(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI);

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells; and

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector (and, optionally, a minimal promoter) and the NOI into a target locus and wherein the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector.

Transient expression or transient upregulation of a nucleotide sequence encoding a selector enables the specific selection of cells in which the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) has been inserted into the target locus from those cells in which the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) have not been inserted into the target locus.

In one aspect, the present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and, optionally, a minimal promoter;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells;

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector and the NOI and, optionally, the minimal promoter into a target locus; and wherein the ETT polypeptide binds to at least one ETT binding site operably linked to the target locus in the genome of the cell and (I) activates an endogenous promoter operably linked to the target locus or (II) activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus such that the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector; or the donor reporter cassette further comprises a minimal promoter operably linked to a regulatory element, wherein the ETT polypeptide binds to the regulatory element and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus such that the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector.

In one aspect, the present disclosure provides a donor reporter cassette sequentially comprising a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus; a nucleotide sequence of interest (NOI), optionally operably liked to a promoter; a nucleotide sequence encoding a selector, wherein the nucleotide sequence encoding the selector is operably linked to a minimal promoter and the minimal promoter is operably linked to a regulatory element, and wherein the regulatory element comprises at least one engineered transcriptional transactivator (ETT) binding site; and a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus. In another aspect, the

present disclosure provides a use of the donor reporter cassette according to claim 17 for selecting genome edited cells, for the enrichment of genome edited cells in a population of cells, for inserting a NOI into a target locus, and/or for correcting or disrupting a NOI at a target locus, preferably wherein, when the nucleotide sequence encoding the selector is inserted into the target locus in the genome of a cell, binding of an ETT polypeptide to the regulatory element activates the minimal promoter.

The present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and a minimal promoter operably linked to a regulatory element;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

CLAIMS

1. A method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and, optionally, a minimal promoter;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells;

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector and the NOI and, optionally, the minimal promoter, into a target locus; and

wherein:

(i) the ETT polypeptide binds to at least one ETT binding site operably linked to the target locus in the genome of the cell and (I) activates an endogenous promoter operably linked to the target locus or (II) activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus such that the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector; or

(ii) the donor reporter cassette further comprises a minimal promoter operably linked to a regulatory element, wherein the ETT polypeptide binds to the regulatory element and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus such that the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables

transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector.

2. The method according to claim 1(i) wherein:

(a) the endogenous promoter is operably linked to the at least one ETT binding site; or

(b) the minimal promoter is operably linked to the at least one ETT binding site when the nucleotide sequence encoding the selector is inserted into the target locus.

3. The method according to claim 1(i) or claim 2 wherein:

(a) the donor reporter cassette sequentially comprises:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;

(ii) the nucleotide sequence encoding the selector operably linked to the minimal promoter;

(iii) the NOI operably linked to a promoter; and

(iv) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component binds to at least one ETT binding site operably linked to the target locus in the genome of the cell and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus; or

(b) the donor reporter cassette sequentially comprises:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;

(ii) optionally, a splicing acceptor site (SA);

(iii) the NOI;

(iv) the nucleotide sequence encoding the selector operably linked to the minimal promoter; and

(v) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component binds to at least one ETT binding site operably linked to the target locus in the genome of the cell and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus; or

(c) the donor reporter cassette sequentially comprises:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;
(ii) optionally, a splicing acceptor site (SA);
(iii) the NOI;
(iv) optionally, a nucleotide sequence encoding a 2A self-cleaving peptide (2A) or an internal ribosome entry site (IRES) element;
(v) the nucleotide sequence encoding the selector, optionally the nucleotide sequence encoding the selector is operably linked to a minimal promoter; and
(vi) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component binds to at least one ETT binding site operably linked to the target locus in the genome of the cell and activates an endogenous promoter in the target locus.

4. The method according to claim 1(ii), wherein the donor reporter cassette sequentially comprises:
(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;
(ii) the NOI, optionally operably linked to a promoter;
(iii) the nucleotide sequence encoding the selector, wherein the nucleotide sequence encoding the selector is operably linked to a minimal promoter and the minimal promoter is operably linked to a regulatory element; and
(iv) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component binds to the regulatory element and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus and when a modulator is present in the cell or population of cells; or
wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component binds to the regulatory element and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus and when a modulator is not present in the cell or population of cells.

5. The method according to any one of claims 1 to 3 wherein the DBD is a Transcriptional Activator-Like Effector (TALE) DBD, a Zinc finger, catalytically inactive Cpf1 or catalytically inactive Cas (dCas) and wherein the TA domain is selected from the group consisting of VP16, VP64, VP128, VP160, VPR, p65, Rta, HSF1, SAM, and SunTag.

6. The method according to claim 1 or claim 4 wherein the DBD is a TetR or reverseTetR (rTetR) and wherein the TA domain is selected from the group consisting of VP16, VP64, VP128, VP160, VPR, p65, Rta, HSF1, SAM, and SunTag.

7. The method according to any one of the preceding claims wherein the gRNA is capable of binding to one or more of the nucleotide sequences selected from the group consisting of SEQ ID NOs 1 to 31 and sequences having at least 75% identity thereto.

8. The method according to any one of the preceding claims wherein the target locus is a safe harbour.

9. The method according to claim 8 wherein the target locus is adeno-associated virus integration site 1 (AAVS1), a common integration site (CIS) of lentiviral vectors, IL2RG, gp91phox, HBB, RAG1, CD40LG, TRAC, TRBC, STAT, PRF1, a gene encoding for a protein expressed in the skin (such as collagen, keratin, laminin, desmocolin, desmoplachine, desmoglein, placoglobin, placophylline, integrin or other proteins that are involved in desmosomes and hemidesmosomes) or another safe harbour genomic locus.

10. A kit comprising a first component, a second component and a third component as defined in any one of the preceding claims and, optionally, a cell population.

11. A population of genome edited cells produced by the method according to any one of claims 1-9.

12. A pharmaceutical composition comprising the population of genome edited cells according to claim 11.

13. A population of genome edited cells according to claim 11 for use in gene therapy.

14. Use of a population of genome edited cells according to claim 11 for the manufacture of a medicament for the treatment or prevention of X-linked Severe Combined Immunodeficiency (SCID –X1).

15. Use of a population of genome edited cells according to claim 11 for the manufacture of a medicament for hematopoietic stem cell transplantation (HSCT).

16. Use of a population of genome edited cells according to claim 11 for the manufacture of a medicament for tissue repair.

17. A donor reporter cassette sequentially comprising:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;

(ii) a nucleotide sequence of interest (NOI), optionally operably liked to a promoter;

(iii) a nucleotide sequence encoding a selector, wherein the nucleotide sequence encoding the selector is operably linked to a minimal promoter and the minimal promoter is operably linked to a regulatory element, and wherein the regulatory element comprises at least one engineered transcriptional transactivator (ETT) binding site; and

(iv) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus.

18. Use of the donor reporter cassette according to claim 17 for selecting genome edited cells, for the enrichment of genome edited cells in a population of cells, for inserting a NOI into a target locus, and/or for correcting or disrupting a NOI at a target locus, preferably wherein, when the nucleotide sequence encoding the selector is inserted into the target locus in the genome of a cell, binding of an ETT polypeptide to the regulatory element activates the minimal promoter.