PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 39/12, C12N 15/00, 7/00 — A1

(11) International Publication Number: **WO 00/50076**

(43) International Publication Date: 31 August 2000 (31.08.00)

(21) International Application Number: PCT/US00/04711

(22) International Filing Date: 24 February 2000 (24.02.00)

(30) Priority Data:
60/121,647 24 February 1999 (24.02.99) US

(71) Applicant *(for all designated States except US)*: PURDUE RESEARCH FOUNDATION [US/US]; Office of Technology Transfer, 1063 Hovde Hall, West Lafayette, IN 47906 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MITTAL, Suresh, Kumar [CA/US]; 2839 Ashland Street, West Lafayette, IN 47906 (US). AGGARWAL, Neeraj [IN/GB]; 6 Bullswater Common, Pirbright, Near Working, Surrey GU24 0LZ (GB).

(74) Agents: SCHWARTZ, Jason, J. et al.; Woodard, Emhardt, Naughton, Moriarty & McNett, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, IN 46204 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ADENOVIRAL VECTORS, PROTEINS AND METHODS FOR THEIR USE

(57) Abstract

Purified proteins that function in viral replication, especially in controlling the viral latent state, are provided. Nucleotide sequences encoding these proteins are also provided. Both DNA sequences, and the RNA transcripts are encompassed by the invention. The invention also provides recombinant vectors including the nucleotide sequence encoding the novel proteins described herein. In a preferred form of the invention, a plasmid is provided that includes the nucleotide squence encoding the novel proteins described herein, along with an adenoviral origin of replication. Further provided are host cells that include the recombinant vectors described herein, adenoviral vectors and methods of expressing viral proteins and other desired proteins utilizing the nucleotide sequences, recombinant vectors and host cells described herein.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

ADENOVIRAL VECTORS, PROTEINS AND METHODS FOR THEIR USE

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Patent Application Serial Number 60/121,647, filed on February 24, 1999 which is hereby incorporated by reference in its entirety.

This invention was made with government support under Public Health Service grant number GM55168-01 awarded by the National Institutes of Health/National Institute of General Medical Sciences. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Adenoviruses are non-enveloped DNA viruses that infect a wide variety of cells and some of them typically cause acute respiratory and ocular infections, as well as hemorrhagic cystitis and infantile gastroenteritis. After adenoviruses infect a cell, their DNA does not become incorporated into the genome of the infected cell, but remains episomal. As adenoviruses infect a wide variety of host cells, and have the ability to replicate in both dividing and non-dividing cells, recombinant adenoviruses are frequently used as viral vectors for gene delivery applications, especially to deliver recombinant vaccines.

Porcine adenoviruses (PAds) usually cause inapparent infections. However, they are associated with enteric, respiratory, kidney, and central nervous system infections in swine [Derbyshire J. B. et al. (1975) *J. Comp. Path.* 85:437-443; Hirahara T., et al. (1990) *Jpn. J. Vet. Sci.* 52:407-409; Hirahara T., et al. (1990) *Jpn J. Vet Sci.* 52:1089-1091]. PAds are classified into five currently known serotypes, PAd-1, -2, -3, -4, & -5 [Derbyshire J. B. et al. (1975) *J. Comp. Path.* 85:437-443; Hirahara T., et al. (1990) *Jpn. J. Vet. Sci.* 52:1089-1091]. Oronasal inoculation of pigs or colostrum-deprived

piglets with PAd-1, -2, or -3 did not result in clinical signs or gross lesions, [Derbyshire J. B. et al. (1975) *J. Comp. Path.* 85:437-443; Sharpe H. B. A. and Jesset D. M. (1967) *J. Comp. Pathol.* 77:45-50; Tuboly, T. et al. (1993) *Res. Vet. Sci.* 54:345-350] suggesting that these serotypes usually lead to subclinical infections. PAd3 was initially isolated from a fecal sample collected from a healthy pig [Clarke M. C. et al. (1967) *Arch. Ges. Virusforsch.* 21:91-97]. A cross-hybridization experiment using labeled human adenovirus type 2 (HAd2) DNA as a probe resulted in hybridization with PAd3 DNA fragments mainly in the region corresponding to structural proteins [Benko M. et al. (1990) *J. Gen. Virol.* 71:465-469]. The genome of PAd3 is approximately 35 Kb in size, restriction maps for a number of enzymes have been reported [Garwes D. J. and Xuan H. (1989) *Intervirology.* 30:234-236; Reddy P. S. et al. (1993) *Intervirology* 36:161-168] and the early region 3 (E3) has been sequenced [Reddy P. S. et al. (1995) *Virus Res.* 36:97-106].

Following adenovirus infection, the E1 genes are the first set of genes to be expressed. The E1 region includes two transcription units, E1A and E1B, each of which expresses multiple proteins as a result of differential splicing and use of different translation reading frames [Berk A.J. and Sharp P. A. (1978) *Cell.* 14:695-711]. E1 gene products of several HAds have been shown to transactivate a variety of viral and cellular promoters, and transform and immortalize cultured rodent cells [Berk A.J. (1986) *Ann. Rev. Genet.* 20:45-79; Whyte P. et al. (1988) *Nature.* 334:124-129; Whyte, P. et al. (1989) *Cell.* 56:67-75; Lillie J.W. and Green M. R. (1989) *Nature.* 338:39-44; Lundblad J. R. et al. (1995) *Nature.* 374:85-88; Arany Z. et al. (1995) *Nature.* 374:81-84].

Recent studies involving HAd E1 insertion vectors as recombinant vaccine delivery vehicles have demonstrated that they may be better than HAd E3 insertion vectors [Eloit M. and Adam M. (1995) *J. Gen. Virol.* 76:1583-1589; Fooks A. R. et al. (1995) *Virology* 210:456-465; Mittal S. K. et al. (1996) *Virology* 222:299-309; Xiang Z. Q. et al. (1996) *Virology* 219:220-

227]. Since E1 insertion vectors do not actively replicate in their natural hosts, they should be safer as virus excretion from vaccinated animals would not occur. These vectors may also be useful for vaccinating immunocompromised hosts since they could not spread *in vivo*. Titers of E1-deleted vectors in E1-complementing cells are similar to those of E3-deleted vectors, therefore, the cost of production of either vector system would be similar.

However, many of these adenovirus vectors stimulate an immune response that results in elimination of the cells carrying the vectors. Therefore, one of the drawbacks of many of the currently used adenovirus vectors as gene delivery vehicles is that the transgene is expressed for only a short period of time. This loss in expression can not be overcome with frequent rounds of vaccination, as the host-immune response often leads to neutralization of the virus before it enters target cells. A need therefore exists for adenovirus vectors that exhibit prolonged transgene expression. The present invention addresses this need.

SUMMARY OF THE INVENTION

Open reading frames in nucleotide sequences have been discovered in porcine adenovirus that are expected to encode proteins involved in controlling viral replication, especially in controlling the viral latent phase. Accordingly, the present invention provides these purified proteins.

In yet another aspect of the invention, isolated nucleic acid molecules that encode the proteins recited above are provided. The nucleic acid molecules may be incorporated into a vector to form a recombinant nucleic acid molecule. Moreover, such recombinant nucleic acid molecules may be introduced into a host cell.

Further provided are adenoviral vectors that include the nucleic acid molecules described herein. In one form of the invention, the vectors may include a foreign promoter, preferably at least one foreign promoter, that is operably linked to a terminal 5' end of the nucleic acid molecule. The vectors may further include a second nucleic acid molecule of interest, such as one having a nucleotide sequence encoding a desired protein. The vector advantageously may also include a second promoter that is operably linked to a terminal 5' end of the second desired nucleic acid molecule, and may further include an adenoviral origin of replication and an inverted terminal repeat sequence. In other forms of the invention, replication-defective porcine adenoviral vectors, especially type 3 vectors, are provided that include a porcine adenovirus genome, such as a type 3 genome, having a functional deletion in the E1 region. These vectors may also include a nucleotide sequence encoding a protein of interest inserted into the E1 region. The vector also preferably includes a foreign promoter operably linked to a terminal 5' end of the nucleotide sequence encoding the protein of interest, and may include one or more of the viral nucleotide sequences provided herein, preferably with at least one foreign promoter operably linked to a terminal 5' end of these novel nucleotide sequences.

In another aspect of the invention, methods of introducing a desired nucleic acid into a cell are provided. In one form of the invention, a method includes infecting a host cell with an adenoviral vector having an isolated first nucleic acid molecule encoding one or more, preferably at least one, of the novel proteins as described herein and a second desired nucleic acid molecule that may advantageously encode a protein of interest. Each of the nucleic acid molecules preferably independently include a foreign promoter operably linked to their terminal 5' end.

In a further aspect of the invention, a method of making an adenovirus vector is provided. In one form, the method includes introducing into a cell an isolated nucleic acid molecule encoding the proteins described herein and a foreign promoter, wherein the foreign promoter is operably linked to a terminal 5' end of the molecule.

In other aspects of the invention, methods of expressing a desired protein are provided. A method may include infecting a host cell with an adenoviral vector having a first isolated nucleic acid molecule encoding a protein, such as a viral protein, as described herein, and a second desired nucleic acid molecule encoding a desired protein, and culturing the host cells under conditions effective in achieving expression of the desired protein. The adenoviral vector may further include a foreign promoter, preferably at least one, that is operably linked to a terminal 5' end of the first isolated nucleic acid molecule and another foreign promoter so linked to the second desired nucleotide sequence. The desired protein may then be purified by conventional techniques.

In another aspect of the invention, methods of expressing viral proteins are provided. In one embodiment, a method includes introducing into a host cell an isolated first nucleotide sequence as described herein and a foreign promoter, preferably at least one, that is operably linked to a terminal 5' end of said first nucleotide sequence and culturing under conditions to achieve expression of said protein.

One object of the invention is to provide proteins and RNA involved in viral replication, especially those expected to function in viral latency, as well as nucleotide sequences encoding these gene products.

A further object of the invention is to provide a porcine adenoviral type 3 vector having a functional E1 deletion.

A further object of the invention is to provide an adenoviral vector that may be used to increase the duration of transgene expression.

Yet another object of the invention is to provide methods of utilizing the nucleotide and amino acid sequences described herein.

These and other objects and advantages of the present invention will be apparent from the descriptions herein.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1G depict the nucleotide sequence and major open reading frames (ORFs) of the PAd3 genome between map unit (m.u.) 0 and 13.7 determined as discussed in Example 1. The first 144 nucleotides (nt) represent an inverted terminal repeat sequence. The conserved region of replication (bold), the putative packaging signals (underlined), a putative nuclear factor III (NFIII) binding site (bold, italics and underlined), and putative SP1-binding sites (italics and underlined) are indicated. PAd3 E1A, E1B-202R, E1B-411R, pIX ORF, novel ORF 184R on the upper strand and an incomplete IVa2 ORF, and four unique ORFs (97R,162R,163R* and 288R) on the lower-strand are shown in bold. The probable TATA motifs and polyadenylation signals are indicated as bold and underlined, and bold and doubled underlined, respectively.

FIG. 2 depicts open reading frames (ORFs) in PAd3 E1 and pIX regions of the PAd genome. The left-end PAd3 genome between m.u. 0 and 13.7 was sequenced as described in Example 1 to provide a sequence of 4769 nt. Three frames of ORFs on the upper-strand are indicated as a, b, and c. Similarly, three frames of ORFs on the lower-strand are indicated as d, e, and f. The direction of ORFs are shown by an open arrow on the left. All ORFs of 50 or more amino acids following methionine are shown. ORFs that have demonstrated homology with known adenovirus proteins are indicated by hatched boxes and novel ORFs that have homology with EBNA1, EBNA2 and other transcriptional initiation factors are indicated by bricked boxes. The putative ORF 163R* did not have an initiation codon.

FIG. 3 depicts a comparison of the amino acid sequence of four important functional regions in the E1A proteins of PAd3, PAd4, HAd5, and BAd3 showing the areas of homology. Residues that are identical in all four E1A proteins are in bold. The residue numbers for each of the viruses are shown.

FIG. 4 depicts a comparison of amino acid sequences of the putative PAd3 E1B proteins as described in Example 1. A) homology between the BAd3 E1B-19k and the PAd3 E1B-202R; B & C) homologous regions of the PAd3 E1B-202R, BAd3 E1B-19k, and HAd5 E1B-19k; and D) homology comparison among C-terminal regions of HAd5 E1B-55k homologs of four adenoviruses are presented. Identical residues are shown in bold. The residue numbers for each of the viral proteins are shown.

FIG. 5 shows a homology comparison among N-terminal regions of HAd5 pIX homologs of three adenoviruses. Identical residues are shown in bold. The residue numbers for each of the viruses are shown.

FIG. 6 depicts a homology comparison among C-terminal regions of HAd5 IVa2 homologs of three adenoviruses. Identical residues are shown in bold. The residue numbers for each of the viruses are shown.

FIGS. 7A-7B show comparisons of the amino acid sequence of the PAd3 five novel ORFs. FIG. 7A, 1) region of homology between PAd3 184R and bovine Na+/Ca+,K+ exchanging protein (PIR S20969†); FIG. 7A, 2) region of homology between PAd3 184R and BICP4 (GI L14321†); FIG. 7A, 3) region of homology between PAd3 97R and EHV membrane glycoprotein (DBJ D88734†); FIG. 7A, 4) region of homology between PAd3 97R and EHV transcriptional control protein 57 (PIR S55652†); FIG. 7A, 5) region of homology between 162R and equine herpesvirus (EHV) membrane glycoprotein (DBJ D88734†); FIG. 7A, 6) region of homology between 162R and trans-acting transcriptional protein ICP0 of herpes simplex virus, (SP P28284†); FIG. 7B, 7) region of homology between PAd3 288R and EBNA1; FIG. 7B, 8) region of homology between PAd3 288R and the transcription factor GATA-4 of mice (SP Q083691†); FIG. 7B, 9) region of homology between PAd3 163R* and EBNA2 (Pir| |542447†);

and FIG. 7B, 10) region of homology between PAd3 163R* and the human transcription initiation factor II (TFII) 130k subunit (SP|O002681†) are presented. Identical residues are shown in bold. The residue numbers for each of the proteins are shown. †Protein sequence database number.

FIG. 8 depicts an SDS-polyacrylamide gel from the purification of PAd3 162R protein expressed in bacteria according to the protocol in Example 2. A plasmid containing PAd3 162R ORF under the control of an inducible bacteriophage T7 promoter was used to transform bacteria expressing T7 RNA polymerase. Bacterial suspension cultures at approximately 0.6 O.D. were treated with 1.5 mM of IPTG to induce the expression of the 162R protein as a his-tag fusion protein. Bacterial cultures were subjected to cellular disruption using a French press and insoluble inclusions containing the fusion protein were isolated and solubilized in a 6M gaunidinium HCl solution. The solution was passed through a 0.2 μm filter and the filtrate was applied onto a Ni^{++} affinity column. The his-tag fusion protein bound to Ni^{++} column was eluted following the manufacturer's instructions using a kit (Novagen, Inc.). All protein fractions were separated by SDS-PAGE and then stained with Coomassie blue. The PAd3 162R fusion protein is indicated by an arrow. The molecular weight (MW) markers are shown on the left.

FIG. 9 depicts a Western dot-blot analysis of IPTG-induced or uninduced bacterial cell extracts using HRP-conjugated S-tag. DE3 bacteria containing pET30-162R were grown in the presence or absence of IPTG. Total bacterial protein, soluble or insoluble (pellet) protein extracts were analyzed by Western blot using HRP-conjugated S-tag. The PAd3 162R fusion protein is indicated by an arrow. The molecular weight (MW) markers are shown on the left.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to preferred embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications of the invention, and such further applications of the principles of the invention as illustrated herein, being contemplated as would normally occur to one skilled in the art to which the invention relates.

Novel nucleotide sequences expected to encode proteins involved in controlling viral latency have been identified in porcine adenovirus. As the proteins, and/or RNA encoded by these nucleotide sequences, may advantageously maintain the adenovirus in a latent state, an adenoviral vector including such sequences, along with a transgene of interest, may not elicit an immune response after being introduced into a host cell. This may increase the duration of expression of the transgene in the cell. In yet other embodiments, other nucleotide sequences are provided that are expected to encode other proteins involved in viral replication, including proteins involved in stimulating DNA synthesis, transactivation of transcription, packing of the full-length genome into virions and shutting down of host cell protein synthesis. Recombinant nucleic acid molecules are also provided that include the nucleotide sequences encoding the proteins described herein. The nucleic acid molecules may be incorporated into a host cell. In another aspect of the invention, methods of expressing a desired protein are also provided, as well as methods of expressing the proteins recited herein.

In a first aspect of the invention, purified proteins are provided that are expected to function in adenoviral replication. In one preferred embodiment, the proteins are expected to function in viral latency as more fully discussed in example 1. Amino acid sequences of these proteins,

from a porcine adenovirus in one form of the invention, are set forth in SEQ ID NOS:1, 2, 3, 4, and 5 (97R, 162R, 163R*, 288R and 184R, respectively) and also shown in FIGS. 1A-1G.

In yet another embodiment, the proteins have amino acid sequences from a porcine adenovirus, as set forth in SEQ ID NOS:6, 7, 8, 9, and 10 (E1A, E1B-202, E1B-474, pIX and IVa2, respectively) and also shown in FIGS. 1A-1G. The polypeptides described herein are substantially pure (i.e., the novel proteins are essentially free, e.g., at least about 95% free, from other proteins with which they naturally occur).

Although the invention is described with reference to porcine adenovirus amino acid sequences, it is understood that the invention is not limited to the specific amino acid sequences set forth in SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. Skilled artisans will recognize that, through the process of mutation and/or evolution, polypeptides of different lengths and having differing constituents, e.g., with amino acid insertions, substitutions, deletions, and the like, may arise that are related to, or sufficiently similar to, a sequence set forth herein by virtue of amino acid sequence homology and advantageous functionality as described herein. The term "adenovirus-related protein" is used to refer generally to proteins having the features described herein and preferred examples include polypeptides having the amino acid sequences set forth in SEQ ID NOS:1, 2, 3, 4, and 5 and SEQ ID NOS:6, 7, 8, 9, and 10. Also included within this definition, and in the scope of the invention, are variants of the polypeptides which function as described herein, especially variants involved in controlling the latent state of adenovirus.

It is well known a wide variety of adenoviral serotypes commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and yet which effectively provide similar function. For example, an amino acid sequence isolated from another serotype may differ to a certain degree from the sequences set forth in SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, and yet have similar

functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although not being limited by theory, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly located and will have the desired activity. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have good activity, even where alterations exist in other portions thereof.

In this regard, variants of the proteins described herein are expected to be functionally similar to that set forth in SEQ ID NOS:1, 2, 3, 4, and 5 and SEQ ID NOS:6, 7, 8, 9, and 10, for example, if they include amino acids which are conserved among a variety of serotypes or if they include non-conserved amino acids which exist at a given location in another serotype that expresses proteins having the functions described herein.

Another manner in which similarity may exist between two amino acid sequences is where a given amino acid of one group (such as a non-polar amino acid, an uncharged polar amino acid, a charged polar acidic amino acid or a charged polar basic amino acid) is substituted with another amino acid from the same amino acid group. For example, it is known that the uncharged polar amino acid serine may commonly be substituted with the uncharged polar amino acid threonine in a polypeptide without substantially altering the functionality of the polypeptide. Whether a given substitution will affect the functionality of the enzyme may be determined without undue experimentation using synthetic techniques and screening assays known in the art.

The invention therefore also encompasses polypeptides having amino acid sequences similar to the amino acid sequences set forth in

SEQ ID NOS:1, 2, 3, 4 and 5 that have at least about 50%, 40%, 60%, 50% and 40% identity thereto, respectively, that preferably function as described herein. Preferably, the amino acid sequences have at least about 70%, 60%, 70%, 70%, and 60% identity to the sequences set forth in SEQ ID NOS:1, 2, 3, 4, and 5, respectively, more preferably at least about 80% identity to SEQ ID NOS:1, 2,3, 4 and 5 and most preferably at least about 90% identity to SEQ ID NOS:1, 2, 3, 4, and 5. In one specific form of the invention, the amino acid sequences include those set forth in SEQ ID NO:2 from amino acid 1 to amino acid 38, in SEQ ID NO:3 from amino acid 41 to amino acid 163, and in SEQ ID NO:4 from amino acid 106 to amino acid 146 having at least the percentage identity to these sequences described above.

Similarly, with respect to the amino acid sequences set forth in SEQ ID NOS:6, 7, 8, 9, and 10, the invention also encompasses amino acid sequences similar to these amino acid sequences having at least about 50%, 40%, 40%, 30% and 90% identity thereto, respectively, that preferably function in viral replication. Preferably, the amino acid sequences have at least about 60%, 60%, 60%, and 50%, identity to the sequences set forth in SEQ ID NOS:6, 7, 8 and 9, respectively, and further preferably at least about 80%, 80%, 80% and 70% identity to the sequences set forth in SEQ ID NOS:6, 7, 8 and 9, respectively. In most preferred embodiments, the amino acid sequences encompassed by the invention include amino acid sequences having at least about 90% identity to the sequences set forth in SEQ ID NOS:6, 7, 8 and 9.

In another specific form of the invention, the amino acid sequences include those set forth in SEQ ID NO:8 from amino acid 1 to amino acid 367, and in SEQ ID NO:9 from amino acid 1 to amino acid 135, and sequences having at least the percentage identity to these sequences described above.

Percent identity between two nucleotide sequences or two amino acid sequences may be determined, for example, by comparing sequence

information over the entire sequence using the Gap program, version 9, available from Genetics Computer Group (GCG) (Madison, Wisconsin). Default parameters are preferably used, with the exception that end gaps are penalized using the end weight option of the program.

In another aspect of the invention, isolated nucleic acid molecules, originally isolated from porcine adenovirus, are provided that encode the proteins having the amino acid sequences described herein, including those amino acid sequences having the aforementioned percent identities to the respective amino acid sequences. In one embodiment, the nucleotide sequences encode proteins that are expected to control viral latency and are set forth in SEQ ID NOS:1, 2, 3, 4, and 5, as well as in FIGS. 1A-1G, or similar nucleotide sequences discussed herein. In other embodiments, the nucleotide sequences encode other proteins involved in viral replication, and are set forth in SEQ ID NOS:6, 7, 8, 9, and 10 and FIGS. 1A-1G or similar nucleotide sequences discussed herein.

In one form of the invention, nucleotide sequences are provided that encode a protein that includes an amino acid sequence having at least about 50%, 40%, 60%, 50%, and 40% identity to the amino acid sequence set forth in SEQ ID NOS:1, 2, 3 , 4 and 5, respectively. Preferably, amino acid sequences have at least about 70%, 60%, 70%, 70%, and 60% identity to the sequences set forth in SEQ ID NOS:1, 2, 3, 4, and 5, respectively, more preferably at least about 80% identity to SEQ ID NOS:1, 2, 3, 4 and 5 and most preferably at least about 90% identity to SEQ ID NOS:1, 2, 3, 4, and 5. In one specific form of the invention, the nucleotide sequence encodes a protein having an amino acid sequence set forth in SEQ ID NO:2 from amino acid 1 to amino acid 38, in SEQ ID NO:3 from amino acid 41 to amino acid 163, and in SEQ ID NO:4 from amino acid 106 to amino acid 146 having at least the percentage identity to these sequences described above for SEQ ID NOS:2, 3, and 4.

Similarly, the invention also encompasses nucleotide sequences that encode proteins that include amino acid sequences having at least

about 50%, 40%, 40%, 30% and 90% identity to the amino acid sequences set forth in SEQ ID NOS:6, 7, 8, 9, and 10, respectively, that preferably function in viral replication. Preferably, the nucleotide sequences encode a protein having an amino acid sequence that has at least about 60%, 60%, 60%, and 50%, identity to the amino acid sequences set forth in SEQ ID NOS:6, 7, 8 and 9, respectively, and further preferably at least about 80%, 80%, 80% and 70% identity to the amino acid sequences set forth in SEQ ID NOS:6, 7, 8 and 9, respectively. In most preferred embodiments, the nucleotide sequences encode a protein having an amino acid sequence having at least about 90% identity to the amino acid sequences set forth in SEQ ID NOS:6, 7, 8 and 9. In one specific form of the invention, the nucleotide sequence encodes a protein having an amino acid sequence set forth in SEQ ID NO:8 from amino acid 1 to amino acid 367, and in SEQ ID NO:9 from amino acid 1 to amino acid 135, and sequences having at least the percent identity to these sequences described above for SEQ ID NOS:8 and 9.

The nucleotide sequences provided herein are also preferably transcribed to form an RNA nucleotide sequence, such as an mRNA transcript. Therefore, the RNA nucleotide sequences corresponding to the DNA sequences recited herein in SEQ ID NOS: 1, 2, 3, 4 and 5, as well as in SEQ ID NOS:6, 7, 8, 9, and 10, wherein deoxythymidine ("T") is replaced by uridine ("U"), are also provided herein. RNA nucleotide sequences substantially similar to the transcripts are also encompassed in the invention, as well as RNA transcripts having the percent identity to these sequences as described herein for the DNA sequences.

The term "isolated nucleic acid," as used herein, is intended to refer to nucleic acid which is not in its native environment. For example, the nucleic acid is separated from other contaminants that naturally accompany it, such as proteins, lipids and other nucleic acid sequences. The term includes nucleic acid which has been removed or purified from its naturally-

occurring environment or clone library, and further includes recombinant or cloned nucleic acid isolates and chemically synthesized nucleic acid.

The term "nucleotide sequence," as used herein, is intended to refer to a natural or synthetic linear and sequential array of nucleotides and/or nucleosides, including deoxyribonucleic acid and ribonucleic acid, and derivatives thereof. The terms "encoding" and "coding" refer to the process by which a nucleotide sequence, through the mechanisms of transcription and translation, provides the information to a cell from which a series of amino acids can be assembled into a specific amino acid sequence to produce a functional polypeptide, such as, for example, an active enzyme or other protein that has a specific function. The process of encoding a specific amino acid sequence may involve DNA sequences having one or more base changes (i.e., insertions, deletions, substitutions) that do not cause a change in the encoded amino acid, or which involve base changes which may alter one or more amino acids, but do not eliminate the functional properties of the polypeptide encoded by the DNA sequence.

It is therefore understood that the invention encompasses more than the specific exemplary nucleotide sequences provided herein. For example, nucleic acid sequences encoding variant amino acid sequences, as discussed above, are within the scope of the invention. Modifications to a sequence, such as deletions, insertions, or substitutions in the sequence, which produce "silent" changes that do not substantially affect the functional properties of the resulting polypeptide molecule are expressly contemplated by the present invention. For example, it is understood that alterations in a nucleotide sequence which reflect the degeneracy of the genetic code, or which result in the production of a chemically equivalent amino acid at a given site, are contemplated. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for

another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, may also be expected to produce a biologically equivalent product.

Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the encoded polypeptide molecule would also not generally be expected to alter the activity of the polypeptide. In some cases, it may in fact be desirable to make mutations in the sequence in order to study the effect of alteration on the biological activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art.

In a preferred embodiment, the nucleotide sequences have substantial similarity to the sequences set forth in SEQ ID NOS:1, 2, 3, 4, and 5, and, in other preferred embodiments, to SEQ ID NOS:6, 7, 8, 9, and 10, and variants described herein. The term "substantial similarity" is used herein with respect to a nucleotide sequence to designate that the nucleotide sequence is sufficiently similar to a reference nucleotide sequence that it will hybridize therewith under moderately stringent conditions. This method of determining similarity is well known in the art to which the invention pertains. Briefly, moderately stringent conditions are defined in Sambrook et al., *Molecular Cloning: A Laboratory Manua*l, 2nd ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989) as including the use of a prewashing solution of 5X SSC (a sodium chloride/sodium citrate solution), 0.5% sodium dodecyl sulfate (SDS), 1.0 mM ethylene diaminetetraacetic acid (EDTA) (pH 8.0) and hybridization and washing conditions of 55°C, 5x SSC. Thus, encompassed as part of the invention are nucleic acid sequences which are complementary to the sequences specifically shown in the sequence listing. A further requirement of the preferred polynucleotide is that it must encode a polypeptide having similar functionality to the proteins described herein.

In a preferred form of the invention, the nucleotide sequences are deoxyribonucleic acid (DNA) sequences. In other forms of the invention,

the nucleotide sequences are RNA sequences, preferably an RNA transcript, such as an mRNA transcript, produced from transcribing the nucleotide sequences provided herein. Such RNA sequences, including those transcribed from SEQ ID NOS:1, 2, 3, 4, and 5, may also be involved in controlling viral latency.

In yet another embodiment, nucleotide sequences having selected percent identities to the nucleotide sequence set forth in SEQ ID NO:1, 2, 3, 4, and 5 are provided. In one preferred form, nucleotide sequences are provided that have at least about 50% identity, preferably at least about 70% identity, more preferably at least about 80% identity, and most preferably at least about 90% identity to the nucleotide sequences set forth in SEQ ID NOS:1, 2, 3, 4, and 5. The nucleotide sequences may further have the percent identities mentioned herein to a nucleotide sequence of substantial length within the porcine adenoviral genome. For example, such length may be less than about 100, 200, 500, 600 or 900 nucleotides in SEQ ID NOS:1, 2, 3, 4, and 5, or may be the length of the entire sequence in these SEQ ID NOS. A further requirement is that the nucleotide sequences described in SEQ ID NOS:1, 2, 3, 4, and 5 encode proteins or RNA, preferably of adenoviral origin, that function as described herein.

In alternative embodiments, nucleotide sequences are provided that have at least about 50% identity, preferably at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to the nucleotide sequences set forth in SEQ ID NOS:6, 7, 8, 9, and 10. The nucleotide sequences may further have the percent identities mentioned herein to a nucleotide sequence of substantial length within the porcine adenoviral genome. For example, such length may be less than about 600, 700 and 1500 nucleotides, for SEQ ID NOS:6, 7, 8, 9 and 10, or may be the length of the entire sequence in these SEQ ID NOS. Furthermore, the nucleotides sequences provided in SEQ ID NOS:6, 7, 8,

9, and 10 must encode proteins or RNA, preferably of adenoviral origin, that function as described herein.

The percent identity may be determined, for example, by comparing sequence information using the Gap program described above.

In one specific form of the invention, the nucleotide sequences include those having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:2 from nucleotide 1 to nucleotide 114, those set forth in SEQ ID NO:3 from nucleotide 121 to nucleotide 489 and those set forth in SEQ ID NO:4 from nucleotide 316 to nucleotide 438. Nucleotide sequences having the selected percent identities to these sequences, as discussed for SEQ ID NOS:2, 3 and 4 above, are also provided, as are nucleotide sequences which are substantially similar to these nucleotide sequences.

In another specific form of the invention, nucleotide sequences include those having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:8 from nucleotide 1 to nucleotide 1101 and those set forth in SEQ ID NO:9 from nucleotide 1 to nucleotide 405 .

It is not intended that the present invention be limited to these exemplary nucleotide sequences, but include sequences having substantial similarity thereto and sequences which encode variant forms of the proteins as discussed above and as further discussed below.

A suitable DNA sequence may be obtained by cloning techniques using cDNA libraries. Such cDNA libraries are available commercially or may be constructed using standard methods known in the art. Suitable nucleotide sequences may be isolated from DNA libraries obtained from a wide variety of adenoviral serotypes by means of nucleic acid hybridization or polymerase chain reaction (PCR) procedures, using as probes or primers nucleotide sequences selected in accordance with the invention, such as those set forth in SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, nucleotide sequences having substantial similarity thereto, or portions thereof as described herein.

Alternately, a suitable sequence may be made by techniques which are well known in the art. For example, nucleic acid sequences encoding the proteins described herein, or acting as templates for the RNA transcripts, may be constructed by recombinant DNA technology, for example, by cutting or splicing nucleic acids using restriction enzymes and DNA ligase. Furthermore, nucleic acid sequences may be constructed using chemical synthesis, such as solid-phase phosphoramidate technology. PCR may be used to increase the quantity of nucleic acid produced. Moreover, if the particular nucleic acid sequence is of a length which makes chemical synthesis of the entire length impractical, the sequence may be broken up into smaller segments which may be synthesized and ligated together to form the entire desired sequence by methods known in the art. RNA sequences may also be produced from the DNA templates by methods known to the skilled artisan.

In a further aspect of the invention, recombinant nucleic acid molecules are provided. In certain embodiments, the nucleic acid molecules include nucleotide sequences encoding the proteins described herein. The nucleotide sequences have substantial similarity or identity, both as defined above, to the nucleotide sequences set forth in either SEQ ID NOS:1, 2, 3, 4, and 5 or SEQ ID NOS:6, 7, 8, 9, and 10, or the selected sequences within these sequences as described above, or combinations thereof. The proteins produced have the amino acid sequences set forth in SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or variants thereof as described above.

Recombinant nucleic acid molecules may be constructed by incorporating the desired nucleotide sequence within a vector according to methods well known to the skilled artisan and as described for example, in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Laboratory, Cold Springs Harbor, New York (1982) and *Current Protocols in Molecular Biology*, John Wiley and Sons, edited by Ausubel et al. (1988). A wide variety of vectors are known that may be used in the invention. For

example, various plasmid and phage vectors are known that are ideally suited for use in the invention. For example, pET30 and λGT11 may be used in the invention. In one embodiment, the desired recombinant vector may be constructed by ligating DNA linker sequences to the 5' and 3' ends of the desired nucleotide insert, cleaving the insert with a restriction enzyme that specifically recognizes sequences present in the linker sequences and the desired vector, cleaving the vector with the same restriction enzyme, mixing the cleaved vector with the cleaved insert and using DNA ligase to incorporate the insert into the vector as known in the art.

Preferred vectors are plasmid vectors. In a further preferred form of the invention, the plasmid vectors include at least one of the nucleotide sequences set forth in SEQ ID NOS:1, 2, 3, 4, and 5, and preferably an adenoviral origin of replication. In a more preferred embodiment, a plasmid vector includes at least one nucleotide sequence selected from the nucleotide sequences set forth in SEQ ID NOS:3 and 4. Such vectors may advantageously be used to introduce the nucleotide sequences into a cell for production of adenoviral vectors as described below.

The vectors may include other nucleotide sequences, such as those encoding selectable markers, including those for antibiotic resistance or color selection. The vectors also preferably include a transcriptional regulatory nucleotide sequence, such as a promoter. The desired nucleic acid insert is preferably operably linked to the promoter. A nucleic acid is "operably linked" to a another nucleic acid sequence, such as a promoter sequence, when it is placed in a specific functional relationship with the other nucleic acid sequence. The functional relationship between a promoter and a desired nucleic acid insert typically involves the nucleic acid and the promoter sequences being contiguous such that transcription of the nucleic acid sequence will be facilitated. Two nucleic acid sequences are further said to be operably linked if the nature of the linkage between the two sequences does not (1) result in the introduction of a frame-shift-

mutation; (2) interfere with the ability of the promoter region sequence to direct the transcription of the desired nucleotide sequence, or (3) interfere with the ability of the desired nucleotide sequence to be transcribed by the promoter sequence region. Typically, the promoter element is generally upstream (i.e., at the 5' end) of the nucleic acid insert coding sequence.

A wide variety of promoters are known in the art, including cell-specific promoters, inducible promoters, and constitutive promoters. The promoters may further be selected such that they require activation by activating elements known in the art, so that production of the gene products, including the protein encoded by the nucleic acid sequence insert as well as the RNA transcript, may be regulated as desired. In further preferred embodiments, the promoter is a foreign promoter. A "foreign promoter" is defined herein as a promoter other than the natural, or native, promoter involved in transcribing a particular region of DNA.

The vectors may further include other regulatory elements, such as enhancer sequences, which cooperate with the promoter to achieve transcription of the nucleic acid insert coding sequence. By "enhancer" is meant nucleotide sequence elements which can stimulate promoter activity in a cell, such as a bacterial or eukaryotic host cell.

Moreover, the vectors may include another nucleotide sequence insert that encodes a protein that may aid in purification of the desired protein encoded by the desired nucleotide sequence. The additional nucleotide sequence is positioned in the vector such that a fusion, or chimeric, protein is obtained. For example, a protein described herein may be produced having at its C-terminal end several histidine molecules joined to the protein. Therefore, the additional nucleotide sequence may include, for example, the nucleotide sequence encoding multiple histidines. The protein may be isolated on a nickel chromatography column, which will bind the histidine in the protein being purified. After purification procedures known to the skilled artisan, the additional amino acid sequence may be cleaved with an appropriate enzyme, such as a protease. The desired

protein may then be isolated from the other proteins, or fragments thereof, by methods known in the art.

The preferred recombinant vectors may be used to transform, or introduce nucleic acid into, a host cell, and thus may include a nucleic acid having a nucleotide sequence, for example, that encodes a protein of interest. Such methods include, for example, those described in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Laboratory, Cold Springs Harbor, New York (1982), as well as *Current Protocols in Molecular Biology*, John Wiley and Sons, edited by Ausubel et al. (1988). Once the desired nucleic acid has been introduced into the host cell, the host cell may produce the desired protein, RNA, or variants thereof as described above. Accordingly, in yet another aspect of the invention, a host cell is provided that includes the preferred recombinant vectors described above. In a preferred embodiment, the recombinant vectors are introduced into a host cell to advantageously form an adenoviral vector as described below. The adenoviral vector may then be used to introduce a desired nucleotide sequence into a cell.

Although a wide variety of host cells may be used in the invention, including prokaryotic and eukaryotic host cells, eukaryotic host cells are preferred. Further preferred are animal host cells, including swine, bovine, murine and avian host cells. Human host cells are most preferred. The host cells may include cell lines that transiently or stably produce the adenoviral vectors herein described.

A further aspect of the invention provides recombinant adenoviral vectors. Such adenoviral vectors are preferred vectors for introducing into a host cell a nucleotide sequence encoding a protein of interest. In one form of the invention, an adenoviral vector may include the nucleotide sequences described herein, especially the nucleotide sequences set forth in SEQ ID NOS:1, 2, 3, 4, and 5, that is preferably replication-defective so that it will not reproduce when introduced into a host cell. For example, the adenoviral vector may include at least one of the nucleotide sequences

described herein, such as those set forth in SEQ ID NOS:1, 2, 3, 4, and 5, preferably a nucleotide sequence encoding a desired protein product and typically a promoter, preferably at least one, as described above.

In another form of the invention, a recombinant adenoviral vector, preferably a porcine adenovirus type 3 vector, is provided that includes an adenoviral genome having a functional deletion in its E1 region. By "functional deletion" is meant a deletion that results in a replication-defective adenovirus. Thus, various portions of the E1 region may be deleted, including, for example, portions of the E1A and E1B genes. In certain forms of the invention, the entire E1 region may be deleted. In preferred embodiments of the invention, the deleted portions of the E1 region, or other applicable region of the genome, are advantageously replaced with a nucleotide sequence of interest. The nucleotide sequence preferably includes a gene that encodes a protein of interest. A promoter sequence may also be operably linked to a terminal 5' end of the nucleotide sequence encoding a protein of interest, as discussed above. The vector having the functional E1 deletion in its genome may further include at least one of the introduced nucleotide sequences described herein, especially those set forth in SEQ ID NOS:1, 2, 3, 4, and 5, and preferably includes at least one promoter sequence operably linked to a terminal 5' end of these nucleotide sequences.

Further provided herein are methods for making such adenoviral vectors. General methods for making adenoviral vectors that include selected deletions of, and additions to, its genome are well known in the art, including direct cloning and homologous recombination methods. As non-limiting examples, methods for producing such vectors may be found in the following references: Yeh, P. and Perricaudet, M. (1997) *FASEB J.* 11:615-623; Wang, Q. et al. (1997) *Gene Ther.* 4:393-400; Gilgenkrantz, H. et al. (1995) *Hum. Gene Ther.* 6:1265-1274; and U.S. Patent Nos. 5,518,913; 5,585,362 and 5,698,202. In one form of the invention, a method typically includes introducing into a cell a transcriptional regulatory

nucleotide sequence and an isolated nucleic acid molecule as described herein, such as SEQ ID NOS:1, 2, 3, 4, and 5 and SEQ ID NOS:6, 7, 8, 9, and 10. In other forms of the invention, a method typically involves making a functional deletion in the E1 region of the adenoviral genome and may also include replacing the deleted region of the genome with a nucleotide sequence of interest as described above.

In yet another aspect of the invention, methods of introducing a desired nucleic acid molecule into a cell is provided. In one embodiment, a method includes introducing into a host cell a nucleic acid molecule encoding a desired protein and an isolated nucleic acid molecule having a nucleotide sequence having the percent identity or similarity as described herein to the nucleotide sequence selected from SEQ ID NOS:1, 2, 3, 4, and 5 and SEQ ID NOS:6, 7, 8, 9, and 10, and combinations thereof. Each of the nucleic acid molecules independently preferably include a foreign promoter, further preferably at least one foreign promoter, operably linked to their terminal 5' end. The nucleotide sequences are preferably carried by an adenoviral vector as described above. In preferred embodiments, the desired nucleic acid molecule is introduced by infecting the cell with an adenoviral vector as described herein. The adenoviral vector may be constructed as described herein. For example, the adenoviral vector may include an adenoviral genome with a functional deletion in its E1 region which is replaced by the nucleotide sequence of interest, and may also include one or more of the viral nucleotide sequences described herein. Alternatively, the vector may include one or more of the nucleotide sequences described herein, especially those selected from SEQ ID NOS:1, 2, 3, 4, and 5, an inverted terminal repeat (ITR) sequence, an adenoviral origin of replication and the nucleotide sequence, such as a transgene, of interest. After introducing the nucleic acid into the host cell, the cells may be cultured under conditions effective to achieve production of the RNA transcript, and/or expression of the desired protein, depending on whether RNA or protein is desired.

In other aspects of the invention, methods of expressing a desired protein are provided that include introducing into a host cell an isolated nucleic acid molecule that includes a first nucleotide sequence described herein, especially those set forth in SEQ ID NOS:1, 2, 3, 4, or 5, or combinations thereof, and further preferably includes at least one promoter sequence, such as a foreign promoter sequence, operably linked to a terminal 5' end of the isolated nucleotide sequence. A second nucleotide sequence encoding a desired protein is also introduced into the host cell. The protein may typically be one that provides some benefit to the host cell, including replacing the function of a non-functional protein, or one of decreased function, in the host cell. The nucleotide sequence encoding the desired protein preferably includes a promoter sequence operably linked to a terminal 5' end of the nucleotide sequence. The nucleotide sequences are preferably introduced into the host cell by first incorporating the sequences into an adenoviral vector. The desired cells may be infected with the adenoviral vector according to known procedures in the art so that the desired nucleotide sequence may be introduced into the cell. The host cells are then cultured under conditions, well known to the skilled artisan, effective to achieve expression of the desired polypeptide. The desired polypeptide may then be purified using conventional techniques. In a further preferred form of the invention, the transgene may be expressed by utilizing a promoter as described herein while the virus is in a latent state, and, when it is desired that the virus be cleared from the system, the virus is then returned to a non-latent state by regulating production of the RNA and/or proteins described herein. This provides a method of sequential expression of the transgene according to the need.

In a further aspect of the invention, methods of expressing viral proteins are provided. In one form, a method includes introducing into a host cell an isolated nucleotide sequence as described herein, preferably having at least one foreign promoter operably linked to its terminal 5' end.

The cells may then be cultured under conditions to achieve expression of the protein.

Reference will now be made to specific examples illustrating the invention described above. It is to be understood that the examples are provided to illustrate preferred embodiments and that no limitation to the scope of the invention is intended thereby.

EXAMPLE 1

SEQUENCE ANALYSIS OF PORCINE ADENOVIRUS

Cell Culture and Virus

The swine testicle (ST) cell line obtained from American Type Culture Collection (ATCC), was grown as monolayer cultures using Eagles minimum essential medium (MEM) [Life Technologies Inc.] supplemented with 10% fetalClone III (HyClone Laboratories, Inc.) and 50 µg/ml gentamicin. PAd3, kindly supplied by Dr. W. Mengeling, USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA, was grown in ST cells and purified by cesium chloride density-gradient centrifugation [Graham F. L. and Prevec L. (1991) in Murray E. J. (ed): *Methods in Molecular Biology: Gene Transfer and Expression Protocols,* Clifton, Humana Press, Vol. 7, pp 109-128].

Plasmid Construction

DNA was extracted from purified virions following a technique described previously [Graham F. L. and Prevec L. (1991) in Murray E. J. (ed): *Methods in Molecular Biology: Gene Transfer and Expression Protocols,* Clifton, Humana Press, Vol. 7, pp 109-128]. The terminal protein attached to PAd3 DNA was removed by sodium hydroxide treatment. PAd3 DNA was digested with *BamHI*, separated on an agarose gel by electrophoresis and a 4.8 kb fragment containing the left-end of PAd3 genome between m.u. 0 and 13.7 was purified and inserted at the *HincII-BamHI* site of pUC18 to obtain pPAd-E1. A large scale purification

of pPAd-E1 was performed by alkaline lysis and cesium chloride purification [Sambrook J. et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press].

DNA Sequencing and Sequence Analysis

Initially, pPAd-E1 was sequenced using universal and reverse primers and then the entire sequence on both strands was obtained by primer walking following the dideoxy nucleotide chain termination technique [Sanger F. et al (1977) *Proc. Natl. Acad. Sci. USA* 74:5463-5467] using an Applied Biosystems 373A automated sequencer. All sequence analyses were done using the GCG sequence analysis program.

Results

A sequence of 4769 nucleotides (nt) of PAd3 genome between m.u. 0 and 13.7 was obtained. This sequence was compared with the similar sequences reported for HAd5 [Chroboczek J. et al. (1992) *Virology* 186:280-285], bovine adenovirus type 3 (BAd3) [Zheng B. et al (1994) *Virus Res.* 31:163-186; Elgadi M. et al. (1993) *Intervirology* 36:113-120; Reddy P. S. et al (1998) *J. Virol.* 72:1394-1402] and PAd4 [Kleiboeker S. B. (1995) *Virus Res.* 31:17-25]. On the basis of protein homology, ORFs homologous to HAd5 E1A, E1B-19k, E1B-55k, pIX, pIVa2 and five novel ORFs (184R, 97R, 162R, 163R* and 288R) were identified in the PAd3 genome (Fig. 1A, 1B and 2). Our PAd3 sequence yielded approximately 41% and 99% homology at the nucleotide level with the E1 regions of PAd4 [Kleiboeker S. B. (1995) *Virus Res.* 31:17-25] and recently published sequence of PAd3 [Reddy P. S. et al. (1998) *Virus Res.* 58:97-106], respectively.

Left-end Untranslated Region

The inverted terminal repeat (ITR) sequence from nt 1-144 was identical to the published sequence of PAd3 ITR [Reddy P. S. (1995)

Virology 212:237-239]. The PAd3 ITR also contains a 10-nt (8-17 nt) sequence that may be important for virus DNA replication [Shinagawa M. et al (1987) *Gene* 55:85-93]. It has been demonstrated that there are seven A-repeat packaging domains located within nt 194-380 of the Had5 genome which are involved in viral DNA packaging [Grable M. and Hearing P. (1990) *J. Virol.* 64:2047-2056; Schmid S. I. and Hearing P. (1997) *J. Virol.* 71:3375-3384]. PAd3 at least has two TTTTG domains that are usually a part of A-repeat packaging domains and are located at nt 37-41 and 265-269. In the PAd3 ITR, the consensus nuclear factor III (NFIII)-binding site [Hatfield L. and Hearing P. (1993) *J. Virol.* 67:3931-3939] is located at nt 130-140. Four GC-rich regions similar to the SP-I binding site were present at nt 68-75, 77-82, 198-204, and 213-219.

E1A Region

Within the 288R E1A protein of HAd, binding sites for a number of cellular proteins including p300, retinoblastoma protein (Rb), cyclin A, an associated protein kinase $p33^{cdk2}$ and other unassigned proteins have been defined [Whyte P. et al. (1988) *Nature* 334:124-129; Whyte P. et al. (1989) *Cell* 56:67-75; Lundblad J. R. et al. (1995) *Nature* 374:85-88; Arany Z. et al. (1995) *Nature* 374:81-84]. The predicted PAd3 E1A ORF contains 170 amino acid residues (R) with an initiation codon at nt 533 followed by a stop codon at nt 1046 (Fig. 1A and 2). This ORF showed approximately 27%, 26%, and 41% amino acid identity and approximately 34%, 35%, and 45% amino acid similarity with E1A of BAd3, HAd5 and PAd4, respectively. A probable intron of 100 bp with its splice donor and splice acceptor sites at nt 1044, and 1145, respectively may result in continuation of E1A ORF until a stop codon appears at 1221. The resultant putative PAd3 E1A is expected to be of 195R. The putative E1A 'TATA' box and the polyadenylation signal are located at nt 449 and 1287, respectively. The conserved region 3 (CR3) of the 288R E1A of HAd5 includes the activation region, the metal binding region, and the promoter binding region [Lillie J. W. and Green M. R. (1989)

Nature 338:39-44]. The predicted activation, metal binding, and the promoter binding regions of PAd3 E1A showed homology with similar E1A sequences of HAd5, BAd3, and PAd4 (Fig. 3A-C). There was a complete conservation of the metal binding (**C**-X_2-**C**-X_{13}-**C**-X_2-**C**) motif of PAd3 E1A. The putative PAd3 E1A region between amino acids 112 and 126 had sufficient homology with the Rb binding region [Dyson N. et al. (1990) *J. Virol.* 64:1353-1356] of E1A of HAd5 and BAd3 (Fig. 3D). However, the putative PAd4 E1A [Kleiboeker S. B. (1995) *Virus Res.* 31:17-25] did not seem to contain either the promoter binding domain or the Rb binding domain.

E1B Region

The E1B transcription unit of HAd5 consists of two major messages to produce 19k and 55k proteins. Both of these proteins independently inhibit p53-mediated apoptosis by different mechanisms [Wold W. S. M. et al. (1994) *Trends Microbiol.* 2:437-443]. Coexpression of both E1A and E1B (19k and or 55k) is required for high frequency transformation of cultured rodent cells [Wold W. S. M. et al. (1994) *Trends Microbiol.* 2:437-443]. The putative first PAd3 E1B ORF begins at nt 1462 to the termination codon at nt 2068. This ORF defines a polypeptide of 202R that had approximately 23%, and 26% amino acid identity and approximately 31%, and 39% amino acid similarity with BAd3 E1B-19k (Fig. 4A) and HAd5 E1B-19k, respectively. The two other regions of HAd5 E1B-19k that are conserved in PAd3 E1B-202R are also shown (Fig. 4B & C). It was surprising to note that the published PAd4 E1 region sequence [Kleiboeker S. B. (1995) *Virus Res.* 31:17-25] did not contain an ORF similar to PAd3 E1B-202R.

The putative second PAd3 E1B ORF starts at nt 1830 and terminates at nt 3255. This ORF defines a polypeptide of 475R that had approximately 32%, 28% and 30% amino acid identity and approximately 43%, 37%, and 36% amino acid similarity with E1B-55k homologs of BAd3, HAd5 and PAd4, respectively (Fig. 4D). The putative E1B TATA motif and the polyadenylation signal are located at nt 1330 and 3340, respectively.

pIX ORF

The HAd5 pIX protein is a minor structural protein that is essential for the packaging of full-length genomes [Ghosh-Choudhury G. et al. (1987) *EMBO J* 6:1733-1739]. An ORF starting at nt 3395 and terminating at nt 3992 represents a protein of 198R. This putative PAd3 pIX showed 22%, and 23% amino acid identity and 30%, and 30% amino acid similarity with pIX of BAd3 and HAd5, respectively. The N-terminal portion of pIX of both HAd5 and BAd3 showed extensive homology with the predicted PAd3 pIX (Fig. 5). The putative PAd3 pIX TATA motif and the polyadenylation signal are present at nt 3339 and 4085, respectively. The sequence information for the putative PAd4 pIX ORF is not available.

IVa2 and Novel ORFs

The HAd IVa2 message transcribed from the lower-strand, is involved in adenovirus replication [Swaminathan S. and Thimmapaya B. (1995) in Doerfler W. and Bohm P. (ed) The Molecular Repertoire of Adenoviruses III: Biology and Pathogenesis, Curr Topics Microbiol. Immunol., Berlin, Spring-Verlag, Vol 1999/III, pp 177-194]. The lower-strand of PAd3 genome between m.u. 13.7 and 11.7 contains an incomplete ORF representing an approximately 50% C-terminal portion of the predicted PAd3 IVa2 protein (SEQ ID NO:10 and FIG. 2). This ORF demonstrated approximately 77%, and 72% identity with IVa2 proteins of HAd5 and BAd3, respectively (FIG. 6). The putative PAd3 IVa2 polyadenylation signal is located at nt 4075. The sequence information for the putative PAd4 IVa2 ORF is not available. The E1A, E1B-202R, E1B-475R showed 100% protein identity whereas pIX and IVa2 showed approximately 92 and 99% protein identity respectively with the recently published sequence of PAd3 [Reddy P. S. et al. (1998) *Virus Res.* 58:97-106].

Following the IVa2 ORF, there are four unique ORFs of 97R, 162R , 163R* and 288R. Following the pIX ORF, there is a novel ORF of a 184R

putative protein that exhibits 31% identity with bovine Na+/Ca+,K+-exchanging protein (FIG. 7A,1) [Reilaender H. et al. (1992) *EMBO J* 11:1689-1695] and 34% identity with bovine herpesvirus type 1 immediate early transcriptional control protein (BIC4) (Fig. 7A, 2) [Schwyzer M. et al. (1993) *Virology* 197:349-357]. The 97R predicted protein has 33% identity with equine herpesvirus (EHV) membrane glycoprotein (Fig. 7A, 3.) [Kirisawa R. et al. (1996) *J. Equine Sci.* 7:79-87] and 43% identity with EHV transcriptional control protein 57 (FIG. 7A, 4.) [Telford E. A. R. et al. (1995) *J. Mol. Biol.* 249:520-528]. The 162R putative protein has shows 28% identity with (EHV) membrane glycoprotein (Fig. 7A, 5.) [Kirisawa R. et al. (1996) *J. Equine Sci.* 7:79-87] and 32% identity trans-acting transcriptional protein ICP0 of herpes simplex virus (Fig. 7A, 6.) [Mcgeoch D. J. et al. (1991) *J. Gen. Virol.* 72:3057-3075]. The C-terminal of putative 288R protein demonstrated 44% identity with herpesvirus papio Epstein- Barr virus nuclear antigen 1 (EBNA1) (Fig. 7B, 7.) [Yates J. L. et al. (1996) *Virology* 222:1-13] and 36% identity with the GATA-4 transcription factor of mice (Fig. 7B, 8.) [Arceci R. J. et al. (1993) *Mol. Cell Biol.* 13:2235-46]. Herpesvirus papio infects baboons and is similar to Epstein–Barr virus (EBV) of human [Heller M. and Kieff E. (1981) *J. Virol.* 37:821-826]. EBNA1 is involved in maintaining the EBV genome during a latent phase by providing a stable and regulated replication [Yates J. L. et al. (1984) *Proc. Natl. Acad .Sci. USA* 81:3806-3810; Yates J. L. et al. (1985) *Nature* 313:812-815]. GATA-4 is a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally-derived tissues and heart and is involved in gene expression in these tissues [Arceci R. J. et al. (1993) *Mol. Cell. Biol.* 13:2235-46]. On the lower strand, there is a unique ORF of 163R* without a start codon. The 163R* predicted protein contains a proline- and alanine-rich region (83-179 residues) that showed 54% identity with the EBV nuclear antigen (FIG. 7B-9) (EBNA2) [Sample J. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5096-6000] and 40% identity with a human transcription initiation factor II (TFII) (Fig. 7B, 10.) [Tanese N. Et al. (1996) *Proc. Natl. Acad. Sci. USA*

93:13611-13616]. EBNA2 is responsible for transforming B lymphocytes [Jones M. D. et al. (1984) *EMBO J* 3:813-821] and also associated with the survival of the EBV genome during latency [Sample J. et al. (1992) *J. Virol.* 66:4654-4661]. ORFs similar to the potential PAd3 184R, 97R, 162R, 163R* and 288R proteins in the HAd5 or BAd3 genome between m.u. 0 and 15 were not identified. These proteins may thus be involved in maintaining the adenovirus genome in the latent state and/or may act as transcription factors in controlling adenovirus gene expression.

Since these novel ORFs showed homology either with EBNA antigens or other cellular and viral transcription factors, these putative proteins appear to be stimulating or inhibiting viral replication. For example, PAd3 162R and 97R putative proteins may be trans-activating adenovirus replication similar to trans-activating immediate early proteins of herpesviruses. These predicted proteins may play a role in initial viral replication, virus latent phase or virus replication during reactivation. Some of these PAd3 putative proteins may function as a complex with other viral or cellular protein/s.

EXAMPLE 2

Expression of PAd3 162R in Bacteria

Experimental

A 709 bp HindIII fragment containing the ORF of 162R was isolated from pPAd-E1 and cloned into the HindIII site of pUC18. The resultant plasmid was digested with BsmBI and cohesive ends were filled in using T4 DNA polymerase. The resulting fragment was digested with HindIII, a 0.7 Kb fragment was isolated and was inserted into the EcoRV - HindIII site of pET-30a(+) expression vector. The resultant plasmid pET162R was used for protein expression in DE3 bacteria. The protein expression was induced with IPTG. The His-tag fusion protein was purified on Nickel affinity chromatography.

Results

The ORF of putative protein 162R was expressed in a bacterial expression vector. The expression of His-tag fusion protein was induced by IPTG and purified by Nickel affinity chromatography. The protein samples were analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and visualized by staining with Coomassie blue (FIG. 8.). The fusion protein was identified by western blotting using S-protein coupled to horseradish peroxidase (FIG.9.) suggesting that the 162R putative proteins may be expressed in virus infected cells.

While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected. In addition, all references cited herein are indicative of the level of skill in the art and are hereby incorporated by reference in their entirety.

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule, comprising:

(a) a first nucleotide sequence selected from the group consisting of a nucleotide sequence having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5; and

(b) at least one foreign promoter, said foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence.

2. The nucleic acid molecule of claim 1, wherein said first nucleotide sequence consists essentially of a nucleotide sequence selected from the group consisting of a nucleotide sequence having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.

3. The molecule of claim 1, wherein said nucleotide sequence encodes a protein comprised of an amino acid sequence selected from the group consisting of an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:2, an amino acid sequence having at least about 60% identity to the amino acid sequence set forth in SEQ ID NO:3, an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:4, and an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:5.

4. The molecule of claim 3, wherein said protein has an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.

5. The molecule of claim 1, wherein said nucleotide sequence is selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO: 4, and SEQ ID NO:5.

6. The molecule of claim 1, wherein said nucleotide sequence consists essentially of a nucleotide sequence having substantial similarity to a nucleotide sequence selected from the group consisting of nucleotide 1 to nucleotide 114 of SEQ ID NO:2, nucleotide 121 to nucleotide 489 of SEQ ID NO:3 and nucleotide 316 to nucleotide 438 of SEQ ID NO:4.

7. The molecule of claim 1, wherein said first nucleotide sequence is a DNA sequence.

8. A nucleic acid molecule, comprising an RNA nucleotide sequence corresponding to the DNA nucleotide sequence of claim 7.

9. An isolated nucleic acid molecule, comprising:

(a) a first nucleotide sequence encoding a protein, said protein having an amino acid sequence selected from the group consisting of an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:2, an amino acid sequence having at least about 60% identity to the amino acid sequence set forth in SEQ ID NO:3, an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in

SEQ ID NO:4, and an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:5; and

(b) at least one foreign promoter, said foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence.

10. An isolated nucleic acid molecule, comprising:

(a) a first nucleotide sequence selected from the group consisting of a nucleotide sequence having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; and

(b) at least one foreign promoter, said foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence.

11. A recombinant nucleic acid molecule, comprising:

(a) an isolated first nucleotide sequence having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and combinations thereof; and

(b) at least one foreign promoter, said foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence.

12. The molecule of claim 11, wherein said nucleotide sequence encodes a protein comprised of an amino acid sequence selected from the group consisting of an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:2, an amino acid sequence having at least about 60% identity to the amino acid sequence set forth in SEQ ID NO:3, an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:4, and an amino acid

sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:5.

13. The molecule of claim 11, wherein said promoter is selected from the group consisting of a constitutive promoter, an inducible promoter, and a cell-specific promoter.

14. The molecule of claim 11, wherein said molecule further comprises a nucleotide sequence encoding a desired protein.

15. The molecule of claim 11, wherein said recombinant nucleic acid molecule comprises a plasmid.

16. The molecule of claim 15, wherein said plasmid comprises an adenoviral origin of replication.

17. The molecule of claim 11, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and combinations thereof

18. A host cell, comprising:

(a) an isolated and introduced first nucleic acid molecule having a nucleotide sequence having substantial similarity to the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and combinations thereof; and

(b) at least one foreign promoter, said foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence.

19. The host cell of claim 18, wherein each of said nucleotide sequences encode a protein, said protein having an amino acid sequence

selected from the group consisting of an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:2, an amino acid sequence having at least about 60% identity to the amino acid sequence set forth in SEQ ID NO:3, an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:4, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:5, and combinations thereof.

20. A purified protein, comprising an amino acid sequence selected from the group consisting of an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:2, an amino acid sequence having at least about 60% identity to the amino acid sequence set forth in SEQ ID NO:3, an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:4, and an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:5.

21. The protein of claim 20, wherein said amino acid sequence is selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.

22. An adenoviral vector, comprising:

(a) an introduced first nucleic acid molecule comprising a first nucleotide sequence having substantial similarity to the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID

NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and combinations thereof; and

(b) at least one foreign promoter, said foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence.

23. The vector of claim 22, wherein said vector further comprises a second nucleotide sequence encoding a protein of interest.

24. The vector of claim 22, wherein said vector further comprises an adenoviral origin of replication and an inverted terminal repeat sequence.

25. A replication-defective porcine adenoviral type 3 vector, comprising a porcine adenovirus type 3 genome having a functional deletion in the E1 region.

26. The vector of claim 25, further comprising:

(a) a nucleotide sequence encoding a protein of interest, said nucleotide sequence inserted into said E1 region; and

(b) a promoter operably linked to a terminal 5' end of said nucleotide sequence encoding said protein of interest.

27. A method of introducing a first desired nucleic acid molecule into a host cell, comprising infecting said host cells with an adenoviral vector, said vector comprising:

(a) a first isolated nucleic acid molecule, said isolated nucleic acid molecule comprising a first nucleotide sequence having substantial similarity to the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and combinations thereof;

(b) at least one first foreign promoter operably linked to a terminal 5' end of said first isolated nucleic acid molecule;

(c) a second desired nucleic acid molecule encoding a desired protein; and

(d) a second foreign promoter operably linked to a terminal 5' end of said second desired nucleic acid molecule.

28. The method of claim 27, wherein said first nucleotide sequence encodes a protein having an amino acid sequences selected from the group consisting of an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:2, an amino acid sequence having at least about 60% identity to the amino acid sequence set forth in SEQ ID NO:3, an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:4, and an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:5.

29. The method of claim 27, wherein said amino acid sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

30. A method of making an adenovirus vector, comprising introducing into a cell a foreign promoter and an isolated nucleic acid molecule, said isolated nucleic acid molecule comprising a first nucleotide sequence having substantial similarity to a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and combinations thereof, said foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence.

31. The method of claim 30, wherein said isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein having an amino acid sequence selected from the group consisting of an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:1, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:2, an amino acid sequence having at least about 60% identity to the amino acid sequence set forth in SEQ ID NO:3, an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID NO:4, an amino acid sequence having at least about 40% identity to the amino acid sequence set forth in SEQ ID NO:5.

32. A method of expressing a desired protein, comprising:

(a) infecting a host cell with an adenoviral vector having an isolated nucleic acid molecule comprising a first nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and combinations thereof, a second nucleotide sequence encoding a desired protein, at least one foreign promoter operably linked to a terminal 5' end of said first nucleotide sequence and a second promoter operably linked to a terminal 5' end of said second nucleotide sequence; and

(b) culturing under conditions to achieve expression of said protein.

33 The method of claim 32, wherein said promoters are inducible promoters.

34. The method of claim 32, further comprising inducing said second promoter to achieve expression of said desired protein.

35. The method of claim 32, wherein said desired protein is an antigen.

36. A method of expressing a viral protein, comprising:

(a) introducing into a host cell an isolated first nucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and combinations thereof, and a foreign promoter, said foreign promoter operably linked to a terminal 5' end of said isolated nucleotide sequence; and

(b) culturing under conditions to achieve expression of said protein

37. The method of claim 36, wherein said nucleotide sequence comprises a nucleotide sequence having substantial similarity to a nucleotide sequence selected from the group consisting of nucleotide 1 to nucleotide 114 of SEQ ID NO:2, nucleotide 121 to nucleotide 489 of SEQ ID NO:3 and nucleotide 316 to nucleotide 438 of SEQ ID NO:4.

```
CATCATCAATAATATACCGCACACTTTTATTGCCCCTTTTGTGGCGTGGTGATTGGCGGA  60
GAGGGTTGGGGGGCGGCGGGCGGTGATTGGTGGAGAGGGGTGTGACGTAGCGTGGGAACGT  120
GACGTCGCGTGGGAAAATGACGTGTGATGACGTCCCGTGGGAACGGGTCAAAGTCCAAGG  180
GGAAGGGGTGGAGCCCTGGGGCGGTCCTCCGCGGGGCGGGGCCGAGCGGCGGAAATTCCC  240
GCACAGGTGGAGAGTACCGCGGGATTTTGTGCCCTCTGGACCGGACCTTCGCCCTCCGGT  300
GTGGCACTTCCGCACCACACGTCCGCGGCCCGGTATTCCCCACCTGACGACGGTGACATC  360
ACTCACCTGAGCGGGGTGTCCTTCGCGCTGAGAGGTCCGCGGCGGCCGCCCGAGATGACG  420
TGTGTGGGTGTATTTTTTCCCCTCAGTGTATATAGTCCGCGCAGCGCCCGAGAGTCACTA  480
CTCTTGAGTCCGAAGGGAGTAGAGTTTTCTCTCAGCGGAACAGACCCTCGACATGGCGAA  540
                                                  E1A→M  A  N
CAGACTTCACCTGGACTGGGACGGAAACCCCGAGGTGGTGCCGGTGCTGGAATGGGACCC  600
 R  L  H  L  D  W  D  G  N  P  E  V  V  P  V  L  E  W  D  P
GGTGGATCTGCGCGACCCCTCTCCGGGGGATGAGGGCTTCTGTGAGCCGTGCTGGGAGAG  660
 V  D  L  R  D  P  S  P  G  D  E  G  F  C  E  P  C  W  E  S
TCTGGTCGATGGACTGCCGGACGAGTGGCTGGACAGTGTGGACGAGGTGGAGGTGATTGT  720
 L  V  D  G  L  P  D  E  W  L  D  S  V  D  E  V  E  V  I  V
GACTGAGGGGGGTGAGTCAGAGGACAGTGGTGGGAGTGCCGCTGGTGACTCAGGTGGCTC  780
 T  E  G  G  E  S  E  D  S  G  G  S  A  A  G  D  S  G  G  S
TCAGGGGGTCTTTGAGATGGACCCCCCAGAAGAGGGGGACAGTAATGAGGAGGATATCAG  840
 Q  G  V  F  E  M  D  P  P  E  E  G  D  S  N  E  E  D  I  S
CGCGGTGGCTGCGGAGGTGCTGTCTGAACTGGCTGATGTGGTGTTTGAGGACCCACTTGC  900
 A  V  A  A  E  V  L  S  E  L  A  D  V  V  F  E  D  P  L  A

GCCACCCTCTCCGTTTGTGTTGGACTGCCCCGAGGTACCTGGTGTGAACTGCCGCTCTTG  960
 P  P  S  P  F  V  L  D  C  P  E  V  P  G  V  N  C  R  S  C
TGATTACCATCGCTTTCACTCCAAGGACCCCAATCTGAAGTGCAGTCTGTGCTACATGAG 1020
 D  Y  H  R  F  H  S  K  D  P  N  L  K  C  S  L  C  Y  M  R
GGATGCATGCCTTTGCTGTCTATGGTGAGTGTTTTTGGACATTTGTGGGATTATGTGGAA 1080
 D  A  C  L  C  C  L  W  *
```

Fig. 1A

```
AAAAAGGAAAAAGTGCTTGTAAGAAATCTCATGTGCTATTTCCCATTTTTTGTCTTTTTA 1140
GAAGCTGTTTCTCCAGCACCTCACAGGTCGGGTTCCCCGGGACTTGGAGACCTGCCAGGA 1200
CGCAAGAGGAAGTACTGCTATGACTCATGCAGCGAACAACCTTTGGACCTGTCTATGAAG 1260
CGCCCCCGCGATTAATCATTAACCTCAATAAACAGCATGTGATGATGACTGATTGTCTGT 1320
GTCTCTGCCTATATATACCCTTGTGGTTTGCAGGGAAGGGATGTGGTGACTGAGCTATTC 1380
CTCAGCATCATCATCGCTCTGCTTTTTTCTACTGCAGGCTATTTCTTGCTAGCTCGCTGT 1440
CCCTTTTCTTTTTCTGTGGGCATGGACTATCAACTTCTGGCCAAGCTTACTAACGTGAAC 1500

          E1B-202R→M  D  Y  Q  L  L  A  K  L  T  N  V  N

TACCTTAGGAAGGTGATAGTACAGGGGTCTCAGAACTGCCCTTGGTGGAAAAAGATTTTT 1560

Y  L  R  K  V  I  V  Q  G  S  Q  N  C  P  W  W  K  K  I  F

TCGGACAGGTTTATCAAGGTAGTAGCAGAGGCCAGGAGGCAGTACGGGCAAGAGTTGATT 1620

S  D  R  F  I  K  V  V  A  E  A  R  R  Q  Y  G  Q  E  L  I

GAGATTTTTGTGGAGGGTGAGAGGGGCTTTGGTCCTGAGTTCCTGCGGGAAGGGGGACTG 1680

E  I  F  V  E  G  E  R  G  F  G  P  E  F  L  R  E  G  G  L

TACGAAGAGGCCGTTCTGAAAGAGTTGGATTTCAGCACCTTGGGACGCACCGTAGCTAGT 1740

Y  E  E  A  V  L  K  E  L  D  F  S  T  L  G  R  T  V  A  S

GTGGCTCTGGTCTGCTTCATTTTTGAGAAGCTTCAGAAGCACAGCGGGTGGACTGACGAG 1800

V  A  L  V  C  F  I  F  E  K  L  Q  K  H  S  G  W  T  D  E
GGTATTTTAAGTCTTCTGGTGCCGCCACTATGTTCCCTGCTGGAGGCGCGAATGATGGCG 1860

G  I  L  S  L  L  V  P  P  L  C  S  L  L  E  A  R  M  M  A

                E1B-474R→M  F  P  A  G  G  A  N  D  G  G

GAGCAGGTGCGGCAGGGGCTGTGCATCATCAGGATGCCGAGCGCGGAGCGGGAGATGCTG 1920

E  Q  V  R  Q  G  L  C  I  I  R  M  P  S  A  E  R  E  M  L

  A  G  A  A  G  A  V  H  H  Q  D  A  E  R  G  A  G  D  A  V

TTGCCCAGTGGGTCATCCGGCAGTGGCAGCGGGGCCGGGATGCGGGACCAGGTGGTGCCC 1980

L  P  S  G  S  S  G  S  G  S  G  A  G  M  R  D  Q  V  V  P

  A  Q  W  V  I  R  Q  W  Q  R  G  R  D  A  G  P  G  G  A  Q

AAGCGCCCGCGGGAGCAGGAAGAGGAGGAGGAGGACGAGGATGGGATGGAAGCGAGCGGG 2040

K  R  P  R  E  Q  E  E  E  E  E  D  E  D  G  M  E  A  S  G

  A  P  A  G  A  G  R  G  G  G  G  R  G  W  D  G  S  E  R  A
CGCAGGCTCGAAGGGCCGGATCTGGTTTAGATCGCCGCCGGCCCGGGGGAGCGGGTGGAG 2100

R  R  L  E  G  P  D  L  V  *
```

Fig. 1B

```
  Q  A  R  R  A  G  S  G  L  D  R  R  R  P  G  G  A  G  G  E
AGGGGAGCGGGGAGGAGGCGGGGGGGTCTTCCATGGTTAGCTATCAGCAGGTGCTTTCTG 2160
  G  S  G  E  E  A  G  G  S  S  M  V  S  Y  Q  Q  V  L  S  E
AGTATCTGGAGAGTCCTCTGGAGATGCATGAGCGCTACAGCTTTGAGCAGATTAGGCCCT 2220
  Y  L  E  S  P  L  E  M  H  E  R  Y  S  F  E  Q  I  R  P  Y
ATATGCTTCAGCCGGGGGATGATCTGGGGGAGATGATAGCCCAGCACGCCAAGGTGGAGT 2280
  M  L  Q  P  G  D  D  L  G  E  M  I  A  Q  H  A  K  V  E  L
TGCAGCCGGGCACGGTGTACGAGCTGAGGCGCCCGATCACCATCCGCAGCATGTGTTACA 2340
  Q  P  G  T  V  Y  E  L  R  R  P  I  T  I  R  S  M  C  Y  I
TCATCGGGAACGGGGCCAAGATCAAGATTCGGGGGAATTACACGGAGTACATCAACATAG 2400
  I  G  N  G  A  K  I  K  I  R  G  N  Y  T  E  Y  I  N  I  E
AGCCGCGTAACCACATGTGTTCCATTGCGGGCATGTGGTCGGTGACTATCACGGATGTGG 2460
  P  R  N  H  M  C  S  I  A  G  M  W  S  V  T  I  T  D  V  V
TTTTTGATCGGGAGCTACCGGCCCGGGGTGGTCTGATTTTAGCCAACACGCACTTCATCC 2520
  F  D  R  E  L  P  A  R  G  G  L  I  L  A  N  T  H  F  I  L
                                   *  G  V  R  V  E  D
TGCACGGCTGCAACTTCCTGGGCTTTCTGGGCTCGGTAATAACGGCGAACGCCGGGGGGG 2580
  H  G  C  N  F  L  G  F  L  G  S  V  I  T  A  N  A  G  G  V
  Q  V  A  A  V  E  Q  A  K  Q  A  R  Y  Y  R  R  V  G  P  P
TGGTGCGGGGATGCTACTTTTTCGCCTGCTACAAGGCGCTTGACCACCGGGGGCGGCTGT 2640
  V  R  G  C  Y  F  F  A  C  Y  K  A  L  D  H  R  G  R  L  W
  H  H  P  S  A  V  K  E  G  A  V  L  R  K  V  V  P  P  P  Q
GGCTGACGGTGAACGAGAACACGTTTGAAAAGTGTGTGTACGCGGTGGTCTCTGCGGGGC 2700
  L  T  V  N  E  N  T  F  E  K  C  V  Y  A  V  V  S  A  G  R
  P  Q  R  H  V  L  V  R  K  F  L  T  H  V  R  H  D  R  R  P
GTTGCAGGATCAAGTACAACTCCTCCCTGTCCACCTTCTGCTTCTTGCACATGAGCTATA 2760
  C  R  I  K  Y  N  S  S  L  S  T  F  C  F  L  H  M  S  Y  T
  T  A  P  D  L  V  V  G  G  Q  G  G  E  A  E  Q  V  H  A  I
```

Fig. 1C

```
CGGGCAAGATAGTGGGGAACAGCATCATGAGCCCTTACACGTTCAGCGACGACCCCTACG 2820
  G  K  I  V  G  N  S  I  M  S  P  Y  T  F  S  D  D  P  Y  V

 R  A  L  Y  H  P  V  A  D  H  A  R  V  R  E  A  V  V  G  V

TGGACCTGGTGTGCTGCCAGAGCGGGATGGTGATGCCCCTGAGCACGGTGCACATCGCTC 2880
  D  L  V  C  C  Q  S  G  M  V  M  P  L  S  T  V  H  I  A  P

 H  V  Q  H  A  A  L  A  P  H  H  H  G  Q  A  R  H  V  D  S

CCTCGTCTCGCCTGCCCTACCCTGAGTTCCGCAAGAATGTGCTCCTCCGCAGCACCATGT 2940
  S  S  R  L  P  Y  P  E  F  R  K  N  V  L  L  R  S  T  M  F

 G  R  R  A  Q  G  V  R  L  E  A  L  I  H  E  E  A  A  G  H

TTGTGGGCGGCCGCCTGGGCAGCTTCAGCCCCAGCCGCTGCTCCTACAGCTACAGCTCCC 3000
  V  G  G  R  L  G  S  F  S  P  S  R  C  S  Y  S  Y  S  S  L

 K  H  A  A  A  Q  A  A  E  A  G  A  A  A  G  V  A  V  A  G

TGGTGGTGGACGAGCAGTCCTACCGGGGTCTGAGTGTGACCTGCTGCTTCGATCAGACCT 3060
  V  V  D  E  Q  S  Y  R  G  L  S  V  T  C  C  F  D  Q  T  C

 Q  H  H  V  L  L  G  V  P  T  Q  T  H  G  A  A  E  I  L  G

GTGAGATGTACAAGCTGCTGCAGTGTACGGAGGCGGACGAGATGGAGACGGATACCTCTC 3120
  E  M  Y  K  L  L  Q  C  T  E  A  D  E  M  E  T  D  T  S  Q

 T  L  H  V  L  Q  Q  L  T  R  L  R  V  L  H  L  R  I  G  R

AGCAGTACGCCTGCCTGTGCGGGGACAATCACCCCTGGCCGCAGGTGCGGCAGATGAAAG 3180
  Q  Y  A  C  L  C  G  D  N  H  P  W  P  Q  V  R  Q  M  K  V

 L  L  V  G  A  Q  A  P  V  I  V  G  P  R  L  H  P  L  H  F

TGACAGACGCGCTGCGGGCCCCCCGGTCCCTGGTGAGCTGCAACTGGGGGGAGTTCAGCG 3240
  T  D  A  L  R  A  P  R  S  L  V  S  C  N  W  G  E  F  S  D

 H  C  V  R  Q  P  G  G  P  G  Q  H  A  A  V  P  P  L  E  A

ATGACGATGACTGAGGATGAGTCACCCCCTCCCCTCCTCTTGCAGGTACGTGGCCCCGCC 3300
  D  D  D  *

 I  V  I  V  S  S  S  D  G  G  G  R  R  K  C  T  R  P  G  A

CAGTGGGATGGGCTTTGGATGGGGGAGGGGTGTTCCCTATAAAAGGGGGGATGGGGGTGGA 3360
 W  H  S  P  S  Q  I  P  S  P  H  E  R  Y  F  P  S  P  P  P
```

Fig. 1D

```
GGCATGCAGCCCCACGGGGAAGCTTGTGTGGAGGATGTCTTCCGAGGGTGAGATCCGGAC 3420
                          pIX→ M  S  S  E  G  E  I  R  T
 P  M ←288R
CTGCTTCATTTCAGCTCGTCTTCCCAGCTGGGCCGGCGTGCGTCAGGGAGTGGCCGGGAC 3480
 C  F  I  S  A  R  L  P  S  W  A  G  V  R  Q  G  V  A  G  T
       *  K  L  E  D  E  W  S  P  R  R  A  D  P  L  P  R  S
GAATGTGAACGGCGGAGTGGTGGGCGCCCCTGCCCAGAGCGGGGTGCTGGCCTACTCCCG 3540
 N  V  N  G  G  V  V  G  A  P  A  Q  S  G  V  L  A  Y  S  R
 S  H  S  R  R  L  P  P  R  G  Q  G  S  R  P  A  P  R  S  G
CTTCGTTCAGCAGCAACAGCAGCAGCCGGGGACGGCGGCGACGGGGTCTGTGTTCCGGGC 3600
 F  V  Q  Q  Q  Q  Q  Q  P  G  T  A  A  T  G  S  V  F  R  A
 S  R  E  A  A  V  A  A  A  P  S  P  P  S  P  T  Q  T  G  P
       *  C  C  C  C  C  G  P  V  A  A  V  P  D  T  N  R  A
GGTGTTTCCATCGGTGGATCTGAGCGCGGAGGTGGGCATGATGCGGCAGGCGCTGGCGGA 3660
 V  F  P  S  V  D  L  S  A  E  V  G  M  M  R  Q  A  L  A  E
 P  T  E  M  P  P  D  S  R  P  P  P  C  S  A  A  P  A  P  P
  T  N  G  D  T  S  R  L  A  S  T  P  M  I  R  C  A  S  A  S
GCTGCGGCAGCAGCTGCAGGAGCTGCGGGAGGTGGTGGAGATACAGCTGCGGGCCACGGC 3720
 L  R  Q  Q  L  Q  E  L  R  E  V  V  E  I  Q  L  R  A  T  A
 A  A  A  A  A  A  P  A  A  P  P  P  P  S  V  A  A  P  W  P
  S  R  C  C  S  C  S  S  R  S  T  T  S  I  C  S  R  A  V  A
CTCGGAGGCGGCCGAGGAGGAAGAGGAGGAGGAGATTGTGGTGGACGAGGAGGTGGCGCC 3780
  S  E  A  A  E  E  E  E  E  E  E  I  V  V  D  E  E  V  A  P
  R  P  P  P  R  P  P  L  P  P  P  S  Q  P  P  R  P  P  P  A
   E  S  A  A  S  S  S  S  S  S  S  I  T  T  S  S  S  T  A  G
CGGCGCTGGAGCGAACACCATGGAAGAGGAGGAGGATGAGATGGTCCTGACGATGACTGT 3840
  G  A  G  A  N  T  M  E  E  E  E  D  E  M  V  L  T  M  T  V
  R  R  Q  L  S  C  W  P  L  P  P  P  H  S  P  G  S  S  S  Q
   P  A  P  A  F  V  M  S  S  S  S  S  S  I  T  R  V  I  V  T
GGTGGGGGACCCTGAGCCTGCTGGAGTGGAAGCCCAGCCGCCACCACCACCCACCCCGGA 3900
```

Fig. 1E

```
 V  G  D  P  E  P  A  G  V  E  A  Q  P  P  P  P  P  T  P  E
 P  P  P  G  Q  A  Q  Q  L  P  L  G  A  A  V  V  V  W  G  P
  T  P  S  G  S  G  A  P  T  S  A  W  G  G  G  G  G  V  G  S
GAGCGACCCTGCGGTGCCTGCTACTACCACTACCCCGAGCGGCGCGGCTACGGAGGAGCA 3960
 S  D  P  A  V  P  A  T  T  T  T  P  S  G  A  A  T  E  E  Q
 S  R  G  Q  P  A  ← 163R*
  L  S  G  A  T  G  A  V  V  V  V  G  L  P  A  A  V  S  S  C
AGAGGAGCGGTCCATGCGCGGAGACAACTGAGGCGGACTGTGGGGGGAAGAAGGGGGAGG 4020
 E  E  R  S  M  R  G  D  N  *
  S  S  R  D  M  R  P  S  L  Q  P  P  S  H  P  S  S  P  P  P
AGGAAAGAAGACCATGGAGACGGGTGTTTGTCTTTTTCCAGCCCAACTTTATTGAGAATA 4080
  P  F  F  V  M  ← 162R
                                          *  Q  S  Y
ATAATAAAGCTTATGGATGTTTGGAACGATAATAGCGTGTCCAGCGTTCTCTGTCTTGCA 4140
          184R →M  F  G  T  I  I  A  C  P  A  F  S  V  L  Q
             *  P  H  K  S  R  Y  Y  R  T  W  R  E  R  D  Q
  Y  Y  L  K  H  I  N  P  V  I  I  A  H  G  A  N  E  T  K  C
GGGTCTTGTGTATCTTCTCGAGGCAGCGGTAGACCTGGTGTTGGACGTTGAGATACATGG 4200
 G  L  V  Y  L  L  E  A  A  V  D  L  V  L  D  V  E  I  H  G
L  T  K  H  I  K  E  L  C  R  Y  V  Q  H  Q  V  N  L  Y  M
  P  R  T  Y  R  R  S  A  A  T  S  R  T  N  S  T  S  I  C  P
GCATGAGTCCCTCGGCGGGGTGCAGGTAGAGCCACTGGAGGGCTGGGTGCGGGGGGCAGG 4260
 H  E  S  L  G  G  V  Q  V  E  P  L  E  G  W  V  R  G  A  G
P  M  L  G  E  A  P  H  L  Y  L  W  Q  L  A  P  H  P  P  C
  C  S  D  R  P  P  T  C  T  S  G  S  S  P  Q  T  R  P  A  P
TGCAGTAGATGATCCAGTCATAGGCGTTCTGGTTGCGGTGGTGGTTGAAGATGTCCTTGA 4320
 A  V  D  D  P  V  I  G  V  L  V  A  V  V  V  E  D  V  L  E
T  C  Y  I  I  W  D  Y  A  N  Q  N  R  H  H  N  F  I  D  K
```

Fig. 1F

```
  A  T  S  S  G  T  M  P  T  R  T  A  T  T  T  S  S  T  R  S

GGAGCAGGCTGATGGCGGTGGGCAGACCCTTGGTGTAGGCATTGATGAAGCGGTTGAGCT 4380

 E  Q  A  D  G  G  G  Q  T  L  G  V  G  I  D  E  A  V  E  L

L  L  L  S  I  A  T  P  L  G  K  T  Y  A  N  I  F  R  N  L

  S  C  A  S  P  P  P  C  V  R  P  T  P  M ← 97R

GGGCGGGCTGCATGAGGGGGGACATGATGTGGTACTTGGCCTGGATCTTGAGGTTGGAGA 4440

 G  G  L  H  E  G  G  H  D  V  V  L  G  L  D  L  E  V  G  D

Q  A  P  Q  M  L  P  S  M  I  H  Y  K  A  Q  I  K  L  N  S

TGTTGCCGCTCTGGTCGCGGCGGGGGTTCATGTTGTGGAGGACGACGAGGACGGCGTAGC 4500

 V  A  A  L  V  A  A  G  V  H  V  V  E  D  D  E  D  G  V  A

I  N  G  S  Q  D  R  R  P  N  M  N  H  L  V  V  L  V  A  Y

CGGTGCAGCGGGGGAAGCGGGCGTGCAGCTTGGAGGGGAAGGCGTGGAAGAACTTGGCGA 4560

 G  A  A  G  E  A  G  V  Q  L  G  G  E  G  V  E  E  L  G  D

G  T  C  R  P  F  R  A  H  L  K  S  P  F  A  H  F  F  K  A

CCCCCTTGTGTCCGCCGAGGTCCTCCATGCACTCGTCGAGGACGATGGCGATGGGTCCGC 4620

 P  L  V  S  A  E  V  L  H  A  L  V  E  D  D  G  D  G  S  A

V  G  K  H  G  G  L  D  E  M  C  E  D  L  V  I  A  I  P  G

GGGCGGCGGCGCGGGCGAAGACGTTGCGTGAGTCAGTGACATCATAGTTGTGCTCCTGCA 4680

 G  G  G  A  G  E  D  V  A  *

R  A  A  A  R  A  F  V  N  R  S  D  T  V  D  Y  N  H  E  Q

TGAGGTCCTGGTAGCTCATGCGGACAAAGTCTGGCATGAGGGTGGCGGTCTGGGGGATTA 4740

M  L  D  Q  Y  S  M  R  V  F  D  P  M  L  T  A  T  Q  P  I

GGGTGTGGTCCGGACCGCTGCGGTAGTTG 4769

L  T  H  D  P  G  S  R  Y  N ←IVa2
```

Fig. 1G

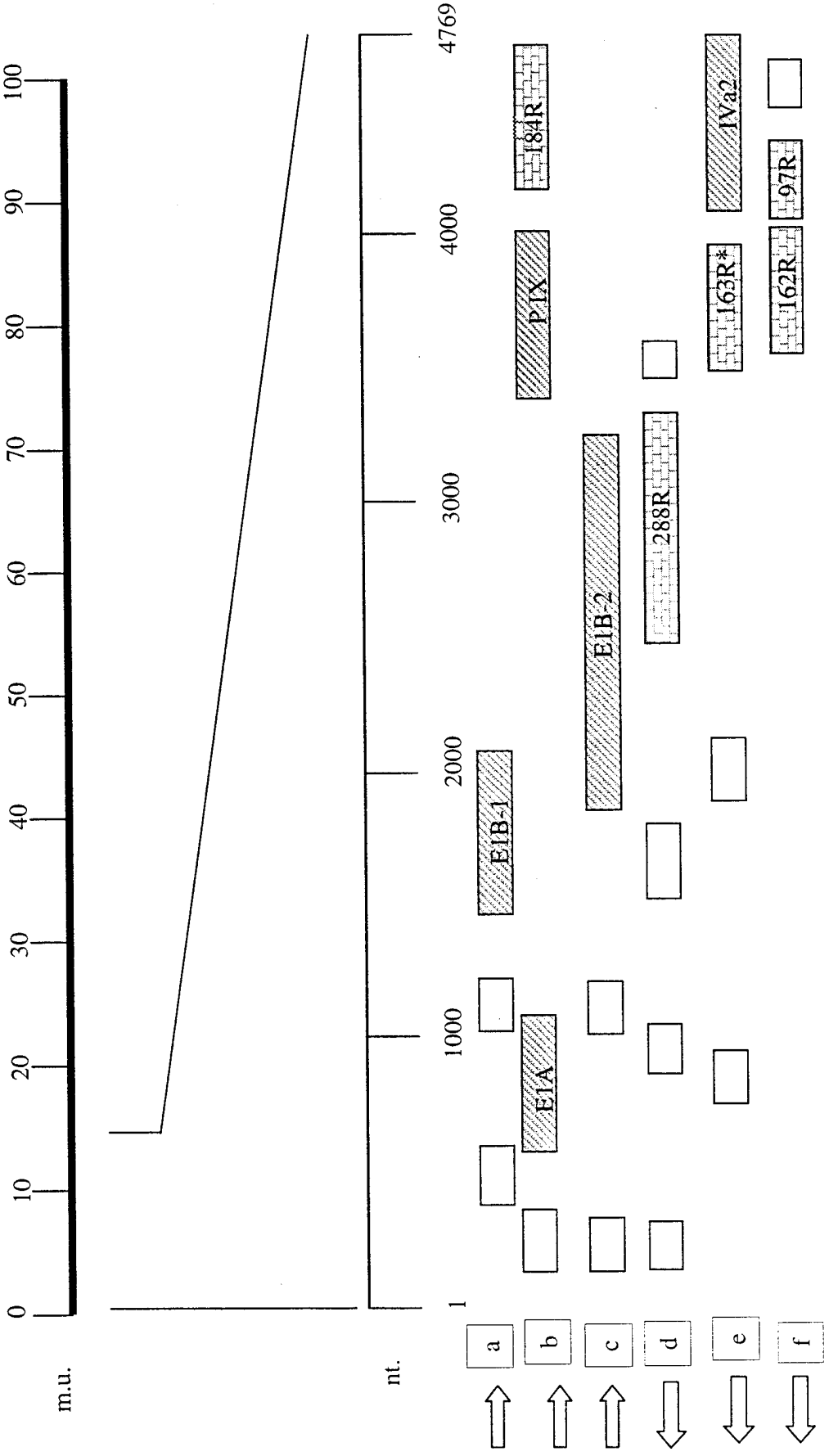
m.u.
0
10
20
30
40
50
60
70
80
90
100
nt.
1
1000
2000
3000
4000
4769
E1A
E1B-1
E1B-2
288R
P IX
184R
IVa2
163R*
162R
97R
a
b
c
d
e
f

Fig. 2

A) Activation region

```
PAd3 128 FVLDCPEVPGVN 139
PAd4  58 FQLDCPPVPGHN 69
HAd5 142 FVLDYVEHPGHG 153
BAd3 161 FFLDCPEDPSRE 172
```

B) Metal binding region

```
PAd3 140 CRSCDYHRFHSKDPNLKCSLC 160
PAd4  70 RASCNYHRDRLGDPNVTCALC 90
HAd5 154 CRSCHYHRRNTGDPDIMCSLC 174
BAd3 173 CSSCGFHQAQSGIPGIMCSLC 193
```

C) Promoter binding region

```
PAd3 161 YMRDA..CL 167
HAd5 175 YMRT...CG 180
BAd3 194 YMRQTYHCI 202
```

D) Rb binding region

```
PAd3 112 ELADVVF.EDPLAPPS 126
HAd5 118 EVIDLTCHEAGF.PPS 132
BAd3  25 EV.DLECHEVL..PPS 37
```

Fig. 3

A)

```
PAd3 1 MDYQLLAKLTNVNYLRKVIVQGSQNCPWWK.KIFSDRFIKVVAEARRQYG 49
BAd3 1 MDH..LSVLLDLKLLRSIVAGASNRTGVWKRRLWLGRLTQLVHDTCVENE 48

PAd3 QELIEIFVEGERGFGPEFLREGGLYEEAVLKELDFSTLGRTVASVALVCF 99
BAd3 SIFLNSLPGNEAFLRLLRSGYFEVFDVFVVPELHLDTPGRVVAALALLVF 98

PAd3 IFEKLQKHSGWTDEGILSLLVPPLCSLLEARMMAEQVRQGLCIIRMPSAE 149
BAd3 ILNDLDANSA.SSGFDSGFLVDRLCVPLWLKARAFKITQSSRSTSQPSSS 147

PAd3 REMLLPSGSS 159
BAd3 PDKTTQTTSQ 157
```

B)

```
PAd3 82 LDFSTLGR TVASVALVCFI 100
HAd5 82 LDFSTPGR AAAAVAFLSFI 100
BAd3 83 LD..TPGR VVAALALLVFI 99
```

C)

```
PAd3 157 GSSGSGS 163
HAd5 20  QSSNSTS 26
BAd3 136 QSSRSTS 142
```

D)

```
PAd3 116 PLEMHE....RYSFEQIRPYMLQPGDDLGEMIAQHAKVELQPGTVYELRR 161
PAd4 52  RDLDARDPESDLEFCEIETVEVGPEDDLGAMIRLHAKVALRPGAEYVLSS 101
HAd5 145 LDLLAQ....KYSIEQLTTYWLQPGDDFEEAIRVYAKVALRPDCKYKISK 190
BAd3 69  HDVFYE....RYSFEDIKSYEALPEDNLEQLIAMHAKIKLLPGREYELTQ 114

PAd3     PITIRSMCYIIGNGAKIKIRGNYTEYINIEPRNHMCSIAGMWSVTIIDVV 211
PAd4     GFCISTCCYVIGRSATLILPAGVGP.ISVMPLSPGPLVQGLYGVTFLDVR 150
HAd5     LVNIRNCCYISGNGAEVEIDTEDRVAFRCSMINMWPGVLGMDGVVIMNVR 240
BAd3     PLNITSCAYVLGNGATIRVTGEASPAIRVGAMAVGPCVTGMTGVTFYNCR 164

PAd3     FDRELPARGG...LILANTHFILHGCNFLGFLGSVITANAGGVVRGCYFF 258
PAd4     FEQRDHSQDESSILFHCDTAARFVGCSFMGFSGVCLKLQMGGELFGCYFS 200
HAd5     FTGPN.FSGT...VFLANTNLILHGVSFYGFNNTCVEAWTDVRVRGCAFY 286
BAd3     FERESTIRGS...LIRASTHVLFHGCYFMGIMGTCIEVGAGAYIRGCEFV 211

PAd3     ACYKALDHRGRLWLTVNENTFEKCVYAVVSAGRCRIKYNSSLSTFCFLHM 308
PAd4     ANYKCRESHGAGKVVLRSCTFNKYMLGVIGHGPVEIRFCTFLETHAAVVA 250
HAd5     CCWKGVVCRPKSRASIKKCLFERCTLGILSEGNSRVRHNVASDCGCFMLV 336
BAd3     GCYRGICSTSNRDIKVRQCNFDKCLLGITCKGDYRLSGNVCSETFCFAHL 261

PAd3     SYTGKIVGNSIMSPYTFSDDPYVDLVCCQSGMVMPLSTVHIAPSSRLPYP 358
PAd4     HSGLVFDANSVVFPNTVTDASDFSMLCCANMVPVPLCTVHVVHDPNKKPA 300
HAd5     KSVAVIKHNMVCGN...CEDRASQMLTCSDGNCHLLKTIHVASHSRKAWP 383
BAd3     EGEGLVKNNTVKSPSRWTSESGFSMITCADGRVTPLGSLHIVGNRCRRWP 311
```

Fig. 4

```
PAd3 1 MSS...EGEIRTCFISARLPSWAGVRQGVAGTNVNGGVVGAPAQSGVLAY 47
HAd5 1 MSTNSFDGSIVSSYLTTRMPPWAGVRQNVMGSSIDGRPV.LPANSTTLTY 49
BAd3 1 MAE...EGRIYVPYVTARLPKWSGSVQDKTGSNMLGGVVLPPNSQAHRTE 47
```

Fig. 5

```
PAd3  1   NYRSGPDHTLIPQTATLMPDFVRMSYQDLMQEHNYDVTDSRNVFARAAAR 50
HAd5  226 NYAPGPDGTIIPQSGTLRPRFVKMAYDDLILEHNYDVSDPRNIFAQAAAR 275
BAd3  149 NYTPGPTGTLVPQSSTLKPAFKIMSYEDLTMDYNYDVAHPQNVFAQAAKT 198

PAd3      GPIAIVLDECMEDLGGHKGVAKFFHAFPSKLHARFPRCTGYAVLVVLHNM 100
HAd5      GPIAIIMDECMENLGGHKGVSKFFHAFPSKLHDKFPKCTGYTVLVVLHNM 325
BAd3      GPIAIIMDECMEDLGRHKAISKFFHAFPSKLHDKFPKCTGYSVFVVLHNM 248

PAd3      NPRRDQSGNISNLKIQAKYHIMSPLMQPAQLNRFINAYTKGLPTAISLLL 150
HAd5      NPRRDMAGNIANLKIQSKMHLISPRMHPSQLNRFVNTYTKGLPLAISLLL 375
BAd3      NPRKDLGGNIANLKIQAKLHLISPRMHPSQLNRFINTYTKSLPLPITLLL 298

PAd3      KDIFNHHRNQNAYDWIIYCTCPPHPALQWLYLHPAEGLMPMYLNVQHQVY 200
HAd5      KDIFRHHAQRSCYDWIIYNTTPQHEALQWCYLHPRDGLMPMYLNIQSHLY 425
BAd3      KDIFSFHAQHSQYDWIIYNTLPPHESLQWLYLNPSEGLMPMYLNIHAMVY 348

PAd3      RCLEKIHKTLQDRERWTRYYRSKHP    225
HAd5      HVLEKIHRTLNDRDRWSRAYRARKTPK  452
BAd3      EALLRMHRTLIDRARWTRYYHRKNKEFY 376
```

Fig. 6

1)

```
   PAd3 184R 114 GDVAALVAAGVHVVEDDEDGVAGAAGEAGVQLGGEGVEELGDPLVSAEVL 163
 Na+/Ca+-K+  896 GDEGEIQAGEAGEVEGEDGEVEGGEDEGEIQAGEGGEGETGEQELNAEI. 944
 Exchanger
 protein
             164 HALVEDDGDGSAGGGAGE 181
             945 QGEAKDDEEGVDGEGGGD 962
```

2)

```
   PAd3 184R 147 GEGVEELGDPLVSAEVLHALVEDDGDGSAGGGAGEDVA 184
     BICP4   561 GEAVRGAGGPGVRAPRRHGLAAEREARGPGPAAGAPAA 598
```

3)

```
   PAd3  97R   2 PTPRVCPPPSACSSRTSST.TTATRTPMTGSSTAPAPRTQPSSGSTCTPP 50
   membrane  104 PTSTHTSSPSSTSTQSSSTAATSSSAPSTASSTTSIPTSTSTETTTTTPT 153
    protein

              51 RDSCPCISTSNTRSTAAS 68
             154 ASTTTPTTTTAAPTTAAT 171
```

4)

```
   PAd3 97R     9 PPSACSSRTSSTTTATRTPMTGSSTAPAPRTQPSSGSTCTPPRD 52
   Transcri-   42 PPSNVSSSLSSGRASNSRPRNNSSLSNANRGRPSSNSSSGPPRE 85
ptional control
protein
```

5)

```
   PAd3 162R   41 TAGSLSGVGGGGGWASTPAGSGSPTTVIVRTISSSSSSMVFAPAPGATSS 90
 membrane      68 TTSSSSTSGSGQSTSSGTTNSSSSPTTSPPTTSSSPPTSTHTSSPSSTST 117
 protein
               91 STTISSSSSSSAASEAVARSCISTTSRSSCSCCRSSASACRIMPTSALRS 140
              118 QSSSTAATSSSAPSTASSTTSIPTSTSTETTTTTPTASTTTPTTTTAAPT 167

              141 TDGNTARNT 149
              168 TAATTTAVT 176
```

6)

```
   PAd3 162R   24 SSCSSVAAPLGVVVVAGTAGSLSGVGGGGGWASTPAGSGSPTTVIVRTIS 73
   ICP0       526 SSASSSAAPRSPLAPQGVGAKRAAPRRAPDSDSGDRGHGPLAPASAGAAP 575

               74 SSSSSMVFAPAPGATSSSTTISSSSSSSAASEAVARSCISTTSRSSCSCC 123
              576 PSASPSSQAAVAAASSSSASSSSASSSSASSSSASSSSASSSSASSSSAS 625

              124 RSSASA 129
              626 SSAGGA 631
```

Fig. 7A

7)
PAd3 288R 149 **GAAAG**AEA**A**Q**A**AAHKH**GA** 167
EBNA1 98 **GAGAG**GSG**AGA**GGSGA**GA** 116

8)
PAd3 288R 127 **EAAGHTQTPVGLLVHHQGAVAVG**AAAG**A**EA**A**QAA**A**HKH**GA** 166
GATA-4 95 **EGA**AYT**P**PP**PV**SPRFSFP**G**TTGSL**AAAAAAAAA**ARE**A**AAY**GS** 134

9)
PAd3 163R* 96 **PPPLPPRPPPRPWPAAVSPPPPAAPAAAAAAAPPAPAASCPPPRSDPP** 142
EBNA2 59 **PPPLPPPPPPPPPPPPPPPPPPPP**.....**P**PPP**P**SPPP**PPP**PPPPP 100

METPPG**TQTPSP** 154
QRRDA**WTQEPSP** 112

10)
PAd3 163R* 83 **L**QRR**A**PP**P**RPP**Q**S**PP**PLPPRP**PP**RPW**PAAVSPP**PPPA**APAAAAAAAPP** 128
TFII 226 **L**PKP**AA**P**P**GTVI**Q**T**PP**FVGAAA**PP**A..**PAAPSPPAAPAPAAPAAAPP** 269

A**P**AASCPPPRSD**P**PMETP**P**G**T**QT**P**SPP**P**S**PAAA**VAAERS**G**SR**PAP**RSGQ**G**R**P** 179
PPPPAPATLARP**P**GHPAG**PPT**AA**P**AV**PP**P**AAA**QNGGSA**G**AA**PAP**APAA**GGP** 320

Fig. 7B

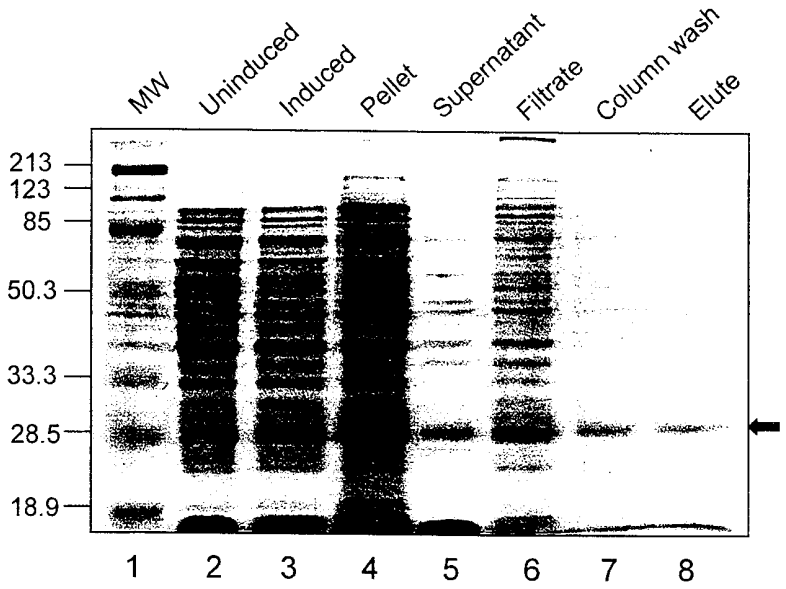
MW
Uninduced
Induced
Pellet
Supernatant
Filtrate
Column wash
Elute
213
123
85
50.3
33.3
28.5
18.9
1
2
3
4
5
6
7
8

Fig. 8

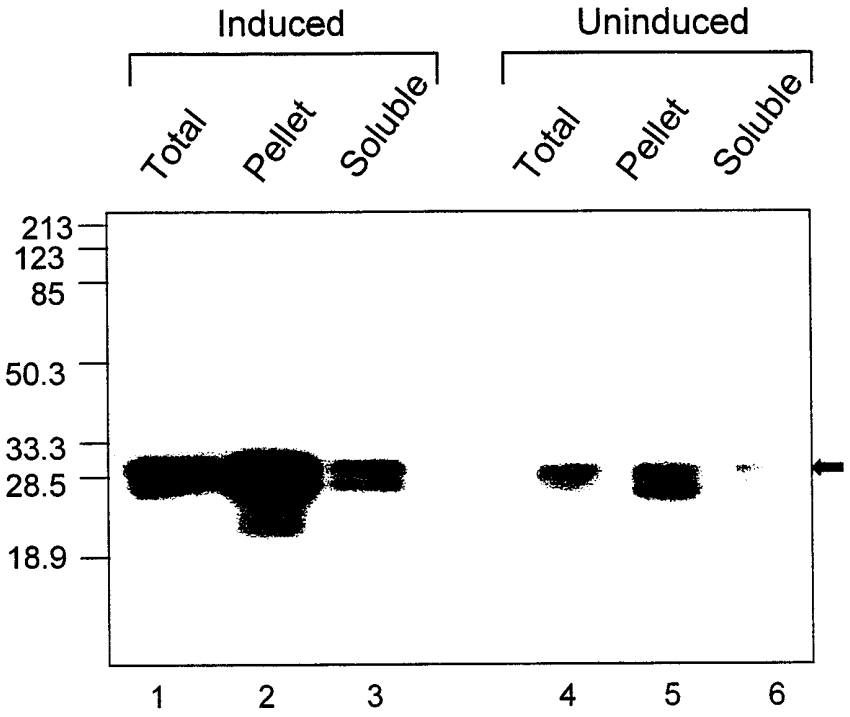
Induced
Uninduced
Total
Pellet
Soluble
Total
Pellet
Soluble
213
123
85
50.3
33.3
28.5
18.9
1
2
3
4
5
6

Fig. 9

SEQUENCE LISTING

<110> Mittal, Suresh K
Aggarwal, Neeraj

<120> Adenoviral Vectors, Proteins and Methods for Their Use

<130> 7024-447

<140> Unknown
<141> 2000-02-24

<150> US 60/121,647
<151> 1999-02-24

<160> 10

<170> PatentIn Ver. 2.1

<210> 1
<211> 294
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 1

```
atg cct aca cca agg gtc tgc cca ccg cca tca gcc tgc tcc tca agg   48
Met Pro Thr Pro Arg Val Cys Pro Pro Pro Ser Ala Cys Ser Ser Arg
  1               5                  10                  15

aca tct tca acc acc acc gca acc aga acg cct atg act gga tca tct   96
Thr Ser Ser Thr Thr Thr Ala Thr Arg Thr Pro Met Thr Gly Ser Ser
             20                  25                  30

act gca cct gcc ccc cgc acc cag ccc tcc agt ggc tct acc tgc acc   144
Thr Ala Pro Ala Pro Arg Thr Gln Pro Ser Ser Gly Ser Thr Cys Thr
         35                  40                  45

ccg ccg agg gac tca tgc cca tgt atc tca acg tcc aac acc agg tct   192
Pro Pro Arg Asp Ser Cys Pro Cys Ile Ser Thr Ser Asn Thr Arg Ser
     50                  55                  60

acc gct gcc tcg aga aga tac aca aga ccc tgc aag aca gag aac gct   240
Thr Ala Ala Ser Arg Arg Tyr Thr Arg Pro Cys Lys Thr Glu Asn Ala
 65                  70                  75                  80
```

gga cac gct att atc gtt cca aac atc cat aag ctt tat tat tat tct 288
Gly His Ala Ile Ile Val Pro Asn Ile His Lys Leu Tyr Tyr Tyr Ser
85 90 95

caa taa 294
Gln

<210> 2
<211> 489
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 2
atg gtc ttc ttt cct cct ccc cct tct tcc ccc cac agt ccg cct cag 48
Met Val Phe Phe Pro Pro Pro Pro Ser Ser Pro His Ser Pro Pro Gln
1 5 10 15

ttg tct ccg cgc atg gac cgc tcc tct tgc tcc tcc gta gcc gcg ccg 96
Leu Ser Pro Arg Met Asp Arg Ser Ser Cys Ser Ser Val Ala Ala Pro
20 25 30

ctc ggg gta gtg gta gta gca ggc acc gca ggg tcg ctc tcc ggg gtg 144
Leu Gly Val Val Val Val Ala Gly Thr Ala Gly Ser Leu Ser Gly Val
35 40 45

ggt ggt ggt ggc ggc tgg gct tcc act cca gca ggc tca ggg tcc ccc 192
Gly Gly Gly Gly Gly Trp Ala Ser Thr Pro Ala Gly Ser Gly Ser Pro
50 55 60

acc aca gtc atc gtc agg acc atc tca tcc tcc tcc tct tcc atg gtg 240
Thr Thr Val Ile Val Arg Thr Ile Ser Ser Ser Ser Ser Ser Met Val
65 70 75 80

ttc gct cca gcg ccg ggc gcc acc tcc tcg tcc acc aca atc tcc tcc 288
Phe Ala Pro Ala Pro Gly Ala Thr Ser Ser Ser Thr Thr Ile Ser Ser
85 90 95

tcc tct tcc tcc tcg gcc gcc tcc gag gcc gtg gcc cgc agc tgt atc 336
Ser Ser Ser Ser Ser Ala Ala Ser Glu Ala Val Ala Arg Ser Cys Ile
100 105 110

tcc acc acc tcc cgc agc tcc tgc agc tgc tgc cgc agc tcc gcc agc 384
Ser Thr Thr Ser Arg Ser Ser Cys Ser Cys Cys Arg Ser Ser Ala Ser
115 120 125

```
gcc tgc cgc atc atg ccc acc tcc gcg ctc aga tcc acc gat gga aac   432
Ala Cys Arg Ile Met Pro Thr Ser Ala Leu Arg Ser Thr Asp Gly Asn
    130                 135                 140

acc gcc cgg aac aca gac ccc gtc gcc gcc gtc ccc ggc tgc tgc tgt   480
Thr Ala Arg Asn Thr Asp Pro Val Ala Ala Val Pro Gly Cys Cys Cys
145                 150                 155                 160

tgc tgc tga                                                      489
Cys Cys
```

<210> 3
<211> 492
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 3

```
gca ccg cag ggt cgc tct ccg ggg tgg gtg gtg gtg gcg gct ggg ctt   48
Ala Pro Gln Gly Arg Ser Pro Gly Trp Val Val Val Ala Ala Gly Leu
  1               5                  10                  15

cca ctc cag cag gct cag ggt ccc cca cca cag tca tcg tca gga cca   96
Pro Leu Gln Gln Ala Gln Gly Pro Pro Pro Gln Ser Ser Ser Gly Pro
             20                  25                  30

tct cat cct cct cct ctt cca tgg tgt tcg ctc cag cgc cgg gcg cca   144
Ser His Pro Pro Pro Leu Pro Trp Cys Ser Leu Gln Arg Arg Ala Pro
         35                  40                  45

cct cct cgt cca cca caa tct cct cct cct ctt cct cct cgg ccg cct   192
Pro Pro Arg Pro Pro Gln Ser Pro Pro Pro Leu Pro Pro Arg Pro Pro
     50                  55                  60

ccg agg ccg tgg ccc gca gct gta tct cca cca cct ccc gca gct cct   240
Pro Arg Pro Trp Pro Ala Ala Val Ser Pro Pro Pro Pro Ala Ala Pro
 65                  70                  75                  80

gca gct gct gcc gca gct ccg cca gcg cct gcc gca tca tgc cca cct   288
Ala Ala Ala Ala Ala Ala Pro Pro Ala Pro Ala Ala Ser Cys Pro Pro
                 85                  90                  95

ccg cgc tca gat cca ccg atg gaa aca ccg ccc gga aca cag acc ccg   336
Pro Arg Ser Asp Pro Pro Met Glu Thr Pro Pro Gly Thr Gln Thr Pro
            100                 105                 110
```

```
tcg ccg ccg tcc ccg gct gct gct gtt gct gct gaa cga agc ggg agt   384
Ser Pro Pro Ser Pro Ala Ala Ala Val Ala Ala Glu Arg Ser Gly Ser
        115                 120                 125

agg cca gca ccc cgc tct ggg cag ggg cgc cca cca ctc cgc cgt tca   432
Arg Pro Ala Pro Arg Ser Gly Gln Gly Arg Pro Pro Leu Arg Arg Ser
    130                 135                 140

cat tcg tcc cgg cca ctc cct gac gca cgc cgg ccc agc tgg gaa gac   480
His Ser Ser Arg Pro Leu Pro Asp Ala Arg Arg Pro Ser Trp Glu Asp
145                 150                 155                 160

gag ctg aaa tga                                                  492
Glu Leu Lys
```

<210> 4
<211> 867
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 4

```
atg cct cca ccc cca tcc ccc ttt tat agg gaa cac ccc tcc ccc atc   48
Met Pro Pro Pro Pro Ser Pro Phe Tyr Arg Glu His Pro Ser Pro Ile
  1               5                  10                  15

caa agc cca tcc cac tgg gcg ggg cca cgt acc tgc aag agg agg gga   96
Gln Ser Pro Ser His Trp Ala Gly Pro Arg Thr Cys Lys Arg Arg Gly
            20                  25                  30

ggg ggt gac tca tcc tca gtc atc gtc atc gct gaa ctc ccc cca gtt   144
Gly Gly Asp Ser Ser Ser Val Ile Val Ile Ala Glu Leu Pro Pro Val
        35                  40                  45

gca gct cac cag gga ccg ggg ggc ccg cag cgc gtc tgt cac ttt cat   192
Ala Ala His Gln Gly Pro Gly Gly Pro Gln Arg Val Cys His Phe His
    50                  55                  60

ctg ccg cac ctg cgg cca ggg gtg att gtc ccc gca cag gca ggc gta   240
Leu Pro His Leu Arg Pro Gly Val Ile Val Pro Ala Gln Ala Gly Val
65                  70                  75                  80

ctg ctg aga ggt atc cgt ctc cat ctc gtc cgc ctc cgt aca ctg cag   288
Leu Leu Arg Gly Ile Arg Leu His Leu Val Arg Leu Arg Thr Leu Gln
                85                  90                  95
```

```
cag ctt gta cat ctc aca ggt ctg atc gaa gca gca ggt cac act cag   336
Gln Leu Val His Leu Thr Gly Leu Ile Glu Ala Ala Gly His Thr Gln
            100                 105                 110

acc ccg gta gga ctg ctc gtc cac cac cag gga gct gta gct gta gga   384
Thr Pro Val Gly Leu Leu Val His His Gln Gly Ala Val Ala Val Gly
        115                 120                 125

gca gcg gct ggg gct gaa gct gcc cag gcg gcc gcc cac aaa cat ggt   432
Ala Ala Ala Gly Ala Glu Ala Ala Gln Ala Ala Ala His Lys His Gly
    130                 135                 140

gct gcg gag gag cac att ctt gcg gaa ctc agg gta ggg cag gcg aga   480
Ala Ala Glu Glu His Ile Leu Ala Glu Leu Arg Val Gly Gln Ala Arg
145                 150                 155                 160

cga ggg agc gat gtg cac cgt gct cag ggg cat cac cat ccc gct ctg   528
Arg Gly Ser Asp Val His Arg Ala Gln Gly His His His Pro Ala Leu
                165                 170                 175

gca gca cac cag gtc cac gta ggg gtc gtc gct gaa cgt gta agg gct   576
Ala Ala His Gln Val His Val Gly Val Val Ala Glu Arg Val Arg Ala
            180                 185                 190

cat gat gct gtt ccc cac tat ctt gcc cgt ata gct cat gtg caa gaa   624
His Asp Ala Val Pro His Tyr Leu Ala Arg Ile Ala His Val Gln Glu
        195                 200                 205

gca gaa ggt gga cag gga gga gtt gta ctt gat cct gca acg ccc cgc   672
Ala Glu Gly Gly Gln Gly Gly Val Val Leu Asp Pro Ala Thr Pro Arg
    210                 215                 220

aga gac cac cgc gta cac aca ctt ttc aaa cgt gtt ctc gtt cac cgt   720
Arg Asp His Arg Val His Thr Leu Phe Lys Arg Val Leu Val His Arg
225                 230                 235                 240

cag cca cag ccg ccc ccg gtg gtc aag cgc ctt gta gca ggc gaa aaa   768
Gln Pro Gln Pro Pro Pro Val Val Lys Arg Leu Val Ala Gly Glu Lys
                245                 250                 255

gta gca tcc ccg cac cac ccc ccc ggc gtt cgc cgt tat tac cga gcc   816
Val Ala Ser Pro His His Pro Pro Gly Val Arg Arg Tyr Tyr Arg Ala
            260                 265                 270

cag aaa gcc cag gaa gtt gca gcc gtg cag gat gaa gtg cgt gtt ggc   864
Gln Lys Ala Gln Glu Val Ala Ala Val Gln Asp Glu Val Arg Val Gly
        275                 280                 285
```

taa 867

<210> 5
<211> 555
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 5

```
atg ttt gga acg ata ata gcg tgt cca gcg ttc tct gtc ttg cag ggt   48
Met Phe Gly Thr Ile Ile Ala Cys Pro Ala Phe Ser Val Leu Gln Gly
  1               5                  10                  15

ctt gtg tat ctt ctc gag gca gcg gta gac ctg gtg ttg gac gtt gag   96
Leu Val Tyr Leu Leu Glu Ala Ala Val Asp Leu Val Leu Asp Val Glu
             20                  25                  30

ata cat ggg cat gag tcc ctc ggc ggg gtg cag gta gag cca ctg gag   144
Ile His Gly His Glu Ser Leu Gly Gly Val Gln Val Glu Pro Leu Glu
         35                  40                  45

ggc tgg gtg cgg ggg gca ggt gca gta gat gat cca gtc ata ggc gtt   192
Gly Trp Val Arg Gly Ala Gly Ala Val Asp Asp Pro Val Ile Gly Val
     50                  55                  60

ctg gtt gcg gtg gtg gtt gaa gat gtc ctt gag gag cag gct gat ggc   240
Leu Val Ala Val Val Val Glu Asp Val Leu Glu Glu Gln Ala Asp Gly
 65                  70                  75                  80

ggt ggg cag acc ctt ggt gta ggc att gat gaa gcg gtt gag ctg ggc   288
Gly Gly Gln Thr Leu Gly Val Gly Ile Asp Glu Ala Val Glu Leu Gly
                 85                  90                  95

ggg ctg cat gag ggg gga cat gat gtg gta ctt ggc ctg gat ctt gag   336
Gly Leu His Glu Gly Gly His Asp Val Val Leu Gly Leu Asp Leu Glu
            100                 105                 110

gtt gga gat gtt gcc gct ctg gtc gcg gcg ggg gtt cat gtt gtg gag   384
Val Gly Asp Val Ala Ala Leu Val Ala Ala Gly Val His Val Val Glu
        115                 120                 125

gac gac gag gac ggc gta gcc ggt gca gcg ggg gaa gcg ggc gtg cag   432
Asp Asp Glu Asp Gly Val Ala Gly Ala Ala Gly Glu Ala Gly Val Gln
    130                 135                 140
```

```
ctt gga ggg gaa ggc gtg gaa gaa ctt ggc gac ccc ctt gtg tcc gcc   480
Leu Gly Gly Glu Gly Val Glu Glu Leu Gly Asp Pro Leu Val Ser Ala
145                 150                 155                 160

gag gtc ctc cat gca ctc gtc gag gac gat ggc gat ggg tcc gcg ggc   528
Glu Val Leu His Ala Leu Val Glu Asp Asp Gly Asp Gly Ser Ala Gly
                165                 170                 175

ggc ggc gcg ggc gaa gac gtt gcg tga                              555
Gly Gly Ala Gly Glu Asp Val Ala
            180                 185
```

<210> 6
<211> 516
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 6

```
atg gcg aac aga ctt cac ctg gac tgg gac gga aac ccc gag gtg gtg   48
Met Ala Asn Arg Leu His Leu Asp Trp Asp Gly Asn Pro Glu Val Val
  1               5                  10                  15

ccg gtg ctg gaa tgg gac ccg gtg gat ctg cgc gac ccc tct ccg ggg   96
Pro Val Leu Glu Trp Asp Pro Val Asp Leu Arg Asp Pro Ser Pro Gly
            20                  25                  30

gat gag ggc ttc tgt gag ccg tgc tgg gag agt ctg gtc gat gga ctg   144
Asp Glu Gly Phe Cys Glu Pro Cys Trp Glu Ser Leu Val Asp Gly Leu
        35                  40                  45

ccg gac gag tgg ctg gac agt gtg gac gag gtg gag gtg att gtg act   192
Pro Asp Glu Trp Leu Asp Ser Val Asp Glu Val Glu Val Ile Val Thr
    50                  55                  60

gag ggg ggt gag tca gag gac agt ggt ggg agt gcc gct ggt gac tca   240
Glu Gly Gly Glu Ser Glu Asp Ser Gly Gly Ser Ala Ala Gly Asp Ser
65                  70                  75                  80

ggt ggc tct cag ggg gtc ttt gag atg gac ccc cca gaa gag ggg gac   288
Gly Gly Ser Gln Gly Val Phe Glu Met Asp Pro Pro Glu Glu Gly Asp
                85                  90                  95

agt aat gag gag gat atc agc gcg gtg gct gcg gag gtg ctg tct gaa   336
Ser Asn Glu Glu Asp Ile Ser Ala Val Ala Ala Glu Val Leu Ser Glu
            100                 105                 110
```

```
ctg gct gat gtg gtg ttt gag gac cca ctt gcg cca ccc tct ccg ttt   384
Leu Ala Asp Val Val Phe Glu Asp Pro Leu Ala Pro Pro Ser Pro Phe
        115                 120                 125

gtg ttg gac tgc ccc gag gta cct ggt gtg aac tgc cgc tct tgt gat   432
Val Leu Asp Cys Pro Glu Val Pro Gly Val Asn Cys Arg Ser Cys Asp
    130                 135                 140

tac cat cgc ttt cac tcc aag gac ccc aat ctg aag tgc agt ctg tgc   480
Tyr His Arg Phe His Ser Lys Asp Pro Asn Leu Lys Cys Ser Leu Cys
145                 150                 155                 160

tac atg agg gat gca tgc ctt tgc tgt cta tgg tga                   516
Tyr Met Arg Asp Ala Cys Leu Cys Cys Leu Trp
                165                 170
```

<210> 7
<211> 609
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 7

```
atg gac tat caa ctt ctg gcc aag ctt act aac gtg aac tac ctt agg   48
Met Asp Tyr Gln Leu Leu Ala Lys Leu Thr Asn Val Asn Tyr Leu Arg
  1               5                  10                  15

aag gtg ata gta cag ggg tct cag aac tgc cct tgg tgg aaa aag att   96
Lys Val Ile Val Gln Gly Ser Gln Asn Cys Pro Trp Trp Lys Lys Ile
            20                  25                  30

ttt tcg gac agg ttt atc aag gta gta gca gag gcc agg agg cag tac   144
Phe Ser Asp Arg Phe Ile Lys Val Val Ala Glu Ala Arg Arg Gln Tyr
        35                  40                  45

ggg caa gag ttg att gag att ttt gtg gag ggt gag agg ggc ttt ggt   192
Gly Gln Glu Leu Ile Glu Ile Phe Val Glu Gly Glu Arg Gly Phe Gly
    50                  55                  60

cct gag ttc ctg cgg gaa ggg gga ctg tac gaa gag gcc gtt ctg aaa   240
Pro Glu Phe Leu Arg Glu Gly Gly Leu Tyr Glu Glu Ala Val Leu Lys
65                  70                  75                  80
```

```
gag ttg gat ttc agc acc ttg gga cgc acc gta gct agt gtg gct ctg   288
Glu Leu Asp Phe Ser Thr Leu Gly Arg Thr Val Ala Ser Val Ala Leu
                85                  90                  95

gtc tgc ttc att ttt gag aag ctt cag aag cac agc ggg tgg act gac   336
Val Cys Phe Ile Phe Glu Lys Leu Gln Lys His Ser Gly Trp Thr Asp
            100                 105                 110

gag ggt att tta agt ctt ctg gtg ccg cca cta tgt tcc ctg ctg gag   384
Glu Gly Ile Leu Ser Leu Leu Val Pro Pro Leu Cys Ser Leu Leu Glu
        115                 120                 125

gcg cga atg atg gcg gag cag gtg cgg cag ggg ctg tgc atc atc agg   432
Ala Arg Met Met Ala Glu Gln Val Arg Gln Gly Leu Cys Ile Ile Arg
    130                 135                 140

atg ccg agc gcg gag cgg gag atg ctg ttg ccc agt ggg tca tcc ggc   480
Met Pro Ser Ala Glu Arg Glu Met Leu Leu Pro Ser Gly Ser Ser Gly
145                 150                 155                 160

agt ggc agc ggg gcc ggg atg cgg gac cag gtg gtg ccc aag cgc ccg   528
Ser Gly Ser Gly Ala Gly Met Arg Asp Gln Val Val Pro Lys Arg Pro
                165                 170                 175

cgg gag cag gaa gag gag gag gag gac gag gat ggg atg gaa gcg agc   576
Arg Glu Gln Glu Glu Glu Glu Glu Asp Glu Asp Gly Met Glu Ala Ser
            180                 185                 190

ggg cgc agg ctc gaa ggg ccg gat ctg gtt tag                      609
Gly Arg Arg Leu Glu Gly Pro Asp Leu Val
        195                 200
```

<210> 8
<211> 1425
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

```
<400> 8
atg ttc cct gct gga ggc gcg aat gat ggc gga gca ggt gcg gca ggg   48
Met Phe Pro Ala Gly Gly Ala Asn Asp Gly Gly Ala Gly Ala Ala Gly
  1               5                  10                  15

gct gtg cat cat cag gat gcc gag cgc gga gcg gga gat gct gtt gcc   96
Ala Val His His Gln Asp Ala Glu Arg Gly Ala Gly Asp Ala Val Ala
            20                  25                  30
```

```
cag tgg gtc atc cgg cag tgg cag cgg ggc cgg gat gcg gga cca ggt   144
Gln Trp Val Ile Arg Gln Trp Gln Arg Gly Arg Asp Ala Gly Pro Gly
         35                  40                  45

ggt gcc caa gcg ccc gcg gga gca gga aga gga gga gga gga cga gga   192
Gly Ala Gln Ala Pro Ala Gly Ala Gly Arg Gly Gly Gly Gly Arg Gly
     50                  55                  60

tgg gat gga agc gag cgg gcg cag gct cga agg gcc gga tct ggt tta   240
Trp Asp Gly Ser Glu Arg Ala Gln Ala Arg Arg Ala Gly Ser Gly Leu
 65                  70                  75                  80

gat cgc cgc cgg ccc ggg gga gcg ggt gga gag ggg agc ggg gag gag   288
Asp Arg Arg Arg Pro Gly Gly Ala Gly Gly Glu Gly Ser Gly Glu Glu
                 85                  90                  95

gcg ggg ggg tct tcc atg gtt agc tat cag cag gtg ctt tct gag tat   336
Ala Gly Gly Ser Ser Met Val Ser Tyr Gln Gln Val Leu Ser Glu Tyr
            100                 105                 110

ctg gag agt cct ctg gag atg cat gag cgc tac agc ttt gag cag att   384
Leu Glu Ser Pro Leu Glu Met His Glu Arg Tyr Ser Phe Glu Gln Ile
        115                 120                 125

agg ccc tat atg ctt cag ccg ggg gat gat ctg ggg gag atg ata gcc   432
Arg Pro Tyr Met Leu Gln Pro Gly Asp Asp Leu Gly Glu Met Ile Ala
    130                 135                 140

cag cac gcc aag gtg gag ttg cag ccg ggc acg gtg tac gag ctg agg   480
Gln His Ala Lys Val Glu Leu Gln Pro Gly Thr Val Tyr Glu Leu Arg
145                 150                 155                 160

cgc ccg atc acc atc cgc agc atg tgt tac atc atc ggg aac ggg gcc   528
Arg Pro Ile Thr Ile Arg Ser Met Cys Tyr Ile Ile Gly Asn Gly Ala
                165                 170                 175

aag atc aag att cgg ggg aat tac acg gag tac atc aac ata gag ccg   576
Lys Ile Lys Ile Arg Gly Asn Tyr Thr Glu Tyr Ile Asn Ile Glu Pro
            180                 185                 190

cgt aac cac atg tgt tcc att gcg ggc atg tgg tcg gtg act atc acg   624
Arg Asn His Met Cys Ser Ile Ala Gly Met Trp Ser Val Thr Ile Thr
        195                 200                 205

gat gtg gtt ttt gat cgg gag cta ccg gcc cgg ggt ggt ctg att tta   672
Asp Val Val Phe Asp Arg Glu Leu Pro Ala Arg Gly Gly Leu Ile Leu
    210                 215                 220
```

```
gcc aac acg cac ttc atc ctg cac ggc tgc aac ttc ctg ggc ttt ctg   720
Ala Asn Thr His Phe Ile Leu His Gly Cys Asn Phe Leu Gly Phe Leu
225                 230                 235                 240

ggc tcg gta ata acg gcg aac gcc ggg ggg gtg gtg cgg gga tgc tac   768
Gly Ser Val Ile Thr Ala Asn Ala Gly Gly Val Val Arg Gly Cys Tyr
                245                 250                 255

ttt ttc gcc tgc tac aag gcg ctt gac cac cgg ggg cgg ctg tgg ctg   816
Phe Phe Ala Cys Tyr Lys Ala Leu Asp His Arg Gly Arg Leu Trp Leu
            260                 265                 270

acg gtg aac gag aac acg ttt gaa aag tgt gtg tac gcg gtg gtc tct   864
Thr Val Asn Glu Asn Thr Phe Glu Lys Cys Val Tyr Ala Val Val Ser
        275                 280                 285

gcg ggg cgt tgc agg atc aag tac aac tcc tcc ctg tcc acc ttc tgc   912
Ala Gly Arg Cys Arg Ile Lys Tyr Asn Ser Ser Leu Ser Thr Phe Cys
    290                 295                 300

ttc ttg cac atg agc tat acg ggc aag ata gtg ggg aac agc atc atg   960
Phe Leu His Met Ser Tyr Thr Gly Lys Ile Val Gly Asn Ser Ile Met
305                 310                 315                 320

agc cct tac acg ttc agc gac gac ccc tac gtg gac ctg gtg tgc tgc  1008
Ser Pro Tyr Thr Phe Ser Asp Asp Pro Tyr Val Asp Leu Val Cys Cys
                325                 330                 335

cag agc ggg atg gtg atg ccc ctg agc acg gtg cac atc gct ccc tcg  1056
Gln Ser Gly Met Val Met Pro Leu Ser Thr Val His Ile Ala Pro Ser
            340                 345                 350

tct cgc ctg ccc tac cct gag ttc cgc aag aat gtg ctc ctc cgc agc  1104
Ser Arg Leu Pro Tyr Pro Glu Phe Arg Lys Asn Val Leu Leu Arg Ser
        355                 360                 365

acc atg ttt gtg ggc ggc cgc ctg ggc agc ttc agc ccc agc cgc tgc  1152
Thr Met Phe Val Gly Gly Arg Leu Gly Ser Phe Ser Pro Ser Arg Cys
    370                 375                 380

tcc tac agc tac agc tcc ctg gtg gtg gac gag cag tcc tac cgg ggt  1200
Ser Tyr Ser Tyr Ser Ser Leu Val Val Asp Glu Gln Ser Tyr Arg Gly
385                 390                 395                 400

ctg agt gtg acc tgc tgc ttc gat cag acc tgt gag atg tac aag ctg  1248
Leu Ser Val Thr Cys Cys Phe Asp Gln Thr Cys Glu Met Tyr Lys Leu
                405                 410                 415
```

```
ctg cag tgt acg gag gcg gac gag atg gag acg gat acc tct cag cag   1296
Leu Gln Cys Thr Glu Ala Asp Glu Met Glu Thr Asp Thr Ser Gln Gln
            420                 425                 430

tac gcc tgc ctg tgc ggg gac aat cac ccc tgg ccg cag gtg cgg cag   1344
Tyr Ala Cys Leu Cys Gly Asp Asn His Pro Trp Pro Gln Val Arg Gln
        435                 440                 445

atg aaa gtg aca gac gcg ctg cgg gcc ccc cgg tcc ctg gtg agc tgc   1392
Met Lys Val Thr Asp Ala Leu Arg Ala Pro Arg Ser Leu Val Ser Cys
    450                 455                 460

aac tgg ggg gag ttc agc gat gac gat gac tga                      1425
Asn Trp Gly Glu Phe Ser Asp Asp Asp Asp
465                 470                 475
```

<210> 9
<211> 597
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 9

```
atg tct tcc gag ggt gag atc cgg acc tgc ttc att tca gct cgt ctt   48
Met Ser Ser Glu Gly Glu Ile Arg Thr Cys Phe Ile Ser Ala Arg Leu
  1               5                  10                  15

ccc agc tgg gcc ggc gtg cgt cag gga gtg gcc ggg acg aat gtg aac   96
Pro Ser Trp Ala Gly Val Arg Gln Gly Val Ala Gly Thr Asn Val Asn
            20                  25                  30

ggc gga gtg gtg ggc gcc cct gcc cag agc ggg gtg ctg gcc tac tcc   144
Gly Gly Val Val Gly Ala Pro Ala Gln Ser Gly Val Leu Ala Tyr Ser
        35                  40                  45

cgc ttc gtt cag cag caa cag cag cag ccg ggg acg gcg gcg acg ggg   192
Arg Phe Val Gln Gln Gln Gln Gln Gln Pro Gly Thr Ala Ala Thr Gly
    50                  55                  60

tct gtg ttc cgg gcg gtg ttt cca tcg gtg gat ctg agc gcg gag gtg   240
Ser Val Phe Arg Ala Val Phe Pro Ser Val Asp Leu Ser Ala Glu Val
65                  70                  75                  80
```

```
ggc atg atg cgg cag gcg ctg gcg gag ctg cgg cag cag ctg cag gag   288
Gly Met Met Arg Gln Ala Leu Ala Glu Leu Arg Gln Gln Leu Gln Glu
                 85                  90                  95

ctg cgg gag gtg gtg gag ata cag ctg cgg gcc acg gcc tcg gag gcg   336
Leu Arg Glu Val Val Glu Ile Gln Leu Arg Ala Thr Ala Ser Glu Ala
            100                 105                 110

gcc gag gag gaa gag gag gag gag att gtg gtg gac gag gag gtg gcg   384
Ala Glu Glu Glu Glu Glu Glu Glu Ile Val Val Asp Glu Glu Val Ala
        115                 120                 125

ccc ggc gct gga gcg aac acc atg gaa gag gag gag gat gag atg gtc   432
Pro Gly Ala Gly Ala Asn Thr Met Glu Glu Glu Glu Asp Glu Met Val
    130                 135                 140

ctg acg atg act gtg gtg ggg gac cct gag cct gct gga gtg gaa gcc   480
Leu Thr Met Thr Val Val Gly Asp Pro Glu Pro Ala Gly Val Glu Ala
145                 150                 155                 160

cag ccg cca cca cca ccc acc ccg gag agc gac cct gcg gtg cct gct   528
Gln Pro Pro Pro Pro Pro Thr Pro Glu Ser Asp Pro Ala Val Pro Ala
                165                 170                 175

act acc act acc ccg agc ggc gcg gct acg gag gag caa gag gag cgg   576
Thr Thr Thr Thr Pro Ser Gly Ala Ala Thr Glu Glu Gln Glu Glu Arg
            180                 185                 190

tcc atg cgc gga gac aac tga                                      597
Ser Met Arg Gly Asp Asn
        195
```

<210> 10
<211> 679
<212> DNA
<213> Mastadenovirus porcine adenovirus 3

<220>

<400> 10

```
c aac tac cgc agc ggt ccg gac cac acc cta atc ccc cag acc gcc acc 49
  Asn Tyr Arg Ser Gly Pro Asp His Thr Leu Ile Pro Gln Thr Ala Thr
    1               5                  10                  15

ctc atg cca gac ttt gtc cgc atg agc tac cag gac ctc atg cag gag   97
Leu Met Pro Asp Phe Val Arg Met Ser Tyr Gln Asp Leu Met Gln Glu
            20                  25                  30
```

```
cac aac tat gat gtc act gac tca cgc aac gtc ttc gcc cgc gcc gcc   145
His Asn Tyr Asp Val Thr Asp Ser Arg Asn Val Phe Ala Arg Ala Ala
         35                  40                  45

gcc cgc gga ccc atc gcc atc gtc ctc gac gag tgc atg gag gac ctc   193
Ala Arg Gly Pro Ile Ala Ile Val Leu Asp Glu Cys Met Glu Asp Leu
     50                  55                  60

ggc gga cac aag ggg gtc gcc aag ttc ttc cac gcc ttc ccc tcc aag   241
Gly Gly His Lys Gly Val Ala Lys Phe Phe His Ala Phe Pro Ser Lys
 65                  70                  75                  80

ctg cac gcc cgc ttc ccc cgc tgc acc ggc tac gcc gtc ctc gtc gtc   289
Leu His Ala Arg Phe Pro Arg Cys Thr Gly Tyr Ala Val Leu Val Val
                 85                  90                  95

ctc cac aac atg aac ccc cgc cgc gac cag agc ggc aac atc tcc aac   337
Leu His Asn Met Asn Pro Arg Arg Asp Gln Ser Gly Asn Ile Ser Asn
            100                 105                 110

ctc aag atc cag gcc aag tac cac atc atg tcc ccc ctc atg cag ccc   385
Leu Lys Ile Gln Ala Lys Tyr His Ile Met Ser Pro Leu Met Gln Pro
        115                 120                 125

gcc cag ctc aac cgc ttc atc aat gcc tac acc aag ggt ctg ccc acc   433
Ala Gln Leu Asn Arg Phe Ile Asn Ala Tyr Thr Lys Gly Leu Pro Thr
    130                 135                 140

gcc atc agc ctg ctc ctc aag gac atc ttc aac cac cac cgc aac cag   481
Ala Ile Ser Leu Leu Leu Lys Asp Ile Phe Asn His His Arg Asn Gln
145                 150                 155                 160

aac gcc tat gac tgg atc atc tac tgc acc tgc ccc ccg cac cca gcc   529
Asn Ala Tyr Asp Trp Ile Ile Tyr Cys Thr Cys Pro Pro His Pro Ala
                165                 170                 175

ctc cag tgg ctc tac ctg cac ccc gcc gag gga ctc atg ccc atg tat   577
Leu Gln Trp Leu Tyr Leu His Pro Ala Glu Gly Leu Met Pro Met Tyr
            180                 185                 190

ctc aac gtc caa cac cag gtc tac cgc tgc ctc gag aag ata cac aag   625
Leu Asn Val Gln His Gln Val Tyr Arg Cys Leu Glu Lys Ile His Lys
        195                 200                 205

acc ctg caa gac aga gaa cgc tgg aca cgc tat tat cgt tcc aaa cat   673
Thr Leu Gln Asp Arg Glu Arg Trp Thr Arg Tyr Tyr Arg Ser Lys His
    210                 215                 220
```

cca taa 679
Pro
225

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04711

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/12; C12N 15/00, 7/00

US CL : 424/199.1, 204.1, 205.1; 435/320.1, 235.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/199.1, 204.1, 205.1; 435/320.1, 235.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, MEDLINE, CAPLUS

search terms: porcine, adenovirus, expression vector, gene deliv?,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	REDDY et al. Sequence and transcription map analysis of early region-1 of porcine adenovirus type-3. Virus Research. 1998, Vol. 58, pages 97-106, see the entire document.	1-37
Y	KLEIBOEKER, S. B. Identification and sequence analysis of the E1 genomic region of a porcine adenovirus. Virus Research. 1995, Vol. 36, pages 259-268, see the abstract.	1-37
Y	KLEIBOEKER, S. B. Sequence analysis of the fiber genomic region of a porcine adenovirus predicts a novel fiber protein. Virus Research. 1995, Vol. 39, pages 299-309, see the entire document.	1-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 MAY 2000	Date of mailing of the international search report 23 MAY 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ALI R. SALIMI Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★