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(54) Title: ADENOVIRUS POLYNUCLEOTIDES AND POLYPEPTIDES

(57) Abstract: There is provided inter alia an isolated polynucleotide, wherein the polynucleotide encodes a polypeptide selected from the group consisting of: (a) a polypeptide having the amino acid sequence according to SEQ ID NO: 1, (b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1, and (c) a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

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ADENOVIRUS POLYNUCLEOTIDES AND POLYPEPTIDES

FIELD OF THE INVENTION

The present invention relates to isolated polynucleotide and polypeptide sequences derived from novel chimp adenovirus ChAd155, as well as to recombinant polynucleotides, vectors, adenoviruses, cells and compositions comprising said polynucleotide and polypeptide sequences.

BACKGROUND OF THE INVENTION

Adenovirus has been widely used for gene transfer applications due to its ability to achieve highly efficient gene transfer in a variety of target tissues and large transgene capacity. Conventionally, E1 genes of adenovirus are deleted and replaced with a transgene cassette consisting of the promoter of choice, cDNA sequence of the gene of interest and a poly A signal, resulting in a replication defective recombinant virus.

Recombinant adenoviruses are useful in gene therapy and as vaccines. Viral vectors based on chimpanzee adenovirus represent an alternative to the use of human derived Ad vectors for the development of genetic vaccines. Adenoviruses isolated from chimpanzees are closely related to adenoviruses isolated from humans as demonstrated by their efficient propagation in cells of human origin. However, since human and chimp adenoviruses are close relatives, serologic cross reactivity between the two virus species is possible.

There is a demand for vectors which effectively deliver molecules to a target and minimize the effect of pre-existing immunity to selected adenovirus serotypes in the population. One aspect of pre-existing immunity that is observed in humans is humoral immunity, which can result in the production and persistence of antibodies that are specific for adenoviral proteins. The humoral response elicited by adenovirus is mainly directed against the three major structural capsid proteins: fiber, penton and hexon.

Vectors, compositions and methods of the present invention may have one or more following improved characteristics over the prior art, including but not limited to higher productivity, improved immunogenicity and increased transgene expression.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an isolated recombinant adenoviral polynucleotide, wherein the polynucleotide encodes a polypeptide comprising: (a) a polypeptide having the amino acid sequence according to SEQ ID NO: 3; (b) a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and (c) a polypeptide selected from the group consisting of (i) a polypeptide having the amino acid sequence according to SEQ ID NO: 1; and (ii) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1; wherein the polynucleotide comprises a mutation or deletion which renders non-functional at least one gene of an adenoviral genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

In a further aspect, the present invention provides an isolated recombinant adenoviral polynucleotide, wherein the polynucleotide encodes a polypeptide comprising: (a) a polypeptide having the amino acid sequence according to SEQ ID NO: 1; (b) a polypeptide having the amino acid sequence according to SEQ ID NO: 3; and (c) a polypeptide having the amino acid sequence according to SEQ ID NO: 5; wherein the polynucleotide comprises a mutation or deletion which renders non-functional at least one gene of an adenoviral genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

Also described herein is an isolated polynucleotide, wherein the polynucleotide encodes a polypeptide selected from the group consisting of:

(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 1,

(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1, and

(c) a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

Also described herein is a recombinant polynucleotide comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1,

(b) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 , wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1, and

(c) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

Also described herein is a recombinant vector comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1,

(b) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1, and

(c) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

In a further aspect the present invention provides a recombinant adenovirus comprising a polynucleotide encoding a polypeptide comprising: (a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3; (b) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and (c) a polynucleotide selected from the group consisting of (i) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1 and (ii) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1; wherein the adenovirus comprises a mutation or deletion which renders non-functional at least one gene of a genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

Also described herein is a recombinant adenovirus comprising at least one polynucleotide or polypeptide selected from the group consisting of:

(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1,

(b) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1,

(c) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3,

(d) a polypeptide having the amino acid sequence according to SEQ ID NO: 1,

(e) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1, and
(f) a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

In a further aspect the present invention provides a composition comprising a polynucleotide encoding a polypeptide comprising: (a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3; (b) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and (c) a polynucleotide selected from the group consisting of (i) an isolated recombinant polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1; and (ii) an isolated recombinant polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1; wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1; and a pharmaceutically acceptable excipient, wherein the composition comprises a mutation or deletion which renders non-functional at least one gene of a genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

Also described herein is a composition comprising at least one of the following:
(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1,
(b) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 , wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1,
(c) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3,
(d) a polypeptide having the amino acid sequence according to SEQ ID NO: 1,
(e) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1, (f) a polypeptide having the amino acid sequence according to SEQ ID NO: 3,
(g) a vector comprising a polynucleotide as described in (a), (b) or (c) above, and

(h) a recombinant adenovirus comprising a polynucleotide as described in (a), (b) or (c) above,

and a pharmaceutically acceptable excipient.

In a further aspect the present invention provides a cell comprising: (a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3; (b) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and (c) a polynucleotide selected from the group consisting of (i) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1; and (ii) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1; wherein the cell is a host cell that expresses at least one adenoviral gene selected from the group consisting of E1A, E1B, E2A, E2B, E3 E4, L1, L2, L3, L4 and L5.

Also described herein is a cell comprising at least one of the following:

(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1,

(b) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 , wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1,

(c) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3,

(d) a polypeptide having the amino acid sequence according to SEQ ID NO: 1,

(e) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1,

(f) a polypeptide having the amino acid sequence according to SEQ ID NO: 3,

(g) a vector comprising a polynucleotide as described in (a), (b) or (c) above, and

(h) a recombinant adenovirus comprising a polynucleotide as described in (a), (b) or (c) above,
and a pharmaceutically acceptable excipient.

Also provided is an isolated adenoviral polypeptide selected from the group consisting of:
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 1 ,
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 , wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1 , and
(c) a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

Also provided is an isolated polynucleotide, vector, recombinant adenovirus, composition or cell comprising or consisting of the sequence according to SEQ ID NO: 6.

Throughout the description and claims of the specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

DESCRIPTION OF THE FIGURES

Figure 1A-C - Alignment of fiber protein sequences from the indicated simian adenoviruses.
ChAd3 (SEQ ID NO:27)
PanAd3 (SEQ ID NO:28)
ChAd17 (SEQ ID NO:29)
ChAd19 (SEQ ID NO:30)
ChAd24 (SEQ ID NO:31)
ChAd155 (SEQ ID NO:1)
ChAd11 (SEQ ID NO:32)
ChAd20 (SEQ ID NO:33)
ChAd31 (SEQ ID NO:34)
PanAd1 (SEQ ID NO:35)

Figure 11 – Expression levels of PanAd3 and ChAd155 vectors expressing an HIV Gag transgene – Western Blot

Figure 12 – Immunogenicity of ChAd3 and ChAd155 vectors expressing an HIV Gag transgene – IFN-gamma ELISpot

Figure 13 – Immunogenicity of PanAd3 and ChAd155 vectors expressing an HIV Gag transgene – IFN-gamma ELISpot

DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 - Polypeptide sequence of ChAd155 fiber
SEQ ID NO: 2 - Polynucleotide sequence encoding ChAd155 fiber
SEQ ID NO: 3 - Polypeptide sequence of ChAd155 penton
SEQ ID NO: 4 - Polynucleotide sequence encoding ChAd155 penton
SEQ ID NO: 5 - Polypeptide sequence of ChAd155 hexon
SEQ ID NO: 6 - Polynucleotide sequence encoding ChAd155 hexon

SEQ ID NO: 7 - Polynucleotide sequence encoding ChAd155#1434
SEQ ID NO: 8 - Polynucleotide sequence encoding ChAd155#1390
SEQ ID NO: 9 - Polynucleotide sequence encoding ChAd155#1375
SEQ ID NO: 10 - Polynucleotide sequence encoding wild type ChAd155
SEQ ID NO: 11 - Polynucleotide sequence encoding ChAd155/RSV
SEQ ID NO: 12 - Polynucleotide sequence encoding the CASI promoter
SEQ ID NO: 13 - Ad5orf6 primer 1 polynucleotide sequence
SEQ ID NO: 14 - Ad5orf6 primer 2 polynucleotide sequence
SEQ ID NO: 15 - BAC/CHAd155 ΔE1_TetO hCMV RpsL-Kana primer 1 polynucleotide sequence
SEQ ID NO: 16 - BAC/CHAd155 ΔE1_TetO hCMV RpsL-Kana (#1375) primer 2 polynucleotide sequence
SEQ ID NO: 17 - 1021-FW E4 Del Step1 primer polynucleotide sequence
SEQ ID NO: 18 - 1022-RW E4 Del Step1 primer polynucleotide sequence
SEQ ID NO: 19 - 1025-FW E4 Del Step2 primer polynucleotide sequence
SEQ ID NO: 20 - 1026-RW E4 Del Step2 primer polynucleotide sequence
SEQ ID NO: 21 - 91-SubMonte FW primer polynucleotide sequence
SEQ ID NO: 22 - 890-BghPolyA RW primer polynucleotide sequence
SEQ ID NO: 23 - CMVfor primer polynucleotide sequence
SEQ ID NO: 24 - CMVrev primer polynucleotide sequence
SEQ ID NO: 25 - CMVFAM-TAMRA qPCR probe polynucleotide sequence
SEQ ID NO: 26 - Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) polynucleotide sequence
SEQ ID NO: 27 - Amino acid sequence for the fiber protein of ChAd3
SEQ ID NO: 28 - Amino acid sequence for the fiber protein of PanAd3

SEQ ID NO: 29 - Amino acid sequence for the fiber protein of ChAd17
SEQ ID NO: 30 - Amino acid sequence for the fiber protein of ChAd19
SEQ ID NO: 31 - Amino acid sequence for the fiber protein of ChAd24
SEQ ID NO: 32 - Amino acid sequence for the fiber protein of ChAd11
SEQ ID NO: 33 - Amino acid sequence for the fiber protein of ChAd20
SEQ ID NO: 34 - Amino acid sequence for the fiber protein of ChAd31
SEQ ID NO: 35 - Amino acid sequence for the fiber protein of PanAd1
SEQ ID NO: 36 - Amino acid sequence for the fiber protein of PanAd2
SEQ ID NO: 37 - RSV FΔTM amino acid sequence
SEQ ID NO: 38 - HIV Gag polynucleotide sequence

DETAILED DESCRIPTION OF THE INVENTION

Adenovirus

Adenoviruses have a characteristic morphology with an icosahedral capsid comprising three major proteins, hexon (II), penton base (III) and a knobbed fiber (IV), along with a number of other minor proteins, VI, VIII, IX, IIIa and IVa2. The virus genome is a linear, double-stranded DNA. The virus DNA is intimately associated with the highly basic protein VII and a small peptide pX (formerly termed mu). Another protein, V, is packaged with this DNA-protein complex and provides a structural link to the capsid via protein VI. The virus also contains a virus-encoded protease, which is necessary for processing of some of the structural proteins to produce mature infectious virus.

The adenoviral genome is well characterized. There is general conservation in the overall organization of the adenoviral genome with respect to specific open reading frames being similarly positioned, e.g. the location of the E1A, E1B, E2A, E2B, E3, E4, L1, L2, L3, L4 and L5 genes of each virus. Each extremity of the adenoviral genome comprises a sequence known as an inverted terminal repeat (ITR), which is necessary for viral replication. The virus also comprises a virus-encoded protease, which is necessary for processing some of the structural proteins required to produce infectious virions. The structure of the adenoviral genome is described on the basis of the order in which the viral genes are expressed following host cell transduction. More specifically, the viral genes are referred to as early (E) or late (L) genes according to whether transcription occurs prior to or after onset of DNA replication. In the early phase of transduction, the E1A, E1B, E2A, E2B, E3 and E4 genes of adenovirus are expressed to prepare the host cell for viral replication. During the late phase of infection, expression of the late genes L1-L5, which encode the structural components of the virus particles, is activated.

Adenoviruses are species-specific and different serotypes, i.e., types of viruses that are not cross-neutralized by antibodies, have been isolated from a variety of mammalian

species. For example, more than 50 serotypes have been isolated from humans which are divided into six subgroups (A–F; B is subdivided into B1 and B2) based on sequence homology and on their ability to agglutinate red blood cells (Tatsis and Ertl *Molecular Therapy* (2004) 10:616–629). Numerous adenoviruses have been isolated from nonhuman simians such as chimpanzees, bonobos, rhesus macaques and gorillas, and they are classified into the same human groups based on phylogenetic relationships based on hexon or fiber sequences (Colloca *et al.* (2012) ScienceTranslational Medicine 4:1-9; Roy *et al.* (2004) *Virology* 324: 361-372; Roy *et al.* (2010) *Journal of Gene Medicine* 13:17-25).

Adenovirus Capsid Proteins Including the Fiber Protein and Polynucleotides Encoding These Proteins

As outlined above, the adenoviral capsid comprises three major proteins, hexon, penton and fiber. The hexon accounts for the majority of the structural components of the capsid, which consists of 240 trimeric hexon capsomeres and 12 penton bases. The hexon has three conserved double barrels, while the top has three towers, each tower containing a loop from each subunit that forms most of the capsid. The base of hexon is highly conserved between adenoviral serotypes, while the surface loops are variable (Tatsis and Ertl *Molecular Therapy* (2004) 10:616–629).

Penton is another adenoviral capsid protein that forms a pentameric base to which fiber attaches. The trimeric fiber protein protrudes from the penton base at each of the 12 vertices of the capsid and is a knobbed rod-like structure. A remarkable difference in the surface of adenovirus capsids compared to that of most other icosahedral viruses is the presence of the long, thin fiber protein. The primary role of the fiber protein is the tethering of the viral capsid to the cell surface via its interaction with a cellular receptor.

The fiber proteins of many adenovirus serotypes share a common architecture: an N-terminal tail, a central shaft made of repeating sequences, and a C-terminal globular knob domain (or “head”). The central shaft domain consists of a variable number of beta-repeats. The beta-repeats connect to form an elongated structure of three intertwined spiralling strands that is highly rigid and stable. The shaft connects the N-terminal tail with the globular knob structure, which is responsible for interaction with the target cellular receptor. The globular nature of the adenovirus knob domain presents large surfaces for binding the receptor laterally and apically. The effect of this architecture is to project the receptor-binding site far from the virus capsid, thus freeing the virus from steric constraints presented by the relatively flat capsid surface.

Although fibers of many adenovirus serotypes have the same overall architecture, they have variable amino acid sequences that influence their function as well as structure. For example, a number of exposed regions on the surface of the fiber knob present an easily

adaptable receptor binding site. The globular shape of the fiber knob allows receptors to bind at the sides of the knob or on top of the fiber knob. These binding sites typically lie on surface-exposed loops connecting beta-strands that are poorly conserved among human adenoviruses. The exposed side chains on these loops give the knob a variety of surface features while preserving the tertiary and quaternary structure. For example, the electrostatic potential and charge distributions at the knob surfaces can vary due to the wide range of isoelectric points in the fiber knob sequences, from pI approximately 9 for Ad 8, Ad 19, and Ad 37 to approximately 5 for subgroup B adenoviruses. As a structurally complex virus ligand, the fiber protein allows the presentation of a variety of binding surfaces (knob) in a number of orientations and distances (shaft) from the viral capsid.

One of the most obvious variations between some serotypes is fiber length. Studies have shown that the length of the fiber shaft strongly influences the interaction of the knob and the virus with its target receptors. Further, fiber proteins between serotypes can also vary in their ability to bend. Although beta-repeats in the shaft form a highly stable and regular structure, electron microscopy (EM) studies have shown distinct hinges in the fiber. Analysis of the protein sequence from several adenovirus serotype fibers pinpoints a disruption in the repeating sequences of the shaft at the third beta-repeat from the N-terminal tail, which correlates strongly with one of the hinges in the shaft, as seen by EM. The hinges in the fiber allow the knob to adopt a variety of orientations relative to the virus capsid, which may circumvent steric hindrances to receptor engagement requiring the correct presentation of the receptor binding site on the knob. For example, the rigid fibers of subgroup D Ads thus require a flexible receptor or one prepositioned for virus attachment, as they are unable to bend themselves. (Nicklin et al *Molecular Therapy* 2005 12:384–393)

The identification of specific cell receptors for different Ad serotypes and the knowledge of how they contribute to tissue tropism have been achieved through the use of fiber pseudotyping technology. Although Ads of some subgroups use CAR as a primary receptor, it is becoming clear that many Ads use alternate primary receptors, leading to vastly different tropism in vitro and in vivo. The fibers of these serotypes show clear differences in their primary and tertiary structures, such as fiber shaft rigidity, the length of the fiber shaft, and the lack of a CAR binding site and/or the putative HSPG binding motif, together with the differences in net charge within the fiber knob. Pseudotyping Ad 5 particles with an alternate fiber shaft and knob therefore provides an opportunity to remove important cell binding domains and, in addition, may allow more efficient (and potentially more cell-selective) transgene delivery to defined cell types compared to that achieved with Ad 5. Neutralization of fiber-pseudotyped Ad particles may also be reduced if the fibers used are from Ads with lower seroprevalence in humans or experimental models, a situation that favours successful administration of the vector (Nicklin et al *Molecular Therapy* (2005) 12:384–393). Furthermore, full length fiber as well as isolated fiber knob

regions, but not hexon or penton alone, are capable of inducing dendritic cell maturation and are associated with induction of a potent CD8+ T cell response (Molinier-Frenkel et al. *J. Biol. Chem.* (2003) 278:37175-37182). Taken together, adenoviral fiber plays an important role in at least receptor-binding and immunogenicity of adenoviral vectors.

Illustrating the differences between the fiber proteins of Group C simian adenoviruses is the alignment provided in Figure 1. A striking feature is that the fiber sequences of these adenoviruses can be broadly grouped into having a long fiber, such as ChAd155, or a short fiber, such as ChAd3. This length differential is due to a 36 amino acid deletion at approximately position 321 in the short fiber relative to the long fiber. In addition, there are a number of amino acid substitutions that differ between the short versus long fiber subgroup yet are consistent within each subgroup. While the exact function of these differences have not yet been elucidated, given the function and immunogenicity of fiber, they are likely to be significant. It has been shown that one of the determinants of viral tropism is the length of the fiber shaft. It has been demonstrated that an Ad5 vector with a shorter shaft has a lower efficiency of binding to CAR receptor and a lower infectivity (Ambriović-Ristov A. et al.: Virology. (2003) 312(2):425-33): It has been speculated that this impairment is the results of an increased rigidity of the shorter fiber leading to a less efficient attachment to the cell receptor (Wu, E et al.: J Virol. (2003) 77(13): 7225–7235). These studies may explain the improved properties of ChAd155 carrying a longer and more flexible fiber in comparison with the previously described ChAd3 and PanAd3 carrying a fiber with a shorter shaft.

In one aspect of the invention there is provided isolated fiber, penton and hexon capsid polypeptides of chimp adenovirus ChAd155 and isolated polynucleotides encoding the fiber, penton and hexon capsid polypeptides of chimp adenovirus ChAd155.

All three capsid proteins are expected to contribute to low seroprevalence and can, thus, be used independently from each other or in combination to suppress the affinity of an adenovirus to preexisting neutralizing antibodies, e.g. to manufacture a recombinant adenovirus with a reduced seroprevalence. Such a recombinant adenovirus may be a chimeric adenovirus with capsid proteins from different serotypes with at least a fiber protein from ChAd155.

The ChAd155 fiber polypeptide sequence is provided in SEQ ID NO: 1.
The ChAd155 penton polypeptide sequence is provided in SEQ ID NO: 3.
The ChAd155 hexon polypeptide sequence is provided in SEQ ID NO: 5.

<u>Polypeptides, Recombinant Adenoviruses, Compositions or Cells Comprising Polypeptide Sequences of ChAd155 Fiber or a Functional Derivative Thereof</u>

Suitably the isolated polypeptide, recombinant adenovirus, composition or cell of the invention comprises a polypeptide having the amino acid sequence according to SEQ ID NO: 1.

Suitably the polypeptide, recombinant adenovirus, composition or cell of the invention comprises a polypeptide which is a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1. Suitably the functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 has an amino acid sequence which is at least 80% identical, such as at least 85.0% identical, , such as at least 90% identical, such as at least 91.0% identical, such as at least 93.0% identical, such as at least 95.0% identical, such as at least 97.0% identical, such as at least 98.0% identical, such as at least 99.0% identical, such as at least 99.2% identical, such as at least 99.4% identical, such as 99.5% identical, such as at least 99.6% identical, such as at least 99.8% identical, such as 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 1. Alternatively the functional derivative has no more than 130, more suitably no more than 120, more suitably no more than 110, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 1.

Suitably the polypeptide, recombinant adenovirus, composition or cell according to the invention further comprises:
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 3; or
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 3, wherein the functional derivative has an amino acid sequence which is at least 50.0% identical over its entire length to the amino acid sequence of SEQ ID NO: 3, and/or
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 5; or
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 5, wherein the functional derivative has an amino acid sequence which is at least 50% identical over its entire length to the amino acid sequence of SEQ ID NO: 5.

Suitably the functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 3 has an amino acid sequence which is at least 60.0%, such as at least 70.0%, such as at least 80.0%, such as at least 85.0%, such as at least 90.0%, such as at least 91.0% identical, such as at least 93.0% identical, such as at least 95.0%

identical, such as at least 97.0% identical, such as at least 98.0% identical, such as at least 99.0%, such as at least 99.2%, such as at least 99.4%, such as 99.5% identical, such as at least 99.6%, such as 99.7% identical such as at least 99.8% identical, such as 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 3. Alternatively the functional derivative has no more than 300, more suitably no more than 250, more suitably no more than 200, more suitably no more than 150, more suitably no more than 125, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 3.

Suitably the functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 5 has an amino acid sequence which is at least 60.0%, such as at least 70.0%, such as at least 80.0%, such as at least 85.0%, such as at least 90.0%, such as at least 91.0% identical, such as at least 93.0% identical, such as at least 95.0% identical, such as at least 97.0% identical, such as at least 98.0% identical, such as at least 99.0%, such as at least 99.2%, such as at least 99.4%, such as 99.5% identical, such as at least 99.6%, such as 99.7% identical such as at least 99.8% identical, such as 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 5. Alternatively the functional derivative has no more than 500, more suitably no more than 400, more suitably no more than 450, more suitably no more than 300, more suitably no more than 250, more suitably no more than 200, more suitably no more than 150, more suitably no more than 125, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 5.

Polypeptides, Recombinant Adenoviruses, Compositions or Cells Comprising Polypeptide Sequences of ChAd155 Penton

Suitably the polypeptide, recombinant adenovirus, composition or cell of the invention comprises a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

Suitably the polypeptide, recombinant adenovirus, composition or cell of the invention further comprises:

(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 1; or
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1
5 and/or
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 5; or
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 5, wherein the functional derivative has an amino acid sequence which is at least 60% identical over its entire length to the amino acid sequence of SEQ ID NO: 5.
10

Suitably the functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 has an amino acid sequence which is at least 60.0% identical, such as at least 70.0% identical, such as at least 80.0% identical, such as at least 85.0% identical, such as at least 87.0% identical, such as at least 89.0% identical, such as at
15 least 91.0% identical, such as at least 93.0% identical, such as at least 95.0% identical, such as at least 97.0% identical, such as at least 98.0% identical, such as at least 99.0% identical, such as at least 99.2%, such as at least 99.4%, such as 99.5% identical, such as at least 99.6%, such as at least 99.8% identical, such as 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 1. Alternatively the functional derivative
20 has no more than 130, more suitably no more than 120, more suitably no more than 110, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5,
25 more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 1.

Suitably the functional derivative of a polypeptide having the amino acid sequence
30 according to SEQ ID NO: 5 has an amino acid sequence which is at least 60.0%, such as at least 70.0%, such as at least 80.0%, such as at least 85.0%, such as at least 90.0%, such as at least 95.0%, such as at least 97.0%, such as at least 99.0%, such as at least 99.0%, such as at least 99.2%, such as at least 99.4%, such as 99.5% identical, such as at least 99.6%, such as at least 99.8% identical, such as 99.9% identical over its entire
35 length to the amino acid sequence of SEQ ID NO:5. Alternatively the functional derivative has no more than 500, more suitably no more than 400, more suitably no more than 450, more suitably no more than 300, more suitably no more than 250, more suitably no more than 200, more suitably no more than 150, more suitably no more than 125, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more
40 suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more

than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 5.

5 Isolated Polynucleotides, Vectors, Recombinant Adenoviruses, Compositions or Cells comprising Polynucleotides Encoding ChAd155 Fiber or a Functional Derivative Thereof

Suitably the isolated polynucleotide, vector, recombinant adenovirus, composition or cell of the invention comprises a polynucleotide which encodes a polypeptide having the amino 10 acid sequence according to SEQ ID NO: 1. Suitably the polynucleotide has a sequence according to SEQ ID NO: 2.

Alternatively, the polynucleotide, vector, recombinant adenovirus, composition or cell of the invention comprises a polynucleotide which encodes a functional derivative of a 15 polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1. Suitably the functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 has an amino acid sequence which is at least 80% identical, such as at least 85.0% 20 identical, such as at least 90% identical, such as at least 91.0% identical, such as at least 93.0% identical, such as at least 95.0% identical, such as at least 97.0% identical, such as at least 98.0% identical, such as at least 99.0% identical, such as at least 99% identical, such as at least 99.4% identical, such as at least 99.6% identical, such as at least 99.8% identical over its entire length to the amino acid sequence of SEQ ID NO: 1. Alternatively 25 the functional derivative has no more than 130, more suitably no more than 120, more suitably no more than 110, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more 30 suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 1.

Suitably the polynucleotide, vector, recombinant adenovirus, composition or cell of the 35 invention further comprises a polynucleotide encoding:
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 3; or
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 3, wherein the functional derivative has an amino acid sequence which is at least 50.0% identical over its entire length to the amino acid sequence of SEQ ID NO: 3, 40 and/or
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 5; or

(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 5, wherein the functional derivative has an amino acid sequence which is at least 50% identical over its entire length to the amino acid sequence of SEQ ID NO: 5.

Suitably the functional derivative of the polypeptide having the amino acid sequence according to SEQ ID NO: 3 has an amino acid sequence which is at least 60.0%, such as at least 70.0%, such as at least 80.0%, such as at least 85.0%, such as at least 90.0%, such as at least 91.0% identical, such as at least 93.0% identical, such as at least 95.0% identical, such as at least 97.0% identical, such as at least 98.0% identical, such as at least 99.0%, such as at least 99%, such as at least 99.4%, such as at least 99.6%, such as at least 99.8% identical over its entire length to the amino acid sequence of SEQ ID NO: 3. Alternatively the functional derivative has no more than 300, more suitably no more than 250, more suitably no more than 200, more suitably no more than 150, more suitably no more than 125, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 3.

Suitably the functional derivative of the polypeptide having the amino acid sequence according to SEQ ID NO: 5 has an amino acid sequence which is at least 60.0%, such as at least 70.0%, such as at least 80.0%, such as at least 85.0%, such as at least 90.0%, such as at least 95.0%, such as at least 97.0%, such as at least 98.0%, such as at least 99.0%, such as at least 99.2%, such as at least 99.4%, such as 99.5% identical, such as at least 99.6%, such as 99.7% identical such as at least 99.8% identical, such as 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 5. Alternatively the functional derivative has no more than 500, more suitably no more than 400, more suitably no more than 450, more suitably no more than 300, more suitably no more than 250, more suitably no more than 200, more suitably no more than 150, more suitably no more than 125, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 5.

<u>Isolated Polynucleotides, Vectors, Recombinant Adenoviruses, Compositions or Cells comprising Polynucleotides Encoding ChAd155 Penton</u>

Suitably the isolated polynucleotide, vector, recombinant adenovirus, composition or cell of the invention comprises a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3. Suitably the polynucleotide has a sequence according to SEQ ID NO: 4.

Suitably the polynucleotide, vector, recombinant adenovirus, composition or cell of the invention further comprises a polynucleotide encoding:
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 1; or
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 50% identical over its entire length to the amino acid sequence of SEQ ID NO: 1
and/or
(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 5; or
(b) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 5, wherein the functional derivative has an amino acid sequence which is at least 50% identical over its entire length to the amino acid sequence of SEQ ID NO: 5.

Suitably the functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1 has an amino acid sequence which is at least 60.0% identical, such as at least 70.0% identical, such as at least 80.0% identical, such as at least 85.0% identical, such as at least 87.0% identical, such as at least 89.0% identical, such as at least 91.0% identical, such as at least 93.0% identical, such as at least 95.0% identical, such as at least 97.0% identical, such as at least 98.0% identical, such as at least 99.0%, such as at least 99.2%, such as at least 99.4%, such as 99.5% identical, such as at least 99.6%, such as 99.7% identical such as at least 99.8% identical, such as 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 1. Alternatively the functional derivative has no more than 130, more suitably no more than 120, more suitably no more than 110, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 1.

Suitably the functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 5 has an amino acid sequence which is at least 60.0%, such as at least 70.0%, such as at least 80.0%, such as at least 85.0%, such as at least 90.0%, such as at least 95.0%, such as at least 97.0%, such as at least 98.0%, such as at least 99.0%, such as at least 99.2%, such as at least 99.4%, such as 99.5% identical, such as

at least 99.6%, such as 99.7% identical such as at least 99.8% identical, such as 99.9% identical over its entire length to the amino acid sequence of SEQ ID NO: 5. Alternatively the functional derivative has no more than 500, more suitably no more than 400, more suitably no more than 450, more suitably no more than 300, more suitably no more than 250, more suitably no more than 200, more suitably no more than 150, more suitably no more than 125, more suitably no more than 100, more suitably no more than 90, more suitably no more than 80, more suitably no more than 70, more suitably no more than 60, more suitably no more than 50, more suitably no more than 40, more suitably no more than 30, more suitably no more than 20, more suitably no more than 10, more suitably no more than 5, more suitably no more than 4, more suitably no more than 3, more suitably no more than 2, more suitably no more than 1 addition(s), deletion(s) or substitutions(s) compared to SEQ ID NO: 5.

ChAd155 Backbones

The invention provides isolated polynucleotide sequences of chimp adenovirus ChAd155, including that of wild type, unmodified ChAd155 (SEQ ID NO: 10) and modified backbone constructs of ChAd155. These modified backbone constructs include ChAd155#1434 (SEQ ID NO: 7), ChAd155#1390 (SEQ ID NO: 8) and ChAd155#1375 (SEQ ID NO: 9). ChAd155 backbones may be used in the construction of recombinant replication-competent or replication-incompetent adenoviruses for example for the delivery of transgenes.

Annotation of the ChAd155 wild type sequence (SEQ ID NO: 10) sequence is provided below.

```
LOCUS       ChAd155                 37830 bp    DNA     linear       10-JUN-2015
DEFINITION  Chimp adenovirus 155, complete genome.
COMMENT     Annotation according to alignment of ChAd155 against the human
            Adenovirus 2 reference strain NC_001405
            Two putative ORFs in the E3 region added manually
FEATURES             Location/Qualifiers
     source          1..37830
                     /organism="Chimpanzee adenovirus 155"
                     /mol_type="genomic DNA"
                     /acronym="ChAd155"
     repeat_region   1..101
                     /standard_name="ITR"
                     /rpt_type=inverted
     gene            466..1622
                     /gene="E1A"
     TATA_signal     466..471
                     /gene="E1A"
     prim_transcript 497..1622
                     /gene="E1A"
     CDS             join(577..1117,1231..1532)
                     /gene="E1A"
                     /product="E1A_280R"
```

```
CDS             join(577..979,1231..1532)
                /gene="E1A"
                /product="E1A_243R"
polyA_signal    1600..1605
                /gene="E1A"
gene            1662..4131
                /gene="E1B"
TATA_signal     1662..1667
                /gene="E1B"
prim_transcript 1692..4131
                /gene="E1B"
CDS             1704..2267
                /gene="E1B"
                /product="E1B_19K"
CDS             2009..3532
                /gene="E1B"
                /product="E1B_55K"
gene            3571..4131
                /gene="IX"
TATA_signal     3571..3576
                /gene="IX"
prim_transcript 3601..4131
                /gene="IX"
CDS             3628..4092
                /gene="IX"
                /product="IX"
polyA_signal    4097..4102
                /note="E1B, IX"
gene            complement(4117..27523)
                /gene="E2B"
prim_transcript complement(4117..27494)
                /gene="E2B"
gene            complement(4117..5896)
                /gene="IVa2"
prim_transcript complement(4117..5896)
                /gene="IVa2"
CDS             complement(join(4151..5487,5766..5778))
                /gene="IVa2"
                /product="E2B_IVa2"
polyA_signal    complement(4150..4155)
                /note="IVa2, E2B"
CDS             complement(join(5257..8838,14209..14217))
                /gene="E2B"
                /product="E2B_polymerase"
gene            6078..34605
                /gene="L5"
gene            6078..28612
                /gene="L4"
gene            6078..22658
                /gene="L3"
gene            6078..18164
                /gene="L2"
gene            6078..14216
                /gene="L1"
TATA_signal     6078..6083
                /note="L"
prim_transcript 6109..34605
                /gene="L5"
prim_transcript 6109..28612
                /gene="L4"
prim_transcript 6109..22658
                /gene="L3"
prim_transcript 6109..18164
                /gene="L2"
prim_transcript 6109..14216
```

```
                     /gene="L1"
CDS                  join(8038..8457,9722..9742)
                     /gene="L1"
                     /product="L1_13.6K"
CDS                  complement(join(8637..10640,14209..14217))
                     /gene="E2B"
                     /product="E2B_pTP"
gene                 10671..10832
                     /gene="VAI"
misc_RNA             10671..10832
                     /gene="VAI"
                     /product="VAI"
gene                 10902..11072
                     /gene="VAII"
misc_RNA             10902..11072
                     /gene="VAII"
                     /product="VAII"
CDS                  11093..12352
                     /gene="L1"
                     /product="L1_52K"
CDS                  12376..14157
                     /gene="L1"
                     /product="L1_pIIIa"
polyA_signal         14197..14202
                     /gene="L1"
CDS                  14254..16035
                     /gene="L2"
                     /product="L2_penton"
CDS                  16050..16646
                     /gene="L2"
                     /product="L2_pVII"
CDS                  16719..17834
                     /gene="L2"
                     /product="L2_V"
CDS                  17859..18104
                     /gene="L2"
                     /product="L2_pX"
polyA_signal         18143..18148
                     /gene="L2"
CDS                  18196..18951
                     /gene="L3"
                     /product="L3_pVI"
CDS                  19063..21945
                     /gene="L3"
                     /product="L3_hexon"
CDS                  21975..22604
                     /gene="L3"
                     /product="L3_protease"
polyA_signal         22630..22635
                     /gene="L3"
gene                 complement(22632..27523)
                     /gene="E2A"
prim_transcript      complement(22632..27494)
                     /gene="E2A"
gene                 complement(22632..26357)
                     /gene="E2A-L"
prim_transcript      complement(22632..26328)
                     /gene="E2A-L"
polyA_signal         complement(22649..22654)
                     /note="E2A, E2A-L"
CDS                  complement(22715..24367)
                     /gene="E2A"
                     /note="DBP; genus-common; DBP family"
                     /codon_start=1
                     /product="E2A"
```

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CDS             24405..26915
                /gene="L4"
                /product="L4_100k"
TATA_signal     complement(26352..26357)
                /gene="E2A-L"
CDS             join(26602..26941,27147..27529)
                /gene="L4"
                /product="L4_33K"
CDS             26602..27207
                /gene="L4"
                /product="L4_22K"
TATA_signal     complement(27518..27523)
                /note="E2A, E2B; nominal"
CDS             27604..28287
                /gene="L4"
                /product="L4_pVIII"
gene            27969..32686
                /gene="E3B"
gene            27969..31611
                /gene="E3A"
TATA_signal     27969..27974
                /note="E3A, E3B"
prim_transcript 27998..32686
                /gene="E3B"
prim_transcript 27998..31611
                /gene="E3A"
CDS             28288..28605
                /gene="E3A"
                /product="E3 ORF1"
polyA_signal    28594..28599
                /gene="L4"
CDS             29103..29303
                /gene="E3A"
                /product="E3 ORF2"
CDS             29300..29797
                /gene="E3A"
                /product="E3 ORF3"
CDS             29826..30731
                /gene="E3A"
                /product="E3 ORF4"
CDS             30728..31579
                /gene="E3A"
                /product="E3 ORF5"
CDS             31283..31579
                /gene="E3A"
                /product="E3 ORF6"
polyA_signal    31578..31584
                /gene="E3A"
CDS             31591..31863
                /gene="E3B"
                /product="E3 ORF7"
CDS             31866..32264
                /gene="E3B"
                /product="E3 ORF8"
CDS             32257..32643
                /gene="E3B"
                /product="E3 ORF9"
polyA_signal    32659..32664
                /gene="E3B"
gene            complement(<32678..32838)
                /gene="U"
CDS             complement(<32678..32838)
                /gene="U"
                /note="exon encoding C terminus unidentified;
                genus-common"
```

```
                 /product="protein U"
CDS              32849..34585
                 /gene="L5"
                 /product="L5_fiber"
polyA_signal     34581..34586
                 /gene="L5"
gene             complement(34611..37520)
                 /gene="E4"
prim_transcript  complement(34611..37490)
                 /gene="E4"
polyA_signal     complement(34625..34630)
                 /gene="E4"
CDS              complement(join(34794..35069,35781..35954))
                 /gene="E4"
                 /product="E4 ORF7"
CDS              complement(35070..35954)
                 /gene="E4"
                 /product="E4 ORF6"
CDS              complement(35875..36219)
                 /gene="E4"
                 /product="E4 ORF4"
CDS              complement(36235..36582)
                 /gene="E4"
                 /product="E4 ORF3"
CDS              complement(36579..36971)
                 /gene="E4"
                 /product="E4 ORF2"
CDS              complement(37029..37415)
                 /gene="E4"
                 /product="E4 ORF1"
TATA_signal      complement(37515..37520)
                 /gene="E4"
repeat_region    37740..37830
                 /standard_name="ITR"
                 /rpt_type=inverted
```

In one embodiment, fragments of the sequences of SEQ ID NO: 7, 8, 9, 10 and their complementary strands, cDNA and RNA complementary thereto are provided. Suitably, fragments are at least 15 nucleotides in length, more suitably 30 nucleotides in length, more suitably 60 nucleotides in length, more suitably 120 nucleotides in length, more suitably 240, more suitably 480 nucleotides in length and encompass functional fragments, i.e., fragments which are of biological interest. For example, a functional fragment can express a desired adenoviral product or may be useful in production of recombinant viral vectors. Such fragments include the gene sequences listed above.

Gene products of the ChAd155 adenovirus, such as proteins, enzymes, and fragments thereof, which are encoded by the adenoviral nucleic acids described herein are provided. Such proteins include those encoded by the open reading frames identified above and the proteins encoded by the polynucleotides provided in the Sequence Listing.

Further ChAd155 Polynucleotides and Polypeptides

In some embodiments the polynucleotide of the invention comprises a polynucleotide encoding a fiber polypeptide; a penton polypeptide; a hexon polypeptide and penton polypeptide; a hexon polypeptide and fiber polypeptide; penton polypeptide and fiber polypeptide; or hexon polypeptide, penton polypeptide and fiber polypeptide of the invention; and may further comprise additional adenoviral polynucleotides, suitably ChAd155 polynucleotides. Thus, suitably the polynucleotide according to the invention comprises one or more of the following, the sequence coordinates relative to SEQ ID NO:10 provided in the previous annotation:

(a) an adenoviral 5'-inverted terminal repeat (ITR);

(b) an adenoviral E1A region, or a fragment thereof selected from among the E1A_280R and E1A_243R regions;

(c) an adenoviral E1B or IX region, or a fragment thereof selected from among the group consisting of the E1B_19K, E1B_55K and IX regions;

(d) an adenoviral E2B region; or a fragment thereof selected from among the group consisting of the E2B_pTP, E2B_polymerase and E2B_IVa2 regions;

(e) an adenoviral L1 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the L1_13.6K, L1_52K and L1_pIIIa protein;

(f) an adenoviral L2 region or a L2 region comprising a polynucleotide encoding the penton protein of the invention, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the L2_penton protein, the L2_pVII protein, the L2_V protein and the L2_pX protein;

(g) an adenoviral L3 region or a L3 region comprising a polynucleotide encoding the hexon protein of the invention, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the L3_pVI protein, the L3_hexon protein and the L3_protease protein;

(h) an adenoviral E2A region;

(i) an adenoviral L4 region, or a fragment thereof said fragment encoding an adenoviral protein selected from the group consisting of the L4_100k protein, the L4_33K protein, the L4_22K protein and protein L4_VIII;

(j) an adenoviral E3 region, or a fragment thereof selected from the group consisting of

E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, and E3 ORF9;

(k) an adenoviral L5 region or a L5 region comprising a polynucleotide encoding the L5_fiber fiber polypeptide of the invention

(l) an adenoviral (such as Ad5) E4 region, or a fragment thereof selected from the group consisting of E4 ORF7, E4 ORF6, E4 ORF4, E4 ORF3, E4 ORF2, and E4 ORF1; in particular ORF6 of said E4 region;

(m) an adenoviral 3'-ITR; and/or

(n) an adenoviral VAI or VAII RNA region, preferably an adenoviral VAI or VAII RNA region from an adenovirus other than ChAd155, more preferably from Ad5.

Definitions

Suitably the polynucleotides or polypeptides of the invention are isolated. An “isolated” polynucleotide is one that is removed from its original environment. For example, a naturally-occurring polynucleotide is isolated if it is separated from some or all of the coexisting materials in the natural system. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of its natural environment or if it is comprised within cDNA.

Suitably the polynucleotides of the invention are recombinant. Recombinant means that the polynucleotide is the product of at least one of cloning, restriction or ligation steps, or other procedures that result in a polynucleotide that is distinct from a polynucleotide found in nature. A recombinant adenovirus is an adenovirus comprising a recombinant polynucleotide. A recombinant vector is a vector comprising a recombinant polynucleotide. ‘A recombinant virus’ includes progeny of the original recombinant virus. ‘A recombinant vector’ includes replicates of the original recombinant vector. ‘A recombinant polynucleotide’ includes replicates of the original recombinant polynucleotide.

Suitably, the polypeptide sequence of the present invention contains at least one alteration with respect to a native sequence. Suitably, the polynucleotide sequences of the present invention contain at least one alteration with respect to a native sequence. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species (and often a different genus, subfamily or family) is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is a heterologous promoter. A specific recombination site that has been cloned into a genome of a virus or viral vector, wherein the genome of the virus does not naturally contain it, is a heterologous recombination site. A heterologous nucleic acid sequence also includes a sequence naturally found in an adenoviral genome, but located at a non-native position within the adenoviral vector.

Typically, "heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared. A heterologous nucleic acid sequence refers to any nucleic acid sequence that is not isolated from, derived from, or based upon a naturally occurring nucleic acid sequence of the adenoviral vector. "Naturally occurring" means a sequence found in nature and not synthetically prepared or modified. A sequence is "derived" from a source when it is isolated from a source but modified (e.g., by

deletion, substitution (mutation), insertion, or other modification), suitably so as not to disrupt the normal function of the source gene.

A "functional derivative" of a polypeptide suitably refers to a modified version of a polypeptide, e.g. wherein one or more amino acids of the polypeptide may be deleted, inserted, modified and/or substituted. A derivative of an unmodified adenoviral capsid protein is considered functional if, for example:

(a) an adenovirus comprising the derivative capsid protein within its capsid retains substantially the same or a lower seroprevalence compared to an adenovirus comprising the unmodified capsid protein and/or
(b) an adenovirus comprising the derivative capsid protein within its capsid retains substantially the same or a higher host cell infectivity compared to an adenovirus comprising the unmodified capsid protein and/or
(c) an adenovirus comprising the derivative capsid protein within its capsid retains substantially the same or a higher immunogenicity compared to an adenovirus comprising the unmodified capsid protein and or
(d) an adenovirus comprising the derivative capsid protein within its capsid retains substantially the same or a higher level of transgene productivity compared to an adenovirus comprising the unmodified capsid protein.

Properties (a)-(d) above may suitably be measured using the methods described in the Examples section below.

Suitably, the polypeptide, vector or recombinant adenovirus has a low seroprevalence in a human population. "Low seroprevalence" may mean having a reduced pre-existing neutralizing antibody level as compared to human adenovirus 5 (Ad5). Similarly or alternatively, "low seroprevalence" may mean less than about 20% seroprevalence, less than about 15% seroprevalence, less than about 10% seroprevalence, less than about 5% seroprevalence, less than about 4% seroprevalence, less than about 3% seroprevalence, less than about 2% seroprevalence, less than about 1% seroprevalence or no detectable seroprevalence. Seroprevalence can be measured as the percentage of individuals having a clinically relevant neutralizing titre (defined as a 50% neutralisation titer >200) using methods as described in Aste-Amézaga *et al.*, Hum. Gene Ther. (2004) 15(3):293-304.

The terms polypeptide, peptide and protein are used interchangeably herein.

The term "simian" is typically meant to encompass nonhuman primates, for example Old World monkeys, New World monkeys, apes and gibbons. In particular, simian may refer to nonhuman apes such as chimpanzees (*Pan troglodyte*), bonobos (*Pan paniscus*) and gorillas (genus *Gorilla*). Non-ape simians may include rhesus macaques (*Macaca mulatta*)

Sequence Comparison

For the purposes of comparing two closely-related polynucleotide or polypeptide sequences, the “% identity" between a first sequence and a second sequence may be calculated using an alignment program, such as BLAST® (available at blast.ncbi.nlm.nih.gov, last accessed 09 March 2015) using standard settings. The % identity is the number of identical residues divided by the number of residues in the reference sequence, multiplied by 100. The % identity figures referred to above and in the claims are percentages calculated by this methodology. An alternative definition of % identity is the number of identical residues divided by the number of aligned residues, multiplied by 100. Alternative methods include using a gapped method in which gaps in the alignment, for example deletions in one sequence relative to the other sequence, are accounted for in a gap score or a gap cost in the scoring parameter. For more information, see the BLAST® fact sheet available at ftp.ncbi.nlm.nih.gov/pub/factsheets/HowTo_BLASTGuide.pdf, last accessed on 09 March 2015.

Sequences that preserve the functionality of the polynucleotide or a polypeptide encoded thereby are likely to be more closely identical. Polypeptide or polynucleotide sequences are said to be the same as or identical to other polypeptide or polynucleotide sequences, if they share 100% sequence identity over their entire length.

A “difference” between sequences refers to an insertion, deletion or substitution of a single amino acid residue in a position of the second sequence, compared to the first sequence. Two polypeptide sequences can contain one, two or more such amino acid differences. Insertions, deletions or substitutions in a second sequence which is otherwise identical (100% sequence identity) to a first sequence result in reduced percent sequence identity. For example, if the identical sequences are 9 amino acid residues long, one substitution in the second sequence results in a sequence identity of 88.9%. If the identical sequences are 17 amino acid residues long, two substitutions in the second sequence results in a sequence identity of 88.2%. If the identical sequences are 7 amino acid residues long, three substitutions in the second sequence results in a sequence identity of 57.1%. If first and second polypeptide sequences are 9 amino acid residues long and share 6 identical residues, the first and second polypeptide sequences share greater than 66% identity (the first and second polypeptide sequences share 66.7% identity). If first and second polypeptide sequences are 17 amino acid residues long and share 16 identical residues, the first and second polypeptide sequences share greater than 94% identity (the first and second polypeptide sequences share 94.1% identity). If first and second polypeptide sequences are 7 amino acid residues long and share 3 identical residues, the first and

second polypeptide sequences share greater than 42% identity (the first and second polypeptide sequences share 42.9% identity).

Alternatively, for the purposes of comparing a first, reference polypeptide sequence to a second, comparison polypeptide sequence, the number of additions, substitutions and/or deletions made to the first sequence to produce the second sequence may be ascertained. An addition is the addition of one amino acid residue into the sequence of the first polypeptide (including addition at either terminus of the first polypeptide). A substitution is the substitution of one amino acid residue in the sequence of the first polypeptide with one different amino acid residue. A deletion is the deletion of one amino acid residue from the sequence of the first polypeptide (including deletion at either terminus of the first polypeptide).

For the purposes of comparing a first, reference polynucleotide sequence to a second, comparison polynucleotide sequence, the number of additions, substitutions and/or deletions made to the first sequence to produce the second sequence may be ascertained. An addition is the addition of one nucleotide residue into the sequence of the first polynucleotide (including addition at either terminus of the first polynucleotide). A substitution is the substitution of one nucleotide residue in the sequence of the first polynucleotide with one different nucleotide residue. A deletion is the deletion of one nucleotide residue from the sequence of the first polynucleotide (including deletion at either terminus of the first polynucleotide).

Suitably substitutions in the sequences of the present invention may be conservative substitutions. A conservative substitution comprises the substitution of an amino acid with another amino acid having a chemical property similar to the amino acid that is substituted (see, for example, Stryer et al, *Biochemistry*, 5th Edition 2002, pages 44-49). Preferably, the conservative substitution is a substitution selected from the group consisting of: (i) a substitution of a basic amino acid with another, different basic amino acid; (ii) a substitution of an acidic amino acid with another, different acidic amino acid; (iii) a substitution of an aromatic amino acid with another, different aromatic amino acid; (iv) a substitution of a non-polar, aliphatic amino acid with another, different non-polar, aliphatic amino acid; and (v) a substitution of a polar, uncharged amino acid with another, different polar, uncharged amino acid. A basic amino acid is preferably selected from the group consisting of arginine, histidine, and lysine. An acidic amino acid is preferably aspartate or glutamate. An aromatic amino acid is preferably selected from the group consisting of phenylalanine, tyrosine and tryptophane. A non-polar, aliphatic amino acid is preferably selected from the group consisting of glycine, alanine, valine, leucine, methionine and isoleucine. A polar, uncharged amino acid is preferably selected from the group consisting of serine, threonine, cysteine, proline, asparagine and glutamine. In contrast to a conservative amino acid substitution, a non-conservative amino acid substitution is the

exchange of one amino acid with any amino acid that does not fall under the above-outlined conservative substitutions (i) through (v).

Vectors and Recombinant Adenovirus

The ChAd155 sequences of the invention are useful as therapeutic agents and in construction of a variety of vector systems, recombinant adenovirus and host cells. Suitably the term “vector” refers to a nucleic acid that has been substantially altered (*e.g.*, a gene or functional region that has been deleted and/or inactivated) relative to a wild type sequence and/or incorporates a heterologous sequence, *i.e.*,nucleic acid obtained from a different source (also called an “insert”), and replicating and/or expressing the inserted polynucleotide sequence, when introduced into a cell (e.g., a host cell). For example, the insert may be all or part of the ChAd155 sequences described herein. In addition or alternatively, a ChAd155 vector may be a ChAd155 adenovirus comprising one or more deletions or inactivations of viral genes, such as E1 or other viral gene or functional region described herein. Such a ChAd155, which may or may not comprise a heterologous sequence, is often called a "backbone" and may be used as is or as a starting point for additional modifications to the vector.

A vector may be any suitable nucleic acid molecule including naked DNA, a plasmid, a virus, a cosmid, phage vector such as lambda vector, an artificial chromosome such as a BAC (bacterial artificial chromosome), or an episome. Alternatively, a vector may be a transcription and/or expression unit for cell-free *in vitro* transcription or expression, such as a T7-compatible system. The vectors may be used alone or in combination with other adenoviral sequences or fragments, or in combination with elements from non-adenoviral sequences. The ChAd155 sequences are also useful in antisense delivery vectors, gene therapy vectors, or vaccine vectors. Thus, further provided are gene delivery vectors, and host cells which contain the ChAd155 sequences.

The term "replication-competent" adenovirus refers to an adenovirus which can replicate in a host cell in the absence of any recombinant helper proteins comprised in the cell. Suitably, a "replication-competent" adenovirus comprises the following intact or functional essential early genes: E1A, E1B, E2A, E2B, E3 and E4. Wild type adenoviruses isolated from a particular animal will be replication competent in that animal.

The term "replication-incompetent" or “replication-defective” adenovirus refers to an adenovirus which is incapable of replication because it has been engineered to comprise at least a functional deletion (or “loss-of-function” mutation), i.e. a deletion or mutation which impairs the function of a gene without removing it entirely, e.g. introduction of artificial stop codons, deletion or mutation of active sites or interaction domains, mutation or deletion of a regulatory sequence of a gene etc, or a complete removal of a gene

encoding a gene product that is essential for viral replication, such as one or more of the adenoviral genes selected from E1A, E1B, E2A, E2B, E3 and E4 (such as E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, E3 ORF9, E4 ORF7, E4 ORF6, E4 ORF4, E4 ORF3, E4 ORF2 and/or E4 ORF1). Particularly suitably E1 and optionally E3 and/or E4 are deleted. If deleted, the aforementioned deleted gene region will suitably not be considered in the alignment when determining % identity with respect to another sequence.

The present invention provides vectors such as recombinant adenovirus that deliver a protein, suitably a heterologous protein, to cells, either for therapeutic or vaccine purposes. A vector may include any genetic element including naked DNA, a phage, transposon, cosmid, episome, plasmid, or a virus. Such vectors contain DNA of ChAd155 as disclosed herein and a minigene. By "minigene" (or "expression cassette") is meant the combination of a selected heterologous gene (transgene) and the other regulatory elements necessary to drive translation, transcription and/or expression of the gene product in a host cell.

Typically, a ChAd155-derived adenoviral vector is designed such that the minigene is located in a nucleic acid molecule which contains other adenoviral sequences in the region native to a selected adenoviral gene. The minigene may be inserted into an existing gene region to disrupt the function of that region, if desired. Alternatively, the minigene may be inserted into the site of a partially or fully deleted adenoviral gene. For example, the minigene may be located in the site of a mutation, insertion or deletion which renders non-functional at least one gene of a genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4. The term "renders non-functional" means that a sufficient amount of the gene region is removed or otherwise disrupted, so that the gene region is no longer capable of producing functional products of gene expression. If desired, the entire gene region may be removed (and suitably replaced with the minigene).

For example, for a production vector useful for generation of a recombinant virus, the vector may contain the minigene and either the 5' end of the adenoviral genome or the 3' end of the adenoviral genome, or both the 5' and 3' ends of the adenoviral genome. The 5' end of the adenoviral genome contains the 5 ' cis-elements necessary for packaging and replication; i.e., the 5' ITR sequences (which function as origins of replication) and the native 5 ' packaging enhancer domains (that contain sequences necessary for packaging linear Ad genomes and enhancer elements for the E1 promoter). The 3' end of the adenoviral genome includes the 3 ' cis-elements (including the ITRs) necessary for packaging and encapsidation. Suitably, a recombinant adenovirus contains both 5' and 3' adenoviral cis-elements and the minigene (suitably containing a transgene) is located between the 5' and 3' adenoviral sequences. A ChAd155-based adenoviral vector may also contain additional adenoviral sequences.

Suitably, ChAd155-based vectors contain one or more adenoviral elements derived from the adenoviral ChAd155 genome of the invention. In one embodiment, the vectors contain adenoviral ITRs from ChAd155 and additional adenoviral sequences from the same adenoviral serotype. In another embodiment, the vectors contain adenoviral sequences that are derived from a different adenoviral serotype than that which provides the ITRs.

As defined herein, a pseudotyped adenovirus refers to an adenovirus in which the capsid proteins of the adenovirus are from a different adenovirus than the adenovirus which provides the ITRs.

Further, chimeric or hybrid adenoviruses may be constructed using the adenoviruses described herein using techniques known to those of skill in the art (e.g., US 7,291,498).

ITRs and any other adenoviral sequences present in the vector of the present invention may be obtained from many sources. A variety of adenovirus strains are available from the American Type Culture Collection, Manassas, Virginia, or available by request from a variety of commercial and institutional sources. Further, the sequences of many such strains are available from a variety of databases including, e.g., PubMed and GenBank. Homologous adenovirus vectors prepared from other chimp or from human adenoviruses are described in the published literature (for example, US 5,240,846). The DNA sequences of a number of adenovirus types are available from GenBank, including type Ad5 (GenBank Accession Number M73370). The adenovirus sequences may be obtained from any known adenovirus serotype, such as serotypes 2, 3, 4, 7, 12 and 40, and further including any of the presently identified human types. Similarly adenoviruses known to infect nonhuman animals (e.g., simians) may also be employed in the vector constructs of this invention (e.g., US 6,083,716). The viral sequences, helper viruses (if needed), and recombinant viral particles, and other vector components and sequences employed in the construction of the vectors described herein may be obtained as described below.

Sequence, Vector and Adenovirus Production

The sequences of the invention may be produced by any suitable means, including recombinant production, chemical synthesis, or other synthetic means. Suitable production techniques are well known to those of skill in the art. Alternatively, peptides can also be synthesized by well known solid phase peptide synthesis methods.

The adenoviral plasmids (or other vectors) may be used to produce adenoviral vectors. In one embodiment, the adenoviral vectors are adenoviral particles which are replication-incompetent. In one embodiment, the adenoviral particles are rendered replication-incompetent by deletions in the E1A and/or E1B genes. Alternatively, the adenoviruses are rendered replication-incompetent by another means, optionally while retaining the E1A

and/or E1B genes. Similarly, in some embodiments, reduction of an immune response to the vector may be accomplished by deletions in the E2B and/or DNA polymerase genes. The adenoviral vectors can also contain other mutations to the adenoviral genome, e.g., temperature-sensitive mutations or deletions in other genes. In other embodiments, it is desirable to retain an intact E1A and/or E1B region in the adenoviral vectors. Such an intact E1 region may be located in its native location in the adenoviral genome or placed in the site of a deletion in the native adenoviral genome (e.g., in the E3 region).

In the construction of adenovirus vectors for delivery of a gene to a mammalian (such as human) cell, a range of modified adenovirus nucleic acid sequences can be employed in the vectors. For example, all or a portion of the adenovirus delayed early gene E3 may be eliminated from the adenovirus sequence which forms a part of the recombinant virus. The function of E3 is believed to be irrelevant to the function and production of the recombinant virus particle. Adenovirus vectors may also be constructed having a deletion of at least the ORF6 region of the E4 gene, and more desirably because of the redundancy in the function of this region, the entire E4 region. Still another vector of the invention contains a deletion in the delayed early gene E2A. Deletions may also be made in any of the late genes L1 to L5 of the adenovirus genome. Similarly, deletions in the intermediate genes IX and IVa2 may be useful for some purposes. Other deletions may be made in the other structural or non-structural adenovirus genes. The above discussed deletions may be used individually, i.e., an adenovirus sequence for use as described herein may contain deletions in only a single region. Alternatively, deletions of entire genes or portions thereof effective to destroy their biological activity may be used in any combination. For example, in one exemplary vector, the adenovirus sequence may have deletions of the E1 genes and the E4 gene, or of the E1, E2A and E3 genes, or of the E1 and E3 genes, or of E1, E2A and E4 genes, with or without deletion of E3, and so on. Any one or more of the E genes may suitably be replaced with an E gene (or one or more E gene open reading frames) sourced from a different strain of adenovirus. Particularly suitably the ChAd155 E1 and E3 genes are deleted and the ChAd155E4 gene is replaced with E4Ad5orf6. As discussed above, such deletions and/or substitutions may be used in combination with other mutations, such as temperature-sensitive mutations, to achieve a desired result.

An adenoviral vector lacking one or more essential adenoviral sequences (e.g., E1A, E1B, E2A, E2B, E4 ORF6, L1, L2, L3, L4 and L5) may be cultured in the presence of the missing adenoviral gene products which are required for viral infectivity and propagation of an adenoviral particle. These helper functions may be provided by culturing the adenoviral vector in the presence of one or more helper constructs (e.g., a plasmid or virus) or a packaging host cell.

Complementation of Replication-Incompetent Vectors

To generate recombinant adenoviruses deleted in any of the genes described above, the function of the deleted gene region, if essential to the replication and infectivity of the virus, must be supplied to the recombinant virus by a helper virus or cell line, i.e., a complementation or packaging cell line.

Helper Viruses

Depending upon the adenovirus gene content of the viral vectors employed to carry the minigene, a helper adenovirus or non-replicating virus fragment may be used to provide sufficient adenovirus gene sequences necessary to produce an infective recombinant viral particle containing the minigene. Useful helper viruses contain selected adenovirus gene sequences not present in the adenovirus vector construct and/or not expressed by the packaging cell line in which the vector is transfected. In one embodiment, the helper virus is replication-defective and contains adenovirus genes in addition, suitably, to one or more of the sequences described herein. Such a helper virus is suitably used in combination with an E1 expressing (and optionally additionally E3 expressing) cell line.

A helper virus may optionally contain a reporter gene. A number of such reporter genes are known to the art as well as described herein. The presence of a reporter gene on the helper virus which is different from the transgene on the adenovirus vector allows both the adenoviral vector and the helper virus to be independently monitored. This reporter is used to enable separation between the resulting recombinant virus and the helper virus upon purification.

Complementation Cell Lines

In many circumstances, a cell line expressing the one or more missing genes which are essential to the replication and infectivity of the virus, such as human E1, can be used to transcomplement a chimp adenoviral vector. This is particularly advantageous because, due to the diversity between the chimp adenovirus sequences of the invention and the human adenovirus sequences found in currently available packaging cells, the use of the current human E1-containing cells prevents the generation of replication-competent adenoviruses during the replication and production process.

Alternatively, if desired, one may utilize the sequences provided herein to generate a packaging cell or cell line that expresses, at a minimum, the E1 gene from ChAd155 under the transcriptional control of a promoter for expression in a selected parent cell line. Inducible or constitutive promoters may be employed for this purpose. Examples of such promoters are described in detail elsewhere in this document. A parent cell is selected for the generation of a novel cell line expressing any desired ChAd155 gene. Without

limitation, such a parent cell line may be HeLa [ATCC Accession No. CCL 2], A549 [ATCC Accession No. CCL 185], HEK 293, KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells, among others. These cell lines are all available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209.

Such E1-expressing cell lines are useful in the generation of recombinant adenovirus E1 deleted vectors. Additionally, or alternatively, cell lines that express one or more adenoviral gene products, e.g., E1A, E1B, E2A, E3 and/or E4, can be constructed using essentially the same procedures as used in the generation of recombinant viral vectors. Such cell lines can be utilised to transcomplement adenovirus vectors deleted in the essential genes that encode those products, or to provide helper functions necessary for packaging of a helper-dependent virus (e.g., adeno-associated virus). The preparation of a host cell involves techniques such as assembly of selected DNA sequences.

In another alternative, the essential adenoviral gene products are provided in trans by the adenoviral vector and/or helper virus. In such an instance, a suitable host cell can be selected from any biological organism, including prokaryotic (e.g., bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells.

Host cells may be selected from among any mammalian species, including, without limitation, cells such as A549, WEHI, 3T3, 10ĭĭ/2, HEK 293 cells or Per.C6 (both of which express functional adenoviral E1) [Fallaux, FJ et al, (1998), Hum Gene Ther, 9:1909-1917], Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster.

A particularly suitable complementation cell line is the Procell92 cell line. The Procell92 cell line is based on HEK 293 cells which express adenoviral E1 genes, transfected with the Tet repressor under control of the human phosphoglycerate kinase-1 (PGK) promoter, and the G418-resistance gene (Vitelli et al. *PLOS One* (2013) 8(e55435):1-9). Procell92.S is adapted for growth in suspension conditions and is useful for producing adenoviral vectors expressing toxic proteins (www.okairos.com/e/inners.php?m=00084, last accessed 13 April 2015).

<u>Assembly of a Viral Particle and Transfection of a Cell Line</u>

Generally, when delivering the vector comprising the minigene by transfection, the vector is delivered in an amount from about 5 µg to about 100 µg DNA, and preferably about 10 to about 50 µg DNA to about 1 x 10^4 cells to about 1 x 10^{13} cells, and preferably about 10^5 cells. However, the relative amounts of vector DNA to host cells may be adjusted, taking

into consideration such factors as the selected vector, the delivery method and the host cells selected.

Introduction into the host cell of the vector may be achieved by any means known in the art, including transfection, and infection. One or more of the adenoviral genes may be stably integrated into the genome of the host cell, stably expressed as episomes, or expressed transiently. The gene products may all be expressed transiently, on an episome or stably integrated, or some of the gene products may be expressed stably while others are expressed transiently.

Introduction of vectors into the host cell may also be accomplished using techniques known to the skilled person. Suitably, standard transfection techniques are used, e.g., CaPC transfection or electroporation.

Assembly of the selected DNA sequences of the adenovirus (as well as the transgene and other vector elements) into various intermediate plasmids, and the use of the plasmids and vectors to produce a recombinant viral particle are all achieved using conventional techniques. Such techniques include conventional cloning techniques of cDNA, use of overlapping oligonucleotide sequences of the adenovirus genomes, polymerase chain reaction, and any suitable method which provides the desired nucleotide sequence. Standard transfection and co-transfection techniques are employed, e.g., CaPC precipitation techniques. Other conventional methods employed include homologous recombination of the viral genomes, plaquing of viruses in agar overlay, methods of measuring signal generation, and the like.

For example, following the construction and assembly of the desired minigene-containing viral vector, the vector is transfected in vitro in the presence of a helper virus into the packaging cell line. Homologous recombination occurs between the helper and the vector sequences, which permits the adenovirus-transgene sequences in the vector to be replicated and packaged into virion capsids, resulting in the recombinant viral vector particles. The resulting recombinant adenoviruses are useful in transferring a selected transgene to a selected cell. In in vivo experiments with the recombinant virus grown in the packaging cell lines, the E1-deleted recombinant adenoviral vectors of the invention demonstrate utility in transferring a transgene to a non-simian mammal, preferably a human, cell.

Transgenes

The transgene is a nucleic acid sequence, heterologous to the vector sequences flanking the transgene, which encodes a protein of interest. The nucleic acid coding sequence is

operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a host cell.

The composition of the transgene sequence will depend upon the use to which the resulting vector will be put. For example, the transgene may be a therapeutic transgene or an immunogenic transgene. Alternatively, a transgene sequence may include a reporter sequence, which upon expression produces a detectable signal. Such reporter sequences include, without limitation, DNA sequences encoding β-lactamase, β-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art, to which high affinity antibodies directed thereto exist or can be produced by conventional means, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or Myc. These coding sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence or other spectrographic assays, fluorescent activating cell sorting assays and immunological assays, including enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunohistochemistry.

In one embodiment, the transgene is a non-marker sequence encoding a product which is useful in biology and medicine, such as a therapeutic transgene or an immunogenic transgene such as proteins, RNA, enzymes, or catalytic RNAs. Desirable RNA molecules include tRNA, dsRNA, ribosomal RNA, catalytic RNAs, and antisense RNAs. One example of a useful RNA sequence is a sequence which extinguishes expression of a targeted nucleic acid sequence in the treated animal.

The transgene may be used for treatment, e.g., of genetic deficiencies, as a cancer therapeutic or vaccine, for induction of an immune response, and/or for prophylactic vaccine purposes. As used herein, induction of an immune response refers to the ability of a protein to induce a T cell and/or a humoral immune response to the protein.

Regulatory Elements

In addition to the transgene the vector also includes conventional control elements which are operably linked to the transgene in a manner that permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (poly A) signals including rabbit beta-globin polyA; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. Among other sequences, chimeric introns may be used.

In some embodiments, the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) (Zuffrey et al. (1999) J Virol; 73(4):2886-9) may be operably linked to the transgene. An exemplary WPRE is provided in SEQ ID NO: 26.

A "promoter" is a nucleotide sequence that permits binding of RNA polymerase and directs the transcription of a gene. Typically, a promoter is located in the 5 ' non-coding region of a gene, proximal to the transcriptional start site of the gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. Examples of promoters include, but are not limited to, promoters from bacteria, yeast, plants, viruses, and mammals (including humans). A great number of expression control sequences, including promoters which are internal, native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

Examples of constitutive promoters include, without limitation, the TBG promoter, the retroviral Rous sarcoma virus LTR promoter (optionally with the enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer, see, e.g., Boshart et al, Cell, 41:521-530 (1985)), the CASI promoter, the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1a promoter (Invitrogen).

In some embodiments, the promoter is a CASI promoter (see, for example, WO2012/115980). The CASI promoter is a synthetic promoter which contains a portion of the CMV enhancer, a portion of the chicken beta-actin promoter, and a portion of the UBC enhancer. In some embodiments, the CASI promoter can include a nucleic acid sequence having at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or more, sequence identity to SEQ ID NO: 12. In some embodiments, the promoter comprises or consists of a nucleic acid sequence of SEQ ID NO: 12.

Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are

available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. For example, inducible promoters include the zinc-inducible sheep metallothionine (MT) promoter and the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter. Other inducible systems include the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No et al, Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)), the tetracycline-repressible system (Gossen et al, Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)), the tetracycline-inducible system (Gossen et al, Science, 378:1766-1769 (1995), see also Harvey et al, Curr. Opin. Chem. Biol, 2:512-518 (1998)). Other systems include the FK506 dimer, VP16 or p65 using castradiol, diphenol murislerone, the RU486-inducible system (Wang et al, Nat. Biotech., 15:239-243 (1997) and Wang et al, Gene Ther., 4:432-441 (1997)) and the rapamycin-inducible system (Magari et al, J. Clin. Invest., 100:2865-2872 (1997)). The effectiveness of some inducible promoters increases over time. In such cases one can enhance the effectiveness of such systems by inserting multiple repressors in tandem, e.g., TetR linked to a TetR by an IRES.

In another embodiment, the native promoter for the transgene will be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

The transgene may be operably linked to a tissue-specific promoter. For instance, if expression in skeletal muscle is desired, a promoter active in muscle should be used. These include the promoters from genes encoding skeletal β-actin, myosin light chain 2A, dystrophin, muscle creatine kinase, as well as synthetic muscle promoters with activities higher than naturally occurring promoters (see Li et al, Nat. Biotech., 17:241-245 (1999)). Examples of promoters that are tissue-specific are known for liver (albumin, Miyatake et al, J. Virol, 71:5124-32 (1997); hepatitis B virus core promoter, Sandig et al, Gene Ther., 3:1002-9 (1996); alpha-fetoprotein (AFP), Arbuthnot et al., Hum. Gene Ther., 7: 1503-14 (1996)), bone osteocalcin (Stein et al, Mol. Biol. Rep., 24:185-96 (1997)); bone sialoprotein (Chen et al., J. Bone Miner. Res., 11:654-64 (1996)), lymphocytes (CD2, Hansal et al, J. Immunol, 161:1063-8 (1998); immunoglobulin heavy chain; T cell receptor chain), neuronal such as neuron-specific enolase (NSE) promoter (Andersen et al, Cell. Mol. Neurobiol, 13:503-15 (1993)), neurofilament light-chain gene (Piccioli et al, Proc. Natl. Acad. Sci. USA, 88:5611-5 (1991)), and the neuron-specific vgf gene (Piccioli et al, Neuron, 15:373-84 (1995)), among others.

Optionally, vectors carrying transgenes encoding therapeutically useful or immunogenic products may also include selectable markers or reporter genes which may include sequences encoding geneticin, hygromicin or purimycin resistance, among others. Such selectable reporters or marker genes (preferably located outside the viral genome to be packaged into a viral particle) can be used to signal the presence of the plasmids in bacterial cells, such as ampicillin resistance. Other components of the vector may include an origin of replication.

These vectors are generated using the techniques and sequences provided herein, in conjunction with techniques known to those of skill in the art. Such techniques include conventional cloning techniques of cDNA such as those described in texts, use of overlapping oligonucleotide sequences of the adenovirus genomes, polymerase chain reaction, and any suitable method which provides the desired nucleotide sequence.

Therapeutics and Prophylaxis

The recombinant ChAd155-based vectors are useful for gene transfer to a human or non-simian mammal in vitro, ex vivo, and in vivo.

The recombinant adenovirus vectors described herein can be used as expression vectors for the production of the products encoded by the heterologous transgenes in vitro. For example, the recombinant replication-incompetent adenovirus containing a transgene may be transfected into a complementation cell line as described above.

A ChAd155-derived recombinant adenoviral vector provides an efficient gene transfer vehicle that can deliver a selected transgene to a selected host cell in vivo or ex vivo even where the organism has neutralizing antibodies to one or more adenovirus serotypes. In one embodiment, the vector and the cells are mixed ex vivo; the infected cells are cultured using conventional methodologies; and the transduced cells are re-infused into the patient. These techniques are particularly well suited to gene delivery for therapeutic purposes and for immunisation, including inducing protective immunity.

<u>Immunogenic Transgenes</u>

The recombinant ChAd155 vectors may also be as administered in immunogenic compositions. An immunogenic composition as described herein is a composition comprising one or more recombinant ChAd155 vector capable of inducing an immune response, for example a humoral (e.g., antibody) and/or cell-mediated (e.g., a cytotoxic T cell) response, against a transgene product delivered by the vector following delivery to a mammal, suitably a human. A recombinant adenovirus may comprise (suitably in any of

its gene deletions) a gene encoding a desired immunogen and may therefore be used in a vaccine. The recombinant adenoviruses can be used as prophylactic or therapeutic vaccines against any pathogen for which the antigen(s) crucial for induction of an immune response and able to limit the spread of the pathogen has been identified and for which the cDNA is available.

Such vaccine or other immunogenic compositions may be formulated in a suitable delivery vehicle. Generally, doses for the immunogenic compositions are in the range defined below under 'Delivery Methods and Dosage'. The levels of immunity of the selected gene can be monitored to determine the need, if any, for boosters. Following an assessment of antibody titers in the serum, optional booster immunizations may be desired.

Optionally, a vaccine or immunogenic composition of the invention may be formulated to contain other components, including, e.g., adjuvants, stabilizers, pH adjusters, preservatives and the like. Examples of suitable adjuvants are provided below under 'Adjuvants'. Such an adjuvant can be administered with a priming DNA vaccine encoding an antigen to enhance the antigen-specific immune response compared with the immune response generated upon priming with a DNA vaccine encoding the antigen only. Alternatively, such an adjuvant can be administered with a polypeptide antigen which is administered in an administration regimen involving the ChAd155 vectors of the invention (as described below under 'Administration Regimens'.

The recombinant adenoviruses are administered in an immunogenic amount, that is, an amount of recombinant adenovirus that is effective in a route of administration to transfect the desired target cells and provide sufficient levels of expression of the selected gene to induce an immune response. Where protective immunity is provided, the recombinant adenoviruses are considered to be vaccine compositions useful in preventing infection and/or recurrent disease.

The recombinant vectors described herein are expected to be highly efficacious at inducing cytolytic T cells and antibodies directed to the inserted heterologous antigenic protein expressed by the vector.

Immunogens expressed by the inventive vectors which are useful to immunize a human or non-human animal against other pathogens include, e.g., bacteria, fungi, parasitic microorganisms or multicellular parasites which infect human and non-human vertebrates, or from a cancer cell or tumor cell. For example, immunogens may be selected from a variety of viral families. Examples of viral families against which an immune response would be desirable include respiratory viruses such as respiratory syncytial virus (RSV) and other paramyxoviruses such as human metapneumovirus, hMPV and parainfluenza viruses (PIV).

Suitable antigens of RSV which are useful as immunogens to immunize a human or non-human animal can be selected from: the fusion protein (F), the attachment protein (G), the matrix protein (M2) and the nucleoprotein (N). The term “F protein” or “fusion protein” or “F protein polypeptide” or “fusion protein polypeptide” refers to a polypeptide or protein having all or part of an amino acid sequence of an RSV Fusion protein polypeptide. Similarly, the term “G protein” or “G protein polypeptide” refers to a polypeptide or protein having all or part of an amino acid sequence of an RSV Attachment protein polypeptide. The term “M protein” or “matrix protein” or “M protein polypeptide” refers to a polypeptide or protein having all or part of an amino acid sequence of an RSV Matrix protein and may include either or both of the M2-1 (which may be written herein as M2.1) and M2-2 gene products. Likewise, the term “N protein” or “Nucleocapsid protein” or “N protein polypeptide” refers to a polypeptide or protein having all or part of an amino acid sequence of an RSV Nucleoprotein.

Two groups of human RSV strains have been described, the A and B groups, based mainly on differences in the antigenicity of the G glycoprotein. Numerous strains of RSV have been isolated to date, any of which are suitable in the context of the antigens of the immunogenic combinations disclosed herein. Exemplary strains indicated by GenBank and/or EMBL Accession number can be found in US published application number 2010/0203071 (WO2008114149), which is incorporated herein by reference for the purpose of disclosing the nucleic acid and polypeptide sequences of RSV F and G proteins suitable for use in present invention. In an embodiment, the RSV F protein can be an ectodomain of an RSV F Protein (FΔTM).

Exemplary M and N protein nucleic acids and protein sequences can be found, e.g., in US published application number 2014/0141042 (WO2012/089833), which are incorporated herein for purpose of disclosing the nucleic acid and polypeptide sequences of RSV M and N proteins suitable for use in present invention.

Suitably, for use with in present invention, a nucleic acid encodes an RSV F antigen and RSV, M and N antigens. More specifically, the nucleic acid encodes an RSV FΔTM antigen and RSV M2-1 and N antigens, wherein a self-cleavage site is included between the RSV FΔTM antigen and the RSV M2-1 and a flexible linker is included between the RSV M2-1 and N antigens. In one embodiment a suitable nucleic acid encodes the polypeptide represented by SEQ ID NO:37

In one embodiment, the immunogen may be from a retrovirus, for example a lentivirus such as the Human Immunodeficiency Virus (HIV). In such an embodiment, immunogens may be derived from HIV-1 or HIV-2.

The HIV genome encodes a number of different proteins, each of which can be immunogenic in its entirety or as a fragment when expressed by vectors of the present invention. Envelope proteins include gp120, gp41 and Env precursor gp160, for example. Non-envelope proteins of HIV include for example internal structural proteins such as the products of the gag and pol genes and other non-structural proteins such as Rev, Nef, Vif and Tat. In an embodiment the vector of the invention encodes one or more polypeptides comprising HIV Gag.

The Gag gene is translated as a precursor polyprotein that is cleaved by protease to yield products that include the matrix protein (p17), the capsid (p24), the nucleocapsid (p9), p6 and two space peptides, p2 and p1, all of which are examples of fragments of Gag.

The Gag gene gives rise to the 55-kilodalton (kD) Gag precursor protein, also called p55, which is expressed from the unspliced viral mRNA. During translation, the N terminus of p55 is myristoylated, triggering its association with the cytoplasmic aspect of cell membranes. The membrane-associated Gag polyprotein recruits two copies of the viral genomic RNA along with other viral and cellular proteins that triggers the budding of the viral particle from the surface of an infected cell. After budding, p55 is cleaved by the virally encoded protease (a product of the pol gene) during the process of viral maturation into four smaller proteins designated MA (matrix [p17]), CA (capsid [p24]), NC (nucleocapsid [p9]), and p6, all of which are examples of fragments of Gag. In one embodiment, the vectors of the present invention comprise a Gag polypeptide of SEQ ID NO: 38.

Adjuvants

An “adjuvant” as used herein refers to a composition that enhances the immune response to an immunogen. Examples of such adjuvants include but are not limited to inorganic adjuvants (e.g. inorganic metal salts such as aluminium phosphate or aluminium hydroxide), organic adjuvants (e.g. saponins, such as QS21, or squalene), oil-based adjuvants (e.g. Freund's complete adjuvant and Freund's incomplete adjuvant), cytokines (e.g. IL-1β, IL-2, IL-7, IL-12, IL-18, GM-CFS, and INF-γ) particulate adjuvants (e.g. immuno-stimulatory complexes (ISCOMS), liposomes, or biodegradable microspheres), virosomes, bacterial adjuvants (e.g. monophosphoryl lipid A, such as 3-de-O-acylated monophosphoryl lipid A (3D-MPL), or muramyl peptides), synthetic adjuvants (e.g. non-ionic block copolymers, muramyl peptide analogues, or synthetic lipid A), synthetic polynucleotides adjuvants (e.g polyarginine or polylysine) and immunostimulatory oligonucleotides containing unmethylated CpG dinucleotides ("CpG").

One suitable adjuvant is monophosphoryl lipid A (MPL), in particular 3-de-O-acylated monophosphoryl lipid A (3D-MPL). Chemically it is often supplied as a mixture of 3-de-O-

acylated monophosphoryl lipid A with either 4, 5, or 6 acylated chains. It can be purified and prepared by the methods taught in GB 2122204B, which reference also discloses the preparation of diphosphoryl lipid A, and 3-O-deacylated variants thereof. Other purified and synthetic lipopolysaccharides have been described (U.S. Pat. No. 6,005,099 and EP 0 729 473 B1; Hilgers et al., 1986, Int.Arch.Allergy.Immunol., 79(4):392-6; Hilgers et al., 1987, Immunology, 60(1):141-6; and EP 0 549 074 B1I).

Saponins are also suitable adjuvants (see Lacaille-Dubois, M and Wagner H, A review of the biological and pharmacological activities of saponins. Phytomedicine vol 2 pp 363-386 (1996)). For example, the saponin Quil A (derived from the bark of the South American tree Quillaja Saponaria Molina), and fractions thereof, are described in U.S. Pat. No. 5,057,540 and Kensil, Crit. Rev. Ther. Drug Carrier Syst., 1996, 12:1-55; and EP 0 362 279 B1. Purified fractions of Quil A are also known as immunostimulants, such as QS21 and QS17; methods of their production is disclosed in U.S. Pat. No. 5,057,540 and EP 0 362 279 B1. Also described in these references is QS7 (a non-haemolytic fraction of Quil-A). Use of QS21 is further described in Kensil et al. (1991, J. Immunology, 146: 431-437). Combinations of QS21 and polysorbate or cyclodextrin are also known (WO 99/10008). Particulate adjuvant systems comprising fractions of QuilA, such as QS21 and QS7 are described in WO 96/33739 and WO 96/11711.

Another adjuvant is an immunostimulatory oligonucleotide containing unmethylated CpG dinucleotides ("CpG") (Krieg, Nature 374:546 (1995)). CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. CpG is known as an adjuvant when administered by both systemic and mucosal routes (WO 96/02555, EP 468520, Davis et al, J.Immunol, 1998, 160:870-876; McCluskie and Davis, J.Immunol., 1998, 161:4463-6). CpG, when formulated into vaccines, may be administered in free solution together with free antigen (WO 96/02555) or covalently conjugated to an antigen (WO 98/16247), or formulated with a carrier such as aluminium hydroxide (Brazolot-Millan et al., Proc. Natl. Acad. Sci., USA, 1998, 95:15553-8).

Adjuvants such as those described above may be formulated together with carriers, such as liposomes, oil in water emulsions, and/or metallic salts (including aluminum salts such as aluminum hydroxide). For example, 3D-MPL may be formulated with aluminum hydroxide (EP 0 689 454) or oil in water emulsions (WO 95/17210); QS21 may be formulated with cholesterol containing liposomes (WO 96/33739), oil in water emulsions (WO 95/17210) or alum (WO 98/15287); CpG may be formulated with alum (Brazolot-Millan, supra) or with other cationic carriers.

Combinations of adjuvants may be utilized in the present invention, in particular a combination of a monophosphoryl lipid A and a saponin derivative (see, e.g., WO 94/00153; WO 95/17210; WO 96/33739; WO 98/56414; WO 99/12565; WO 99/11241),

more particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a composition where the QS21 is quenched in cholesterol-containing liposomes (DQ) as disclosed in WO 96/33739. Alternatively, a combination of CpG plus a saponin such as QS21 is an adjuvant suitable for use in the present invention. A potent adjuvant formulation involving QS21, 3D-MPL & tocopherol in an oil in water emulsion is described in WO 95/17210 and is another formulation for use in the present invention. Saponin adjuvants may be formulated in a liposome and combined with an immunostimulatory oligonucleotide. Thus, suitable adjuvant systems include, for example, a combination of monophosphoryl lipid A, preferably 3D-MPL, together with an aluminium salt (e.g. as described in WO00/23105). A further exemplary adjuvant comprises comprises QS21 and/or MPL and/or CpG. QS21 may be quenched in cholesterol-containing liposomes as disclosed in WO 96/33739.

Other suitable adjuvants include alkyl Glucosaminide phosphates (AGPs) such as those disclosed in WO9850399 or U.S. Pat. No. 6,303,347 (processes for preparation of AGPs are also disclosed), or pharmaceutically acceptable salts of AGPs as disclosed in U.S. Pat. No. 6,764,840. Some AGPs are TLR4 agonists, and some are TLR4 antagonists. Both are thought to be useful as adjuvants.

It has been found (WO 2007/062656, which published as US 2011/0293704 and is incorporated by reference for the purpose of disclosing invariant chain sequences) that the fusion of the invariant chain to an antigen which is comprised by an expression system used for vaccination increases the immune response against said antigen, if it is administered with an adenovirus. Accordingly, in one embodiment of the invention, the immunogenic transgene may be co-expressed with invariant chain in a recombinant ChAd155 viral vector.

In another embodiment, the invention provides the use of the capsid of ChAd155 (optionally an intact or recombinant viral particle or an empty capsid is used) to induce an immunomodulatory effect response, or to enhance or adjuvant a cytotoxic T cell response to another active agent by delivering a ChAd155 capsid to a subject. The ChAd155 capsid can be delivered alone or in a combination regimen with an active agent to enhance the immune response thereto. Advantageously, the desired effect can be accomplished without infecting the host with an adenovirus.

Administration Regimens

Commonly, the ChAd155 recombinant adenoviral vectors will be utilized for delivery of therapeutic or immunogenic molecules (such as proteins). It will be readily understood for both applications, that the recombinant adenoviral vectors of the invention are particularly well suited for use in regimens involving repeat delivery of recombinant adenoviral vectors.

Such regimens typically involve delivery of a series of viral vectors in which the viral capsids are alternated. The viral capsids may be changed for each subsequent administration, or after a pre-selected number of administrations of a particular serotype capsid (e.g. one, two, three, four or more). Thus, a regimen may involve delivery of a recombinant adenovirus with a first capsid, delivery with a recombinant adenovirus with a second capsid, and delivery with a recombinant adenovirus with a third capsid. A variety of other regimens which use the adenovirus capsids of the invention alone, in combination with one another, or in combination with other adenoviruses (which are preferably immunologically non-crossreactive) will be apparent to those of skill in the art. Optionally, such a regimen may involve administration of recombinant adenovirus with capsids of other non-human primate adenoviruses, human adenoviruses, or artificial sequences such as are described herein.

The adenoviral vectors of the invention are particularly well suited for therapeutic regimens in which multiple adenoviral-mediated deliveries of transgenes are desired, e.g., in regimens involving redelivery of the same transgene or in combination regimens involving delivery of other transgenes. Such regimens may involve administration of a ChAd155 adenoviral vector, followed by re-administration with a vector from the same serotype adenovirus. Particularly desirable regimens involve administration of a ChAd155 adenoviral vector, in which the source of the adenoviral capsid sequences of the vector delivered in the first administration differs from the source of adenoviral capsid sequences of the viral vector utilized in one or more of the subsequent administrations. For example, a therapeutic regimen involves administration of a ChAd155 vector and repeat administration with one or more adenoviral vectors of the same or different serotypes.

In another example, a therapeutic regimen involves administration of an adenoviral vector followed by repeat administration with a ChAd155 vector which has a capsid which differs from the source of the capsid in the first delivered adenoviral vector, and optionally further administration with another vector which is the same or, preferably, differs from the source of the adenoviral capsid of the vector in the prior administration steps. These regimens are not limited to delivery of adenoviral vectors constructed using the ChAd155 sequences. Rather, these regimens can readily utilize other adenoviral sequences, including, without limitation, other adenoviral sequences including other non-human primate adenoviral sequences, or human adenoviral sequences, in combination with the ChAd155 vectors.

In a further example, a therapeutic regimen may involve either simultaneous (such as co-administration) or sequential (such as a prime-boost) delivery of (i) one or more ChAd155 adenoviral vectors and (ii) a further component such as non-adenoviral vectors, non-viral vectors, and/or a variety of other therapeutically useful compounds or molecules such as antigenic proteins optionally simultaneously administered with adjuvant. Examples of co-

administration include homo-lateral co-administration and contra-lateral co-administration (further described below under 'Delivery Methods and Dosage').

Suitable non-adenoviral vectors for use in simultaneous or particularly in sequential delivery (such as prime-boost) with one or more ChAd155 adenoviral vectors include one or more poxviral vectors. Suitably, the poxviral vector belongs to the subfamily chordopoxvirinae, more suitably to a genus in said subfamily selected from the group consisting of orthopox, parapox, yatapox, avipox (suitably canarypox (ALVAC) or fowlpox (FPV)) and molluscipox. Even more suitably, the poxviral vector belongs to the orthopox and is selected from the group consisting of vaccinia virus, NYVAC (derived from the Copenhagen strain of vaccinia), Modified Vaccinia Ankara (MVA), cowpoxvirus and monkeypox virus. Most suitably, the poxviral vector is MVA.

"Simultaneous" administration suitably refers to the same ongoing immune response. Preferably both components are administered at the same time (such as simultaneous administration of both DNA and protein), however, one component could be administered within a few minutes (for example, at the same medical appointment or doctor's visit), within a few hours. Such administration is also referred to as co-administration. In some embodiments, co-administration may refer to the administration of an adenoviral vector, an adjuvant and a protein component. In other embodiments, co-administration refers to the administration of an adenoviral vector and another viral vector, for example a second adenoviral vector or a poxvirus such as MVA. In other embodiments, co-administration refers to the administration of an adenoviral vector and a protein component, which is optionally adjuvanted.

A prime-boost regimen may be used. Prime-boost refers to two separate immune responses: (i) an initial priming of the immune system followed by (ii) a secondary or boosting of the immune system many weeks or months after the primary immune response has been established.

Such a regimen may involve the administration of a recombinant ChAd155 vector to prime the immune system to second, booster, administration with a traditional antigen, such as a protein (optionally co-administered with adjuvant), or a recombinant virus carrying the sequences encoding such an antigen (e.g., WO 00/11140). Alternatively, an immunization regimen may involve the administration of a recombinant ChAd155 vector to boost the immune response to a vector (either viral or DNA-based) encoding an antigen. In another alternative, an immunization regimen involves administration of a protein followed by booster with a recombinant ChAd155 vector encoding the antigen. In one example, the prime-boost regimen can provide a protective immune response to the virus, bacteria or other organism from which the antigen is derived. In another embodiment, the prime-boost regimen provides a therapeutic effect that can be measured using conventional

assays for detection of the presence of the condition for which therapy is being administered.

Preferably, a boosting composition is administered about 2 to about 27 weeks after administering the priming composition to the subject. The administration of the boosting composition is accomplished using an effective amount of a boosting composition containing or capable of delivering the same antigen or a different antigen as administered by the priming vaccine. The boosting composition may be composed of a recombinant viral vector derived from the same viral source or from another source. Alternatively, the boosting composition can be a composition containing the same antigen as encoded in the priming vaccine, but in the form of a protein, which composition induces an immune response in the host. The primary requirements of the boosting composition are that the antigen of the composition is the same antigen, or a cross-reactive antigen, as that encoded by the priming composition.

Delivery Methods and Dosage

The vector may be prepared for administration by being suspended or dissolved in a pharmaceutically or physiologically acceptable carrier such as isotonic saline; isotonic salts solution or other formulations that will be apparent to those skilled in the art. The appropriate carrier will be evident to those skilled in the art and will depend in large part upon the route of administration. The compositions described herein may be administered to a mammal in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels and liposomes.

In some embodiments, the recombinant adenovirus of the invention is administered to a subject by intramuscular injection, intravaginal injection, intravenous injection, intraperitoneal injection, subcutaneous injection, epicutaneous administration, intradermal administration, nasal administration or oral administration.

If the therapeutic regimen involves co-administration of one or more ChAd155 adenoviral vectors and a further component, each formulated in different compositions, they are favourably administered co-locationally at or near the same site. For example, the components can be administered (e.g. via an administration route selected from intramuscular, transdermal, intradermal, sub-cutaneous) to the same side or extremity (“co-lateral” administration) or to opposite sides or extremities (“contra-lateral” administration).

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective adult human or veterinary dosage of the viral vector

generally contains $1x10^5$ to $1x10^{15}$ viral particles, such as from $1x10^8$ to $1x10^{12}$ (e.g., $1x10^8$, $2.5x10^8$, $5x10^8$, $1x10^9$, $1.5x10^9$, $2.5x10^9$, $5x10^9$, $1x10^{10}$, $1.5x10^{10}$, $2.5x10^{10}$, $5x10^{10}$, $1x10^{11}$ $1.5x10^{11}$, $2.5x10^{11}$, $5x10^{11}$, $1x10^{12}$ particles). Alternatively, a viral vector can be administered at a dose that is typically from $1x10^5$ to $1x10^{10}$ plaque forming units (PFU), such as $1x10^5$ PFU, $2.5x10^5$ PFU, $5x10^5$ PFU, $1x10^6$ PFU, $2.5x10^6$ PFU, $5x10^6$ PFU, $1x10^7$ PFU, $2.5x10^7$ PFU, $5x10^7$ PFU, $1x10^8$ PFU, $2.5x10^8$ PFU, $5x10^8$ PFU, $1x10^9$ PFU, $2.5x10^9$ PFU, $5x10^9$ PFU, or $1x10^{10}$ PFU. Dosages will vary depending upon the size of the animal and the route of administration. For example, a suitable human or veterinary dosage (for about an 80 kg animal) for intramuscular injection is in the range of about 1×10^9 to about 5×10^{12} particles per mL, for a single site. Optionally, multiple sites of administration may be used. In another example, a suitable human or veterinary dosage may be in the range of about 1×10^{11} to about 1×10^{15} particles for an oral formulation.

The viral vector can be quantified by Quantitative PCR Analysis (Q-PCR), for example with primers and probe designed on CMV promoter region using as standard curve serial dilution of plasmid DNA containing the vector genome with expression cassette including HCMV promoter. The copy number in the test sample is determined by the parallel line analysis method. Alternative methods for vector particle quantification can be analytical HPLC or spectrophotometric method based on A_{260} nm.

An immunologically effective amount of a nucleic acid may suitably be between 1 ng and 100 mg. For example, a suitable amount can be from 1 µg to 100 mg. An appropriate amount of the particular nucleic acid (e.g., vector) can readily be determined by those of skill in the art. Exemplary effective amounts of a nucleic acid component can be between 1 ng and 100 µg, such as between 1 ng and 1µg (e.g., 100 ng-1µg), or between1 µg and 100 µg, such as 10 ng, 50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 500 ng, 750 ng, or 1 µg. Effective amounts of a nucleic acid can also include from 1µg to 500 µg, such as between 1 µg and 200 µg, such as between 10 and 100 µg, for example 1 µg, 2 µg, 5 µg, 10 µg, 20 µg, 50 µg, 75 µg, 100 µg, 150 µg, or 200 µg. Alternatively, an exemplary effective amount of a nucleic acid can be between 100 µg and 1 mg, such as from 100 µg to 500 µg, for example, 100 µg, 150 µg, 200 µg, 250 µg, 300 µg, 400 µg, 500 µg, 600 µg, 700 µg, 800 µg, 900 µg or 1 mg.

Generally a human dose will be in a volume of between 0.1ml and 2 ml. Thus the composition described herein can be formulated in a volume of, for example 0.1, 0.15, 0.2, 0.5, 1.0, 1.5 or 2.0 ml human dose per individual or combined immunogenic components.

One of skill in the art may adjust these doses, depending on the route of administration and the therapeutic or vaccine application for which the recombinant vector is employed. The levels of expression of the transgene, or for an adjuvant, the level of circulating antibody, can be monitored to determine the frequency of dosage administration.

If one or more priming and/or boosting steps are used, this step may include a single dose that is administered hourly, daily, weekly or monthly, or yearly. As an example, mammals may receive one or two doses containing between about 10 µg to about 50 µg of plasmid in carrier. The amount or site of delivery is desirably selected based upon the identity and condition of the mammal.

The therapeutic levels of, or level of immune response against, the protein encoded by the selected transgene can be monitored to determine the need, if any, for boosters. Following an assessment of CD8+ T cell response, or optionally, antibody titers, in the serum, optional booster immunizations may be desired. Optionally, the recombinant ChAd155 vectors may be delivered in a single administration or in various combination regimens, e.g., in combination with a regimen or course of treatment involving other active ingredients or in a prime-boost regimen.

The present invention will now be further described by means of the following non-limiting examples.

EXAMPLES

Example 1: Isolation of ChAd155

Wild type chimpanzee adenovirus type 155 (ChAd155) was isolated from a healthy young chimpanzee housed at the New Iberia Research Center facility (New Iberia Research Center; The University of Louisiana at Lafayette) using standard procedures as described in Colloca *et al.* (2012) and WO 2010086189, which is hereby incorporated by reference for the purpose of describing adenoviral isolation and characterization techniques

Example 2: ChAd155 Vector Construction

The ChAd155 viral genome was then cloned in a plasmid or in a BAC vector and subsequently modified (Figure 2) to carry the following modifications in different regions of the ChAd155 viral genome:

a) deletion of the E1 region (from bp 449 to bp 3529) of the viral genome;
b) deletion of the E4 region (from bp 34731 to bp 37449) of the viral genome;
c) insertion of the E4orf6 derived from human Ad5.

<u>2.1: Deletion of E1 region: Construction of BAC/ChAd155 ΔE1 TetO hCMV RpsL-Kana#1375</u>

The ChAd155 viral genome was cloned into a BAC vector by homologous recombination in *E. coli* strain BJ5183 electroporation competent cells (Stratagene catalog no. 2000154) co-transformed with ChAd155 viral DNA and Subgroup C BAC Shuttle (#1365). As shown in the schematic of Figure 3, the Subgroup C Shuttle is a BAC vector derived from pBeloBAC11 (GenBank U51113, NEB) and which is dedicated to the cloning of ChAd belonging to species C and therefore contains the pIX gene and DNA fragments derived from right and left ends (including right and left ITRs) of species C ChAd viruses.

The Species C BAC Shuttle also contains a RpsL-Kana cassette inserted between left end and the pIX gene. In addition, an Amp-LacZ-SacB selection cassette, flanked by IScel restriction sites, is present between the pIX gene and right end of the viral genome. In particular, the BAC Shuttle comprised the following features: Left ITR: bp 27 to 139, hCMV(tetO) RpsL-Kana cassette: bp 493 to 3396, pIX gene: bp 3508 to 3972, IScel restriction sites: bp 3990 and 7481, Amp-LacZ-SacB selection cassette: bp 4000 to 7471, Right ITR: bp 7805 to 7917.

BJ5183 cells were co-transformed by electroporation with ChAd155 purified viral DNA and Subgroup C BAC Shuttle vector digested with IScel restriction enzyme and then purified from gel. Homologous recombination occurring between pIX gene and right ITR sequences (present at the ends of Species C BAC Shuttle linearized DNA) and homologous sequences present in ChAd155 viral DNA lead to the insertion of ChAd155 viral genomic DNA in the BAC shuttle vector. At the same time, the viral E1 region was deleted and substituted by the RpsL-Kana cassette, generating BAC/ChAd155 ΔE1/ TetO hCMV RpsL-Kana#1375.

2.2: Plasmid construction by homologous recombination in E.coli BJ5183

2.2.1: Deletion of E4 region - Construction of pChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RpsL-Kana (#1434)

To improve propagation of the vector, a deletion of the E4 region spanning from nucleotide 34731-37449 (ChAd155 wild type sequence) was introduced in the vector backbone by replacing the native E4 region with Ad5 E4orf6 coding sequence using a strategy involving several steps of cloning and homologous recombination in *E.coli*. The E4 coding region was completely deleted while the E4 native promoter and polyadenylation signal were conserved. To this end, a shuttle vector was constructed to allow the insertion of Ad5orf6 by replacing the ChAd155 native E4 region by homologous recombination in *E.coli* BJ5183 as detailed below.

Construction of pARS SpeciesC Ad5E4orf6-1

A DNA fragment containing Ad5orf6 was obtained by PCR using Ad5 DNA as template, with the oligonucleotides 5'-ATACGGACTAGTGGAGAAGTACTCGCCTACATG-3' (SEQ ID NO: 13) and 5'-ATACGGAAGATCTAAGACTTCAGGAAATATGACTAC-3' (SEQ ID NO: 14). The PCR fragment was digested with BglII and SpeI and cloned into Species C RLD-EGFP shuttle digested with BglII and SpeI, generating the plasmid pARS Species C Ad5orf6-1. Details regarding the shuttle can be found in Colloca *et al*, Sci. Transl. Med. (2012) 4:115ra.

Construction of pARS SpeciesC Ad5E4orf6-2

To delete the E4 region, a 177 bp DNA fragment spanning bp 34586 to bp 34730 of the ChAd155 wt sequence (SEQ ID NO: 10) was amplified by PCR using the plasmid BAC/ChAd155 ΔE1_TetO hCMV RpsL-Kana (#1375) as a template with the following oligonucleotides: 5'-ATTCAGTGTACAGGCGCGCCAAAGCATGACGCTGTTGATTTGATTC-3' (SEQ ID NO: 15) and 5'-ACTAGGACTAGTTATAAGCTAGAATGGGGCTTTGC-3' (SEQ ID NO: 16). The PCR fragment was digested with BsrGI and SpeI and cloned into pARS SubGroupC Ad5orf6-1 digested with BsrGI and SpeI, generating the plasmid pARS SpeciesC Ad5orf6-2 (#1490). A schematic diagram of this shuttle plasmid is provided in Figure 4. In particular, the shuttle plasmid comprised the following features: Left ITR: bp 1 to 113, Species C first 460bp: bp 1 to 460, ChAd155 wt (bp 34587 to bp 34724 of SEQ ID NO:10) : bp 516 to 650, Ad5 orf6: bp 680 and 1561, Species C last 393 bp: bp 1567 to 1969, Right ITR: bp 1857 to 1969.

Construction of pChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RpsL-Kana (#1434)

The resulting plasmid pARS SubGroupC Ad5orf6-2 was then used to replace the E4 region within the ChAd155 backbone with Ad5orf6. To this end the plasmid BAC/ChAd155 ΔE1_TetO hCMV RpsL-Kana (#1375) was digested with PacI/PmeI and co-transformed into BJ5183 cells with the digested plasmid pARS SubGroupC Ad5orf6-2 BsrGI/AscI, to obtain the pChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RpsL-Kana (#1434) pre-adeno plasmid.

2.2.2: Insertion of RSV Expression Cassette - Construction of pChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RSV

An RSV cassette was cloned into a linearised pre-adeno acceptor vector via homologous recombination in *E.coli* by exploiting the homology existing between HCMV promoter and BGH polyA sequences. The plasmid pvjTetOhCMV-bghpolyA_RSV was cleaved with SfiI and SpeI to excise the 4,65 Kb fragment containing the HCMV promoter with tetO, RSV and BGHpolyA sequence. The resulting RSV 4,65 Kb fragment was cloned by

homologous recombination into the pChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RpsL-Kana (#1434) acceptor vector carrying the RpsL-Kana selection cassette under control of HCMV and BGHpA. The acceptor pre-adeno plasmid was linearized with the restriction endonuclease SnaBI. The resulting construct was the pChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RSV vector (Figure 5).

2.3: BAC vector construction by recombineering

2.3.1: Deletion of E4 region - Construction of BAC/ChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RpsL-Kana#1390

A deletion of the E4 region spanning from nucleotide 34731-37449 of the ChAd155 wt sequence was introduced in the vector backbone by replacing this native E4 region with the Ad5 E4orf6 coding sequence using a strategy involving two different steps of recombineering in *E.Coli* SW102 competent cells.

The first step resulted in insertion of a selection cassette including the suicide gene SacB, ampicillin–R gene and lacZ (Amp-LacZ-SacB selection cassette) in the E4 region of ChAd155, for the purpose of positive/negative selection of recombinants.

First step – Substitution of ChAd155 native E4 region with Amp-LacZ-SacB selection cassette

The Amp-LacZ-SacB selection cassette was amplified by PCR using the oligonucleotides provided below containing E4 flanking sequences to allow homologous recombination: 1021-FW E4 Del Step1 (5'-TTAATAGACACAGTAGCTTAATAGACCCAGTAGTGCAAAGCCCCATTCTAGCTTATAACCCCTATTTGTTTATTTTTCT-3') (SEQ ID NO: 17) and 1022-RW E4 Del Step1 (5'-ATATATACTCTCTCGGCACTTGGCCTTTTACACTGCGAAGTGTTGGTGCTGGTGCTGCGTTGAGAGATCTTTATTTGTTAACTGTTAATTGTC-3') (SEQ ID NO: 18).

The PCR product was used to transform *E.Coli* SW102 competent cells containing the pAdeno plasmid BAC/ChAd155 (DE1) tetO hCMV - RpsLKana#1375. The transformation of SW102 cells allowed the insertion of the selection cassette in the E4 region of ChAd155 via lambda (λ) Red-mediated homologous recombination, thus obtaining BAC/ChAd155 (DE1) TetOhCMV - RpsL Kana #1379 (including Amp-LacZ-SacB cassette by substituting ChAd155 native E4 region).

Second step – Substitution of Amp-lacZ-SacB selection cassette with Ad5E4orf6 region

The resulting plasmid BAC/ChAd155 (DE1) TetOhCMV - RpsL Kana #1379 (with Amp-LacZ-SacB cassette in place of ChAd155 E4 region) was then manipulated to replace the Amp-lacZ-SacB selection cassette with Ad5orf6 within the ChAd155 backbone. To this end, a DNA fragment containing the Ad5orf6 region was obtained by PCR, using the oligonucleotides 1025-FW E4 Del Step2 (5'-TTAATAGACACAGTAGCTTAATA-3') (SEQ ID NO: 19) and 1026-RW E4 Del Step2 (5'-GGAAGGGAGTGTCTAGTGTT-3') (SEQ ID NO: 20). The resulting DNA fragment was introduced into *E. coli* SW102 competent cells containing the pAdeno plasmid BAC/ChAd155 (DE1) TetOhCMV - RpsL Kana)#1379, resulting in a final plasmid BAC/ChAd155 (ΔE1, E4 Ad5E4orf6) TetOhCMV - RpsL Kana#1390 containing Ad5orf6 substituting the native ChAd155 E4 region.

2.3.2: Insertion of RSV Expression Cassette: Construction of BAC/ChAd155 ΔE1, E4_Ad5E4orf6 /TetOhCMV RSV#1393

An RSV transgene was cloned into the BAC/ChAd155 ΔE1, E4_Ad5E4orf6 /TetOhCMV RSV#1393 vector by substituting the RpsL-Kana selection cassette. The construction strategy was based on two different steps of recombineering in *E.Coli* SW102 competent cells.

First step – Substitution of RpsL-Kana cassette with Amp-LacZ-SacB selection cassette

The Amp-LacZ-SacB selection cassette was obtained from plasmid BAC/ChAd155 (DE1) TetO hCMV Amp-LacZ-SacB#1342 by PCR using the oligonucleotides 91-SubMonte FW (5'-CAATGGGCGTGGATAGCGGTTTGAC-3') (SEQ ID NO: 21) and 890-BghPolyA RW (5'-CAGCATGCCTGCTATTGTC-3') (SEQ ID NO: 22). The product was transformed into *E.Coli* SW102 competent cells containing the pAdeno plasmid BAC/ChAd155 (DE1, E4 Ad5E4orf6) TetOhCMV - RpsL Kana#1390, resulting in BAC/ChAd155 (DE1, E4 Ad5E4orf6) TetOhCMV – Amp-LacZ-SacB#1386.

Second step – Substitution of Amp-lacZ-SacB selection cassette with RSV transgene

The RSV transgene was inserted in plasmid BAC/ChAd155 (DE1, E4 Ad5E4orf6) TetOhCMV – Amp-LacZ-SacB#1386 by replacing the Amp-lacZ-SacB selection cassette by homologous recombination. To this end, the plasmid pvjTetOhCMV-bghpolyA_RSV#1080 (containing an RSV expression cassette) was cleaved with SpeI and SfiI to excise the 4.4 Kb fragment including the HCMV promoter, RSV and BGHpolyA. The resulting RSV 4.4 Kb fragment was transformed into *E.Coli* SW102 competent cells containing the pAdeno plasmid BAC/ChAd155 (DE1, E4 Adr5E4orf6) TetOhCMV – Amp-LacZ-SacB#1386, resulting in the final plasmid BAC/ChAd155 ΔE1, E4_Ad5E4orf6 / TetO hCMV Kana#1390. The structure of the BAC carrying ChAd155/RSV (SEQ ID NO: 11) is illustrated in Figure 6. In particular, ChAd155/RSV comprised the following features:

Species C Left ITR: bp 1 to 113, hCMV(tetO) bp 467 to 1311, RSV gene: bp 1348 to 4785, bghpolyA: bp 4815 to 5032, Ad5E4orf6: bp 36270 to 37151, Species C Right ITR: bp 37447 to 37559.

5 **Example 3: Vector production**

The productivity of ChAd155 was evaluated in comparison to ChAd3 and PanAd3 in the Procell 92 cell line.

10 3.1: Production of vectors comprising an HIV Gag transgene

Vectors expressing the HIV Gag protein were prepared as described above (ChAd155/GAG) or previously (ChAd3/GAG Colloca *et al*, Sci. Transl. Med. (2012) 4:115ra). ChAd3/GAG and ChAd155/GAG were rescued and amplified in Procell 92 until
15 passages 3 (P3); P3 lysates were used to infect 2 T75 flasks of Procell 92 cultivated in monolayer with each vector. A multiplicity of infection (MOI) of 100 vp/cell was used for both infection experiments. The infected cells were harvested when full CPE was evident (72 hours post-infection) and pooled; the viruses were released from the infected cells by 3 cycles of freeze/thaw (-70°/37°C) then the lysate was clarified by centrifugation. The
20 clarified lysates were quantified by Quantitative PCR Analysis with primers and probe complementary to the CMV promoter region. The oligonucleotide sequences are the following: CMVfor 5'-CATCTACGTATTAGTCATCGCTATTACCA-3' (SEQ ID NO: 23), CMVrev 5'-GACTTGGAAATCCCCGTGAGT-3' (SEQ ID NO: 24), CMVFAM-TAMRA probe 5'-ACATCAATGGGCGTGGATAGCGGTT-3' (SEQ ID NO: 25) (QPCRs were run on ABI
25 Prism 7900 Sequence detector – Applied Biosystem). The resulting volumetric titers (vp/ml) measured on clarified lysates and the specific productivity expressed in virus particles per cell (vp/cell) are provided in Table 1 below and illustrated in Figure 7.

Table 1. Vector productivity from P3 lysates.

30

Vector	vp/ml	Total vp (20 ml conc.)	vp/cell
ChAd3/GAG	9,82E+09	1,96E+11	6,61E+03
ChAd155/GAG	1,11E+10	2,22E+11	7,46E+03

To confirm the higher productivity of the ChAd155 vector expressing HIV Gag transgene, a second experiment was performed by using purified viruses as inoculum. To this end, Procell 92 cells were seeded in a T25 Flask and infected with ChAd3/GAG and
35 ChAd155/GAG when the confluence of the cells was about 80%, using a MOI=100 vp/cell

of infection. The infected cells were harvested when full CPE was evident; the viruses were released from the infected cells by freeze/thaw and clarified by centrifugation. The clarified lysates were quantified by Quantitative PCR Analysis by using following primers and probe: CMVfor 5'-CATCTACGTATTAGTCATCGCTATTACCA-3' (SEQ ID NO: 23), CMV rev GACTTGGAAATCCCCGTGAGT (SEQ ID NO: 24), CMV FAM-TAMRA probe 5'-ACATCAATGGGCGTGGATAGCGGTT-3' (SEQ ID NO: 25) complementary to the CMV promoter region (samples were analysed on an ABI Prism 7900 Sequence detector-Applied Biosystems). The resulting volumetric titers (vp/ml) measured on clarified lysates and the specific productivity expressed in virus particles per cell (vp/cell) are provided in Table 2 below and illustrated in Figure 8.

Table 2. Vector productivity from purified viruses.

Vector	vp/ml	Total vp/T25 flask (5ml of lysate)	vp/cell
ChAd3/GAG	1,00E+10	5,00E+10	1,67E+04
ChAd155/GAG	1,21E+10	6,05E+10	2,02E+04

3.2: Production of vectors comprising an RSV transgene

A different set of experiments were performed to evaluate the productivity of RSV vaccine vectors in Procell 92.S cultivated in suspension. The experiment compared PanAd3/RSV (described in WO2012/089833) and Chad155/RSV in parallel by infecting Procell 92.S at a cell density of $5x10^5$ cells/ml. The infected cells were harvested 3 days post infection; the virus was released from the infected cells by 3 cycles of freeze/thaw and the lysate was clarified by centrifugation. The clarified lysates were then quantified by Quantitative PCR Analysis as reported above. The volumetric productivity and the cell specific productivity are provided in Table 3 below and illustrated in Figure 9.

Table 3

Virus	Volumetric productivity (Vp/ml)	Total vp	Cell specific productivity (vp/cell)
PanAd3/RSV	5,82E+09	2,91E+11	1,16E+4
ChAd155/RSV	3,16E+10	1,58E+12	6,31E+04

Example 4: Transgene Expression Levels

4.1: Expression level of HIV Gag transgene

Expression levels were compared in parallel experiments by infecting HeLa cells with ChAd3 and ChAd155 vectors comprising an HIV Gag transgene. HeLa cells were seeded in 24 well plates and infected in duplicate with ChAd3/GAG and ChAd155/GAG purified viruses using a MOI=250 vp/cell. The supernatants of HeLa infected cells were harvested 48 hours post-infection, and the production of secreted HIV GAG protein was quantified by using a commercial ELISA Kit (HIV-1 p24 ELISA Kit, PerkinElmer Life Science). The quantification was performed according to the manufacturer's instruction by using an HIV-1 p24 antigen standard curve. The results, expressed in pg/ml of GAG protein, are illustrated in Figure 10.

4.1: Expression level of RSV F transgene

Expression levels were compared in parallel experiments by infecting HeLa cells with the above-described PanAd3 and ChAd155 vectors comprising an RSV F transgene. To this end, HeLa cells were seeded in 6 well plates and infected in duplicate with PanAd3/RSV and ChAd155/RSV purified viruses using a MOI=500 vp/cell. The supernatants were harvested 48 hours post-infection, and the production of secreted RSV F protein was quantified by ELISA. Five different dilutions of the supernatants were transferred to microplate wells which are coated with a commercial mouse anti-RSV F monoclonal antibody. The captured antigen was revealed using a secondary anti-RSV F rabbit antiserum followed by Biotin-conjugated anti-rabbit IgG, then by adding Streptavidin-AP conjugate (BD Pharmingen cat. 554065). The quantification was performed by using an RSV F protein (Sino Biological cat. 11049-V08B) standard curve. The results obtained, expressed as ug/ml of RSV F protein, are provided in Table 4 below.

Table 4

Sample	µg/ml RSV F protein
ChAd155/RSV	5,9
PanAd3/RSV	4

A western blot analysis was also performed to confirm the higher level of transgene expression provided by the ChAd155 RSV vector relative to the PanAd3 RSV vector. HeLa cells plated in 6 well plates were infected with PanAd3/RSV and ChAd155/RSV purified viruses using MOI=250 and 500 vp/cell. The supernatants of HeLa infected cells were harvested and the production of secreted RSV F protein were analysed by non-reducing SDS gel followed by Western Blot analysis. Equivalent quantities of supernatants were loaded on non-reducing SDS gel; after electrophoresis separation, the proteins were

transferred to a nitrocellulose membrane to be probed with an anti-RSV F mouse monoclonal antibody (clone RSV-F-3 catalog no: ABIN308230 available at antibodies-online.com (last accessed 13 April 2015). After the incubation with primary antibody, the membrane was washed and then incubated with anti-mouse HRP conjugate secondary antibody. Finally the assay was developed by electrochemiluminescence using standard techniques (ECL detection reagents Pierce catalog no W3252282). The Western Blot results are shown in Figure 11. A band of about 170 kD indicated by the arrow was revealed by monoclonal antibody mAb 13 raised against the F protein, which corresponds to the expected weight of trimeric F protein. It can be seen that the ChAd155 RSV vector produced a darker band at both MOI=250 and 500vp/cell.

Example 5: Evaluation of immunological potency by mouse immunization experiments

5.1: Immunogenicity of vectors comprising the HIV Gag transgene

The immunogenicity of the ChAd155/GAG vector was evaluated in parallel with the ChAd3/GAG vector in BALB/c mice (5 per group). The experiment was performed by injecting 10^6 viral particles intramuscularly. T-cell response was measured 3 weeks after the immunization by ex vivo IFN-gamma enzyme-linked immunospot (ELISpot) using a GAG CD8+ T cell epitope mapped in BALB/c mice. The results are shown in Figure 12, expressed as IFN-gamma Spot Forming Cells (SFC) per million of splenocytes. Each dot represents the response in a single mouse, and the line corresponds to the mean for each dose group. Injected dose in number of virus particles and frequency of positive mice to the CD8 immunodominant peptide are shown on the x axis.

5.2 Immunogenicity of vectors comprising the RSV transgene

The immunological potency of the PanAd3/RSV and ChAd155/RSV vectors was evaluated in BALB/c mice. Both vectors were injected intramuscularly at doses of 10^8, 10^7 and $3X10^6$ vp. Three weeks after vaccination the splenocytes of immunized mice were isolated and analyzed by IFN-gamma-ELISpot using as antigens immunodominant peptide F and M epitopes mapped in BALB/c mice. The levels of immune-responses were reduced in line with decreasing dosage (as expected) but immune responses were clearly higher in the groups of mice immunized with ChAd155/RSV vector compared to the equivalent groups of mice immunized with PanAd3/RSV vaccine (Figure 13). In Figure 13, symbols show individual mouse data, expressed as IFN-gamma Spot Forming Cells (SFC)/million splenocytes, calculated as the sum of responses to the three immunodominant epitopes (F_{51-66} F_{85-93} and $M2\text{-}1_{282-290}$) and corrected for background. Horizontal lines represent the mean number of IFN-gamma SFC/million splenocytes for each dose group.

Conclusion

Taken together the results reported above demonstrated that ChAd155 is an improved adenoviral vector in comparison to ChAd3 and PanAd3 vectors. ChAd155 was shown to be more productive therefore facilitating the manufacture process, able to express higher level of transgene in vitro and also in vivo providing a stronger T-cell response against the antigens expressed in animal models.

DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 - Polypeptide sequence of ChAd155 fiber

MKRTKTSDESFNPVYPYDTESGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDITTASPPLKKTKTNLSLETSSPLTVSTSGALTVAAAAPLAVAGTSLTMQSEAPL
TVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPLSTSNGSLGIDMQAPIYTTNGKL
GLNFGAPLHVVDSLNALTVVTGQGLTINGTALQTRVSGALNYDTSGNLELRAAGGMRVDANGQLIL
DVAYPFDAQNNLSLRLGQGPLFVNSAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAI
NAGDGLQFDSGSDTNPLKTKLGLGLDYDSSRAIIAKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDP
SPNCRIYSEKDAKFTLVLTKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLLSNSSLDPQ
YWNYRKGDLTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKSKPMTLTITLNGTNET
GDATVSTYSMSFSWNWNGSNYINETFQTNSFTFSYIAQE

SEQ ID NO: 2 - Polynucleotide sequence encoding ChAd155 fiber

ATGAAGCGCACCAAAACGTCTGACGAGAGCTTCAACCCCGTGTACCCCTATGACACGGAAAGCG
GCCCTCCCTCCGTCCCTTTCCTCACCCCTCCCTTCGTGTCTCCCGATGGATTCCAAGAAAGTCCCC
CCGGGGTCCTGTCTCTGAACCTGGCCGAGCCCCTGGTCACTTCCCACGGCATGCTCGCCCTGAAA
ATGGGAAGTGGCCTCTCCCTGGACGACGCTGGCAACCTCACCTCTCAAGATATCACCACCGCTA
GCCCTCCCCTCAAAAAAACCAAGACCAACCTCAGCCTAGAAACCTCATCCCCCCTAACTGTGAG
CACCTCAGGCGCCCTCACCGTAGCAGCCGCCGCTCCCCTGGCGGTGGCCGGCACCTCCCTCACC
ATGCAATCAGAGGCCCCCCTGACAGTACAGGATGCAAAACTCACCCTGGCCACCAAAGGCCCCC
TGACCGTGTCTGAAGGCAAACTGGCCTTGCAAACATCGGCCCCGCTGACGGCCGCTGACAGCAG
CACCCTCACAGTCAGTGCCACACCACCCCTTAGCACAAGCAATGGCAGCTTGGGTATTGACATG
CAAGCCCCCATTTACACCACCAATGGAAAACTAGGACTTAACTTTGGCGCTCCCCTGCATGTGGT
AGACAGCCTAAATGCACTGACTGTAGTTACTGGCCAAGGTCTTACGATAAACGGAACAGCCCTA
CAAACTAGAGTCTCAGGTGCCCTCAACTATGACACATCAGGAAACCTAGAATTGAGAGCTGCAG
GGGGTATGCGAGTTGATGCAAATGGTCAACTTATCCTTGATGTAGCTTACCCATTTGATGCACAA
AACAATCTCAGCCTTAGGCTTGGACAGGGACCCCTGTTTGTTAACTCTGCCCACAACTTGGATGT
TAACTACAACAGAGGCCTCTACCTGTTCACATCTGGAAATACCAAAAAGCTAGAAGTTAATATC
AAAACAGCCAAGGGTCTCATTTATGATGACACTGCTATAGCAATCAATGCGGGTGATGGGCTAC
AGTTTGACTCAGGCTCAGATACAAATCCATTAAAAACTAAACTTGGATTAGGACTGGATTATGA
CTCCAGCAGAGCCATAATTGCTAAACTGGGAACTGGCCTAAGCTTTGACAACACAGGTGCCATC
ACAGTAGGCAACAAAAATGATGACAAGCTTACCTTGTGGACCACACCAGACCCATCCCCTAACT
GTAGAATCTATTCAGAGAAAGATGCTAAATTCACACTTGTTTTGACTAAATGCGGCAGTCAGGT

GTTGGCCAGCGTTTCTGTTTTATCTGTAAAAGGTAGCCTTGCGCCCATCAGTGGCACAGTAACTA
GTGCTCAGATTGTCCTCAGATTTGATGAAAATGGAGTTCTACTAAGCAATTCTTCCCTTGACCCT
CAATACTGGAACTACAGAAAAGGTGACCTTACAGAGGGCACTGCATATACCAACGCAGTGGGA
TTTATGCCCAACCTCACAGCATACCCAAAAACACAGAGCCAAACTGCTAAAAGCAACATTGTAA
GTCAGGTTTACTTGAATGGGGACAAATCCAAACCCATGACCCTCACCATTACCCTCAATGGAAC
TAATGAAACAGGAGATGCCACAGTAAGCACTTACTCCATGTCATTCTCATGGAACTGGAATGGA
AGTAATTACATTAATGAAACGTTCCAAACCAACTCCTTCACCTTCTCCTACATCGCCCAAGAA

SEQ ID NO: 3 - Polypeptide sequence of ChAd155 penton

MRRAAMYQEGPPPSYESVVGAAAAAPSSPFASQLLEPPYVPPRYLRPTGGRNSIRYSELAPLFDTTRV
YLVDNKSADVASLNYQNDHSNFLTTVIQNNDYSPSEASTQTINLDDRSHWGGDLKTILHTNMPNVN
EFMFTNKFKARVMVSRSHTKEDRVELKYEWVEFELPEGNYSETMTIDLMNNAIVEHYLKVGRQNG
VLESDIGVKFDTRNFRLGLDPVTGLVMPGVYTNEAFHPDIILLPGCGVDFTYSRLSNLLGIRKRQPFQE
GFRITYEDLEGGNIPALLDVEAYQDSLKENEAGQEDTAPAASAAAEQGEDAADTAAADGAEADPA
MVVEAPEQEEDMNDSAVRGDTFVTRGEEKQAEAEAAAEEKQLAAAAAAAALAAAEAESEGTKPA
KEPVIKPLTEDSKKRSYNLLKDSTNTAYRSWYLAYNYGDPSTGVRSWTLLCTPDVTCGSEQVYWSL
PDMMQDPVTFRSTRQVSNFPVVGAELLPVHSKSFYNDQAVYSQLIRQFTSLTHVFNRFPENQILARPP
APTITTVSENVPALTDHGTLPLRNSIGGVQRVTVTDARRRTCPYVYKALGIVSPRVLSSRTF

SEQ ID NO: 4 - Polynucleotide sequence encoding ChAd155 penton

ATGCGGCGCGCGGCGATGTACCAGGAGGGACCTCCTCCCTCTTACGAGAGCGTGGTGGGCGCGG
CGGCGGCGGCGCCCTCTTCTCCCTTTGCGTCGCAGCTGCTGGAGCCGCCGTACGTGCCTCCGCGC
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CCACAGCAATTTTTTGACCACGGTCATCCAGAACAATGACTACAGCCCGAGCGAGGCCAGCACC
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ACATGCCCAACGTGAACGAGTTCATGTTCACCAATAAGTTCAAGGCGCGGGTGATGGTGTCGCG
CTCGCACACCAAGGAAGACCGGGTGGAGCTGAAGTACGAGTGGGTGGAGTTCGAGCTGCCAGA
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AAAGTGGGCAGGCAGAACGGGGTCCTGGAGAGCGACATCGGGGTCAAGTTCGACACCAGGAAC
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CTCCTGGGCATCCGCAAGCGGCAGCCCTTCCAGGAGGGCTTCAGGATCACCTACGAGGACCTGG
AGGGGGGCAACATCCCCGCGCTCCTCGATGTGGAGGCCTACCAGGATAGCTTGAAGGAAAATG
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AGGAGGAGGACATGAATGACAGTGCGGTGCGCGGAGACACCTTCGTCACCCGGGGGGAGGAAA
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TGCTGTGCACGCCGGACGTGACCTGCGGCTCGGAGCAGGTGTACTGGTCGCTGCCCGACATGAT
GCAAGACCCCGTGACCTTCCGCTCCACGCGGCAGGTCAGCAACTTCCCGGTGGTGGGCGCCGAG
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SEQ ID NO: 5 - Polypeptide sequence of ChAd155 hexon

MATPSMMPQWSYMHISGQDASEYLSPGLVQFARATDSYFSLSNKFRNPTVAPTHDVTTDRSQRLTL
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EIGVGNNFAMEINLSANLWRNFLYSNVALYLPDKLKYNPSNVEISDNPNTYDYMNKRVVAPGLVDC
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AANMLYPIPANATNVPISIPSRNWAAFRGWAFTRLKTKETPSLGSGFDPYYTYSGSIPYLDGTFYLNH
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SEQ ID NO: 6 - Polynucleotide sequence encoding ChAd155 hexon

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SEQ ID NO: 7 - Polynucleotide sequence encoding ChAd155#1434 backbone construct

CATCATCAATAATATACCTTATTTTGGATTGAAGCCAATATGATAATGAGATGGGCGGCGCGGG
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SEQ ID NO: 8 - Polynucleotide sequence encoding ChAd155#1390 backbone construct

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SEQ ID NO: 9 - Polynucleotide sequence encoding ChAd155#1375 backbone construct

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SEQ ID NO: 11 - Polynucleotide sequence encoding ChAd155/RSV

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GTCCCCTGCTAGTGGAGCGGGAGCGGGGTCCCTGTGTCCTAACTATCGCCTGCAACTGCCCTAAC
CCTGGATTACATCAAGATCTTTGCTGTCATCTCTGTGCTGAGTTTAATAAACGCTGAGATCAGAA
TCTACTGGGGCTCCTGTCGCCATCCTGTGAACGCCACCGTCTTCACCCACCCCGACCAGGCCCAG
GCGAACCTCACCTGCGGTCTGCATCGGAGGGCCAAGAAGTACCTCACCTGGTACTTCAACGGCA
CCCCCTTTGTGGTTTACAACAGCTTCGACGGGGACGGAGTCTCCCTGAAAGACCAGCTCTCCGGT
CTCAGCTACTCCATCCACAAGAACACCACCCTCCAACTCTTCCCTCCCTACCTGCCGGGAACCTA
CGAGTGCGTCACCGGCCGCTGCACCCACCTCACCCGCCTGATCGTAAACCAGAGCTTTCCGGGA
ACAGATAACTCCCTCTTCCCCAGAACAGGAGGTGAGCTCAGGAAACTCCCCGGGGACCAGGGCG
GAGACGTACCTTCGACCCTTGTGGGGTTAGGATTTTTTATTACCGGGTTGCTGGCTCTTTTAATCA
AAGTTTCCTTGAGATTTGTTCTTTCCTTCTACGTGTATGAACACCTCAACCTCCAATAACTCTACC
CTTTCTTCGGAATCAGGTGACTTCTCTGAAATCGGGCTTGGTGTGCTGCTTACTCTGTTGATTTTT
TTCCTTATCATACTCAGCCTTCTGTGCCTCAGGCTCGCCGCCTGCTGCGCACACATCTATATCTAC
TGCTGGTTGCTCAAGTGCAGGGGTCGCCACCCAAGATGAACAGGTACATGGTCCTATCGATCCT
AGGCCTGCTGGCCCTGGCGGCCTGCAGCGCCGCCAAAAAAGAGATTACCTTTGAGGAGCCCGCT
TGCAATGTAACTTTCAAGCCCGAGGGTGACCAATGCACCACCCTCGTCAAATGCGTTACCAATC
ATGAGAGGCTGCGCATCGACTACAAAAACAAAACTGGCCAGTTTGCGGTCTATAGTGTGTTTAC
GCCCGGAGACCCCTCTAACTACTCTGTCACCGTCTTCCAGGGCGGACAGTCTAAGATATTCAATT
ACACTTTCCCTTTTTATGAGTTATGCGATGCGGTCATGTACATGTCAAAACAGTACAACCTGTGG
CCTCCCTCTCCCCAGGCGTGTGTGGAAAATACTGGGTCTTACTGCTGTATGGCTTTCGCAATCAC
TACGCTCGCTCTAATCTGCACGGTGCTATACATAAAATTCAGGCAGAGGCGAATCTTTATCGATG
AAAAGAAAATGCCTTGATCGCTAACACCGGCTTTCTATCTGCAGAATGAATGCAATCACCTCCCT
ACTAATCACCACCACCCTCCTTGCGATTGCCCATGGGTTGACACGAATCGAAGTGCCAGTGGGG
TCCAATGTCACCATGGTGGGCCCCGCCGGCAATTCCACCCTCATGTGGGAAAAATTTGTCCGCA
ATCAATGGGTTCATTTCTGCTCTAACCGAATCAGTATCAAGCCCAGAGCCATCTGCGATGGGCA
AAATCTAACTCTGATCAATGTGCAAATGATGGATGCTGGGTACTATTACGGGCAGCGGGGAGAA
ATCATTAATTACTGGCGACCCCACAAGGACTACATGCTGCATGTAGTCGAGGCACTTCCCACTAC
CACCCCCACTACCACCTCTCCCACCACCACCACCACTACTACTACTACTACTACTACTACTACTA
CTACCACTACCGCTGCCCGCCATACCCGCAAAAGCACCATGATTAGCACAAAGCCCCCTCGTGC
TCACTCCCACGCCGGCGGGCCCATCGGTGCGACCTCAGAAACCACCGAGCTTTGCTTCTGCCAAT
GCACTAACGCCAGCGCTCATGAACTGTTCGACCTGGAGAATGAGGATGTCCAGCAGAGCTCCGC
TTGCCTGACCCAGGAGGCTGTGGAGCCCGTTGCCCTGAAGCAGATCGGTGATTCAATAATTGAC
TCTTCTTCTTTTGCCACTCCCGAATACCCTCCCGATTCTACTTTCCACATCACGGGTACCAAAGAC
CCTAACCTCTCTTTCTACCTGATGCTGCTGCTCTGTATCTCTGTGGTCTCTTCCGCGCTGATGTTA
CTGGGGATGTTCTGCTGCCTGATCTGCCGCAGAAAGAGAAAAGCTCGCTCTCAGGGCCAACCAC
TGATGCCCTTCCCCTACCCCCCGGATTTTGCAGATAACAAGATATGAGCTCGCTGCTGACACTAA
CCGCTTTACTAGCCTGCGCTCTAACCCTTGTCGCTTGCGACTCGAGATTCCACAATGTCACAGCT
GTGGCAGGAGAAAATGTTACTTTCAACTCCACGGCCGATACCCAGTGGTCGTGGAGTGGCTCAG
GTAGCTACTTAACTATCTGCAATAGCTCCACTTCCCCCGGCATATCCCCAACCAAGTACCAATGC
AATGCCAGCCTGTTCACCCTCATCAACGCTTCCACCCTGGACAATGGACTCTATGTAGGCTATGT

ACCCTTTGGTGGGCAAGGAAAGACCCACGCTTACAACCTGGAAGTTCGCCAGCCCAGAACCACT
ACCCAAGCTTCTCCCACCACCACCACCACCACCACCATCACCAGCAGCAGCAGCAGCAGCAGCC
ACAGCAGCAGCAGCAGATTATTGACTTTGGTTTTGGCCAGCTCATCTGCCGCTACCCAGGCCATC
TACAGCTCTGTGCCCGAAACCACTCAGATCCACCGCCCAGAAACGACCACCGCCACCACCCTAC
5 ACACCTCCAGCGATCAGATGCCGACCAACATCACCCCCTTGGCTCTTCAAATGGGACTTACAAG
CCCCACTCCAAAACCAGTGGATGCGGCCGAGGTCTCCGCCCTCGTCAATGACTGGGCGGGGCTG
GGAATGTGGTGGTTCGCCATAGGCATGATGGCGCTCTGCCTGCTTCTGCTCTGGCTCATCTGCTG
CCTCCACCGCAGGCGAGCCAGACCCCCCATCTATAGACCCATCATTGTCCTGAACCCCGATAAT
GATGGGATCCATAGATTGGATGGCCTGAAAAACCTACTTTTTTCTTTTACAGTATGATAAATTGA
10 GACATGCCTCGCATTTTCTTGTACATGTTCCTTCTCCCACCTTTTCTGGGGTGTTCTACGCTGGCC
GCTGTGTCTCACCTGGAGGTAGACTGCCTCTCACCCTTCACTGTCTACCTGCTTTACGGATTGGTC
ACCCTCACTCTCATCTGCAGCCTAATCACAGTAATCATCGCCTTCATCCAGTGCATTGATTACAT
CTGTGTGCGCCTCGCATACTTCAGACACCACCCGCAGTACCGAGACAGGAACATTGCCCAACTT
CTAAGACTGCTCTAATCATGCATAAGACTGTGATCTGCCTTCTGATCCTCTGCATCCTGCCCACC
15 CTCACCTCCTGCCAGTACACCACAAAATCTCCGCGCAAAAGACATGCCTCCTGCCGCTTCACCCA
ACTGTGGAATATACCCAAATGCTACAACGAAAAGAGCGAGCTCTCCGAAGCTTGGCTGTATGGG
GTCATCTGTGTCTTAGTTTTCTGCAGCACTGTCTTTGCCCTCATAATCTACCCCTACTTTGATTTG
GGATGGAACGCGATCGATGCCATGAATTACCCCACCTTTCCCGCACCCGAGATAATTCCACTGC
GACAAGTTGTACCCGTTGTCGTTAATCAACGCCCCCCATCCCCTACGCCCACTGAAATCAGCTAC
20 TTTAACCTAACAGGCGGAGATGACTGACGCCCTAGATCTAGAAATGGACGGCATCAGTACCGAG
CAGCGTCTCCTAGAGAGGCGCAGGCAGGCGGCTGAGCAAGAGCGCCTCAATCAGGAGCTCCGA
GATCTCGTTAACCTGCACCAGTGCAAAAGAGGCATCTTTTGTCTGGTAAAGCAGGCCAAAGTCA
CCTACGAGAAGACCGGCAACAGCCACCGCCTCAGTTACAAATTGCCCACCCAGCGCCAGAAGCT
GGTGCTCATGGTGGGTGAGAATCCCATCACCGTCACCCAGCACTCGGTAGAGACCGAGGGGTGT
25 CTGCACTCCCCCTGTCGGGGTCCAGAAGACCTCTGCACCCTGGTAAAGACCCTGTGCGGTCTCAG
AGATTTAGTCCCCTTTAACTAATCAAACACTGGAATCAATAAAAAGAATCACTTACTTAAAATC
AGACAGCAGGTCTCTGTCCAGTTTATTCAGCAGCACCTCCTTCCCCTCCTCCCAACTCTGGTACT
CCAAACGCCTTCTGGCGGCAAACTTCCTCCACACCCTGAAGGGAATGTCAGATTCTTGCTCCTGT
CCCTCCGCACCCACTATCTTCATGTTGTTGCAGATGAAGCGCACCAAAACGTCTGACGAGAGCTT
30 CAACCCCGTGTACCCCTATGACACGGAAAGCGGCCCTCCCTCCGTCCCTTTCCTCACCCCTCCCT
TCGTGTCTCCCGATGGATTCCAAGAAAGTCCCCCCGGGGTCCTGTCTCTGAACCTGGCCGAGCCC
CTGGTCACTTCCCACGGCATGCTCGCCCTGAAAATGGGAAGTGGCCTCTCCCTGGACGACGCTG
GCAACCTCACCTCTCAAGATATCACCACCGCTAGCCCTCCCCTCAAAAAAACCAAGACCAACCT
CAGCCTAGAAACCTCATCCCCCCTAACTGTGAGCACCTCAGGCGCCCTCACCGTAGCAGCCGCC
35 GCTCCCCTGGCGGTGGCCGGCACCTCCCTCACCATGCAATCAGAGGCCCCCCTGACAGTACAGG
ATGCAAAACTCACCCTGGCCACCAAAGGCCCCCTGACCGTGTCTGAAGGCAAACTGGCCTTGCA
AACATCGGCCCCGCTGACGGCCGCTGACAGCAGCACCCTCACAGTCAGTGCCACACCACCCCTT
AGCACAAGCAATGGCAGCTTGGGTATTGACATGCAAGCCCCCATTTACACCACCAATGGAAAAC
TAGGACTTAACTTTGGCGCTCCCCTGCATGTGGTAGACAGCCTAAATGCACTGACTGTAGTTACT
40 GGCCAAGGTCTTACGATAAACGGAACAGCCCTACAAACTAGAGTCTCAGGTGCCCTCAACTATG
ACACATCAGGAAACCTAGAATTGAGAGCTGCAGGGGGTATGCGAGTTGATGCAAATGGTCAACT

TATCCTTGATGTAGCTTACCCATTTGATGCACAAAACAATCTCAGCCTTAGGCTTGGACAGGGAC
CCCTGTTTGTTAACTCTGCCCACAACTTGGATGTTAACTACAACAGAGGCCTCTACCTGTTCACA
TCTGGAAATACCAAAAAGCTAGAAGTTAATATCAAAACAGCCAAGGGTCTCATTTATGATGACA
CTGCTATAGCAATCAATGCGGGTGATGGGCTACAGTTTGACTCAGGCTCAGATACAAATCCATT
AAAAACTAAACTTGGATTAGGACTGGATTATGACTCCAGCAGAGCCATAATTGCTAAACTGGGA
ACTGGCCTAAGCTTTGACAACACAGGTGCCATCACAGTAGGCAACAAAAATGATGACAAGCTTA
CCTTGTGGACCACACCAGACCCATCCCCTAACTGTAGAATCTATTCAGAGAAAGATGCTAAATT
CACACTTGTTTTGACTAAATGCGGCAGTCAGGTGTTGGCCAGCGTTTCTGTTTTATCTGTAAAAG
GTAGCCTTGCGCCCATCAGTGGCACAGTAACTAGTGCTCAGATTGTCCTCAGATTTGATGAAAAT
GGAGTTCTACTAAGCAATTCTTCCCTTGACCCTCAATACTGGAACTACAGAAAAGGTGACCTTAC
AGAGGGCACTGCATATACCAACGCAGTGGGATTTATGCCCAACCTCACAGCATACCCAAAAACA
CAGAGCCAAACTGCTAAAAGCAACATTGTAAGTCAGGTTTACTTGAATGGGGACAAATCCAAAC
CCATGACCCTCACCATTACCCTCAATGGAACTAATGAAACAGGAGATGCCACAGTAAGCACTTA
CTCCATGTCATTCTCATGGAACTGGAATGGAAGTAATTACATTAATGAAACGTTCCAAACCAACT
CCTTCACCTTCTCCTACATCGCCCAAGAATAAAAAGCATGACGCTGTTGATTTGATTCAATGTGT
TTCTGTTTTATTTTCAAGCACAACAAAATCATTCAAGTCATTCTTCCATCTTAGCTTAATAGACAC
AGTAGCTTAATAGACCCAGTAGTGCAAAGCCCCATTCTAGCTTATAACTAGTGGAGAAGTACTC
GCCTACATGGGGGTAGAGTCATAATCGTGCATCAGGATAGGGCGGTGGTGCTGCAGCAGCGCGC
GAATAAACTGCTGCCGCCGCCGCTCCGTCCTGCAGGAATACAACATGGCAGTGGTCTCCTCAGC
GATGATTCGCACCGCCCGCAGCATAAGGCGCCTTGTCCTCCGGGCACAGCAGCGCACCCTGATC
TCACTTAAATCAGCACAGTAACTGCAGCACAGCACCACAATATTGTTCAAAATCCCACAGTGCA
AGGCGCTGTATCCAAAGCTCATGGCGGGGACCACAGAACCCACGTGGCCATCATACCACAAGCG
CAGGTAGATTAAGTGGCGACCCCTCATAAACACGCTGGACATAAACATTACCTCTTTTGGCATGT
TGTAATTCACCACCTCCCGGTACCATATAAACCTCTGATTAAACATGGCGCCATCCACCACCATC
CTAAACCAGCTGGCCAAAACCTGCCCGCCGGCTATACACTGCAGGGAACCGGGACTGGAACAAT
GACAGTGGAGAGCCCAGGACTCGTAACCATGGATCATCATGCTCGTCATGATATCAATGTTGGC
ACAACACAGGCACACGTGCATACACTTCCTCAGGATTACAAGCTCCTCCCGCGTTAGAACCATA
TCCCAGGGAACAACCCATTCCTGAATCAGCGTAAATCCCACACTGCAGGGAAGACCTCGCACGT
AACTCACGTTGTGCATTGTCAAAGTGTTACATTCGGGCAGCAGCGGATGATCCTCCAGTATGGTA
GCGCGGGTTTCTGTCTCAAAAGGAGGTAGACGATCCCTACTGTACGGAGTGCGCCGAGACAACC
GAGATCGTGTTGGTCGTAGTGTCATGCCAAATGGAACGCCGGACGTAGTCATATTTCCTGAAGT
CTTAGATCTCTCAACGCAGCACCAGCACCAACACTTCGCAGTGTAAAAGGCCAAGTGCCGAGAG
AGTATATATAGGAATAAAAAGTGACGTAAACGGGCAAAGTCCAAAAAACGCCCAGAAAAACCG
CACGCGAACCTACGCCCCGAAACGAAAGCCAAAAAACACTAGACACTCCCTTCCGGCGTCAACT
TCCGCTTTCCCACGCTACGTCACTTGCCCCAGTCAAACAAACTACATATCCCGAACTTCCAAGTC
GCCACGCCCAAAACACCGCCTACACCTCCCCGCCCGCCGGCCCGCCCCCAAACCCGCCTCCCGC
CCCGCGCCCCGCCCCGCGCCGCCCATCTCATTATCATATTGGCTTCAATCCAAAATAAGGTATAT
TATTGATGATG

SEQ ID NO: 12 - Polynucleotide sequence encoding the CASI promoter

GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC
CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCA
ATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTT
ATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGA
GCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCG
GGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG
CGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGC
GCTCCCTATCAGTGATAGAGATCTCCCTATCAGTGATAGAGATCGTCGACGAGCTCGCGGCGGG
CGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCC
CGGCTCTGACTGACCGCGTTACTAAAACAGGTAAGTCCGGCCTCCGCGCCGGGTTTTGGCGCCTC
CCGCGGGCGCCCCCCTCCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGCGAGCGTCC
TGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCTTAGAACCC
CAGTATCAGCAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCC
AGAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTG
GGGCGGTGAACGCCGATGATGCCTCTACTAACCATGTTCATGTTTTCTTTTTTTTCTACAGGTCC
TGGGTGACGAACAG

SEQ ID NO: 13 - Ad5orf6 primer 1 polynucleotide sequence
ATACGGACTAGTGGAGAAGTACTCGCCTACATG

SEQ ID NO: 14 - Ad5orf6 primer 2 polynucleotide sequence
ATACGGAAGATCTAAGACTTCAGGAAATATGACTAC

SEQ ID NO: 15 - BAC/CHAd155 ΔE1_TetO hCMV RpsL-Kana primer 1 polynucleotide sequence
ATTCAGTGTACAGGCGCGCCAAAGCATGACGCTGTTGATTTGATTC

SEQ ID NO: 16 - BAC/CHAd155 ΔE1_TetO hCMV RpsL-Kana (#1375) primer 2 polynucleotide sequence
ACTAGGACTAGTTATAAGCTAGAATGGGGCTTTGC

SEQ ID NO: 17 - 1021-FW E4 Del Step1 primer polynucleotide sequence
TTAATAGACACAGTAGCTTAATAGACCCAGTAGTGCAAAGCCCCATTCTAGCTTATAACCCCTAT
TTGTTTATTTTTCT

SEQ ID NO: 18 - 1022-RW E4 Del Step1 primer polynucleotide sequence
ATATATACTCTCTCGGCACTTGGCCTTTTACACTGCGAAGTGTTGGTGCTGGTGCTGCGTTGAGA
GATCTTTATTTGTTAACTGTTAATTGTC

SEQ ID NO: 19 - 1025-FW E4 Del Step2 primer polynucleotide sequence

TTAATAGACACAGTAGCTTAATA

SEQ ID NO: 20 - 1026-RW E4 Del Step2 primer polynucleotide sequence
GGAAGGGAGTGTCTAGTGTT

SEQ ID NO: 21 - 91-SubMonte FW primer polynucleotide sequence
CAATGGGCGTGGATAGCGGTTTGAC

SEQ ID NO: 22 - 90-BghPolyA RW primer polynucleotide sequence
CAGCATGCCTGCTATTGTC

SEQ ID NO: 23 - CMVfor primer polynucleotide sequence
CATCTACGTATTAGTCATCGCTATTACCA

SEQ ID NO: 24 - CMVrev primer polynucleotide sequence
GACTTGGAAATCCCCGTGAGT

SEQ ID NO: 25 - CMVFAM-TAMRA qPCR probe polynucleotide sequence
ACATCAATGGGCGTGGATAGCGGTT

SEQ ID NO: 26 - Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) polynucleotide sequence
TAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTT
TACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCAT
TTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCA
ACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCT
GTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCT
GCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGG
GAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTT
CTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGC
GGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCG
CCT

SEQ ID NO: 27 - ChAd3 fiber amino acid sequence
MKRTKTSDESFNPVYPYDTESGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDITTASPPLKKTKTNLSLETSSPLTVSTSGALTVAAAAPLAVAGTSLTMQSEAPL
TVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPINVSSGSLGLDMEDPMYTHDGK
LGIRIGGPLRVVDSLHTLTVVTGNGLTVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLI
LNVAYPFDAQNNLSLRLGQGPLYINTDHNLDLNCNRGLTTTTTNNTKKLETKISSGLDYDTNGAVIIK
LGTGLSFDNTGALTVGNTGDDKLTLWTTPDPSPNCRIHSDKDCKFTLVLTKCGSQILASVAALAVSG
NLASITGTVASVTIFLRFDQNGVLMENSSLDRQYWNFRNGNSTNAAPYTNAVGFMPNLAAYPKTQS

QTAKNNIVSQVYLNGDKSKPMTLTITLNGTNESSETSQVSHYSMSFTWAWESGQYATETFATNSFTF
SYIAEQ

SEQ ID NO: 28- PanAd3 fiber amino acid sequence

MKRAKTSDETFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLRLSEPLVTSHGMLALKMGN
GLSLDDAGNLTSQDVTTVTPPLKKTKTNLSLQTSAPLTVSSGSLTVAAAAPLAVAGTSLTMQSQAPL
TVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSTLTVGTTPPISVSSGSLGLDMEDPMYTHDGK
LGIRIGGPLQVVDSLHTLTVVTGNGITVANNALQTKVAGALGYDSSGNLELRAAGGMRINTGGQLIL
DVAYPFDAQNNLSLRLGQGPLYVNTNHNLDLNCNRGLTTTTSSNTTKLETKIDSGLDYNANGAIIAK
LGTGLTFDNTGAITVGNTGDDKLTLWTTPDPSPNCRIHADKDKFTLVLTKCGSQILASVAALAVSGN
LSSMTGTVSSVTIFLRFDQNGVLMENSSLDKEYWNFRNGNSTNATPYTNAVGFMPNLSAYPKTQSQ
TAKNNIVSEVYLHGDKSKPMILTITLNGTNESSETSQVSHYSMSFTWSWDSGKYATETFATNSFTFSY
IAEQ

SEQ ID NO: 29 - ChAd17 fiber amino acid sequence

MKRTKTSDESFNPVYPYDTESGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDITSTTPPLKKTKTNLSLETSSPLTVSTSGALTVAAAAPLAVAGTSLTMQSEAPL
AVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSSTPPISVSSGSLGLDMEDPMYTHDGK
LGIRIGGPLRVVDSLHTLTVVTGNGLTVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLI
LDVAYPFDAQNNLSLRLGQGPLYVNTDHNLDLNCNRGLTTTTTNNTKKLETKISSGLDYDTNGAVII
KLGTGLSFDNTGALTVGNTGDDKLTLWTTPDPSPNCRIHSDKDCKFTLVLTKCGSQILASVAALAVS
GNLASITGTVASVTIFLRFDQNGVLMENSSLDKQYWNFRNGNSTNAAPYTNAVGFMPNLAAYPKTQ
SQTAKNNIVSQVYLNGDKSKPMTLTITLNGTNESSETSQVSHYSMSFTWAWESGQYATETFATNSFT
FSYIAEQ

SEQ ID NO: 30 - ChAd19 fiber amino acid sequence

MKRTKTSDKSFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDVTTTTPPLKKTKTNLSLETSAPLTVSTSGALTLAAAAPLAVAGTSLTMQSEAPL
TVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPISVSSGSLGLDMEDPMYTHDGK
LGIRIGGPLRVVDSLHTLTVVTGNGIAVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLIL
DVAYPFDAQNNLSLRLGQGPLYVNTDHNLDLNCNRGLTTTTTNNTKKLETKIGSGLDYDTNGAVIIK
LGTGVSFDSTGALSVGNTGDDKLTLWTTPDPSPNCRIHSDKDCKFTLVLTKCGSQILASVAALAVSG
NLASITGTVSSVTIFLRFDQNGVLMENSSLDKQYWNFRNGNSTNATPYTNAVGFMPNLAAYPKTQS
QTAKNNIVSQVYLNGDKSKPMTLTITLNGTNESSETSQVSHYSMSFTWAWESGQYATETFATNSFTF
SYIAEQ

SEQ ID NO: 31 - ChAd24 fiber amino acid sequence

MKRTKTSDESFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDVTTTTPPLKKTKTNLSLETSAPLTVSTSGALTLAAAAPLAVAGTSLTMQSEAPL
TVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPINVSSGSLGLDMENPMYTHDGK
LGIRIGGPLRVVDSLHTLTVVTGNGIAVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLIL
DVAYPFDAQNNLSLRLGQGPLYVNTDHNLDLNCNRGLTTTTTNNTKKLETKIGSGLDYDTNGAVIIK

LGTGVSFDSTGALSVGNTGDDKLTLWTTPDPSPNCRIHSDKDCKFTLVLTKCGSQILASVAALAVSG
NLASITGTVSSVTIFLRFDQNGVLMENSSLDKQYWNFRNGNSTNATPYTNAVGFMPNLAAYPKTQS
QTAKNNIVSQVYLNGDKSKPMILTITLNGTNESSETSQVSHYSMSFTWAWESGQYATETFATNSFTFS
YIAEQ

SEQ ID NO: 32 - ChAd11 fiber amino acid sequence

MKRTKTSDESFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDVTTTTPPLKKTKTNLSLETSAPLTVSTSGALTLAAAVPLAVAGTSLTMQSEAPL
TVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTISATPPLSTSNGSLGIDMQAPIYTTNGKLG
LNFGAPLHVVDSLNALTVVTGQGLTINGTALQTRVSGALNYDSSGNLELRAAGGMRVDANGKLILD
VAYPFDAQNNLSLRLGQGPLFVNSAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAIN
PGDGLEFGSGSDTNPLKTKLGLGLEYDSSRAIIAKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDPSP
NCRIYSEKDAKFTLVLTKCGSQVLASVSVLSVKGSLAPISGTVTSAQIILRFDENGVLLSNSSLDPQYW
NYRKGDLTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKSKPMILTITLNGTNETGD
ATVSTYSMSFSWNWNGSNYINETFQTNSFTFSYIAQE

SEQ ID NO: 33 - ChAd20 fiber amino acid sequence

MKRTKTSDESFNPVYPYDTESGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDITTASPPLKKTKTNLSLETSSPLTVSTSGALTVAAAAPLAVAGTSLTMQSEAPL
TVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPLSTSNGSLGIDMQAPIYTTNGKL
GLNFGAPLHVVDSLNALTVVTGQGLTINGTALQTRVSGALNYDTSGNLELRAAGGMRVDANGQLIL
DVAYPFDAQNNLSLRLGQGPLFVNSAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAI
NAGDGLQFDSGSDTNPLKTKLGLGLDYDSSRAIIAKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDP
SPNCRIYSEKDAKFTLVLTKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLLSNSSLDPQ
YWNYRKGDLTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKSKPMTLTITLNGTNET
GDATVSTYSMSFSWNWNGSNYINETFQTNSFTFSYIAQE

SEQ ID NO: 34 - ChAd31 fiber amino acid sequence

MKRTKTSDESFNPVYPYDTESGPPSVPFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGS
GLSLDDAGNLTSQDITTASPPLKKTKTNLSLETSSPLTVSTSGALTVAAAAPLAVAGTSLTMQSEAPL
TVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPLSTSNGSLGIDMQAPIYTTNGKL
GLNFGAPLHVVDSLNALTVVTGQGLTINGTALQTRVSGALNYDTSGNLELRAAGGMRVDANGQLIL
DVAYPFDAQNNLSLRLGQGPLFVNSAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAI
NAGDGLQFDSGSDTNPLKTKLGLGLDYDSSRAIIAKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDP
SPNCRIYSEKDAKFTLVLTKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLLSNSSLDPQ
YWNYRKGDLTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKSKPMTLTITLNGTNET
GDATVSTYSMSFSWNWNGSNYINETFQTNSFTFSYIAQE

SEQ ID NO: 35 - PanAd1 fiber amino acid sequence

MKRAKTSDETFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLRLSEPLVTSHGMLALKMGN
GLSLDDAGNLTSQDVTTVTPPLKKTKTNLSLQTSAPLTVSSGSLTVAAAAPLAVAGTSLTMQSQAPL
TVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSTLTVSATPPLSTSNGSLSIDMQAPIYTTNGKL

ALNIGAPLHVVDTLNALTVVTGQGLTINGRALQTRVTGALSYDTEGNIQLQAGGGMRIDNNGQLILN
VAYPFDAQNNLSLRLGQGPLIVNSAHNLDLNLNRGLYLFTSGNTKKLEVNIKTAKGLFYDGTAIAIN
AGDGLQFGSGSDTNPLQTKLGLGLEYDSNKAIITKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDPS
PNCRINSEKDAKLTLVLTKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLLSNSSLDPQY
WNYRKGDSTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKTKPMTLTITLNGTNETG
DATVSTYSMSFSWNWNGSNYINDTFQTNSFTFSYIAQE

SEQ ID NO: 36 - PanAd2 fiber amino acid sequence

MKRAKTSDETFNPVYPYDTENGPPSVPFLTPPFVSPDGFQESPPGVLSLRLSEPLVTSHGMLALKMGN
GLSLDDAGNLTSQDVTTVTPPLKKTKTNLSLQTSAPLTVSSGSLTVAAAAPLAVAGTSLTMQSQAPL
TVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSTLTVSATPPLSTSNGSLSIDMQAPIYTTNGKL
ALNIGAPLHVVDTLNALTVVTGQGLTINGRALQTRVTGALSYDTEGNIQLQAGGGMRIDNNGQLILN
VAYPFDAQNNLSLRLGQGPLIVNSAHNLDLNLNRGLYLFTSGNTKKLEVNIKTAKGLFYDGTAIAIN
AGDGLQFGSGSDTNPLQTKLGLGLEYDSNKAIITKLGTGLSFDNTGAITVGNKNDDKLTLWTTPDPS
PNCRINSEKDAKLTLVLTKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLLSNSSLDPQY
WNYRKGDSTEGTAYTNAVGFMPNLTAYPKTQSQTAKSNIVSQVYLNGDKTKPMTLTITLNGTNETG
DATVSTYSMSFSWNWNGSNYINDTFQTNSFTFSYIAQE

SEQ ID NO: 37 - RSV FΔTM amino acid sequence

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCN
GTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKR
RFLGFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQ
LLPIVNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLNDMPITNDQKKL
MSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGW
YCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITS
LGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIIN
FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNRKRRAPVKQTLNFDLLKLAG
DVESNPGPMALSKVKLNDTLNKDQLLSSSKYTIQRSTGDSIDTPNYDVQKHINKLCGMLLITEDANH
KFTGLIGMLYAMSRLGREDTIKILRDAGYHVKANGVDVTTHRQDINGKEMKFEVLTLASLTTEIQINI
EIESRKSYKKMLKEMGEVAPEYRHDSPDCGMIILCIAALVITKLAAGDRSGLTAVIRRANNVLKNEM
KRYKGLLPKDIANS
FYEVFEKYPHFIDVFVHFGIAQSSTRGGSRVEGIFAGLFMNAYGAGQVMLRWGVLAKSVKNIMLGH
ASVQAEMEQVVEVYEYAQKLGGEAGFYHILNNPKASLLSLTQFPHFSSVVLGNAAGLGIMGEYRGT
PRNQDLYDAAKAYAEQLKENGVINYSVLDLTAEELEAIKHQLNPKDNDVELGGGGSGGGGMSRRN
PCKFEIRGHCLNGKRCHFSHNYFEWPPHALLVRQNFMLNRILKSMDKSIDTLSEISGAAELDRTEEYA
LGVVGVLESYIGSINNITKQSACVAMSKLLTELNSDDIKKLRDNEELNSPKIRVYNTVISYIESNRKNN
KQTIHLLKRLPADVLKKTIKNTLDIHKSITINNPKESTVSDTNDHAKNNDTT

SEQ ID NO: 38 - HIV Gag polynucleotide sequence

ATGGGTGCTAGGGCTTCTGTGCTGTCTGGTGGTGAGCTGGACAAGTGGGAGAAGATCAGGCTGA
GGCCTGGTGGCAAGAAGAAGTACAAGCTAAAGCACATTGTGTGGGCCTCCAGGGAGCTGGAGA
GGTTTGCTGTGAACCCTGGCCTGCTGGAGACCTCTGAGGGGTGCAGGCAGATCCTGGGCCAGCT
CCAGCCCTCCCTGCAAACAGGCTCTGAGGAGCTGAGGTCCCTGTACAACACAGTGGCTACCCTG
TACTGTGTGCACCAGAAGATTGATGTGAAGGACACCAAGGAGGCCCTGGAGAAGATTGAGGAG

GAGCAGAACAAGTCCAAGAAGAAGGCCCAGCAGGCTGCTGCTGGCACAGGCAACTCCAGCCAG
GTGTCCCAGAACTACCCCATTGTGCAGAACCTCCAGGGCCAGATGGTGCACCAGGCCATCTCCC
CCCGGACCCTGAATGCCTGGGTGAAGGTGGTGGAGGAGAGGCCTTCTCCCCTGAGGTGATCCCC
ATGTTCTCTGCCCTGTCTGAGGGTGCCACCCCCCAGGACCTGAACACCATGCTGAACACAGTGG
GGGGCCATCAGGCTGCCATGCAGATGCTGAAGGAGACCATCAATGAGGAGGCTGCTGAGTGGG
ACAGGCTGCATCCTGTGCACGCTGGCCCCATTGCCCCCGGCCAGATGAGGGAGCCCAGGGGCTC
TGACATTGCTGGCACCACCTCCACCCTCCAGGAGCAGATTGGCTGGATGACCAACAACCCCCCC
ATCCCTGTGGGGGAAATCTACAAGAGGTGGATCATCCTGGGCCTGAACAAGATTGTGAGGATGT
ACTCCCCCACCTCCATCCTGGACATCAGGCAGGGCCCCAAGGAGCCCTTCAGGGACTATGTGGA
CAGGTTCTACAAGACCCTGAGGGCTGAGCAGGCCTCCCAGGAGGTGAAGAACTGGATGACAGA
GACCCTGCTGGTGCAGAATGCCAACCCTGACTGCAAGACCATCCTGAAGGCCCTGGGCCCTGCT
GCCACCCTGGAGGAGATGATGACAGCCTGCCAGGGGGTGGGGGGCCCTGGTCACAAGGCCAGG
GTGCTGGCTGAGGCCATGTCCCAGGTGACCAACTCCGCCACCATCATGATGCAGAGGGGCAACT
TCAGGAACCAGAGGAAGACAGTGAAGTGCTTCAACTGTGGCAAGGTGGGCCACATTGCCAAGA
ACTGTAGGGCCCCCAGGAAGAAGGGCTGCTGGAAGTGTGGCAAGGAGGGCCACCAGATGAAGG
ACTGCAATGAGAGGCAGGCCAACTTCCTGGGCAAAATCTGGCCCTCCCACAAGGGCAGGCCTGG
CAACTTCCTCCAGTCCAGGCCTGAGCCCACAGCCCCTCCCGAGGAGTCCTTCAGGTTTGGGGAG
GAGAAGACCACCCCCAGCCAGAAGCAGGAGCCCATTGACAAGGAGCTGTACCCCCTGGCCTCCC
TGAGGTCCCTGTTTGGCAACGACCCCTCCTCCCAGTAA

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated recombinant adenoviral polynucleotide, wherein the polynucleotide encodes a polypeptide comprising:

(a) a polypeptide having the amino acid sequence according to SEQ ID NO: 3;

(b) a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and

(c) a polypeptide selected from the group consisting of

(i) a polypeptide having the amino acid sequence according to SEQ ID NO: 1; and

(ii) a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1;

wherein the polynucleotide comprises a mutation or deletion which renders non-functional at least one gene of an adenoviral genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

2. A recombinant adenovirus comprising a polynucleotide encoding a polypeptide comprising:

(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3;

(b) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and

(c) a polynucleotide selected from the group consisting of

(i) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1 and

(ii) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1;

wherein the adenovirus comprises a mutation or deletion which renders non-functional at least one gene of a genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

3. A composition comprising a polynucleotide encoding a polypeptide comprising:

(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3;

(b) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and

(c) a polynucleotide selected from the group consisting of

(i) an isolated recombinant polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1; and

(ii) an isolated recombinant polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1;
wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1;

and a pharmaceutically acceptable excipient, wherein the composition comprises a mutation or deletion which renders non-functional at least one gene of a genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

4. A cell comprising:
(a) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3;
(b) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 5; and
(c) a polynucleotide selected from the group consisting of
(i) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1; and
(ii) a polynucleotide which encodes a functional derivative of a polypeptide having the amino acid sequence according to SEQ ID NO: 1, wherein the functional derivative has an amino acid sequence which is at least 80% identical over its entire length to the amino acid sequence of SEQ ID NO: 1;

wherein the cell is a host cell that expresses at least one adenoviral gene selected from the group consisting of E1A, E1B, E2A, E2B, E3 E4, L1, L2, L3, L4 and L5.

5. The host cell according to claim 4, wherein the host cell is able to grow in suspension.

6. The polynucleotide according to claim 1, the recombinant adenovirus according to claim 2, the composition according to claim 3, or the cell according to claim 4, wherein the polynucleotide comprises at least one of the following:
(a) an adenoviral 5' inverted terminal repeat;
(b) an adenoviral EIA region, or a fragment thereof selected from among the E1A_280R and E1A_243R regions;
(c) an adenoviral EIB or IX region, or a fragment thereof selected from among the group consisting of the E1B_19K, E1B_55K or IX regions;
(d) an adenoviral E2b region; or a fragment thereof selected from among the group consisting of the E2B_pTP, E2B_Polymerase and E2B_IVa2 regions;
(e) an adenoviral L1 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the L1_13.6k protein, L1_52k and L1_IIIa protein;

(f) an adenoviral L2 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the L2_penton protein according to claim 3, L2_pVII, L2_V, and L2_pX protein;

(g) an adenoviral L3 region, or a fragment thereof, said fragment encoding an adenoviral protein selected from the group consisting of the L3_pVI protein, L3_hexon protein according to claim 2 and L3_protease;

(h) an adenoviral E2A region;

(i) an adenoviral L4 region, or a fragment thereof said fragment encoding an adenoviral protein selected from the group consisting of the L4_100k protein, the L4_33k protein and protein L4_VIII;

(j) an adenoviral E3 region, or a fragment thereof selected from the group consisting of E3 ORF1, E3 ORF2, E3 ORF3, E3 ORF4, E3 ORF5, E3 ORF6, E3 ORF7, E3 ORF8, and E3 ORF9;

(k) an adenoviral L5 region, or a fragment thereof said fragment encoding the L5_fiber fiber protein according to claim 1;

(l) an adenoviral E4 region, or a fragment thereof selected from the group consisting of E4 ORF7, E4 ORF6, E4 ORF4, E4 ORF3, E4 ORF2, and E4 ORF1; and/or

(m) an adenoviral 3'-end, preferably an adenoviral 3' inverted terminal repeat.

7. The polynucleotide according to claim 6, further comprising an adenoviral VAI or VAII RNA region.

8. The polynucleotide according to claim 7, wherein the VAI or VAII RNA region is from Ad5.

9. The polynucleotide according to claim 1, the recombinant adenovirus according to claim 2, or the composition according to claim 3, wherein the genomic regions are E1A and/or E1B.

10. The recombinant adenovirus according to claim 2, wherein the recombinant adenovirus is replication-incompetent.

11. The replication-incompetent recombinant adenovirus according to claim 10, wherein the adenovirus comprises a heterologous nucleic acid sequence encoding a protein and wherein the heterologous nucleic acid sequence is operatively linked to one or more sequences which direct expression of the protein in a host cell.

12. The replication-incompetent recombinant adenovirus according to claim 11, wherein the protein is an antigenic protein or a fragment thereof.

13. The recombinant adenovirus according to claim 2, wherein the adenovirus has a seroprevalence of less than 10% in human subjects.

14. The recombinant adenovirus according to claim 13, wherein the adenovirus has a seroprevalence of less than 5% in human subjects.

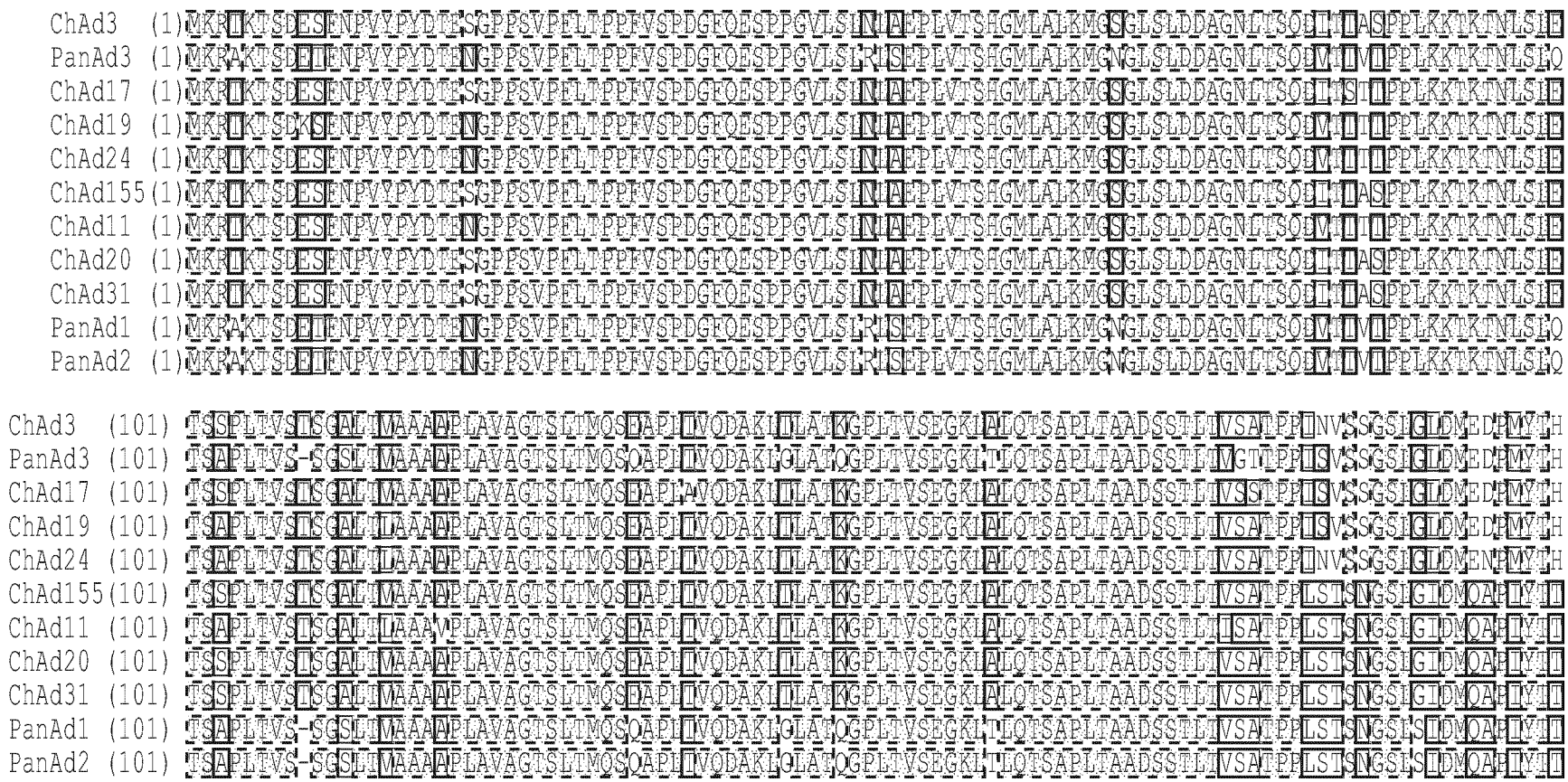
15. A method of inducing an immune response in a subject comprising administering the composition of claim 3 to a mammalian subject.

16. An isolated recombinant adenoviral polynucleotide, where in the polynucleotide encodes a polypeptide comprising:
    (a) a polypeptide having the amino acid sequence according to SEQ ID NO: 1;
    (b) a polypeptide having the amino acid sequence according to SEQ ID NO: 3; and
    (c) a polypeptide having the amino acid sequence according to SEQ ID NO: 5;

wherein the polynucleotide comprises a mutation or deletion which renders non-functional at least one gene of an adenoviral genomic region selected from the group consisting of E1A, E1B, E2A, E2B, E3 and E4.

17. The isolated recombinant adenoviral polynucleotide of claim 16 further comprising a heterologous nucleic acid sequence encoding a protein and wherein the heterologous nucleic acid sequence is operatively linked to one or more sequences that direct expression of the protein in a host cell.

18. Use of a composition of claim 3 in the manufacture of a medicament for inducing an immune response in a subject.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PanAd3 (1)
ChAd17 (1)
ChAd19 (1)
ChAd24 (1)
ChAd155 (1)
ChAd11 (1)
ChAd20 (1)
ChAd31 (1)
PanAd1 (1)
PanAd2 (1)
ChAd3 (101)
PanAd3 (101)
ChAd17 (101)
ChAd19 (101)
ChAd24 (101)
ChAd155 (101)
ChAd11 (101)
ChAd20 (101)
ChAd31 (101)
PanAd1 (101)
PanAd2 (101)

FIG. 1A

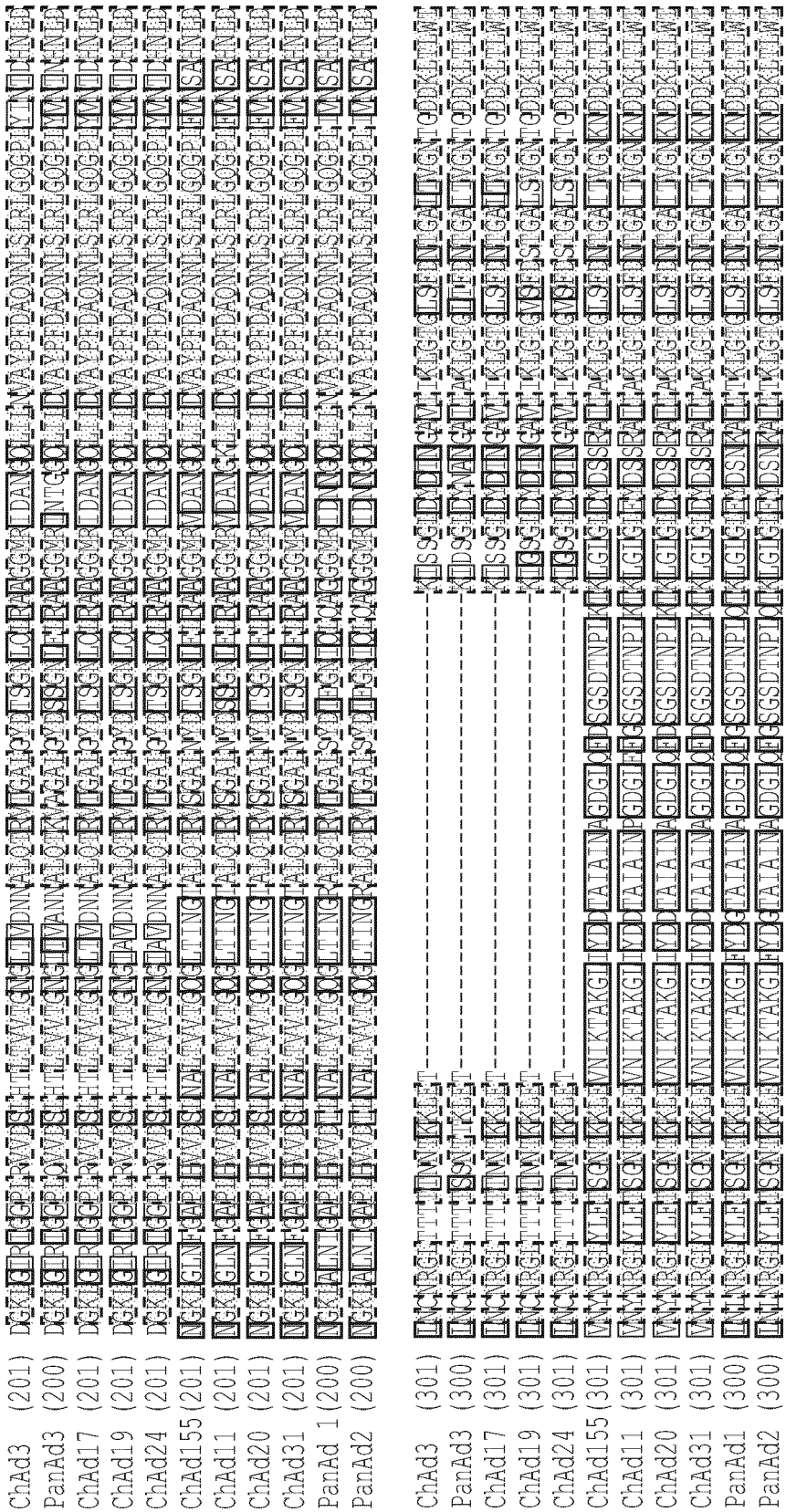
ChAd3 (201)
PanAd3 (200)
ChAd17 (201)
ChAd19 (201)
ChAd24 (201)
ChAd155 (201)
ChAd11 (201)
ChAd20 (201)
ChAd31 (201)
PanAd 1 (200)
PanAd2 (200)
ChAd3 (301)
PanAd3 (300)
ChAd17 (301)
ChAd19 (301)
ChAd24 (301)
ChAd155 (301)
ChAd11 (301)
ChAd20 (301)
ChAd31 (301)
PanAd1 (300)
PanAd2 (300)

FIG. 1B

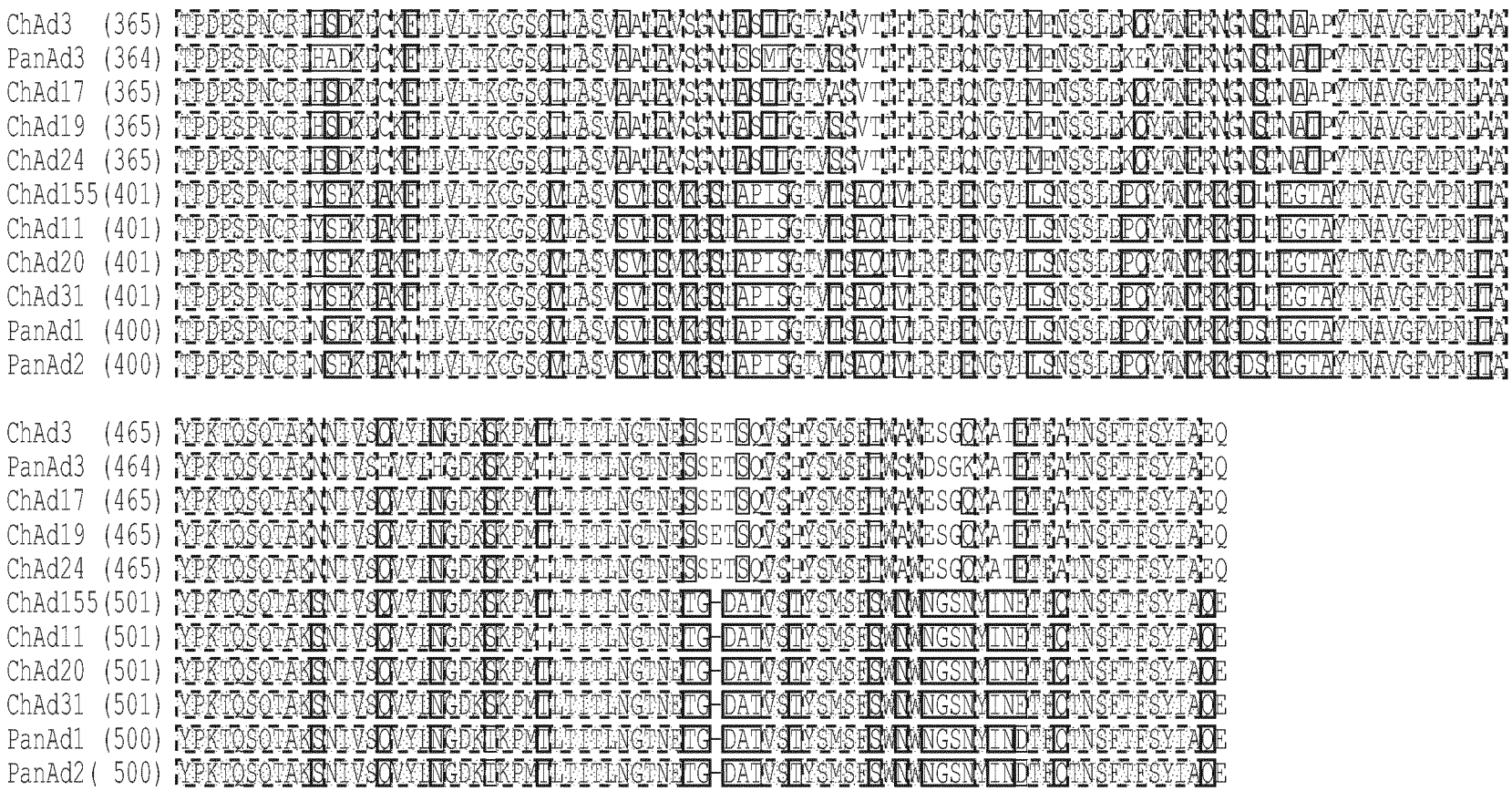
ChAd3 (365)
PanAd3 (364)
ChAd17 (365)
ChAd19 (365)
ChAd24 (365)
ChAd155(401)
ChAd11 (401)
ChAd20 (401)
ChAd31 (401)
PanAd1 (400)
PanAd2 (400)
ChAd3 (465)
PanAd3 (464)
ChAd17 (465)
ChAd19 (465)
ChAd24 (465)
ChAd155(501)
ChAd11 (501)
ChAd20 (501)
ChAd31 (501)
PanAd1 (500)
PanAd2(500)

FIG. 1C

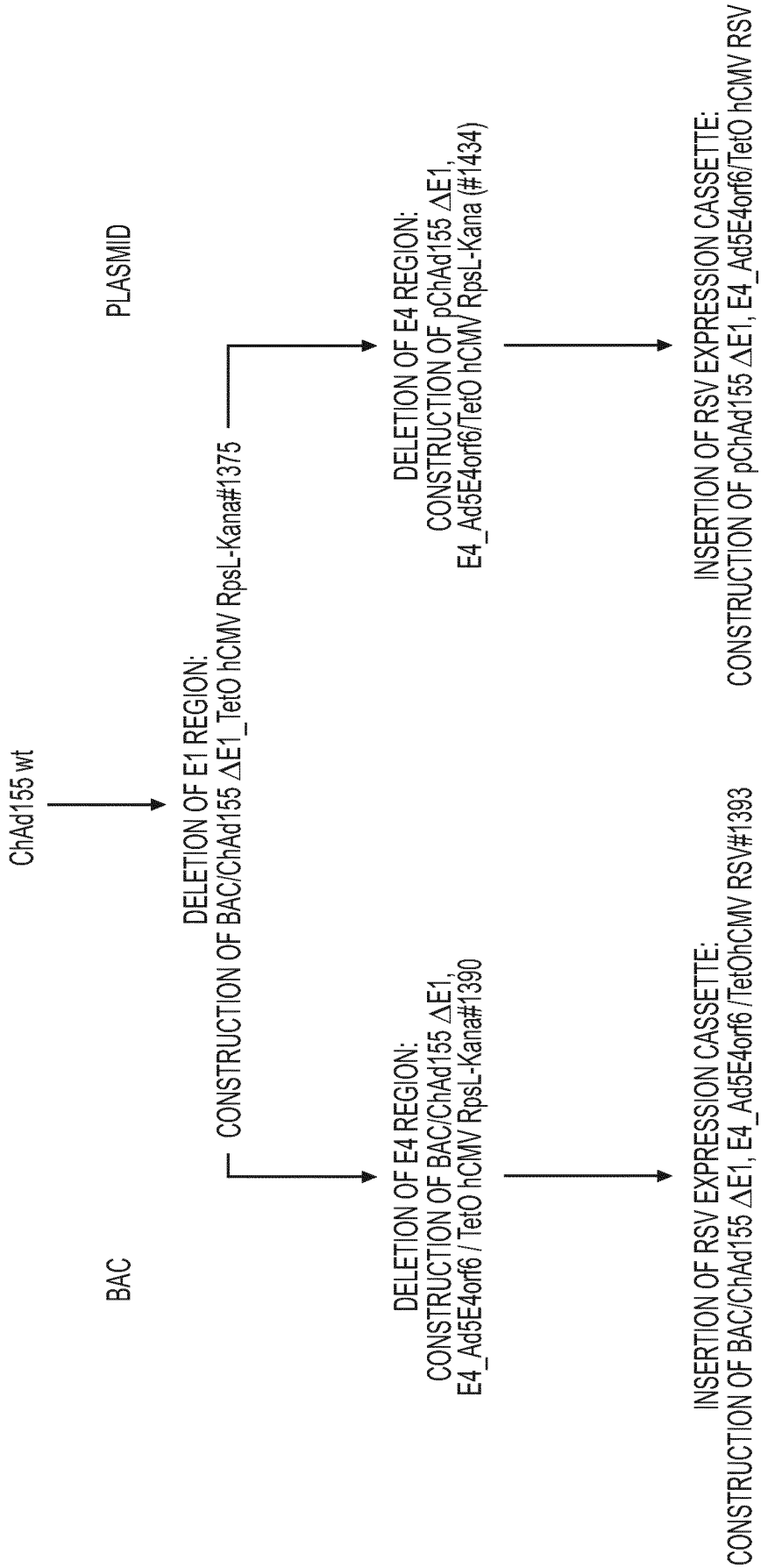
ChAd155 wt
BAC
PLASMID
DELETION OF E1 REGION:
CONSTRUCTION OF BAC/ChAd155 ΔE1_TetO hCMV RpsL-Kana#1375
DELETION OF E4 REGION:
CONSTRUCTION OF BAC/ChAd155 ΔE1,
E4_Ad5E4orf6 / TetO hCMV RpsL-Kana#1390
DELETION OF E4 REGION:
CONSTRUCTION OF pChAd155 ΔE1,
E4_Ad5E4orf6/TetO hCMV RpsL-Kana (#1434)
INSERTION OF RSV EXPRESSION CASSETTE:
CONSTRUCTION OF BAC/ChAd155 ΔE1, E4_Ad5E4orf6 /TetOhCMV RSV#1393
INSERTION OF RSV EXPRESSION CASSETTE:
CONSTRUCTION OF pChAd155 ΔE1, E4_Ad5E4orf6/TetO hCMV RSV

FIG. 2

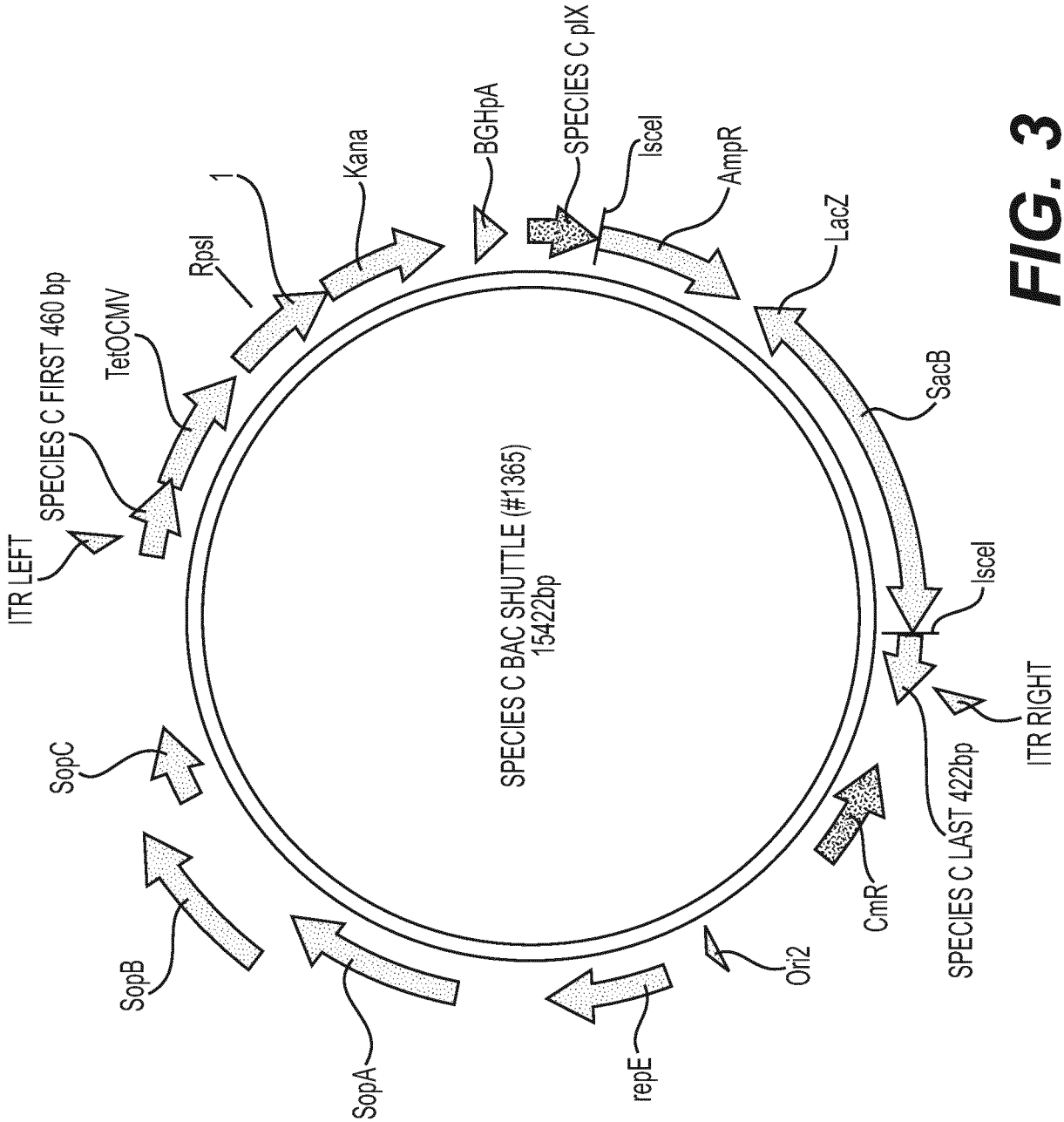
SPECIES C BAC SHUTTLE (#1365)
15422bp
ITR LEFT
SPECIES C FIRST 460 bp
TetOCMV
RpsI
1
Kana
BGHpA
SPECIES C pIX
IsceI
AmpR
LacZ
SacB
IsceI
ITR RIGHT
SPECIES C LAST 422bp
CmR
Ori2
repE
SopA
SopB
SopC

FIG. 3

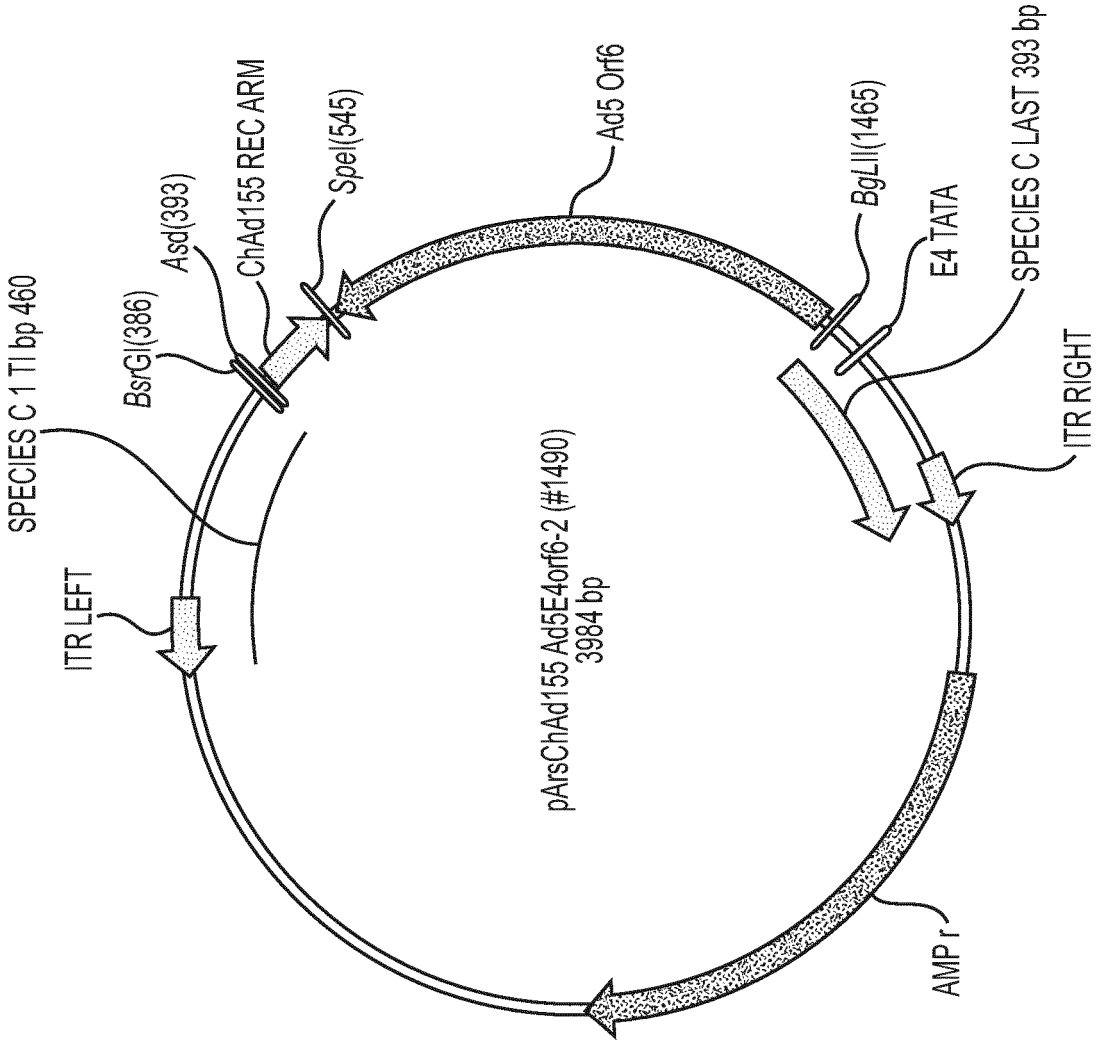
SPECIES C 1 TI bp 460
ITR LEFT
BsrGI(386)
Asd(393)
ChAd155 REC ARM
SpeI(545)
pArsChAd155 Ad5E4orf6-2 (#1490)
3984 bp
Ad5 Orf6
BglII(1465)
E4 TATA
SPECIES C LAST 393 bp
ITR RIGHT
AMP r

FIG. 4

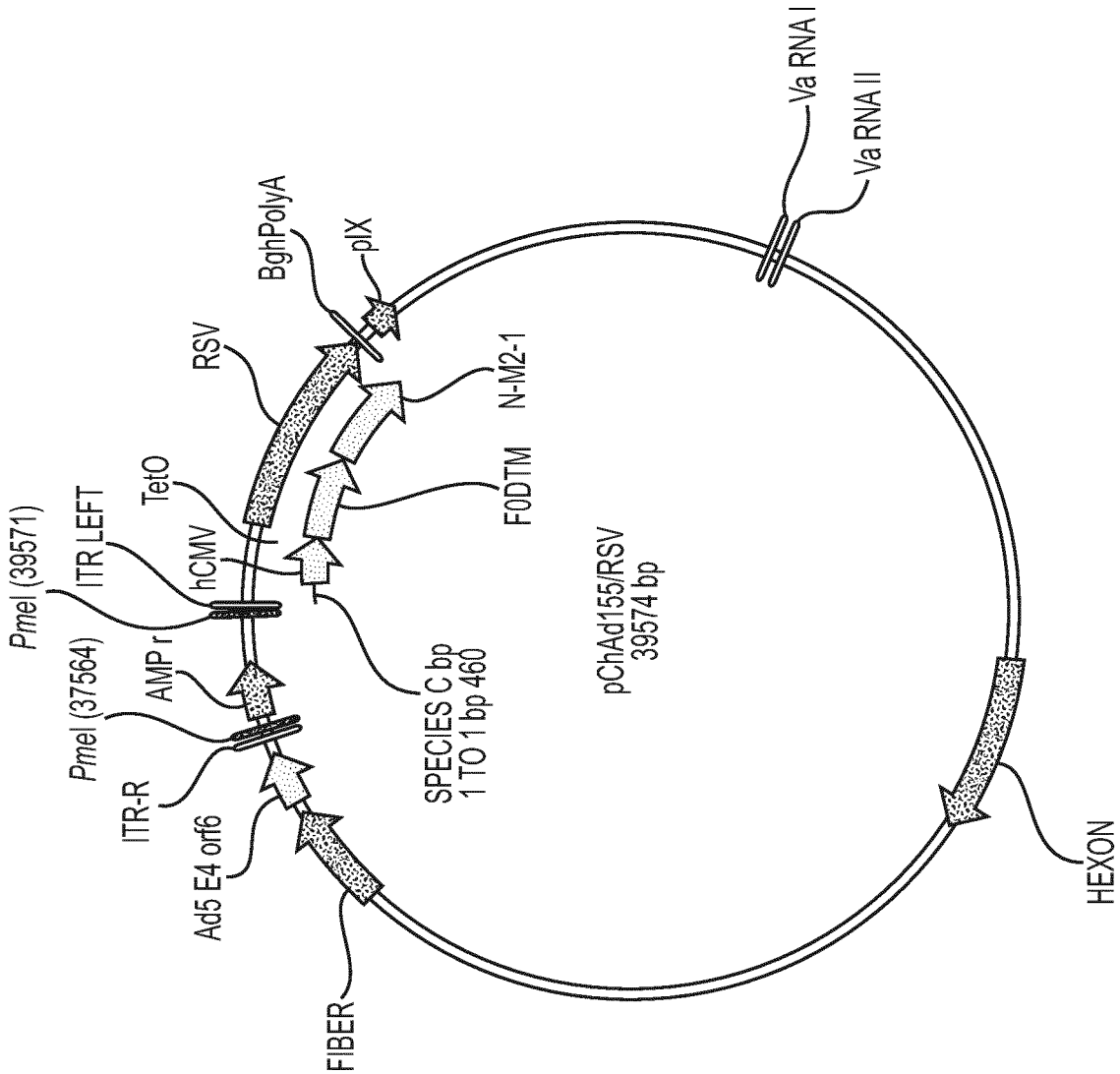
PmeI (39571)
ITR LEFT
TetO
RSV
BghPolyA
pIX
Va RNA I
Va RNA II
PmeI (37564)
AMPr
hCMV
N-M2-1
ITR-R
Ad5 E4 orf6
SPECIES C bp
1 TO 1 bp 460
F0DTM
pChAd155/RSV
39574 bp
FIBER
HEXON

FIG. 5

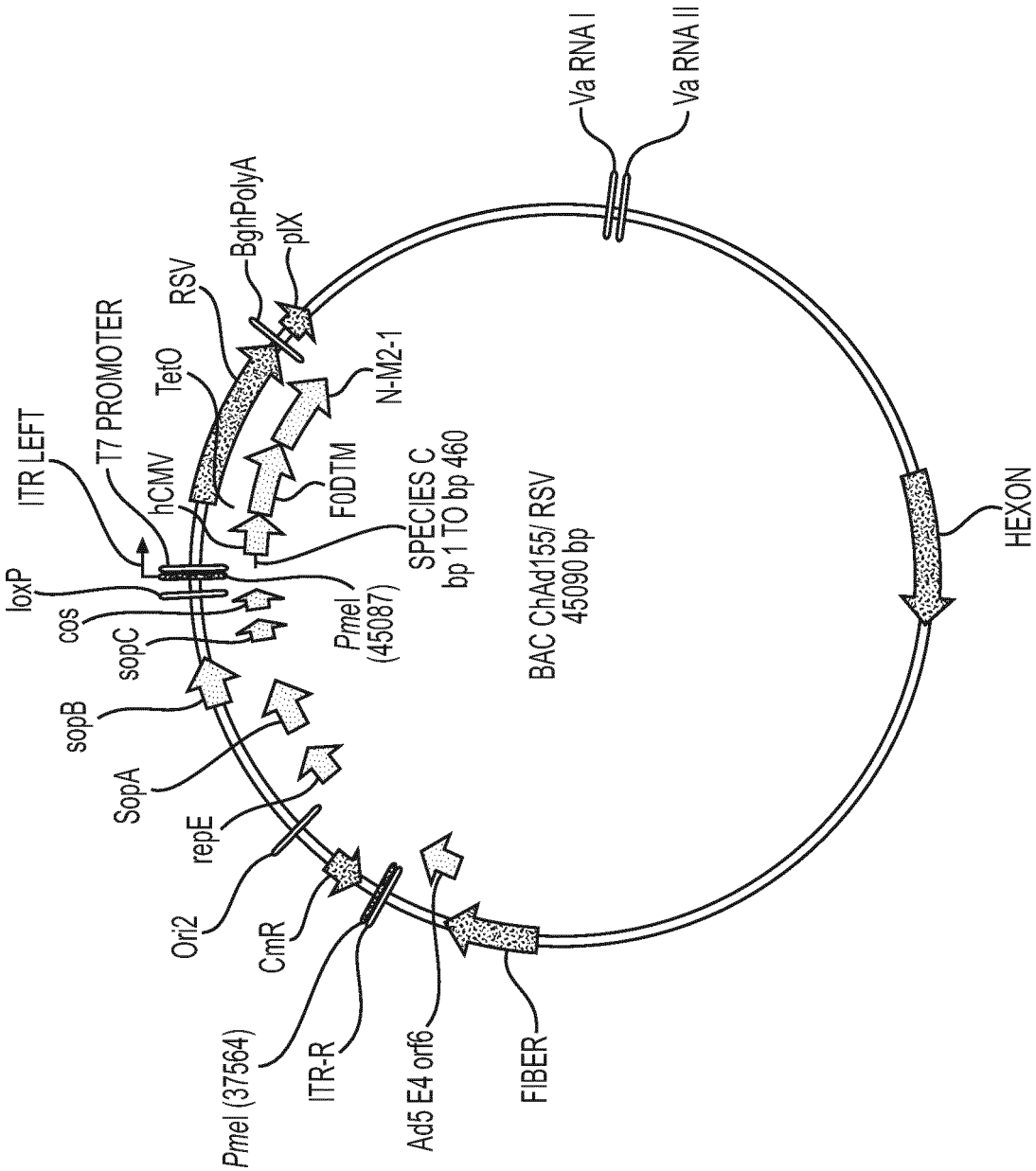
Va RNA I
Va RNA II
BgnPolyA
pIX
RSV
TetO
T7 PROMOTER
ITR LEFT
hCMV
N-M2-1
F0DTM
SPECIES C
bp 1 TO bp 460
BAC ChAd155/ RSV
45090 bp
HEXON
loxP
cos
sopC
sopB
SopA
repE
Ori2
CmR
ITR-R
Ad5 E4 orf6
FIBER
PmeI (37564)
PmeI
(45087)

FIG. 6

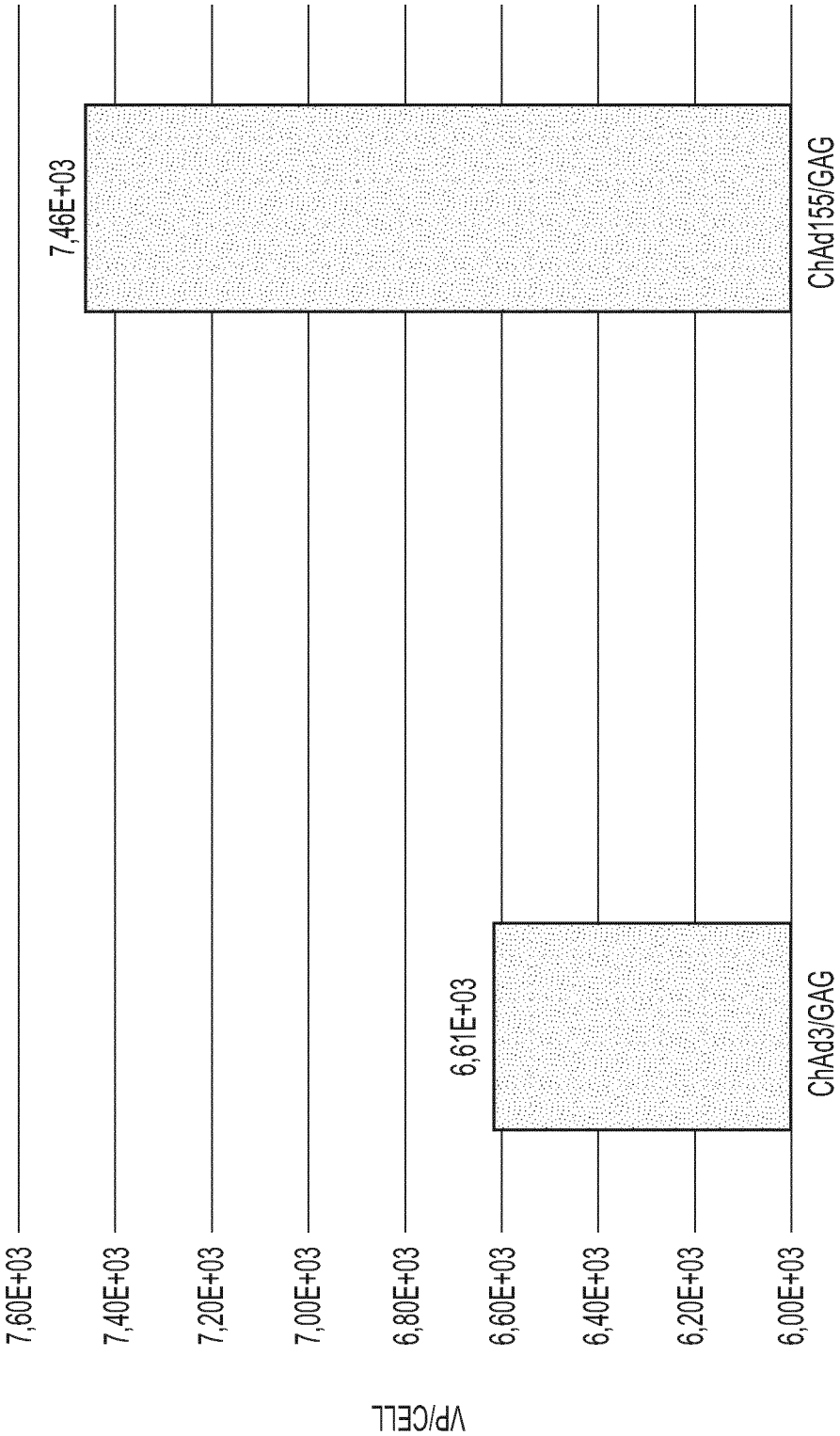
7,60E+03
7,40E+03
7,20E+03
7,00E+03
6,80E+03
6,60E+03
6,40E+03
6,20E+03
6,00E+03
VP/CELL
7,46E+03
6,61E+03
ChAd155/GAG
ChAd3/GAG

FIG. 7

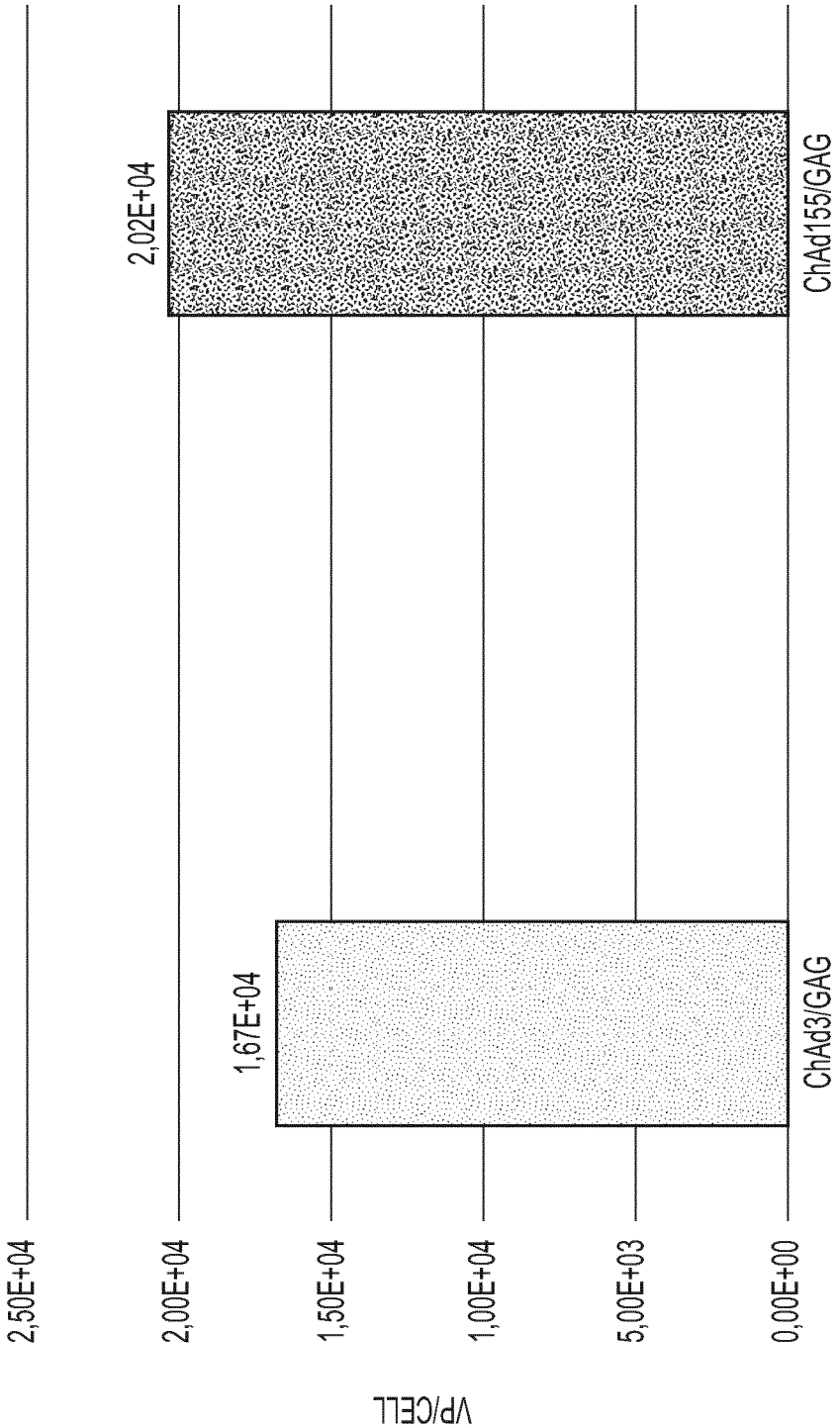
2,50E+04
2,00E+04
1,50E+04
1,00E+04
5,00E+03
0,00E+00
VP/CELL
1,67E+04
2,02E+04
ChAd3/GAG
ChAd155/GAG

FIG. 8

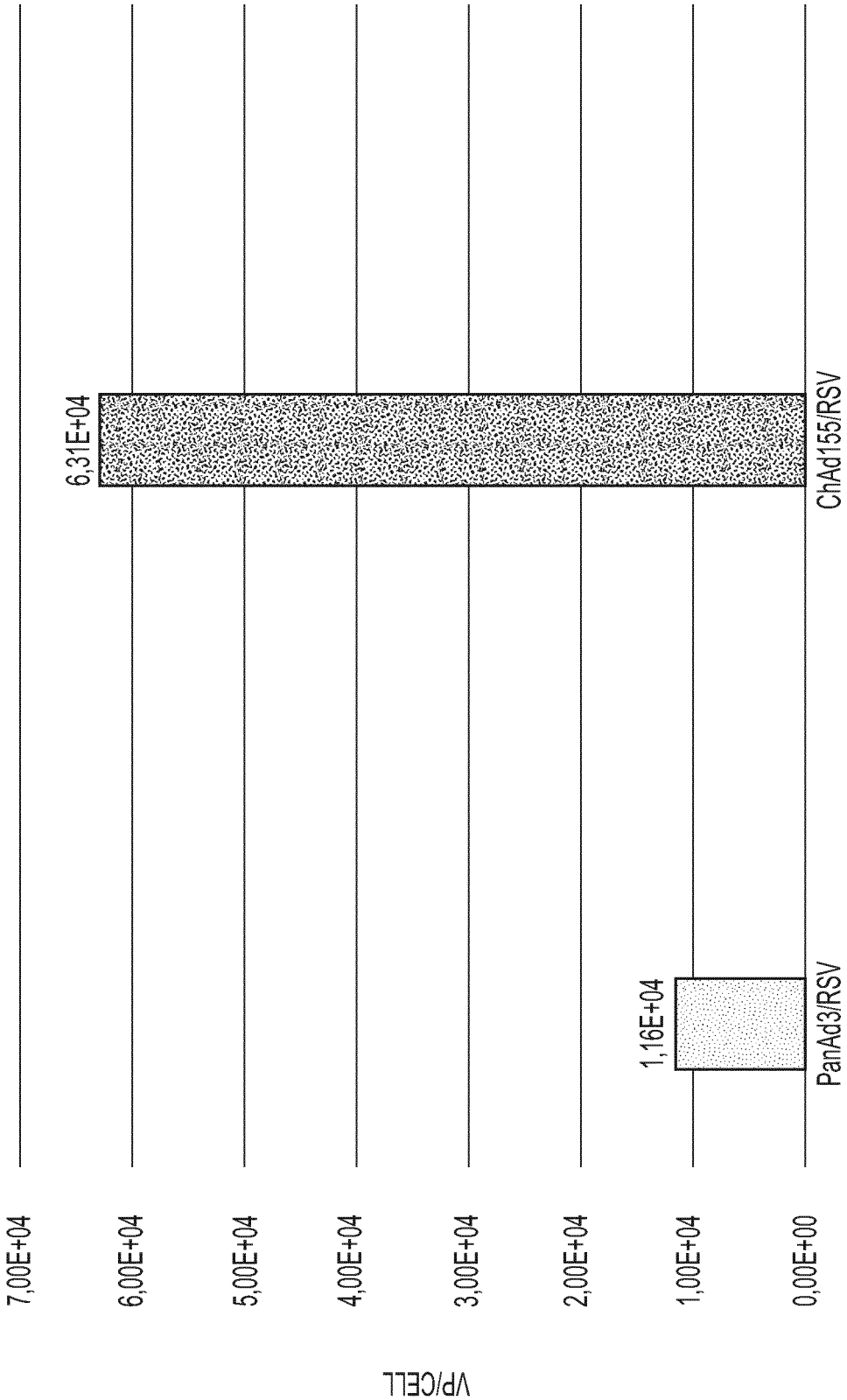
7,00E+04
6,00E+04
5,00E+04
4,00E+04
3,00E+04
2,00E+04
1,00E+04
0,00E+00
VP/CELL
6,31E+04
1,16E+04
ChAd155/RSV
PanAd3/RSV

FIG. 9

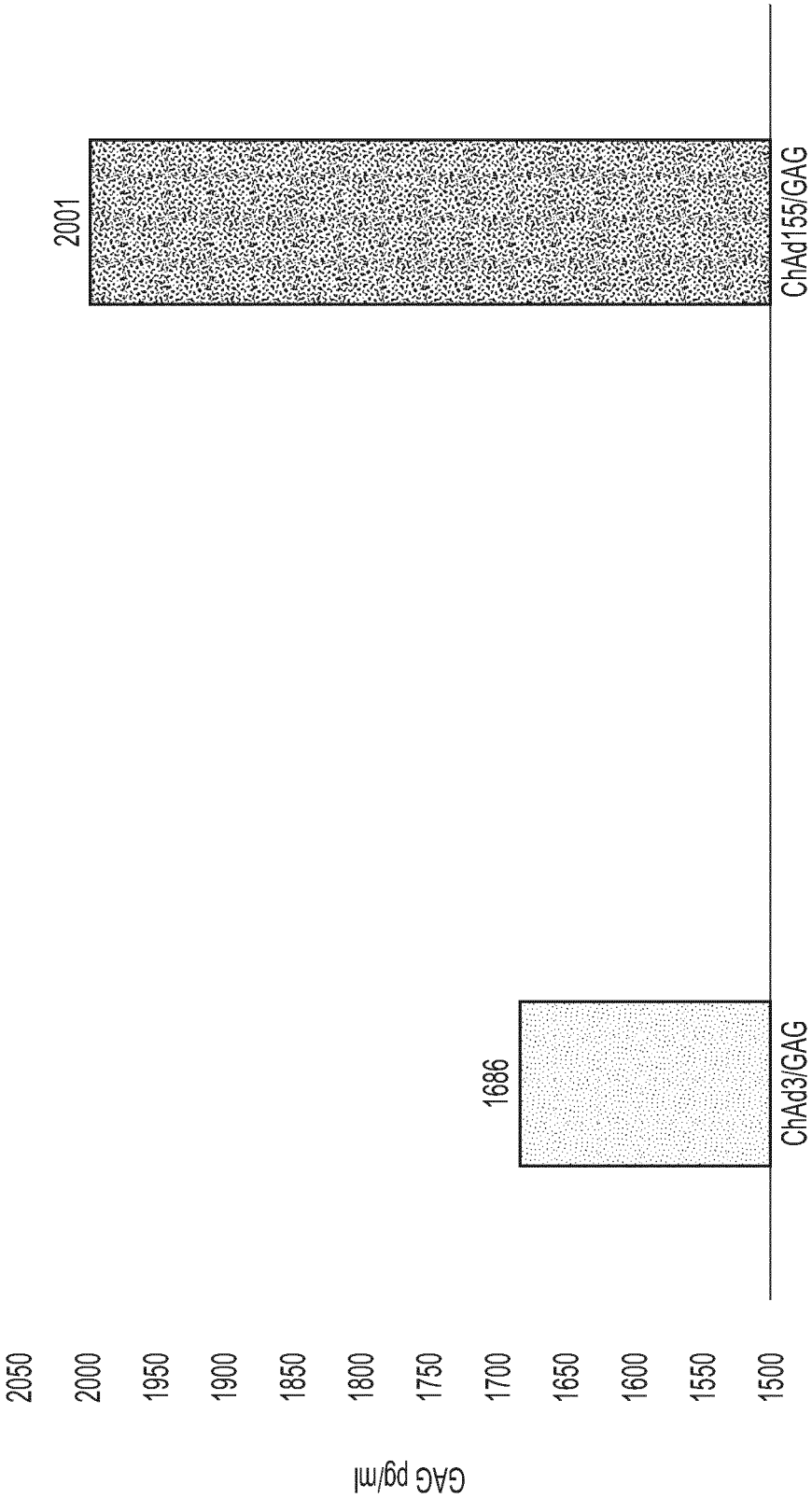
2001
1686
ChAd155/GAG
ChAd3/GAG
2050
2000
1950
1900
1850
1800
1750
1700
1650
1600
1550
1500
GAG pg/ml

FIG. 10

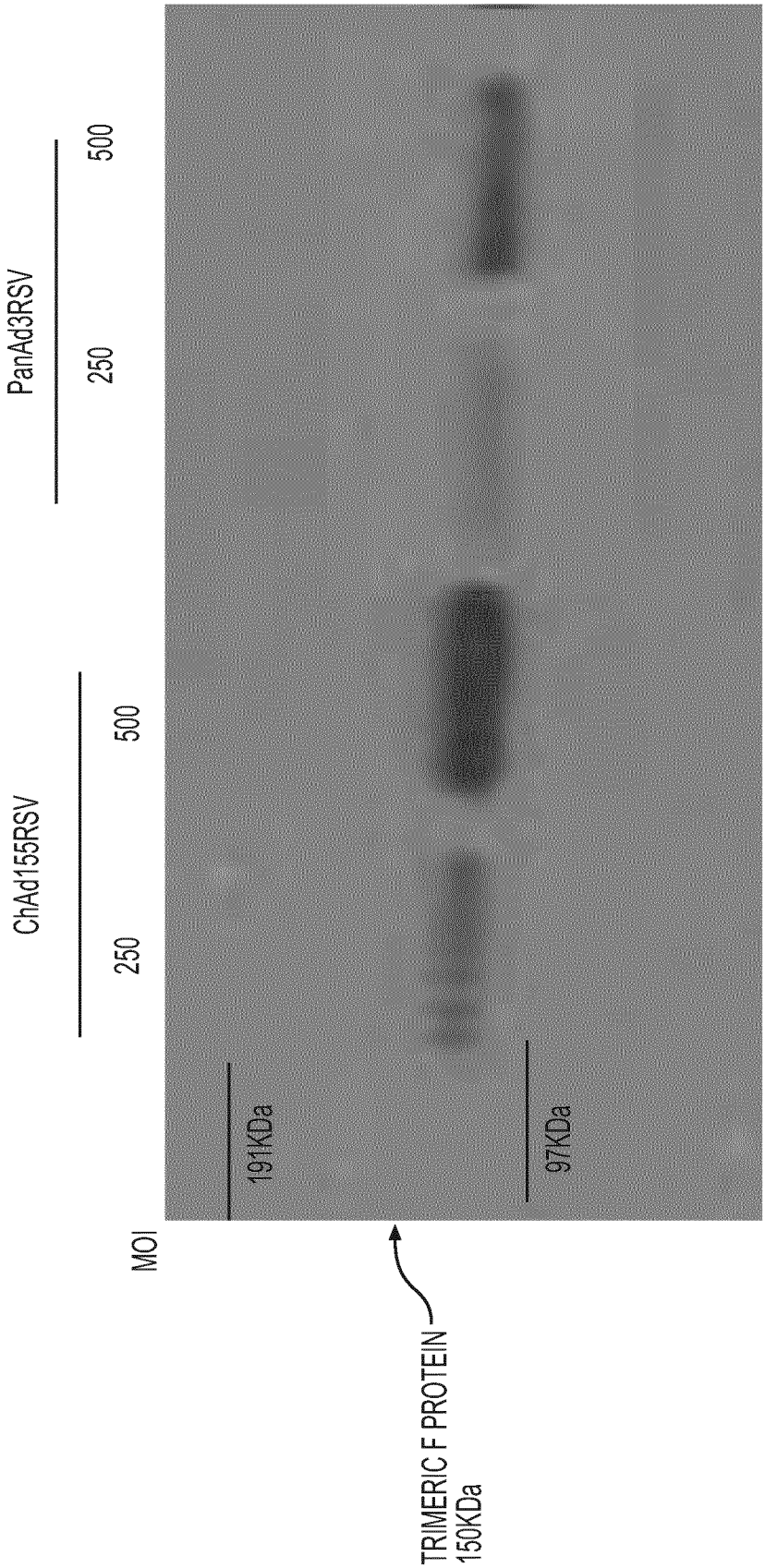
ChAd155RSV
PanAd3RSV
MOI
250
500
250
500
191KDa
97KDa
TRIMERIC F PROTEIN
150KDa

FIG. 11

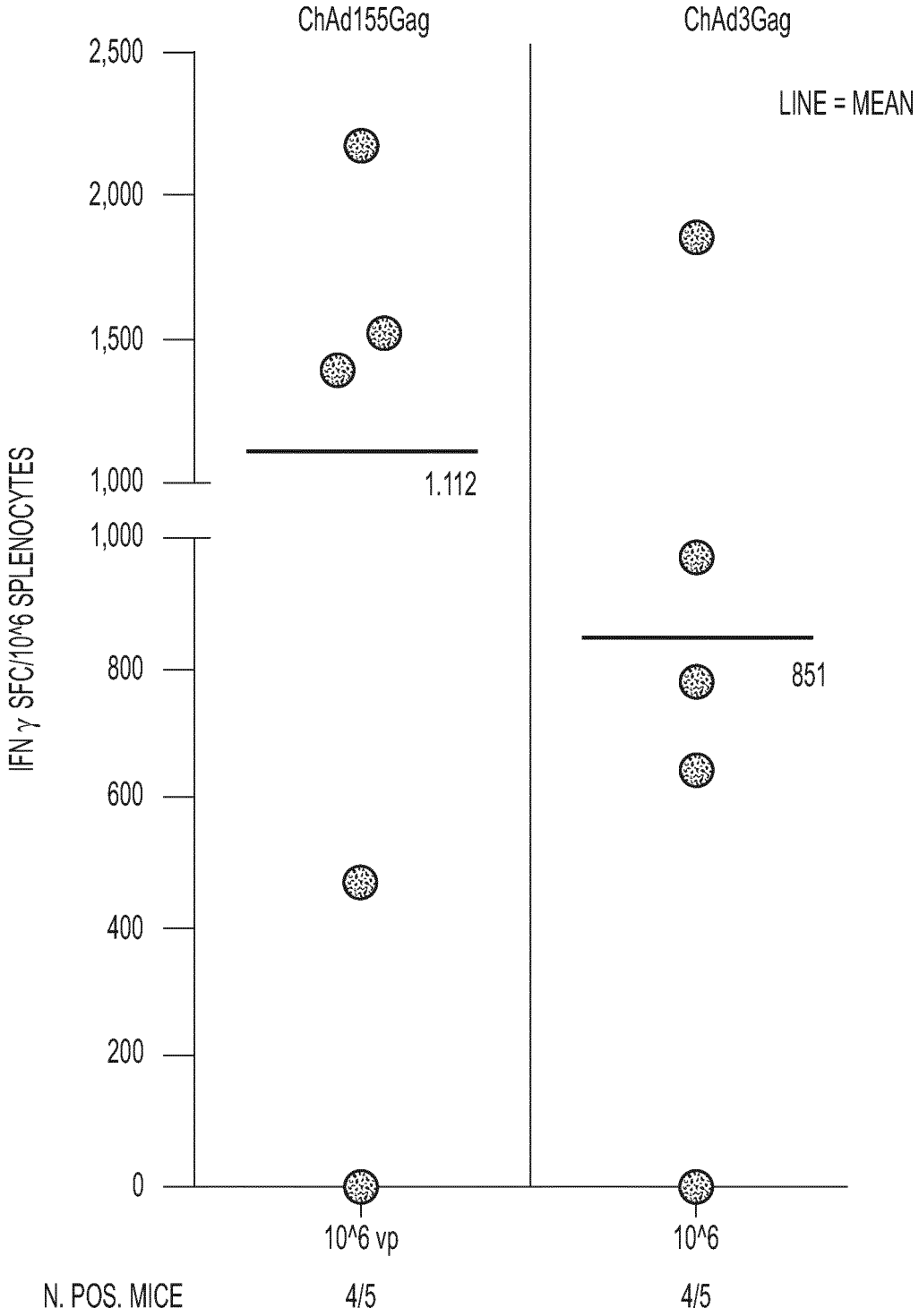
ChAd155Gag
ChAd3Gag
LINE = MEAN
IFN γ SFC/10^6 SPLENOCYTES
2,500
2,000
1,500
1,000
1,000
800
600
400
200
0
1.112
851
10^6 vp
10^6
N. POS. MICE
4/5
4/5

FIG. 12

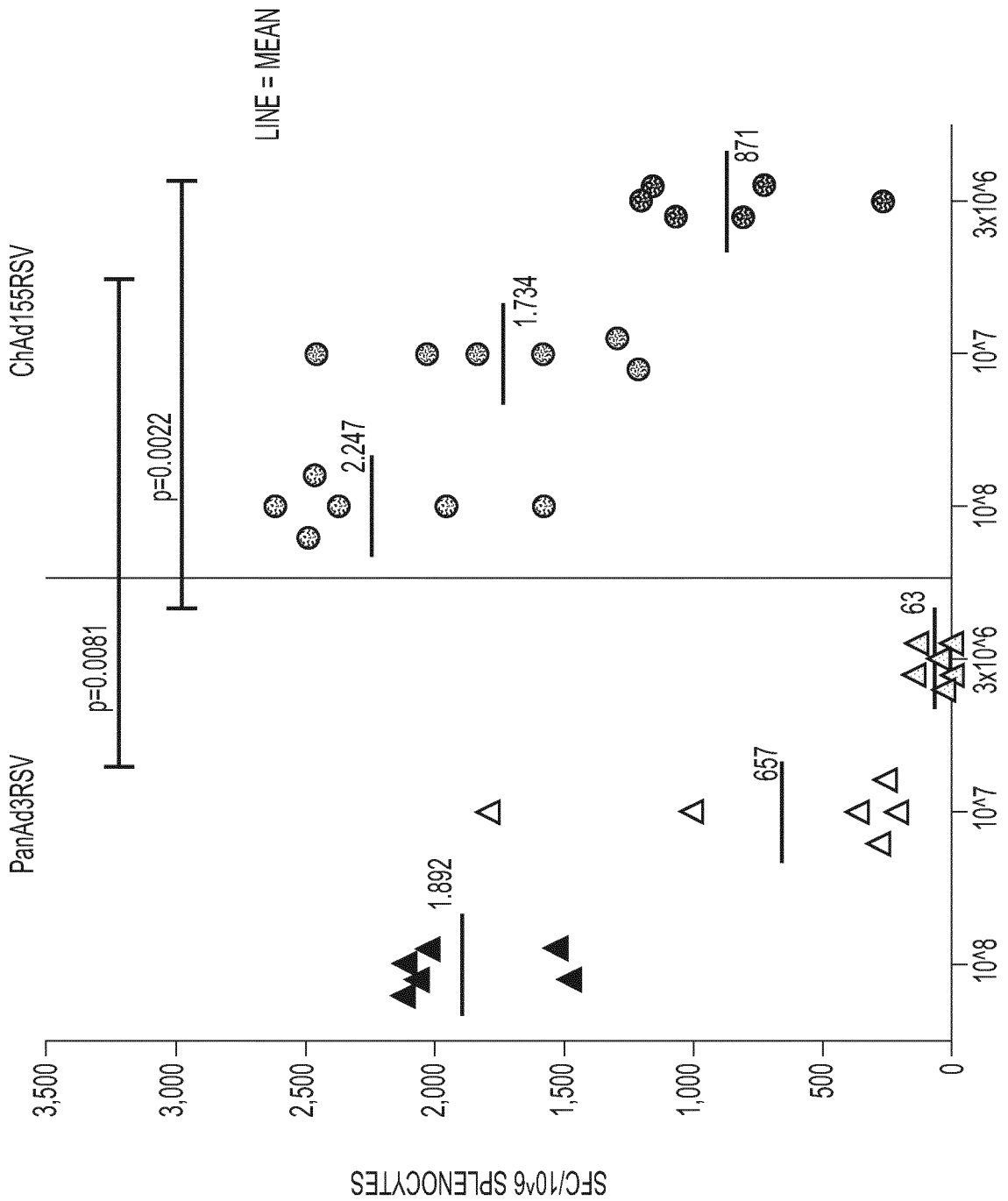
PanAd3RSV
ChAd155RSV
LINE = MEAN
p=0.0081
p=0.0022
1.892
657
63
2.247
1.734
871
3,500
3,000
2,500
2,000
1,500
1,000
500
0
10^8
10^7
3x10^6
10^8
10^7
3x10^6
SFC/10^6 SPLENOCYTES

FIG. 13

SEQUENCE LISTING

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<130> VU65698 PCT

<140>
<141>

<150> UK 1514772.1
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<151> 2015-06-12

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<170> PatentIn version 3.5

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Tyr Asp Thr Glu Ser Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Ile Thr Thr Ala Ser Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ser Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val
165 170 175

Ser Ala Thr Pro Pro Leu Ser Thr Ser Asn Gly Ser Leu Gly Ile Asp
180 185 190

Met Gln Ala Pro Ile Tyr Thr Thr Asn Gly Lys Leu Gly Leu Asn Phe
195 200 205

Gly Ala Pro Leu His Val Val Asp Ser Leu Asn Ala Leu Thr Val Val
210 215 220

Thr Gly Gln Gly Leu Thr Ile Asn Gly Thr Ala Leu Gln Thr Arg Val
225 230 235 240

Ser Gly Ala Leu Asn Tyr Asp Thr Ser Gly Asn Leu Glu Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Val Asp Ala Asn Gly Gln Leu Ile Leu Asp Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Val Asn Ser Ala His Asn Leu Asp Val Asn Tyr Asn
290 295 300

Arg Gly Leu Tyr Leu Phe Thr Ser Gly Asn Thr Lys Lys Leu Glu Val
305 310 315 320

Asn Ile Lys Thr Ala Lys Gly Leu Ile Tyr Asp Asp Thr Ala Ile Ala
325 330 335

Ile Asn Ala Gly Asp Gly Leu Gln Phe Asp Ser Gly Ser Asp Thr Asn
340 345 350

Pro Leu Lys Thr Lys Leu Gly Leu Gly Leu Asp Tyr Asp Ser Ser Arg
355 360 365

Ala Ile Ile Ala Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly
370 375 380

Ala Ile Thr Val Gly Asn Lys Asn Asp Asp Lys Leu Thr Leu Trp Thr
385 390 395 400

Thr Pro Asp Pro Ser Pro Asn Cys Arg Ile Tyr Ser Glu Lys Asp Ala
405 410 415

Lys Phe Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Val Leu Ala Ser
420 425 430

Val Ser Val Leu Ser Val Lys Gly Ser Leu Ala Pro Ile Ser Gly Thr
435 440 445

Val Thr Ser Ala Gln Ile Val Leu Arg Phe Asp Glu Asn Gly Val Leu
450 455 460

Leu Ser Asn Ser Ser Leu Asp Pro Gln Tyr Trp Asn Tyr Arg Lys Gly
465 470 475 480

Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met Pro
485 490 495

Asn Leu Thr Ala Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Ser Asn
500 505 510

Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Thr
515 520 525

Leu Thr Ile Thr Leu Asn Gly Thr Asn Glu Thr Gly Asp Ala Thr Val
530 535 540

Ser Thr Tyr Ser Met Ser Phe Ser Trp Asn Trp Asn Gly Ser Asn Tyr
545 550 555 560

Ile Asn Glu Thr Phe Gln Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala
565 570 575

Gln Glu

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<211> 1734
<212> DNA
<213> Simian adenovirus

<400> 2

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caagatatca ccaccgctag ccctcccctc aaaaaaacca agaccaacct cagcctagaa 300

acctcatccc ccctaactgt gagcacctca ggcgccctca ccgtagcagc cgccgctccc 360

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caaacatcgg ccccgctgac ggccgctgac agcagcaccc tcacagtcag tgccacacca 540

ccccttagca caagcaatgg cagcttgggt attgacatgc aagcccccat ttacaccacc 600

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aatggagttc tactaagcaa ttcttccctt gaccctcaat actggaacta cagaaaaggt 1440

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tacccaaaaa cacagagcca aactgctaaa agcaacattg taagtcaggt ttacttgaat 1560

ggggacaaat ccaaacccat gaccctcacc attaccctca atggaactaa tgaaacagga 1620

gatgccacag taagcactta ctccatgtca ttctcatgga actggaatgg aagtaattac 1680

attaatgaaa cgttccaaac caactccttc accttctcct acatcgccca agaa 1734

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<212> PRT
<213> Simian adenovirus

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Ser Val Val Gly Ala Ala Ala Ala Ala Pro Ser Ser Pro Phe Ala Ser
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Gln Leu Leu Glu Pro Pro Tyr Val Pro Pro Arg Tyr Leu Arg Pro Thr
35 40 45

Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe Asp
50 55 60

Thr Thr Arg Val Tyr Leu Val Asp Asn Lys Ser Ala Asp Val Ala Ser
65 70 75 80

Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile Gln
85 90 95

Asn Asn Asp Tyr Ser Pro Ser Glu Ala Ser Thr Gln Thr Ile Asn Leu
100 105 110

Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His Thr
115 120 125

Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys Ala
130 135 140

Arg Val Met Val Ser Arg Ser His Thr Lys Glu Asp Arg Val Glu Leu
145 150 155 160

Lys Tyr Glu Trp Val Glu Phe Glu Leu Pro Glu Gly Asn Tyr Ser Glu
165 170 175

Thr Met Thr Ile Asp Leu Met Asn Asn Ala Ile Val Glu His Tyr Leu
180 185 190

Lys Val Gly Arg Gln Asn Gly Val Leu Glu Ser Asp Ile Gly Val Lys
195 200 205

Phe Asp Thr Arg Asn Phe Arg Leu Gly Leu Asp Pro Val Thr Gly Leu
210 215 220

Val Met Pro Gly Val Tyr Thr Asn Glu Ala Phe His Pro Asp Ile Ile
225 230 235 240

Leu Leu Pro Gly Cys Gly Val Asp Phe Thr Tyr Ser Arg Leu Ser Asn
245 250 255

Leu Leu Gly Ile Arg Lys Arg Gln Pro Phe Gln Glu Gly Phe Arg Ile
260 265 270

Thr Tyr Glu Asp Leu Glu Gly Gly Asn Ile Pro Ala Leu Leu Asp Val

275 280 285

Glu Ala Tyr Gln Asp Ser Leu Lys Glu Asn Glu Ala Gly Gln Glu Asp
290 295 300

Thr Ala Pro Ala Ala Ser Ala Ala Ala Glu Gln Gly Glu Asp Ala Ala
305 310 315 320

Asp Thr Ala Ala Ala Asp Gly Ala Glu Ala Asp Pro Ala Met Val Val
325 330 335

Glu Ala Pro Glu Gln Glu Glu Asp Met Asn Asp Ser Ala Val Arg Gly
340 345 350

Asp Thr Phe Val Thr Arg Gly Glu Glu Lys Gln Ala Glu Ala Glu Ala
355 360 365

Ala Ala Glu Glu Lys Gln Leu Ala Ala Ala Ala Ala Ala Ala Ala Leu
370 375 380

Ala Ala Ala Glu Ala Glu Ser Glu Gly Thr Lys Pro Ala Lys Glu Pro
385 390 395 400

Val Ile Lys Pro Leu Thr Glu Asp Ser Lys Lys Arg Ser Tyr Asn Leu
405 410 415

Leu Lys Asp Ser Thr Asn Thr Ala Tyr Arg Ser Trp Tyr Leu Ala Tyr
420 425 430

Asn Tyr Gly Asp Pro Ser Thr Gly Val Arg Ser Trp Thr Leu Leu Cys
435 440 445

Thr Pro Asp Val Thr Cys Gly Ser Glu Gln Val Tyr Trp Ser Leu Pro
450 455 460

Asp Met Met Gln Asp Pro Val Thr Phe Arg Ser Thr Arg Gln Val Ser
465 470 475 480

Asn Phe Pro Val Val Gly Ala Glu Leu Leu Pro Val His Ser Lys Ser
485 490 495

Phe Tyr Asn Asp Gln Ala Val Tyr Ser Gln Leu Ile Arg Gln Phe Thr
500 505 510

Ser Leu Thr His Val Phe Asn Arg Phe Pro Glu Asn Gln Ile Leu Ala
515 520 525

Arg Pro Pro Ala Pro Thr Ile Thr Thr Val Ser Glu Asn Val Pro Ala
530 535 540

Leu Thr Asp His Gly Thr Leu Pro Leu Arg Asn Ser Ile Gly Gly Val
545 550 555 560

Gln Arg Val Thr Val Thr Asp Ala Arg Arg Arg Thr Cys Pro Tyr Val
565 570 575

Tyr Lys Ala Leu Gly Ile Val Ser Pro Arg Val Leu Ser Ser Arg Thr
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Phe

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<211> 1779
<212> DNA
<213> Simian adenovirus

<400> 4
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cctccgcgct acctgcggcc tacgggggg agaaacagca tccgttactc ggagctggcg 180

cccctgttcg acaccacccg ggtgtacctg gtggacaaca agtcggcgga cgtggcctcc 240

ctgaactacc agaacgacca cagcaatttt ttgaccacgg tcatccagaa caatgactac 300

agcccgagcg aggccagcac ccagaccatc aatctggatg accggtcgca ctggggcggc 360

gacctgaaaa ccatcctgca caccaacatg cccaacgtga acgagttcat gttcaccaat 420

aagttcaagg cgcgggtgat ggtgtcgcgc tcgcacacca aggaagaccg ggtggagctg 480

aagtacgagt gggtggagtt cgagctgcca gagggcaact actccgagac catgaccatt 540

gacctgatga acaacgcgat cgtggagcac tatctgaaag tgggcaggca gaacggggtc 600

ctggagagcg acatcggggt caagttcgac accaggaact tccgcctggg gctggacccc 660

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accaacaccg cgtaccgcag ctggtacctg gcctacaact acggcgaccc gtcgacgggg 1320

gtgcgctcct ggaccctgct gtgcacgccg gacgtgacct gcggctcgga gcaggtgtac 1380

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cagcgagtga ccgttactga cgccagacgc cgcacctgcc cctacgttta caaggccttg 1740

ggcatagtct cgccgcgcgt cctttccagc cgcactttt 1779

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<211> 964
<212> PRT
<213> Simian adenovirus

<400> 5
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Gly Gln Asp Ala Ser Glu Tyr Leu Ser Pro Gly Leu Val Gln Phe Ala
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Arg Ala Thr Asp Ser Tyr Phe Ser Leu Ser Asn Lys Phe Arg Asn Pro
35 40 45

Thr Val Ala Pro Thr His Asp Val Thr Thr Asp Arg Ser Gln Arg Leu
50 55 60

Thr Leu Arg Phe Ile Pro Val Asp Arg Glu Asp Thr Ala Tyr Ser Tyr
65 70 75 80

Lys Ala Arg Phe Thr Leu Ala Val Gly Asp Asn Arg Val Leu Asp Met
85 90 95

Ala Ser Thr Tyr Phe Asp Ile Arg Gly Val Leu Asp Arg Gly Pro Thr
100 105 110

Phe Lys Pro Tyr Ser Gly Thr Ala Tyr Asn Ser Leu Ala Pro Lys Gly
115 120 125

Ala Pro Asn Ser Cys Glu Trp Glu Gln Glu Glu Thr Gln Thr Ala Glu
130 135 140

Glu Ala Gln Asp Glu Glu Glu Asp Glu Ala Glu Ala Glu Glu Glu Met
145 150 155 160

Pro Gln Glu Glu Gln Ala Pro Val Lys Lys Thr His Val Tyr Ala Gln
165 170 175

Ala Pro Leu Ser Gly Glu Lys Ile Thr Lys Asp Gly Leu Gln Ile Gly
180 185 190

Thr Asp Ala Thr Ala Thr Glu Gln Lys Pro Ile Tyr Ala Asp Pro Thr
195 200 205

Phe Gln Pro Glu Pro Gln Ile Gly Glu Ser Gln Trp Asn Glu Ala Asp
210 215 220

Ala Ser Val Ala Gly Gly Arg Val Leu Lys Lys Thr Thr Pro Met Lys
225 230 235 240

Pro Cys Tyr Gly Ser Tyr Ala Arg Pro Thr Asn Ala Asn Gly Gly Gln
245 250 255

Gly Val Leu Val Glu Lys Asp Gly Gly Lys Met Glu Ser Gln Val Asp
260 265 270

Met Gln Phe Phe Ser Thr Ser Glu Asn Ala Arg Asn Glu Ala Asn Asn

275 280 285

Ile Gln Pro Lys Leu Val Leu Tyr Ser Glu Asp Val His Met Glu Thr
290 295 300

Pro Asp Thr His Ile Ser Tyr Lys Pro Ala Lys Ser Asp Asp Asn Ser
305 310 315 320

Lys Val Met Leu Gly Gln Gln Ser Met Pro Asn Arg Pro Asn Tyr Ile
325 330 335

Gly Phe Arg Asp Asn Phe Ile Gly Leu Met Tyr Tyr Asn Ser Thr Gly
340 345 350

Asn Met Gly Val Leu Ala Gly Gln Ala Ser Gln Leu Asn Ala Val Val
355 360 365

Asp Leu Gln Asp Arg Asn Thr Glu Leu Ser Tyr Gln Leu Leu Leu Asp
370 375 380

Ser Met Gly Asp Arg Thr Arg Tyr Phe Ser Met Trp Asn Gln Ala Val
385 390 395 400

Asp Ser Tyr Asp Pro Asp Val Arg Ile Ile Glu Asn His Gly Thr Glu
405 410 415

Asp Glu Leu Pro Asn Tyr Cys Phe Pro Leu Gly Gly Ile Gly Val Thr
420 425 430

Asp Thr Tyr Gln Ala Ile Lys Thr Asn Gly Asn Gly Asn Gly Gly Gly
435 440 445

Asn Thr Thr Trp Thr Lys Asp Glu Thr Phe Ala Asp Arg Asn Glu Ile
450 455 460

Gly Val Gly Asn Asn Phe Ala Met Glu Ile Asn Leu Ser Ala Asn Leu
465 470 475 480

Trp Arg Asn Phe Leu Tyr Ser Asn Val Ala Leu Tyr Leu Pro Asp Lys
485 490 495

Leu Lys Tyr Asn Pro Ser Asn Val Glu Ile Ser Asp Asn Pro Asn Thr
500 505 510

Tyr Asp Tyr Met Asn Lys Arg Val Val Ala Pro Gly Leu Val Asp Cys
515 520 525

Tyr Ile Asn Leu Gly Ala Arg Trp Ser Leu Asp Tyr Met Asp Asn Val
530 535 540

Asn Pro Phe Asn His His Arg Asn Ala Gly Leu Arg Tyr Arg Ser Met
545 550 555 560

Leu Leu Gly Asn Gly Arg Tyr Val Pro Phe His Ile Gln Val Pro Gln
565 570 575

Lys Phe Phe Ala Ile Lys Asn Leu Leu Leu Leu Pro Gly Ser Tyr Thr
580 585 590

Tyr Glu Trp Asn Phe Arg Lys Asp Val Asn Met Val Leu Gln Ser Ser
595 600 605

Leu Gly Asn Asp Leu Arg Val Asp Gly Ala Ser Ile Lys Phe Glu Ser
610 615 620

Ile Cys Leu Tyr Ala Thr Phe Phe Pro Met Ala His Asn Thr Ala Ser
625 630 635 640

Thr Leu Glu Ala Met Leu Arg Asn Asp Thr Asn Asp Gln Ser Phe Asn
645 650 655

Asp Tyr Leu Ser Ala Ala Asn Met Leu Tyr Pro Ile Pro Ala Asn Ala
660 665 670

Thr Asn Val Pro Ile Ser Ile Pro Ser Arg Asn Trp Ala Ala Phe Arg
675 680 685

Gly Trp Ala Phe Thr Arg Leu Lys Thr Lys Glu Thr Pro Ser Leu Gly
690 695 700

Ser Gly Phe Asp Pro Tyr Tyr Thr Tyr Ser Gly Ser Ile Pro Tyr Leu
705 710 715 720

Asp Gly Thr Phe Tyr Leu Asn His Thr Phe Lys Lys Val Ser Val Thr
725 730 735

Phe Asp Ser Ser Val Ser Trp Pro Gly Asn Asp Arg Leu Leu Thr Pro
740 745 750

Asn Glu Phe Glu Ile Lys Arg Ser Val Asp Gly Glu Gly Tyr Asn Val
755 760 765

Ala Gln Cys Asn Met Thr Lys Asp Trp Phe Leu Ile Gln Met Leu Ala
770 775 780

Asn Tyr Asn Ile Gly Tyr Gln Gly Phe Tyr Ile Pro Glu Ser Tyr Lys
785 790 795 800

Asp Arg Met Tyr Ser Phe Phe Arg Asn Phe Gln Pro Met Ser Arg Gln
805 810 815

Val Val Asp Glu Thr Lys Tyr Lys Asp Tyr Gln Gln Val Gly Ile Ile
820 825 830

His Gln His Asn Asn Ser Gly Phe Val Gly Tyr Leu Ala Pro Thr Met
835 840 845

Arg Glu Gly Gln Ala Tyr Pro Ala Asn Phe Pro Tyr Pro Leu Ile Gly
850 855 860

Lys Thr Ala Val Asp Ser Val Thr Gln Lys Lys Phe Leu Cys Asp Arg
865 870 875 880

Thr Leu Trp Arg Ile Pro Phe Ser Ser Asn Phe Met Ser Met Gly Ala
885 890 895

Leu Thr Asp Leu Gly Gln Asn Leu Leu Tyr Ala Asn Ser Ala His Ala
900 905 910

Leu Asp Met Thr Phe Glu Val Asp Pro Met Asp Glu Pro Thr Leu Leu
915 920 925

Tyr Val Leu Phe Glu Val Phe Asp Val Val Arg Val His Gln Pro His
930 935 940

Arg Gly Val Ile Glu Thr Val Tyr Leu Arg Thr Pro Phe Ser Ala Gly
945 950 955 960

Asn Ala Thr Thr

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<211> 2880
<212> DNA
<213> Simian adenovirus

<400> 6

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tatgcagacc ctacattcca gcccgaaccc caaatcgggg agtcccagtg gaatgaggca 660

gatgctacag tcgccggcgg tagagtgcta aagaaatcta ctcccatgaa accatgctat 720

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<212> DNA
<213> Simian adenovirus

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gaaacgggga agtgaaatct gattaatttt gcgttagtca taccgcgtaa tatttgtcta 360

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cagcacagca ccacaatatt gttcaaaatc ccacagtgca aggcgctgta tccaaagctc 36540

atggcgggga ccacagaacc cacgtggcca tcataccaca agcgcaggta gattaagtgg 36600

cgacccctca taaacacgct ggacataaac attacctctt ttggcatgtt gtaattcacc 36660

acctcccggt accatataaa cctctgatta aacatggcgc catccaccac catcctaaac 36720

cagctggcca aaacctgccc gccggctata cactgcaggg aaccgggact ggaacaatga 36780

cagtggagag cccaggactc gtaaccatgg atcatcatgc tcgtcatgat atcaatgttg 36840

gcacaacaca ggcacacgtg catacacttc ctcaggatta caagctcctc ccgcgttaga 36900

accatatccc agggaacaac ccattcctga atcagcgtaa atcccacact gcagggaaga 36960

cctcgcacgt aactcacgtt gtgcattgtc aaagtgttac attcgggcag cagcggatga 37020

tcctccagta tggtagcgcg ggtttctgtc tcaaaaggag gtagacgatc cctactgtac 37080

ggagtgcgcc gagacaaccg agatcgtgtt ggtcgtagtg tcatgccaaa tggaacgccg 37140

gacgtagtca tatttcctga agtcttagat ctctcaacgc agcaccagca ccaacacttc 37200

gcagtgtaaa aggccaagtg ccgagagagt atatatagga ataaaaagtg acgtaaacgg 37260

gcaaagtcca aaaaacgccc agaaaaaccg cacgcgaacc tacgccccga aacgaaagcc 37320

aaaaaacact agacactccc ttccggcgtc aacttccgct ttcccacgct acgtcacttg 37380

ccccagtcaa acaaactaca tatcccgaac ttccaagtcg ccacgcccaa aacaccgcct 37440

acacctcccc gcccgccggc ccgcccccaa acccgcctcc cgccccgcgc cccgccccgc 37500

gccgcccatc tcattatcat attggcttca atccaaaata aggtatatta ttgatgatg 37559

<210> 12
<211> 1109
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 12
ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc ccaacgaccc 60

ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag ggactttcca 120

ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac atcaagtgta 180

tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg cctggcatta 240

tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg tattagtcat 300

cgctattacc atggtcgagg tgagccccac gttctgcttc actctcccca tctccccccc 360

ctccccaccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc 420

gggggggggg gggggcgcgc gccaggcggg gcggggcggg gcgaggggcg gggcggggcg 480

aggcggagag gtgcggcggc agccaatcag agcggcgcgc tccgaaagtt tccttttatg 540

gcgaggcggc ggcggcggcg gccctataaa aagcgaagcg ctccctatca gtgatagaga 600

tctccctatc agtgatagag atcgtcgacg agctcgcggc gggcgggagt cgctgcgcgc 660

tgccttcgcc ccgtgccccg ctccgccgcc gcctcgcgcc gcccgccccg gctctgactg 720

accgcgttac taaaacaggt aagtccggcc tccgcgccgg gttttggcgc ctcccgcggg 780

cgcccccctc ctcacggcga gcgctgccac gtcagacgaa gggcgcagcg agcgtcctga 840

tccttccgcc cggacgctca ggacagcggc ccgctgctca taagactcgg ccttagaacc 900

ccagtatcag cagaaggaca ttttaggacg ggacttgggt gactctaggg cactggtttt 960

ctttccagag agcggaacag gcgaggaaaa gtagtccctt ctcggcgatt ctgcggaggg 1020

atctccgtgg ggcggtgaac gccgatgatg cctctactaa ccatgttcat gttttctttt 1080

tttttctaca ggtcctgggt gacgaacag 1109

<210> 13
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

primer"

<400> 13
atacggacta gtggagaagt actcgcctac atg 33

<210> 14
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 14
atacggaaga tctaagactt caggaaatat gactac 36

<210> 15
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 15
attcagtgta caggcgcgcc aaagcatgac gctgttgatt tgattc 46

<210> 16
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 16

actaggacta gttataagct agaatggggc tttgc 35

<210> 17
<211> 79
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 17
ttaatagaca cagtagctta atagacccag tagtgcaaag ccccattcta gcttataacc 60

cctatttgtt tatttttct 79

<210> 18
<211> 93
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 18
atatatactc tctcggcact tggcctttta cactgcgaag tgttggtgct ggtgctgcgt 60

tgagagatct ttatttgtta actgttaatt gtc 93

<210> 19
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 19
ttaatagaca cagtagctta ata 23

<210> 20
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 20
ggaagggagt gtctagtgtt 20

<210> 21
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 21
caatgggcgt ggatagcggt ttgac 25

<210> 22
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 22
cagcatgcct gctattgtc 19

<210> 23
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 23
catctacgta ttagtcatcg ctattacca 29

<210> 24
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
primer"

<400> 24
gacttggaaa tccccgtgag t 21

<210> 25
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
probe"

<400> 25
acatcaatgg gcgtggatag cggtt 25

<210> 26
<211> 592

<212> DNA
<213> Woodchuck hepatitis virus

<400> 26
taatcaacct ctggattaca aaatttgtga aagattgact ggtattctta actatgttgc 60

tccttttacg ctatgtggat acgctgcttt aatgcctttg tatcatgcta ttgcttcccg 120

tatggctttc attttctcct ccttgtataa atcctggttg ctgtctcttt atgaggagtt 180

gtggcccgtt gtcaggcaac gtggcgtggt gtgcactgtg tttgctgacg caacccccac 240

tggttggggc attgccacca cctgtcagct cctttccggg actttcgctt tccccctccc 300

tattgccacg gcggaactca tcgccgcctg ccttgcccgc tgctggacag gggctcggct 360

gttgggcact gacaattccg tggtgttgtc ggggaaatca tcgtcctttc cttggctgct 420

cgcctgtgtt gccacctgga ttctgcgcgg gacgtccttc tgctacgtcc cttcggccct 480

caatccagcg gaccttcctt cccgcggcct gctgccggct ctgcggcctc ttccgcgtct 540

tcgccttcgc cctcagacga gtcggatctc cctttgggcc gcctccccgc ct 592

<210> 27
<211> 543
<212> PRT
<213> Simian adenovirus

<400> 27
Met Lys Arg Thr Lys Thr Ser Asp Glu Ser Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Ser Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Ile Thr Thr Ala Ser Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ser Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val
165 170 175

Ser Ala Thr Pro Pro Ile Asn Val Ser Ser Gly Ser Leu Gly Leu Asp
180 185 190

Met Glu Asp Pro Met Tyr Thr His Asp Gly Lys Leu Gly Ile Arg Ile
195 200 205

Gly Gly Pro Leu Arg Val Val Asp Ser Leu His Thr Leu Thr Val Val
210 215 220

Thr Gly Asn Gly Leu Thr Val Asp Asn Asn Ala Leu Gln Thr Arg Val
225 230 235 240

Thr Gly Ala Leu Gly Tyr Asp Thr Ser Gly Asn Leu Gln Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Ile Asp Ala Asn Gly Gln Leu Ile Leu Asn Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Tyr Ile Asn Thr Asp His Asn Leu Asp Leu Asn Cys Asn
290 295 300

Arg Gly Leu Thr Thr Thr Thr Thr Asn Asn Thr Lys Lys Leu Glu Thr
305 310 315 320

Lys Ile Ser Ser Gly Leu Asp Tyr Asp Thr Asn Gly Ala Val Ile Ile
325 330 335

Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly Ala Leu Thr Val
340 345 350

Gly Asn Thr Gly Asp Asp Lys Leu Thr Leu Trp Thr Thr Pro Asp Pro
355 360 365

Ser Pro Asn Cys Arg Ile His Ser Asp Lys Asp Cys Lys Phe Thr Leu
370 375 380

Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Ser Val Ala Ala Leu
385 390 395 400

Ala Val Ser Gly Asn Leu Ala Ser Ile Thr Gly Thr Val Ala Ser Val
405 410 415

Thr Ile Phe Leu Arg Phe Asp Gln Asn Gly Val Leu Met Glu Asn Ser
420 425 430

Ser Leu Asp Arg Gln Tyr Trp Asn Phe Arg Asn Gly Asn Ser Thr Asn
435 440 445

Ala Ala Pro Tyr Thr Asn Ala Val Gly Phe Met Pro Asn Leu Ala Ala
450 455 460

Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Asn Asn Ile Val Ser Gln
465 470 475 480

Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Thr Leu Thr Ile Thr
485 490 495

Leu Asn Gly Thr Asn Glu Ser Ser Glu Thr Ser Gln Val Ser His Tyr
500 505 510

Ser Met Ser Phe Thr Trp Ala Trp Glu Ser Gly Gln Tyr Ala Thr Glu
515 520 525

Thr Phe Ala Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala Glu Gln
530 535 540

<210> 28
<211> 541
<212> PRT
<213> Simian adenovirus

<400> 28
Met Lys Arg Ala Lys Thr Ser Asp Glu Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Asn Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Val Thr Thr Val Thr Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Gln Thr Ser Ala Pro Leu Thr Val Ser Ser Gly Ser Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Gly Leu
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Thr Leu Gln
145 150 155 160

Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val Gly
165 170 175

Thr Thr Pro Pro Ile Ser Val Ser Ser Gly Ser Leu Gly Leu Asp Met
180 185 190

Glu Asp Pro Met Tyr Thr His Asp Gly Lys Leu Gly Ile Arg Ile Gly
195 200 205

Gly Pro Leu Gln Val Val Asp Ser Leu His Thr Leu Thr Val Val Thr
210 215 220

Gly Asn Gly Ile Thr Val Ala Asn Asn Ala Leu Gln Thr Lys Val Ala
225 230 235 240

Gly Ala Leu Gly Tyr Asp Ser Ser Gly Asn Leu Glu Leu Arg Ala Ala
245 250 255

Gly Gly Met Arg Ile Asn Thr Gly Gly Gln Leu Ile Leu Asp Val Ala
260 265 270

Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln Gly
275 280 285

Pro Leu Tyr Val Asn Thr Asn His Asn Leu Asp Leu Asn Cys Asn Arg
290 295 300

Gly Leu Thr Thr Thr Thr Ser Ser Asn Thr Thr Lys Leu Glu Thr Lys
305 310 315 320

Ile Asp Ser Gly Leu Asp Tyr Asn Ala Asn Gly Ala Ile Ile Ala Lys
325 330 335

Leu Gly Thr Gly Leu Thr Phe Asp Asn Thr Gly Ala Ile Thr Val Gly
340 345 350

Asn Thr Gly Asp Asp Lys Leu Thr Leu Trp Thr Thr Pro Asp Pro Ser
355 360 365

Pro Asn Cys Arg Ile His Ala Asp Lys Asp Lys Phe Thr Leu Val Leu
370 375 380

Thr Lys Cys Gly Ser Gln Ile Leu Ala Ser Val Ala Ala Leu Ala Val

385 390 395 400

Ser Gly Asn Leu Ser Ser Met Thr Gly Thr Val Ser Ser Val Thr Ile
405 410 415

Phe Leu Arg Phe Asp Gln Asn Gly Val Leu Met Glu Asn Ser Ser Leu
420 425 430

Asp Lys Glu Tyr Trp Asn Phe Arg Asn Gly Asn Ser Thr Asn Ala Thr
435 440 445

Pro Tyr Thr Asn Ala Val Gly Phe Met Pro Asn Leu Ser Ala Tyr Pro
450 455 460

Lys Thr Gln Ser Gln Thr Ala Lys Asn Asn Ile Val Ser Glu Val Tyr
465 470 475 480

Leu His Gly Asp Lys Ser Lys Pro Met Ile Leu Thr Ile Thr Leu Asn
485 490 495

Gly Thr Asn Glu Ser Ser Glu Thr Ser Gln Val Ser His Tyr Ser Met
500 505 510

Ser Phe Thr Trp Ser Trp Asp Ser Gly Lys Tyr Ala Thr Glu Thr Phe
515 520 525

Ala Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala Glu Gln
530 535 540

<210> 29
<211> 543
<212> PRT
<213> Simian adenovirus

<400> 29

Met Lys Arg Thr Lys Thr Ser Asp Glu Ser Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Ser Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Ile Thr Ser Thr Thr Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ser Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Ala Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val
165 170 175

Ser Ser Thr Pro Pro Ile Ser Val Ser Ser Gly Ser Leu Gly Leu Asp

180 185 190

Met Glu Asp Pro Met Tyr Thr His Asp Gly Lys Leu Gly Ile Arg Ile
195 200 205

Gly Gly Pro Leu Arg Val Val Asp Ser Leu His Thr Leu Thr Val Val
210 215 220

Thr Gly Asn Gly Leu Thr Val Asp Asn Asn Ala Leu Gln Thr Arg Val
225 230 235 240

Thr Gly Ala Leu Gly Tyr Asp Thr Ser Gly Asn Leu Gln Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Ile Asp Ala Asn Gly Gln Leu Ile Leu Asp Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Tyr Val Asn Thr Asp His Asn Leu Asp Leu Asn Cys Asn
290 295 300

Arg Gly Leu Thr Thr Thr Thr Thr Asn Asn Thr Lys Lys Leu Glu Thr
305 310 315 320

Lys Ile Ser Ser Gly Leu Asp Tyr Asp Thr Asn Gly Ala Val Ile Ile
325 330 335

Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly Ala Leu Thr Val
340 345 350

Gly Asn Thr Gly Asp Asp Lys Leu Thr Leu Trp Thr Thr Pro Asp Pro
355 360 365

Ser Pro Asn Cys Arg Ile His Ser Asp Lys Asp Cys Lys Phe Thr Leu
370 375 380

Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Ser Val Ala Ala Leu
385 390 395 400

Ala Val Ser Gly Asn Leu Ala Ser Ile Thr Gly Thr Val Ala Ser Val
405 410 415

Thr Ile Phe Leu Arg Phe Asp Gln Asn Gly Val Leu Met Glu Asn Ser
420 425 430

Ser Leu Asp Lys Gln Tyr Trp Asn Phe Arg Asn Gly Asn Ser Thr Asn
435 440 445

Ala Ala Pro Tyr Thr Asn Ala Val Gly Phe Met Pro Asn Leu Ala Ala
450 455 460

Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Asn Asn Ile Val Ser Gln
465 470 475 480

Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Thr Leu Thr Ile Thr
485 490 495

Leu Asn Gly Thr Asn Glu Ser Ser Glu Thr Ser Gln Val Ser His Tyr
500 505 510

Ser Met Ser Phe Thr Trp Ala Trp Glu Ser Gly Gln Tyr Ala Thr Glu
515 520 525

Thr Phe Ala Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala Glu Gln
530 535 540

<210> 30
<211> 543
<212> PRT
<213> Simian adenovirus

<400> 30
Met Lys Arg Thr Lys Thr Ser Asp Lys Ser Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Asn Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Val Thr Thr Thr Thr Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ala Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Leu Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val
165 170 175

Ser Ala Thr Pro Pro Ile Ser Val Ser Ser Gly Ser Leu Gly Leu Asp
180 185 190

Met Glu Asp Pro Met Tyr Thr His Asp Gly Lys Leu Gly Ile Arg Ile
195 200 205

Gly Gly Pro Leu Arg Val Val Asp Ser Leu His Thr Leu Thr Val Val
210 215 220

Thr Gly Asn Gly Ile Ala Val Asp Asn Asn Ala Leu Gln Thr Arg Val
225 230 235 240

Thr Gly Ala Leu Gly Tyr Asp Thr Ser Gly Asn Leu Gln Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Ile Asp Ala Asn Gly Gln Leu Ile Leu Asp Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Tyr Val Asn Thr Asp His Asn Leu Asp Leu Asn Cys Asn
290 295 300

Arg Gly Leu Thr Thr Thr Thr Thr Asn Asn Thr Lys Lys Leu Glu Thr
305 310 315 320

Lys Ile Gly Ser Gly Leu Asp Tyr Asp Thr Asn Gly Ala Val Ile Ile
325 330 335

Lys Leu Gly Thr Gly Val Ser Phe Asp Ser Thr Gly Ala Leu Ser Val
340 345 350

Gly Asn Thr Gly Asp Asp Lys Leu Thr Leu Trp Thr Thr Pro Asp Pro
355 360 365

Ser Pro Asn Cys Arg Ile His Ser Asp Lys Asp Cys Lys Phe Thr Leu
370 375 380

Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Ser Val Ala Ala Leu
385 390 395 400

Ala Val Ser Gly Asn Leu Ala Ser Ile Thr Gly Thr Val Ser Ser Val
405 410 415

Thr Ile Phe Leu Arg Phe Asp Gln Asn Gly Val Leu Met Glu Asn Ser
420 425 430

Ser Leu Asp Lys Gln Tyr Trp Asn Phe Arg Asn Gly Asn Ser Thr Asn
435 440 445

Ala Thr Pro Tyr Thr Asn Ala Val Gly Phe Met Pro Asn Leu Ala Ala
450 455 460

Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Asn Asn Ile Val Ser Gln
465 470 475 480

Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Thr Leu Thr Ile Thr
485 490 495

Leu Asn Gly Thr Asn Glu Ser Ser Glu Thr Ser Gln Val Ser His Tyr
500 505 510

Ser Met Ser Phe Thr Trp Ala Trp Glu Ser Gly Gln Tyr Ala Thr Glu
515 520 525

Thr Phe Ala Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala Glu Gln
530 535 540

<210> 31
<211> 543
<212> PRT
<213> Simian adenovirus

<400> 31
Met Lys Arg Thr Lys Thr Ser Asp Glu Ser Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Asn Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Val Thr Thr Thr Thr Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ala Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Leu Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val
165 170 175

Ser Ala Thr Pro Pro Ile Asn Val Ser Ser Gly Ser Leu Gly Leu Asp
180 185 190

Met Glu Asn Pro Met Tyr Thr His Asp Gly Lys Leu Gly Ile Arg Ile
195 200 205

Gly Gly Pro Leu Arg Val Val Asp Ser Leu His Thr Leu Thr Val Val
210 215 220

Thr Gly Asn Gly Ile Ala Val Asp Asn Asn Ala Leu Gln Thr Arg Val
225 230 235 240

Thr Gly Ala Leu Gly Tyr Asp Thr Ser Gly Asn Leu Gln Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Ile Asp Ala Asn Gly Gln Leu Ile Leu Asp Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Tyr Val Asn Thr Asp His Asn Leu Asp Leu Asn Cys Asn
290 295 300

Arg Gly Leu Thr Thr Thr Thr Thr Asn Asn Thr Lys Lys Leu Glu Thr
305 310 315 320

Lys Ile Gly Ser Gly Leu Asp Tyr Asp Thr Asn Gly Ala Val Ile Ile
325 330 335

Lys Leu Gly Thr Gly Val Ser Phe Asp Ser Thr Gly Ala Leu Ser Val
340 345 350

Gly Asn Thr Gly Asp Asp Lys Leu Thr Leu Trp Thr Thr Pro Asp Pro
355 360 365

Ser Pro Asn Cys Arg Ile His Ser Asp Lys Asp Cys Lys Phe Thr Leu
370 375 380

Val Leu Thr Lys Cys Gly Ser Gln Ile Leu Ala Ser Val Ala Ala Leu
385 390 395 400

Ala Val Ser Gly Asn Leu Ala Ser Ile Thr Gly Thr Val Ser Ser Val
405 410 415

Thr Ile Phe Leu Arg Phe Asp Gln Asn Gly Val Leu Met Glu Asn Ser
420 425 430

Ser Leu Asp Lys Gln Tyr Trp Asn Phe Arg Asn Gly Asn Ser Thr Asn
435 440 445

Ala Thr Pro Tyr Thr Asn Ala Val Gly Phe Met Pro Asn Leu Ala Ala
450 455 460

Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Asn Asn Ile Val Ser Gln
465 470 475 480

Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Ile Leu Thr Ile Thr

485 490 495

Leu Asn Gly Thr Asn Glu Ser Ser Glu Thr Ser Gln Val Ser His Tyr
500 505 510

Ser Met Ser Phe Thr Trp Ala Trp Glu Ser Gly Gln Tyr Ala Thr Glu
515 520 525

Thr Phe Ala Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala Glu Gln
530 535 540

<210> 32
<211> 578
<212> PRT
<213> Simian adenovirus

<400> 32
Met Lys Arg Thr Lys Thr Ser Asp Glu Ser Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Asn Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Val Thr Thr Thr Thr Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ala Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Leu Ala Ala Ala Val Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Ile
165 170 175

Ser Ala Thr Pro Pro Leu Ser Thr Ser Asn Gly Ser Leu Gly Ile Asp
180 185 190

Met Gln Ala Pro Ile Tyr Thr Thr Asn Gly Lys Leu Gly Leu Asn Phe
195 200 205

Gly Ala Pro Leu His Val Val Asp Ser Leu Asn Ala Leu Thr Val Val
210 215 220

Thr Gly Gln Gly Leu Thr Ile Asn Gly Thr Ala Leu Gln Thr Arg Val
225 230 235 240

Ser Gly Ala Leu Asn Tyr Asp Ser Ser Gly Asn Leu Glu Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Val Asp Ala Asn Gly Lys Leu Ile Leu Asp Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln

275 280 285

Gly Pro Leu Phe Val Asn Ser Ala His Asn Leu Asp Val Asn Tyr Asn
290 295 300

Arg Gly Leu Tyr Leu Phe Thr Ser Gly Asn Thr Lys Lys Leu Glu Val
305 310 315 320

Asn Ile Lys Thr Ala Lys Gly Leu Ile Tyr Asp Asp Thr Ala Ile Ala
325 330 335

Ile Asn Pro Gly Asp Gly Leu Glu Phe Gly Ser Gly Ser Asp Thr Asn
340 345 350

Pro Leu Lys Thr Lys Leu Gly Leu Gly Leu Glu Tyr Asp Ser Ser Arg
355 360 365

Ala Ile Ile Ala Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly
370 375 380

Ala Ile Thr Val Gly Asn Lys Asn Asp Asp Lys Leu Thr Leu Trp Thr
385 390 395 400

Thr Pro Asp Pro Ser Pro Asn Cys Arg Ile Tyr Ser Glu Lys Asp Ala
405 410 415

Lys Phe Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Val Leu Ala Ser
420 425 430

Val Ser Val Leu Ser Val Lys Gly Ser Leu Ala Pro Ile Ser Gly Thr
435 440 445

Val Thr Ser Ala Gln Ile Ile Leu Arg Phe Asp Glu Asn Gly Val Leu
450 455 460

Leu Ser Asn Ser Ser Leu Asp Pro Gln Tyr Trp Asn Tyr Arg Lys Gly
465 470 475 480

Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met Pro
485 490 495

Asn Leu Thr Ala Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Ser Asn
500 505 510

Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Ile
515 520 525

Leu Thr Ile Thr Leu Asn Gly Thr Asn Glu Thr Gly Asp Ala Thr Val
530 535 540

Ser Thr Tyr Ser Met Ser Phe Ser Trp Asn Trp Asn Gly Ser Asn Tyr
545 550 555 560

Ile Asn Glu Thr Phe Gln Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala
565 570 575

Gln Glu

<210> 33
<211> 578
<212> PRT
<213> Simian adenovirus

<400> 33
Met Lys Arg Thr Lys Thr Ser Asp Glu Ser Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Ser Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro

20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Ile Thr Thr Ala Ser Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ser Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val
165 170 175

Ser Ala Thr Pro Pro Leu Ser Thr Ser Asn Gly Ser Leu Gly Ile Asp
180 185 190

Met Gln Ala Pro Ile Tyr Thr Thr Asn Gly Lys Leu Gly Leu Asn Phe
195 200 205

Gly Ala Pro Leu His Val Val Asp Ser Leu Asn Ala Leu Thr Val Val
210 215 220

Thr Gly Gln Gly Leu Thr Ile Asn Gly Thr Ala Leu Gln Thr Arg Val
225 230 235 240

Ser Gly Ala Leu Asn Tyr Asp Thr Ser Gly Asn Leu Glu Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Val Asp Ala Asn Gly Gln Leu Ile Leu Asp Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Val Asn Ser Ala His Asn Leu Asp Val Asn Tyr Asn
290 295 300

Arg Gly Leu Tyr Leu Phe Thr Ser Gly Asn Thr Lys Lys Leu Glu Val
305 310 315 320

Asn Ile Lys Thr Ala Lys Gly Leu Ile Tyr Asp Asp Thr Ala Ile Ala
325 330 335

Ile Asn Ala Gly Asp Gly Leu Gln Phe Asp Ser Gly Ser Asp Thr Asn
340 345 350

Pro Leu Lys Thr Lys Leu Gly Leu Gly Leu Asp Tyr Asp Ser Ser Arg
355 360 365

Ala Ile Ile Ala Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly
370 375 380

Ala Ile Thr Val Gly Asn Lys Asn Asp Asp Lys Leu Thr Leu Trp Thr
385 390 395 400

Thr Pro Asp Pro Ser Pro Asn Cys Arg Ile Tyr Ser Glu Lys Asp Ala
405 410 415

Lys Phe Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Val Leu Ala Ser
420 425 430

Val Ser Val Leu Ser Val Lys Gly Ser Leu Ala Pro Ile Ser Gly Thr
435 440 445

Val Thr Ser Ala Gln Ile Val Leu Arg Phe Asp Glu Asn Gly Val Leu
450 455 460

Leu Ser Asn Ser Ser Leu Asp Pro Gln Tyr Trp Asn Tyr Arg Lys Gly
465 470 475 480

Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met Pro
485 490 495

Asn Leu Thr Ala Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Ser Asn
500 505 510

Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Thr
515 520 525

Leu Thr Ile Thr Leu Asn Gly Thr Asn Glu Thr Gly Asp Ala Thr Val
530 535 540

Ser Thr Tyr Ser Met Ser Phe Ser Trp Asn Trp Asn Gly Ser Asn Tyr
545 550 555 560

Ile Asn Glu Thr Phe Gln Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala
565 570 575

Gln Glu

<210> 34
<211> 578
<212> PRT
<213> Simian adenovirus

<400> 34
Met Lys Arg Thr Lys Thr Ser Asp Glu Ser Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Ser Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Asn Leu Ala Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Ser Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Ile Thr Thr Ala Ser Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Glu Thr Ser Ser Pro Leu Thr Val Ser Thr Ser Gly Ala
100 105 110

Leu Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu
115 120 125

Thr Met Gln Ser Glu Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Thr
130 135 140

Leu Ala Thr Lys Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu
145 150 155 160

Gln Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val
165 170 175

Ser Ala Thr Pro Pro Leu Ser Thr Ser Asn Gly Ser Leu Gly Ile Asp
180 185 190

Met Gln Ala Pro Ile Tyr Thr Thr Asn Gly Lys Leu Gly Leu Asn Phe
195 200 205

Gly Ala Pro Leu His Val Val Asp Ser Leu Asn Ala Leu Thr Val Val
210 215 220

Thr Gly Gln Gly Leu Thr Ile Asn Gly Thr Ala Leu Gln Thr Arg Val
225 230 235 240

Ser Gly Ala Leu Asn Tyr Asp Thr Ser Gly Asn Leu Glu Leu Arg Ala
245 250 255

Ala Gly Gly Met Arg Val Asp Ala Asn Gly Gln Leu Ile Leu Asp Val
260 265 270

Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Val Asn Ser Ala His Asn Leu Asp Val Asn Tyr Asn
290 295 300

Arg Gly Leu Tyr Leu Phe Thr Ser Gly Asn Thr Lys Lys Leu Glu Val
305 310 315 320

Asn Ile Lys Thr Ala Lys Gly Leu Ile Tyr Asp Asp Thr Ala Ile Ala
325 330 335

Ile Asn Ala Gly Asp Gly Leu Gln Phe Asp Ser Gly Ser Asp Thr Asn
340 345 350

Pro Leu Lys Thr Lys Leu Gly Leu Gly Leu Asp Tyr Asp Ser Ser Arg
355 360 365

Ala Ile Ile Ala Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly
370 375 380

Ala Ile Thr Val Gly Asn Lys Asn Asp Asp Lys Leu Thr Leu Trp Thr
385 390 395 400

Thr Pro Asp Pro Ser Pro Asn Cys Arg Ile Tyr Ser Glu Lys Asp Ala
405 410 415

Lys Phe Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Val Leu Ala Ser
420 425 430

Val Ser Val Leu Ser Val Lys Gly Ser Leu Ala Pro Ile Ser Gly Thr
435 440 445

Val Thr Ser Ala Gln Ile Val Leu Arg Phe Asp Glu Asn Gly Val Leu
450 455 460

Leu Ser Asn Ser Ser Leu Asp Pro Gln Tyr Trp Asn Tyr Arg Lys Gly
465 470 475 480

Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met Pro

485 490 495

Asn Leu Thr Ala Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Ser Asn
500 505 510

Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Thr
515 520 525

Leu Thr Ile Thr Leu Asn Gly Thr Asn Glu Thr Gly Asp Ala Thr Val
530 535 540

Ser Thr Tyr Ser Met Ser Phe Ser Trp Asn Trp Asn Gly Ser Asn Tyr
545 550 555 560

Ile Asn Glu Thr Phe Gln Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala
565 570 575

Gln Glu

<210> 35
<211> 577
<212> PRT
<213> Simian adenovirus

<400> 35
Met Lys Arg Ala Lys Thr Ser Asp Glu Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Asn Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Val Thr Thr Val Thr Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Gln Thr Ser Ala Pro Leu Thr Val Ser Ser Gly Ser Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Gly Leu
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Thr Leu Gln
145 150 155 160

Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val Ser
165 170 175

Ala Thr Pro Pro Leu Ser Thr Ser Asn Gly Ser Leu Ser Ile Asp Met
180 185 190

Gln Ala Pro Ile Tyr Thr Thr Asn Gly Lys Leu Ala Leu Asn Ile Gly
195 200 205

Ala Pro Leu His Val Val Asp Thr Leu Asn Ala Leu Thr Val Val Thr
210 215 220

Gly Gln Gly Leu Thr Ile Asn Gly Arg Ala Leu Gln Thr Arg Val Thr

225 230 235 240

Gly Ala Leu Ser Tyr Asp Thr Glu Gly Asn Ile Gln Leu Gln Ala Gly
245 250 255

Gly Gly Met Arg Ile Asp Asn Asn Gly Gln Leu Ile Leu Asn Val Ala
260 265 270

Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln Gly
275 280 285

Pro Leu Ile Val Asn Ser Ala His Asn Leu Asp Leu Asn Leu Asn Arg
290 295 300

Gly Leu Tyr Leu Phe Thr Ser Gly Asn Thr Lys Lys Leu Glu Val Asn
305 310 315 320

Ile Lys Thr Ala Lys Gly Leu Phe Tyr Asp Gly Thr Ala Ile Ala Ile
325 330 335

Asn Ala Gly Asp Gly Leu Gln Phe Gly Ser Gly Ser Asp Thr Asn Pro
340 345 350

Leu Gln Thr Lys Leu Gly Leu Gly Leu Glu Tyr Asp Ser Asn Lys Ala
355 360 365

Ile Ile Thr Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly Ala
370 375 380

Ile Thr Val Gly Asn Lys Asn Asp Asp Lys Leu Thr Leu Trp Thr Thr
385 390 395 400

Pro Asp Pro Ser Pro Asn Cys Arg Ile Asn Ser Glu Lys Asp Ala Lys
405 410 415

Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Val Leu Ala Ser Val
420 425 430

Ser Val Leu Ser Val Lys Gly Ser Leu Ala Pro Ile Ser Gly Thr Val
435 440 445

Thr Ser Ala Gln Ile Val Leu Arg Phe Asp Glu Asn Gly Val Leu Leu
450 455 460

Ser Asn Ser Ser Leu Asp Pro Gln Tyr Trp Asn Tyr Arg Lys Gly Asp
465 470 475 480

Ser Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met Pro Asn
485 490 495

Leu Thr Ala Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Ser Asn Ile
500 505 510

Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Met Thr Leu
515 520 525

Thr Ile Thr Leu Asn Gly Thr Asn Glu Thr Gly Asp Ala Thr Val Ser
530 535 540

Thr Tyr Ser Met Ser Phe Ser Trp Asn Trp Asn Gly Ser Asn Tyr Ile
545 550 555 560

Asn Asp Thr Phe Gln Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala Gln
565 570 575

Glu

<210> 36
<211> 577
<212> PRT
<213> Simian adenovirus

<400> 36
Met Lys Arg Ala Lys Thr Ser Asp Glu Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Asn Gly Pro Pro Ser Val Pro Phe Leu Thr Pro Pro
20 25 30

Phe Val Ser Pro Asp Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser His Gly Met Leu Ala Leu
50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Asp Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asp Val Thr Thr Val Thr Pro Pro Leu Lys Lys Thr Lys Thr Asn
85 90 95

Leu Ser Leu Gln Thr Ser Ala Pro Leu Thr Val Ser Ser Gly Ser Leu
100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Ala Val Ala Gly Thr Ser Leu Thr
115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val Gln Asp Ala Lys Leu Gly Leu
130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Thr Leu Gln
145 150 155 160

Thr Ser Ala Pro Leu Thr Ala Ala Asp Ser Ser Thr Leu Thr Val Ser
165 170 175

Ala Thr Pro Pro Leu Ser Thr Ser Asn Gly Ser Leu Ser Ile Asp Met
180 185 190

Gln Ala Pro Ile Tyr Thr Thr Asn Gly Lys Leu Ala Leu Asn Ile Gly
195 200 205

Ala Pro Leu His Val Val Asp Thr Leu Asn Ala Leu Thr Val Val Thr
210 215 220

Gly Gln Gly Leu Thr Ile Asn Gly Arg Ala Leu Gln Thr Arg Val Thr
225 230 235 240

Gly Ala Leu Ser Tyr Asp Thr Glu Gly Asn Ile Gln Leu Gln Ala Gly
245 250 255

Gly Gly Met Arg Ile Asp Asn Asn Gly Gln Leu Ile Leu Asn Val Ala
260 265 270

Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln Gly
275 280 285

Pro Leu Ile Val Asn Ser Ala His Asn Leu Asp Leu Asn Leu Asn Arg
290 295 300

Gly Leu Tyr Leu Phe Thr Ser Gly Asn Thr Lys Lys Leu Glu Val Asn
305 310 315 320

Ile Lys Thr Ala Lys Gly Leu Phe Tyr Asp Gly Thr Ala Ile Ala Ile
325 330 335

Asn Ala Gly Asp Gly Leu Gln Phe Gly Ser Gly Ser Asp Thr Asn Pro
340 345 350

Leu Gln Thr Lys Leu Gly Leu Gly Leu Glu Tyr Asp Ser Asn Lys Ala
355 360 365

Ile Ile Thr Lys Leu Gly Thr Gly Leu Ser Phe Asp Asn Thr Gly Ala
370 375 380

Ile Thr Val Gly Asn Lys Asn Asp Asp Lys Leu Thr Leu Trp Thr Thr
385 390 395 400

Pro Asp Pro Ser Pro Asn Cys Arg Ile Asn Ser Glu Lys Asp Ala Lys
405 410 415

Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Val Leu Ala Ser Val
420 425 430

Ser Val Leu Ser Val Lys Gly Ser Leu Ala Pro Ile Ser Gly Thr Val
435 440 445

Thr Ser Ala Gln Ile Val Leu Arg Phe Asp Glu Asn Gly Val Leu Leu
450 455 460

Ser Asn Ser Ser Leu Asp Pro Gln Tyr Trp Asn Tyr Arg Lys Gly Asp
465 470 475 480

Ser Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly Phe Met Pro Asn
485 490 495

Leu Thr Ala Tyr Pro Lys Thr Gln Ser Gln Thr Ala Lys Ser Asn Ile
500 505 510

Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys Pro Met Thr Leu
515 520 525

Thr Ile Thr Leu Asn Gly Thr Asn Glu Thr Gly Asp Ala Thr Val Ser
530 535 540

Thr Tyr Ser Met Ser Phe Ser Trp Asn Trp Asn Gly Ser Asn Tyr Ile
545 550 555 560

Asn Asp Thr Phe Gln Thr Asn Ser Phe Thr Phe Ser Tyr Ile Ala Gln
565 570 575

Glu

<210> 37
<211> 1145
<212> PRT
<213> Respiratory syncytial virus

<400> 37
Met Glu Leu Leu Ile Leu Lys Ala Asn Ala Ile Thr Thr Ile Leu Thr
1 5 10 15

Ala Val Thr Phe Cys Phe Ala Ser Gly Gln Asn Ile Thr Glu Glu Phe
20 25 30

Tyr Gln Ser Thr Cys Ser Ala Val Ser Lys Gly Tyr Leu Ser Ala Leu
35 40 45

Arg Thr Gly Trp Tyr Thr Ser Val Ile Thr Ile Glu Leu Ser Asn Ile
50 55 60

Lys Glu Asn Lys Cys Asn Gly Thr Asp Ala Lys Val Lys Leu Ile Lys
65 70 75 80

Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu
85 90 95

Met Gln Ser Thr Pro Ala Thr Asn Asn Arg Ala Arg Arg Glu Leu Pro
100 105 110

Arg Phe Met Asn Tyr Thr Leu Asn Asn Ala Lys Lys Thr Asn Val Thr
115 120 125

Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe Leu Leu Gly Val
130 135 140

Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu
145 150 155 160

Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu Ser Thr Asn Lys
165 170 175

Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val
180 185 190

Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu Pro Ile Val Asn
195 200 205

Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val Ile Glu Phe Gln
210 215 220

Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val Asn
225 230 235 240

Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser Glu
245 250 255

Leu Leu Ser Leu Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys Leu
260 265 270

Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile Met
275 280 285

Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro Leu
290 295 300

Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser Pro Leu
305 310 315 320

Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr Arg Thr
325 330 335

Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe Pro
340 345 350

Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys Asp Thr
355 360 365

Met Asn Ser Leu Thr Leu Pro Ser Glu Val Asn Leu Cys Asn Val Asp
370 375 380

Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr Asp
385 390 395 400

Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser Cys Tyr
405 410 415

Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile Ile Lys
420 425 430

Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Val Asp Thr

435 440 445

Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly Lys
450 455 460

Ser Leu Tyr Val Lys Gly Glu Pro Ile Ile Asn Phe Tyr Asp Pro Leu
465 470 475 480

Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn Glu
485 490 495

Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu Leu
500 505 510

His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Arg Lys Arg Arg Ala
515 520 525

Pro Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp
530 535 540

Val Glu Ser Asn Pro Gly Pro Met Ala Leu Ser Lys Val Lys Leu Asn
545 550 555 560

Asp Thr Leu Asn Lys Asp Gln Leu Leu Ser Ser Ser Lys Tyr Thr Ile
565 570 575

Gln Arg Ser Thr Gly Asp Ser Ile Asp Thr Pro Asn Tyr Asp Val Gln
580 585 590

Lys His Ile Asn Lys Leu Cys Gly Met Leu Leu Ile Thr Glu Asp Ala
595 600 605

Asn His Lys Phe Thr Gly Leu Ile Gly Met Leu Tyr Ala Met Ser Arg
610 615 620

Leu Gly Arg Glu Asp Thr Ile Lys Ile Leu Arg Asp Ala Gly Tyr His
625 630 635 640

Val Lys Ala Asn Gly Val Asp Val Thr Thr His Arg Gln Asp Ile Asn
645 650 655

Gly Lys Glu Met Lys Phe Glu Val Leu Thr Leu Ala Ser Leu Thr Thr
660 665 670

Glu Ile Gln Ile Asn Ile Glu Ile Glu Ser Arg Lys Ser Tyr Lys Lys
675 680 685

Met Leu Lys Glu Met Gly Glu Val Ala Pro Glu Tyr Arg His Asp Ser
690 695 700

Pro Asp Cys Gly Met Ile Ile Leu Cys Ile Ala Ala Leu Val Ile Thr
705 710 715 720

Lys Leu Ala Ala Gly Asp Arg Ser Gly Leu Thr Ala Val Ile Arg Arg
725 730 735

Ala Asn Asn Val Leu Lys Asn Glu Met Lys Arg Tyr Lys Gly Leu Leu
740 745 750

Pro Lys Asp Ile Ala Asn Ser Phe Tyr Glu Val Phe Glu Lys Tyr Pro
755 760 765

His Phe Ile Asp Val Phe Val His Phe Gly Ile Ala Gln Ser Ser Thr
770 775 780

Arg Gly Gly Ser Arg Val Glu Gly Ile Phe Ala Gly Leu Phe Met Asn
785 790 795 800

Ala Tyr Gly Ala Gly Gln Val Met Leu Arg Trp Gly Val Leu Ala Lys
805 810 815

Ser Val Lys Asn Ile Met Leu Gly His Ala Ser Val Gln Ala Glu Met
820 825 830

Glu Gln Val Val Glu Val Tyr Glu Tyr Ala Gln Lys Leu Gly Gly Glu
835 840 845

Ala Gly Phe Tyr His Ile Leu Asn Asn Pro Lys Ala Ser Leu Leu Ser
850 855 860

Leu Thr Gln Phe Pro His Phe Ser Ser Val Val Leu Gly Asn Ala Ala
865 870 875 880

Gly Leu Gly Ile Met Gly Glu Tyr Arg Gly Thr Pro Arg Asn Gln Asp
885 890 895

Leu Tyr Asp Ala Ala Lys Ala Tyr Ala Glu Gln Leu Lys Glu Asn Gly
900 905 910

Val Ile Asn Tyr Ser Val Leu Asp Leu Thr Ala Glu Glu Leu Glu Ala
915 920 925

Ile Lys His Gln Leu Asn Pro Lys Asp Asn Asp Val Glu Leu Gly Gly
930 935 940

Gly Gly Ser Gly Gly Gly Gly Met Ser Arg Arg Asn Pro Cys Lys Phe
945 950 955 960

Glu Ile Arg Gly His Cys Leu Asn Gly Lys Arg Cys His Phe Ser His
965 970 975

Asn Tyr Phe Glu Trp Pro Pro His Ala Leu Leu Val Arg Gln Asn Phe
980 985 990

Met Leu Asn Arg Ile Leu Lys Ser Met Asp Lys Ser Ile Asp Thr Leu
995 1000 1005

Ser Glu Ile Ser Gly Ala Ala Glu Leu Asp Arg Thr Glu Glu Tyr
1010 1015 1020

Ala Leu Gly Val Val Gly Val Leu Glu Ser Tyr Ile Gly Ser Ile
1025 1030 1035

Asn Asn Ile Thr Lys Gln Ser Ala Cys Val Ala Met Ser Lys Leu
1040 1045 1050

Leu Thr Glu Leu Asn Ser Asp Asp Ile Lys Lys Leu Arg Asp Asn
1055 1060 1065

Glu Glu Leu Asn Ser Pro Lys Ile Arg Val Tyr Asn Thr Val Ile
1070 1075 1080

Ser Tyr Ile Glu Ser Asn Arg Lys Asn Asn Lys Gln Thr Ile His
1085 1090 1095

Leu Leu Lys Arg Leu Pro Ala Asp Val Leu Lys Lys Thr Ile Lys
1100 1105 1110

Asn Thr Leu Asp Ile His Lys Ser Ile Thr Ile Asn Asn Pro Lys
1115 1120 1125

Glu Ser Thr Val Ser Asp Thr Asn Asp His Ala Lys Asn Asn Asp
1130 1135 1140

Thr Thr

1145

<210> 38
<211> 1502
<212> DNA
<213> Human immunodeficiency virus

<400> 38
atgggtgcta gggcttctgt gctgtctggt ggtgagctgg acaagtggga gaagatcagg 60

ctgaggcctg gtggcaagaa gaagtacaag ctaaagcaca ttgtgtgggc ctccagggag 120

ctggagaggt ttgctgtgaa ccctggcctg ctggagacct ctgaggggtg caggcagatc 180

ctgggccagc tccagccctc cctgcaaaca ggctctgagg agctgaggtc cctgtacaac 240

acagtggcta ccctgtactg tgtgcaccag aagattgatg tgaaggacac caaggaggcc 300

ctggagaaga ttgaggagga gcagaacaag tccaagaaga aggcccagca ggctgctgct 360

ggcacaggca actccagcca ggtgtcccag aactacccca ttgtgcagaa cctccagggc 420

cagatggtgc accaggccat ctccccccgg accctgaatg cctgggtgaa ggtggtggag 480

gagaggcctt ctcccctgag gtgatcccca tgttctctgc cctgtctgag ggtgccaccc 540

cccaggacct gaacaccatg ctgaacacag tgggggggcca tcaggctgcc atgcagatgc 600

tgaaggagac catcaatgag gaggctgctg agtgggacag gctgcatcct gtgcacgctg 660

gccccattgc ccccggccag atgagggagc ccaggggctc tgacattgct ggcaccacct 720

ccaccctcca ggagcagatt ggctggatga ccaacaaccc ccccatccct gtgggggaaa 780

tctacaagag gtggatcatc ctgggcctga acaagattgt gaggatgtac tcccccacct 840

ccatcctgga catcaggcag ggccccaagg agcccttcag ggactatgtg gacaggttct 900

acaagaccct gagggctgag caggcctccc aggaggtgaa gaactggatg acagagaccc 960

tgctggtgca gaatgccaac cctgactgca agaccatcct gaaggccctg ggccctgctg 1020

ccaccctgga ggagatgatg acagcctgcc agggggtggg gggccctggt cacaaggcca 1080

gggtgctggc tgaggccatg tcccaggtga ccaactccgc caccatcatg atgcagaggg 1140

gcaacttcag gaaccagagg aagacagtga agtgcttcaa ctgtggcaag gtgggccaca 1200

ttgccaagaa ctgtagggcc cccaggaaga agggctgctg gaagtgtggc aaggagggcc 1260

accagatgaa ggactgcaat gagaggcagg ccaacttcct gggcaaaatc tggccctccc 1320

acaagggcag gcctggcaac ttcctccagt ccaggcctga gcccacagcc cctcccgagg 1380

agtccttcag gtttggggag gagaagacca cccccagcca gaagcaggag cccattgaca 1440

aggagctgta ccccctggcc tccctgaggt ccctgtttgg caacgacccc tcctcccagt 1500

aa 1502