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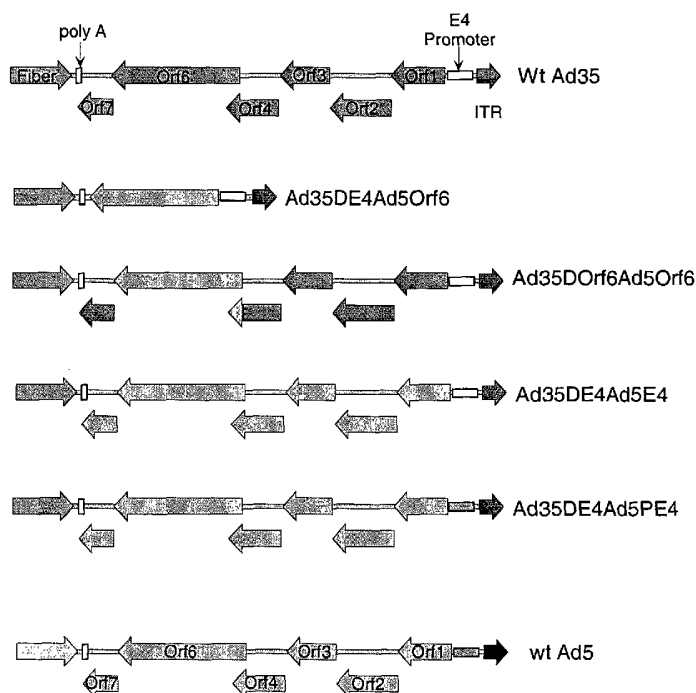
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(54) Title: METHODS FOR PROPAGATING ADENOVIRUS AND VIRUS PRODUCED THEREBY



(57) Abstract: Various methods for propagating and rescuing multiple serotypes of replication-defective adenovirus in a single adenoviral E1-complementing cell line are disclosed. Typically, replication-defective adenovirus vectors propagate only in cell lines which express E1 proteins of the same serotype or subgroup as the vector. The disclosed methods offer the ability to propagate vectors derived from multiple adenoviral serotypes in a single production cell line which expresses E1 proteins from a single serotype. Propagation in this manner is accomplished by providing all or a portion of an E4 region *in cis* within the genome of the replication-defective adenovirus. The added E4 region or portion thereof is cloned from a virus of the same or highly similar serotype as that of the E1 gene product(s) of the complementing cell line. Interaction between the expressed E1 of the cell line and the heterologous E4 of the replication-defective adenoviral vectors enables their propagation and rescue. The invention bypasses a need in the art to customize specific cell lines to the serotype or subgroup of the adenoviral vector being propagated and enables one to easily and rapidly develop alternative adenoviral serotypes as gene delivery vectors for use as vaccines or as a critical component in gene therapy.



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TITLE OF THE INVENTION

METHODS FOR PROPAGATING ADENOVIRUS AND VIRUS PRODUCED THEREBY

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application claims the benefit of application serial nos. 60/458,825, filed March 28, 2003; 60/455,312, filed March 17, 2003; 60/455,234, filed March 17, 2003; and 60/405,182, filed August 22, 2002.

FIELD OF THE INVENTION

10 The present invention concerns various methods to propagate and rescue multiple serotypes of replication-defective adenovirus in a single adenoviral E1-complementing cell line. Typically, replication-defective adenovirus vectors propagate only in cell lines which express E1 proteins of the same serotype or subgroup as the vector. The methods disclosed herein offer the ability to propagate vectors derived from multiple serotypes in a single cell line expressing E1
15 proteins from a single serotype. Such propagation of a wide range of vectors in one cell line is accomplished by providing all or a portion of an E4 region *in cis* within the genome of the replication-defective adenovirus. The added E4 region or portion thereof is cloned from a virus of the same or highly similar serotype as that of the E1 gene product(s) of the complementing cell line. Interaction between the E1 gene products of the cell line and the heterologous E4 gene
20 products of the replication-defective adenoviral vector enables the propagation and rescue of the recombinant replication-defective adenovirus vectors. The invention, therefore, bypasses an existing need in the art to customize complementing cell lines to the specific serotype or subgroup of the adenoviral vector being propagated or, alternatively, to have to transfect a cell line with an E4 region and then regulate the expression *in trans* of the E4 region within the E1
25 complementing cell line.

BACKGROUND OF THE INVENTION

Beginning with the first human adenoviruses (Ads) isolated over four decades ago (Rowe *et al.*, *Proc. Soc. Exp. Biol. Med.*, 84:570-579, 1953), over 100 distinct serotypes of
30 adenovirus have been isolated which infect various mammalian species, 51 of which are of human origin (Straus, Adenovirus infections in humans. In *The Adenoviruses*. 451-498, 1984; Hierholzer *et al.*, *J. Infect. Dis.*, 158: 804-813, 1988; Schnurr and Dondero, *Intervirology*, 36: 79-83, 1993; Jong *et al.*, *J Clin Microbiol.*, 37:3940-3945:1999). The human serotypes have been categorised into six subgenera (A-F) based on a number of biological, chemical,
35 immunological and structural criteria; criteria which include hemagglutination properties of rat

and rhesus monkey erythrocytes, DNA homology, restriction enzyme cleavage patterns, percentage of G+C content and oncogenicity (Straus, Adenovirus infections in humans. In *The Adenoviruses*. 451-498, 1984; Horwitz, Adenoviridae and their replication, *In Virology*: 1679-172, 1990).

5 Deletion of an essential E1 region common to the various adenovirus serotypes has enabled the use of adenovirus vectors as gene transfer vectors for vaccine and gene therapy purposes. Resultant replication-defective vectors are propagated in cell lines that provide the deleted E1 gene products *in trans*. Supplementation of the essential E1 gene products *in trans* in this manner works well when the E1 gene products are from the same or a highly similar
10 serotype. As such, E1-deleted group C serotypes (Ad1, Ad2, Ad5 and Ad6) grow well in 293 or PER.C6 cells which contain and express the Ad5 E1 region. In contrast, E1-deleted serotypes other than group C, for example those from subgroups A, B, D, E, and F (e.g., Ad3, Ad4, and Ad7 to Ad51), do not replicate efficiently in 293 or PER.C6 cells. The Ad5 E1 sequences in 293 and PER.C6 cells do not fully complement the replication of these alternative serotypes. This
15 presents a challenge due to the fact that the most characterized and studied complementing cell lines available for growth and propagation of adenovirus are based on E1 sequence from adenovirus serotype 5.

This inability to fully complement the replication of serotypes other than group C adenovirus in Ad5 E1 complementing cell lines has been attributed to the inability of Ad5 (group
20 C) E1b 55K gene product to functionally interact with the E4 gene products of non-group C serotypes. While the interaction is conserved within members of the same subgroup, it is not well conserved between subgroups.

Hence, cell lines expressing both Ad5 E1 and ORF6 were generated and proved useful in complementing alternative adenovirus serotypes; *see, e.g., Abrahamsen et al., 1997 J. Virol.* 8946-8951. Such incorporation of E4 (or ORF6) into Ad 5 complementing cell lines as
25 was done in Abrahamsen *et al., supra*, is known.

U.S. Patent No. 5,849,561 discloses complementation of an E1-deleted non-group C adenovirus vector in an Ad5-E1 complementing cell line which also expresses portions of the Ad5-E4 gene.

30 U.S. Patent No. 6,127,175, issued to Vigne, *et al.*, discloses a stably transfected mammalian cell line which expresses a portion of the E4 region of adenovirus, preferably ORF6 or ORF6/7. Such a cell line is useful for complementation of recombinant Ad genomes deficient in the E4 region.

European Application EP 1 054 064 A1 discloses recombinant, replication
35 deficient adenovirus 35 (Ad35) vectors and cell lines which complement *in trans* the growth of

these vectors. A cell line which expresses Ad5E1A and E2A genes (PER.C6) was shown to complement an Ad35-E1 deleted vector upon co-expression of Ad35-E1B proteins.

U.S. Patent No. 6,270,996, issued to Wilson, *et al.*, discloses E1/E4 deleted adenovirus vectors and E1/E4(ORF6) cell lines which complement *in trans* virus growth without
5 resulting in cell toxicity.

U.S. Patent No. 6,202,060, issued to Mehtali, *et al.*, discloses adenoviral vectors wherein portions of the early genes are under control of an inducible promoter. The '060 patent also discloses complementing cell lines which may be used in tandem with these Ad vectors.

The generation of serotype-specific cell lines providing a complementing
10 serotype-specific E1 gene product(s) *in trans* is known as well.

Although Ad5-based vectors have been used extensively in a number of gene therapy trials, there may be limitations on the use of Ad5 and other group C adenoviral vectors due to preexisting immunity in the general population due to natural infection. Ad5 and other group C members tend to be among the most seroprevalent serotypes. Immunity to existing
15 vectors may develop as a result of exposure to the vector during treatment. These types of preexisting or developed immunity to seroprevalent gene delivery vectors may limit the effectiveness of gene therapy or vaccination efforts. Alternative adenovirus serotypes, thus, constitute very important targets in the pursuit of gene delivery systems capable of evading the host immune response.

There remains both a practical and commercial need for an adenovirus-based
20 vaccine and/or gene therapy delivery system which allows for the production of multiple serotype recombinant adenovirus vectors in a single source complementing mammalian cell line. The present invention addresses and overcomes this deficiency in the art by disclosing novel methods for propagating multiple serotype recombinant Ad vectors in a single complementing
25 cell line where the required serotype-specific sequences are provided *in cis*.

SUMMARY OF THE INVENTION

The present invention relates to an enhanced means for propagating replication-defective adenovirus in an E1-complementing cell line(s) where the E1 gene product(s) being
30 expressed is not native to the adenovirus being propagated. The method is based on Applicants' finding that supply, *in cis*, of a nucleic acid sequence encoding all or a portion of a heterologous adenoviral E4 region which is native to a virus of the same or highly similar serotype as the E1 gene product(s) of the complementing cell line enables the growth of adenoviral vectors of varying serotype in any single complementing cell line, despite the fact the cell line is not
35 customized for the particular serotype of vector being propagated. This is of particular

importance given that existing and settled adenoviral E1-complementing cell lines (such as PER.C6™ and 293) are based on one of the most prominent adenovirus serotypes (Ad5) and are not suited for the large-scale propagation and rescue of alternative serotypes.

The basic steps involved in the propagation of adenoviral vectors in accordance with the methods of the instant invention are as follows: First, all or a portion of a heterologous adenoviral E4 region comprising nucleic acid sequence encoding at least open reading frame 6 (ORF6) is inserted into a replication-defective adenoviral vector. By "heterologous", Applicants mean that the nucleic acid sequence is not native to the viral vector being propagated, *i.e.*, not normally present within a virus of the same or highly similar serotype. As will be described, the adenoviral E4 region or portion thereof can be either a nucleic acid sequence encoding ORF 6 or any larger portion of the E4 region, and includes nucleic acid comprising the complete E4 region with E4 promoter. The region into which the nucleic acid is incorporated is not limited, *i.e.*, the insertion can be made into the complete E4 region with E4 promoter or into a smaller portion narrowing into the ORF6 region. Alternatively, the heterologous E4 region or portion thereof can be inserted into different areas of the genome such as the E1 or E3 regions. Further, the native E4 region or portion thereof can be deleted and replaced, or left intact. This is not deemed a critical element of the instant invention. What is a critical element is that the heterologous E4 region or portion thereof being inserted is native to a virus of the same or highly similar serotype as the E1 gene product(s) expressed by the complementing cell line.

Following the modification of the adenoviral vector of interest, the recombinant adenovirus is then introduced into an adenoviral E1-complementing cell line and allowed to propagate. The adenovirus is subsequently harvested and rescued from the complementing cell line.

The resultant virus can be studied and used in various gene therapy and vaccine efforts. The virus, therefore, forms an important aspect of the instant invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 illustrates a transcription map for adenovirus serotype 5. The linear genome is divided into 100 map units as well as into r- and l- strands which designate the direction of transcription. Early transcription units are designated with an E and are active prior to viral DNA replication. Late transcription units are designated with and L and are active primarily after DNA replication. Promoters are represented as brackets and polyadenylation sites as arrowheads. The tripartite leader is designated 1, 2, and 3.

FIGURES 2A-1 through 2A-10 illustrate the nucleic acid sequence for the wild-type adenovirus 35 (SEQ ID NO: 1) utilized in the Examples.

FIGURE 3 illustrates the homologous recombination scheme utilized to recover pAd35ΔE1.

FIGURE 4 illustrates the various configurations of the E4 regions (or portions) within the alternative serotype recombinants.

FIGURE 5 illustrates the homologous recombination scheme utilized to recover pAd35ΔE1ΔE4Ad5Orf6.

FIGURE 6 illustrates the nucleic acid sequence encoding the gag expression cassette (SEQ ID NO: 2). The various regions of the figure are as follows: (1) a first underlined segment of nucleic acid sequence encoding the immediate early gene promoter region from human cytomegalovirus; (2) a first segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; (3) a region in caps which contains the coding sequence of HIV-1 gag; (4) a second segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; and (5) a second underlined segment, this segment containing nucleic acid sequence encoding a bovine growth hormone polyadenylation signal sequence.

FIGURE 7 illustrates the nucleic acid sequence encoding the SEAP expression cassette (SEQ ID NO: 3). The various regions of the figure are as follows: (1) a first underlined segment of nucleic acid sequence encoding the immediate early gene promoter region from human cytomegalovirus; (2) a first segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; (3) a region in caps which contains the coding sequence of the human placental SEAP gene; (4) a second segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; and (5) a second underlined segment, this segment containing nucleic acid sequence encoding a bovine growth hormone polyadenylation signal sequence.

FIGURE 8 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10^{10} vp doses of Ad35 vectors. This experiment was designed to address any effects of E3 deletion. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Shown are geometric means for each cohort of 5 mice.

FIGURE 9 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10^{10} vp doses of Ad35 vectors. This experiment was designed to address any effects of Ad5 sequence insertion into the Ad35 genome. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Two extra cohorts received 10^{10} vp and 10^9 vp of Ad5 vector. Shown are geometric means for each cohort of 5 mice.

FIGURES 10A-B illustrate *in vivo* SEAP expression using MRKAd5-based (A) and Ad35ΔE1ΔE4Ad5Orf6-based (B) vector in rhesus macaques. Shown are the serum antigen

levels for individual monkeys following a single intramuscular (i.m.) injection of 10^{11} vp MRKAd5SEAP (filled circles), 10^9 vp MRKAd5SEAP (open boxes) or 10^{11} vp Ad35 Δ E1SEAP Δ E4Ad5Orf6.

FIGURE 11 illustrates *in vivo* SEAP expression in African green monkeys using Ad5- and Ad35-based vectors. Shown are the antigen levels for each animal in serum samples collected two days after the treatment.

FIGURE 12 illustrates the homologous recombination scheme utilized to recover pAd24 Δ E1.

FIGURE 13 illustrates the homologous recombination scheme utilized to recover pAd24 Δ E1Ad5Orf6.

FIGURE 14 illustrates the configuration of E4 regions in the Ad24 recombinants generated.

FIGURE 15 illustrates the growth kinetics of the Ad24-based vectors in PER.C6 cells.

FIGURES 16A-1 through 16A-10 illustrate the nucleic acid sequence for wild-type adenovirus serotype 24 (SEQ ID NO: 5). The ATCC product number for Ad24 is VR-259.

FIGURE 17 illustrates, in tabular format, gag-specific T cell responses in monkeys immunized with MRKAd5-HIVgag and Ad24 HIV vectors. Shown are the numbers of spot-forming cells per million PBMC following incubation in the absence (mock) or presence of Gag peptide pool. The pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

FIGURE 18 illustrates, in tabular format, the characterization of the gag-specific T cells in monkeys immunized with 10^{11} vp of MRKAd5-HIV1gag and Ad24 Δ E1gag Δ Orf6Ad5Orf6. Shown are the percentages of CD3⁺ T cells that are either gag-specific CD4⁺ or gag-specific CD8⁺ cells. These values were corrected for mock values (<0.03%).

FIGURE 19 illustrates individual anti-p24 titers (in mMU/mL) in macaques immunized with gag-expressing adenovirus vectors.

FIGURE 20 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10^{10} vp doses of Ad24 vectors. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Two extra cohorts received 10^{10} vp and 10^9 vp of Ad5 vector. Shown are geometric means for each cohort of 5 mice.

FIGURE 21 illustrates *in vivo* SEAP expression using MRKAd5 and Ad24 vectors in rhesus macaques. Shown are the geometric means of the SEAP levels for cohorts of 3 monkeys. In bars are the standard errors of the geometric means.

FIGURE 22 illustrates a homologous recombination scheme to be utilized to recover pAd24ΔE1ΔE4Ad5Orf6.

FIGURE 23 illustrates gag-specific T cell responses in rhesus macaques immunized following a heterologous Ad5/Ad6 prime-Ad24 boost regimen. a: Mock, no peptide: gag, 20-mer peptide pool encompassing entire gag sequence; b: Peak response after 2 or 3 doses of the priming vaccine; c: 3 wks prior to boost; d: 4 wks after boost; e: ND, not determined.

FIGURE 24 illustrates, in tabular format, the percentages of CD3⁺ T lymphocytes that are gag-specific CD8⁺ cells or gag-specific CD4⁺ cells determined after the Ad24 Boost Immunization (wk 60). Numbers reflect the percentages of circulating CD3⁺ lymphocytes that are either gag-specific CD4⁺ or gag-specific CD8⁺ cells. Mock values (equal to or less than 0.01%) have been subtracted.

FIGURE 25 illustrates gag-specific T cell responses in rhesus macaques immunized following a heterologous Ad 24 prime-Ad5 boost regimen. a: Mock, no peptide: gag, 20-mer peptide pool encompassing entire gag sequence; b: Peak response after 2 doses of the priming vaccine; c: Wk 24; d: 4 wks after boost; e: ND, not determined.

FIGURE 26 illustrates the homologous recombination scheme utilized to recover pAd34ΔE1ΔE4Ad5Orf6.

FIGURE 27 illustrates the homologous recombination scheme utilized to recover pMRKAd34ΔE1ΔE4Ad5Orf6.

FIGURES 28A-1 to 28A-9 illustrate a nucleic acid sequence for wild-type adenovirus serotype 34 (SEQ ID NO: 12). The ATCC product number for Ad34 is VR-716.

FIGURE 29 illustrates the time course of SEAP expression using MRKAd5 and Ad34 vectors in rhesus macaques. Data represent cohort geometric means.

FIGURE 30 illustrates, in tabular format, T cell responses induced using MRKAd5 and Ad34 vectors expressing HIV-1 gag. Data are expressed in numbers of spot-forming cells per million PBMC (SFC/10⁶ PBMC). "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 31 illustrates, in tabular format, the levels of CD4⁺ and CD8⁺ Gag-specific T cells in Ad34-immunized macaques at week 12. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 32 illustrates, in tabular format, T cell responses induced using a heterologous Ad34 prime/Ad35 boost regimen in macaques. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 33 illustrates, in tabular format, the levels of CD4+ and CD8+ Gag-specific T cells in Ad34 primed/Ad35 boosted macaques at week 28. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

5 DETAILED DESCRIPTION OF THE INVENTION

The present invention details an efficient strategy for the propagation and rescue of alternative adenoviral serotypes utilizing available adenovirus production cell lines, nullifying the need to customize available cell lines for a specific serotype of interest. This is enabled by the incorporation of a critical E4 region into the adenovirus to be propagated.

10 The critical E4 region in the instant invention comprises, in the minimum, nucleic acid sequence encoding E4 ORF6 and can comprise the entire region of E4, inclusive of the promoter region. An important characteristic of the imported E4 region is that it is native to a virus of the same or highly similar serotype as the E1 gene product(s) (particularly E1B 55K) of the E1-complementing cell line, but heterologous to (*i.e.*, non-native to a virus of the same
15 serotype as) the adenoviral vector being propagated. As will be detailed below, the heterologous E4 region or portion thereof can be varied and can be inserted into the vector backbone at numerous locations.

The heterologous E4 region or portion thereof can, for instance, be a nucleic acid sequence encoding the entire open reading frame of the non-native E4. This segment of nucleic
20 acid sequence can, in turn, be incorporated into the "native" entire E4 open reading frame of the recipient virus. In such an embodiment, the promoter native to the adenoviral vector would drive the expression of the non-native E4 region within the recombinant replication-defective adenoviral vector. Alternatively, the nucleic acid sequence encoding the entire open reading frame can be inserted into a different region of the adenoviral vector genome, such as for
25 example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

In another embodiment, the heterologous E4 region comprises a nucleic acid sequence encoding the entire open reading frame of E4 and includes a non-native E4 promoter. In this type of embodiment, the E4 region can be inserted into the location of the combined
30 native E4 and E4 promoter region. The non-native E4 region in this embodiment would be driven by expression of the non-native E4 promoter. Alternatively, the nucleic acid sequence encoding the entire open reading frame and the non-native E4 promoter can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

An alternative and further embodiment exists wherein the heterologous E4 region or portion thereof comprises nucleic acid sequence encoding a partial E4 region comprising ORF6 (one aspect of which is a region solely encoding ORF6). In this particular aspect of the invention, the heterologous non-native E4 protein can, in certain embodiments, replace the non-native ORF6 region or the entire E4-encoding region of the native virus. In the latter situation, the promoter driving expression of the non-native ORF6 can either be the native E4 promoter or a heterologous, non-native promoter operatively linked to the non-native ORF6, while in the latter, the expression of the non-native ORF6 would generally be driven by the native E4 promoter. Alternatively, the nucleic acid sequence encoding a partial E4 region comprising ORF6 can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

As one of skill in the art can appreciate, there are various ways in which one can envision the supply of a heterologous E4 nucleic acid sequence *in cis* to an adenoviral vector and thereby enable its growth based on Applicants' novel findings herein. Moreover, as one of skill in the art can appreciate, either native or non-native promoters can be utilized to drive expression of the heterologous E4 region or portion thereof.

Adenovirus pre-plasmids (plasmids comprising the genome of the replication-defective adenovirus with desired deletions and insertions) can be generated by homologous recombination using adenovirus backbones and an appropriate shuttle vector (designed to target specific deletions and incorporate desired restriction sites into the resultant plasmid). Shuttle vectors of use in this process can be generated using general methods widely understood and appreciated in the art, *e.g.*, PCR of the adenoviral terminal ends taking into account the desired deletions, and the sequential cloning of the respective segments into an appropriate cloning plasmid. The adenoviral pre-plasmid can then be digested and transfected into the complementing cell line via calcium phosphate co-precipitation or other suitable means. Virus replication and amplification then occurs, a phenomenon made evident by notable cytopathic effect. Infected cells and media are then harvested after viral replication is complete (generally, 7-10 days post-transfection).

It is to be noted that various alternative adenoviral serotypes can be developed in accordance with the disclosed methods and, particularly, alternative adenoviral serotype vectors that were previously unable to be propagated or very inefficiently propagated utilizing existing adenoviral production cell lines based on subgroup C complementing E1 sequence. The various adenoviral vectors that can be developed in accordance with the instant methods include adenoviral vectors of subgroups A-F (for instance, serotypes of subgroups A, B (*e.g.*, serotypes

11, 14, 16, 21, 34 and 35), C (e.g., serotypes 2 and 5), D (e.g., serotypes 24, 26 and 36), E (e.g., serotype 4) and F.

In preferred embodiments, the various non-group C family members can be developed with heterologous E4 supplied from a subgroup C member such as adenovirus serotype 5. Particular embodiments of the instant invention utilize a development scheme wherein the heterologous E4 protein is derived from a wildtype adenovirus serotype 5 sequence; see, e.g., a viral sequence which has been deposited with the American Type Culture Collection ("ATCC") under ATCC Deposit No. VR-5 (for which a transcription map can be found in Figure 1). A particular example of this type of embodiment is wherein an adenovirus of subgroup B (or any non-C subgroup) comprising heterologous E4 proteins *in cis* from Ad5 is propagated in Ad5 E1-complementing cell lines, for instance, PER.C6™ or 293. Applicants have, in fact, successfully propagated E1- serotypes 10, 24, 34, and 35 via use of this particular embodiment.

One of skill in the art can readily identify alternative adenovirus serotypes (e.g., alternative serotypes of subgroups A, B (e.g., serotypes 11, 14, 16, 21, 34 and 35), C, (e.g., serotypes 2 and 5), D (e.g., serotypes 24, 26 and 36), E (e.g., serotype 4) and F) for the supply of the heterologous E4 protein. As long as the heterologous E4 region (or portion thereof comprising ORF6) of the vector is native to a virus of the same or highly similar serotype as the E1 region of the complementing cell line, the methods of the instant invention are widely applicable to the propagation and rescue of adenovirus of all serotypes. In light of the present disclosure, one can readily envision, for instance, how a complementing cell line based on a non-subgroup C adenovirus (e.g., the Ad35 cell line of EP 1 054 064 A1) can be utilized to propagate a virus of an adenoviral vector of subgroup C (e.g., adenovirus serotype 5) provided that the appropriate nucleic acid sequence encoding an E4 protein provided *in cis* is native to a virus of the same or highly similar serotype as that of the E1 expressed by the complementing cell line (i.e., an Ad35 E4 protein).

Complementing cell lines of use in the instant invention are available in the art and are not limited to any specific type. The critical feature, again, is that the heterologous segment of E4-encoding nucleic acid sequence provided *in cis* to the replication-defective vector being propagated be native to a virus of the same or highly similar serotype as the E1 expressed by the complementing cell line. One aspect of the instant invention employs E1-complementing cell lines wherein the expressed E1 is of serotype 5; e.g., PER.C6™ and 293 cell lines. Both these cell lines express the adenoviral E1 gene product. PER.C6™ is described in Fallaux *et al.*, 1998 *Human Gene Therapy* 9:1909-1917, hereby incorporated by reference. 293 cell lines are described in Graham *et al.*, 1977 *J. Gen. Virol.* 36:59-72, hereby incorporated by reference.

Another aspect of the instant invention are the adenoviral vectors of any serotype falling with adenoviral subgroups A, B, C, D, E and F (for instance, alternative serotypes of subgroups A, B (*e.g.*, serotypes 11, 14, 16, 21, 34 and 35), C (*e.g.*, serotype 2), D (*e.g.*, serotypes 24, 26 and 36), E (*e.g.*, serotype 4) and F) which are modified to contain a non-native E4-
5 encoding nucleic acid sequence *in cis* which comprises, in whole or in part, nucleic acid sequence encoding open reading frame 6 (ORF6). Virus in accordance with this description can be propagated in accordance with the above-described methods and rescued using any suitable means known in the art.

Another aspect of the instant invention is a vector in accordance with the instant
10 invention which comprises a heterologous passenger gene in addition to that of the heterologous E4 nucleic acid sequence. In specific embodiments, the passenger gene encodes an antigen.

As one of ordinary skill in the art will appreciate, the instant methods are not limited by the heterologous gene that can be incorporated. The instant invention relates generally to a means by which to propagate multiple serotypes of adenovirus in a single
15 complementing cell line and the recombinant virus that make the process possible. In preferred embodiments, the passenger gene is incorporated into the E1 deletion. In alternatively preferred embodiments, the passenger gene is inserted in an E3-deleted region. The position of the passenger gene, as one of ordinary skill in the art will appreciate, can be varied according to the specific complementing cell utilized and the specific deletions present within the replication-
20 defective adenovirus genome.

In specific embodiments the passenger gene can encode an HIV-1 antigen, and in more preferred embodiments selected from the group consisting of genes encoding HIV-1 gag, pol, nef and env. In alternative embodiments, the passenger gene can be a reporter gene, such as secreted alkaline phosphatase (SEAP).

The passenger gene preferably exists in the form of an expression cassette. A
25 gene expression cassette preferably comprises (a) a nucleic acid sequence encoding a protein of interest; (b) a promoter operatively linked to the nucleic acid sequence encoding the protein; and (c) a transcription termination sequence. The transcriptional promoter of the adenoviral vector is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the
30 promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman *et al.*, 1991 *Nucl. Acids Res.* 19:3979-3986), which is hereby incorporated by reference), in certain embodiments without intronic sequences. Those skilled in the art, however, will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters

may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

The promoter may comprise a regulatable sequence such as the Tet operator sequence. This is extremely useful, for example, in cases where the gene products are affecting a result other than that desired and repression is sought.

Transcription termination sequences can also be utilized within the gene expression cassettes. Preferred termination sequences are, for instance, the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:4).

Further embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Construction and Rescue

An E1- Ad35-based pre-adenovirus plasmid was constructed in order to determine whether an E1- Ad35 vector (a representative group B serotype) could be propagated in a group C E1-complementing cell line. The general strategy used to recover Ad35 as a bacterial plasmid is illustrated in Figure 3. Cotransformation of BJ5183 bacteria with purified wild-type Ad35 viral DNA and a second DNA fragment termed the Ad35 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome (*see* Figures 2A-1 to 2A-10) separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad5 457 to 3402 with a unique *Swa* I site located in the deletion. The Ad35 sequences in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which recombination can occur. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1. Pre-Adenovirus plasmid pAd35ΔE1 contains Ad35 sequences from 4 to 456 and bp 3403 to 34793.

To determine if pre-adenovirus plasmid pAd35ΔE1 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into a T-25 flask of PER.C6 cells using the calcium phosphate co-precipitation technique. *Pme* I digestion releases the viral genome from the plasmid sequences allowing viral replication to occur after entry into 293 cells. Viral cytopathic effect (CPE), indicating that virus replication and amplification is occurring, was never observed. Cells and media from the transfection were harvested at 14 days post transfection, freeze-thawed three times, clarified by centrifugation and used to infect new PER.C6 cells but no virus was ever amplified. Following multiple attempts, we have been unable to rescue and amplify pAd35ΔE1 in PER.C6 cells.

EXAMPLE 2

Insertion of Ad5 Orf 6 and Ad5 E4 into the Ad5 Genome

To refine the strategy of including Ad5 Orf6 in the genome of an alternative serotype so that propagation could take place in a Ad5/group C complementing cell line four additional strategies were developed. In the first strategy, the entire alternative serotype E4 region (not including the E4 promoter) was deleted and replaced with Ad5 Orf6. In the second strategy, just the alternative serotype Orf6 gene was deleted and replaced with Ad5 Orf6. In the third strategy, the entire alternative serotype E4 coding region (not including the E4 promoter) was deleted and replaced with the Ad5 E4 coding region (not including the Ad5 E4 promoter) and, in the final strategy, the entire alternative serotype E4 coding and promoter region was deleted and replaced with the Ad5 E4 promoter and coding region. The configuration of the E4 regions generated by the four strategies is diagramed in Figure 4. For each of these strategies the desired pre-Adenovirus plasmid was generated by bacterial recombination. Cotransformation of BJ 5183 bacteria with purified wild-type viral DNA and the appropriately constructed ITR cassette resulted in the circularization of the viral genome by homologous recombination. The construction of each pre-Ad plasmid, based on Ad35, is outlined below:

To construct pAd35ΔE1ΔE4Ad5Orf6 (An Ad35 pre-Ad plasmid containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31913 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 31913 and 34419 generating pNEBAd35-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a

bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31912 to 34418 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct, Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31913 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria (Figure 5). The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme*I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5Orf6. Pre-Adenovirus plasmid pAd35ΔE1ΔE4Ad5Orf6 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31913 and bp 34419 to bp 34793 with Ad5Orf6 cloned between bp 31913 and bp 34419.

To construct pAd35ΔE1ΔOrf6Ad5Orf6 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 Orf6 substituted with Ad5 Orf6), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 32081 and bp 32990 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-10. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 32081 and 32990 generating pNEBAd35-10Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and a deletion of E4 Orf6 from Ad35 bp 32082 to 32989 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct, Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 32081 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔOrf6Ad5Orf6. Pre-Adenovirus plasmid pAd35ΔE1ΔOrf6Ad5Orf6 contains Ad35

sequences from bp 4 to 456; bp 3403 to bp 32081 and bp 32990 to bp 34793 with Ad5Orf6 cloned between bp 32081 and bp 32990.

To construct pAd35 Δ E1 Δ E4Ad5E4 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 substituted with Ad5 E4), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31838 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-7. Next the Ad5 E4 coding region was generated by PCR and cloned between Ad35 bp 31838 and 34419 generating pNEBAd35-7Ad5E4-2 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31839 to 34418 into which the Ad5 E4 coding region was introduced in an E4 parallel orientation. In this construct, the Ad5 E4 region is expressed using the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31838 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35 Δ E1 Δ E4Ad5E4. Pre-Adenovirus plasmid pAd35 Δ E1 Δ E4Ad5E4 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31838 and bp 34419 to bp 34793 with the Ad5 E4 coding region (Ad 5 bp 32914 to bp 35523) cloned between bp 31838 and bp 34419.

To construct pAd35 Δ E1 Δ E4Ad5PE4 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 coding region and promoter substituted with Ad5 E4 coding region and promoter), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31838 and bp 34660 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-8. Next the Ad5 E4 promoter and coding region was generated by PCR and cloned between Ad35 bp 31838 and 34660 generating pNEBAd35-8Ad5E4PC (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication,

ampicillin resistance gene, and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31839 to 34659 into which the Ad5 E4 promoter and coding region was introduced in an E4 parallel orientation. In this construct, the Ad5 E4 region is expressed using the Ad5 E4 promoter. The Ad35 sequences (bp 31599 to 31838 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5PE4. Pre-Adenovirus plasmid pAd35ΔE1ΔE4Ad5PE4 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31838 and bp 34660 to bp 34793 with the Ad5 E4 promoter and coding region (Ad 5 bp 32914 to bp 35826) cloned between bp 31838 and bp 34660.

EXAMPLE 3

Rescue of pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4 into Virus

In order to determine if pre-adenovirus plasmids pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmids were each digested with *Pme* I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique; Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc. *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring, was observed for all construct. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect aT-225 flasks of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then

digested with *Hind*III and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with *Pme*I/*Hind*III prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 4

Insertion of an Expression Cassette into pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4

In order to introduce a gag or SEAP expression cassette into the E1 region of the various Ad35 pre-Adenovirus plasmids described above (pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4) bacterial recombination was again used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence (Figure 6), was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2 (a precursor to the Ad35 ITR cassettes described above), generating pNEBAd35CMVgagBGHpA. pNEBAd35-2 contains Ad35 sequences from the left end of the genome (bp 4 to 456 and bp 3403 to 3886) with a unique *Swa*I site between bp 456 and 3403 at the position of the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by *Eco*RI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pNEBAd35-2. This cloning step resulted in the gag expression cassette being cloned into the E1 deletion between bp 456 and 3403 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad35 bp 4 to 456 and bp 3403 to 3886 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad35 pre-Ad plasmids (pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4, pAd35ΔE1ΔE4Ad5PE4), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of corresponding Ad35 gag-containing pre-Adenovirus plasmids (pAd35ΔE1gagΔE4Ad5Orf6, pAd35ΔE1gagΔOrf6Ad5Orf6, pAd35ΔE1gagΔE4Ad5E4, and pAd35ΔE1gagΔE4Ad5PE4) by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad35 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence (Figure 7) was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2, generating pNEBAd35CMVSEAPBGHPA. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SmaI site in pNEBAd35-2. The transgene was then recombined into the various Ad35 backbones generating pAd35ΔE1SEAPΔE4Ad5Orf6, pAd35ΔE1SEAPΔOrf6Ad5Orf6, pAd35ΔE1SEAPΔE4Ad5E4, and pAd35ΔE1SEAPΔE4Ad5PE4 as described above for the gag transgene. All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

EXAMPLE 5

In vivo Transgene Expression

A. Immunization

Female mice were between 4-10 weeks old. The total dose of each vaccine was suspended in 0.1 mL of buffer. The vectors were given to both quadriceps of each animal with a volume of 50 μL per quad and using 0.3-mL 28G1/2 insulin syringes (Becton-Dickinson, Franklin Lakes, NJ). The rhesus macaques and African green monkeys were between 2-5 kg in weight. For the primates, the total dose of each vaccine was suspended in 1 mL of buffer. The monkeys were anesthetized (ketamine/xylazine mixture) and the vaccines were delivered i.m. in 0.5-mL aliquots into two muscle sites using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Serum samples were collected at defined intervals and stored frozen until the assay date. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

Serum samples were analyzed for circulating SEAP levels using TROPIX phospho-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 μL aliquots of each serum were mixed with 45 μL of kit-supplied dilution buffer in a 96-well white DYNEX plate.

Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey or mouse serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the well for 30 minutes at 65 °C. Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorder using DYNEX luminometer. RLU readings are converted to ng/mL SEAP using a log-log regression analyses.

C. Rodent Results

In the first mouse experiment, cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^{10} vp Ad35 Δ E1SEAP Δ E4Ad5Orf6; (2) 10^{10} vp Ad35 Δ E1SEAP Δ E3 Δ E4Ad5Orf6; or (3) 10^{10} vp Ad35 Δ E1SEAP. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 8. Results indicate that (1) the Ad35 constructs are all capable of expressing the SEAP transgene and that (2) the introduction of Ad5Orf6 sequence where the deleted Ad35E4 was did not significantly affect the transgene expression relative to Ad35 Δ E1SEAP. Ad35 Δ E1SEAP Δ E3 Δ E4Ad5Orf6 also yielded a similar expression profile as Ad35 Δ E1SEAP. The levels of SEAP in the serum dropped after day 2 and were at background levels by day 12.

The second mouse experiment evaluates the effect of a full Ad5E4 replacement instead of an Ad5Orf6 substitution for the Ad35 E4 cassette. Here, cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^{10} vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; (3) 10^{10} vp Ad35 Δ E1SEAP Δ E4Ad5Orf6; (4) 10^{10} vp Ad35 Δ E1SEAP Δ E4Ad5E4; or (5) 10^{10} vp Ad35 Δ E1SEAP Δ E4Ad5PE4. The introduction of Ad5E4 or Ad5PE4 resulted in comparable if not, slightly improved expression levels compared to the vector with the Ad5Orf6 insertion (Figure 9). The peak levels for the Ad35 constructs are lower than those produced by Ad5SEAP (at least 10-fold).

D. Primate Results

Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the following vectors: (1) 10^{11} vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; or (3) 10^{11} vp Ad35 Δ E1SEAP Δ E4Ad5Orf6. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results for the individual monkeys are shown in Figures 10A-B. Results indicate that the peak level of SEAP product produced by the alternative adenovirus serotype was lower than but were within 3-fold of that of MRKAd5SEAP at the same

high dose level of 10^{11} vp. The levels observed from the Ad35 vector were about 50-fold higher than those observed using 10^9 vp of MRKAd5SEAP. The levels of SEAP in the serum dropped after day 10 and were close to background as early as day 15.

5 A separate experiment using African green monkeys was conducted to examine the effect of the additional E3 deletion or the full Ad5E4 substitution on in vivo gene expression. In here, cohorts of 2-3 African green macaques were given single intramuscular injections of one of the following vectors: (1) 10^{11} vp MRKAd5-SEAP; (2) 10^{10} vp MRKAd5-SEAP; (3) 10^9 vp MRKAd5-SEAP; (4) 10^{10} vp Ad35 Δ E1SEAP Δ E4Ad5Orf6; (5) 10^{10} vp Ad35 Δ E1SEAP Δ E3 Δ E4Ad5Orf6; or (6) 10^{10} vp Ad35 Δ E1SEAP Δ E4Ad5E4. Results (Figure 10 11) indicate that the peak levels of SEAP product produced by Ad35 Δ E1SEAP Δ E3 Δ E4Ad5Orf6 and Ad35 Δ E1SEAP Δ E4Ad5E4 were comparable if not, slightly improved compared to Ad35 Δ E1SEAP Δ E4Ad5Orf6.

EXAMPLE 6

15 In vivo Immunogenicity

A. Immunization

Cohorts of 3-6 animals were given intramuscular injections at wk 0 and wk 4 of either of the following constructs: (1) 10^{11} vp MRKAd5-HIV1 gag; or (2) 10^{11} vp of 20 Ad35 Δ E1gag Δ E4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson). Sera and peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the 25 immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

30 B. ELISPOT Assay

The IFN- γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., 35 Dublin, CA). To each well, 50 μ L of $2-4 \times 10^5$ peripheral blood mononuclear cells (PBMCs)

were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 μ L of media or the gag peptide pool at 8 μ g/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO₂ for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10⁶ cell input.

C. Intracellular Cytokine Staining

To 1 ml of 2 x 10⁶ PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μ g/mL. For gag-specific stimulation, 10 μ L of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μ L of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO₂, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μ L per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μ L anti-hCD8-PerCP, clone SK1 (Becton Dickinson, Franklin Lakes, NJ); and 20 μ L anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μ L 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μ g of FITC-anti-hIFN- γ , clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both CD4⁺ and CD8⁺ populations, and for both mock and gag-peptide reaction tubes of a sample.

D. Results

PBMCs collected at regular 4-wk intervals were analyzed in an ELISPOT assay. Results (Table 1) indicate that the Ad35 Δ E1gag Δ E4Ad5Orf6 is able to induce in non-human primates significant levels of gag-specific T cells. After a single dose (wk 4), the Ad35-induced responses were about 5-fold lower than that of MRKAd5-HIV1 gag. After the second dose (wk

8), the responses between both cohorts were comparable; the differences became pronounced in the succeeding time points.

Table 1. Gag-specific T cell response in monkeys immunized with MRKAd5-HIV1 gag and Ad35ΔE1gagΔE4Ad5Orf6. Shown is the number of spot-forming cells per million PBMC following incubation in the absence (mock) or presence of Gag H peptide pool. The H pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

Grp	Vaccine Wk 0, Wk 4	Monkey ID	Pre		Wk 4		Wk 8		Wk 12		Wk 16	
			Mock	Gag H	Mock	Gag H	Mock	Gag H	Mock	Gag H	Mock	Gag H
1	MRKAd5-HIV1 gag 10 ¹¹ vp	00C018	1	5	13	1025	0	824	3	753	1	533
		00C034	0	4	5	219	5	404	0	491	1	350
		00C058	4	4	3	1086	0	440	0	439	0	599
2	Ad35ΔE1gagΔE4Ad5Orf6 10 ¹¹ vp	00D045	1	1	3	168	5	645	4	178	0	91
		00D067	1	4	5	89	0	103	0	76	0	19
		00D068	1	4	10	34	5	365	3	143	0	95
		00D054	3	15	10	195	0	501	3	350	0	124
		00D075	3	5	18	275	13	716	3	158	0	103
		00D073	14	26	1	241	3	485	3	278	0	148
3	Naive	00D087	1	1	3	3	8	54	3	5	3	1

Intracellular IFN- γ staining analyses of PBMC collected at wk 8 suggest that the Ad35-based vaccine is able to induce both HIV-specific CD4⁺ and CD8⁺ T cells (Table 2).

Table 2. Characterization of the gag-specific T cells in monkeys immunized with MRKAd5-HIV1 gag and Ad35ΔE1gagΔE4Ad5Orf6. Shown are the percentages of CD3⁺ T cells that are either gag-specific CD4⁺ or gag-specific CD8⁺ cells. These values were corrected for mock values (<0.02%).

Grp	Vaccine Wk 0, Wk 4	Monkey ID	Wk 8	
			%CD4 ⁺ CD3 ⁺	%CD8 ⁺ CD3 ⁺
1	MRKAd5-HIV1 gag 10 ¹¹ vp	00C018	0.08	0.37
		00C034	0.09	0.06
		00C058	0.03	0.21
2	Ad35ΔE1gagΔE4Ad5Orf6 10 ¹¹ vp	00D045	0.06	0.08
		00D067	0.02	0.02
		00D068	0.15	0.02
		00D054	0.05	0.08
		00D075	0.08	0.05
		00D073	0.09	0.06

In a separate experiment, 3 different Ad35 constructs expressing HIV-1 gag were evaluated for their immunogenicity in macaques. Here, cohorts of 3 macaques were given immunizations at wk 0 and 4 of either of the following vectors: (1) 10¹⁰ vp Ad35ΔE1gagΔE4Ad5Orf6; (2) 10¹⁰

vp Ad35ΔE1gagΔE3ΔE4Ad5Orf6; or (3) 10¹⁰ vp Ad35ΔE1gagΔE4Ad5E4. The levels of T cell immunity induced by all 3 vectors were comparable at this stage (Table 2), suggesting that the additional E3 deletion or full Ad5E4 substitution does not appear to impair the immunogenic properties of the vector.

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Table 3. Gag-specific T cell response in monkeys immunized with several Ad35ΔE1ΔE4-based vectors. Shown is the number of spot-forming cells per million PBMC following incubation in the absence (mock) or presence of Gag H peptide pool. The H pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

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Grp	Vaccine Wk 0, Wk 4	Monkey ID	Pre		Wk 4		Wk 8	
			Mock	Gag H	Mock	Gag H	Mock	Gag H
1	Ad35ΔE1gagΔE4Ad5Orf6 10 ¹⁰ vp	00C047	4	1	0	20	0	189
		00C157	8	5	1	81	1	833
		00C078	3	1	0	46	4	349
2	Ad35ΔE1gagΔE3ΔE4Ad5Orf6 10 ¹⁰ vp	00C091	1	1	1	118	3	315
		00C122	3	0	0	31	1	138
		00D177	3	3	1	45	1	64
3	Ad35ΔE1gagΔE4Ad5E4 10 ¹⁰ vp	00D018	3	19	29	120	23	193
		00D046	8	5	1	21	10	143
		00D063	3	4	0	63	4	371
Naïve	none	00D363	0	5	ND	ND	0	0

EXAMPLE 7

Construction and Rescue of pAd24ΔE1.

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An E1- Ad24-based pre-adenovirus plasmid was constructed in order to determine whether an E1- Ad24 vector (a representative group D serotype) could be propagated in an Ad5/group C E1-complementing cell line. Since at the time the vector construction was initiated the complete sequence of Ad24 (*see* Figures 16A-1 through 16A-10; subject of copending application serial no. 60/455, 312, filed March 17, 2003) was unknown we took advantage of some sequence homology between Ad24 and Ad17. The general strategy used to recover Ad24 as a bacterial plasmid is illustrated in Figure 12 and described below.

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Cotransformation of BJ5183 bacteria with purified wild-type Ad24 viral DNA and a second DNA fragment termed the Ad17 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 34469 to 35098) and left (bp 4 to 414 and bp 3373 to 4580) end of the Ad17 genome (Accession No. AF108105) separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad17

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(bp 415 to 3372) with a unique *Swa* I site located in the deletion. The Ad17 sequences in the ITR cassette provide regions of homology with the purified Ad24 viral DNA in which recombination can occur. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the Ad24 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd24ΔE1. pAd24ΔE1 contains Ad17 sequences from bp 4 to 414 and from bp 3373 to 4580, Ad24 bp 4588 to 34529, and Ad17 bp 34469 to 35098 (bp numbers refer to the wt sequence for both Ad17 and Ad24). pAd24ΔE1 contains the coding sequences for all Ad24 virion structural proteins that constitute its serotype specificity. This approach can be used to circularize any group D serotype into plasmid form which has sufficient homology to Ad17.

To determine if pre-adenovirus plasmid pAd24ΔE1 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into a 6 cm dish of 293 cells using the calcium phosphate co-precipitation technique. *Pme* I digestion releases the viral genome from the plasmid sequences allowing viral replication to occur after entry into 293 cells. Viral cytopathic effect (CPE), indicating that virus replication and amplification is occurring, was very slow to arise. Following multiple attempts, we were successful at rescuing and amplifying Ad24ΔE1 but the virus grew to lower titers and took more passages to amplify than a similar Ad5 based vector. In order to verify the genetic structure of the virus, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hind*III and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pme*I/*Hind*III prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

EXAMPLE 8

Insertion of Ad5 Orf 6 into the E1 region of Ad24

In order to determine if the insertion of Ad5 E4 Orf6 into the Ad24 genome would allow more efficient propagation in a group C E1 complementing cell line we constructed an Ad24 based pre-adenovirus plasmid containing Ad5 Orf6 in the E1 region. In order to introduce Ad5 Orf6 in to the E1 region of pAd24ΔE1, bacterial recombination was used. An Ad5 Orf6 transgene consisting of the Ad5 Orf6 coding region flanked by the HCMV promoter and pA was cloned into the E1 deletion in an Ad17 shuttle vector (a precursor to the Ad17 ITR cassette). The Ad5 Orf6 transgene was cloned between bp 414 and 3373 in the E1 anti-parallel

orientation. The shuttle vector containing the Ad5 Orf6 transgene was digested to generate a DNA fragment consisting of the transgene flanked by Ad17 sequences (bp 4 to 414 and bp 3373 to 4580) and the fragment was purified after electrophoresis on an agarose gel.

Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pAd24ΔE1, which had been linearized in the E1 region by digestion with *Swa*I, resulted in the generation of pAd24ΔE1Ad5Orf6 by homologous recombination (Figure 13). Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1Ad5Orf6.

In order to determine if pre-adenovirus plasmid pAd24ΔE1Ad5Orf6 could be rescued into virus and propagated in an Ad5/group C E1 complementing cell line, pAd24ΔE1Ad5Orf6 was digested with *Pme* I and transfected into a 6 cm dish of 293 cells using the calcium phosphate co-precipitation technique. *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into 293 cells. Once complete viral cytopathic effect (CPE) was observed at approximately 7-10 days post transfection, the infected cells and media were freeze/thawed three times and the cell debris pelleted. The virus was amplified in two additional passages in 293 cells and then purified from the final infection by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the virus, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hind*III and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pme*I/*Hind*III prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

EXAMPLE 9

Insertion of Ad5 Orf 6 into the E4 region of Ad24

To refine the strategy of including Ad5 Orf6 in the genome of an alternative serotype so that propagation could take place in an Ad5/group C complementing cell line two additional strategies were developed. In the first strategy, the entire alternative serotype E4 region (not including the E4 promoter) was deleted and replaced with Ad5 Orf6. In the second strategy, just the alternative serotype Orf6 gene was deleted and replaced with Ad5 Orf6. The configuration of the E4 regions generated by the two strategies is diagramed in Figure 14. For each of these strategies the desired pre-Adenovirus plasmid was generated by bacterial recombination. Cotransformation of BJ 5183 bacteria with pAd24ΔOrf6BstZ17I and the

appropriately constructed Ad24 E4 shuttle plasmid resulted in the generation of the desired Ad24 based pre-Ad plasmid. PAd24ΔOrf6BstZ17I, a derivative of pAd24ΔE1, was constructed so that the E4 region in the Ad24 pre-Ad plasmid could be easily modified using bacterial recombination. PAd24ΔOrf6BstZ17I contains a deletion in the E4 region from Ad24 bp 32373 to bp 33328 with a unique *Bst*Z17I site located at the position of the deletion. The complete sequence of pAd24ΔOrf6BstZ17I consists of Ad17 sequences from bp 4 to 414 and from bp 3373 to 4580, Ad24 bp 4588 to 32372 and from 33329 to 34529, and Ad17 bp 34469 to 35098 (bp numbers refer to the wt sequence for both Ad17 and Ad24).

To construct pAd24ΔE1ΔE4Ad5Orf6 (An Ad24 pre-Ad plasmid containing an E1 deletion and a deletion of E4 substituted with Ad5 Orf6), an Ad24 E4 shuttle plasmid was constructed by digesting pAd24ΔE1 with *Pme*I and *Bsr*GI and cloning the restriction fragment representing the E4 region (bp 31559 to bp 35164) into pNEB193, generating pNEBAd24E4. PNEBAd24E4 was then digested with *Acc*I and *Eco*NI to remove the E4 coding sequences and ligated with an oligo designed to contain *Bgl*II and *Xho*I sites (underlined) (5' ACTCGAGATGTATAGATCT (SEQ ID NO: 6); 5' CTAGATCTATACATCTCGAG (SEQ ID NO: 7)), generating pNEBAd24ΔE4. PNEBAd24ΔE4 was then digested with *Bgl*II and *Xho*I and ligated with the Ad5 Orf6 gene, which was PCR amplified, generating pNEBAd24ΔE4Ad5Orf6. The PCR primers used to amplify the Ad5 Orf6 gene (5' GCACAGATCTTTGCTTCAGGAATATG (SEQ ID NO: 8); 5' GAGAACTCGAGGCCTACATGGGGGTAGAG (SEQ ID NO: 9)) were designed to contain *Bgl*II and *Xho*I sites (underlined above) for ligation with the pNEBAd24ΔE4 fragment. In the final step pNEBAd24ΔE4Ad5Orf6 E4 shuttle plasmid was digested with *Pvu*I and *Pme*I, the restriction fragments were size fractionated by agarose gel electrophoresis and the desired fragment containing Ad5Orf6 flanked by Ad24 sequences was gel purified. Cotransformation of BJ 5183 bacteria with E4 shuttle fragment and pAd24ΔOrf6BstZ17I, which had been linearized in the E4 region by digestion with *Bst*Z17I, resulted in the generation of pAd24ΔE1ΔE4Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1ΔE4Ad5Orf6.

To construct pAd24ΔE1ΔOrf6Ad5Orf6 (An Ad24 pre-Ad plasmid containing an E1 deletion and a deletion of E4 Orf6 substituted with Ad5 Orf6), an Ad24 E4 shuttle plasmid was constructed in which the Ad24 Orf6 gene was replaced by Ad5 Orf6. To do this the *Eco*R1 restriction fragment representing bp 32126 to bp 33442 of the Ad24 genome (encompassing the E4 Orf6 coding region), was subcloned into the *Eco*RI site in pNEB193, generating pNEBAd24Orf6. In order to delete the E4 Orf6 gene in pNEBAd24Orf6 and replace it with Ad5 Orf6, pNEBAd24Orf6 was digested with *Sty*I and treated with Klenow to blunt the ends and then

digested with to *Eag*I. The desired pNEBAd24Orf6 fragment was then ligated with a PCR product representing the Ad5 Orf6 gene from Ad5 bp 33193 to bp 24125, generating pNEBAd24ΔOrf6Ad5Orf6. The PCR primers used to generate the Ad5 Orf6 fragment (5'CGAGACGGCCGACGCAGATCTGTTTG (SEQ ID NO: 10);

5 5'GAAGTCCCGGGCTACATGGGGGTAG (SEQ ID NO: 11)) were designed to contain *Eag*I and *Sma*I sites (underlined above) for ligation with the pNEBAd24Orf6 fragment. In the final step pNEBAd24ΔOrf6Ad5Orf6 was digested with *Eco*RI, the restriction fragments were size fractionated by agarose gel electrophoresis and the desired fragment containing Ad5Orf6 flanked by Ad24 sequences was gel purified. Cotransformation of BJ 5183 bacteria with the *Eco*RI
10 fragment and pAd24ΔOrf6BstZ17I, which had been linearized in the E4 region by digestion with *Bst*Z17I, resulted in the generation of pAd24ΔE1ΔOrf6Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1ΔOrf6Ad5Orf6.

15 EXAMPLE 10

Rescue of pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6, into Virus

In order to determine if pre-adenovirus plasmids pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6, could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmids were each digested with *Pme* I and transfected into T-25
20 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique; (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring, was observed for both constructs. When CPE was complete, approximately 7-10 days post transfection, the
25 infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect T-225 flasks of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell
30 lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hind*III and treated with Klenow fragment to end-label the restriction
35 fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared

with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with *Pme*I/*Hind*III prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

5 EXAMPLE 11

Comparison of the Growth Kinetics of Ad24 based vectors.

In order to compare the growth kinetic of Ad24ΔE1, Ad24ΔE1Ad5Orf6, Ad24ΔE1ΔE4Ad5Orf6 and Ad24ΔE1ΔOrf6Ad5Orf6 one step growth curves were preformed (Figure 15). PER.C6 cells in 60 mm dishes were infected at 1 vp per cell with either Ad24ΔE1, Ad24ΔE1Ad5Orf6, Ad24ΔE1ΔE4Ad5Orf6 or Ad24ΔE1ΔOrf6Ad5Orf6. Cells and media were then harvested at various times post infection, freeze thawed three times and clarified by centrifugation. The amount of virus present in the samples was determined by quantitative PCR and is illustrated in Figure 15. This study demonstrates that Ad24 vectors that incorporate Ad5 Orf6 have a distinct growth advantage over Ad24ΔE1 in PER.C6 cells. The instant invention can be practiced with recombinant Ad24 vectors absent a heterologous Orf 6 region where the E1-complementing cell line expresses an Ad24 E1 region or, alternatively, E1 and E4 regions of the same serotype (such as Ad5E1/E4-expressing cell lines).

EXAMPLE 12

Insertion of an Expression Cassette into pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6.

In order to introduce a gag or SEAP expression cassette (*see* Figures 6 and 7, respectively) into the E1 region of the Ad24 pre-Adenovirus plasmids described above (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6) bacterial recombination was used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, was cloned into the E1 deletion in Ad17 shuttle plasmid, pABSA17-3, generating pABSA17HCMVgagBGHpA. The ITR cassette contains sequences from the right (bp 34469 to 35098) and left (bp 4 to 414 and bp 3373 to 4580) end of the Ad17 genome separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad17 (bp 415 to 3372) with a unique *Swa* I site located in the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by *Eco*RI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pABSA17-3. This cloning step resulted in the gag expression cassette being

cloned into the E1 deletion between bp 414 and 3373 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad17 bp 4 to 414 and bp 3373 to 4580 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad24 pre-Ad plasmids (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the corresponding Ad24 gag-containing pre-Adenovirus plasmids (pAd24ΔE1gagΔE4Ad5Orf6, pAd24ΔE1gagΔOrf6Ad5Orf6) by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad24 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence was cloned into the E1 deletion in Ad17 shuttle plasmid, pABSAd17-3, generating pABSAd17HCMVSEAPBGH. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by *Eco*RI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pABSAd17-3. The shuttle vector containing the SEAP transgene was digested to generate a DNA fragment consisting of the SEAP expression cassette flanked by Ad17 bp 4 to 414 and bp 3373 to 4580 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad24 pre-Ad plasmids (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the corresponding Ad24 SEAP-containing pre-Adenovirus plasmids (pAd24ΔE1SEAPΔE4Ad5Orf6, pAd24ΔE1SEAPΔOrf6Ad5Orf6) by homologous recombination. Potential clones were screened by restriction analysis. All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

EXAMPLE 13

In Vivo Immunogenicity

A. Immunization

Cohorts of 3-6 animals were given intramuscular injections at wk 0 and wk 4 of either of the following constructs: (1) 10^{11} vp MRKAd5-HIV1 gag; (2) 10^{10} vp MRKAd5-HIV1 gag; (3) 10^{11} vp of Ad24ΔE1gagΔOrf6Ad5Orf6; (4) 10^{10} vp of

Ad24ΔE1gagΔOrf6Ad5Orf6; or (5) 10^{10} vp of Ad24ΔE1gagΔE4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points (typically 4 wk intervals) during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. ELISPOT Assay

The IFN- γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen et al., 2001 *J. Virol.* 75(2):738-749; Casimiro et al., 2002 *J. Virol.* 76:185-94), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 μ L of $2-4 \times 10^5$ peripheral blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 μ L of media or the gag peptide pool at 8 μ g/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO₂ for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10^6 cell input.

C. Intracellular Cytokine Staining

To 1 ml of 2×10^6 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μ g/mL. For gag-specific stimulation, 10 μ L of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μ L of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO₂, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μ L per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μ L anti-hCD8-PerCP, clone SK1 (Becton Dickinson);

and 20 μ L anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μ L 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μ g of FITC-anti-hIFN- γ , clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both CD4⁺ and CD8⁺ populations, and for both mock and gag-peptide reaction tubes of a sample.

D. Anti-p24 ELISA

A modified competitive anti-p24 assay was developed using reagents from the Coulter p24 Antigen Assay kit (Beckman Coulter, Fullerton, CA). Briefly, to a 250- μ L serum sample, 20 μ L of Lyse Buffer and 15 μ L of p24 antigen (9.375 pg) from the Coulter kit were added. After mixing, 200 μ L of each sample were added to wells coated with a mouse anti-p24 mAb from the Coulter kit and incubated for 1.5 hr at 37°C. The wells were then washed and 200 μ L of Biotin Reagent (polyclonal anti-p24-biotin) from the Coulter kit was added to each well. After a 1 hr, 37°C incubation, detection was achieved using streptavidin-conjugated horseradish peroxidase and TMB substrate as described in the Coulter Kit. OD450nm values were recorded. A 7-point standard curve was generated using a serial 2-fold dilution of serum from an HIV-seropositive individual. The lower cut-off for the assay is arbitrarily set at 10 milli Merck units/mL (mMU/mL) defined by a dilution of the seropositive human serum. This cutoff falls at approximately 65% of the maximum bound control signal which corresponds to that obtained with the diluent control only and with no positive analyte.

E. Results

PBMCs collected at regular 4-wk intervals were analyzed in an ELISPOT assay (Figure 17). Both Ad24 Δ E1gag Δ Orf6Ad5Orf6 and Ad24 Δ E1gag Δ E4Ad5Orf6 were able to induce significant levels of gag-specific T cells in non-human primates. At 10¹¹ vp dose level, the Ad24-induced responses were within 2-3-fold of those of MRKAd5-HIV1 gag. Both Ad24 vectors were also able to induce detectable levels of gag-specific T cells at 10¹⁰ vp but were lower than those observed using MRKAd5gag at the same dose.

PBMCs collected at wk 12 from the vaccinees were analyzed for intracellular IFN- γ staining after the priming immunizations. The assay results provided information on the relative amounts of CD4⁺ and CD8⁺ gag-specific T cells in the peripheral blood (Figure 18). The

results indicated that the prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4⁺ and CD8⁺ T cells.

F. Humoral Immune Responses

5 The Ad24-based vaccine vector was able to generate detectable levels of circulating anti-gag antibodies at the reasonably high dose level (Figure 19). No detectable titers were observed at equal to or lower than 10¹⁰ vp, suggesting the existence of a dose-dependent response.

10 EXAMPLE 14

In Vivo Transgene Expression

A. Immunization

15 Cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10¹⁰ vp Ad24ΔE1SEAPΔE4Ad5Orf6; (2) 10¹⁰ vp Ad24ΔE1SEAPΔOrf6Ad5Orf6; (3) 10¹⁰ vp MRKAd5SEAP; and (4) 10⁹ vp MRKAd5SEAP. Female mice were between 4-10 weeks old. The total dose of each vaccine was suspended in 0.1 mL of buffer. The vectors were given to both quadriceps of each of the animals with a volume of 50 uL per quad and using 0.3-mL 28G1/2 insulin syringes (Becton-Dickinson, Franklin Lakes, NJ). For the primates, the total dose of each vaccine was suspended in 1 mL of buffer. The monkeys were anesthetized (ketamine/xylazine mixture) and the vaccines were delivered i.m. in 0.5-mL aliquots into two muscle sites using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Serum samples were collected at defined intervals and stored frozen until the assay date. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

30 Serum samples were analyzed for circulating SEAP levels using TROPIX phospho-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 uL aliquots of each serum were mixed with 45 uL of kit-supplied dilution buffer in a 96-well white DYNEX plate. Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the wells for 30 minutes at 65 °C.

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Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorder using DYNEX luminometer. RLU readings are converted to ng/mL SEAP using a log-log regression analyses.

5 C. Rodent Results

Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 20. Results indicate that (1) both Ad24 constructs are all capable of expressing the SEAP transgene in vivo to comparable levels; and that (2) the level of expression achieved using the Ad24 vectors are comparable to that of Ad5 at 10-fold
10 lower dose. The levels of SEAP in the serum dropped dramatically after day 2 and were at background levels by day 12.

D. Primate Results

Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the following vectors: (1) 10^{11} vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; (3) 10^{11} vp Ad24 Δ E1SEAP Δ Orf6Ad5Orf6; or (4) 10^{11} vp Ad24 Δ E1SEAP Δ E4Ad5Orf6. Serum
15 samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 21.

Results indicate that the peak levels of SEAP product produced by adenovirus serotype 24 were lower than but were within 3-fold of that of MRKAd5 at the same high dose
20 level of 10^{11} vp (Figure 21). The levels observed with adenovirus serotype 24 are generally 50-fold higher than those observed using 10^9 vp of MRKAd5. The levels of SEAP in the serum dropped dramatically after day 10 and were close to background as early as day 15. These observations strongly indicate that adenovirus serotype 24 is very efficient in expressing a transgene following intramuscular administration in a primate.

25

EXAMPLE 15

Construction of pMRKAd24 Δ E1 Δ E4Ad5Orf6

To construct pMRKAd24 Δ E1 Δ E4Ad5Orf6 (An Ad24 pre-Ad plasmid, composed entirely of Ad24 sequence and containing an E1 deletion and an E4 deletion substituted with
30 Ad5 Orf6), an Ad24 ITR cassette was constructed containing sequences from the right (bp 31978 to 32264 and bp 34713 to 35164) and left (bp 4 to 450 and bp 3364 to 3799) end of the Ad24 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd24-4. Next the Ad5 Orf6 open reading frame (Ad5 bp 31192
35 to bp 34078) was generated by PCR and cloned between Ad24 bp 32264 and 34713 generating

pNEBAd24E-Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad24 bp 451 to 3363 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad24 bp 32265 to 34712 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5 Orf6 expression is driven by the Ad24 E4 promoter. The Ad24 sequences (bp 31978 to 32264 and bp 3464 to 3799) in the ITR cassette provide regions of homology with the purified Ad24 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria (Figure 22). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion will release the recombinant Ad24 genome from plasmid sequences. Potential clones will be screened by restriction analysis and one clone was selected as pMRKAd24ΔE1ΔE4Ad5Orf6. Pre-Adenovirus plasmid pMRKAd24ΔE1ΔE4Ad5Orf6 should contain Ad24 sequences from bp 4 to 450; bp 3364 to bp 32264 and bp 34713 to bp 35164 with Ad5Orf6 cloned between bp 32264 and bp 34713. The bp numbering in the above description refers to the wt sequence for both Ad24 and Ad5.

EXAMPLE 16

20 Insertion of HIV-1 gag and SEAP transgenes into pAd24ΔE1ΔE4Ad5Orf6

In order to introduce a gag or SEAP expression cassettes into the E1 region of pMRKAd24ΔE1ΔE4Ad5Orf6, bacterial recombination will be used. An HIV-1 gag expression cassette will consist of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, in the E1 deletion of an Ad24 shuttle plasmid, pNEBAd24-2 (a precursor to the Ad24 ITR cassette described above), generating pNEBAd24CMVgagBGHpA. PNEBAd24-2 contains Ad24 sequences from the left end of the genome (bp 4 to 450 and bp 3364 to 3799) that define the E1 deletion. The gag expression cassette will be obtained from a previously constructed plasmid and cloned into the E1 deletion between bp 450 and 3364 in the E1 parallel orientation. The shuttle vector containing the gag transgene will be digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad24 bp 4 to 450 and bp 3364 to 3799 and the fragment will be purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pMRKAd24ΔE1ΔE4Ad5Orf6 which was linearized in the E1 region by digestion with SwaI, should result in the generation of Ad24 gag-

containing pre-Adenovirus plasmids pMRKAd24ΔE1gagΔE4Ad5Orf6 by homologous recombination. Potential clones will be screened by restriction analysis.

A similar strategy will be used to generate Ad24 pre-Ad plasmids containing a SEAP expression cassette. In this case, a SEAP expression cassette will consist of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence cloned into the E1 deletion of an Ad24 shuttle plasmid, pNEBAd24-2, generating pNEBAd24CMVSEAPBGHpA. The transgene will then be recombined into pMRKAd24ΔE1ΔE4Ad5Orf6 as described above for the gag transgene.

EXAMPLE 17

In Vivo Immunogenicity

A. Immunization

Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. T Cell Responses

Ad24 Vaccine Vector as a Heterologous Booster: Cohort of 4 rhesus macaques was immunized initially with 3 doses (wk 0, 4, 26) of either 10^7 or 10^9 vp of MRKAd5-gag (*see*, PCT/US01/28861, published March 21, 2002) or MRKAd6-gag. At wk 56, the animals received a booster vaccine of 10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6. A separate cohort of naïve animals received a single dose of the booster vaccine. The results of the IFN- γ ELISPOT analyses of PBMC collected during the course of the studies are shown in Figure 23. It is apparent that the Ad24 HIV vectors can be utilized to amplify the existing pools of HIV-specific T cells. The increases in the levels of gag-specific T cells from the pre-boost levels to those measured at 4 wks post boost were consistently larger than the levels induced by the same booster vaccine in naïve animals. PBMCs from the vaccinees of the heterologous MRKAd5/MRKAd6-Ad24 boost

regimen were analyzed for intracellular IFN- γ staining after the priming immunizations (wk 60). The assay results provided information on the relative amounts of CD4⁺ and CD8⁺ gag-specific T cells in the peripheral blood (Figure 24). The results indicated that heterologous prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4⁺ and CD8⁺ T cells.

Ad24 Vaccine Vector as a Heterologous Primer: In a separate study, a cohort of 3 rhesus macaques was immunized initially with 2 doses (wk 0, 4) of 10^{11} vp Ad24 Δ E1gag Δ Orf6Ad5Orf6 and boosted at wk 24 with 10^7 vp of MRKAd5-gag. The low dose of MRKAd5-gag is selected to mimic the effect of pre-existing neutralizing immunity to the vector in a subject. A separate cohort of naïve animals was given a single dose of 10^7 vp MRKAd5-gag. The results of the IFN- γ ELISPOT analyses of PBMC collected during the course of the studies are shown in Figure 25.

The Ad24-based vaccine was able to prime effectively for HIV-specific T cell responses in macaques. Boosting with a low dose MRKAd5-gag resulted in a significant increase in the levels of gag-specific T cells. The increases in 2 out of 3 animals exceed the levels typically observed after treatment of naïve animals with the same low dose of MRKAd5-gag.

EXAMPLE 18

Construction of pAd34 Δ E1 Δ E4Ad5Orf6

To generate an E1- Ad34 based vector that can propagate in existing group C/Ad5 E1 complementing cell lines (293, PER.C6), Ad5 Orf6 was inserted in place of the native E4 region. Since at the time, the complete sequence of Ad34 (*see* Figures 28A-1 to 28A-9; subject of copending application serial no. 60/458,825, filed March 28, 2003) was unknown, advantage was taken of the sequence homology between Ad34 and Ad35 in order to construct the Ad34 pre-Adenovirus plasmid. Cotransformation of BJ 5183 bacteria with purified wild-type Ad34 viral DNA and the appropriately constructed Ad35 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The construction of the pre-Ad plasmid based on Ad34, is outlined below:

To construct pAd34 Δ E1 Δ E4Ad5Orf6 (An Ad34 pre-Ad plasmid containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), we utilized an Ad35 ITR cassette. We anticipated that sequence homology between Ad34 and Ad35 would allow homologous recombination to occur. The Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31913 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome (*see* Figures 2A-1 to 2A-10) separated by plasmid sequences containing a

bacterial origin of replication and an ampicillin resistance gene. The four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 31913 and 34419 generating pNEBAd35-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31914 to 34418 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31913 and bp 3403 to 3886) in the ITR cassette provided regions of homology with the purified Ad34 viral DNA in which bacterial recombination could occur following cotransformation into BJ 5183 bacteria (Figure 26). The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme*I) located at the end of the viral ITR's so that digestion would release the recombinant Ad34 genome from the plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd34ΔE1ΔE4Ad5Orf6.

EXAMPLE 19

Rescue of pAd34ΔE1ΔE4Ad5Orf6 into Virus

In order to determine if pre-adenovirus plasmid pAd34ΔE1ΔE4Ad5Orf6, could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc). *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring was observed following transfection. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect a T-225 flask of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE, the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment

followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hind*III and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with *Pme*I/*Hind*III prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 20

10 Insertion of an Expression Cassette into pAd34ΔE1ΔE4Ad5Orf6

In order to introduce a gag or SEAP expression cassette (*see* Figures 6 and 7, respectively) into the E1 region of pAd34ΔE1ΔE4Ad5Orf6, bacterial recombination was again used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2 (a precursor to the Ad35 ITR cassettes described above), generating pNEBAd35CMVgagBGHpA. pNEBAd35-2 contains Ad35 sequences from the left end of the genome (bp 4 to 456 and bp 3403 to 3886) with a unique *Swa*I site between bp 456 and 3403 at the position of the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by *Eco*RI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pNEBAd35-2. This cloning step resulted in the gag expression cassette being inserted into the E1 deletion between bp 456 and 3403 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad35 bp 4 to 456 and bp 3403 to 3886 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pAd34ΔE1ΔE4Ad5Orf6, linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the Ad34 gag-containing pre-Adenovirus plasmid pAd34ΔE1gagΔE4Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad34 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence was

cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2, generating pNEBAd35CMVSEAPBGHpA. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by *EcoRI* digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *SwaI* site in pNEBAd35-2. The transgene was then recombined into the pAd34ΔE1ΔE4Ad5Orf6, generating pAd34ΔE1SEAPΔE4Ad5Orf6 as described above for the gag transgene.

All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

EXAMPLE 21

Construction of pMRKAd34ΔE1ΔE4Ad5Orf6

To construct an Ad34 pre-Ad plasmid that was composed entirely of Ad34 sequences, an Ad34 ITR cassette was generated. The Ad34 ITR cassette was constructed containing sequences from the right (bp 31584 to 31895 and bp 34409 to 34772) and left (bp 4 to 456 and bp 3402 to 3885) end of the Ad34 genome (*see* Figures 28A-1 to 28A-9) separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd34-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad34 bp 31895 and 34409 generating pNEBAd34-4Ad5Orf6 (the ITR cassette).

pNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad34 bp 457 to 3401 with a unique *Swa I* restriction site located in the deletion and an E4 deletion from Ad34 bp 31896 to 34408 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5Orf6 expression is driven by the Ad34 E4 promoter. The Ad34 sequences (bp 31584 to 31895 and bp 3402 to 3885) in the ITR cassette provided regions of homology with the purified Ad34 viral DNA in which bacterial recombination could occur following cotransformation into BJ 5183 bacteria (Figure 27). The ITR cassette was also designed to contain unique restriction enzyme sites (*PmeI*) located at the end of the viral ITR's so that digestion would release the recombinant Ad34 genome from the plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pMRKAd34ΔE1ΔE4Ad5Orf6.

EXAMPLE 22

In Vivo StudiesA. Immunization

5 Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the two vectors: (1) 10^{11} vp MRKAd5-SEAP (in MRKAd vector backbone disclosed in PCT/US01/28861, published March 21, 2002); and (2) 10^{11} vp Ad34 Δ E1SEAP Δ E4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the
10 vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide*
15 *for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

20 Serum samples were analyzed for circulating human secreted alkaline phosphatase (SEAP) levels using TROPIX phospho-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 μ L aliquots of each serum were mixed with 45 μ L of kit-supplied dilution buffer in a 96-well white DYNEX plate. Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated
25 by heating the well for 30 minutes at 65 °C. Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorded using DYNEX luminometer. RLU readings were converted to ng/mL SEAP using a log-log regression analyses.

C. ELISPOT Assay

30 The IFN- γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp.,
35 Dublin, CA). To each well, 50 μ L of $2-4 \times 10^5$ peripheral blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower

size cut-off set at 80 femtoliters ("fL"). Either 50 μ L of media or the gag peptide pool at 8 μ g/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO₂ for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10⁶ cell input.

D. Intracellular Cytokine Staining (ICS)

To 1 ml of 2 x 10⁶ PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μ g/mL. For gag-specific stimulation, 10 μ L of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μ L of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO₂, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μ L per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μ L anti-hCD8-PerCP, clone SK1 (Becton Dickinson); and 20 μ L anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μ L 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μ g of FITC-anti-hIFN- γ , clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both CD4⁺ and CD8⁺ populations, and for both mock and gag-peptide reaction tubes of a sample.

E. Results

Expression: Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 29. Results indicate that the peak levels of SEAP protein produced by the alternative adenovirus serotype were lower than but were within 3-fold of that of MRKAd5 at the same high dose level of 10¹¹ vp (Figure 29). The levels of SEAP in the serum dropped dramatically after day 10 and were close to background as early as day 15. These observations strongly indicate that the Ad34-based vector is efficient in expressing a transgene following intramuscular administration in a primate.

Immunogenicity: Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 30; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool or the mock (no peptide) control.

Immunization with gag-expressing Ad34 vector induced detectable levels of circulating gag-specific T cells immediately after a single dose of the vector. The responses improved following a second dose given at wk 4. Overall, the responses to the Ad34-based vector were slightly lower than those induced by the same dose of MRKAd5-gag. The results strongly indicate the Ad34-based vector can prime effectively for HIV-specific T cell responses.

IFN- γ ICS analyses of the PBMC from the Ad34-immunized animals revealed that the vector can induce detectable levels of both CD4⁺ and CD8⁺ HIV-specific T cells (Figure 31).

EXAMPLE 23

Heterologous Immunization

Cohorts of 3 monkeys were immunized (at wks 0, 4) with 10^{11} vp Ad34 Δ E1gag Δ E4Ad5Orf6 followed by a booster at week 24 with 10^{10} vp Ad35 Δ E1gag Δ E4Ad5Orf6. Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 32; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool or the mock (no peptide) control.

Immunization with gag-expressing Ad34 vector induced detectable levels of circulating gag-specific T cells that decreased to between 94-139 SFC/ 10^6 PBMC at the time of the boost. Heterologous immunization with an Ad35-based HIV vector resulted in as much as a 3-fold increase in T cell responses.

IFN- γ ICS analyses of the PBMCs from the Ad34 primed/Ad35 boosted animals at week 28 revealed that the vector can induce detectable levels of both CD4⁺ and CD8⁺ HIV-specific T cells (Figure 33).

WHAT IS CLAIMED IS:

1. A means for propagating replication-defective adenovirus in an adenoviral E1-complementing cell line expressing E1 gene product(s) which are non-native to the adenovirus, which comprises:
 - (a) inserting all or a portion of a heterologous adenoviral E4 region comprising nucleic acid sequence encoding open reading frame 6 (ORF6) into a replication-defective adenovirus; wherein the E4 region or portion thereof inserted into the adenovirus is native to a virus of the same adenovirus serotype as the E1 gene product(s) expressed by the complementing cell line;
 - (b) introducing the replication-defective adenovirus into the adenoviral E1-complementing cell line;
 - (c) allowing the replication-defective adenovirus to propagate in the adenoviral E1-complementing cell line; and
 - (d) rescuing the propagated adenovirus.
2. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof comprises the complete adenoviral E4-encoding region.
3. A means in accordance with claim 2 wherein the heterologous adenoviral E4 region or portion thereof comprises the complete adenoviral E4-encoding region and native E4 promoter.
4. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is inserted into the replication-defective virus in place of nucleic acid sequence encoding open reading frame 6 (ORF6).

5. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is inserted into the replication-defective virus in place of nucleic acid sequence encoding the complete adenoviral E4-encoding region.

6. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is derived from a subgroup C adenovirus.

7. A means in accordance with claim 1 wherein the subgroup C adenovirus is adenovirus of serotype 5.

8. A means in accordance with claim 7 wherein the replication-defective adenovirus is an adenovirus of subgroup B.

9. A means in accordance with claim 7 wherein the replication-defective adenovirus is an adenovirus of serotype 35.

10. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is operatively linked to a heterologous promoter.

11. A means in accordance with claim 1 wherein the adenoviral E1-complementing cell line is a PER.C6® cell line.

12. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6).

13. A replication-defective adenovirus in accordance with claim 12 wherein the adenovirus comprises a heterologous gene of interest.

14. A replication-defective adenovirus in accordance with claim 13 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.

15. A replication-defective adenovirus in accordance with claim 14 wherein the HIV-1 antigen is selected from the group consisting of HIV-1 gag, pol, nef and env.

16. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6) and a gene encoding HIV-1 gag.

5 17. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6) in place of a native E4 region or portion thereof comprising ORF6.

18. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a complete heterologous E4 region in place of a complete native E4 region.

10 19. A replication-defective adenovirus comprising a heterologous E4 region or portion thereof comprising a complete heterologous E4 region including E4 promoter in place of a complete native E4 region.

20. Adenovirus propagated in accordance with the means of claim 1.

15 21. A means in accordance with claim 1 wherein the replication-defective adenovirus comprises a heterologous gene of interest.

22. A means in accordance with claim 21 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.

23. A means in accordance with claim 22 wherein the HIV-1 antigen is selected from the group consisting of: HIV-1 gag, pol, nef and env.

20 24. A replication-defective adenovirus of serotype 35 comprising all or a portion of an adenovirus serotype 5 E4 region comprising open reading frame 6 (ORF6) and a heterologous gene of interest.

25. A replication-defective adenovirus in accordance with claim 24 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.

26. A replication-defective adenovirus in accordance with claim 25 wherein the HIV-1 antigen is selected from the group consisting of: HIV-1 gag, pol, nef and env.

27. A replication-defective adenovirus of serotype 35 comprising all or a portion of an adenovirus serotype 5 E4 region comprising open reading frame 6 (ORF6) and a
5 gene encoding HIV-1 gag.

28. A recombinant adenoviral vector of serotype 24 which comprises an E4 gene or a segment of an E4 gene comprising open reading frame 6 ("ORF6") of an alternative serotype.

29. A population of cells comprising the recombinant adenoviral vector of
10 claim 28.

30. A method for producing recombinant, replication-defective adenovirus particles comprising:

(a) introducing a recombinant adenoviral vector of claim 28 into a population of cells expressing adenovirus E1; and

15 (b) harvesting the resultant recombinant, replication-defective adenovirus.

31. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 30.

32. A composition comprising purified recombinant adenovirus particles in accordance with claim 31.

20 33. A composition in accordance with claim 32 which comprises a physiologically acceptable carrier.

34. A recombinant adenoviral vector in accordance with claim 28 which is at least partially deleted in E1 and devoid of E1 activity and comprises a heterologous nucleic acid.

35. A composition comprising purified recombinant adenoviral particles in accordance with claim 31 which are at least partially deleted in E1 and devoid of E1 activity and comprise a heterologous nucleic acid.

36. A method for effecting the delivery and expression of heterologous nucleic acid comprising administering the composition of claim 35 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

37. A method in accordance with claim 36 wherein the composition is preceded or followed by administration of heterologous nucleic acid with an adenovirus of a different serotype.

38. A composition in accordance with claim 35 wherein the heterologous nucleic acid encodes an HIV antigen.

39. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 38.

40. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

41. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

42. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

43. A recombinant adenoviral vector of serotype 24 which is at least partially deleted in E1 and devoid of E1 activity; wherein said vector comprises an E4 gene or a segment of an E4 gene from adenovirus serotype 5 comprising open reading frame 6 ("ORF6"), and a heterologous nucleic acid.

44. A population of cells comprising the recombinant adenoviral vector of claim 43.

45. A method for producing recombinant, replication-defective adenovirus particles comprising:

5 (a) introducing a recombinant adenoviral vector of claim 43 into a population of cells expressing adenovirus serotype 5 E1; and

(b) harvesting the resultant recombinant, replication-defective adenovirus.

46. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 45.

10 47. A composition comprising purified recombinant adenovirus particles in accordance with claim 46.

48. A composition in accordance with claim 47 which comprises a physiologically acceptable carrier.

49. A method for effecting the delivery and expression of the heterologous
15 nucleic acid comprising administering the composition of claim 48 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

50. A method in accordance with claim 49 above wherein the composition is preceded or followed by administration of the heterologous nucleic acid with an adenovirus of a different serotype.

20 51. A composition in accordance with claim 48 wherein the heterologous nucleic acid encodes an HIV antigen.

52. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 51.

53. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

54. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

5 55. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

56. A recombinant adenoviral vector of serotype 34 which comprises an E4 gene or a segment of an E4 gene comprising open reading frame 6 ("ORF6") of an alternative serotype.

10 57. A population of cells comprising the recombinant adenoviral vector of claim 56.

58. A method for producing recombinant, replication-defective adenovirus particles comprising:

15 (a) introducing a recombinant adenoviral vector of claim 56 into a population of cells expressing adenovirus E1; and

(b) harvesting the resultant recombinant, replication-defective adenovirus.

59. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 58.

20 60. A composition comprising purified recombinant adenovirus particles in accordance with claim 59.

61. A composition in accordance with claim 60 which comprises a physiologically acceptable carrier.

62. A recombinant adenoviral vector in accordance with claim 56 which is at least partially deleted in E1 and devoid of E1 activity and comprises a heterologous nucleic acid.

63. A composition comprising purified recombinant adenoviral particles in accordance with claim 59 which are at least partially deleted in E1 and devoid of E1 activity and comprise a heterologous nucleic acid.

64. A method for effecting the delivery and expression of heterologous nucleic acid comprising administering the composition of claim 63 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

65. A method in accordance with claim 64 wherein the composition is preceded or followed by administration of heterologous nucleic acid with an adenovirus of a different serotype.

66. A composition in accordance with claim 63 wherein the heterologous nucleic acid encodes an HIV antigen.

67. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 66.

68. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

69. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

70. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

71. A recombinant adenoviral vector of serotype 34 which is at least partially deleted in E1 and devoid of E1 activity; wherein said vector comprises an E4 gene or a segment of an E4 gene from adenovirus serotype 5 comprising open reading frame 6 ("ORF6"), and a heterologous nucleic acid.

72. A population of cells comprising the recombinant adenoviral vector of claim 71.

73. A method for producing recombinant, replication-defective adenovirus particles comprising:

5 (a) introducing a recombinant adenoviral vector of claim 71 into a population of cells expressing adenovirus serotype 5 E1; and

(b) harvesting the resultant recombinant, replication-defective adenovirus.

74. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 73.

10 75. A composition comprising purified recombinant adenovirus particles in accordance with claim 74.

76. A composition in accordance with claim 75 which comprises a physiologically acceptable carrier.

15 77. A method for effecting the delivery and expression of the heterologous nucleic acid comprising administering the composition of claim 76 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

78. A method in accordance with claim 77 above wherein the composition is preceded or followed by administration of the heterologous nucleic acid with an adenovirus of a different serotype.

20 79. A composition in accordance with claim 76 wherein the heterologous nucleic acid encodes an HIV antigen.

80. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 79.

81. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

82. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

5 83. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

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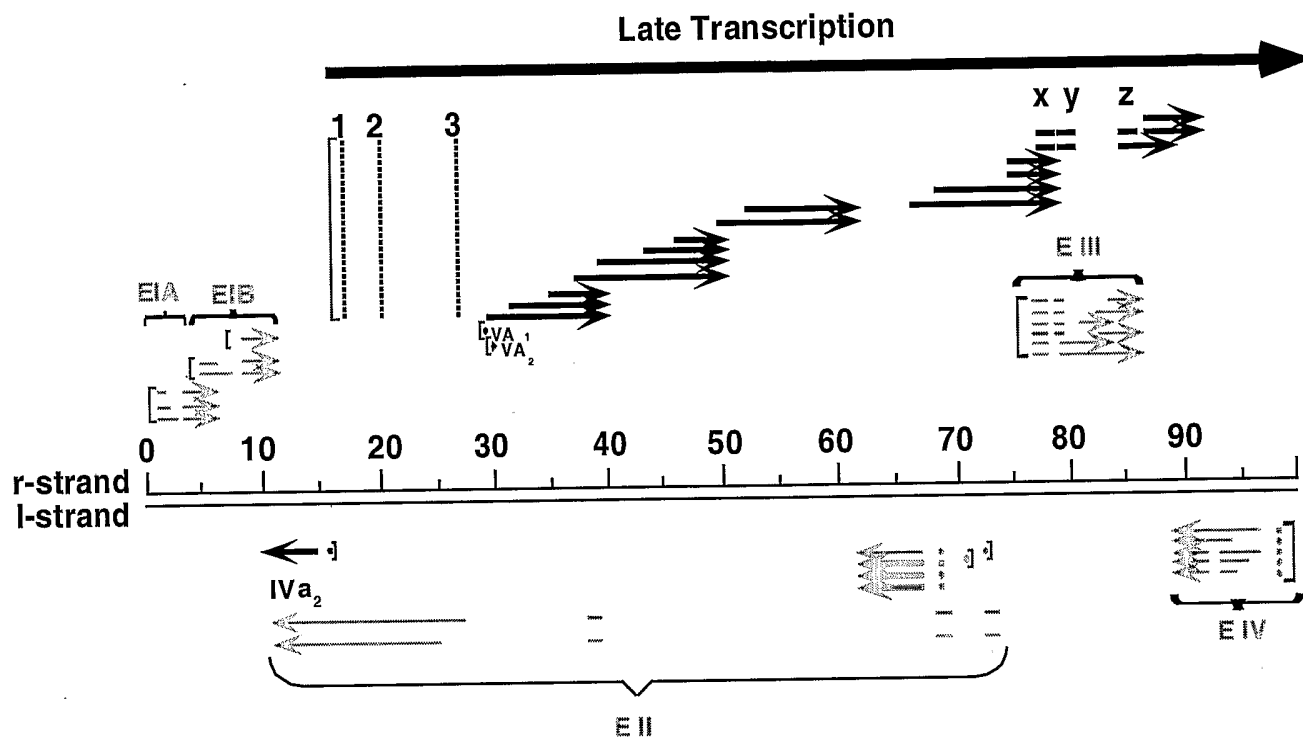


FIG. 1

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FIG. 2A-1

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FIG. 2A-2

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11161 gcccgcgaag aagttaccct tggtttgatg ctttttggg tggtggtgca acacagcaga
11221 cagaacccta ctagcaaacc tctgaccgcc cagctgtttc aacctcaccg gagatggttg
11281 gacaatgagg ctttcagaga ggcgctgctg aacctcaccg gagatggttg

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FIG. 2A-3

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11341 tatgatctta tcaacattct acagagtatc atagtgcagg agcggagcct gggcctggcc
11401 gagaaggtag ctgccatcaa ttactcgggt ttgagcttgg gaaaatatta cgctcgcaaa
11461 atctacaaga ctccatacgt tcccatagac aaggaggtga agatagatgg gttctacatg
11521 cgcatgacgc tcaaggctctt gaccctgagc gatgatcttg ggggtgatcg caatgacaga
11581 atgcatacgc cggtttagcgc cagcaggagg cgcgagttaa gcgacaggga actgatgcac
11641 agttttgcaaa gagctctgac tggagctgga accgaggggtg agaattactt cgacatggga
11701 gctgacttgc agtggcagcc tagtcgcagg gctctgagcg ccgcgacggc aggatgtgag
11761 cttccttaca tagaagaggc ggatgaaggc gaggaggaag agggcgagta cttggaagac
11821 tgatggcaca acccgtgttt tttgctagat ggaacagcaa gcaccggatc ccgcaatgcg
11881 ggcggcgctg cagagccagc cgtccggcat taactcctcg gacgattgga ccagggccat
11941 ccagcgtgat atggcggtga cgactcgcga cccggaagcc tttagacagc aaccccgaggc
12001 caaccgtcta tcggccatca tggaaagctgt agtgccttcc cgatctaata ccactcatga
12061 gaaggctcctg gccatcgtga acgcgttggg ggagaacaaa gctattcgtc cagatgaggc
12121 cggactggta tacaacgctc tcttagaacg cgtggctcgc tacaacagta gcaatgtgca
12181 aaccaatttg gaccgtatga taacagatgt acgcgaagcc gtgtctcagc gcgaaaggtt
12241 ccagcgtgat gccaacctgg gttcgttggg ggcgttaaat gctttcttga gtactcagcc
12301 tgctaatttg ccgcgtggctc aacaggatta tactaacttt ttaagtgttt tgagactgat
12361 ggtatcagaa gtacctcaga gcgaagtgtg tcagtcgggt cctgattact tctttcagac
12421 tagcagacag ggcttgacaga cggtaaatct gagccaagct tttaaaaacc ttaaaggttt
12481 gtggggagtg catgccccgg taggagaaag agcaaccgtg tctagcttgt taactccgaa
12541 ctcccgctg ttattactga tttcaccgac ttcaccgac agcggtagca tcggaggttaa
12601 ttcctatttg ggttacctac taaacctgta tcgcgaagcc atagggcaaa gtcagggtgga
12661 cgagcagacc tatcaagaaa ttaccaagt cagtcgcgct ttgggacagg aagacactgg
12721 cagtttgtaa gccactctga acttcttgct taccaatcgg tctcaaaaga tccctcctca
12781 atatgctctt actgcgaggg aggagaggat ccttagatat gtgcagcaga gcgtgggatt
12841 gtttctgatg caagaggggg caactccgac tgagcactg gacatgacag acagtaaatat
12901 ggagcccagc atgtatgcca gtaaccgacc tttcattaac aaactgctgg cactattgca
12961 cagagctgcc gctatgaact ctgattatgt caccaatgcc atcttaaacc cgcactggct
13021 gccccacact ggtttctaca cgggcgaata tgacatgcc gaccctaata acggtttct
13081 gtgggacgac gtggacagcg atgttttttc acctctttct gatcatcgca cgtggaaaaa
13141 ggaaggcggt gatagaatgc attcttctgc atcgctgtcc ggggtcatgg gtgtaccgc
13201 gctgagccc gagtctgcaa gtccttttcc tagtctacc tttctctac acagtgtacg
13261 tagcagcgaa gtgggtagaa taagtcgccc gagtttaatg ggcgaagagg agtacctaaa
13321 cgattccttg ctacagaccg caagagaaaa aaatttccca aacaatggaa tagaaagttt
13381 ggtggataaa atgagtagat ggaagactta tgctcaggat cacagagacg agcctgggat
13441 catggggact acaagtagag cgagccgtag acgccagcgc catgacagac agaggggtct
13501 tgtgtgggac gatgaggatt cggccgatga tagcagctg ttggacttgg gtgggagagg
13561 aaggggcaac ccgtttgctc atttgcgccc ggtatgttgt gtaaaaaaat gtaaaaaaat
13621 aaaaaagaaa aactcaccaa ggccatggcg acgagcgtac gttcgttctt ctttattatc
13681 tgtgtctagt ataatgaggc gagtcgtgct agggcgagcg gtggtgtatc cggaggggtcc
13741 tcctccttcg tacgagagcg tgatgcagca gcagcaggcg acggcggtga tgcaatcccc
13801 actggaggct ccctttgtgc ctcgcgata cctggcacct acggagggca gaaacagcat
13861 tcgttactcg gaactggcac ctacagaca taccaccagg ttgtatctgg ttagacaaca
13921 gtcggcgagc attgcttctc tgaactatca gaatgaccac agcaacttct tgaccacggt
13981 ggtgcagaac aatgacttta cccctacgga agccagcacc cagaccatta actttgatga
14041 acgatcgcgg tggggcggtc agctaaagac acttaacatgc actaacatgc caaacgtgaa
14101 cgagtatatg tttagtaaca agttcaaagc gctgtgatg gtgtccagaa aacctcccga
14161 cgggtgctgca gttggggata cttatgatca caagcaggat attttggaat atgagtgggt
14221 cgagtttact ttgccagaag gcaacttttc actattgatt actattgatt tgatgaacaa
14281 tgccatcata gataattact tgaaagtggg tagacagaat ggagtgtctg aaagtacat
14341 tgggtgttaag ttcgacacca ggaacttcaa gctgggatgg gatcccgaat ccaagttgat
14401 catgcctgga gtgtatacgt atgaagcctt ccactctgac attgtcttac tgcctggctg
14461 cggagtggat tttaccgaga gtcgtttgag caacttctt ggtatcagaa aaaaacagcc
14521 atttcaagag ggttttaaga ttttgtatga agatttagaa ggtggtaata ttccggccct
14581 cttggatgta gatgcctatg agaacagtaa gaaagaacaa aaagccaaaa tagaagctgc
14641 tacagctgct gcagaagcta aggcaaacat gactctacaa ggggttgctaa
14701 cgctggagag gtcagaggag acaattttgc gccaacacct gttccgactg cagaatcatt
14761 attggccgat gtgtctgatg gaacggacgt gaaactcact attcaacctg tagaaaaaga
14821 tagtaagaat agaagctata atgtgttggg agacaaaatc aacacagcct atcgagttg
14881 gtatctttcg tacaattatg gcgatcccg aaaaaggagt cgttcctgga cattgctcac
14941 cacctcagat gtcacctgcg gagcagagca ggtttactgg tgcgttccag acatgatgaa
15001 ggatcctgtc actttccgct ccactagaca agtcagtaac taccctgtgg tgggtgcaga
15061 gcttatgccc gtcttctcaa agagcttcta caacgaacaa gctgtgtact cccagcagct

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FIG. 2A-4

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15121 ccgccagtc acctcgetta cgcacgtctt caaccgcttt cctgagaacc agattttaat
15181 ccgtccgccc gcgcccacca ttaccaccgt cagtgaatac gttcctgctc tcacagatca
15241 cgggaccctg ccgttgcgca gcagtatccg gggagtccaa cgtgtgaccg ttactgacgc
15301 cagacgccgc acctgtccct acgtgtacaa ggcactgggc atagtgcac cgcgctcct
15361 ttcaagccgc actttctaaa aaaaaatgt ccattcttat ctgcccagt aataacaccg
15421 gttggggtct gcgcgctoca agcaagatgt acggaggcgc acgcaacgt tctaccaac
15481 atcccggtcg tggtcgcgga ctttttcgcg ctccatgggg tgccctcaag ggccgcactc
15541 gcgttcgaac caccgtcgat gatgtaatcg atcagggtgg cagtgtagt gctgacgctc
15601 ctccctactgc gcctacatct actgtgggat ggcgcattgc cagacgccac cgagctacca
15661 gcaactatgc tcgacgtaag agccggcgaa ggcgcattgc acgctgggg cgaagagcca
15721 ctgcccgtcg agccgcaaga gctctgctac gaagagctag cgcgtgggg cgcagggtcc
15781 tgcttagggc ggccagacgt gcagcttcgg gcgccagcgc cggcagggtc cgcaggcaag
15841 cagccgctgt cgacggcgcg actattgccg acatggccca atcgcaaga ggcaatgtat
15901 actgggtgcg tgacgtgccg accggtcaac gtgtaccctg ggcacccgt cccctcgca
15961 cttagaagat actgagcagt ctccgatgtt gtgtcccagc ggcgaggat ggcgagcca
16021 aatacaagga agaatgctg caggttatcg cactgaagt ctacggccaa ccgttgaagg
16081 atgaaaaaaaa accccgcaaa atcaagcggg ttaaaaagga caaaaagaa gaggaagatg
16141 gcgatgatgg gctggcggag tttgtgcgcg agtttgccc acggcgacgc gtgcaatggc
16201 gtgggcgcaa agttcgacat gtgttgagac ctggaacttc ggtggtcttt acaccggcg
16261 agcgttcaag cgctactttt aagcgttctt atgatgaggt gtacggggat gatgatattc
16321 ttgagcaggc ggctgaccga ttaggcgagt ttgcttatgg caagcgtagt agaataactt
16381 ccaaggatga gacagtgtca atacccttgg atcatggaaa tcccaccctt agtcttaaac
16441 cggctcacttt gcagcaagtg ttaccctgta ctccgcgaac aggtgttaaa cgcgaagggtg
16501 aagatttgta tcccactatg caactgatgg taccctgagg aacctgaggt taaagtgaga cccattaagc
16561 tggagaaagt aaaagtggat ccagatatct gtacaaactg tagacattaa gattcccact tccaagcgca
16621 aggtagcgcc tggctgggg gtacaaactg aagcctactg ccacctccac tgaagtgcaa acggatccat
16681 aagtgcgaac tgaaccgca actgacgcgc cgggtcccac tgaagatcc cgacgaaagt
16741 ggatgcccat gcctattaca atgcccactt atgttgtaga gccgaaacag occatctatt attcctactc
16801 acggtccagc aagtctgttg aggcactcgc tactatcgca cgcgaaacag tacctcccgc cgtcgccgca
16861 ctggttaccg aaatcgcagt agtgtagcgc aatggtatgt cggaaccttt gacactccgc cctggcgctt
16921 agacacctgc atcatcact tatcactact catcactggg taccgaggaa gaaactcgcg cgttagaaga
16981 tggtgcccga gacgcggaat ggcacgtctac aggcgacggc gtgctatccg caagcaattg cgcgatacca
17041 accatccgag ttcgcgttcc cgcacgtctac attatcgctg ctgcaattgg gacattggaa gaaaacgta
17101 acttgctgcc ttcgcgttcc cgcacgtctac attatcgctg ctgcaattgg gacattggaa gaaaacgta
17161 gggatgttgg gacgcggaat ggcacgtctac aggcgacggc gtgctatccg caagcaattg cgcgatacca
17221 cggggtggtt ttttaccagc cgtggtgggt gcagcgtctc caacgacatt ctgtgactat cgtgacgaag
17281 ggcataagctt cgtggtgggt ggcacgtctc caacgacatt ctgtgactat cgtgacgaag
17341 taaataaaaa aaatacaaat catcaatttt acgagccaac tgaacggggg cgcttcaat tggagcagta
17401 agatggaaga catcaatttt acgagccaac tgaacggggg cgcttcaat tggagcagta
17461 gcacctggag cgacatcggc tttgggtcaa ccataaaaa acagcttggg caaagcttgg
17521 tctggagcgg gcttaaaaaa cttagaaata aacttaaga gagtggtaga tttggctaac caggctgtgc
17581 acagcagtag aggcagggcg gatagcttcc ggcacaaatg tgccagcaac cccaggtgaa atgcaagtgg
17641 tagtcaatgg aaacagtcgt ttggaccgca gaaacacgag gccacaagcg tccgcgtccc gatttggaag
17701 agaaaagat aaacagtcgt ttggaccgca gaaacacgag gccacaagcg tccgcgtccc gatttggaag
17761 aggaagaaat tcttcgcca gacgcgcgta gatgaaccgc cttcttatga ggaagcaacg aagcttggaa
17821 agacgtgggt tagaccgata gcccacatgg ttgccccttc cccctgctgc tactgtgta tctcagttgc
17881 tgcccaccac atcgaccgtg cacttggat ccagtcgccc tagccaggtc acgtcccggg gggctagggc
17941 atcgaccgtg accttggat ccagtcgccc tagccaggtc acgtcccggg gggctagggc
18001 agcctgtcgc tgccccgaaa gcaactgcaa aatactctga acagcatcgt cttacttga ggaagcaacg
18061 gtccaaatgc gcaactgcaa aatactctga acagcatcgt cttacttga ggaagcaacg
18121 taaaacgcgc tcgctgcttt taattaaata tggagtagcg agaggaaaaa aggaagaggt
18181 atatgtgtca ttacacgccg ggcaccccca tcgatgctgc ccaatgggc atacatgcac
18241 cgctgagtta ctttcaagat ggagtagctt ggagtagctt tttgagtagc cccccgcgc
18301 atcgccggac aggatgcttc gggaaataag tttagaaatc ccacgtagc gcccgcgcc
18361 acagacacct acttcaatct cccagcggtc atgttgcgct tgggagtagc cccacgtagc
18421 gatgtgacca ccgaccgtag ccagcggtc agtggtgctt accctggcgg tgggagtagc
18481 gacaatacat actcttcaaa agtgcggtac accctggcgg tgggagtagc
18541 gatattggca gcacgttctt tgacattagg ggcgtgttgg acagaggtcc cagtttcaaa
18601 cctattctcg gtacggctta caactctctg gtcctaaaag gcgttcaaa tgcattctca
18661 tggattgcaa aaggcgctac aactgcagca gccgcaggca atggtgaaga agaactgaa
18721 acagaggaga aaactgctac ttacactttt gccaatgctc ctgtaaaagc cgaggctcaa
18781 attacaaaag agggcttacc aataggtttg gagatttcag ctgaaaacga atctaaaccc
18841 atctatgcag ataaacttta tcagccagaa cctcaagtgg gagatgaaac ttggactgac

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FIG. 2A-5

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18901 ctagacggaa aaaccgaaga gtatggaggc agggctctaa agcctactac taacatgaaa
18961 ccctgttacg ggtcctatgc gaagcctact aattttaaag gtggctcaggc aaaaccgaaa
19021 aactcggaac cgtcgagtga aaaaattgaa tatgatattg acatggaatt ttttgataac
19081 tcacgcgcaa gaacaaactt cagtcctaaa attgtcatgt atgcagaaaa tgtagggttg
19141 gaaacgccag acactcatgt agtgtacaaa cctggaacag aagacacaag ttccgaagct
19201 aatttgggac aacagtctat gcccaacaga cccaactaca ttggcttcag agataacttt
19261 attggactca tgtactataa cagtactggt aacatggggg tgctggctgg tcaagcgtct
19321 cagttaaatg cagtggttga cttgcaggac agaaacacag aactttctta ccaactcttg
19381 cttgactctc tgggcgcacag aaccagatac ttttagcatgt ggaatcaggc tgtggacagt
19441 tatgatcctg atgtacgtgt tattgaaaat catggtgtgg aagatgaact tcccaactat
19501 tgttttccac tggacggcat aggtgttcca acaaccagtt acaaatcaat agttccaaat
19561 ggagaagata ataataattg gaaagaacct gaagtaaatt gaacaagtga gatcggacag
19621 ggtaatttgt ttgccatgga aattaacctt caagccaatc tatggcgaag tttcctttat
19681 tccaatgtgg ctctgtatct ccagactcgc tacaataaca ccccgccaa tgtcactctt
19741 ccagaaaaca aaaacaccta cgactacatg aacgggcggg tgggtgccgc atctctagta
19801 gacacctatg tgaacattgg tgccagggtg tctctggatg ccattggaca tgtcaacctc
19861 ttcaaccacc accgtaacgc tggcttgcgt taccgatcta tgcttctggg taacggacgt
19921 tatgtgcctt tccacataca agtgcctcaa aaattcttcg ctgttaaaaa cctgctgctt
19981 ctcccaggct cctacactta tgagtggaaac tttaggaagg atgtgaacat ggttctacag
20041 agttccctcg gtaacgacct gcgggtagat ggcgccagca tcagtttcac gagcatcaac
20101 ctctatgcta cttttttccc catggtctac aacaccgctt ccacccttga agccatgctg
20161 cggaaatgaca ccaatgatca gtcattcaac gactacctat ctgcagctaa catgctctac
20221 cccattcctg ccaatgcaac caatatccc atttccattc cttctcgcaa ctgggcggct
20281 ttcagaggct ggtcatttac cagactgaaa accaaagaaa ctccctcttt ggggtctgga
20341 tttgaccctt actttgtcta ttctggttct attccctacc tggatggtag cttctacctg
20401 aaccacactt ttaagaaggt ttccatcatg tttgactctt cagtggactg ccttggaatc
20461 gacaggttac tatctcctaa cgaatttgaa ataaagcgca ctgtggatgg gcaaggctac
20521 aacgtagccc aatgcaacat gaccaaagac tggttcttgg tacagatgct cgccaactac
20581 aacatcggct atcagggtt ctacattcca gaaggataga aagatcgcat gtattcattt
20641 ttcagaaact tccagcccat gaggaggcag gtggttgatg aggtcaatta caaagacttc
20701 aaggccgtcg ccatacccta ccaacacaac aactctggct ttgtgggtta ctgggtccg
20761 accatgcgcc aaggtcaacc ctatcccgct aactatccct atcccaactc tggaaacact
20821 gccgtaaatg gtgttacgca gaaaaagttc ttgtgtgaca gaacctgtg gcgcataacc
20881 ttctcgagca acttcatgtc tatgggggccc cttacagact tgggacagaa tatgctctat
20941 gccaactcag ctcatgctct ggacatgacc tttgagggtg atcccatgga tgagccacc
21001 ctgcttttatc ttctcttcga agttttcgac gtggtcagag tgcacagacc acaccgccc
21061 atcatcgagg cagtctacct gcgtacaccg ttctcgcccg gtaacgctac cacgtaagaa
21121 gcttcttgct tcttgcaaat agcagctgca accatggcct gcggatccca aaacggctcc
21181 agcgagcaag agctcagagc cattgtccaa gacctgggtt gcggacccta ttttttggga
21241 acctacgata agcgcttccc ggggttcatt gcccccgata agctcgccctg tgccattgta
21301 aatacggccg gacgtgagac ggggggagag cactggttgg ctttcggttg gaaccacagt
21361 tctaacacct gctacctttt tgatcctttt ggattctcgg atgatcgtct caaacagatt
21421 taccagtttg aatatgaggg tctcctgcgc cgcagcgctc ttgctaccaa ggaccgctgt
21481 attacgctgg aaaaatctac ccagaccgtg gttctgccgc ctgctgccc accgtcccat
21541 ttctgctgca tgttccctca cgcctttgtg cactggcctg accgtcccat ggacggaaac
21601 cccaccatga aattgctaac tggagtgcc aacaacatgc ttcattctcc taaagtccag
21661 cccaccctgt gtgacaatca aaaagcactc taccattttc ttaataccca ttcgccttat
21721 tttcgctctc atcgtaacac catcgaaagg gccactgcgt tgcaccgtat ggtgttcaa
21781 taatgactca tgtaaacaac gtgttcaata aacatcactt tattttttta catgtatcaa
21841 ggctctggat tacttattta tttacaagtc gaatgggttc tgacgagaat cagaatgacc
21901 cgcaggcagc gatacgttgc ggaactgata cttgggttgc cacttgaatt cgggaaatcac
21961 caacttgagg accggtatat cgggcaggat gtcactccac agctttcttg tcagctgcaa
22021 agctccaagc aggtcaggag ccgaaatctt gaaatcacaa ttaggaccag tgccttgagc
22081 gcgagagttg cggtacaccg gattcgagca ctgaaacacc atcagcgacg gatgtctcac
22141 gcttgccagc acggtgggat ctgcaatcat gccacatcc agatcttcag cattggcaat
22201 gctgaacggg gtcactcttg aggtctgcct acccatggcg ggcacccaat taggcttggt
22261 gttgcaatcg cagtgcaggg ggatcagtat catcttgccc tgatcctgtc tgattccttg
22321 atacacggct ctcatgaaag catcatattg cttgaaagcc tgctgggctt tactaccctc
22381 ggtataaaac atcccgcagg acctgctcga aaactgggtt gctgcacagc cgtctcattt
22441 cacacagcag cgggcgctcat tgttggctat ttgcaccaca cttctgcccc agcgggtttt
22501 ggtgattttg gttcgctcgg gattctcctt taaggctcgt tgtccgttct cgctggccac
22561 atccatctcg ataactctgt ccttctgaat cataatattg ccatgcaggc acttcagctt
22621 gccctcataa tcattgcagc catgaggcca caacgcacag cctgtacatt cccaattatg

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FIG. 2A-6

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22681 gtgggcgatc tgagaaaaag aatgtatcat tccctgcaga aatcttccca tcatcggtct
22741 cagtgtcttg tgactagtga aagttaactg gatgcctcgg tgctcttcgt ttacgtactg
22801 gtgacagatg cgcttgattt gttcgtgttg ctcaggcatt agtttaaaac aggttctaag
22861 ttcgttatcc agcctgtact tctccatcag cagacacatc acttccatgc ctttctccca
22921 agcagacacc aggggcaagc taatcggatt cttaacagtg caggcagcag ctcctttagc
22981 cagagggtca tcttttagcga tcttctcaat gcttcttttg ccctccttct caacgatgcg
23041 cacgggcggg tagctgaaac ccactgctac aagttgcgcc tcttctcttt cttcttcgct
23101 gtcttgactg atgtcttgca tggggatatg tttgggtctc cttgggtctt ttttgggggg
23161 tatcggagga ggaggactgt cgctccgttc cgggagacagg gaggattgtg acgtttcgct
23221 caccattacc aactgactgt cggtagaaga acctgacccc acacggcgac aggtgttttt
23281 cttcgggggc agagggtggag gcgattgcga agggctgcgg tccgacctgg aaggcggatg
23341 actggcagaa ccccttccgc gttcgggggt gtgctccctg tggcggtcgc ttaactgatt
23401 tccttcgcgg ctggccattg tgttctccta ggcagagaaa caacagacat ggaaactcag
23461 ccattgctgt caacatcgcc acgagtgcca tcacatctcg tctcagcga cgaggaaaag
23521 gagcagagct taagcattcc accgccagt cctgccacca cctctacctt agaagataag
23581 gaggtcgacg catctcatga catgcagaat aaaaaagcga aagagtctga gacagacatc
23641 gagcaagacc cgggctatgt gacaccggtg gaacacgagg aagagttgaa acgctttcta
23701 gagagagagg atgaaaactg cccaaaacag cgagcagata actatcacca agatgctgga
23761 aatagggatc agaacaccga ctacctcata gggcttgacg gggaagacgc gctccttaaa
23821 catctagcaa gacagtcgct catagtcaag gatgcattat tggacagaac tgaagtgcct
23881 atcagtgtgg aagagctcag ctgcgcctac gagcttaacc ttttttcacc tcgtactccc
23941 cccaaacgtc agccaaacgg cacctgcgag ccaaactctc gcttaaaact ttatccagct
24001 tttgctgtgc cagaagtact ggctacctat cacatctttt ttaaaaatca aaaaattcca
24061 gtctcctgcc gcgctaactg caccgcgcc gatgccctac tcaatctggg acctggttca
24121 cgcttacctg atatatgctt cttggaagag gttccaaaaga tcttcgaggg tctgggcaat
24181 aatgagactc gggccgcaaa tgctctgcaa aaggagaaaa atggcatgga atggcatcac
24241 agcgttctgg tgggaattgga aggcgataat gccagactcg cagtactcaa gcgaagcgtc
24301 gaggtcacac acttcgcata tcccgtgtgc aacctgcccc ctaaagtcac gacggcggtc
24361 atggaccagt tactcattaa gcgcgcaagt cccctttcag aagacatgca tgaccagat
24421 gcctgtgatg agggtaaacc agtggtcagt gatgagcagc taacccgatg gctgggcacc
24481 gactctcccc gggatttggg agagcgtcgc aagcttatga tggcgtgtgt cgtggttacc
24541 gtagaactag agtgtctccg acgtttcttt accgattcag aaactctgaa ctaactcgaa
24601 gagaactctg actacacttt tagacacggc tttgtgcggc aggcattgcaa gatattctaac
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24721 agcgtgctgc acagcaccct taaggggaa gcccgccgtg attacatccg cgatttgttc
24781 tatctctacc tgtgccacac gtggcaaacc ggcatgggtg tatggcagca atgtttagaa
24841 gaacagaact tgaaagagct tgacaagctc ttacagaaat ctcttaaggt tctgtggaca
24901 gggttcgacg agcgaccgt cgcttcgac ctggcagacc tcactctccc agagcgtctc
24961 agggttactt tgcgaaacgg attgcctgac tttatgagcc agagcatgct taacaatttt
25021 cgctctttca tcttggaaac ctccggtatc ctgcccgcga cctgctgcgc actgccctcc
25081 gacttttgtg ctctcaccta ccgcgagtgc ccccgccgc tatggagtca ctgctacctg
25141 ttccgtctgg ccaactatct ctcctaccac tcggatgtga tgcgggagac gagcggagac
25201 ggcttctgg agtgccactg ccgttgcaat ctgtgcacgc cccaccggtc cctagcttgc
25261 aacccccagt tgatgagcga aaccagata ataggcacct ttgaattgca agggccagc
25321 agccaaggcg atgggtcttc tcttgggcaa agtttaaaac tgaccccggy actgtggacc
25381 tccgcctact tgcgcaagtt tgctccggaa gattaccacc cctatgaaat caagttctat
25441 gaggaccaat cacagcctcc aaaggccgaa ctttcggctt gcgtcatcac ccagggggca
25501 attctggccc aattgcaagc catccaaaaa tcccgccaag aatttctact gaaaaagggg
25561 aaggggggtc accttgaccc ccagaccggc gaggaactca acacaagggt ccctcaggat
25621 gtcccaacga cgagaaaaca agaagttgaa ggtgcagccg cggcccccag aagatatgga
25681 ggaagattgg gacagtcagg cagaggaggc ggaggaggac agtctggagg acagtctgga
25741 ggaagacagt ttggaggagg aaaacgagga ggcagaggag gtggaagaag taaccgccga
25801 caaacagtta tctcggctg cggagacaag caacagcgct accatctccg ctccgagtcg
25861 aggaacccgg cggtgcctca gcagtagatg ggacgagacc ggacgcttcc cgaacccaac
25921 cagcgcttcc aagaccggtg agaaggatcg gcagggatac aagtcctggc gggggcataa
25981 gaatgccatc atctcctgct tgcatgagtg cgggggcaac atatccttca cgcggcgcta
26041 cttgctatcc caccatgggg tgaacttttc ttgcattact accgtcacct
26101 ccacagcccc tactatagcc agcaaatccc gacagtctcg acagataaag acagcggcgg
26161 cgacctccaa cagaaaacca gcagggcgag ttagaaaata cacaacaagt gcagcaacag
26221 gaggattaaa gattacagcc aacgagccag cgcaaaccgg agagttaaga aatcggatct
26281 ttccaaccct gtatgccatc ttccagcaga gtcgggggtc agagcaggaa ctgaaaataa
26341 aaaaccgatc tctgcgttcg ctcaccagaa gttgtttgta tcacaagagc gaagatcaac
26401 ttcagcgcac tctcgaggac gccgaggctc tcttcaacaa gtactgcgcg ctgactctta

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FIG. 2A-7

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26461 aagagtaggc agcgaccgcg cttattcaaa aaaggcggga attacatcat cctcgacatg
26521 agtaaaagaaa ttcccacgcc ttacatgtgg agttatcaac cccaaatggg attggcagca
26581 ggcgcctccc aggactactc caccgcgatg aattgggtca ggcgcggggc ttctatgatt
26641 tctcgagtta atgatatacy cgcctaccga aaccaaatac ttttgaaca gtcagctctt
26701 accaccacgc cccgccaaca ccttaatccc agaaattggc cgcgcgcctt agtgtaccag
26761 gaaagtcccg ctcccaccac tgtattactt cctcgagacg cccaggccga agtccaaatg
26821 actaatgcag gtgcgcagtt agctggcggc tccaccctat gtcgtcacag gcctcggcat
26881 aatataaaac gcctgatgat cagaggccga ggtatccagc tcaacgacga gtcggtgagc
26941 tctccgcttg gtctacgacc agacggaatc tttcagattg ccggctgcgg gagatcttcc
27001 ttcacccctc gtcaggctgt tctgactttg gaaagtctgt tttactccct ctgtctactt
27061 ggaatcgggg cggttcaatt tgtagaggag tttactccct ctgtctactt caaccccttc
27121 tccggatctc ctgggcacta cccggacgag ttcataccga acttcgacgc gattagcgag
27181 tcaagtggacg gctacgattg atgtctgggt acgcggctga gctatctcgg ctggcacatc
27241 tagaccactg ccgccgcttt cgctgctttg cccgggaact tattgagttc atctacttcg
27301 aactcccaa ggatcacctt caaggtccgg cccacggagt gcggattact atcgaaggca
27361 aaatagactc tcgcctgcaa cacggtttcc atctactgca tttgtaatca ccccgattg
27421 agggaaacac gatgtgtact gagtttaata aaaactgaat taagactctc ctacggactg
27481 tttgctgtct cgtcttcttc aaccgggatt ttacaaccag aagaacaaa cttttcctgt cgtccaggac
27541 ccgcttcttc tctgttaact tcaccttttc tactcaciaa ctagaagctc aacgactaca cgccttttcc
27601 agaagcattt tccctactaa tactactttc agcgggcctt gtagtactag gaattcttgc
27661 agaaaaaac cttgggtgga atacacacct tgcttactt tcctagtggg
27721 gtgattatcc tttgctacct atactagtct tgcttgtttt actttcgctt
27781 tgggttaaaa aatggggccc ttacgatcca tgtctagact ttgaccaga aaactgcaca
27841 gttctgccaa ttacgatcca tgtggagtcc ttattaagtg cggatgggaa
27901 caccgcgac acacaataac aaaacctgga acaatacctt atccaccaca
27961 ttgaaattac gagttcccg gtgtgacact gtctctgtcc gaggtcctga
28021 gagttcccg cattttttct gaaatgtgcy atctggccat gttcatgagc
28081 acaacacttt tcctagcaag gacaacatcg taacgttctc cattgcttat
28141 ctctatggcc tctgtcttct tactgtctta ctgtgcgtat gcataacact
28201 cttgccttct tctgtcttct aaaatgcctt aacctcttcc tgtttacaga
28261 aaaacgcaa taacaaagaa aactgcctt actgccgtc acggacaaac
28321 cttacatctc tcataattgt cagcattgtc ggaccccaa tcacttcaga
28381 atcccactag gacataatta cactctcata gacaccccaa acaaaacaaa
28441 accaaactgg gaagcgttga ttactttgat ataactgtta gcaaagttaa
28501 gtaacttgca acatacaaaa tcttacattg attaatgtta gcaaagttaa
28561 tattatgggt atgacagata cagtagtcaa tatagaaatt acttggttcg
28621 ttgaaaacca cgaaaatgcc aaatatggca aagattcgat ccgatgacaa
28681 acttttacat ctcccaccac acccgacgaa aaaaacatcc cagattcaat
28741 gttgcagcgg tggcagtggt gatggcacta ataataatat gcatgtttt
28801 cgctacaaaa agtttcatcc taaaacaa gatctcctac taaggcttaa
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28921 tgacttctgc tcgctcacac ctactgttaa ctataggctc aaactgcaca
28981 ctcaagggtg tcatgtcttt tggtaggagaa tatatgacaa tggatgggtt
29041 gtgaccaacc tggtagattt ttctgcaacg gcagagacct aaccattatc
29101 caaatgacaa aggttcttat actataaaag actataaaag tagtttagat
29161 ttgtactgcc atctaccact ccagcaccct gcacaactac tttctctagc
29221 ctaacaatac aatttccaat ccaacctttg cgcgcgtttt aaaacgcact
29281 ctacaacttc acatacaaca atttccactt caacaatcag catcatcgct
29341 ttggaatatc tattcttgtt tttaccataa cctactacgc ctgctgctat
29401 aacataaagg tgatccatta cttagatttg atatttaatt tgttcttttt
29461 agtatgggtg acaccaatca tggtagctag aaatttcttc ttcaccatac
29521 ttttaattgt tgcgctactt tcacagcagt agccacagca accccagact
29581 atttgcttcc tatgcacttt ttgcttttgt tacttgcatc tgcgtatgta
29641 cctgggttatt aattttttcc aacttctaga ctggatcctt gtgcgaattg
29701 ccaccatccc gaataccgca accaaaatat cgcggcactt cttagactca
29761 tgcaggctat actaccaata tttttgcttc tattgcttcc ctacgctgtc
29821 ctgcctatag tactccacca gaacacctta gaaaatgcaa attccaacaa
29881 ttcttgcttg ctatcgagaa aaatcagaaa tcccccaaa ttaataatg
30001 taattaatat aatctgttgc accataattt catttttgat ataccctcta
30061 gctggaatgc tccaatgca catgatcatc cacaagacct agaggacac
30121 aaaacatgca acatocaata gcgctaatag attacgaaag tgaaccacaa
30181 tccctgctat tagttacttc aacctaaccg gcggagatga ctgaaacact

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FIG. 2A-8

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30241 aattccgccc aggatctgct cgatatggac ggccgcgtct cagaacaacg acttgcccaa
30301 ctacgcatcc gccagcagca ggaacgcgtg gccaaagagc tcagagatgt catccaaatt
30361 caccaatgca aaaaaggcat attctgtttg gtaaaacaag ccaagatatc ctacgagatc
30421 accgctactg accatcgccct ctcttacgaa cttggccccc aacgacaaaa atttacctgc
30481 atgggtgggaa tcaaccccat agttatcacc caacaaagtg gagatactaa ggggttgcat
30541 cactgctcct gcgattccat cgagtgcacc tacaccctgc tgaagaccct atgcggccta
30601 agagacctgc taccaatgaa ttaaaaaaaa atgattaata aaaaatcact tacttgaaat
30661 cagcaataag gtctctgttg aaattttctc ccagcagcac ctcaactccc tcttcccaac
30721 tctgggtattc taaaccccggt tcagcggcat actttctcca tactttaaag gggatgtcaa
30781 attttagctc ctctcctgta cccacaatct tcattgtctt cttcccgatg gaccaagaga
30841 gtccgggtcgt gtgactcctt caacctgtgc taccctatg aagatgaaag cacctcccaa
30901 caccctttta taaacccagg gtttatttcc ccaaattggct tcacacaaag cccagacgga
30961 gttcttactt taaaatgttt aacccacta acaaccacag gcggatctct acagctaaaa
31021 gtgggagggg gacttacagt ggatgacact gatggtacct tacaagaaaa catacgtgct
31081 acagcaccca ttactaaaaa taatcactct gtagaactat ccattggaaa tggattagaa
31141 actcaaaaaca ataaactatg tgccaaattg ggaattgggt taaaatttaa caacggtgac
31201 atttgatataa aggatagtat taacacctta tggactggaa taaacctctc acctactgt
31261 caaattgtgg aaaacactaa tacaatgat ggcaaactta ctttagtatt agtaaaaaat
31321 ggagggtctg ttaatggcta cgtgtctcta gttggtgat cagacactgt gaacaaatg
31381 ttcacacaaa agacagcaaa catccaatta agattatatt ttgactcttc tggaaactga
31441 ttaactgagg aatcagactt aaaaattcca cttaaaaata aatcttctac agcgaccagt
31501 gaaactgtag ccagcagcaa agcctttatg ccaagtacta cagcttatcc cttcaacacc
31561 actactaggg atagtgaata ctacattcat ggaatatgtt actacatgac tagttatgat
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31741 atagctacgc tgaccacatc cccctttttc ttttcttaca ttacagaaga ttacaaactaa
31801 aataaagttt aagtgttttt attttaaate acaaaattcg agtagttatt ttgcctccac
31861 cttcccattht gacagaatac accaatctct cccacgcac agctttaaac atttggtatc
31921 cattagagat agacattgtt ttagattcca cattccaaac agtttcagag cgagccaatc
31981 tggggtcagt gatagataaa aatccatcgc gatagtcttt taaagcgctt tcacagtcca
32041 actgctgcgg atgcgactcc ggagtttgga tcacggtcat ctggaagaag aacgatggga
32101 atcataatcc gaaaacggtg tcggagcatt gtgtctcatc aaaccacaaa cgacgcgtg
32161 tctgcgtcgc tccgtgcgac tccactttct ggtgcgatgc gcgcagcaac gcattctgat
32221 gattttaata gcccttaaca tcaactttct cattattaca atattgttta ataaaccata
32281 ttactcaaaa tctttgcagt aggtacaaca cttatataat gcccttgcac gaccatcata
32341 attaaaagcg ctccagccaa aactcatatc tgatataatc acactacca cctacatgat
32401 ccaaagttha atataaatta aatgacgttc cctcaaaaac ggacaacggt gctccccag
32461 ctcttttggc atgtgcatac taacaatctg tctgtaccat gctccccag gctccccag
32521 gcaacccaat ataaccttcc ggaaccacac aagaacccaa ttctctcgac cgtgaatcac
32581 aagtgaaccc tgctgattac aatgacaatg acatagacat aaatgcagtc atcttctcat
32641 ttgagaatga aaaatatcta tagtggcaca atcccaggga ataggaagct cttgcagaac
32701 aatttttaac tcctcaggat ttagaacatc aacacaactt acactatgca tagtcatagt
32761 agtaaagctg gcagaacaag gaagaccagc agtcatagaa gctcgggttt cattttctc
32821 atcacaatct ggcaacagcg ggtggtcttc gtgatgtctg gcgcagatg togagcgtgc
32881 acaacgtggt aactgggctc tgggtgaagg gtgacattctc gtattttgta tagcaaaacg
32941 gcgcaacctt gtcataatgg agttgcttcc ttctatcctg ccgcttagcg tgttccgtgt
33001 cggccctggc agaacacact actcttaagt tggcctaaag aatgctggct tcagttgtaa
33061 gatagttcaa gtacagccac attgttctga ggaaatcatc cacggtagca tatgcaaatc
33121 tcaaaactcc atcgcatcta attgttctga gattgcgttt caagcaggag aggagaggga
33181 ccaaccaagc aatgcaactg ccaaacgatc tcgcagtaac tcaaatgtta gatcgcgcag
33241 gaaccatgtt aattttttatt tctgttgggt aaaaagcaca gctaaatcaa aagaaatgcg
33301 atggcatctc tcgccccac tggcttccaa caaagcctcc acgcgcacat ccaagaacaa
33361 attttcaagg tgctcaacgg tggcttccaa cttctcaatc atcatattac attcctgcac
33421 aagaatacca aaagaaggag cattttctaa ctctcaatc cgtgtcagtt cttgtggtaa
33481 cattocccaga taattttcag ctttccagcc ttgaattatt tccaccacca ttcttaaaaa
33541 atccaatcca cacattacaa acaggtcccg gagggcgccc gtatgcgaatt gagaatggca
33601 caccctcata atgacaaaaa atcttgcctc tcttctttaa gttctagtgt taaaactct
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33781 acagtgcagt acaagcgag acctcccaa aatataatc aaatataatc aggcagagtt
33841 gcatattggg aaccaccagt aatatcatcg aaaaaattt gccaaaaaaa cattcaaac
33901 tctttagtaa attgaataaa agaaaaattt gctgcgctcc aacattgtta gttttgaatt
33961 caaatgcaat aggttaccgc gctgcgctcc aacattgtta gttttgaatt agtctgcaaa

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FIG. 2A-9

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34021 aataaaaaaa aaacaagcgt catatcatag tagcctgacg aacaggtgga taaatcagtc
34081 tttccatcac aagacaagcc acaggggtctc cagctcgacc ctcgtaaaac ctgtcatcgt
34141 gattaaacaa cagcaccgaa agttcctcgc ggtgaccagc atgaataagt cttgatgaag
34201 catacaatcc agacatgtta gcatcagtta aggagaaaaa acagccaaca tagcctttgg
34261 gtataattat gcttaatcgt aagtatagca aagccacccc tcgcggtac aaagtaaaag
34321 gcacaggaga ataaaaaata taattatttc tctgctgctg tttaggcaac gtgcggcccg
34381 gtccctctaa atacacatac aaagcctcat cagccatggc ttaccagaga aagtacagcg
34441 ggcacacaaa ccacaagctc taaagtcact ctccaacctc tccacaatat atatacacia
34501 gccctaaact gacgtaatgg gactaaagtg taaaaaatcc cgccaaaccc aacacacacc
34561 ccgaaactgc gtcaccaggg aaaagtacag ttctacttcc gcaatcccaa caagcgtcac
34621 ttctcttttc tcacggtacg tcacatccca ttaacttaca acgtcatttt cccacggccg
34681 cgccgcccct tttaaccgtt aacccacag ccaatcacca cacggcccac actttttaaa
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SEQ ID NO: 1

FIG. 2A-10

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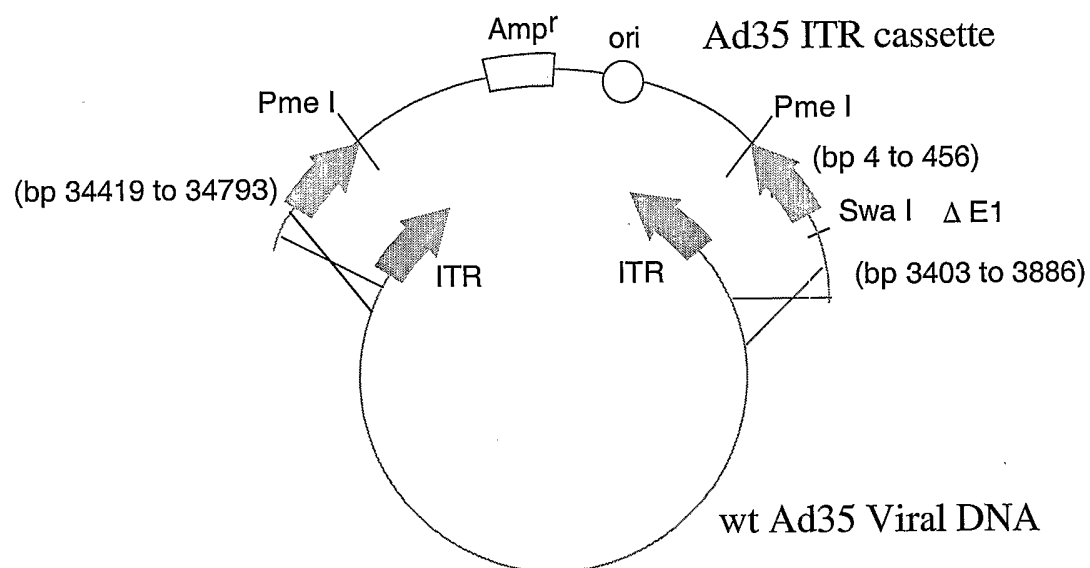


FIG. 3

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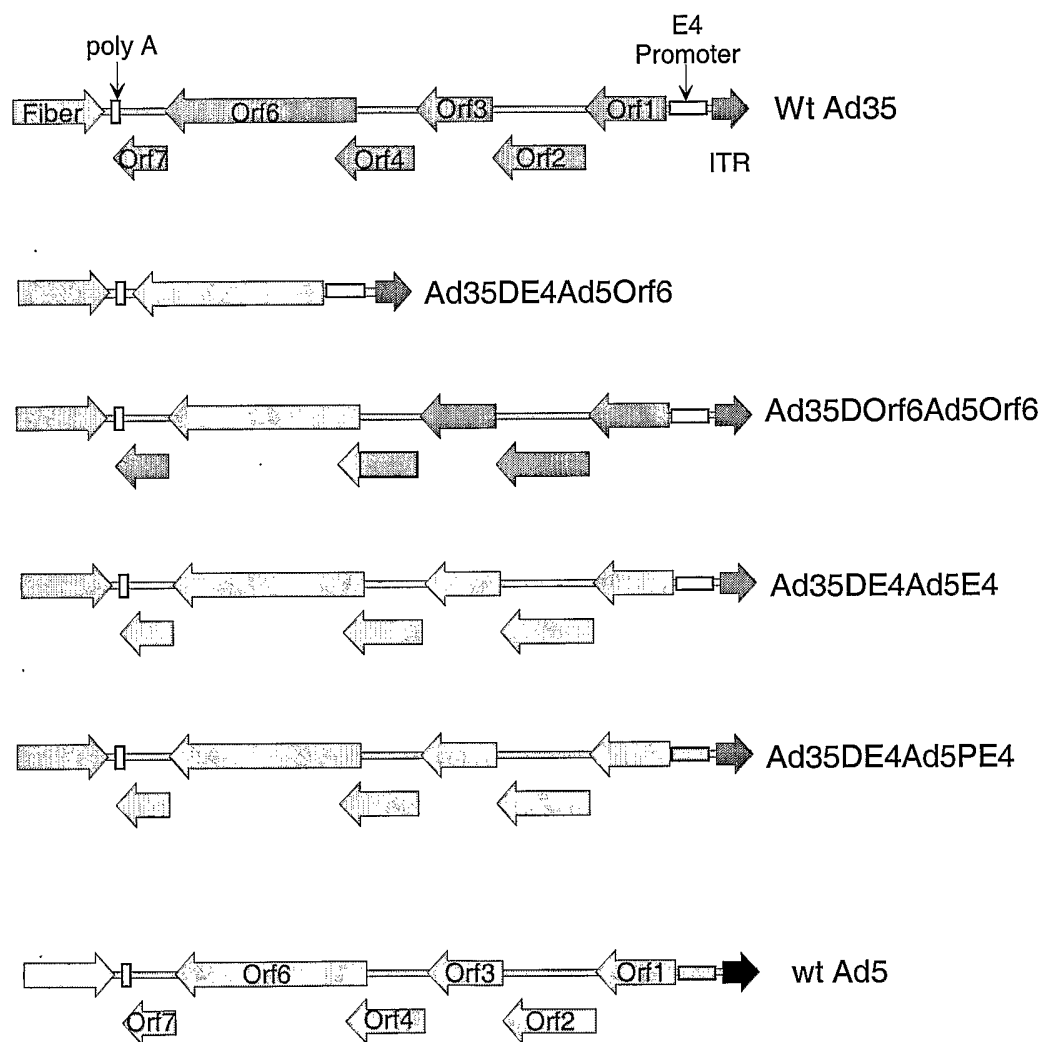


FIG. 4

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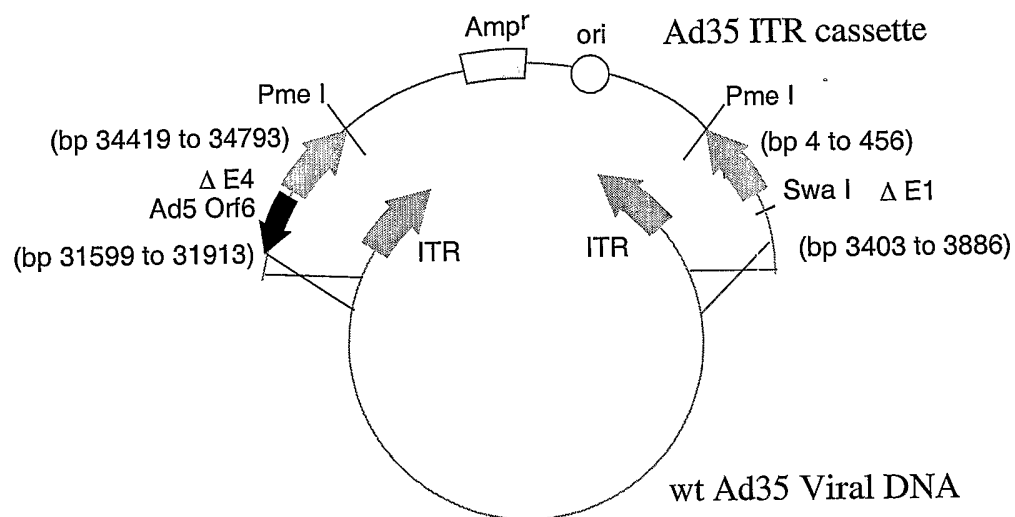


FIG. 5

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1 ccattgcata cgttgatatcc atatcataat atgtacattt atattggctc atgtccaaca
61 ttaccgccat gttgacattg attattgact agttattaat agtaataat tacgggggtca
121 ttagttcata gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgccct
181 ggctgaccgc ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta
241 acgccaatag ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac
301 ttggcagtac atcaagtgtg tcatatgcca agtacgcccc ctattgacgt caatgacggg
361 aaatggcccg cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag
421 tacatctacg tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat
481 gggcgtggat agcgggttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat
541 gggagtttgt tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc
601 ccattgacgc aaatgggcgg taggcgtgta cgggtggagg tctatataag cagagctcgt
661 ttagtgaacc gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga
721 caccgggacc gatccagcct ccgcgcccg gaacggtgca ttggaacgcg gattccccgt
781 gccaagagtg agatctacca TGGGTGCTAG GGCTTCTGTG CTGTCTGGTG GTGAGCTGGA
841 CAAGTGGGAG AAGATCAGGC TGAGGCCTGG TGGCAAGAAG AAGTACAAGC TAAAGCACAT
901 TGTGTGGGCC TCCAGGGAGC TGGAGAGGTT TGCTGTGAAC CCTGGCCTGC TGGAGACCTC
961 TGAGGGGTGC AGGCAGATCC TGGGCCAGCT CCAGCCCTCC CTGCAAACAG GCTCTGAGGA
1021 GCTGAGGTCC CTGTACAACA CAGTGGCTAC CCTGTACTGT GTGCACCAGA AGATTGATGT
1081 GAAGGACACC AAGGAGGCC TGGAGAAGAT TGAGGAGGAG CAGAACAAGT CCAAGAAGAA
1141 GGCCAGCAG GCTGCTGCTG GCACAGGCAA CTCCAGCCAG GTGTCCCAGA ACTACCCCAT
1201 TGTGCAGAAC CTCCAGGGCC AGATGGTGCA CCAGGCCATC TCCCCCGGA CCCTGAATGC
1261 CTGGGTGAAG GTGGTGGAGG AGAAGGCCTT CTCCCCTGAG GTGATCCCCA TGTCTCTGTC
1321 CCTGTCTGAG GGTGCCACCC CCCAGGACCT GAACACCATG CTGAACACAG TGGGGGGCCA
1381 TCAGGCTGCC ATGCAGATGC TGAAGGAGAC CATCAATGAG GAGGCTGCTG AGTGGGACAG
1441 GCTGCATCCT GTGCACGCTG GCCCCATTGC CCCCAGGCCAG ATGAGGGAGC CCAGGGGCTC
1501 TGACATTGCT GGCACCACCT CCACCCTCCA GGAGCAGATT GGCTGGATGA CCAACAACCC
1561 CCCCATCCCT GTGGGGGAAA TCTACAAGAG GTGGATCATC CTGGGCCTGA ACAAGATTGT
1621 GAGGATGTAC TCCCCACCT CCATCCTGGA CATCAGGCAG GGCCCCAAGG AGCCCTTCAG
1681 GGACTATGTG GACAGGTTCT ACAAGACCCT GAGGGCTGAG CAGGCCCTCC AGGAGGTGAA
1741 GAACTGGATG ACAGAGACCC TGCTGGTGCA GAATGCCAAC CCTGACTGCA AGACCATCCT
1801 GAAGGCCCTG GGCCCTGCTG CCACCCTGGA GGAGATGATG ACAGCCTGCC AGGGGGTGGG
1861 GGGCCCTGGT CACAAGGCCA GGGTGCTGGC TGAGGCCATG TCCCAGGTGA CCAACTCCGC
1921 CACCATCATG ATGCAGAGGG GCAACTTCAG GAACCAGAGG AAGACAGTGA AGTGCTTCAA
1981 CTGTGGCAAG GTGGGCCACA TTGCCAAGAA CTGTAGGGCC CCCAGGAAGA AGGGCTGCTG
2041 GAAGTGTGGC AAGGAGGGCC ACCAGATGAA GGAAGTCAAT GAGAGGCAGG CCAACTTCCT
2101 GGGCAAAATC TGGCCCTCCC ACAAGGGCAG GCCTGGCAAC TTCTCCAGT CCAGGCCCTGA
2161 GCCCACAGCC CCTCCCGAGG AGTCCTTCAG GTTTGGGGAG GAGAAGACCA CCCCAGCCA
2221 GAAGCAGGAG CCCATTGACA AGGAGCTGTA CCCCCTGGCC TCCCTGAGGT CCCTGTTTGG
2281 CAACGACCCC TCCTCCCAGT AAaataaagc ccgggcagat ctgatctgct gtgccttcta
2341 gttgccagcc atctgttggt tgccccctccc ccgtgccttc cttgaccctg gaagggtgcca
2401 ctcccactgt cctttcctaa taaaatgagg aaattgcac gcattgtctg agtaggtgtc
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2521 gcaggcatgc tggggatgag gtgggctcta
SEQ ID NO: 2

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FIG. 6

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1 ccattgcata cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca
61 ttaccgccat gttgacattg attattgact agttattaat agtaatcaat tacgggggtca
121 ttagttcata gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgct
181 ggctgaccgc ccaacgaccc cggccattg acgtcaataa tgacgtatgt tcccatagta
241 acgccaatag ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac
301 ttggcagtac atcaagtgt tcatatgcc agtacgccc ctattgacgt caatgacggt
361 aaatggcccg cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag
421 tacatctacg tatttagtcat cgctattacc atgggtgatgc ggttttggca gtacatcaat
481 gggcggtggat agcgggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat
541 gggagtttgt tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caatccgccc
601 ccattgacgc aaatgggccc taggcgtgta cgggtgggagg tctatataag cagagctcgt
661 ttagtgaacc gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga
721 caccgggacc gatccagcct cgcgggcccga gaacgggtgca ttggaacgcg gattccccgt
781 gccaaagagt agatcgatct aagtaagctt CCTGCATGCT GCTGCTGCTG CTGCTGCTGG
841 GCCTGAGGCT ACAGCTCTCC CTGGGCATCA TCCCAGTTGA GGAGGAGAAC CCGGACTTCT
901 GGAACCGCGA GGCAGCCGAG GCCCTGGGTG CCGCAAGAA GCTGCAGCCT GCACAGACAG
961 CCGCCAAGAA CCTCATCATC TTCCTGGGCG ATGGGATGGG GGTGTCTACG GTGACAGCTG
1021 CCAGGATCCT AAAAGGGCAG AAGAAGGACA AACTGGGGCC TGAGATACCC CTGGCCATGG
1081 ACCGCTTCCC ATATGTGGCT CTGTCCAAGA CATAACAATGT AGACAAACAT GTGCCAGACA
1141 GTGGAGCCAC AGCCACGGCC TACCTGTGCG GGGTCAAGGG CAACTTCCAG ACCATTGGCT
1201 TGATGTCAGC CGCCCGCTTT AACCAGTGCA ACACGACACG CGGCAACGAG GTCATCTCCG
1261 TGATGAATCG GGCCAAGAAA GCAGGGAAGT CAGTGGGAGT GGTAAACCACC ACACGAGTGC
1321 AGCACGCCCT GCCAGCCGGC ACCTACGCCC ACACGGTGAA CCGCAACTGG TACTCGGACG
1381 CCGACGTGCC TGCTTCCGCC CGCCAGGAGG GGTGCCAGGA CATCGCTACG CAGCTCATCT
1441 CCAACATGGA CATTGACGTG ATCCTAGGTG GAGGCCGAAA GTACATGTTT CGCATGGGAA
1501 CCCCAGACCC TGAGTACCCA GATGACTACA GCCAAGGTGG GACCAGGCTG GACGGGAAGA
1561 ATCTGGTGCA GGAATGGCTG GCGAAGCGCC AGGGTGCCCG GTATGTGTGG AACCACACTG
1621 AGCTCATGCA GGCTTCCCTG GACCCGTCTG TGACCCATCT CATGGGTCTC TTTGAGCCTG
1681 GAGACATGAA ATACGAGATC CACCGAGACT CCACACTGGA CCCCTCCCTG ATGGAGATGA
1741 CAGAGGCTGC CCTGCGCCTG CTGAGCAGGA ACCCCCGCGG CTTCTTCCCTC TTCGTGGAGG
1801 GTGGTCGCAT CGACCATGGT CATCATGAAA GCAGGGCTTA CCGGGCACTG ACTGAGACGA
1861 TCATGTTCGA CGACGCCATT GAGAGGGCGG GCCAGCTCAC CAGCGAGGAG GACACGCTGA
1921 GCCTCGTCAC TGCCGACCAC TCCCACGTCT TCTCCTTCGG AGGCTACCCC CTGCGAGGGA
1981 GCTCCATCTT CGGGCTGGCC CCTGGCAAGG CCGGGGACAG GAAGGCCTAC ACGGTCTCTC
2041 TATACGGAAA CGGTCCAGGC TATGTGCTCA AGGACGGCGC CCGGCCGGAT GTTACCGAGA
2101 GCGAGAGCGG GAGCCCCGAG TATCGGCAGC AGTCAGCAGT GCCCTTGGAC GAAGAGACCC
2161 ACGCAGGCGA GGACGTGGCG GTGTTGCGCG GCGGCCCGCA GGCACACTG GTTACAGGCG
2221 TGCAGGAGCA GACCTTCATA GCGCACGTCA TGGCCTTCGC CGCCTGCCTG GAGCCCTACA
2281 CCGCCTGCGA CCTGGCGCCC CCGCCCGGCA CCACCGACGC CGCGCACCCG GGTAAAcceg
2341 tggccccgc gttgcttcct ctgctggccg ggacatcagg tggccccgc tgaattggaa
2401 tcgatcagaa ttgatctgat ctgctgtgcc ttctagttgc cagccatctg ttgtttgccc
2461 ctcccccgct ccttccttga ccctggaagg tgccactccc actgtccttt cctaataaaa
2521 tgaggaaatt gcatcgcat gtctgagtag gtgtcattct attctggggg gtgggggtggg
2581 gcagcacagc aagggggagg attgggaaga caatagcagg catgctgggg atgcggtggg
2641 ctcta
SEQ ID NO: 3

```

FIG. 7

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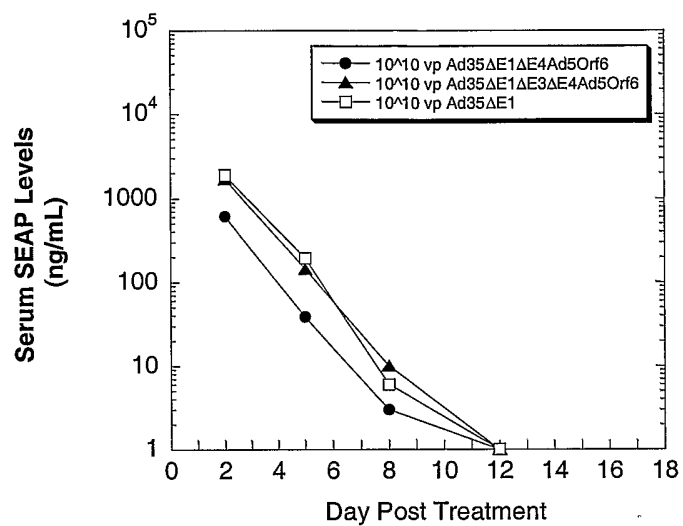


FIG. 8

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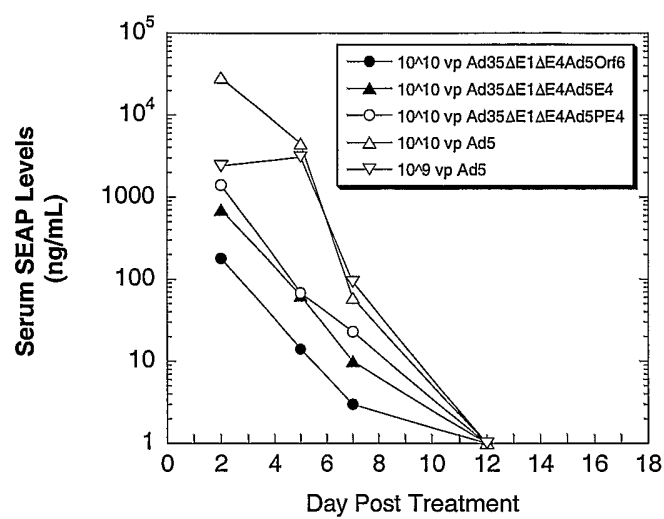


FIG. 9

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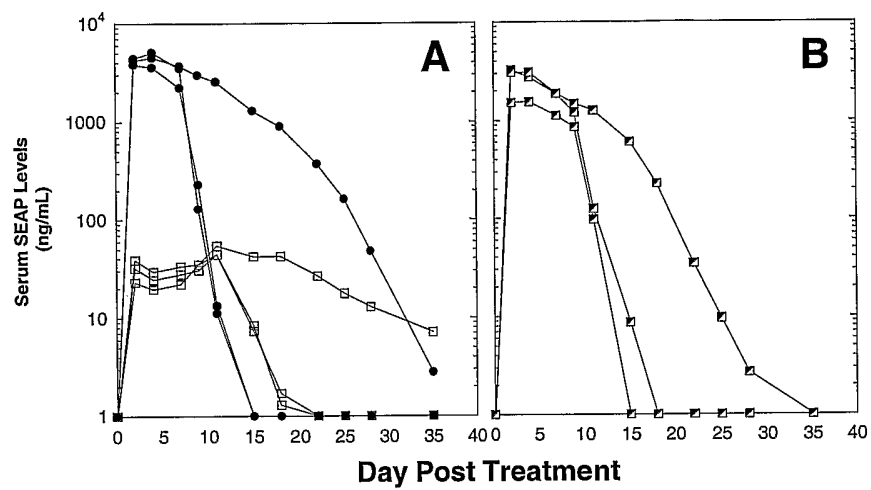


FIG. 10A-B

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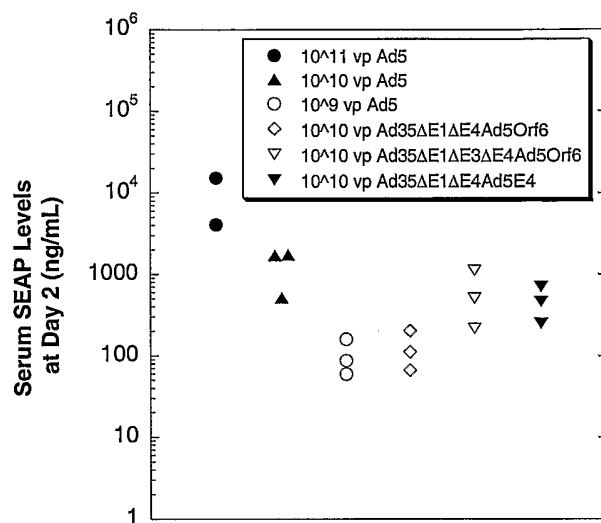


FIG. 11

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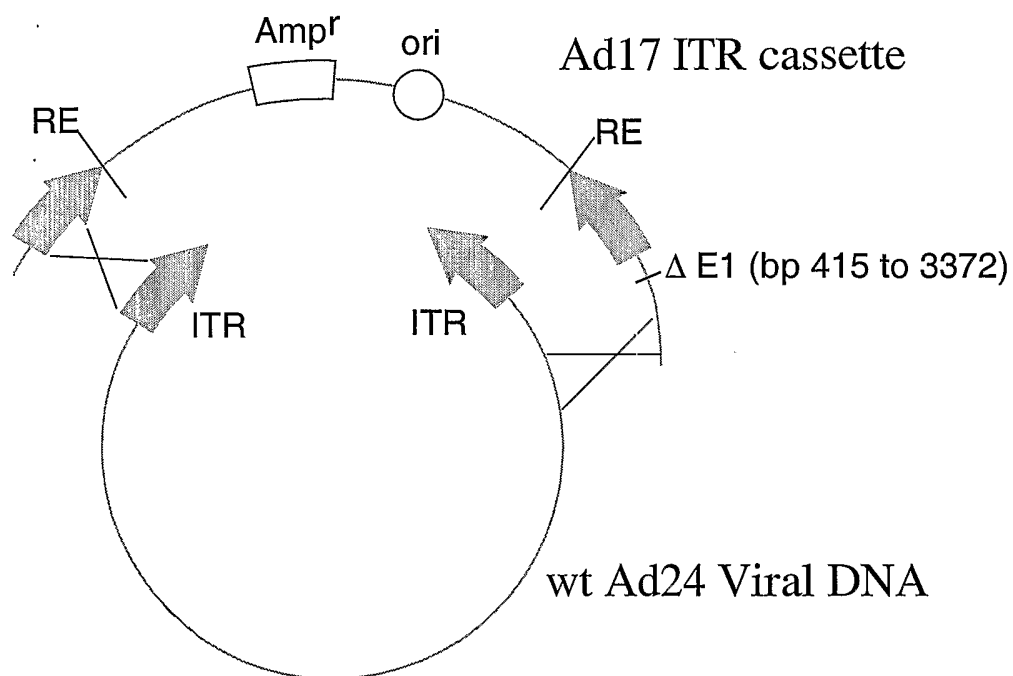


FIG. 12

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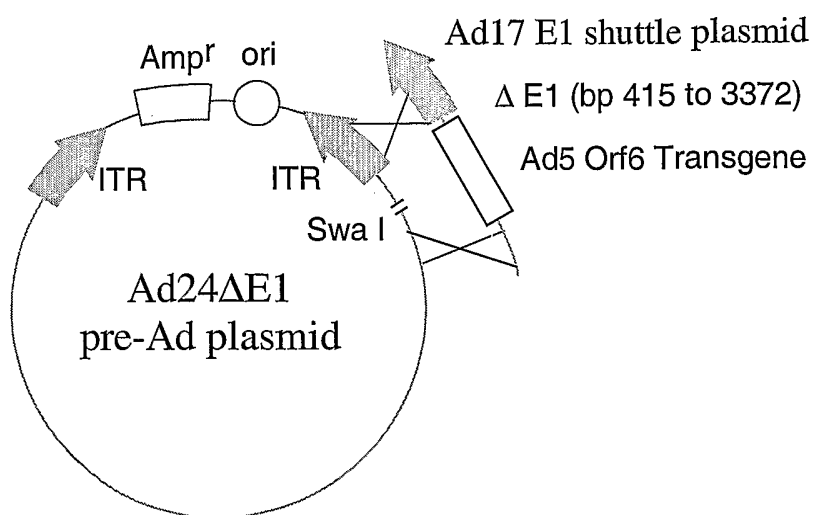


FIG. 13

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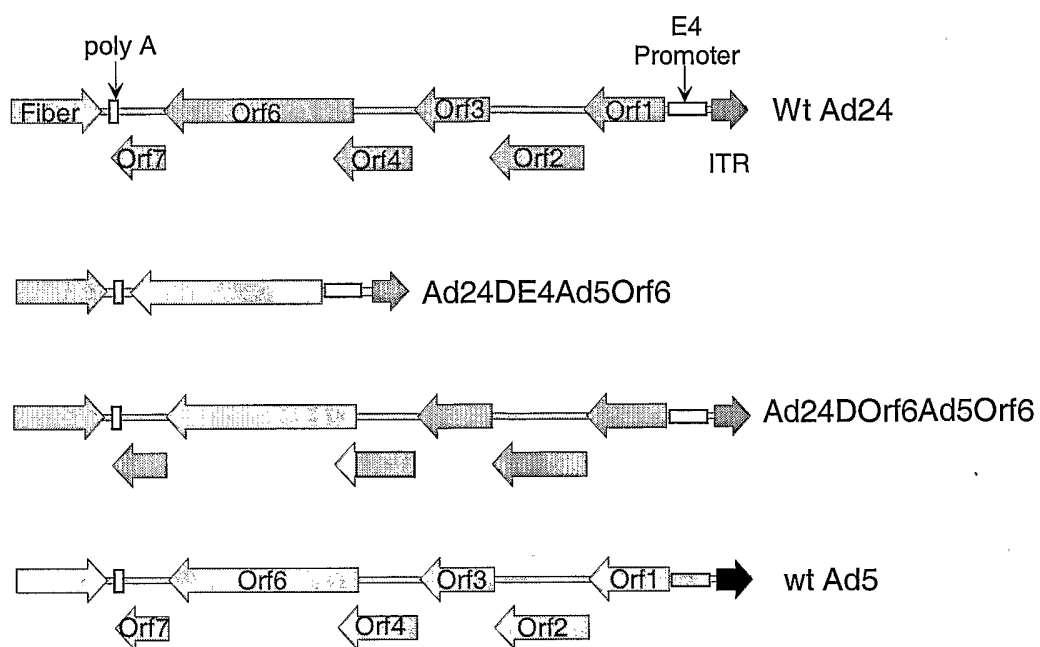


FIG. 14

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Growth Curve Comparison of Ad24 Based Vectors

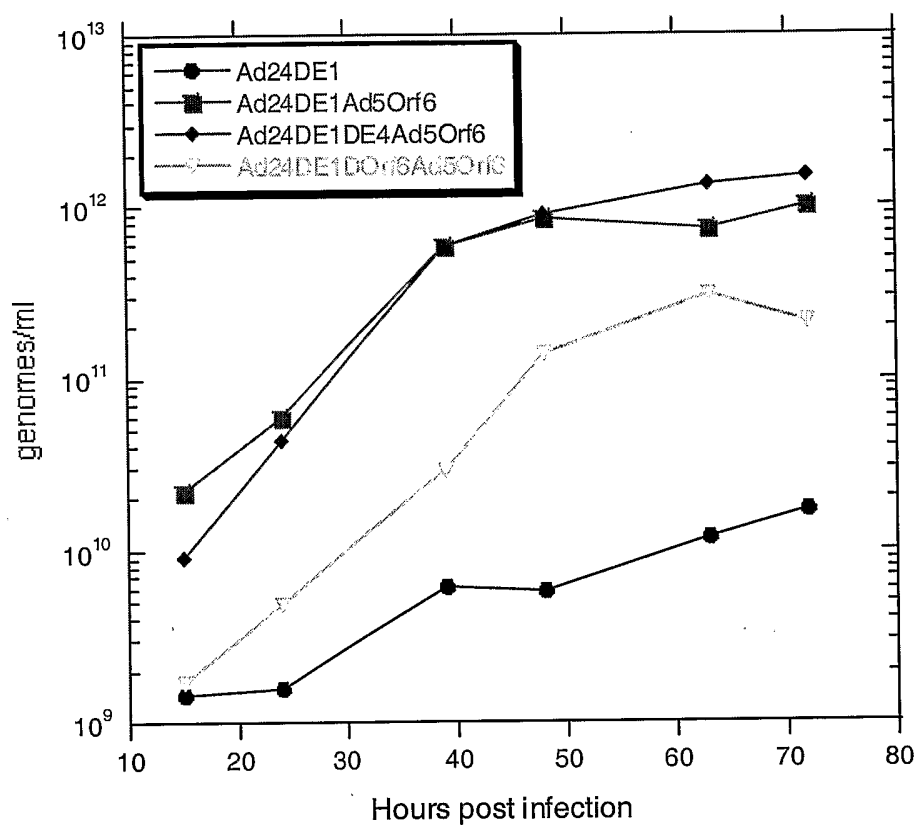


FIG. 15

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1 catcatcaat aatatacccc acaaagtaaa caaaagttaa catgcaaagt agcttttgaa
61 tttagggcgg ggccagcgct gattggacga gagaagatga tgcaaagtac gtcacgacgc
121 acggctaacg gtcgcccgcg aggcgtggcc tagcccggaa gcaagtcgcg gggctgatga
181 cgtataaaaa agcggacttt agacccggaa acggccgatt ttcccgcggc cagcccggga
241 tatgaggtaa ttctgggcgg atgcaagtaa aattagggtca ttttggcgcg aaaactgaat
301 gaggaagtga aaagtgaaaa ataccggctc cggccagggc ggaattatta ccgagggccg
361 agagactttg accgattacg tgggggtttc gattgcggtg ttttttcgcg aatttcgcg
421 tccgtgtcaa agtccggtgt ttatgtcaca gatcagctga tccacagggt atttaaacca
481 gtcgagcccg tcaagaggcc actcttgagt gccagcgagt agagatttct ctgagctccg
541 ctcccagagt ctgagaaaaa tgagacacct ggcctcctt tcttcaactg tgcctattga
601 catggccgca ttattgctgg aggattatgt gagtacaata ttggaggacg aactgcatcc
661 atctccattt gagctgggac ctacacttca ggacctatat gatttggagg tagactgaga
721 tgatgacgac ccgaacgaag aggctgtgaa ttaataattt ccagaatctc tgattcttca
781 ggcgtgacata gccagcgaag ctgtacctac accacttcat acaccgactc tgtcacccat
841 acctgaattg gaagaggagg acgagctaga cctccgatgt tatgaggaag gttttcctcc
901 cagcgattca gaggacgaac agggtagagca gagcatggct ctaatctcaa aatatgcttg
961 tgtggttgtg gaagagcatt ttgtgttgga caatcctgag gtgcccgggc aaggctgtag
1021 atcctgccag taccaccggg ataagaccgg agacacgaac gcctcctgcg ctctgtgtta
1081 catgaaaaag aacttcagct ttattttacag taagtggagt gaatgtgaga gatctgagt
1141 gcttaacaca taactgggta atgcttaaac agctgtgcta agtgtggttt attttgttt
1201 ctaggctcgg tgtcagagga tgagtcatca cctcagaag aagaccaccc gtgtccctct
1261 gagctgtcag gcgaaacgcc cctgcaagtg cacagaccca cccagtcag acccagtggc
1321 gagaggcgag cagctgttga aaaaattgag gacttggtac atgacatggg tggggatgaa
1381 cctttggacc tgagcttgaa acgcccagg aactaggctc agctgtgctt agtcatgtgt
1441 aaataaagtt gtacaataaa agtatatgtg acgcatgcaa ggtgtggttt atgactcatg
1501 ggcgtggctt agtcctatat aagtggcaac acctgggcac tggggcacag accttcaggg
1561 agttcctgat ggatgtgtgg actatccttg cagactttag caagacacgc cggctgttag
1621 aggatagttc agacgggtgc tccgggttct ggagacactg gtttggaaact cctctatctc
1681 gtctgtgtga cacagttaag aaggattata acgaggaatt tgaaaatctt tttgtgtatt
1741 gctctggcct gctagattct ctaaatctcg gccaccagtc ccttttccag gaaagggtag
1801 tccacagcct tgatttttca agcccagggc gactacagc cggggttgct tttgtggttt
1861 ttctggttga caaatggagc cagaacaccc aactgagcag gggctacatt ctggacttct
1921 cagccatgca cctgtggagg gcatgggtga ggcagcgggg acagagaatc ttgaactact
1981 ggcttataca gccagcagct ccgggtcttc ttcgtctaca cagacaaaca tccatgttgg
2041 aggaagaaat gaggcaggcc atggacgaga acccgaggag cggcctggac cctccgtcgg
2101 aagaggagct ggattgaatc aggtatccag cctgtaccca gagcttagca ggggtgtgac
2161 atccatggcc aggggagtgag agagggagag gagcgatggg ggcaataacc ggatgatgac
2221 cgagctgacg gccagcctga tgaatcgcaa gcgtccagag cgcattacct ggcacgagct
2281 acagatggag ttaggggatg aggtgggcct gatgcaggat aaatatggcc tggagcagat
2341 aaaaaccacac tggttgaacc cagatgagga ttgggaggag gccattaaga aatatgccaa
2401 gatagccctg cgcccagatt gcaagtacag ggtgaccaag acggtgaata acggtgaata
2461 ctgctacatc tcggggaacg gggcagaggt ggtcatcgat accctggaca aggccgcctt
2521 caggtgttgc atgatgggaa tgagagccgg agtgatgaat atgaattcca tgattttcat
2581 gaacatgaag ttcaatggag agaagtttaa tggggtgatg ttcatggcca acagtcacat
2641 gaccctgcac ggctgcagtt tcttcggctt caacaatatg tgcgagagg tctggggcgc
2701 tgctaagatc aggggatgta agttttatgg ctgctggatg ggcgtggtcg gaagacccaa
2761 gagcgagatg tctgtgaagc agtgtgtgtt tgagaaatgc tacctgggag tctctaccga
2821 gggcaatgct agagtgaagc attgtctctt cctggagacg ggctgcttct gcctggtgaa
2881 gggcacagcc tctctgaagc ataatatggt gaagggtgct acggatgagc gcatgtacaa
2941 catgctgaca tgcgactcgg gggctctgcca tatcctgaag aacatccatg tgacctccca
3001 cccccggaag aagtggccag tgtttgagaa taacctactg atcaagtgcc acatgcacct
3061 gggcgccaga aggggacact tccagccgta ccagtgaac ttagccaga ccaagctgct
3121 gctggagaac gatgccttct ccagggtgaa cctgaacggc atctttgaca tggatgtctc
3181 ggtgtacaag atcctgagat acgatgagac caagtccagg gtgcgcgctt gcgagtgcgg
3241 gggcagacac accaggatgc aaccagtggc cctggatgtg accgaggagc tgaggcccga
3301 ccacctggtg atggcttgta ccgggaccca gttcagctcc agtggggagg acacagatta
3361 gaggtagggt gagtattagt gggcgtggct aagggtgacta taaaggcggg tgtcttacga
3421 ggggtctttt gcttttctgc agacatcatg aacgggactg gcggggcctt cgaagggggg
3481 ctttttagcc cttatttgac aaccgcctg ccgggatggg ccggagtctc tcagaatgtg
3541 atgggatcga cgttgacggg gcgtccagtg cttccagcaa attcctcgac catgacctac
3601 gcgaccgtgg ggaactcgtc gctcgacagc accgccgcag ccgcccgcag cgcagccgcc

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FIG. 16A-1

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3661 atgacagcga cgagactggc ttcgagctac atgcccagca gcagcagtag cccctctgtg
3721 cccagttcca tcatcgccga ggagaaactg ctggccctgc tggccgagct ggaagccctg
3781 agccgccagc tggccgccct gacccagcag gtgtccgagc tccgcgaaca gcagcagcag
3841 caaaataaat gattcaataa acacagattc tgattcaaac agcaaagcat ctttattatt
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3961 tttttttccg gacccggtag aggtgggatt ggatgttgag gtacatgggc atgagcccg
4021 cccgggggtg gaggtagcac cactgcacat cctcgtgctc tggggtcgtg ttgtagatga
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4261 gatccgcctt ggggttcatg ttgtgcagga ccaccagaac ggtgtagccc gtgcacttgg
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4741 ccccgatgac ggggttgacg tggtagtcca aggacatgca gctgccgtcg tcccggagg
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4981 agagctcggt gacgtgctct acggcatctc gatccagcag acttctcgtt tcggggggtt
5041 gggacgactg cgaactgtag gcacgagacg atgggctgcc agcgtgcca gcgtcatgtc
5101 cttccagggt ctcaagtgtc gcgtgagcgt ggtctccgtc acggtgaagg ggtgggcccc
5161 gggctgtgct cttgcaaggg tgcgcttgag actcatcctg ctggtgctga aacgggcacg
5221 gtcttcgccc tgcgctcgcc cgagatagca gttgaccatg agctcgtagt tgagggcctc
5281 ggcggcggtg ccttggcgc ggagcttgcc cttggaagag cgcccgcagg cgggacagag
5341 gagggattgc agggcgtaga gcttggtgct gagaaagacg gactcggggg cgaagcatc
5401 cgctccgcag tgggcgcaga cggctctgca ctcgaccagc caggtagact cgggctgctc
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5581 gggcctgtcc tgcaggggcg tcccgcggct ctctcgtag agaaactcgg accactctga
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5701 gtccaccagg ggtccacct tttccagggt atgcagacac atgtccccct cctccgcac
5761 caagaagggt attggcttgt aggtgtaggc cacgtgacct ggggtccccg acgggggggt
5821 ataaaagggg gcgggtctgt gctcgtctc actctcttc ccgtcgtgt ccacgagcgc
5881 cagctgttgg ggtaggtatt ccttttcgag agcgggcatg acctcggcac tcaggttgtc
5941 agtttctaga aacgaggagg atttgatgtt ggcttgccct gccgcaatgc tttttaggag
6001 actttcatcc atctggtcag aaaagactat ttttttattg tcaagcttgg tggcgaagga
6061 gccatagagg gcgttgagga gaagcttgcc gatggatctc atggtctgat tttgtcacg
6121 gtcggctcgc tccttgcccg cgatgttgag ctggacatac tgcgcgcga cgcacttcca
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6241 cagggtgacc agatccacgc tgggtggccac ctgcgcgcgc aggggctcgt tggccagca
6301 gaggcgtccg cccttgccgc agcagaacgg gggcagcaca tcaagcagat gctcgtcagg
6361 ggggtccgca tcgatgtgga agatgcccgg acagagttcc ttgtcaaaat aatcgatttt
6421 tgaggatgca tcatccaagg ccatctgcca ctgcggggcg gccagcgctc gctcgtaggg
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6781 gtcgcggatg aagtgcgcgt aggagtcttg cagcttgggc acgagctcgg cggtagcag
6841 gacgtccatg gcgcagtagt ccagcgtttc gcggatgatg tcataaccgg cctctccttt
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7081 ggtgtgcgtc agggcgaagg tatccctgac catgactttc aagaactggt acttgaaatc
7141 cgagtcgtcg cagccgccgt gctcccagag ctcgaaatcg gtgcgcttct tcgagagggg
7201 gttaggcaga gcgaaagtga cgtcattgaa gagaatcttg cctgcccgcg gcatgaaatt
7261 gcgggtgatg cggaaagggc ccgggacgga ggctcgggtg ttgatgacct gggcggcgag

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FIG. 16A-2

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7321 gacgatctcg tcgaagccgt tgatgttggtg cccgacgatg tagagttcca tgaatcgcgg
7381 gcggccttta atgtgcggca gctttttgag ctccctcgtag gtgaggtcct cggggcaatg
7441 cagtccgtgc tgctcgagcg cccactcctg gagatgtggg ttggcttgca tgaatgaagc
7501 ccagagctcg cgggccataa gggctctggag ctcgtcgcga aagaggcgga actgctggcc
7561 cacggccatc ttttctgggg tgacgcagta gaaagtaagg ggtcccgt cccagcgtc
7621 ccagcgtaag cgcacggcta gatcgcgagc gagggcgacc agctctgggt ccccgagaa
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8461 cggttgacat cctggatctg ccgctctcgc gtgaagacca ccggccccgt gactttgaac
8521 ctgaaagaca gttcaacaga atcaatctcg gcgtcattga cggcgccctg acgcaggatc
8581 tcttgacagt cggccgagtt gtccctggtag gcgatctcgg acatgaactg ctcgatctcc
8641 tccctctgga gatcgccgcg gcccgcgcgc tccacggtag cggcgaggtc attggagatg
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8761 acgtccccgt cggcgctcgc cgcgcgcagt accacctgcg cgaggttgag ctccacgtgc
8821 cgcgtgaaga cggcgtagtt gcgcaggcgc tggaaagagg agtttagggg ggtggcgatg
8881 tgctcgggta cgaagaagta catgatccag cggcgaggg gcactcgcgt gatgtcgccg
8941 atggcctcca gcctttccat ggctcgtag aaatccacag cgaagttgaa aaactgggcg
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10021 gccggcgcg cgggggcccag gtcctcgagc atgaggcggt ggtagccgta gaggtagcgg
10081 gacatccagg tgatgccggc ggcgggtggtg agggcgcgcg ggaactcgcg gacgcggtc
10141 cagatgttgc gcagcggcag gaaatagttc atggtcggca cggctcgcc ggtgagacgc
10201 gcgcagtcac tgacgtctta gaggcaaaaa cgaaagcggt tgagcgggct cttctccgt
10261 agcctggcgg aacgcaaacg ggttaggcgg cgtgtgtacc cgggttcgag tccctcgaa
10321 tcaggctgga gccgcgacta acgtggtatt ggcactccc tctcgacccg agcccgatag
10381 ccgccaggat acggcggaga gccctttttg ccgaccgagg ggagtgcgta gacttgaaag
10441 cggccgaaaa cccgcgcggg tagtggtctc cgcccgtagt ctggagaagc tttgccaggg
10501 ttgagtcgcg gcagaacccg gttcgcggac ggccgcggcg agcgggactt ggtcaccccc
10561 ccgatttaaa gaccacagc cagccgactt ctccagttac gggagccgac ccccttttt
10621 ctttttgcca gatgcatccc gtctgcgccc aaatgcgtcc cacccccgtc cggcgacca
10681 cctgtaccgc ggccgtagca ggcgcggcg ctgtagcccc gccacagcag acagagatgg
10741 acttggaaga gggcgaaggg ctggcgagac tgggggcgcc gtccccggag cgacaccccc
10801 gcgtgcagct gcagaaggac gtgcgcccgg cgtacgtgcc tgccgagAAC ctgttcaggg
10861 accgcagcgg ggaggagccc gaggagatgc gcgactgcc ttttcggggc ggcagggagc
10921 tgcgcgaggg cctggaccgc cagcgcgtgc tgccgcagca ggatttcgag ccgaacgagc

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FIG. 16A-3

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10981 agacggggat cagccccgcg cgcgcgcacg tggcggcggc caacctgggtg acggcctacg
11041 agcagacgggt gaagcaggag cgcaacttcc aaaagagttt caacaacccat gtgcgcacgc
11101 taatcgcgcg cgaggaggtg gccctgggct tgatgcacct gtgggacctg gcgaggcca
11161 tcgtgcagaa cccggacagc aagcctctga cggcgcagct gttcctgggtg gtgcagcaca
11221 gcagggacaa cgaggcgctt agggaggcgc tgctaaacat cgccgagccc gagggccgct
11281 ggctgctgga gctgatcaac atcttgacga gcatcgtagt gcaggagcgc agcctagacc
11341 tggccgagaa ggtggcggct atcaactact cggtgctgag cctgggcaag ttttacgcgc
11401 gcaagattta caagacgccc tacgtgcccc tagacaagga ggtgaagata gacagctttt
11461 acatgcgcat ggcgctcaag gtgctgacgc tgagcgacga cctgggcgtg taccgcaacg
11521 accgcatcca caaggccgtg agcgcgagcc ggcggcgcga gctgagcgac cgcgagctga
11581 tgctgagtct gcgccgggcg ctggtagggg gcgccgccgg cggtgaggag tcctacttcg
11641 acatgggggc ggacctgcat tggcagccga gccggcgcgc cttggaggcc gcctacggtc
11701 cagaggactt ggatgaggat gaggaagagg agggagatgc acccgctgcg ggggtctgac
11761 gcctccgtga tgtgttttta gatgcagcaa gctccggac gactgggagg ccgcgatgca acgcatcatg
11821 caagcagc cgtccggtct cgcgaaccc cgagtctttt agacaacagc cgcaggccaa cagactctcg
11881 gccctgacga cccgcaaccc aggcgggtgt cccctctcgg accaacccca cgcacgagaa ggtgctggcg
11941 gccattcttg aggcgggtgt cgctggcggg gaacaaggcc atccgtcccg acgaggccgg gctggtgtac
12001 atcgtgaacg cgctggcggg gggccgctac aacagcacia acgtgcagtc caacctggac
12061 aacgccctgc tggagcgcgt cggacgtgcg cgaggccgtg gcgcagcgcg agcggttcaa gaacgagggc
12121 cggtggtgta cggacgtgcg tggtggcgct gaacgccttc ctggcgacgc agccgcgagc ggcggcgcg
12181 ctgggctcgt tgggtggcgct ctttatcagc gcgctgcggc tgatggtgac cgagggtccc
12241 gggcaggagc attacaccaa ctttatcagc gggcccagac tactttttcc agacgagccg gcagggtctg
12301 cagagcgagg tgtaccagtc gggcccagac aatctgcgcg ggctgtgggg cgtgcaggcg
12361 cagacgggtg acctaaagcca ggctttcaag atgtgacgc ttgctaacgc ccaactcgcg gctgctgctg
12421 cccgtgggcg accggtcgac ggtgagcagc cgacagcggc agcgtgaacc gcaactcgta cctggggccac
12481 ctgctgatcg cgcccttcac ggccataggg caggcgaggc tggacgagca gacctccag
12541 ctgctgacgc tttaccgcga ggcctagggc cagacgagca cgcagagtct cgcagtagcg gctgtcggcc
12601 gagatcacta gcgtgagccg cgcgctgggt tagacagcag aagattccgg cgcagtagcg gctgtcggcc
12661 ctgaacttct tgctgacaaa tagacagcag atatgtgcag cagagcgtag ggcttttctt gatgcaggag
12721 gaggaggagc gcctcctgag gctggacatg accgcgcgca acatggaacc tagcatgtac
12781 ggggccaccc ccagcgccgc gctggacatg atggactacc tgcaccgcgc ggctgccatg
12841 gccgccaccc ggccgttcat caataagctg agcgtgaacc gcaactcgta cctggggccac
12901 aactcggact actttactaa tgctatacta aaccgcgact ggctcccgcc gccgggggtt
12961 tacacgggcg agtaacgacat gcccgaaccc aacgatgggt tcctgtggga cgacgtggac
13021 agcgcgggtg tctccccgac cttgcaaaaag cgccaggagg cggtagcgac cggcgcgagc
13081 gagggcgcgg tgggtcggag cccctttcct agcttaggga gtttgcatag cttgccgggc
13141 tcggtgaaca gcggcagggg gagccggccg cgcttgctgg gcgaggacga gtacctgaac
13201 gactcgctgc tgcagccgcc gggggtcaag aacgccatgg ccaataacgg gatagagagt
13261 ctggtggaca aactgaaccg ctggaagacc tacgctcagg accataggga tgcgcccgcg
13321 ccgcgcgac agcgccacga ccggcagcgg ggcctggtgt gggacgacga ggactcggcc
13381 gacgatagca gcgtgttgga cttgggcggg agcgggtggg ccaaccgctt cgcgcatctg
13441 cagcccagac tggggcgacg gatgttttga atgaaataaa actcaccaag actcacagct
13501 gcgttctctt ccttggttaga gatgagcgc gcgggtggtg cttcctctcc tcctccctcg
13561 tacgagagcg tgatggcgca ggcaaccctg gaggttccgt ttgtgcctcc gcggtatatg
13621 gctcctacgg agggcagaaa cagcattcgt tactcggaac tggctccgca gtacgacacc
13681 actcgcgtgt acttggtgga caacaagtcg gcggacatcg cttccctgaa ctacaaaaac
13741 gaccacagca acttccctgac cacggtgggt cagacaacg atttcacccc cgccgaggcc
13801 agcacgcaga cgataaattt tgacgagcgg tcgcggtggg gcggtgattt gaagaccatt
13861 ctgcacacca acatgcccac tgtgaacgag tacatgttca ccagcaagt taaggcgcg
13921 gtgatgggtg ctaggaaggt ggtgatcag aatgatagga gcaaggatga gttaaaatat
13981 gagtgggttg agttttaccct gcccaggggc aacttttccg agaccatgac catagacctg
14041 atgaacaacg ccatcttgga aaactacttg caagtggggc ggcaaaatgg cgtgctggag
14101 agcgatatcg gagtcaagtt tgacagcagg aatttcaagc tgggctggga cccggtaacc
14161 aagctggtga tgcctggggt ctacacctac gaggccttcc acccgagcgt tgtgctgctg
14221 ccgggctgcg ggttggaact caccgagagc cgcttgagca acctcctggg cattcgcaag
14281 aagcaacctt tccaagaggg cttcaggatc atgtatgagg atctcgaggg tggtaacatc
14341 cccgccctcc tggatgtcaa gcaatatatt gatagtaaaa agaatcttga agaatcaaca
14401 cagaatgcaa ccagggtgc tggagatatc agaggagaca gtcatatccc aagagctgtg
14461 gaacaagcgg ctgaaaagga tctggtcatt gtaccagtaa cacaagatga aagtaagaga
14521 agctataatg tcatagatgg caccatgac accctctacc gaagtgggta cctgtcctat
14581 acctacgggg accccgagaa gggggtgcag tcgtggacgc tgctcaccac cccggacgct

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FIG. 16A-4

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14641 acctgccccg cgagcaagt ctactggtcg ctgccccgacc tcatgcaaga ccccgtcacc
14701 ttccgctcta cccagcaagt cagcaactac cccgtggttg gcgcgagag catgcccttc
14761 cgcgcgaaga gcttttataa cgacctcgcc gtctactccc agctcatccg cagctacacc
14821 tccctcacc acgtcttcaa ccgcttcccc gacaaccaga tctctgcccg tccgcccgcg
14881 cccaccatca ccacggtcag tgaacacgtg cctgctctca cagatcacgg gacgctaccg
14941 ctgcgacaga gtatccgagg agtccagcga gtgaccgtca ctgacgcccg tcgcccacc
15001 tgtccctacg tctacaaggc cctgggcata gtgccccgcg gcgtgctttc cagtcgcacc
15061 ttctaaaaaa tgtctattct catctcgccc agcaataaca cgggtggggg tcttactagg
15121 cccagcacca tgtacggagg agccaagaag cgctcccagc agcacccegt cgcgctccgc
15181 ggccacttcc gcgctccctg gggcgcttac aagcgccggc ggacttctac cgcgcgcgtg
15241 cgcaccaccg tcgacgacgt catcgactcg gtggtcgccg acgcgcgcaa ctataccccc
15301 gccccctcca ccgtggacgc ggtcatcgac agcgtggttg ccgacgcgcg cgactagcc
15361 agacgcaaga gccggcgggc acggatcgcc aggcgccacc ggagtacggc cgcgagtcg
15421 gccgcccggg ctctgctgcg ccgcccagca cgcacggggc gccgggccc atgatgcgagc
15481 gcgcgcgcgc ccgccactgc acccccgcga ggcaggactc gcagacgagc ggccgcccgc
15541 gctgcccgcg ccatttctag catgaccaga cccaggcgcg gaaacgtgta ctgggtgccc
15601 gactccgtca cgggctgctg cgtgcccgtg cgcaccgcgt ctctctgctc ctgatctaat
15661 gcttgtgtcc tccccgcgaa gcgacgatgt caaagcgcaa aatcaaggag gagatgctcc
15721 aggtcgctgc cccggagatt tacggaccac cccaggcgga ccagaaaccc cgcaaatca
15781 agcgggttaa aaaaaaggat gaggtggagc agggggcagt agagtgtgtg cgcgagttcg
15841 ctccgcccgc gcgcgtaaat tggaaagggc gcagggtgca gcgcgtgttg cggcccggca
15901 cggcggtggt gtttacgccc ggcgagcggt cctcggtcag gagcaagcgt agctatgacg
15961 aggtgtacgg cgacgacgac atcctggacc aggcggcgga gcggcgggc gagttgcct
16021 acgggaagcg gtcgcgcgaa gaggagctga tctcgttgcc gctggacgag agcaacccca
16081 cgcctagcct gaagcccgtg accctgcagc aggtgctgcc ccaagcagtg ctgctgccga
16141 gccgcggggg caagcgcgag ggcgagaata tgtaccgac catgcagatc atggtgcca
16201 agcgccggcg cgtggaagaa gtgctggaca ccgtgaaat ggatgtggag cccgaggtca
16261 aggtgcgccc catcaagcag tggcgccggc ccctgggctg gcagaccgtg gacattcaga
16321 tccccaccga catggatgtt gacaaaaaac cctcgaccag catcgaggtg cagaccgacc
16381 cctggctccc agcctccacc gctgcccgtc ccacttctac gcgccccag gctaccgagc
16441 ctcccagaag gcgaagatgg ggccttgcca accggtgatg gcccaactac gtattgcac
16501 cttccattat cccgacgccc ggctatcgcg gcacccggtg ctacgccagc cgcaggcgcc
16561 cagccagcaa acgcccgcgc cgcaccgcca cccgcccgcg tctggcccc ccccgcgtgc
16621 gccgcgtaac cagcgcccg ggcgctcgc tcgttctgcc caccgtgccc taccgccc
16681 gcatccttta atccgtgtgc tgtgatactg ttgcagagag atggctctca cttgcccct
16741 gcgcatcccc gtcccgaatt accgaggaag atcccgcgc aggagaggca tggcaggcag
16801 cggcctcaac cgcgcgcggc ggcgggccc atgagtgccg ctgagtgccg gctttctgcc
16861 cgcgctcatc cccataatcg cggcgcccat cggcacgatc ccgggcatag cttccgttgc
16921 gctgcaggcg tcgcagcgcc gttgatgtgc gaataaagcc tctttagact ctgacacacc
16981 tggctcctgta tatttttaga atggaagaca tcaattttgc gtccctggct cgcggcgacg
17041 gcacgcggcc gttcatgggc acctggaacg agatcggcac cagcagctg aacggggcg
17101 ctttcaattg gagcagtgct tggagcgggc ttaaaaaatt cggctcgacg cccgggacct
17161 atgggaacaa ggcctggaat agtagcaggg ggcagttgtt aagggaaaag ctcaaagacc
17221 agaacttcca gcagaagggt gtggacggcc tagcctcggg cattaacggg gtggtggaca
17281 tagcaaacca ggccgtgcag cgcgagataa acagccgcct ggaccgcgg ccgcccacgg
17341 tgggtggagat ggaagatgca actcctccgc cgcccaaggg cgagaagcgg ccgcggccc
17401 acgcgaggga gacgatcctg caggtggacg agccgccctc gtacgaggag gccgtcaagg
17461 ccggcatgcc caccacgcgt atcatcgcg cactggccac tgggtgtaat aaacccgcca
17521 cccttgacct gcctccgcca cccacgcgcg ctcaccgaa ggcatccg gttgtgcagc
17581 cccctcctgt ggcgaccgcc gtgcccgcg tccccgcgg ccgccaggcc gttgtgcagc
17641 agagcacgct gcacagtatc gtgggcctgg gagtgaagag tctgaagcgc cgccgatgct
17701 attgagagag aggaaagagg aactaaagg gagagcttaa cttgtatgtg cttaccgccc
17761 agagaacgcg cgaagatggc taccctcctg atgatccgc agtggcgta catgcacatc
17821 gccgggagg acgcctcgga gtacctgagc ccgggtcttg tgcagtttgc ccgcccacc
17881 gacacgtact tcagcctggg caacaagttt aggaacccca cggtggtccc caccacgat
17941 gtgaccacgg accggtcccc gcgtctgacg ctgctcttgg tgcccgtaga tgcgaggac
18001 accagctact cgtacaaggc cgtcttact ctggcctgg ggcacaaccg ggtgtagac
18061 atggccagca cttactttga catccgccc gtcctggacc gcggtcccag cttcaaacc
18121 tactcgggca cggcttaca cagcctggcc cccaaaggcg ccccaactc tagtcagtg
18181 gaacaagcta aagctaccaa tgccggtcaa aaggaaactc acacatttgg agtagccgt
18241 atgggcggag aagacattac agtgaaaggt cttcaaattg gaactgatga aactaaggaa

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FIG. 16A-5

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18301 gatggagagg atgaaatttt tgcagatcaa acattccagc cagaacctca agtgggagaa
18361 cagaactggc aagaaacgtt tgttttctat ggaggcagag ctcttaagaa agaaaccaa
18421 atgaagccat gttatggctc ttatgcgaga cccacaaatg aaaagggagg acaggctaaa
18481 ttacactttg atgaaaaagg tcagccaacc aaaattcctg atattacaat ggatttcttt
18541 gatagtccac aagatgatac atcaggtgta actaataagc cagatattgt catgtatgca
18601 gaaaatgtaa atttagaagc tcctgacaca catgtagttt acaaaccagg caaagatgat
18661 tctagttctt cgcctaacct cacacaacag gccatgccta acagaccgaa ctacatcggg
18721 ttcagagaca actttgtggg tcttatgtac tacaatagta ctggcaacat ggggtgtgtg
18781 gctggtcagg cctctcagtt gaatgctgtg gtcgacttgc aagacagaaa caccgagctg
18841 tcttaccagc tattgctaga ttctctgggt gacagaacca gatactttag catgtggaat
18901 tctgcagtgg acagctatga cccgatgtc aggatcattg agaatcacgg tgtggaagat
18961 gaacttccaa actattgctt cccactgaat ggcagtgggt ctaacagcac atacaaaggt
19021 gttaaagctg gaactggaaa caattgggat gacgatgaaa atgttgcaag acaaaatcag
19081 attggcactg gcaacctgtt cgctatggag atcaacctcc aggccaaagt atggaagagt
19141 tttctgtact cgaacgtggc cctgtacttg cccgactcct acaagtaaac gccggccaac
19201 gtcacgctgc ccaccaacac caacacctac gactacatga acggccgctg ggtagccccc
19261 tcgctgggtg acgcctacat caacattggc gcccgctggg cgctggaccc catggacaat
19321 gtcaatccct tcaaccacca ccgcaacgcy ggcctgcgct accgctccat gctcctgggc
19381 aacggccgct acgtgccctt ccacatccaa gtgccccaaa agttctttgc catcaagaac
19441 ctgcttctgc tccccggttc ctacacctac gactggaact tccgcaagga cgtcaactg
19501 atcctgcaga gttccctcgg caacgacctg cgcgtcgacg gcgctccgt cgcttcgac
19561 agcgtcaacc tctacgccac cttcttcccc atggcgcaca acaccgctc caccctggaa
19621 gccatgctgc gcaacgacac caacgaccag tccttcaacg actacctctc ggccgccaac
19681 atgctctacc ccatcccggc caaggccacc aacgtgcca tctccatccc ctgcgcaac
19741 tgggcccgcct tccgcggtg gagtttcacc cggctcaaga ccaaggaaac tccctccctc
19801 ggctcgggtt tcgaccccta ctttgtctac tcgggtcca tccctacct cgacgggacc
19861 ttctacctca accacacctt caagaaggtc tccatcatgt tcgactcctc ggtcagctgg
19921 cccggcaacg accggctgct cagccgaac gagttcgaga tcaagcgag cgttgacggg
19981 gagggtcata acgtggccca atgcaacatg accaaggact ggttcctcgt ccagatgctc
20041 tcccactaca acatcggcta ccagggtctt cactgccccg agggctacaa ggaccgcatg
20101 tactccttct tccgcaactt ccagcccatg agcaggcagg tggctgatga gatcaactac
20161 aaggactaca aggcgcgtac cctacccttc cagcacaaca actcgggctt caccggctac
20221 cttgcgcca ccatgcgcca ggggcagccc taccgcgcca acttccccta cccgctcatc
20281 ggctccaccg cagttccctc cgtcaccag aaaaagttcc tctgcgacag ggtcatgtgg
20341 cgcateccat tctccagcaa ctttatgtcc atgggcgccc tcaccgacct ggttgacggg
20401 atgctctatg ccaactcggc ccacgcgtc gacatgacct ttgagggtgga ccccatggat
20461 gagcccaacc tctctatct tctcttcgaa gttttcgacg tggctcagagt gcaccagccg
20521 caccgcgggc tcatcgaggc cgtctacctg cgcacgccc tctccgccc gatcgtgcgc
20581 acttaagcat gagcggctcc agcgaacaag agctcgcggc catcgtgcgc tggcttccct
20641 gcgggcccta ctttttggga acccacgaca agcgttccc tggcttccct gccggcgaca
20701 agctggcctg cgccatcgtc aacacggccg gccgcgagac cggaggcgtg cactggctcg
20761 cctttggctg gaatccgcgc tcgcgcacct gctacatgtt cgacccttt gggttctcgg
20821 accgcccggc caagcagatt tacagcttcg actacgaggc catgctcgc cgaagcgcgc
20881 ttgcctcctc gcccgaccgc tgtctcagcc tcgagcagtc caccagacc gtgcaggggc
20941 ccgactccgc cgctgcgga cttttttgtt gcatgttttt gcatgccttc gtgcactggc
21001 ccgaccgacc catggacgga aaccccacca tgaacttgct gacgggggtg ccaaaccgga
21061 tgctacaatc gccacaggtg ctgcccaccc tcaggcgcaa ccaggaggag ctctaccgct
21121 tcctcgcgcy ccaactccct tactttcgat cccaccgcgc cgccatcgaa aacgccaccg
21181 cttttgataa aatgaaacaa ctgctgtgat ctcaataaac agcactttat ttacatgca
21241 ctggagtata tgcaagttat ttaaaagtcg aaggggttct cgcgctcgtc gttgtgcgc
21301 gcgctgggga gggccacgtt gcggtactgg tacttgggaa gccacttgaa ctcggggatc
21361 accagtttgg gcactggggg ctcgggggag gtctcgctcc acatgcgccc gctcatctgc
21421 agggcgccca gcatgtccgg gccggagatc ttgaaatcac aattggggcc ggtgctctgc
21481 gcgcgcgagt tgcggtacac ggggttgag cactggaaca ccattagact ggggtacttc
21541 acactggcaa gcacgctctt gtcgctgatc tgatccttgt ccaggctctc ggcgttgctc
21601 aggcgaacg ggtcatctt gcacagctgg cggcccagga agggcacgct ctgaggcttg
21661 tggttacact cgcagtgcac gggcatcagc atcatcccc cgccgcgctg catattctgg
21721 tgagggcct tgacgaaggc cgtgatctgc ttgaaagctt gctgggctt agccccctg
21781 ctgaaaaaca ggcgcgagct cttcccgtc aactggttat tccgcaccc ggcacatgc
21841 acgcagcagc gcgcgtcatg gctggctcag tgcaccacgc tacgtcccca gcggttctg
21901 gtcaccttgg ccttgctggg ctgctccttc aacgcgcgct gccggttctc gctggtcaca

FIG. 16A-6

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21961 tccatctcca ccacgtggtc cttgtggatc atcacccgtcc catgcagaca cttgagctga
22021 ccctcgacat cgcagcagcc atgatccac agggcgagc cgggtgactc ccagttctta
22081 tgcgcgatcc cgctgtggct gaagatgtaa ccttgcaaca ggcgacccat gacggtgcta
22141 aatgctttct ggggtggtgaa ggtcagttgc agaccgcggg cctcctcggt catccaggtc
22201 tggcacatct tttggaagat ctcggtctgc tcgggcatga gcttgtaagc atcgcgcagg
22261 ccgctgtcga cgcggtagcg ttccatcagc acgttcatgg tatccatgcc cttctcccag
22321 gacgagacca gaggcagact caggggggtt cgcacgttca ggacaccggg ggtcgcaggc
22381 tcgacgatgc gttttccgtc cttgccttcc ttcaacagaa cgggaggctg gctgaatccc
22441 actcccacga ttacggcatc ttcttggggc atctcttcgt cggggtctac cttggtcaca
22501 tgcttgggtc ttctggcttg cttctttttt ggagggtgtt ccacggggac cacgtcctcc
22561 tcggaagacc cggagcccac ccgctgatac tttcggcgct tgggtggcgag aggaggtggt
22621 ggcggcgagg ggtcctctc ctgctccggc ggatagcgcg ccgaccctgt gccccggggc
22681 ggagtggcct ctcgctccat gaaccggcgc acgtcctgac tgccgcgggc cattgtttcc
22741 taggggaaga tggaggagca gccgcgtaag caggagcagg aggagactt aaccaccac
22801 gagcaaccga aaatcgagca ggacctggc ttcgaagagc cggctcgtct agaaccacca
22861 caggatgaac aggagcacga gcaagacgca ggccaggagg agaccgacgc tgggctccag
22921 catggctacc tgggaggaga ggaggatgtg ctgctaaaac acttgacgcy ccaatccatc
22981 atcctccggg acgcccctgg cgaccggagc gaaaccctc tcagcgctga ggagctgtgt
23041 cgggcctacg agctcaacct cttctcgccg cgcgtgcccc ccaaacgcca gcccaacggc
23101 acctgcgagc ccaaccgcg tctcaacttc tatcccgctt ttgcggtccc cgaggcccta
23161 gccacctatc acatcttttt caagaaccaa aagatccccg tctcctggcg ccaccaaccg
23221 acccgcgccg acgcgctcct cgtctctggg cccggcgcg ccatacctga tatcgcttcc
23281 ctggaagagg tgcccaagat cttcgaaggg ctcggctcgg acgagacgcy cgcggcaaac
23341 gctctgaaag aaacagcaga ggaagagggt cacactagcy ccttggtaga gttggaaggc
23401 gacaacgcca ggctggccgt gctcaagcgc agcgtcgagc tcaccactt cgcctacccc
23461 gccgtcaacc tcccgcacca ggtcatgcgt cgcacatagg atcagctcat catgccccac
23521 atcgaggccc tcgatgaaag tcaggagcag cgcgccgagg acgcccggcc cgtggtcagc
23581 gacgagcagc tcgcgcgttg gctcgggacc cgcgaccccc aggttttggg acagcggcgc
23641 aagctcatgc tgcccggtgt cctgtgcacc ctcgagctcg aatgcacgcy ccgcttcttc
23701 agcgaccccg agaccctgcy taaggtcgag gagaccctgc actacacttt caggcacggt
23761 ttcgtcaggc aggcctgcaa gatctccaac gtggagctga ccaacctggt ctcatgcctg
23821 gggatcctgc acgagaaccg cctgggacag accgtgctcc actctactct gaagggcgag
23881 gcgcgtcgcy actatgtccg cgactgtgta tttctcttta tctgccacac ctggcaagca
23941 gccatgggcy tgtggcagca gtgtctcgag gacgaaaatc tgaaggagct ggacaagctt
24001 cttgctagaa accttaaaaa gctgtggacg ggcttcgagc agcgacccgt agcgcggcgc
24061 ctggccgaga tcgtttttcc agaagcctg aggcagacgc tgaaaggcgg gctgcccagc
24121 ttcgatggcc agagcatggt gcaaaactac cgcactttca ttctcgagcy atctgggatg
24181 ctaccgcgca cctgcaacgc attccctccc gactttgtcc cgctgagcta ccgcgagtgt
24241 ccccgccgcy tgtggagcca ctgctatctc ttgcagctgg ccaactacat cgcctaccac
24301 tcggacgtga tcgaggacgt gagcgcgagc gggcttctcy agtgccactg ccgctgcaac
24361 ctgtgtctcc cgcaccgctc cctggtctgc aacccccagc ttctgagcga gaccagggtc
24421 atcggtacct tcgagctgca aggtccgcag gaggccaccg ctccgctgaa actcacgccc
24481 gggttgtgga cttccgcgta cctgcgcaaa ttgtaccg aggactacca cccccatgaa
24541 ataaagttct tcgaggacca atcgcgccca cagcacgcyg atctcacggc ctgcgtcatc
24601 acccagggcy cgatcctcgc ccaattgcac gccatccaaa aatcccgcga agagtttctt
24661 ctaaaaaagg gtagaggggt ctacctggac cccagacgcy gcgaggtgct caaccggggt
24721 ctccccagc atgccgagga agaagcagga gccgctagtg gagcagatgg aagaagaatg
24781 ggacagccag gcagaggagg acgaatggga ggaggagaca gaggaggaag aattggaaga
24841 ggtggaagag gagcaggaaa cagagcagcc cgtcgccgca ccatccgcyg cggcagcccc
24901 gccggtcacg gatacaacct ccacagctcc ggccaagcct cctcgtagat gggatcgagt
24961 gaaggggtgac ggtaagcacg agcggcaggc ctaccgatca tggagggtcc acaaagccgc
25021 gatcatcgcc tgcttgcaag actgcggggg gaacatcgct ttcgcccgcg gctacctgct
25081 cttccaccgc ggggtgaaca tccccgcga cgtgttgcat tactaccgcy accttcacag
25141 ctaagaaaaa gcaagtaaga ggagtgcgcy gaggaggcct gaggatcgcy gcgaacgagc
25201 cctcgaccac cagggagctg aggaaccgga tcttccccac tctttatgcc atttttcagc
25261 agagtgcagg tcagcagcaa gaactgaaag taaaaaacg gtctctgcgc tcgctcacc
25321 gcagttgctt gtaccacaaa aacgaagatc agctgcagcy cactctgaa gacggcgagc
25381 ctctgttcca caagtactgc gcgtcactc ttaaagacta aggcgcgccc acccgaaaaa
25441 aaggcgggaa ttacctcatc gccaccatga gcaaggagat tcccaccctt tacatgtgga
25501 gctatcagcc ccagatgggc ctggccgcyg gcgcctccca ggactactcc acccgcatga
25561 actgggtcag tgccggcccc tcgatgatct cagggtcaa cggggtccgt aaccatcgaa

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FIG. 16A-7

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25621 accagatatt gttggagcag ggggcggtca cctccacgcc cagggcaaag ctcaaccgcg
25681 gtaattggcc ctccaccctg gtgtatcagg aaatccccgg gccgactacc gtactacttc
25741 cgcgtgacgc actggccgaa gtccgcatga ctaactcagg tgtccagctg gccggcggcg
25801 cttcccgggtg cccgctccgc ccacaatcgg gtataaaaaa cctgggtgatc cgaggcagag
25861 gcacacagct caacgacgag ttggtgagct cttcgatcgg tctgcgaccg gacggagtgt
25921 tccaactagc cggagccggg agatcgctct tcaactccaa ccaggcctac ctgaccttgc
25981 agagcagctc ttcggagcct cgctccggag gcatcggaac cctccagttc gtggaggagt
26041 ttgtgccctc ggtctacttc aacccttctc cgggatcgcc aggcctctac ccggacgagt
26101 ttataccgaa cttcgacgca gtgagagaag cgggtggacg ctacgactga atgtcccatt
26161 gtgactcggc tgagctcgct cggttgaggg atctggacca ctgccgccgc ctgctgctgt
26221 tcgcccggga gagctcgcca ctcactactt ttgagtttcc cgaggagcac cccaacggcc
26281 ctgcacacgg agtgccgcat accgttctct gtgcagcggg gcaccaccga gtctcacctg gtcaggttct
26341 tccaccagca acccttctct gtgcagcggg accggggagc taccacctac accgtctact
26401 gcatctgtcc taccccaagc ttgcattgag attttttgct tactctttgt ggtgagttta
26461 ataaaagctg aactaagaac cttctttgga atcccttgct atcatcaaat caacaagacc
26521 atcaacttca cttttgagga acaggtgaac tttacctgca agccacacaa gaagtacatc
26581 atctggtttt atcacaacac tactctagca gtagccaaca cctgctcgaa cgacggtgtt
26641 ctctaccta acaatctcac cagtggacta accttctcag ttaaaagggc aaagctaatt
26701 cttcatcgcc ctattgtaga aggaacttac cagtgtcaga gcggaacctg cttccacagt
26761 ttcactttgg tgaacgttac cggcagcagc acagccgctc cagaacatc taaccttctt
26821 tctgatacta acaaacctcg tgcggaggtg gagctttggg ttccatctct aacagaggtt
26881 gggagtctta ttgaagtggg tgggtatttg attttagggg tggctatttg tgggtgcata
26941 gcagtgtctg atcaacttcc ttgctgggtc gaaatcaggg tatttatctg ctgggtcaga
27001 cattgtgggg aggaaccatg aaggggctct tgctgattat cctttccctg gtggggggtg
27061 tgctgtcatg ccacgaacag ccacgatgta acattaccac aggcaatgag aggaacgact
27121 gctctgtagt tatcaaatgc gagcaccatt gtccctctca catcacattc aagaatpaga
27181 ccatgggaaa tgtatgggtg ggaattctgg aaccaggaga tgagcagaac tacacgggtc
27241 ctgtccattg tagcgatggc aatcacactt tcggtttcaa attcattttt gaagtcatgt
27301 gtgatatcac actacatgtg gctagacttc atggcttggt gccccctacc aaggagaaca
27361 tgggtgggtt ttctttggct ttgtgatca tggcctgctt gatgtcaggt ctgctggtag
27421 gggctctagt gtggtttctg aaacgcaagc ccaggtagcg aatgaggag aaggaaaaat
27481 tgctataaat tctttttctc ttgcacaac catgaatata gtgttccgta tcgtgctgct
27541 ctctcttctt gtagctttcg gtcaggcagg aattcatatt attaatgcta catggtggga
27601 taatataact ttagtgggag cctcagatac tccagttacc tggatgatg gcaagggtt
27661 gcaattttgt gacggaagta cagttaagaa tccgcagatc agacattttt gaatgatca
27721 aaacttaact ctgattcatg ttaacaaaac ccatgaaaga acatacatgg gttacagaca
27781 tgacagtaag ggaaaagtag actataaggt tacagtcatt ccacctctc ctgctactgt
27841 aaagccacaa ccagatccag aaaatgtctt tgtttatatg ggaaataatg taacttttagt
27901 tggacctcca ggaattccag ttagttggta ttatcataat ggcacacagt tctgcgatgg
27961 agataaaatt attcatccag aattcaacca cacctgtgat aaacaaaacc ttacactgct
28021 gtttgtaaac ttacacatg atggaggcta tcttggattc aattacaaag gtactcagag
28081 aattcagtat gaggttatag ttttagatcg atttccaaat tctggtcaga tgaaaattga
28141 agaacaaagt gaggaaacag aacagaaaca tactgagcat aataaggctg gacaaaagca
28201 gggatatagat acaaatcaaa agaaagctaa taacagacaa aagccatctc aaaggccatc
28261 aagaagacgg ccgacaaaca ctctgagac aaaacaactt acagtgtcta ttgggtctaa
28321 cttaacttta gttggtccag atggaaaagt cacttggtat gatggtgatt taaaaagacc
28381 atgtgaagaa caaaactata ggcttcaca tcagtgtagt gctcagaact taactttaat
28441 taatgtaact aaatctcatg agggaactta ctatggcact aatgacaaag acgaaagcaa
28501 aagatacaga gtgaaagtga acactacaaa ttctcaagct gtaaaaatta acccatatac
28561 cagacctact actcctgac agaaacacag atttgaatta caaattgaaa ataattgaaa
28621 tgatgaagaa tcaaaaattc catctactac tgtggcaatc gtggtgggag tgattgcggg
28681 cttcataact ataactattg tcattctgtg ctacatctgc tgccgcaagc gtcccagggc
28741 atacaatcat atggtagacc cactactcag cttctcttac tgagactcag tcactttcat
28801 ttcagaacca tgaaggcttt cacagcttgc gttctgttta acataatcac acttagtgta
28861 gctgcaaatg gttttaaaca tgttaatggt accagattaa gtaatgtaac actgacagga
28921 gctggaatta atactacatg gacaggtgat ttaatgagg gtccaaaagg aaaaaattggg
28981 tggatgaata ttgcacatg gggcgatcct agatatgtgt gccattgaaa tagcagtact
29041 attactaatc ttacagttgt ggcacttcta aatttaacca ctaacagaag atttaagca
29101 gaaagtttta ctagtaacga tggttatgaa actaccagtg caaaatttta tgaaattaaa
29161 attattgagc ttccaacaac tagagcacc accacagtta ggacaacaca gcctaccact
29221 gtgcccacta cacatccaac caccacagtc agtacaacta ttgagaccac tactcatact

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FIG. 16A-8

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29281 acacagctag acacaacagt gcagaatact actttattga ttgggttttt actgagagga
29341 aatgaaagta ctactgaaca gacagaggct acctcaagt ccttcagcag cactgcaaat
29401 ttaacttcgc ttgcttggac taatgaaacc ggagtatcat tgatgaatcg acagccttac
29461 tcagggtttg atattcaaat tacttttctg gttgtctgtg ggatctttat tcttgcggtt
29521 cttctgtact ttgtctgtcg caaagccaga gagaaatcta ggcgccccat atacaggcca
29581 gtaatcgggg aacctcagcc tctccaagt gatggaggct taaggaaatct tctcttctct
29641 tttacagtat ggtgatcagc catgattcct aggttcttcc tatttaacat cctgttctgt
29701 ctcttcaaca tctgtgtcgc cttcgcgccc gtctcgcacg cctcgcccga ctgtctaggg
29761 cctttcccaa catacctcct ctttgccctg ctaacctgca cctgcgtctg cagcattgtc
29821 tgggtggtca tcacctttct gcagctcatc gactgggtgt cgcgcgcgta caattatctc
29881 caccacagtc ccgaatacag ggacgagaac gtacccagaa tcttaaggct catctgacca
29941 tgcagcctct gctcatgctg atatccctcc tatccctgtc ccttgccact tctgtgtatt
30001 actctaaatg caaatctcgc gacatatgga atttcttaga ttgctatcag gagaaattg
30061 atatgccctc ctattacttg gtgattgttg gggtagtcat ggtctgtctc tgcactttct
30121 ttgccattat gatctacccc tgttttaatc ttggctggaa ctctgttgag gcattcacat
30181 acacactaga aaacagttca ctagcctcca cgccaccacc cacaccgctt ccccgcagaa
30241 atcagttccc tatgattcag tacttagaag agccccctcc ccggccccct tccactgtta
30301 gctactttca cataaccggc ggcgatgact gaccacctgg acctcgagat ggacggccag
30361 gcctccgagc agcgcacatc gcaactgcgc gtccgacagc agcaggagcg gggcgccaag
30421 gagctcctcg atgccatcaa catccaccag tgcaagaagg gcactctctg cctggtaaac
30481 caggcaaaaga tcacctacga gctcgtgtcc ggcggcaagc agcatcgctt cgcctatgag
30541 ctaccccgagc agaagcaaaa gttaacctgc atgggtggcg tcaaccccat agtcatcacc
30601 cagcagtcgg gcgagaccaa cggtgcacac cactgctcct gcgaaagccc cgagtgcac
30661 tactccctcc tcaagaccct ttgcggactc cgcgacctcc tccccatgaa ctgatgttga
30721 ttaaaagccc aaaaaccaat caaaccttcc cccaattact cataagaata aatcattgga
30781 actaatcatt caataaagat cacttacttg aaatctgaaa gtatgtctct ggtgtagtgt
30841 ttcagcagca cctcggaacc ctctcccaga ctctggtact ccagtcctcg gcgggcgggc
30901 aacttctctc acaccttgaa agggatgtca aattcctggt ccacaatttt cattgtcttc
30961 cctcagatga caaagaggct ccgggtggaa gatgacttca acccgtctta cccctatggc
31021 tacgcgcgga atcagaatat cccttccctt actccccctt ttgtttcttc cgatggattc
31081 caaaacttcc cacctggggt cctgtcactc aaactggctg acccaatcgc catcactaat
31141 ggggatgttt cactcaaggt gggagggggt cttactgttg aaaaagatag tggaaatcta
31201 aagggtgaacc ctaaggctcc cttgcaagtt acaactgata aacagttgga aattgcactg
31261 gcttatccat ttgaagtcag taatggcaag cttggcataa aagcaggtca tggattgaaa
31321 gtcattgaca aaattgctgg ttggaaggt ttggcaggta cgcttgtagt ttgactgga
31381 aaaggaatag gtactgaaaa tcttgaaaac agtgatgggt caagtagagg agttggtata
31441 aacgtaagac ttgctaaaga tggaggtctg tcttttgata aaaaggtgta tttagttgct
31501 tggaaataaac atgatgacag acgcactcta tggacaactc ccgacctatc cccaaattgt
31561 acaatcgatc aggaagggga ttcaaagctc actttagtat taacaaaatg tggcagtcaa
31621 attttggtcta atgtctcttt acttgttgta aaaggaaaat ttagtaacat aaacaataat
31681 actaatccaa ctgataaaaa aatcacagta aagctacttt ttaatgaaa gggagtatta
31741 atggacagtt cgacacttaa gaaagaatat tggaaactaa gaaatgataa tctactgta
31801 tctcaggcct atgataatgc agttcctttt atgccaaaaca taaaagctta tcctaaacct
31861 accacagaca cttcggttaa accagaagat aaaaaaagt ctgctaaaag atacattgtg
31921 agcaatgtct atattggagg cttgccagat aaaactgttg ttataactat taagtttaat
31981 gcagaaactg aatgtgttta ttcgattacc tttgaattca catgggcaaa aacctttgaa
32041 gatgtgcagt ttgattcctc ctcttttacc ttttctata ttgcccaga aaatgaggac
32101 gaagacaaat aaaatgtttt aaaatgaatt catgtatctt tattgatttt tacaccagca
32161 cggttagtca gtctcccacc accagcccat ttcacagtgt aaacgattct ctcagcacgg
32221 gtggccttaa atagggaaat gttctgatta gtgcgggaac tggacttggg gtctataatc
32281 cacacagttt cctggcgagc caaacggggg tcggtgattg agatgaagcc gtcctctgaa
32341 aagtcattcca agcgggcctc acagtccaag gtcacagtct ggtgaaacga gaagaacgca
32401 cagattcata ctcggaaaac aggatgggtc tgtgcctctc catcagcgcc ctcaacagtc
32461 tctgccgccc gggctcggtg cggctgctgc agatgggatc gggatcacia gtctctctga
32521 ctatgatccc cacagccttc agcatcagtc tcttggtgcg tcgggcacag caccgcatcc
32581 tgatctcgct catgttctca cagtaagtgc agcacataat caccatgtaa tctgacgccc
32641 cataattcag ggtgtccag ccaaaactca tgggaacccc gatggaaacc acgtgacct
32701 cgtaccagat gcggcagtat atcagatgcc tgccccctcat gaacacactg cccatataca
32761 tgatctcttt ggcagtgct ctgttcacaa tctgacggta ccagggaaag cgctggttga
32821 acatgcaccc gtaaatgact ctctgaacc acacggccag cagggtgcct cccgcccgc
32881 actgcagga gcccggggat gaacagtggc aatgcaggat ccagcgctcg taccgctca

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FIG. 16A-9

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32941 ccattctgagc tctcaccaag tccagggttag cggggcacag gcacactgac atacatcttt
33001 ttaaaatctt tatttctctt ggagtcaaga tcatatccca ggggactgga aactcttgga
33061 gcagggtaaa gccagcagca catggtaatc cacggacaga acttacatta tgataatctg
33121 catgatcaca atcaggcaac aggggatgtt gttcagtcag tgaagccctg gtttcctcat
33181 cagatcgtgg taaacggggc ctgcatatg gatgatggcg gagcagctg gattgaatct
33241 cggtttgcat tgtagtgat tctcttgctg accttgctgt acttctgcca gcagaaatgg
33301 gcccttgaac agcagatacc cctcctgctg ccgtcctttc gctgctgccc ctcatcatc
33361 caactgaagt acatccattc tcgaagattc tggagaagtt cctctgcatc tgatgaaaca
33421 aaaaacccgt ccatgcgaat tcccctcatc acatcagcca ggactctgta ggccatcccc
33481 atccagttaa tgctgccttg tctatcattc agagggggcg gtggcaggat tggagaacc
33541 atttttattc caaacggtct cgaaggacga taaagtcaa gtcacgcagg tgacagcggt
33601 cccctccgct gtgctggtgg aaacagacag ccaggcaca acccactcta ttttcaagg
33661 ggttcgacgt ggcttcgagc agtggctcta cgcgtacatc cagcataaga atcacattaa
33721 aggctggccc tccatcgatt tcatcaatca tcagggttaca ttctgacac atccccagg
33781 aattctcatt tttccagcct tggattatct ctacaaattg ttggtgtaag tccactccgc
33841 acatgtggaa aagctccac agtgccccct ccactttcat aatcaggcag acctcataa
33901 tagaaacaga tctgtctgct ccaccacctg cagcgtgttc aaaacaacaa gattcaataa
33961 ggttctgccc tccgccctga gctcgcgctc caatgtcagc tgcaaaaaat cacttaagtc
34021 ctggggcact acagctgaca attcagagcc agggctaagc gtgggactgg caagcgtaag
34081 ggaaaacttt aatgctccaa agttagcacc caaaaactgc atgctggaat aagctctctt
34141 tgtgtctccg gtgatgcctt ccaaaatgtg agtgataaag cgtggtagtt tttctttaat
34201 catttgcgta atagaaaagt cctgtaaata agtactagg accccaggga ccacaatgtg
34261 gtagcttaca ccgcgtcgct gaagcatggt tagtagagat gagagtctga aaaacagaaa
34321 gcatgcacta aactaagggtg gctatcttca ctgaaggaaa aatcactctc tccaacaaca
34381 gggtagccac tgggtggccc ttggggacat acaaaaatcg gtccgtgtga ttaaaaagca
34441 gcacagtaag ttcctgtctt cttccggcaa aaatcacatc ggactgggtt agtatgtccc
34501 tggcatggta gtcattcaag gccataaatc tgccctgata tccagtagga accagcacac
34561 tcacttttag gtgaagcaat accaccccat gcggaggaat gtggaagat tcagggcaaa
34621 aaaaattata tctattgcta gtcccttcct ggacgggagc aatccctcca ggactatcta
34681 tgaaagcata cagagattca gccatagctc agcccgtta ccagtagaca gagagcacag
34741 cagtacaagc gccaacagca gcgactgact acccactgac ccagctccct atttaaggc
34801 gccttacact gacgtaatga ccaaaggctc aaaaaccccg ccaaaaaaaa acacacacgc
34861 cctgggtggt ttttgcgaaa acacttcgcg gttctcactt cctcgtattg atttcgtgac
34921 ttaacttcg gggtccacg ttacgtcact tctgccctta catgtaactc agtcgtaggg
34981 cgccatcttg ccacgtcca aaatggcttc catgtccagc cagcctccg cggcgaccgt
35041 tagccgtgcg tcgtgacgct atttgcatca tcttctctcg tccaatcagc gctggccccg
35101 ccctaaattc aaaagctcat ttgcatgtta acttttgttt actttgtggg gtatattatt
35161 gatgatc
SEQ ID NO: 5

FIG. 16A-10

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Grp	Vaccine at Wk 0, Wk 4	Monkey ID	Pre		Wk 4		Wk 8		Wk 12	
			Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
1	Ad24ΔE1gagΔOrf6Ad5Orf6 10 ⁴ 11 vp	00C072	3	4	4	381	3	150	3	68
		00C178	3	3	1	559	1	743	0	635
		00C222	0	3	1	369	1	753	0	670
		00D011	1	9	9	211	4	273	0	520
		00D023	0	6	0	295	1	459	1	368
		00D031	15	5	10	103	1	101	1	40
2	Ad24ΔE1gagΔOrf6Ad5Orf6 10 ⁴ 10 vp	99C168	4	6	0	118	5	241	3	209
		99C170	10	5	5	241	3	141	3	103
		99C173	1	3	0	23	0	14	0	21
3	Ad24ΔE1gagΔE4Ad5Orf6 10 ⁴ 10 vp	99C154	0	3	0	93	0	60	1	53
		99C158	1	0	1	141	0	101	1	120
		99C177	0	0	0	45	0	39	0	79
4	MRKAd5-HIVgag 10 ⁴ 11 vp	00C018	1	5	13	1025	0	824	3	753
		00C034	0	4	5	219	5	404	0	491
		00C058	4	4	3	1086	0	440	0	439
5	MRKAd5-HIVgag 10 ⁴ 10 vp	99C218	0	3	5	2500	0	1580	10	1655
		99C227	6	1	4	529	5	365	5	1004
		99D185	ND	ND	0	425	0	310	0	271

FIG. 17

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Vaccine at Wk 0, Wk 4	Monkey ID	Gag-Specific (Wk 12)	
		%CD4	%CD8
Ad24ΔE1gagΔOrf6Ad5Orf6 10 ¹¹ vp	00C072	0.02	0.02
	00C178	0.05	0.38
	00C222	0.02	0.40
	00D011	0.02	0.27
	00D023	0.01	0.11
	00D031	0.01	0.01
MRKAd5-HIVgag 10 ¹¹ vp	00C018	0.05	0.41
	00C034	0.06	0.18
	00C058	0.02	0.28

FIG. 18

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Grp	Vaccine at Wk 0, Wk 4	Monkey ID	Wk 4	WK 8
1	Ad24ΔE1gagΔOrf6Ad5Orf6 10 ¹¹ vp	00C072	<10	77
		00C178	<10	26
		00C222	<10	423
		00D011	<10	98
		00D023	<10	<10
		00D031	<10	<10
2	Ad24ΔE1gagΔOrf6Ad5Orf6 10 ¹⁰ vp	99C168	<10	<10
		99C170	<10	<10
		99C173	<10	<10
3	Ad24ΔE1gagΔE4Ad5Orf6 10 ¹⁰ vp	99C154	<10	<10
		99C158	<10	<10
		99C177	<10	<10
4	MRKAd5-HIVgag 10 ¹¹ vp	00C018	34	1017
		00C034	14	423
		00C058	46	934
5	MRKAd5-HIVgag 10 ¹⁰ vp	99C218	20	99
		99C227	40	767
		99D185	17	342

FIG. 19

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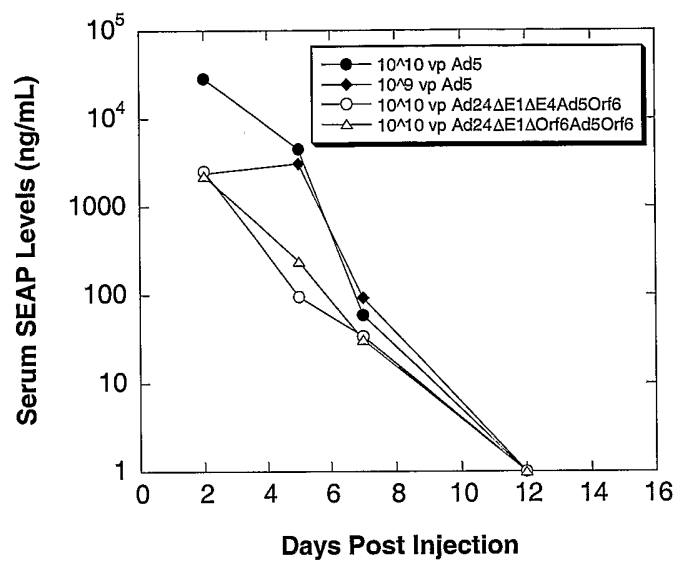


FIG. 20

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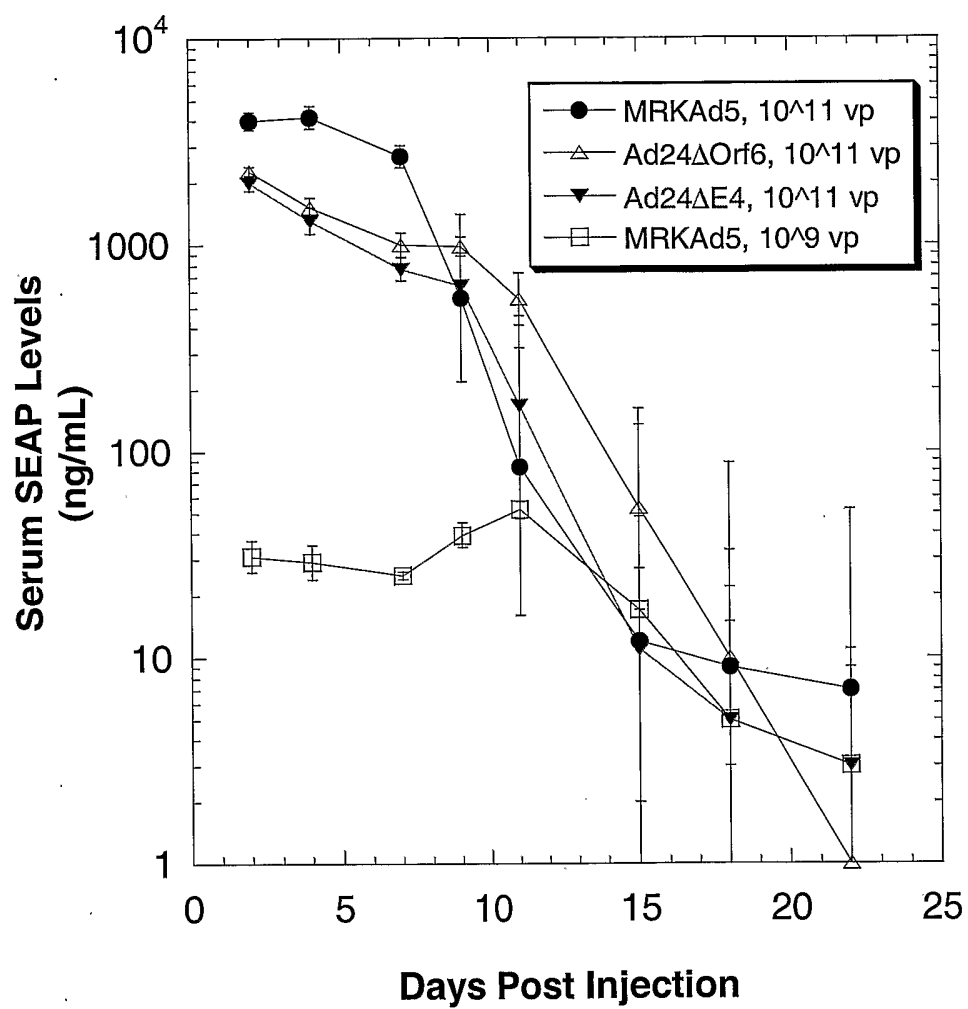


FIG. 21

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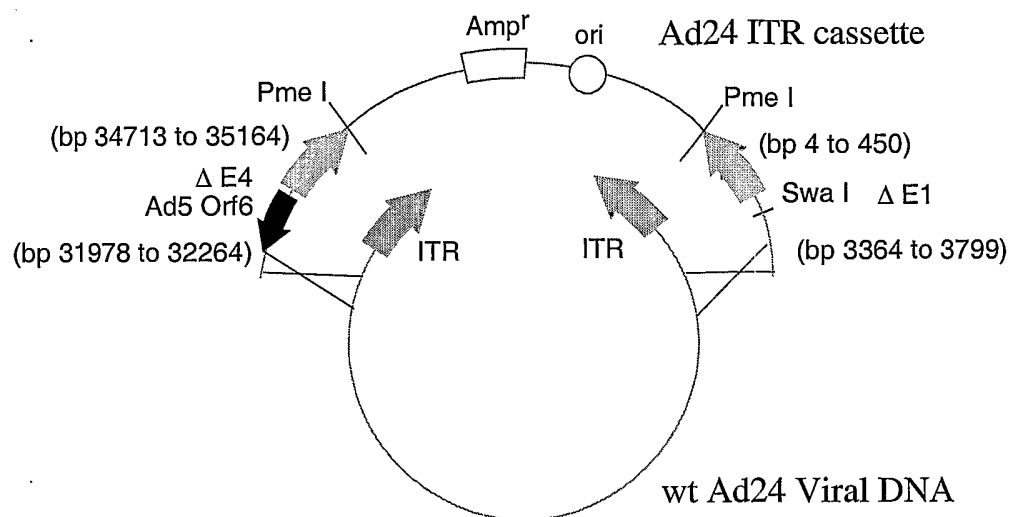


FIG. 22

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Animal	Prime (Wk 0, 4, 26)	Boost (Wk 56)	Pre		Prime ^b		Pre-Boost ^c		Post-Boost ^d	
			Mock ^a	Gag ^a	Mock	Gag	Mock	Gag	Mock	Gag
Monkey 1	10 ⁸ vp MRKAd5-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	18	16	1	244	3	74	3	1235
Monkey 2	10 ⁷ vp MRKAd5-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	10	9	4	83	0	18	0	856
Monkey 3	10 ⁸ vp MRKAd6-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	1	1	0	219	9	69	0	703
Monkey 4	10 ⁷ vp MRKAd6-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	1	1	3	59	1	20	0	419
Monkey 5	none	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	3	4	ND*	ND	ND	ND	4	558
Monkey 6	none	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	0	3	ND	ND	ND	ND	1	295
Monkey 7	none	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	1	9	ND	ND	ND	ND	9	103
Monkey 8	none	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	3	3	ND	ND	ND	ND	1	381
Monkey 9	none	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	0	6	ND	ND	ND	ND	0	369
Monkey 10	none	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	15	5	ND	ND	ND	ND	10	211

FIG. 23

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Animal	Prime (Wk 0, 4, 26)	Boost (Wk 56)	Gag-Specific T cells (Wk 60)	
			%CD4	%CD8
Monkey 1	10 ⁹ vp MRKAd5-gag	10 ¹¹ vp Ad24ΔE1 gagΔOrf6Ad5Orf6	0.06	0.37
Monkey 2	10 ⁷ vp MRKAd5-gag	10 ¹¹ vp Ad24ΔE1 gagΔOrf6Ad5Orf6	0.01	0.56
Monkey 3	10 ⁹ vp MRKAd6-gag	10 ¹¹ vp Ad24ΔE1 gagΔOrf6Ad5Orf6	0.07	0.06
Monkey 4	10 ⁷ vp MRKAd6-gag	10 ¹¹ vp Ad24ΔE1 gagΔOrf6Ad5Orf6	0.04	0.20

FIG. 24

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Animal	Prime (Wk 0, 4)	Boost (Wk 24)	Pre		Prime ^b		Pre-Boost ^c		Post-Boost ^d	
			Mock ^a	Gag ^a	Mock	Gag	Mock	Gag	Mock	Gag
Monkey 11	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	10 ⁷ vp MRKAd5-gag	3	4	3	150	4	28	0	188
Monkey 12	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	10 ⁷ vp MRKAd5-gag	0	3	1	753	4	554	0	1029
Monkey 13	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	10 ⁷ vp MRKAd5-gag	1	9	4	273	0	370	0	1520
Monkey 14	none	10 ⁷ vp MRKAd5-gag	0	0	ND ^e	ND	ND	ND	4	94
Monkey 15	none	10 ⁷ vp MRKAd5-gag	0	0	ND	ND	ND	ND	1	168
Monkey 16	none	10 ⁷ vp MRKAd5-gag	8	3	ND	ND	ND	ND	8	149

FIG. 25

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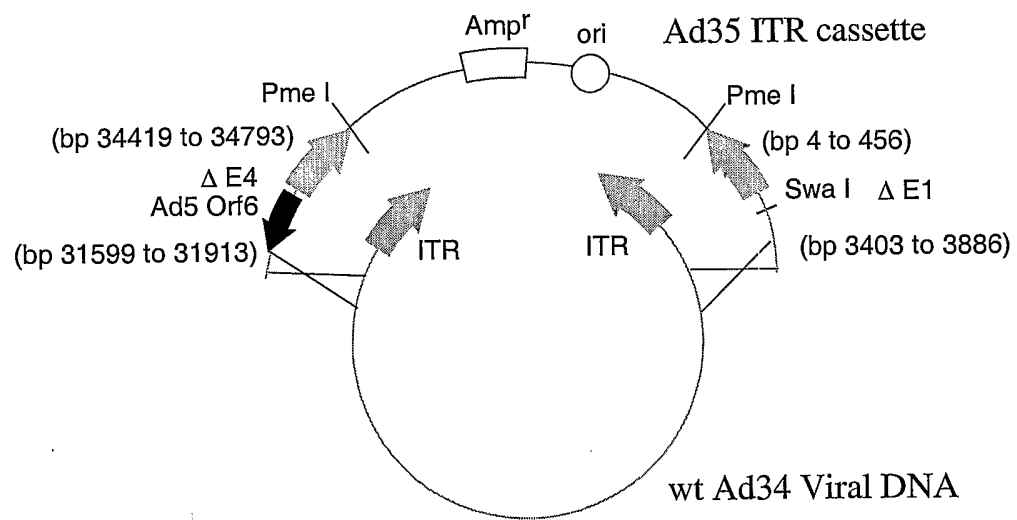


FIG. 26

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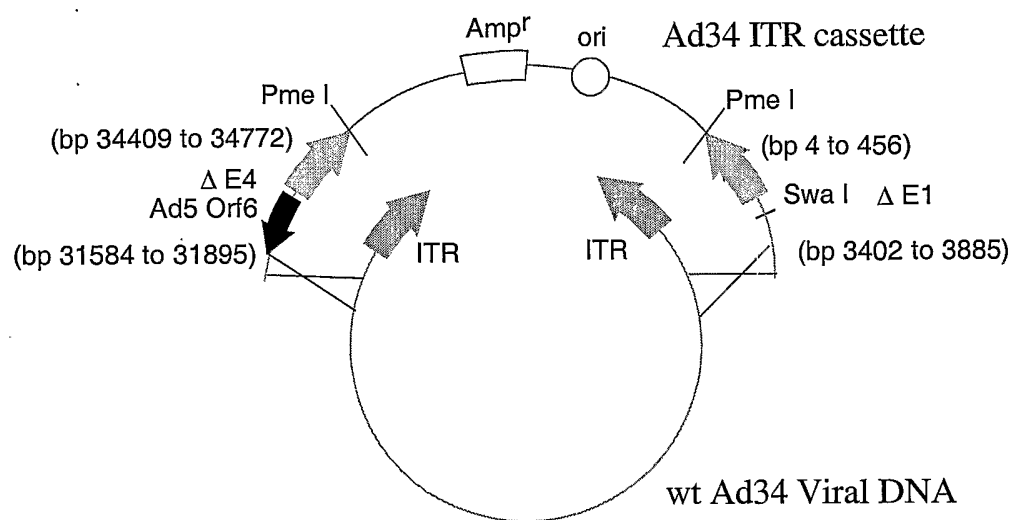


FIG. 27

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1 catcatcaat aatatacctt atagatggaa tgggtgccaat atgtaaatga ggtgattttta
61 aaaattgtgg ggtgtgtggt gattggctgt ggggttaacg gctaaacggg gggcgcgggc
121 cgtgggaaaa tgacgtttttg tgggggtgga gtttttttgc aagtgtctgc gggaaatgtg
181 acgcataaaa aggcctttttt tctcacggaa ctactgactt tccccacggg atttaacagg
241 aaatgaggta gttttgaccg gatgcaagtg aaaattgctg atttgcgcg gaaaactgaa
301 tgaggaagtg tttttctgaa taatgtggta tttatggcag ggtggagtat ttgttcaggg
361 ccaggtagac tttgacccat tacgtggagg tttcgattac cgtgtttttt acctgaattt
421 ccgcgtaccg tgtcaaagtc ttctgttttt acgtaggtgt cagctgatcg ctacggttatt
481 tatacctcag ggtttgtgtc aagaggccac tcttgagtgc cagcgagaag agttttctcc
541 tctgcgccgg cagtttaata ataaaaaaat gagagatttg cgattttctgc ctcaggaaat
601 aatttctgct gagactggaa atgaaatact ggagcttggt gtgcacgccc tgatgggaga
661 cgatccggag ccacctgtgc agcttttttga gcctcctacg cttcaggaaac tgtatgattt
721 agaggtagag ggatcggagg attctaataa ggaagctgtg aatggctttt ttaccgattc
781 tatgctttta gctgctaata aaggattaga attagatccg cctttggaca ctttcgatac
841 tccaggggtg attgtggaaa gcggtacagg tctaagaaaa ttacctgatt tgggttccgt
901 ggcactgtgt ttgcactgct atgaagacgg gtttcctccg agtgatgagg aggacctatg
961 aaaggagcag tctatgcaga ctgcagcggg tgagggagtg aaggctgcca gtgttggttt
1021 tcagttggat tgcccggagc ttcttggaac gctttgttat atgagagcgc actgccactt
1081 aaatactgga gtaaaggaac tgttatgttc ttaaaggaat atgctgtttt tcacatgtat
1141 tatttacagt aagtgtgttt aagttaaaaa aggtcctgtg tctgatgctg atagtcaacc
1201 attgagtggg agttttgtgc ttcttattat gattcaagca cctgttctcg tggacgtgcg
1261 atctcctgat tctactacct ttaagcctgg gaaacgtcca gcagtggaaa aacttgagga
1321 caagccatt cctgtgaagc gaccttttga cttgagtaca cggaaacggc caagacaata
1381 cttgttacag ggtggggacg acttaaggtg acgtcaatat ttgtgtgaga gtgcaatgta
1441 agtgttccat atccgtgttt cactggtttt tattgctttt tgggcgggga ctaggtata
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1561 taagtagaag ggaagacctt agaaagacta ggcaactgtt agaggacgct atggaggttt
1621 gggccatttt ttggagattc tggttcgcta gtgaattagc tagggtagtt ttaggataa
1681 tctccggttt taaagaagaa tttgaaaagt ttttggtaga ttgccagga ctttttgaag
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1861 tcccgcagac tcatttcagc aggggatacg ttttgattt cgtagccaca gcattgtgga
1921 gaacatggaa ggttcgcaag atgaggacaa tcttaggtta ctggccagtg cagcctttgg
1981 gtgtagcggg aatcctgagg catccaccgg tcatgccagc ggttctggag gaggaacagc
2041 aagaggacaa cccgagagcc ggcctggacc ctccagtgga ggaggcggag tagctgactt
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2221 gagtcgcaga cgtcctgaaa ccatttggtg gcatgaggtc cagaaagagg gaagggatga
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3541 tccaaccgca caattcttca acgctgacct atgctacttt aagttcttca cctttggacg
3601 cagctgcagc cgccgccgcc gcctctgttg ccgctaacac tgtgcttggg atgggttact
3661 atggaagtat cgtggctaata tccacttctc ctaataaacc ttctaccctg actcaggaca
3721 agttacttgt ccttttggcc cactggagg ctttgaccca acgtccctgt gaactttatc
3781 agcaggtggc cgagttgcga gtacaaactg agtctgctgt cggcacggca aagtctaaat

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FIG. 28A-1

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3901 aaaaaaaaaat tccacaatca atgaataaat aaacgagcctt gttgttgatt taaaatcaag
3961 tgttttttatt tcatTTTTtCG cgcacgggat gccctagacc accgatctcg atcattgaga
4021 acacgggtgga ttttttccag aatcctatag aggtgggatt gaatgtttag atacatgggc
4081 attaggccat ctttgggggtg gagatagctc cattgaaggg attcatgctc cggggtagtg
4141 ttgtaaatca cccagtcata acaaggtcgc agtgcatggg gttgcacaat atcttttaga
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6541 catatgggtt aaggggactg ccccagggca tgggatgggt gagtgcagag gcatacatgc
6601 cacagatgtc atagacgtag atgggatcct caaagatgcc tatataggtt ggatagcatc
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FIG. 28A-2

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9121 gtttcgcgcg gacacggta attcctctc gagaagacgg atgagttcgg ctatggtggc
9181 cgttacttcc cgttcgaagg ctcccgggat ctcttcttcc tcttctatct cttcttccac
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9301 caccggcaaa cggtcgatga atcgttcaat gacctctccg cggcggcgcc gcatggtttc
9361 agtgacggcg cggccgttct cgcgcggctc cagagtaaaa acaccgccc gcatctcctt
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10141 cagatactgg taacctataa gaaaatgcgg cggtggttgg cggtagagag gccatcggtc
10201 tgtagctgga gcgcggggg caggtgattc agaggtcttc cggtgatagc cgtagatgta
10261 cctggacatc caggtgattc ctgcggcggt agtagaagcc cgcgtacgcg cgcgtacgcg
10321 gttccaaaatg ttgcgtagcg gcatgaagta gttcattgta ggcacggttt gaccagttag
10381 gcgcgcgcag tcattgatgc tctatagaca cggagaaaaat gaaagcgttc agcactcga
10441 ctccgtagcc tggaggaaac tgaacgggtt gggtcgcggt gtaccccggt tcgagacttg
10501 tactcgagcc ggcgcggagc ggcgctaacc tggatttggc actcccgtct cgacccagcc
10561 tacaaaaatc caggatacgg aatcagatcg ttttgctggt tgccgaatgg cagggaagtg
10621 agtccatatt tttttttttg cegctcagat ctgcgcagag ccatcccgtg tgctcccca
10681 acaacagccc ccctcgcagc agcagcaacc gtccctgcaa ctactgcaac
10741 tgccgctgtg agcgggtgcg gacagcccgc ctatgatctg gacttggaag agggcgaagg
10801 actggcacgt ctagggtgcg cttcgcgccg ctatgatctg cgagttcaac tgaaaaaaga
10861 ttctcgcgag gcgtatgtgc cccaacagaa cctattttaga gacagaagcg gcgaggagcc
10921 ggaggagatg cgagcttccc gctttaaacc gggtcgtgag ctgcgtcacg gtttggacag
10981 aagacgagtg ttgcgggacg aggtatttca gtgtgatgaa gtgacaggga tcagtcctgc
11041 cagggcacac gtggctgcag ccaaccttgt atcggcttac gaacagacag taaaggaaga
11101 gcgtaatttc caaaagtctt ttaataatca tgtgcgaacc ctcatgccc gcgaagaagt
11161 cacccttggg ttgatgcatt tgtgggattt gatggaagct atcattcaga accctactag
11221 caaacctctg accgcacagc tgtttcttgg ggtgcaacac agcagagaca atgaggcttt
11281 cagagagcgc ctgctcaaca tcaccgaacc tggtttagat tggtttagat atcttatcaa
11341 cattctacag agtatcatag tgcaggagcg gagcctgggc ctggccgaga aggtggctgc
11401 catcaattac tcggttttga gcttgggaaa gtattacgct cgcaagatct acaagactcc
11461 atacgttccc atagacaagg aggtgaagat agatgggttc tacatgcgca tgacgtgaa
11521 ggtgttgacc ctgagcgatg atcttggggg gtaccgcaat gacagaatgc atcgcgcggt
11581 gagcgccagc aggagggcgc aggttaagcg cagggaactg atgcacagtt tgcaagagc
11641 tctaactgga gctggaaccg aggttgagaa ttactttgat atgggagctg acttgcagtg

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FIG. 28A-3

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11701 gcagcctagt cgcaggggctc tgaacgccgc gacggcagga tgtgagcttc cttacataga
11761 agaggcggat gaaggcgagg aggaagaggg cgagtacttg gaagactgat ggcacaaccc
11821 gtgttttttg ctagatggaa cagcaagcac cggatccccg aatgcgggcg ggcgtgcaga
11881 gccagccgtc cggcattaac tcctcggacg attggaccca ggccatgcaa cgtatcatgg
11941 cgttgacgac tcgcaacccc gaagccttta gacagcaacc ccaggccaac cgtctatcgg
12001 ccatcatgga agctgtagtg ccttcccgct ctaatcccac tcatgagaag gtcctggcca
12061 tcgtgaacgc gttggtggag aacaaagcta ttcgtccaga tgaggccgga ctggtataca
12121 acgctctctt agaacgcgtg gctcgtaca acagtagcaa tgtgcaaacc aatttggacc
12181 gtatgataac agatgtacgc gaagccgtgt ctcagcgcg aaggttccag cgcgatgcca
12241 acctgggttc gctggtggcg ttaaattgctt tcttgagtac tcagcctgct aatgtgccgc
12301 gtggtcaaca ggattatact aactttttaa gtgctttgag actgatggta tcagaagtac
12361 ctcagagcga agtatatcag tcctggtcctg attacttctt tcagactagc agacagggct
12421 tgcagacggg aaatctgagc caagccttta aaaaccttaa aggtttgtgg tccgaactcc
12481 ccccggtagg agaaagagca accgtgtcta gcttgttaac tccgaactcc cgttaattcc
12541 tactgttggg agctcctttc accgacagcg gtagcatcga ccgtaattcc tatttgggtt
12601 acctactaaa cctgtatcgc gaagccatag ggcaaaagtc ggtggacgag cagacctatc
12661 aagaaattac ccaagtcagt cgcgctttgg gacaggaaga cactggcagt ttggaagcca
12721 ctctgaactt cttgcttacc aatcgggtctc aaaagatccc tcttcaatat gctcttactg
12781 cggaggagga gaggatcctt agatatgtgc agcagagcgt gggattgttt ctgatgcaag
12841 aggggggcaa tccgactgca gcaactggga tgacagcgcg aaatatggag cccagcatgt
12901 atgccagtaa ccgacctttc attaacaacac tgcctggacta cttgcacaga cttgcccgtc
12961 tgaactctga ttatttcacc aatgccatct taaacccgca ctggctgccc ccacctgggt
13021 tctacacggg cgaatatgac atgcccgacc ctaatgacgg atttctgtgg gacgacgtgg
13081 acagcgatgt tttttcacct ctttctgatc atcgacgtg gaaaaaggaa ggcggcgata
13141 gaatgcattc ttctgcatcg ctgtccgggg tcattgggtg taccgcggtc gagcccagat
13201 ctgcaagtcc ttttcttagt ctacctttt ctctacacag tgtacagtgg agcgaagtgg
13261 gtagaataag tcgcccagat ttaatgggcy aagaggagta cctaaacgat tccttgctca
13321 gaccggcaag agaaaaaaat ttcccaaca atggaataga aagtttgggt gataaaatga
13381 gtagatggaa gacttatgct caggatcaca gagacgagcc tgggatcatg gggactacaa
13441 gtagagcgag ccgtagacgc cagcgccatg acagacagag ggtcttgtg tgggacgatg
13501 aggattcggc cgatgatagc agcgtattgg acttgggtgg gagaggaagg ggcaacccgt
13561 ttgtctatth cgcgccctgc ttgggtggta tgtgttaaaa aaaaaataaaa agagaaaaac
13621 tcaccaaggc catggcgacg agcgtacgtt cgttcttctt tattatctgt gtctagtata
13681 atgaggcgag tcgtgctagg cggagcgggt gtgtatccgg agggctctcc tccttcgtac
13741 gagagcgtga tgcagcagca gcaggcgacg gcggtgatgc aatccccact ggaggtcccc
13801 tttgtgcctc cgcgatacct ggcacctagc gagggcagaa acagcattcg ttactcgaaa
13861 ctggcacctc agtacgatac caccaggttg tatctggtgg acaacaagtc ggcggacatt
13921 gcttctctga actatcagaa tgaccacagc aacttcttga ccacggtggt gcaaaacaat
13981 gactttaccc ctacggaagc cagcaccagc accattaact ttgatgaacg atcgcggtgg
14041 ggcggtcagc taaaaacat catgcatact aacatgccca acgtgaacga gtatatgttt
14101 agtaacaagt tcaaagcgcg tgtgatggtg tccagaaaaa ctctgaggg tgtagagta
14161 gacgataatt atgatcataa gcaagatatg ctaaaatacg agtggttcga gtttactttg
14221 ccagaaggca acttttcggt cactatgact atcgacttga tgaacaatgc catcatagac
14281 aattacttga aagtgggcag acagaatgga gtgttggaag gtgacattgg tgtaagttc
14341 gacactagga acttcaagtt gggatgggat ccagaaacta agttgatcat gcttgggggt
14401 tacacctatg aggccttcca tcctgacatc gtattgctgc ctggctgcgg agtggaactt
14461 accgaaagcc gtctgagcaa ccttcttggc attagaaaga aacaccatt ccaagagggt
14521 ttttaagatc tgtatgagga ttttagaggg ggaatatatt cagccctttt ggtgtagat
14581 gcttatgaga acagcaagaa agatcaaaaa gccaaaatag aagctgctgc agaagctaaa
14641 gcaaacatag ttgccaacga tccggttaagg gtggctaacg ctagtgaat caggggagac
14701 agttttgccc caacatccgt tccgactaaa gaatcattat tggatgatgt gtctcaaaac
14761 atagagttaa aactcactat taagcctgtg gaaaaagatg gcaaaaacag aagttacaat
14821 gtgttgggag ataaaaatcaa caccggcctat cgagttggt acctttcgta caattatggc
14881 gaccccgaaa aaggagtgcg ttcttggaac ttgtcacca cctcagatgt cacctgcgga
14941 gcggagcagg tctactggtc gcttccagac atgatgcagg atcctgtcac tttccgctcc
15001 actagacaag tcagtaacta ccctgtggtg ggtgcagagc ttatgcccg ttttcaaag
15061 agcttctaca acgaacaagc tgtgtactcc cagcagctcc gccagtcac ctcgcttacg
15121 cacgtcttca accgcttttc tgagaaacag attttaatcc gtcgcccggc gcccacaatt
15181 accaccgcca gtgaaaacgt tctgtctctc acagatcacg ggaccctgcc gttgcgcagc
15241 agtatccggg gagtccaacg tgtgaccgtt actgacgcca gacgccgcac ctgtccctac
15301 gtgtacaagg cactgggcat agtcgcaccg cgcgtccttt caagccgcac tttctaaaaa
15361 aaaaaaaaaa atgtccgttc ttatctcgcc cagtaataac accggttggg gtctgcgcgc
15421 tcccagcaag atgtacggag gcgcagccea acgttctacc caacatccc tgcggttcg
15481 cgggcatttt cgcgctccat ggggtgcctt caagggccgc actcgcgttc gaaccacgt
15541 cgatgatgta atcgatcagg tggttgccga cgcccgtaat tatactccta ctgcgcctac

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FIG. 28A-4

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15601 atctactgtg gacgcagtta ttgacagtgt agtggctgac gctcgcaact atgctcgacg
15661 taagagccgg cgaaggcgca ttgccagacg tcaccgagct accactgcca tgcgagcagc
15721 aagagctctg ctacgaagag ctacagcgct ggggcgaaga gccatgctta gggcgccag
15781 acgtgcagct tcgggcgcca gcgcggcgag gtcccgagg caagcagccg ctgtcgcagc
15841 ggcgactatt gccgacatgg cccaatcgcg aagaggcaat gtatactggg tgcgtgacgc
15901 tgccaccggt caacgtgtac ccgtgcgcac ccgtccccct cgcacttaga agatactgag
15961 cagtctccga tgttgtgtcc cagcggcgag gatgtccaag cgcaaataca aggaagaaat
16021 gctgcagggt atcgcacctg aagtctacgg ccaaccgttg aaggatgaaa aaaaaccccg
16081 caaaatcaag cgggtaaaaa aggacaaaaa agaagaggaa gatggcgatg atgggctggc
16141 ggagtttgtg cgcgagtttg cccacggcg acgctgcaa tggcgtgggc gcaaagttcg
16201 agatgtgttg agacctggaa cttcgggtgg ttttacaccc ggcgagcggt caagcgctac
16261 ttttaagcgt tcctatgatg aggtgtacgg ggatgatgat attcttgagc aggcagctga
16321 ccgattaggg gagtttgctt atggcaagcg tagtagaata aatcccaagg atgaaacagt
16381 gtccataccc ttggatcatg gaaatcccac ccctagtctt aaaccggtca ctttgcagca
16441 agtgttaccg gtaactccgc gaacaggtgt taaacgcgaa ggtagaagatt tgcattccac
16501 tatgcaactg atggtgcccc aacgccagaa gttggaggac gttttggaga agtaaaaagt
16561 ggatccagat attcaacctg aggttaaagt gagaccatt aagcaggtag cgcctgggtc
16621 gggagtacaa actgtagaca ttaaaattcc cactgaaagt atggaagtgc aaactgaacc
16681 cgcaaagcct actgccacct ccactgaagt gcaaacggac ccatggatgc ccatgcctat
16741 tacaactgac gccgtcggtc ccactcgaag atcccgacga aagtagcgtc cagcaagctc
16801 gttgatgccc aactatgtcg tacaccatc tattattcct actcctgggt accagggcac
16861 tcgctactat cgcagccgaa acagtacttc ccgcgtcgc cgcaagacac ctgcaaatcg
16921 cagtcgtcgc cgtagacgca caagcaaac gattcccgcc gccctgggtg ggcaagtgtg
16981 ccgcaatggg agtgcggaac ctttgacact gccgcgtgcy cgttaccatc ctagtatcat
17041 cacttaatat atgttgccgc tgcctccttg cagatatggc cctcacttgt cgccttcgcy
17101 ttcccatcac tggttaccga ggaagaaact cgcgcgtag atgaggatg ttggggcgcy
17161 gaatgcgacg ctacaggcga cggcgtgcta tccgcaagca attgcggggg ggttttttgc
17221 cagccttaat tccaattatc gctgctgcca ttggcgcaat accaggcata gcttccgtgg
17281 cggttcaggg ctcgcaacga cattgacatt ggaaaaaaa aaaacgtata aataaaaaat
17341 acaatggagt ctgacactcc tggtagctgt actatgtttt cttagagatg gaagacatca
17401 atttttcac cttggctccg cgacacggca cgaagccgta catgggcacc tggagcgaca
17461 tccggcacgag ccaactgaac gggggcgcc tcaattggag cagtatctgg agcggtcta
17521 aaaattttgg ctcaaccata aaaacatacg ggaacaaagc ttggaacagc agtacaggac
17581 aggcgcttag aaataaactt aaagaccaga acttccaaca aaaagtagtc gatgggtag
17641 cttccgggtat caatggagtg gtatagtttg ctaaccaggc tgtgcagaaa aagataaaca
17701 gtcgttttga cccgccgcca gcaaccccg gtgaaatgca agtgaggaa gaaattcctc
17761 cgccagaaaa acgaggcgac aagcgtccgc gtcccgattt ggaagagacg ctggtgacgc
17821 gcgtagatga accgccttct tatgaggaag caacgaagct tggaatgcc accactagac
17881 cgatagcccc tatggccacc ggggtgatga aaccttctca gttgcatcga cccgtcacct
17941 tggattttgc ccctcctcct gctgctactg ctgtaccgcg ttctaagcct gtcgctgccc
18001 cgaaaccagt cgccgtagcc aggtcacgct ccgggggcgc tcctcgcca aatgcacat
18061 ggcataatac tctgaacagc atcgctgggtc taggcgtgca aggtgtaaaa cgccgtcgct
18121 gcttttaatt aaatatggag tagcgcttaa cttgcctatc tgtgtatatg tgtcattaca
18181 cgccgtcaca gcatcagagg aaaaaaggaa gaggtcgtgc gtcgacgctg agttactttc
18241 aagatggcca ccccatcgat gctgccccaa tgggcataca tgcacatcgc cggacaggat
18301 gcttcggagt acctgagtcg ggggtcgttg cagttcgccc gcgccacaga caccacttcc
18361 aatctgggaa ataagtttag aaactctacc gtagcgccga cccacgatgt gaccaccgat
18421 cgtagccagc ggctcatggt gcgcttcgtg cccgttgacc gggaggacaa tacatactct
18481 tacaagtgc ggtacacctt ggccgtgggc gacaacagag tgctggatat ggccagcacg
18541 ttctttgaca ttaggggcgt gttggacaga ggtcccagtt ttaaacccta ttctggtacg
18601 gcttacaact ccctggctcc taaaggcgct ccaatgcat ctcagtgggt ggataaggga
18661 gttacaagca ctggcctagt ggacgacggc aatactgat atggggaaga agccaaaaaa
18721 gcaacataca cttttggtaa tgctccagta aaagccgagg ctgaaatcac aaaagacgga
18781 ttgccggtgg gcttgggaagt ttcaactgaa ggtcctaaac caatctatgc tgataagctt
18841 tatcagccag aacctcaagt gggagacgaa acttgactg acctagacgg aaaaaccgaa
18901 gagtatggag ggaggggtct taaacctgaa actaaaatga aaccctgcta cggatctttt
18961 gctaaacctc ctaatattaa aggaggtcag gcaaaggtaa aaccaaaaga agacgatggc
19021 actaacaaca tcgagtatga cattgacatg aacttctttg acttaagatc acaaagatca
19081 gaactcaaac ctaaaattgt aatgtatgca gaaaatgtgg acctggaatg tccagatact
19141 catgttgtgt acaaacctgg agtttcagat gctagtcttg agaccaatct tggacaacag
19201 tctatgcccc acagacccaa ctacattggc ttcagagata acttcatcgg acttatgtac
19261 tataacagta ctggcaacat gggggctact gctggccaag cgtctcagtt gaatgcagtg
19321 gttgacttgc aggacagaaa cacagaactg tcttaccac tcttgcttga ctctctgggc
19381 gacagaacca gatactttag catgtggaat caggctgtgg acagttatga tcctgatgtg
19441 cgtgttattg aaaatcatgg tgtggaagat gaacttccca actattgttt tccgttggat

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FIG. 28A-5

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19501 ggtgtcgggtc cgcgaaacaga tagttacaag gagattaagc caaatggaga ccaatctact
19561 tggacaaatg tagacccaac tggcagcagt gaacttgcta aggggaaatcc atttgccatg
19621 gaaattaacc ttcaagccaa tctatggcga agtttccttt attccaatgt ggctctatat
19681 ctcccagact cgtacaaata caccctgccc aatgtcactc ttccagaaaa caaaaacacc
19741 tacgactaca tgaacgggcg ggtgggtgccc ccactctctag tagacaccta tgtgaacatt
19801 ggtgccaggt ggtctcttgga tgccatggac aatgtcaacc cattcaacca ccaccgtaac
19861 gctggcttgc gttaccgatc catgcttctg ggtaacggac gttatgtgcc ttccacata
19921 caagtgcctc aaaaattctt cgctgttaaa aacctgctgc ttctcccagg ctctacact
19981 tatgagtgga acttttaggaa ggaatgtaaac atggttctac agagttccct cggtaacgac
20041 ctacgggtag atggcgccag catcagtttt acctctatgc accctctatgc tacttttttc
20101 cccatggctc acaacaccgc ttccaccctt gaagccatgc tgcggaatga caccaatgat
20161 cagtcatcca acgactacct atctgcagct aacatgctct accccattcc tgccaatgca
20221 accaatattc ccatttccat tccttctcgc aactgggcgg ctttcagagg ctggtcattt
20281 accagactga aaaccaaaga aactccctct ttggggctcg gatttgacct ctacttcgtc
20341 tattctgggt ctattcccta cctggatggt accttctacc tgaaccacac ttttaagaag
20401 gtttccatca tgtttgactc ttcagtgaac tggcctggaa atgacagggt actatctcct
20461 aacgaatttg aaataaagcg cactgtggat ggcaaggct acaacgtagc ccaatgcaac
20521 atgaccaaag actggttctt ggtacagatg ctgcgcaact acaacatcgg ctatcaggggc
20581 ttctacattc cagaaggata caaagatcgc atgtattcat ttttcagaaa cttccagccc
20641 atgacagcgg aggtggttga tgaggtcaat tacaaagact tcaaggccgt cgccatcccc
20701 taacaacaca acaactctgg ctttgtgggt tacatggctc cgaccgtcg ctcaaggtaa
20761 ccctatcccg ctaactatcc ctatccactc attggaacaa ctgccgtaaa tagtggtacg
20821 cagaaaaagt tcttgtgtga cagaacatg tggcgcatat cgttctcaag caacttcatg
20881 tctatgggag cccttacaga cttgggacag aacatgctct atgccaactc agctcatgct
20941 ctggacatga cctttgaggt ggatcccatg gatgagccca ccctgcttta tcttctcttc
21001 gaagttttcg acgtggtcag agtgcatcag ccacaccgcy gcatctcga ctcagtctac
21061 ctgcgtacac cgttctcggc cggtaacgct accacgtaag aagcttcttg cttcttgcaa
21121 acagcagctg caaccatggc ctgcgatccc caaacggct ccagcgagca agagctcaga
21181 gccattgtcc aagacctggg ttgcggaaca tatttttttg gaacctttga taagcgcttc
21241 ccggggttca tggcccccga taagctcgcc tgtgccattg taaatacggc cggacgtgag
21301 acggggggag agcattggtt ggccttcggt ttggaaccac gttctaacc ctgctacctt
21361 tttgatccct ttggattctc ggatgatcgt ctcaaacaga tttaccagtt tgaatatgag
21421 ggtctcctgc gccgcagcgc tcttgcctac aaggaccggt gtattacgct ggaaaaatct
21481 acccagaccg tgcagggccc ccgttctgcc gcctgcggac ttttctgctg catgttccct
21541 catgcctttg tgcactggcc tgaccgtccc atggacggaa accccaccat gaaattgcta
21601 actggagtgc caaacaacat gcttcattct cctaagctcc agcccaaat atgtgacaat
21661 caaaaagcac tctaccattt tctcaatacc cattcgctt attttcgctc tcatcgta
21721 cacatcgaaa gggccactgc gttcgaccgt atggatgtgc aataatgatt catgtaaaca
21781 acgtgttcaa taaacagcac tttatttttt acatgtatcg aggcctctga ttactatatt
21841 atttacaagt cgaatgggtt ctgacgagaa tcagaatgac ccgcaggcag tgatacgttg
21901 cggaactgat acttgggttg ccacttgaat tcgggaatca ccaacttggg aaccggtata
21961 tcgggcagga tgcactcca cagcttctct gtcagctgca aagctcccag caggtcagga
22021 gccgaaatct tgaaatcaca attaggacca gtgctctgag cgcgagagtt gcggtapacc
22081 ggattgcagc actgaaacac catcagcgac ggatgtctta cgcttgccag cacggtggga
22141 tctgcaatca tgcccacatc cagatcttca gcattggcaa tgctgaacgg ggtcatcttg
22201 caggtctgcc taccatggc gggcacccta ttaggcttgt ggttacaatc gcagtcgagg
22261 gggatcagta tcatcttggc ctgattcctg ctgattcctg gatacacggc tctcatgaaa
22321 gcatcatatt gcttgaaagc ctgctgggct ttactacctt cgggtataaaa catcccgag
22381 gacctgctcg aaaactggtt agctgcgcag ccggcatcat tcacacagca gcggcgctca
22441 ttgttggtca tttgcaccac acttctgccc cagcggtttt ggggtgattt catccatctc
22501 ggatttctct tcaaggctcg ttgtccgttc tcgctggcca cacttcagct tgccctcata
22561 tccttctgaa tcataatatt gccatgcaag cacttcagct tgccctcata atcattgcag
22621 ccatgaggcc acaacgcaca cctgtacat tccaattat ggtgggcgat ctgagaaaaa
22681 gaatgtatca ttccctgcag aaatcttccc atcatcgtgc tcagtgtctt gtgactagt
22741 aaagttaact ggatgcctcg gtgctcctcg ttcacgtact ggtgacagat gcgcttgat
22801 tgttcgtgct gctcaggcat tagtttaaaa gaggttctaa gttcgttatc gttcgttatc
22861 ttctccatca gcagacacat cacttccatg cctttctccc aagcagacac caggggcaag
22921 ctaatcggat tcttaacagt gcaggcgca gctcctttag ccagaggttc atcttggcg
22981 atcttctcaa tgcctctttt gccatccttc tcaacgatgc gcacgggccc gtagctgaaa
23041 cccactgcta caagttgcgc ctcttctctt tcttcttcgc tgtcttgact gatgtcttg
23101 atggggacat gtttgggtct ccttggcttc ttttctgggg gtatcggagg aggaggactg
23161 tcgctccggt ccggagacag ggaggattgt gacgtttcgc tcaccattac caactgactg
23221 tcggtagaag aacctgaccc caccggcgga caggtgtttc tcttcggggg cagaggtgga
23281 gcgattgcg aagggctgcg gtccgacctg gaaggcggat gactggcaga acccttccg
23341 cgttcggggg tgtgctccct gtggcggtcg cttaactgat ttccttcgcy gctggccatt

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FIG. 28A-6

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23401 gtgtttctcct aggcagagaa acaacagaca tggaaactca gccattgctg tcaacatcgc
23461 cacgagtgcc atcacatctc gtcctcagcg acgaggaaaa ggagcagagc ttaagcattc
23521 caccgcccag tcctgccacc acctctaccc tagaagataa ggaggtcgac gcattctcatg
23581 acatgcagaa taaaaaagcg aaagagtctg agccagacat cgaacaagac ccgggctatg
23641 tgacaccggg ggaacacgag gaagagttga aacgctttct agagagagag gatgaaaact
23701 gccccaaaaca gcaagcggat aactatcacc aagatgctgg aaatagggat cagaacaccg
23761 actacctcat agggcttgac ggggaagacg cgctccttaa acatctagca agacagtcac
23821 tcatagtcaa ggatgcatta ttggacagaa ctgaagtgcc catcagtgtc gaagagctca
23881 gccgcgccta cgagcttaac ctattttcac ctcgactcc ccccaaactg cagccaaacg
23941 gcacctgcga gccaaatcct cgtttaaact tttatccagc ttttgctgtg ccagaagtac
24001 tggctacctt tcacatcttt tttaaaaatc aaaaaattcc agtctcctgc cgcgctaatac
24061 gcacccgcgc cgatgcccta ctcaatctgg gacctggttc acgcttacct gatatagctt
24121 ccttggaaga ggttccaaag atcttcgagg gtctgggcaa taatgagact cgggccgcaa
24181 atgctctgca aaagggagaa aatggcatgg atgagcatca cagcgttctg gtggaattgg
24241 aagcgataaa tgccagactc gcagtactca agcgaagcgt cgaggtcaca cactttgcat
24301 accccgctgt caacctgccc cctaaagtca tgacggccgt catggaccag ttactcatta
24361 agcgcgcaag tcccccttca gaagacatgc atgaccaga tgctgtgat gagggtaaac
24421 cagtggtcag tgatgagcag ctaacccgat ggctgggcac cgactctccc cgggatttgg
24481 aagagcgtcg caagcttatg atggcctgg tgctggttac cgtagaacta gactgtcttc
24541 ggcgtttctt taccgattca gaaactctgc gcaaactcga agagaactct agtacactt
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24781 cgtggcaaac cggcatgggt gtatggcagc aatgtttaga agaacagaac ctgaaagagc
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25261 aaacccagat aataggcacc tttgaattgc aaggccccag cagccaaggc gatgggtctt
25321 ctctgggca aagtttaaaa ctgaccccg gactgtggac ctccgcctac ttgcaagt
25381 ttgccccgga agattaccac ccctatgaaa tcaagttcta tgaggacca caatttgcaag
25441 cgaaagccga actttcggcc tgcgtcatca cccagggggc aattctggcc taatttgacc
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25561 cccagaccgg cgaggaactc aacacaaggt tccctcagga tgtcccaacg acgagaaagc
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25681 gcagaggaag cggaggagga ggacagtctg gaggacagtc tggaggaaga cagtgtggag
25741 gaggaaaacg aggaggcaga ggaggtggaa gaagtaaccg ccgacaaaca gttatcctcg
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25861 cccagcagta gatgggacga gccgggacgc ttcccgaacc caaccagcgc tccaagacc
25921 ggtaagaagg atcggcaggg atacaagtcc tggcgggggc ataagaatgc catcatctcc
25981 tgcttgcatg agtgcggggg caacatatcc ttcacgcggc gctacttgct attccaccat
26041 ggggtgaact ttccgcgcaa tgttttgcac tactaccgtc acctccacag cccctactat
26101 agccagcaaa tcccggcagt ctgcagatg aaagacagcg gcggcgacct ccaacagaaa
26161 accagcagcg gcagttagaa aatacacaa acaggaggt acaggaggt taaagattac
26221 agccaacgag ccagcgcaaa cccgagagtt aagaaatcgg atctttccaa cctgtatgc
26281 catcttccag cagagtcggg gccaaagagc ggaactgaaa ataaaaaacc gatctctgag
26341 ttcgctcacc agaagttgtt tgtatcacia gagcgaagat caacttcagc gcactctcga
26401 ggacgccgag gctctcttca acaagtactg cgcgctgact cttaaagagt aggcagcgac
26461 cgcgcttatt caaaaaaggg ggggaattaca tcatcctcga catgagtaaa gaaattccca
26521 cgccttacat gtggagttat cagcccaaaa tgggattggc ggcaggcgcc tcccaggact
26581 actccacccg catgaattgg ctgagcgccg ggccttctat gatttctcga gttaatgata
26641 tacgcgccta ccgaaaccaa atacttttgg aacagtcagc tcttaccacc acgccccgc
26701 aacaccttaa tcccagaaat tggcccgcgc ccctagtgtg ccaggaaagt cccgctccca
26761 cactgtatt acttctctga gacgcccagg ccgaagtcca aatgactaat gcaggtgcgc
26821 agttagcggg cggtccacc ctatgtctgc acaggcctcg gcataatata cagggtcga
26881 tgatcagagg ccgaggtatc cagctcaacg acgagtcggg gagctctccg cttggtctac
26941 gaccagacgg aatctttcag attgcccgtt gcgggagatc ttccttcacc cctcgtcagg
27001 ctgttctgac tttggaaagt tegtcttcgc aaccccgtc gggcggaatc gggaccgttc
27061 aatttgtgga ggagtttact cctctgtctt acttcaacc cttctccgga tctctgggc
27121 actaccgga cgagttcata ccgaacttcg acgcgattag cgaggtacg cagcgtacg
27181 attgatgtct ggtgacgggg ctgagctatc tcgggtgcga catctagacc actgccgcg
27241 ctttcgctgc tttgcccggg aactcattga gttcatctac ttcgaactcc ccaaggatca

FIG. 28A-7

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27301 ccctcaaggt cccgcccacg gaggcgcat tactatcgaa ggcaaaatac actctcgcct
27361 gcaacgaatt ttctcccagc ggcccgtgct gatcgagcga gaccagggaa acaccacggt
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27541 gatttttaca ccagaagaac gaaacttttc ctgtcgtcca ggactctggt aacttcacct
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27661 ctaatactac tttcaaaacc ggaggtgagc tccaaggtct tcctacagaa aacccttggg
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28561 aaaactttac attgattaat gttagcaaag ttacagcggg ttactattat gggtatgaca
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29641 cactttttgc ttttgttact tgcactctgc tatgtagcat agtctgcctg gttattaatt
29701 ttttccaact tctagactgg atccttgtgc gaattgccta cctgcgccac catcccgaat
29761 accgcaacca aaatatcgcg gcatctcta gactcatcta aaacctgca ggctatacta
29821 ccaatatttt tgcttctatt gcttccctac gctgtctcaa ccccagctgc ctatagtact
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29941 cgagaaaaat cagaaattcc cccaaattta ataagtattg ctggaataat taatataatc
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30361 aggcataattc tgtttggtaa aacaagccaa gatatcctac gagatcaccg ctactgacca
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31081 aaataatcac tctgtagaac tctcatttgg aaatggatta gaaactcaaa acaataaact
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FIG. 28A-8

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31921 gtttttagatt ccacattcca aacagtttca gagcgagcca atctggggtc agtgatagat
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32281 agtaggtaca acacattatt acaatattgt ttaataaacc ataattaaaa gcgctccagc
32341 caaaactcat atctgatata atcgccccctg catgaccatc ataccaaagt ttaataaaaa
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32761 aaggaagacc acgaacacaa cttacactat gcatagtcat agtatcaca tctggcaaca
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34561 aaagtacagt ttcacttccg caatcccaac aagcgtcact tcctcttctt cacggtacgt
34621 cacatcccat taacttgcga cgtcattttc ccacggccgc tccgcccgtt ttacgcttta
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34741 caccattcca tctataaggt atattattga tgatg

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SEQ ID NO: 12

FIG. 28A-9

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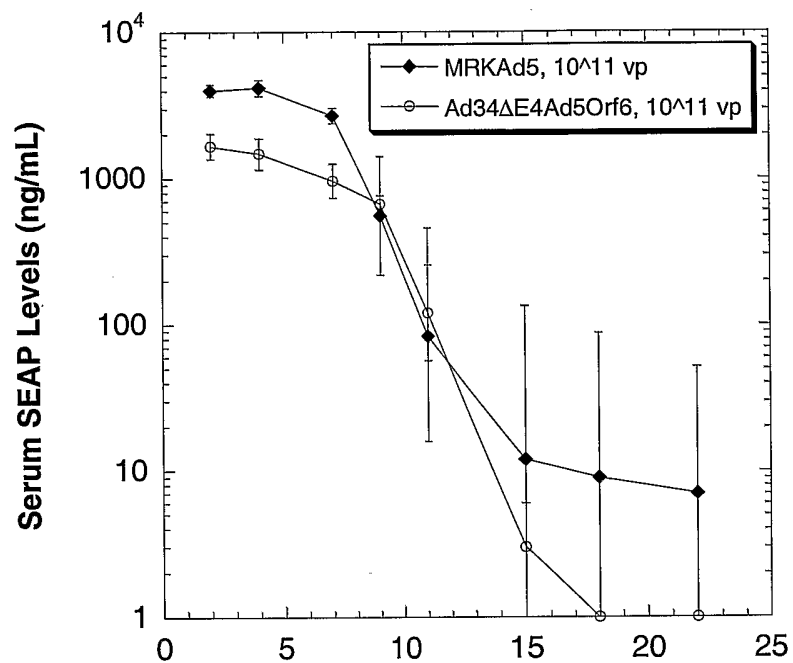


FIG. 29

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Vaccine Wk 0, 4, 24	Monkey ID	Pre		Wk 4		Wk 8		Wk 24		Wk 28		Wk 36	
		Mock	Gag ^a	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
MRKAd5gag, 10 ⁴ 11 vp	00C018	1	5	13	1025	0	824	8	756	0	474	0	383
MRKAd5gag, 10 ⁴ 11 vp	00C034	0	4	5	219	5	404	3	445	3	339	0	216
MRKAd5gag, 10 ⁴ 11 vp	00C058	4	4	3	1086	0	440	4	1439	0	2338	0	940
Ad34ΔE1gagΔE4Ad5Orf6, 10 ⁴ 11 vp	00D038	6	8	5	111	1	301	0	224	1	536	0	233
Ad34ΔE1gagΔE4Ad5Orf6, 10 ⁴ 11 vp	00D042	6	30	4	89	4	264	1	73	0	181	0	69
Ad34ΔE1gagΔE4Ad5Orf6, 10 ⁴ 11 vp	00D086	3	18	1	118	1	816	0	429	0	439	0	273

FIG. 30

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Vaccine	Monk ID	IFN- γ ⁺ CD4 ⁺ CD3 ⁺ per 10 ⁶ Lymphocytes		IFN- γ ⁺ CD8 ⁺ CD3 ⁺ per 10 ⁶ Lymphocytes	
		Mock	Gag ^a	Mock	Gag ^a
Ad34ΔE1gagΔE4Ad5Orf6	00D038	22	154	130	450
	00D042	32	118	96	171
	00D066	12	238	150	442

FIG. 31

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Vaccine T=0, 4 wks	Vaccine T=24 wks	Monkey ID	Pre		T=4 wks		T=8 wks		T=24 wks		T=28 wks		T=32 wks	
			Mock	Gag ^A	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
Ad34ΔE1gagΔE4Ad5Orf6, 10 ¹¹ vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ¹⁰ vp	00D018	4	6	1	84	5	334	5	99	0	306	3	244
Ad34ΔE1gagΔE4Ad5Orf6, 10 ¹¹ vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ¹⁰ vp	00D044	1	1	8	79	0	374	8	136	0	493	1	253
Ad34ΔE1gagΔE4Ad5Orf6, 10 ¹¹ vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ¹⁰ vp	00D064	4	6	1	125	8	655	6	145	0	351	1	236
Naïve		00D087	1	1	3	3	8	54	6	8	5	5	3	0

FIG. 32

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Vaccine (T=0, 4 Wks)	Vaccine (T=24 Wk)	Monkey ID	IFN- γ ⁺ CD4 ⁺ CD3 ⁺ per 10 ⁶ Lymphocytes		IFN- γ ⁺ CD8 ⁺ CD3 ⁺ per 10 ⁶ Lymphocytes	
			Mock	Gag	Mock	Gag
Ad34ΔE1gagΔE4Ad5Orf6, 10 ¹¹ vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ¹⁰ vp	00D016	62	433	176	1288
Ad34ΔE1gagΔE4Ad5Orf6, 10 ¹¹ vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ¹⁰ vp	00D044	136	593	323	1871
Ad34ΔE1gagΔE4Ad5Orf6, 10 ¹¹ vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ¹⁰ vp	00D064	188	785	292	992

FIG. 33