Claims

1. A recombinant adenoviral helper genome comprising:

a 5′ Ad35 inverted terminal repeat (ITR);

a 3′ Ad35 ITR; and

recombinase direct repeats flanking an Ad35 packaging sequence, wherein the recombinase direct repeats comprise a first recombinase direct repeat between nucleotide positions corresponding to 155 and 171, 161 and 181, 185 and 205, or 214 and 234 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 377 and 504 or 3175 and 3225 of GenBank Accession No. AY128640.

2. The helper genome of claim 1, wherein the recombinase direct repeats comprise:

(i) a first recombinase direct repeat between nucleotide positions corresponding to 155 and 171, 161 and 181, 185 and 205, or 214 and 234 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 392 and 412 or 469 and 489 of GenBank Accession No. AY128640; or

(ii) a first recombinase direct repeat between nucleotide positions corresponding to 155 and 171, 161 and 181, 185 and 205, or 214 and 234 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 3190 and 3210 of GenBank Accession No. AY128640.

3. The helper genome of claim 1 or claim 2, wherein the recombinase direct repeats comprise:

(i) a first recombinase direct repeat between nucleotide positions corresponding to 155 and 171 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 3190 and 3210 of GenBank Accession No. AY128640;

(ii) a first recombinase direct repeat between nucleotide positions corresponding to 161 and 181 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 392 and 412 of GenBank Accession No. AY128640;

(iii) a first recombinase direct repeat between nucleotide positions corresponding to 185 and 205 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 469 and 489 of GenBank Accession No. AY128640; or
(iv) a first recombinase direct repeat between nucleotide positions corresponding to 214 and 234 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 392 and 412 of GenBank Accession No. AY128640.

4. The helper genome of any one of claims 1-3, wherein the recombinase direct repeats comprise a first recombinase direct repeat at a nucleotide position corresponding to 161, 171, 195, or 224 of GenBank Accession No. AY128640 and a second recombinase direct repeat at a nucleotide position corresponding to 402, 479, or 3200 of GenBank Accession No. AY128640;

optionally wherein the recombinase direct repeats comprise:

(i) a first recombinase direct repeat at a nucleotide position corresponding to 161 of GenBank Accession No. AY128640 and a second recombinase direct repeat at a nucleotide position corresponding to 3200 of GenBank Accession No. AY128640;

(ii) a first recombinase direct repeat at a nucleotide position corresponding to 171 of GenBank Accession No. AY128640 and a second recombinase direct repeat at a nucleotide position corresponding to 402 of GenBank Accession No. AY128640;

(iii) a first recombinase direct repeat at a nucleotide position corresponding to 195 of GenBank Accession No. AY128640 and a second recombinase direct repeat at a nucleotide position corresponding to 479 of GenBank Accession No. AY128640;

(iv) a first recombinase direct repeat at a nucleotide position corresponding to 224 of GenBank Accession No. AY128640 and a second recombinase direct repeat at a nucleotide position corresponding to 402 of GenBank Accession No. AY128640.

5. The helper genome of any one of claims 1-4, wherein the Ad35 helper genome comprises an inverted packaging sequence.

6. A recombinant adenoviral helper genome comprising:

a 5′ Ad35 inverted terminal repeat (ITR);

a 3′ Ad35 ITR; and

recombinase direct repeats flanking an Ad35 packaging sequence, wherein the recombinase direct repeats comprise a first recombinase direct repeat between nucleotide positions corresponding to 136 and 249 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 377 and 504 or 3175 and 3225 of GenBank Accession No. AY128640,

wherein the Ad35 helper genome comprises an inverted packaging sequence.

7. The helper genome of claim 6, wherein the recombinase direct repeats comprise:

(i) a first recombinase direct repeat between nucleotide positions corresponding to 151 and 171, 161 and 181, 185 and 205, or 214 and 234 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 392 and 412 or 469 and 489 of GenBank Accession No. AY128640; or

(ii) a first recombinase direct repeat between nucleotide positions corresponding to 151 and 171, 161 and 181, 185 and 205, or 214 and 234 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 3190 and 3210 of GenBank Accession No. AY128640; or

(iii) a first recombinase direct repeat between nucleotide positions corresponding to 151 and 171 of GenBank Accession No. AY128640 and a second recombinase direct repeat between nucleotide positions corresponding to 3190 and 3210 of GenBank Accession No. AY128640.

8. The helper genome of any one of claims 5-7, wherein the inverted packaging sequence comprises the Ad35 packaging sequence and one or both of the first recombinase direct repeat and the second recombinase direct repeat.

9. The helper genome of any one of claims 5-8, wherein the inverted packaging sequence comprises, or comprises a first end point at, a nucleotide position between nucleotide positions corresponding to 119 and 169 of AY128640 or 134 and 154 of AY128640, optionally wherein the inverted packaging sequence comprises, or comprises a first end point at, a nucleotide position corresponding to position 144 of AY128640.

10. The helper genome of any one of claims 5-9, wherein the inverted packaging sequence comprises, or comprises a second end point at:

(i) a nucleotide position between nucleotide positions corresponding to 3175 and 3225 of AY128640 or 3190 and 3210 of AY128640, optionally wherein the inverted packaging sequence comprises, or comprises a second end point at, a nucleotide position corresponding to position 3200 of AY128640; and/or

(ii) a nucleotide position between nucleotide positions corresponding to 455 and 505 of AY128640 or 470 and 490 of AY128640, optionally wherein the inverted packaging sequence comprises, or comprises a second end point at, a nucleotide position corresponding to position 480 of AY128640.

11. The helper genome of any one of claims 1-7, wherein the recombinase direct repeats that flank the Ad35 packaging sequence are FRT, loxP, rox, vox, AttB, or AttP sites; optionally wherein the recombinase direct repeats that flank the Ad35 packaging sequence are loxP sites.

12. A recombinant adenoviral helper vector comprising the Ad35 helper genome of any one of claims 1-11.

13. A recombinant adenoviral vector production system comprising:

(i) the Ad35 helper genome of any one of claims 1-11 or the helper vector of claim 12, and

(ii) an HDAd35 donor genome, the HDAd35 donor genome comprising:

a 5′ Ad35 inverted terminal repeat (ITR);

a 3′ Ad35 ITR;

an Ad35 packaging sequence; and

a nucleic acid sequence encoding at least one heterologous expression product.

14. A method of producing a recombinant helper-dependent adenoviral donor vector, the method comprising isolating the recombinant helper-dependent Ad35 donor vector from a culture of cells, wherein the cells comprise:

a recombinant Ad35 helper genome of any one of claims 1-11 or a recombinant adenoviral helper vector of claim 12; and

a recombinant helper-dependent Ad35 donor genome comprising:

a 5′ Ad35 inverted terminal repeat (ITR);

a 3′ Ad35 ITR;

an Ad35 packaging sequence; and

a nucleic acid sequence encoding at least one heterologous expression product.

15. The helper genome, helper vector, system, or method of any one of claims 1-14, wherein the helper genome comprises a nucleic acid sequence that encodes an Ad35 fiber knob;

optionally wherein the Ad35 fiber knob comprises:

(i) a mutation that increases affinity with CD46; and/or

(ii) one or more mutations:

selected from Ile192Val, Asp207Gly (or Glu207Gly), Asn217Asp, Thr226Ala, Thr245Ala, Thr254Pro, Ile256Leu, Ile256Val, Arg259Cys, and Arg279His; or

comprising each of mutations Ile192Val, Asp207Gly (or Glu207Gly), Asn217Asp, Thr226Ala, Thr245Ala, Thr254Pro, Ile256Leu, Ile256Val, Arg259Cys, and Arg279His.

16. The helper genome, helper vector, system, or method of any one of claims 1-15, wherein the helper genome is present in a cell that comprises a nucleic acid encoding a recombinase for recombination of the direct repeats;

optionally wherein the recombinase is a Flp, Cre, Dre, Vika, or PhiC31 recombinase;

optionally wherein the cell is a HEK293 cell, further optionally wherein the cell is a HEK293 cell that encodes or expresses Cre recombinase, even further optionally wherein the HEK293 cell that encodes or expresses Cre recombinase is a 116 cell.

17. A recombinant recombinase site-flanked adenoviral serotype 35 (Ad35) packaging sequence, wherein recombinase direct repeats flank an Ad35 packaging sequence, wherein the Ad35 packaging sequence corresponds to a fragment of GenBank Accession No. AY128640 having a first end point between nucleotide positions corresponding to 136 and 249 of GenBank Accession No. AY128640 and a second end point between nucleotide positions corresponding to 377 and 504 or 3175 and 3225 of GenBank Accession No. AY128640.

18. The recombinant packaging sequence of claim 17, wherein:

(i) the first end point is between nucleotide positions corresponding to 155 and 171, 161 and 181, 185 and 205, or 214 and 234 of GenBank Accession No. AY128640 and the second end point is between nucleotide positions corresponding to 392 and 412 or 469 and 489 of GenBank Accession No. AY128640; or

(ii) the first end point is between nucleotide positions corresponding to 155 and 171, 161 and 181, 185 and 205, or 214 and 234 of GenBank Accession No. AY128640 and the second end point is between nucleotide positions corresponding to 3190 and 3210 of GenBank Accession No. AY128640;

optionally wherein:

(a) the first end point is between nucleotide positions corresponding to 155 and 171 of GenBank Accession No. AY128640 and the second end point is between nucleotide positions corresponding to 3190 and 3210 of GenBank Accession No. AY128640;

(b) the first end point is between nucleotide positions corresponding to 161 and 181 of GenBank Accession No. AY128640 and the second end point is between nucleotide positions corresponding to 392 and 412 of GenBank Accession No. AY128640;

(c) the first end point is between nucleotide positions corresponding to 185 and 205 of GenBank Accession No. AY128640 and the second end point is between nucleotide positions corresponding to 469 and 489 of GenBank Accession No. AY128640; or

(d) the first end point is between nucleotide positions corresponding to 214 and 234 of GenBank Accession No. AY128640 and the second end point is between nucleotide positions corresponding to 392 and 412 of GenBank Accession No. AY128640.

19. The recombinant packaging sequence of claim 17 or claim 18, wherein the first end point is at a nucleotide position corresponding to 161, 162, 171, 172, 195, 196, 224, or 225 of GenBank Accession No. AY128640 and the second end point is at a nucleotide position corresponding to 402, 479, or 3200 of GenBank Accession No. AY128640;

optionally wherein:

(i) the first end point is at a nucleotide position corresponding to 161 or 162 of GenBank Accession No. AY128640 and the second end point is at a nucleotide position corresponding to 3200 of GenBank Accession No. AY128640;

(ii) the first end point is at a nucleotide position corresponding to 171 or 172 of GenBank Accession No. AY128640 and the second end point is at a nucleotide position corresponding to 402 of GenBank Accession No. AY128640;

(iii) the first end point is at a nucleotide position corresponding to 195 or 196 of GenBank Accession No. AY128640 and the second end point is at a nucleotide position corresponding to 479 of GenBank Accession No. AY128640; or

(iv) the first end point is at a nucleotide position corresponding to 224 or 225 of GenBank Accession No. AY128640 and the second end point is at a nucleotide position corresponding to 402 of GenBank Accession No. AY128640.

20. The recombinant packaging sequence of any one of claims 17-19, wherein the packaging sequence is present in an adenoviral genome and is inverted, optionally wherein the packaging sequence is inverted as compared to a 5′ ITR of the adenoviral genome.

21. A recombinant adenoviral helper genome comprising:

a 5′ Ad35 inverted terminal repeat (ITR);

a 3′ Ad35 ITR; and

an inverted sequence comprising an Ad35 packaging sequence,

wherein the inverted sequence comprises, or comprises a first end point at, a nucleotide position between nucleotide positions corresponding to 119 and 169 of AY128640 or 134 and 154 of AY128640, optionally wherein the inverted sequence comprises, or comprises a first end point at, a nucleotide position corresponding to position 144 of AY128640, and

wherein (i) the inverted sequence comprises, or comprises a second end point at, a nucleotide position between nucleotide positions corresponding to 3175 and 3225 of AY128640 or 3190 and 3210 of AY128640, optionally wherein the inverted sequence comprises, or comprises a second end point at, a nucleotide position corresponding to position 3200 of AY128640, or (ii) the inverted sequence comprises, or comprises a second end point at, a nucleotide position between nucleotide positions corresponding to 455 and 505 of AY128640 or 470 and 490 of AY128640,

optionally wherein the inverted sequence comprises, or comprises a second end point at, a nucleotide position corresponding to position 480 of AY128640;
optionally wherein recombinase direct repeats flank the Ad35 packaging sequence.