	CAICAACCCC	TTCAAACCTT	TCIGGATATT	ATAAAATACC	AGTTAATGAT	ATTAAAATAG	ATTGTTTAAAG ^{23/24}
CTATAATTAT	TAAATGAATT	GCTACAATTA	TCTGAATAAG	GTAGTTGGGG	AAGTTTGGAA	AGACCTAATA	TATTTTAATG	TCAATTACTA	TAATTTTATC	TAACAAATTC
3660	3670	3680	3690	3700	3710	3720	3730	3740	3750	3760
* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *
AGAIGTAAAT	AATTATTGGG	AGGTAAAGGA	TATAAAATTA	GTCATCTTTT	CACATGGGAA	TGAATTACCT	AATATTAAATA	ATTATGATAG	GAATTTTTTA	GGATTACAG
TCTACATTTA	TTAATAAACC	TCCATTTCCT	ATATTTTAAAT	CAGATAGAAA	GGTGATCCCT	ACTTAAATGGA	TTATAATTAT	TAATACTATC	CTTAAAAAAT	CCTAAATGTC
3770	3780	3790	3800	3810	3820	3830	3840	3850	3860	3870
* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *
CIGTTATAIG	TATCAACAAT	ACAGGCAGAT	CTAIGGTTAT	GGTAAACAC	TGTAAACGGG	AGCAGCATTC	TAIGGTAACT	GGCTAATGTT	TAAATAGCCAG	ATCAATTTIAC
GACAATATAC	ATAGTTTGTTA	TGTCGGTCTA	GATACCAATA	CCATTTTGTG	ACATTTGCCCT	TCGTGCTAAG	ATACCAATTGA	CCGGATACAA	ATTATCGGTC	TAGTAAAAATG
3880	3890	3900	3910	3920	3930	3940	3950	3960	3970	3980
* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *
TCTATAAACA	TTTTACCACA	AATAATAGGA	TCCCTCTAGAT	ATTAAATATT	ATATCTAACA	ACAACAATAA	AATTTAAGGA	TGTATGGCCA	GAAGTATTTT	CTACIAATAA
AGATAATTTGT	AAAATGGTGT	TTATTATCCT	AGGAGATCTA	TAAATATATA	TATAGATTTGT	TGTGTGTTTT	TTAAATTTGCT	ACATACCCGT	CTTCATAAAA	GATGATTATT

FIG. 6H

3990	4000	4010	4020	4030	4040	4050	4060	4070	4080	4090
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
AGATAAAGAT	AGTCTATCTT	ATCTACAAGA	TAIGAAGAA	GATAATCATT	TAGTAGTAGC	TACTAATATG	GAAAGAAATG	TATACAAAAA	CGTGGGAAGCI	TTTATATATIAA
TCTATTICTA	TCAGATAGAA	TAGATGTTCT	ATACTTTCTT	CTATTAGTAA	ATCATCATCG	ATGATTATAC	CTTCTTTTAC	ATATGTTTTT	GCACCTTCGA	AAATATAATT
4100	4110	4120	4130	4140	4150	4160	4170	4180	4190	4200
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
ATAGCATATT	ACTAGAAGAT	TTAAAATCTA	GACTTAGTAT	AACAAAACAG	TTAAATGCCA	ATAICGATTC	TATATTTTCAT	CATAACAGTA	GTACATTAAAT	CAGTGAATATA
TATCGTATAA	TGATCTTCTA	AAATTTAGAT	CTGAATCATA	TTGTTTTGIC	AAATTTACGGT	TATAGCTAAG	ATATAAGTA	GTATTTGTCAT	CATGTAAATTA	GTCACATATAT
4210	4220	4230	4240	4250	4260	4270	4280	4290	4300	4310
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
CTGAAACGAT	CTACAGACIC	AACTATGCCAA	GGAATAAGCA	ATAIGCCCAAT	TATGTCTAAT	ATTTTAACTT	TAGAACTAAA	ACGTTCTACC	AATACIAAAA	ATAGGATACG
GACTTTGCTA	GAIGTCTGAG	TTGATACGTT	CCATTATTCGT	TATACGGTTA	ATACAGATTA	TAAAATTGAA	ATCTTGATTT	TGCAAGATGG	TTATGATTTT	TATCCTATGC
4320	4330	4340	4350	4360	4370	4380	4390	4400	4410	4420
* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
TGATAGGCTG	TTAAAAGCTG	CAATAAATAG	TAAGGATGTA	GAAGAAATAC	TTTGTTCIAT	ACCTTCGGAG	GAAAGAACIT	TAGAACAACI	TAAGTTTAAAT	CAAACTTIGTA
ACTATCCGAC	AAATTTTCGAC	GTATTTTATC	ATTCCTACAT	CTTCITTAIG	AAACAAGATA	TGGAAGCCIC	CTTCTCTTGA	ATCTTGTGTA	ATTCAAAATTA	GTTTGAACAT
4430	* *									
TTTATGAAGG	TACC									
AAATACTTCC	ATGG									

24/24