samples of the electrophoresis gel were loaded as follows: 1) Marker; 2) –EP +H; 3) c+g+o-H; 4) c+g+o+H; 5) –EP +H; 6) c+g+o-H; 7) c+g+o+H. Lanes 2-4 represent genomic DNA from the AAVS1 locus and lanes 5-7 represent genomic DNA from SCD locus. The cut products of an integrated AAVS1 site are 298 and 170 basepairs, and the parental band is 468 base pairs. The integration rate was calculated as (density of digested bands)/[(density of digested bands + density of parental band). K562 cells transfected with DNA oligo and a AAVS1 locus-specific guide RNA integrates specifically into the AAVS1 site and not the SCD locus. The integration rate at the AAVS1 locus was 20%.

[0064] FIG. 15A-B: Site-specific inetegration using two guide RNAs targeting the AAVS1 (FIG. 15A) and SCD locus (FIG. 15B). As shown in FIG. 15B, site-specific integration of donor DNA was achieved at the SCD locus. These results are further described in Example 2.

[0065] FIG. 16A-B: Example donor DNA oligo with sequence modification region (uppercase and not shaded) and homologous region (lower case and shaded). FIG. 16A shows an example where a stop codon is inserted as an addition into a target genomic DNA. FIG. 16B shown an example where a single base is changed in the target genomic DNA (SEQ ID NOS:1-4, 9 and 49).

[0066] FIG. 17: Efficient transfection of HSC with mRNA encoding eGFP 1d post transfection. Control cells (without transfection, left two micrographs) and the transfected cells are presented (rgiht two micrograph). Cells are viable for both control and transfected cells. The close to 100% expression of eGFP (bottom, right) demonstrates the efficient transfection efficiency with mRNA transfection.

[0067] FIG. 18: Electroporation mediated efficient gene editing at AAVS1 site in HSC. HSC was transfected with cas9 (c) and gRNA (g) in mRNA formulation. Cel-1 assay was performed for the analysis of gene editing. Lane 1 is marker. Lane 2 is the control HSC (-EP). Lane 3 is GFP-mRNA transfected HSC. Lane 4 to 7 are the quadrate transfections of HSC with Cas9/gRNA.

[0068] FIG. 19: The most prevalent mutation (a 'hotspot') in gp91phox is the position 676C to T mutation in Exon 7. With the use of CRISPR and donor DNA single-stranded oligo to correct the T mutation back to C, from stop codon in CGD back to Arg at amino site of 226 after correction, it will restore the gp91 expression. By using EBV-transformed B cells derived from CGD patients, the cotranasfection actually restored the gp91 expression when assayed at 5d post Transfection with FITC-conjugated antibody against pg91 from 1% basal

ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:	ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:
Age I	ACCGGT		Hinc II	GTYRAC	
Ahd I	GACNNNNNGTC	8	Hind III	AAGCTT	
Alu I	AGCT		Hinf I	GANTC	
Alw I	GGATC		HinP1 I	GCGC	
AlwN I	CAGNNNCTG		Hpa I	GTTAAC	
Apa I	GGGCCC		Hpa II	CCGG	
ApaL I	GTGCAC		Hph I	GGTGA	
Apo I	RAATTY		Kas I	GGCGCC	
Asc I	GGCGCGCC		Kpn I	GGTACC	
Ase I	ATTAAT		Mbo I	GATC	
Ava I	CYCGRG		Mbo II	GAAGA	
Ava II	GGWCC		Mfe I	CAATTG	
Avr II	CCTAGG		Mlu I	ACGCGT	
			Mly I	GAGTCNNNNN	18
BamH I	GGATCC		Mnl I	CCTC	
Ban I	GGYRCC		Msc I	TGGCCA	
Ban II	GRGCYC		Mse I	TTAA	
Bbs I	GAAGAC		Msl I	CAYNNNNRTG	19
Bbv I	GCAGC		MspA1 I	CMGCKG	
BbvC I	CCTCAGC		Msp I	CCGG	
Bcg I	CGANNNNNNTGC	10	Mwo I	GCNNNNNNNGC	20
BciV I	GTATCC		Nae I	GCCGGC	
Bcl I	TGATCA		Nar I	GGCGCC	
Bfa I	CTAG		Nci I	CCSGG	
Bgl I	GCCNNNNNGGC	11	Nco I	CCATGG	
Bgl II	AGATCT		Nde I	CATATG	
Blp I	GCTNAGC		NgoMI V	GCCGGC	
Bmr I	ACTGGG		Nhe I	GCTAGC	
Bpm I	CTGGAG		Nla III	CATG	
BsaA I	YACGTR		Nla IV	GGNNCC	

CLAIMS

1. An ex vivo or in vitro method for site-specific sequence modification of a target genomic DNA region in mammalian hematopoietic stem or progenitor cells comprising:

transfecting the cells by electroporation with a non-viral composition comprising (a) a single-stranded DNA oligo of 70-100 nucleotides; (b) a Cas9 nuclease encoded on a RNA; and (c) a guide RNA, wherein the guide RNA is capped and polyadenylated;

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 and wherein the DNA oligo and the targeting RNA are complementary to the same strand of the target genomic DNA region; and wherein the concentration of the DNA oligo in the composition is 100-200 μg/mL.

2. The method of claim 1, wherein electroporation is flow electroporation using a flow electroporation device.

3. The method of claim 1 or 2, wherein the cells comprise mammalian cells and wherein the mammalian cells comprise peripheral blood lymphocytes, B cells, expanded T cells, hematopoietic stem cells, or mesenchymal stem cells.

4. The method of claim 3, wherein the mammalian cells are primary mammalian cells.

5. The method of claim 1 or 2, wherein the cells are cells isolated from a subject.

6. The method of claim 5, wherein the cell viability after electroporation is at least 40%.

7. The method of claim 5 or 6, wherein the cells are isolated from the bone marrow of the subject.

8. The method of any one of claims 5-7, wherein the cells comprise stem cells or hematopoietic stem cells.

9. The method of 3 or 4, wherein the cells are cells isolated from a subject.

10. The method of claim 9, wherein the cell viability after electroporation is at least 40%.

11. The method of claim 9 or 10, wherein the cells are isolated from the bone marrow of the subject.