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

(57) **Abrégé/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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(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 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.

5 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
10 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
15 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.

20 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.

25 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
30 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-
35 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.

5 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.

25 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.

35 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).

25 The passenger gene preferably exists in the form of an expression cassette. A 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-TTTTGTGTG (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 pAd35 Δ E1 Δ E4Ad5Orf6. Pre-Adenovirus plasmid pAd35 Δ E1 Δ E4Ad5Orf6 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 pAd35 Δ E1 Δ Orf6Ad5Orf6 (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 pAd35 Δ E1 Δ Orf6Ad5Orf6. Pre-Adenovirus plasmid pAd35 Δ E1 Δ Orf6Ad5Orf6 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.

15 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 animals 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.

30 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.

5 Table 1. Gag-specific T cell response in monkeys immunized with MRKAd5-HIV1gag 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	Naïve	00D087	1	1	3	3	8	54	3	5	3	1

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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).

15 Table 2. Characterization of the gag-specific T cells in monkeys immunized with MRKAd5-HIV1gag 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

20

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) 10^{10} 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.

5

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

pending 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.

20

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
5 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
10 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
15 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
20 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
25 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
30 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
35 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. PAd24 Δ Orf6BstZ17I, a derivative of pAd24 Δ E1, was constructed so that the E4 region in the Ad24 pre-Ad plasmid could be easily modified using bacterial recombination. PAd24 Δ Orf6BstZ17I contains a deletion in the E4 region from Ad24 bp 32373 to
 5 bp 33328 with a unique *Bst*Z17I site located at the position of the deletion. The complete sequence of pAd24 Δ Orf6BstZ17I 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 pAd24 Δ E1 Δ E4Ad5Orf6 (An Ad24 pre-Ad plasmid containing an E1
 10 deletion and a deletion of E4 substituted with Ad5 Orf6), an Ad24 E4 shuttle plasmid was constructed by digesting pAd24 Δ E1 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'
 15 ACTCGAGATGTATAGATCT (SEQ ID NO: 6); 5' CTAGATCTATACATCTCGAG (SEQ ID NO: 7)), generating pNEBAd24 Δ E4. PNEBAd24 Δ E4 was then digested with *Bgl*II and *Xho*I and ligated with the Ad5 Orf6 gene, which was PCR amplified, generating pNEBAd24 Δ E4Ad5Orf6. The PCR primers used to amplify the Ad5 Orf6 gene (5'
 20 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 pNEBAd24 Δ E4 fragment. In the final step pNEBAd24 Δ E4Ad5Orf6 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
 25 BJ 5183 bacteria with E4 shuttle fragment and pAd24 Δ Orf6BstZ17I, which had been linearized in the E4 region by digestion with *Bst*Z17I, resulted in the generation of pAd24 Δ E1 Δ E4Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24 Δ E1 Δ E4Ad5Orf6.

To construct pAd24 Δ E1 Δ Orf6Ad5Orf6 (An Ad24 pre-Ad plasmid containing an
 30 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
 35 Orf6, pNEBAd24Orf6 was digested with *Sty*I and treated with Klenow to blunt the ends and then

digested with to *EagI*. 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 *EagI* and *SmaI* sites (underlined above) for ligation with the pNEBAd24Orf6 fragment. In the final step pNEBAd24ΔOrf6Ad5Orf6 was digested with *EcoRI*, 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 *EcoRI* fragment and pAd24ΔOrf6BstZ17I, which had been linearized in the E4 region by digestion with *BstZ17I*, 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 *PmeI* 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.). *PmeI* 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 lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following
30 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 *HindIII* and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by
35 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 Ad24 Δ E1, Ad24 Δ E1Ad5Orf6, Ad24 Δ E1 Δ E4Ad5Orf6 and Ad24 Δ E1 Δ Orf6Ad5Orf6 one step growth curves were preformed (Figure 15). PER.C6 cells in 60 mm dishes were infected at 1 vp per cell with either Ad24 Δ E1, Ad24 Δ E1Ad5Orf6, Ad24 Δ E1 Δ E4Ad5Orf6 or Ad24 Δ E1 Δ Orf6Ad5Orf6. 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 Ad24 Δ E1 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 pAd24 Δ E1 Δ E4Ad5Orf6, pAd24 Δ E1 Δ Orf6Ad5Orf6,

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 (pAd24 Δ E1 Δ E4Ad5Orf6, pAd24 Δ E1 Δ Orf6Ad5Orf6) 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, pABSAd17-3, generating pABSAd17HCMVgagBGHpA. 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 pABSAd17-3. 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 (pAd24 Δ E1 Δ E4Ad5Orf6, pAd24 Δ E1 Δ Orf6Ad5Orf6), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the corresponding Ad24 gag-containing pre-Adenovirus plasmids (pAd24 Δ E1gag Δ E4Ad5Orf6, pAd24 Δ E1gag Δ Orf6Ad5Orf6) 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, pABSA17-3, generating pABSA17HCMVSEAPBGH. 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 pABSA17-3. 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 (pAd24 Δ E1 Δ E4Ad5Orf6, pAd24 Δ E1 Δ Orf6Ad5Orf6), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the corresponding Ad24 SEAP-containing pre-Adenovirus plasmids (pAd24 Δ E1SEAP Δ E4Ad5Orf6, pAd24 Δ E1SEAP Δ Orf6Ad5Orf6) 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 Ad24 Δ E1gag Δ Orf6Ad5Orf6; (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 strepavidin-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.

35

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 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 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 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 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 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 *HindIII* 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 *PmeI/HindIII* prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 20

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 *SwaI* 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 *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. 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
5 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.

10 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
15 456 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).
20 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
25 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
30 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

Serum samples were analyzed for circulating human secreted alkaline
20 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

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
5 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
10 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
15 (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
20 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.

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.

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.

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.

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
5 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.

10 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.

15 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.

20 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.

15 49. A method for effecting the delivery and expression of the heterologous 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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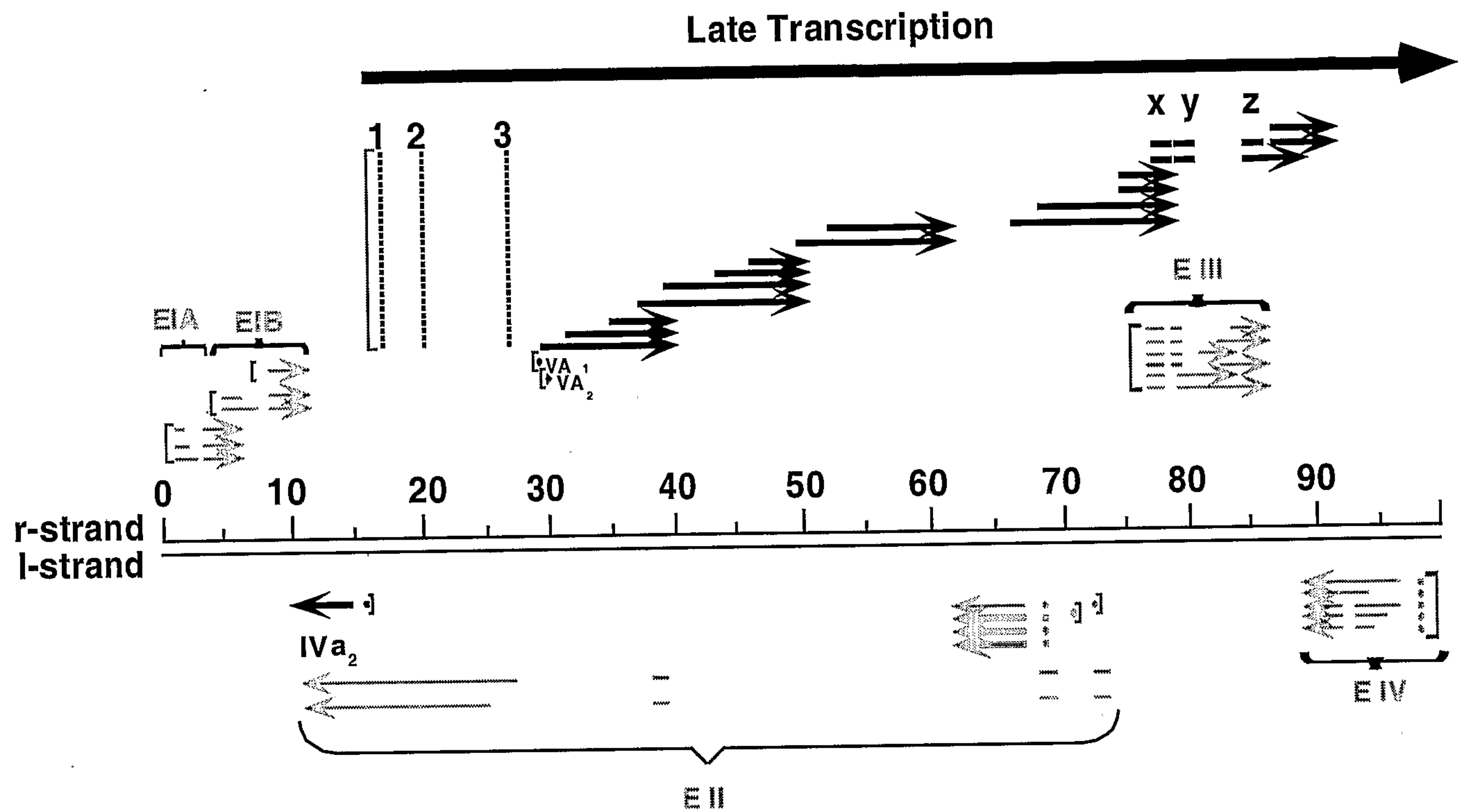


FIG. 1

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FIG. 2A-1

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FIG. 2A-2

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8581 gtcgattgac gtcttgatc tgacgtctct gggtagaagc taccggcccc gtgagcttga
8641 acctgaaaga gagttcaaca gaatcaattt cggtagcgtt aacggcagct tgtctcagta
8701 tttcttgtag gtcaccagag ttgtcctggg aggcgatctc cgccatgaac tgctcgattt
8761 cttcctcctg aagatctccg cgaccgcctc tttcgacggg ggccgcgagg tcattggaga
8821 tacggcccat gagttgggag aatgcattca tgccgcctc gttccagacg cggctgtaaa
8881 ccacggcccc ctccgaggtc cttgcgcgca tcaccacctg agcgagggtta agctccacgt
8941 gtctggtgaa gaccgcatag ttgcataggc gctgaaaaag gtagttgagt gtggtggcaa
9001 tgtgttcggc gacgaagaaa tacatgatcc atcgtctcag cggcatttcg ctaacatcgc
9061 ccagagcttc caagcgctcc atggcctcgt agaagtccac ggcaaaatta aaaaactggg
9121 agtttcgcgc ggacacggtc aattcctcct cgagaagacg gatgagttcg gctatgggtg
9181 cccgtacttc gcgttcgaag gctcccggga tctcttcttc ctcttctatc tcttcttcca
9241 ctaacatctc ttcttcgtct tcaggcgggg gcggaggggg cacgcggcga cgtcgacggc
9301 gcacgggcaa acggtcgatg aatcgttcaa tgacctctcc gcggcggcgg cgcattggtt
9361 cagtgcgggc gcggccgttc tcgcgcggtc gcagagtaaa aacaccgccg cgcattctct
9421 taaagtgggt actgggagggt tctccgtttg ggaggagag ggctgatt atacatttta
9481 ttaattggcc cgtagggact gcgcgcagag atctgatcgt gtcaagatcc acgggatctg
9541 aaaacctttc gacgaaagcg tctaaccagt cacagtcaca aggtaggctg agtacggctt
9601 cttgtgggcg ggggtggtta tgtgttcggt ctgggtcttc tgtttcttct tcattctcgg
9661 aaggtgagac gatgctgctg gtgatgaaat taaagtaggc agttctaaga cggcggatgg
9721 tggcgaggag caccaggtct ttgggtccgg cttgctggat acgcaggcga ttggccattc
9781 cccaagcatt atcctgacat ctagcaagat cttttagtag gtcttgcatg agccgttcta
9841 cgggcacttc ttcctcacc gttctgcat gcatacgtgt gactccaaat ccgcgcattg
9901 gttgtaccag tgccaagtca gctacgactc tttcggcgag gatggcttgc tgtacttggg
9961 taagggtggc ttgaaagtca tcaaaatcca caaagcgggt gtaagccctt gtattaatgg
10021 tgtaagcaca gttggccatg actgaccagt taactgtctg gtgaccaggg cgcacgagct
10081 cgggtgtattt aaggcgcgaa taggcgcggg tgtcaaagat gtaatcggtt cagggtgcgca
10141 ccagatactg gtaccctata agaaaatgcg gcggtggttg gcggtagaga ggccatcggt
10201 ctgtagctgg agcgcacagg gcgaggtctt ccaacataag gcggtgatag ccgtagatgt
10261 acctggacat ccaggtgatt cctgcggcgg tagtagaagc ccgaggaaac tcgcgtacgc
10321 ggttccaaat gttgcgtagc ggcattgaagt agttcattgt aggcacgggt tgaccagtga
10381 ggcgcgcgca gtcattgatg ctctatagac acggagaaaa tgaaagcgtt cagcgactcg
10441 actccgtagc ctggaggaaac gtgaacgggt tgggtcgcgg tgtaccccgg ttcgagactt
10501 gtactcgagc cggccgggag gcgcgctaac gtggtattgg cactcccgtc tcgaccacgc
10561 ctacaaaaat ccaggatacg gaatcgagtc gttttgctgg tttccgaatg gcagggaagt
10621 gagtcctatt tttttttttt ttttgccgct cagatgcata ccgtgctgcg acagatgcgc
10681 ccccaacaac agccccctc gcagcagcag cagcagcagc aaccacaaaa ggctgtccct
10741 gcaactactg caactgccgc cgtgagcggg gcgggacagc ccgcctatga tctggacttg
10801 gaagagggcg aaggactggc acgtctaggt gcgccttcgc ccgagcggca tccgcgagtt
10861 caactgaaaa aagattctcg cgaggcgtat gtgccccaac agaacctatt tagagacaga
10921 agcggcgagg agccggagga gatgcgagct tcccgtttta acgcgggtcg tgagctgcgt
10981 cacggtttgg accgaagacg agtggtgcga gacgaggatt tcgaagttga tgaagtgaca
11041 gggatcagtc ctgccagggc acacgtggct gcagccaacc ttgtatcggc ttacgagcag
11101 acagtaaagg aagagcgtaa cttccaaaag tcttttaata atcatgtgcg aaccctgatt
11161 gccgcggaag aagttaccct tggtttgatg catttggtgg atttgatgga agctatcatt
11221 cagaacccta ctagcaaac tctgaccgcc cagctgtttc tggtgggtgca acacagcaga
11281 gacaatgagg ctttcagaga ggcgctgctg aacatcaccg aaccggaggg gagatgggtg

FIG. 2A-3

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11341	tatgatctta	tcaacattct	acagagtatc	atagtgcagg	agcggagcct	gggcctggcc
11401	gagaaggtag	ctgccatcaa	ttactcgggt	ttgagcttgg	gaaaatatta	cgctcgc aaa
11461	atctacaaga	ctccatacgt	tcccatagac	aaggaggtga	agatagatgg	gttctacatg
11521	cgc atgacgc	tcaaggtcct	gaccctgagc	gatgatcttg	gggtgtatcg	caatgacaga
11581	atgcatcgcg	cggttagcgc	cagcaggagg	cgcgagttaa	gcgacaggga	actgatgcac
11641	agtttgcaaa	gagctctgac	tggagctgga	accgaggggtg	agaattactt	cgacatggga
11701	gctgacttgc	agtggcagcc	tagtcgcagg	gctctgagcg	ccgcgacggc	aggatgtgag
11761	cttccttaca	tagaagaggg	ggatgaaggc	gaggaggaag	agggcgagta	cttgggaagac
11821	tgatggcaca	accogtggtt	tttgctagat	ggaacagcaa	gcaccggatc	ccgcaatgcg
11881	ggcggcgctg	cagagccagc	cgtccggcat	taactcctcg	gacgattgga	cccaggccat
11941	gcaacgtatc	atggcggttg	cgactcgcaa	ccccgaagcc	tttagacagc	aaccccaggc
12001	caaccgtcta	tcggccatca	tgggaagctgt	agtgccttcc	cgatctaate	ccactcatga
12061	gaaggctcctg	gccatcgtga	acgcgttggg	ggagaacaaa	gctattcgtc	cagatgaggc
12121	cggactggta	tacaacgctc	tcttagaacg	cgtggctcgc	tacaacagta	gcaatgtgca
12181	aaccaatttg	gaccgtatga	taacagatgt	acgcgaagcc	gtgtctcagc	gcgaaagggt
12241	ccagcgtgat	gccaacctgg	gttcgctggg	ggcggttaaat	gctttcttga	gtactcagcc
12301	tgctaagtgtg	ccgcgtgggtc	aacaggatta	tactaacttt	ttaagtgttt	tgagactgat
12361	ggtatcagaa	gtacctcaga	gcgaagtgtg	tcagtccggg	cctgattact	tctttcagac
12421	tagcagacag	ggcttgacga	cggtaaatct	gagccaagct	tttaaaaacc	ttaaagggtt
12481	gtggggagtg	catgccccgg	taggagaaaag	agcaaccgtg	tctagcttgt	taactccgaa
12541	ctcccgcttg	ttattactgt	tggtagctcc	tttcaccgac	agcggtagca	tgcaccgtaa
12601	ttcctatattg	ggttacctac	taaacctgta	tcgcgaagcc	atagggcaaa	gtcaggtgga
12661	cgagcagacc	tatcaagaaa	ttaccaaggt	cagtcgcgct	ttgggacagg	aagacactgg
12721	cagtttgga	gccactctga	acttcttgct	taccaatcgg	tctcaaaaga	tccctcctca
12781	atatgctctt	actgcggagg	aggagaggat	ccttagatat	gtgcagcaga	gcgtgggatt
12841	gtttctgatg	caagaggggg	caactccgac	tgcagcactg	gacatgacag	cgcgaaatat
12901	ggagcccagc	atgtatgcca	gtaaccgacc	tttcattaac	aaactgctgg	actacttgca
12961	cagagctgcc	gctatgaact	ctgattatgt	caccaatgcc	atcttaaacc	cgactgggt
13021	gccccacct	ggtttctaca	cgggcgaata	tgacatgccc	gaccctaata	acggatttct
13081	gtgggacgac	gtggacagcg	atgttttttc	acctctttct	gatcatcgca	cgtggaaaaa
13141	ggaaggcggg	gatagaatgc	attcttctgc	atcgtgtgct	ggggtcatgg	gtgctaccgc
13201	ggctgagccc	gagtctgcaa	gtccttttcc	tagtctaccc	ttttctctac	acagtgtacg
13261	tagcagcgaa	gtgggtagaa	taagtcgccc	gagtttaaat	ggcgaagagg	agtacctaaa
13321	cgattccttg	ctcagaccgg	caagagaaaa	aaatttccca	aacaatggaa	tagaaagttt
13381	ggtggataaa	atgagtagat	ggaagactta	tgctcaggat	cacagagacg	agcctgggat
13441	catggggact	acaagtagag	cgagccgtag	acgccagcgc	catgacagac	agaggggtct
13501	tgtgtgggac	gatgaggatt	cggccgatga	tagcagcgtg	ttggacttgg	gtgggagagg
13561	aaggggcaac	ccgtttgctc	atttgcgccc	tcgcttgggt	ggtatgttgt	gaaaaaaaat
13621	aaaaaagaaa	aactcaccaa	ggccatggcg	acgagcgtac	gttcgttctt	ctttattatc
13681	tgtgtctagt	ataatgaggc	gagtcgtgct	aggcggagcg	gtgggtgtatc	cggagggtcc
13741	tcctccttcg	tacgagagcg	tgatgcagca	gcagcaggcg	acggcgggtga	tgcaatcccc
13801	actggagggt	ccctttgtgc	ctccgcgata	cctggcacct	acggagggga	gaaacagcat
13861	tcgttactcg	gaactggcac	ctcagtacga	taccaccagg	ttgtatctgg	tggacaacaa
13921	gtcggcggac	attgcttctc	tgaactatca	gaatgaccac	agcaacttct	tgaccacggt
13981	ggtgcagaac	aatgacttta	cccctacgga	agccagcacc	cagaccatta	actttgatga
14041	acgatcgcg	tggggcgggtc	agctaaagac	catcatgcat	actaacatgc	caaacgtgaa
14101	cgagtatatg	tttagtaaca	agttcaaagc	gcgtgtgatg	gtgtccagaa	aacctcccga
14161	cgggtgctgca	gttggggata	cttatgatca	caagcaggat	atthttggaat	atgagtgggt
14221	cgagtttact	ttgccagaag	gcaacttttc	agttactatg	actattgatt	tgatgaacaa
14281	tgccatcata	gataattact	tgaaagtggg	tagacagaat	ggagtgtgtg	aaagtgcacat
14341	tggtgttaag	ttcgacacca	ggaacttcaa	gctgggatgg	gatcccga aa	ccaagttgat
14401	catgcctgga	gtgtatacgt	atgaagcctt	ccatcctgac	attgtcttac	tgcttggtcg
14461	cggagtggat	tttaccgaga	gtcgtttgag	caaccttctt	ggtatcagaa	aaaaacagcc
14521	atthtcaagag	ggtttttaaga	ttttgtatga	agattttagaa	ggtggtaata	ttccggccct
14581	cttggatgta	gatgcctatg	agaacagtaa	gaaagaacaa	aaagccaaaa	tagaagctgc
14641	tacagctgct	gcagaagcta	aggcaaacat	agttgccagc	gactctacaa	gggttgctaa
14701	cgctggagag	gtcagaggag	acaattttgc	gccaacacct	gttccgactg	cagaatcatt
14761	attggccgat	gtgtctgatg	gaacggacgt	gaaactcact	attcaacctg	tagaaaaaga
14821	tagtaagaat	agaagctata	atgtgttgga	agacaaaatc	aacacagcct	atcgcagttg
14881	gtatctttcg	tacaattatg	gcgatcccga	aaaaggagtg	cgttcctgga	cattgctcac
14941	cacctcagat	gtcacctgcg	gagcagagca	ggtttactgg	tcgcttccag	acatgatgaa
15001	ggatcctgtc	actttccgct	ccactagaca	agtcagtaac	taccctgtgg	tgggtgcaga
15061	gcttatgccc	gtcttctcaa	agagcttcta	caacgaacaa	gctgtgtact	cccagcagct

FIG. 2A-4

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15121	ccgccagtcc	acctcgctta	cgcacgtctt	caaccgcttt	cctgagaacc	agattttaat
15181	ccgtccgccg	gcgcccacca	ttaccaccgt	cagtgaaaac	gttcctgctc	tcacagatca
15241	cgggaccctg	ccgttgcgca	gcagtatccg	gggagtccaa	cgtgtgaccg	ttactgacgc
15301	cagacgccgc	acctgtccct	acgtgtacaa	ggcactgggc	atagtcgcac	cgcgcgctct
15361	ttcaagccgc	actttctaaa	aaaaaaatgt	ccattcttat	ctcgcccagt	aataacaccg
15421	gttgggggtct	gcgcgctcca	agcaagatgt	acggaggcgc	acgcaaacgt	tctacccaac
15481	atcccgtgcg	tgttcgcgga	cattttcgcg	ctccatgggg	tgccctcaag	ggccgcactc
15541	gcgttcgaac	caccgtcgat	gatgtaatcg	atcagggtgg	tgccgacgcc	cgtaattata
15601	ctcctactgc	gcctacatct	actgtggatg	cagttattga	cagtgtagtg	gctgacgctc
15661	gcaactatgc	tcgacgtaag	agccggcgaa	ggcgcatatg	cagacgccac	cgagctacca
15721	ctgccatgcg	agccgcaaga	gctctgctac	gaagagctag	acgcgtgggg	cgaagagcca
15781	tgcttagggc	ggccagacgt	gcagcttcgg	gcgccagcgc	cggcagggtcc	cgcaggcaag
15841	cagccgctgt	cgcagcggcg	actattgccg	acatggccca	atcgcgaaaga	ggcaatgtat
15901	actgggtgcg	tgacgctgcc	accggtcaac	gtgtaccctg	gcgcaccctg	ccccctcgca
15961	cttagaagat	actgagcagt	ctccgatgtt	gtgtcccagc	ggcgaggatg	tccaagcgca
16021	aatacaagga	agaaatgctg	caggttatcg	cacctgaagt	ctacggccaa	ccgttgaagg
16081	atgaaaaaaa	accccgc aaa	atcaagcggg	ttaaaaagga	caaaaaagaa	gaggaagatg
16141	gcgatgatgg	gctggcgagg	tttgtgcgcg	agtttgcccc	acggcgacgc	gtgcaatggc
16201	gtgggcgcaa	agttcgacat	gtgttgagac	ctggaacttc	ggtggtcttt	acaccggcg
16261	agcgttcaag	cgctactttt	aagcgttcct	atgatgaggt	gtacggggat	gatgatatcc
16321	ttgagcaggc	ggctgaccga	ttaggcgagt	ttgcttatgg	caagcgtagt	agaataactt
16381	ccaaggatga	gacagtgtca	atacccttgg	atcatggaaa	tcccaccctt	agtcttaaac
16441	cggtcacttt	gcagcaagtg	ttaccctgaa	ctccgcgaac	aggtgttaaa	cgcgaaagtg
16501	aagatttgta	tcccactatg	caactgatgg	tacccaaacg	ccagaagtgt	gaggacgttt
16561	tggagaaagt	aaaagtggat	ccagatatcc	aacctgaggt	taaagtgaga	cccattaagc
16621	aggtagcgcc	tggtctgggg	gtacaaactg	tagacattaa	gattcccact	gaaagtatgg
16681	aagtgcaaac	tgaaccgcga	aagcctactg	ccacctccac	tgaagtgcaa	acggatccat
16741	ggatgcccac	gcctattaca	actgacgcgc	ccggtcccac	tcgaagatcc	cgacgaaagt
16801	acgggtccagc	aagtctgttg	atgcccaatt	atgttgtaga	cccatctatt	attcctactc
16861	ctgggttaccg	aggcactcgc	tactatcgca	gccgaaacag	tacctcccgc	cgtcgccgca
16921	agacacctgc	aaatcgcagt	cgtcgccgta	gacgcacaag	caaaccgact	cccggcgccc
16981	tggtgcgggc	agtgtaccgc	aatggtagtg	cggaaccttt	gacactgccg	cgtgcgcgtt
17041	accatccgag	tatcatcact	taatcaatgt	tgccgctgcc	tccttgagca	tatggccctc
17101	acttgtcgcc	ttcgcgttcc	catcactggg	taccgaggaa	gaaactcgcg	ccgtagaaga
17161	gggatgttgg	gacgcggaat	gcgacgctac	aggcgacggc	gtgctatccg	caagcaattg
17221	cggggtgggt	ttttaccagc	cttaattcca	attatcgctg	ctgcaattgg	cgcgatacca
17281	ggcatagctt	ccgtggcggt	tcaggcctcg	caacgacatt	gacattggaa	aaaaaacgta
17341	taaataaaaa	aaaatacaat	ggactctgac	actcctggtc	ctgtgactat	gttttcttag
17401	agatggaaga	catcaatttt	tcatecttgg	ctccgcgaca	cggcacgaag	ccgtacatgg
17461	gcacctggag	cgacatcggc	acgagccaac	tgaacggggg	cgccttcaat	tggagcagta
17521	tctggagcgg	gcttaaaaaa	tttgggtcaa	ccataaaaaa	atacgggaac	aaagcttgga
17581	acagcagtag	aggacaggcg	cttagaaata	aacttaaaaga	ccagaacttc	caacaaaaag
17641	tagtcgatgg	gatagcttcc	ggcatcaatg	gagtggtaga	tttgggtaac	caggctgtgc
17701	agaaaaagat	aaacagtcgt	ttggaccgcg	cgccagcaac	cccagggtgaa	atgcaagtgg
17761	aggaagaaat	tcctccgcca	gaaaaacgag	gcgacaagcg	tcgcgctccc	gatttggaag
17821	agacgctggg	gacgcgcgta	gatgaaccgc	cttcttatga	ggaagcaacg	aagcttgga
17881	tgcccaccac	tagaccgata	gccccaatgg	ccaccggggg	gatgaaacct	tctcagttgc
17941	atcgaccctg	caccttggat	ttgccccctc	cccttgctgc	tactgctgta	cccgttctta
18001	agcctgtcgc	tgccccgaaa	ccagtcgcgc	tagccagggtc	acgtcccggg	ggcgctcctc
18061	gtccaaatgc	gcactggcaa	aataactctga	acagcatcgt	gggtctaggc	gtgcaaagtg
18121	taaaacgccg	tcgctgcttt	taattaaata	tggagtagcg	cttaacttgc	ctatctgtgt
18181	atatgtgtca	ttacacgccg	tcacagcagc	agaggaaaaa	aggaagaggt	cgtgcgtcga
18241	cgctgagtta	ctttcaagat	ggccacccca	tcgatgctgc	cccaatgggc	atacatgcac
18301	atcgccggac	aggatgcttc	ggagtacctg	agtccgggtc	tggtgcagtt	cgcccgcgcc
18361	acagacacct	acttcaatct	gggaaataag	tttagaaatc	ccaccgtagc	gccgaccac
18421	gatgtgacca	ccgaccgtag	ccagcggctc	atgttgcgct	tcgtgcccgt	tgaccgggag
18481	gacaatacat	actcttacia	agtgcgggtac	accctggccg	tgggcgacaa	cagagtgtctg
18541	gatattggcca	gcacgttctt	tgacattagg	ggcgtgttgg	acagagggtcc	cagtttcaaa
18601	ccctattctg	gtacggctta	caactctctg	gctcctaaag	gcgctccaaa	tgcatctcaa
18661	tggattgcaa	aaggcgtacc	aactgcagca	gccgcaggca	atggtgaaga	agaacatgaa
18721	acagaggaga	aaactgctac	ttacactttt	gccaatgctc	ctgtaaaagc	cgaggctcaa
18781	attacaaaag	agggcttacc	aataggtttg	gagatttcag	ctgaaaacga	atctaaaccc
18841	atctatgcag	ataaacttta	tcagccagaa	cctcaagtgg	gagatgaaac	ttggactgac

FIG. 2A-5

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18901	ctagacggaa	aaaccgaaga	gtatggaggc	agggctctaa	agcctactac	taacatgaaa
18961	ccctgttacg	ggtcctatgc	gaagcctact	aattttaaag	gtggtcaggc	aaaaccgaaa
19021	aactcggaac	cgtcagagtga	aaaaattgaa	tatgatattg	acatggaatt	ttttgataac
19081	tcatcgcaaa	gaacaaactt	cagtcctaaa	attgtcatgt	atgcagaaaa	tgtaggtttg
19141	gaaacgccag	acactcatgt	agtgtacaaa	cctggaacag	aagacacaag	ttccgaagct
19201	aatttgggac	aacagtctat	gcccacacaga	cccaactaca	ttggcttcag	agataacttt
19261	attggactca	tgtactataa	cagtactggg	aacatggggg	tgctggctgg	tcaagcgtct
19321	cagttaaagt	cagtgggtga	cttgcaggac	agaaacacag	aactttctta	ccaactcttg
19381	cttgactctc	tgggcgacag	aaccagatac	tttagcatgt	ggaatcaggc	tgtggacagt
19441	tatgatcctg	atgtacgtgt	tattgaaaat	catgggtgtg	aagatgaact	tcccaactat
19501	tgttttccac	tggacggcat	aggtgttcca	acaaccagtt	acaaatcaat	agttccaaat
19561	ggagaagata	ataataattg	gaaagaacct	gaagtaaagt	gaacaagtga	gatcggacag
19621	ggtaatttgt	ttgccatgga	aattaacctt	caagccaatc	tatggcgaag	tttcctttat
19681	tccaatgtgg	ctctgtatct	cccagactcg	tacaaataca	ccccgtccaa	tgtcactctt
19741	ccagaaaaca	aaaacaccta	cgactacatg	aacgggcggg	tggtgccgcc	atctctagta
19801	gacacctatg	tgaacattgg	tgccagggtg	tctctggatg	ccatggacaa	tgtcaacca
19861	ttcaaccacc	accgtaacgc	tggcttgctg	taccgatcta	tgcttctggg	taacggacgt
19921	tatgtgcctt	tccacataca	agtgcctcaa	aaattcttcg	ctgttaaaaa	cctgctgctt
19981	ctcccaggct	cctacactta	tgagtggaa	tttaggaagg	atgtgaacat	ggttctacag
20041	agttccctcg	gtaacgacct	gcgggtagat	ggcgccagca	tcagtttcac	gagcatcaac
20101	ctctatgcta	cttttttccc	catggctcac	aacaccgctt	ccacccttga	agccatgctg
20161	cggaatgaca	ccaatgatca	gtcattcaac	gactacctat	ctgcagctaa	catgctctac
20221	cccattcctg	ccaatgcaac	caatattccc	atttccattc	cttctcgcaa	ctgggcggct
20281	ttcagaggct	ggtcattttac	cagactgaaa	accaaagaaa	ctccctcttt	ggggtctgga
20341	tttgaccctt	actttgtcta	ttctgggtct	attccctacc	tggatggtac	cttctacctg
20401	aaccacactt	ttaagaaggt	ttccatcatg	tttgactctt	cagtgaagct	gcctggaaat
20461	gacagggttac	tatctcctaa	cgaatttgaa	ataaagcgca	ctgtggatgg	cgaaggctac
20521	aacgtagccc	aatgcaacat	gaccaaagac	tggttcttgg	tacagatgct	cgccaactac
20581	aacatcggct	atcagggtct	ctacattcca	gaaggataca	aagatcgcat	gtattcattt
20641	ttcagaaact	tccagcccat	gagcaggcag	gtgggtgatg	aggtcaatta	caaagacttc
20701	aaggccgctg	ccatacccta	ccaacacaac	aactctggct	ttgtgggtta	catggctccg
20761	accatgcgcc	aaggtaacc	ctatcccgtt	aactatccct	atccactcat	tggacaact
20821	gccgtaaata	gtgttacgca	gaaaaagttc	ttgtgtgaca	gaaccatgtg	gcgcataccg
20881	ttctcgagca	acttcatgtc	tatggggggc	cttacagact	tgggacagaa	tatgctctat
20941	gccaaactcag	ctcatgctct	ggacatgacc	tttgagggtg	atcccatgga	tgagcccacc
21001	ctgctttatc	ttctcttcga	agtttttcgac	gtgggtcagag	tgcacagcc	acaccgcggc
21061	atcatcgagg	cagtctacct	gcgtacaccg	ttctcggccg	gtaacgctac	cacgtaagaa
21121	gcttcttgct	tcttgcaaat	agcagctgca	accatggcct	gcggatccca	aaacggctcc
21181	agcgagcaag	agctcagagc	cattgtccaa	gacctggggt	gcggacccta	ttttttggga
21241	acctacgata	agcgcttccc	gggggttcac	gcccccgata	agctcgccct	tgccattgta
21301	aatacggccg	gacgtgagac	gggggggagag	cactgggttg	ctttcggttg	gaaccacagt
21361	tctaacacct	gctacctttt	tgatcctttt	ggattctcgg	atgatcgtct	caaacagatt
21421	taccagtttg	aatatgaggg	tctcctgctg	cgcagcgcct	ttgctaccaa	ggaccgctgt
21481	attacgctgg	aaaaatctac	ccagaccgtg	cagggccccc	gttctgccc	ctgcggactt
21541	ttctgctgca	tgttccttca	cgcctttgtg	cactggcctg	accgtcccat	ggacggaaac
21601	cccaccatga	aattgctaac	tggagtgcc	aacaacatgc	ttcattctcc	taaagtccag
21661	cccaccctgt	gtgacaatca	aaaagcactc	taccattttt	ttaataccca	ttcgcttat
21721	tttcgctctc	atcgtaacac	catcgaaagg	gccactgcgt	tcgaccgtat	ggatgttcaa
21781	taatgactca	tgtaaacac	gtgttcaata	aacatcactt	tattttttta	catgtatcaa
21841	ggctctggat	tacttattta	tttacaagtc	gaatgggttc	tgacgagaat	cagaatgacc
21901	cgcaggcagt	gatacgttgc	ggaactgata	cttgggttgc	cacttgaatt	cggaatcac
21961	caacttggga	accggtatat	cgggcaggat	gtcactccac	agctttcttg	tcagctgcaa
22021	agctccaagc	aggtcaggag	ccgaaatctt	gaaatcacia	ttaggaccag	tgctctgagc
22081	gcgagagttg	cggtaacacc	gattgcagca	ctgaaacacc	atcagcgacg	gatgtctcac
22141	gcttgccagc	acggtgggat	ctgcaatcat	gccacatcc	agatcttcag	cattggcaat
22201	gctgaacggg	gtcatcttgc	aggtctgcct	acccatggcg	ggcaccat	taggcttggt
22261	gttgcaatcg	cagtgcaggg	ggatcagtat	catcttggcc	tgatcctgtc	tgattcctgg
22321	atacacggct	ctcatgaaag	catcatattg	cttgaaagcc	tgctgggctt	tactaccctc
22381	ggtataaaac	atcccgagc	acctgctcga	aaactgggtta	gctgcacagc	cggcatcatt
22441	cacacagcag	cgggcgctcat	tggtggctat	ttgcaccaca	cttctgcccc	agcggttttg
22501	ggtgattttg	gttcgctcgg	gattctcctt	taaggctcgt	tgtccgttct	cgctggccac
22561	atccatctcg	ataatctgct	ccttctgaat	cataatattg	ccatgcaggc	acttcagctt
22621	gccctcataa	tcattgcagc	catgaggcca	caacgcacag	cctgtacatt	cccaattatg

FIG. 2A-6

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22681 gtgggcgatc tgagaaaaag aatgtatcat tccctgcaga aatcttccca tcatcgtgct
22741 cagtgtcttg tgactagtga aagttaactg gatgcctcgg tgctcttcgt ttacgtactg
22801 gtgacagatg cgcttgattt gttcgtgttg ctcaggcatt agttttaaag aggttctaag
22861 ttcgttatcc agcctgtact tctccatcag cagacacatc acttccatgc ctttctccca
22921 agcagacacc aggggcaagc taatcggatt cttaacagtg caggcagcag ctcctttagc
22981 cagaggggtca tcttttagcga tcttctcaat gcttcttttg ccaccttctt caacgatgcg
23041 cacgggcggg tagctgaaac ccactgctac aagttgcgcc tcttctcttt cttcttcgct
23101 gtcttgactg atgtcttgca tggggatatg tttgggtctt cttgggttct ttttgggggg
23161 tatcggagga ggaggactgt cgctccgttc cggagacagg gaggattgtg acgtttcgct
23221 caccattacc aactgactgt cggtagaaga acctgacccc acacggcgac aggtgttttt
23281 cttcggggggc agaggtggag gcgattgcga agggctgcgg tccgacctgg aaggcggatg
23341 actggcagaa ccccttccgc gttcgggggt gtgctccctg tggcggtcgc ttaactgatt
23401 tccttcgcgg ctggccattg tgttctccta ggcagagaaa caacagacat ggaaactcag
23461 ccattgctgt caacatcgcc acgagtcca tcacatctcg tctcagcga cgaggaaaag
23521 gagcagagct taagcattcc accgccagt cctgccacca cctctaccct 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 gacagtgcgt catagtcaag gatgcattat tggacagaac tgaagtgcc
23881 atcagtgtgg aagagctcag ctgctgctac gagcttaacc ttttttcacc tcgtactccc
23941 cccaaacgtc agccaaacgg cacctgcgag ccaaactctc gcttaaactt ttatccagct
24001 tttgctgtgc cagaagtact ggctacctat cacatctttt ttaaaaatca aaaaattcca
24061 gtctcctgcc gcgctaactg caccgcgcc gatgccctac tcaatctggg acctggttca
24121 cgcttacctg atatatgttc cttggaagag gttccaaaga tcttcgaggg tctgggcaat
24181 aatgagactc gggccgcaaa tgctctgcaa aaggagaaaa atggcatgga tgagcatcac
24241 agcgttctgg tggaaattgga aggcgataat gccagactcg cagtactcaa gcgaagcgtc
24301 gaggtcacac acttcgcata tcccgtgttc aacctgcccc cttaaagtcac gacggcggtc
24361 atggaccagt tactcattaa gcgcgcaagt cccctttcag aagacatgca tgaccagat
24421 gcctgtgatg agggtaaacc agtggtcagt gatgagcagc taaccgatg gctgggcacc
24481 gactctcccc gggatttggg agagcgtcgc aagcttatga tggcgtggt gctggttacc
24541 gtagaactag agtgtctccg acgtttcttt accgattcag aaaccttgcg caaactcgaa
24601 gagaatctgc actacacttt tagacacggc tttgtgcggc aggcattgcaa gatataaac
24661 gtggaactca ccaacctggg ttcctacatg ggtattctgc atgagaatcg cctaggacaa
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24961 agggttactt tgcgaaacgg attgcctgac tttatgagcc agagcatgct taacaatttt
25021 cgctctttca tcctggaacg ctccggtatc ctgcccgcga cctgctgcgc actgccctcc
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25141 ttccgtctgg ccaactatct ctctaccac tcggatgtga tgcaggatgt gagcggagac
25201 ggcttgctgg agtgccactg ccgctgcaat ctgtgcacgc cccaccggtc cctagcttgc
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25321 agccaaggcg atgggtcttc tcctgggcaa agttttaaag tgaccccggt actgtggacc
25381 tccgcctact tgcgcaagtt tgctccggaa gattaccacc cctatgaaat caagttctat
25441 gaggaccaat cacagcctcc aaaggccgaa ctttcggctt gcgtcatcac ccagggggca
25501 attctggccc aattgcaagc catccaaaaa tcccgccaag aatttctact gaaaaagggt
25561 aaggggggtc accttgaccc ccagaccggc gaggaactca acacaagggt ccctcaggat
25621 gtcccaacga cgagaaaaca agaagttgaa ggtgcagccg ccgccccagc aagatatgga
25681 ggaagattgg gacagtcagg cagaggaggc ggaggaggac agtctggagg acagtctgga
25741 ggaagacagt ttggaggagg aaaacgagga ggcagaggag gtggaagaag taaccgccga
25801 caaacagtta tctcggctg cgagacaag caacagcgct accatctccg ctccgagtgc
25861 aggaaccggc cggcgtccca gcagtagatg ggacgagacc ggacgcttcc cgaacccaac
25921 cagcgcttcc aagaccggtg agaaggatcg gcagggatac aagtctgggc gggggcataa
25981 gaatgccatc atctcctgct tgcattgagt cgggggcaac atatccttca cgcggcgcta
26041 cttgctattc caccatgggg tgaactttcc gcgcaatggt ttgcattact accgtcacct
26101 ccacagcccc tactatagcc agcaaattcc gacagtctcg acagataaag acagcggcgg
26161 cgacctccaa cagaaaacca gcagcggcag ttagaaaata cacaacaagt gcagcaacag
26221 gaggattaaa gattacagcc aacgagccag cgcaaaccgg agagttaaga aatcggatct
26281 ttccaacctt gtatgccatc ttccagcaga gtcgggggtc agagcaggaa ctgaaaataa
26341 aaaaccgatc tctgcgttcg ctcaccagaa gttgtttgta tcacaagagc gaagatcaac
26401 ttcagcgcac tctcgaggac gccgagggtc tcttcaacaa gtactgcgcg ctgactctta

FIG. 2A-7

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26461 aagagtaggc agcgaccgcg cttattcaaa aaaggcggga attacatcat cctcgacatg
26521 agtaaagaaa ttcccacgcc ttacatgtgg agttatcaac cccaaatggg attggcagca
26581 ggcgcctccc aggactactc caccgcgatg aattgggtca gcgccggggc ttctatgatt
26641 tctcgagtta atgatatacg cgcctaccga aaccaaatac ttttggaaca gtcagctctt
26701 accaccacgc cccgccaaaca ccttaatccc agaaattggc cgcgccccct 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 ttcaccccctc gtcaggctgt tctgactttg gaaagtccgt cttcgcaacc ccgctcgggc
27061 ggaatcggga ccgttcaatt tgtagaggag tttactccct ctgtctactt caacccttc
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27181 tcagtggacg gctacgattg atgtctgggt acgcggctga gctatctcgg ctgcgacatc
27241 tagaccactg ccgcgcgttt cgctgctttg cccgggaact tattgagttc atctacttcg
27301 aactccccaa ggatcacccct caaggtccgg cccacggagt gcggattact atcgaaggca
27361 aaatagactc tcgcctgcaa cgaattttct cccagcggcc cgtgctgatc gagcgagacc
27421 agggaaacac cacggtttcc atctactgca tttgtaatca ccccgattg catgaaagcc
27481 tttgctgtct tatgtgtact gagtttaata aaaactgaat taagactctc ctacggactg
27541 ccgcttcttc aaccgggatt ttacaaccag aagaacaaaa cttttcctgt cgtccaggac
27601 tctgttaact tcacctttcc tactcacaaa ctagaagctc aacgactaca ccgcttttcc
27661 agaagcattt tccctactaa tactactttc aaaaccggag gtgagctcca cggctctccct
27721 acagaaaacc cttgggtgga agcgggcctt gtagtactag gaattcttgc ggggtggcctt
27781 gtgattattc tttgctacct atacacacct tgcttcaact tcctagtggg gttgtgggat
27841 tggtttataa aatggggccc atactagtct tgcttgtttt actttcgtct ttggaaccgg
27901 gttctgccaa ttacgatcca tgtctagact ttgaccaga aaactgcaca cttacttttg
27961 caccgcacac aagccgcac tgtggagttc ttattaagtg cggatgggaa tgcaggctccg
28021 ttgaaattac acacaataac aaaacctgga acaatacctt atccaccaca tgggagccag
28081 gagttcccga gtggtacact gtctctgtcc gaggtcctga cggttccatc cgcattagta
28141 acaacacttt cattttttct gaaatgtgcg atctggccat gttcatgagc aaacagtatt
28201 ctctatggcc tcctagcaag gacaacatcg taacgttctc cattgcttat tgcttgtgcg
28261 cttgccttct tactgcttta ctgtgcgtat gcatacacct gcttgtaacc actcgcatca
28321 aaaacgcaa taacaaagaa aaaatgcctt aacctctttc tgtttacaga catggcttct
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28681 ttgaaaacca cgaaaatgcc aaatatggca aagattcgat ccgatgacaa ttctctagaa
28741 acttttacat ctcccaccac acccgacgaa aaaaacatcc cagattcaat gattgcaatt
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29161 caaatgacaa aggcttctat tatggaaccg actataaaag tagtttagat tataacatta
29221 ttgtactgcc atctaccact ccagcacccc gcacaactac tttctctagc agcagtgtcg
29281 ctaacaatac aatttccaat ccaacctttg ccgcgctttt aaaacgcact gtgaataatt
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29461 aacataaagg tgatccatta cttagatttg atatttaatt ttttatttac tcatctgtgc
29521 agtatgggtg acaccaatca tggtagctag aaatttcttc ttcaccatac gtataggagc
29581 ttttaattgt tgcgctactt tcacagcagt agccacagca accccagact gcatagctcg
29641 atttgcttcc tatgcacttt ttgcttttgt tacttgcatc tgcgtatgta cctacctgcg
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29761 ccaccatccc gaataccgca accaaaatat cgcggcactt tattgcttcc ctacgctgtc tcaaccacag
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29941 ttcttgcttg ctatcgagaa aatcagaaa tctttttgat ataccctcta tttgattttg
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30061 gctggaatgc tcccaatgca catgatcatc cacaagacct agaggaacac attccccac
30121 aaaacatgca acatccaata gcgctaatag attacgaaag tgaaccacaa cccccactac
30181 tccctgctat tagttacttc aacctaaccg gcggagatga ctgaaacact caccacctcc

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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 accatcgctt ctcttacgaa cttggccccc aacgacaaaa atttacctgc
30481 atggtgggaa tcaaccccat agttatcacc caacaaagtg gagatactaa ggggtgcatt
30541 cactgctcct gcgattccat cgagtgcacc tacaccctgc tgaagaccct atgcggccta
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30721 tctggtattc taaaccccg tccagcggcat actttctcca tactttaag gggatgtcaa
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30961 gttcttactt taaaatgttt aacccacta acaaccacag gcggatctct acagctaaaa
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31621 agaagtctat ttcccttgaa catttctata atgctaaaca gccgtatgat ttcttccaat
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31921 cattagagat agacattgtt ttagattcca cattccaaac agtttcagag cgagccaatc
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33541 atccaatcca cacattacaa acaggtcccg gagggcgccc tccaccacca ttcttaacaa
33601 caccctcata atgacaaaat atcttgctcc tgtgtcacct gtagcgaatt gagaatggca
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33781 acagtgcagt acaagcgcag acctcccaa ttggctccag caaaaacaag attggaataa
33841 gcataattgg aaccaccagt aatatcatcg aagttgctgg aaatataatc aggcagagtt
33901 tctttagtaa attgaataaa agaaaaatth gccaaaaaaa cattcaaac ctctgggatg
33961 caaatgcaat aggttaccgc gctgcgctcc aacattgtta gttttgaatt agtctgcaaa

```

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 tagccttttg
34261 gtataattat gcttaatcgt aagtatagca aagccacccc tcgcggatac aaagtaaaag
34321 gcacaggaga ataaaaaata taattatttc tctgctgctg tttaggcaac gtcgcccccg
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 tttcacttcc gcaatcccaa caagcgtcac
34621 ttcctctttc tcacggtacg tcacatccca ttaacttaca acgtcathtt cccacggccg
34681 cgccgcccct tttaaccgtt aaccccacag ccaatcacca cacggcccac actttttaaa
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SEQ ID NO: 1
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FIG. 2A-10

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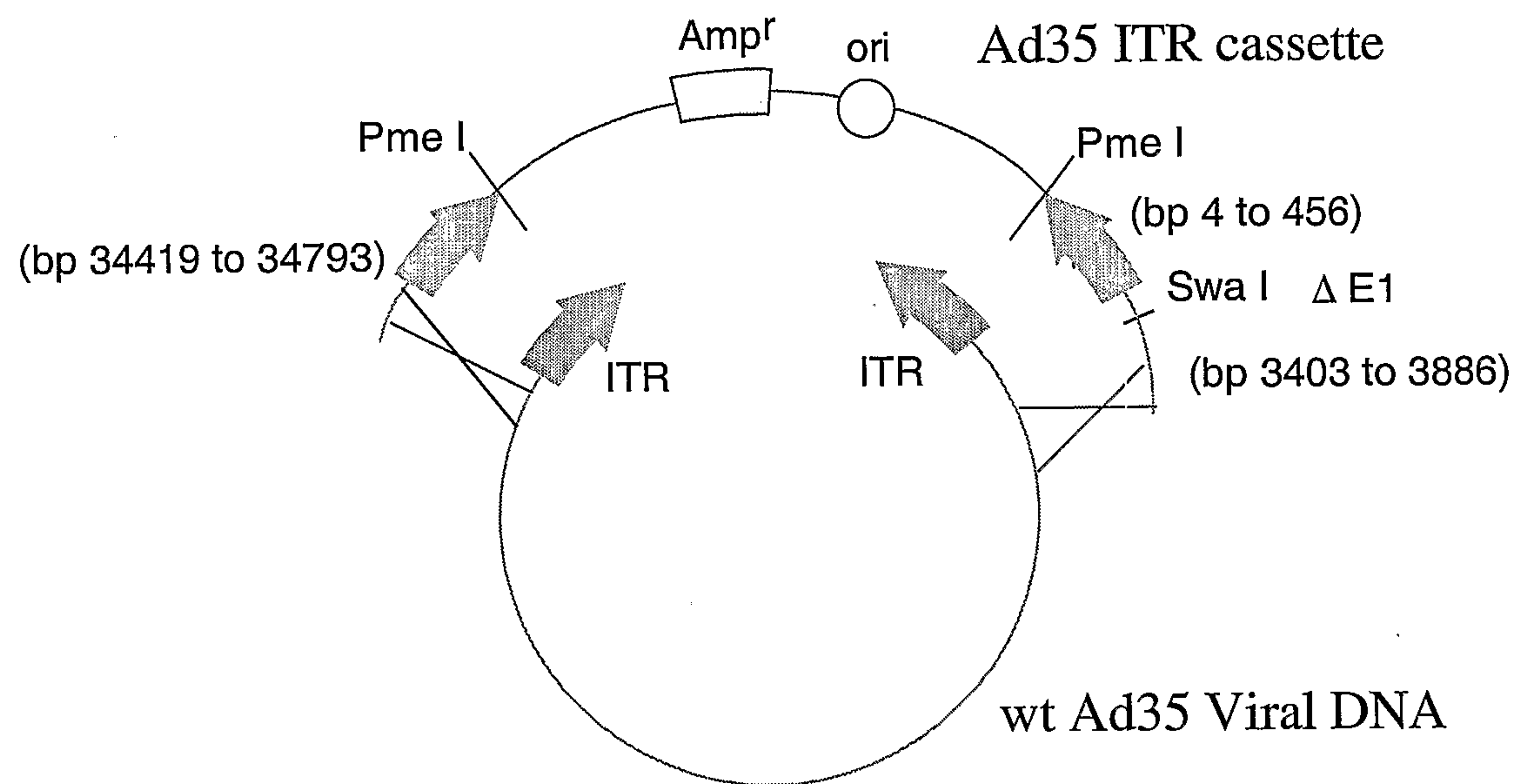


FIG. 3

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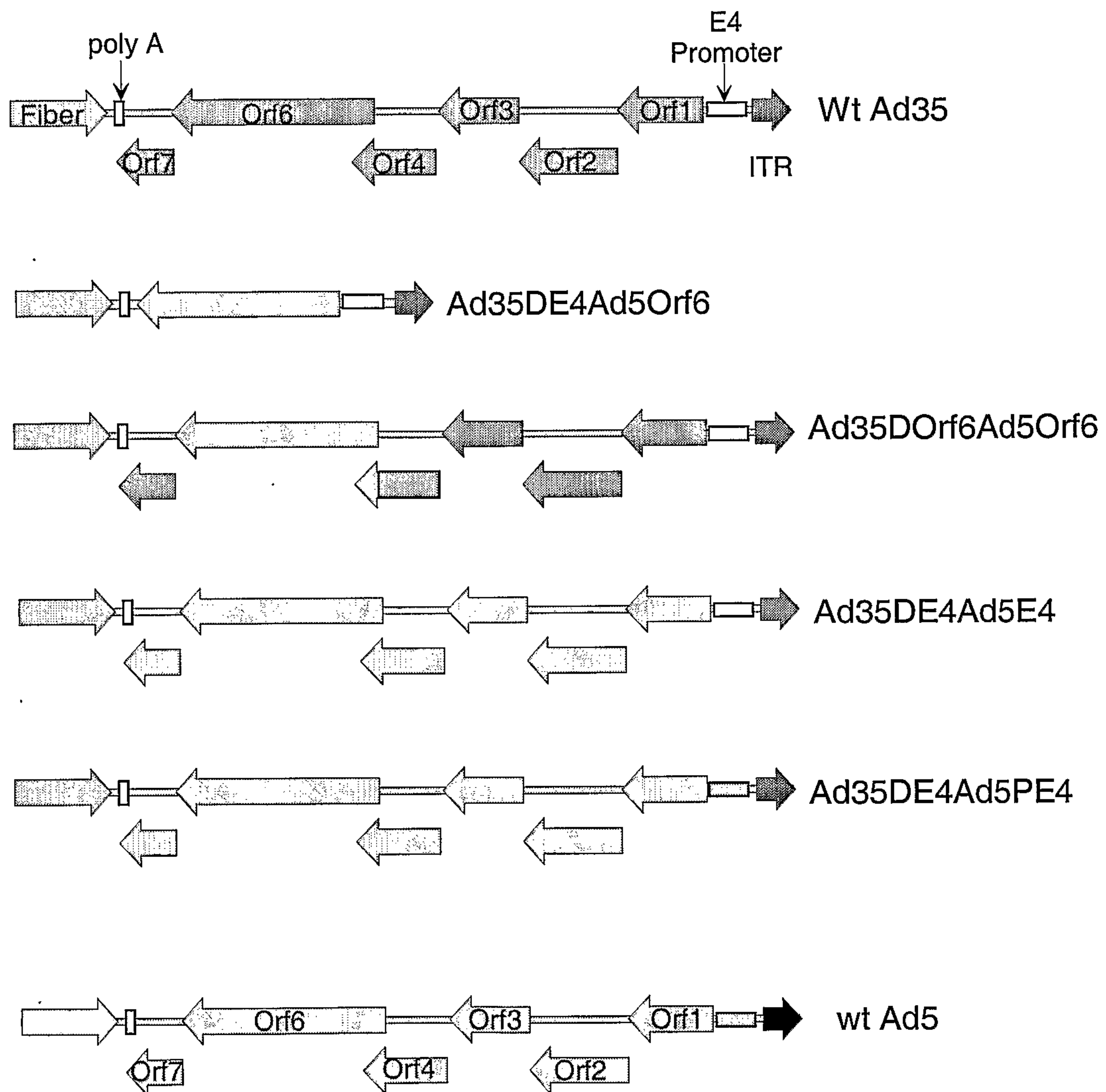


FIG. 4

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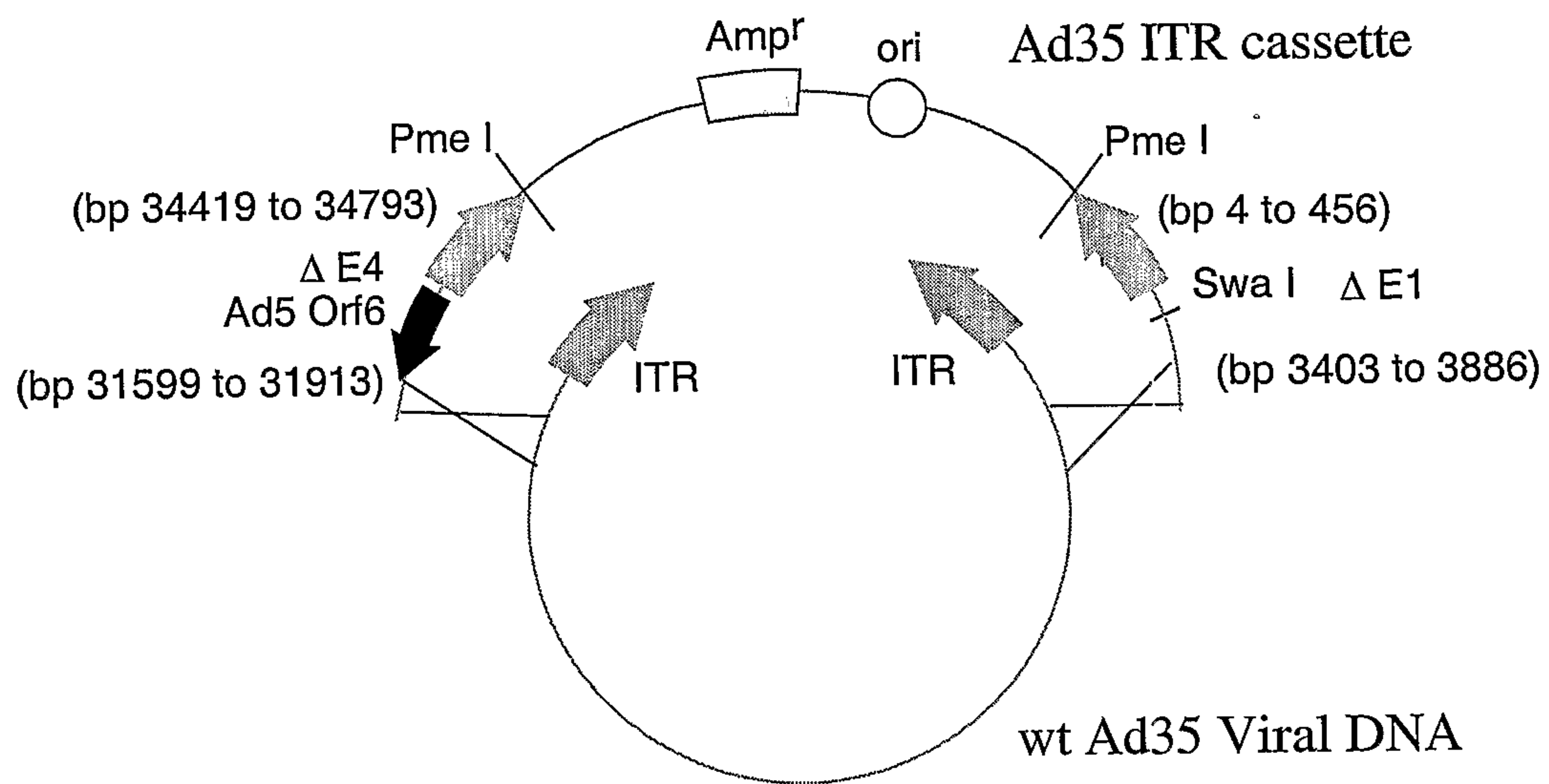


FIG. 5

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1 ccattgcata cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca
61 ttaccgccat gttgacattg attattgact agttattaat agtaatcaat tacgggggtca
121 ttagttcata gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgct
181 ggctgaccgc ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta
241 acgccaatag ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac
301 ttggcagtac atcaagtgt tcatatgcca agtacgcccc ctattgacgt caatgacggt
361 aaatggccc cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag
421 tacatctacg tattagtcac cgctattacc atgggtgatgc ggttttggca gtacatcaat
481 gggcgtggat agcgggttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat
541 gggagtttgt tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc
601 ccattgacgc aaatgggcgg taggcgtgt cgggtggagg tctatataag cagagctcgt
661 ttagtgaacc gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga
721 caccgggacc gatccagcct ccgcgcccg gaacgggtgca ttggaacgcg gattccccgt
781 gccaaagagt 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 CTCCCTGAG GTGATCCCCA TGTCTCTGCTG
1321 CCTGTCTGAG GGTGCCACCC CCCAGGACCT GAACACCATG CTGAACACAG TGGGGGGCCA
1381 TCAGGCTGCC ATGCAGATGC TGAAGGAGAC CATCAATGAG GAGGCTGCTG AGTGGGACAG
1441 GCTGCATCCT GTGCACGCTG GCCCCATTGC CCCCAGCCAG ATGAGGGAGC CCAGGGGCTC
1501 TGACATTGCT GGCACCACCT CCACCCTCCA GGAGCAGATT GGCTGGATGA CCAACAACCC
1561 CCCCATCCCT GTGGGGGAAA TCTACAAGAG GTGGATCATC CTGGGCCTGA ACAAGATTGT
1621 GAGGATGTAC TCCCCACCT CCATCCTGGA CATCAGGCAG GGCCCCAAGG AGCCCTTCAG
1681 GGAATATGTG GACAGGTTCT ACAAGACCCT GAGGGCTGAG CAGGCCTCCC AGGAGGTGAA
1741 GAACTGGATG ACAGAGACCC TGCTGGTGCA GAATGCCAAC CCTGACTGCA AGACCATCCT
1801 GAAGGCCCTG GGCCCTGCTG CCACCCTGGA GGAGATGATG ACAGCCTGCC AGGGGGTGGG
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1921 CACCATCATG ATGCAGAGGG GCAACTTCAG GAACCAGAGG AAGACAGTGA AGTGCTTCAA
1981 CTGTGGCAAG GTGGGCCACA TTGCCAAGAA CTGTAGGGCC CCCAGGAAGA AGGGCTGCTG
2041 GAAGTGTGGC AAGGAGGGCC ACCAGATGAA GGAATGCAAT GAGAGGCAGG CCAACTTCCT
2101 GGGCAAAATC TGGCCCTCCC ACAAGGGCAG GCCTGGCAAC TTCCTCCAGT CCAGGCCTGA
2161 GCCCAGAGCC CCTCCCGAGG AGTCCTTCAG GTTTGGGGAG GAGAAGACCA CCCCAGCCA
2221 GAAGCAGGAG CCCATTGACA AGGAGCTGTA CCCCCTGGCC TCCCTGAGGT CCCTGTTTGG
2281 CAACGACCCC TCCTCCAGT AAaataaagc ccgggcagat ctgatctgct gtgccttcta
2341 gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg gaagggtgcca
2401 ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg agtaggtgtc
2461 attctattct ggggggtggg gtgggggcagc acagcaaggg ggaggattgg gaagacaata
2521 gcaggcatgc tggggatgcg gtgggctcta

SEQ ID NO: 2

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 tggcccgctt
181 ggctgaccgc ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta
241 acgccaatag ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac
301 ttggcagtac atcaagtgtg tcatatgcc agtacgcccc ctattgacgt caatgacggt
361 aaatggcccg cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag
421 tacatctacg tattagtcac cgctattacc atgggtgatgc ggttttggca gtacatcaat
481 gggcgtggat agcgggttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat
541 gggagtttgt tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc
601 ccattgacgc aaatgggagg taggcgtgta cgggtgggagg tctatataag cagagctcgt
661 ttagtgaacc gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga
721 caccgggacc gatccagcct ccgcgcccgga gaacggtgca ttggaacgcg gattccccgt
781 gccaagagtg agatcgatct aagtaagctt CCTGCATGCT GCTGCTGCTG CTGCTGCTGG
841 GCCTGAGGCT ACAGCTCTCC CTGGGCATCA TCCCAGTTGA GGAGGAGAAC CCGGACTTCT
901 GGAACCGCGA GGCAGCCGAG GCCCTGGGTG CCGCCAAGAA GCTGCAGCCT GCACAGACAG
961 CCGCCAAGAA CCTCATCATC TTCCTGGGCG ATGGGATGGG GGTGTCTACG GTGACAGCTG
1021 CCAGGATCCT AAAAGGGCAG AAGAAGGACA AACTGGGGCC TGAGATACCC CTGGCCATGG
1081 ACCGCTTCCC ATATGTGGCT CTGTCCAAGA CATACAATGT AGACAAACAT GTGCCAGACA
1141 GTGGAGCCAC AGCCACGGCC TACCTGTGCG GGGTCAAGGG CAACTTCCAG ACCATTGGCT
1201 TGAGTGCAGC CGCCCGCTTT AACCAGTGCA ACACGACACG CGGCAACGAG GTCATCTCCG
1261 TGATGAATCG GGCCAAGAAA GCAGGGAAGT CAGTGGGAGT GGTAACCACC ACACGAGTGC
1321 AGCACGCCTC GCCAGCCGGC ACCTACGCCC ACACGGTGAA CCGCAACTGG TACTCGGACG
1381 CCGACGTGCC TGCCTCCGCC CGCCAGGAGG GGTGCCAGGA CATCGCTACG CAGCTCATCT
1441 CCAACATGGA CATTGACGTG ATCCTAGGTG GAGGCCGAAA GTACATGTTT CGCATGGGAA
1501 CCCCAGACCC TGAGTACCCA GATGACTACA GCCAAGGTGG GACCAGGCTG GACGGGAAGA
1561 ATCTGGTGCA GGAATGGCTG GCGAAGCGCC AGGGTGCCCG GTATGTGTGG AACCGCCTG
1621 AGCTCATGCA GGCTTCCCTG GACCCGTCTG TGACCCATCT CATGGGTCTC TTTGAGCCTG
1681 GAGACATGAA ATACGAGATC CACCGAGACT CCACACTGGA CCCCTCCCTG ATGGAGATGA
1741 CAGAGGCTGC CCTGCGCCTG CTGAGCAGGA ACCCCCGCGG CTTCTTCTC TTCGTGGAGG
1801 GTGGTCGCAT CGACCATGGT CATCATGAAA GCAGGGCTTA CCGGGCACTG ACTGAGACGA
1861 TCATGTTCGA CGACGCCATT GAGAGGGCGG GCCAGCTCAC CAGCGAGGAG GACACGCTGA
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1981 GCTCCATCTT CGGGCTGGCC CCTGGCAAGG CCCGGGACAG GAAGGCCTAC ACGGTCCTCC
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2101 GCGAGAGCGG GAGCCCCGAG TATCGGCAGC AGTCAGCAGT GCCCTGGAC GAAGAGACCC
2161 ACGCAGGCGA GGACGTGGCG GTGTTTCGCG GCGGCCCGCA GGCGCACCTG GTTACGGCG
2221 TGCAGGAGCA GACCTTCATA GCGCACGTCA TGGCCTTCGC CGCCTGCCTG GAGCCCTACA
2281 CCGCCTGCGA CCTGGCGCCC CCCGCCGGCA CCACCGACGC CGCGCACCCG GGTAAccccg
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2461 ctcccccggtg ccttccttga ccctggaagg tgccactccc actgtccttt cctaataaaa
2521 tgaggaaatt gcatcgcatt gtctgagtag gtgtcattct attctggggg gtgggggtggg
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2641 ctcta
SEQ ID NO: 3

FIG. 7

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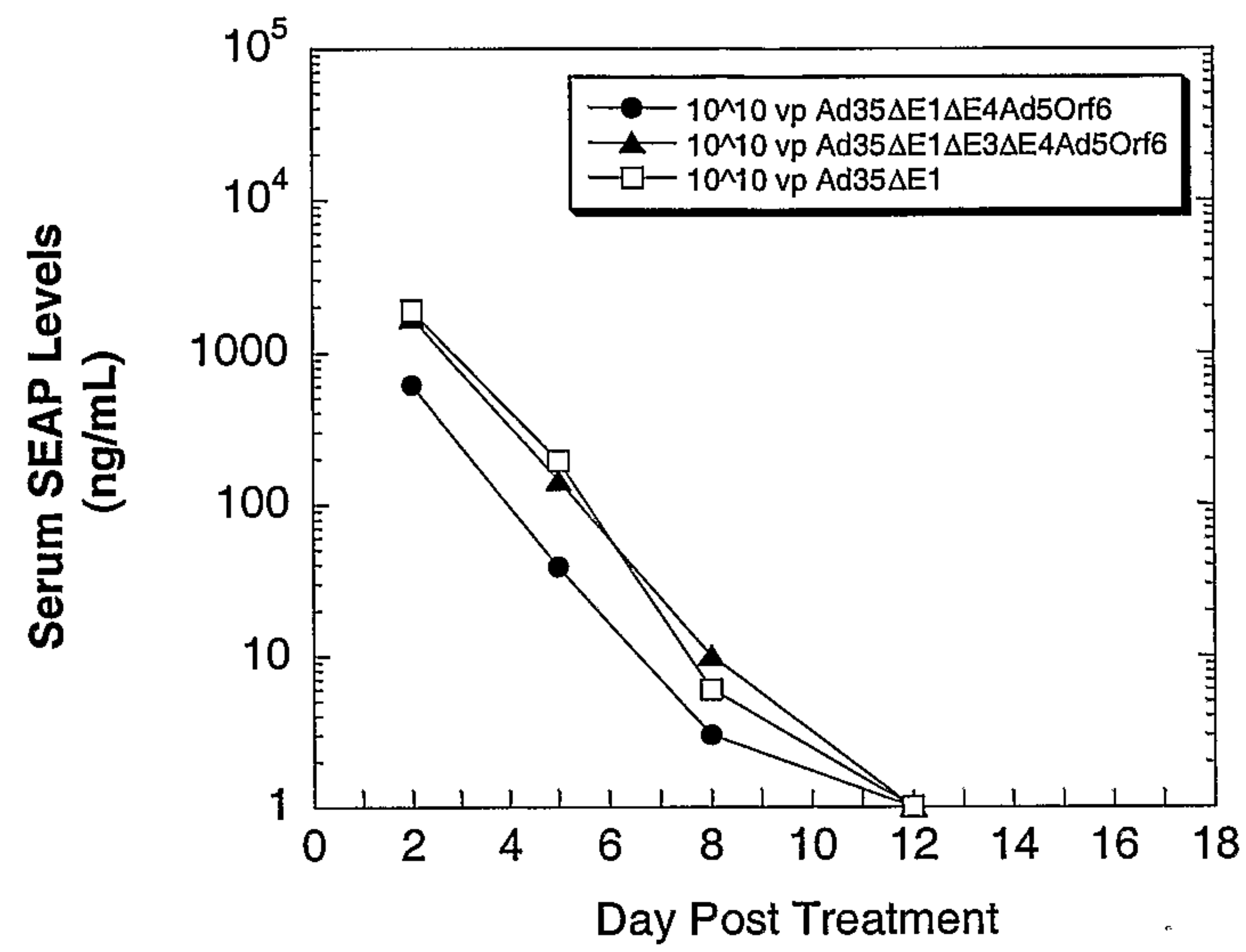


FIG. 8

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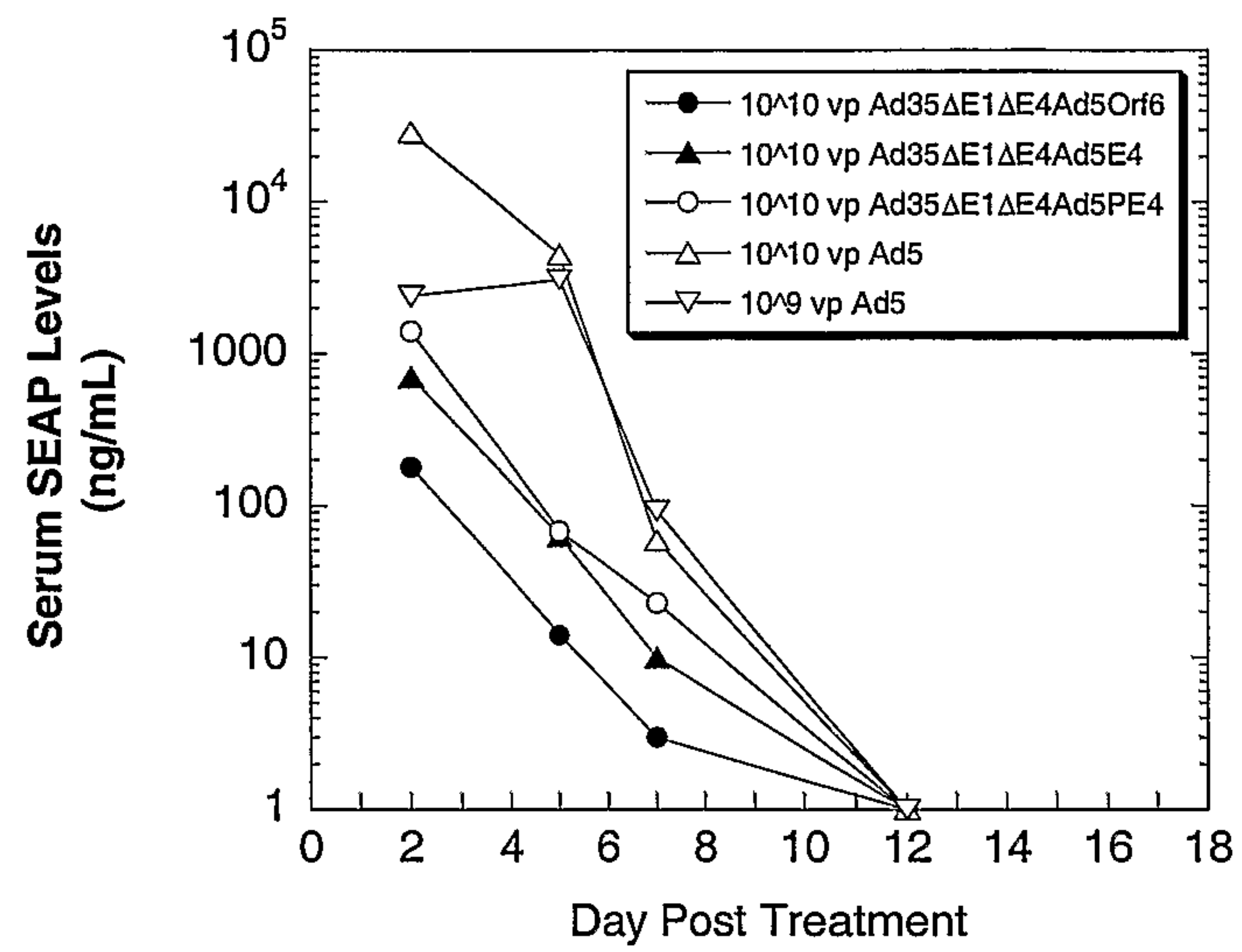


FIG. 9

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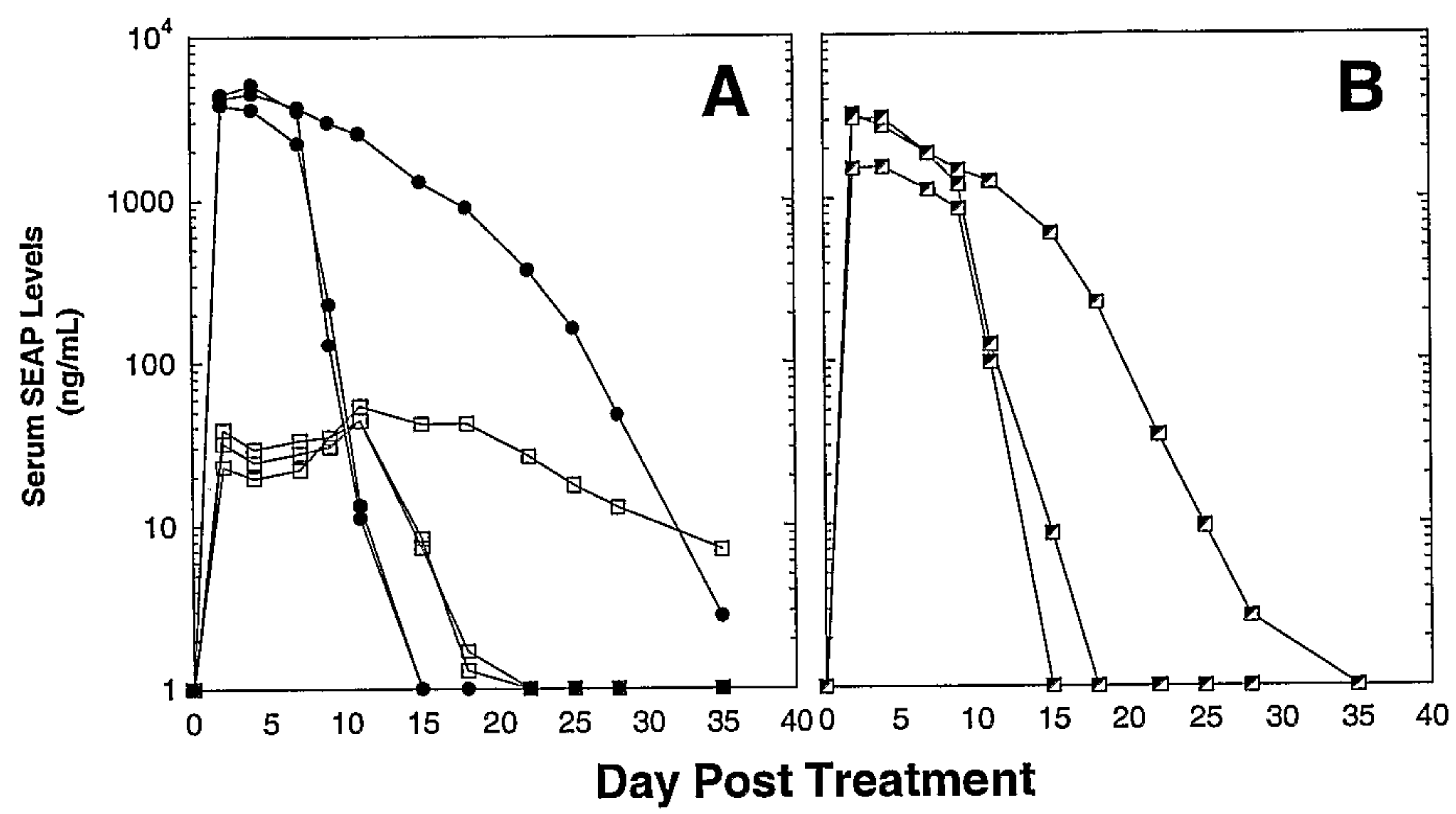


FIG. 10A-B

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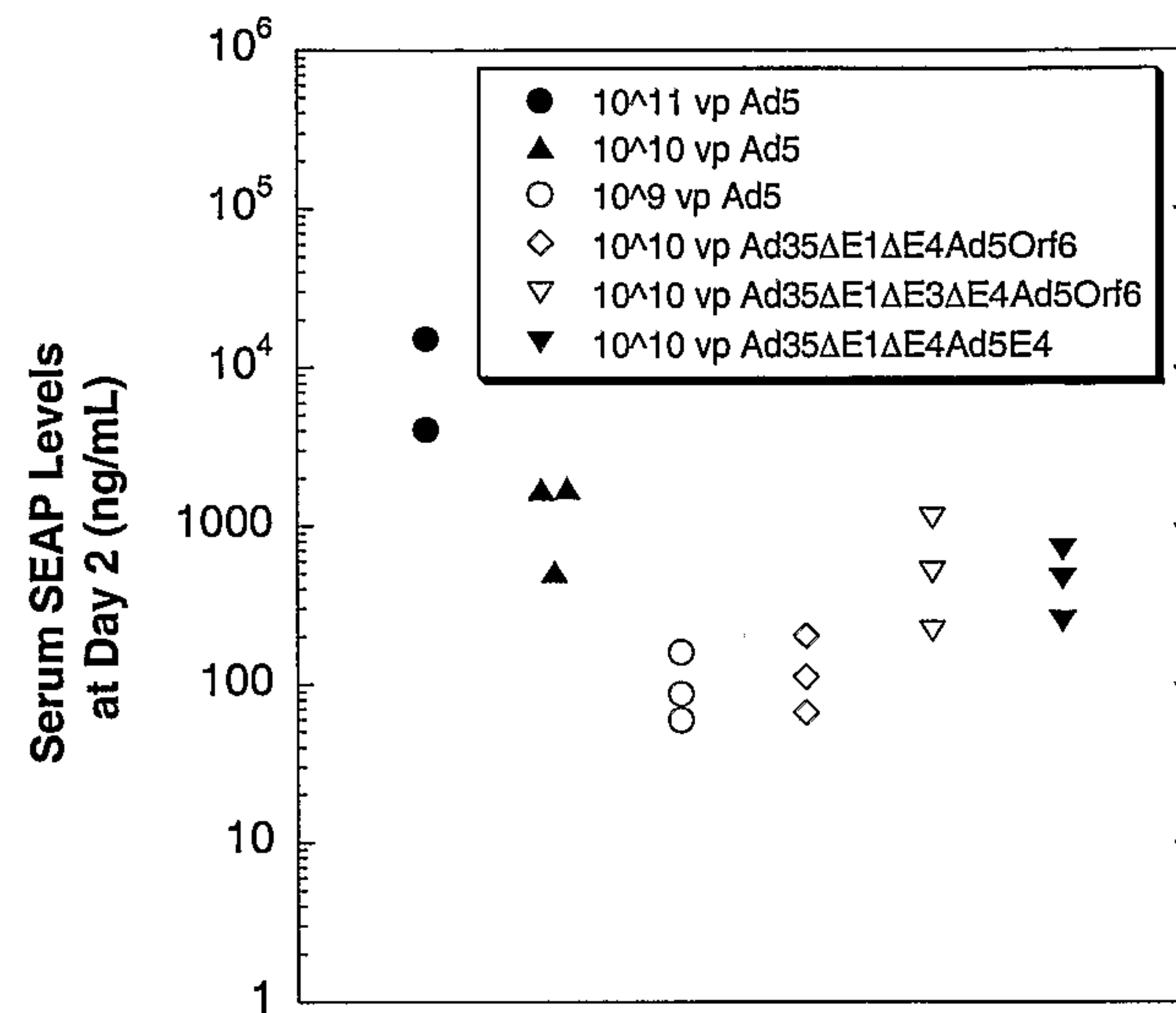


FIG. 11

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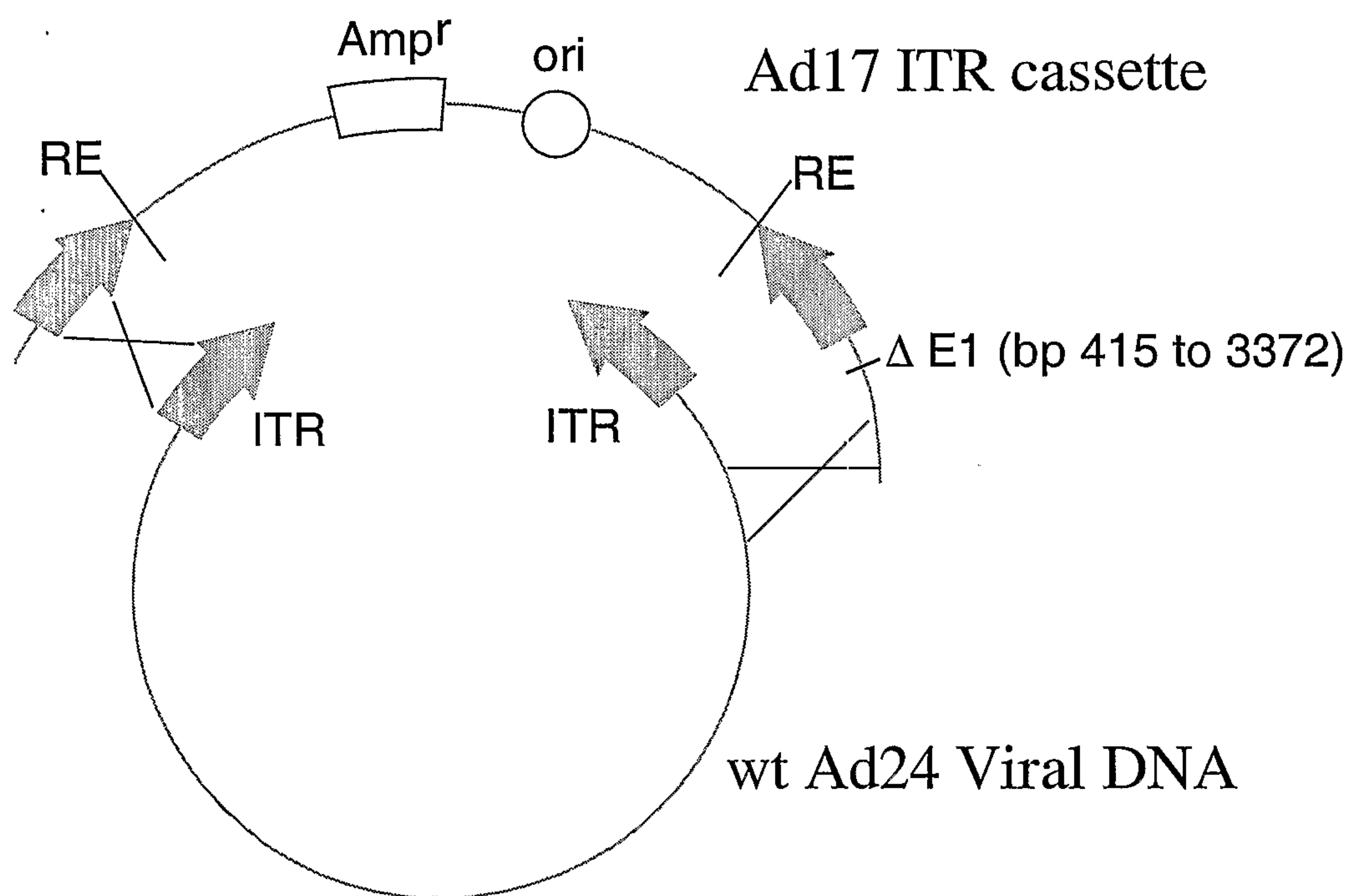


FIG. 12

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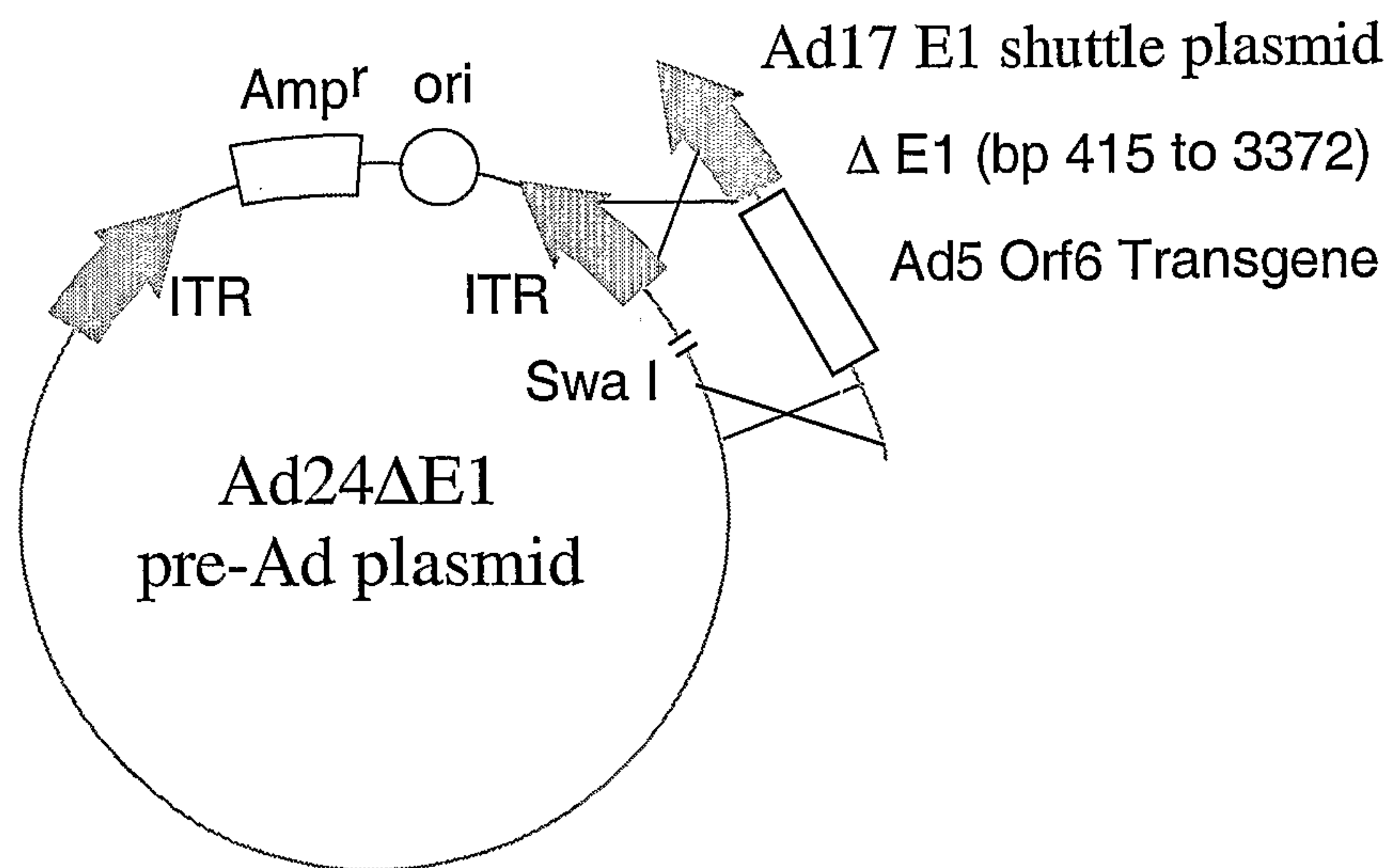


FIG. 13

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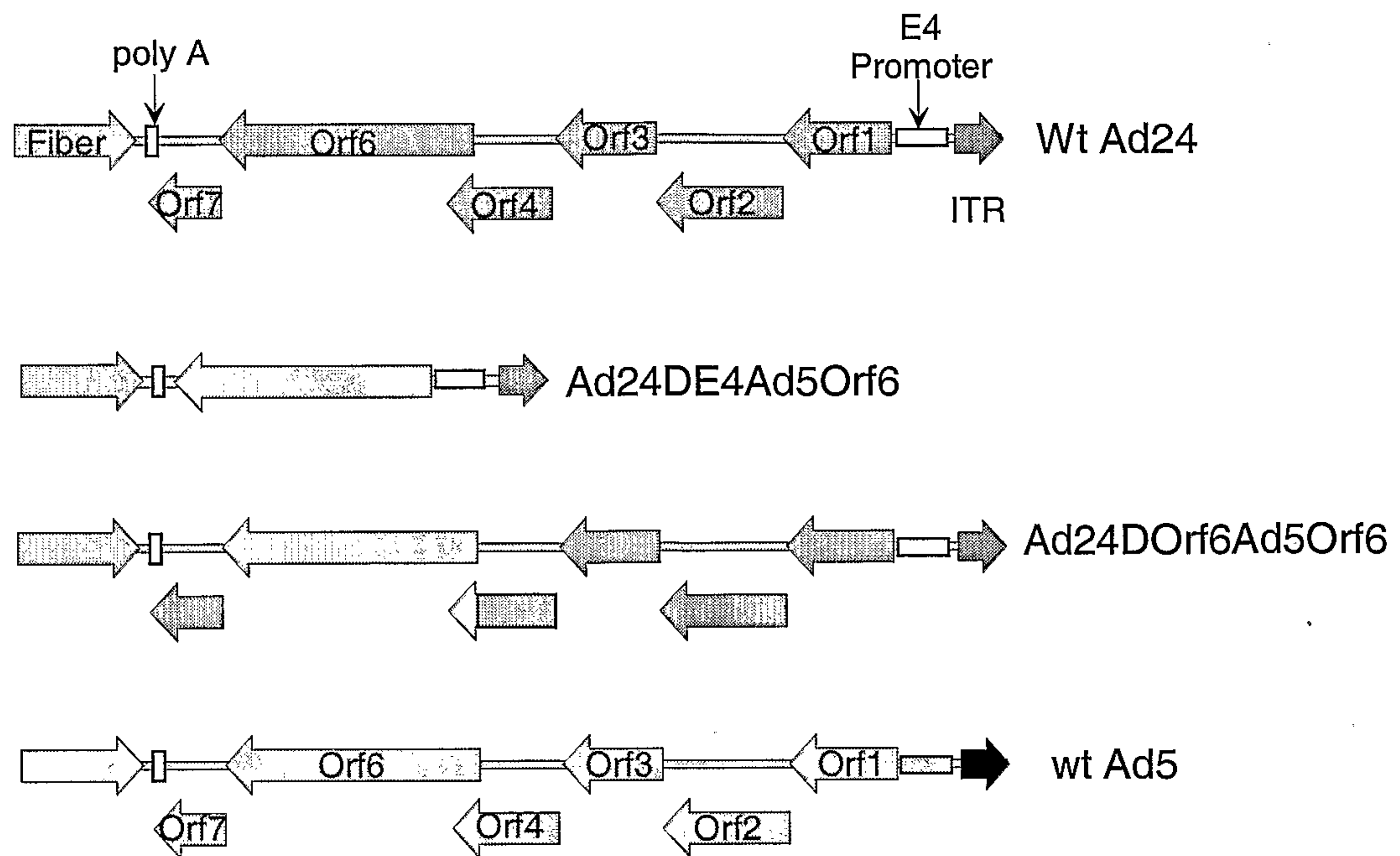


FIG. 14

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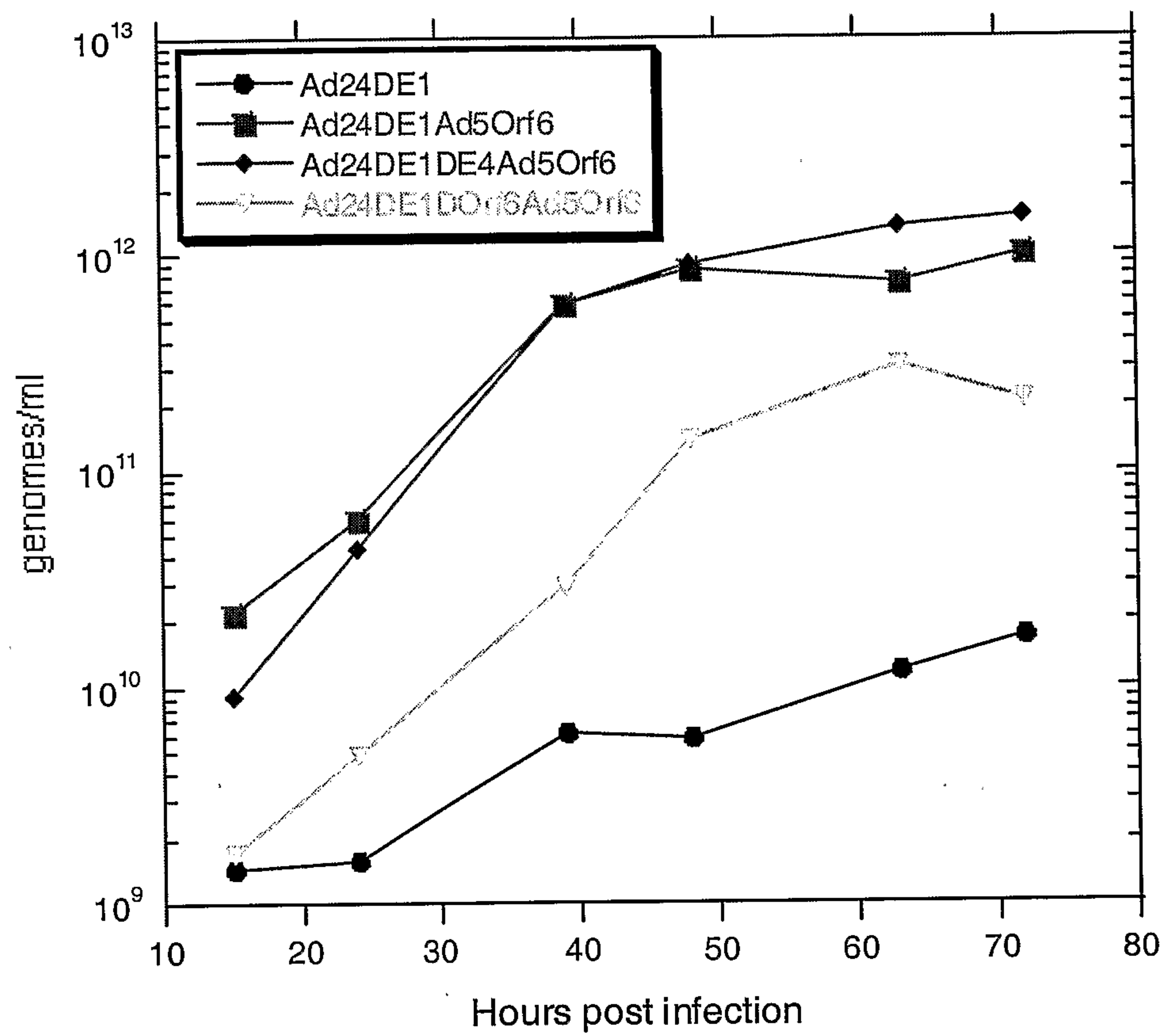
Growth Curve Comparison of
Ad24 Based Vectors

FIG. 15

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```

1 catcatcaat aatatacccc acaaagtaaa caaaagttaa catgcaaattg agctttttgaa
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121 acggctaacg gtcgcccgcg aggcggtggcc tagccccgaa gcaagtcgcg gggctgatga
181 cgtataaaaa agcggacttt agaccggaa acggccgatt tccccgcggc cacgcccgga
241 tatgaggtaa ttctgggcgg atgcaagtaa aattagggtca ttttggcgcg aaaactgaat
301 gaggaagtga aaagtgaaaa ataccgggtcc cgcccagggc ggaatatatta ccgaggggccc
361 agagactttg accgattacg tgggggtttc gattgcggtg ttttttcgcg aatttccgcg
421 tccgtgtcaa agtccgggtg ttatgtcaca gatcagctga tccacagggt atttaaacca
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1741 gctctggcct gctagattct ctaaactctc gccaccagtc ccttttccag gaaagggtag
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2161 atccatggcc aggggagtg agagggagag gagcgatggg ggcaataacc ggatgatgac
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2581 gaacatgaag ttcaatggag agaagtttaa tggggtgatg ttcattggcc acagtcacat
2641 gaccctgcac ggctgcagtt tcttcggcct caacaatatg tgcgagagg tctggggcgc
2701 tgctaagatc aggggatgta agttttatgg ctgctggatg ggcgtggtcg gaagacccaa
2761 gagcgagatg tctgtgaagc agtgtgtgtt tgagaaatgc tacctgggag tctctaccga
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FIG. 16A-1

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3661 atgacagcga cgagactggc ttccagctac atgcccagca gcagcagtag cccctctgtg
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5761 caagaagggt attggcttgt aggtgtaggc cacgtgacct ggggtccccg acgggggggt
5821 ataaaagggg gcgggtctgt gctcgtctc actctcttcc gcgtcgtgt ccacgagcgc
5881 cagctgttgg ggtaggtatt ccctttcgag agcgggcatg acctcggcac tcaggttgtc
5941 agtttctaga aacgaggagg atttgatgtt ggcttgccct gccgcaatgc ttttaggag
6001 actttcatcc atctggtcag aaaagactat ttttttattg tcaagcttgg tggcgaagga
6061 gccatagagg gcgttggaga gaagcttggc gatggatctc atggtctgat tttgtcacg
6121 gtcggctcgc tccttggccg cgatgttgag ctggacatac tcgcgcgcga cgcacttcca
6181 ttcggggaag acggtggtgc gctcgtcggg cacgacctg acgcgccagc cgcggttatg
6241 cagggtgacc agatccacgc tgggtggccac ctgcgccgcg aggggctcgt tgggtccagca
6301 gaggcgtccg cccttgcgcg agcagaacgg gggcagcaca tcaagcagat gctcgtcagg
6361 ggggtccgca tcgatggtag agatgcccg acagagttcc ttgtcaaaat aatcgatttt
6421 tgaggatgca tcatccaagg ccatctgcc a ctcgcgggcg gccagcgtc gctcgtaggg
6481 gttgaggggc ggaccccagg gcatgggatg cgtcagggcg gaggcgtaca tgccgcagat
6541 gtcgtagaca tagatgggct ccgagaggat gccgatgtag gtgggataac agcggcccc
6601 gcggatgctg gcgcgcacgt agtcatacaa ctctgtcgag ggggccaaga aggcggggcc
6661 gagattggtg cgctggggct gctcggcgcg gaagacgatc tggcgaaaga tggcatgcga
6721 gttggaggag atggtgggccc gttggaagat gttaaagtgg gcatgaggca gacgaaccga
6781 gtcgcggatg aagtgcgcgt aggagtcttg cagcttggcg acgagctcgg cggtagcag
6841 gacgtccatg gcgcagtagt ccagcgtttc gcggatgatg tcataaccg cctctcctt
6901 cttctcccat agctcgcggg tgagggcgta ctccctcgtc tccttccagt actcccggag
6961 cgggaatcct cgatcgtccg cacggtaaga gccagcatg tagaaatggg tcacggcctt
7021 gtagggacag cagccccttct ccacggggag ggcgtaagct tgagcggcct tgccggagcga
7081 ggtgtgcgtc agggcgaagg tatccctgac catgactttc aagaactggg acttgaaatc
7141 cgagtcgtcg cagccgccgt gctcccagag ctcgaaatcg gtgcgcttct tcgagagggg
7201 gttaggcaga gcgaaagtga cgtcattgaa gagaatcttg cctgcccgcg gcatgaaat
7261 gcgggtgatg cggaaagggc ccgggacgga ggctcgggtt ttgatgacct gggcggcgag

FIG. 16A-2

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7321 gacgatctcg tcgaagccgt tgatgttgtg cccgacgatg tagagttcca tgaatcgcg
7381 gcggccttta atgtgcggca gctttttgag ctctctgtag gtgaggtcct cggggcaatg
7441 cagtccgtgc tgctcgagcg cccactcctg gagatgtggg ttggcttgca tgaatgaagc
7501 ccagagctcg cgggccataa gggctctggag ctctctcgca aagaggcgga actgctggcc
7561 cacggccatc ttttctgggg tgacgcagta gaaagtaagg gggctccgct cccagcgatc
7621 ccagcgtaag cgcacggcta gatcgcgagc gagggcgacc agctctgggt ccccgagaa
7681 tttcataacc agcataaagg ggacgagctg cttgccgaag gaccccatcc aggtgtaggt
7741 ttctacatcg taggtgacaa agagccgctc cgtgcgagga tgagagccga ttgggaagaa
7801 ctggatttcc tgccaccagt tggacgagtg gctgttgatg tgatgaaagt agaaatccc
7861 ccggcgaacc gagcactcgt gctgatgctt gtaaaagcgt ccgcagtact cgcagcgctg
7921 cacgggctgt acctcatcca cgagatacac agcgcgtccc ttgaggagga acttcaggag
7981 tggcggccct ggctgggtgg tttcatgttc gcctgcgtgg gactcaccct ggggctcctc
8041 gaggacggag aggtgacga gccgcgcgg gagccaggtc cagatctcgg cgcggcgggg
8101 gcggagagcg aagacgaggg cgcgcgagtg ggagctgtcc atggtgtcgc ggagatccag
8161 gtccgggggc aggttctga ggttgacctc gtagaggcgg gtgaggcggt gcttgagatg
8221 cagatggtac ttgatctcca cgggtgagtt ggtggctgtg tccacgcatt gcatgagccc
8281 gtagctgcgc ggggccacga ccgtgccgcg gtgcgctttt agaagcgggt tgcgggacgc
8341 gctcccggcg gcagcggcgg ttccggcccc gcgggcaggg gcggcagagg cacgtcggcg
8401 tggcgctcgg gcaggtcccg gtgctgcgcc ctgagagcgc tggcgtgcgc gacgacgcgg
8461 cggttgacat cctggatctg ccgcctctgc gtgaagacca ccggccccgt gactttgaac
8521 ctgaaagaca gttcaacaga atcaatctcg gcgtcattga cggcggcctg acgcaggatc
8581 tcttgacagt cgcgcgagtt gtcttggtag gcgatctcgg acatgaactg ctcgatctcc
8641 tctcctgga gatcgccgcg gccgcgcgcg tccacgggtg cggcgaggtc attggagatg
8701 cgacccatga gctgcgagaa ggcgcccagg ccgctctcat tccagacgcg gctgtagacc
8761 acgtcccctg cggcgctcgc cgcgcgcatg accacctgcg cgaggttgag ctccacgtgc
8821 cgcgtgaaga cggcgtagtt gcgcaggcgc tggagaggt agtttagggg ggtggcgatg
8881 tgctcggtga cgaagaagta catgatccag cggcgcgagg gcacctcgct gatgtcgccg
8941 atggcctcca gcctttccat ggctcgtag aaatccacag cgaagttaga aaactgggcg
9001 ttgcggggcg agaccgtgag ctctgctccc aggagcctga tgagttcggc gatggtggcg
9061 cgcacctcgc gctcgaaatc cccggggggc tctcctctct cctcttcttc catgacgacc
9121 tcttcttcta tttcttctc tgggggcggt ggtggtggcg gggcccgcgc acgacggcga
9181 cgcaccggga gacggtcgac gaagcgctcg atcatctccc cgcggcgggc acgcatggtt
9241 tcggtgacgg cgcgaccccc ttgcgcgagga cgcagcgtag agacgccgcc ggtcatctcc
9301 cggtaatggg gcgggtcccc gttgggcagc gagagggcgc tgacgatgca tcttatcaat
9361 tgcggtgtag gggacgtgag cgcgctcgaga tcgaccggat cggagaatct ttcgaggaaa
9421 gcgtctagcc aatcgagtc gcaaggtaag ctcaaacacg tagcagccct gtggacgctg
9481 ttagaattgc ggttgctgat gatgtaattg aagtaggcgt ttttaaggcg gcggatggtg
9541 gcgaggagga ccaggtcctt gggctccgct tgctggatgc gaagccgctc ggccatgccc
9601 caggcctggc cctgacaccg gctcagggtc ttgtagtagt catgcatgag cctctcaatg
9661 tcatcactgg cggaggcgga gtcttccatg cgggtgaccc cgacgcccct gagcggctgc
9721 acgagcgcca ggctcggcgac gacgcgctcg gcgaggatgg cctgttgacac gcgggtgagg
9781 gtgtcctgga agtcgtccat gtcgacgaag cgggtggtagg ccccggtgtt gatggtgtag
9841 gtgcagttgg ccatgagcga ccagttgacg gtctgcaggc cgggttgacac gacctctgag
9901 tacctgagcc gcgagaaggc gcgcgagtcg aagacatagt cgttgaggtt gcgcacgagg
9961 tactggtatc caactaggaa gtgcggcggc ggctggcggt agagcggccca gcgctgggtg
10021 gccggcgcg gcggggccag gtcctcgagc atgaggcggt ggtagccgta gaggtagcgg
10081 gacatccagg tgatgccggc ggcggtggtg gaggcgcgcg ggaactcgcg gacgcggttc
10141 cagatgttgc gcagcggcag gaaatagtcc atggtcggca cggctctggc ggtgagacgc
10201 gcgcagtcac tgacgctcta gaggcaaaaa cgaaagcggg tgagcgggct cttcctccgt
10261 agcctggcgg aacgcaaacg ggttaggccg cgtgtgtacc ccggttcgag tcccctcgaa
10321 tcaggctgga gccgcgacta acgtggtatt ggcactcccc tctcgacccc agcccgatag
10381 ccgccaggat acggcgagga gccctttttg ccgaccgagg ggagtcgcta gacttgaaag
10441 cggccgaaaa ccccgccggg tagtggtctg cgcctcgtag ctggagaagc tttgccaggg
10501 ttgagtcgcg gcagaacccg gttcgcgagc ggccgcggcg agcgggactt ggtcaccccc
10561 ccgattttaa gaccacagc cagccgactt ctccagttac gggagcgagc cccctttttt
10621 ctttttgcca gatgcatccc gtcctgcgcc aatgcgtcc cacccccct cccggcgacca
10681 ccgcgaccgc ggccgtagca ggccgcggcg ctgtagcccc gccacagcag acagagatgg
10741 acttggaaga gggcgaaggc ctggcgagac tggggggcgcc gtccccggag cgacaccccc
10801 gcgtgcagct gcagaaggac gtgcgcccgc cgtacgtgcc tgcgcagaa ctgttcaggg
10861 accgcagcgg ggaggagccc gaggagatgc gcgactgcgc ttttcgggcg ggcagggagc
10921 tgcgcgaggg cctggaccgc cagcgcgctg tgcgcgacga ggatttcgag ccgaacgagc

FIG. 16A-3

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10981 agacggggat cagccccgcg cgcgcgacg tggcgggcggc caacctgggtg acggcctacg
11041 agcagacggt gaagcaggag cgcaacttcc aaaagagttt caacaacccat gtgcgcacgc
11101 taatcgcgcg cgaggaggtg gccctgggct tgatgcacct gtgggacctg gcgaggcca
11161 tcgtgcagaa cccggacagc aagcctctga cgcgcgagct gttcctgggtg gtgcagcaca
11221 gcagggacaa cgaggcggtc agggaggcgc tgctaaacat cgccgagccc gagggccgct
11281 ggctgctgga gctgatcaac atcttgacga gcatcgtagt gcaggagcgc agcctgagcc
11341 tggccgagaa ggtggcggtc atcaactact cggtgctgag cctgggcaag ttttacgcgc
11401 gcaagattta caagacgccg tacgtgcca tagacaagga ggtgaagata gacagctttt
11461 acatgcgcat ggcgctcaag gtgctgacgc tgagcgacga cctgggcggtg taccgcaacg
11521 accgcatcca caaggccgtg agcgcgagcc ggcgcgcgga gctgagcgac cgcgagctga
11581 tgctgagtct gcgcccggcg ctggtagggg gcgcccggcg cggtagaggag tcctacttcg
11641 acatggggggc ggacctgcat tggcagccga gccggcgcg cttggaggcc gcctacggtc
11701 cagaggactt ggatgaggat gaggaagagg aggaggatgc acccgctgcg gggtagtgac
11761 gcctccgtga tgtgttttta gatgcagcaa gcccgggacc ccgccataag ggcggcgctg
11821 caaagccagc cgtccggtct agcatcgac gactgggagg ccgcatgca acgcatcatg
11881 gccctgacga cccgcaaccc cgagtccttt agacaacagc cgcaggccaa cagactctcg
11941 gccattctgg aggcggtggt cccctctcgg accaacccca cgcacgagaa ggtgctggcg
12001 atcgtgaacg cgctggcgga gaacaaggcc atccgtcccg acgaggccgg gctggtgtac
12061 aacgccctgc tggagcgcg tggccgctac aacagcacia acgtgcagtc caacctggac
12121 cggctggtga cggacgtgc cgaggccgtg gcgcagcgcg agcgggttcaa gaacgagggc
12181 ctgggctcgt tgggtggcgct gaacgccttc ctggcgacgc agccggcgaa cgtgccgcgc
12241 gggcaggacg attacaccaa ctttatcagc gcgctgcggc tgatggtgac cgagggtccc
12301 cagagcgagg tgtaccagtc gggcccagac tactttttcc agacgagccg gcagggtctg
12361 cagacggtga acctaaagcca ggctttcaag aatctgcgcg ggctgtgggg cgtgcaggcg
12421 cccgtgggcg accggtcgac ggtgagcagc ttgctaacgc ccaactcgcg gctgctgctg
12481 ctgctgatcg cgcccttcac cgacagcggc agcgtgaacc gcaactcgtc cctgggcccac
12541 ctgctgacgc tttaccgcga ggccataggg caggcgaggg tggacgagca gaccttccag
12601 gagatcacta gcgtgagccg cgcgctgggt cagaacgaca ccgacagtct gagagccacc
12661 ctgaacttct tgctgacaaa tagacagcag aagattccgg cgagtagcgc gctgtcggcc
12721 gaggaggagc gcatcctgag atatgtgcag cagagcgtag ggcttttcc gatgcaggag
12781 ggggccaccc ccagcgccgc gctggacatg acccgcgcca acatggaacc tagcatgtac
12841 gccgccaacc ggccgttcat caataagctg atggactacc tgcaccgcgc ggctgccatg
12901 aactcggact actttactaa tgctatacta aaccgcact ggctcccgcg gccgggggtc
12961 tacacgggcg agtacgacat gcccgaaccc aacgatgggt tcctgtggga cgacgtggac
13021 agcgcggtgt tctccccgac cttgcaaaag cgccaggagg cggtagcac gcccgcgagc
13081 gagggcgcgg tgggtcggag cccctttcct agcttaggga gtttgcatag cttgccgggc
13141 tcggtgaaca gcggcagggt gagccggccg cgcttgctgg gcgaggacga gtacctgaac
13201 gactcgctgc tgcagccgcc gcgggtcaag aacgccatgg ccaataacgg gatagagagt
13261 ctggtggaca aactgaaccg ctggaagacc tacgctcagg accataggga tgcgcccgcg
13321 ccgcggcgac agcgccacga ccggcagcgg ggcctgggtg gggacgacga ggactcggcc
13381 gacgatagca gcgtggttga cttgggcggg agcgggtggg ccaaccggtt cgcgcatctg
13441 cagcccagac tggggcgacg gatgttttga atgaaataaa actaccaag gccatagcgt
13501 gcgttctctt ccttggttaga gatgaggcgc gcggtggtgt cttcctctcc tcctccctcg
13561 tacgagagcg tgatggcgca ggcaaccctg gaggttccgt ttgtgcctcc gcggtatatg
13621 gctcctacgg agggcagaaa cagcattcgt tactcggaac tggctccgca gtacgacacc
13681 actcgcggtg acttggtgga caacaagtcg gcggacatcg cttccctgaa ctacaaaac
13741 gaccacagca acttcctgac cacggtggtg cagaacaacg atttcacc cccgaggcc
13801 agcacgcaga cgataaattt tgacgagcgg tcgcggtggg gcggtgattt gaagaccatt
13861 ctgcacacca acatgcccaa tgtgaacgag tacatgttca ccagcaagtt taaggcgcg
13921 gtgatggtgg ctaggaaggt ggtgatcag aatgatagga gcaaggatga gttaaaatat
13981 gagtggtttg agtttaccct gcccgagggc aacttttccg agacctgac catagacctg
14041 atgaacaacg ccatcttgga aaactacttg caagtggggc ggcaaatgg cgtgctggag
14101 agcgatatcg gagtcaagtt tgacagcagg aatttcaagc tgggctggga cccggtaac
14161 aagctggtga tgcctgggg tctacacctac gaggccttcc acccgacgt tgtgctgctg
14221 ccgggctgcg ggggtggactt caccgagagc cgctgagca acctcctggg cattcgcaag
14281 aagcaacctt tccaagaggg cttcaggatc atgtatgagg atctcgaggg tggtaacatc
14341 cccgccctcc tggatgtcaa gcaatatatt gatagtaaaa agaagcttga ggaggcaaca
14401 cagaatgcaa ccagggtgc tggagatatc agaggagaca gtcataattc aagagctgtg
14461 gaacaagcgg ctgaaaagga tctggtcatt gtaccagtaa cacaagatga aagtaagaga
14521 agctataatg tcatagatgg caccatgac accctctacc gaagttggta cctgtcctat
14581 acctacgggg accccgagaa gggggtgcag tcgtggacgc tgctcaccac cccggacgtc

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FIG. 16A-4

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14641 acctgcgggcg cggagcaagt ctactgggtcg ctgcccggacc tcatgcaaga ccccgtcacc
14701 ttccgctcta cccagcaagt cagcaactac cccgtgggtg gcgccgagct catgcccttc
14761 cgcgcccaaga gctttttacaa cgacctcgcc gtctactccc agctcatccg cagctacacc
14821 tccctcaccac acgtcttcaa ccgcttcccc gacaaccaga tctcttgccg tccgcccgcg
14881 cccaccatca ccacggtcag tgaaaacgtg cctgctctca cagatcacgg gacgctaccg
14941 ctgcgagca gtatccgcgg agtccagcga gtgaccgtca ctgacgcccg tcgcccacc
15001 tgtccctacg tctacaaggc cctgggcata gtcgcccgc gcgtgctttc cagtgcacc
15061 ttctaaaaaa tgtctattct catctcgccc agcaataaca ccggctgggg tcttactagg
15121 cccagcacca tgtacggagg agccaagaag cgctcccagc agcaccccg tccgctccgc
15181 ggccacttcc gcgctccctg gggcgcttac aagcgccggc ggacttctac cgccgcccgtg
15241 cgcaccaccg tcgacgacgt catcgactcg gtggctcgcc acgcgcgcaa ctataccccc
15301 gccccctcca ccgtggacgc ggtcatcgac agcgtgggtg ccgacgcgcg cgactatgcc
15361 agacgcaaga gccggcggcg acggatcgcc aggcgccacc ggagtacgcc cgccatgcgc
15421 gccgcccggg ctctgctgcg ccgcccaga cgcacggggc gccggggccat gatgcgagcc
15481 gcgcccgcgc ccgccactgc acccccgcga ggcaggactc gcagacgagc ggccgcccgc
15541 gctgcccgcg ccatttctag catgaccaga cccaggcgcg gaaacgtgta ctgggtgcgc
15601 gactccgtca cgggcgtgcg cgtgcccgtg cgcacccgtc ctctcgtcc ctgatcta
15661 gcttgtgtcc tccccgcaa gcgacgatgt caaagcgcaa aatcaaggag gagatgctcc
15721 aggtcgtcgc ccggagatt tacggaccac cccaggcgga ccagaaacc cgcaaatca
15781 agcgggttaa aaaaaaggat gaggtggacg agggggcagt agagtgtgtg cgcgagttcg
15841 ctccgcggcg gcgcgtaaat tggaaggggc gcagggtgca gcgcgtgttg cggcccggca
15901 cggcggtggt gtttacgccc ggcgagcggg cctcggtcag gagcaagcgt agctatgacg
15961 aggtgtacgg cgacgacgac atcctggacc aggcggcgga gccggcgggc gagttcgct
16021 acgggaagcg gtcgcgcgaa gaggagctga tctcgttgcc gctggacgag agcaaccaca
16081 cgcctagcct gaagcccgtg accctgcagc aggtgctgcc ccaagcagt ctgctgccga
16141 gccgcggggg caagcgcgag ggcgagaata tgtaccgac catgcagatc atggtgcca
16201 agcgcggcg cggtggaaga gtgctggaca ccgtgaaaat ggatgtggag cccgaggtca
16261 aggtgcgccc catcaagcag gtggcgccgg gcctgggcgt gcagaccgtg gacattcaga
16321 tccccaccga catggatgtt gacaaaaaac cctcgaccag catcgaggtg cagaccgacc
16381 cctggctccc agcctccacc gctgcccgtc ccacttctac cgccgccacg gctaccgagc
16441 ctcccagaag gcgaagatgg ggccctgcca accggctgat gcccaactac gtattgcac
16501 cttccattat cccgacgccc ggctatcgcg gcacccggt tctggccccc gcccgctgc
16561 cagccagcaa acgcccgcgc cgcaccgcca cccgcccgcg tctggccccc gcccgctgc
16621 gccgcgtaac cacgcgcggg ggccgctcgc tcgttctgcc caccgtgcgc taccaccaca
16681 gcatccttta atccgtgtgc tgtgatactg ttgcagagag atggctctca cttgccgct
16741 gcgcatcccc gtcccgaatt accgaggaag atcccgccgc aggagaggca tggcaggcag
16801 cggcctcaac cgccgcccgc ggccggccat gcgcaggcgc ctgagtggcg gctttctgcc
16861 cgcgctcatc ccataatcg cggcgcccat cggcacgac cccggccatag cttccgttgc
16921 gctgcaggcg tcgcagcgcc gttgatgtgc gaataaagcc tcttttagact ctgacacacc
16981 tggctcctgta tatttttaga atggaagaca tcaattttgc gtccctggct ccgcccgcag
17041 gcacgcggcc gttcatgggc acctggaacg agatcggcac cagccagctg aacgggggag
17101 ccttcaattg gagcagtgtc tggagcgggc ttaaaaattt cggctcgacg ctccggacct
17161 atgggaacaa ggcctggaat agtagcacgg ggcagttgtt aagggaagag ctcaaagacc
17221 agaacttcca gcagaagggt gtggacggcc tagcctcggg cattaacggg gtggtggaca
17281 tagcaaacca ggccgtgcag cgcgagataa acagccgcct ggacccgcgg ccgcccacgg
17341 tgggtggagat ggaagatgca actcctccgc cgcccaaggg cgagaagcgg ccgcccgcgg
17401 acgcggagga gacgatcctg cagggtggac agccgcccct gtacgaggag gccgtcaagg
17461 ccggcatgcc caccacgcgt atcatcgcg cactggccac tgggtgtaatg aaaccgcca
17521 cccttgacct gcctccgcca cccacgcccg ctccaccgaa ggcagctccg gttgtgcagc
17581 cccctcctgt ggcgaccgcc gtgcccgcgc tccccgccc cccgaggcc cagaactggc
17641 agagcacgct gcacagtatc gtgggcctgg gagtgaaaag tctgaagcgc cgccgatgct
17701 attgagagag aggaagagg acactaaagg gagagcttaa cttgtatgtg cttaccgcc
17761 agagaacgcg cgaagatggc taccctcctg atgatccgc agtgggcgta catgcacatc
17821 gccgggcagg acgcctcgga gtacctgagc ccgggtctgg tgcagtttgc ccgcccacc
17881 gacacgtact tcagcctggg caacaagttt aggaacccca cgggtggctcc caccacgat
17941 gtgaccacgg accggtccca gcgtctgacg ctgcccgttg tggccgtgga tcgcgaggac
18001 accacgtact cgtacaaggc gcgcttccat ctggccgtgg gcgacaaccg ggtgctagac
18061 atggccagca cttactttga catccgcggc gtcctggacc gcggtcccag cttcaaacc
18121 tactcgggca cggcttacia cagcctggcc ccaaaggcg ccccaactc tagtcagtgg
18181 gaacaagcta aagctaccaa tgccggtcaa aaggaaactc acacatttgg agtagccgct
18241 atgggcggag aagacattac agtgaaagg cttcaaattg gaactgatga aactaaggaa

FIG. 16A-5

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18301 gatggagagg atgaaatthtt tgcagatcaa acattccagc cagaacctca agtgggagaa
18361 cagaactggc aagaaacgth tgtthttctat ggaggcagag ctcttaagaa agaaaccaa
18421 atgaagccat gttatggctc ttatgcgaga cccacaaatg aaaaggagg acaggctaaa
18481 ttacacttg atgaaaaagg tcagccaacc aaaattcctg atattacaat ggatttcttt
18541 gatagtccac aagatgatac atcagggtgta actaataagc cagatattgt catgtatgca
18601 gaaaatgtaa atttagaagc tcctgacaca catgtagtht acaaaccagg caaagatgat
18661 tctagthtctt ccgctaacct cacacaacag gccatgccta acagaccgaa ctacatcggg
18721 ttcagagaca actthtgthg tcttatgtac tacaatagta ctggcaacat gggthtgctg
18781 gctggthcagg cctctcagth gaatgctgtg gthcagctthg aagacagaaa caccgagctg
18841 tcttaccagc tathgtctaga thctctgggt gacagaacca gatactthtag catgtggaat
18901 tctgcagthg acagctatga ccccgatgth aggatcathg agaathcagth tgtggaagat
18961 gaactthcaa actathgctt cccactgaat ggcagthgth ctaacagcac atacaaaggt
19021 gthaaagctg gaactggaaa caathgggat gacgatgaaa atgthgcaag acaaatcag
19081 atthgthcag gcaacctgth cgcctaggag atcaacctcc aggccaacct atggaagagth
19141 thtctgtact cgaacgthgg cctgtacctg cccgactcct acaagthcac gccggccaac
19201 gthcagctgc ccaccaacac caacacctac gactacatga acggccgctg ggtagcccc
19261 thgctggthg acgctacat caacathggc gccgctgthg cgtgggacct catggacaat
19321 gthcaatccct tcaaccacca ccgcaacgth ggctgctgct accgctccat gctcctggg
19381 aacggccgct acgthgccct ccacathcaa gtgccccaaa agthctthtg catcaagaac
19441 ctgctthctg tccccgtht ctacacctac gagthggaact tccgcaagga cgtcaacatg
19501 atcctgcaga gthccctcgg caacgacctg cgcgthcag ggcctcctg ccgcttcgac
19561 agcgtcaacc tctacgcccac cthctthccc atggcgcaac acaccgctc caccctggaa
19621 gccatgctgc gcaacgacac caacgaccag tctthcaacg actacctctc ggccgccaac
19681 atgctctacc ccatcccgth caaggccacc aacgthgcca thctcatccc thcgcgcaac
19741 thggccgctt tccgcgctg gagththcacc cggctcaaga ccaaggaaac thctcctc
19801 ggctcggtht thgacctcta cththgtctac thgggctcca thctcctacc cgacgggacc
19861 thctacctca accacacct caagaaggtc thcatcatgt thcactcctc ggtcagctgg
19921 cccggcaacg accggtgct cacgcccgaac gagthcagga tcaagcgag cgttgacggg
19981 gagggctaca acgthggcca atgcaacatg accaaggact ggtthctcgt ccagatgctc
20041 thccactaca acatcggtc ccagggtth cagthgcccg agggctacaa ggaccgcatg
20101 tactccttht tccgcaact ccagcccath agcaggcagg thggtcagat gatcaactac
20161 aaggactaca aggcgthcac cctacctth cagcacaaca actcgggctt caccggctac
20221 cthgcgcca ccatgthgca ggggcagccc thccccgcca actthcccta cccgctcatc
20281 ggctccaccg cagthccctc cgtcacccag aaaaagtht tctgthcag ggtcatgtg
20341 cgcathccat thctcagcaa cththatgtc atgggthgccc thaccgacct gggthcagaac
20401 atgctctatg ccaactcggc ccacgthctc gacatgacct thgaggtgga ccccatggat
20461 gagcccaccc thctctatct thctthcga gthththcag thggtcagag gcaccagccg
20521 caccgthgthg thcatcaggg cgtctacctg cgcacgccc thctcggcgg thctcggcgg caacgctacc
20581 acttaagcat gagcggctcc agcgaacaag agctcggthg catcgtgthg gacctgggth
20641 gcgggccccta cthththggga acccagcaca agcgtthccc thggtthctt gccggcgaca
20701 agctggcctg cgcctatgth aacacgthg gccgthgag cggagthgth cactggctc
20761 cthththgct gaathcgtc thcgthcact gctacatgt thgacctth gggththctg
20821 accgthgthg caagcagath thagctthc thgagcagth caccagacc thgagggg gthgagggg
20881 thgctcctc cggcagcggc cththththg gcatgththth gcatgctth gthgactggc
21001 cgcacgacc catggacgga aacccacca thgactthg gacggggthg ccaaagcga
21061 thgctacaat gccacaggtg cthgcccacc thaggcgcaa ccaggaggag thctaccgct
21121 thctcgtcgg ccatccct thctthcag cccaccgth cgcctcga aacgthcgg
21181 cthththgata aatgaaacaa cthgctgth thcaataaac agcacttht thtcatgca
21241 cthggagtata thgaagtht thaaaagthc aagggththt cgtgctcgt gthgthgthg
21301 gctgthggga gggccagth gctgthgth thctthggga gccactthga thctgggthg
21361 accagththg gactgthgg gthgthgth cthgthgthg acatgthgth gthctatctg
21421 agggcgccc gcatgthcgg gccgthgth thgaaatcac aattggggth gthgctctg
21481 gthgthgthg thgthgthc gggththgth cactggaaca ccatthgth gggthactth
21541 aactgthgaa gthgthctt gthgthgth thgactthth ccagthctc gthgthgth
21601 agggcgaaag gggthcatct gthgthgth cggcccagga agggcagct thgagggthg
21661 thgthgthc cgtgthgth gggthctgth atcatcccc cgtgthgth catathcgg
21721 thgagggthg thgthgthg cgtgthctg thgaaagth gthgthgth agccccctg
21781 cthgaaacaa gggcgthgth cthctcggth aactgthth thctcggth gthgthgth
21841 acgthgthg gthgthgth gthgthgth thgthgthg thgthgthg gthgthgth
21901 gthgthgth cthgthgth cthgthgth aacgthgth gthgthgth gthgthgth

FIG. 16A-6

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21961 tccatctcca ccacgtggtc cttgtggatc atcacccgtcc catgcagaca cttgagctga
22021 ccctcgacat cgcagcagcc atgatccac agggcgcagc cgggtgcactc ccagttctta
22081 tgcgcgatcc cgctgtggct gaagatgtaa ccttgcaaca ggcgacccat gacgggtgcta
22141 aatgctttct ggggtgggtgaa ggtcagttgc agaccgcggg cctcctcgtt catccaggtc
22201 tggcacatct tttggaagat ctccgtctgc tcgggcatga gcttgtaagc atcgcgcagg
22261 ccgctgtcga cgcggtagcg ttccatcagc acgttcattg tatccatgcc cttctcccag
22321 gacgagacca gaggcagact caggggggtg cgcacgttca ggacaccggg ggtcgcaggc
22381 tcgacgatgc gttttccgct cttgccttcc ttcaacagaa ccggaggctg gctgaatccc
22441 actcccacga ttacggcatc ttcttggggc atctcttcgt cggggtctac cttgggtcaca
22501 tgcttgggtct ttctggcttg cttctttttt ggagggtgtt ccacggggac cacgtcctcc
22561 tcggaagacc cggagcccac ccgctgatac ttccggcgct tgggtgggcag aggaggtggt
22621 ggcggcgagg ggctcctctc ctgctccggc ggatagcgcg ccgaccctg gccccggggc
22681 ggagtggcct ctccgtccat gaaccggcg cagtcctgac tgccgcccgc cattgtttcc
22741 taggggaaga tggaggagca gccgcgtaag caggagcagg aggaggactt aaccaccac
22801 gagcaacca aaatcgagca ggacctgggc ttcaagagc cggctcgtct agaaccacca
22861 caggatgaac aggagcacga gcaagacgca ggccaggagg agaccgacgc tgggctccag
22921 catggctacc tgggaggaga ggaggatgtg ctgctaaaac acttgacgag ccaatccatc
22981 atcctccggg acgcccgtgc cgaccggagc gaaaccctc tcagcgtcga ggagctgtgt
23041 cgggcctacg agctcaacct cttctcgcgc cgctgcccc ccaaaccgca gcccaacggc
23101 acctgcgagc ccaaccgcgc tctcaacttc tatcccgctt ttgcggtccc cgaggcccta
23161 gccacctatc acatcttttt caagaaccaa aagatccccg tctcctgcgc cgccaaccgc
23221 acccgcgccg acgcgtcctc cgctctgggg cccggcgcg cgcatacctga tatcgcttcc
23281 ctggaagagg tgcccaagat cttcgaaggg ctccgtcggg acgagacgcg cgcggcaaac
23341 gctctgaaag aaacagcaga ggaagagggt cacactagcg ccctggtaga gttggaaggc
23401 gacaacgcca ggctggccgt gctcaagcgc agcgtcgcgc tcaccactt cgcctacccc
23461 gccgtcaacc tcccgcccaa ggtcatgcgt cgcattcatg atcagctcat catgcccac
23521 atcgaggccc tcgatgaaag tcaggagcag cgcgccgagg acgcccggcc cgtggtcagc
23581 gacgagcagc tcgcgcgttg gctcgggacc cgcgaccccc aggctttgga acagcggcg
23641 aagctcatgc tggccgtggt cctgggtcacc ctccagctcg aatgcattgc cgccttcttc
23701 agcgcacccc agaccctgcg taaggctcag gagaccctgc actacacttt caggcacggt
23761 ttccgtcaggc aggcctgcaa gatctccaac gtggagctga ccaacctggt ctcatgcctg
23821 gggatcctgc acgagaaccg cctgggacag accgtgctcc actctactct gaagggcgag
23881 gcgcgtcggg actatgtccg cgactgtgta tttctcttta tctgccacac ctggcaagca
23941 gccatgggcg tgtggcagca gtgtctcgcg gacgaaaatc tgaaggagct ggacaagctt
24001 cttgctagaa accttaaaaa gctgtggacg ggcttcgcgc agcgcaccgt cgcctcggac
24061 ctggccgaga tcgtttttcc agaacgcctg aggcagacgc tgaaaggcgg gctgcccgac
24121 ttcatgagcc agagcatggt gcaaaactac cgcactttca ttctcgagcg atctgggatg
24181 ctaccgcgca cctgcaacgc attcccctcc gactttgtcc cgctgagcta ccgcgagtgt
24241 cccccgcgc tgtggagcca ctgctatctc ttgcagctgg ccaactacat cgcctaccac
24301 tcggacgtga tcgaggacgt gagcggcgag gggcttctcg agtgccactg ccgctgcaac
24361 ctgtgctccc cgcaccgctc cctgggtctgc aacccccagc ttctgagcga gaccaggtc
24421 atcggtacct tcgagctgca aggtccgcag gagtccaccg ctccgctgaa actcacgccc
24481 ggggtgtgga cttccgcgta cctgcgcaaa tttgtaccgg aggactacca cgcccatgaa
24541 ataaagtctc tcgaggacca atcgcgcgca cagcacgcgg atctcacggc ctgcgtcatc
24601 acccagggcg cgatcctcgc ccaattgcac gccatccaaa aatcccgcca agagtttctt
24661 ctaaaaaagg gtagagggtt ctacctggac ccccagacgg gcgagggtgt caaccgggt
24721 ctccccagc atgccgagga agaagcagga gccgctagtg gagcagatgg aagaagaatg
24781 ggacagccag gcagaggagg acgaatggga ggaggagaca gaggaggaag aattggaaga
24841 ggtggaagag gagcaggaaa cagagcagcc cgctgcgcga ccacccgcgc cggcagcccc
24901 gccggtcacg gatacaacct ccacagctcc ggccaagcct cctcgtagat gggatcgagt
24961 gaaggggtgac ggtaagcacg agcggcaggg ctaccgatca tggagggtcc acaaagccgc
25021 gatcatcgcc tgcttgcaag actgcggggg gaacatcgct ttcccccgc gctacctgt
25081 cttccaccgc ggggtgaaca tccccgcaa cgtgttgcat tactaccgtc accttcacag
25141 ctaagaaaaa gcaagtaaga ggagtgcgc gaggaggcct gaggatcgcg gcgaacgagc
25201 cctcgaccac cagggagctg aggaaccgga tcttccccac tctttatgcc atttttcagc
25261 agagtcgagg tcagcagcaa gaactgaaag taaaaaacgg gtctctgcgc tcgctacccc
25321 gcagttgctt gtaccacaaa aacgaagatc agctgcagcg cactctcgaa gacgccgagg
25381 ctctgttcca caagtactgc gcgctcactc tttaaagacta aggcgcgccc acccggaaaa
25441 aaggcgggaa ttacctcatc gccaccatga gcaaggagat tcccaccctt tacatgtgga
25501 gctatcagcc ccagatgggc ctggccgcgc gcgcctccca ggactactcc acccgcatga
25561 actggctcag tgccggcccc tcgatgatct cacgggtcaa cggggtccgt aaccatcgaa

FIG. 16A-7

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25621 accagatatt gttggagcag gcggcgggtca cctccacgcc cagggcaaag ctcaacccgc
25681 gtaattggcc ctccaccctg gtgtatcagg aaatccccgg gccgactacc gtactacttc
25741 cgcgtgacgc actggccgaa gtccgcatga ctaactcagg tgtccagctg gccggcggcg
25801 cttcccggtg cccgctccgc ccacaatcgg gtataaaaac cctgggtgatc cgaggcagag
25861 gcacacagct caacgacgag ttggtgagct cttcgatcgg tctgcgaccg gacggagtgt
25921 tccaactagc cggagccggg agatcgtcct tcactcccaa ccaggcctac ctgaccttgc
25981 agagcagctc ttcggagcct cgctccggag gcacccgaac cctccagttc gtggaggagt
26041 ttgtgccctc ggtctacttc aacccttctt cgggatcgcc aggcctctac ccggacgagt
26101 ttataccgaa cttcgacgca gtgagagaag cgggtggacgg ctacgactga atgtcccatg
26161 gtgactcggc tgagctcgct cgggtgaggg atctggacca ctgccgccgc ctgctgtgct
26221 tcgcccggga gagctgcgga ctcatctact ttgagtttcc cgaggagcac cccaacggcc
26281 ctgcacacgg agtgccgcat accgtagagg gcaccaccga gtctcacctg gtcaggttct
26341 tcacccagca acccttcctg gtcgagcggg accggggagc taccacctac accgtctact
26401 gcactctgtc taccgccgaag ttgcatgaga atttttgctg tactctttgt ggtgagttta
26461 ataaaagctg aactaagaac cttcttttga atcccttgct atcatcaa atcaacaagacc
26521 atcaacttca cctttgagga acaggtgaac tttacctgca agccacacaa gaagtacatc
26581 atctggtttt atcacaacac tactctagca gtagccaaca cctgctcgaa cgacgggtgtt
26641 ctcctaccta acaatctcac cagtggacta accttctcag ttaaaagggc aaagctaatt
26701 cttcatcgcc ctattgtaga aggaacttac cagtgtcaga gcggaccttg cttccacagt
26761 ttcacttttg tgaacgttac cggcagcagc acagccgctc cagaaacatc taaccttctt
26821 tctgatacta acaaacctcg tgtcggaggt gagctttggg ttccatctct aacagagggt
26881 gggagttcta ttgaagtggg tgggtatattg attttagggg tgggtcattg tgggtgcata
26941 gcagtgtgtg atcaacttcc ttgctgggtc gaaatcaggg tatttatctg ctgggtcaga
27001 cattgtgggg aggaacctat aaggggctct tgctgattat cctttccctg gtgggggggtg
27061 tgctgtcatg ccacgaacag ccacgatgta acattaccac aggcaatgag aggaacgact
27121 gctctgtagt tatcaaatgc gagcaccatt gtcctctcaa catcacattc aagaatpaga
27181 ccatgggaaa tgtatgggtg ggattctggc aaccaggaga tgagcagaac tacacgggtca
27241 ctgtccatgg tagcgatggc aatcacactt tcggtttcaa attcattttt gaagtcatgt
27301 gtgatatcac actacatgtg gctagacttc atggcttggt gccccctacc aaggagaaca
27361 tgggtgggttt ttctttggct tttgtgatca tggcctgctt gatgtcagggt ctgctggtag
27421 gggctctagt gtggtttctg aaacgcaagc ccagggtacgg aatgaggag aaggaaaaat
27481 tgctataaat tctttttctc ttgcacaaac catgaatata gtgttccgta tcgtgctgct
27541 ctctcttctt gtagcttttcg gtcaggcagg aattcatatt attaattgcta catggtggga
27601 taatataact ttagtgggac cctcagatac tccagttacc tgggtatgat gcaagggatt
27661 gcaattttgt gacggaagta cagttaagaa tccgcagatc agacatactt gtaatgatca
27721 aaacttaact ctgattcatg ttaacaaaac ccatgaaaga acatacatgg gttacagaca
27781 tgacagtaag ggaaaagtag actataaggt tacagtcatt ccacctctc ctgctactgt
27841 aaagccacaa ccagatccag aaaatgtctt tggtttatat ggaaataatg taactttagt
27901 tggacctcca ggaattccag ttagttggta ttatcataat ggcacacagt tctgcatggg
27961 agataaaaatt attcatccag aattcaacca cacctgtgat aaacaaaacc ttacttgct
28021 gtttgtaaac ttacacatg atggaggcta tcttggtatc aattacaaag gtactcagag
28081 aattcagtat gaggttatag ttttagatcg atttccaaat tctgggtcaga tgaaaattga
28141 agaacaaagt gaggaacag 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 ggcttcacaa tcagtgtagt gctcagaact taactttaat
28441 taatgtaact aaatctcatg agggaaactta ctatggcact aatgacaaag acgaaagcaa
28501 aagatacaga gtgaaagtga aactacaaa ttctcaagct gtaaaaatta acccatatac
28561 cagacctact actcctgatc agaaacacag atttgaatta caaattgaaa ataattgcaa
28621 tgatgaagaa tcaaaaattc catctactac tgtggcaatc gtgggtgggag tgattgcggg
28681 cttcataact ataattcattg tcattctgtg ctacatctgc tgccgcaagc gtcccagggc
28741 atacaatcat atggtagacc cactactcag cttctcttac tgagactcag tcactttcat
28801 ttcagaacca tgaaggcttt cacagcttgc gttctgttta acataatcac acttagtgta
28861 gctgcaaatg gtttttaaca tgtaaatgtt accagattaa gtaatgtaac actgacagga
28921 gctggaatta atactacatg gacagggtat tttaatgagg gtccaaaagg aaaaaatggg
28981 tggatgaata tttgcacatg gggcgatcct agatatgtgt gccatggaaa tagcagtact
29041 attactaatc ttacagtgtg ggcacttcta aatttaacca ctaacagaag atttaagca
29101 gaaagtttta ctagtaacga tggttatgaa actaccagtg caaaatttta tgaaatttaa
29161 attattgagc ttccaacaac tagagcacc accacagtta ggacaacaca gcctaccact
29221 gtgcccacta cacatccaac caccacagtc agtacaacta ttgagaccac tactcatact

FIG. 16A-8

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29281 acacagctag acacaacagt gcagaatact actttattga ttgggttttt actgagagga
29341 aatgaaagta ctactgaaca gacagaggct acctcaagt ccttcagcag cactgcaa
29401 ttaacttcgc ttgcttggac taatgaaacc ggagtatcat tgatgaatcg acagccttac
29461 tcaggtttgg atattcaa atacttttctg gttgtctgtg ggatctttat tcttgcggtt
29521 cttctgtact ttgtctgtg caaagccaga gagaaatcta ggccggcccat atacaggcca
29581 gtaatcgggg aacctcagcc tctccaagt gatggaggct taaggaatct tctcttctct
29641 tttacagtat ggtgatcagc catgattcct aggttcttcc tatttaacat cctgttctgt
29701 ctcttcaaca tctgtgctgc cttcgcggcc gtctcgcacg cctcgcccga ctgtctaggg
29761 cctttcccaa catacctcct ctttgccctg ctaacctgca cctgcgtctg cagcattgtc
29821 tgcgtggtca tcacctttct gcagctcatc gactggtgct gcgcgcgcta caattatctc
29881 caccacagtc ccgaatacag ggacgagaac gtagccagaa tcttaaggct catctgacca
29941 tgcagcctct gctcatgctg atatccctcc tatccctgct ccttgccact tctgctgatt
30001 actctaaatg caaattcgcg gacatatgga atttcttaga ttgctatcag gagaaaattg
30061 atatgccctc ctattacttg gtgattgttg gggtagtcat ggtctgctca tgcactttct
30121 ttgccattat gatctacccc tgttttaatc ttggctggaa ctctgttgag gcattcacat
30181 acacactaga aaacagttca ctacgtctca cgccaccacc cacaccgctt cccgcagaa
30241 atcagttccc tatgattcag tacttagaag agccctctcc ccggcccccct tccactgtta
30301 gctactttca cataaccggc ggcgatgact gaccacctgg acctcgagat ggacggccag
30361 gcctccgagc agcgcacccct gcaactgcgc gtccgacagc agcaggagcg ggccgccaag
30421 gagtcctcgc atgccatcaa catccaccag tgcaagaagg gcatcttctg cctggtcaag
30481 caggcaaaga tcacctacga gctcgtgtcc ggccggcaagc agcatcgcct cgcctatgag
30541 ctaccccagc agaagcaaaa gttcacctgc atgggtggcg tcaaccccat agtcatcacc
30601 cagcagtcgg gcgagaccaa cggctgcac cactgctcct gcgaaagccc cgagtgcac
30661 tactccctcc tcaagaccct ttgcggactc cgcgacctcc tccccatgaa ctgatgttga
30721 ttaaaagccc aaaaaccaat caaaccttc cccaattact cataagaata aatcattgga
30781 actaatcatt caataaagat cacttacttg aaatctgaaa gtatgtctct ggtgtagttg
30841 ttcagcagca cctcgggaacc ctctcccag ctctgggtact ccagtccccg gcgggcggcg
30901 aacttcctcc acaccttgaa agggatgtca aattcctggt ccacaatttt cattgtcttc
30961 cctcagatga caaagaggct cgggtggaa gatgacttca acccgtctta cccctatggc
31021 tacgcgcgga atcagaatat ccccttccct actccccct ttgtttcttc cgatggattc
31081 caaaacttcc cacctgggggt cctgtcactc aaactggctg acccaatcgc catcactaat
31141 ggggatgttt cactcaagggt gggaggggggt cttactgttg aaaaagatag tggaaatcta
31201 aagggtgaacc ctaaggctcc cttgcaagtt acaactgata aacagttgga aattgcactg
31261 gcttatccat ttgaagtcag taatggcaag cttggcataa aagcaggtca tggattgaaa
31321 gtcattgaca aaattgctgg tttggaagggt ttggcaggta cgctttagt tttgactgga
31381 aaaggaatag gtactgaaaa tcttgaaaac agtgatgggt caagtagagg agttggtata
31441 aacgtaagac ttgctaaaga tggagggtctg tcttttgata aaaagggtga tttagttgct
31501 tggataaata atgatgacag acgcaactta tggacaactc ccgacccatc cccaaattgt
31561 acaatcgatc aggaaaggga ttcaaagctc acttttagtat taacaaaatg tggcagtcac
31621 attttggcta atgtctcttt acttggtgta aaaggaaaat ttagtaacat aaacaataat
31681 actaatccaa ctgataaaaa aatcacagta aagctacttt ttaatgaaa gggagtatta
31741 atggacagtt cgacacttaa gaaagaatat tggaaactaca gaaatgataa ttctactgta
31801 tctcaggcct atgataatgc agttcctttt atgccaacaa taaaagctta tcctaaacct
31861 accacagaca cttcggctaa accagaagat aaaaaaagt ctgctaaaag atacattgtg
31921 agcaatgtct atattggagg cttgccagat aaaactgttg ttataactat taagtttaat
31981 gcagaaactg aatgtgctta ttcgattacc tttgaattca catgggcaaa aacctttgaa
32041 gatgtgcagt ttgattcctc ctcttttacc ttttccata ttgccaaga aatgaggac
32101 gaagacaaat aaaatgtttt aaaatgaatt catgtatctt tattgatttt tacaccagca
32161 cgggtagtca gtctcccacc accagcccat ttcacagtgt aaacgattct ctcagcacgg
32221 gtggccttaa atagggaaat gttctgatta gtgcgggaac tggacttggg gtctataatc
32281 cacacagttt cctggcgagc caaacggggg tcggtgattg agatgaagcc gtcctctgaa
32341 aagtcattca agcgggcctc acagtccaag gtcacagtct ggtgaaacga gaagaacgca
32401 cagattcata ctcggaaaac aggatgggtc tgtgcctctc catcagcgcc ctcaacagtc
32461 tctgccgccc gggctcgggtg cggctgctgc agatgggatc gggatcacia gtctctctga
32521 ctatgatccc cacagccttc agcatcagtc tctggtgctg tcgggcacag caccgcatcc
32581 tgatctcgct catgttctca cagtaagtgc agcacataat caccatgtta ttcagcagcc
32641 cataattcag ggtgctccag ccaaaactca tgttggggat gatggaaccc acgtgaccat
32701 cgtaccagat gcggcagtat atcagatgcc tgccctcat gaacacactg cccatataca
32761 tgatctcttt gggcatgtct ctgttcacaa tctgacggta ccagggaag cgctggttga
32821 acatgcaccc gtaaatagact ctctgaacc acacggccag cagggtgcct cccgcccagc
32881 actgcaggga gcccggggat gaacagtggt aatgcaggat ccagcgctcg taccgctca

FIG. 16A-9

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32941 ccattctgagc tctcaccaag tccagggtag cggggcacag gcacactgac atacatcttt
33001 ttaaaatttt tatttcctct ggagtcaaga tcatatccca ggggactgga aactcttgga
33061 gcagggtaaa gccagcagca catggtaatc cacggacaga acttacatta tgataatctg
33121 catgatcaca atcaggcaac aggggatgtt gttcagtcag tgaagccctg gtttcctcat
33181 cagatcgtgg taaacgggcc ctgcgatatg gatgatggcg gagcgagctg gattgaatct
33241 cggtttgcat tgtagtggat tctcttgctg accttgctgt acttctgcca gcagaaatgg
33301 gcccttgaac agcagatacc cctcctgcgg ccgtcctttc gctgctgccg ctcagtcac
33361 caactgaagt acatccattc tcgaagattc tggagaagtt cctctgcatc tgatgaaaca
33421 aaaaacccgt ccattgcgaat tcccctcatc acatcagcca ggactctgta ggccatcccc
33481 atccagttaa tgctgccttg tctatcattc agagggggcg gtggcaggat tggaagaacc
33541 atttttattc caaacggtct cgaaggacga taaagtgcga gtcacgcagg tgacagcgtt
33601 cccctccgct gtgctgggtg aaacagacag ccagggtcaaa acccactcta ttttcaaggt
33661 gctcgaccgt ggcttcgagc agtggctcta cgcgtacatc cagcataaga atcacattaa
33721 aggctggccc tccatcgatt tcatcaatca tcagggtaca ttcctgcacc atccccaggt
33781 aattctcatt tttccagcct tggattatct ctacaaattg ttggtgtaag tccactccgc
33841 acatgtggaa aagctccac agtggccctt ccactttcat aatcaggcag accttcataa
33901 tagaaacaga tcctgctgct ccaccacctg cagcgtgttc aaaacaacaa gattcaataa
33961 ggttctgccc tccgccctga gctcgcgcct caatgtcagc tgcaaaaaat cacttaagtc
34021 ctggggccact acagctgaca attcagagcc agggctaagc gtgggactgg caagcgtaag
34081 ggaaaacttt aatgctccaa agctagcacc caaaaactgc atgctggaat aagctctctt
34141 tgtgtctccg gtgatgcctt caaaaatgtg agtgataaag cgtggtagtt tttctttaat
34201 catttgcgta atagaaaagt cctgtaaata agtcactagg accccaggga ccacaatgtg
34261 gtagcttaca ccgcgtcgct gaagcatggt tagtagagat gagagtctga aaaacagaaa
34321 gcatgcacta aactaagggt gctattttca ctgaaggaaa aatcactctc tccaacaaca
34381 gggtagccac tgggtggccc ttgctggacat acaaaaatcg gtccgtgtga ttaaaaagca
34441 gcacagtaag ttcctgtctt cttccggcaa aaatcacatc ggactgggtt agtatgtccc
34501 tggcatggta gtcattcaag gccataaatc tgccctgata tccagtagga accagcacac
34561 tcacttttag gtgaagcaat accaccccat gcggaggaat gtggaaagat tcagggcaaa
34621 aaaaattata tctattgcta gtcccttctt ggacgggagc aatccctcca ggactatcta
34681 tgaaagcata cagagattca gccatagctc agcccgttta ccagtagaca gagagcacag
34741 cagtacaagc gccaacagca gcgactgact acccactgac ccagctccct atttaaaggc
34801 gccttacact gacgtaatga ccaaaggctt aaaaaccccg ccaaaaaaaa acacacacgc
34861 cctgggtggt ttttgcgaaa acacttccgc gttctcactt cctcgtattg atttcgtgac
34921 ttaacttccg gggtcccacg ttacgtcact tctgccctta catgtaactc agtcgtaggg
34981 cgccatcttg cccacgtcca aaatggcttc catgtccagc cacgcctccg cggcgaccgt
35041 tagccgtgcg tcgtgacgtc atttgcatca tcttctctcg tccaatcagc gctggccccg
35101 ccctaaattc aaaagctcat ttgcatgtta acttttgttt actttgtggg gtatatattt
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 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 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 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 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 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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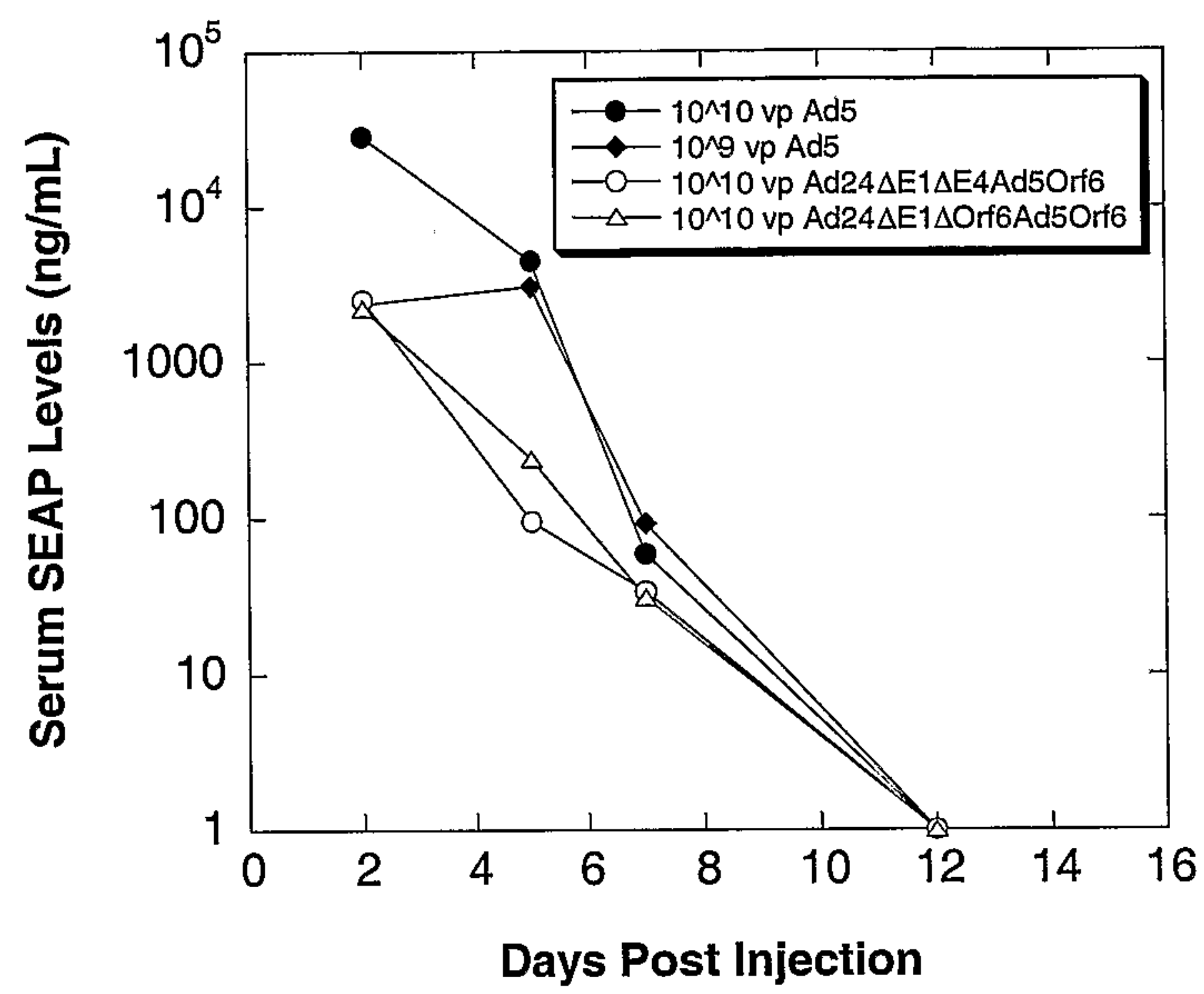


FIG. 20

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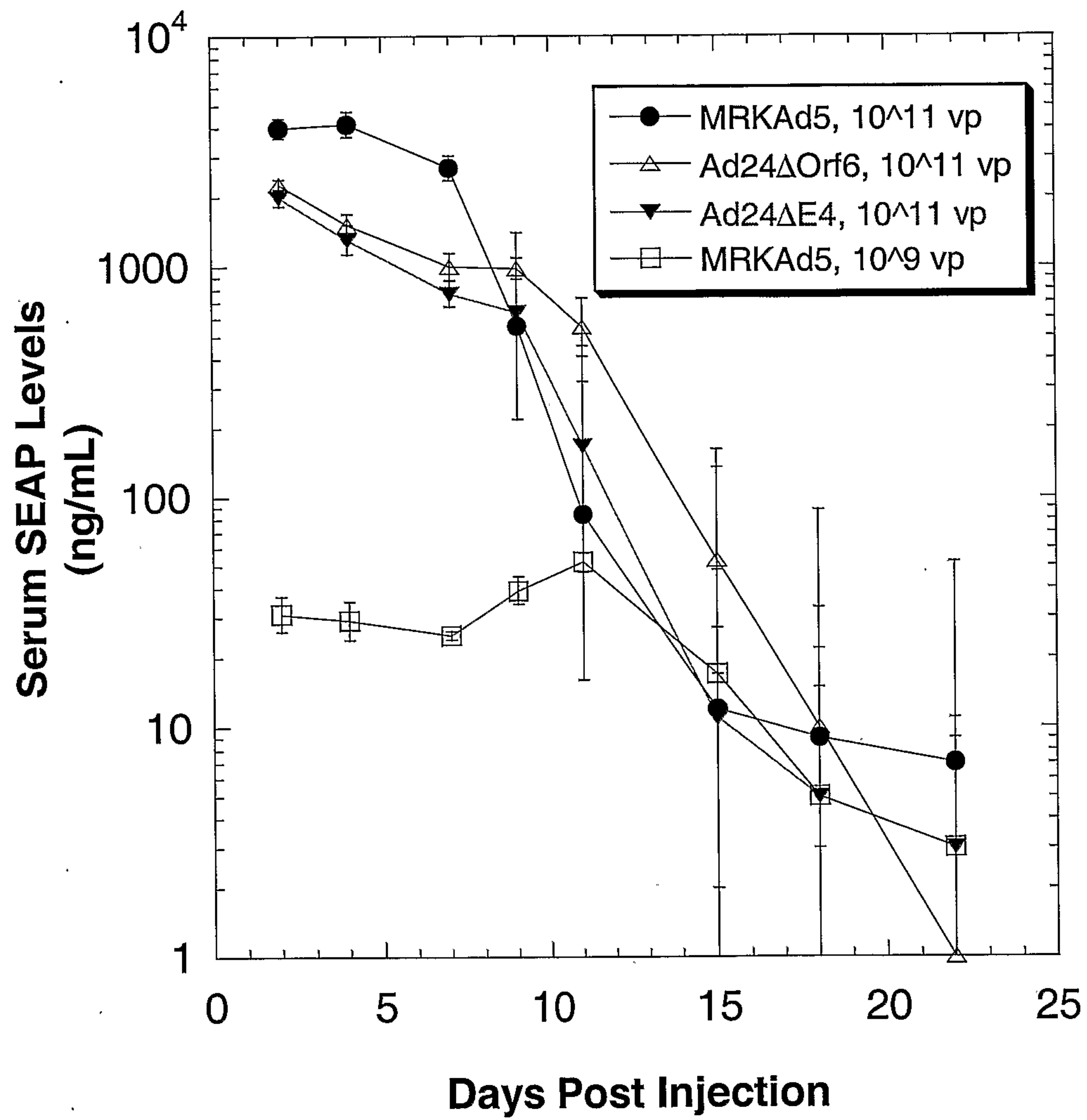


FIG. 21

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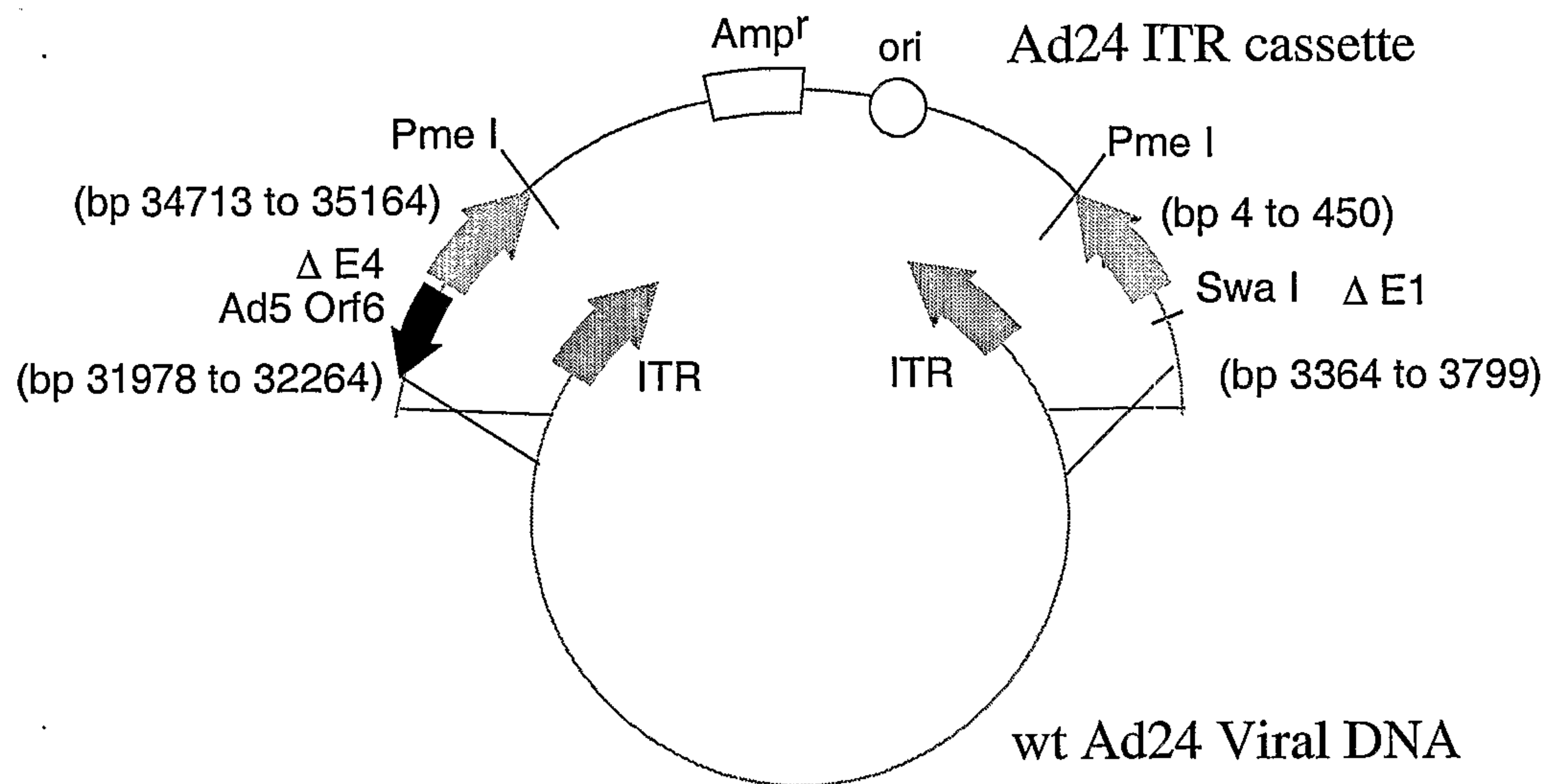


FIG. 22

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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 ⁹ 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 ⁹ 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 ^a	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 Ad24ΔE1gagΔOrf6Ad5Orf6	0.06	0.37
Monkey 2	10 ⁷ vp MRKAd5-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	0.01	0.56
Monkey 3	10 ⁹ vp MRKAd6-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	0.07	0.06
Monkey 4	10 ⁷ vp MRKAd6-gag	10 ¹¹ vp Ad24ΔE1gagΔOrf6Ad5Orf6	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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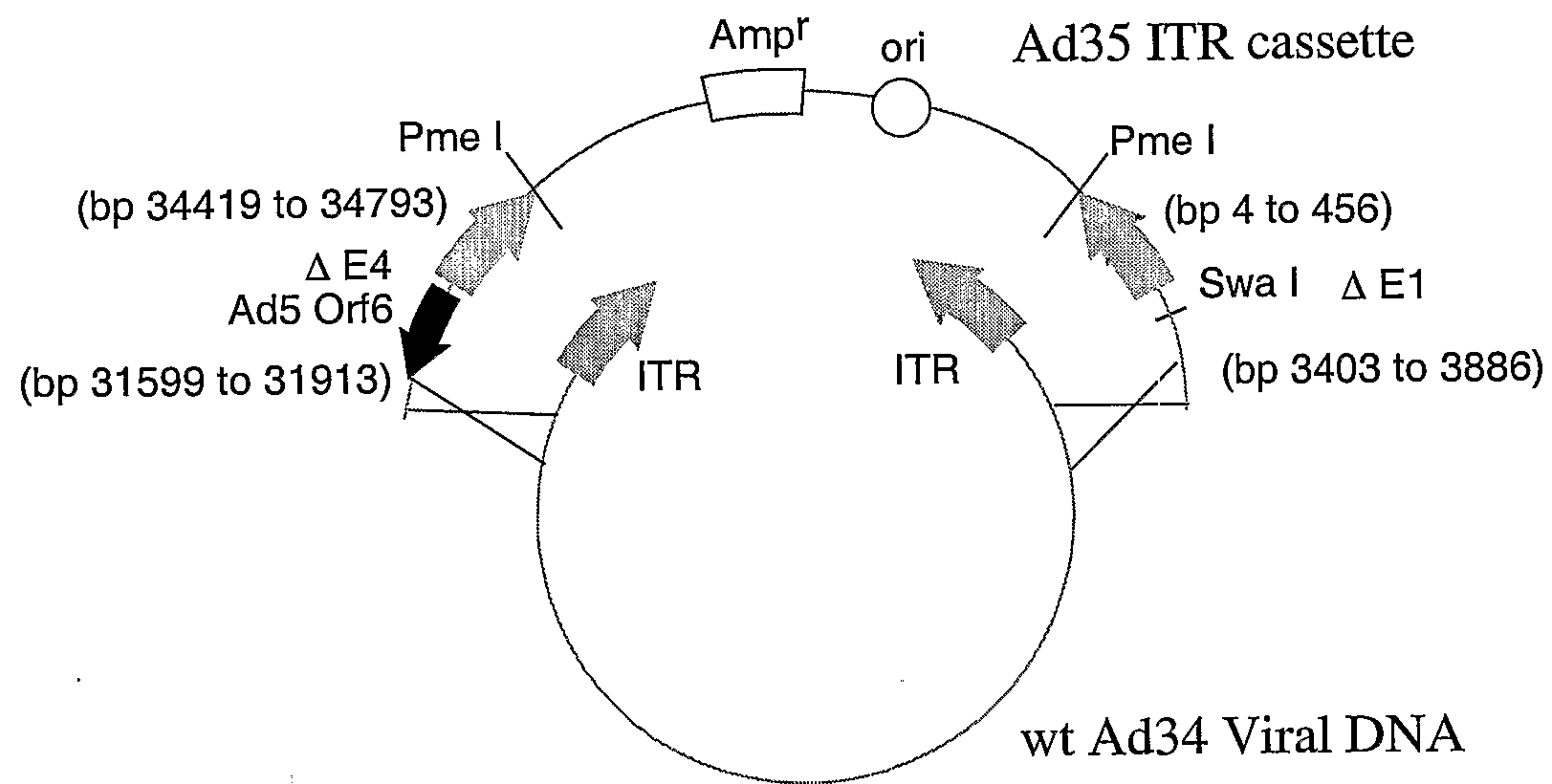


FIG. 26

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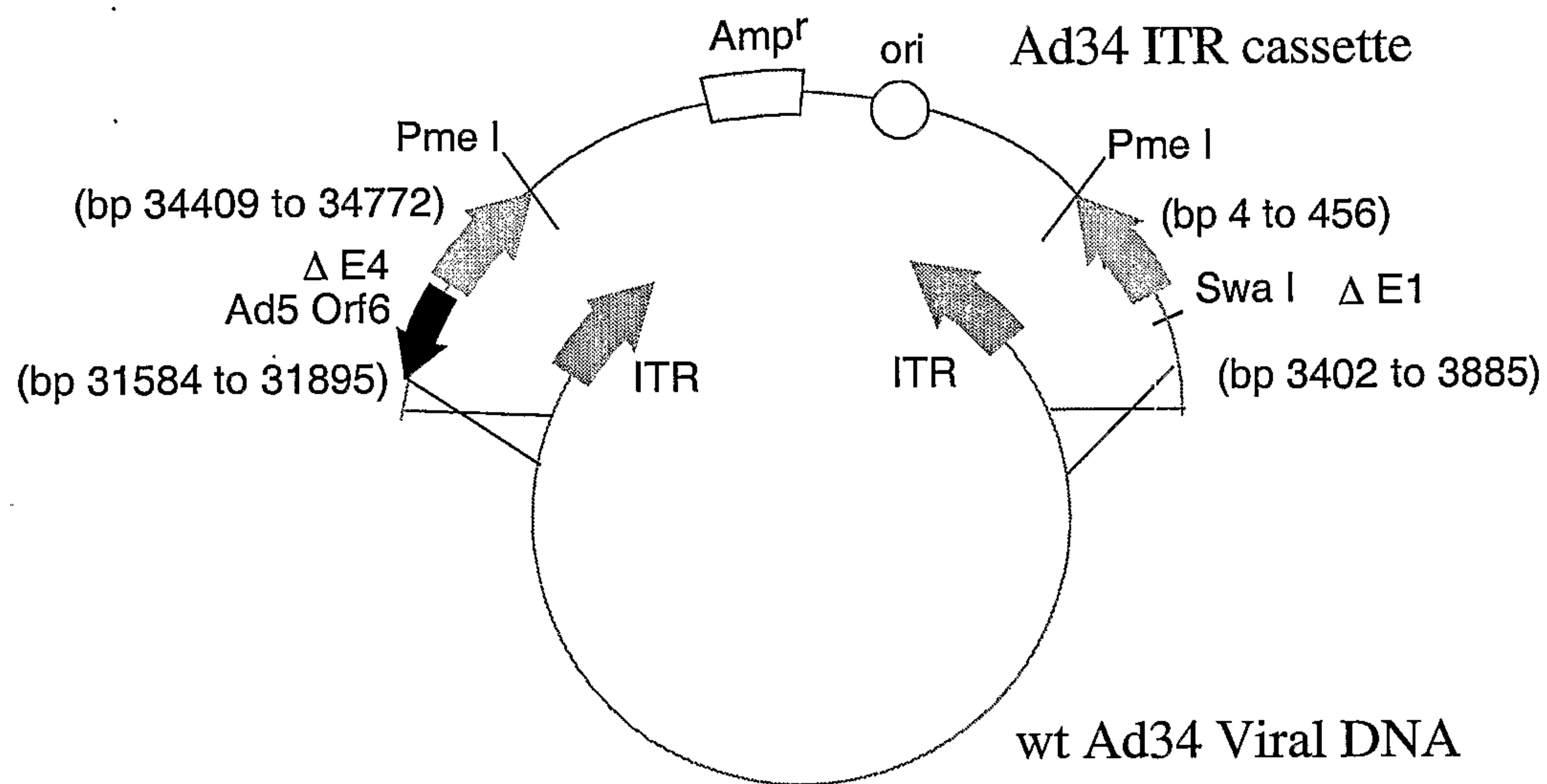


FIG. 27

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1 catcatcaat aatatacctt atagatggaa tgggtgccaat atgtaaata ggtgatttta
61 aaaattgtgg ggtgtgtggt gattggctgt ggggttaacg gctaaacggg gcggcgcggc
121 cgtgggaaaa tgacgttttg tgggggtgga gtttttttgc aagtgtcgc gggaaatgtg
181 acgcataaaa aggcctttttt tctcacggaa ctactgactt tcccacggg atttaacagg
241 aaatgaggta gttttgaccg gatgcaagtg aaaattgctg atttgcgcgc gaaaactgaa
301 tgaggaagtg tttttctgaa taatgtggta tttatggcag ggtggagtat ttgttcaggg
361 ccaggtagac tttgacccat tacgtggagg tttcgattac cgtgtttttt acctgaattt
421 ccgcgtaccg tgtcaaagtc ttctgttttt acgtagggtg cagctgatcg ctacgggtatt
481 tatacctcag ggtttgtgtc aagaggccac tcttgagtgc cagcgagaag agttttctcc
541 tctgcgccgg cagtttaata ataaaaaat gagagatttg cgatttctgc ctcaggaaat
601 aatttctgct gagactggaa atgaaatact ggagcttgtg gtgcacgcc tgatgggaga
661 cgatccggag ccacctgtgc agctttttga gcctcctacg cttcaggaa cttatgattt
721 agaggtagag ggatcggagg attctaataa ggaagctgtg aatggctttt ttaccgattc
781 tatgctttta gctgctaata aaggattaga attagatccg cctttggaca ctttcgatac
841 tccaggggtg attgtggaaa gcggtacagg tgtaagaaaa ttacctgatt tgggttccgt
901 ggactgtgat ttgcactgct atgaagacgg gtttcctccg agtgatgagg aggaccatga
961 aaaggagcag tctatgcaga ctgcagcggg tgagggagtg aaggctgcca gtgttggtt
1021 tcagttggat tgcccggagc ttcttggaac tggctgtaag tcttgtgaat ttcacaggaa
1081 aaatactgga gtaaaggaa cgtttatgtt gctttgttat atgagagcgc actgccactt
1141 tatttacagt aagtgtgttt aagttaaaat ttaaaggaa atgctgtttt tcacatgtat
1201 attgagtggg agttttgtgc ttcttattat aggtcctgtg tctgatgctg atgagtcacc
1261 atctcctgat tctactacct cacctcctga gattcaagca cctgttcctg tggacgtgcg
1321 caagcccatt cctgtgaagc ttaagcctgg gaaacgtcca gcagtggaaa aacttgagga
1381 cttgttacag ggtggggacg gacctttgga cttgagtaca cggaaacggc caagacaata
1441 agtgttccat atccgtgttt acttaagggt acgtcaatat ttgtgtgaga gtgcaatgta
1501 ataaaaatat gttaactgtt cactggtttt tattgctttt tgggcgggga ctcaggatata
1561 taagtagaag cagacctgta tggtagctc ataggagctg gctttcatcc atggaggtt
1621 gggccatttt ggaagacctt agaaagacta ggcaactgtt agaggacgct tcggacggag
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1741 aacaggacta taaagaagaa tttgaaaagt tgtaggtaga ttgcccagga ctttttgaag
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1921 tcccgcagac tcatttcagc aggggatacg ttttgattt cgtagccaca gcattgtgga
1981 gaacatggaa ggttcgcaag atgaggacaa tcttaggtta ctggccagtg cagccttttg
2041 gtgtagcggg aatcctgagg catccaccgg tcatgccagc ggttctggag gaggaacagc
2101 aagaggacaa cccgagagcc ggcctggacc ctccagtgga ggaggcggag tagctgactt
2161 gtctcctgaa ctgcaacggg tgcttactgg atctacgtcc actggacggg ataggggctg
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2461 acagtataag attactagac ggattaatat ccggaatgct tgttacatat ctggaaatgg
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3361 tggagcagag ttcggatcca gtggagaaga aactgactaa ggtgagtatt gggaaaactt
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3661 cagctgcagc cgccgccgcc gcctctgttg ccgctaacac tgtgcttggg atgggttact
3721 atggaagtat cgtggctaata tccacttctt ctaataaccc ttctaccctg actcaggaca
3781 agttacttgt ccttttggcc cagctggagg ctttgacca acgtctgggt gaactttatc
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FIG. 28A-1

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3901 aaaaaaaaaat tccacaatca atgaataaat aaacgagctt gttgttgatt taaaatcaag
3961 tgttttttatt tcattttttcg cgcacgggtat gccctagacc accgatctcg atcattgaga
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5881 ctgggggggt ataaaagggg gcggttcttt gctcttcctc actgtcttcc ggatcgctgt
5941 ccaggaacgt cagctgttgg ggtaggtatt cctctcga ggcgggcatg acctctgcac
6001 tcaggttgtc agtttctaag aacgaggagg atttgatatt gacagtgccg gttgagatgc
6061 ctttcatgag gttttcgtcc atttggtcag aaaacacaaat ttttttattg tcaagtttgg
6121 tggcaaatga tccatacagg gcgttggata aaagtthggc aatggatcgc atggttgggt
6181 tcttttctt gtccgcgcgc tctttggcag cgatgttgag ttggacatac tgcggtgcta
6241 ggcacttcca ttccgggaag atagttgtca attcatctgg cacgattctc acttgccacc
6301 ctcgattatg caaggtaatt aaatccacac tgggtggccac ctgcctcga aggggttctg
6361 tgggtccaaca gagcctacct cctttcctag aacagaaagg gggagtggtg tctagcataa
6421 gttcatcggg agggctctgca tccatggtaa agattcccgg aagtaaatcc ttatcaaat
6481 agctgatggg agtgggggtc tctaaggcca tttgccattc tcgagctgcc agtgcacgct
6541 catatgggtt aaggggactg cccaggggca tgggatgggt gagtgcagag gcatacatgc
6601 cacagatgtc atagacgtag atgggatcct caaagatgcc tatatagggt ggatagcatc
6661 gccccctct gatacttgct cgcacatagt catatagtth catatagtgg gctagcaacc
6721 ccggacccaa gttggtgcga ttgggttttt ctgttctgta gacaatctgg cgaaagatgg
6781 cgtgagaatt ggaagagatg gtgggtcttt gaaaaatgtt gaaatgggca tgaggtagac
6841 ctacagagtc tctgacaaag tgggcataag attcttgaag cttgggtacc agttcggcgg
6901 tgacaagtac gtctagggcg cagtagtcaa gtgtttcttg aatgatgtca taacctggtt
6961 ggtttttctt ttcccacagt tgcgggttga gaaggatthc ttcgcgatcc ttccagtact
7021 cttctagcgg aaaccctctt ttgtctgcac ggtaagatcc tagcatgtag aactgattaa
7081 ctgccttgta agggcagcag cccttctcta cgggtagaga gtatgcttga gcagcttttc
7141 gcagcgaagc gtgagtaagg gcgaagggtg ctctgaccat gactttgaga aattgggtatt
7201 tgaagtccat gtcgtcacag gctccctgtt cccagagttg gaagtctacc cgtttcttgt
7261 aggcgggggt gggcaaagcg aaagtaacat cgttgaagag aatcttaccg gctctgggca
7321 taaaattgcg agtgatgcgg aaaggctgtg gtacttccgc tcgattgttg atcacctggg
7381 cagctaggac gatctcgtcg aaaccgttga tgttgtgtcc tacgatgtat aattctatga
7441 aacgcggcgt gcctttgacg tgaggtagct tattgagctc atcaaagggt aggtctgtag
7501 ggtcagataa ggcgtagtgt tcgagagccc attcgtgcag gtgaggattt gcatgtagga
7561 atgatgacca aagatccacc gccagtgtg tttgtaactg gtcccagatac tgacgaaaat
7621 gctggccaat tgccattttt tctggagtga cacagtagaa ggttctgggg tcttgttgcc
7681 atcgatccca ctttagttta atggctagat cgtgggcat gttgacgaga cgctcttctc
7741 ctgagagttt catgaccagc atgaaaggaa ctagtthgtt gccaaaggac cccatccagg

FIG. 28A-2

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7801 tgtaagtttc cacatcgtag gtcaggaaga gtctttctgt gcgaggatga gagccgatcg
7861 ggaagaactg gatttcctgc caccagttgg aggattggct gttgatgtga tggaaagtaga
7921 agtttctgcg gcgcgccgag cattcgtgtt tgtgcttgta cagacggccg cagtagtcgc
7981 agcgttgcaac gggttgtatc tcgtgaatga gctgtacctg gcttcccttg acgagaaatt
8041 tcagtgggaa gccgaggcct ggcgattgta tctcgtgctc ttctatatcc gctgtatcgg
8101 cctgttcatc ttctgttttc gtggtggtca tgctgacgag ccccgccggg aggcaagtcc
8161 agacctcggc gcgggagggg cggagctgaa ggaccagagc gcgcaggctg gagctgtcca
8221 gagtccctgag acgctgcgga ctacaggttag taggtaggga cagaagatta acttgcataga
8281 tcttttccag ggcgtgcggg aggttcagat ggtacttgat ttccacaggc tcgtttgtag
8341 agatgtcaat ggcttgacag gtcccggtgc ctttgggcgc cactaccgta cctttgtttt
8401 ttcttttgat cgggtggtggc tctcttgctt cttgcatgct cagaagcgat gacggggacg
8461 cgccgccggg ggaagcgggt gtcccgacc cggaggcatg gctggtagtg gcacgtcggc
8521 gccgcgcacg ggcagggtct ggtactgcgc tctgagaaga cttgcgtgcg ccaccacgcg
8581 tcgattgacg tcttgatctt gacgtctctg ggtgaaagct accggccccc tgagcttgaa
8641 cctgaaagag agttcaacag aatcaatttc ggtatcgtta acggcagctt gtctcagtat
8701 ttcttgtagc tcaccagagt tgcctggta ggcgatctcc gccatgaact gctcgatttc
8761 ttctcctga agatctccgc gaccgctct ctcgacgggt gccgcgaggc cattggagat
8821 acggcccatg agttgggaga atgcagtcac gccgcctcg ttccagacgc ggctgtaaac
8881 cacggccccc tcggagtctc ttgcgcgcac caccacctga gcgagggtta gctccacgtg
8941 tctggtgaag accgcatagt tgcataggcg ctgaaaaagg tagttgagtg tgggtggcaat
9001 gtgttcggcg acgaagaaat acatgatcca tcgtctcagc ggcatcttcg tgacatcgcc
9061 cagagcttcc aagcgcctca tggcctcgtg gaagtccacg gcaaaattaa aaaactggga
9121 gtttcgcgcg gacacggtca attcctcctc gagaagacgg atgagttcgg ctatggtggc
9181 ccgtacttcg cgttcgaagg ctcccgggat ctcttcttcc tcttctatct cttcttccac
9241 taacatctct tcttcgtctt caggcggggg cggagggggc acacggcgac gtcgacggcg
9301 cacgggcaaa cggtcgatga atcgttcaat gacctctccg cggcggcggc gcatggtttc
9361 agtgacggcg cggccgttct cgcccggtcg cagagtaaaa acaccgccgc gcatctcctt
9421 aaagtgggta ctgggaggtt ctccgtttgg gagggagagg gcgctgatta tacattttat
9481 taattggccc gtagggactg cgccagaga tctgatcgtg tcaagatcca cgggatctga
9541 aaacctttcg acgaaagcgt ctaaccagtc acagtcacaa ggtaggctga gtacggcttc
9601 ttgtgggccc ggggtggttat gtgttcggtc tgggtcttct gtttcttctt catctcggga
9661 aggtgagacg atgctgctgg tgatgaaatt aaagtaggca gttctaagac ggcggatggt
9721 ggcgaggagc accaggtctt tgggtccggc ttgctggata cgcaggcgat tggccattcc
9781 ccaagcatta tcctgacatc tagcaagatc tttgtagtag tcttgcatga gccgttctac
9841 gggcacttct tcctcaccgc ttctgccatg catacgtgtg agtccaaacc cgccattggg
9901 ttgtaccagt gccaaagtcag ctacgactct ttccggcagg atggcttgct gtacttgggt
9961 gaggggtggc tgaaagtcac caaaatccac aaagcgggtg taagccccgg tattaatggt
10021 gtaagcacag ttggccatga ctgaccagtt aactgtctgg tgaccagggc gcacgagctc
10081 ggtgtattta aggcgcgaat aggcgcgggt gtcaaagatg taatcggtgc aggtgcgcac
10141 cagatactgg taacctataa gaaaatgcgg cgggtggttg cggtagagag gccatcgctc
10201 tgtagctgga gcgcggggg cgaggtcttc caacataagg cggtagatag cgtagatgta
10261 cctggacatc caggtgattc ctgcggcggg agtagaagcc cgaggaaact cgcgtacgcg
10321 gttccaaatg ttgcgtagcg gcatgaagta gttcattgta ggcacggttt gaccagttag
10381 gcgcgcgcag tcattgatgc tctatagaca cggagaaaaat gaaagcgttc agcgactcga
10441 ctccgtagcc tggaggaacg tgaacgggtt gggtcgcggg gtaccccggt tcgagacttg
10501 tactcgagcc ggccggagcc gcggctaacc tgggtattggc actcccgctc cgaccagcc
10561 tacaaaaatc caggatacgg aatcgagtcg ttttgctggg tgccgaatgg cagggaaagt
10621 agtcctattt tttttttttg ccgctcagat gcatcccgtg ctgcgacaga tgcgtccca
10681 acaacagccc ccctcgcagc agcagcaacc acaaaaggct gtccctgcaa ctactgcaac
10741 tgccgctgtg agcgggtgcg gacagcccgc ctatgatctg gacttggaag agggcgaagg
10801 actggcacgt ctaggtgcgc cttcgcccga gcggcatccg cgagttcaac tgaaaaaaga
10861 ttctcgcgag gcgtatgtgc cccaacagaa cctatttaga gacagaagcg gcgaggagcc
10921 ggaggagatg cgagcttccc gctttaacgc gggtcgtgag ctgcgtcacg gtttgagacg
10981 aagacgagtg ttgcgggacg aggatttcga agttgatgaa 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 tgtttctggt ggtgcaacac agcagagaca atgaggcttt
11281 cagagaggcg ctgctcaaca tcaccgaacc cgaggggaga tgggtgtatg 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 atcgccgggt
11581 gagcgccagc aggaggcgcg agttaagcga cagggaactg atgcacagtt tgcaaagagc
11641 tctaactgga gctggaaccg agggtagaaa ttactttgat atgggagctg acttgcagtg

FIG. 28A-3

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11701	gcagcctagt	cgcaggggctc	tgaacgccgc	gacggcagga	tgtgagcttc	cttacataga
11761	agaggcgat	gaaggcgagg	aggaagaggg	cgagtacttg	gaagactgat	ggcacaaccc
11821	gtgttttttg	ctagatggaa	cagcaagcac	cggatcccgc	aatgcgggcg	gcgctgcaga
11881	gccagccgtc	cggcattaac	tcctcggacg	attggaccca	ggccatgcaa	cgtatcatgg
11941	cgttgacgac	tcgcaacccc	gaagccttta	gacagcaacc	ccaggccaac	cgtctatcgg
12001	ccatcatgga	agctgtagtg	ccttcccgc	ctaataccac	tcatagagaag	gtcctggcca
12061	tcgtgaacgc	gttggtggag	aacaaagcta	ttcgtccaga	tgaggccgga	ctggtataca
12121	acgctctctt	agaacgcgtg	gctcgctaca	acagtagcaa	tgtgcaaacc	aatttggacc
12181	gtatgataac	agatgtacgc	gaagccgtgt	ctcagcgcg	aagggtccag	cgcatgcca
12241	acctgggttc	gctgggtggc	ttaaatgctt	tcttgagtac	tcagcctgct	aatgtgccgc
12301	gtgggtcaaca	ggattatact	aactttttta	gtgctttgag	actgatggta	tcagaagtac
12361	ctcagagcga	agtatatcag	tccgggtcctg	attacttctt	tcagactagc	agacagggct
12421	tgcagacggt	aaatctgagc	caagctttta	aaaaccttaa	aggtttgtgg	ggagtgcattg
12481	ccccggtagg	agaaagagca	accgtgtcta	gcttggttaac	tccgaactcc	cgcctattat
12541	tactgttggg	agctcctttc	accgacagcg	gtagcatcga	ccgtaattcc	tatttggggtt
12601	acctactaaa	cctgtatcgc	gaagccatag	ggcaaagtca	ggtggacgag	cagacctatc
12661	aagaaattac	ccaagtacgt	cgcgcttttg	gacaggaaga	cactggcagt	ttggaagcca
12721	ctctgaactt	cttgcttacc	aatcgggtctc	aaaagatccc	tcctcaatat	gctcttactg
12781	cggaggagga	gaggatcctt	agatatgtgc	agcagagcgt	gggattgttt	ctgatgcaag
12841	agggggcaac	tccgactgca	gcactggaca	tgacagcgcg	aaatatggag	cccagcatgt
12901	atgccagtaa	ccgacctttc	attaacaaac	tgctggacta	cttgacaga	gctgccgcta
12961	tgaactctga	ttatttccacc	aatgccatct	taaaccgcga	ctggctgccc	ccacctgggtt
13021	tctacacggg	cgaatatgac	atgcccgcacc	ctaatagacg	atttctgtgg	gacgacgtgg
13081	acagcgatgt	tttttccact	ctttctgatc	atcgcacgtg	gaaaaaggaa	ggcggcgata
13141	gaatgcattc	ttctgcatcg	ctgtccgggg	tcattgggtgc	taccgcggct	gagcccgagt
13201	ctgcaagtcc	ttttcctagt	ctaccctttt	ctctacacag	tgtacgtagc	agcgaagtgg
13261	gtagaataag	tcgccccgagt	ttaatgggcg	aagaggagta	cctaaacgat	tccttgctca
13321	gaccggcaag	agaaaaaat	ttcccaaaca	atggaataga	aagtttgggtg	gataaaatga
13381	gtagatggaa	gacttatgct	caggatcaca	gagacgagcc	tgggatcatg	gggactacaa
13441	gtagagcgag	ccgtagacgc	cagcgccatg	acagacagag	gggtcttgtg	tgggacgatg
13501	aggattcggc	cgatgatagc	agcgtattgg	acttgggtgg	gagaggaagg	ggcaaccctg
13561	ttgctcattt	gcgcccctcg	ttgggtggta	tggtgtaaaa	aaaaataaaa	aagaaaaaac
13621	tcaccaaggc	catggcgacg	agcgtacgtt	cgttcttctt	tattatctgt	gtctagtata
13681	atgaggcgag	tcgtgctagg	cggagcggtg	gtgtatccgg	agggctctcc	tccttcgtac
13741	gagagcgtga	tgcagcagca	gcaggcgacg	gcgggtgatg	aatccccact	ggaggctccc
13801	tttgtgcctc	cgcgatacct	ggcacctacg	gagggcagaa	acagcattcg	ttactcggaa
13861	ctggcacctc	agtacgatac	caccaggttg	tatctgggtg	acaacaagtc	ggcggacatt
13921	gcttctctga	actatcagaa	tgaccacagc	aacttcttga	ccacgggtgg	gcaaaacaat
13981	gactttaccc	ctacggaagc	cagcaccag	accatctaact	ttgatgaacg	atcgcgggtg
14041	ggcggtcagc	taaaaacat	catgcatact	aacatgccc	acgtgaacga	gtatatgttt
14101	agtaacaagt	tcaaagcgcg	tgtgatgggtg	tccagaaaac	ctcctgaggg	tgtagagta
14161	gacgataatt	atgatcataa	gcaagatatt	ctaaaatacg	agtggttcga	gtttactttg
14221	ccagaaggca	acttttccgt	cactatgact	atcgacttga	tgaacaatgc	catcatagac
14281	aattacttga	aagtgggcag	acagaatgga	gtgttggaaa	gtgacattgg	tgtaagtctc
14341	gacactagga	acttcaagtt	gggatgggat	ccagaaacta	agttgatcat	gcctgggggtt
14401	tacacctatg	aggccttcca	tcctgacatc	gtattgctgc	ctggctgcgg	agtggacttt
14461	accgaaagcc	gtctgagcaa	ccttcttggc	attagaaaga	aacacccatt	ccaagagggt
14521	tttaagatct	tgtatgagga	tttagaagga	ggaaatattc	cagccctttt	ggatgtagat
14581	gcttatgaga	acagcaagaa	agatcaaaaa	gccaaaatag	aagctgctgc	agaagctaaa
14641	gcaaacatag	ttgccaacga	tccggtaagg	gtggctaacg	ctagtgaat	caggggagac
14701	agttttgccg	caacatccgt	tccgactaaa	gaatcattat	tggatgatgt	gtctcaaaac
14761	atagagttaa	aactcactat	taagcctgtg	gaaaaagatg	gcaaaaacag	aagttacaat
14821	gtgttggaag	ataaaaatcaa	cacggcctat	cgcagttggt	acctttcgta	caattatggc
14881	gaccccgaaa	aaggagtgcg	ttcctggaca	ttgctcacca	cctcagatgt	cacctgcgga
14941	gcggagcagg	tctactggtc	gcttccagac	atgatgcagg	atcctgtcac	tttccgctcc
15001	actagacaag	tcagtaacta	ccctgtgggtg	ggtgcagagc	ttatgcccgt	cttttcaaag
15061	agcttctaca	acgaacaagc	tgtgtactcc	cagcagctcc	gccagtccac	ctcgcttacg
15121	cacgtcttca	accgcttttc	tgagaaccag	attttaatcc	gtccgcccgc	gcccacaatt
15181	accaccgtca	gtgaaaacgt	tcctgctctc	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	gcgcacgcaa	acgttctacc	caacatccc	tgcggtgtcg
15481	cgggcatttt	cgcgctccat	ggggtgccct	caagggccgc	actcgcgttc	gaaccaccgt
15541	cgatgatgta	atcgatcagg	tggttgccga	cgcgccgta	tatactccta	ctgcgcctac

FIG. 28A-4

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15601	atctactgtg	gacgcagtta	ttgacagtgt	agtggctgac	gctcgcaact	atgctcgacg
15661	taagagccgg	cgaaggcgca	ttgccagacg	tcaccgagct	accactgcca	tgcgagcagc
15721	aagagctctg	ctacgaagag	ctagacgcgt	ggggcggaaga	gccatgctta	gggcgggccag
15781	acgtgcagct	tcgggcgcca	gcgccggcag	gtcccgcagg	caagcagccg	ctgtcgcagc
15841	ggcgactatt	gccgacatgg	cccaatcgcg	aagaggcaat	gtatactggg	tgcgtgacgc
15901	tgccaccggt	caacgtgtac	ccgtgcgcac	ccgtccccct	cgcacttaga	agatactgag
15961	cagtctccga	tgttgtgtcc	cagcggcgag	gatgtccaag	cgcaaatata	aggaagaaat
16021	gctgcagggt	atcgcacctg	aagtctacgg	ccaaccgttg	aaggatgaaa	aaaaaccccg
16081	caaaatcaag	cgggtaaaaa	aggacaaaaa	agaagaggaa	gatggcgatg	atgggctggc
16141	ggagtttgtg	cgcgagtttg	ccccacggcg	acgcgtgcaa	tggcgtgggc	gcaaagttcg
16201	acatgtgttg	agacctggaa	cttcgggtgg	ctttacaccc	ggcgagcgtt	caagcgctac
16261	ttttaagcgt	tcctatgatg	aggtgtacgg	ggatgatgat	attcttgagc	aggcagctga
16321	ccgattaggg	gagtttgctt	atggcaagcg	tagtagaata	aatcccaagg	atgaaacagt
16381	gtccataccc	ttggatcatg	gaaatcccac	ccctagtctt	aaaccgggtc	ctttgcagca
16441	agtgttacct	gtaactccgc	gaacaggtgt	taaacgcgaa	ggtgaagatt	tgtatcccac
16501	tatgcaactg	atggtgcccc	aacgccagaa	ggtggaggac	gttttggaga	aagtaaaagt
16561	ggatccagat	attcaacctg	aggttaaagt	gagaccatt	aagcaggtag	cgcttgggtc
16621	gggagtacaa	actgtagaca	ttaaaatttc	cactgaaagt	atggaagtgc	aaactgaacc
16681	cgcaaagcct	actgccacct	ccactgaagt	gcaaacggac	ccatggatgc	ccatgcctat
16741	tacaactgac	gccgtcggtc	ccactcgaag	atcccagcga	aagtacggtc	cagcaagtct
16801	gttgatgccc	aactatgtcg	tacacccatc	tattattcct	actcctgggt	accgaggcac
16861	tcgctactat	cgcagccgaa	acagtacttc	ccgccgtcgc	cgcaagacac	ctgcaaatcg
16921	cagtcgtcgc	cgtagacgca	caagcaaacc	gattcccggc	gccctgggtc	ggcaagtgtg
16981	ccgcaatggg	agtgcggaac	ctttgacact	gccgcgtgcg	cgttaccatc	ctagtatcat
17041	cacttaatca	atggtgccgc	tgcctccttg	cagatatggc	cctcacttgt	cgcttcgcgc
17101	ttcccatcac	tgggtaccga	ggaagaaact	cgcgccgtag	aagaggggat	ttggggcgcg
17161	gaatgcgacg	ctacaggcga	cggcgtgcta	tccgcaagca	attgcggggg	ggttttttgc
17221	cagccttaat	tccaattatc	gctgctgcga	ttggcgcaat	accaggcata	gcttccgtgg
17281	cggttcaggc	ctcgcaacga	cattgacatt	ggaaaaaaa	aaaacgtata	aataaaaaat
17341	acaatggact	ctgacactcc	tggtagctgt	actatgtttt	cttagagatg	gaagacatca
17401	atthtttcac	cttggctccg	cgacacggca	cgaagccgta	catgggcacc	tggagcgaca
17461	tcggcacgag	ccaactgaac	gggggcgcc	tcaattggag	cagtatctgg	agcgggctta
17521	aaaatthttg	ctcaaccata	aaaacatacg	ggaacaaagc	ttggaacagc	agtacaggac
17581	aggcgcttag	aaataaactt	aaagaccaga	acttccaaca	aaaagtagtc	gatgggatag
17641	cttccggtat	caatggagtg	gtagattttg	ctaaccaggc	tgtgcagaaa	aagataaaca
17701	gtcgttttga	cccgcgcgca	gcaaccccag	gtgaaatgca	agtggaggaa	gaaattcctc
17761	cgccagaaaa	acgaggcgac	aagcgtccgc	gtcccgatth	ggaagagacg	ctgggtgacgc
17821	gcgtagatga	accgccttct	tatgaggaag	caacgaagct	tggaatgccc	accactagac
17881	cgatagcccc	tatggccacc	gggggtgatga	aacctttctc	gttgcatcga	cccgtcacct
17941	tggatthtgc	ccctcctcct	gctgctactg	ctgtaccgcg	ttctaagcct	gtcgtgcccc
18001	cgaaaccagt	cgccgtagcc	aggtcacgtc	ccggggggcg	tcctcgtcca	aatgcacact
18061	ggcaaaatac	tctgaacagc	atcgtgggtc	taggcgtgca	aagtgtaaaa	cgccgtcgtc
18121	gctthttaatt	aaatatggag	tagcgcttaa	cttgcctatc	tgtgtatatg	tgtcattaca
18181	cgccgtcaca	gcatcagagg	aaaaaaggaa	gaggtcgtgc	gtcgacgctg	agttactttc
18241	aagatggcca	ccccatcgat	gctgccccaa	tgggcataca	tgcacatcgc	cggacaggat
18301	gcttcggagt	acctgagtc	gggtctgggt	cagttcgccc	gcgccacaga	cacctacttc
18361	aatctgggaa	ataagtthtag	aaatcctacc	gtagcgccga	cccacgatgt	gaccaccgat
18421	cgtagccagc	ggctcatgtt	gcgcttcgtg	cccgttgacc	gggaggacaa	tacatactct
18481	tacaaagtgc	ggtacaccct	ggccgtgggc	gacaacagag	tgctggatat	ggccagcagc
18541	ttctthtgaca	ttagggggcg	gttgagacaga	ggtcccagtt	ttaaacccta	ttctggtagc
18601	gcttacaact	ccctggctcc	taaaggcgct	ccaatgcat	ctcagtgggt	ggataaggga
18661	gttacaagca	ctggcctagt	ggacgacggc	aatactgatg	atgggggaag	agccaaaaaa
18721	gcaacataca	ctthttggtaa	tgctccagta	aaagccgagg	ctgaaatcac	aaaagacgga
18781	ttgccgggtg	gcttggaagt	ttcaactgaa	ggctcctaac	caatctatgc	tgataagctt
18841	tatcagccag	aacctcaagt	gggagacgaa	acttggaact	acctagacgg	aaaaaccgaa
18901	gagtatggag	ggaggggttct	taaacctgaa	actaaaatga	aaccctgcta	cggatcttht
18961	gctaaacct	ctaataatga	aggaggtcag	gcaaaggtaa	aaccaaaaga	agacgatggc
19021	actaacaaca	tcgagtatga	cattgacatg	aactthctth	acttaagatc	acaaagatca
19081	gaactcaaac	ctaaaattgt	aatgtatgca	gaaaatgtgg	acctggaatg	tccagatact
19141	catgttgtgt	acaaacctgg	agthttcagat	gctagthtct	agaccaatct	tggacaacag
19201	tctatgccc	acagacccaa	ctacattggc	ttcagagata	acttcatcgg	acttatgtac
19261	tataacagta	ctggcaacat	gggggtactg	gctggccaag	cgtctcagtt	gaatgcagtg
19321	gttgactthg	aggacagaaa	cacagaactg	tcttaccac	tcttgcttga	ctctctgggc
19381	gacagaacca	gatactthtag	catgtggaat	caggctgtgg	acagthtatga	tcctgatgta
19441	cgtgttattg	aaaatcatgg	tgtggaagat	gaactthcca	actattgtht	tccgttggat

FIG. 28A-5

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19501	ggtgtcggtc	cgcgaaacaga	tagttacaag	gagattaagc	caaattggaga	ccaatctact
19561	tggacaaatg	tagacccaac	tggcagcagt	gaacttgcta	agggaaatcc	atgtgccatg
19621	gaaattaacc	ttcaagccaa	tctatggcga	agtttccttt	attccaatgt	ggctctatat
19681	ctcccagact	cgtacaaata	caccccgctc	aatgtcactc	ttccagaaaa	caaaaacacc
19741	tacgactaca	tgaacgggcg	ggtgggtgcc	ccatctctag	tagacaccta	tgtgaacatt
19801	ggtgccaggt	ggtctctgga	tgccatggac	aatgtcaacc	cattcaacca	ccaccgtaac
19861	gctggcttgc	gttaccgatc	catgcttctg	ggtaacggac	gttatgtgcc	tttccacata
19921	caagtgcctc	aaaaattctt	cgctgttaaa	aacctgctgc	ttctcccagg	ctcctacact
19981	tatgagtgga	actttaggaa	ggatgtaaac	atggttctac	agagttccct	cggtaacgac
20041	ctacgggtag	atggcgccag	catcagtttt	acgagcatca	acctctatgc	tacttttttc
20101	cccatggctc	acaacaccgc	ttccaccctt	gaagccatgc	tgcggaatga	caccaatgat
20161	cagtcattca	acgactacct	atctgcagct	aacatgctct	acccatttcc	tgccaatgca
20221	accaatattc	ccattttccat	tcctttctcgc	aactggggcgg	ctttcagagg	ctgggtcattt
20281	accagactga	aaaccaaaga	aactccctct	ttgggggtctg	gatttgaccc	ctacttcgtc
20341	tattctgggt	ctattcccta	cctggatggt	accttctacc	tgaaccacac	ttttaagaag
20401	gtttccatca	tgtttgactc	ttcagtgage	tggcctggaa	atgacaggtt	actatctcct
20461	aacgaatttg	aaataaagcg	cactgtggat	ggcgaaggct	acaacgtagc	ccaatgcaac
20521	atgaccaaag	actggttctt	ggtacagatg	ctcgccaact	acaacatcgg	ctatcagggc
20581	ttctacattc	cagaaggata	caaagatcgc	atgtattcat	ttttcagaaa	cttcagagcc
20641	atgagcaggc	aggtggttga	tgaggatcaat	tacaaagact	tcaaggccgt	cgccataccc
20701	taccaacaca	acaactctgg	ctttgtgggt	tacatggctc	cgaccatgcg	tcaagggtcaa
20761	ccctatcccg	ctaactatcc	ctatccactc	attggaacaa	ctgccgtaaa	tagtggttacg
20821	cagaaaaagt	tcttgtgtga	cagaaccatg	tggcgcatac	cgttctcaag	caacttcatg
20881	tctatgggag	cccttacaga	cttgggacag	aacatgctct	atgccaaactc	agctcatgct
20941	ctggacatga	cctttgaggt	ggatcccacg	gatgagccca	ccctgcttta	tcttctcttc
21001	gaagttttcg	acgtgggtcag	agtgcacacg	ccacaccgcg	gcatcatcga	ggcagtcctac
21061	ctgcgtacac	cgttctcggc	cggtaacgct	accacgtaag	aagcttcttg	cttcttgcaa
21121	acagcagctg	caacctatggc	ctgcggatcc	caaaacggct	ccagcgagca	agagctcaga
21181	gccattgtcc	aagacctggg	ttgcggacca	tatttttttg	gaacctttga	taagcgcttc
21241	ccgggggttca	tggcccccca	taagctcgcc	tgtgccattg	taaatacggc	cggacgtgag
21301	acgggggggag	agcactgggt	ggctttcggg	tggaaaccac	gttctaacac	ctgctacctt
21361	tttgatcctt	ttggattctc	ggatgatcgt	ctcaaacaga	tttaccagtt	tgaatatgag
21421	ggtctcctgc	gccgcagcgc	tcttgctacc	aaggaccggt	gtattacgct	ggaaaaatct
21481	accagaccg	tgcagggccc	ccgttctgcc	gcctgcggac	ttttctgctg	catgttcttc
21541	catgcctttg	tgcactggcc	tgaccgtccc	atggacggaa	acccaccat	gaaattgcta
21601	actggagtgc	caaacaacat	gcttcattct	cctaaagtcc	agcccaccct	gtgtgacaat
21661	caaaaagcac	tctaccattt	tctcaatacc	cattcgcctt	attttcgctc	tcatcgtaca
21721	cacatcgaaa	gggccactgc	gttcgaccgt	atggatgtgc	aataatgatt	catgtaaaca
21781	acgtgttcaa	taaacagcac	tttatttttt	acatgtatcg	aggctctgga	ttacttattt
21841	atttacaagt	cgaatgggtt	ctgacgagaa	tcagaatgac	ccgcaggcag	tgatacgttg
21901	cggaaactgat	acttgggttg	ccacttgaat	tcgggaatca	ccaacttggg	aaccggtata
21961	tcgggcagga	tgtcactcca	cagctttctg	gtcagctgca	aagctcccag	caggtcagga
22021	gccgaaatct	tgaaatcaca	attaggacca	gtgctctgag	cgcgagagtt	gcggtacacc
22081	ggattgcagc	actgaaacac	catcagcgcg	ggatgtctta	cgcttgccag	cacggtggga
22141	tctgcaatca	tgcccacatc	cagatcttca	gcattggcaa	tgctgaacgg	ggtcatcttg
22201	caggtctgcc	tacccatggc	gggcacccaa	ttaggcttgt	ggttacaatc	gcagtgcagg
22261	gggatcagta	tcatcttggc	ctgatcctgt	ctgattcctg	gatacacggc	tctcatgaaa
22321	gcatcatatt	gcttgaaagc	ctgctgggct	ttactaccct	cggtataaaa	catcccgcag
22381	gacctgctcg	aaaactgggt	agctgcgcag	ccggcatcat	tcacacagca	gcgggcgtca
22441	ttgttggcta	tttgaccac	acttctgccc	cagcggtttt	gggtgatttt	ggttcgctcg
22501	ggattctcct	tcaaggctcg	ttgtccgttc	tcgctggcca	catccatctc	gataatctgc
22561	tccttctgaa	tcataatatt	gccatgcaag	cacttcagct	tgccctcata	atcattgcag
22621	ccatgaggcc	acaacgcaca	gcctgtacat	tcccaattat	ggtgggcgat	ctgagaaaaa
22681	gaatgtatca	ttccctgcag	aaatcttccc	atcatcgtgc	tcagtgtctt	gtgactagtg
22741	aaagttaact	ggatgcctcg	gtgctcctcg	ttcacgtact	ggtgacagat	gcgcttgat
22801	tgttcgtgct	gctcaggcat	tagtttaaaa	gaggttctaa	gttcgttatc	cagcctgtac
22861	ttctccatca	gcagacacat	cacttccatg	cctttctccc	aagcagacac	caggggcaag
22921	ctaatacgat	tcttaacagt	gcaggcagca	gctcctttag	ccagagggtc	atctttggcg
22981	atcttctcaa	tgcttctttt	gccatccttc	tcaacgatgc	gcacgggcgg	gtagctgaaa
23041	cccactgcta	caagttgcgc	ctcttctctt	tcttcttcgc	tgtcttgact	gatgtcttgc
23101	atggggacat	gtttggctct	ccttggcttc	tttttcgggg	gtatcggagg	aggaggactg
23161	tcgctccggt	ccggagacag	ggaggattgt	gacgtttcgc	tcaccattac	caactgactg
23221	tcggtagaag	aacctgaccc	cacacggcga	caggtgtttc	tcttcggggg	cagaggtgga
23281	ggcgattgcg	aagggtgcg	gtccgacctg	gaaggcggat	gactggcaga	accccttccg
23341	cgttcggggg	tgtgctccct	gtggcggtcg	cttaactgat	ttccttcgcg	gctggccatt

FIG. 28A-6

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23401 gtgtttctcct aggcagagaa acaacagaca tggaaactca gccattgctg tcaacatcgc
23461 caccgagtgcc atcacatctc gtcctcagcg acgaggaaaa ggagcagagc ttaagcattc
23521 caccgcccag tcctgccacc acctctaccc tagaagataa ggaggctcgac gcattctcatg
23581 acatgcagaa taaaaaagcg aaagagtctg agccagacat cgaacaagac ccgggctatg
23641 tgacaccggt ggaacacgag gaagagtga aacgctttct agagagagag gatgaaaact
23701 gcccaaaaca gcaagcggat aactatcacc aagatgctgg aaatagggat cagaacaccg
23761 actacctcat agggcttgac ggggaagacg cgctccttaa acatctagca agacagtcac
23821 tcatagtcaa ggatgcatta ttggacagaa ctgaagtgcc catcagtgtc gaagagctca
23881 gccgcgccta cgagcttaac ctattttcac ctctactcc ccccaaactg cagccaaacg
23941 gcacctgcga gccaaatcct cgcttaaaact tttatccagc ttttgctgtg ccagaagtac
24001 tggctaccta tcacatcttt tttaaaaatc aaaaaattcc agtctcctgc cgcgctaata
24061 gcaccgcgcg cgatgcccta ctcaatctgg gacctgggtc acgcttacct gatatagctt
24121 ccttgggaaga ggttccaaag atcttcgagg gtctgggcaa taatgagact cgggcccga
24181 atgctctgca aaagggagaa aatggcatgg atgagcatca cagcgttctg gtggaattgg
24241 aaggcgataa tgccagactc gcagtactca agcgaagcgt cgaggtcaca cactttgcat
24301 acccgcgtgt caacctgccc cctaaagtca tgacggccgt catggaccag ttactcatta
24361 agcgcgcaag tcccctttca gaagacatgc atgaccaga tgcctgtgat gagggtaa
24421 cagtggtcag tgatgagcag ctaaccgat ggctgggcac cgactctccc cgggatttgg
24481 aagagcgtcg caagcttatg atggccgtgg tgctgggttac cgtagaacta gagtgtcttc
24541 ggcgtttctt taccgattca gaaaccttgc gcaaactcga agagaatctg cactacactt
24601 ttagacacgg ctttgtgcgg caggcatgca agatatctaa cgtggaactc accaacttgg
24661 tttcctacat gggatattct catgagaatc gcctaggaca aagcgtgctg cacagcacc
24721 ttaaggggga agcccgccgt gattacatcc gcgattgtgt ttatctctac ctgtgccaca
24781 cgtggcaaac cggcatgggt gtatggcagc aatgtttaga agaacagaac ctgaaagagc
24841 taaacaagct cttacagaaa tctcttaagg ttctgtggac agggttcgac gagcgcaccg
24901 tcgcttccga cctggcagac ctcatcttcc cagagcgtct cagggttact ttgcgaaacg
24961 gactgcctga ctttatgagc cagagcatgc ttaacaattt tcgctcttcc atcctggaac
25021 gctccggtat cctgcccgcc acctgctgcg cactgccctc cgactttgtg cctctcacct
25081 accgcgaatg cccccgcgg ctatggagtc actgctacct gttccgtctg gccaactacc
25141 tctcctacca ctccgatgtg atcgaggatg tgagcggaga cggcttgctg gagtgtcact
25201 gccgctgcaa tctgtgcacg cccaccgggt ccctagcttg caacccccag ttgatgagcg
25261 aaaccagat aataggcacc tttgaattgc aaggccccag cagccaaggc gatgggtctt
25321 ctccctggga aagtttaaaa ctgaccccg gactgtggac ctccgcctac ttgcgcaagt
25381 ttgccccgga agattaccac ccctatgaaa tcaagttcta tgaggaccaa tcacagcctc
25441 cgaaagccga actttcggcc tgcgtcatca cccagggggc aattctggcc caattgcaag
25501 ccatccaaaa atcccgccaa gaatttctac tgaaaaaggg taaggggggtc taccttgacc
25561 cccagaccgg cgaggaactc aacacaaggt tccctcagga tgtcccaacg acgagaaagc
25621 aagaagttga aggtgcagcc gccgccccca gaagatatgg aggaagattg ggacagtcag
25681 gcagaggaag cggaggagga ggacagtctg gaggacagtc tggaggaaga cagtgtggag
25741 gaggaaaacg aggaggcaga ggaggtggaa gaagtaaccg cgcagaaaca gttatcctcg
25801 gctgcggaga caagcaacag cgctaccatc tccgctccga gtcgaggaac ccggcggcgt
25861 cccagcagta gatgggacga gaccggacgc ttcccgaacc caaccagcgc ttccaagacc
25921 ggtaagaagg atcggcaggg atacaagtcc tggcgggggc ataagaatgc catcatctcc
25981 tgcttgcatg agtgcggggg caacatatcc ttacgcggc gctacttgct attccaccat
26041 ggggtgaact ttccgcgcaa tgttttgcac tactaccgtc acctccacag cccctactat
26101 agccagcaaa tcccggcagt ctgcacagat aaagacagcg gcggcgacct ccaacagaaa
26161 accagcagcg gcagttagaa aatacacaac aagtgcagca acaggaggat taaagattac
26221 agccaacgag ccagcgcaaa cccgagagtt aagaaatcgg atctttccaa ccctgtatgc
26281 catcttccag cagagtccgg agaagtgtgt tgtatcacia gagcgaagat caacttcagc
26341 ttcgctcacc gctctcttca acaagtactg cgcgctgact cttaaagagt aggcagcgac
26401 ggacgccgag gctctcttca ggaattaca tcatcctcga catgagtaaa gaaattccca
26461 cgcgcttatt caaaaaaggg gtggagttat cagcccaaaa ggcaggcgcc tcccaggact
26521 cgccttacat gtggagttat ctgagcgccg ggccttctat gatttctcga gttaatgata
26581 actccacccg catgaattgg atacttttgg aacagtcagc tcttaccacc acgccccgcc
26641 tacgcgccta ccgaaaccaa atacttttgg tggcccgcgg ccctagtgtg ccaggaaagt cccgctccca
26701 aacaccttaa tcccagaaat gagccccagg ccgaagtcca aatgactaat gcagggtgcgc
26761 ccactgtatt acttcctcga ctatgtcgtc acaggcctcg gcataatata aaacgcctga
26821 agttagcggg cggctccacc cagctcaacg acgagtcggt gagctctccg cttgggtctac
26881 tgatcagagg cagaggtatc attgccggct gcgggagatc ttccttcacc cctcgtcagg
26941 gaccagacgg aatctttcag tcgtcttctgc aaccccgctc gggcggaatc gggaccgttc
27001 ctgttctgac tttggaaagt cctctgtctt acttcaaccc cttctccgga tctcctgggc
27061 aatttgtgga ggagtttact ccgaacttcg acgcgattag cgagtcagtg gacggctacg
27121 actaccgga ggtgacgcgg ctgagctatc tcggctgcga catctagacc actgccgcgg
27181 attgatgtct ggtgacgcgg aactcattga gttcatctac ttcgaactcc ccaaggatca

FIG. 28A-7

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27301 ccctcaaggt ccggcccacg gagtgcggat tactatcgaa ggcaaaatac actctcgcct
27361 gcaacgaatt ttctcccagc ggcccgtgct gatcgagcga gaccagggaa acaccacggt
27421 ttccatctac tgcatttgta atcaccccggt attgcatgaa agcctttgct gtcttatgtg
27481 tactgagttt aataaaaact gaattaagac tctcctacgg actgccgctt cttcaacccg
27541 gatttttacia ccagaagaac gaaacttttc ctgtcgtcca ggactctggt aacttcacct
27601 ttctactca caaactagaa gctcaacgac tacaccgctt ttccagaagc attttcccta
27661 ctaatactac tttcaaaacc ggaggtgagc tccaaggtct tctacagaa aacccttggg
27721 tggaagcggg ccttgtagtg ctaggaattc ttgcgggtgg gcttggtgatt attctttgct
27781 acctatacac accttgcttc actttcctag tgggtgtgtg gtattggttt aaaaaatggg
27841 gcccatacta gtcttgcttg ttttactttc gcttttgtaa ccgggttctg ccaattacga
27901 tccatgtcta gacttcgacc cagaaaactg cacacttact tttgcacccg acacaagccg
27961 catctgtgga gttcttatta agtgcggatg ggaatgcagg tccgttgaaa ttacacacaa
28021 taacaaaacc tggaacaata ccttatccac cacatgggag ccaggagttc ccgagtggta
28081 cactgtctct gtccgaggtc ctgacgggtc catccgcatt agtaacaaca ctttcatttt
28141 ttctgaaatg tgcgatctgg ccatgttcat gagcaaacag tattctctat ggcctcctag
28201 caaggacaac atcgtaacgt tctccattgc ttattgcttg tgcgcttgcc ttcttactgc
28261 tttactgtgc gtatgcatac acctgcttgt aaccactcgc atcaaaaacg ccaataacaa
28321 agaaaaaatg ccttaacctc tttctgttta cagacatggc ttctcttaca tctctcatat
28381 ttgtcagcat tgtcactgcc gctcacggac aaacagtcgt ctctatccct ctaggacata
28441 attacactct cataggacct ccaatcactt cagaggtcat ctggaccaa ctgggaagcg
28501 ttgattactt tgatataatc tgcaacaaaa caaaaccaat aatagtaact tgcaacatac
28561 aaaatcttac attgattaat gttagcaaag tttacagcgg ttactattat gggtatgaca
28621 gatacagtag tcaatataga aattacttgg ttcgtgttac ccagttaaaa accacgaaaa
28681 tgccaaatat ggcaaagatt cgatccgatg acaattctct agaaactttt acatctccca
28741 ccacacccga cgaaaaaac atcccagatt caatgattgc aattgttgca gcggtggcag
28801 tgggtgatggc actaataata atatgcattc ttttatatgc ttgtcgtac aaaaagtttc
28861 atcctaaaaa acaagatctc ctactaaggc ttaacattta atttcttttt atacagccat
28921 ggtttccact accacattcc ttatgcttac tagtcttgca actctgactt ctgctcgtc
28981 acacctcact gtaactatag gctcaaactg cacactaaaa ggacctcaag gtggtcatgt
29041 cttttgggtgg agaatatatg acaatggatg gtttataaaa ccatgtgacc aacctggtag
29101 atttttctgc aacggcagag acctaacctat tatcaacgtg acagcaaatg acaaaggctt
29161 ctattatgga accgactata aaagtagttt agattataac attattgtac tgccatctac
29221 cactccagca ccccgacaaa ctactttctc tagcagcagt gtcgctaaca atacaatttc
29281 caatccaacc tttgccgcgc ttttaaaacg cactgtgaat aattctacaa cttcacatac
29341 aacaatttcc acttcaacaa tcagcattat cgctgcagtg acaattggaa tatctattct
29401 tgtttttacc ataacctact acgcctgctg ctatagaaaa gacaaacata aaggtgatcc
29461 attacttaga tttgatattt aatttgttct tttttttttt atttacagta tgggtgaacac
29521 caatcatggt acctagaaat ttcttcttca ccatactcat ttgtgcattt aatgtttgcg
29581 ctactttcac agcagtagcc acagcaaccc cagactgtat aggagcattt gcttcctatg
29641 cactttttgc ttttggttact tgcactctgc tatgtagcat agtctgcctg gttattaatt
29701 ttttccaact tctagactgg atccttgtgc gaattgccta cctgcgccac gttatccgaat
29761 accgcaacca aaatatcgcg gcacttctta gactcatcta aaacctgca ggctatacta
29821 ccaatatattt tgcttctatt gcttccctac gctgtctcaa cccagctgc ctatagtact
29881 ccaccagaac acctagaaa atgcaaatte caacaaccgt ggtcatttct tgcttgctat
29941 cgagaaaaat cagaaattcc cccaaattta ataattgatt ctggaataat taatatatac
30001 tgttgaccca taatttcatt tttgatatac cccctatttg attttggctg gaatgctccc
30061 aatgcacatg atcatccaca agaccagag gaacacattc ccctacaaaa catgcaacat
30121 ccaatagcgc taatagatta cgaaagtga aacactcacc cactactccc tgctattagt
30181 tacttcaacc taaccggcgg agatgactga acactcacc acctccaatt ccgccgagga
30241 tctgctcgat atggacggcc gctctcaga acagcgactt gcccaactac gcatccgcca
30301 gcagcaggaa cgcgcgcca aagagctcag agatgtcatc caaattcacc aatgcaaaaa
30361 aggcatattc tgtttggtaa aacaagccaa gatatactac gagatcaccg ctactgacca
30421 tcgcctctct tacgaacttg gcccccaacg acaaaaattt acctgcatgg tgggaatcaa
30481 ccccatagtt atcacccagc aaagtggaga tactaagggg tgcattcact gctcctgcga
30541 ttccatcgag tgcacctaca ccctgctgaa gaccctatgc ggcctaagag acctgctacc
30601 aatgaattaa aaaatgatta ataaaaaatc acttacttga aatcagcaat aaggtctctg
30661 ttgaaatttt ctcccagcag cacctcactt ccctcttccc aactctggta ttctaaccct
30721 cgttcagcgg catactttct ccatacttta aaggggatgt caaatttttag ctctctcct
30781 gtaccacaa tcttcatgtc tttcttccca gatgaccaag agagtccggc tcagtactc
30841 cttcaaccct gtctaccct atgaagatga aagcacctcc caacaccct ttataaaccct
30901 agggtttatt tccccaaatg gcttcacaca aagcccagac ggagttctta ctttaaaatg
30961 ttttaacccta ctaacaacca caggcggatc tctacagcta aaagtgggag ggggacttac
31021 agtggatgac actgatggta ccttacaaga aaacatacgt gctacagcac ccattactaa
31081 aaataatcac tctgtagaac tatccattgg aaatggatta gaaactcaaa acaataaact
31141 atgtgccaaa ttgggaaatg ggttaaaatt taacaacggt gacatttgta taaaggatag

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FIG. 28A-8

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31201 tattaacacc ttatggactg gaataaaccc tccacctaac tgtcaaattg tggaaaacac
31261 taatacaaat gatggcaaac ttacttttagt attagtaaaa aacggagggc ttgttaatgg
31321 ctacgtgtct ctagttgggtg tatcagacac tgtgaaccaa atgttcacac aaaagacagc
31381 aaacatccaa ttaagattat attttgactc ttctggaaat ctattaactg atgaatcaga
31441 cttaaaaatt ccacttaaaa ataaatcttc tacagcgacc agtgaaactg tagccagcag
31501 caaagccttt atgccaagta ctacagctta tcccttcaac accactacta gggatagtga
31561 aaactacatt catggaatat gttactacat gactagttaa gatagaagtc tatttccctt
31621 gaacatttct ataattgctaa acagccgtat gatttcttcc aatgttgcct atgccatata
31681 atttgaatgg aatctaaatg caagtgaatc tccagaaagc aacatagcta cgctgaccac
31741 atcccccttt ttcttttctt acattacaga agacgacaac taaaataaag ttttaagtgtt
31801 tttattttaa atcacaaaat tctgagtagt attttgcctc caccttccca tttgacagaa
31861 tacaccaatc tctccccacg cacagcttta aacatttgga taccattaga gatagacatt
31921 gtttttagatt ccacattcca aacagtttca gagcgagcca atctgggggtc agtgatagat
31981 aaaaatccat cgcgatagtc ttttaaagcg ctttcacagt ccaactgctg cggatgcgaa
32041 tccggagtct ggatcacggt catctggaag aagaacgatg ggaatcataa tccgaaaacg
32101 gtatcggacg attgtgtctc atcaaaccca caagcagccg ctgtctgcgt cgctccgtgc
32161 aactgctgtt tatgggatca ggggtccacag tgtcctgaag catgatttta atagccctta
32221 acatcaactt tctgggtgca tgcgcgcagc aacgcattct gatttcactc aaatctttgc
32281 agtaggtaca acacattatt acaatattgt ttaataaacc ataattaaaa gcgctccagc
32341 caaaactcat atctgatata atcgccctg catgaccatc ataccaaagt ttaataataa
32401 ttaaatgacg ttccctcaaa aacacactac ccacatacat gatctctttt ggcatgtgca
32461 tattaacaat ctgtctgtac catggacaac gttgggttaat catgcaaccc aatataacct
32521 tccggaacca cactgccaac accgctcccc cagccatgca ttgaagtga cctgtctgat
32581 tacaatgaca atgaagaacc caattctctc gaccgtgaat cacttgagaa tgaaaaatat
32641 ctatagtggc acaacataga cataaatgca tgcattcttct cataattttt aactcctcag
32701 gatttagaaa catatcccag ggaataggaa gctcttgtag aacagtaaag ctggcagaac
32761 aaggaagacc acgaacacaa cttacactat gcatagtcat agtatcaca tctggcaaca
32821 gcgggtgggtc ttcagtcata gaagctcggg tttcattttc ctcacaacgt ggtaactggg
32881 ctctgggtgta aggggtgatgt ctggcgcag atgtcgagcg tgcgcgcaac cttgtcataa
32941 tggagttgct tcctgacatt ctctgatttt gtatagcaaa acgcggccct ggcagaacac
33001 actcttcttc gccttctatc ctgcccgtta gctgtttccg tgtgatagtt caagtacagc
33061 cacactctta agttgggtcaa aagaatgctg gcttcagttg taatcaaaac tccatcgcat
33121 ctaattgttc tgaggaaatc atccacggta gcatatgcaa atcccaacca agcaatgcaa
33181 ctggattgctg tttcaagcag gagaggagag ggaagagacg gaagaacat gtttaattttt
33241 attccaaacg atctcgcagt acttcaaatt gtagatcgcg cagatggcat ctctcgcccc
33301 cactgtgttg gtgaaaaagc acagctaaat caaaagaaat gcgattttca aggtgctcaa
33361 cgggtggcttc caacaaagcc tccacgcgca catccaagaa caaaagaata ccaaagaag
33421 gagcattttc taactcctca atcatcatat tacattcctg caccattccc agataatttt
33481 cagctttcca gccttgaatt attcgtgtca gttcttgtgg taaatccaat ccacacatta
33541 caaacaggtc ccggagggcg ccctccacca ccattcttaa acacaccctc ataatagcaa
33601 aatatcttgc tcctgtgtca cctgtagcga attgagaatg gcaacatcaa ttgacatgcc
33661 cttggctcta agttcttctt taagttctag ttgtaaaaac tctctcatat tatcaccaaa
33721 ctgcttagcc agaagccccc cggaacaag agcaggggac gctacagtgc agtacaagcg
33781 cagacctccc caattggctc cagcaaaaac aagattggaa taagcatatt ggggaaccgc
33841 agtaatatca tcgaagttgc tggaaatata atcaggcaga gtttcttgta aaaattgaat
33901 aaaagaaaaa tttgccaaaa aaacattcaa aacctctggg atgcaaatgc aataggttac
33961 cgcgctgcgc tccaacattg ttagttttga attagtctgc aaaaataaaa aaaaaaaca
34021 gcgtcatatc atagtagcct gacgaacagg tggataaatc agtctttcca tcacaagaca
34081 agccacaggg tctccagctc gaccctcgta aaacctgtca tgggtattaa acaacagcac
34141 cgaaagtccc tcgcggtgac cagcatgaat aattcttgat gaagcataca atccagacat
34201 gtttagcatca gttaacgaga aaaaacagcc aacatagcct ttgggtataa ttatgcttaa
34261 tcgtaagtat agcaaagcca cccctcgcg atacaaagta aaaggcacag gagaataaaa
34321 aatataatta tttctctgct gctgttcagg caacgtcgcc cccggtcctt ctaaatacac
34381 atacaaagcc tcatcagcca tggcttacca gacaaagtac agcgggcacg cacaagctct
34441 aaagtactc tccaacctct ccacaatata tatacacaag ccctaaactg acgtaatggg
34501 agtaaagtgt aaaaaatccc gccaaaccca acacacaccc cgaaactgcg tcaccaggga
34561 aaagtacagt ttcacttccg caatcccaac aagcgtcact tcctctttct cacggtacgt
34621 cacatcccat taacttgcaa cgtcattttc ccacggccgc gccgccccgt ttagccgtta
34681 accccacagc caatcaccac acaccccaca attttttaaaa tcacctcatt tacatattgg
34741 caccattcca tctataaggt atattattga tgatg

SEQ ID NO: 12

FIG. 28A-9

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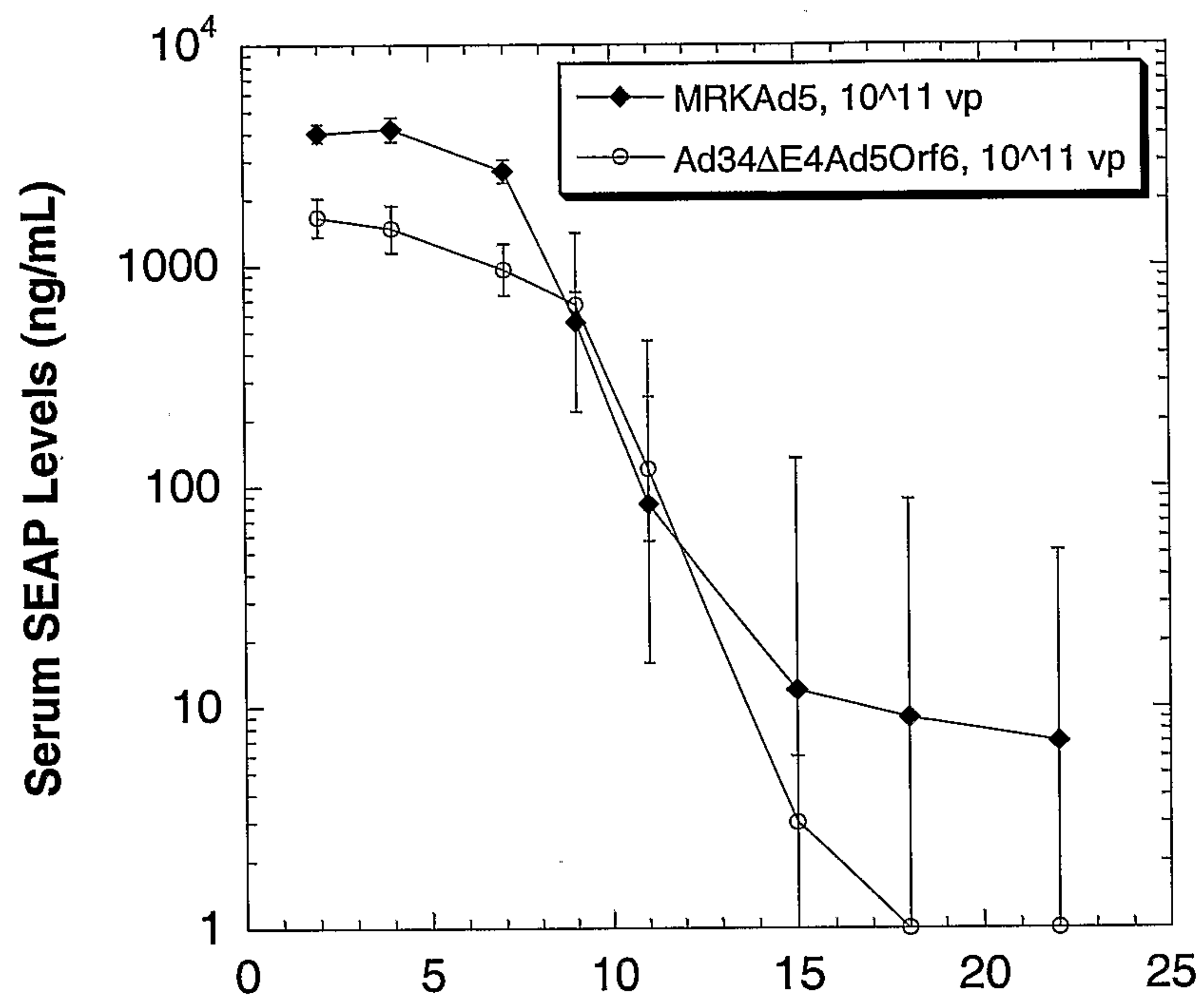


FIG. 29

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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	00D066	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
Ad34 Δ E1gag Δ E4Ad5Orf6	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 ⁴ 11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ⁴ 10 vp	00D016	4	6	1	84	5	334	5	99	0	306	3	244
Ad34ΔE1gagΔE4Ad5Orf6, 10 ⁴ 11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ⁴ 10 vp	00D044	1	1	8	79	0	374	8	136	0	493	1	253
Ad34ΔE1gagΔE4Ad5Orf6, 10 ⁴ 11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10 ⁴ 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