

Having the ability to detect one or more mutations present on both strands of a double stranded nucleic acid (*e.g.*, true somatic mutations) provides a unique and unrealized opportunity to accurately and efficiently assess multiple mutations simultaneously and at an affordable cost. Using methods and materials described herein to detect the presence of one or more mutations present on both strands of a double stranded nucleic acid (*e.g.*, SaferSeqS methods) can permit the identification of rare mutations with high confidence, while minimizing the amount of DNA damage, the amount of PCR to be performed, and/or the number of DNA damage artifacts. It is noted that the terms "Watson strand" and "Crick strand" are used simply to distinguish between the two strands of a double-stranded starting nucleic acid sequence. Either of the strands can be denoted "Watson" or "Crick," and then the other strand is denoted with the other name.

In some embodimentexamples, provided herein are methods of comprising: a) forming a reaction mixture comprising: i) a plurality of double-stranded DNA fragments that are dephosphorylated and blunt-ended, wherein each of the double-stranded DNA fragments comprises Watson and Crick strands; ii) a plurality of adaptors, wherein each of the adaptors comprises, in the 5' to 3' direction: A) a barcode, and B) a universal 3' adaptor sequence; and iii) a ligase; and b) incubating the reaction mixture such that: i) adaptors are ligated to the 3' ends of the Watson and Crick strands, and ii) adaptors are not ligated to the 5' ends of either the Watson or Crick strands, thereby generating double-stranded ligation products.

In certain embodimentexamples, each of the plurality of adaptors comprises a unique barcode. In further embodimentexamples, each of the double-stranded ligation products comprises a Watson strand with only one barcode and a Crick strand with only one barcode that is different from the barcode on the Watson strand. In further embodimentexample, the methods further comprise the step of: c) sequencing at least a portion of the double-stranded ligation products.

In particular embodimentexamples, provided herein are methods comprising: a) attaching partially double-stranded 3' adaptors (3'PDSAs) to 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments in an analyte DNA sample, wherein a first strand of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and, optionally, (ii) a 3' blocking group, b) annealing 5' adaptors to the annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor

sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor; c) extending the 5' adaptors across the exogenous UID sequence and the first segment, thereby generating the complement of the exogenous UID sequence and complement of the first segment, and d) covalently linking the 3' end of the complement of the first segment to the 5' ends of the Watson and Crick strands of the double-stranded DNA fragments, thereby generating a plurality of adaptor-ligated double-stranded DNA fragments.

In one aspect, the disclosure provides a method, comprising:

a) attaching partially double-stranded 3' adaptors (3'PDSAs) to 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments in an analyte DNA sample,

wherein a first strand of each of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence that is optionally 8-50 nucleotides in length, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and

wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group,

b) annealing 5' adaptors to said annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor;

c) extending the 5' adaptors across the exogenous UID sequence and said first segment, thereby generating the complement of said exogenous UID sequence and complement of said first segment, and

d) covalently linking the 3' end of said complement of said first segment to the 5' ends of the Watson and Crick strands of the double-stranded DNA fragments, thereby generating a plurality of adaptor-ligated double-stranded DNA fragments.

In some **embodiment examples**, provided herein are methods comprising: a) attaching partially double-stranded 3' adaptors (3'PDSAs) to 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments in an analyte DNA sample, wherein a first strand of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and optionally (ii) a 3' blocking group, b) annealing 5' adaptors to the annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor; c) extending the 5' adaptors across the exogenous UID sequence, thereby generating the complement of the exogenous UID sequence, and d) covalently linking the 3' end of the complement of the exogenous UID sequence to the 5' ends of the segment complementary to the first segment on each of the Watson and Crick strands of the double-stranded DNA fragments, thereby generating a plurality of adaptor-ligated double-stranded DNA fragments.

In some **embodiment examples**, provided herein are methods comprising: a) attaching partially double-stranded 3' adaptors (3'PDSAs) to 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments in an analyte DNA sample, wherein a first strand of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and optionally (ii) a 3' blocking group, b) annealing 5' adaptors to the annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor

sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor; c) extending the 5' adaptors across the exogenous UID sequence and the first segment of the 3'PDSA, thereby generating the complement of the exogenous UID sequence and the complement of the first segment of the 3'PDSA, and d) covalently linking the 3' end of the complement of the first segment of the 3'PDSA to the 5' ends of the Watson and Crick strands of the double-stranded DNA fragments, thereby generating a plurality of adaptor-ligated double-stranded DNA fragments.

In some embodiment examples, provided here are systems and kits and compositions comprising: a) a population of partially double-stranded 3' adaptors (3'PDSAs) configured to be ligated to the 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments, wherein a first strand of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group; and b) a population of 5' adaptors configured to anneal to the annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 3' adaptor.

In another aspect, the disclosure provides a system, comprising:

a) a population of partially double-stranded 3' adaptors (3'PDSAs) configured to be ligated to the 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments,

wherein a first strand of each of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence that is optionally 8-50 nucleotides in length, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and

wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group; and

b) a population of 5' adaptors configured to anneal to said annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 3' adaptor.

In further embodiment examples, the systems, kits, and compositions further comprise: c) the population of double-stranded DNA fragments from a biological sample, and/or c) reagents for degrading the second strand of the 3'PDSAs to generate single-stranded 3' adaptors (3'SSAs); and/or c) a first primer complementary to the universal 3' adaptor sequence, and second primer complementary to the complement of the universal 5' adaptor sequence; and/or c) a sequencing system; and/or c) a Watson anchor primer complimentary to the universal 3' adapter sequence, and d) a Crick anchor primer complimentary to the complement of the universal 5' adapter sequence; and/or c) a first set of Watson target-selective primer pairs, comprising (i) one or more first Watson target-selective primers comprising a sequence complementary to a portion of the universal 3' adapter sequence, and (ii) one or more second Watson target selective primers, each of the one or more second Watson target-selective primers comprising a target-selective sequence; and/or c) a first set of Crick target-selective primer pairs, comprising (i) one or more Crick target-selective

primers comprising a sequence complementary to a portion of the universal 5' adaptor sequence, and (ii) one or more second Crick target-selective primers, each of the one or more second Crick target selective primers comprising the same target-selective sequence as the second Watson target-selective primer sequence.

5 In some [embodimentexamples](#), the methods further comprise: amplifying the plurality of adaptor-ligated, double-stranded DNA fragments with a first primer complementary to the universal 3' adaptor sequence, and second primer complementary to the complement of the universal 5' adaptor sequence, thereby generating amplicons, wherein the amplicons comprise a plurality of double-stranded Watson templates and a plurality of double-stranded
10 Crick templates. In certain [embodimentexamples](#), the methods further comprise: selectively amplifying said double-stranded Watson templates with a first set of Watson target-selective primer pairs, the first set of Watson target-selective primer pairs comprising: (i) a first Watson target-selective primer comprising a sequence complementary to a portion of the universal 3' adaptor sequence, and (ii) a second Watson target-selective primer comprising a
15 target-selective sequence, thereby creating target Watson amplification products. In further [embodimentexamples](#), the methods further comprise: selectively amplifying said double-stranded Crick templates with a first set of Crick target-selective primer pairs, the first set of Crick target-selective primer pairs comprising: (i) a first Crick target-selective primer comprising a sequence complementary to the complement of a portion of the universal 5'
20 adaptor sequence, and (ii) a second Crick target-selective primer comprising the same target-selective sequence as the second Watson target-selective primer sequence, thereby creating target Crick amplification products. In certain [embodimentexamples](#), the population of double-stranded DNA fragments are incubated with a mixture of uracil-DNA glycosylase and DNA glycosylase-lyase Endonuclease VIII, prior to ligating any adapters.

25 In some [embodimentexamples](#), the polymerase employed (e.g., for extending the 5' adaptor sequences) has 5' to 3' exonuclease activity (e.g., which can digest the second strand of the 3'PDSAs). In other [embodimentexamples](#), the polymerase employed (e.g., for extending the 5' adaptor sequences) does not have 5' to 3' exonuclease activity.

30 In other [embodimentexamples](#), the methods further comprise: removing the second strand of the 3'PDSAs to generate single-stranded 3' adaptors (3'SSAs). In other [embodimentexamples](#), wherein the removing the second strand occurs after step b), or before step b), or during step b). In some [embodimentexamples](#), the removing the second strand of the 3'PDSAs comprises contacting the 3' duplex adapter with uracil-DNA glycosylase (UDG) to degrade the second

strand. In further [embodimentexamples](#), the removing the second strand is accomplished by a polymerase with exonuclease activity, wherein the polymerase extends the 5' adaptors across the exogenous UID sequence and the first segment.

In further [embodimentexamples](#), the methods further comprise: determining sequence reads of one or more of the amplicons. In other [embodimentexamples](#), the methods further comprise: assigning the sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence. In particular [embodimentexamples](#), the methods further comprise: assigning sequence reads of each UID family into a Watson subfamily and Crick subfamily based on spatial relationship of the exogenous UID sequence to the R1 and R2 read sequence. In other [embodimentexamples](#), the methods further comprise: identifying a nucleotide sequence as accurately representing a Watson strand of an analyte DNA fragment when at least 50% (e.g., 50 ... 75 ... 95%) of the Watson subfamily contain the sequence. In other [embodimentexamples](#), the methods further comprise: identifying a nucleotide sequence as accurately representing a Crick strand of an analyte DNA fragment when at least 50% (e.g., 50 ... 75 ... 90%) of the Crick subfamily contain the sequence.

In some [embodimentexamples](#), the methods further comprise: identifying a mutation in the nucleotide sequence as accurately representing a the Watson strand when the sequence accurately representing the Watson strand differs from a reference sequence that lacks the mutation. In additional [embodimentexamples](#), the methods further comprise: identifying a mutation in the nucleotide sequence as accurately representing the Crick Strand when the sequence accurately representing the Crick Strand differs from a reference sequence that lacks the mutation. In other [embodimentexamples](#), the methods further comprise: identifying a mutation in the analyte DNA fragment when the mutation in the nucleotide sequence accurately representing the Watson strand and the mutation in the nucleotide sequence accurately representing the Crick strand are the same mutation. In some [embodimentexamples](#), each member of a UID family further comprises the same endogenous UID sequence, wherein the endogenous UID sequence comprises an end of a double-stranded DNA fragment from the population. In other [embodimentexamples](#), the population of double-stranded DNA fragments has blunt ends.

Provided herein are for detecting the presence or absence of a mutation in a target region of a double stranded DNA template obtained from a sample from a mammal, and determining whether the mutation is present on both strands of the double stranded DNA template, wherein the method comprises: A) generating double stranded DNA fragments each having a duplex molecular barcode on each end of the double stranded DNA fragment; B)

amplifying the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment to generate an amplified duplex sequencing library, wherein the amplifying comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with a universal primer pair under whole-genome PCR conditions; C) optionally, generating a single stranded DNA library of Watson strands from the amplified duplex sequencing library; D) optionally, generating a single stranded DNA library of Crick strands from the amplified duplex sequencing library; E) amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Watson strands using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; F) amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Crick strands using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter; G) sequencing the target region amplified from the DNA library (e.g., the single stranded DNA library) (e.g., the DNA library (e.g., the single stranded DNA library)) of Watson strands to generate a sequencing read and to detect the presence or absence of the mutation in a Watson strand of the target region; H) sequencing the target region amplified from the DNA library (e.g., the single stranded DNA library) (e.g., the DNA library (e.g., the single stranded DNA library)) of Crick strands to generate a sequencing read and detect the presence or absence of the mutation in a Crick strand of the target region; and I) grouping the sequencing reads by the molecular barcode present in each sequencing read to determining whether the mutation is present on both strands of the double stranded DNA template. In some embodiment examples, generating the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment comprises: i) ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template, wherein the 3' duplex adapter comprises a) a first oligonucleotide comprising a 5' phosphate, a first molecular barcode, and a 3' oligonucleotide annealed to b) a second oligonucleotide comprising a degradable 3' blocking group, wherein the 3' oligonucleotide and the second oligonucleotide sequence are complementary; ii) degrading the degradable 3' blocking group; iii) ligating a 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template, wherein the 5' duplex adapter comprises an oligonucleotide comprising a second molecule barcode, wherein the second molecular barcode is different

from the first molecular barcode, wherein the 5' adapter is ligated onto the double stranded DNA fragment upstream of the first molecular barcode and leaving a gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter; and iv) filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter to generate the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment. In some embodimentexamples, generating the DNA library (e.g., the single stranded DNA library) (e.g., the DNA library (e.g., the single stranded DNA library)) of Watson strands from the amplified duplex sequencing library comprises: i) amplifying a first aliquot of the amplified duplex sequencing library using a primer pair consisting of a first primer and a second primer, wherein the first primer is capable of hybridizing to the Watson strand, and wherein the first primer comprises a tag, to generate double stranded amplification products having tagged Watson strands; ii) denaturing the double stranded amplification products having tagged Watson strands to generate single stranded tagged Watson strands and single stranded Crick strands; and iii) recovering the single stranded tagged Watson strands to generate the DNA library (e.g., the single stranded DNA library) of Watson strands from the amplified duplex sequencing library.

In some embodimentexamples, the double stranded DNA template is obtained from a sample from a mammal the generating the DNA library (e.g., the single stranded DNA library) of Crick strands from the amplified duplex sequencing library comprises: i) amplifying a second aliquot of the amplified duplex sequencing library using a primer pair consisting of a first primer and a second primer, wherein the first primer is capable of hybridizing to the Crick strand, and wherein the first primer comprises a tag, to generate double stranded amplification products having tagged Crick strands; ii) denaturing the double stranded amplification products having tagged Crick strands to generate single stranded tagged Crick strands and single stranded Watson strands; and iii) recovering the single stranded tagged Crick strands to generate the DNA library (e.g., the single stranded DNA library) of Crick strands from the amplified duplex sequencing library. In some embodimentexamples, the mammal is a human.

In some embodimentexamples, the method further comprises, prior to the generating a double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment: fragmenting the double stranded DNA to generate double stranded DNA fragments; dephosphorylating 5' ends of the double stranded DNA fragments; and

blunting the ends of double stranded DNA fragments. In some [embodimentexamples](#),
ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from
the double stranded DNA template comprises contacting the 3' duplex adapter and the
double stranded DNA fragment obtained from the double stranded DNA template in the
5 presence of a ligase. In some [embodimentexamples](#), the ligase is a T4 DNA ligase.

In some [embodimentexamples](#), degrading the degradable 3' blocking group
comprises contacting the 3' duplex adapter with uracil-DNA glycosylase (UDG). In some
[embodimentexamples](#), ligating the 5' adapter to each dephosphorylated 5' end of the double
stranded DNA fragment obtained from the double stranded DNA template comprises
10 contacting the 5' adapter and the double stranded DNA fragment obtained from the double
stranded DNA template in the presence of a ligase. In some [embodimentexamples](#), the ligase
is an *Escherichia coli* ligase.

In some [embodimentexamples](#), filling in the gap of single stranded nucleic acid
between the 5' end of the double stranded DNA fragment and the 5' adapter comprises
15 contacting the 5' end of the double stranded DNA fragment and the 5' adapter in the
presence of a polymerase and dNTPs. In some [embodimentexamples](#), the polymerase is a
Taq polymerase.

In some [embodimentexamples](#), the ligating the 5' adapter to each 5' end of the double
stranded DNA fragment, and the filling in the gap between the 5' end of the double stranded
20 DNA fragment and the 5' adapter are performed concurrently. In some
[embodimentexamples](#), amplifying the double stranded DNA fragment comprising the duplex
molecular barcode on each end of the double stranded DNA fragment to generate an
amplified duplex sequencing library comprises contacting the double stranded DNA
fragment comprising the duplex molecular barcode on each end of the double stranded DNA
25 fragment with a universal primer pair under PCR conditions. In some [embodimentexamples](#),
the amplifying comprises whole-genome PCR. In some [embodimentexamples](#), the tagged
primers are biotinylated primers, and wherein the biotinylated primers can generate
biotinylated single stranded Watson strands and biotinylated single stranded Crick strands. In
some [embodimentexamples](#), the denaturing steps comprise NaOH denaturation, heat
30 denaturation, or a combination of both.

In some [embodimentexamples](#), the recovering steps comprise contacting the tagged
Watson strands with streptavidin-functionalized beads and contacting the tagged Crick
strands with streptavidin-functionalized beads. In some [embodimentexamples](#), the
recovering steps further comprise denaturing non-tagged Watson strands and denaturing non-
35 tagged Watson strands. In some [embodimentexamples](#), the recovering steps further comprise
releasing the biotinylated single

stranded Watson strands from the streptavidin-functionalized beads and releasing the biotinylated single stranded Crick strands from the streptavidin-functionalized beads. In some [embodimentexamples](#), the tagged primers are phosphorylated primers, and the phosphorylated primers can generate phosphorylated single stranded Watson strands and phosphorylated single stranded Crick strands. In some [embodimentexamples](#), the denaturing steps comprises lambda exonuclease digestion.

In some [embodimentexamples](#), amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Watson strands further comprises second amplification using a second primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; and wherein the amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Crick strands further comprises a second amplification using a second primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter. In some [embodimentexamples](#), the sequencing steps comprise paired-end sequencing.

Also provided herein are methods for detecting the presence or absence of a mutation in a target region of a double stranded DNA template obtained from a sample from a mammal, and determining whether the mutation is present on both strands of the double stranded DNA template, wherein the method comprises: A) generating double stranded DNA fragments each having a duplex molecular barcode on each end of the double stranded DNA fragment; B) generating a DNA library (e.g., the single stranded DNA library) of Watson strands and a DNA library (e.g., the single stranded DNA library) of Crick strands from the amplified duplex sequencing library from the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment; C) amplifying the target region from the single stranded Watson strands using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; D) amplifying the target region from the single stranded Crick strands using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter; E) sequencing the target region amplified from the DNA library (e.g., the single stranded DNA library) of Watson strands to generate a sequencing read and to detect the presence or absence of the mutation in a Watson strand of the target region; F) sequencing the target region amplified from the DNA library (e.g., the single stranded DNA library) of Crick strands to generate a

sequencing read and detect the presence or absence of the mutation in a Crick strand of the target region; and G) grouping the sequencing reads by the molecular barcode present in each sequencing read to determining whether the mutation is present on both strands of the double stranded DNA template.

5 In some embodiment examples, the double stranded DNA template is a genomic DNA sample and generating the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment comprises: i) ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template, wherein the 3' duplex adapter comprises a) a first oligonucleotide comprising
10 a 5' phosphate, a first molecular barcode, and a 3' oligonucleotide annealed to b) a second oligonucleotide comprising a degradable 3' blocking group, wherein the 3' oligonucleotide and the second oligonucleotide sequence are complementary; ii) degrading the degradable 3' blocking group; iii) ligating a 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template, wherein the 5'
15 duplex adapter comprises an oligonucleotide comprising a second molecule barcode, wherein the second molecular barcode is different from the first molecular barcode, wherein the 5' adapter is ligated onto the double stranded DNA fragment upstream of the first molecular barcode and leaving a gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter; and iv) filling in the gap of single stranded
20 nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter to generate the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment.

In some embodiment examples, the double stranded DNA template is a cell-free DNA sample and generating the DNA library (e.g., the single stranded DNA library) of Watson
25 strands and the DNA library (e.g., the single stranded DNA library) of Crick strands from the amplified duplex sequencing library from the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment comprises: i) amplifying the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment using a universal primer pair consisting of a first
30 primer and a second primer, wherein the amplifying comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with the primer pair under whole-genome PCR conditions, wherein the first primer is capable of hybridizing to the Watson strand, and wherein the first primer is

biotinylated, to generate double stranded amplification products having biotinylated Watson strands; ii) contacting the double stranded amplification products having biotinylated Watson strands with streptavidin-functionalized beads under conditions where the biotinylated Watson strands bind to the streptavidin-functionalized beads; iii) denaturing the double stranded amplification products having biotinylated Watson strands to leave single stranded biotinylated Watson strands bound to the streptavidin-functionalized beads and to release single stranded Crick strands; iv) collecting the single stranded Crick strands; v) releasing the single stranded biotinylated Watson strands from the streptavidin-functionalized beads; and vi) collecting the single stranded biotinylated Watson strands.

10 In some [embodimentexamples](#), the double stranded DNA template is obtained from a sample from a mammal. In some [embodimentexamples](#), the mammal is a human.

In some [embodimentexamples](#), the method further comprises, prior to the generating a double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment: fragmenting the double stranded DNA to generate double stranded DNA fragments; dephosphorylating 5' ends of the double stranded DNA fragments; 15 and blunting the ends of double stranded DNA fragments.

In some [embodimentexamples](#), ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 3' duplex adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase. In some [embodimentexamples](#), 20 the ligase is a T4 DNA ligase. In some [embodimentexamples](#), the degradable 3' blocking group comprises contacting the 3' duplex adapter with uracil-DNA glycosylase (UDG). In some [embodimentexamples](#), ligating the 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template comprises 25 contacting the 5' adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase. In some [embodimentexamples](#), the ligase is an *Escherichia coli* ligase.

In some [embodimentexamples](#), filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter comprises 30 contacting the 5' end of the double stranded DNA fragment and the 5' adapter in the presence of a polymerase and dNTPs. In some [embodimentexamples](#), the polymerase is a Taq-B polymerase. In some [embodimentexamples](#), ligating the 5' adapter to each 5' end of the double stranded DNA fragment,

and the filling in the gap between the 5' end of the double stranded DNA fragment and the 5' adapter are performed concurrently.

In some embodimentexamples, amplifying the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on

each end of the double stranded DNA fragment with the primer pair under PCR conditions. In some embodimentexamples, amplifying comprises whole-genome PCR. In some embodimentexamples, amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Watson strands further comprises second amplification using a second primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; and wherein the amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Crick strands further comprises a second amplification using a second primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter. In some embodimentexamples, the sequencing steps comprise paired-end sequencing or single-end sequencing.

Also provided herein are methods for detecting the presence or absence of a mutation in a target region of a double stranded DNA template obtained from a sample from a mammal, and determining whether the mutation is present on both strands of the double stranded DNA template, wherein the method comprises: A) generating double stranded DNA fragments each having a duplex molecular barcode on each end of the double stranded DNA fragment; B) amplifying the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment using a universal primer pair, wherein the amplifying comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with the primer pair under whole-genome PCR conditions; C) amplifying the target region from a Watson strand of the amplified double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; D) amplifying the target region from a Crick strand of the amplified double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer

capable of hybridizing to the 5' adapter; E) sequencing the target region amplified from the Watson strand to generate a sequencing read and to detect the presence or absence of the mutation in a Watson strand of the target region; F) sequencing the target region amplified from the Crick strand to generate a sequencing read and detect the presence or absence of the mutation in a Crick strand of the target region; G) grouping the sequencing reads by the molecular barcode present in each sequencing read to determining whether the mutation is present on both strands of the double stranded DNA template.

In some embodimentexamples, the double stranded DNA template is a genomic DNA sample and generating the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment comprises: i) ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template, wherein the 3' duplex adapter comprises a) a first oligonucleotide comprising a 5' phosphate, a first molecular barcode, and a 3' oligonucleotide annealed to b) a second oligonucleotide comprising a degradable 3' blocking group, wherein the 3' oligonucleotide and the second oligonucleotide sequence are complementary; ii) degrading the degradable 3' blocking group; iii) ligating a 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template, wherein the 5' duplex adapter comprises an oligonucleotide comprising a second molecule barcode, wherein the second molecular barcode is different from the first molecular barcode, wherein the 5' adapter is ligated onto the double stranded DNA fragment upstream of the first molecular barcode and leaving a gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter; and iv) filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter to generate the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment. In some embodimentexamples, the double stranded DNA template is a cell-free DNA sample. In some embodimentexamples, the double stranded DNA template is a genomic DNA sample. In some embodimentexamples, the mammal is a human.

In some embodimentexamples, the method further comprises, prior to the generating a double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment: fragmenting the double stranded DNA to generate double stranded DNA fragments; dephosphorylating 5' ends of the double stranded DNA fragments; and blunting the ends of double stranded DNA fragments.

In some [embodimentexamples](#), ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 3' duplex adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase. In some [embodimentexamples](#), the ligase is a T4 DNA ligase. In some [embodimentexamples](#), degrading the degradable 3' blocking group comprises contacting the 3' duplex adapter with uracil-DNA glycosylase (UDG). In some [embodimentexamples](#), ligating the 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 5' adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase. In some [embodimentexamples](#), the ligase is an *Escherichia coli* ligase.

In some [embodimentexamples](#), filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter comprises contacting the 5' end of the double stranded DNA fragment and the 5' adapter in the presence of a DNA polymerase and dNTPs. In some [embodimentexamples](#), the DNA polymerase is a Taq-B polymerase.

In some [embodimentexamples](#), ligating the 5' adapter to each 5' end of the double stranded DNA fragment, and the filling in the gap between the 5' end of the double stranded DNA fragment and the 5' adapter are performed concurrently. In some [embodimentexamples](#), amplifying the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with the primer pair under PCR conditions. In some [embodimentexamples](#), amplifying comprises whole-genome PCR. In some [embodimentexamples](#), amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Watson strands further comprises second amplification using a second primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; and wherein the amplifying the target region from the DNA library (e.g., the single stranded DNA library) of Crick strands further comprises a second amplification using a second primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter. In some [embodimentexamples](#), the sequencing steps comprise paired-end sequencing.

Also provided herein are methods, comprising:

- j. identifying a mutation in the nucleotide sequence accurately representing the Watson Strand when the sequence accurately representing the Watson Strand differs from a reference sequence that lacks the mutation;
- k. identifying a mutation in the nucleotide sequence accurately representing the Crick Strand when the sequence accurately representing the Crick Strand differs from a reference sequence that lacks the mutation; and
- l. identifying a mutation in the analyte DNA fragment when the mutation in the nucleotide sequence accurately representing the Watson strand and the mutation in the nucleotide sequence accurately representing the Crick strand are the same mutation.

In some embodimentexamples, each member of a UID family further comprises the same endogenous UID sequence, wherein the endogenous UID sequence comprises an end of a double-stranded DNA fragment from the population. In some embodimentexamples, the endogenous UID sequence comprising the end of the double-stranded DNA fragment comprises at least 8, 10, or 15 bases. In some embodimentexamples, exogenous UID sequence is unique to each double-stranded DNA fragment. In some embodimentexamples, the exogenous UID sequence is not unique to each double-stranded DNA fragment. In some embodimentexamples, each member of a UID family comprises the same endogenous UID sequence and the same exogenous UID sequence. In some embodimentexamples, step (d) comprises no more than 11 cycles of PCR amplification. In some embodimentexamples, step (d) comprises no more than 7 cycles of PCR amplification. In some embodimentexamples, step (d) comprises no more than 5 cycles of PCR amplification. In some embodimentexamples, step (d) comprises at least 1 cycles of PCR amplification.

In some embodimentexamples, prior to determining the sequence reads, the amplicons are enriched for one or more target polynucleotides. In some embodimentexamples, the enriching comprises:

- a. selectively amplifying amplicons of Watson strands comprising the target polynucleotide sequence with a first set of Watson target-selective primer pairs, the first set of Watson target-selective primer pairs comprising: (i) a first Watson target-selective primer comprising a sequence complementary to a portion of the universal 3' adapter sequence, optionally wherein the portion of the universal 3' adapter sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) a second Watson target-selective

primer comprising a target-selective sequence, thereby creating target Watson amplification products; and

- b. selectively amplifying amplicons of Crick strands comprising the same target polynucleotide sequence with a first set of Crick target-selective primer pairs, the first set of Crick target-selective primer pairs comprising: (i) a first Crick target-selective primer comprising a sequence complementary to a portion of the universal 5' adapter sequence, optionally wherein the portion of the universal 5' adapter sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) a second Crick target-selective primer comprising the same target-selective sequence as the second Watson target-selective primer sequence, thereby creating target Crick amplification products.

In some [embodimentexamples](#), the method further comprises purifying the target Watson amplification products and the target Crick amplification products from non-target polynucleotides. In some [embodimentexamples](#), the method further comprises purifying comprises attaching the target Watson amplification products and the target Crick amplification products to a solid support. In some [embodimentexamples](#), the first Watson target-selective primer and first Crick target-selective primer comprises a first member of an affinity binding pair, and wherein the solid support comprises a second member of the affinity binding pair. In some [embodimentexample](#), the first member is biotin and the second member is streptavidin. In some [embodimentexamples](#), the solid support comprises a bead, well, membrane, tube, column, plate, sepharose, magnetic bead, or chip. In some [embodimentexamples](#), the method further comprises removing polynucleotides that are not attached to the solid support.

In some [embodimentexamples](#), the method further comprises:

- a. further amplifying the target Watson amplification products with a second set of Watson target-selective primers, the second set of Watson target-selective primers comprising (i) a third Watson target-selective primer comprising a sequence complementary to a portion of the universal 3' adapter sequence, optionally wherein the portion of the universal 3' adapter sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) a fourth Watson target-selective primer comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target-selective sequence selective for the same target polynucleotide, thereby creating target Watson library members;

b. further amplifying the target Crick amplification products with a second set of Crick target-selective primers, the second set of Crick target-selective primers comprising (i) a third Crick target-selective primer comprising a sequence complementary to a portion of the universal 5' adapter sequence, optionally wherein the portion of the universal 5' adapter sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) a fourth Crick target-selective primer comprising, in the 5' to 3' direction, an R2 sequencing primer site and the target-selective sequence selective for the same target polynucleotide of the fourth Watson target-selective primer, thereby creating target Crick library members.

In some embodimentexamples, the third Watson and Crick target-selective primers further comprise a sample barcode sequence. In some embodimentexamples, the third Watson target-selective primer further comprises a first grafting sequence that enables hybridization to a first grafting primer on a sequencer and wherein the third Crick target-selective primer further comprises a second grafting sequence that enables hybridization to a second grafting primer on the sequencer. In some embodimentexamples, the fourth Watson target-selective primer further comprises the second grafting sequence and wherein the fourth Crick target-selective primer further comprises the first grafting sequence. In some embodimentexamples, the first grafting sequence is a P7 sequence and wherein the second grafting sequence is a P5 sequence. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 50% of the target polynucleotides in the population of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 70% of the target polynucleotides in the population of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 80% of the target polynucleotides in the population of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 90% of the target polynucleotides in the population of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 50% of the population of total DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 70% of the population of total DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 80%

of the population of total DNA fragments. In some embodiment examples, the target Watson library members and the target Crick library members represent at least 90% of the population of total DNA fragments.

Also provided herein are methods comprising:

- 5 a. attaching adaptors to a population of double-stranded DNA fragments in an analyte DNA sample, wherein the adaptors comprise a double-stranded portion comprising an exogenous UID and a forked portion comprising (i) a single-stranded 3' adaptor sequence comprising an R2 sequencing primer site and (ii) a single-stranded 5' adaptor sequence comprising an R1 sequencing
10 primer site;
- b. performing an initial amplification to amplify the adaptor-ligated, double-stranded DNA fragments to produce amplicons;
- c. selectively amplifying amplicons of Watson strands comprising a target polynucleotide sequence with a first set of Watson target-selective primer
15 pairs, the first set of Watson target-selective primer pairs comprising: (i) a first Watson target-selective primer comprising a sequence complementary to a portion of the universal 3' adapter sequence, optionally wherein the portion of the universal 3' adapter sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) a second Watson target-selective
20 primer comprising a target-selective sequence, thereby creating target Watson amplification products;
- d. selectively amplifying amplicons of Crick strands comprising the same target polynucleotide sequence with a first set of Crick target-selective primer pairs, the first set of Crick target-selective primer pairs comprising: a first Crick
25 target-selective primer comprising a sequence a sequence complementary to a portion of the universal 5' adapter sequence, optionally wherein the portion of the universal 5' adapter sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) a second Crick target-selective primer comprising the same target-selective sequence as the second Crick target-selective primer sequence, thereby creating target Crick amplification
30 products.
- e. determining sequence reads of the target Watson amplification products and the target Crick amplification products;

- f. assigning the sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence;
- g. assigning sequence reads of each UID family into a Watson subfamily and Crick subfamily based on spatial relationship of the exogenous UID sequence to the R1 and R2 read sequence;
- h. identifying a nucleotide sequence as accurately representing a Watson strand of an analyte DNA fragment when a threshold percentage of members of the Watson family contain the sequence;
- i. identifying a nucleotide sequence as accurately representing a Crick strand of an analyte DNA fragment when a threshold percentage of members of the Crick family contain the sequence; and
- j. identifying a mutation in the analyte DNA fragment when the nucleotide sequence accurately representing the Watson strand and the nucleotide sequence accurately representing the Crick strand both contain the same mutation.

In some embodimentexamples, the method further comprises purifying the target Watson amplification products and the target Crick amplification products from non-target polynucleotides. In some embodimentexamples, the method further comprises attaching the target Watson amplification products and the target Crick amplification products to a solid support. In some embodimentexamples, the first Watson target-selective primer and first Crick target-selective primer comprises a first member of an affinity binding pair, and wherein the solid support comprises a second member of the affinity binding pair. In some embodimentexamples, the first member is biotin and the second member is streptavidin. In some embodimentexamples, the solid support comprises a bead, well, membrane, tube, column, plate, sepharose, magnetic bead, or chip. In some embodimentexamples, the method further comprises removing polynucleotides that are not attached to the solid support.

In some embodimentexamples, the method further comprises:

- a. further amplifying the target Watson amplification products with a second set of Watson target-selective primers, the second set of Watson target-selective primers comprising (i) a third Watson target-selective primer comprising a sequence complementary to the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) a fourth Watson target-selective primer comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target-selective

sequence selective for the same target polynucleotide, thereby creating target Watson library members;

- b. further amplifying the target Crick amplification products with a second set of Crick target-selective primers, the second set of Crick target-selective primers comprising (i) a third Crick target-selective primer comprising a sequence complementary to the R1 sequencing primer site of the universal 3' adaptor sequence, and (ii) a fourth Crick target-selective primer comprising, in the 5' to 3' direction, an R2 sequencing primer site and the target-selective sequence selective for the same target polynucleotide of the fourth Watson target-selective primer, thereby creating target Crick library members , thereby creating target Crick library members.

In some [embodimentexamples](#), the third Watson and Crick target-selective primers further comprise a sample barcode sequence. In some [embodimentexamples](#), the third Watson target-selective primer further comprises a first grafting sequence that enables hybridization to a first grafting primer on a sequencer and wherein the third Crick target-selective primer further comprises a second grafting sequence that enables hybridization to a second grafting primer on the sequencer. In some [embodimentexamples](#), the fourth Watson target-selective primer further comprises the second grafting sequence and wherein the fourth Crick target-selective primer further comprises the first grafting sequence. In some [embodimentexamples](#), the first grafting sequence is a P7 sequence and wherein the second grafting sequence is a P5 sequence. In some [embodimentexamples](#), the attaching comprises attaching A-tailed adaptors to the population of double-stranded DNA fragments. In some [embodimentexamples](#), the attaching comprises attaching A-tailed adaptors to both ends of DNA fragments in the population.

In some [embodimentexamples](#), the attaching comprises:

- a. attaching partially double-stranded 3' adaptors to 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments, wherein a first strand of the partially double-stranded 3' adaptor comprises, in the 5'-3' direction, (i) a first segment, (ii) optionally, an exogenous UID sequence, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and wherein the second strand of the partially double-stranded 3' adaptor comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group, optionally wherein the second strand is degradable; and

- b. annealing 5' adaptors to the 3' adaptors via the annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor; and
- c. performing a nick translation-like reaction to extend the 5' adaptors across the 3' adaptors (e.g., using a DNA polymerase) and covalently link the extended 5' adaptor to the 5' ends of the Watson and Crick strands of the double-stranded DNA fragments (e.g. using a ligase).

In some embodimentexamples, the UID sequence comprises an endogenous UID sequence comprising an end of a double-stranded DNA fragment from the population. In some embodimentexamples, the endogenous UID sequence comprising the end of the double-stranded DNA fragment comprises at least 8, 10, or 15 bases. In some embodimentexamples, the exogenous UID sequence is unique to each double-stranded DNA fragment. In some embodimentexamples, the exogenous UID sequence is not unique to each double-stranded DNA fragment. In some embodimentexamples, each member of a UID family comprises the same endogenous UID sequence and the same exogenous UID sequence.

In some embodimentexamples, amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises no more than 11 cycles of PCR amplification. In some embodimentexamples, amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises no more than 7 cycles of PCR amplification. In some embodimentexamples, amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises no more than 5 cycles of PCR amplification. In some embodimentexamples, amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises at least 1 cycles of PCR amplification. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 50% of the target polynucleotides in the population of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 70% of the target polynucleotides in the population of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 80% of the target polynucleotides in the population of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 90% of the target polynucleotides in the population

of double-stranded DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 50% of the population of total DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 70% of the population of total DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 80% of the population of total DNA fragments. In some embodimentexamples, the target Watson library members and the target Crick library members represent at least 90% of the population of total DNA fragments.

In some embodimentexamples, the determination of the sequence reads enables sequence determination of both ends of template molecules. In some embodimentexamples, the determination of both ends of template molecules comprises paired end sequencing. In some embodimentexamples, the determination of the sequence reads comprises single read sequencing across the length of the templates for generating the sequence reads. In some embodimentexamples, the determination of the sequence reads comprises sequencing with a massively parallel sequencer. In some embodimentexamples, the massively parallel sequencer is configured to determine sequence reads from both ends of template polynucleotides. In some embodimentexamples, the population of double-stranded DNA fragments comprises one or more fragments that are about 50-600 nt in length. In some embodimentexamples, the population of double-stranded DNA fragments comprises one or more fragments that are less than 2000, less than 1000, less than 500, less than 400, less than 300, or less than 250 nt in length.

In some embodimentexamples, methods provided herein further comprise, after the initial amplification and before the selective amplification, preparing single stranded (ss) DNA libraries corresponding to sense and antisense strands of the amplicons. In some embodimentexamples, the ss DNA library preparation comprises:

- a. performing an amplification reaction utilizing two primers, wherein only one of the two primers comprises a first member of an affinity binding pair, thereby creating amplification products comprising a strand comprising the first member of the affinity binding pair and a strand not comprising the first member of the affinity binding pair;
- b. contacting the amplification products with a solid support, wherein the solid support comprises the second member of the affinity binding pair;

- c. denaturing the amplification products to separate the strand comprising the first member of the affinity binding pair from the strand not comprising the first member of the affinity binding pair; and
- d. purifying the separated strands comprising the first member of the affinity binding pair and the separated strands not comprising the first member of the affinity binding pair.

In some embodimentexamples, the first member of the affinity binding pair is biotin and the second member of the affinity binding pair is streptavidin. In some

embodimentexamples, the ss DNA library preparation comprises:

- a. partitioning the amplicons into two amplification reactions, each amplification reaction utilizing a forward primer and a reverse primer, wherein only one of the two primers is phosphorylated, thereby creating amplification products comprising a phosphorylated strand and a non-phosphorylated strand;
- b. contacting the amplification products with an exonuclease that selectively digests the strands with the 5' phosphate.

In some embodimentexamples:

- a. in the first amplification reaction, the forward primer is phosphorylated and the reverse primer is non-phosphorylated;
- b. in the second amplification reaction, the reverse primer is phosphorylated and the forward primer is non-phosphorylated.

In some embodimentexamples, the exonuclease is a lambda exonuclease. In some embodimentexamples, the phosphorylation is at the 5' site.

In some embodimentexamples, the initial amplification comprises:

- a. amplifying with a primer pair, in which only one of the two primers in the primer pair comprises a first member of an affinity binding pair, thereby creating amplification products comprising a strand comprising the first member of the affinity binding pair and a strand not comprising the first member of the affinity binding pair;
- b. contacting the amplification products with a solid support, wherein the solid support comprises the second member of the affinity binding pair;
- c. denaturing the amplification products to separate the strand comprising the first member of the affinity binding pair from the strand not comprising the first member of the affinity binding pair; and

- d. purifying the separated strands comprising the first member of the affinity binding pair and the separated strands not comprising the first member of the affinity binding pair.

In some [embodimentexamples](#), the first member of the affinity binding pair is biotin and the second member of the affinity binding pair is streptavidin. In some [embodimentexamples](#), sequence reads of a UID family are assigned to a Watson subfamily when the exogenous UID sequence is downstream of the R2 sequence and upstream of the R1 sequence. In some [embodimentexamples](#), sequence reads of a UID family are assigned to a Crick subfamily when the exogenous UID sequence is downstream of the R1 sequence and upstream of the R2 sequence. In some [embodimentexamples](#), sequence reads of a UID family are assigned to a Watson subfamily when the exogenous UID sequence is in greater proximity to the R2 sequence and lesser proximity to the R1 sequence. In some [embodimentexamples](#), sequence reads of a UID family are assigned to a Crick subfamily when the exogenous UID sequence is in greater proximity to the R1 sequence and in lesser proximity to the R2 sequence. In some [embodimentexamples](#), sequence reads of a UID family are assigned to a Watson subfamily when the exogenous UID sequence is immediately downstream or within 1-300, 1-70, 1-60, 1-50, 1-40, 1-30, 1-20, 1-10, or 1-5 nucleotides of the R2 sequence. In some [embodimentexamples](#), sequence reads of a UID family are assigned to a Crick subfamily when the exogenous UID sequence is immediately downstream or within 1-300, 1-70, 1-60, 1-50, 1-40, 1-30, 1-20, 1-10, or 1-5 nucleotides of the R1 sequence.

In some [embodimentexamples](#), the population of double-stranded DNA fragments are from a biological sample. In some [embodimentexamples](#), the biological sample is obtained from a subject.

In some [embodimentexamples](#), the subject is a human subject. In some [embodimentexamples](#), the biological sample is a fluid sample. In some [embodimentexamples](#), the fluid sample is selected from whole blood, plasma, serum sputum, urine, sweat, tears, ascites, semen, and bronchoalveolar lavage. In some [embodimentexamples](#), the fluid sample is a cell-free or essentially cell-free sample. In some [embodimentexamples](#), the biological sample is a solid biological sample. In some [embodimentexamples](#), the solid biological sample is a tumor sample.

In some [embodimentexamples](#), the identified mutation is present in the population of double-stranded DNA fragments at a frequency of 0.1% or less. In some [embodimentexamples](#), the identified mutation is present in the population of double-stranded DNA fragments at a frequency of 0.1% to 0.00001%. In some [embodimentexamples](#), the identified mutation is present in the population of double-stranded DNA fragments at a frequency of 0.1% to 0.01%. In some [embodimentexamples](#),

the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 50% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample. In some [embodimentexamples](#), the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 70% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample. In some [embodimentexamples](#), the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 80% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample. In some [embodimentexamples](#), the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 90% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample. In some [embodimentexamples](#), the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 50% of the double-stranded DNA fragments in the analyte DNA sample. In some [embodimentexamples](#), the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 70% of the double-stranded DNA fragments in the analyte DNA sample. In some [embodimentexamples](#), the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 80% of the double-stranded DNA fragments in the analyte DNA sample. In some [embodimentexamples](#), the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 90% of the double-stranded DNA fragments in the analyte DNA sample.

In some [embodimentexamples](#), the error rate associated with the identification of one or more mutations in analyte DNA fragments according to a method of any one of the preceding claims is reduced by at least 2-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold, as compared to an alternative method of identifying mutations that does not require the mutation to be detected in both Watson and Crick strands of an analyte DNA fragment. In some [embodimentexamples](#), the alternative method comprises standard molecular barcoding or standard PCR-based molecular barcoding. In some [embodimentexamples](#), the alternative method comprises:

- a. attaching adaptors to a population of double-stranded DNA fragments in an analyte DNA sample, wherein the adaptors comprise a unique exogenous UID;

- b. performing an initial amplification to amplify the adaptor-ligated, double-stranded DNA fragments to produce amplicons;
- c. determining sequence reads of one or more amplicons of the one or more of the adaptor-ligated, double-stranded DNA fragments;
- 5 d. assigning the sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence;
- e. identifying a nucleotide sequence as accurately representing an analyte DNA fragment when a threshold percentage of members of a UID family contain the sequence; and
- 10 f. identifying a mutation in the analyte DNA fragment when the sequence identified as accurately representing an analyte DNA fragment differs from a reference sequence that lacks the mutation.

In some embodiment examples, the error rate associated with the identification of one or more mutations in analyte DNA fragments according to a method of any one of the preceding

15 claims is no more than 1×10^{-2} , no more than 1×10^{-3} , no more than 1×10^{-4} , no more than 1×10^{-5} , no more than 1×10^{-6} , no more than 5×10^{-6} , or no more than 1×10^{-7} .

Also provided herein are computer readable media comprising computer executable instructions for analyzing sequence read data from a nucleic acid sample, wherein the data is generated by a method of any one of the preceding claims. In one aspect, the disclosure provides a computer readable medium comprising computer executable instructions for analyzing sequence read data from a nucleic acid sample, wherein the data is generated by a method as disclosed herein.

20 In some embodiment examples, the computer readable medium comprises executable instructions for

- a. assigning sequence reads into UID families, wherein each member of a UID
- 25 family comprises the same exogenous UID sequence;
- b. assigning sequence reads of each UID family into Watson and Crick subfamilies based on spatial relationship of the exogenous UID sequence to the R1 and R2 read sequence;
- c. identifying a nucleotide sequence as accurately representing a Watson strand
- 30 of an analyte DNA fragment when a threshold percentage of members of the Watson subfamily contain the sequence;
- d. identifying a nucleotide sequence as accurately representing a Crick strand of an analyte DNA fragment when a threshold percentage of members of the Crick subfamily contain the sequence;

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- e. identifying a mutation in the nucleotide sequence accurately representing the Watson Strand when the sequence accurately representing the Watson Strand differs from a reference sequence that lacks the mutation;
- f. identifying a mutation in the nucleotide sequence accurately representing the Crick Strand when the sequence accurately representing the Crick Strand differs from a reference sequence that lacks the mutation;
- g. identifying a mutation in the analyte DNA fragment when the mutation in the nucleotide sequence accurately representing the Watson strand and the mutation in the nucleotide sequence accurately representing the Crick strand are the same mutation.

In some embodimentexamples, the computer readable medium comprises executable instructions for assigning UID family members to the Watson subfamily when the exogenous UID sequence is immediately downstream or within 1-300 nucleotides of the R2 sequencing primer binding site. In some embodimentexamples, the computer readable medium comprises executable instructions for assigning UID family members to the Crick subfamily when the exogenous UID sequence is immediately downstream or within 1-300 nucleotides of the R1 sequencing primer binding site. In some embodimentexamples, the computer readable medium comprises executable instructions for mapping the sequence reads to a reference genome. In some embodimentexamples, the reference genome is a human reference genome.

In some embodimentexamples, the computer readable medium further comprises computer executable instructions to generate a report of therapeutic options based on the presence, absence, or amount of a mutation in the sample. In some embodimentexamples, the computer readable medium further comprises computer executable code that enables transmission of the data over a network.

Also provided herein are computer systems, comprising:

- a. a memory unit configured to receive sequence data from a nucleic acid sample, wherein the data is generated by a method of any one of the preceding claims;
- b. a processor communicatively coupled to the memory unit, wherein the processor comprises a computer readable medium of any of the preceding claims.

In some embodimentexamples, the computer system further comprises a sequencing system configured to communicate the data to the memory unit. In some embodimentexamples, the computer

system further comprises a user interface configured to communicate or display the report to a user. In some embodiment examples, the computer system further comprises a digital processor configured to transmit results of the data analysis over a network.

Also provided herein are systems, comprising:

- 5 a. a population of double-stranded DNA fragments from a biological sample;
- b. a population of 3' adaptors as described in any one of the preceding claims;
- c. a population of 5' adaptors as described in any one of the preceding claims;
- d. reagents for performing nick translation-like reaction (e.g., with a DNA polymerase, cohesive end-specific ligase, and uracil-DNA glycosylase);
- 10 e. reagents for enriching amplicons for one or more target polynucleotides; and
- f. a sequencing system.

In some embodiment examples, the system further comprises a computer system of any one of the preceding claims.

Also provided herein are kits, comprising:

- 15 a. a first set of Watson target-selective primer pairs, comprising (i) one or more first Watson target-selective primers comprising a sequence complementary to a portion of the universal 3' adapter sequence, optionally wherein the portion of the universal 3' adapter sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) one or more second Watson target-
- 20 selective primers, each of the one or more second Watson target-selective primers comprising a target-selective sequence;
- b. a first set of Crick target-selective primer pairs, comprising (i) one or more Crick target-selective primers comprising a sequence a sequence complementary to a portion of the universal 5' adapter sequence, optionally
- 25 wherein the portion of the universal 5' adapter sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) one or more second Crick target-selective primers, each of the one or more second Crick target-selective primers comprising the same target-selective sequence as the second Watson target-selective primer sequence;
- 30 c. a second set of Watson target-selective primer pairs, comprising (i) one or more third Watson target-selective primers comprising a sequence complementary to the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) one or more fourth Watson target-selective primers, each of

the one or more fourth Watson target-selective primers comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target-selective sequence selective for the same target polynucleotide; and

- d. a second set of Crick target-selective primers comprising (i) one or more third Crick target-selective primers comprising a sequence complementary to the R1 sequencing primer site of the universal 3' adaptor sequence, and (ii) one or more fourth Crick target-selective primers, each of the one or more fourth Crick target-selective primers comprising, in the 5' to 3' direction, an R2 sequencing primer site and a target-selective sequence selective for the same target polynucleotide.

In one aspect, the present disclosure provides a kit, comprising:

- a. a first set of Watson target-selective primer pairs, comprising (i) one or more first Watson target-selective primers comprising a sequence complementary to a portion of the universal 3' adaptor sequence, optionally wherein the portion of the universal 3' adaptor sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) one or more second Watson target-selective primers, each of the one or more second Watson target-selective primers comprising a target-selective sequence;
- b. a first set of Crick target-selective primer pairs, comprising (i) one or more Crick target-selective primers comprising a sequence a sequence complementary to a portion of the universal 5' adaptor sequence, optionally wherein the portion of the universal 5' adaptor sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) one or more second Crick target-selective primers, each of the one or more second Crick target-selective primers comprising the same target-selective sequence as the second Watson target-selective primer sequence;

- c. a second set of Watson target-selective primer pairs, comprising (i) one or more third Watson target-selective primers comprising a sequence complementary to the R2 sequencing primer site of the universal 3' adaptor sequence and optionally a Watson sample index barcode, and (ii) one or more fourth Watson target-selective primers, each of the one or more fourth Watson target-selective primers comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target-selective sequence selective for the same target polynucleotide; and
- d. a second set of Crick target-selective primers comprising (i) one or more third Crick target-selective primers comprising a sequence complementary to the R1 sequencing primer site of the universal 3' adaptor sequence and optionally a Crick sample index barcode, and (ii) one or more fourth Crick target-selective primers, each of the one or more fourth Crick target-selective primers comprising, in the 5' to 3' direction, an R2 sequencing primer site and a target-selective sequence selective for the same target polynucleotide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this ~~invention~~ disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the ~~invention~~ disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more ~~embodiment~~ examples of the ~~invention~~ disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the ~~invention~~ disclosure will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 contains a schematic of an exemplary duplex anchored PCR method. Duplex adapters with molecular barcodes are ligated onto the ends of nucleic acid fragments having blunted ends, to generate a duplex sequencing library, and the duplex sequencing library is subjected to PCR to generate an amplified duplex sequencing library. The amplification products in the amplified duplex sequencing library are divided into two aliquots and each aliquot is subjected to PCR where the Watson strand is amplified from the first aliquot, and the Crick strand is amplified from the second aliquot.

Figure 2 contains a schematic of an exemplary second round of library amplification where the Watson strands amplified from the first aliquot in Figure 1 are subjected to PCR using a primer pair where a first primer is biotinylated and a second primer is non-

WHAT IS CLAIMED IS:

1. A method, comprising:

a) attaching partially double-stranded 3' adaptors (3'PDSAs) to 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments in an analyte DNA sample,

wherein a first strand of each of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence that is optionally 8-50 nucleotides in length, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and

wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group,

b) annealing 5' adaptors to said annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor;

c) extending the 5' adaptors across the exogenous UID sequence and said first segment, thereby generating the complement of said exogenous UID sequence and complement of said first segment, and

d) covalently linking the 3' end of said complement of said first segment to the 5' ends of the Watson and Crick strands of the double-stranded DNA fragments, thereby generating a plurality of adaptor-ligated double-stranded DNA fragments.

2. The method of claim 1, further comprising: amplifying said plurality of adaptor-ligated, double-stranded DNA fragments with a first primer complementary to said universal 3' adaptor sequence, and second primer complementary to the complement of said universal 5' adaptor sequence, thereby generating a duplex sequencing library~~amplicons~~, wherein said duplex sequencing library ~~amplicons~~ comprises a plurality of double-stranded Watson templates and a plurality of double-stranded Crick templates.

3. The method of claim 2, further comprising: selectively

A) amplifying a first aliquot of said duplex sequencing library ~~said double-stranded Watson templates~~ with a first set of Watson target-selective primer pairs, the first set of Watson target-selective primer pairs comprising: (i) a first Watson target-selective primer comprising a sequence complementary to a portion of the universal 3' adapter sequence, and (ii) a second Watson target-selective primer comprising a target-selective sequence, thereby creating target Watson amplification products; and

B) optionally amplifying the target Watson amplification products with a second set of Watson target-selective primers, the second set of Watson target-selective primers comprising

(i) a third Watson target-selective primer comprising a sequence complementary to a portion of the universal 3' adapter sequence and a Watson sample barcode index, optionally wherein the portion of the universal 3' adapter sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and

(ii) a fourth Watson target-selective primer comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target-selective sequence selective for the same target polynucleotide, thereby creating target Watson library members.

4. The method of claim 3, further comprising: ~~selectively~~

A) amplifying a second aliquot of said duplex sequencing library ~~said double-stranded Crick templates~~ with a first set of Crick target-selective primer pairs, the first set of Crick target-selective primer pairs comprising: (i) a first Crick target-selective primer comprising a sequence complementary to the complement of a portion of the universal 5' adapter sequence, and (ii) a second Crick target-selective primer comprising the same target-selective sequence as the second Watson target-selective primer sequence, thereby creating target Crick amplification products; and

B) optionally amplifying the target Crick amplification products with a second set of Crick target-selective primers, the second set of Crick target-selective primers comprising

(i) a third Crick target-selective primer comprising a sequence complementary to a portion of the universal 5' adapter sequence and a Crick sample barcode index, optionally wherein the portion of the universal 5' adapter sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and

(ii) a fourth Crick target-selective primer comprising, in the 5' to 3' direction, a R2 sequencing primer site and the target-selective sequence selective for the same target polynucleotide of the fourth Watson target-selective primer, thereby creating target Crick library members.

5. The method of any one of claims 1-4, further comprising: removing said second strand of said 3'PDSAs to generate single-stranded 3' adaptors (3'SSAs).
6. The method of claim 5, wherein said removing said second strand occurs after step b), or before step b), or during step b).
7. The method of any one of claims 5-6, wherein said second strand comprises one or more deoxyuridines, and wherein said removing said second strand of said 3'PDSAs comprises contacting the 3' duplex adapter with uracil-DNA glycosylase (UDG) to degrade said second strand.
8. The method of claim 5, wherein said removing said second strand is accomplished by a polymerase with exonuclease activity, wherein said polymerase extends the 5' adaptors across the exogenous UID sequence and said first segment.
9. The method of claim 42, further comprising: determining sequence reads of one or more of said target Watson amplification products and target Crick amplification products and/or said target Watson library members and said target Crick library members~~amplicons~~.
10. The method of claim 9, further comprising: assigning the sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence.
11. The method of claim 10, further comprising: assigning sequence reads of each UID family into a Watson subfamily and Crick subfamily based on: i) spatial relationship of the exogenous UID sequence to the R1 and R2 read sequence, and/or ii) the presence of said Watson sample index barcode or said Crick sample index barcode.

12. The method of claim 11, further comprising: identifying a nucleotide sequence as accurately representing a Watson strand of an analyte DNA fragment when at least 50% of the Watson subfamily contain the sequence.

13. The method of claim 12, further comprising: identifying a nucleotide sequence as accurately representing a Crick strand of an analyte DNA fragment when at least 50% of the Crick subfamily contain the sequence.

14. The method of claim 12, further comprising: identifying a mutation in the nucleotide sequence as accurately representing the Watson strand when the sequence accurately representing the Watson strand differs from a reference sequence that lacks the mutation.

15. The method of claim 14, further comprising: identifying a mutation in the nucleotide sequence as accurately representing the Crick Strand when the sequence accurately representing the Crick Strand differs from a reference sequence that lacks the mutation.

16. The method of claim 15, further comprising: identifying a mutation in the analyte DNA fragment when the mutation in the nucleotide sequence accurately representing the Watson strand and the mutation in the nucleotide sequence accurately representing the Crick strand are the same mutation.

~~17. The method of claim 10, wherein each member of a UID family further comprises the same endogenous UID sequence, wherein the endogenous UID sequence comprises an end of a double stranded DNA fragment from the population.~~

~~18. The method of claim 1, wherein said population of double stranded DNA fragments has blunt ends.~~

179. A system, comprising:

a) a population of partially double-stranded 3' adaptors (3'PDSAs) configured to be ligated to the 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments,

wherein a first strand of each of the 3'PDSAs comprises, in the 5'-3' direction, (i) a first segment, (ii) an exogenous UID sequence that is optionally 8-50 nucleotides in length, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and

wherein the second strand of the 3'PDSAs comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group; and

b) a population of 5' adaptors configured to anneal to said annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 3' adaptor.

~~20. —The system of claim 19, further comprising: c) said population of double-stranded DNA fragments from a biological sample.~~

~~21. —The system of claim 20, wherein said population of double-stranded DNA fragments has blunt ends.~~

~~22. —The system of claim 19, further comprising: c) reagents for degrading said second strand of said 3'PDSAs to generate single-stranded 3' adaptors (3'SSAs).~~

~~23. —The system of claim 19, further comprising: c) a first primer complementary to said universal 3' adaptor sequence, and second primer complementary to the complement of said universal 5' adaptor sequence.~~

~~24. —The system of claim 19, further comprising: c) a Watson anchor primer complementary to said universal 3' adapter sequence, and d) a Crick anchor primer complementary to the complement of said universal 5' adapter sequence.~~

~~25. —The system of claim 19, further comprising:~~

~~—— c) a first set of Watson target selective primer pairs, comprising (i) one or more first Watson target selective primers comprising a sequence complementary to a portion of the~~

universal 3' adapter sequence, and (ii) one or more second Watson target selective primers, each of the one or more second Watson target selective primers comprising a target selective sequence, and

——— d) a first set of Crick target selective primer pairs, comprising (i) one or more Crick target selective primers comprising a sequence complementary to the complement of a portion of the universal 5' adapter sequence, and (ii) one or more second Crick target selective primers, each of the one or more second Crick target selective primers comprising the same target selective sequence as the second Watson target selective primer sequence.

26. — A method comprising:

——— a) — forming a reaction mixture comprising:

——— i) — a plurality of double stranded DNA fragments that are

——— dephosphorylated and blunt ended, wherein each of said double stranded DNA

——— fragments comprises Watson and Crick strands;

——— ii) — a plurality of adaptors, wherein each of said adaptors comprises, in the 5' — to 3' direction: A) a barcode, and B) a universal 3' adaptor sequence; and

——— iii) — a ligase; and

——— b) — incubating said reaction mixture such that: i) adaptors are ligated to the 3' ends of the Watson and Crick strands, and ii) adaptors are not ligated to the 5' ends of either the Watson or Crick strands, thereby generating double stranded ligation products.

27. — The method of claim 26, wherein each of said plurality of adaptors comprises a unique barcode.

28. — The method of claim 27, wherein each of said double stranded ligation products comprises a Watson strand with only one barcode and a Crick strand with only one barcode that is different from said barcode on said Watson strand.

29. — A method for detecting the presence or absence of a mutation in a target region of a double stranded DNA template obtained from a sample from a mammal, and determining whether the mutation is present on both strands of the double stranded DNA template, wherein the method comprises:

~~———A) generating double stranded DNA fragments each having a duplex molecular barcode on each end of the double stranded DNA fragment;~~

~~B) amplifying the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment to generate an amplified duplex sequencing library, wherein the amplifying comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with a universal primer pair under whole genome PCR conditions;~~

~~———C) optionally, generating a single stranded DNA library of Watson strands from the amplified duplex sequencing library;~~

~~———D) optionally, generating a single stranded DNA library of Crick strands from the amplified duplex sequencing library;~~

~~———E) amplifying the target region from the DNA library of Watson strands using a primer pair comprising a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter;~~

~~———F) amplifying the target region from the DNA library of Crick strands using a primer pair comprising a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter;~~

~~———G) sequencing the target region amplified from the DNA library of Watson strands to generate a sequencing read and to detect the presence or absence of the mutation in a Watson strand of the target region;~~

~~———H) sequencing the target region amplified from the DNA library of Crick strands to generate a sequencing read and detect the presence or absence of the mutation in a Crick strand of the target region;~~

~~———I) grouping the sequencing reads by the molecular barcode present in each sequencing read to determining whether the mutation is present on both strands of the double stranded DNA template.~~

30. — The method of claim 29, wherein generating the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment comprises:

~~———i) ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template, wherein the 3' duplex adapter comprises a) a first oligonucleotide comprising a 5' phosphate, a first molecular barcode, and a 3'~~

oligonucleotide annealed to b) a second oligonucleotide comprising a degradable 3' blocking group, wherein the 3' oligonucleotide and the second oligonucleotide sequence are complementary;

—— ii) degrading the degradable 3' blocking group;

—— iii) ligating a 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template, wherein the 5' duplex adapter comprises an oligonucleotide comprising a second molecule barcode, wherein the second molecular barcode is different from the first molecular barcode, wherein the 5' adapter is ligated onto the double stranded DNA fragment upstream of the first molecular barcode and leaving a gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter; and

—— iv) filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter to generate the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment.

31. — The method of claim 29, wherein generating the DNA library of Watson strands from the amplified duplex sequencing library comprises:

—— i) amplifying a first aliquot of the amplified duplex sequencing library using a primer pair consisting of a first primer and a second primer, wherein the first primer is capable of hybridizing to the Watson strand, and wherein the first primer comprises a tag, to generate double stranded amplification products having tagged Watson strands;

—— ii) denaturing the double stranded amplification products having tagged Watson strands to generate single stranded tagged Watson strands and single stranded Crick strands; and

—— iii) recovering the single stranded tagged Watson strands to generate the DNA library of Watson strands from the amplified duplex sequencing library.

32. — The method of any one of claims 29-31, wherein the double stranded DNA template is obtained from a sample from a mammal the generating the DNA library of Crick strands from the amplified duplex sequencing library comprises:

—— i) amplifying a second aliquot of the amplified duplex sequencing library using a primer pair comprising a first primer and a second primer, wherein the first primer is capable

of hybridizing to the Crick strand, and wherein the first primer comprises a tag, to generate double stranded amplification products having tagged Crick strands;

—— ii) denaturing the double stranded amplification products having tagged Crick strands to generate single stranded tagged Crick strands and single stranded Watson strands; and

—— iii) recovering the single stranded tagged Crick strands to generate the DNA library of Crick strands from the amplified duplex sequencing library.

33. — The method of any one of claims 29-32, wherein the mammal is a human.

34. — The method of any one of claims 29-33, wherein the method further comprises, prior to the generating a double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment:

—— fragmenting the double stranded DNA to generate double stranded DNA fragments;

—— dephosphorylating 5' ends of the double stranded DNA fragments; and

—— blunting the ends of double stranded DNA fragments.

35. — The method of any one of claims 29-34, wherein the ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 3' duplex adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase.

36. — The method of claim 35, wherein the ligase is a T4 DNA ligase.

37. — The method of any one of claims 29-36, wherein the degrading the degradable 3' blocking group comprises contacting the 3' duplex adapter with uracil DNA glycosylase (UDG).

38. — The method of any one of claims 29-37, wherein the ligating the 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 5' adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase.

39. — The method of claim 38, wherein the ligase is an *Escherichia coli* ligase.

40. — ~~The method of any one of claims 29-39, wherein the filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter comprises contacting the 5' end of the double stranded DNA fragment and the 5' adapter in the presence of a polymerase and dNTPs.~~

41. — ~~The method of claim 40, wherein the polymerase is a Taq polymerase.~~

42. — ~~The method of any one of claims 29-31, wherein the ligating the 5' adapter to each 5' end of the double stranded DNA fragment, and the filling in the gap between the 5' end of the double stranded DNA fragment and the 5' adapter are performed concurrently.~~

43. — ~~The method of any one of claims 29-42, wherein amplifying the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment to generate an amplified duplex sequencing library comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with a universal primer pair under PCR conditions.~~

44. — ~~The method of claim 43, wherein the amplifying comprises whole genome PCR.~~

45. — ~~The method of any one of claims 29-44, wherein the tagged primers are biotinylated primers, and wherein the biotinylated primers can generate biotinylated single stranded Watson strands and biotinylated single stranded Crick strands.~~

46. — ~~The method of claim 45, wherein the denaturing steps comprise NaOH denaturation, heat denaturation, or a combination of both.~~

47. — ~~The method of claim 45 or claim 46, wherein the recovering steps comprise contacting the tagged Watson strands with streptavidin-functionalized beads and contacting the tagged Crick strands with streptavidin-functionalized beads.~~

48. — ~~The method of claim 47, wherein the recovering steps further comprise denaturing non-tagged Watson strands and denaturing non-tagged Watson strands.~~

49. — ~~The method of claim 47 or claim 48, wherein the recovering steps further comprise releasing the biotinylated single stranded Watson strands from the streptavidin-functionalized beads and releasing the biotinylated single stranded Crick strands from the streptavidin-functionalized beads.~~

50. — ~~The method of any one of claims 29-44, wherein the tagged primers are phosphorylated primers, and wherein the phosphorylated primers can generate phosphorylated single stranded Watson strands and phosphorylated single stranded Crick strands.~~

51. — ~~The method of claim 50, wherein the denaturing steps comprises lambda-exonuclease digestion.~~

52. — ~~The method of any one of claims 29-51, wherein amplifying the target region from the DNA library of Watson strands further comprises second amplification using a second primer pair comprising a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; and wherein the amplifying the target region from the DNA library of Crick strands further comprises a second amplification using a second primer pair comprising a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter.~~

53. — ~~The method of any one of claims 29-52, wherein the sequencing steps comprise paired-end sequencing.~~

54. — ~~A method for detecting the presence or absence of a mutation in a target region of a double stranded DNA template obtained from a sample from a mammal, and determining whether the mutation is present on both strands of the double stranded DNA template, wherein the method comprises:~~

— ~~A) generating double stranded DNA fragments each having a duplex molecular barcode on each end of the double stranded DNA fragment;~~

~~——— B) generating a DNA library of Watson strands and a DNA library of Crick strands from the amplified duplex sequencing library from the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment;~~

~~——— C) amplifying the target region from the single stranded Watson strands using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter;~~

~~——— D) amplifying the target region from the single stranded Crick strands using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter;~~

~~——— E) sequencing the target region amplified from the DNA library of Watson strands to generate a sequencing read and to detect the presence or absence of the mutation in a Watson strand of the target region;~~

~~——— F) sequencing the target region amplified from the DNA library of Crick strands to generate a sequencing read and detect the presence or absence of the mutation in a Crick strand of the target region;~~

~~——— G) grouping the sequencing reads by the molecular barcode present in each sequencing read to determining whether the mutation is present on both strands of the double stranded DNA template.~~

~~55. ——— The method of claim 54, wherein the double stranded DNA template is a genomic DNA sample and generating the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment comprises:~~

~~——— i) ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template, wherein the 3' duplex adapter comprises a) a first oligonucleotide comprising a 5' phosphate, a first molecular barcode, and a 3' oligonucleotide annealed to b) a second oligonucleotide comprising a degradable 3' blocking group, wherein the 3' oligonucleotide and the second oligonucleotide sequence are complementary;~~

~~——— ii) degrading the degradable 3' blocking group;~~

~~——— iii) ligating a 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template, wherein the 5' duplex adapter comprises an oligonucleotide comprising a second molecule barcode, wherein the second molecular barcode is different from the first molecular barcode, wherein the 5' adapter is~~

ligated onto the double stranded DNA fragment upstream of the first molecular barcode and leaving a gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter; and

——— iv) filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter to generate the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment.

56. — The method of claim 54, wherein the double stranded DNA template is a cell free DNA sample and generating the DNA library of Watson strands and the DNA library of Crick strands from the amplified duplex sequencing library from the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment comprises:

——— i) amplifying the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment using a universal primer pair consisting of a first primer and a second primer, wherein the amplifying comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with the primer pair under whole genome PCR conditions, wherein the first primer is capable of hybridizing to the Watson strand, and wherein the first primer is biotinylated, to generate double stranded amplification products having biotinylated Watson strands;

——— ii) contacting the double stranded amplification products having biotinylated Watson strands with streptavidin functionalized beads under conditions where the biotinylated Watson strands bind to the streptavidin functionalized beads;

——— iii) denaturing the double stranded amplification products having biotinylated Watson strands to leave single stranded biotinylated Watson strands bound to the streptavidin functionalized beads and to release single stranded Crick strands;

——— iv) collecting the single stranded Crick strands;

——— v) releasing the single stranded biotinylated Watson strands from the streptavidin functionalized beads; and

——— vi) collecting the single stranded biotinylated Watson strands.

57. — The method of any one of claims 54-56, wherein the double stranded DNA template is obtained from a sample from a mammal.

~~58. —The method of any one of claims 54-57, wherein the mammal is a human.~~

~~59. —The method of any one of claims 54-58, wherein the method further comprises, prior to the generating a double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment:~~

~~—— fragmenting the double stranded DNA to generate double stranded DNA fragments;~~

~~—— dephosphorylating 5' ends of the double stranded DNA fragments; and~~

~~—— blunting the ends of double stranded DNA fragments.~~

~~60. —The method of any one of claims 54-59, wherein ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 3' duplex adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase.~~

~~61. —The method of claim 60, wherein the ligase is a T4 DNA ligase.~~

~~62. —The method of any one of claims 54-61, wherein degrading the degradable 3' blocking group comprises contacting the 3' duplex adapter with uracil DNA glycosylase (UDG).~~

~~63. —The method of any one of claims 54-62, wherein ligating the 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 5' adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase.~~

~~64. —The method of claim 63, wherein the ligase is an *Escherichia coli* ligase.~~

~~65. —The method of any one of claims 54-64, wherein filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter comprises contacting the 5' end of the double stranded DNA fragment and the 5' adapter in the presence of a polymerase and dNTPs.~~

66. — ~~The method of claim 65, wherein the polymerase is a Taq B polymerase.~~

67. — ~~The method of any one of claims 54-66, wherein ligating the 5' adapter to each 5' end of the double stranded DNA fragment, and the filling in the gap between the 5' end of the double stranded DNA fragment and the 5' adapter are performed concurrently.~~

68. — ~~The method of any one of claims 54-67, wherein amplifying the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with the primer pair under PCR conditions.~~

69. — ~~The method of claim 68, wherein amplifying comprises whole-genome PCR.~~

70. — ~~The method of any one of claims 54-69, wherein amplifying the target region from the DNA library of Watson strands further comprises second amplification using a second primer pair comprising a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter; and wherein the amplifying the target region from the DNA library of Crick strands further comprises a second amplification using a second primer pair comprising a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter.~~

71. — ~~The method of any one of claims 54-70, wherein the sequencing steps comprise paired-end sequencing or single-end sequencing.~~

72. — ~~A method for detecting the presence or absence of a mutation in a target region of a double stranded DNA template obtained from a sample from a mammal, and determining whether the mutation is present on both strands of the double stranded DNA template, wherein the method comprises:~~

— ~~A) generating double stranded DNA fragments each having a duplex molecular barcode on each end of the double stranded DNA fragment;~~

— ~~B) amplifying the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment using a universal primer pair;~~

~~wherein the amplifying comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with the primer pair under whole-genome PCR conditions;~~

~~———C) amplifying the target region from a Watson strand of the amplified double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 3' duplex adapter;~~

~~———D) amplifying the target region from a Crick strand of the amplified double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment using a primer pair consisting of a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter;~~

~~———E) sequencing the target region amplified from the Watson strand to generate a sequencing read and to detect the presence or absence of the mutation in a Watson strand of the target region;~~

~~———F) sequencing the target region amplified from the Crick strand to generate a sequencing read and detect the presence or absence of the mutation in a Crick strand of the target region;~~

~~———G) grouping the sequencing reads by the molecular barcode present in each sequencing read to determining whether the mutation is present on both strands of the double stranded DNA template.~~

73. — The method of claim 72, wherein the double stranded DNA template is a genomic DNA sample and generating the double stranded DNA fragments each having the duplex molecular barcode on each end of the double stranded DNA fragment comprises:

~~———i) ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template, wherein the 3' duplex adapter comprises a) a first oligonucleotide comprising a 5' phosphate, a first molecular barcode, and a 3' oligonucleotide annealed to b) a second oligonucleotide comprising a degradable 3' blocking group, wherein the 3' oligonucleotide and the second oligonucleotide sequence are complementary;~~

~~———ii) degrading the degradable 3' blocking group;~~

~~— iii) ligating a 5' adapter to each dephosphorylated 5' end of the double stranded DNA fragment obtained from the double stranded DNA template, wherein the 5' duplex adapter comprises an oligonucleotide comprising a second molecule barcode, wherein the second molecular barcode is different from the first molecular barcode, wherein the 5' adapter is ligated onto the double stranded DNA fragment upstream of the first molecular barcode and leaving a gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter; and~~

~~— iv) filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter to generate the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment.~~

~~74. — The method of claim 73, wherein the double stranded DNA template is a cell free DNA sample.~~

~~75. — The method of any one of claims 72-74, wherein the double stranded DNA template is a genomic DNA sample.~~

~~76. — The method of any one of claims 72-75, wherein the mammal is a human.~~

~~77. — The method of any one of claims 72-76, wherein the method further comprises, prior to the generating a double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment:~~

~~— fragmenting the double stranded DNA to generate double stranded DNA fragments;~~

~~— dephosphorylating 5' ends of the double stranded DNA fragments; and~~

~~— blunting the ends of double stranded DNA fragments.~~

~~78. — The method of any one of claims 72-77, wherein ligating a 3' duplex adapter to each 3' end of a double stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 3' duplex adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase.~~

~~79. — The method of claim 50, wherein the ligase is a T4 DNA ligase.~~

80. — ~~The method of any one of claims 72-79, wherein degrading the degradable 3' blocking group comprises contacting the 3' duplex adapter with uracil-DNA glycosylase (UDG).~~

81. — ~~The method of any one of claims 72-80, wherein ligating the 5' adapter to each dephosphorylated 5' end of the double-stranded DNA fragment obtained from the double stranded DNA template comprises contacting the 5' adapter and the double stranded DNA fragment obtained from the double stranded DNA template in the presence of a ligase.~~

82. — ~~The method of claim 81, wherein the ligase is an *Escherichia coli* ligase.~~

83. — ~~The method of any one of claims 72-82, wherein filling in the gap of single stranded nucleic acid between the 5' end of the double stranded DNA fragment and the 5' adapter comprises contacting the 5' end of the double stranded DNA fragment and the 5' adapter in the presence of a DNA polymerase and dNTPs.~~

84. — ~~The method of claim 83, wherein the DNA polymerase is a Taq-B polymerase.~~

85. — ~~The method of any one of claims 72-84, wherein ligating the 5' adapter to each 5' end of the double stranded DNA fragment, and the filling in the gap between the 5' end of the double stranded DNA fragment and the 5' adapter are performed concurrently.~~

86. — ~~The method of any one of claims 72-85, wherein amplifying the double stranded DNA fragment having the duplex molecular barcode on each end of the double stranded DNA fragment comprises contacting the double stranded DNA fragment comprising the duplex molecular barcode on each end of the double stranded DNA fragment with the primer pair under PCR conditions.~~

87. — ~~The method of claim 86, wherein amplifying comprises whole-genome PCR.~~

88. — ~~The method of any one of claims 72-87, wherein amplifying the target region from the DNA library of Watson strands further comprises second amplification using a second primer pair comprising a first primer capable of hybridizing to the target region and a second~~

~~primer capable of hybridizing to the 3' duplex adapter; and wherein the amplifying the target region from the DNA library of Crick strands further comprises a second amplification using a second primer pair comprising a first primer capable of hybridizing to the target region and a second primer capable of hybridizing to the 5' adapter.~~

~~89. The method of any one of claims 72-88, wherein the sequencing steps comprise paired-end sequencing.~~

~~90. A method, comprising~~

- ~~— a. attaching partially double-stranded 3' adaptors to 3' ends of both Watson and Crick strands of a population of double-stranded DNA fragments in an analyte DNA sample, wherein a first strand of the partially double-stranded 3' adaptor comprises, in the 5'–3' direction, (i) a first segment, (ii) an exogenous UID sequence, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and wherein the second strand of the partially double-stranded 3' adaptor comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group, optionally wherein the second strand is degradable;~~
- ~~— b. annealing 5' adaptors to the 3' adaptors via the annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor;~~
- ~~— c. performing a nick translation like reaction to extend the 5' adaptors across the exogenous UID sequence of the 3' adaptors and covalently link the extended 5' adaptor to the 5' ends of the Watson and Crick strands of the double-stranded DNA fragments;~~
- ~~— d. performing an initial amplification to amplify the adaptor-ligated, double-stranded DNA fragments to produce amplicons;~~
- ~~— e. determining sequence reads of one or more amplicons of the one or more of the adaptor-ligated, double-stranded DNA fragments;~~

- ~~—f. — assigning the sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence;~~
- ~~—g. — assigning sequence reads of each UID family into a Watson subfamily and Crick subfamily based on spatial relationship of the exogenous UID sequence to the R1 and R2 read sequence;~~
- ~~—h. — identifying a nucleotide sequence as accurately representing a Watson strand of an analyte DNA fragment when a threshold percentage of members of the Watson subfamily contain the sequence;~~
- ~~—i. — identifying a nucleotide sequence as accurately representing a Crick strand of an analyte DNA fragment when a threshold percentage of members of the Crick subfamily contain the sequence;~~
- ~~—j. — identifying a mutation in the nucleotide sequence accurately representing the Watson Strand when the sequence accurately representing the Watson Strand differs from a reference sequence that lacks the mutation;~~
- ~~—k. — identifying a mutation in the nucleotide sequence accurately representing the Crick Strand when the sequence accurately representing the Crick Strand differs from a reference sequence that lacks the mutation; and~~
- ~~—l. — identifying a mutation in the analyte DNA fragment when the mutation in the nucleotide sequence accurately representing the Watson strand and the mutation in the nucleotide sequence accurately representing the Crick strand are the same mutation.~~

~~91. The method of claim 90, wherein each member of a UID family further comprises the same endogenous UID sequence, wherein the endogenous UID sequence comprises an end of a double stranded DNA fragment from the population.~~

~~92. The method of claim 91, wherein the endogenous UID sequence comprising the end of the double stranded DNA fragment comprises at least 8, 10, or 15 bases.~~

~~93. The method of any one of claims 90-92, wherein the exogenous UID sequence is unique to each double stranded DNA fragment.~~

94. The method of any one of claims 90-92, wherein the exogenous UID sequence is not unique to each double-stranded DNA fragment.
95. The method of any one of claims 91-94, wherein each member of a UID family comprises the same endogenous UID sequence and the same exogenous UID sequence.
96. The method of any one of the preceding claims, wherein step (d) comprises no more than 11 cycles of PCR amplification.
97. The method of claim 96, wherein step (d) comprises no more than 7 cycles of PCR amplification.
98. The method of claim 97, wherein step (d) comprises no more than 5 cycles of PCR amplification.
99. The method of any one of the preceding claims, wherein step (d) comprises at least 1 cycles of PCR amplification.
100. The method of any one of the preceding claims, wherein, prior to determining the sequence reads, the amplicons are enriched for one or more target polynucleotides.
101. The method of claim 100, wherein the enriching comprises:
- a. — selectively amplifying amplicons of Watson strands comprising the target polynucleotide sequence with a first set of Watson target-selective primer pairs, the first set of Watson target-selective primer pairs comprising:
 - (i) a first Watson target-selective primer comprising a sequence complementary to a portion of the universal 3' adapter sequence, optionally wherein the portion of the universal 3' adapter sequence is the R2-sequencing primer site of the universal 3' adaptor sequence, and
 - (ii) a second Watson target-selective primer comprising a target-selective sequence, thereby creating target Watson amplification products; and
 - b. — selectively amplifying amplicons of Crick strands comprising the same target polynucleotide sequence with a first set of Crick target-selective primer

pairs, the first set of Crick target selective primer pairs comprising: (i) a first Crick target selective primer comprising a sequence complementary to a portion of the universal 5' adaptor sequence, optionally wherein the portion of the universal 5' adaptor sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) a second Crick target selective primer comprising the same target selective sequence as the second Watson target selective primer sequence, thereby creating target Crick amplification products.

102. — The method of claim 101, comprising purifying the target Watson amplification products and the target Crick amplification products from non-target polynucleotides.

103. — The method of claim 102, wherein the purifying comprises attaching the target Watson amplification products and the target Crick amplification products to a solid support.

104. — The method of claim 103, wherein the first Watson target selective primer and first Crick target selective primer comprises a first member of an affinity binding pair, and wherein the solid support comprises a second member of the affinity binding pair.

105. — The method of claim 104, wherein the first member is biotin and the second member is streptavidin.

106. — The method of any one of claims 102-105, wherein the solid support comprises a bead, well, membrane, tube, column, plate, sepharose, magnetic bead, or chip.

107. — The method of any one of claims 102-106, comprising removing polynucleotides that are not attached to the solid support.

108. — The method of any one of claims 101-107, comprising:

- a. — further amplifying the target Watson amplification products with a second set of Watson target selective primers, the second set of Watson target selective primers comprising (i) a third Watson target selective primer

comprising a sequence complementary to a portion of the universal 3' adapter sequence, optionally wherein the portion of the universal 3' adapter sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) a fourth Watson target selective primer comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target selective sequence selective for the same target polynucleotide, thereby creating target Watson library members;

— b. — further amplifying the target Crick amplification products with a second set of Crick target selective primers, the second set of Crick target selective primers comprising (i) a third Crick target selective primer comprising a sequence complementary to a portion of the universal 5' adapter sequence, optionally wherein the portion of the universal 5' adapter sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) a fourth Crick target selective primer comprising, in the 5' to 3' direction, an R2 sequencing primer site and the target selective sequence selective for the same target polynucleotide of the fourth Watson target selective primer, thereby creating target Crick library members.

109. — The method of claim 108, wherein the third Watson and Crick target selective primers further comprise a sample barcode sequence.

110. — The method of claim 108 or 109, wherein the third Watson target selective primer further comprises a first grafting sequence that enables hybridization to a first grafting primer on a sequencer and wherein the third Crick target selective primer further comprises a second grafting sequence that enables hybridization to a second grafting primer on the sequencer.

111. — The method of any one of claims 108–110, wherein the fourth Watson target selective primer further comprises the second grafting sequence and wherein the fourth Crick target selective primer further comprises the first grafting sequence.

112. — The method of claim 110 or 111, wherein the first grafting sequence is a P7 sequence and wherein the second grafting sequence is a P5 sequence.

- ~~113.—The method of any one of claims 101–112, wherein the target Watson library members and the target Crick library members represent at least 50% of the target polynucleotides in the population of double-stranded DNA fragments.~~
- ~~114.—The method of claim 113, wherein the target Watson library members and the target Crick library members represent at least 70% of the target polynucleotides in the population of double-stranded DNA fragments.~~
- ~~115.—The method of claim 114, wherein the target Watson library members and the target Crick library members represent at least 80% of the target polynucleotides in the population of double-stranded DNA fragments.~~
- ~~116.—The method of claim 115, wherein the target Watson library members and the target Crick library members represent at least 90% of the target polynucleotides in the population of double-stranded DNA fragments.~~
- ~~117.—The method of any one of claims 101–112, wherein the target Watson library members and the target Crick library members represent at least 50% of the population of total DNA fragments.~~
- ~~118.—The method of claim 117, wherein the target Watson library members and the target Crick library members represent at least 70% of the population of total DNA fragments.~~
- ~~119.—The method of claim 118, wherein the target Watson library members and the target Crick library members represent at least 80% of the population of total DNA fragments.~~
- ~~120.—The method of claim 119, wherein the target Watson library members and the target Crick library members represent at least 90% of the population of total DNA fragments.~~
- ~~121.—A method, comprising:~~
- ~~—a. —attaching adaptors to a population of double-stranded DNA fragments in an analyte DNA sample, wherein the adaptors comprise a double-stranded portion comprising an exogenous UID and a forked portion comprising (i) a single-stranded 3' adaptor sequence comprising an R2 sequencing primer site~~

- and (ii) a single stranded 5' adaptor sequence comprising an R1 sequencing primer site;
- b. — performing an initial amplification to amplify the adaptor ligated, double-stranded DNA fragments to produce amplicons;
 - c. — selectively amplifying amplicons of Watson strands comprising a target polynucleotide sequence with a first set of Watson target selective primer pairs, the first set of Watson target selective primer pairs comprising:
 - (i) a first Watson target selective primer comprising a sequence complementary to a portion of the universal 3' adaptor sequence, optionally wherein the portion of the universal 3' adaptor sequence is the R2 sequencing primer site of the universal 3' adaptor sequence, and (ii) a second Watson target selective primer comprising a target selective sequence, thereby creating target Watson amplification products;
 - d. — selectively amplifying amplicons of Crick strands comprising the same target polynucleotide sequence with a first set of Crick target selective primer pairs, the first set of Crick target selective primer pairs comprising: a first Crick target selective primer comprising a sequence a sequence complementary to a portion of the universal 5' adaptor sequence, optionally wherein the portion of the universal 5' adaptor sequence is the R1 sequencing primer site of the universal 5' adaptor sequence, and (ii) a second Crick target selective primer comprising the same target selective sequence as the second Crick target selective primer sequence, thereby creating target Crick amplification products.
 - e. — determining sequence reads of the target Watson amplification products and the target Crick amplification products;
 - f. — assigning the sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence;
 - g. — assigning sequence reads of each UID family into a Watson subfamily and Crick subfamily based on spatial relationship of the exogenous UID sequence to the R1 and R2 read sequence;
 - h. — identifying a nucleotide sequence as accurately representing a Watson strand of an analyte DNA fragment when a threshold percentage of members of the Watson family contain the sequence;

- ~~—i. — identifying a nucleotide sequence as accurately representing a Crick strand of an analyte DNA fragment when a threshold percentage of members of the Crick family contain the sequence; and~~
- ~~—j. — identifying a mutation in the analyte DNA fragment when the nucleotide sequence accurately representing the Watson strand and the nucleotide sequence accurately representing the Crick strand both contain the same mutation.~~

~~122. — The method of claim 121, comprising purifying the target Watson amplification products and the target Crick amplification products from non-target polynucleotides.~~

~~123. — The method of claim 122, wherein the purifying comprises attaching the target Watson amplification products and the target Crick amplification products to a solid support.~~

~~124. — The method of claim 123, wherein the first Watson target-selective primer and first Crick target-selective primer comprises a first member of an affinity binding pair, and wherein the solid support comprises a second member of the affinity binding pair.~~

~~125. — The method of claim 124, wherein the first member is biotin and the second member is streptavidin.~~

~~126. — The method of any one of claims 122–125, wherein the solid support comprises a bead, well, membrane, tube, column, plate, sepharose, magnetic bead, or chip.~~

~~127. — The method of any one of claims 122–126, comprising removing polynucleotides that are not attached to the solid support.~~

~~128. — The method of any one of claims 121–127, comprising:~~

- ~~—a. — further amplifying the target Watson amplification products with a second set of Watson target-selective primers, the second set of Watson target-selective primers comprising (i) a third Watson target-selective primer comprising a sequence complementary to the R2-sequencing primer site of the universal 3' adaptor sequence, and (ii) a fourth Watson target-selective primer~~

comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target selective sequence selective for the same target polynucleotide, thereby creating target Watson library members;

- b. — further amplifying the target Crick amplification products with a second set of Crick target selective primers, the second set of Crick target selective primers comprising (i) a third Crick target selective primer comprising a sequence complementary to the R1 sequencing primer site of the universal 3' adaptor sequence, and (ii) a fourth Crick target selective primer comprising, in the 5' to 3' direction, an R2 sequencing primer site and the target selective sequence selective for the same target polynucleotide of the fourth Watson target selective primer, thereby creating target Crick library members, thereby creating target Crick library members.

129. — The method of claim 128, wherein the third Watson and Crick target selective primers further comprise a sample barcode sequence.

130. — The method of claim 128 or 129, wherein the third Watson target selective primer further comprises a first grafting sequence that enables hybridization to a first grafting primer on a sequencer and wherein the third Crick target selective primer further comprises a second grafting sequence that enables hybridization to a second grafting primer on the sequencer.

131. — The method of any one of claims 128-130, wherein the fourth Watson target selective primer further comprises the second grafting sequence and wherein the fourth Crick target selective primer further comprises the first grafting sequence.

132. — The method of claim 130 or 131, wherein the first grafting sequence is a P7 sequence and wherein the second grafting sequence is a P5 sequence.

133. — The method of any one of claims 121-131, wherein the attaching comprises attaching A-tailed adaptors to the population of double stranded DNA fragments.

134. ~~The method of claim 133, wherein the attaching comprises attaching A tailed adaptors to both ends of DNA fragments in the population.~~
135. ~~The method of any one of claims 121–131, wherein the attaching comprises:~~
- ~~— a. — attaching partially double stranded 3' adaptors to 3' ends of both Watson and Crick strands of a population of double stranded DNA fragments, wherein a first strand of the partially double stranded 3' adaptor comprises, in the 5'–3' direction, (i) a first segment, (ii) optionally, an exogenous UID sequence, (iii) an annealing site for a 5' adaptor, and (iv) a universal 3' adaptor sequence comprising an R2 sequencing primer site, and wherein the second strand of the partially double stranded 3' adaptor comprises, in the 5' to 3' direction, (i) a segment complementary to the first segment, and (ii) a 3' blocking group, optionally wherein the second strand is degradable; and~~
 - ~~— b. — annealing 5' adaptors to the 3' adaptors via the annealing site, wherein the 5' adaptors comprise, in the 5' to 3' direction, (i) a universal 5' adaptor sequence that is not complementary to the universal 3' adaptor sequence and that comprises an R1 sequencing primer site, and (ii) a sequence complementary to the annealing site for the 5' adaptor; and~~
 - ~~— c. — performing a nick translation like reaction to extend the 5' adaptors across the 3' adaptors and covalently link the extended 5' adaptor to the 5' ends of the Watson and Crick strands of the double stranded DNA fragments.~~
136. ~~The method of any one of claims 121–135, wherein the UID sequence comprises an endogenous UID sequence comprising an end of a double stranded DNA fragment from the population.~~
137. ~~The method of claim 136, wherein the endogenous UID sequence comprising the end of the double stranded DNA fragment comprises at least 8, 10, or 15 bases.~~
138. ~~The method of any one of claims 121–136, wherein the exogenous UID sequence is unique to each double stranded DNA fragment.~~
139. ~~The method of any one of claims 121–136, wherein the exogenous UID sequence is not unique to each double stranded DNA fragment.~~

- ~~140.—The method of any one of claims 136–139, wherein each member of a UID family comprises the same endogenous UID sequence and the same exogenous UID sequence.~~
- ~~141.—The method of any one of claims 121–140, wherein the amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises no more than 11 cycles of PCR amplification.~~
- ~~142.—The method of claim 141, wherein the amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises no more than 7 cycles of PCR amplification.~~
- ~~143.—The method of claim 142, wherein the amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises no more than 5 cycles of PCR amplification.~~
- ~~144.—The method of any one of the preceding claims, wherein the amplifying the adaptor-ligated, double-stranded DNA fragments to produce amplicons comprises at least 1 cycles of PCR amplification.~~
- ~~145.—The method of any one of claims 121–143, wherein the target Watson library members and the target Crick library members represent at least 50% of the target polynucleotides in the population of double-stranded DNA fragments.~~
- ~~146.—The method of claim 145, wherein the target Watson library members and the target Crick library members represent at least 70% of the target polynucleotides in the population of double-stranded DNA fragments.~~
- ~~147.—The method of claim 146, wherein the target Watson library members and the target Crick library members represent at least 80% of the target polynucleotides in the population of double-stranded DNA fragments.~~

- ~~148.—The method of claim 147, wherein the target Watson library members and the target Crick library members represent at least 90% of the target polynucleotides in the population of double-stranded DNA fragments.~~
- ~~149.—The method of any one of claims 121–143, wherein the target Watson library members and the target Crick library members represent at least 50% of the population of total DNA fragments.~~
- ~~150.—The method of claim 149, wherein the target Watson library members and the target Crick library members represent at least 70% of the population of total DNA fragments.~~
- ~~151.—The method of claim 150, wherein the target Watson library members and the target Crick library members represent at least 80% of the population of total DNA fragments.~~
- ~~152.—The method of claim 151, wherein the target Watson library members and the target Crick library members represent at least 90% of the population of total DNA fragments.~~
- ~~153.—The method of any one of the preceding claims, wherein the determination of the sequence reads enables sequence determination of both ends of template molecules.~~
- ~~154.—The method of claim 153, wherein the determination of both ends of template molecules comprises paired-end sequencing.~~
- ~~155.—The method of any one of the preceding claims, wherein the determination of the sequence reads comprises single read sequencing across the length of the templates for generating the sequence reads.~~
- ~~156.—The method of any one of the preceding claims, wherein the determination of the sequence reads comprises sequencing with a massively parallel sequencer.~~
- ~~157.—The method of claim 156, wherein the massively parallel sequencer is configured to determine sequence reads from both ends of template polynucleotides.~~

~~158.—The method of any one of the preceding claims, wherein the population of double-stranded DNA fragments comprises one or more fragments that are about 50–600 nt in length.~~

~~159.—The method of any one of the preceding claims, wherein the population of double-stranded DNA fragments comprises one or more fragments that are less than 2000, less than 1000, less than 500, less than 400, less than 300, or less than 250 nt in length.~~

~~160.—The method of any one of claims 101–159, further comprising, after the initial amplification and before the selective amplification, preparing single stranded (ss) DNA libraries corresponding to sense and antisense strands of the amplicons.~~

~~161.—The method of claim 160, wherein the ss DNA library preparation comprises:~~

- ~~—a. — performing an amplification reaction utilizing two primers, wherein only one of the two primers comprises a first member of an affinity binding pair, thereby creating amplification products comprising a strand comprising the first member of the affinity binding pair and a strand not comprising the first member of the affinity binding pair;~~
- ~~—b. — contacting the amplification products with a solid support, wherein the solid support comprises the second member of the affinity binding pair;~~
- ~~—c. — denaturing the amplification products to separate the strand comprising the first member of the affinity binding pair from the strand not comprising the first member of the affinity binding pair; and~~
- ~~—d. — purifying the separated strands comprising the first member of the affinity binding pair and the separated strands not comprising the first member of the affinity binding pair.~~

~~—~~

~~162.—The method of claim 161, wherein the first member of the affinity binding pair is biotin and the second member of the affinity binding pair is streptavidin.~~

~~163.—The method of claim 160, wherein the ss DNA library preparation comprises:~~

- ~~—a. — partitioning the amplicons into two amplification reactions, each amplification reaction utilizing a forward primer and a reverse primer;~~

~~wherein only one of the two primers is phosphorylated, thereby creating amplification products comprising a phosphorylated strand and a non-phosphorylated strand;~~

- ~~— b. — contacting the amplification products with an exonuclease that selectively digests the strands with the 5' phosphate.~~

~~—~~

~~164. — The method of claim 163, wherein:~~

- ~~— a. — in the first amplification reaction, the forward primer is phosphorylated and the reverse primer is non-phosphorylated;~~
- ~~— b. — in the second amplification reaction, the reverse primer is phosphorylated and the forward primer is non-phosphorylated.~~

~~—~~

~~165. — The method of claim 163, wherein the exonuclease is a lambda-exonuclease.~~

~~166. — The method of any one of claims 163–165, wherein the phosphorylation is at the 5' site.~~

~~167. — The method of any one of claims 90–153, wherein the initial amplification comprises:~~

- ~~— a. — amplifying with a primer pair, in which only one of the two primers in the primer pair comprises a first member of an affinity binding pair, thereby creating amplification products comprising a strand comprising the first member of the affinity binding pair and a strand not comprising the first member of the affinity binding pair;~~
- ~~— b. — contacting the amplification products with a solid support, wherein the solid support comprises the second member of the affinity binding pair;~~
- ~~— c. — denaturing the amplification products to separate the strand comprising the first member of the affinity binding pair from the strand not comprising the first member of the affinity binding pair; and~~
- ~~— d. — purifying the separated strands comprising the first member of the affinity binding pair and the separated strands not comprising the first member of the affinity binding pair.~~

~~—~~

- 168.—~~The method of claim 167, wherein the first member of the affinity binding pair is biotin and the second member of the affinity binding pair is streptavidin.~~
- 169.—~~The method of any one of the preceding claims, wherein sequence reads of a UID family are assigned to a Watson subfamily when the exogenous UID sequence is downstream of the R2 sequence and upstream of the R1 sequence.~~
- 170.—~~The method of any one of the preceding claims, wherein sequence reads of a UID family are assigned to a Crick subfamily when the exogenous UID sequence is downstream of the R1 sequence and upstream of the R2 sequence.~~
- 171.—~~The method of any one of the preceding claims, wherein sequence reads of a UID family are assigned to a Watson subfamily when the exogenous UID sequence is in greater proximity to the R2 sequence and lesser proximity to the R1 sequence.~~
- 172.—~~The method of any one of the preceding claims, wherein sequence reads of a UID family are assigned to a Crick subfamily when the exogenous UID sequence is in greater proximity to the R1 sequence and in lesser proximity to the R2 sequence.~~
- 173.—~~The method of any one of the preceding claims, wherein sequence reads of a UID family are assigned to a Watson subfamily when the exogenous UID sequence is immediately downstream or within 1-300, 1-70, 1-60, 1-50, 1-40, 1-30, 1-20, 1-10, or 1-5 nucleotides of the R2 sequence.~~
- 174.—~~The method of any one of the preceding claims, wherein sequence reads of a UID family are assigned to a Crick subfamily when the exogenous UID sequence is immediately downstream or within 1-300, 1-70, 1-60, 1-50, 1-40, 1-30, 1-20, 1-10, or 1-5 nucleotides of the R1 sequence.~~
- 175.—~~The method of any one of the preceding claims, wherein the population of double-stranded DNA fragments are from a biological sample.~~
- 176.—~~The method of claim 175, wherein the biological sample is obtained from a subject.~~

- ~~177.—The method of claim 176, wherein the subject is a human subject.~~
- ~~178.—The method of claim of any one of claims 175–177, wherein the biological sample is a fluid sample.~~
- ~~179.—The method of claim 178, wherein the fluid sample is selected from whole blood, plasma, serum sputum, urine, sweat, tears, ascites, semen, and bronchoalveolar lavage.~~
- ~~180.—The method of claim 178, wherein the fluid sample is a cell-free or essentially cell-free sample.~~
- ~~181.—The method of any one of claims 175–177, wherein the biological sample is a solid biological sample.~~
- ~~182.—The method of claim 181, wherein the solid biological sample is a tumor sample.~~
- ~~183.—The method of any one of the preceding claims, wherein the identified mutation is present in the population of double-stranded DNA fragments at a frequency of 0.1% or less.~~
- ~~184.—The method of claim 183, wherein the identified mutation is present in the population of double-stranded DNA fragments at a frequency of 0.1% to 0.00001%.~~
- ~~185.—The method of claim 183, wherein the identified mutation is present in the population of double-stranded DNA fragments at a frequency of 0.1% to 0.01%.~~
- ~~186.—The method of any one of the preceding claims, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 50% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample.~~

~~187.—The method of claim 186, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 70% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample.~~

~~188.—The method of claim 187, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 80% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample.~~

~~189.—The method of claim 188, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 90% of the double-stranded DNA fragments comprising a target polynucleotide in the analyte DNA sample.~~

~~190.—The method of any one of the preceding claims, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 50% of the double-stranded DNA fragments in the analyte DNA sample.~~

~~191.—The method of any one of the preceding claims, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 70% of the double-stranded DNA fragments in the analyte DNA sample.~~

~~192.—The method of any one of the preceding claims, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 80% of the double-stranded DNA fragments in the analyte DNA sample.~~

~~193.—The method of any one of the preceding claims, wherein the determining of sequence reads comprises determining sequence reads from both Watson and Crick strands of at least 90% of the double-stranded DNA fragments in the analyte DNA sample.~~

~~194.—The method of any one of the preceding claims, wherein the error rate associated with the identification of one or more mutations in analyte DNA fragments according to a~~

method of any one of the preceding claims is reduced by at least 2 fold, 4 fold, 5 fold, 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as compared to an alternative method of identifying mutations that does not require the mutation to be detected in both Watson and Crick strands of an analyte DNA fragment.

195.—The method of claim 194, wherein the alternative method comprises standard molecular barcoding or standard PCR-based molecular barcoding.

196.—The method of claim 195, wherein the alternative method comprises:

- a.—attaching adaptors to a population of double-stranded DNA fragments in an analyte DNA sample, wherein the adaptors comprise a unique exogenous UID;
- b.—performing an initial amplification to amplify the adaptor-ligated, double-stranded DNA fragments to produce amplicons;
- c.—determining sequence reads of one or more amplicons of the one or more of the adaptor-ligated, double-stranded DNA fragments;
- d.—assigning the sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence;
- e.—identifying a nucleotide sequence as accurately representing an analyte DNA fragment when a threshold percentage of members of a UID family contain the sequence; and
- f.—identifying a mutation in the analyte DNA fragment when the sequence identified as accurately representing an analyte DNA fragment differs from a reference sequence that lacks the mutation.

197.—The method of any one of the preceding claims, wherein the error rate associated with the identification of one or more mutations in analyte DNA fragments according to a method of any one of the preceding claims is no more than 1×10^{-2} , no more than 1×10^{-3} , no more than 1×10^{-4} , no more than 1×10^{-5} , no more than 1×10^{-6} , no more than 5×10^{-6} , or no more than 1×10^{-7} .

1898. A computer readable medium comprising computer executable instructions for analyzing sequence read data from a nucleic acid sample, wherein the data is generated by a method of any one of the preceding claims.

1999. The computer readable medium of claim 18-198, comprising executable instructions for

- a. assigning sequence reads into UID families, wherein each member of a UID family comprises the same exogenous UID sequence;
- b. assigning sequence reads of each UID family into Watson and Crick subfamilies based on spatial relationship of the exogenous UID sequence to the R1 and R2 read sequence;
- c. identifying a nucleotide sequence as accurately representing a Watson strand of an analyte DNA fragment when a threshold percentage of members of the Watson subfamily contain the sequence;
- d. identifying a nucleotide sequence as accurately representing a Crick strand of an analyte DNA fragment when a threshold percentage of members of the Crick subfamily contain the sequence;
- e. identifying a mutation in the nucleotide sequence accurately representing the Watson Strand when the sequence accurately representing the Watson Strand differs from a reference sequence that lacks the mutation;
- f. identifying a mutation in the nucleotide sequence accurately representing the Crick Strand when the sequence accurately representing the Crick Strand differs from a reference sequence that lacks the mutation;
- g. identifying a mutation in the analyte DNA fragment when the mutation in the nucleotide sequence accurately representing the Watson strand and the mutation in the nucleotide sequence accurately representing the Crick strand are the same mutation.

~~200. The computer readable medium of claim 199, comprising assigning UID family members to the Watson subfamily when the exogenous UID sequence is immediately downstream or within 1-300 nucleotides of the R2 sequencing primer binding site.~~

- ~~201. The computer readable medium of any one of the preceding claims, comprising assigning UID family members to the Crick subfamily when the exogenous UID sequence is immediately downstream or within 1-300 nucleotides of the R1 sequencing primer binding site.~~
- ~~202. The computer readable medium of any one of the preceding claims, comprising mapping the sequence reads to a reference genome.~~
- ~~203. The computer readable medium of claim 202, wherein the reference genome is a human reference genome.~~
- ~~204. The computer readable medium of any one of the preceding claims, further comprising computer executable instructions to generate a report of therapeutic options based on the presence, absence, or amount of a mutation in the sample.~~
- ~~205. The computer readable medium of any one of the preceding claims, further comprising computer executable code that enables transmission of the data over a network.~~
- ~~206. A computer system, comprising:~~
- ~~— a. a memory unit configured to receive sequence data from a nucleic acid sample, wherein the data is generated by a method of any one of the preceding claims;~~
 - ~~— b. a processor communicatively coupled to the memory unit, wherein the processor comprises a computer readable medium of any of the preceding claims.~~
 - ~~—~~
- ~~207. The computer system of claim 206, further comprising a sequencing system configured to communicate the data to the memory unit.~~
- ~~208. The computer system of any one of the preceding claims, further comprising a user interface configured to communicate or display the report to a user.~~

~~209. The computer system of any one of the preceding claims, further comprising a digital processor configured to transmit results of the data analysis over a network.~~

~~210. A system, comprising:~~

- ~~— a. a population of double stranded DNA fragments from a biological sample;~~
- ~~— b. a population of 3' adaptors as described in any one of the preceding claims;~~
- ~~— c. a population of 5' adaptors as described in any one of the preceding claims;~~
- ~~— d. reagents for performing nick translation like reaction;~~
- ~~— e. reagents for enriching amplicons for one or more target polynucleotides; and~~
- ~~— f. a sequencing system.~~

~~211. The system of claim 210, further comprising a computer system of any one of the preceding claims.~~

2012. A kit, comprising:

- a. a first set of Watson target-selective primer pairs, comprising (i) one or more first Watson target-selective primers comprising a sequence complementary to a portion of the universal 3' adapter sequence, optionally wherein the portion of the universal 3' adapter sequence is the R2 sequencing primer site of the universal 3' adapter sequence, and (ii) one or more second Watson target-selective primers, each of the one or more second Watson target-selective primers comprising a target-selective sequence;
- b. a first set of Crick target-selective primer pairs, comprising (i) one or more Crick target-selective primers comprising a sequence a sequence complementary to a portion of the universal 5' adapter sequence, optionally wherein the portion of the universal 5' adapter sequence is the R1 sequencing primer site of the universal 5' adapter sequence, and (ii) one or more second Crick target-selective primers, each of the one or more second Crick target-

selective primers comprising the same target-selective sequence as the second Watson target-selective primer sequence;

- c. a second set of Watson target-selective primer pairs, comprising (i) one or more third Watson target-selective primers comprising a sequence complementary to the R2 sequencing primer site of the universal 3' adaptor sequence and optionally a Watson sample index barcode, and (ii) one or more fourth Watson target-selective primers, each of the one or more fourth Watson target-selective primers comprising, in the 5' to 3' direction, an R1 sequencing primer site and a target-selective sequence selective for the same target polynucleotide; and
- d. a second set of Crick target-selective primers comprising (i) one or more third Crick target-selective primers comprising a sequence complementary to the R1 sequencing primer site of the universal 3' adaptor sequence and optionally a Crick sample index barcode, and (ii) one or more fourth Crick target-selective primers, each of the one or more fourth Crick target-selective primers comprising, in the 5' to 3' direction, an R2 sequencing primer site and a target-selective sequence selective for the same target polynucleotide.