

WHAT IS CLAIMED IS:

1. A method of amplifying DNA comprising:
 - a) providing linear double stranded DNA molecules having complementary sequences;
 - b) attaching single-stranded adaptors to both ends of the linear double stranded DNA molecules to produce single stranded, covalently closed DNA molecules; wherein the adaptors have a hairpin structure; and
 - c) amplifying the single-stranded, covalently closed DNA molecules in a single operation by (i) rolling circle amplification and (ii) multiple displacement amplification wherein rolling circle amplification produces a concatenated molecule that folds back on itself, forming double-stranded segments based on the complementary sequences, and wherein multiple displacement amplification comprises using a primase/polymerase to prime strand extension from the adaptors on the double stranded segments of the folded, concatenated molecules; and strand extension with a polymerase having strand displacement activity.
2. The method of claim 1, wherein the linear double stranded DNA molecules comprise apoptotic cell free DNA molecules, e.g., having sizes less than 480 bp, less than 320 bp, or between about 140 bp and 180 bp (e.g., averaging about 160 bp).
- ~~3. The method of claim 1, wherein providing the linear double stranded DNA comprises fragmenting chromosomal DNA.~~
- ~~4.3. The method of any of the previous claims~~ claim 1 or 2, wherein the linear double stranded DNA is derived from one or more bodily fluids from mammals, e.g., selected from CSF, blood, plasma, serum, ascites, urine, saliva, tear drops, milk, semen and synovial fluid.
- ~~5. The method of any of the previous claims, wherein providing the linear double stranded DNA comprises isolating the linear double stranded DNA from other cellular components in a biological sample.~~
- ~~6.4. The method of any of the previous claims~~ claims 1 to 3, wherein providing the linear double stranded DNA comprises end-repair and/or dA-tailing.

~~7. The method of claim 5, wherein end repair is performed using one or more of T4 polynucleotide kinase (PNK), T4 DNA polymerase Klenow fragment and T4 DNA polymerase large fragment.~~

~~8. The method of claim 5, comprising performing dA tailing using Taq polymerase.~~

~~9. The method of any of the previous claims, wherein the adaptors comprise single-stranded DNA molecules.~~

~~10. The method of any of the previous claims, wherein the adaptors have a hairpin structure.~~

~~11.5. The method of any of the previous claims~~claim 1, comprising attaching adaptors having the following sequence: 5TAACATTT GTT G GCCACT C AG GCCAACAAAT GTTAT3' [SEQ ID NO: 1].

~~12.6. The method of~~ claim 1 or 5~~any of the previous claims~~, wherein the adaptors comprise a primase/polymerase recognition sequence.

~~13.7. The method of~~ claim 1, 5 or 6~~any of the previous claims~~, wherein attaching the adaptors comprises blunt-end ligation or sticky-end ligation.

~~14.8. The method of~~ claim 1~~any of the previous claims~~, comprising:
providing apoptotic cell free DNA molecules;
performing end repair and dA tailing of the cell free DNA molecules; and ligating hairpin adaptors comprising a dT overhang to the end repaired, dA tailed cell free DNA molecules.

9. The method of claim 1, wherein amplification comprises strand extension using Phi29 polymerase.

~~15. The method of any of the previous claims, wherein amplification is primed by a primase/polymerase.~~

~~16.10. The method of any one of claims 1 to 9~~any of the previous claims, wherein amplification is primed by Thermus thermophilus primase/polymerase (TthPrimPol).

- ~~17. The method of any of the previous claims, wherein amplification is primed by a TthPrimPol having the sequence of SEQ ID NO: 10.~~
- ~~18. The method of any of the previous claims, wherein amplification is primed by human primase/polymerase (HsPrimPol).~~
- ~~19. The method of any of the previous claims, wherein amplification is primed with random synthetic primers (e.g., random hexamer sequences).~~
- ~~20. The method of any of the previous claims wherein amplification comprises strand extension using a polymerase having strand displacement activity.~~
- ~~21. The method of any of the previous claims wherein amplification comprises strand extension using Phi29 polymerase.~~
- ~~22. The method of any of the previous claims wherein amplification comprises primase-initiated multiple displacement amplification.~~
- ~~23. The method of any of the previous claims comprising using a primase/polymerase to generate primers on the DNA and using a polymerase having strand displacement activity to extend the primers.~~
- ~~24. The method of claim 23, wherein the primase/polymerase comprises TthPrimPol and the polymerase comprises Phi29 polymerase.~~
- ~~25. The method of any of the previous claims further comprising:
d) sequencing the amplified DNA.~~
- ~~26. The method of claim 25, wherein sequencing comprises fragmenting the amplified DNA and attaching to the fragmented DNA sequencing platform-specific adaptors.~~
- ~~27. The method of claim 25, wherein sequencing is performed on selected sequences (amplicons), exomes, transcriptome or whole genome.~~

~~28. The method of claim 25, further comprising:-~~

~~e) detecting one or a plurality of genetic variants in the sequenced, amplified DNA.~~

~~29. The method of any of the preceding claims, further comprising quantifying the amplified DNA.~~

~~30. A method of amplifying DNA comprising:-~~

~~a) providing linear double stranded DNA molecules;-~~

~~b) performing end repair and dA tailing on the DNA molecules;-~~

~~c) ligating hairpin adaptors to the end-repaired dA-tailed DNA molecules to produce adaptor-tagged DNA molecules; and~~

~~d) amplifying the adaptor-tagged DNA molecules by (i) rolling circle amplification and (ii) multiple displacement amplification ("MDA").~~

~~31. The method of claim 30, wherein amplification comprises random-primed MDA, using Phi29 DNA polymerase and random synthetic primers.~~

~~32. The method of claim 30, wherein amplification comprises priming with a primase/polymerase (e.g., TthPrimPol).~~

~~33. A method of amplifying DNA comprising:-~~

~~a) providing linear double stranded DNA molecules;-~~

~~b) attaching single stranded adaptors to both ends of the linear double stranded DNA molecules to produce single stranded, covalently closed DNA molecules; and~~

~~c) amplifying the single stranded, covalently closed DNA molecules by any combination of a thermostable DNA polymerase, e.g., Taq polymerase, random or degenerate primers and an optional ligase.~~

~~34. The method of claim 33, wherein amplification comprises Degenerate oligonucleotide-primed PCR (DOP-PCR), linker-adaptor PCR (LA-PCR), Primer Extension Pre-amplification PCR (PEP-PCR / I-PEP-PCR), and variations thereof.~~

~~35. A method of amplifying DNA comprising:-~~

- ~~a) providing linear double stranded DNA molecules;-~~
- ~~b) attaching single stranded adaptors to both ends of the linear double stranded DNA molecules to produce single stranded, covalently closed DNA molecules; and~~
- ~~c) amplifying the single stranded, covalently closed DNA molecules using any combination of a thermostable DNA polymerase (e.g., Taq polymerase) and a highly processive strand displacement DNA polymerase (e.g., Phi29 polymerase or Bacillus stearothermophilus (Bst) polymerase).~~

~~36. The method of claim 35, wherein amplification comprises multiple annealing and looping based amplification cycles (MALBAC).~~

~~37.11. A kit for use in the method of any one of claims 1 to 10 comprising:~~

- ~~(a) a the primase/polymerase;~~
- ~~(b) a the polymerase having strand displacement activity;~~
- ~~(c) deoxyribonucleotide triphosphates provided and used as substrates in step (c) of claim 1; and~~
- ~~(d) a the single stranded hairpin-adaptor.~~

~~38. The kit of claim 37, wherein the primase/polymerase is TthPrimPol.~~

~~39. The kit of claim 37, wherein the polymerase is Phi29 DNA polymerase.~~

~~40. The kit of claim 37, wherein the single strand adaptor has the sequence: 5'
T AACATTT GTTGGCC ACTCAG GCCAAC AAAT GTT AT 3' [SEQ ID NO: 1]~~

~~41. The kit of claim 37, wherein the kit further comprises:-~~

- ~~d) one, two or three elements selected from:-~~
 - ~~(i) one or more enzymes for dsDNA end repair (e.g., T4 DNA polymerase, klenow fragment and/or Taq polymerase);-~~
 - ~~(ii) one or more enzymes for DNA ligation;~~
 - ~~(iii) optionally, a reagent to increase DNA ligation efficiency;~~
 - ~~(iv) reaction buffer; and~~
 - ~~(v) a buffer for use with any of the aforementioned elements.~~

42. ~~The kit of any of the preceding claims wherein the kit further comprises:~~

~~(a) reagents for isolation of apoptotic cell free DNA.~~

43. ~~A DNA library comprising a population of adapter tagged, single stranded, covalently closed DNA molecules, wherein each DNA molecule comprises first, second, third and fourth regions, wherein the second and fourth regions are complementary to each other and the first and third regions are not complementary to each other.~~

44. ~~The DNA library of claim 43, wherein, the first and third regions have identical sequences.~~

45. ~~The DNA library of claim 43 or claim 44, wherein the second and fourth regions comprise a segment of genomic DNA, e.g., apoptotic cfDNA.~~