CLAIMS

What is claimed is:

1. A system for modifying at least one target DNA, comprising:

   two or more endonucleases, or one or more nucleic acid(s) encoding said endonucleases, wherein each endonuclease nicks one strand of the target DNA at a target nick, and at least one donor DNA comprising a sequence of interest to be incorporated to a target DNA site.

2. The system of claim 1, wherein the target DNA is a double-stranded DNA; and/or

   the target DNA is a genomic DNA; preferably, the target DNA is part of the DNA genome within a cell.

3. The system of claim 1 or 2, wherein the target nicks of the two or more endonucleases are on the same DNA strand or on different DNA strands; and/or

   two of the target nicks are separated by 1 to 3,000,000 bases; preferably, 10 to 2,000 bases; more preferably, 10 to 200 bases; more preferably, 10 to 100 bases; more preferably, 20 to 60 bases; more preferably, 30 to 50 bases; more preferably, 35 to 40 bases; or preferably, 100 to 2,000 bases.

4. The system of any one of claims 1-3, wherein the donor DNA is a single-stranded DNA; or is a double-stranded DNA; or the donor DNA comprises one or more portions of single-stranded DNA and one or more portions of double-stranded DNA; and/or

   the donor DNA is a circularized DNA; and/or

   the donor DNA is 6-nucleotide to 100,000-nucleotide long, preferably, the donor DNA is 50-nucleotide to 4,000-nucleotide long; or the donor DNA having the single-stranded DNA and double-stranded DNA has a total length of 6-nucleotide to 100,000-nucleotide long, preferably, the single-stranded and double-stranded mixed donor DNA has a total length of 50-nucleotide to 4,000-nucleotide long.

5. The system of any one of claims 1-4, wherein endonuclease targeting sites comprising the target nicks on the target DNA are distant for 1 to 3,000,000 bases; preferably, the endonuclease targeting sites comprising the target nicks on the target DNA are distant for 10 to 2,000 bases, more preferably, the endonuclease targeting sites comprising the target nicks on the target DNA are distant for 10 to 200 bases, more preferably, the endonuclease targeting sites on the target DNA are separated by 10 to 100 bases, more preferably, the endonuclease targeting sites on the target DNA are separated by 20 to 60 bases, more preferably, the endonuclease targeting sites on the target DNA are

separated by 30 to 50 bases; more preferably, the endonuclease targeting sites on the target DNA are separated by 35 to 40 bases; or preferably, the endonuclease targeting sites on the target DNA are separated by 100 to 2,000 bases; preferably, the target DNA is a genomic DNA; or

the endonuclease targeting sites comprising the target nicks on the target DNA are on two different chromosomes, and the target DNA is a genomic DNA.

6. The system of any one of claims 1-5, wherein the donor DNA comprises one or more homologous portions, each having a sequence homologous to the one or more endonuclease targeting sites comprising the target nicks; or a first donor DNA comprises a homologous portion with a sequence homologous to a first targeting site, and a second donor DNA comprises a homologous portion with a sequence homologous to a second targeting site;

   preferably,

   the homologous portion has 20-1,000 nucleotides in length, preferably, the homologous portion has 20-800 nucleotides in length; and/or

   the homologous portion is located at the 5' end of the donor DNA and/or 3’end of the donor DNA; and/or

   the homologous portion of the donor DNA is configured to be capable of base-pairing with the target DNA site; and/or the homologous portion of the donor DNA is configured to be filled in a single-stranded portion of the target DNA site; and/or the homologous portion of the donor DNA is configured to be capable of stranding in and displacing a nicked target DNA; and/or the homologous portion of the donor DNA is configured to base-pair with the target DNA on the complementary strand to a nicked strand; and/or the homologous portion of the donor DNA is configured to base-pair with the target DNA on the complementary strand to a nicked strand at its 5' end; and/or the homologous portion of the donor DNA is configured to base-pair with the target DNA on the complementary strand to a nicked strand at its 3' end; and/or the homologous portion of the donor DNA is configured to base-pair with the target DNA on the complementary strand to a nicked strand and spanning the target nicks.

7. The system of any one of claims 1-6, wherein the endonuclease is an RNA-guided nickase and an associated guide RNA;

   preferably,

   the guide RNA comprises a portion that binds the RNA-guided nickase and an MS2 hairpin portion, and the endonuclease contains an MS2 binding motif; and/or

the guide RNA comprises a modified nucleoside; preferably, the modified nucleoside comprises an inverted nucleoside, a locked nucleic acid, a 2' fluoro, a 2' O-alkyl, a methylated cytosine, or a combination thereof.

8. The system of any one of claims 1-7, wherein the system comprises **(a)** at least two associated guide RNAs: a first guide RNA capable of hybridizing to a first target sequence in the target DNA, and a second guide RNA capable of hybridizing to a second target sequence in the target DNA, or **(b)** one or more nucleic acid(s) encoding said associated guide RNAs;

   preferably,

   the first guide RNA and the second guide RNA are configured to be positioned with their PAM sites oriented towards each other (PAM-facing) on the target DNA, optionally, the first and second guide RNAs bind to different strands of the target DNA; or

   the first guide RNA and the second guide RNA are configured to be positioned with their PAM sites oriented away from each other (PAM-backing) on the target DNA, optionally, the guide RNAs bind to different strands of the target DNA; or

   the first guide RNA and the second guide RNA are configured to be positioned with their PAM sites oriented in the same direction (PAM-same-directional), optionally, the guide RNAs bind to the same strand of the target DNA.

9. The system of any one of claims 7-8, wherein each RNA-guided nickase is a CRISPR Cas nickase; preferably, each RNA-guided nickase is a class II CRISPR Cas nickase;

   preferably,

   each RNA-guided nickase is individually a Cas9 nickase; preferably, each Cas9 nickase is individually SpCas9-D10A nickase or SpCas9-H840A nickase; and/or

   at least one RNA-guided nickase is SpCas9-D10A nickase, and at least one RNA-guided nickase is SpCas9-H840A nickase.

10. The system of any one of claims 7-9, wherein the RNA-guided nickases and the guide RNAs are arranged according to any one of the Embodiments in Table 1.

11. The system of any one of claims 1-10, wherein each endonuclease is individually coupled to a DNA ligase, a DNA polymerase, a DNA exonuclease, a HUH endonuclease or an MS2 binding motif, and/or at least two endonucleases are coupled with each other; preferably, the coupling is covalent, preferably, the coupling forms a fusion protein with a linker; or preferably, the coupling is non-covalent; and/or

the donor DNA is coupled to at least one of the endonucleases; preferably, the coupling is covalent, preferably, the coupling is formed by bioconjugation ability of an HUH endonuclease to covalently tether the donor DNA; or the coupling is non-covalent, preferably, the coupling is via complementary pairing of a portion of a guide RNA and a portion of the donor DNA.

12. The system of any one of claims 1 to 11, wherein the donor DNA comprises a modified nucleoside; preferably, the modified nucleoside comprises an inverted nucleoside, a locked nucleic acid, a 2' fluoro, a 2' O-alkyl, a methylated cytosine, or a combination thereof; and/or

    the donor DNA comprises a modified inter-nucleoside linkage; preferably, the modified inter-nucleoside linkage is a phosphorothioate linkage.

13. A method of using the system of any one of claims 1 to 12 for modifying at least one target DNA, preferably, the methodcomprises the steps of: (i) complexing the system in vitro, and (ii) delivering the system into a target cell; or the method comprises the steps of: (i) packaging the system in vivo in a producer cell, and (ii) delivering the system into a target cell.

14. An engineered cell or a population of cells derived therefrom, wherein the engineered cell is produced by the method of claim 13; preferably, the engineered cell is an in vitro cell;

    preferably, the sequence of interest is incorporated into the target DNA of the engineered cell.

15. Use of the system of any one of claims 1 to 12 in the manufacture of a medicament for the treatment of a genetic disease.