CLAIMS

1. A method for amplifying nucleic acid molecules, said method comprising:

providing 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;

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.

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

providing 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;

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.

3. The method of claim 2, 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.

4. The method of claim 2, 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.

5. The method of any of claims 1, 2, 3, or 4, further comprising:

sequencing the plurality of extension products or double stranded extension product fragments, optionally

(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; 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.