

Claims

1. A method for preparing a producer cell for producing a retroviral vector carrying a nucleic acid fragment of interest, comprising:

integrating one or more but not all of, sequences of gag and pol genes of a retrovirus, a coding sequence of a viral envelope protein, and a viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of a host cell using a Sleeping Beauty (SB) transposon system, and further

integrating the remaining one or more of, the sequences of gag and pol genes of the retrovirus, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of the host cell using a PiggyBac (PB) transposon system,

or

integrating one or more but not all of, sequences of gag and pol genes of a retrovirus, a coding sequence of a viral envelope protein, and a viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of a host cell using a PB transposon system, and further

integrating the remaining one or more of, the sequences of gag and pol genes of the retrovirus, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of the host cell using an SB transposon system.

2. A method for preparing a producer cell for producing a retroviral vector carrying a nucleic acid fragment of interest, wherein the retroviral vector is a lentiviral vector, and the method comprises:

integrating one or more but not all of, sequences of gag, pol and rev genes of a lentivirus, a coding sequence of a viral envelope protein, and a viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of a host cell using an SB transposon system, and further

integrating the remaining one or more of, the sequences of gag, pol and rev genes of the lentivirus, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of the host cell using a PB transposon system,

or

integrating one or more but not all of, sequences of gag, pol and rev genes of a lentivirus, a coding sequence of a viral envelope protein, and a viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of a host cell using a PB transposon system, and further

integrating the remaining one or more of, the sequences of gag, pol and rev genes of the lentivirus, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, into the genome of the host cell using a SB transposon system.

3. The method of claim 2, wherein

the integrating of the sequences of gag, pol and rev genes of the lentivirus and the coding sequence of the viral envelope protein into the genome of the host cell is achieved using the SB transposon system, and the further integrating of the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest into the genome of the host cell is achieved using the PB transposon system, or

the integrating of the sequences of gag, pol and rev genes of the lentivirus and the coding sequence of the viral envelope protein into the genome of the host cell is achieved using the PB transposon system, and the further integrating of the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest into the genome of the host cell is achieved using the SB transposon system.

4. The method of claim 2, wherein the sequences of gag, pol and rev genes of the lentivirus, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest are located in two or more constructs.

5. The method of claim 4, wherein the sequences of gag and pol genes are located in one construct, and the sequence of rev gene is located in another construct, and the coding sequence of the viral envelope protein is located in a third construct, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest is located in a fourth construct.

6. The method of claim 2, wherein the gag, pol, and rev genes of the lentivirus are gag, pol, and rev genes of HIV-1 virus.

7. The method of claim 2, wherein the viral envelope protein is a vesicular stomatitis virus glycoprotein (VSV-G).

8. The method of claim 2, wherein SB100X is used as a transposase in the SB system, and/or ePiggyBac is used as a transposase in the PB system.

9. The method of claim 2, wherein the transcription of one or more of the gag, pol and rev genes, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette carrying the nucleic acid fragment of interest is controllable.

10. The method of claim 9, wherein the viral envelope protein is VSV-G and the transcription of the rev gene and the coding sequence of VSV-G is controllable.

11. The method of claim 9, wherein the genes or sequences are placed under a single control of a Tet-On inducible expression system or under a dual control of a Tet-On inducible expression system and a Cumate inducible expression system.

12. The method of claim 11, wherein the viral envelope protein is a VSV-G, and
when under the single control of the Tet-On inducible expression system, (i) the transcription of rev is under the control of a TRE_{3G} sequence, (ii) the transcription of the coding sequence of VSV-G is under the control of a TRE_{3G}-intron sequence, and/or (iii) the transcription of gag and pol is under the control of a eukaryotic promoter-intron sequence or a TRE_{3G}-intron sequence;

when under the dual control of the Tet-On inducible expression system and the Cumate inducible expression system, (i) the transcription of rev is under the control of a TRE_{adv}CuO sequence, a TRE_{adv}CuO-intron sequence, a TRE_{3G} sequence, a TRE_{3G}CuO sequence or a TRE_{3G}CuO-intron sequence, (ii) the transcription of VSV-G is under the control of a TRE_{adv}CuO sequence, a TRE_{adv}CuO-intron sequence, a TRE_{3G}-intron sequence or a TRE_{3G}CuO-intron sequence, and/or (iii) the transcription of gag and pol is under the control of a eukaryotic promoter-intron sequence, a TRE_{adv}-intron sequence, a TRE_{adv}CuO-intron sequence, a TRE_{3G}-intron sequence or a TRE_{3G}CuO-intron sequence.

13. The method of claim 11, wherein a coding sequence of a Tet-On transactivator protein, or

a coding sequence of a Tet-On transactivator protein and a coding sequence of a repressor CymR protein of Cumate operon, is/are integrated into the genome of the host cell using an SB transposon system or a PB transposon system.

14. The method of claim 11, wherein,

when under the single control of the Tet-On inducible expression system, (i) the sequences of gag, pol and rev genes of the lentivirus, the coding sequence of the viral envelope protein, and the coding sequence of the Tet-On transactivator protein are integrated into the genome of the host cell using an SB transposon system, and then the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest is further integrated into the genome of the host cell using a PB transposon system; or (ii) the sequences of gag, pol and rev genes of the lentivirus, the coding sequence of the viral envelope protein, and the coding sequence of the Tet-On transactivator protein are integrated into the genome of the host cell using a PB transposon system, and then the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest is further integrated into the genome of the host cell using an SB transposon system;

when under the dual control of the Tet-On inducible expression system and the Cumate inducible expression system, (i) the sequences of gag, pol and rev genes of the lentivirus, the coding sequence of the viral envelope protein, and the coding sequence of the Tet-On transactivator protein and the coding sequence of the repressor CymR protein of Cumate operon are integrated into the genome of the host cell using an SB transposon system, and then the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest is further integrated into the genome of the host cell using a PB transposon system; or (ii) the sequences of gag, pol and rev genes of the lentivirus, the coding sequence of the viral envelope protein, and the coding sequence of the Tet-On transactivator protein and the coding sequence of the repressor CymR protein of Cumate operon are integrated into the genome of the host cell using a PB transposon system, and then the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest is further integrated into the genome of the host cell using an SB transposon system.

15. A producer cell for producing a retroviral vector carrying a nucleic acid fragment of interest, wherein the producer cell is integrated in the genome thereof with a sequence of gag and pol genes of a retrovirus, a coding sequence of a viral envelope protein, and a viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest; and

wherein each of, the sequence of the gag and pol genes, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, has IR/DR sequences for recognition by an SB transposase or ITR sequences for recognition by a PB transposase at both ends thereof, and both the IR/DR sequences and the ITR sequences are present in the producer cell.

16. The producer cell of claim 15, wherein

each of, the sequence of the gag and pol genes and the coding sequence of the viral envelope protein, has IR/DR sequences for recognition by an SB transposase at both ends thereof, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest has ITR sequences for recognition by a PB transposase at both ends thereof, or

each of, the sequence of the gag and pol genes and the coding sequence of the viral envelope protein, has ITR sequences for recognition by a PB transposase at both ends thereof, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest has IR/DR sequences for recognition by an SB transposase at both ends thereof.

17. A producer cell for producing a lentiviral vector carrying a nucleic acid fragment of interest, wherein the producer cell is integrated in the genome thereof with a sequence of gag and pol genes of a lentivirus, a sequence of rev gene of a lentivirus, a coding sequence of a viral envelope protein, and a viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest; and

wherein each of, the sequence of gag and pol genes, the sequence of rev gene, the coding sequence of the viral envelope protein, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest, has IR/DR sequences for recognition by an SB transposase or ITR sequences for recognition by a PB transposase at both ends thereof, and both the IR/DR sequences and the ITR sequences are present in the producer cell.

18. The producer cell of claim 17, wherein

each of, the sequence of the gag and pol genes, the sequence of the rev gene and the coding sequence of the viral envelope protein, has IR/DR sequences for recognition by an SB transposase at both ends thereof, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest has ITR sequences for recognition by a PB transposase at both ends thereof; or

each of, the sequence of the gag and pol genes, the sequence of the rev gene and the coding sequence of the viral envelope protein, has ITR sequences for recognition by a PB transposase at both ends thereof, and the viral genome transcriptional cassette sequence carrying the nucleic acid fragment of interest has IR/DR sequences for recognition by an SB transposase at both ends thereof.

19. The producer cell of claim 15 or 17, wherein the nucleic acid fragment of interest has recognition sequences for a site-specific recombinase system at both ends thereof and/or the coding sequence of the envelope protein has recognition sequences for a site-specific recombinase system at both ends thereof.