

WHAT IS CLAIMED IS:

1. A synthetic chimeric poxvirus (scPV) that is replicated and reactivated from DNA derived from synthetic DNA, the viral genome of said virus differing from a wild type genome of said virus in that it is characterized by one or more modifications, the modifications being derived from a group comprising chemically-synthesized DNA, cDNA or genomic DNA.
2. The scPV of claim 1, wherein the synthetic DNA is selected from one or more of: chemically synthesized DNA, PCR amplified DNA, engineered DNA and polynucleotides comprising nucleoside analogs.
3. The scPV of claim 1, wherein the synthetic DNA is chemically synthesized DNA.
4. The scPV of any one of claims 1 to 3, wherein the one or more modifications comprise one or more deletions, insertions, substitutions, or a combination thereof.
5. The scPV of any one of claims 1 to 4, wherein the one or more modifications comprise one or more modifications to introduce one or more unique restriction sites.
6. The scPV of any one of claims 1 to 5, wherein the viral genome comprises heterologous terminal hairpin loops.
7. The scPV of any one of claims 1 to 6, wherein the viral genome comprises terminal hairpin loops derived from vaccinia virus.
8. The scPV of claim 7, wherein the left and right terminal hairpin loops a) comprise the slow form and the fast form of the vaccinia virus terminal hairpin loop, respectively, b) comprise the fast form and the slow form of the vaccinia virus terminal hairpin loop, respectively, c) both comprise the slow form of the vaccinia virus terminal hairpin loop, or d) both comprise the fast form of the vaccinia virus terminal loop.

9. The scPV of any one of claims 1 to 8, wherein the virus is replicated and reactivated from overlapping chemically-synthesized DNA fragments that correspond to substantially all of the viral genome of the scPV.
10. The scPV of claim 9, wherein the virus is replicated and reactivated from 1-14 overlapping fragments.
11. The scPV of claim 9, wherein the virus is replicated and reactivated from 8-12 overlapping fragments.
12. The scPV of claim 9, wherein the virus is replicated and reactivated from 10 overlapping fragments.
13. The scPV of any one of claims 1 to 12, wherein the virus is reactivated using leporipox virus-catalyzed recombination and reactivation.
14. The scPV of claim 13, wherein the leporipox virus is selected from the group consisting of: Shope fibroma virus (SFV), hare fibroma virus, rabbit fibroma virus, squirrel fibroma virus, and myxoma virus.
15. A synthetic chimeric orthopox virus (scOPV) that is replicated and reactivated from DNA derived from synthetic DNA, the viral genome of said virus differing from a wild type genome of said virus in that it is characterized by one or more modifications, the modifications being derived from a group comprising chemically-synthesized DNA, cDNA or genomic DNA.
16. The scOPV of claim 15, wherein the synthetic DNA is selected from one or more of: chemically synthesized DNA, PCR amplified DNA, engineered DNA and polynucleotides comprising nucleoside analogs.
17. The scOPV of claim 15, wherein the synthetic DNA is chemically synthesized DNA.
18. The scOPV of any one of claims 15 to 17, wherein the viral genome of the scOPV is based on an OPV selected from the group consisting of: camelpox (CMLV) virus, cowpox virus (CPXV), ectromelia virus (ECTV), horsepox virus

(HPXV), monkeypox virus (MPXV), vaccinia virus (VACV), variola virus (VARV), rabbitpox virus (RPXV), raccoon poxvirus, skunkpox virus, Taterapox virus, Uasin Gishu disease virus, and volepox virus.

19. The scOPV of any one of claims 15 to 18, wherein the viral genome of the
5 scOPV is based on a VACV.

20. The scOPV of claim 19, wherein the viral genome of the scOPV is based on the genome of VACV strain ACAM2000 and differs from the ACAM2000 genome in that it is characterized by one or more modifications.

21. The scOPV of claim 19, wherein the viral genome of the scOPV is based on
10 the genome of VACV strain IOC and differs from the IOC genome in that it is characterized by one or more modifications.

22. The scOPV of claim 19, wherein the viral genome of the scOPV is based on the genome of VACV strain MVA and differs from the MVA genome in that it is characterized by one or more modifications.

15 23. The scOPV of claim 19, wherein the viral genome of the scOPV is based on the genome of VACV strain MVA-BN and differs from the MVA-BN genome in that it is characterized by one or more modifications

24. The scOPV of claim 19, wherein the wild type VACV genome is the genome of a strain selected from the group consisting of: Western Reserve, Clone 3, Tian
20 Tian, Tian Tian clone TT9, Tian Tian clone TP3, NYCBH, Wyeth, Copenhagen, Lister 107, Lister-LO, IHD-W, LC16m18, Lederle, Tashkent clone TKT3, Tashkent clone TKT4, USSR, Evans, Praha, LIVP, Ikeda, EM-63, Malbran, Duke, 3737, CV-1, Connaught Laboratories, Serro 2, CM-01, Dryvax clone DPP13, Dryvax clone DPP15, Dryvax clone DPP20, Dryvax clone DPP17, Dryvax clone
25 DPP21, and chorioallantois vaccinia virus Ankara.

25. The scOPV of any one of claims 15 to 24, wherein the one or more modifications comprise one or more deletions, insertions, substitutions, or a combination thereof.

26. The scOPV of any one of claims 15 to 25, wherein the one or more modifications comprise one or more modifications to introduce one or more unique restriction sites.
27. The scOPV of any one of claims 15 to 26, wherein the one or more
5 modifications comprise one or more modifications to eliminate one or more restriction sites.
28. The scOPV of claim 27, wherein the one or more modifications comprise one or more modifications to eliminate one or more *AarI* restriction sites.
29. The scOPV of claim 28, wherein the one or more modifications comprise one
10 or more modifications to eliminate all *AarI* restriction sites.
30. The scOPV of any one of claims 15 to 29, wherein the one or more modifications comprise one or more modifications to eliminate one or more *BsaI* restriction sites.
31. The scOPV of any one of claims 15 to 30, wherein the viral genome comprises
15 heterologous terminal hairpin loops.
32. The scOPV of any one of claims 15 to 30, wherein the viral genome comprises terminal hairpin loops derived from vaccinia virus.
33. The scOPV of claim 32, wherein the left and right terminal hairpin loops a) comprise the slow form and the fast form of the vaccinia virus terminal hairpin
20 loop, respectively, b) comprise the fast form and the slow form of the vaccinia virus terminal hairpin loop, respectively, c) both comprise the slow form of the vaccinia virus terminal hairpin loop, or d) both comprise the fast form of the vaccinia virus terminal loop.
34. The scOPV of claim 33, wherein the slow form comprises a nucleotide
25 sequence that is at least 85% identical to the nucleotide sequence of SEQ ID NO: 11 and the fast form comprises a nucleotide sequence that is at least 85% identical to the nucleotide sequence of SEQ ID NO: 12.

35. The scOPV of claim 33, wherein the slow form comprises a nucleotide sequence that is at least 90% identical to the sequence of SEQ ID NO: 11 and the fast form comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 12.
- 5 36. The scOPV of claim 33, wherein the slow form comprises a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 11 and the fast form comprises a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 12.
- 10 37. The scOPV of claim 33, wherein the slow form consists of the nucleotide sequence of SEQ ID NO: 11 and the fast form consists of the nucleotide sequence of SEQ ID NO: 12.
38. The scOPV of any one of claims 15 to 37, wherein the virus is replicated and reactivated from overlapping chemically-synthesized DNA fragments that correspond to substantially all of the viral genome of the OPV.
- 15 39. The scOPV of claim 38, wherein the virus is replicated and reactivated from 1-14 overlapping fragments.
40. The scOPV of claim 38, wherein the virus is replicated and reactivated from 8-12 overlapping fragments.
- 20 41. The scOPV of claim 38, wherein the virus is replicated and reactivated from 10 overlapping fragments.
42. The scOPV of any one of claims 15 to 41, wherein the virus is reactivated using leporipox virus-catalyzed recombination and reactivation.
43. The scOPV of claim 42, wherein the leporipox virus is selected from the group consisting of: Shope fibroma virus (SFV), hare fibroma virus, rabbit fibroma virus, 25 squirrel fibroma virus, and myxoma virus.
44. A synthetic chimeric horsepox virus (schPXV) that is replicated and reactivated from synthetic DNA, the viral genome differing from a wild type

genome of HPXV in that it is characterized by one or more modifications, the modifications being derived from a group comprising chemically-synthesized DNA, cDNA or genomic DNA.

45. The scHPXV of claim 44, wherein the synthetic DNA is selected from one or
5 more of: chemically synthesized DNA, PCR amplified DNA, engineered DNA and polynucleotides comprising nucleoside analogs.

46. The scHPXV of claim 44, wherein the synthetic DNA is chemically synthesized DNA.

47. The scHPXV of any one of claims 44 to 46, wherein the viral genome is based
10 on the genome of HPXV strain MNR-76 and differs from the MNR-76 genome in that it is characterized by one or more modifications.

48. The scHPXV of any one of claims 44 to 47, wherein the one or more modifications comprise one or more deletions, insertions, substitutions, or a combination thereof.

49. The scHPXV of any one of claims 44 to 48, wherein the one or more
15 modifications comprise one or more modifications to introduce one or more unique restriction sites.

50. The scHPXV of claim 49, wherein the one or more modifications are present in *HPXV044* or *HPXV095*.

20 51. The scHPXV of any one of claims 44 to 50, wherein the one or more modifications comprise one or more mutations listed in Table 3.

52. The scHPXV of any one of claims 44 to 51, wherein the one or more modifications comprise one or more modifications to eliminate one or more restriction sites.

25 53. The scHPXV of claim 52, wherein the one or more modifications comprise one or more modifications to eliminate one or more *AarI* restriction sites.

54. The scHPXV of claim 53, wherein the one or more modifications comprise one or more modifications to eliminate all *AarI* restriction sites.
55. The scHPXV of any one of claims 44 to 54, wherein the one or more modifications comprise one or more modifications to eliminate one or more *BsaI* restriction sites.
56. The scHPXV of any one of claims 44 to 55, wherein the one or more modifications comprise one or more mutations listed in Table 2.
57. The scHPXV of any one of claims 44 to 56, wherein the viral genome comprises heterologous terminal hairpin loops.
58. The scHPXV of claim 57, wherein the viral genome comprises terminal hairpin loops derived from vaccinia virus.
59. The scHPXV of claim 58, wherein the left and right terminal hairpin loops a) comprise the slow form and the fast form of the vaccinia virus terminal hairpin loop, respectively, b) comprise the fast form and the slow form of the vaccinia virus terminal hairpin loop, respectively, c) both comprise the slow form of the vaccinia virus terminal hairpin loop, or d) both comprise the fast form of the vaccinia virus terminal loop.
60. The scHPXV of claim 59, wherein the slow form comprises a nucleotide sequence that is at least 85% identical to the sequence of SEQ ID NO: 11 and the fast form comprises a nucleotide sequence that is at least 85% identical to the nucleotide sequence of SEQ ID NO: 12.
61. The scHPXV of claim 59, wherein the slow form comprises a nucleotide sequence that is at least 90% identical to the sequence of SEQ ID NO: 11 and the fast form comprises a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO: 12.
62. The scHPXV of claim 59, wherein the slow form comprises a nucleotide sequence that is at least 95% identical to the sequence of SEQ ID NO: 11 and the

fast form comprises a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO: 12.

63. The scHPXV of claim 59, wherein the slow form consists of the nucleotide sequence of SEQ ID NO: 11 and the fast form consists of the nucleotide sequence of SEQ ID NO: 12.

64. The scHPXV of claim 57, wherein the viral genome comprises terminal hairpin loops derived from camelpox virus, cowpox virus, ectromelia virus, monkeypox virus, variola virus, rabbitpox virus, raccoon poxvirus, skunkpox virus, Taterapox virus, Uasin Gishu disease virus, or volepox virus.

65. The scHPXV of any one of claims 44 to 64, wherein the virus is replicated and assembled from overlapping chemically-synthesized DNA fragments that correspond to substantially all of the viral genome of HPXV.

66. The scHPXV of claim 65, wherein the virus is replicated and reactivated from 1-14 overlapping fragments.

67. The scHPXV of claim 65, wherein the virus is replicated and reactivated from 8-12 overlapping fragments.

68. The scHPXV of claim 65, wherein the virus is replicated and reactivated from 10 overlapping fragments.

69. The scHPXV of any one of claims 44 to 68, wherein the virus is reactivated using leporipox virus-catalyzed recombination and reactivation.

70. The scHPXV of claim 69, wherein the leporipox virus is selected from the group consisting of: Shope fibroma virus (SFV), hare fibroma virus, rabbit fibroma virus, squirrel fibroma virus, and myxoma virus.

71. A method of producing a synthetic chimeric poxvirus (scPV) comprising the steps of:

(i) chemically synthesizing overlapping DNA fragments that correspond to substantially all of the viral genome of the poxvirus;

- (ii) transfecting the overlapping DNA fragments into helper virus-infected cells;
 - (iii) culturing said cells to produce a mixture of helper virus and synthetic chimeric poxviral particles in said cells; and
 - (iv) plating the mixture on host cells specific to the scPV to recover the scPV.
- 5 72. The method of claim 71, wherein the helper virus is a leporipox virus.
73. The method of claim 72, wherein the leporipox virus is selected from the group consisting of: Shope fibroma virus (SFV), hare fibroma virus, rabbit fibroma virus, squirrel fibroma virus, and myxoma virus.
74. The method of claim 73, wherein the leporipox virus is SFV.
- 10 75. The method of claim 71, wherein the helper virus is fowlpox virus.
76. The method of claim 71, wherein the helper virus is a psoralen-inactivated helper virus.
77. The method of any one of claims 71 to 74, wherein the helper virus-infected cells are BGMK cells.
- 15 78. The method of any one of claims 71 to 74, wherein step (i) further comprises chemically synthesizing terminal hairpin loops from a poxvirus and ligating them onto the fragments comprising the left and right termini of the viral genome.
79. A method of producing a synthetic chimeric orthopox virus (scOPV) comprising the steps of:
- 20 (i) chemically synthesizing overlapping DNA fragments that correspond to substantially all of the viral genome of the OPV;
- (ii) transfecting the overlapping DNA fragments into helper virus-infected cells;
- (iii) culturing said cells to produce a mixture of helper virus and scOPV particles in said cells; and
- 25 (iv) plating the mixture on OPV-specific host cells to recover the scOPV.
80. The method of claim 79, wherein the helper virus is a leporipox virus.

81. The method of claim 80, wherein the leporipox virus is selected from the group consisting of: Shope fibroma virus (SFV), hare fibroma virus, rabbit fibroma virus, squirrel fibroma virus, and myxoma virus.
82. The method of claim 81, wherein the leporipox virus is SFV.
- 5 83. The method of claim 79, wherein the helper virus is fowlpox virus.
84. The method of claim 79, wherein the helper virus is a psoralen-inactivated helper virus.
85. The method of any one of claims 79 to 82, wherein the helper virus-infected cells are BGMK cells.
- 10 86. The method of any one of claims 79 to 85, wherein the OPV-specific host cells are BSC-40 cells.
87. The method of any one of claims 79 to 86, wherein the OPV is selected from the group consisting of: camelpox virus, cowpox virus, ectromelia virus, horsepox virus, monkeypox virus, vaccinia virus, variola virus, rabbitpox virus, raccoon
- 15 poxvirus, skunkpox virus, Taterapox virus, Uasin Gishu disease virus, volepox virus.
88. The method of any one of claims 79 to 87, wherein step (i) further comprises chemically synthesizing terminal hairpin loops from an OPV and ligating them onto the fragments comprising the left and right termini of the viral genome.
- 20 89. A method of producing a synthetic chimeric horsepox virus (scHPXV) comprising the steps of:
- (i) chemically synthesizing overlapping DNA fragments that correspond to substantially all of the HPXV genome;
- (ii) transfecting the overlapping DNA fragments into helper virus-infected cells;
- 25 (iii) culturing said cells to produce a mixture of helper virus and scHPXV particles in said cells; and
- (iv) plating the mixture on HPXV-specific host cells to recover the scHPXV.

90. The method of claim 89, wherein the helper virus is a leporipox virus.
91. The method of claim 90, wherein the leporipox virus is selected from the group consisting of: Shope fibroma virus (SFV), hare fibroma virus, rabbit fibroma virus, squirrel fibroma virus, and myxoma virus.
- 5 92. The method of claim 91, wherein the leporipox virus is SFV.
93. The method of claim 89, wherein the helper virus is fowlpox virus.
94. The method of claim 89, wherein the helper virus is a psoralen-inactivated helper virus.
95. The method of any one of claims 89 to 92, wherein the helper virus-infected
10 cells are BGMK cells.
96. The method of any one of claims 89 to 95, wherein the HPXV-specific host cells are BSC-40 cells.
97. The method of any one of claims 89 to 96, wherein step (i) further comprises chemically synthesizing terminal hairpin loops from an OPV and ligating them
15 onto the fragments comprising the left and right termini of the HPXV genome.
98. The method of any one of claims 89 to 97, wherein the overlapping DNA fragments comprise:
- i) nucleotide sequences that are at least 85% identical to the sequences of SEQ ID NOs: 1–10;
- 20 ii) nucleotide sequences that are at least 90% identical to the sequences of SEQ ID NOs: 1–10;
- (iii) nucleotide sequences that are at least 95% identical to the sequences of SEQ ID NOs: 1–10; or
- (iv) nucleotide sequences that consist of the sequences of SEQ ID NOs: 1–10.
- 25 99. A method of producing a synthetic chimeric horsepox virus (scHPXV) comprising:

- (i) chemically synthesizing overlapping DNA fragments that correspond to substantially all of the HPXV genome;
- (ii) transfecting the overlapping DNA fragments into Shope fibroma virus (SFV)-infected cells;
- 5 (iii) culturing said cells to produce a mixture of SFV and scHPXV particles in said cells; and
- (iv) plating the mixture on HPXV-specific host cells to recover the scHPXV.
100. The method of claim 99, wherein the SFV-infected cells are BGMK cells.
101. The method of claim 99 or 100, wherein the HPXV-specific host cells are
- 10 BSC-40 cells.
102. A synthetic chimeric poxvirus (scPV) generated by the method of any one of claims 71 to 78.
103. A synthetic chimeric orthopox virus (scOPV) generated by the method of any one of claims 79 to 88.
- 15 104. A synthetic chimeric horsepox virus (scHPXV) generated by the method of any one of claims 89 to 101.
105. A composition comprising a pharmaceutically acceptable carrier and the scPV of any one of claims 1 to 14 and 102.
106. A composition comprising a pharmaceutically acceptable carrier and the
- 20 scOPV of any one of claims 15 to 43 and 103.
107. A method of triggering or boosting an immune response against variola virus, comprising administering to a subject in need thereof a composition comprising the scOPV of any one of claims 15 to 43 and 103.
108. A method of triggering or boosting an immune response against vaccinia
- 25 virus, comprising administering to a subject in need thereof a composition comprising the scOPV of any one of claims 15 to 43 and 103.

109. A method of triggering or boosting an immune response against monkeypox virus, comprising administering to a subject in need thereof a composition comprising the scOPV of any one of claims 15 to 43 and 103.
110. A method of immunizing a human subject to protect said subject from variola virus infection, comprising administering to said subject a composition comprising the scOPV of any one of claims 15 to 43 and 103.
111. A method of treating a variola virus infection, comprising administering to a subject in need thereof a composition comprising the scOPV of any one of claims 15 to 43 and 103.
112. A composition comprising a pharmaceutically acceptable carrier and the scHPXV of any one of claims 44 to 70 and 104.
113. A method of triggering or boosting an immune response against variola virus, comprising administering to a subject in need thereof a composition comprising the scHPXV of any one of claims 44 to 70 and 104.
114. A method of triggering or boosting an immune response against vaccinia virus, comprising administering to a subject in need thereof a composition comprising the scHPXV of any one of claims 44 to 70 and 104.
115. A method of triggering or boosting an immune response against monkeypox virus, comprising administering to a subject in need thereof a composition comprising the scHPXV of any one of claims 44 to 70 and 104.
116. A method of immunizing a human subject to protect said subject from variola virus infection, comprising administering to said subject a composition comprising the scHPXV of any one of claims 44 to 70 and 104.
117. A method of treating a variola virus infection, comprising administering to a subject in need thereof a composition comprising the scHPXV of any one of claims 44 to 70 and 104.

118. A kit comprising a composition comprising the scPV of any one of claims 1 to 13 and 102.
119. A kit comprising a composition comprising the OPV of any one of claims 15 to 43 and 103 and instructions for the use thereof.
- 5 120. A kit comprising a composition comprising the scHPXV of any one of claims 44 to 70 and 104 and instructions for the use thereof.
121. The method of any one of claims 107-111 or 113-117, wherein the composition is administered in a poxvirus treatment facility.
122. The method of any one of claims 107-111, 113-117 or 121, wherein the
10 composition is administered by a specialist in smallpox adverse events.
123. The method of claim 122, wherein the smallpox adverse events are selected from: eczema vaccinatum, progressive vaccinia, postvaccinal encephalitis, myocarditis, and dilated cardiomyopathy.
124. A poxvirus treatment facility wherein subjects in need of immunization or
15 treatment with a composition comprising the virus of any one of claims 1-70 and 102-106 or the composition of any one of claims 105, 106, or 112 may be immunized or treated in an environment such that they are sequestered from other subjects not intended to be immunized or treated.
125. The poxvirus treatment facility of claim 124 being characterized by specialists
20 in smallpox adverse events to administer the immunization or treatment.
126. The poxvirus treatment facility of claim 124 or 125, wherein the smallpox adverse events are selected from: eczema vaccinatum, progressive vaccinia, postvaccinal encephalitis, myocarditis, and dilated cardiomyopathy.
127. The poxvirus treatment facility of any one of claims 124-126, wherein the
25 subjects in need of immunization or treatment are immunized or treated according to the methods of any one of claims 107-111 or 113-117.