Summary

In one aspect, the present disclosure refers to a process for producing a modified nucleic acid molecule, the process comprising the steps:

(a) contacting a nucleic acid molecule comprising a mutant *HBB* gene encoding a mutant Hb-β polypeptide with a base editor, wherein the mutant *HBB* gene comprises a first non-wild-type codon coding for a first non-wild-type amino acid; and

(b) incubating the mutant *HBB* gene and base editor under conditions such that the base editor is targeted to the nucleotide sequence of the first non-wild-type codon and wherein the base editor edits one or more nucleotides in the first non-wild-type codon to produce a second non-wild-type codon which codes for a second non-wild-type amino acid, thereby producing a modified nucleic acid molecule comprising an edited *HBB* gene which encodes an edited Hb-β polypeptide,

wherein the edited Hb-β polypeptide has a non-wild-type, yet phenotypically-viable, amino acid sequence.

In another aspect, the present disclosure refers to a population of isolated cells comprising haematopoietic stem cells or progenitor cells comprising edited *HBB* genes, the edited *HBB* genes comprising:

(i) a nucleotide sequence having 90-99.9% nucleotide sequence identity to SEQ ID NO: 1 or a nucleotide sequence encoding an amino acid sequence having 95-99.5% amino acid sequence identity to SEQ ID NO: 2; and wherein

(ii) the nucleotide sequence at the codon which corresponds to codon 7 in SEQ ID NO: 1 codes for glycine or alanine; and/or the nucleotide sequence at the codon which corresponds to codon 27 in SEQ ID NO: 1 codes for glycine.

Detailed Description

In one embodiment, the invention provides a process for producing a modified nucleic acid molecule, the process comprising the steps:

(a) contacting a nucleic acid molecule comprising a mutant *HBB* gene encoding a mutant Hb-β polypeptide with a base editor, wherein the mutant *HBB* gene comprises a first non-wild-type codon coding for a first non-wild-type amino acid; and

(b) incubating the mutant *HBB* gene and base editor under conditions such that the base editor is targeted to the nucleotide sequence of the first non-wild-type codon and wherein the base editor edits one or more nucleotides in the first non-wild-type codon to produce a second non-wild-type codon which codes for a second non-wild-type amino acid, thereby producing a modified nucleic acid molecule comprising an edited *HBB* gene which encodes an edited Hb-β polypeptide,

wherein the edited Hb-β polypeptide has a non-wild-type, yet phenotypically-viable, amino acid sequence.

Preferably, Step (a) comprises contacting a nucleic acid molecule comprising a mutant HBB gene encoding a mutant Hb-β polypeptide with a base editor and a gRNA, wherein the mutant *HBB* gene comprises a first non-wild-type codon coding for a first non-wild-type amino acid and wherein the gRNA is capable of targeting the base editor to the nucleotide sequence of the first non-wild-type codon of the mutant HBB gene; and Step (b) comprises incubating the mutant *HBB* gene, base editor and gRNA under conditions such that the gRNA targets the base editor to the nucleotide sequence of the first non-wild-type codon and wherein the base editor edits one or more nucleotides in the first non-wild-type codon to produce a second non-wild-type codon which codes for a second non-wild-type amino acid, thereby producing a modified nucleic acid molecule comprising an edited *HBB* gene.

In another embodiment, the invention provides a process for producing a modified nucleic acid molecule, the process comprising the steps:

(a) contacting a nucleic acid molecule comprising a mutant *HBB* gene encoding a mutant Hb-β polypeptide with a base editor and a gRNA, wherein the mutant *HBB* gene comprises a first non-wild-type codon coding for a first non-wild-type amino acid and wherein the gRNA is capable of targeting the base editor to the nucleotide sequence of the first non-wild-type codon of the mutant *HBB* gene; and

(b) incubating the mutant *HBB* gene, base editor and gRNA under conditions such that the gRNA targets the base editor to the nucleotide sequence of the first non-wild-