CLAIMS

1. A method of retrieving a nucleic acid sequence from a donor nucleic acid strand *in vitro*, the method comprising:

(a) providing a nucleic acid vector comprising a first nucleic acid strand, in which the first strand comprises:

(i) a first homology arm (HA1) that is homologous to a first nucleotide sequence (NS1) of the donor strand for homologous recombination between the first strand and the donor strand;

(ii) a second homology arm (HA2) that is homologous to a second nucleotide sequence (NS2) of the donor strand for homologous recombination between the first strand and the donor strand; and

(iii) an intervening nucleotide sequence joining the 3' end of HA1 to the 5' end of HA2;

in which HA1 is 5' to HA2 in the first strand; and NS1 is 3' to NS2 in the donor strand, in which the donor strand comprises a retrieval sequence (RS) between NS1 and NS2;

(b) combining the first strand with the donor strand;

(c) nuclease cutting of a first cut site (CS1) of the first strand at the 5' end of HA1, or flanking 5' of the 5' end of HA1; and/or nuclease cutting of a second cut site (CS2) of the first strand at the 3' end of HA2, or flanking 3' of the 3' end of HA2 to provide a cut first strand;

in which steps (b) and (c) are carried out in any order or simultaneously; and

(d) carrying out homologous recombination of the cut first strand with the donor strand, whereby gap repair of the cut first strand produces a product vector in which RS is retrieved between HA1 and HA2; and

in which the product of step (d) is a circular vector.

2. A method according to Claim 1, in which the vector is a BAC, YAC, PAC, cosmid or plasmid.

3. A method according to Claim 1, in which the method uses a respective cutting site motif to direct each the cutting of step (c), in which each cutting site is removed by the homologous recombination.

4. A method according to Claim 1, in which the donor strand is a strand of a chromosome or an episome of a cell.

5. A method according to Claim 1, in which RS is at least 10kb in length.

6. A method according to Claim 1, in which the nuclease cutting is Cas, meganuclease, zinc finger protein-mediated or TALEN-mediated cutting.

7. A method according to Claim 1, in which the nuclease cutting is by a Cas nuclease, and in which in step (a) the vector comprises in 5' to 3' direction:

A. A first protospacer sequence (PS1) comprising CS1;

B. A first PAM (P1) adjacent to the 3' end of PS1; and

C. HA1;

in which a seed sequence (S1) of PS1 joins CS1 to P1; and/or

in which in step (a) the vector comprises in 5' to 3' direction:

D. HA2;

E. A second protospacer sequence (PS2) comprising CS2;

F. A second PAM (P2) adjacent to the 3' end of PS2;

in which a seed sequence (S2) of PS2 joins CS2 to P2.

8. A method according to Claim 7, in which in step (c) the same guide RNA (gRNA1) is used to guide the nuclease cutting of CS1 and CS2 by the Cas nuclease.

9. A method according to Claim 1, in which before or during step (d) the donor strand is cut at the 5' end of NS2 or flanking 5' of the 5' end of NS2; and/or at the 3' end of NS1 or flanking 3' of the 3' end of NS1.

10. A method according to Claim 1, further comprising inserting all or part of the RS of the product vector into a further nucleic acid strand.

11. A method according to Claim 10, in which the further strand is comprised by a cell and the product vector of step (d) is inserted into the cell before insertion of the RS of the product vector into the further nucleic acid strand via homologous recombination.

12. A method according to Claim 10, in which the further strand is comprised by an immune cell or an embryonic stem cell.

13. A method according to Claim 1, in which in step (b) and/or (d) the vector is a closed circular vector.

14. A method according to Claim 13, in which in step (b) the vector is supercoiled.

15. A method according to Claim 1, in which sequence between HA1 and HA2 is deleted from the first strand in step (c) and/or sequence between NS1 and NS2 is deleted from the donor strand in or after step (d).

16. A method according to Claim 15, in which RS is deleted from the donor strand.

17. A method according to Claim 1, in which the nuclease cutting is cutting by a Cas nuclease, in which a protospacer/PAM combination at the 5' end of HA1 or flanking 5' of the 5' end of HA1 is destroyed by step (d), whereby PS1 is rendered non-functional in the first strand; and/or in which a protospacer/PAM combination at the 3' end of HA2 or flanking 3' of the 3' end of HA2 is destroyed by step (d), whereby PS2 is rendered non-functional in the first strand.

18. A method according to Claim 1, in which a donor sequence is inserted into the first strand and/or a sequence is deleted from the first strand, to form a new protospacer/PAM combination or a new marker sequence.

19. A method according to Claim 18, comprising cutting the protospacer of the new combination using a Cas after step (d).

20. A method according to Claim 1, in which a donor sequence is deleted from the donor strand to form a new protospacer/PAM combination or a new marker sequence.

21. A method according to Claim 20, comprising cutting the protospacer of the new combination in the donor strand using a Cas after step (d).

22. A method according to Claim 1, the method comprising isolating or copying the donor strand after step (d) or the cut region of the first strand.

23. A method according to Claim 1, in which RS is inserted into the first strand in step (d) and the inserted RS sequence is functional for expression from the modified first strand.

24. A method according to Claim 1, in which steps (b) to (d) are performed in a host cell, in which RS is deleted from the donor strand in or after step (d) and (i) the deletion is lethal to the host cell, or (ii) the method down-regulates cell viability, growth or proliferation, or (iii) the method up-regulates cell viability, growth or proliferation.

25. A method according to Claim 1, in which the method is carried out in a bacterial or archaeal cell.

26. A method according to Claim 1, in which the method is carried out in a population of prokaryotic cells to kill the cells, in which the cells are of the same species or strain.

27. A method according to Claim 1 , in which steps (b) to (d) are performed in a host cell, in which the nuclease cutting is Cas-mediated cutting, and in step (c) the Cas-mediated cutting is in a protospacer sequence in the first strand, in which the protospacer comprises a seed sequence, in which the seed sequence comprises a nucleotide sequence that is not found in the genome of the host cell.

28. A method according to Claim 1, in which steps (b) to (d) are performed in a host cell.