

CLAIMS

1. ~~A collection of different circular chimeric single stranded nucleic acid constructs, each construct comprising:~~
~~_____ a first single stranded segment of original genomic DNA from a host organism~~
~~and~~
~~_____ a second single stranded synthetic nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, said nucleotide sequence comprising a unique identifier portion, wherein the nucleotide sequence of both the unique identifier portion and the segment of original genomic DNA distinguishes one chimeric single stranded nucleic acid construct in the collection from every other chimeric single stranded nucleic acid construct in the collection, said chimeric single stranded nucleic acid constructs of the collection being circularized and suitable for rolling circle amplification and/or sequencing.~~

21. A method for amplifying nucleic acid molecules, said method comprising:
providing ~~the~~ a collection of different circular chimeric single-stranded nucleic acid constructs, each construct comprising: ~~of claim 1;~~
_____ a first single stranded segment of original genomic DNA from a host organism and
_____ a second single stranded synthetic nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, said nucleotide sequence comprising a unique identifier portion, wherein the nucleotide sequence of both the unique identifier portion and the segment of original genomic DNA distinguishes one chimeric single-stranded nucleic acid construct in the collection from every other chimeric single-stranded nucleic acid construct in the collection, said chimeric single-stranded nucleic acid constructs of the collection being circularized and suitable for rolling circle amplification and/or sequencing;

blending the collection of different circular chimeric single-stranded nucleic acid constructs with a polymerase and a plurality of short primers that are 6 to 10 nucleotides in length and comprise random bases and/or nucleotide analogues, wherein one or more of the short

primers is complementary to a portion of one or more circular chimeric single stranded nucleic acid constructs of the collection, whereby said blending forms an amplification reaction mixture; and

subjecting the amplification reaction mixture to one or more hybridization and extension treatments, wherein the one or more short primers hybridize to the circular chimeric single-stranded nucleic acid constructs and the polymerase extends the hybridized short primers to produce a plurality of extension products, each extension product comprising two or more tandem linear sequences, wherein each tandem linear sequence is complementary to a chimeric single-stranded nucleic acid construct from the collection.

32. A method for generating tandem linear copies of nucleic acid molecules, said method comprising:

providing ~~the~~ a collection of different circular chimeric single-stranded nucleic acid constructs ~~of claim 1~~, each construct comprising:

a first single stranded segment of original genomic DNA from a host organism and

a second single stranded synthetic nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, said nucleotide sequence comprising a unique identifier portion, wherein the nucleotide sequence of both the unique identifier portion and the segment of original genomic DNA distinguishes one chimeric single-stranded nucleic acid construct in the collection from every other chimeric single-stranded nucleic acid construct in the collection, said chimeric single-stranded nucleic acid constructs of the collection being circularized and suitable for rolling circle amplification and/or sequencing;

blending the collection of different circular chimeric single-stranded nucleic acid constructs with a polymerase with strand-displacement activity and one or more primary primers, wherein the one or more primary primers are complementary to a portion of one or more circular chimeric single stranded nucleic acid constructs of the collection, whereby said blending forms a rolling circle extension mixture;

subjecting the rolling circle extension mixture to one or more hybridization and extension treatments, wherein the one or more primary primers hybridize to the circular chimeric single-stranded nucleic acid constructs and the polymerase extends the hybridized primary

primers to produce single-stranded extension products, each single-stranded extension product comprising two or more tandem linear sequences, wherein each tandem linear sequence is complementary to a chimeric single-stranded nucleic acid construct from the collection;

providing one or more secondary primer sets, each secondary primer set comprising (a) a first secondary primer that comprises a nucleotide sequence that is complementary to a first portion of a single-stranded extension product formed from the one or more primary primers, and (b) a second secondary primer that comprises a nucleotide sequence that is complementary to a second portion of a single-stranded extension product, said second portion being different than and displaced from the first portion of the single-stranded extension product formed from the one or more primary primers;

providing an endonuclease that cleaves both the single stranded extension product and first secondary primer when they are hybridized to each other;

blending the single-stranded extension products with a polymerase lacking strand-displacement activity, a ligase, the endonuclease, and the one or more secondary primer sets to form an extension-ligation-cleavage reaction mixture;

subjecting the extension-ligation-cleavage reaction mixture to a hybridization and extension treatment, wherein the first and second secondary primers hybridize to the single-stranded extension products, the polymerase extends the hybridized secondary primers, the ligase ligates the extended hybridized secondary primers to form double stranded extension products, and the endonuclease cleaves the double stranded extension products at positions where the first secondary primer hybridized to the single-stranded extension product to produce double stranded extension product fragments, wherein said double stranded extension product fragments comprise two or more tandem linear sequences complementary to the chimeric single-stranded nucleic acid construct from the collection.

43. The method of claim 32, wherein the number of tandem linear sequences in the double stranded extension product fragment is determined by the ratio of first and second secondary primers in the extension-ligation-cleavage reaction mixture.

54. The method of claim 32, wherein the rolling circle extension mixture further comprises:

a methyl-sensitive restriction endonuclease, wherein during said subjecting the rolling circle extension mixture to the one or more hybridization and extension treatment, the endonuclease cleaves double-stranded DNA at a recognition sequence if said recognition sequence is unmethylated thereby selectively forming single-stranded extension products only from chimeric single-stranded nucleic acid constructs comprising a first single stranded segment of original methylated genomic DNA.

65. The method of any of claims 1, 2, 3, or 4, ~~or 5~~ further comprising:
sequencing the plurality of extension products or double stranded extension product fragments, optionally;

~~7. The method of claim 6 (a) wherein universal linker sequences are appended to 5' and 3' ends of the extension products or fragments thereof, or to 5' and 3' ends of the double stranded extension product fragments prior to said sequencing;~~

~~8. The method of claim 6,; or~~
(b) wherein said sequencing is carried out using a method selected from the group consisting of fluorescent primer hybridization, molecular beacon hybridization, primer extension, exonuclease-based sequencing, ligase detection reaction, ligase chain reaction, pyrosequencing, fluorescence-based sequencing-by-synthesis, fluorescence-based sequencing-by-ligation, nanopore based sequencing, ion-based sequencing-by-synthesis, and ion-based sequencing-by-ligation.

~~9. A system comprising:
a collection of different circular chimeric single stranded nucleic acid constructs,
each construct comprising:
a first single stranded segment of original genomic DNA from a host organism and
a second single stranded nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, said nucleotide sequence comprising a first solid support primer specific portion, a~~

~~second solid support primer specific portion, and a patient identifier sequence, whereby said chimeric single stranded nucleic acid constructs of the collection are circularized and suitable for rolling circle amplification and/or sequencing; and~~

~~————— a collection of extension products, each extension product comprising two or more tandem linear sequences, wherein each tandem linear sequence is complementary to the chimeric single stranded nucleic acid construct from the collection, wherein each extension product in the collection is hybridized to its complementary circular chimeric single stranded nucleic acid construct of the collection.~~

~~————— 10. — The system of claim 9 further comprising:~~

~~————— a solid support on which a plurality of first oligonucleotide primers is immobilized, each first oligonucleotide primer comprising a nucleotide sequence that is the same as the nucleotide sequence of the first solid support primer specific portion of the chimeric single stranded nucleic acid constructs of the collection, wherein one or more of the plurality of first oligonucleotide primers on the solid support are hybridized to an extension product of the collection of extension products.~~

~~————— 11. — A system comprising:~~

~~————— a collection of different circular chimeric single stranded nucleic acid constructs, each construct comprising:~~

~~————— a first single stranded segment of original genomic DNA from a host organism and~~

~~————— a second single stranded nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism, said nucleotide sequence comprising a first solid support primer specific portion, a second solid support primer specific portion, and a patient identifier sequence, whereby said chimeric single stranded nucleic acid constructs of the collection are suitable for rolling circle amplification and/or sequencing;~~

~~————— one or more oligonucleotide amplification primers, each amplification primer comprising at least a first nucleotide sequence that is complementary to a portion of the chimeric single stranded nucleic acid constructs of the collection; and~~

~~a polymerase suitable for rolling circle amplification.~~

~~12. — The system of claim 11, wherein the one or more oligonucleotide amplification primers comprise a first nucleotide sequence that is complementary to the second solid support primer specific portion of the chimeric single stranded nucleic acid constructs of the collection, and said one or more amplification primers are immobilized on a solid support.~~

~~13. — The system of claim 11, wherein the one or more oligonucleotide amplification primers comprise (i) a first nucleotide sequence that is complementary to the first solid support primer specific portion of the chimeric single stranded nucleic acid constructs of the collection and (ii) a second nucleotide sequence that is complementary to the second solid support primer specific portion of the chimeric single stranded nucleic acid constructs of the collection.~~

~~14. — The system of claim 11, wherein the one or more oligonucleotide amplification primers comprise (i) a first nucleotide sequence that is complementary to the original genomic DNA segment of the chimeric single stranded nucleic acid constructs of the collection, (ii) a 3' portion comprising a cleavable nucleotide or nucleotide analogue and a blocking group that blocks 3' polymerase extension of said oligonucleotide amplification primer, and (iii) a 5' portion comprising a cleavable nucleotide or nucleotide analogue and a capture group, said capture group capable of being immobilized to a solid support.~~

~~15. — The system of claim 11, wherein the one or more oligonucleotide amplification primers comprise (a) a first oligonucleotide amplification primer having (i) a first nucleotide sequence that is complementary to a first portion of the original genomic DNA segment of the chimeric single stranded nucleic acid constructs of the collection, and (ii) a 3' portion comprising a cleavable nucleotide or nucleotide analogue and a blocking group that blocks 3' polymerase extension of said first oligonucleotide amplification primer, and (b) a second oligonucleotide amplification primer having (i) a first nucleotide sequence that is complementary to a second portion of the original genomic DNA segment of the chimeric single stranded nucleic acid constructs of the collection, and (ii) a 5' portion comprising a cleavable~~

~~nucleotide or nucleotide analogue and a capture group, said capture group capable of being immobilized to a solid support.~~

~~16. — The system of claim 11, wherein the one or more amplification primers comprise a portion that is capable of being tethered to a solid support.~~

~~17. — The system of claim 11 further comprising:
a solid support on which a plurality of first oligonucleotide primers is immobilized, each first oligonucleotide primer comprising a nucleotide sequence that is the same as the nucleotide sequence of the first solid support primer specific portion of the chimeric single stranded nucleic acid constructs of the collection.~~

~~18. — A method for sequencing a plurality of nucleic acid molecules, said method comprising:
————— providing the system of claim 11;
————— blending one or more oligonucleotide amplification primers hybridized to circular chimeric single stranded nucleic acid constructs of the collection with the polymerase to form a rolling circle amplification reaction mixture;
————— subjecting the rolling circle amplification reaction mixture to an extension treatment where the polymerase extends the one or more hybridized oligonucleotide amplification primers to produce a plurality of primary extension products, each primary extension product comprising one or more tandem linear sequences, wherein each tandem linear sequence is complementary to a circular chimeric single stranded nucleic acid construct in the collection; and
————— sequencing the circular chimeric single stranded nucleic acid constructs directly or said plurality of primary extension products thereof.~~

~~19. — The method of claim 18, wherein the circular chimeric single stranded nucleic acid constructs of the collection are prepared by a method comprising:
providing a sample containing one or more target genomic DNA segments;~~

_____ providing one or more first oligonucleotide probes, each first oligonucleotide probe comprising (a) a 5' target-specific portion, (b) a 3' target-specific portion, and (c) a further portion, said further portion comprising (i) the patient identifier sequence, (ii) the first solid support primer-specific portion, and (iii) the second solid support primer-specific portion;

contacting the sample and the one or more first oligonucleotide probes under conditions effective for the 3' target-specific portion of a first oligonucleotide probes to hybridize in a base specific manner to a complementary 3' end of a target genomic DNA segment and for the 5' target-specific portion of the first oligonucleotide probe to hybridize in a base specific manner to a complementary 5' end of the target genomic DNA segment, if present in the sample;

generating a ligation competent junction between the 3' and 5' ends of each target genomic DNA segment hybridized to the first oligonucleotide probe; and

ligating the ligation junction of each target genomic DNA segment to form different circular chimeric single stranded nucleic acid constructs of the collection.

20. _____ The method of claim 18, wherein the different circular chimeric single stranded nucleic acid constructs of the collection are prepared by a method comprising:

providing a sample containing one or more target genomic DNA segments, wherein said target genomic DNA segments potentially contain one or more base differences or one or more methylated residues;

appending nucleotide linkers to 3' and 5' ends of the target genomic DNA segments to form linker appended target genomic DNA segments;

_____ providing one or more first oligonucleotide probes, each first oligonucleotide probe comprising (a) a 3' portion complementary to the 3' nucleotide linker of the target genomic DNA segment, (b) a 5' portion complementary to the 5' nucleotide linker of the target genomic DNA segment, and (c) a further portion, said further portion comprising (i) the patient identifier sequence, (ii) the first solid support primer-specific portion, and/or (iii) the second solid support primer-specific portion;

contacting the sample and the one or more first oligonucleotide probes under conditions effective for the first oligonucleotide probes to hybridize in a base specific manner to

~~complementary nucleotide linker sequences of the linker appended target genomic DNA segments, if present in the sample;~~

~~generating one or more ligation competent junctions suitable for coupling the 3' and 5' ends of the target genomic DNA segments hybridized to the first oligonucleotide probes; and~~

~~ligating the target genomic DNA segments at the one or more ligation junctions to form different circular chimeric single stranded nucleic acid constructs of the collection.—~~

21. — ~~The method of claim 18, wherein the different circular chimeric single stranded nucleic acid constructs of the collection are prepared by a method comprising:~~

~~providing a sample containing one or more target genomic DNA segments, wherein said target genomic DNA segments have 3' and 5' ends;~~

~~appending linker sequences to the 3' and 5' ends of the target genomic DNA segments, wherein said linker sequences comprise (i) one or more patient identifier sequences, (ii) the first solid support primer specific portion, and (iii) the second solid support primer-specific portion to form linker appended target genomic DNA segments;~~

~~— providing one or more first oligonucleotide probes, each first oligonucleotide probe comprising (a) a portion complementary to the 3' linker sequence of the linker appended target genomic DNA segment, and (b) a portion complementary to the 5' linker sequence of the linker appended target genomic DNA segment; and~~

~~contacting the sample and the one or more first oligonucleotide probes under conditions effective for the first oligonucleotide probes to hybridize in a base specific manner to complementary 3' and 5' linker sequences of the linker appended target genomic DNA segments, if present in the sample;~~

~~generating one or more ligation competent junctions suitable for coupling 3' and 5' ends of the linker appended target genomic DNA segments hybridized to the first oligonucleotide probes; and~~

~~ligating the linker appended target genomic DNA segments at the one or more ligation junctions to form different circular chimeric single stranded nucleic acid construct of the collection.—~~

22. — A method for identifying, in a sample, one or more nucleic acid molecules potentially comprising distinct first target and second target regions coupled to each other, said method comprising:

providing a sample potentially containing one or more nucleic acid molecules comprising distinct first target and second target regions coupled to each other;

providing one or more oligonucleotide probe sets, each probe set comprising (i) a first oligonucleotide probe comprising a 5' first target specific portion, a 3' second target specific portion, and a further portion, and (ii) a second oligonucleotide probe comprising a 5' second target specific portion, a 3' first target specific portion, and a further portion, wherein the further portion of the first or second oligonucleotide probes of a probe set comprises (i) a unique identifier sequence, (ii) a patient identifier sequence, (iii) one or more primer binding sequences, or any combination of (i), (ii), and (iii);

contacting the sample and the one or more oligonucleotide probe sets under conditions effective for first and second oligonucleotide probes of a probe set to hybridize in a base specific manner to their corresponding first and second target regions of the nucleic acid molecule, if present in the sample;

generating one or more ligation competent junctions suitable for coupling 3' ends of first oligonucleotide probes to 5' ends of second oligonucleotide probes of a probe set and for coupling 5' ends of first oligonucleotide probes to 3' ends of second oligonucleotide probes of a probe set when said probe sets are hybridized to complementary first and second target regions of a nucleic acid molecule;

ligating the first and second oligonucleotides of a probe set at the one or more ligation competent junctions to form circular ligated products comprising a nucleotide sequence corresponding to the first and second distinct target regions of a nucleic acid molecule coupled to a further portion; and

— detecting and distinguishing the circular ligated products in the sample thereby identifying the presence, if any, of one or more nucleic acid molecules comprising distinct first target and second target regions coupled to each other in the sample.