CLAIMS

1. A method of retrieving a nucleic acid sequence from a donor nucleic acid strand, 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 strands;

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

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

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

(e) optionally isolating or sequencing the vector or the RS sequence thereof.

2. A method according to Claim 1, in which the product of step (d) is a circular vector.

3. A method according to Claim 1 or 2, in which steps (b) to (d) are performed in a host cell, and optionally the vector is circular in step (a) and the circular vector is introduced into the cell in step (b).

4. A method according to Claim 1, 2 or 3, in which the vector is a BAC, YAC, PAC, cosmid or plasmid.

5. A method according to any preceding claim, in which the method uses a respective cutting site motif to direct each the cutting of step (c), in which each the motif is destroyed by the homologous recombination.

6. A method according to any preceding claim, in which the donor strand is a strand of a chromosome or episome of a or the cell.

7. A method according to any preceding claim, in which RS is at least 10kb in length.

8. A method according to any preceding claim, in which the nuclease cutting is Cas, meganuclease, zinc finger protein-mediated or TALEN-mediated cutting.

9. A method according to any preceding claim, in which the cutting is cutting by a Cas nuclease, 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

optionally in which HA1 comprises S1 and 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; and

optionally in which the remainder of PS2 is at the 3'end of HA2.

10. A method according to any preceding claim, in which in step (c) the same guide RNA (gRNA1) is used to guide Cas cutting of CS1 and CS2.

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

12. A method according to any preceding claim, further comprising inserting all or part of the RS of the product vector into a further nucleic acid strand, optionally in which RS sequence is inserted into the further strand using HA1 and/or HA2 in homologous recombination with the further strand.

13. A method according to Claim 12, in which the further strand is comprised by a cell and the vector product of step (d) is inserted into the cell before the homologous recombination.

14. A method according to Claim 12 or 13, in which the further strand is comprised by an embryonic stem cell or an immune cell; optionally in which the method comprises developing the stem cell product into tissue or a non-human organism.

15. A method according to Claim 12, 13 or 14 for gene therapy *in vitro* or *in vivo*, in which a mutation of the further strand is removed by the insertion of RS into the further strand; the method optionally comprising isolating or sequencing the further strand product or RS in the product.

16. A method according to any of Claims 12 to 15 for correcting a genetic mutation *in vitro* in a target human cell, in which the target cell comprises the further strand, in which the further strand comprises the genetic mutation, the method comprising

(i) obtaining a second human cell and carrying out a method according to any of Claims 1 to 11 inside the second cell, in which RS in the product vector of step (d) comprises the genetic defect;

(ii) using genetic engineering to correct the defect, thereby producing a product vector comprising a corrected RS sequence;

(iii) inserting the corrected RS sequence into the uncorrected target cell, in which the corrected RS sequence supplements or replaces the uncorrected RS sequence, thereby producing a corrected target cell; and

(iv) optionally culturing the corrected cell or developing tissue from the corrected cell or a progeny thereof.

17. A method according to Claim 16, in which the method uses homologous recombination of the uncorrected RS sequence in the target cell with HA1 and/or HA2 flanking the corrected RS sequence, thereby replacing the uncorrected RS sequence; optionally in which the uncorrected and corrected RS sequences differ only by the corrected mutation(s).

18. A method according to Claim 16 or 17, in which in step (iii) a closed circular corrected product vector is inserted into the target cell or progeny; optionally in which the corrected RS is at least 10kb in length.

19. A method according to Claim 16, 17 or 18, in which the cells are cells of the same human subject and optionally the same cell type.

20. A method according to any preceding claim for producing a Chimaeric Antigen Receptor T-cell (CAR-T cell).

21. A method according to any preceding claim, in which in step (b) and/or (d) the vector is a closed circular vector.

22. A method according to Claim 21, in which in step (b) the vector is supercoiled.

23. A method according to any preceding claim, in which sequence between HA1 and HA2 is deleted from the first strand and/or sequence between NS1 and NS2 is deleted from the donor strand.

24. A method according to Claim 23, in which RS is deleted from the donor strand.

25. A method according to any preceding claim, in which the cutting is cutting by a Cas nuclease, in which a protospacer/PAM combination at the 5’ end of HA1 or flanking 5’ of the end 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 end is destroyed by step (d), whereby PS2 is rendered non-functional in the first strand.

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

27. A method according to Claim 26 comprising Cas cutting the protospacer of the new combination after step (d), and optionally subsequently inserting a further sequence into the first strand.

28. A method according to any preceding claim, in which donor sequence is deleted from the donor strand to form a new protospacer/PAM combination or a new marker sequence.

29. A method according to Claim 28 comprising Cas cutting the protospacer of the new combination in the donor strand after step (d), and optionally subsequently inserting or deleting a further sequence into or from the donor strand.

30. A method according to any preceding claim, the method comprising isolating or copying the modified donor strand or the region of donor nucleic acid sequence where deletion occurred.

31. A method according to any preceding claim, in which RS is inserted into the first strand in step (d) and the inserted sequence is functional for expression from the modified first strand, the method optionally comprising detecting or isolating the expression product of the inserted sequence.

32. A method according to any preceding claim, in which steps (b) to (d) are performed in a host cell, in which RS is deleted from the donor strand 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.

33. A method according to any preceding claim, in which the method is carried out in a bacterial or archaeal cell, optionally an *E coli* cell.

34. A method according to any preceding claim, 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.

35. A method according to any preceding claim , in which steps (b) to (d) are performed in a host cell, in which the nuclease is a Cas and in step (c) Cas 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 rare or not found in the cell genome.

36. A corrected cell, corrected vector or corrected RS sequence as recited in any of Claims 16 to 19 for treating a genetic defect in a human.

37. A vector product, nucleic acid strand product, cell product or RS product obtainable by a method according to any of Claims 1 to 35 for administration to a human or animal subject to treat or prevent a disease or condition in the subject.

38. A method for producing a plurality of vectors comprising a collection of retrieved sequences (RSs), in which the collection comprises first and second RS sequences that are different from each other, the method comprising carrying out a method according to any of Claims 1 to 35 to obtain each vector.

39. A method according to Claim 38, in which the vectors are BAC or YAC vectors comprising a collection of genomic fragments retrieved from a cell sample.