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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.



ADENOVIRUS POLYNUCLEOTIDES AND POLYPEPTIDES

FIELD OF THE INVENTION

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

BACKGROUND OF THE INVENTION

10 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
15 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
20 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
25 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.

30 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. Vectors of the present invention may find use in the expression of one or more immunogens useful to immunise a
35 human or non-human animal against a pathogen.

Respiratory syncytial virus (RSV) is a highly contagious human pathogen that causes respiratory tract infections in people of all ages. During the first year of life, 50-70% of infants are infected with RSV and essentially all children have had an RSV infection by their second
40 birthday. The risk for severe RSV-associated lower respiratory tract infections (LRTI) is highest in infants below 6 months of age and is a leading cause for hospitalization. Infection with RSV does not confer full protective immunity. Symptomatic RSV re-infections are common later in life and continue throughout adulthood. These re-infections generally go undiagnosed because

they usually present as common acute upper respiratory tract infections. In more vulnerable persons (e.g., immunocompromised adults or elderly), re-infections can however also lead to severe disease.

- 5 To date, no vaccine is available against RSV and treatment of RSV disease is largely symptomatic and supportive care. The antiviral drug ribavirin is currently the only approved antiviral therapy for RSV treatment, but its use is restricted to severe hospitalized cases due to uncertainties regarding its efficacy, difficulty in administration (aerosol) and high cost [American Academy of Pediatrics Subcommittee on Diagnosis and Management of
- 10 Bronchiolitis, 2006]. RSV-specific monoclonal antibodies (palivizumab, *Synagis*TM, Medimmune) are indicated for the prevention of serious LRTIs requiring hospitalization caused by RSV in children at high risk for RSV disease but are not indicated or recommended in the general, healthy infant population due to high cost and the need for repeated administration.
- 15 In the late 1960s, a formalin-inactivated whole virus RSV vaccine (FI-RSV) tested in clinical trials led to more severe clinical symptoms upon subsequent natural infection with RSV in children under the age of two [Kim, 1969; Chin, 1969]]. This experience has led to heightened safety concerns with pediatric RSV vaccine candidates. Since that time, several investigational vaccines have been and continue to be explored, including live attenuated viral vaccines and
- 20 those based upon purified or recombinant viral proteins. However there is not yet a licensed vaccine for the prevention of RSV disease.

SUMMARY OF THE INVENTION

There is provided an isolated polynucleotide, wherein the polynucleotide encodes a

25 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
- 30 (c) a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

Also provided 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
- 35 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
- 40 (c) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3.

Also provided 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,

5 (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

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

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

15 (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,

20 (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,

25 (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.

Also provided is a composition comprising at least one of the following:

30 (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,

35 (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,

40 (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 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.
- 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.

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)
- PanAd2 (SEQ ID NO:36)

- Figure 2 – Flow diagram for production of specific ChAd155 BAC and plasmid vectors
- Figure 3 – Species C BAC Shuttle #1365 schematic
- Figure 4 – pArsChAd155 Ad5E4orf6-2 (#1490) schematic
- Figure 5 – pChAd155/RSV schematic
- 5 Figure 6 – BAC ChAd155/RSV schematic
- Figure 7 – Productivity of ChAd3 and ChAd155 vectors expressing an HIV Gag transgene (Experiment 1)
- Figure 8 – Productivity of ChAd3 and ChAd155 vectors expressing an HIV Gag transgene (Experiment 2)
- 10 Figure 9 – Productivity of PanAd3 and ChAd155 vectors expressing RSV transgene
- Figure 10 – Expression levels of ChAd3 and ChAd155 vectors expressing an HIV Gag transgene
- Figure 11 – Expression levels of PanAd3 and ChAd155 vectors expressing an HIV Gag transgene – Western Blot
- 15 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
- Figure 14 – Schematic of the synthetic DNA fragment used to express RSV antigens by the
- 20 Figure 15 – Anti-F antibody titers induced by ChAd155-RSV and PanAd3-RSV in BALB/c mice
- Figure 16 – RSV titers in nasal tissues (A) and lung homogenates (B), RSV neutralizing antibody (C) and pathology score (D) after viral challenge
- 25 Figure 17 – Kinetics of induction of bRSV-specific IgG (A) and RSV neutralizing antibodies (B)
- Figure 18 – Kinetics of mean virus titers in nasopharyngeal swabs detected up to day 6 post bRSV challenge
- Figure 19 – Effect of vaccination on bRSV replication in the lower respiratory tract
- 30 Figure 20 – Effect of vaccination on gross pneumonic consolidation 6 days post bRSV challenge
- Figure 21 – Effect of vaccination on the pulmonary inflammatory response 6 days post bRSV challenge

35 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
- 40 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
5	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 Δ E1_TetO hCMV RpsL-Kana primer 1
10		polynucleotide sequence
	SEQ ID NO: 16 -	BAC/CHAd155 Δ E1_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
15	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
20	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
25	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
30	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 F Δ TM-N-M2-1 amino acid sequence
35	SEQ ID NO: 38 -	HIV Gag polynucleotide sequence

DETAILED DESCRIPTION OF THE INVENTION

Adenovirus

40

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)

5 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
10 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
15 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
20 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
25 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
30 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
35 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.

40 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.

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

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.

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.

40

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

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.

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% 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 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 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 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.

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

- 10 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: 1. Suitably the polynucleotide has a sequence according to SEQ ID NO: 2.
- 15 Alternatively, the polynucleotide, vector, recombinant adenovirus, composition or cell of the invention comprises 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. Suitably the functional derivative of a polypeptide having the
- 20 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% identical, such as at least 99.4% identical, such as at least 99.6%
- 25 identical, such as at least 99.8% 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
- 30 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.
- 35 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: 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
- 40 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 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.

Isolated Polynucleotides, Vectors, Recombinant Adenoviruses, Compositions or Cells comprising Polynucleotides Encoding ChAd155 Penton

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.

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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

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		/gene="IX"
	TATA_signal	3571..3576
		/gene="IX"
5	prim_transcript	3601..4131
		/gene="IX"
	CDS	3628..4092
		/gene="IX"
		/product="IX"
10	polyA_signal	4097..4102
		/note="E1B, IX"
	gene	complement(4117..27523)
		/gene="E2B"
	prim_transcript	complement(4117..27494)
		/gene="E2B"
15	gene	complement(4117..5896)
		/gene="IVa2"
	prim_transcript	complement(4117..5896)
		/gene="IVa2"
20	CDS	complement(join(4151..5487,5766..5778))
		/gene="IVa2"
		/product="E2B_IVa2"
	polyA_signal	complement(4150..4155)
		/note="IVa2, E2B"
25	CDS	complement(join(5257..8838,14209..14217))
		/gene="E2B"
		/product="E2B_polymerase"
	gene	6078..34605
		/gene="L5"
30	gene	6078..28612
		/gene="L4"
	gene	6078..22658
		/gene="L3"
	gene	6078..18164
		/gene="L2"
35	gene	6078..14216
		/gene="L1"
	TATA_signal	6078..6083
		/note="L"
40	prim_transcript	6109..34605
		/gene="L5"
	prim_transcript	6109..28612
		/gene="L4"
	prim_transcript	6109..22658
		/gene="L3"
45	prim_transcript	6109..18164
		/gene="L2"
	prim_transcript	6109..14216
		/gene="L1"
50	CDS	join(8038..8457,9722..9742)
		/gene="L1"
		/product="L1_13.6K"
	CDS	complement(join(8637..10640,14209..14217))
		/gene="E2B"
		/product="E2B_pTP"
55	gene	10671..10832
		/gene="VAI"
	misc_RNA	10671..10832
		/gene="VAI"
		/product="VAI"
60	gene	10902..11072
		/gene="VAII"
	misc_RNA	10902..11072
		/gene="VAII"
		/product="VAII"
65	CDS	11093..12352
		/gene="L1"
		/product="L1_52K"
	CDS	12376..14157

		/gene="L1"
		/product="L1_pIIIa"
	polyA_signal	14197..14202
5	CDS	/gene="L1"
		14254..16035
		/gene="L2"
		/product="L2_penton"
	CDS	16050..16646
10		/gene="L2"
		/product="L2_pVII"
	CDS	16719..17834
		/gene="L2"
		/product="L2_v"
15	CDS	17859..18104
		/gene="L2"
		/product="L2_pX"
	polyA_signal	18143..18148
		/gene="L2"
20	CDS	18196..18951
		/gene="L3"
		/product="L3_pVI"
	CDS	19063..21945
		/gene="L3"
		/product="L3_hexon"
25	CDS	21975..22604
		/gene="L3"
		/product="L3_protease"
	polyA_signal	22630..22635
		/gene="L3"
30	gene	complement(22632..27523)
		/gene="E2A"
	prim_transcript	complement(22632..27494)
		/gene="E2A"
	gene	complement(22632..26357)
35		/gene="E2A-L"
	prim_transcript	complement(22632..26328)
		/gene="E2A-L"
	polyA_signal	complement(22649..22654)
		/note="E2A, E2A-L"
40	CDS	complement(22715..24367)
		/gene="E2A"
		/note="DBP; genus-common; DBP family"
		/codon_start=1
		/product="E2A"
45	CDS	24405..26915
		/gene="L4"
		/product="L4_100k"
	TATA_signal	complement(26352..26357)
		/gene="E2A-L"
50	CDS	join(26602..26941,27147..27529)
		/gene="L4"
		/product="L4_33K"
	CDS	26602..27207
		/gene="L4"
55		/product="L4_22K"
	TATA_signal	complement(27518..27523)
		/note="E2A, E2B; nominal"
	CDS	27604..28287
		/gene="L4"
60		/product="L4_pVIII"
	gene	27969..32686
		/gene="E3B"
	gene	27969..31611
		/gene="E3A"
65	TATA_signal	27969..27974
		/note="E3A, E3B"
	prim_transcript	27998..32686
		/gene="E3B"

	prim_transcript	27998..31611	/gene="E3A"
	CDS	28288..28605	/gene="E3A"
5			/product="E3 ORF1"
	polyA_signal	28594..28599	/gene="L4"
	CDS	29103..29303	/gene="E3A"
10			/product="E3 ORF2"
	CDS	29300..29797	/gene="E3A"
			/product="E3 ORF3"
15	CDS	29826..30731	/gene="E3A"
			/product="E3 ORF4"
	CDS	30728..31579	/gene="E3A"
			/product="E3 ORF5"
20	CDS	31283..31579	/gene="E3A"
			/product="E3 ORF6"
	polyA_signal	31578..31584	/gene="E3A"
25	CDS	31591..31863	/gene="E3B"
			/product="E3 ORF7"
	CDS	31866..32264	/gene="E3B"
30			/product="E3 ORF8"
	CDS	32257..32643	/gene="E3B"
			/product="E3 ORF9"
35	polyA_signal	32659..32664	/gene="E3B"
	gene	complement(<32678..32838)	/gene="U"
	CDS	complement(<32678..32838)	/gene="U"
40			/note="exon encoding C terminus unidentified; genus-common"
	CDS	32849..34585	/product="protein U"
			/gene="L5"
45			/product="L5_fiber"
	polyA_signal	34581..34586	/gene="L5"
	gene	complement(34611..37520)	/gene="E4"
50	prim_transcript	complement(34611..37490)	/gene="E4"
	polyA_signal	complement(34625..34630)	/gene="E4"
	CDS	complement(join(34794..35069,35781..35954))	/gene="E4"
55			/product="E4 ORF7"
	CDS	complement(35070..35954)	/gene="E4"
			/product="E4 ORF6"
60	CDS	complement(35875..36219)	/gene="E4"
			/product="E4 ORF4"
	CDS	complement(36235..36582)	/gene="E4"
65			/product="E4 ORF3"
	CDS	complement(36579..36971)	/gene="E4"
			/product="E4 ORF2"


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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

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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.

25 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, 10T1/2, HEK 293 cells or Per.C6 (both of which express functional adenoviral E1) [Fallaux, 1998], 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/innners.php?m=00084, last accessed 13 April 2015).

Assembly of a Viral Particle and Transfection of a Cell Line

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 1×10^4 cells to about 1×10^{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.

Accordingly, in one embodiment the present invention provides a recombinant vector according to the present invention comprising an expression cassette comprising an immunogenic transgene derived from a pathogen. In certain embodiments the pathogen is a respiratory virus. Thus, the present invention provides a recombinant ChAd155-derived adenoviral vector comprising an expression cassette comprising an immunogenic transgene derived from human respiratory syncytial virus (RSV). In one embodiment the recombinant ChAd155-derived adenoviral vector of the present invention comprises an RSV F antigen and RSV M and N antigens. More specifically, the nucleic acid encodes an RSV F Δ TM antigen (fusion (F) protein deleted of the transmembrane and cytoplasmic regions), and RSV M2-1 (transcription anti-termination) and N (nucleocapsid) antigens. In certain embodiments, a recombinant vector comprises a nucleic acid sequence (for example within an expression cassette) which encodes a sequence according to SEQ ID NO: 37. In one embodiment, the recombinant vector consists essentially of a polynucleotide having a sequence according to SEQ IS NO: 11.

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.

- 5 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,
10 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
15 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).

- 20 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
25 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
30 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 metallothioneine (MT) promoter and the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter.
35 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
40 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.

5 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
10 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
15 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);
20 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)),
25 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
30 encoding geneticin, hygromycin 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.

35 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.

40 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.

Immunogenic Transgenes

The recombinant ChAd155 vectors may also be 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.

Accordingly, in one embodiment the present invention provides the use of a recombinant adenovirus according to the present invention in the treatment of disease cause by a pathogen. In one embodiment, such treatment is prophylaxis. In one embodiment, the present invention provides the use of a recombinant adenovirus in the generation of an immune response against a pathogen. In certain embodiments the pathogen is a respiratory virus. Thus, the present invention provides the use of a recombinant ChAd155-derived adenoviral vector comprising an expression cassette comprising an immunogenic transgene derived from human respiratory syncytial virus (RSV) in the treatment or prophylaxis of RSV infection. In one embodiment the recombinant ChAd155-derived adenoviral vector of the present invention comprises an RSV F antigen and RSV M and N antigens. More specifically, the nucleic acid encodes an RSV F Δ TM antigen (fusion (F) protein deleted of the transmembrane and cytoplasmic regions), and RSV M2-1 (transcription anti-termination) and N (nucleocapsid) antigens. Particularly, the transgene encodes an RSV antigen as set out in SEQ ID NO: 37.

In further embodiments, the present invention provides the use of a recombinant adenovirus according to the present invention in the manufacture of a medicament for the generation of an immune response against a pathogen. Thus, the present invention provides the use of a recombinant ChAd155-derived adenoviral vector comprising an expression cassette comprising an immunogenic transgene derived from human respiratory syncytial virus (RSV) in the manufacture of a medicament for the treatment or prophylaxis of RSV infection. More specifically, the transgene encodes an RSV F Δ TM antigen (fusion (F) protein deleted of the transmembrane and cytoplasmic regions), and RSV M2-1 (transcription anti-termination) and N (nucleocapsid) antigens. Particularly, the transgene encodes an RSV antigen as set out in SEQ ID NO: 37, for example, in one embodiment the transgene comprises a polynucleotide of SEQ ID NO: 11.

In one embodiment the present invention provides a method of treatment or prevention of a disease caused by a pathogen, comprising the administration of an effective amount of a recombinant adenovirus according to the present invention, for example a ChAd155-derived adenovirus, comprising an expression cassette comprising an immunogenic transgene derived from the pathogen. In certain embodiments the pathogen is human respiratory syncytial virus (RSV). In one embodiment the present invention provides a method of generating or enhancing an immune response directed against human respiratory syncytial virus (RSV) comprising the administration of a recombinant adenovirus according to the present invention. Particularly, the method of generating or enhancing an immune response comprises the administration of an effective amount of a ChAd155-derived adenovirus comprising a transgene encoding an RSV antigen as set out in SEQ ID NO: 37, for example, in one embodiment the transgene comprises a polynucleotide of SEQ ID NO: 11.

In one embodiment, the present invention provides an immunogenic composition comprising a recombinant adenovirus according to the present invention, for example a ChAd155-derived adenovirus, including an expression cassette comprising an immunogenic transgene derived from a pathogen, for example human respiratory syncytial virus (RSV), and a pharmaceutically acceptable excipient.

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.

In one embodiment an immunogenic composition comprising a recombinant ChAd155-derived adenoviral vector of the present invention comprising an expression cassette containing a transgene encoding an RSV F antigen and RSV M and N antigens. More specifically, the transgene encodes an RSV F Δ TM antigen (fusion (F) protein deleted of the transmembrane and cytoplasmic regions), and RSV M2-1 (transcription anti-termination) and N (nucleocapsid)

antigens. Particularly, the transgene encodes an RSV antigen as set out in SEQ ID NO: 37, for example, in one embodiment the transgene comprises a polynucleotide of SEQ ID NO: 11. 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.

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).

Infection with RSV does not confer full protective immunity. Infection in infancy is followed by symptomatic RSV re-infections which continue throughout adulthood. These re-infections generally go undiagnosed because they usually present as common acute upper respiratory tract infections. In more vulnerable persons (e.g., immunocompromised adults or elderly), re-infections can however also lead to severe disease. Both arms of the immune system (humoral and cellular immunity) are involved in protection from severe disease [Guvenel, 2014].

The humoral immune response is capable of neutralizing the virus and inhibiting viral replication, thereby playing a major role in protection against lower respiratory RSV infection and severe disease [Piedra, 2003]. Passive immunization, in the form of Immunoglobulin G (IgG) RSV-neutralizing monoclonal antibodies (*Synagis*) given prophylactically, has been shown to prevent RSV disease to some extent in premature infants and newborns with bronchopulmonary dysplasia or underlying cardiopulmonary disease [Cardenas, 2005].

T cells are also involved in the control of RSV disease. Lethal RSV infections have been described in patients with low CD8 T cells counts, as in the case of severe combined immunodeficiency, bone marrow and lung transplant recipients [Hertz, 1989]. The histopathology of fatal cases of RSV infection of newborns shows that there is a relative paucity of CD8 T cells in the lung infiltrate [Welliver, 2007]. Moreover, the presence of CD8 T cells producing Interferon-gamma (IFN- γ) has been associated with diminished Th2 responses and reduced eosinophilia in animal models of RSV [Castilow, 2008; Stevens, 2009].

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.

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 (F Δ TM).

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 transgene nucleic acid encodes an RSV F antigen and RSV M and N antigens. More specifically, the nucleic acid encodes an RSV F Δ TM antigen (fusion (F) protein deleted of the transmembrane and cytoplasmic regions), and RSV M2-1 (transcription anti-termination) and N (nucleocapsid) antigens.

Fusion (F) protein deleted of the transmembrane and cytoplasmic regions (F Δ TM)

The RSV F protein is a major surface antigen and mediates viral fusion to target cells. The F protein is an antigen which is highly conserved among RSV subgroups and strains. The F protein is a target for neutralizing antibodies, including the prophylactic RSV-neutralizing monoclonal antibody *Synagis*. Deletion of the transmembrane region and cytoplasmic tail permits secretion of the F Δ TM protein. Neutralizing antibodies including *Synagis*, that recognize this soluble form of the F protein, inhibit RSV infectivity *in vitro* [Magro, 2010].

Nucleocapsid (N) protein

The N protein is an internal (non-exposed) antigen, highly conserved between RSV strains and known to be a source of many T cell epitopes [Townsend, 1984]. The N protein is essential for the replication and transcription of the RSV genome. The primary function of the N protein is to encapsulate the virus genome for the purposes of RNA transcription, replication and packaging and protects it from ribonucleases.

Transcription anti-termination (M2-1) protein

The M2-1 protein is a transcription anti-termination factor that is important for the efficient synthesis of full-length messenger RNAs (mRNAs) as well as for the synthesis of polycistronic readthrough mRNAs, which are characteristic of non-segmented negative-strand RNA viruses. M2-1 is an internal (non-exposed) antigen, which is highly conserved between RSV strains and known to be a source of many T cell epitopes [Townsend, 1984].

In one embodiment, the present invention provides a recombinant ChAd155 vector comprising a transgene encoding an RSV F Δ TM antigen, and RSV M2-1 and N antigens wherein a self-cleavage site is included between the RSV F Δ TM 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 transgene 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 spacer 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-1 β , IL-2, IL-7, IL-12, IL-18, GM-CSF, 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 B1).

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 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 may deliver the same or different therapeutic or immunogenic molecules. 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. These regimens may deliver the same or different therapeutic or immunogenic molecules. 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.

In another embodiment, a therapeutic regimen involves the administration of an immunogenic composition to a pregnant mother and subsequent administration of a further immunogenic composition to the infant after birth, for example 1, 2, 3, 4, 5, 6, 7 or 8 months after birth. Antibodies generated as a result of the maternal immunisation can cross the placenta to provide passive immunisation of the gestational infant. In one embodiment the maternal immunisation is by administration of a recombinant protein antigen and the infant immunisation is by administration of a recombinant adenoviral vector according to the present invention. In another embodiment the maternal immunisation is by administration of a recombinant adenoviral vector according to the present invention and the infant immunisation is by administration of a recombinant protein antigen. In another embodiment both the maternal and infant immunisation is by administration of a recombinant adenoviral vector according to the present invention.

Thus, the present invention provides the use of a recombinant ChAd155-derived adenoviral vector according to the present invention in the generation of an immune response against RSV in an infant, particularly an infant born to a mother immunised against RSV during pregnancy. Accordingly, the present invention provides the use of a recombinant ChAd155-derived adenoviral vector comprising an immunogenic transgene derived from human respiratory syncytial virus (RSV) in the manufacture of a medicament for the generation of an immune response in an infant. Desirably, particularly the infant born to a mother immunised against RSV during her pregnancy. More specifically, the transgene encodes an RSV F Δ TM antigen (fusion (F) protein deleted of the transmembrane and cytoplasmic regions), and RSV M2-1 (transcription anti-termination) and N (nucleocapsid) antigens. Particularly, the transgene encodes an RSV antigen as set out in SEQ ID NO: 37, for example, in one embodiment the transgene comprises a polynucleotide of SEQ ID NO: 11.

A prime-boost regimen may be used. Prime-boost refers to two separate immune responses in the same individual: (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-locally 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 1×10^5 to 1×10^{15} viral particles, such as from 1×10^8 to 1×10^{12} (e.g., 1×10^8 , 2.5×10^8 , 5×10^8 , 1×10^9 , 1.5×10^9 , 2.5×10^9 , 5×10^9 , 1×10^{10} , 1.5×10^{10} , 2.5×10^{10} , 5×10^{10} , 1×10^{11} , 1.5×10^{11} , 2.5×10^{11} , 5×10^{11} , 1×10^{12} particles). Alternatively, a viral vector can be administered at a dose that is typically from 1×10^5 to 1×10^{10} plaque forming units (PFU), such as 1×10^5 PFU, 2.5×10^5 PFU, 5×10^5 PFU, 1×10^6 PFU, 2.5×10^6 PFU, 5×10^6 PFU, 1×10^7 PFU, 2.5×10^7 PFU, 5×10^7 PFU, 1×10^8 PFU, 2.5×10^8 PFU, 5×10^8 PFU, 1×10^9 PFU, 2.5×10^9 PFU, 5×10^9 PFU, or 1×10^{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 μ g 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 μ g, such as between 1 ng and 1 μ g (e.g., 100 ng-1 μ g), or between 1 μ g and 100 μ g, such as 10 ng, 50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 500 ng, 750 ng, or 1 μ g. Effective amounts of a nucleic acid can also include from 1 μ g to 500 μ g, such as between 1 μ g and 200 μ g, such as between 10 and 100 μ g, for example 1 μ g, 2 μ g, 5 μ g, 10 μ g, 20 μ g, 50 μ g, 75 μ g, 100 μ g, 150 μ g, or 200 μ g. Alternatively, an exemplary effective amount of a nucleic acid can be between 100 μ g and 1 mg, such as from 100 μ g to 500 μ g, for example, 100 μ g, 150 μ g, 200 μ g, 250 μ g, 300 μ g, 400 μ g, 500 μ g, 600 μ g, 700 μ g, 800 μ g, 900 μ g 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 μ g to about 50 μ g 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.

2.1: Deletion of E1 region: Construction of BAC/ChAd155 Δ E1 TetO hCMV RpsL-Kana#1375

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 Δ E1/ 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 Δ E1, 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 Δ E1_TetO hCMV RpsL-Kana (#1375) as a template with the following oligonucleotides: 5'-ATTCAAGTGTACAGGCGCGCCAAAGCATGACGCTGTTGATTTGATTC-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 Δ E1, 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 Δ E1_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/Ascl, to obtain the pChAd155 Δ E1, E4_Ad5E4orf6/TetO hCMV RpsL-Kana (#1434) pre-adeno plasmid.

2.2.2: Insertion of RSV Expression Cassette - Construction of pChAd155 Δ E1, E4_Ad5E4orf6/TetO hCMV RSV

The vaccine antigens are computational consensus sequences derived from the alignment of many different subgroup A RSV isolates retrieved from the National Centre for Biotechnology Information (NCBI) database. For each antigen the protein consensus sequence was derived using Multiple Sequence Comparison by Log-Expectation (MUSCLE) version 3.6 by alignment of all non-identical sequences and applying the majority rule. When blasted, the derived consensus sequence of the F protein was found to differ by only one amino acid from a natural annotated variant: embCAA26143.1. The consensus N protein also had only one amino acid different from the annotated variant ID:1494470, while the M2-1 protein was identical to the variant P04545. Finally, each antigenic sequence was codon-optimized for expression in eukaryotic cells, chemically synthesized and assembled. The construct, shown in Figure 14 and SEQ ID NO: 37 contains the aphtovirus Foot and Mouth Disease Virus 2A region (18 amino acids) between the soluble F protein F Δ TM and the other two RSV antigens, which mediates polyprotein processing by a translational effect known as ribosomal skip [Donnelly, 2001]. After transfection into mammalian cells, cleavage occurs and the soluble F protein is detected in the cell culture supernatant. The fusion protein N-M2-1 is instead expressed and detected in the intracellular fraction.

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 Δ E1, 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 Δ E1, 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 Δ E1, E4_Ad5E4orf6/TetO hCMV RpsL-Kana#1390

- 5 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.
- 10 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

- 15 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'-
TTAATAGACACAGTAGCTTAATAGACCCAGTAGTGCAAAGCCCCATTCTAGCTTATAA
CCCCTATTTGTTTATTTTCT-3') (SEQ ID NO: 17) and 1022-RW E4 Del Step1
20 (5'-
ATATATACTCTCTCGGCACTTGGCCTTTTACACTGCGAAGTGTTGGTGCTGGTGCTGCGTT
GAGAGATCTTTATTTGTAACTGTTAATTGTC-3') (SEQ ID NO: 18).

- 25 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).

30

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

- 35 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
40 BAC/ChAd155 (DE1) TetOhCMV - RpsL Kana)#1379, resulting in a final plasmid BAC/ChAd155 (Δ E1, 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 Δ E1, E4_Ad5E4orf6 /TetOhCMV RSV#1393

An RSV transgene was cloned into the BAC/ChAd155 Δ E1, E4_Ad5E4orf6 /TetOhCMV RpsL Kana#1390 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 Ad5E4orf6) TetOhCMV – Amp-LacZ-SacB#1386, resulting in the final plasmid BAC/ChAd155 Δ E1, E4_Ad5E4orf6 / TetO hCMV RSV#1393. 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.

Example 3: Vector production

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

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 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 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 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.

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 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 5'-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 5×10^5 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

Preclinical studies to evaluate the immunogenicity of the vaccine candidate ChAd155-RSV were performed in inbred BALB/c mice (5.2.1). The vaccine efficacy was also evaluated in cotton rats after intranasal (IN) challenge with RSV through measurement of viral load in lower (lung) or upper (nasal tissue) respiratory tract (5.2.2). Finally, the vaccine was tested in young seronegative calves, which is a model that mimics natural RSV infection (5.2.3).

5.2.1 – Inbred Mice

ChAd155-RSV was tested in the BALB/c mouse strain to evaluate its immunological potency. Dose escalation in inbred mice is the standard assay that has enabled the ranking of chimpanzee adenoviral vectors immunological potency in mice with results that have been confirmed consistently across species (non-human primates and humans) [Colloca, 2012].

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 3×10^6 , 10^7 , and 10^8 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 $M2-1_{282-290}$) and corrected for background. Horizontal lines represent the mean number of IFN-gamma SFC/million splenocytes for each dose group. A T cell dose response was observed in ChAd155-RSV immunized mice with all mice responding even at the low 3×10^6 vp dosage. PanAd3-RSV induced comparable responses at the highest dosage, while ChAd155-RSV induced higher responses at the two lower dosages (Figure 13).

In a second study, a group of BALB/c mice received ChAd155-RSV and another group received PanAd3-RSV, IM at a single dose of 5×10^8 vp. The mice ($n=5$ /group) were subsequently bled every two weeks starting from Week 4 post-vaccination up to Week 10, to monitor induction and maintenance of anti F antibodies. Pooled sera from immunized mice were tested in Enzyme-Linked Immunosorbent Assay (ELISA) on coated RSV-F protein. Figure 15 shows RSV F Immunoglobulin G (IgG) titers, measured by ELISA in pooled sera from immunized mice at different time points from vaccination. Pooled sera serial dilutions were plated in RSV-F protein coated ELISA wells, and the binding of specific IgG revealed

using a goat anti-mouse IgG conjugated to Alkaline Phosphatase (AP) and *p*-Nitrophenyl Phosphate (pNPP) substrate. The reaction was allowed to proceed over time and read at 405 nanometres (nm) during fixed time points. Data are expressed as endpoint titers calculated as the dilution of serum giving an optical density (OD)₄₀₅ reading greater than three Standard Deviations (SDs) above the mean of pre-immune sera at a 1:100 dilution. Antibody responses to RSV F protein were induced by ChAd155-RSV and maintained over a period of 10 weeks after a single IM administration of 5×10^8 vp, and antibody titers were 1.5-fold higher at plateau than those induced by PanAd3-RSV (Figure 15).

5.2.2 – Cotton rats

Methodology

Five groups of female, 6-8 weeks old cotton rats (8 rats/group) were immunized by the intramuscular (IM) route with 5×10^8 or 5×10^7 vp of ChAd155-RSV or PanAd3-RSV (see Table 5). A control group was left unvaccinated. Seven weeks after vaccination, the animals were challenged by intranasal (IN) inoculation with a 10^5 pfu standard dose of RSV A (Long strain). Five days after challenge, the animals were sacrificed, the nasal tissue harvested for viral titration, and the lung en bloc collected and bisected for viral titration (left lobes) and histopathology (right lobes, Groups A, D, E only). RSV titers in nasal tissue or lung homogenates collected five days after RSV challenge were determined by a standard plaque assay on permissive cells (HEp-2 cells). FFPE lung sections were stained with Hematoxylin/Eosin. Four parameters of pulmonary inflammation were evaluated: peribronchiolitis (PB), perivascularitis (PV), interstitial pneumonia (IP), and alveolitis (A). Slides were scored blind on a 0-4 severity scale, and values were then converted to a 0-100% histopathology score. The animals were also bled at Day 0 and at the time of challenge, for RSV neutralizing antibody titration by standard plaque assay on permissive cells (Vero cells). Neutralizing antibody titers were determined as the reciprocal of the serum dilutions at which 60% of the virus was neutralized compared to virus control.

Table 5 Dosing scheme of cotton rats

Group	Vaccine	Immunization dose
A	Control	-
B	PanAd3-RSV IM	5×10^8 vp
C	PanAd3-RSV IM	5×10^7 vp
D	ChAd155-RSV IM	5×10^8 vp
E	ChAd155-RSV IM	5×10^7 vp

Immunogenicity and efficacy results

Figure 16 panels A and B show the RSV viral titers from nasal tissue and lung homogenates, respectively, by plaque assay. RSV titers in nasal tissue or lung homogenates collected five days after RSV challenge were determined by a standard plaque assay on permissive cells. Data are expressed as RSV plaque forming units per gram of tissue (pfu/g). intramuscular

ChAd155-RSV at both dosages completely abolished viral replication in the lung, apart from one animal at the lowest dosage. Infection of the upper respiratory tract was also significantly reduced (between 1 and 2 logs lower RSV titers recovered from nasal tissue) in a dose-dependent manner compared to unvaccinated control animals.

It has been previously shown that in cotton rats a serum neutralizing antibody titer of 1:100 or greater confers protection from viral replication in the lung [Prince, 1985]. In this study both vectors administered IM at 5×10^8 vp induced RSV neutralizing antibodies in the range of the protective threshold while titers decreased with a lower vaccine dosage (Figure 16 panel C). Nevertheless, the vaccination prevented viral replication in the lung even when serum antibody levels were below 1:100, suggesting a role for other immune effector mechanisms. RSV neutralizing antibody titers are expressed as the serum dilution reducing plaques by 60% compared to control.

Safety results

Lung histopathology was performed five days post-infection to assess whether vaccination with ChAd155-RSV induced vaccine-enhanced pathology. Four parameters of pulmonary inflammation were evaluated according to the presence of inflammatory cells in different areas of the lung structure: peribronchiolitis (PB, inflammatory cell infiltration around the bronchioles), perivascularitis (PV, inflammatory cell infiltration around the small blood vessels), interstitial pneumonia (IP, inflammatory cell infiltration and thickening of alveolar walls), and alveolitis (A, cells within the alveolar space). Formalin-fixed, paraffin-embedded lung sections were stained with Hematoxylin/Eosin. Slides were scored blind on a 0-4 severity scale, and values were then converted to a 0-100% histopathology score. The dashed line (set at 5%) represents the threshold for significant pathology. Histopathology data for PanAd3-RSV (depicted in grey) derive from a previous study. Among the four parameters, the presence of inflammatory infiltrate in the alveolar walls (interstitial pneumonia [IP]), and more importantly in the alveolar space, (alveolitis [A]), is considered predictive for enhanced disease and lung pathology [Prince, 2001]. The results of the lung histopathology analysis (Figure 16 panel D) showed that IM ChAd155-RSV did not induce significant IP and A pathology scores. The low levels of IP and A observed were consistent with what has been observed during RSV acute infection and secondary RSV re-infection [Boukhvalova, 2013], and were comparable to values observed with PanAd3-RSV in previous studies.

In figure 16, dashed horizontal lines represent limit of detection (LOD) for each assay in panels A, B and C. In panel D The dashed horizontal line (set at 5%) represents the threshold for significant pathology.

5.2.3 – Seronegative calves

Bovine RSV (bRSV) causes respiratory disease in young calves that is very similar to that observed in human infants. In addition, bovine RSV is genetically and antigenically related to

human RSV. The newborn calf bRSV model a relevant animal model for evaluation of safety of human RSV vaccines

Methodology

- 5 Three groups of five colostrum-restricted calves were vaccinated on two occasions, four weeks apart, with 5×10^{10} vp ChAd155-RSV or comparator PanAd3-RSV, as indicated in Table 6.

Table 6 Dosing scheme of newborn calves

Group	Vaccination 1	Dose	Vaccination 2	Dose
A	PanAd3-RSV IM	5×10^{10} vp	PanAd3-RSV IM	5×10^{10} vp
B	ChAd155-RSV IM	5×10^{10} vp	ChAd155-RSV IM	5×10^{10} vp
C	PanAd3-control IM	5×10^{10} vp	PanAd3-control IM	5×10^{10} vp

- 10 Group C was mock-vaccinated with PanAd3 adenovectors containing unrelated antigens. The animals were challenged four weeks after the second vaccination by IN (10 mL) and intratracheal (IT) (10 mL) administration of 10^4 pfu of bRSV Snook strain. Six days after the infection, when the virus replication peaked in the lung and in the nose causing maximal pulmonary pathology, the animals were sacrificed. In this model, due to the IN/IT routes of administration of the challenge inoculum, very few, if any, clinical signs are observed in
15 challenged control animals. Therefore, the effects of vaccination on bRSV challenge were studied by nasopharyngeal excretion of bRSV, analysis of levels of bRSV in lung homogenate, development of gross pneumonic lesions and analysis of leukocytes in broncho-alveolar lavage (BAL). Antibody responses (antibody and neutralizing titers) induced after vaccination and/or challenge were also investigated.

20

Immunogenicity results

Effect of vaccination on induction of bRSV-specific serum IgG

- 25 Kinetics of the mean bRSV-specific serum IgG antibody and human RSV (hRSV) neutralizing titers throughout the course of the vaccination are shown per study group in Figure 17. The graph shows the geometric mean value of each study group. Levels of bRSV-specific IgG were determined using bRSV (Snook strain)-infected fetal calf kidney (FCK) cell lysate as antigen [Taylor, 1995]. A lysate of mock-infected FCK cells was used as a control antigen.

- 30 Two out of five animals in each group receiving an RSV immunogen showed low levels of maternal bRSV-specific antibodies at the start of the vaccination. Nevertheless, all animals responded to the vaccines and reached high levels ($\log_{10}=3-3.5$) of bRSV-specific antibodies after the boost (Figure 17A). All the calves in the control group had detectable bRSV-specific antibodies at the start of the vaccination which declined during the study and were not detectable on the day of the challenge. In conclusion, PanAd3-RSV and ChAd155-RSV
35 displayed similar potency in eliciting antibody responses.

No hRSV neutralizing response could be detected before vaccination. After a single dose, all animals vaccinated with PanAd3-RSV and 2 out of 5 animals vaccinated with ChAd155-RSV had measurable RSV neutralizing antibodies (Figure 17B). A marked boost was observed after the second dose for both vaccines. No further enhancement of the hRSV neutralizing response was observed 1 week after challenge.

Efficacy results

Effect of vaccination on nasopharyngeal excretion of bRSV

Following bRSV challenge, nasopharynx swabs were obtained daily and the bRSV titers within the samples determined. As shown in Figure 18, titers in the control group increased from day 3 to day 6. bRSV replication appeared to be significantly reduced in calves that had received either of the adenovector RSV constructs.

Effect of vaccination on replication of bRSV in the lower respiratory tract.

Six days after bRSV challenge, high titers of bRSV were detected in lung wash cells (LWC) and lung lobe homogenates (RA, RC and LC) of all of the control calves, and from tracheal scrape (TSc) samples in 5 out of 6 of the control calves (Figure 19). In contrast, bRSV was detected at low titers from the TSc samples of 2 and from LWC of 1 of the calves which received ChAd155-RSV, while it was undetectable in all samples from the calves which received PanAd3-RSV.

Effect of vaccination on pulmonary pathology

Six days after bRSV challenge, extensive gross pneumonic consolidation was observed in 6 out of 6 control calves (Figure 20). In contrast, there was little or no gross pneumonic consolidation in the calves that had received either of the adenovector RSV constructs. Figure 21 quantifies the mean proportion $\pm$ SD of lymphocytes (L), macrophages (Mo), polymorphonuclear leukocytes (PMN) and eosinophils (Eo) in BAL, 6 days after bRSV challenge. Approximately 70% of the cells in BAL from control calves were PMN. A lower proportion of PMN was found in BAL from calves which received ChAd155-RSV or PanAd3-RSV, indicating a reduced pulmonary inflammation. Importantly, eosinophils were very few or undetectable in the lungs of calves which received ChAd155-RSV or PanAd3-RSV suggesting the absence of vaccine-induced disease exacerbation.

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 and at least as potent an antibody response against the antigens expressed in animal models. Protective immunity is achieved without signs of vaccine-induced enhanced pulmonary pathology.

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DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 - Polypeptide sequence of ChAd155 fiber

- 30 MKRRTKTSDESNPVYPYDTESGPPSPVFLTPPFVSPDGFQESPPGVLSLNLAEPLVTSHGMLALKMGSGLS
LDDAGNLTSQDITTASPPLKKTCTNLSLETSSPLTVSTSGALTVA AAA PLAVAGTSLTMQSEAPLTVQDAK
LTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPLSTSNGLGIDMQAPIYTTNGKLGLNFGAPLH
VVDLSLNA LTVVTGQGLTINGTALQTRVSGALNYDTSGNLELRAAGGMRVDANGQLILDVAYPFDAQNN
LSLRLGQGGLFVN SAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAINAGDGLQFDSGSDT
35 NPLKTKLGLGLDYDSSRAIIAKLGTGLSFDNTGAITVGNKNDDKLT LWTPDPSPNCRIYSEKDAKFTLVL
TKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLSSNSLDPQYWNRYRKGDLTEGTAYTNAV
GFMPNLTA YPKTQSQTAKSNIVSQVYLN GDKSKPMTLTITLNGTNETGDATVSTYSMSFSWNWNGSNYI
NETFQTNSFTFSYIAQE

- 40 SEQ ID NO: 2 - Polynucleotide sequence encoding ChAd155 fiber

ATGAAGCGCACCAAAACGTCTGACGAGAGCTTCAACCCCGTGTACCCCTATGACACGGAAAGCGGCC
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AAAAACCAAGACCAACCTCAGCCTAGAAACCTCATCCCCCTAACTGTGAGCACCTCAGGCGCCCTC
 ACCGTAGCAGCCGCCGCTCCCCTGGCGGTGGCCGGCACCTCCCTACCATGCAATCAGAGGCCCCC
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 GGCCTTGCAAACATCGGCCCCGCTGACGGCCGCTGACAGCAGCACCTCACAGTCAGTGCCACACCA
 5 CCCCTTAGCACAAGCAATGGCAGCTTGGGTATTGACATGCAAGCCCCCATTTACACCACCAATGGAA
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 10 TTA ACTCTGCCCACA ACTTGGATGT TAACTACAACAGAGGCCTCTACCTGTT CACATCTGGAAATACC
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 15 CCCCTAACTGTAGAATCTATTCAGAGAAAGATGCTAAATTCACACTTGTTTTGACTAAATGCGGCAGT
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 AATACTGGA ACTACAGAAAAGGTGACCTTACAGAGGGCACTGCATATACCAACGCAGTGGGATTTAT
 GCCCAACCTCACAGCATAACCCAAAAACACAGAGCCAACTGCTAAAAGCAACATTGTAAGTCAGGTT
 20 TACTTGAATGGGGACAAATCCAAACCCATGACCCTCACCATTACCCTCAATGGA ACTAATGAAACAG
 GAGATGCCACAGTAAGCACTTACTCCATGTCATTCTCATGGA ACTGGAATGGAAGTAATTACATTAA
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SEQ ID NO: 3 - Polypeptide sequence of ChAd155 penton

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 KFKARVMVSRSHTKEDRVELKYEWVEFELPEGNYSETMTIDLMNNAIVEHYLKVGRQNGVLES DIGVKF
 DTRNFRGLDPTVGLVMPGVYTNEAFHPDIILLPGCGVDFTYSRLSNLLGIRKRQPFQEGFRITYEDLEGG
 NIPALLDVEAYQDSLKENEAGQEDTAPAASAAAEQGEDAADTAAADGA EADPAMVVEAPEQEEDMND
 30 AVRGDTFVTRGEEKQAEAEAAAEKQLAAAAAAAAALAAAEAESEGTKPAKEPVIKPLTEDSKKRSYNLL
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 GVQRVTVDARRRTC PYVYKALGIVSPRVLSSRTF

35 SEQ ID NO: 4 - Polynucleotide sequence encoding ChAd155 penton

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 10 AGCGGCGGCGGCGGCGTGGCCGCGGCGGAGGCTGAGTCTGAGGGGACCAAGCCCGCCAAGGAGCC
 CGTGATTAAGCCCCTGACCGAAGATAGCAAGAAGCGCAGTTACAACCTGCTCAAGGACAGCACCAA
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 TGCAAGACCCCGTGACCTTCCGCTCCACGCGCAGGTCAGCAACTTCCCGGTGGTGGGCGCCGAGCT
 15 GCTGCCCCGTGCACTCCAAGAGCTTCTACAACGACCAGGCCGTCTACTCCAGCTCATCCGCCAGTTCA
 CCTCTCTGACCCACGTGTTCAATCGCTTTCCTGAGAACCAGATTCTGGCGCGCCCCGCCGCCGCCACC
 ATCACCACCGTCAGTGAAAACGTTCTGCTCTCACAGATCACGGGACGCTACCGCTGCGCAACAGCA
 TCGGAGGAGTCCAGCGAGTGACCGTTACTGACGCCAGACGCCGCACCTGCCCCCTACGTTTACAAGGC
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SEQ ID NO: 5 - Polypeptide sequence of ChAd155 hexon

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 30 AGLRYRSMLLGNRGYVPFHIQVPQKFFAIKNLLLLPGSYTYEWNFRKDVNMVLQSSLGNDLRVDGASIKF
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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

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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: 10 - Polynucleotide sequence encoding wild type ChAd155

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

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10 GCCCGCCGGCCCCGCCCAAAACCCGCCTCCCGCCCCGCGCCCCGCGCCGCCATCTCATTATC
ATATTGGCTTCAATCCAAAATAAGGTATATTATTGATGATG

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

15 GGAGTTCGCGTTACATAAATTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCA
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30 TTCTGCGGAGGGATCTCCGTGGGGCGGTGAACGCCGATGATGCCTCTACTAACCATGTTTCATGTTTTT
TTTTTTTTTCTACAGGTCCTGGGTGACGAACAG

SEQ ID NO: 13 - Ad5orf6 primer 1 polynucleotide sequence

ATACGGACTAGTGGAGAAGTACTCGCCTACATG

35

SEQ ID NO: 14 - Ad5orf6 primer 2 polynucleotide sequence

ATACGGAAGATCTAAGACTTCAGGAAATATGACTAC

SEQ ID NO: 15 - BAC/CHAd155 Δ E1_TetO hCMV RpsL-Kana primer 1 polynucleotide sequence

40 ATTCAGTGTACAGGCGCGCCAAAGCATGACGCTGTTGATTTGATTC

SEQ ID NO: 16 - BAC/CHAd155 Δ E1_TetO hCMV RpsL-Kana (#1375) primer 2 polynucleotide sequence

ACTAGGACTAGTTATAAGCTAGAATGGGGCTTTGC

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

TTAATAGACACAGTAGCTTAATAGACCCAGTAGTGCAAAGCCCCATTCTAGCTTATAACCCCTATTG
TTTATTTTCT

5

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

ATATATACTCTCTCGGCACTTGGCCTTTTACACTGCGAAGTGTTGGTGCTGGTGCTGCGTTGAGAGAT
CTTTATTTGTAACTGTAAATTGTC

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

15

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

CAATGGGCGTGGATAGCGGTTTGAC

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

CAGCATGCCTGCTATTGTC

20

SEQ ID NO: 23 - CMVfor primer polynucleotide sequence

CATCTACGTATTAGTCATCGCTATTACCA

SEQ ID NO: 24 - CMVrev primer polynucleotide sequence

GACTTGGAATCCCCGTGAGT

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

ACATCAATGGGCGTGGATAGCGGTT

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**SEQ ID NO: 26 - Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE)
polynucleotide sequence**

TAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTAC
GCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTC
CTCCTTGTATAAATCCTGGTTGCTGTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCG
TGGTGTGCACTGTGTTTGTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTT
TCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAATCATCGCCGCCTGCCTTGCCCGCTG
CTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCGTTGGTGTGTCGGGGAAATCATCGTCCTTTT
CTTGGCTGCTCGCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCTTTCGGCCC
TCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTC
GCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCTCCCCGCCT

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SEQ ID NO: 27 - ChAd3 fiber amino acid sequence

MKRTKTSDESNPVYPYDTESGPPSVPFLTTPPFVSPDGFQESPPGVLSLNLAEPVLTSHGMLALKMGSGLS
 LDDAGNLTSDITTASPPLKKTNTLSLETSSPLTVSTSGALTVAAAAPLAVAGTSLTMQSEAPLTVQDAK
 LTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPINVSSGSLGLDMEDPMYTHDGKLGIRIGGPLR
 VVDSLHTLTVVTGNGLTVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLILNVAYPFDAQNN
 5 LSLRLGQGPLYINTDHNLDLNCNRGLTTTTNTNKKLETKISSGLDYDTNGAVIIKLTGLSFDNTGALT
 GNTGDDKLTWTTDPDPSPCRIHSDKDCKFTLVLTCKGSQILASVAALAVSGNLASITGTVASVTIFLRFD
 QNGVLMENSSLDKQYWNFRNGNSTNAAPYTNAVGFMPNLAAYPKTQSQTAKNIVSQVYLNKDCKSKP
 MTLTITLNGTNESESQVSHYSMSFTWAWESGQYATETFATNSFTFSYIAEQ

10 **SEQ ID NO: 28- PanAd3 fiber amino acid sequence**

MKRAKTSDETNPVYPYDTENGPPSVPFLTTPPFVSPDGFQESPPGVLSRLSEPLVTSHGMLALKMGNGLS
 LDDAGNLTSDVTTVTPLKKTNTLSLQTSAPLTVSSGSLTVAAAAPLAVAGTSLTMQSQAPLTVQDAK
 LGLATQGPLTVSEGKLTQLTSAPLTAADSSTLTVGTPPISVSSGSLGLDMEDPMYTHDGKLGIRIGGPLQ
 VVDSLHTLTVVTGNGITVANALQTKVAGALGYDSSGNLELRAAGGMRINTGGQLILDVAYPFDAQNNL
 15 SLRLGQGPLYVNTNHNLDLNCNRGLTTTTSSNTTKLETKIDSGLDYNANGAIIAKLTGTLTFDNTGAITVG
 NTGDDKLTWTTDPDPSPCRIHADKDKFTLVLTCKGSQILASVAALAVSGNLSSMTGTVSSVTIFLRFDQN
 GVLMESSLDKEYWNFRNGNSTNATPYTNAVGFMPNLSAYPKTQSQTAKNIVSEVYLHGDCKSKPMILT
 ITLNGTNESESQVSHYSMSFTWSWDSGKYATETFATNSFTFSYIAEQ

20 **SEQ ID NO: 29 - ChAd17 fiber amino acid sequence**

MKRTKTSDESNPVYPYDTESGPPSVPFLTTPPFVSPDGFQESPPGVLSLNLAEPVLTSHGMLALKMGSGLS
 LDDAGNLTSDITSTTPPLKKTNTLSLETSSPLTVSTSGALTVAAAAPLAVAGTSLTMQSEAPLAVQDAK
 LTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSSTPPISVSSGSLGLDMEDPMYTHDGKLGIRIGGPLRV
 VDSLHTLTVVTGNGLTVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLILDVAYPFDAQNNL
 25 SLRLGQGPLYVNTDHNLDLNCNRGLTTTTNTNKKLETKISSGLDYDTNGAVIIKLTGLSFDNTGALT
 GNTGDDKLTWTTDPDPSPCRIHSDKDCKFTLVLTCKGSQILASVAALAVSGNLASITGTVASVTIFLRFD
 QNGVLMENSSLDKQYWNFRNGNSTNAAPYTNAVGFMPNLAAYPKTQSQTAKNIVSQVYLNKDCKSKP
 MTLTITLNGTNESESQVSHYSMSFTWAWESGQYATETFATNSFTFSYIAEQ

30 **SEQ ID NO: 30 - ChAd19 fiber amino acid sequence**

MKRTKTSKDSFNPVYPYDTENGPPSVPFLTTPPFVSPDGFQESPPGVLSLNLAEPVLTSHGMLALKMGSGLS
 LDDAGNLTSDVTTTTTPPLKKTNTLSLETSAPLTVSTSGALTLAAAAPLAVAGTSLTMQSEAPLTVQDA
 KLTATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPISVSSGSLGLDMEDPMYTHDGKLGIRIGGPLR
 VVDSLHTLTVVTGNGLIAVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLILDVAYPFDAQNN
 35 LSLRLGQGPLYVNTDHNLDLNCNRGLTTTTNTNKKLETKIGSGLDYDTNGAVIIKLTGTVSFDSTGALS
 VGNTGDDKLTWTTDPDPSPCRIHSDKDCKFTLVLTCKGSQILASVAALAVSGNLASITGTVSSVTIFLRFD
 QNGVLMENSSLDKQYWNFRNGNSTNATPYTNAVGFMPNLAAYPKTQSQTAKNIVSQVYLNKDCKSKP
 MTLTITLNGTNESESQVSHYSMSFTWAWESGQYATETFATNSFTFSYIAEQ

40 **SEQ ID NO: 31 - ChAd24 fiber amino acid sequence**

MKRTKTSDESNPVYPYDTENGPPSVPFLTTPPFVSPDGFQESPPGVLSLNLAEPVLTSHGMLALKMGSGLS
 LDDAGNLTSDVTTTTTPPLKKTNTLSLETSAPLTVSTSGALTLAAAAPLAVAGTSLTMQSEAPLTVQDA
 KLTATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPINVSSGSLGLDMENPMYTHDGKLGIRIGGPL
 RVVDSLHTLTVVTGNGLIAVDNNALQTRVTGALGYDTSGNLQLRAAGGMRIDANGQLILDVAYPFDAQN

NLSRLRGQGPLYVNTDHNLDLNCNRGLTTTTNTKKLETKIGSGLDYDTNGAVIIKLTGTVSFDSTGAL
 SVGNTGDDKLT LWTPDPSPNCRIHSDKDCKFTLVLT KCGSQILASVAALAVSGNLA SITGT VSSVTIFLRF
 DQNGVLMENSSLDKQYWNFRNGNSTNATPYTNAVGFMPNLAAYPKTQSQTAKNNIVSQVYLN GDKSKP
 MILTITLNGTNESETSQVSHYSMSFTWAWESGQYATET FATNSFTFSYIAEQ

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SEQ ID NO: 32 - ChAd11 fiber amino acid sequence

MKRTKTSDESNPVYPYDTENGPPSVPF LTPPFVSPDGFQESPPGVLSLNLAEP LVTSHGMLALKMGSGLS
 LDDAGNLTSQDVTTTTPLKKTKTNLSLET SAPLTVSTSGALTLAAAVPLAVAGTSLTMQSEAPLTVQDA
 KLT LATKGPLTVSEGKLALQTSAPLTAADSSTLTISATPPLSTSNGSLGIDMQAPIYTTNGKLGLNFGAPLH
 10 VVDSL NALT VVTGQGLTINGTALQTRVSGALNYDSSGNLELRAAGGMRVDANGKLILDVAYPFDAQNN
 LSLRLGQGPLFVNSAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAINPGDGLFGSGSDTN
 PLKTKLGLGLE YDSSRAIIAKLTGLSFDNTGAITVGKNDDKLT LWTPDPSPNCRIYSEKDAKFTLVLT
 KCGSQVLASVSVLSVKGSLAPISGTVTSAQIILRFDENGVL LSNSSLDPQYWNYRKGDLTEGTAYTNAVGF
 MPNLTA YPKTQSQTAKSNIVSQVYLN GDKSKP MILTITLNGTNETGDATVSTYSMSFSWNWNGSNYINET
 15 FQTNSFTFSYIAEQ

SEQ ID NO: 33 - ChAd20 fiber amino acid sequence

MKRTKTSDESNPVYPYDTESGPPSVPF LTPPFVSPDGFQESPPGVLSLNLAEP LVTSHGMLALKMGSGLS
 LDDAGNLTSQDITTASPLKKTKTNLSLETSSPLTVSTSGALTVA A APLAVAGTSLTMQSEAPLTVQDAK
 20 LTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPLSTSNGSLGIDMQAPIYTTNGKLGLNFGAPLH
 VVDSL NALT VVTGQGLTINGTALQTRVSGALNYDTSGNLELRAAGGMRVDANGQLILDVAYPFDAQNN
 LSLRLGQGPLFVNSAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAINAGDGLQFDSGSDT
 NPLKTKLGLGLDYDSSRAIIAKLTGLSFDNTGAITVGKNDDKLT LWTPDPSPNCRIYSEKDAKFTLVLT
 TKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVL LSNSSLDPQYWNYRKGDLTEGTAYTNAV
 25 GFMPNLTA YPKTQSQTAKSNIVSQVYLN GDKSKP MTLTITLNGTNETGDATVSTYSMSFSWNWNGSNYI
 NETFQTNSFTFSYIAEQ

SEQ ID NO: 34 - ChAd31 fiber amino acid sequence

MKRTKTSDESNPVYPYDTESGPPSVPF LTPPFVSPDGFQESPPGVLSLNLAEP LVTSHGMLALKMGSGLS
 30 LDDAGNLTSQDITTASPLKKTKTNLSLETSSPLTVSTSGALTVA A APLAVAGTSLTMQSEAPLTVQDAK
 LTLATKGPLTVSEGKLALQTSAPLTAADSSTLTVSATPPLSTSNGSLGIDMQAPIYTTNGKLGLNFGAPLH
 VVDSL NALT VVTGQGLTINGTALQTRVSGALNYDTSGNLELRAAGGMRVDANGQLILDVAYPFDAQNN
 LSLRLGQGPLFVNSAHNLDVNYNRGLYLFTSGNTKKLEVNIKTAKGLIYDDTAIAINAGDGLQFDSGSDT
 NPLKTKLGLGLDYDSSRAIIAKLTGLSFDNTGAITVGKNDDKLT LWTPDPSPNCRIYSEKDAKFTLVLT
 35 TKCGSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVL LSNSSLDPQYWNYRKGDLTEGTAYTNAV
 GFMPNLTA YPKTQSQTAKSNIVSQVYLN GDKSKP MTLTITLNGTNETGDATVSTYSMSFSWNWNGSNYI
 NETFQTNSFTFSYIAEQ

SEQ ID NO: 35 - PanAd1 fiber amino acid sequence

MKRAKTSDETFNPVYPYDTENGPPSVPF LTPPFVSPDGFQESPPGVLSRLSEPLVTSHGMLALKMGNGLS
 40 LDDAGNLTSQDVTTVTPPLKKTKTNLSLQTSAPLTVSSGSLTVAAAAPLAVAGTSLTMQSQA PLTVQDAK
 LGLATQGPLTVSEGKLT LQTSAPLTAADSSTLTVSATPPLSTSNGSLSIDMQAPIYTTNGKLALNIGAPLHV
 VDTL NALT VVTGQGLTINGRALQTRVTGALS YDTEGNIQLQAGGMRIDNNGQLILNVAYPFDAQNNLS
 LRLGQGPLIVNSAHNLDLNLNRGLYLFTSGNTKKLEVNIKTAKGLFYDGT AIAINAGDGLQFGSGSDTNPL

QTKLGLGLEYDSNKAITKLGTGLSFDNTGAITVGKNDDKLTWTTDPSPNCRINSEKDAKLTTLVLTKC
 GSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLSSNSLDPQYWNYRKGDESTEGTAYTNAVGF
 PNLTAYPKTSQTAKSNIQSQVYLNGDKTKPMTLTITLNGTNETGDATVSTYSMSFSWNWNGSNYINDTF
 QTNSFTFSYIAQE

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SEQ ID NO: 36 - PanAd2 fiber amino acid sequence

MKRAKTSDETFNPVYPYDTENGPPSVFPFLTTPPFVSPDGFQESPPGVLSLRLSEPLVTSHGMLALKMGNGLS
 LDDAGNLTSQDVTTVTPLKKTKTNLSLQTSAPLTVSSGSLTVAAPLAVAGTSLTMQSQAPLTVQDAK
 LGLATQGPLTVSEGKLTQLTSAPLTAADSSTLTVSATPPLSTSNGSLSIDMQAPIYTTNGKLALNIGAPLHV
 10 VDTLNALTAVVTGQGLTINGRALQTRVTGALSVDTEGNIQLQAGGMRIDNNGQLILNVAYPFDAQNLS
 LRLGQGPLIVNSAHNLDLNLNRGLYLFTSGNTKKLEVNIKTAKGLFYDGTAINAGDGLQFGSGSDTNPL
 QTKLGLGLEYDSNKAITKLGTGLSFDNTGAITVGKNDDKLTWTTDPSPNCRINSEKDAKLTTLVLTKC
 GSQVLASVSVLSVKGSLAPISGTVTSAQIVLRFDENGVLSSNSLDPQYWNYRKGDESTEGTAYTNAVGF
 PNLTAYPKTSQTAKSNIQSQVYLNGDKTKPMTLTITLNGTNETGDATVSTYSMSFSWNWNGSNYINDTF
 15 QTNSFTFSYIAQE

SEQ ID NO: 37 - RSV F^{ATM}-N-M2-1 amino acid sequence

MELLILKANAITTILTAFTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNGLD
 AKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNVTLSSKKRRRFLGFL
 20 GVGSAIASGVAVSKVLHLEGEVNIKSALLSTNKAVVSLNNGVSVLTSKVLDLKNYIDKQLPIVKNQSCS
 ISNIETVIEFQQKNRLLLEITREFSVNAGVTTTPVSTYMLTNSSELLSLNDMPITNDQKKLMSNNVQIVRQQSY
 SIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAET
 CKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGGTKCTASKN
 RGIKTFNNGCDYVSNKGVDTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN
 25 QSLAFIRKSDELLHNVNAGKSTTNKRRAVPVKQTLNFDLLKLAGDVESNPGPMALS KVKLNDTLNKDQL
 LSSSKYTIQRSTGDSIDTPNYDVQKHINKLCGMLLITEDANHKFTGLIGMLYAMSRLGREDTIKILRDAGY
 HVKANGVDVTTHRQDINGKEMKFEVLTLASLTTEIQINIEIESRKSYYKKMLKEMGEVAPEYRHDS PDCGM
 IILCIAALVITKLAAGDRSGLTAVIRRANNVLKNEMKRYKGLLPKDIANS
 FYEVFEKYPHFIDVFVHFGLAQSSSTRGGS RVEGIFAGLFMNAYGAGQVMLRWGVLA KSVKNIMLGHASV
 30 QAEME QVVEVYEAQKL GGEAGFYHILNNPKASLLSLTQFPHFSSVVLGNAAGLGIMGEYRGTPRNQDL
 YDAAKAYAEQLKENGVINYSVLDLTAEELEAIKHQLNPKDNDVELGGGGSGGGGMSRRNPCKFEIRGHC
 LNGKRCHFHSNYFEWPPHALLVRQNFMLNRILKSMDKSIDTLSEISGAAELDRTEEYALGVVGVLESYIGS
 INNITKQSACVAMSKLLTELNSDDIKLRDNEELNSPKIRVYNTVISYIESNRKNNKQTHLLKRLPADVLK
 KTIKNTLDIHK SITINNPKESTVSDTNDHAKNNDTT

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SEQ ID NO: 38 - HIV Gag polynucleotide sequence

ATGGGTGCTAGGGCTTCTGTGCTGTCTGGTGGTGAGCTGGACAAGTGGGAGAAGATCAGGCTGAGGC
 CTGGTGGCAAGAAGAAGTACAAGCTAAAGCACATTGTGTGGGCCTCCAGGGAGCTGGAGAGGTTTG
 CTGTGAACCCTGGCCTGCTGGAGACCTCTGAGGGGTGCAGGCAGATCCTGGGCCAGCTCCAGCCCTC
 40 CCTGCAAACAGGCTCTGAGGAGCTGAGGTCCCTGTACAACACAGTGGCTACCCTGTACTGTGTGCAC
 CAGAAGATTGATGTGAAGGACACCAAGGAGGCCCTGGAGAAGATTGAGGAGGAGCAGAACAAGTCC
 AAGAAGAAGGCCAGCAGGCTGCTGCTGGCACAGGCAACTCCAGCCAGGTGTCCAGAACTACCCC
 ATTGTGCAGAACCTCCAGGGCCAGATGGTGCACAGGCCATCTCCCCCGGACCCTGAATGCCTGGG
 TGAAGTGGTGGAGGAGAGGCCCTTCTCCCTGAGGTGATCCCCATGTTCTCTGCCCTGTCTGAGGGTG
 45 CCACCCCCAGGACCTGAACACCATGCTGAACACAGTGGGGGCCATCAGGCTGCCATGCAGATGCT
 GAAGGAGACCATCAATGAGGAGGCTGCTGAGTGGGACAGGCTGCATCCTGTGCACGCTGGCCCCATT
 GCCCCCGGCCAGATGAGGGAGCCCAGGGGCTCTGACATTGCTGGCACCACTCCACCCTCCAGGAGC
 AGATTGGCTGGATGACCAACAACCCCCCATCCCTGTGGGGGAAATCTACAAGAGGTGGATCATCCT

GGGCCTGAACAAGATTGTGAGGATGTACTCCCCACCTCCATCCTGGACATCAGGCAGGGCCCCAAG
GAGCCCTTCAGGGACTATGTGGACAGGTTCTACAAGACCCTGAGGGCTGAGCAGGCCTCCAGGAGG
TGAAGAACTGGATGACAGAGACCCTGCTGGTGCAGAATGCCAACCCCTGACTGCAAGACCATCCTGAA
GGCCCTGGGCCCTGCTGCCACCCTGGAGGAGATGATGACAGCCTGCCAGGGGGTGGGGGGCCCTGGT
5 CACAAGGCCAGGGTGCTGGCTGAGGCCATGTCCCAGGTGACCAACTCCGCCACCATCATGATGCAGA
GGGGCAACTTCAGGAACCAGAGGAAGACAGTGAAGTGCTTCAACTGTGGCAAGGTGGGCCACATTG
CCAAGAACTGTAGGGCCCCCAGGAAGAAGGGCTGCTGGAAGTGTGGCAAGGAGGGCCACCAGATGA
AGGACTGCAATGAGAGGCAGGCCAACTTCCTGGGCAAAATCTGGCCCTCCCACAAGGGCAGGCCTG
GCAACTTCCTCCAGTCCAGGCCTGAGCCCACAGCCCCTCCCGAGGAGTCCTTCAGGTTTGGGGAGGA
10 GAAGACCACCCCCAGCCAGAAGCAGGAGCCCATTGACAAGGAGCTGTACCCCTGGCCTCCCTGAG
GTCCCTGTTTGGCAACGACCCCTCCTCCCAGTAA

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CLAIMS

1. A recombinant vector comprising a polynucleotide selected from the group consisting of:
- 5 (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
- 10 (c) a polynucleotide which encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 3.
- 15 2. 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,
- 20 (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,
- 25 (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
- 30 (f) a polypeptide having the amino acid sequence according to SEQ ID NO: 3.
3. 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,
- 35 (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,
- 40 (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 recombinant vector according to claim 1, and

(h) a recombinant adenovirus according to claim 2;

and a pharmaceutically acceptable excipient.

4. The recombinant vector, recombinant adenovirus or composition according to any one of claims 1 to 3, wherein the polynucleotide encodes a polypeptide having the amino acid sequence according to SEQ ID NO: 1.

5. The recombinant vector, recombinant adenovirus or composition according to claim 4, wherein the polynucleotide has a sequence according to SEQ ID NO: 2.

6. The recombinant vector, recombinant adenovirus or composition according to any one of claims 1-3, wherein the polynucleotide 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, such as at least 89.0% identical, such as at least 99.0% identical, such as at least 99.6% identical over its entire length to the amino acid sequence of SEQ ID NO: 1.

7. The recombinant vector, recombinant adenovirus or composition according to any preceding claim, comprising 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,

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.

8. The recombinant vector, recombinant adenovirus or composition according to claim 7, wherein 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 70.0%, such as at least 90.0%, such as at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 3.

9. The recombinant vector, recombinant adenovirus or composition according to any claim 7, wherein 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 70.0%, such as at least 90.0%, such as at least 99% identical over its entire length to the amino acid sequence of SEQ ID NO: 5.

- 5 10. The recombinant vector, recombinant adenovirus or composition according to any preceding claim, wherein the polynucleotide comprises at least one of the following:
 - (a) an adenoviral 5'-end, preferably an adenoviral 5' inverted terminal repeat;
 - (b) an adenoviral E1A region, or a fragment thereof selected from among the E1A_280R and E1A_243R regions;
 - 10 (c) an adenoviral E1B 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_Illa protein;
 - 15 (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, L2_pVII, L2_V, and L2_pX protein;
 - 20 (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 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;
 - 25 (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;
 - 30 (k) an adenoviral L5 region, or a fragment thereof said fragment encoding the L5_fiber fiber protein;
 - (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;
 - 35 (m) an adenoviral 3'-end, preferably an adenoviral 3' inverted terminal repeat; and/or
 - (n) an adenoviral VAI or VAIL RNA region, preferably an adenoviral VAI or VAIL RNA region from an adenovirus other than ChAd155, more preferably from Ad5.
- 40 11. The recombinant vector, recombinant adenovirus or composition according to any preceding claim, wherein the polynucleotide comprises an adenoviral VAI or VAIL RNA region.

12. The recombinant vector, recombinant adenovirus or composition according to claim 11, wherein the VAI or VAII RNA region is from Ad5.
- 5 13. The recombinant vector, recombinant adenovirus or composition according to any one of claims 1-9, wherein the polynucleotide comprises or consists of a polynucleotide which is at least 98.6% identical over its entire length to a reference sequence that consists essentially of SEQ ID NO: 7, 8 or 9.
- 10 14. The recombinant vector, recombinant adenovirus or composition according to claim 13, wherein the polynucleotide comprises or consists of a polynucleotide which is identical over its entire length to the reference sequence.
- 15 15. The recombinant vector, recombinant adenovirus or composition according to any one of claims 1 to 9, wherein the polynucleotide 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.
- 20 16. The recombinant vector, recombinant adenovirus or composition according to claim 15, wherein the polynucleotide lacks at least one gene of a genomic region selected from the group consisting of E1A, and/or E1B.
17. The recombinant adenovirus according to any one of claims 2 and 4 to 16, wherein the recombinant adenovirus is replication-competent.
- 25 18. The recombinant adenovirus according to any one of claims 2 and 4 to 16, wherein the recombinant adenovirus is replication-incompetent.
- 30 19. The recombinant adenovirus according to any one of claims 2 and 4 to 16, wherein the recombinant adenovirus comprises a nucleic acid sequence encoding a heterologous protein, wherein the nucleic acid sequence is operatively linked to one or more sequences which direct expression of said heterologous protein in a host cell.
- 35 20. The recombinant adenovirus according to claim 19, wherein the heterologous protein is an antigenic protein or a fragment thereof.
21. The recombinant adenovirus according to claim 20, wherein the heterologous protein is derived from a virus.
- 40 22. The recombinant adenovirus according to any one of claims 19-21, wherein the heterologous protein is selected from the fusion protein (F), the attachment protein (G), the matrix protein (M2) and the nucleoprotein (N) of respiratory syncytial virus (RSV).

23. The recombinant adenovirus according to claim 19 wherein the nucleic acid sequence encoding a heterologous protein encodes an RSV F antigen and RSV M and N antigens.
- 5 24. The recombinant adenovirus according to claim 23 wherein the nucleic acid sequence encoding a heterologous protein encodes an RSV F Δ TM antigen, an RSV M2-1 antigen and an N antigen.
- 10 25. The recombinant adenovirus according to claim 23 or claim 24 wherein the nucleic acid sequence encoding a heterologous protein encodes a sequence according to SEQ ID NO: 37.
- 15 26. The recombinant adenovirus according to any one of claims 2 and 4 to 25, wherein the adenovirus is capable of infecting a mammalian cell.
- 20 27. The composition according to any one of claims 3 to 16, comprising an adjuvant selected from the list consisting of: 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-1 β , IL-2, IL-7, IL-12, IL-18, GM-CSF, 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").
- 25 28. The composition according to claim 34, wherein the adjuvant is a 3D-MPL and/or QS21.
- 30 29. The recombinant vector according to any one of claims 1 and 4 to 16, wherein the recombinant vector comprises a nucleic acid sequence encoding a heterologous protein, wherein the nucleic acid sequence is operatively linked to one or more sequences which direct expression of said heterologous protein in a host cell.
- 35 30. The recombinant vector according to claim 29, wherein the heterologous protein is selected from the fusion protein (F), the attachment protein (G), the matrix protein (M2) and the nucleoprotein (N) of respiratory syncytial virus (RSV).
- 40 31. The recombinant vector according to claim 29 wherein the nucleic acid sequence encoding a heterologous protein encodes an RSV F Δ TM antigen, an RSV M2-1 antigen and an N antigen.

32. The recombinant vector according to claim 31 wherein the nucleic acid sequence encoding a heterologous protein encodes a sequence according to SEQ ID NO: 37.
- 5 33. A recombinant vector, recombinant adenovirus or composition according to any preceding claim, for use as a medicament.
34. The recombinant vector, recombinant adenovirus or composition for use according to claim 33, for use in the treatment or prophylaxis of respiratory syncytial virus (RSV) infection.
- 10 35. A recombinant vector, recombinant adenovirus or composition for use according to claim 33, for use as a vaccine.
- 15 36. The use of a recombinant vector, recombinant adenovirus or composition according to any one of claims 1 to 32 for the therapy or prophylaxis of a disease.
37. The use of a recombinant vector, recombinant adenovirus or composition according to claim 36, wherein the disease is caused by RSV infection.
- 20 38. The use of a recombinant vector, recombinant adenovirus or composition according to any one of claims 1 to 32 in the manufacture of a medicament for the therapy or prophylaxis of a disease.
- 25 39. The use of a recombinant vector, recombinant adenovirus or composition in the manufacture of a medicament according to claim 38, wherein the disease is caused by RSV infection.
- 30 40. A method of inducing an immune response in a subject comprising administering the recombinant vector, recombinant adenovirus or composition according to any one of claims 1 to 32 to the subject.
41. A method according to claim 40 wherein the immune response is directed against respiratory syncytial virus (RSV).
- 35 42. An isolated polynucleotide comprising or consisting of the sequence according to SEQ ID NO: 11.

Figure 1A

ChAd3	(1)	MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRAKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRTKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRAKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE (1) MKRAKTSDESEFNPVYPYDTESGPPSPVPFLTPPFVSPDGFQESPPGVLSLNLAEPVTSHGMLALKMGSGLSLDDAGNLTSDI TTASPPPLKKTKTNLSLE
PanAd3	(101)	TSSPLTVSTSGALTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSLTIVSATPPINVSSGSLGIDMEDPMYTH TSAPLTVS-SGSLTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSLTIVGTPPLSVSSGSLGIDMEDPMYTH TSSPLTVSTSGALTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSLTIVSSTPPISVSSGSLGIDMEDPMYTH TSAPLTVSTSGALTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSLTIVSATPPISVSSGSLGIDMEDPMYTH TSAPLTVSTSGALTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSLTIVSATPPINVSSGSLGIDMENPNYTH TSSPLTVSTSGALTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSLTIVSATPPPLSTNSGSLGIDMOAPIYTH TSAPLTVSTSGALTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSLTIVSATPPPLSTNSGSLGIDMOAPIYTH TSSPLTVSTSGALTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLTLATKGPLTVSEGKLALQTSAPLTAADSSLTIVSATPPPLSTNSGSLGIDMOAPIYTH TSAPLTVS-SGSLTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSLTIVSATPPPLSTNSGSLGIDMOAPIYTH TSAPLTVS-SGSLTVAAAAA PLAVAGTSLTMQSEAPLTVQDAKLGLATQGPLTVSEGKLTLQTSAPLTAADSSLTIVSATPPPLSTNSGSLGIDMOAPIYTH

Figure 1B

ChAd3 (201)	DGKLGIRIGGPLRVVDSLHTLTIVVTGNGGLTV	DNNALQTRVTGALGYDTSNQLRAAGGMRIDANGQLILNVAYPFDQAQNLSRLGGGPLYINIDHNLD
PanAd3 (200)	DGKLGIRIGGPLQVVDLSLHTLTIVVTGNGGLTV	ANNALQTRVAGALGYDSSGNIELRAAGGMRINTGGQLILDVAYPFDQAQNLSRLGGGPLYVNNHNLD
ChAd17 (201)	DGKLGIRIGGPLRVVDSLHTLTIVVTGNGGLTV	DNNALQTRVTGALGYDTSNQLRAAGGMRIDANGQLILDVAYPFDQAQNLSRLGGGPLYVNIIDHNLD
ChAd19 (201)	DGKLGIRIGGPLRVVDSLHTLTIVVTGNGGLTV	DNNALQTRVTGALGYDTSNQLRAAGGMRIDANGQLILDVAYPFDQAQNLSRLGGGPLYVNIIDHNLD
ChAd24 (201)	DGKLGIRIGGPLRVVDSLHTLTIVVTGNGGLTV	DNNALQTRVTGALGYDTSNQLRAAGGMRIDANGQLILDVAYPFDQAQNLSRLGGGPLYVNIIDHNLD
ChAd155 (201)	NGKLGINFAGPLHVVDLSINALTVVTGCGGLTINGTALQTRVSGALNYDTSNQLRAAGGMRVDANGQLILDVAYPFDQAQNLSRLGGGPLYVNSAHNLD	
ChAd11 (201)	NGKLGINFAGPLHVVDLSINALTVVTGCGGLTINGTALQTRVSGALNYDSSGNIELRAAGGMRVDANGKLLILDVAYPFDQAQNLSRLGGGPLYVNSAHNLD	
ChAd20 (201)	NGKLGINFAGPLHVVDLSINALTVVTGCGGLTINGTALQTRVSGALNYDTSNQLRAAGGMRVDANGQLILDVAYPFDQAQNLSRLGGGPLYVNSAHNLD	
ChAd31 (201)	NGKLGINFAGPLHVVDLSINALTVVTGCGGLTINGTALQTRVSGALNYDTSNQLRAAGGMRVDANGQLILDVAYPFDQAQNLSRLGGGPLYVNSAHNLD	
PanAd 1 (200)	NGKLALNIGAPLHVVDLINALTVVTGCGGLTINGRALQTRVTGALSYDTEGNIQLQAGGGMRI	DNNQQLILNVAYPFDQAQNLSRLGGGPLYVNSAHNLD
PanAd2 (200)	NGKLALNIGAPLHVVDLINALTVVTGCGGLTINGRALQTRVTGALSYDTEGNIQLQAGGGMRI	DNNQQLILNVAYPFDQAQNLSRLGGGPLYVNSAHNLD
ChAd3 (301)	LNCNRGLTTTTNNNTKKLET	-----KISSGLDYDINGAVIIKLGTLSEFNTGALTVGNTGDDKLTLTWT
PanAd3 (300)	LNCNRGLTTTTSNNTKKLET	-----KIDSGLDYNANGAIIAKLGTGLFDNTGALTVGNTGDDKLTLTWT
ChAd17 (301)	LNCNRGLTTTTNNNTKKLET	-----KISSGLDYDINGAVIIKLGTLSEFNTGALTVGNTGDDKLTLTWT
ChAd19 (301)	LNCNRGLTTTTNNNTKKLET	-----KISSGLDYDINGAVIIKLGTLSEFNTGALTVGNTGDDKLTLTWT
ChAd24 (301)	LNCNRGLTTTTNNNTKKLET	-----KISSGLDYDINGAVIIKLGTLSEFNTGALTVGNTGDDKLTLTWT
ChAd155 (301)	MYNRRGLYLFSTSGNTKKLEVNIIKTAKGLIYDDTAIAINAGDGLQFDGSDTNPLKTKLGLGLDYDSSRAIIAKLGTGLSEFNTGALTVGNKNDKLTLTWT	
ChAd11 (301)	MYNRRGLYLFSTSGNTKKLEVNIIKTAKGLIYDDTAIAINAGDGLQFDGSDTNPLKTKLGLGLDYDSSRAIIAKLGTGLSEFNTGALTVGNKNDKLTLTWT	
ChAd20 (301)	MYNRRGLYLFSTSGNTKKLEVNIIKTAKGLIYDDTAIAINAGDGLQFDGSDTNPLKTKLGLGLDYDSSRAIIAKLGTGLSEFNTGALTVGNKNDKLTLTWT	
ChAd31 (301)	MYNRRGLYLFSTSGNTKKLEVNIIKTAKGLIYDDTAIAINAGDGLQFDGSDTNPLKTKLGLGLDYDSSRAIIAKLGTGLSEFNTGALTVGNKNDKLTLTWT	
PanAd1 (300)	LNLNRGLYLFSTSGNTKKLEVNIIKTAKGLFYDGTATAINAGDGLQFDGSDTNPLQTKLGLGLFYDSNKAIIITKLGTLSEFNTGALTVGNKNDKLTLTWT	
PanAd2 (300)	LNLNRGLYLFSTSGNTKKLEVNIIKTAKGLFYDGTATAINAGDGLQFDGSDTNPLQTKLGLGLFYDSNKAIIITKLGTLSEFNTGALTVGNKNDKLTLTWT	

Figure 1C

ChAd3 (365)	TPDPSPNCRIHSDKDKCKFTLVLTCKGSQLASVAALAVSGNLASIIIGTVASVTIFLRFQNGVLMENSSLDQYWNFRNGNSTNAAPYTNNAVGFMPNLAA
PanAd3 (364)	TPDPSPNCRIHADKDKCKFTLVLTCKGSQLASVAALAVSGNLSSMIGTVSSVTIFLRFQNGVLMENSSLDKEYWNFRNGNSTNAIPYTNNAVGFMPNLAA
ChAd17 (365)	TPDPSPNCRIHSDKDKCKFTLVLTCKGSQLASVAALAVSGNLASIIIGTVASVTIFLRFQNGVLMENSSLDQYWNFRNGNSTNAAPYTNNAVGFMPNLAA
ChAd19 (365)	TPDPSPNCRIHSDKDKCKFTLVLTCKGSQLASVAALAVSGNLASIIIGTVSSVTIFLRFQNGVLMENSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
ChAd24 (365)	TPDPSPNCRIHSDKDKCKFTLVLTCKGSQLASVAALAVSGNLASIIIGTVSSVTIFLRFQNGVLMENSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
ChAd155 (401)	TPDPSPNCRIYSEKDAKFTLVLTCKGSQLASVMSLVKGS LAPISGTVLSAQIIVLRFDENGVLLSNSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
ChAd11 (401)	TPDPSPNCRIYSEKDAKFTLVLTCKGSQLASVMSLVKGS LAPISGTVLSAQIILRFDENGVLLSNSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
ChAd20 (401)	TPDPSPNCRIYSEKDAKFTLVLTCKGSQLASVMSLVKGS LAPISGTVLSAQIILRFDENGVLLSNSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
ChAd31 (401)	TPDPSPNCRIYSEKDAKFTLVLTCKGSQLASVMSLVKGS LAPISGTVLSAQIILRFDENGVLLSNSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
PanAd1 (400)	TPDPSPNCRIINSEKDAKFTLVLTCKGSQLASVMSLVKGS LAPISGTVLSAQIILRFDENGVLLSNSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
PanAd2 (400)	TPDPSPNCRIINSEKDAKFTLVLTCKGSQLASVMSLVKGS LAPISGTVLSAQIILRFDENGVLLSNSSLDQYWNFRNGNSTNAIPYTNNAVGFMPNLAA
ChAd3 (465)	YPKTSQTAKNNIVSQVYVYLVNGDKSKPMTLITITLNGTNESETSQVSHYSMSFTWAWESGQYATETTFATNSFTFSYIAEQ
PanAd3 (464)	YPKTSQTAKNNIVSEVYLVHGDGSKPMTLITITLNGTNESETSQVSHYSMSFTWSDSGKYATETTFATNSFTFSYIAEQ
ChAd17 (465)	YPKTSQTAKNNIVSQVYVYLVNGDKSKPMTLITITLNGTNESETSQVSHYSMSFTWAWESGQYATETTFATNSFTFSYIAEQ
ChAd19 (465)	YPKTSQTAKNNIVSQVYVYLVNGDKSKPMTLITITLNGTNESETSQVSHYSMSFTWAWESGQYATETTFATNSFTFSYIAEQ
ChAd24 (465)	YPKTSQTAKNNIVSQVYVYLVNGDKSKPMTLITITLNGTNESETSQVSHYSMSFTWAWESGQYATETTFATNSFTFSYIAEQ
ChAd155 (501)	YPKTSQTAKSNIVSQVYVYLVNGDKSKPMTLITITLNGTNETG-DATVSTYSMSFSWNWNGSNYINETFQTNSTFTFSYIAEQ
ChAd11 (501)	YPKTSQTAKSNIVSQVYVYLVNGDKSKPMTLITITLNGTNETG-DATVSTYSMSFSWNWNGSNYINETFQTNSTFTFSYIAEQ
ChAd20 (501)	YPKTSQTAKSNIVSQVYVYLVNGDKSKPMTLITITLNGTNETG-DATVSTYSMSFSWNWNGSNYINETFQTNSTFTFSYIAEQ
ChAd31 (501)	YPKTSQTAKSNIVSQVYVYLVNGDKSKPMTLITITLNGTNETG-DATVSTYSMSFSWNWNGSNYINETFQTNSTFTFSYIAEQ
PanAd1 (500)	YPKTSQTAKSNIVSQVYVYLVNGDKSKPMTLITITLNGTNETG-DATVSTYSMSFSWNWNGSNYINETFQTNSTFTFSYIAEQ
PanAd2 (500)	YPKTSQTAKSNIVSQVYVYLVNGDKSKPMTLITITLNGTNETG-DATVSTYSMSFSWNWNGSNYINETFQTNSTFTFSYIAEQ

Figure 2

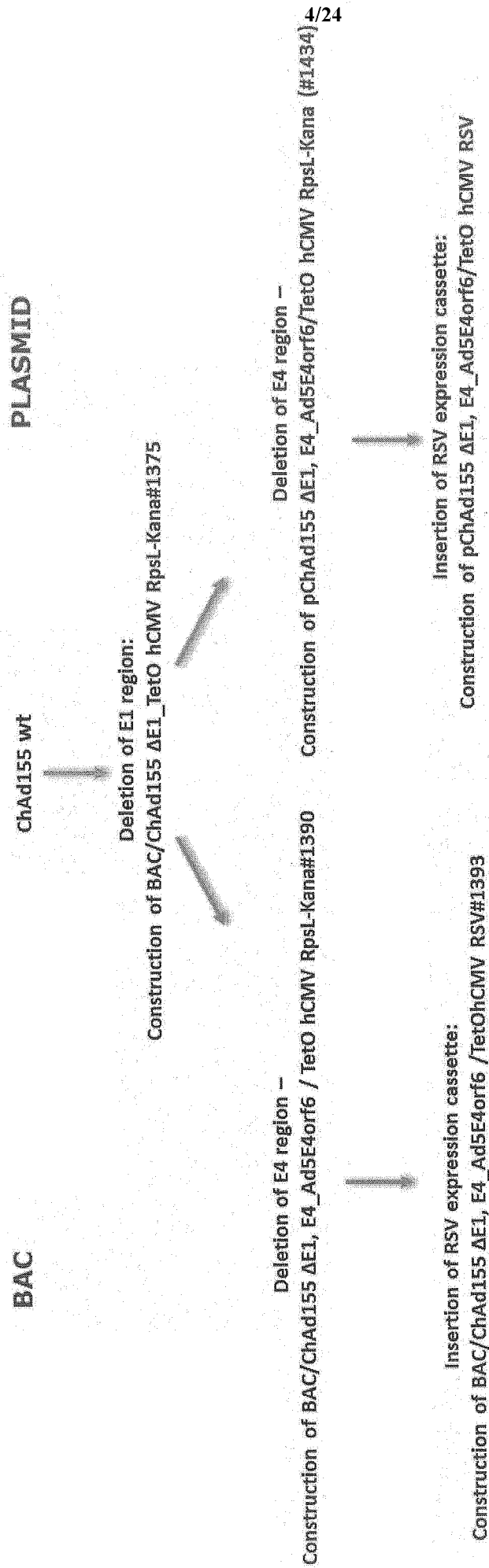


Figure 3

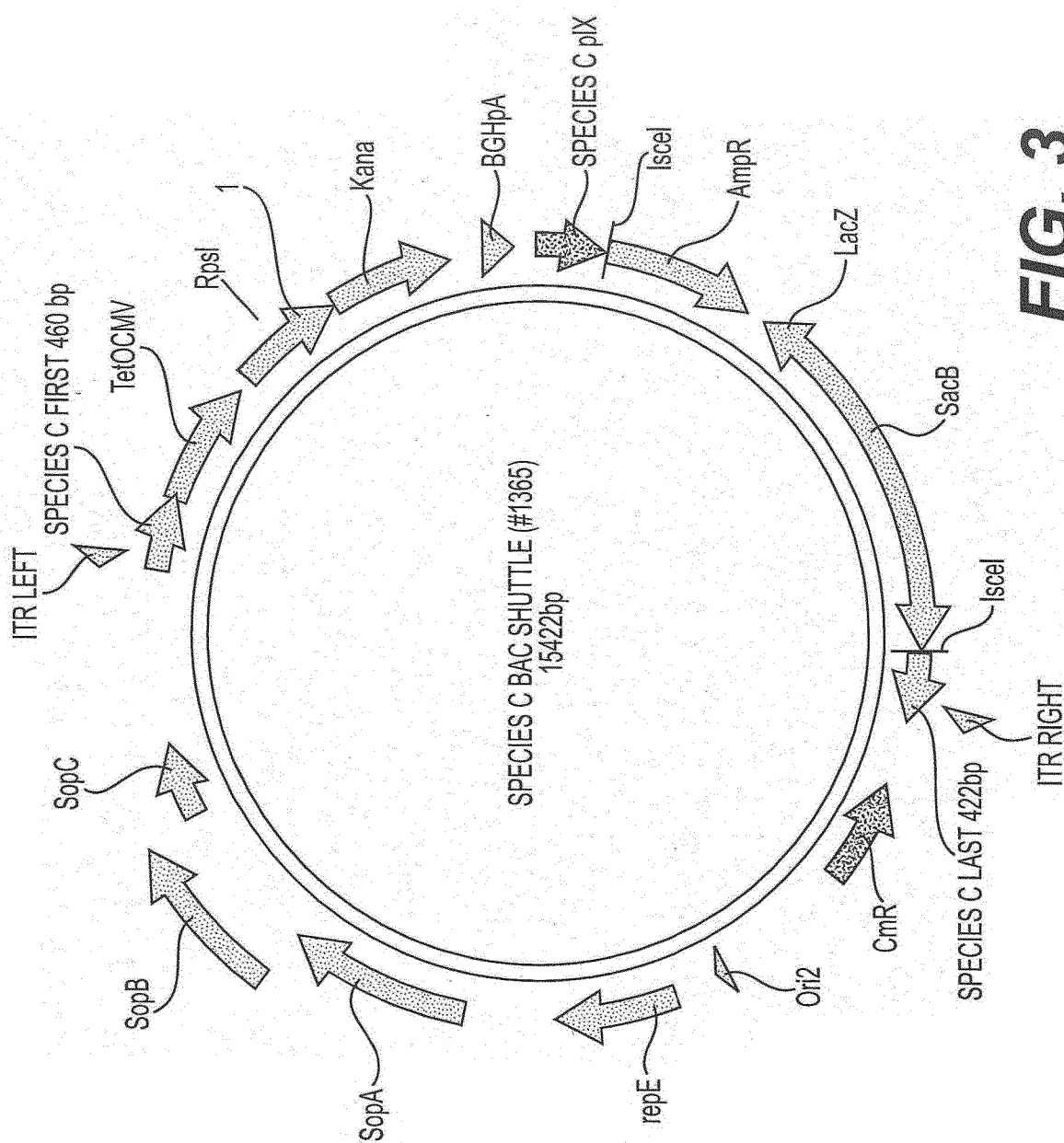


FIG. 3

Figure 4

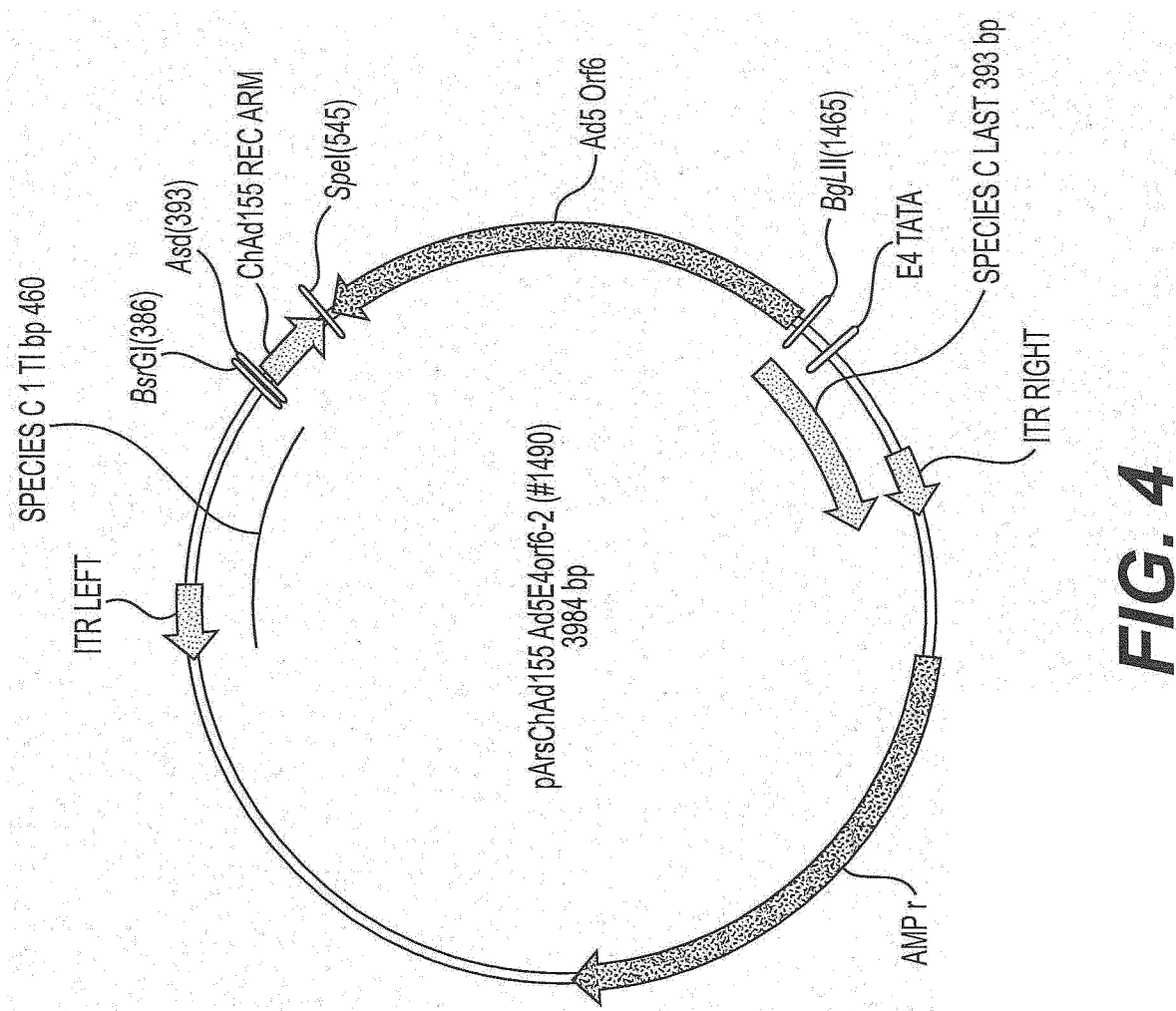


FIG. 4

Figure 5

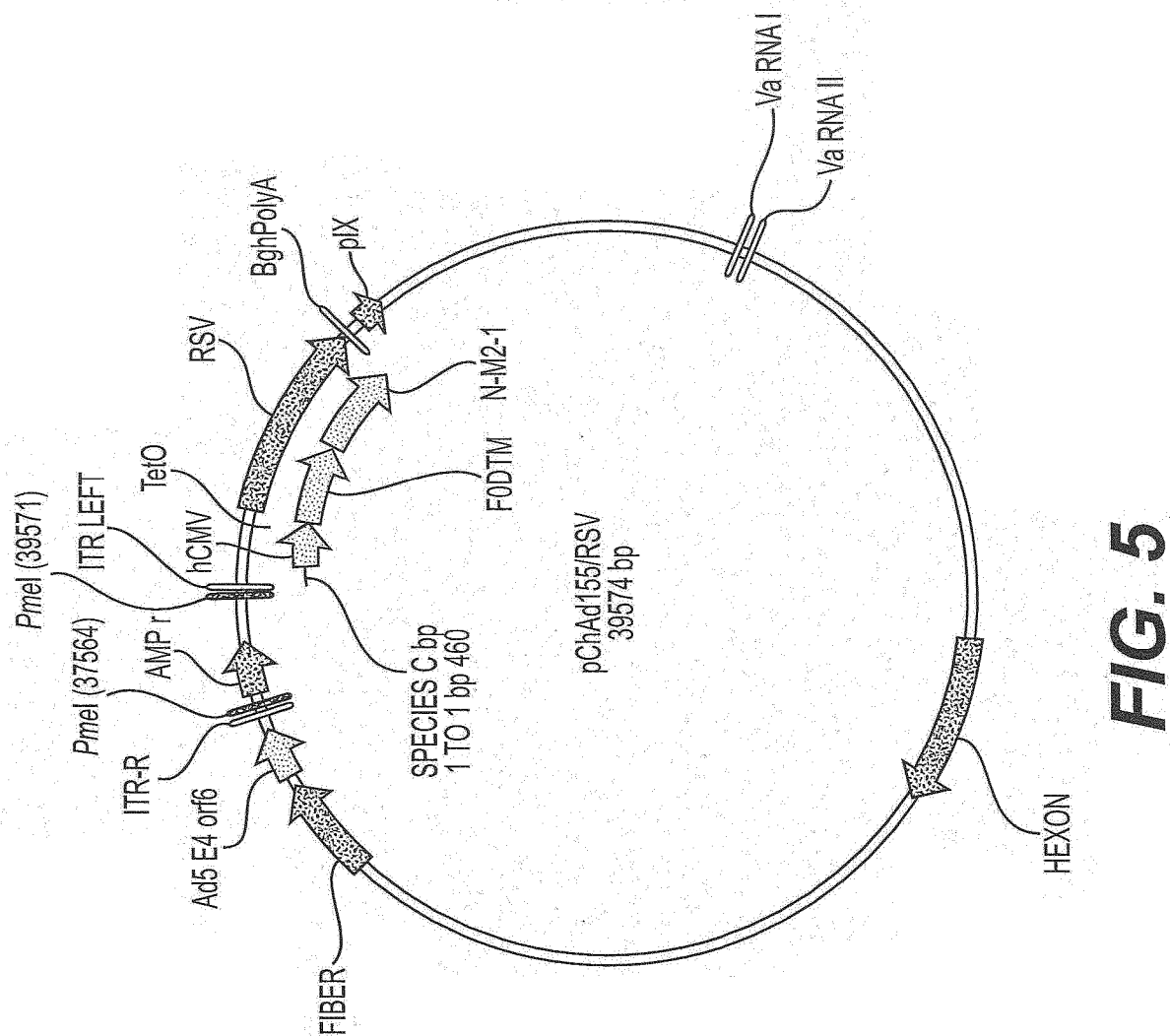


FIG. 5

Figure 6

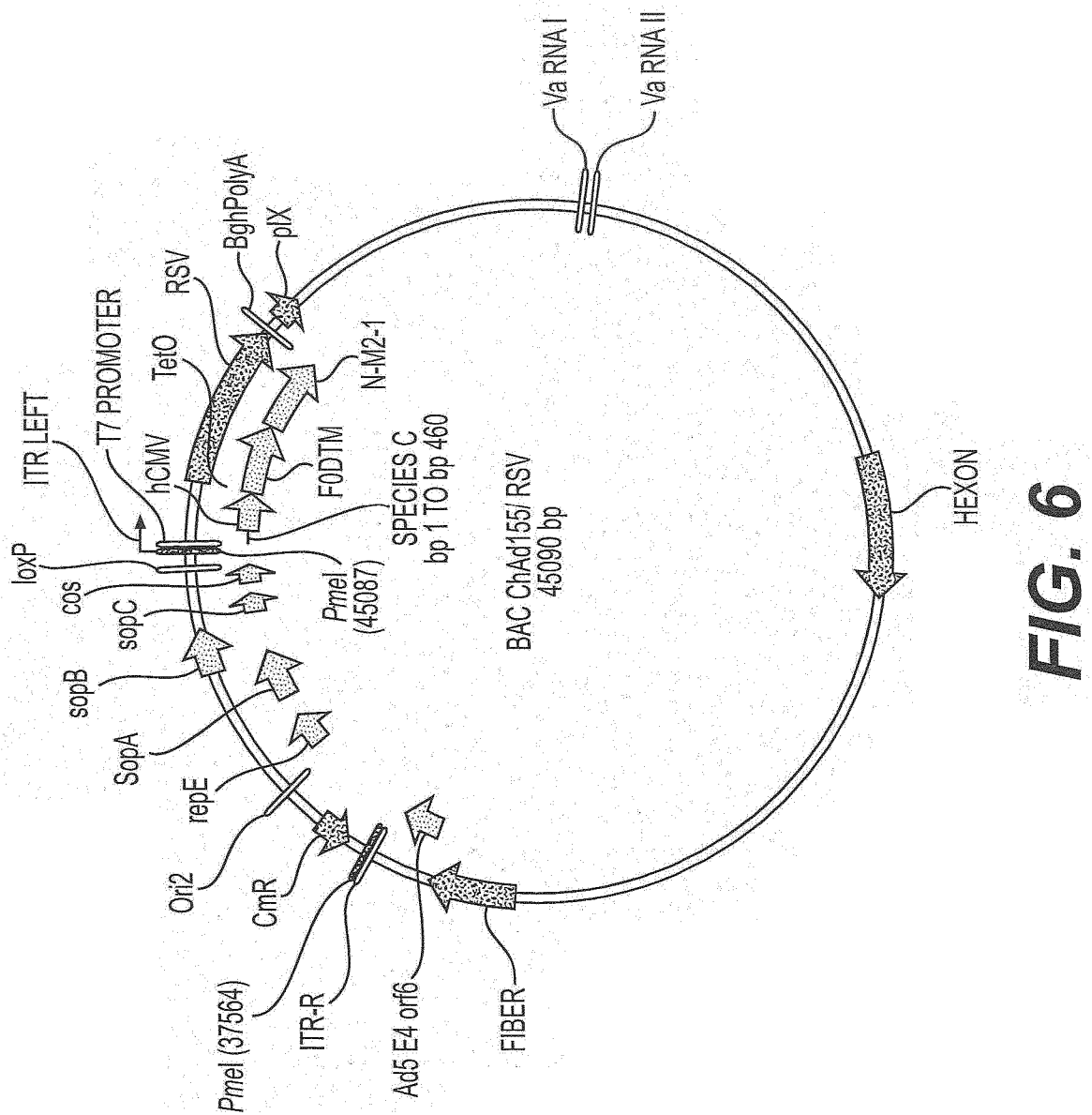


FIG. 6

Figure 7

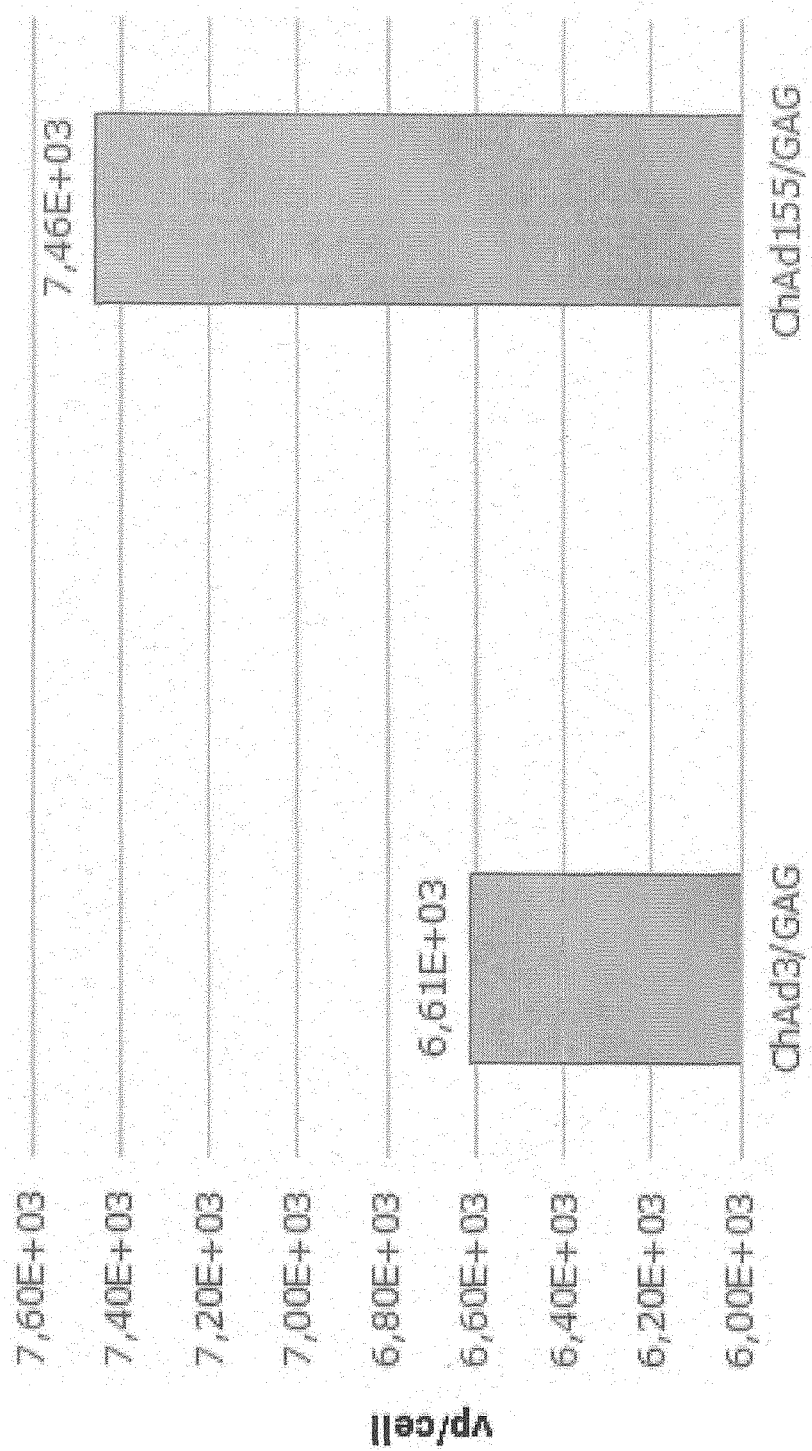


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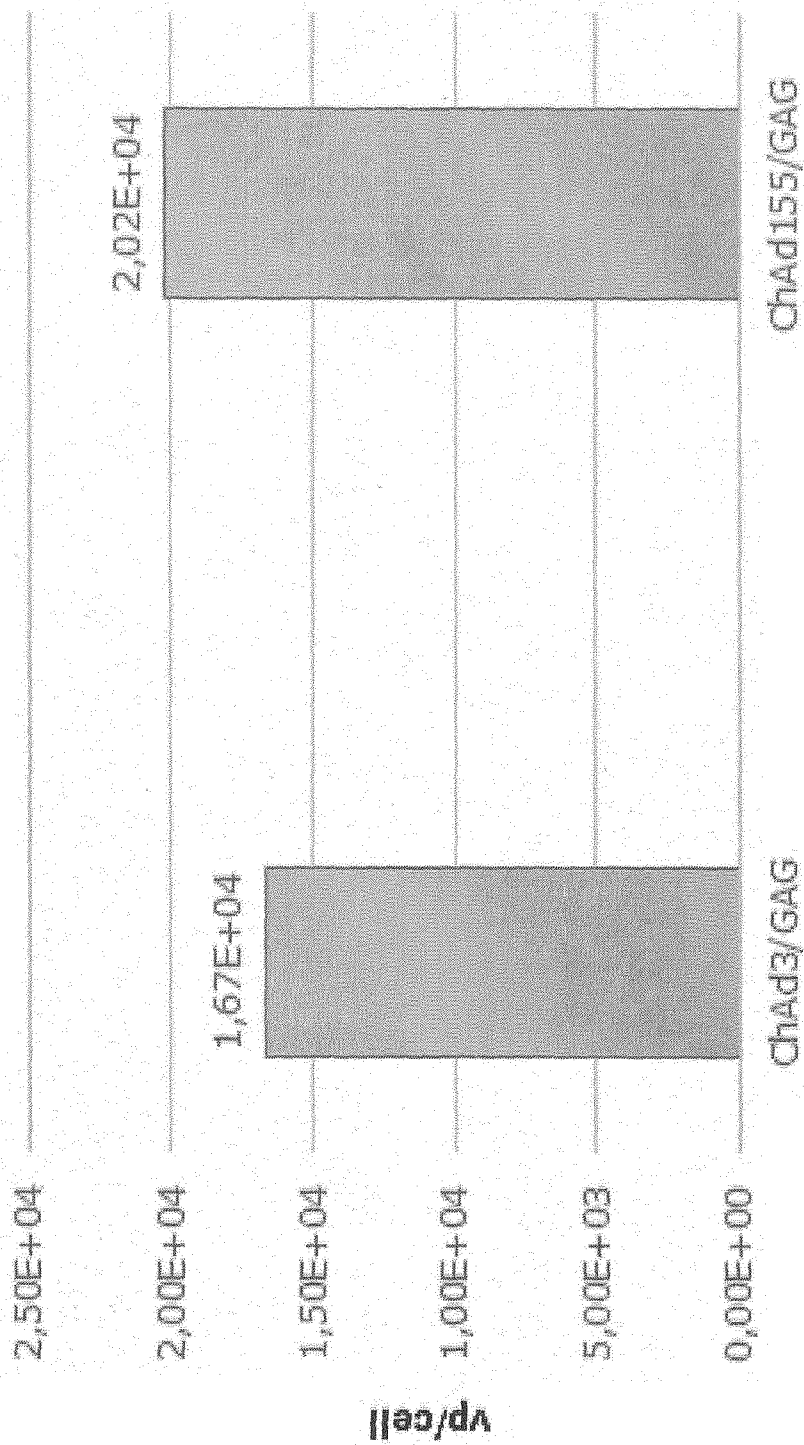


Figure 9

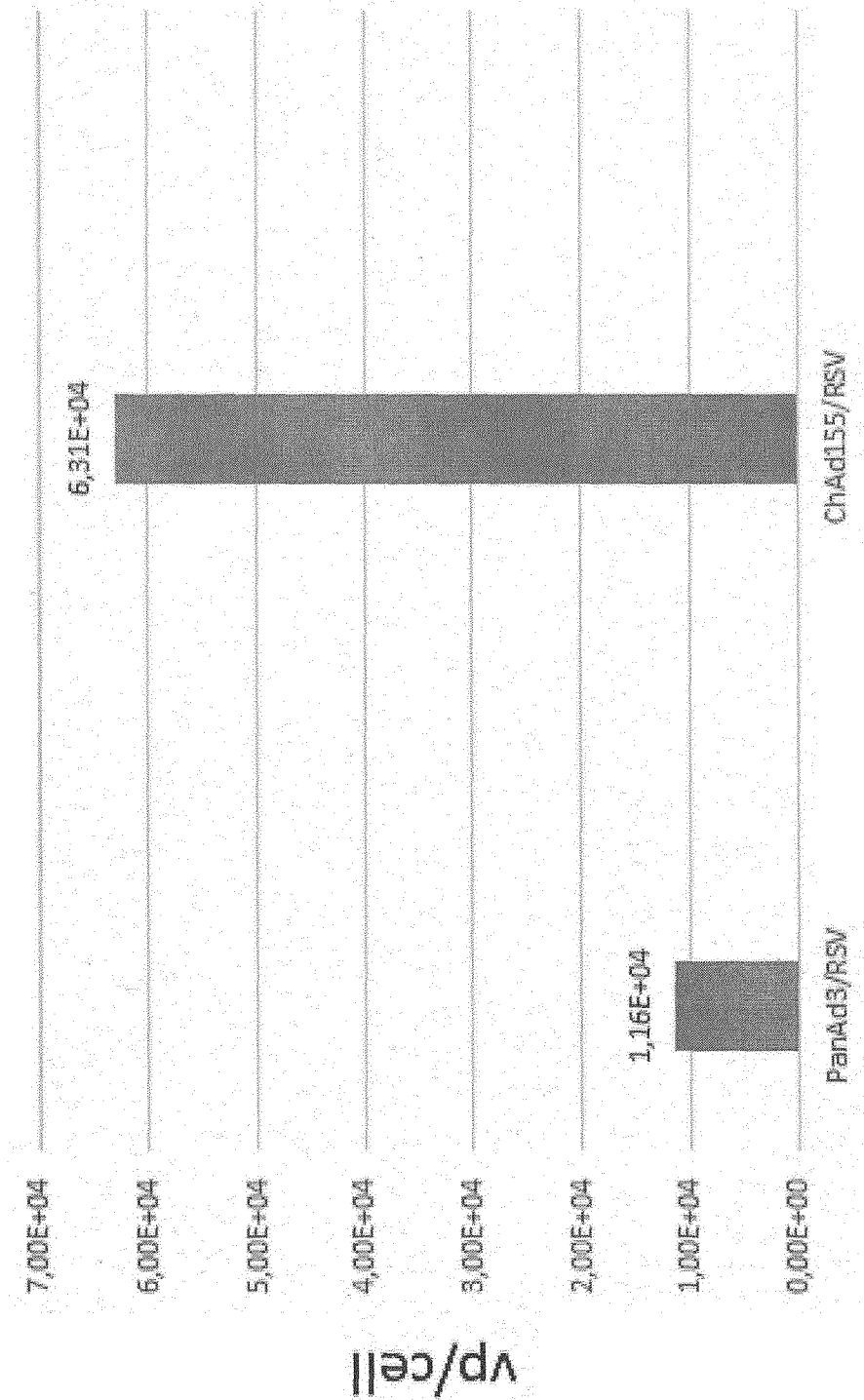


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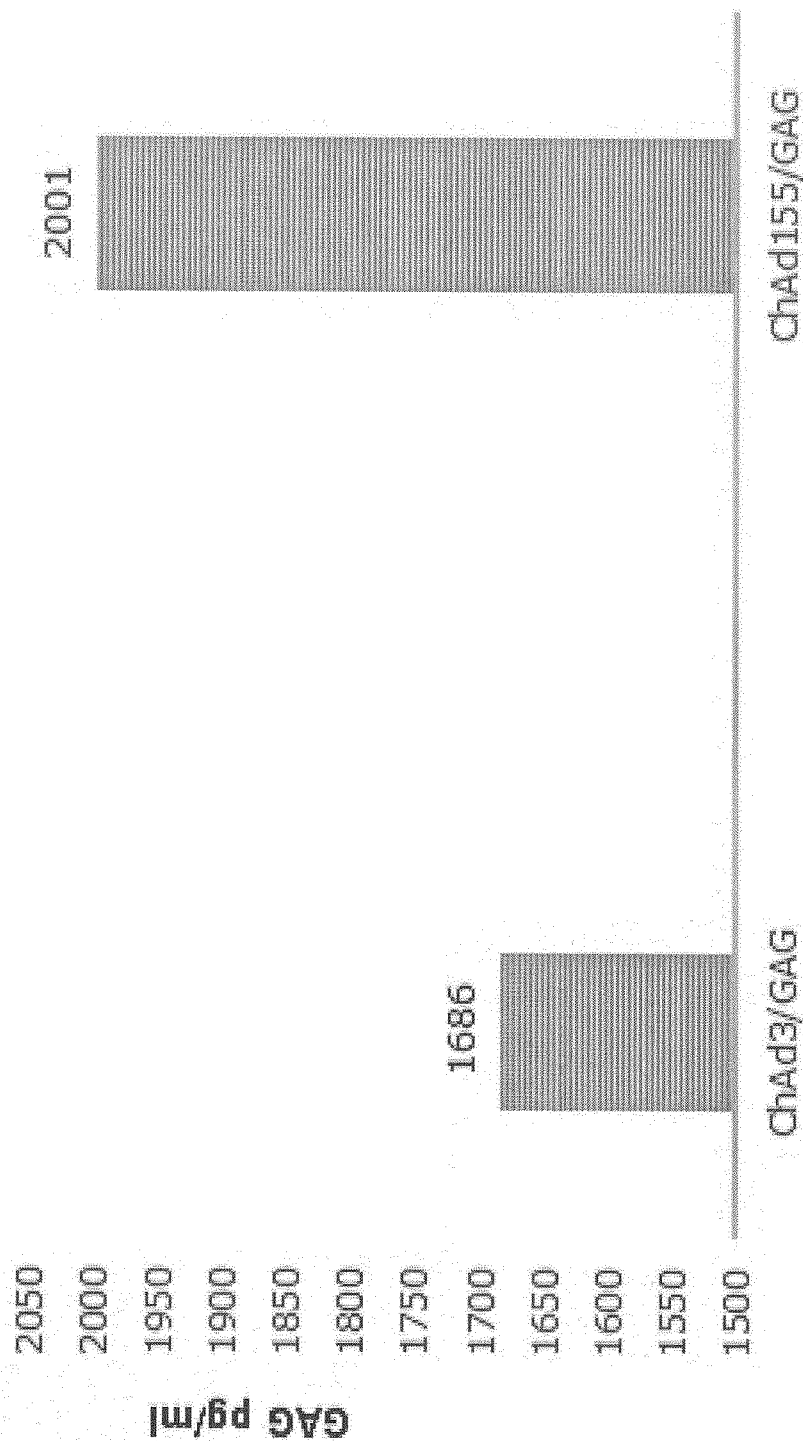


Figure 11

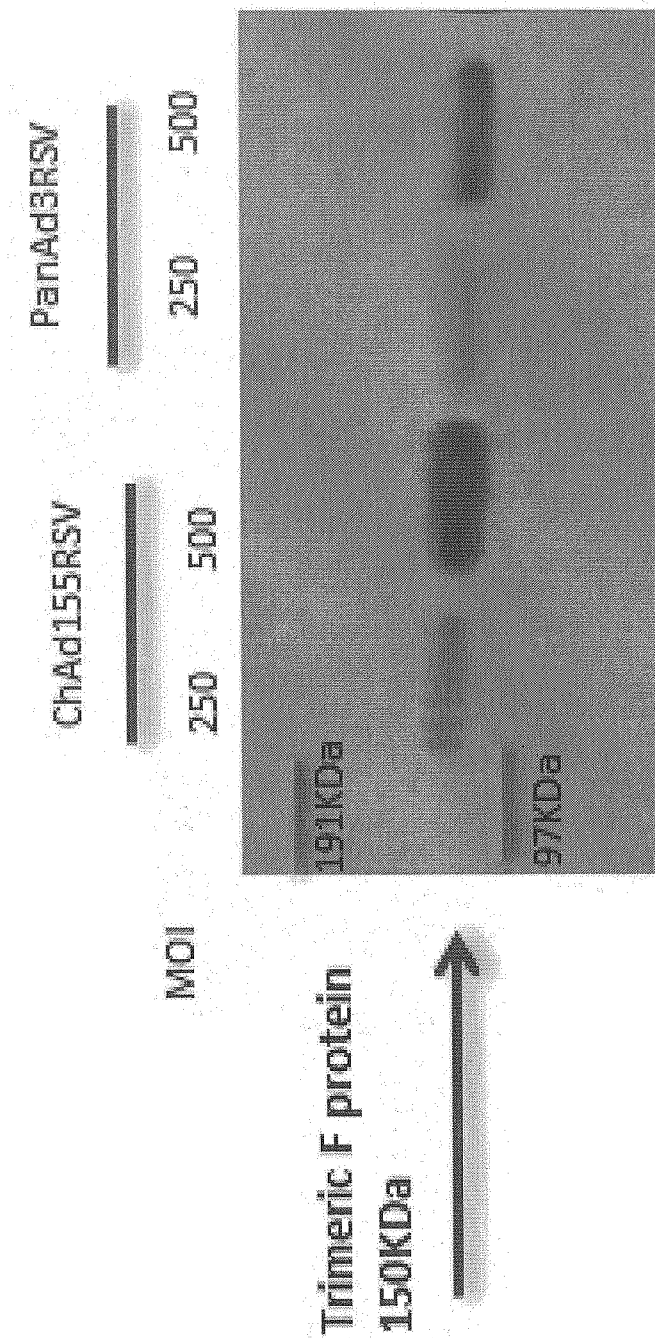


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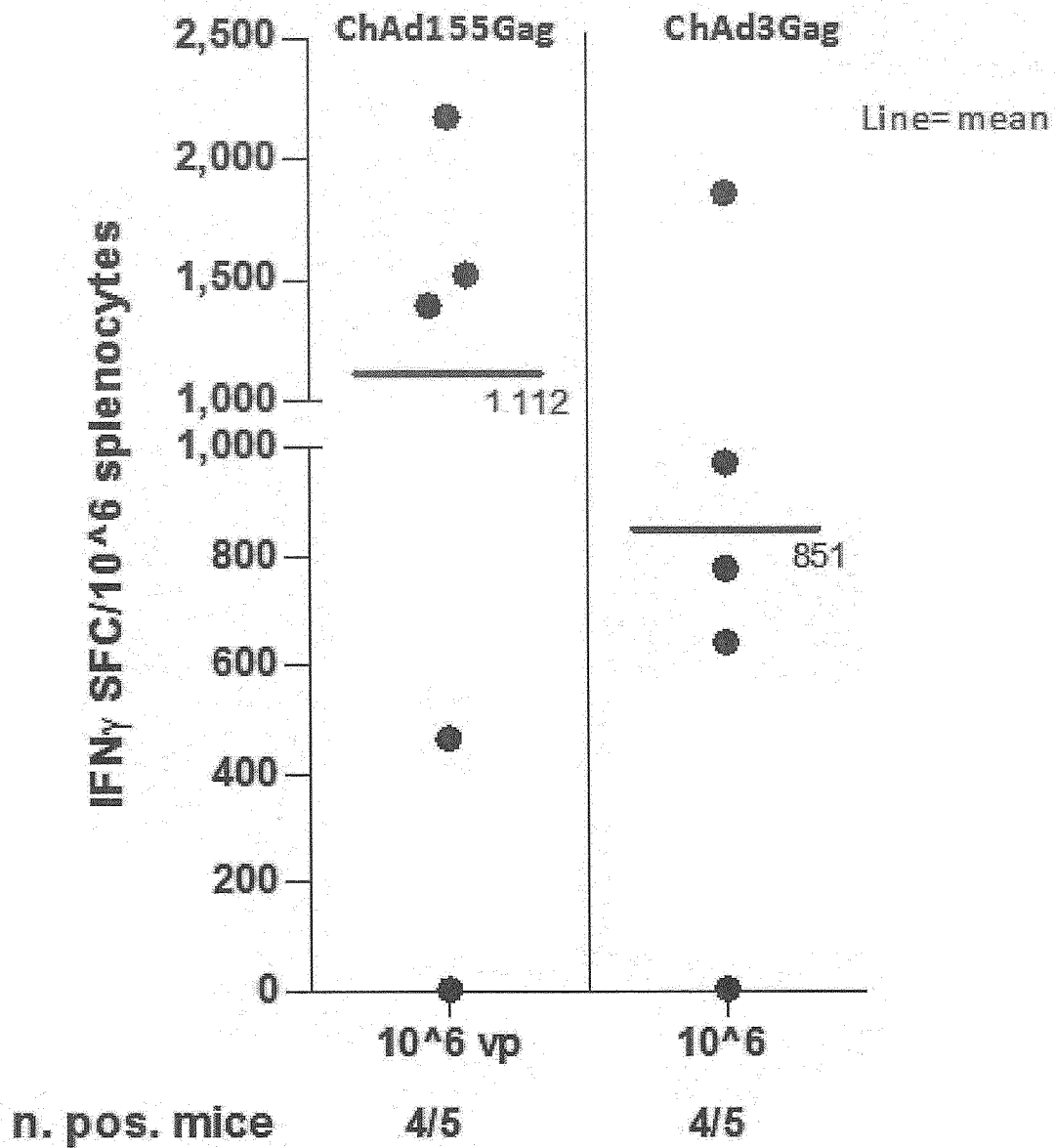


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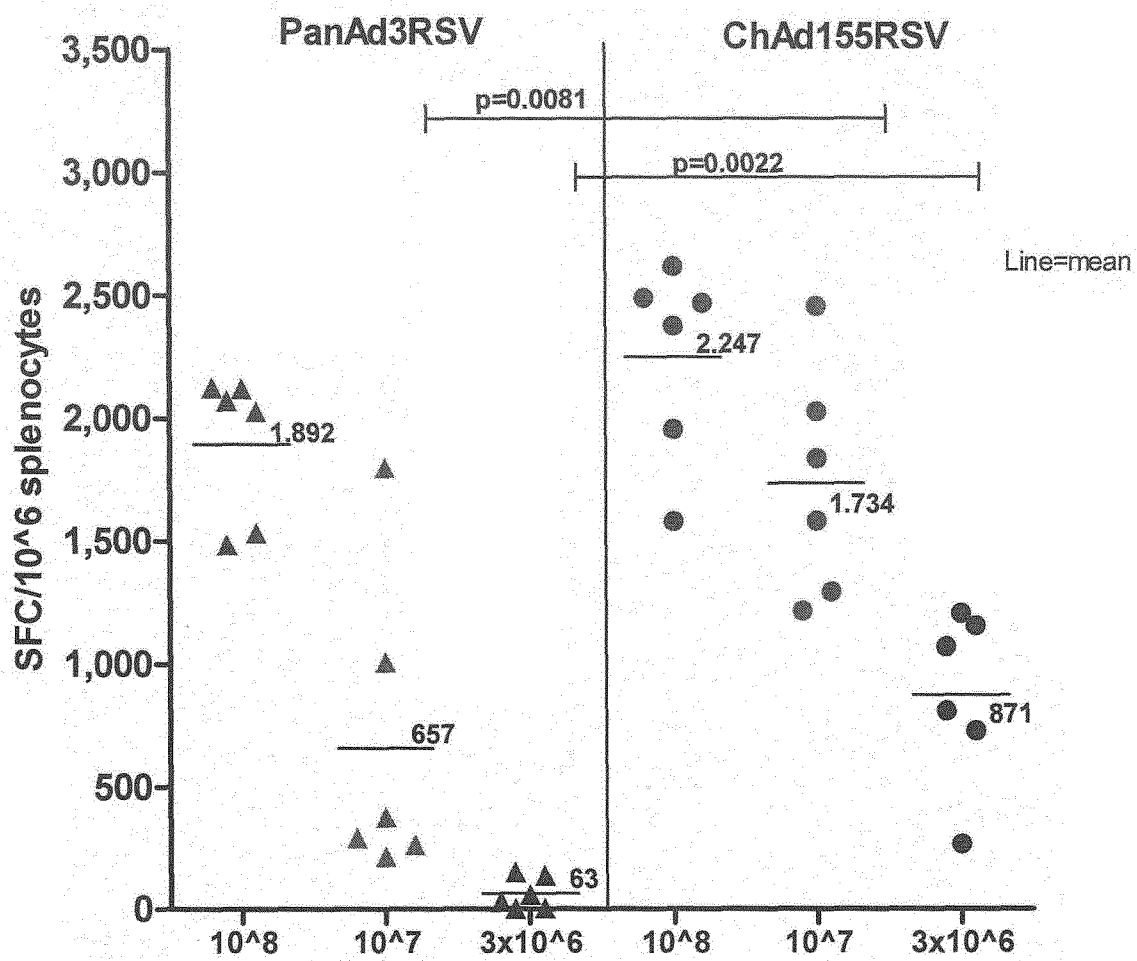


Figure 14

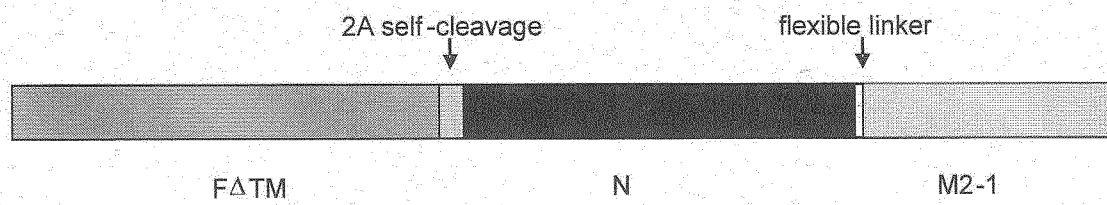


Figure 15

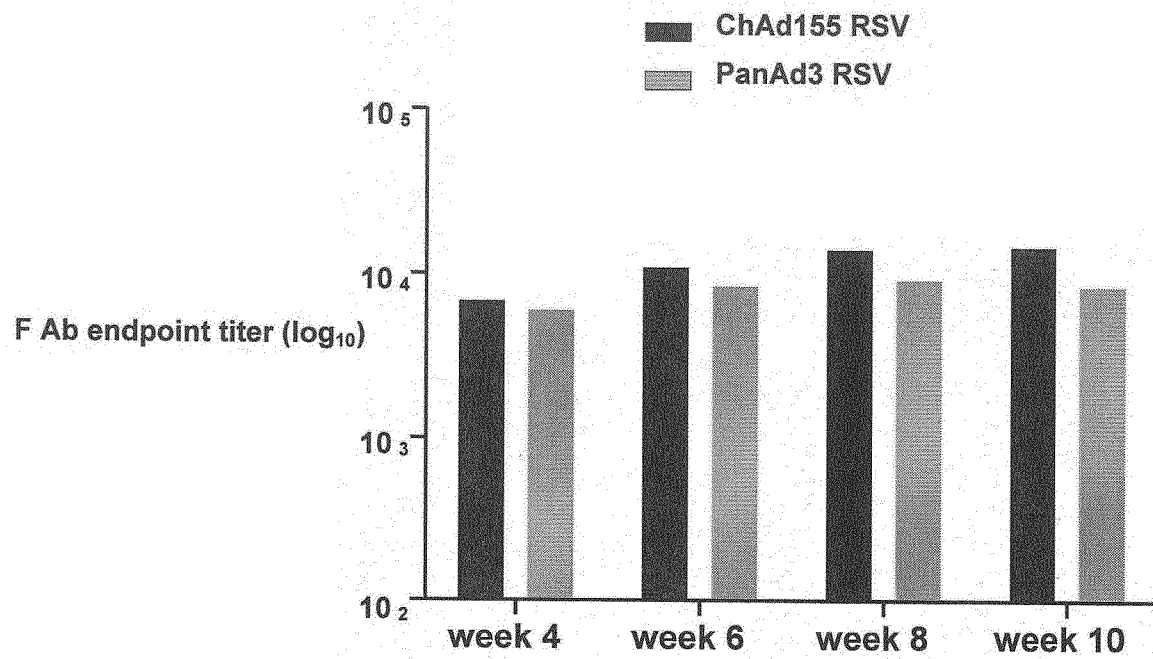


Figure 16

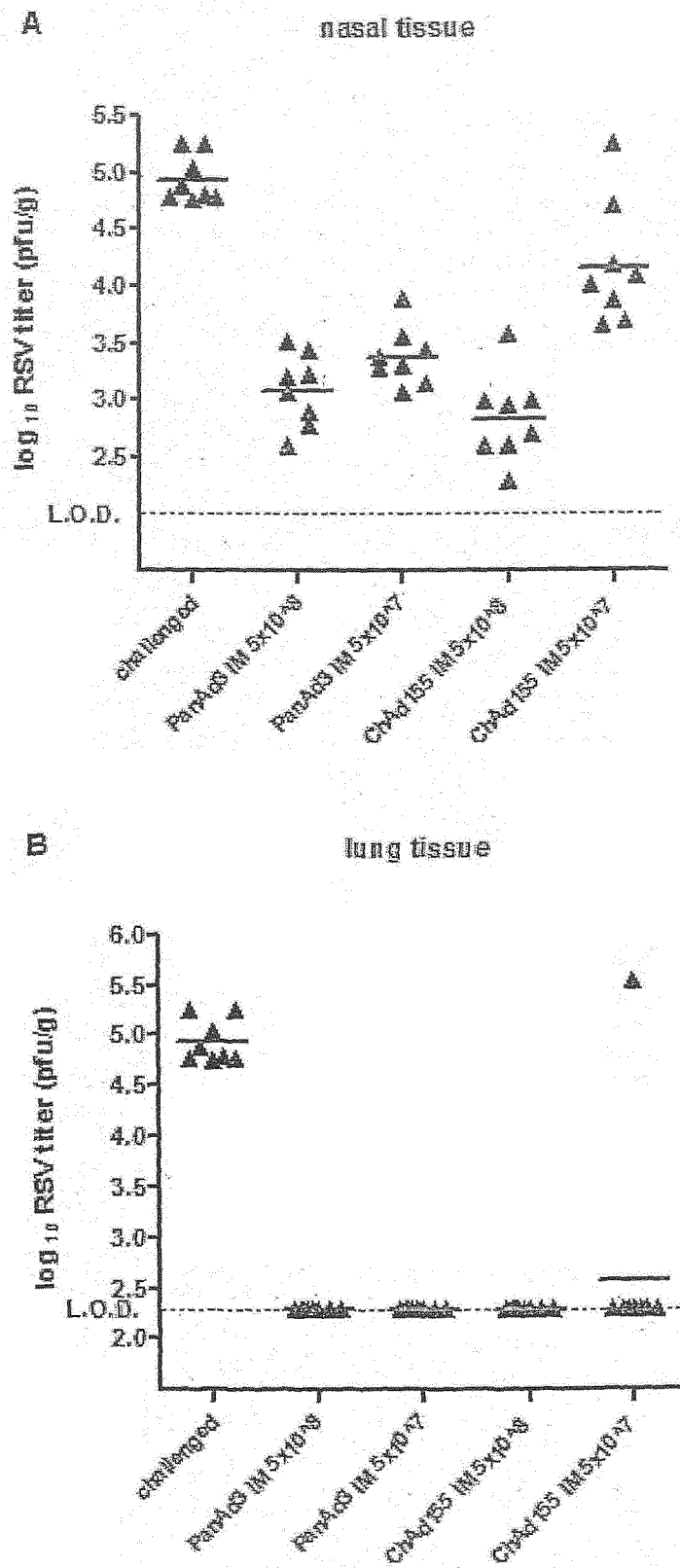


Figure 16

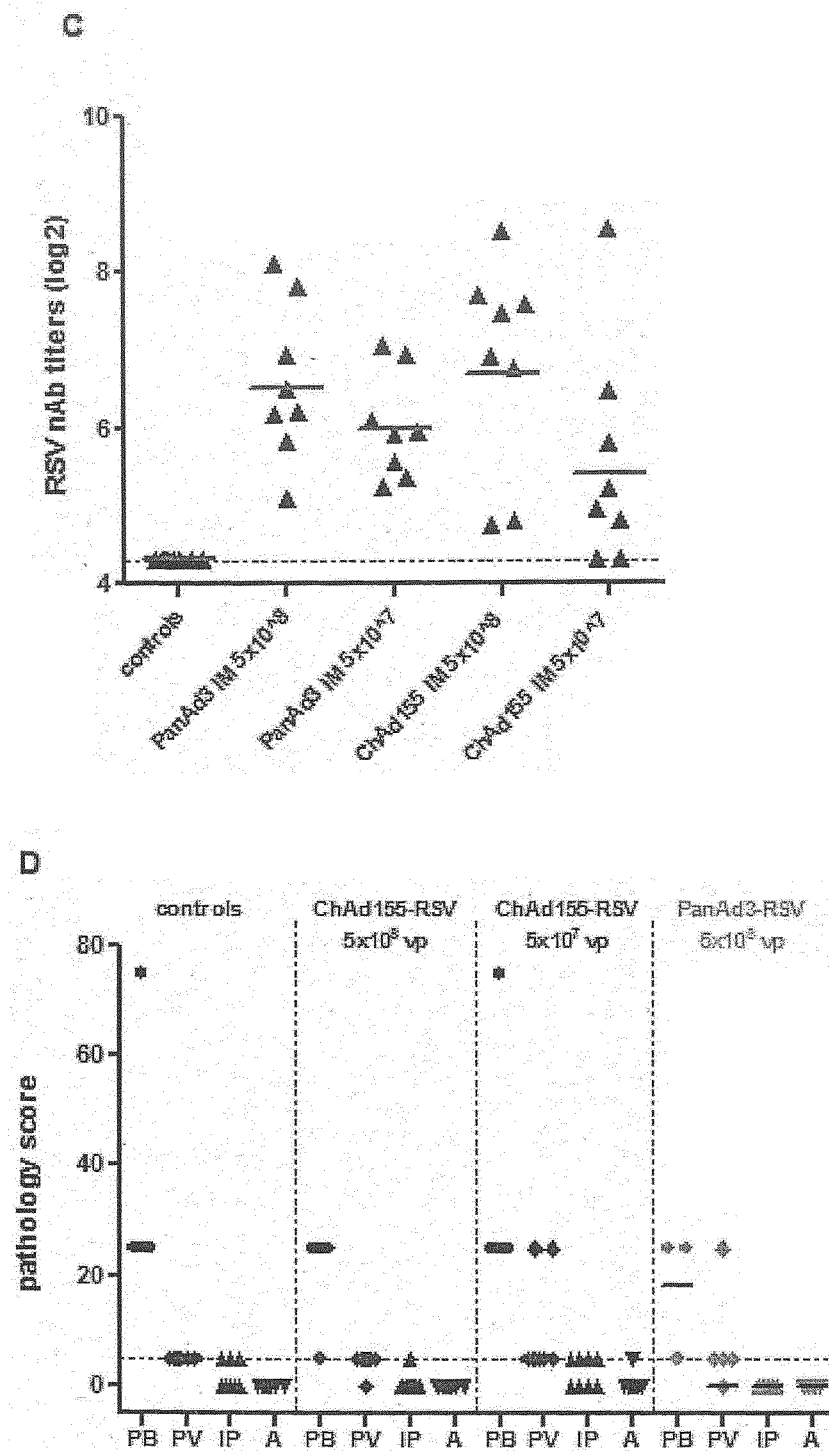
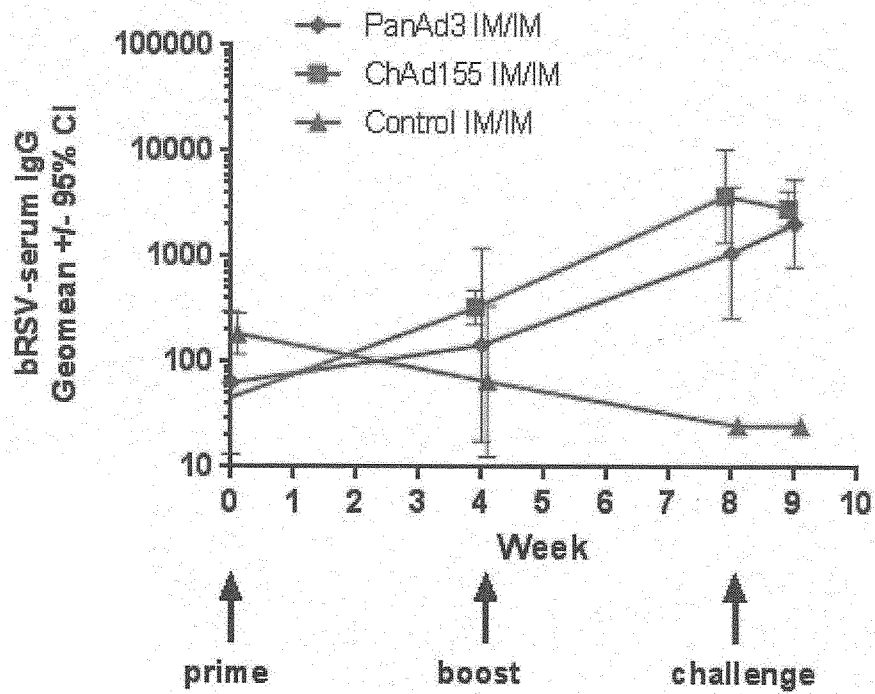
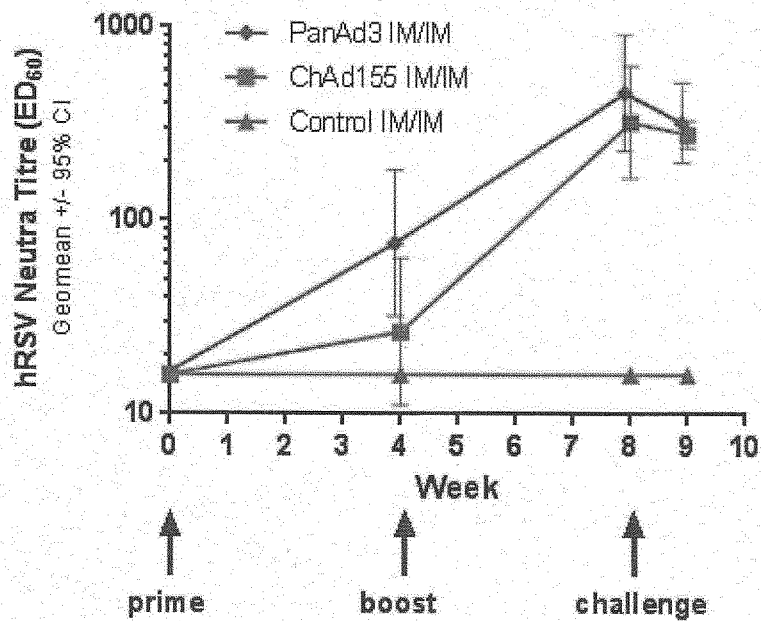


Figure 17

A)



B)



IM: Intramuscular. Symbols represent the geometric mean for each group and the error bars represent 95% confidence intervals of the geometric mean.

Figure 18

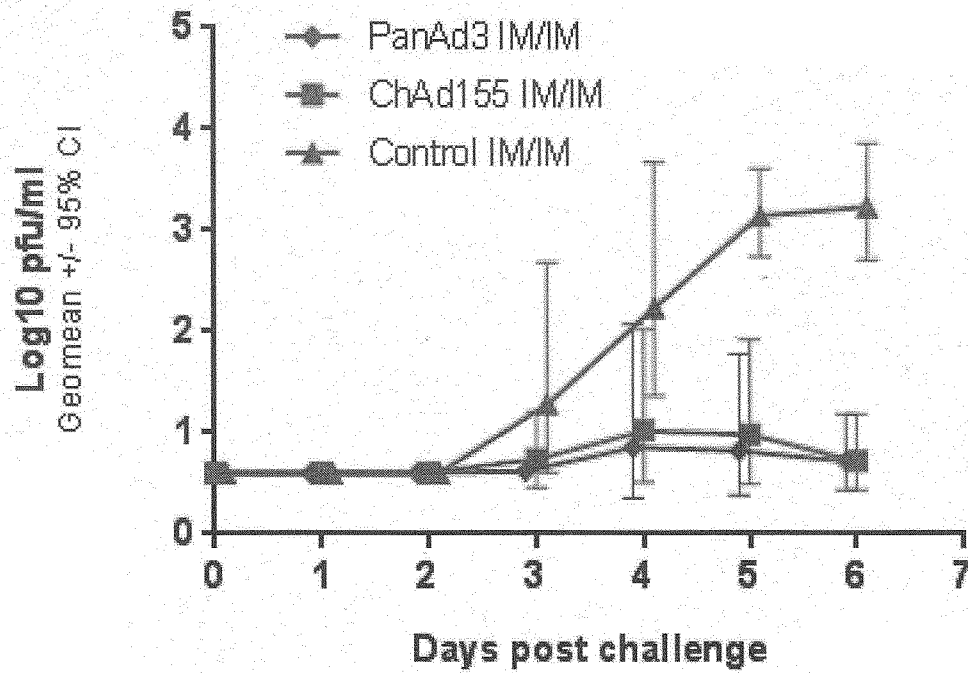
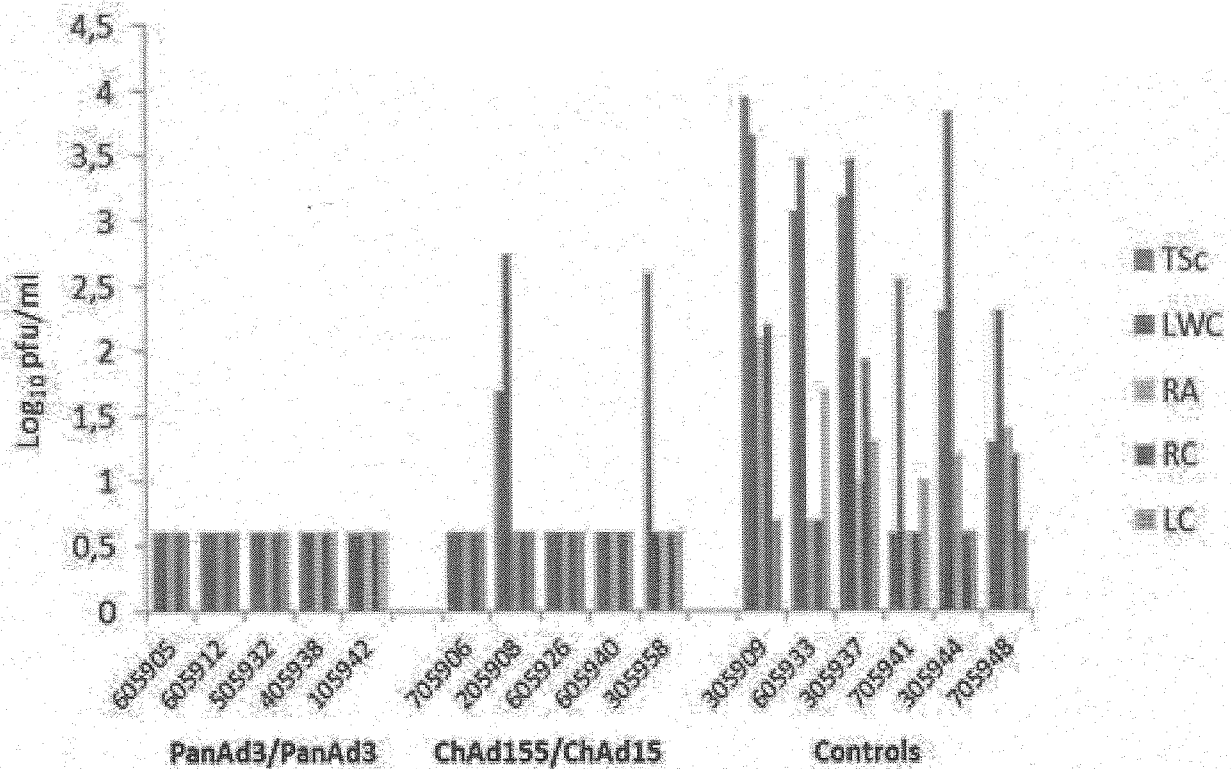


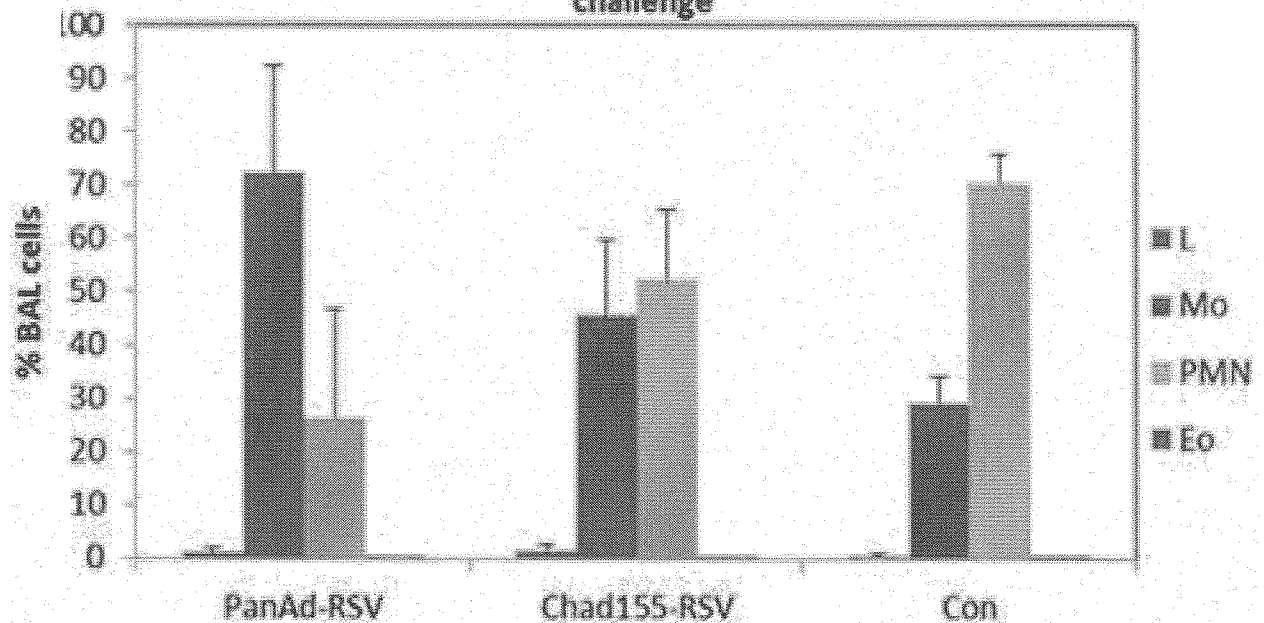
Figure 19



TSc: Tracheal Scrape cells; LWC: Lung Wash cells; RA: Right Apical (lung lobe cells); RC: Right Cardiac (lung lobe cells); LC: Left Cardiac (lung lobe cells)

Figure 21

AR000530: Comparison of IM/IM vaccination with PanAd3-RSV and ChAd155-RSV on pulmonary inflammatory response following BRSV challenge



L: Lymphocytes; Mo: macrophages; PMN: Polymorphonuclear Leukocytes; Eo; Eosinophils;
Con: control

eolf-seql
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<120> ADENOVIRUS POLYNUCLEOTIDES AND POLYPEPTIDES

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35 40 45

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

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Gly Ala Pro Leu His Val Val Asp Ser Leu Asn Ala Leu Thr Val Val
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Thr Gly Gln Gly Leu Thr Ile Asn Gly Thr Ala Leu Gln Thr Arg Val
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Ala Gly Gly Met Arg Val Asp Ala Asn Gly Gln Leu Ile Leu Asp Val
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Ala Tyr Pro Phe Asp Ala Gln Asn Asn Leu Ser Leu Arg Leu Gly Gln
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Gly Pro Leu Phe Val Asn Ser Ala His Asn Leu Asp Val Asn Tyr Asn
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Arg Gly Leu Tyr Leu Phe Thr Ser Gly Asn Thr Lys Lys Leu Glu Val
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Asn Ile Lys Thr Ala Lys Gly Leu Ile Tyr Asp Asp Thr Ala Ile Ala
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Ile Asn Ala Gly Asp Gly Leu Gln Phe Asp Ser Gly Ser Asp Thr Asn
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Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Ser Lys Pro Met Thr
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eolf-seql

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eolf-seql

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Gly Gly Arg Asn Ser Ile Arg Tyr Ser Glu Leu Ala Pro Leu Phe Asp
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Thr Thr Arg Val Tyr Leu Val Asp Asn Lys Ser Ala Asp Val Ala Ser
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Leu Asn Tyr Gln Asn Asp His Ser Asn Phe Leu Thr Thr Val Ile Gln
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Asn Asn Asp Tyr Ser Pro Ser Glu Ala Ser Thr Gln Thr Ile Asn Leu
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Asp Asp Arg Ser His Trp Gly Gly Asp Leu Lys Thr Ile Leu His Thr
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Asn Met Pro Asn Val Asn Glu Phe Met Phe Thr Asn Lys Phe Lys Ala
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Arg Val Met Val Ser Arg Ser His Thr Lys Glu Asp Arg Val Glu Leu
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165 170 175

Thr Met Thr Ile Asp Leu Met Asn Asn Ala Ile Val Glu His Tyr Leu
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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
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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

eolf-seql

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Phe	Tyr	Asn	Asp	Gln	Ala	Val	Tyr	Ser	Gln	Leu	Ile	Arg	Gln	Phe	Thr
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eolf-seq1

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Leu Thr Asp His Gly Thr Leu Pro Leu Arg Asn Ser Ile Gly Gly Val
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Gln Arg Val Thr Val Thr Asp Ala Arg Arg Arg Thr Cys Pro Tyr Val
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Tyr Lys Ala Leu Gly Ile Val Ser Pro Arg Val Leu Ser Ser Arg Thr
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 <213> Simian adenovirus

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eof-seql

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35 40 45

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Thr Leu Arg Phe Ile Pro Val Asp Arg Glu Asp Thr Ala Tyr Ser Tyr
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Lys Ala Arg Phe Thr Leu Ala Val Gly Asp Asn Arg Val Leu Asp Met
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Ala Ser Thr Tyr Phe Asp Ile Arg Gly Val Leu Asp Arg Gly Pro Thr
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Phe Lys Pro Tyr Ser Gly Thr Ala Tyr Asn Ser Leu Ala Pro Lys Gly
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Ala Pro Asn Ser Cys Glu Trp Glu Gln Glu Glu Thr Gln Thr Ala Glu

										eolf-seql							
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Gly	Val	Leu	Val	Glu	Lys	Asp	Gly	Gly	Lys	Met	Glu	Ser	Gln	Val	Asp		
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Ile	Gln	Pro	Lys	Leu	Val	Leu	Tyr	Ser	Glu	Asp	Val	His	Met	Glu	Thr		
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eolf-seql

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

eolf-seql

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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
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Gly Trp Ala Phe Thr Arg Leu Lys Thr Lys Glu Thr Pro Ser Leu Gly
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Asp Gly Thr Phe Tyr Leu Asn His Thr Phe Lys Lys Val Ser Val Thr
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Phe Asp Ser Ser Val Ser Trp Pro Gly Asn Asp Arg Leu Leu Thr Pro
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Asn Glu Phe Glu Ile Lys Arg Ser Val Asp Gly Glu Gly Tyr Asn Val
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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

eof-seq1

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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
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Arg Gly Val Ile Glu Thr Val Tyr Leu Arg Thr Pro Phe Ser Ala Gly
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eolf-seq1

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eolf-seql

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tgacggccgc	tgacagcagc	accctcacag	tcagtgccac	accacccctt	agcacaagca	34920
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ttaacttttg	cgctccccctg	catgtggtag	acagcctaaa	tgcactgact	gtagttactg	35040
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cagcacagca	ccacaatatt	gttcaaaatc	ccacagtgc	aggcgctgta	tccaaagctc	36540
atggcgggga	ccacagaacc	cacgtggcca	tcataccaca	agcgaggtga	gattaagtgg	36600
cgacccctca	taaacacgct	ggacataaac	attacctctt	ttggcatgtt	gtaattcacc	36660

eolf-seql

acctccccggt accatataaaa cctctgatta aacatggcgc catccaccac catcctaaac	36720
cagctggcca aaacctgccc gccggctata cactgcaggg aaccgggact ggaacaatga	36780
cagtggagag cccaggactc gtaacatgg atcatcatgc tcgtcatgat atcaatgttg	36840
gcacaacaca ggcacacgtg catacacttc ctcaggatta caagctcctc ccgcgttaga	36900
accatatccc agggaaacaac ccatttcctga atcagcgtaa atcccacact gcagggaaga	36960
cctcgcacgt aactcacgtt gtgcattgtc aaagtgttac attcgggcag cagcggatga	37020
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ggagtgcgcc gagacaaccg agatcgtgtt ggtcgtagt tcatgccaaa tggaacgccg	37140
gacgtagtca tatttcctga agtccttagat ctctcaacgc agcaccagca ccaacacttc	37200
gcagtgtaaa aggccaagt cccgagagagt atatatagga ataaaaagt 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 acccgccctc cgccccgcgc cccgccccgc	37500
gccgcccac 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

ggagtccgc gttacataac ttacggtaaa tggcccgct ggctgaccgc ccaacgaccc	60
ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag ggactttcca	120
ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac atcaagtgt	180
tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg cctggcatta	240
tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg tattagtc	300
cgctattacc atggtcgagg tgagccccac gttctgcttc actctcccca tctccccccc	360
ctccccaccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc	420
gggggggggg gggggcgcg gccaggcggg gcggggcggg gcgaggggcg gggcggggcg	480
aggcggagag gtgcggcggc agccaatcag agcggcgcg tccgaaagt tccttttatg	540
gcgaggcggc ggcggcggg gccctataaa aagcgaagcg ctccctatca gtgatagaga	600

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tctccctatc agtgatagag atcgtcgacg agctcgcgcc gggcgggagt cgctgcgcgc	660
tgcccttcgcc ccgtgccccg ctccgccgcc gcctcgcgcc gcccgccccg gctctgactg	720
accgcgttac taaaacaggt aagtccggcc tccgcgccgg gttttggcgc ctcccgcggg	780
cgccccctc ctcacggcga gcgctgccac gtcagacgaa gggcgagcg agcgtcctga	840
tccttcgcc cggaagctca ggacagcggc ccgtgctca 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 actcgctac atg	33

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

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

<400> 14	
atacgaaga 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	
attcagtgtgta caggcgcgcc aaagcatgac gctgttgatt tgattc	46

<210> 16
 <211> 35

eolf-seql

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<212> DNA
<213> Artificial Sequence

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

<400> 16
actaggacta gttataagct agaatggggc ttgtgc
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 cccattcta gcttataacc
60
cctatttggt 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 ttatttggtta 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

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eolf-seql

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<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 ggatagcggg 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 ttagtcacg 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

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eolf-seql

<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 tgggtgtgtc ggggaaatca tcgtcctttc cttggctgct 420
 cgctgtgtt 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

eolf-seql

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

eolf-seql																		
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

eolf-seql

<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

eol f - seq l															
245								250				255			
Gly	Gly	Met	Arg 260	Ile	Asn	Thr	Gly	Gly 265	Gln	Leu	Ile	Leu	Asp 270	Val	Ala
Tyr	Pro	Phe 275	Asp	Ala	Gln	Asn	Asn 280	Leu	Ser	Leu	Arg	Leu 285	Gly	Gln	Gly
Pro	Leu 290	Tyr	Val	Asn	Thr	Asn 295	His	Asn	Leu	Asp	Leu 300	Asn	Cys	Asn	Arg
Gly 305	Leu	Thr	Thr	Thr	Thr 310	Ser	Ser	Asn	Thr	Thr 315	Lys	Leu	Glu	Thr	Lys 320
Ile	Asp	Ser	Gly	Leu 325	Asp	Tyr	Asn	Ala	Asn 330	Gly	Ala	Ile	Ile	Ala 335	Lys
Leu	Gly	Thr	Gly 340	Leu	Thr	Phe	Asp	Asn 345	Thr	Gly	Ala	Ile	Thr 350	Val	Gly
Asn	Thr	Gly 355	Asp	Asp	Lys	Leu	Thr 360	Leu	Trp	Thr	Thr	Pro 365	Asp	Pro	Ser
Pro	Asn 370	Cys	Arg	Ile	His	Ala 375	Asp	Lys	Asp	Lys	Phe 380	Thr	Leu	Val	Leu
Thr 385	Lys	Cys	Gly	Ser	Gln 390	Ile	Leu	Ala	Ser	Val 395	Ala	Ala	Leu	Ala	Val 400
Ser	Gly	Asn	Leu	Ser 405	Ser	Met	Thr	Gly	Thr 410	Val	Ser	Ser	Val	Thr 415	Ile
Phe	Leu	Arg	Phe 420	Asp	Gln	Asn	Gly	Val 425	Leu	Met	Glu	Asn	Ser 430	Ser	Leu
Asp	Lys	Glu 435	Tyr	Trp	Asn	Phe	Arg 440	Asn	Gly	Asn	Ser	Thr 445	Asn	Ala	Thr
Pro	Tyr 450	Thr	Asn	Ala	Val	Gly 455	Phe	Met	Pro	Asn	Leu 460	Ser	Ala	Tyr	Pro
Lys 465	Thr	Gln	Ser	Gln	Thr 470	Ala	Lys	Asn	Asn	Ile 475	Val	Ser	Glu	Val	Tyr 480
Leu	His	Gly	Asp	Lys 485	Ser	Lys	Pro	Met	Ile 490	Leu	Thr	Ile	Thr	Leu 495	Asn

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

eolf-seql

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		

eolf-seq1

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

eolf-seql

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			

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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
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60

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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

eolf-seql

<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 245	Tyr	Asp	Ser	Ser	eolf-seql							Gly 250	Asn	Leu	Glu	Leu	Arg 255	Ala
Ala	Gly	Gly	Met 260	Arg	Val	Asp	Ala	Asn 265	Gly	Lys	Leu	Ile	Leu 270	Asp	Val							
Ala	Tyr	Pro 275	Phe	Asp	Ala	Gln	Asn 280	Asn	Leu	Ser	Leu	Arg 285	Leu	Gly	Gln							
Gly	Pro 290	Leu	Phe	Val	Asn	Ser 295	Ala	His	Asn	Leu	Asp 300	Val	Asn	Tyr	Asn							
Arg 305	Gly	Leu	Tyr	Leu	Phe 310	Thr	Ser	Gly	Asn	Thr 315	Lys	Lys	Leu	Glu	Val							
Asn	Ile	Lys	Thr	Ala 325	Lys	Gly	Leu	Ile	Tyr 330	Asp	Asp	Thr	Ala	Ile 335	Ala							
Ile	Asn	Pro	Gly 340	Asp	Gly	Leu	Glu	Phe 345	Gly	Ser	Gly	Ser	Asp 350	Thr	Asn							
Pro	Leu	Lys 355	Thr	Lys	Leu	Gly	Leu 360	Gly	Leu	Glu	Tyr	Asp 365	Ser	Ser	Arg							
Ala	Ile 370	Ile	Ala	Lys	Leu	Gly 375	Thr	Gly	Leu	Ser	Phe 380	Asp	Asn	Thr	Gly							
Ala 385	Ile	Thr	Val	Gly	Asn 390	Lys	Asn	Asp	Asp	Lys 395	Leu	Thr	Leu	Trp	Thr 400							
Thr	Pro	Asp	Pro	Ser 405	Pro	Asn	Cys	Arg	Ile 410	Tyr	Ser	Glu	Lys	Asp 415	Ala							
Lys	Phe	Thr	Leu 420	Val	Leu	Thr	Lys	Cys 425	Gly	Ser	Gln	Val	Leu 430	Ala	Ser							
Val	Ser	Val 435	Leu	Ser	Val	Lys	Gly 440	Ser	Leu	Ala	Pro	Ile 445	Ser	Gly	Thr							
Val	Thr 450	Ser	Ala	Gln	Ile	Ile 455	Leu	Arg	Phe	Asp	Glu 460	Asn	Gly	Val	Leu							
Leu 465	Ser	Asn	Ser	Ser	Leu 470	Asp	Pro	Gln	Tyr	Trp 475	Asn	Tyr	Arg	Lys	Gly 480							
Asp	Leu	Thr	Glu	Gly 485	Thr	Ala	Tyr	Thr	Asn 490	Ala	Val	Gly	Phe	Met 495	Pro							

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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

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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

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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

eolf-seq1

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

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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

eolf-seql

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

eolf-seql

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	

eolf-seql

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

eolf-seql

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

eolf-seql

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

eolf-seql

545 550 555 560

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

Glu

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<210> 37
<211> 1145
<212> PRT
<213> Respiratory syncytial virus
```

<400> 37
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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
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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
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Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu
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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

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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
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Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val Asn
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Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser Glu
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Leu Leu Ser Leu Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys Leu
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Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile Met
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Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro Leu
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Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser Pro Leu
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Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr Arg Thr
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Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe Pro
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Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys Asp Thr
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Met Asn Ser Leu Thr Leu Pro Ser Glu Val Asn Leu Cys Asn Val Asp
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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

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Met Leu Lys Glu Met Gly Glu Val Ala Pro Glu Tyr Arg His Asp Ser
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Pro Asp Cys Gly Met Ile Ile Leu Cys Ile Ala Ala Leu Val Ile Thr
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Lys Leu Ala Ala Gly Asp Arg Ser Gly Leu Thr Ala Val Ile Arg Arg
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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
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Arg Gly Gly Ser Arg Val Glu Gly Ile Phe Ala Gly Leu Phe Met Asn
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Ala Tyr Gly Ala Gly Gln Val Met Leu Arg Trp Gly Val Leu Ala Lys
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Glu Gln Val Val Glu Val Tyr Glu Tyr Ala Gln Lys Leu Gly Gly Glu
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Ala Gly Phe Tyr His Ile Leu Asn Asn Pro Lys Ala Ser Leu Leu Ser
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Leu Thr Gln Phe Pro His Phe Ser Ser Val Val Leu Gly Asn Ala Ala
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Ile Lys His Gln Leu Asn Pro Lys Asp Asn Asp Val Glu Leu Gly Gly
930 935 940

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Glu Ile Arg Gly His Cys Leu Asn Gly Lys Arg Cys His Phe Ser His
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Asn Tyr Phe Glu Trp Pro Pro His Ala Leu Leu Val Arg Gln Asn Phe
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Ser Glu Ile Ser Gly Ala Ala Glu Leu Asp Arg Thr Glu Glu Tyr
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