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).

[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

[0073] FIG. 24 shows the effect of the selection of ssDNA oligomer on mutation repair efficiency. A higher repair efficiency was seen when the applied single stranded DNA (ssDNA) Oligomer and gRNA are complementary to the same strand of the genomic DNA duplex (oligo 1). Lower repair efficiency when seen when the applied ssDNA oligomer and gRNA are complementary to the different strands of the genomic DNA duplex (oligo 2). The gRNA targets to sense in CGD case and antisense for SCID case.

[0074] FIG. 25A-C shows the consistency in gene repair with gRNA made from a different kit. Shown in (a) is the viability of patient B-LCL after CRISPR/ssOND transfection with gRNA made from three kits with different lot numbers. Shown in (b) is the proliferation of patient B-LCL after CRISPR/ssOND transfection with gRNA made from three kits with different lot numbers. Shown in (c) is the gp91 repair of patient B-LCL after CRISPR/ssOND tranfection with gRNA made from three kits with different lot numbers. Consistent and efficient gene repair is demonstrated with gRNAs made by different kits and different lot numbers.

[0075] FIG. 26 shows gp91 repair of patient B-LCL after CRISPR/ssOND tranfection with Poly A tailing gRNA added without enzymatic reaction. The forward primer used was: 5-TTAATACGACTCACTATAGGCACCCAGATGAATTGTACGT-3' (SEQ ID NO:5). The reverse primer for 20T was: 5'-TTTTTTTTTTTTTTTTTTTTAAAAGCACCGACTCGGTGCC-3' (SEQ ID NO:6). The reverse primer for 30T was: 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAAGCACCGACTCGGTGCC-3' (SEQ ID NO:7). This data demonstrates that it is possible to add poly A through adding poly T in the reverse primer used for amplyifying amplicon as a template for the RNA production.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0076] Methods described herein use a DNA oligo and a DNA digesting agent to modify/amend a DNA sequence. It is contemplated that methods described herein provide a low toxicity and a high efficiency of incorporation of the DNA sequence modification.

Nucleic acids

A. Oligo

[0077] Embodiments concern the sequence modification of target genomic DNA sequences by electroporating cells with a composition comprising a DNA oligo and a DNA digesting agent. In some embodiments, the DNA oligo is single-stranded.

in duplex DNA to produce single-strand gaps. Single-stranded DNA is resistant to Exonuclease III. Mung Bean Nuclease degrades single-stranded extensions from the ends of DNA. Mung Bean Nuclease is also an RNA endonuclease. Nuclease BAL 31 degrades both 3' and 5' termini of duplex DNA. Nuclease BAL 31 is also a highly specific single-stranded endonuclease that cleaves at nicks, gaps, and single-stranded regions of duplex DNA and RNA. RNase I is a single strand specific RNA endonuclease that will cleave at all RNA dinucleotide. S1 Nuclease degrades single-stranded DNA and RNA endonucleolytically to yield 5'-phosphoryl-terminated products. Double-stranded nucleic acids (DNA:DNA, DNA:RNA or RNA:RNA) are resistant to S1 nuclease degradation except with extremely high concentrations of enzyme. Lambda Exonuclease catalyzes the removal of 5' mononucleotides from duplex DNA. Its preferred substrate is 5'-phosphorylated double stranded DNA, although Lambda Exonuclease will also degrade single-stranded and non-phosphorylated substrates at a greatly reduced rate. Lambda Exonuclease is unable to initiate DNA digestion at nicks or gaps, RecJ is a single-stranded DNA specific exonuclease that catalyzes the removal of deoxy-nucleotide monophosphates from DNA in the 5' to 3' direction. T7 exonuclease catalyzes the removal of 5' mononucleotides from duplex DNA. T7 Exonuclease catalyzes nucleotide removal from the 5' termini or at gaps and nicks of double-stranded DNA.

[0093] Restriction endonucleases are another example of nucleases that may be used in connection with the methods of the present invention. Non-limiting examples of restriction endonucleases and their recognition sequences are provided in Table 1.

Table 1. Recognition Sequences for Restriction Endonucleases.

ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:	ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:
AatII	GACGTC		Fnu4H I	GCNGC	
Acc65 I	GGTACC		Fok I	GGATG	
Acc I	GTMKAC		Fse I	GGCCGGCC	
Aci I	CCGC		Fsp I	TGCGCA	
Acl I	AACGTT		Hae II	RGCGCY	
Afe I	AGCGCT		Hae III	GGCC	
Afl II	CTTAAG		Hga I	GACGC	
Afl III	ACRYGT		Hha I	GCGC	

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	
Bae I	NACNNNNGTAPYCN	9	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	

ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:	ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:
BsaB I	GATNNNNATC	12	Not I	GCGGCCGC	
BsaH I	GRCGYC		Nru I	TCGCGA	
Bsa I	GGTCTC		Nsi I	ATGCAT	
BsaJ I	CCNNGG		Nsp I	RCATGY	
BsaW I	WCCGGW		Pac I	TTAATTAA	
BseR I	GAGGAG		PaeR7 I	CTCGAG	
Bsg I	GTGCAG		Pci I	ACATGT	
BsiE I	CGRYCG		PflF I	GACNNNGTC	
BsiHKA I	GWGCWC		PflM I	CCANNNNNTGG	21
BsiW I	CGTACG		PleI	GAGTC	
Bsl I	CCNNNNNNNGG	13	Pme I	GTTTAAAC	
BsmA I	GTCTC		Pml I	CACGTG	
BsmB I	CGTCTC		PpuM I	RGGWCCY	
BsmF I	GGGAC		PshA I	GACNNNNGTC	22
Bsm I	GAATGC		Psi I	TTATAA	
BsoB I	CYCGRG		PspG I	CCWGG	
Bsp1286 I	GDGCHC		PspOM I	GGGCCC	
BspD I	ATCGAT		Pst I	CTGCAG	
BspE I	TCCGGA		Pvu I	CGATCG	
BspH I	TCATGA		Pvu II	CAGCTG	
BspM I	ACCTGC		Rsa I	GTAC	
BsrB I	CCGCTC		Rsr II	CGGWCCG	
BsrD I	GCAATG		Sac I	GAGCTC	
BsrF I	RCCGGY		Sac II	CCGCGG	
BsrG I	TGTACA		Sal I	GTCGAC	
Bsr I	ACTGG		Sap I	GCTCTTC	
BssH II	GCGCGC		Sau3A I	GATC	
BssK I	CCNGG		Sau96 I	GGNCC	
Bst4C I	ACNGT		Sbf I	CCTGCAGG	
BssS I	CACGAG		Sca I	AGTACT	

ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:	ENZYME	RECOGNITION SEQUENCE	SEQ ID NO:
BstAP I	GCANNNNNTGC	14	ScrF I	CCNGG	
BstB I	TTCGAA		SexA I	ACCWGGT	
BstE II	GGTNACC		SfaN I	GCATC	
BstF5 I	GGATGNN		Sfc I	CTRYAG	
BstN I	CCWGG		Sfi I	GGCCNNNNNGGCC	23
BstU I	CGCG		Sfo I	GGCGCC	
BstX I	CCANNNNNNTGG	15	SgrA I	CRCCGGYG	
BstY I	RGATCY		Sma I	CCCGGG	
BstZ17 I	GTATAC		Sml I	CTYRAG	
Bsu36 I	CCTNAGG		SnaB I	TACGTA	
Btg I	CCPuPyGG		Spe I	ACTAGT	
Btr I	CACGTG		Sph I	GCATGC	
Cac8 I	GCNNGC		Ssp I	AATATT	
Cla I	ATCGAT		Stu I	AGGCCT	
Dde I	CTNAG		Sty I	CCWWGG	
Dpn I	GATC		Swa I	ATTTAAAT	
Dpn II	GATC		Taq I	TCGA	
Dra I	TTTAAA		Tfi I	GAWTC	
Dra III	CACNNNGTG		Tli I	CTCGAG	
Drd I	GACNNNNNNGTC	16	Tse I	GCWGC	
Eae I	YGGCCR		Tsp45 I	GTSAC	
Eag I	CGGCCG		Tsp509 I	AATT	
Ear I	CTCTTC		TspR I	CAGTG	
Eci I	GGCGGA		Tth111 I	GACNNNGTC	
EcoN I	CCTNNNNNAGG	17	Xba I	TCTAGA	
EcoO109 I	RGGNCCY		Xcm I	CCANNNNNNNNNTGG	24
EcoR I	GAATTC		Xho I	CTCGAG	
EcoR V	GATATC		Xma I	CCCGGG	
Fau I	CCCGCNNNN		Xmn I	GAANNNNTTC	25

[00163] In some embodiments, the method comprises modifying the *HBB* gene genomic DNA sequence in hematopoietic stem cells (a.k.a. hemocytoblasts) or in myeloid progenitor cells. In certain embodiments, the sequence is modified to correct a disease-associated mutation in the genomic sequence. For example, the genomic sequence of a subject with sickle-cell anemia may be modified to correct the E6V mutation. Therefore, methods described herein may be used to correct the genomic sequence of cells from a subject harboring a genomic mutation that produces a β-globin protein with a valine at the sixth position instead of a glutamic acid. Accordingly, in one embodiment, the sequence modification is the correction of the genomic DNA that modifies the sixth codon of the *HBB* gene to a glutamic acid codon.

[00164] The protein coding sequence for the *HBB* gene is exemplified in the following sequence:
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAV
MGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNV
LVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH (SEQ ID NO:26). The underlined glutamic acid represents the amino acid at the sixth position of the protein. At the DNA level, the genomic DNA comprises a GAG to GTG mutation, which results in the E6V mutant protein.

[00165] In some embodiments, the method comprises modifying the gp91phox gene in cells. In some embodiments, the cells are autologous cells from the patient. In some embodiments, the cells are hematopoietic stem cells. In some embodiments, the method is for treating chronic granulomatous disease (CGD).

[00166] The protein coding sequence for the gp91phox gene is exemplified by the following sequence: mgnwavnegl sifvilvwlg lnvflfvwyy rvydippkff ytrkllgsal alarapaacl nfncmlillp vcrnllsflr gssaccstrv rrqldrnltf hkmvawmial hsaihtiahl fnvewcvnar vnnsdpysva lselgdrqne sylnfarkri knpegglyla vtllagitgv vitlclilii tsstktirrs yfevfwythh lfviffigla ihgaerivrg qtaeslavhn itvceqkise wgkikecpip qfagnppmtw kwivgpmfly lcerlvrfwr sqqkvvitkv vthpfktiel qmkkkgfkme vgqyifvkcp kvsklewhpf tltsapeedf fsihirivgd wteglfnacg cdkqefqdaw klpkiavdgp fgtasedvfs yevvmlvgag igvtpfasil ksvwykycnn atnlklkkiy fywlcrdtha fewfadllql lesqmqernn agflsyniyl tgwdesqanh favhhdeekd vitglkqktl ygrpnwdnef ktiasqhpnt rigvflcgpe alaetlskqs isnsesgprg vhfifnkenf (SEQ ID NO:27).

[00167] In some embodiments, the method corrects the nucleotide sequence of the gp91phox gene at Exon 7, position 676C to T. Correcting nucleotide "T" at amino acid site

[00209] Genomic DNA extraction: Genomic DNA was extracted using Purelink genomic DNA Mini kit (Invitrogen, Carlsbad CA). The extracted genomic DNA was stored in -4C refrigerator before use.

[00210] Gene modification assay: Cel-1 assay was performed to assay the gene genomic DNA editing by using SURVEYOR Mutation detection kit (Trangenomic, Omaha NE). The protocol with the kit provided by the company was followed. The integration of HindIII recoganizing 6 nucleotides was assayed by HindIII digestion. The samples were analyzed with 10% TBE gel ((Invitrogen, Carlsbad CA).

[00211] CRISPR (Cas9 and gRNA): The whole kit for Cas9 and gRNA targeting to the specific 5' GGGGCCACTAGGGACAGGAT TGG 3' (SEQ ID NO:28) site on SSAV1 safe harbor site was purchased from Washington University in St. Luise Genome Engineering Center). The primers (**F** – 5' TTCGGGTCACCTCTCACTCC 3' (SEQ ID NO:29); **R** – 5' GGCTCCATCGTAAGCAAACC 3' (SEQ ID NO:30)) for amplify genomic DNA segment containing the gRNA target site were included in the kit. The about 468bp amplicon was used for further Cel-1 assay and HindIII digest assay, the digestion of which will give two bands of about 170 and 298 bp each, if genomic DNA modification occurs.

[00212] CRISPR mRNA: the mRNA was made with mMESSAGE mMACHINE® T7 Ultra Kit (Invitrogen, Carlsbad CA) from template plasmid DNA purchase from Washington University in St Luise.

[00213] Single-stranded Oligomer: the sequence of the oligos are as follows:

Example 2

[00214] The methods described herein may be used to correct the disease-causing mutation(s) in patient hematopoietic stem cells (HSC) to cure genetic diseases. This example describes methods to correct the mutation in Chronic Granulomatous Disease (CGD) for curing this disease. This disease will not only demonstrate the proof-of-concept of the gene therapy methods described herein, but also advance therapeutic approaches for this disease, which, thus far, is still an unmet challenge. Since the genetic mutations in CGD are well known, the techniques of in vitro functional assays of the cells with the disease are matured, the animal model of CGD is established, and low percentage of correction can lead to significant clinical efficacy. Non-viral approaches will be used to deliver messenger RNA (mRNA) encoding CRISPR (Cas9 and gRNA pair) and DNA oligomer targeting the most

[00232] Nucleotide integration by transfection of CRISPR and single-stranded DNA oligomer may be intuitively believed to be different from the nucleotide correction, which does not increase nucleotide length. The gene correction will further be different from the correction of the gene expression of the right functional protein. To further show by proof-of-concept, Applicants demonstrated that mRNA transfection of CRISPR and single-stranded oligomer not only can mediate gene integration, gene correction, but also can mediate phenotype reverse to have functional gene expression. As shown in FIG. 19, Flow electroporation mediated efficient restoration of gp91 expression in CGD patient EBV-transformed B cells. Actual rate of the HindIII-recognizing 6-neucleotide integration in the same EBV-transformed B cells was much higher than the rate of gp91 expressing cells (data not shown). This was also successfully performed in patient HSCs with high efficiency (>5%; data not shown).

[00233] The following methods may be used to correct CGD in patients: The most prevalent mutation (a 'hotspot') in gp91phox in Exon 7 at position 676C to T can first be targeted by first using EBV-transformed B cells derived from the patients. In this special CGD, correcting nucleotide "T" at amino acid site 226 to be "C" restores the site to be the right Arg from the stop codon, and expression from corrected cells can be quantified by the gp91 expression and confirmed by sequencing.

[00234] Four gRNA (g1, g2, g3, and g4) can be first tested and verified for efficacy. These gRNA are listed in the table below:

	gRNA targeting to gp91	SEQ ID NO:
g1	TTTCCTATTACTAAATGATCNGG	31
g2	CACCCAGATGAATTGTACGTNGG	32
g3	TGCCCACGTACAATTCATCTNGG	33
g4	AGTCCAGATCATTTAGTAATNGG	34

[00235] The effect of oligomer size from 50mer to 200mer (As shown in the table below, O1-O4) on the gene correction efficiency can be tested. It is expected that longer

oligomer size may result in a better correction outcome, if the size still does not invoke the DNA sensor inside cells to initiate apoptosis or pyroptosis.

	Single-Stranded Oligo for Gene Correction	SEQ ID NO:
01	5'ACATTTTTCACCCAGATGAATTGTACGTGGGCAGACCGC AGAGAGTTTGGC-3'	35
02	5'CTATTACTAAATGATCTGGACTTACATTTTTCACCCAGAC GAATTGTACGTGGGCAGACCGCAGAGAGTTTGGCTGTGCA TAATATAACAGTTTGTGAA-3'	36
03	5'TCTTTTAATAAAACAATTTAATTTCCTATTACTAAATGAT CTGGACTTACATTTTTCACCCAGATGAATTGTACGTGGGCA GACCGCAGAGAGTTTGGCTGTGCATAATATAACAGTTTGTG AACAAAAAATCTCAGAATGGGGAA-3	37
04	5'CAGAGCACTTAAAATATATGCAGAATCTTTTAATAAAAC AATTTAATTTCCTATTACTAAATGATCTGGACTTACATTTTT CACCCAGATGAATTGTACGTGGGCAGACCGCAGAGAGTTT GGCTGTGCATAATATAACAGTTTGTGAACAAAAAATCTCA GAATGGGGAAAAATAAAGGAATGCCCAATCCCTCA-3	38

[00236] For the first phase of study, oligomer can be used to integrate a HindIII-recognizing site (AAGCTT), and the efficiency of targeting of the four gRNA can be tested using an Indel efficiency by Cel-1 assay and integration efficiency of HindIII-recognizing site by HindIII digesting assay of the PCR amplified amplicon. Oligomer with the HindIII-recognizing sequence removed can then be used, and with T to C change at the mutation site to test the restoration of the gp91 expression for extended long time to understand the persistency of gene correction.

[00237] To confirm the true correction, the three assays listed below may be used: 1) Use antibody against gp91 to assay to check the restoration of gp91 expression; 2) Sorting gp91 positive cells, and sequencing the PCR amplicon to verify the correction; and 3) *In vitro* functional study of $O2^-$ production by measure the enhanced chemiluminescence with the stimulation of phorbol myristate acetate (PMA).

[00238] Since Applicants already have Cas9 produced and tested, Applicants do not expect any problems relating to obtaining efficient gene editing at the site close to the mutation site. A cel-1 assay can be used to check the efficiency of gene editing. A Hind III

with IL2receptor gamma chain mutation, which leads to no IL2receptor gamma chain expression. Successful gene repair will lead to the restoration of gp91 and IL2Rgc expression.

[00250] mMESSAGE mMACHINE® T7 ULTRA Transcription Kit (T7 Ultra) is used, unless specified. Protocol provided by the kit is followed, unless specified. For the study the effect of capping, tail, TranscriptAid T7 High Yield Transcription Kit (TranscriptAid) is used. The protocol from the kit is followed when no capping and poly A are added. For capping, 4:1 ARCA:GTP is used. For poly A, the reagent for poly A addition from

[00251] mMESSAGE mMACHINE® T7 ULTRA Transcription Kit is used.

Cas9 mRNA is made by using linearized cas 9 plasmid. gRNA RNA is made by using PCR amplicon from gRNA plasmid. Single stranded DNA oligo of 100mer size, complementary to the target site, is purchased from IDT.

[00252] Gp91-gRNA targeting sequence: CACCCAGATGAATTGTACGTNGG (SEQ ID NO:39) (Red is the mutated nucleotide).

[00253] The following primers, oligos, and sequences were used in the experiments of this example:

Forward primer for gp91-gRNA template	5-TTAATACGACTCACTATAGGCACCCAGATGAATTGTACGT-3'	SEQ ID NO:40
The reverse primer for gp91-gRNA template	5'-aaaagcaccgactcggtgcc-3'	SEQ ID NO:41
Oligo sequence complementary to the same duplex as gp91-gRNA targets to	5-CTATTACTAAATGATCTGGACTTACATTTTTCACCCAGACGAATTGTACGTGGGCAGACCGCAGAGAGTTTGGCTGTGCATAATATAACAGTTTGTGAAC-3	SEQ ID NO:42
Oligo sequence complementary to the opposite duplex as gp91-gRNA targets to	5-TGTTATATTATGCACAGCCAAACTCTCTGCGGTCTGCCCACGTACAATTCGTCTGGGTGAAAAATGTAAGTCCAGATCATTTAGTAATAGGAAATTAAAT-3	SEQ ID NO: 43

X-SCID-gRNA targeting sequence	5' TTCTCATCGAAAAGTTCCCG 3' (underlined is the mutated nucleotide)	SEQ ID NO:44
Forward primer for X-SCID-gRNA template	5-TTAATACGACTCACTATAGGTTCTCATCGAAAAGTTCCCG-3	SEQ ID NO:45
The reverse primer for X-SCID-gRNA template	5'-aaaagcaccgactcggtgcc-3'	SEQ ID NO:46
Oligo sequence complementary to the same duplex as X-SCID-gRNA targets to	5'-GTTGGCAGTTGATAGACTGCAGCATGCTTATGACAGCGTTCTCACCGAAAAGTTCCCGTGGTATTCAGTAACAAGATCCTCTAGGTTCTTCAGGGTGGGA-3'	SEQ ID NO:47
Oligo sequence complementary to the opposite duplex as X-SCID-gRNA targets to	5-TCCCACCCTGAAGAACCTAGAGGATCTTGTTACTGAATAC CACGGGAACTTTTCGGTGAGAACGCTGTCATAAGCATGCTGCAGTCTATCAACTGCCAAC-3	SEQ ID NO:48

[00254] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.