WHAT IS CLAIMED IS:

1. A method of constructing a library comprising a plurality of polynucleotides of interest, comprising:

(a) cleaving an isolated poxvirus genome to produce a first viral fragment and a second viral fragment, wherein the first fragment is nonhomologous with the second fragment;

(b) providing a population of transfer plasmids each comprising a polynucleotide of interest flanked by a 5′ flanking region and a 3′ flanking region, wherein the 5′ flanking region comprises a region homologous to the 3' end of the first viral fragment and the 3′ flanking region comprises a region homologous to the 5' end of the second viral fragment; and wherein the transfer plasmids are capable of homologous recombination with the first and second viral fragments such that a viable poxvirus genome is formed;

(c) transfecting the transfer plasmids and the first and second viral fragments into a mammalian host cell permissive for poxvirus infectivity, wherein the host cell is maintained in cell culture medium following transfection;

(d) adding an inhibitor of poxvirus assembly;

(e) allowing the transfer plasmid and the first and second viral fragments to undergo homologous recombination, thereby producing a library of viable modified poxvirus genomes each comprising a heterologous nucleic acid; and

(f) recovering the library.

2. The method of claim 1, wherein the inhibitor of poxvirus assembly is rifampicin (rifampin) or a derivative thereof.

3. The method of claim 2, wherein the rifampicin or derivative thereof is added to the cell culture medium comprising the transfected cells at about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 30 hours, about 36 hours, about 42 hours, about 48 hours, about 54 hours, or about 60 hours following transfection.

4. The method of claim 2 or claim 3, wherein the rifampicin or derivative thereof is added to the cell culture medium at a concentration of about 30 μg/ml, about 40

μg/ml, about 50 μg/ml, about 60 μg/ml, about 70 μg/ml, about 80 μg/ml, about 90 μg/ml, about 100 μg/ml, about 110 μg/ml, about 120 μg/ml, or about 130 μg/ml.

5. The method of any one of claims 2 to 4, wherein the rifampicin or derivative thereof is allowed to remain cell culture medium for about one day, about two days, about three days, about four days or about five days, and wherein the cell culture medium is changed following treatment with rifampicin or a derivative thereof, and the transfected host cells further cultured without rifampicin for about one day, about two days, or about three days.

6. The method of any one of claims 1 to 5, wherein the library comprises an increased number of independent modified poxvirus genomes than a library constructed in the absence of the inhibitor of poxvirus assembly, and wherein the number of independent modified poxvirus genomes is increased by at least about one-fold, about five-fold, about ten-fold, about fifteen-fold, about twenty-fold, about twenty-five-fold, or about thirty-fold; or wherein the library comprises an increased virus titer than a library constructed in the absence of the inhibitor of poxvirus assembly.

7. The method of any one of claims 1 to 6, wherein the isolated poxvirus genome is an isolated vaccinia virus genome.

8. The method of claim 7, wherein said isolated vaccinia virus genome is a WR genome or a modified derivative thereof, or a Modified Vaccinia virus Ankara (MVA) genome or a modified derivative thereof.

9. The method of any one of claims 1 to 8, wherein the isolated poxvirus genome comprises a first recognition site for a first restriction endonuclease and a second recognition site for a second restriction endonuclease; and wherein the first and second viral fragments are produced by digesting the viral genome with the first restriction endonuclease and the second restriction endonuclease, and isolating the first and second viral fragments.

10. The method of claim 9, wherein the first restriction enzyme is NotI, and the second restriction enzyme site is ApaI.

11. The method of any one of claims 7 to 10, wherein the isolated vaccinia virus genome is a v7.5/tk virus genome or a vEL/tk virus genome.

12. The method of any one of claims 1 to 11, wherein the mammalian host cell is capable of packaging the modified vaccinia virus genomes into infectious vaccinia virus particles.

13. The method of claim 12, wherein the transfer plasmids and the first and second viral fragments are introduced into the mammalian host cell along with a helper virus, wherein the host cell is non-permissive for the production of infectious virus particles of the helper virus, but supports packaging of the modified vaccinia virus genomes into infectious vaccinia virus particles.

14. The method of any one of claims 1 to 13, wherein the 5′ and 3′ flanking regions of the transfer plasmid are capable of homologous recombination with a vaccinia virus thymidine kinase gene or with a vaccinia virus HindIII J fragment, and wherein the transfer plasmid comprises an insert nucleic acid ligated into a plasmid selected from the group consisting of: pVHE, pVLE, and pVKE.

15. The method of any one of claims 1 to 14, wherein the plurality of polynucleotides of interest each comprise a coding region of a polypeptide of interest capable of expression in a vaccinia virus-infected cell and wherein the transfer plasmid further comprises a poxvirus promoter in operable association with the polynucleotide of interest, wherein the poxvirus promoter functions in the cytoplasm of a vaccinia virus-infected cell.

16. The method of claim 15, wherein the plurality of polynucleotides of interest each encode an antibody subunit polypeptide comprising an antibody heavy chain variable region or antigen-binding fragment thereof, an antibody light chain variable region or antigen-binding fragment thereof, or a combination thereof.

17. The method of claim 16, wherein the antibody subunit polypeptide further comprises a constant region or fragment thereof, a signal peptide capable of directing cell surface expression or secretion of the antibody subunit polypeptide, or a combination thereof.