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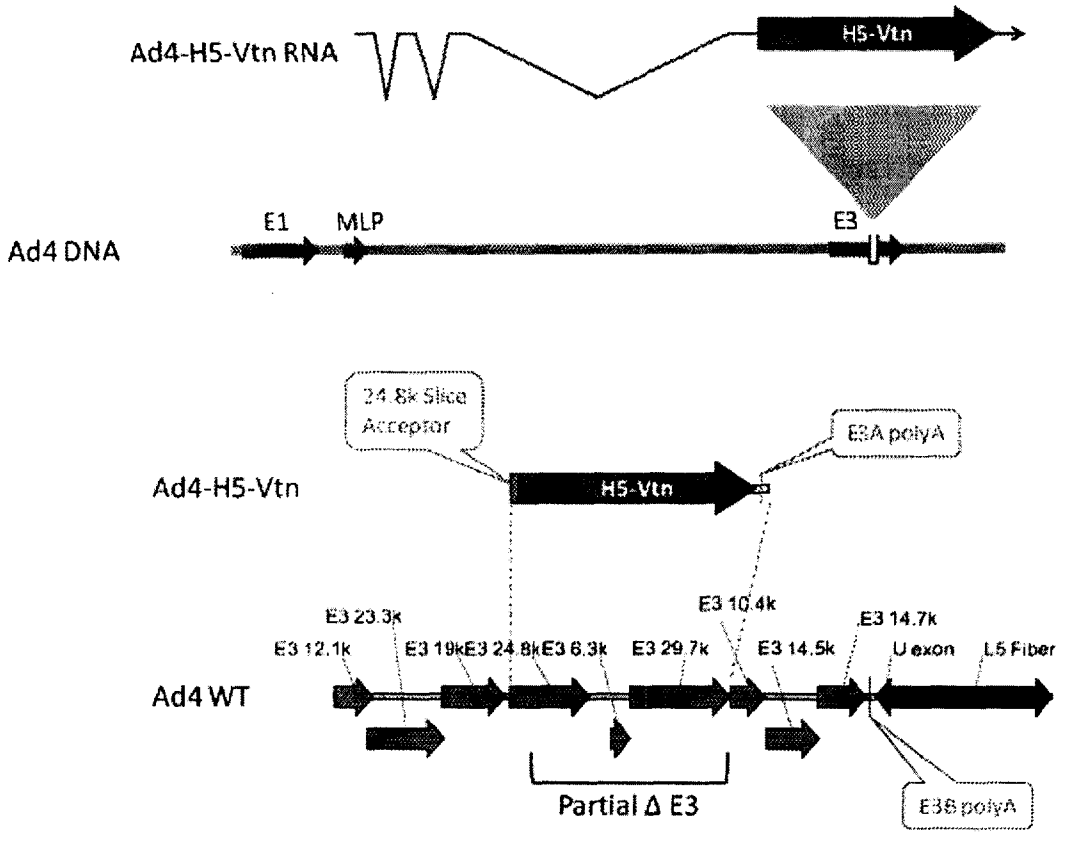
ABSTRACT

ADENOVIRAL-BASED VECTORS

The present invention provides replication competent adenoviral vectors capable of expressing antigens from infectious pathogens, such as influenza virus. The adenoviral vectors can be used to vaccinate subjects against the infectious pathogens. The adenoviral vectors comprise heterologous sequences encoding the antigens. The heterologous sequences can be inserted into various locations in the adenoviral vectors, including in or near specific E3 deletions and/or integrated into the adenoviral hexon coding region. The adenoviral vectors can be derived from any adenoviral serotype, particularly an Ad4 or Ad7 serotype.

FIGURE 15



I/We claim:

1. A vaccine comprising an adenoviral vector comprising a first heterologous sequence, wherein said adenoviral vector is replication competent and has a partial E3 deletion, and wherein the first heterologous sequence is integrated into a location containing the partial E3 deletion.

2. The vaccine of claim 1, wherein the adenoviral vector is derived from Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, or Ad50.

3. The vaccine of claim 1, wherein the adenoviral vector is derived from chimpanzee serotypes Ad C1, Ad C3, Ad C6, Ad C7, or Ad68.

4. The vaccine of claim 1, wherein the partial E3 deletion comprises deletion of at least 1, 2 or 3 open reading frames within the E3 region.

5. The vaccine of claim 1, wherein the partial E3 deletion comprises deletion of at least 1, 2, or 3 open reading frames with unknown function.

6. The vaccine of claim 1, wherein the partial E3 deletion comprises deletion of a region corresponding to ADP region of Ad5.

7. The vaccine of claim 1, wherein the adenoviral vector is derived from Ad4 and the partial E3 deletion comprises a deletion of E3 24.8k, E3 6.3k, and E3 29.7k.

8. The vaccine of claim 1, wherein the adenoviral vector is derived from Ad7 and the partial E3 deletion comprises a deletion of E3 20.1k, E3 20.6k, and E3 7.7k.

9. The vaccine of claim 1, wherein the expression of the first heterologous sequence is under the control of an adenoviral promoter.

10. The vaccine of claim 9, wherein the expression of the first heterologous sequence is under the control of an endogenous adenoviral promoter.

11. The vaccine of claim 10, wherein the expression of the first heterologous sequence is under the control of endogenous Major Late Promoter and tripartite leader.

12. The vaccine of claim 1, wherein the expression of the first heterologous sequence is under the control of a non-adenoviral promoter.

13. The vaccine of claim 12, wherein the expression of the first heterologous sequence is under the control of a cytomegalovirus (CMV) promoter.

14. The vaccine of claim 12, wherein the expression of the first heterologous sequence is under the control of a CMV promoter and an adenoviral tripartite leader.

15. The vaccine of claim 1, wherein the first heterologous sequence is operably linked to an adenoviral splice acceptor.

16. The vaccine of claim 15, wherein the first heterologous sequence is operably linked to a native E3 24.8k splice acceptor.

17. The vaccine of claim 1, wherein the first heterologous sequence is operably linked to an adenoviral polyA signal sequence.

18. The vaccine of claim 17, wherein the first heterologous sequence is operably linked to an Ad5 E3 polyA signal sequence.

19. The vaccine of claim 1, wherein the first heterologous sequence encodes an immunogenic protein of an infectious pathogen.

20. The vaccine of claim 19, wherein the infectious pathogen is selected from the group consisting of a virus, a bacterium, a protist, and a fungus.

21. The vaccine of claim 20, wherein the infectious pathogen is influenza, human immunodeficiency virus, or human papilloma virus.

22. The vaccine of claim 20, wherein the infectious pathogen is *Bacillus*, *Shigella*, *Mycobacterium*, or *Plasmodium*.

23. The vaccine of claim 1, wherein the first heterologous sequence encodes influenza hemaglutinin, influenza neuraminidase, influenza M2, a multimer of M2e, a multimer of HTL epitopes, or a multimer of CTL epitopes.

24. The vaccine of claim 1, wherein the first heterologous sequence comprises a first ORF encoding an immunogenic protein of an infectious pathogen and a second ORF encoding a multimer of epitopes from said infectious pathogen.

25. A vaccine comprising an adenoviral vector comprising a first heterologous sequence, wherein said adenoviral vector is replication competent and wherein the expression of the first heterologous sequence is under the control of an adenoviral promoter.

26. The vaccine of claim 25, wherein the adenoviral promoter is an endogenous adenoviral promoter.

27. The vaccine of claim 25, wherein the first heterologous sequence is under the control of endogenous Major Late Promoter and tripartite leader.

28. The vaccine of claim 25, wherein the adenoviral vector comprises a full or partial E3 deletion and wherein the first heterologous sequence is integrated into a location containing the full or partial E3 deletion.

29. A vaccine comprising an adenoviral vector comprising a first heterologous sequence, wherein said adenoviral vector is derived from Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, Ad50, Ad C1, Ad C3, Ad C6, Ad C7, or Ad68 , is replication competent, and has a full E3 deletion.

30. A vaccine comprising an adenoviral vector comprising a first heterologous sequence and a second heterologous sequence, wherein the second heterologous sequence is integrated into an adenoviral hexon region, wherein the first heterologous sequence is integrated into an adenoviral non-hexon region, and wherein the adenoviral vector is replication competent.

31. The vaccine of claim 30, wherein the adenoviral vector is derived from Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, or Ad50.

32. The vaccine of claim 30, wherein the adenoviral vector is derived from chimpanzee serotypes Ad C1, Ad C3, Ad C6, Ad C7, or Ad68.

33. The vaccine of claim 30, wherein the adenoviral vector comprises a partial E3 deletion and wherein the first heterologous sequence is integrated into a location containing the partial E3 deletion.

34. The vaccine of claim 30, wherein the expression of the first heterologous sequence is under the control of an adenoviral promoter.

35. The vaccine of claim 30, wherein the expression of the first heterologous sequence is under the control of an endogenous adenoviral promoter.

36. The vaccine of claim 30, wherein the second heterologous sequence is integrated into a hexon region of the adenoviral vector.

37. The vaccine of claim 30, wherein the second heterologous sequence is integrated into HVR1, HVR2, HVR4, or HVR5.

38. The vaccine of claim 30, wherein the second heterologous sequence encodes a region of a membrane protein of a virus.

39. The vaccine of claim 30, wherein the second heterologous sequence encodes an extracellular part of a conserved virus membrane protein.

40. The vaccine of claim 30, wherein the second heterologous sequence encodes a region of an influenza M2 protein, an influenza Matrix CTL, an influenza NP epitope, one or more HVRs from an adenovirus of another serotype, or a combination thereof.

41. The vaccine of claim 30, wherein the second heterologous sequence encodes one or more copies of M2e of influenza M2.

42. The vaccine of claim 30, wherein the second heterologous sequence encodes one or more copies of M2e of influenza M2, wherein each M2 copy is integrated into a different HVR, and

wherein the one or more copies of M2e are integrated into HVR1, HVR2, HVR4, HVR5 or a combination thereof.

43. The vaccine of claim 30, wherein the second heterologous sequence comprises the sequence of SEQ ID NO. 318 (H5 M2e), SEQ ID NO. 321 (H7 M2e), SEQ ID NO. 327 (H9 M2e), SEQ ID NO. 312 (Human M2e), SEQ ID NO. 337 (NP), or SEQ ID NO. 336 (Matrix CTL).

44. A vaccine comprising an adenoviral vector comprising a second heterologous sequence, wherein the second heterologous sequence encodes a region of a membrane protein of a virus and is adjacent to an endogenous adenoviral sequence, and wherein the second heterologous sequence is integrated into a hexon region of the adenoviral vector.

45. The vaccine of claim 44, wherein the second heterologous sequence encodes a region of an influenza M2 protein, an influenza Matrix CTL, an influenza NP epitope, one or more HVRs from an adenovirus of another serotype, or a combination thereof.

46. The vaccine of claim 44, wherein the adenoviral vector is derived from Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, or Ad50.

47. The vaccine of claim 44, wherein the adenoviral vector is derived from chimpanzee serotypes Ad C1, Ad C3, Ad C6, Ad C7, or Ad68.

48. A vaccine comprising an adenoviral vector comprising a second heterologous sequence, wherein the second heterologous sequence encodes a region of a membrane protein of a virus flanked by a spacer sequence, and wherein the second heterologous sequence is integrated into a hexon region of the adenoviral vector.

49. The vaccine of claim 48, wherein the spacer sequence encodes an "LGS" peptide.

50. The vaccine of claim 48, wherein the adenoviral vector is derived from Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, or Ad50.

51. The vaccine of claim 48, wherein the adenoviral vector is derived from chimpanzee serotypes Ad C1, Ad C3, Ad C6, Ad C7, or Ad68.

52. The vaccine of claim 1, 25, 29, 30, 44, or 48, which is formulated for oral, intranasal, sublingual, intravesical, rectal, or intravaginal administration.

53. A vaccine of claim 1, 25, 29, 30, 44, or 48, further comprising an acceptable carrier.

54. A dosage unit of the vaccine of claim 1, 25, 29, 30, 44, or 48, wherein a single dose comprises about 10^3 to about 10^{13} adenoviral particles.

55. A method of inducing an immune response to an infectious pathogen in a subject comprising administering to the subject the vaccine of claim 1, 25, 29, 30, 44, or 48.

56. The method of claim 55, wherein one or more doses of the vaccine is administered to the subject.

57. The method of claim 55, wherein the infectious pathogen is influenza, HIV, HPV, *Bacillus*, *Plasmodium*, *Mycobacteria*, or *Shigella*.

58. The method of claim 55, wherein the subject has an infection induced by said infectious pathogen.

Dated **29 February 2012**

AVI GARG
IN/PA-1522
Agent for the Applicant

To,
The Controller of Patents
The Patent Office at **New Delhi**

Applicant(s) Name: **PAX-VAX, INC.**
Application No:

Sheet No.: 1
Total No. of Sheets: 35

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1897/DEL/12

Figure 1.

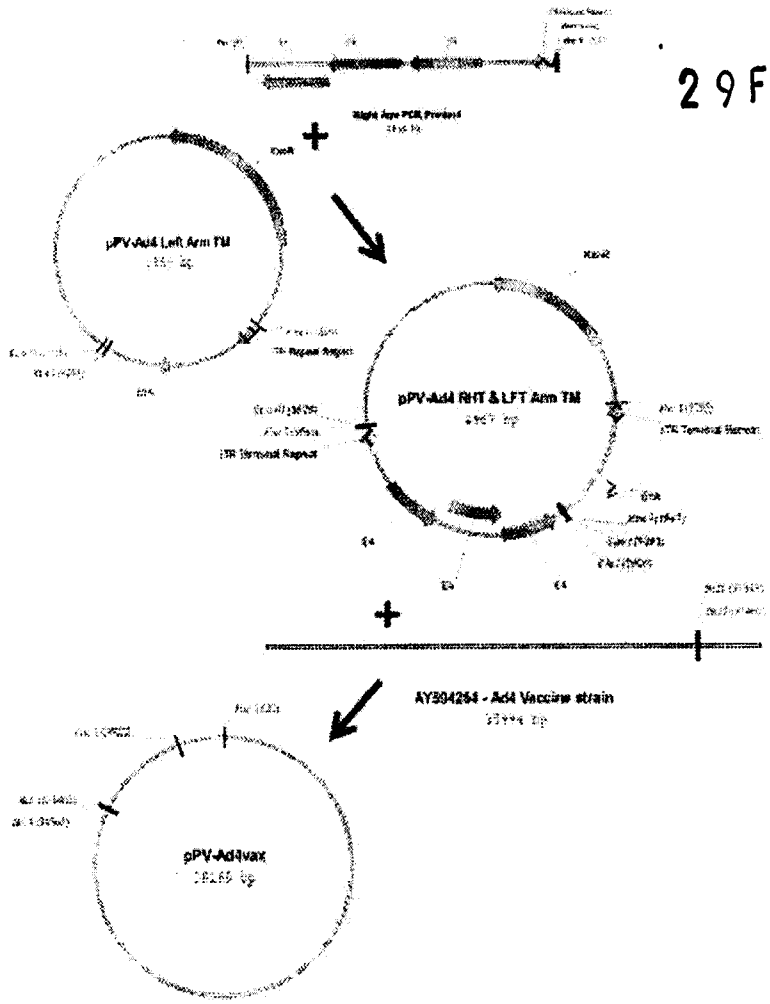

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Figure 2.

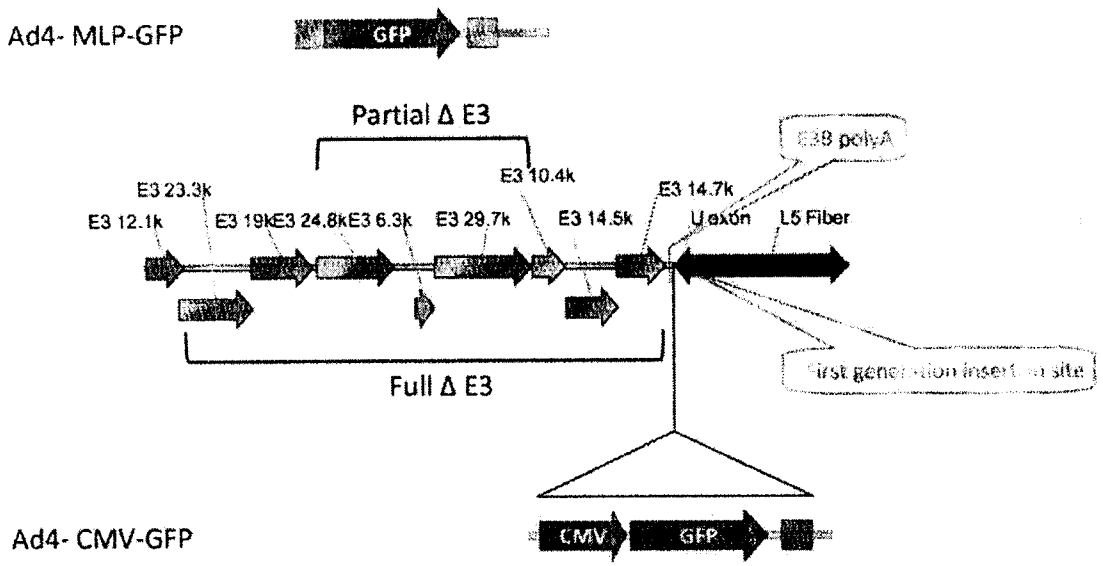

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Figure 3.

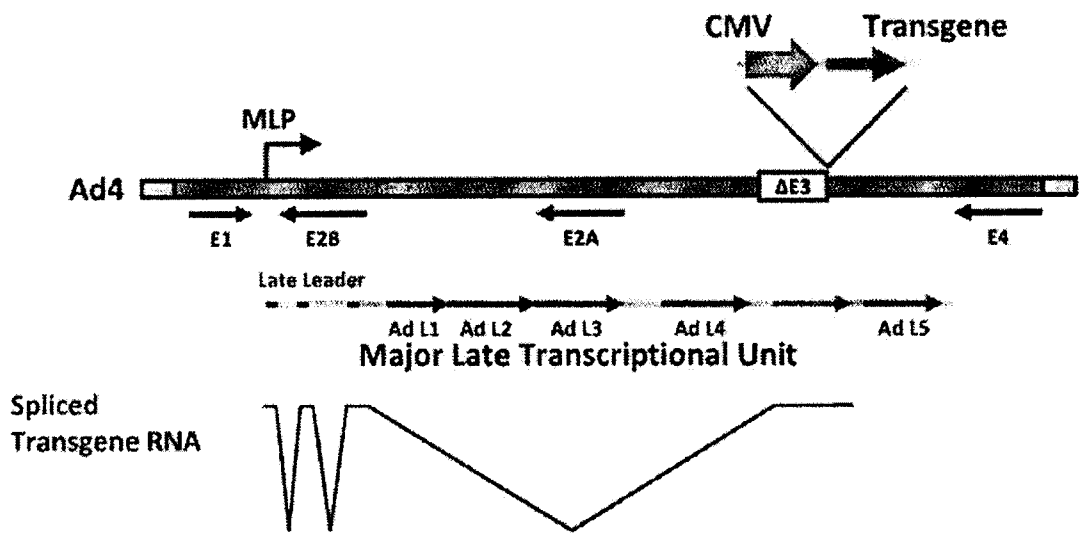

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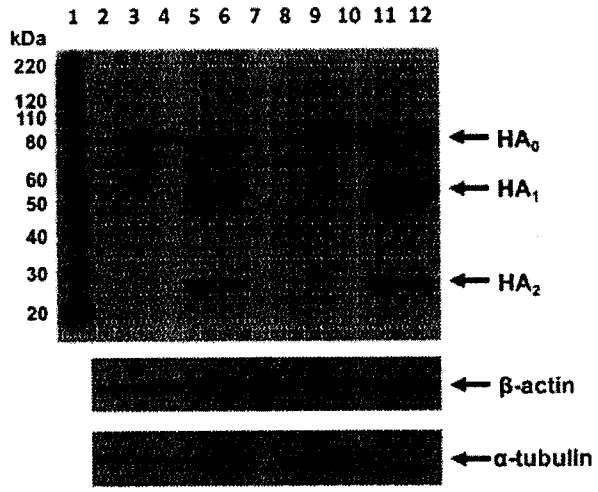
FIGURE 4



	Sample	Time (hrs)
1	Marker	
2	Uninfected A549 cells	24
3	PXVX0103	24
4	PXVX0113	24
5	PXVX0114	24
6	PXVX0124	24
7	—	
8	Uninfected A549 cells	48
9	PXVX0103	48
10	PXVX0113	48
11	PXVX0114	48
12	PXVX0124	48

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Application No:

Sheet No.: 5
Total No. of Sheets: 35

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FIGURE 5

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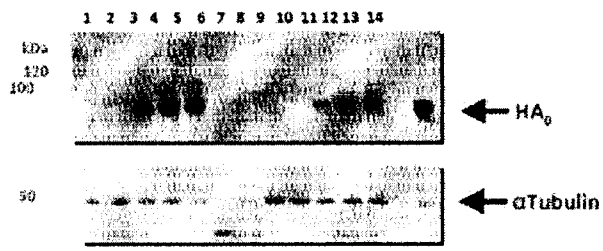
Time Course of Brisbane HA (Native) Expression from PXVX0101 and PXVX0111



Infection
Cells: A549, 5 x 10^5 cells/6-well in suspension
Inoculum: 2.5 x 10^7 P/mL PxVx 0101 clone#1
PxVx 0111 clone#8.1

Harvest: 3,6,18, 24 and 48 hours post infection, resuspended in 200µL RIPA buffer with protease inhibitors

Immunoblot
1° Ab: HA Tag, mouse monoclonal (1/1000, invivogen)
α tubulin, mouse monoclonal(1/5000)
2° Ab: Rat anti mouse IgG1 HRP (1/3000, invitrogen)
Substrate: Chemiluminescence (Pierce)

Samples: 0.6ug of total protein
Control: A/Brisbane/10/2007 100µL virus (HA titer 1:40) *added to 3 x 10^6 A549 cells in suspension and harvested at 19 hrs post infection*

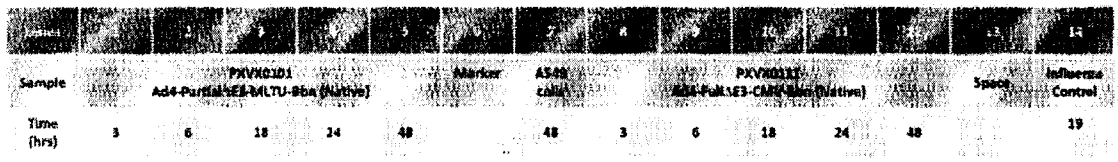


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FIGURE 6

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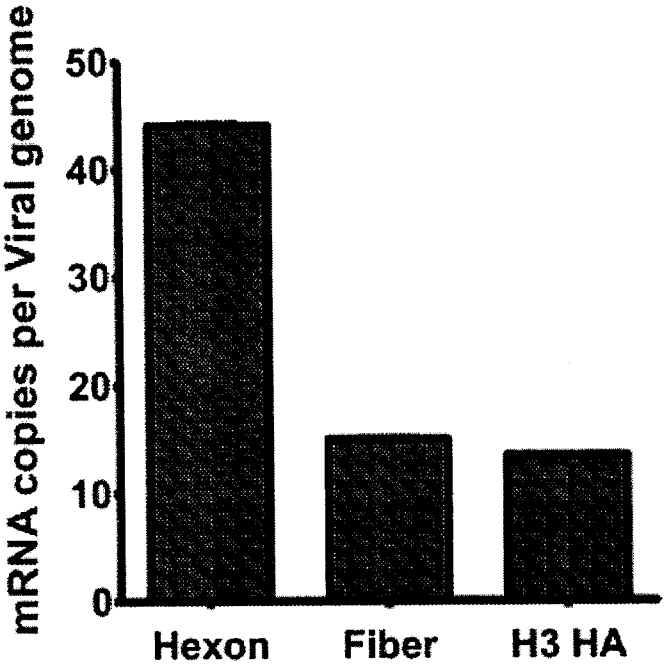

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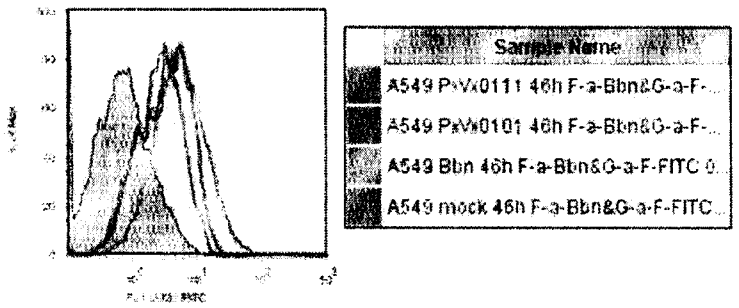
FIGURE 7

Surface Expression of HA (2 days post-infection)

Ab:
1st: Ferret anti-Bbn sera
2nd: Goat anti-Ferret-FITC
(surface staining)

Cells:
A549 (1 x 10^5 cells), infected with:
- PxVx0111: 50 MOI (BROWN)
- PxVx0101: 50 MOI (BLUE)
- Bbn: 100 µl (GREEN)
- Mock: no virus (RED)

PxVx0101: Ad4, PDE3, MLP, Bbn H3, Native;
PxVx0111: Ad4, PDE3, CMV, Bbn H3, Native;
Bbn: Influenza, H3N2, A/Brisbane/10/2007.



Samples	Freq. of FITC+ subset	Mean (Total Viable)	MFI (Δ)
A549 mock 46hr G-a-F-FITC	0.62%	0.97	
A549 mock 46hr F-a-Bbn&G-a-F-FITC	1.25%	1.15	0.18
A549 Bbn 46hr G-a-F-FITC	0.18%	0.85	
A549 Bbn 46hr F-a-Bbn&G-a-F-FITC	38.48%	8.32	7.47
A549 PxVx0101 46hr G-a-F-FITC	0.30%	1.32	
A549 PxVx0101 46hr F-a-Bbn&G-a-F-FITC	20.55%	4.47	3.15
A549 PxVx0111 46hr G-a-F-FITC	0.09%	1.20	
A549 PxVx0111 46hr F-a-Bbn&G-a-F-FITC	9.85%	3.50	2.3

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FIGURE 8A ORIGINAL

One-Step Growth Assay

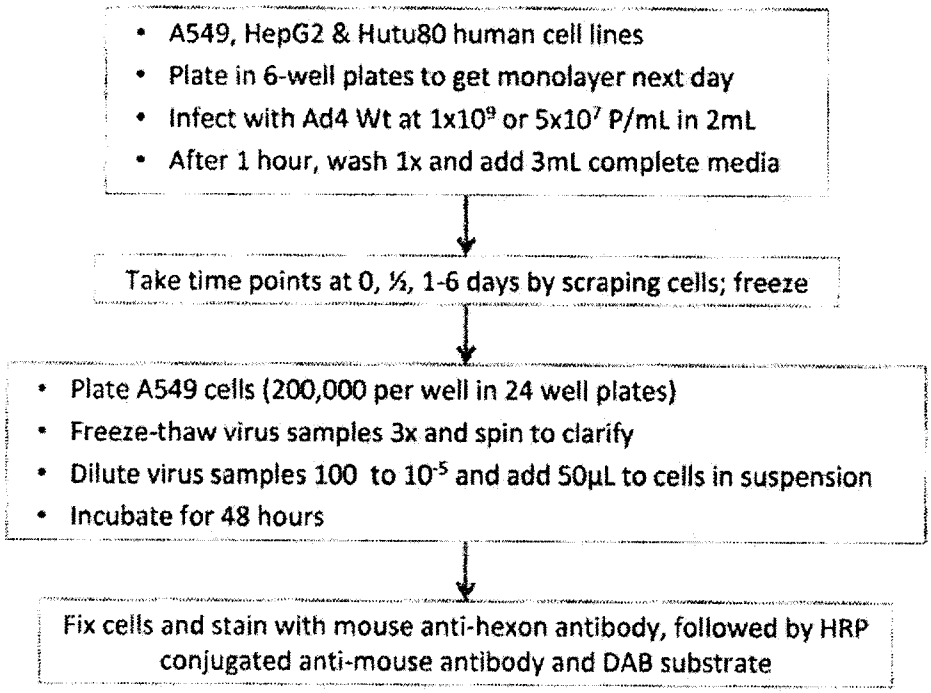

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FIGURE 8B

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One-Step Growth Assay
Comparison of PXVX0101 and PXVX0111

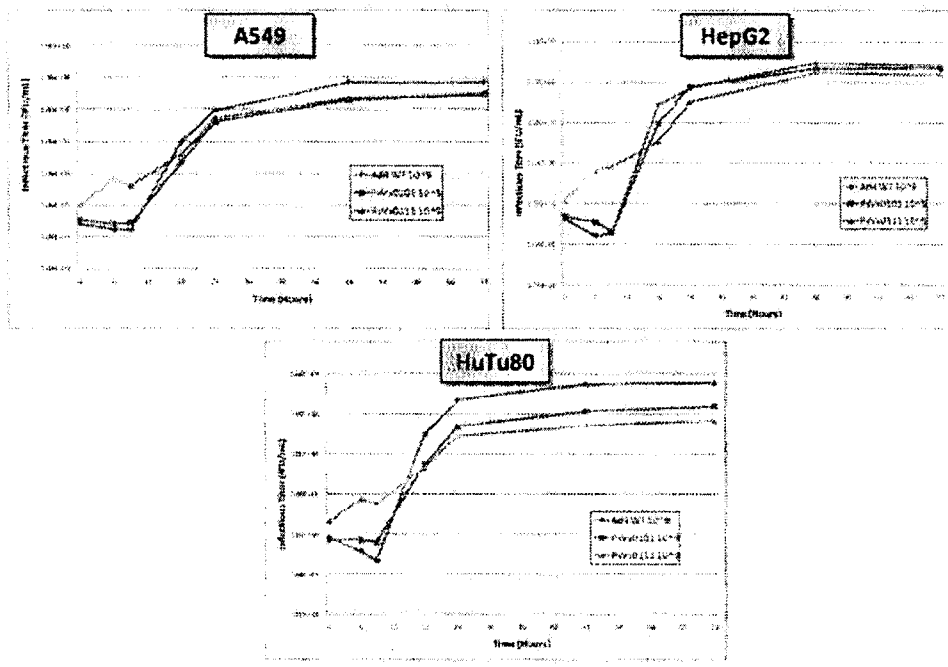

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FIGURE 9A

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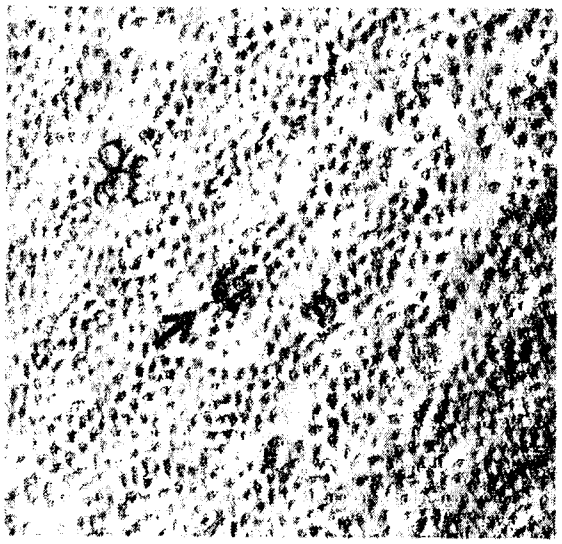
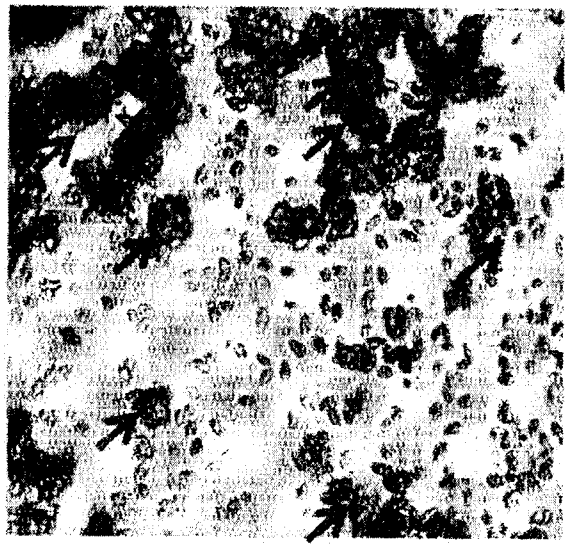
Influenza Virus Infected A549 Cells Plus Chicken RBCs



Flu Bbn (H3N2) Virus

+/-



Flu PR8 (H1N1) Virus

+++++

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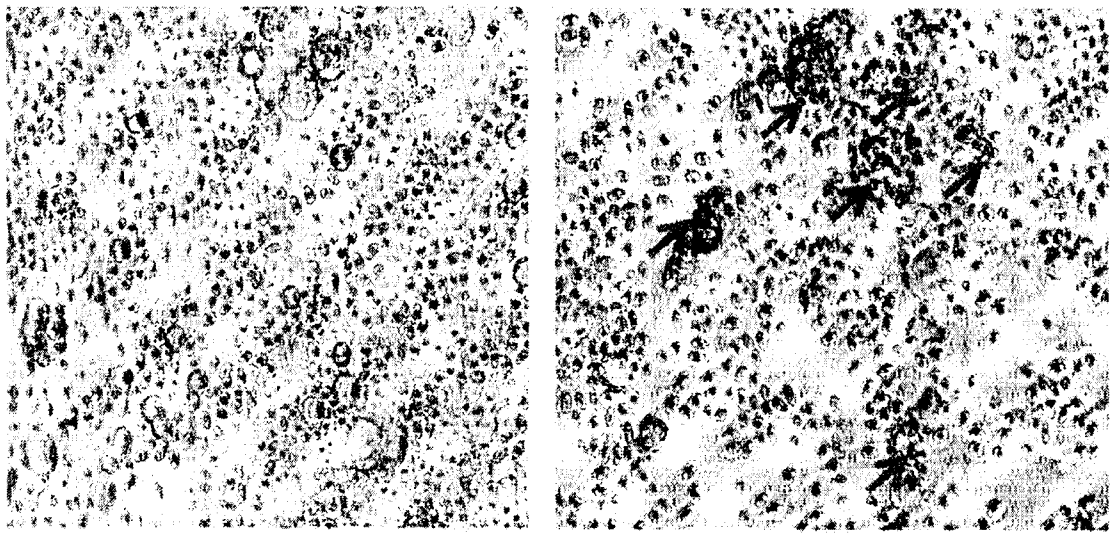
FIGURE 9B

Ad4-HA Infected A549 Cells Plus Chicken RBCs



Ad4 wt

-

PXVX0101 PE3 MLTU-Bbn H3

++

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FIGURE 10

Hexon Engineering

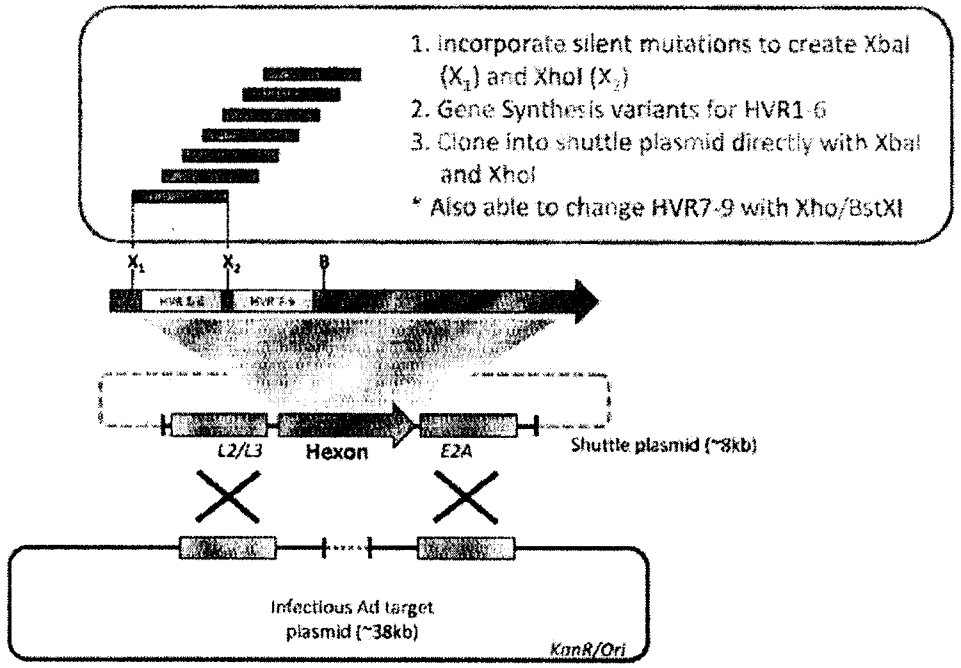

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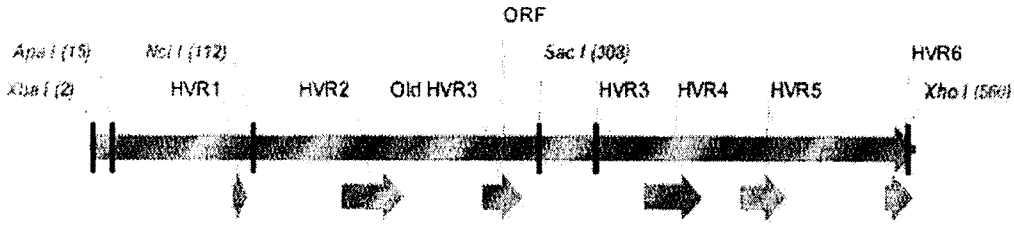
FIGURE 11

Hexon Engineering



HVR 1-6 region (XX)
564 bp

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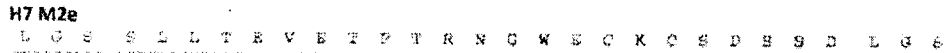
FIGURE 12

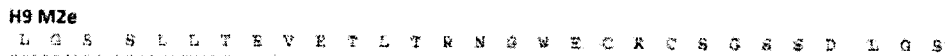
Epitope Sequences

H5 M2e

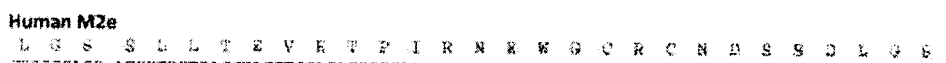
H7 M2e



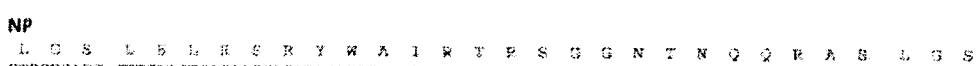
H9 M2e



Human M2e



NP



Matrix CTL 58-66

Legend:
Each peptide is flanked by LGS as a flexible spacer to improve stability
The matrix CTL is also flanked directly by sequences known to improve the processing of CTL peptides

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FIGURE 13

Diagram of insertion sites and sequences

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HVR	1	2	3	4	5	6	7
Hex1					M2e (H5)		
Hex2	M2e (H5)						
Hex3		M2e (H5)					
Hex4				M2e (H5)			
Hex5		M2e (H5)			M2e (H5)		
Hex6	M2e (H5)	M2e (H5)			M2e (H5)		
Hex7	M2e (H5)	M2e (H5)		M2e (H5)	M2e (H5)		
Hex8	M2e (H7)	M2e (H9)			M2e (H5)		
Hex9	M2e (H7)	M2e (H9)		M2e (human)	M2e (H5)		
Hex10	Ad7	Ad7	Ad7	Ad7			
Hex11	Ad7	Ad7	Ad7	Ad7	Ad7	Ad7	Ad7
Hex12	Matrix	NP			H5 (M2e)		
Hex13	Matrix						
Hex14		Matrix					
Hex15					Matrix		
Hex16	Matrix	Matrix			Matrix		
Hex17					NP		

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FIGURE 14

Purified PXVX0103 and PXVX0116 Induce HA-Specific Antibody Responses in Mice

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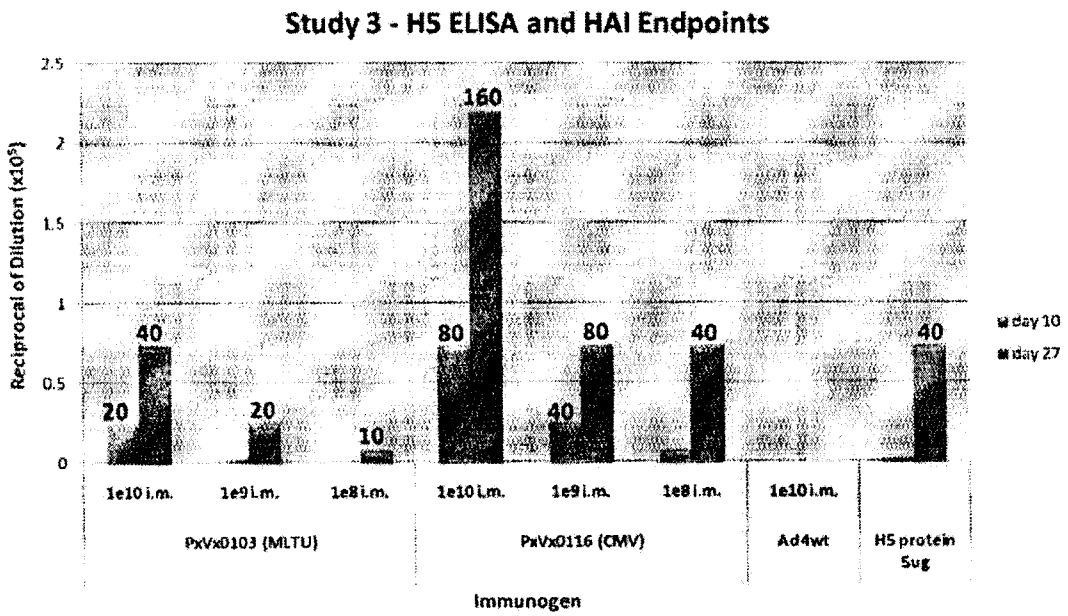

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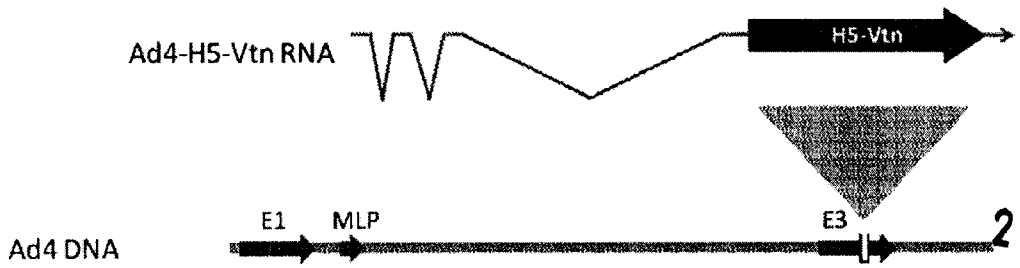
FIGURE 15



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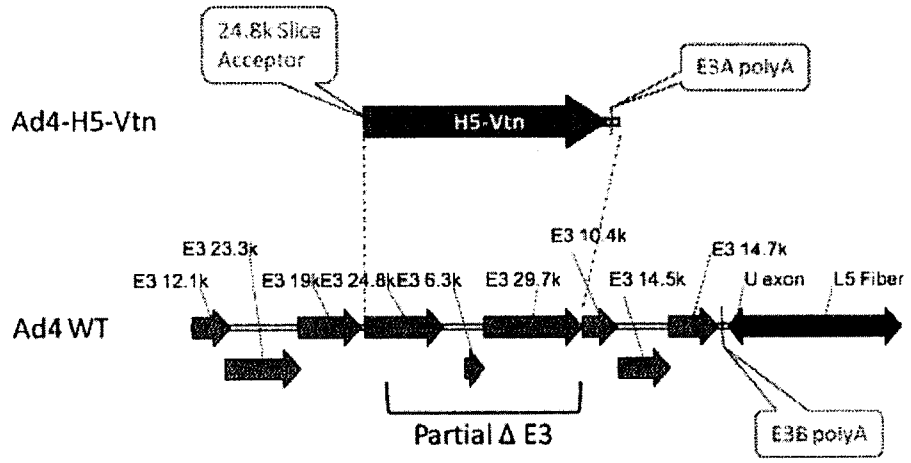

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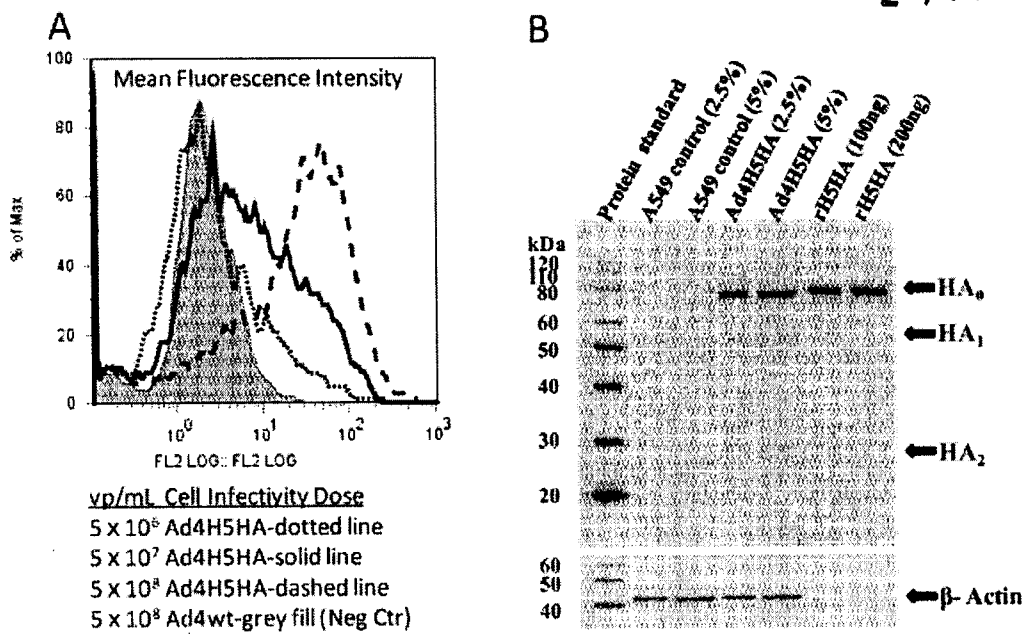
FIGURE 16



vp/mL Cell Infectivity Dose
5 x 10^6 Ad4H5HA-dotted line
5 x 10^7 Ad4H5HA-solid line
5 x 10^8 Ad4H5HA-dashed line
5 x 10^8 Ad4wt-grey fill (Neg Ctr)

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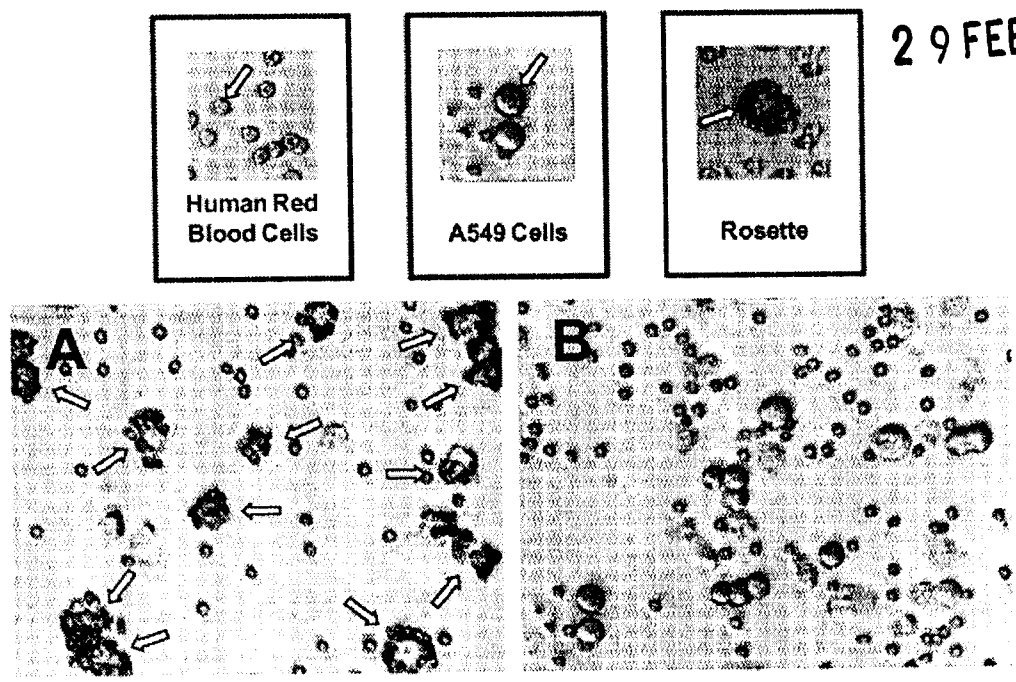
FIGURE 17



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182/DELNP/12

FIGURE 18

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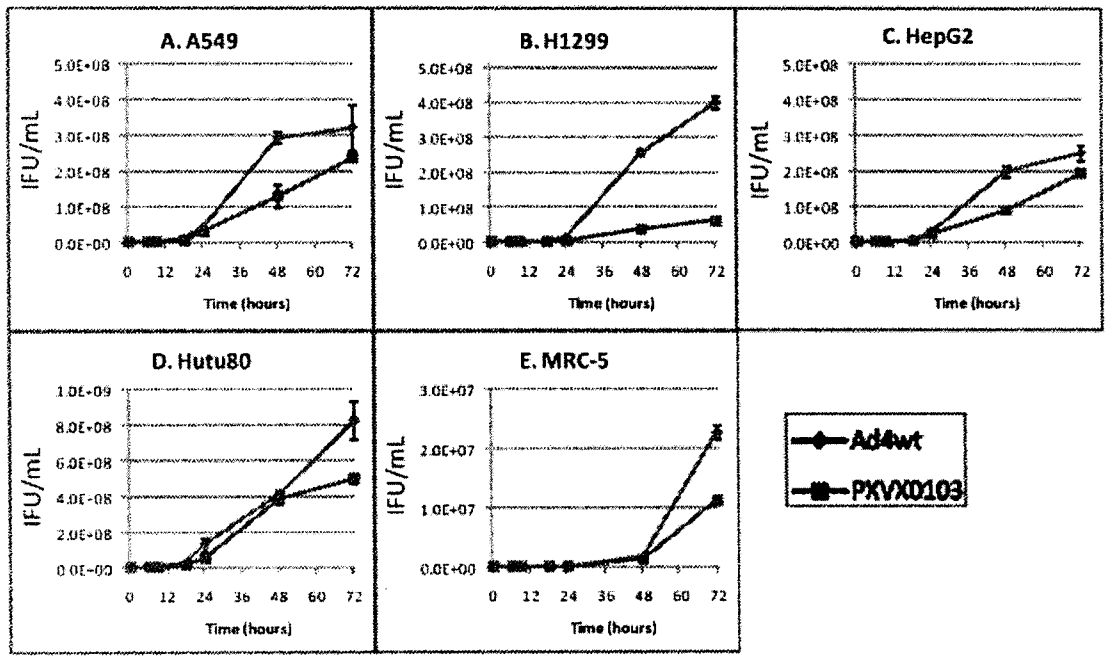

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FIGURE 19

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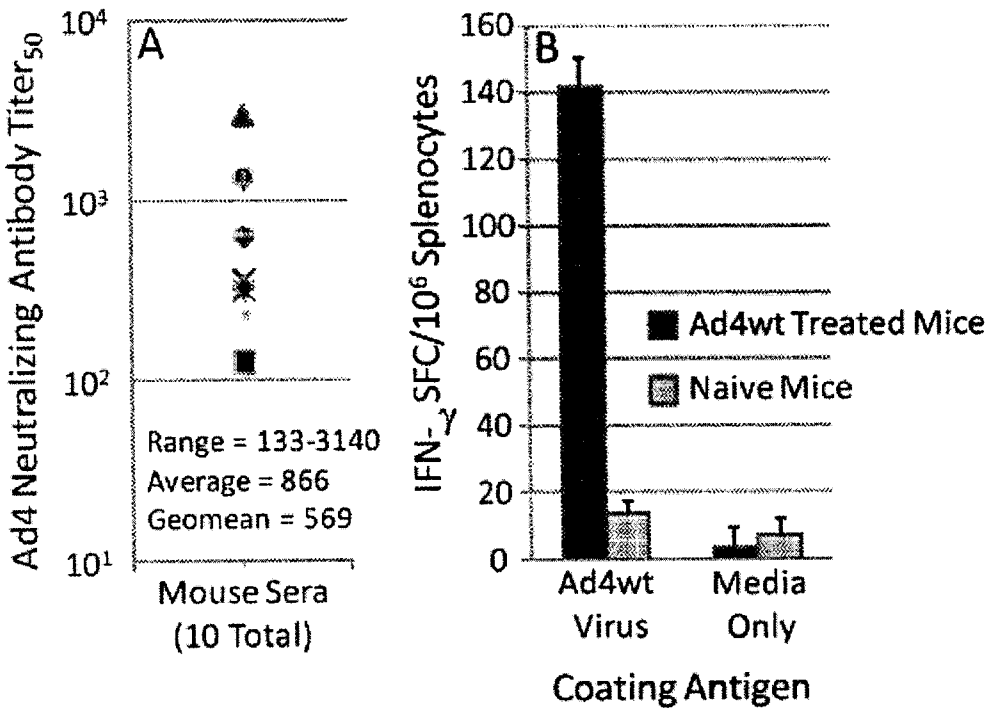

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FIGURE 20

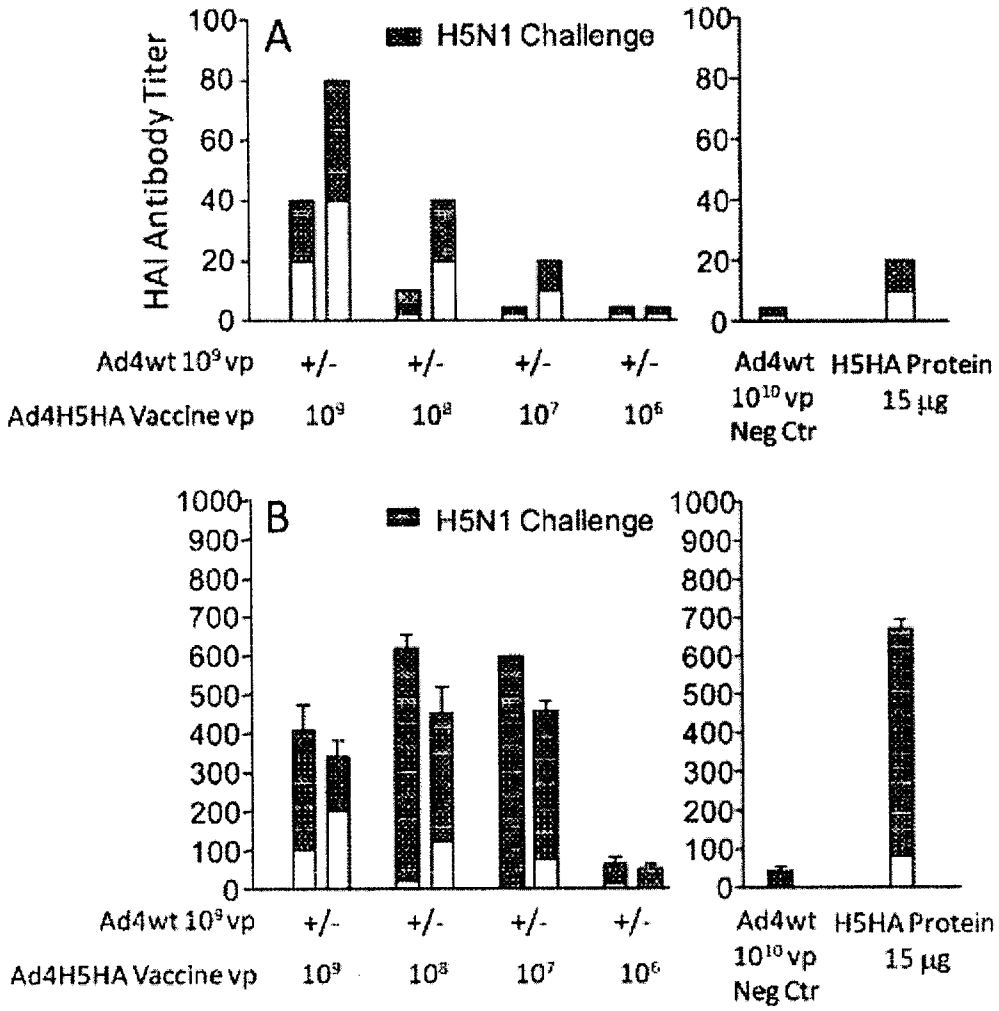

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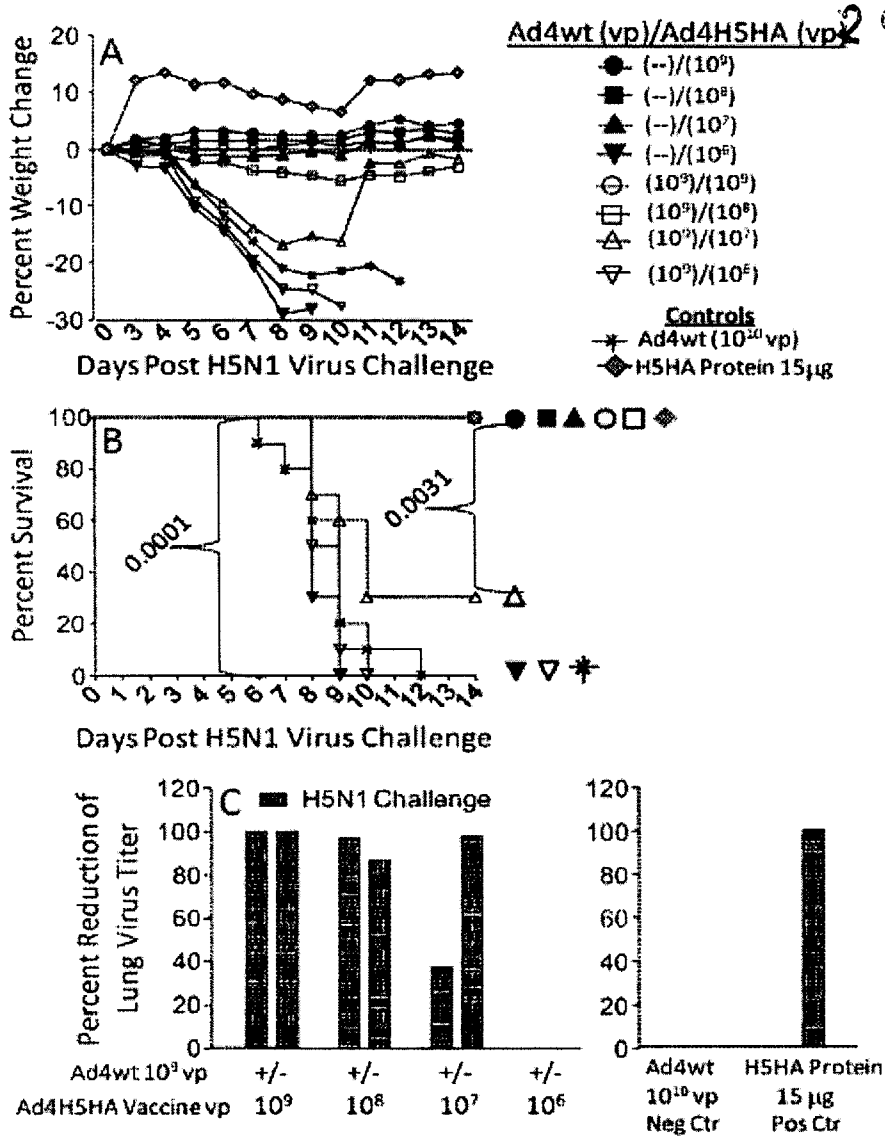
FIGURE 21



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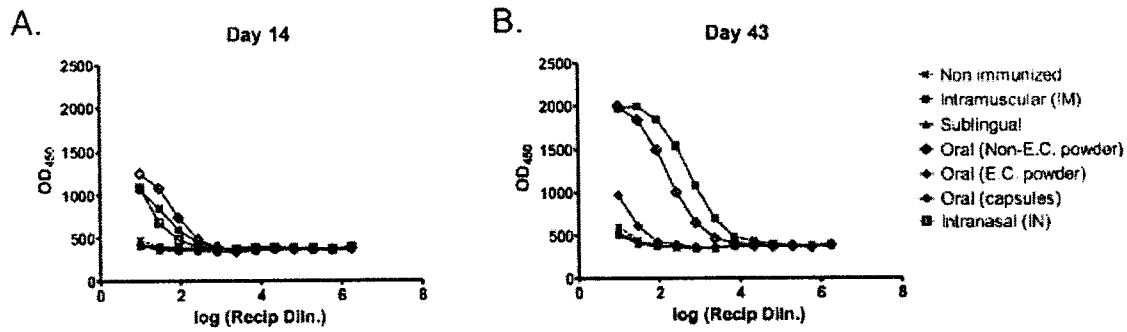
1827/DELNP/19

FIGURE 22

20 FEB 2012



C.

Route	Formulation	Day 14 (Dose 1)	Day 43 (Dose 2)
Non-immunized	—	10	30
I.M.	BDS	270	21870
S.L.	BDS	<10	10
O.G.	Non-E.C. powder	270	2430
O.G.	E.C. powder	<10	30
O.G.	Capsules	<10	10
I.N.	BDS	90	N/A

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FIGURE 23

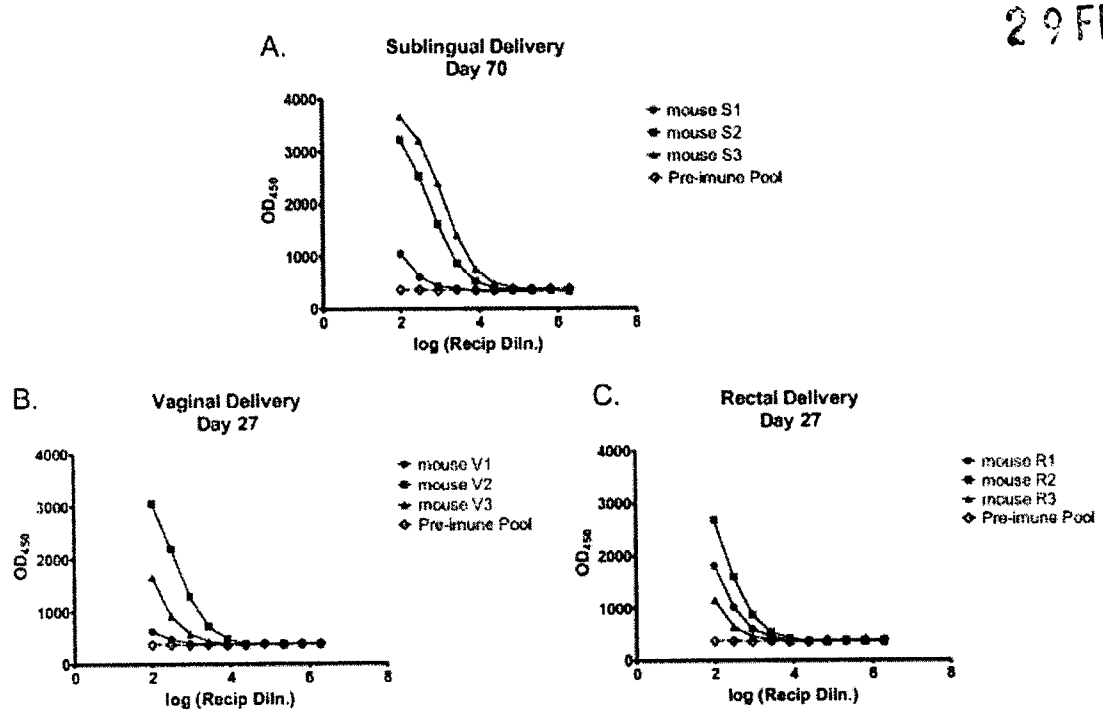

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FIGURE 24

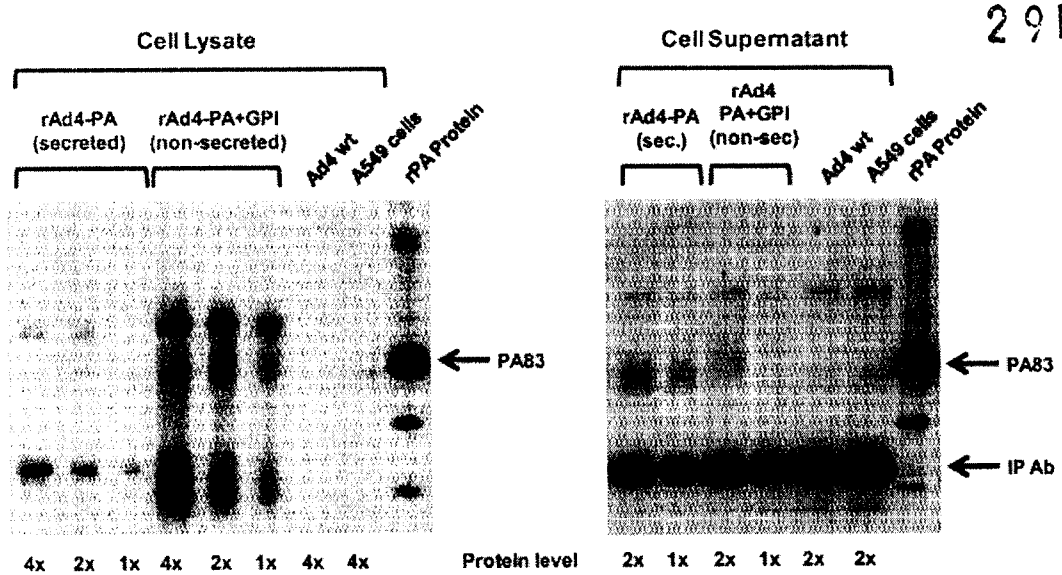

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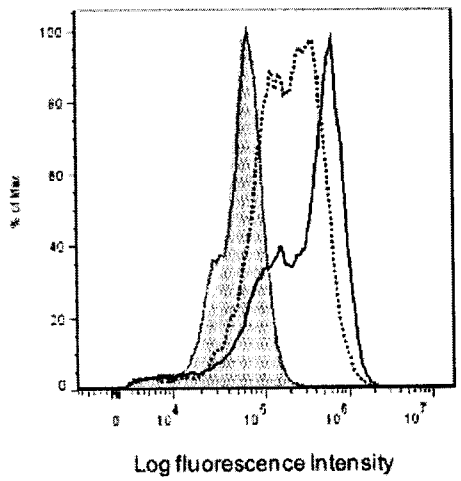
FIGURE 25



	MFI
rAd4- PA (secreted)	26052
rAd4- PA+GPI (non-secreted)	70869
Ad4 WT	3680
A549 cells	3537

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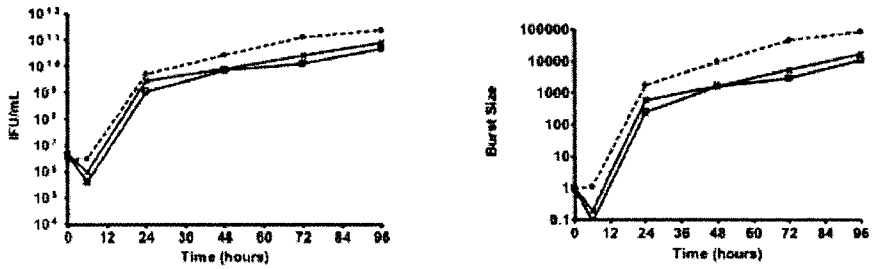
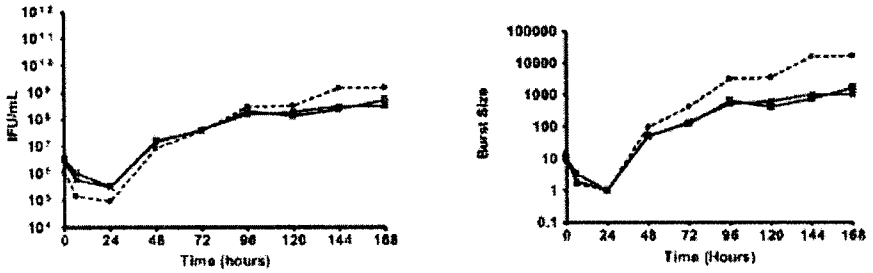
FIGURE 26

A549



MRC-5



WT Ad4 PXVX0212 (PA) PXVX0214 (PA+GPI)

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FIGURE 27

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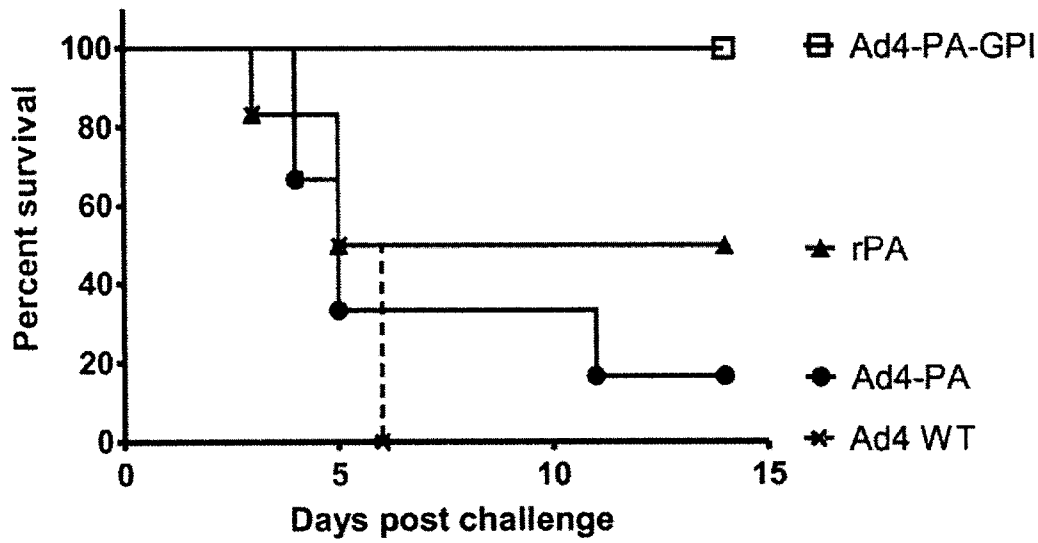

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FIGURE 28

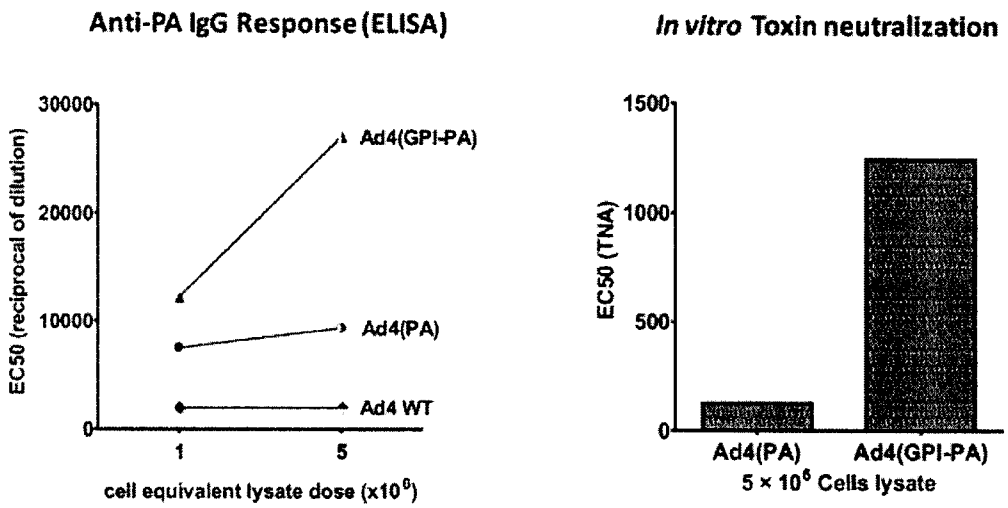

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FIGURE 29

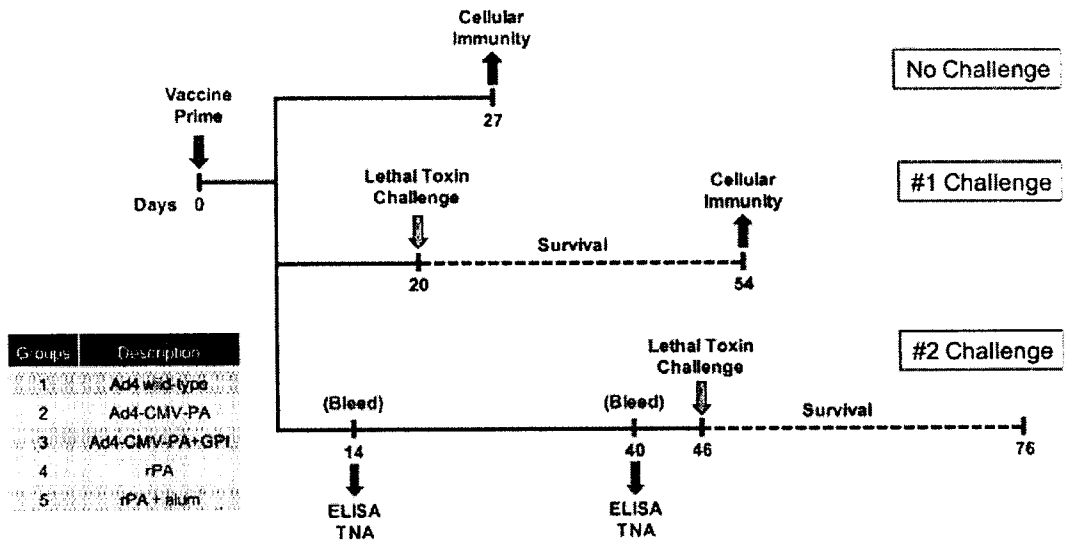

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FIGURE 30

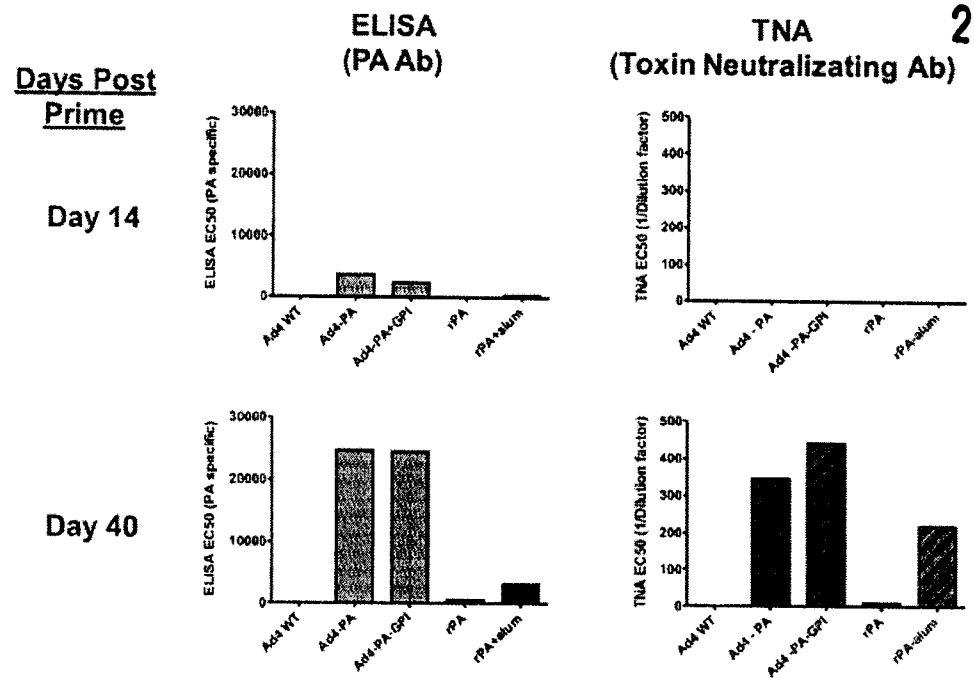

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FIGURE 31

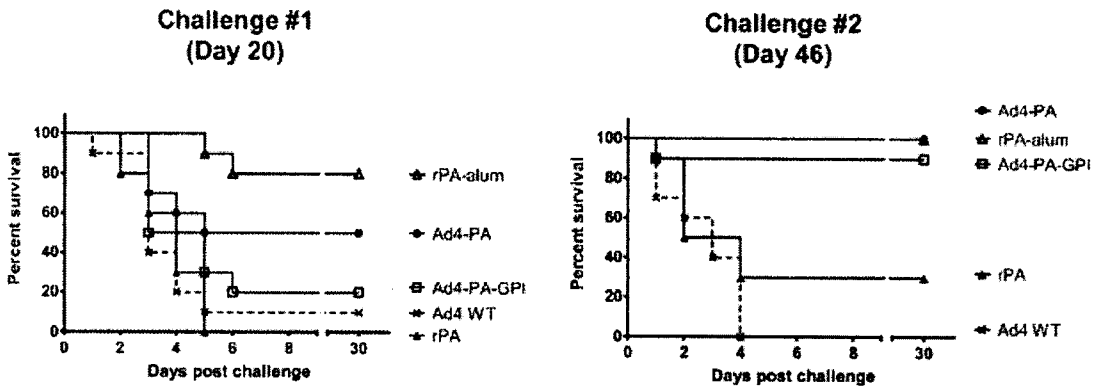

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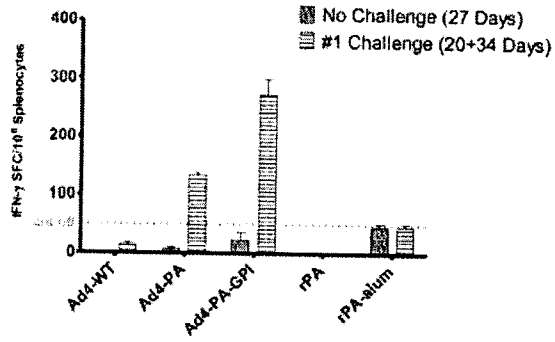
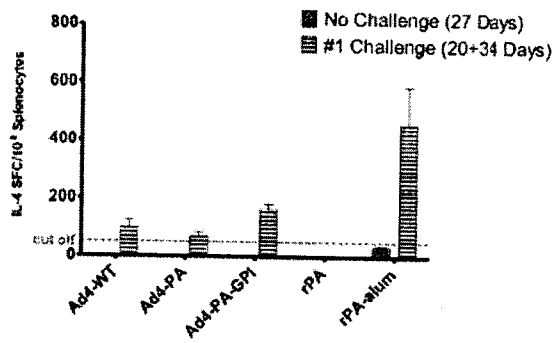
FIGURE 32



IFN-γ
Th1 Response
(T cell proliferation, macrophage activation)



IL-4
Th2 Response
(B cell Ig isotype switching, maintenance of ASC)

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Applicant(s) Name: **PAX-VAX, INC.**
Application No:

Sheet No.: 35
Total No. of Sheets: 35

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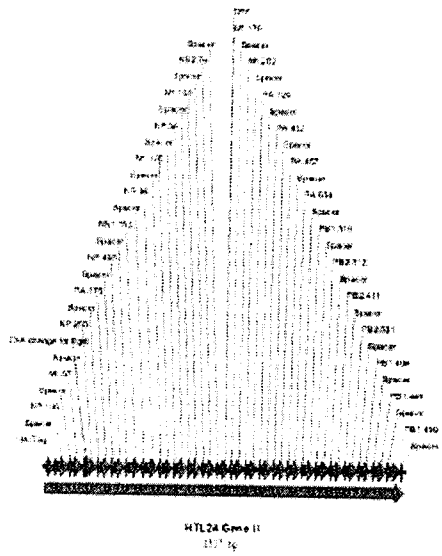
1827 DELNP 12

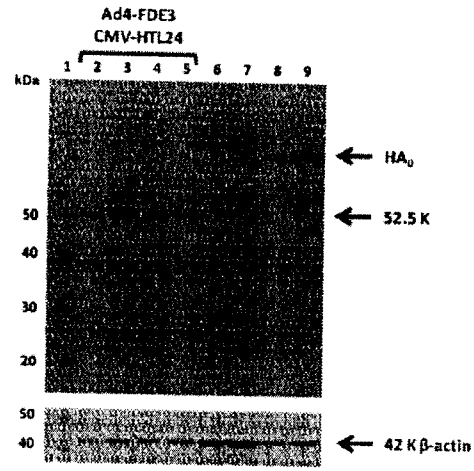
2012

FIGURE 33

A.



B.



Lane	Sample	Quantity
1	Magic marker	2 µL
2	Clone 15/4 CMV-HTL24	1x
3	Clone 15/4 CMV-HTL24	2x
4	Clone 19/1 CMV-HTL24	1x
5	Clone 19/1 CMV-HTL24	2x
6	A549 Cell control	1x
7	A549 Cell control	2x
8	A/Brisbane infected A549	1x
9	A/Brisbane infected A549	2x

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/230,617, filed July 31, 2009, which is herein incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: PAXV_004_01WO_SeqList_ST25.txt, date recorded: July 30, 2010, file size 62 kilobytes).

BACKGROUND OF THE INVENTION

Adenoviruses have been widely studied as infectious agents, as a subject for basic research, and for their potential use in gene therapy and vaccines. Forty-nine human adenoviral serotypes have been identified and they are categorized into six subgenera (A through F) based on nucleic acid comparisons, fiber protein characteristics, and biological properties. For example, group A includes serotypes 12 and 31, group B includes serotypes 3 and 7, group C includes serotypes 2 and 5, group D includes serotypes 8 and 30, group E includes serotype 4, and group F includes serotypes 40 and 41.

In terms of general structure, all adenoviruses examined to date are nonenveloped, regular icosahedrons of about 80 nanometers in diameter. Adenoviruses contain linear, double-stranded DNA that is complexed with core proteins and surrounded by the adenoviral capsid. Individual virions contain about 11 different proteins designated by Roman numerals (II-XII), in order of their decreasing size on SDS gels.

The capsid is composed of seven structural proteins: II (hexon), III (penton), IIIa, IV (fiber), VI, VII, and IX. The capsid comprises 252 capsomeres, of which 240 are hexon capsomeres and 12 are penton capsomeres. Hexon capsomeres, which are trimers of the hexon protein, make up about 75% of the protein of the capsid. Penton capsomeres, which are pentamers of the penton protein, are situated at each of the 12 vertices of the virion. Each penton capsomer is bound to six adjacent hexon capsomeres and a fiber. The fiber, which is usually a trimer of the fiber protein, projects from the penton capsomer. The hexon protein and, to a lesser

extent, the fiber protein comprise the main antigenic determinants of an adenovirus and also determine serotype specificity.

Researchers have examined and compared the structure of the capsid proteins of different adenoviral serotypes, and in particular hexon proteins, in an effort to define the regions of the proteins against which neutralizing antibodies are elicited. The predominant regions in hexon protein against which neutralizing antibodies are directed appear to be in loops 1 and 2 (*i.e.*, LI or l1, and LII or l2, respectively), which project outward from the base of the hexon capsomere. Analysis of loops 1 and 2 from different adenovirus hexon proteins has revealed the presence of seven discrete hypervariable regions (HVR1 to HVR7) corresponding to locations where the hexon proteins differ considerably between serotypes.

The core of an adenovirus virion contains the linear double-stranded DNA genome and associated proteins V, VII, X (mu), IVa2, and terminal protein (TP). The genome organization of different adenoviruses is conserved and has been proposed to have a timing function, wherein the ends of the genome are transcribed first (the immediate early genes E1 and E4 are located at opposite ends of the linear genome). Early transcription of E1 and E4 leads to the opening of the central region of the genome, allowing transcription of the central region.

Adenoviral genomes typically comprise eight RNA polymerase II transcriptional units: five early units, E1A, E1B, E2A-E2B, E3, and E4; two delayed early units, IX and IVa2; and the Major Late transcriptional unit. The Major Late transcriptional unit is further subdivided into L1-L5 regions based upon the use of alternative splicing sites. The transcriptional units often express proteins of similar function. For example, the E1A unit codes for two proteins responsible for activation of transcription and induction of S-phase upon cellular infection; the E1B transcription unit encodes two proteins that inhibit cellular apoptosis; the E3 transcriptional unit is involved in evasion of the immune response; and the Major Late transcriptional unit encodes structural proteins necessary for assembly of the capsid.

For the purpose of gene therapy and vaccination, recombinant adenoviral vectors have been designed to encode and express heterologous genes and antigens. The Ad2 and Ad5 serotypes have been used most extensively in this context. Heterologous sequences have been inserted into the adenoviral genomes, including in the early transcriptional units and in the coding regions of various structural proteins, such as hexon, penton, and fiber. In many cases, deletions in the adenoviral genome (*e.g.*, in the E1 regions) have been used to create replication-

defective adenoviral vectors, which have generally been considered safer for administration to human subjects. Despite such extensive research and development, there remains a need in the art for new recombinant adenoviral vectors suitable, for example, as vaccines for infectious diseases.

SUMMARY OF THE INVENTION

The present invention is directed to recombinant adenoviral vectors that find use as effective vaccines. The invention is based, in part, on the development of novel recombinant adenoviral vectors that express heterologous sequences at high levels. The invention is also based, in part, on the development of novel recombinant adenoviral vectors designed to improve host immune response and circumvent pre-existing neutralizing antibodies. The invention is also based, in part, on the development of novel recombinant adenoviral vectors to be used as antigen-specific and/or universal influenza vaccines.

Accordingly, in one aspect, the invention provides a vaccine comprising an adenoviral vector comprising a first heterologous sequence, wherein the adenoviral vector is replication competent and has a partial E3 deletion. In certain embodiments, the adenoviral vector is derived from an Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, or Ad50 adenovirus. In other embodiments, the adenoviral vector is derived from a chimpanzee adenovirus, for instance, Ad C1, Ad C3, Ad C6, Ad C7, or Ad68. In certain embodiments, the first heterologous sequence is integrated into a location containing the partial E3 deletion. In certain embodiments, the first heterologous sequence is under the control of or operably linked to an adenoviral transcriptional and/or translational control sequence. For example, the first heterologous sequence can be under the control of or operably linked to an adenoviral Major Late Promoter (MLP), an adenoviral tripartite leader (TPL) sequence, an adenoviral splice acceptor sequence, and/or an adenoviral poly-adenylation signal sequence. In certain embodiments, the first heterologous sequence comprises and/or is under the control of an non-adenoviral transcriptional and/or translational control sequence, such as an enhancer, promoter, intron sequence, and/or leader sequence from cytomegalovirus (CMV), rous sarcoma virus (RSV), or simian virus 40 (SV40), or any combination of such elements. In certain embodiments, the first heterologous sequence is modified to increase expression. For example, the first heterologous sequence can be codon optimized and/or modified to include a consensus Kozak sequence. In certain embodiments, the