Claims

1. A method for site-specific sequence modification of a target genomic DNA region in hematopoietic stem cells isolated from the body of a subject comprising:

transfecting the stem cells by flow electroporation with a composition, in particular a non-viral composition, comprising (a) a DNA oligo, in particular a single-stranded DNA oligo, more in particular having 100 nucleotides or less, and (b) a DNA digesting agent, in particular encoded on one or more RNAs, more in particular TALEN, transposase, integrase or nuclease, especially a site specific nuclease, more especially a composition selected from a composition comprising (i) a nuclease and a Cas9, or (ii) a site-specific nuclease and a guided RNA,

wherein the DNA oligo comprises:

(i) a homologous region comprising DNA sequence homologous to the target genomic DNA region; and

(ii) a sequence modification region; and

wherein the genomic DNA sequence is modified specifically at the target genomic DNA region, in particular wherein the DNA sequence modification is one or more stop codons, changes one or more base pairs of the genomic sequence, adds one or more base pairs of the genomic sequence, or deletes one or more base pairs of the genomic sequence,

optionally further comprising screening the cells for the sequence modification, and/or further comprising freezing transfected cells, and/or further comprising expanding transfected cells that were previously frozen.

2. The method of claim 1, wherein the DNA oligo is more than 10 nucleic acids,10-800 nucleic acids, 10-600 nucleic acids, 10-200 nucleic acids, 10-100 nucleic acids, or 10-50 nucleic acids.

3. The method of claim 1 or 2, wherein the concentration of the DNA oligo in the composition is more than 10 µg/mL, from 10 to 500 µg/mL, from 35 to 300 µg/mL, or from 35 to 200 µg/mL.

4. The method of any one of claims 1-3, wherein the cells are mammalian hematopoietic stem cells.

5. The method of any one of claims 1-4, wherein the oligo comprises at least 10 nucleic acids of homologous sequence, at least 20 nucleic acids of homologous sequence, or at least 30 nucleic acids of homologous sequence.

6. The method of any one of claims 1-5, wherein the efficiency of the sequence modification is greater than 3%, greater than 5%, or greater than 10%.

7. The method of any one of claims 1-6, wherein the cell viability after electroporation is at least 30%, at least 40%, or at least 50%.

8. The method of any one of claims 1-7, wherein the composition comprises two or more DNA oligos with different homologous sequences, or wherein the composition comprises two or more DNA oligos with different homologous sequences and two or more DNA digesting agents, in particular wherein the DNA digesting agents are targeted to different genomic sites.

9. The method of any one of claims 1-8, wherein the cells are cells isolated from a patient, were isolated from the patient at a time period of less than one week prior to transfection of the cells, or were isolated from the patient at a time period of less than one day prior to transfection of the cells, in particular wherein the isolated cells have not been frozen, and/or wherein the isolated cells comprise two or more different cell types, and/or wherein the two or more different cell types comprise two or more cell types at different stages of pluripotency.

10. The method of any one of claims 1-9, wherein the efficiency of the sequence modification is greater than 3%, greater than 5%, greater than 10%, and/or wherein the cell viability after electroporation is at least 30%, at least 40%, or at least 50%.

11. The method of any one of claims 1-10, wherein the cells are isolated from the bone marrow of the subject, in particular wherein the hematopoietic stem cells comprise the cell surface marker CD34+.

12. The method of any of claims 1-11, further comprising expanding a clonal isolated and selected cell to produce clonal cells having the DNA sequence modification, in particular wherein the clonal cells are expanded for large scale manufacturing, more in particular wherein the clonal cells are expanded in a volume greater than 1 L, or in a volume of 3 L or more.

13. The method of any of claims 1-12, wherein the cells are cultured in serum-free media.

14. A method for producing a stable cell line comprising a genomic DNA sequence modification of a target genomic DNA sequence, the method comprising:

transfecting hematopoietic stem cells isolated from the body of a subject by flow electroporation with a composition comprising (a) a DNA oligo and (b) a digesting agent;

wherein the donor DNA comprises:

(i) a homologous region comprising nucleic acid sequence homologous to the target genomic DNA region; and

(ii) a sequence modification region; and

screening transfected cells for the genomic DNA sequence modification at the target genomic DNA region;

isolating screened transfected cells by limiting dilution to obtain clonal cells;

expanding isolated transfected cells to produce a stable cell line comprising the genomic DNA sequence modification.