

CLAIMS

1. A method for enriching a target nucleic acid comprising:

providing an endonuclease system having:

a clustered regularly interspaced short palindromic repeat (CRISPR) RNA (crRNA) or a derivative thereof, and

a CRISPR-associated (Cas) protein or a variant thereof,

in which the crRNA or the derivative thereof contains a target-specific nucleotide region complementary to a region of the target nucleic acid;

contacting the target nucleic acid with the endonuclease system to form a complex,

in which the crRNA or the derivative thereof in the complex formed by the CRISPR-Cas endonuclease system is labelled, and

separating the complex using the label, thereby enriching for the target nucleic acid.

2. A method according to Claim 1 for enriching a target double-stranded nucleic acid comprising:

providing an endonuclease system having:

a clustered regularly interspaced short palindromic repeats (CRISPR) RNA (crRNA) or a derivative thereof, and

a CRISPR-associated (Cas) protein or a variant thereof,

in which the crRNA or the derivative thereof contains a target-specific nucleotide region complementary to a region of a first strand of the target double-stranded nucleic acid;

contacting the target double-stranded nucleic acid with the endonuclease system to form a first complex;

hybridizing a labelled nucleic acid to a second strand of the target double-stranded nucleic acid to form a second complex, the second strand of the target double-stranded nucleic acid being non-complementary to the crRNA or the derivative thereof, and

separating the second complex through the labelled nucleic acid, thereby enriching for the target nucleic acid.

3. A method according to Claim 1 or 2

optionally further comprising separating the target nucleic acid from the complex,
 optionally further comprising amplifying the targeted nucleic acid,
 in which

(i) the endonuclease system further comprises a trans-activating crRNA (tracrRNA) or a derivative thereof; or

(ii) the crRNA or the derivative thereof is a polynucleotide comprising a crRNA polynucleotide fused to a tracrRNA polynucleotide; or

(iii) the endonuclease system is a Type II CRISPR-Cas system or a derivative thereof; or

(iv) the target nucleic acid is a double-stranded DNA (dsDNA); or

(v) the endonuclease system is labelled,

optionally

(a) in which the crRNA is labelled with biotin, and the method optionally further comprising adding streptavidin and thereby separating the complex; or

(b) in which the Cas protein or the derivative thereof is labelled with a capture tag.

4. A method according to Claim 1 or 2, in which the Cas protein or the variant thereof is a Cas9 protein or a variant thereof,

optionally

in which the Cas9 protein or the variant thereof retains two nuclease domains and is able to produce a double-stranded DNA break; or

in which the Cas9 protein or the variant thereof contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is complementary to the crRNA, optionally in which the mutation is D10A; or

in which the Cas9 protein or the variant thereof contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the crRNA, optionally in which the mutation is H840A; or

in which the Cas9 protein or the variant thereof contains two inactivated nuclease domains, optionally in which the two inactivated nuclease domains comprise a first mutation in the domain that cleaves the strand complementary to the crRNA and a second mutation in the domain that cleaves the strand non-complementary to the

crRNA, optionally in which the first mutation is D10A and the second mutation is H840A.

5. A method according to Claim 1 or 2 further comprising tagmenting the target nucleic acid.

5 6. A method according to Claim 1 or 2 further comprising adding a transposase, in which the crRNA or the derivative thereof contains a transposon end, optionally in which the transposon end is a mosaic end (ME), and in which the transposase is a Tn5 transposase.

7. A method according to Claim 1 or 2 further comprising:

adding a transposon end to the target nucleic acid, and

10 tagmenting the target nucleic acid,

in which the endonuclease system further comprises a transposase, optionally in which:

(i) the transposase binds to a nucleotide sequence of the endonuclease system; or

(ii) the transposase and the Cas protein form a fusion protein; or

(iii) the transposon end is a mosaic end (ME), and in which the transposase is a

15 Tn5 transposase.

8. A method for enriching a target nucleic acid according to Claim 1, in which the target nucleic acid is obtained from a population of cell free DNA (cfDNA) from a subject's plasma or serum, the population of cell free DNA containing the target nucleic acid.

9. A method according to Claim 8, in which:

20 (A) the target nucleic acid contains a single nucleotide variant (SNV); or

(B) further comprising separating the target nucleic acid from the complex; or

(C) further comprising amplifying the targeted nucleic acid; or

(D) the endonuclease system further comprises a trans-activating crRNA (tracrRNA) or a derivative thereof; or

25 (E) the crRNA or the derivative thereof is a polynucleotide comprising a crRNA polynucleotide fused to a tracrRNA polynucleotide; or

(F) the endonuclease system is a Type II CRISPR-Cas system or a derivative thereof; or

(G) the target nucleic acid is a double-stranded DNA (dsDNA); or

(H) the endonuclease system is labelled optionally in which the endonuclease system is labelled in which the crRNA is labelled with biotin optionally further comprising adding streptavidin and thereby separating the complex; in which the endonuclease system is labelled and in which the Cas protein or the derivative thereof is labelled with a capture tag; or

(I) in which the Cas protein or the variant thereof is a Cas9 protein or a variant thereof, optionally in which

(i) the Cas9 protein or the variant thereof retains two nuclease domains and is able to produce a double-stranded DNA break; or

(ii) the Cas9 protein or the variant thereof contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is complementary to the crRNA, optionally in which the mutation is D10A; or

(iii) the Cas9 protein or the variant thereof contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the crRNA optionally in which the mutation is H840A; or

(iv) the Cas9 protein or the variant thereof contains two inactivated nuclease domains optionally in which the two inactivated nuclease domains comprise a first mutation in the domain that cleaves the strand complementary to the crRNA and a second mutation in the domain that cleaves the strand non-complementary to the crRNA, optionally in which the first mutation is D10A and the second mutation is H840A; or

(J) the target nucleic acid is in a fetal cell fraction of the cell free DNA, and in which the cell free DNA is from maternal plasma.

10. A method for detecting a single nucleotide variant (SNV) in which the target nucleic acid is obtained from a population of cell free DNA from a subject's plasma or serum, the method comprising:

providing a first endonuclease system having:

a first clustered regularly interspaced short palindromic repeats (CRISPR) RNA (crRNA) or a derivative thereof, and

a first CRISPR-associated (Cas) protein or a variant thereof,

in which the first crRNA or the derivative thereof contains a first target-specific nucleotide region complementary to a region of a first target nucleic acid, and in which the first Cas protein has nuclease activity; in which the RNA in the complex formed by the CRISPR-Cas endonuclease is labelled;

cleaving the first target nucleic acid using the endonuclease system, and

amplifying a second target nucleic acid using Polymerase Chain Reaction (PCR), in which the second target nucleic acid contains a single nucleotide variant version of the first target nucleic acid.

11. A method according to Claim 8 or 9, in which the subject is a cancer patient.

12. A method according to Claim 9, in which:

(a) the first endonuclease system further comprises a trans-activating crRNA (tracrRNA) or a derivative thereof; or

(b) the first crRNA or the derivative thereof is a polynucleotide comprising a crRNA polynucleotide fused to a tracrRNA polynucleotide; or

(c) the first endonuclease system is a Type II CRISPR-Cas system or a derivative thereof; or

(d) the first target nucleic acid is a double-stranded DNA (dsDNA); or

(e) the first Cas protein or the variant thereof is a Cas9 protein or a variant thereof; or

(f) further comprising:

providing a second endonuclease system having:

a second clustered regularly interspaced short palindromic repeats (CRISPR) RNA (crRNA) or a derivative thereof, and

a second CRISPR-associated (Cas) protein or a variant thereof,

in which the second crRNA or the derivative thereof contains a second target-specific nucleotide region complementary to a region of the second target nucleic acid;

contacting the second target nucleic acid with the second endonuclease system to form a complex, and

separating the complex and thereby enriching for the second target nucleic acid optionally

(i) further comprising separating the second target nucleic acid from the complex; or

5 in which (ii) the second endonuclease system further comprises a trans-activating crRNA (tracrRNA) or a derivative thereof; or

(iii) the second crRNA or the derivative thereof is a polynucleotide comprising a crRNA polynucleotide fused to a tracrRNA polynucleotide or

10 (iv) the second endonuclease system is a Type II CRISPR-Cas system or a derivative thereof; or

(v) the second target nucleic acid is a double-stranded DNA (dsDNA); or

(vi) the second endonuclease system is labelled optionally in which the second crRNA is labelled with biotin optionally further comprising adding streptavidin and thereby separating the complex or in which the second Cas protein or the derivative thereof is labelled with a capture tag or

15 (vii) in which the second Cas protein or the variant thereof is a Cas9 protein or a variant thereof optionally

in which the Cas9 protein or the variant thereof retains two nuclease domains and is able to produce a double-stranded nucleic acid break or

20 in which the Cas9 protein contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is complementary to the crRNA optionally in which the mutation is D10A or optionally

25 in which the Cas9 protein contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the crRNA optionally in which the mutation is H840A or

30 in which the Cas9 protein contains two inactivated nuclease domains optionally in which the two inactivated nuclease domains comprise a first mutation in the domain that cleaves the strand complementary to the crRNA and a second mutation in the domain that cleaves the strand non-

complementary to the crRNA optionally in which the first mutation is D10A and the second mutation is H840A.

13. A method according to Claim 10, in which the target nucleic acid is in a fetal cell fraction of the cell free DNA, and in which the cell free DNA is from maternal plasma.

5 14. A method for labeling a target nucleic acid comprising:

providing a first nuclease system having:

a first clustered regularly interspaced short palindromic repeats (CRISPR) RNA (crRNA) or a derivative thereof, and

a first CRISPR-associated (Cas) protein or a variant thereof,

10 in which the first crRNA or the derivative thereof contains a first target-specific nucleotide region complementary to a first region of the target nucleic acid, and in which the first Cas protein contains one inactivated nuclease domain;

contacting a double-stranded nucleic acid containing the target nucleic acid with the first nuclease system to generate a first single-stranded nick at the first region of the target nucleic acid, and

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labelling the target nucleic acid optionally

(a) further comprising separating the target nucleic acid through the labeling and thereby enriching the target nucleic acid optionally further comprising amplifying the target nucleic acid or

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in which

(b) the first nuclease system further comprises a trans-activating crRNA (tracrRNA) or

(c) the first crRNA or the derivative thereof is a polynucleotide comprising a crRNA polynucleotide fused to a tracrRNA polynucleotide or

(d) the first nuclease system is a Type II CRISPR-Cas system or a derivative thereof or

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(e) the target nucleic acid is a double-stranded DNA (dsDNA) or

(f) the first Cas protein or the variant thereof is a Cas9 protein or a variant thereof optionally

(i) in which the Cas9 protein or the variant thereof contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic

acid strand that is complementary to the first crRNA optionally in which the mutation is D10A or

(ii) in which the first Cas9 protein or the variant thereof contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the first crRNA optionally in which the mutation is H840A.

15. A method according to Claim 14 further comprising performing a nick translation optionally in which

(a) the nick translation is performed by using a nick translation polymerase selected from a group consisting of DNA Pol 1, Bst, and Taq; or

(b) the nick translation is performed in a reaction mixture containing biotinylated dNTPs optionally in which the biotinylated dNTPs are biotinylated dUTPs; or

(c) further comprising adding magnetic streptavidin beads to enrich biotinylated target nucleic acid or

(d) further comprising:

providing a second nuclease system having:

a second crRNA or a derivative thereof, and

a second Cas protein or a variant thereof,

in which the second crRNA or the derivative thereof contains a second target-specific nucleotide region complementary to a second region of the target nucleic acid, and in which the second Cas protein contains one inactivated nuclease domain, and

contacting the double-stranded nucleic acid containing the target nucleic acid with the second nuclease system to generate a second single-stranded nick at the second region of the target nucleic acid,

in which the first region of the target nucleic acid is different from the second region of the target nucleic acid optionally in which the first single-stranded nick and the second single-stranded nick are on the same strand of the target nucleic acid optionally in which

(i) the space between the first single-stranded nick and the second single-stranded nick on the same strand of the target nucleic acid is 1 bp to 20 bp; or

(ii) further comprising performing a nick translation optionally in which the nick translation is performed by using a nick translation polymerase Phi29; or

(iii) the first region of the target nucleic acid and the second region of the target nucleic acid are on the same strand of the target nucleic acid;

5 in which the first Cas protein or the variant thereof is a first Cas9 protein containing one inactivated nuclease domain comprising a first mutation in the domain that cleaves a target nucleic acid strand that is complementary to the first crRNA, and

10 in which the second Cas protein or the variant thereof is a second Cas9 protein containing one inactivated nuclease domain containing a second mutation in the domain that cleaves a target nucleic acid strand that is complementary to the second crRNA optionally in which the first mutation and the second mutation are both D10A; or

(iv) in which:

15 the first region of the target nucleic acid and the second region of the target nucleic acid are on the same strand of the target nucleic acid;

20 in which the first Cas protein or the variant thereof is a first Cas9 protein containing one inactivated nuclease domain comprising a first mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the first crRNA, and

25 in which the second Cas protein or the variant thereof is a second Cas9 protein containing one inactivated nuclease domain containing a second mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the second crRNA optionally in which the first mutation and the second mutation are both H840A; or

(v) in which:

the first region of the target nucleic acid and the second region of the target nucleic acid are on different strands of the target nucleic acid;

30 the first Cas protein or the variant thereof is a first Cas9 protein containing one inactivated nuclease domain comprising a first mutation in the domain

that cleaves a target nucleic acid strand that is complementary to the first crRNA, and

the second Cas protein or the variant thereof is a second Cas9 protein containing one inactivated nuclease domain comprising a second mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the second crRNA optionally in which the first mutation is D10A, and the second mutation is H840A optionally in which the capture probe is a biotinylated probe.

16. A method according to Claim 14, in which the method further comprises:

providing a second nuclease system having:

a second crRNA or a derivative thereof, and

a second Cas protein or a variant thereof,

in which the second crRNA or the derivative thereof contains a second target-specific nucleotide region complementary to a second region of the target nucleic acid, and in which the second Cas protein contains one inactivated nuclease domain, and

contacting the double-stranded nucleic acid containing the target nucleic acid with the second nuclease system to generate a second single-stranded nick at the second region of the target nucleic acid,

in which the first region of the target nucleic acid is different from the second region of the target nucleic acid, and

in which the space between the first single-stranded nick and the second single-stranded nick is from 20 bp to 500 bp; or

further comprising:

adding a capture probe; and

exchanging a single-stranded nucleic acid product between the first single-stranded nick and the second single-stranded nick with the capture probe,

in which the capture probe is able to hybridize to a nucleic acid complementary to the single-stranded nucleic acid product.

17. A method according to Claim 16:

in which the sequence of the capture probe is 10% to 100% identical to the sequence of the single-stranded nucleic acid product or

in which the capture probe contains an overhang nucleotide sequence, and the overhang nucleotide sequence is complementary to an oligonucleotide immobilized on a surface.

18. A method according to Claim 16, further comprising adding magnetic streptavidin beads to enrich the target nucleic acid.

19. A method according to Claim 9, in which the Cas protein or the variant thereof is a Cas9 protein or a variant thereof in which the Cas9 protein or the variant thereof contains one inactivated nuclease domain comprising a mutation in the domain that cleaves a target nucleic acid strand that is complementary to the crRNA in which the first single-stranded nick and the second single-stranded nick are on opposite strands of the target nucleic acid, thereby generating a first double-stranded nucleic acid break end optionally in which

(a) the first region of the target nucleic acid and the second region of the target nucleic acid are on the same strand of the target nucleic acid;

the first Cas protein or the variant thereof is a first Cas9 protein or a variant thereof containing one inactivated nuclease domain comprising a first mutation in the domain that cleaves a target nucleic acid strand that is complementary to the first crRNA, and

the second Cas protein or the variant thereof is a second Cas9 protein or a variant thereof containing one inactivated nuclease domain comprising a second mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the second crRNA; or

(b) the first mutation is D10A, and the second mutation is H840A; or

(c) the first region of the target nucleic acid and the second region of the target nucleic acid are on opposite strands of the target nucleic acid;

the first Cas protein or the variant thereof is a first Cas9 protein or a variant thereof containing one inactivated nuclease domain comprising a first mutation in the domain that cleaves a target nucleic acid strand that is complementary to the first crRNA, and

the second Cas protein or the variant thereof is a second Cas9 protein or a variant thereof containing one inactivated nuclease domain containing a second mutation in the domain that cleaves a target nucleic acid strand that is complementary to the second crRNA optionally in which the first mutation and the second mutation are both D10A; or

(d) the first region of the target nucleic acid and the second region of the target nucleic acid are on opposite strands of the target nucleic acid;

the first Cas protein or the variant thereof is a first Cas9 protein or a variant thereof containing one inactivated nuclease domain comprising a first mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the first crRNA, and

the second Cas protein or the variant thereof is a second Cas9 protein or a variant thereof containing one inactivated nuclease domain containing a second mutation in the domain that cleaves a target nucleic acid strand that is non-complementary to the second crRNA optionally in which the first mutation and the second mutation are both H840A; or

(e) further comprising ligating an adapter to the first double-stranded DNA break end optionally in which the adapter is biotinylated optionally further comprising adding magnetic streptavidin beads to enrich the target nucleic acid.

20. A method according to Claim 14 further comprising:

providing a second nuclease system having:

a second crRNA or a derivative thereof, and

a second Cas protein or a variant thereof,

in which the second crRNA or the derivative thereof contains a second target-specific nucleotide region complementary to a second region of the target nucleic acid, and in which the second Cas protein or the variant thereof contains one inactivated nuclease domain, and

contacting the double-stranded nucleic acid containing the target nucleic acid with the second nuclease system to generate a second single-stranded nick at the second region of the target nucleic acid,

in which the first region of the target nucleic acid is different from the second region of the target nucleic acid further comprising

providing a third nuclease system having:

a third crRNA or a derivative thereof, and

5 a third Cas protein or a variant thereof,

in which the third crRNA or the derivative thereof contains a third target-specific nucleotide region substantially complementary to a third region of the target nucleic acid, and in which the third Cas protein or the variant thereof contains one inactivated nuclease domain;

10 providing a fourth nuclease system having:

a fourth crRNA or a derivative thereof, and

a fourth Cas protein or a variant thereof,

15 in which the fourth crRNA or the derivative thereof contains a fourth target-specific nucleotide region substantially complementary to a fourth region of the target nucleic acid, and in which the fourth Cas protein or the variant thereof contains one inactivated nuclease domain; and

20 contacting the double-stranded nucleic acid containing the target nucleic acid with the third and fourth nuclease systems to generate a third single-stranded nick at the third region of the target nucleic acid and a fourth single-stranded nick at the fourth region of the target nucleic acid, in which in the third single-stranded nick and the fourth single-stranded nick are on opposite strands of the target nucleic acid, thereby generating a second double-stranded nucleic acid break end, the second double-stranded nucleic acid break end being different from the first double-stranded nucleic acid break end.

21. A method according to Claim 19 further comprising ligating an adapter to the first
25 double-stranded DNA break end and further comprising ligating an adapter to the second double-stranded nucleic acid break end.