CLAIMS

What is claimed is:

1. A recombinant multigenome retroviral vector preparation comprising:

a. a first retroviral particle comprising two copies of a first retroviral vector genome comprising a first sequence of interest; wherein the first sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

b. a second retroviral particle comprising two copies of a second retroviral vector genome comprising a second sequence of interest; wherein the second sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

c. a third retroviral particle comprising one copy of the first retroviral vector genome comprising a first sequence of interest and one copy of the second retroviral vector genome comprising a second sequence of interest;

wherein the first and second sequences of interest are different; and

wherein the retroviral vector preparation is pseudotyped with a heterologous envelope glycoprotein.

2. The recombinant multigenome retroviral vector preparation of claim 1, wherein the heterologous envelope glycoprotein is selected from the group consisting of VSVg, measles envelope glycoprotein, and a Sindbis envelope glycoprotein.

3. The recombinant multigenome retroviral vector preparation of any one of claims 1-2, wherein the retroviral vector preparation is a lentiviral vector preparation.

4. The recombinant multigenome retroviral vector preparation of any one of claims 1-3, wherein the first sequence of interest encodes a tumor-associated antigen or one or more neoantigens and the second sequence of interest encodes an immunomodulatory molecule.

5. The recombinant multigenome retroviral vector preparation of any one of claims 1-3, wherein the first sequence of interest encodes a tumor-associated antigen and the second sequence of interest encodes a different tumor-associated antigen.

6. A retroviral vector packaging system for producing a pseudotyped multigenome retroviral vector preparation, comprising:

a. a first nucleic acid molecule encoding a viral envelope protein;

b. a second nucleic acid molecule encoding gag and pol proteins;

c. a third nucleic acid molecule encoding rev;

d. a fourth nucleic acid molecule comprising a first retroviral vector genome comprising a first sequence of interest; wherein the first sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

e. a fifth nucleic acid molecule comprising a second retroviral vector genome comprising a second sequence of interest; wherein the second sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

f. a sixth nucleic acid molecule encoding vpx; and

g. a packaging cell or cell line.

7. The retroviral vector packaging system of claim 6 wherein the retroviral vector preparation is a lentiviral vector preparation.

8. The retroviral vector packaging system of claim 6 or claim 7 wherein the first sequence of interest encodes a tumor-associated antigen or one or more neoantigens and the second sequence of interest encodes an immunomodulatory molecule.

9. The retroviral vector packaging system of claim 6 wherein the first sequence of interest encodes a tumor-associated antigen and the second sequence of interest encodes a different tumor-associated antigen.

10. The retroviral vector packaging system of any one of claims 6-9 wherein the viral envelope protein is selected from the group consisting of VSVg, measles envelope glycoprotein, and an alphavirus envelope glycoprotein.

11. The retroviral vector packaging system of any one of claims 6-10 wherein equal amounts of the fourth nucleic acid molecule and the fifth nucleic acid molecule are used.

12. The retroviral vector packaging system of any one of claims 6-10 wherein the relative input ratio of the fourth nucleic acid molecule to the fifth nucleic acid molecule used in the packaging system is 3:2.

13. The retroviral vector packaging system of any one of claims 6-12 wherein the first lentiviral vector genome and the second lentiviral vector genome comprise a palindromic dimer initiation site (DIS) sequence.

14. The retroviral vector packaging system of any one of claims 6-12 wherein the first lentiviral vector genome comprises a first palindromic DIS sequence and the second lentiviral vector genome comprise a second palindromic DIS sequence.

15. The retroviral vector packaging system of any one of claims 6-12 wherein the palindromic DIS sequence in the first lentiviral vector genome and the second lentiviral vector genome is the same.

16. The retroviral vector packaging system of any one of claims 6-15 wherein the pol protein has a non-functional integrase.

17. The recombinant multigenome retroviral vector preparation of any one of claims 1-5, further comprising:

e. a fourth retroviral particle comprising two copies of a third retroviral vector genome comprising a third sequence of interest;

f. a fifth retroviral particle comprising one copy of the first retroviral vector genome comprising a first sequence of interest and one copy of the third retroviral vector genome comprising the third sequence of interest; and

g. a sixth retroviral particle comprising one copy of the second retroviral vector genome comprising a second sequence of interest and one copy of the third retroviral vector genome comprising the third sequence of interest.

18. The recombinant multigenome retroviral vector preparation of any one of claims 1-5 or claim 17 wherein the first retroviral vector genome comprises about 50% of the vector genomes in the multigenome vector preparation.

19. A multigenome retroviral vector packaging system, comprising:

a. at least two nucleic acid molecules each comprising a retroviral vector genome comprising a unique sequence of interest; wherein the unique sequence of interest encodes a heterologous protein selected from the group consisting of a tumor antigen, a neoantigen, an infectious disease antigen, an immunomodulatory molecule, a chimeric antigen receptor, a recombinant T cell receptor, and an immunoglobulin antigen-binding domain;

b. one or more nucleic acid molecules encoding the components necessary to generate pseudotyped retroviral vector particles; wherein the components necessary to generate pseudotyped retroviral vector particles are selected from the group consisting of gag, pol, envelope, rev, and vpx proteins; and

c. a packaging cell line.

20. The multigenome retroviral vector packaging system of claim 19, consisting of 2 to 8 nucleic acid molecules each comprising a retroviral vector genome comprising a unique sequence of interest.

21. The multigenome retroviral vector packaging system of claim 20, consisting of 5 nucleic acid molecules each comprising a retroviral vector genome comprising a unique sequence of interest.

22. The multigenome retroviral vector packaging system of claim 21, wherein the relative input ratio of the 5 nucleic acid molecules each comprising a retroviral vector genome comprising a unique sequence of interest is selected from one of the input ratios shown in Table 3.

23. The multigenome retroviral vector packaging system of claim 21, wherein the relative input ratio of the 5 nucleic acid molecules each comprising a retroviral vector genome comprising a unique sequence of interest is 4:1:1:1:1.

24. The multigenome retroviral vector packaging system of any one of claims 19-23, wherein the unique sequences of interest are selected from the group consisting of the sequences encoding MAGEA1, MAGEA4, NYESO1, MAGEA3, MAGEA10, ScFvanti-PD1, IL12, IL23, CD40, ScFvanti-PDL1, and ScFvanti-CTLA4, or an immunogenic variant of any of the foregoing having at least 80%-90% identity to the wild type sequence.

25. The multigenome retroviral vector packaging system of any one of claims 19-24 wherein the retroviral vector genome is a lentiviral vector genome.

26. A method for producing a pseudotyped multigenome retroviral vector preparation comprising culturing the packaging cell line of any one of claims 19-24 transfected with the nucleic acids of (a) and (b) and optionally (c).

27. A pseudotyped multigenome retroviral vector preparation produced by the method of claim 26.

28. A method of inducing an immune response in a subject comprising administering the multigenome retroviral vector preparation of any one of claims 1-5, 17, 18 and 27.

29. A method of treating cancer in a subject comprising administering the multigenome retroviral vector preparation of any one of claims 1-5, 17, 18 and 27.

30. A method for delivering and expressing multiple sequences of interest to a subject in vivo comprising administering the multigenome retroviral vector preparation of any one of the preceding claims.

31. A recombinant multigenome retroviral vector preparation of any of the preceding claims for use in therapy.

32. A recombinant multigenome retroviral vector preparation of any of the preceding claims for use in a method of treatment of a patient.

33. A recombinant multigenome retroviral vector preparation of any of the preceding claims for use in a method of treating cancer in a patient.

34. A recombinant multigenome retroviral vector preparation of any of the preceding claims for use in a method of stimulating an immune response in a patient.