

WHAT IS CLAIMED IS:

- 1 1. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a plurality of nucleic acid molecules, the plurality of nucleic acid
3 molecules being cell-free, each nucleic acid molecule of the plurality of nucleic acid molecules
4 being double-stranded with a first strand having a first portion and a second strand, wherein the
5 first portion of the first strand of at least some of the plurality of nucleic acid molecules has no
6 complementary portion from the second strand, is not hybridized to the second strand, and is at a
7 first end of the first strand, the method comprising:
8 for each nucleic acid molecule of the plurality of nucleic acid molecules:
9 measuring a property of the first strand and/or the second strand that is
10 proportional to a length of the first strand that overhangs the second strand;
11 determining a jagged end value using the measured properties of the plurality of
12 nucleic acid molecules, wherein the jagged end value provides a collective measure that a strand
13 overhangs another strand in the plurality of nucleic acid molecules;
14 comparing the jagged end value to a reference value; and
15 determining a level of a condition of the individual based on the comparison.
- 1 2. The method of claim 1, wherein the condition comprises a disease, a
2 disorder, or a pregnancy.
- 1 3. The method of claim 2, wherein the condition is a cancer, an auto-immune
2 disease, or a pregnancy-related condition.
- 1 4. The method of claim 1, wherein the first end is a 5' end.
- 1 5. The method of any one of claims 1 to 4, further comprising:
2 measuring sizes of nucleic acid molecules, wherein the plurality of nucleic acid
3 molecules has sizes within a specified range.
- 1 6. The method of claim 5, wherein the specified range is 140 to 160 bp.
- 1 7. The method of claim 5, wherein:

2 the plurality of nucleic acid molecules is a first plurality of nucleic acid molecules,
3 and
4 the specified range is a first specified range,
5 the method further comprising:
6 measuring the property of a strand of each nucleic acid molecule of a
7 second plurality of nucleic acid molecules, wherein the second plurality of nucleic acid
8 molecules has sizes with a second specified range,
9 wherein determining the jagged end value comprises calculating a ratio using the
10 measured properties of the first plurality of nucleic acid molecules and the measured properties
11 of the second plurality of nucleic acid molecules.

1 8. The method of any one of claims 1 to 4, wherein the property is a
2 methylation status at one or more sites at end portions of the first strands and/or second strands
3 of each of the plurality of nucleic acid molecules, and wherein the jagged end value includes a
4 methylation level over the plurality of nucleic acid molecules at one or more sites of end portions
5 of the first strands and/or second strands.

1 9. The method of claim 8, wherein a higher methylation level is correlated
2 with a longer length of the first strand that overhangs the second strand.

1 10. The method of any one of claims 1 to 4, further comprising:
2 analyzing nucleic acid molecules to produce reads,
3 aligning the reads to a reference genome,
4 wherein:
5 the plurality of nucleic acid molecules have reads within a certain distance
6 range relative to a transcription start site.

1 11. The method of any one of claims 1 to 10, wherein the measured property
2 is length.

1 12. The method of claim 1, wherein the reference value is determined using
2 one or more reference samples of subjects that have the condition.

1 13. The method of claim 1, wherein the reference value is determined using
2 one or more reference samples of subjects that do not have the condition.

1 14. The method of claim 1, wherein a machine learning model is used to
2 perform the comparing of the jagged end value to the reference value and the determining of the
3 level of the condition of the individual.

1 15. A method of determining a fraction of clinically-relevant DNA in a
2 biological sample obtained from an individual, the biological sample including a plurality of
3 nucleic acid molecules, the plurality of nucleic acid molecules being cell-free, each nucleic acid
4 molecule of the plurality of nucleic acid molecules being double-stranded with a first strand
5 having a first portion and a second strand, wherein the first portion of the first strand of at least
6 some of the plurality of nucleic acid molecules has no complementary portion from the second
7 strand, is not hybridized to the second strand, and is at a first end of the first strand, the method
8 comprising:
9 for each nucleic acid molecule of the plurality of nucleic acid molecules:
10 measuring a property of the first strand and/or the second strand that is
11 proportional to a length of the first strand that overhangs the second strand;
12 determining a jagged end value using the measured properties of the plurality of
13 nucleic acid molecules, wherein the jagged end value provides a collective measure that a strand
14 overhangs another strand in the plurality of nucleic acid molecules;
15 comparing the jagged end value to a reference value; and
16 determining the fraction of clinically-relevant DNA in the biological sample
17 based on the comparison.

1 16. The method of claim 15, further comprising:
2 treating the plurality of nucleic acid molecules by a protocol before measuring the
3 property of the first strand and/or the second strand,
4 wherein:
5 the reference value is obtained using nucleic acid molecules from one or
6 more reference subjects having a known fraction of clinically-relevant DNA, and

7 the nucleic acid molecules from the one or more reference subjects are
8 treated by the protocol.

1 17. The method of claim 15, wherein the clinically-relevant DNA comprises
2 fetal DNA, tumor DNA, or transplant DNA.

1 18. The method of claim 15, wherein the plurality of nucleic acid molecules
2 have sizes within a particular size range.

1 19. The method of claim 15, wherein the reference value is determined from
2 one or more calibration samples having a known fraction of clinically-relevant DNA and whose
3 jagged end value has been measured.

1 20. The method of claim 15, wherein the reference value is determined from a
2 calibration curve that is fit to calibration data points of a plurality of calibration samples, each of
3 the calibration data points including a measured jagged end value and a measured fraction of
4 clinically-relevant DNA of one of the plurality of calibration samples.

1 21. A method of determining an age of an individual using a biological sample
2 obtained from the individual, the biological sample including a plurality of nucleic acid
3 molecules, the plurality of nucleic acid molecules being cell-free, each nucleic acid molecule of
4 the plurality of nucleic acid molecules being double-stranded with a first strand having a first
5 portion and a second strand, wherein the first portion of the first strand of at least some of the
6 plurality of nucleic acid molecules has no complementary portion from the second strand, is not
7 hybridized to the second strand, and is at a first end of the first strand, the method comprising:
8 for each nucleic acid molecule of the plurality of nucleic acid molecules:
9 measuring a property of the first strand and/or the second strand that is
10 proportional to a length of the first strand that overhangs the second strand;
11 determining a jagged end value using the measured properties of the plurality of
12 nucleic acid molecules, wherein the jagged end value provides a collective measure that a strand
13 overhangs another strand in the plurality of nucleic acid molecules;
14 comparing the jagged end value to a reference value; and
15 determining the age of the individual based on the comparison.

1 22. The method of claim 21, wherein the reference value is obtained using
2 nucleic acid molecules from one or more reference subjects having known ages.

1 23. The method of claim 21, wherein the plurality of nucleic acid molecules
2 have sizes within a particular size range.

1 24. The method of claim 21, wherein the reference value is determined from a
2 calibration curve that is fit to calibration data points of a plurality of calibration samples, each of
3 the calibration data points including a measured jagged end value and a known age of a subject
4 from which one of the plurality of calibration samples was obtained.

1 25. A method of analyzing a tissue type by analyzing a biological sample
2 obtained from an individual, the biological sample including a plurality of nucleic acid molecules,
3 the plurality of nucleic acid molecules being cell-free, each nucleic acid molecule of the plurality
4 of nucleic acid molecules being double-stranded with a first strand having a first portion at an
5 end and a second strand, wherein the first portion of the first strand of at least some of the
6 plurality of nucleic acid molecules has no complementary portion from the second strand, is not
7 hybridized to the second strand, and is at a first end of the first strand, the method comprising:
8 for each nucleic acid molecule of the plurality of nucleic acid molecules:
9 measuring a property of the first strand and/or the second strand that is
10 proportional to a length of the first strand that overhangs the second strand,
11 sequencing the nucleic acid molecule to produce one or more reads, and
12 determining a genomic location of the nucleic acid molecule;
13 identifying a set of nucleic acid molecules having genomic locations in open
14 chromatin regions and non-open chromatin regions associated with a first tissue type;
15 for the set of nucleic acid molecules:
16 calculating a first value of a parameter using a first plurality of measured
17 properties of a first plurality of first portions, wherein the first plurality of first portions
18 are from nucleic acid molecules located in the open chromatin regions of the first tissue
19 type,
20 calculating a second value of the parameter using a second plurality of
21 measured properties of a second plurality of first portions, wherein the second plurality of

22 first portions are from nucleic acid molecules located in the non-open chromatin regions
23 of the first tissue type,
24 calculating a separation value between the first value of the parameter and
25 the second value of the parameter,
26 comparing the separation value to a reference value, and
27 determining whether the first tissue type exhibits a cancer based on
28 comparing the separation value to a reference value.

1 26. The method of claim 25, wherein the open chromatin regions include
2 transcription start sites (TSS).

1 27. The method of claim 25, wherein determining the genomic location
2 includes aligning the one or more reads to a reference sequence.

1 28. The method of claim 25, further comprising:
2 retrieving the open chromatin regions and non-open chromatin regions associated
3 with the first tissue type from a database.

1 29. The method of claim 25, wherein the separation value includes a ratio of
2 the first value and the second value.

1 30. The method of claim 25, wherein the reference value is determined using
2 one or more reference samples from one or more reference subjects known to have cancer
3 affecting the first tissue type.

1 31. The method of claim 25, wherein the reference value is determined using
2 one or more reference samples from reference subjects known to not have cancer affecting the
3 first tissue type.

1 32. The method of claim 25, wherein the first tissue type is blood, liver, lung,
2 kidney, heart, or brain.

1 33. The method of claim 25, wherein the cancer is HCC.

1 34. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a plurality of nucleic acid molecules, the plurality of nucleic acid
3 molecules being cell-free, each nucleic acid molecule of the plurality of nucleic acid molecules
4 being double-stranded with a first strand having a first portion at an end and a second strand,
5 wherein the first portion of the first strand of at least some of the plurality of nucleic acid
6 molecules has no complementary portion from the second strand, is not hybridized to the second
7 strand, and is at a first end of the first strand, the method comprising:

8 adding, to the biological sample, a set of first compounds, wherein:

9 the set of first compounds comprise oligonucleotides of different
10 nucleotide lengths,

11 each oligonucleotide of a subset of the oligonucleotides comprises
12 nucleotides complementary to at least one of a plurality of the first portions,

13 each first compound of the set of first compounds comprises an identifier
14 molecule, the identifier molecule indicating a length of the oligonucleotide of the first
15 compound, and

16 for each nucleic acid molecule of the plurality of nucleic acid molecules:

17 hybridizing the oligonucleotide of a first compound of the set of first
18 compounds to the first portion of the first strand to form an elongated second strand that
19 is part of an aggregate molecule and includes the identifier molecule,

20 analyzing the aggregate molecule to detect the identifier molecule, and

21 determining the length of the first portion based on the identifier molecule.

1 35. The method of claim 34, wherein each oligonucleotide of the subset of the
2 oligonucleotides comprise nucleotides of an Alu sequence.

1 36. The method of claim 34, further comprising:

2 sequencing the elongated second strand to produce reads corresponding to the
3 identifier molecule for each nucleic acid molecule of the plurality of nucleic acid molecules.

1 37. The method of claim 34, further comprising:

2 determining a jagged end value using a statistical value calculated from the
3 plurality of determined lengths.

1 38. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a plurality of nucleic acid molecules, the plurality of nucleic acid
3 molecules being cell-free, each nucleic acid molecule of the plurality of nucleic acid molecules
4 being double-stranded with a first strand having a first portion at an end and a second strand,
5 wherein the first portion of the first strand of at least some of the plurality of nucleic acid
6 molecules has no complementary portion from the second strand, is not hybridized to the second
7 strand, and is at a first end of the first strand, the method comprising:

8 measuring a methylation status for each of a plurality of sites of the first and
9 second strands of the plurality of nucleic acid molecules, wherein each site of the plurality of
10 sites corresponds to a cycle of a sequencing process, and wherein the plurality of sites cover ends
11 of the first and second strands;

12 for each of the plurality of sites, determining a methylation level based on an
13 amount of the methylation statuses that indicate methylation at the site;

14 identifying, in a direction toward the ends of the first and second strands, a first
15 change in the methylation levels to a first value at a first site of the plurality of sites;

16 determining a first distance of the first site relative to an outermost nucleotide at
17 the first end of the first strand based on the corresponding cycle of the sequencing process;

18 determining a first magnitude of the first change in the methylation level;

19 determining a first length of a first plurality of first portions using the first
20 distance of the first site; and

21 determining a first amount of nucleic acid molecules using the first magnitude of
22 the first change in the methylation level, the first amount of nucleic acid molecules comprising
23 first portions with lengths less than or equal to the first length.

1 39. The method of claim 38, further comprising:

2 for each nucleic acid molecule of the plurality of nucleic acid molecules:

3 hybridizing a first compound comprising one or more nucleotides to the
4 first portion of the first strand, the first compound attached to a first end of the second
5 strand to form an elongated second strand with a first end comprising the first compound,
6 the first compound having a first end not contacting the second strand, the one or more
7 nucleotides being unmethylated,

8 separating the first strand from the elongated second strand.

1 40. The method of claim 38, further comprising:
2 identifying, in the direction toward the ends of the first and second strands, a
3 second change in the methylation level to a second value at a second site of the plurality of sites,
4 wherein:

5 the second site is at a second distance relative to the outermost nucleotide
6 at the first end of the first strand,

7 the second distance is less than the first distance, and

8 the second value is lower than the first value,

9 determining a second magnitude of the second change in methylation level,

10 determining a second length of a second plurality of first portions using the
11 second distance of the second site, and

12 determining a second amount of nucleic acid molecules using the second
13 magnitude of the second change in the methylation level, wherein:

14 the second amount of nucleic acid molecules comprises first portions with
15 lengths less than or equal to the second length of the second plurality of first portions,
16 and

17 the first amount comprises first portions with lengths greater than the
18 second length.

1 41. The method of claim 38 or 40, further comprising:
2 determining a jagged end value using at least one of the first amount and the
3 second amount.

1 42. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a double-stranded nucleic acid molecule, the double-stranded
3 nucleic acid molecules being cell-free, the double-stranded nucleic acid molecule having a first
4 strand and a second strand, the double-stranded nucleic acid molecule having a first end and a
5 second end opposite the first end, the method comprising:

6 circularizing the double-stranded nucleic acid molecule using oligonucleotides
7 having known patterns, thereby producing a circular nucleic acid molecule;

8 cleaving the circular nucleic acid molecule to form a single-stranded nucleic acid
9 molecule;
10 analyzing the single-stranded nucleic acid molecule to produce a first read and a
11 second read, wherein:
12 the single-stranded nucleic acid molecule comprises:
13 a first section comprising a first pattern of nucleotides of the first
14 strand at the first end to which the first read corresponds,
15 a first oligonucleotide having a first known pattern of nucleotides,
16 and
17 a second section comprising a second pattern of nucleotides of the
18 second strand at the first end to which the second read corresponds;
19 aligning the first read and the second read to a reference sequence or to each other;
20 and
21 determining, using the aligning of the first read and the second read, whether the
22 double-stranded nucleic acid molecule comprises a portion of the first strand not hybridized to
23 the second strand.

1 43. The method of claim 42, further comprising determining the length of the
2 portion of the first strand not hybridized to the second strand.

1 44. The method of claim 43, further comprising determining a jagged end
2 value using a statistical value calculated from the plurality of the determined lengths.

1 45. The method of claim 42, wherein:
2 the reference sequence is a human reference genome.

1 46. The method of any one of claims 42 to 45, further comprising:
2 attaching the first oligonucleotide to the first strand and the second strand at the
3 first end, and
4 attaching a second oligonucleotide to the first strand and the second strand at the
5 second end, wherein the second oligonucleotide comprises a second known pattern of
6 nucleotides,
7 wherein:

8 the circular nucleic acid molecule comprises the first strand, the second
9 strand, the first oligonucleotide, and the second oligonucleotide.

10 analyzing the single-stranded nucleic acid molecule comprises:
11 random tagging of the single-stranded nucleic acid molecule,
12 annealing a third oligonucleotide to the single-stranded nucleic
13 acid molecule, and
14 amplifying the single-stranded nucleic acid molecule to add
15 sequencing adapters.

1 47. The method of any one of claims 42 to 45, further comprising:
2 attaching the first oligonucleotide to the first strand and the second strand at the
3 first end, and
4 attaching a second oligonucleotide to the first strand and the second strand at the
5 second end, wherein the second oligonucleotide comprises a second known pattern of
6 nucleotides,
7 sequencing the single-stranded nucleic acid molecule to produce the first read and
8 the second read.

1 48. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a double-stranded nucleic acid molecule, the double-stranded
3 nucleic acid molecules being cell-free, the double-stranded nucleic acid molecule having a first
4 strand and a second strand, the double-stranded nucleic acid molecule having a first end and a
5 second end opposite the first end, the method comprising:
6 circularizing the double-stranded nucleic acid molecule using oligonucleotides
7 having known patterns, thereby producing a circular nucleic acid molecule;
8 analyzing the circular nucleic acid molecule to produce a first read and a second
9 read, wherein:
10 the circular nucleic acid molecule comprises:
11 a first section comprising a first pattern of nucleotides of the first
12 strand at the first end to which the first read corresponds,
13 a first oligonucleotide having a first known pattern of nucleotides,
14 and

15 a second section comprising a second pattern of nucleotides of the
16 second strand at the first end to which the second read corresponds;
17 aligning the first read and the second read to a reference sequence or to each other;
18 and
19 determining, using the aligning of the first read and the second read, whether the
20 double-stranded nucleic acid molecule comprises a portion of the first strand not hybridized to
21 the second strand.

1 49. The method of claim 48, further comprising determining the length of the
2 portion of the first strand not hybridized to the second strand.

1 50. The method of claim 49, further comprising determining a jagged end
2 value using a statistical value calculated from the plurality of the determined lengths.

1 51. The method of claim 48 or 49, wherein:
2 the reference sequence is a human reference genome.

1 52. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a plurality of nucleic acid molecules, the plurality of nucleic acid
3 molecules being cell-free, each nucleic acid molecule of the plurality of nucleic acid molecules
4 being double-stranded with a first strand and a second strand, each first strand comprising a first
5 portion not hybridized to the second strand, each first portion being at a first end of the first
6 strand, the method comprising:
7 for each nucleic acid molecule of the plurality of nucleic acid molecules:
8 hybridizing a first compound comprising one or more nucleotide analogs
9 to the first portion of the first strand, wherein the first compound and the second strand
10 form an elongated second strand, and wherein the one or more nucleotide analogs
11 hybridize to any nucleotide; and
12 separating the first strand from the first compound and the second strand;
13 sequencing each elongated second strand of the plurality of elongated second
14 strands to produce nucleotide signals at each of a plurality of positions on the elongated
15 second strand, wherein the sequencing includes clonal amplification of the elongated
16 second strand;

17 for each elongated second strand of the plurality of elongated second strands:
18 identifying a first position of an end of the corresponding second strand by
19 detecting a change in intensity of a maximum nucleotide signal from the first position
20 to a subsequent position, wherein the change in intensity is greater than a threshold.

1 53. The method of claim 52, wherein the change in intensity greater than the
2 threshold is required to be sustained for N position relative to the first position, wherein N is an
3 integer greater than one.

1 54. The method of claim 52, wherein the change in intensity of a maximum
2 nucleotide signal is relative to a second highest nucleotide signal.

1 55. The method of claim 54, wherein the change in intensity of a maximum
2 nucleotide signal is measured as a quality score of a base call at the first position.

1 56. The method of claim 52, wherein the sequencing comprises:
2 performing a plurality of sequencing cycles;
3 determining a fluorescence intensity for each of a plurality of nucleotides after
4 each cycle of the plurality of sequencing cycles, and wherein identifying the first position of the
5 end of the corresponding second strand includes:
6 identifying a first decrease in a maximum fluorescence intensity to below the
7 threshold at a first number of sequencing cycles.

1 57. The method of claim 52, wherein the sequencing comprises:
2 performing a plurality of sequencing cycles;
3 determining an electrical intensity for each of a plurality of nucleotides after each
4 cycle of the plurality of sequencing cycles, and wherein identifying the first position of the end
5 of the corresponding second strand includes:
6 identifying a first decrease in a maximum electrical intensity to below the
7 threshold at a first number of sequencing cycles.

1 58. The method of claim 52, wherein the one or more nucleotide analogs
2 comprise inosine.

1 59. The method of claim 52, further comprising determining a jagged end
2 value using a statistical value calculated from the changes of intensity.

1 60. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a plurality of nucleic acid molecules, the plurality of nucleic acid
3 molecules being cell-free, each nucleic acid molecule of the plurality of nucleic acid molecules
4 being double-stranded with a first strand having a first portion at an end and a second strand,
5 wherein the first portion of the first strand of at least some of the plurality of nucleic acid
6 molecules has no complementary portion from the second strand, is not hybridized to the second
7 strand, and is at a first end of the first strand, the method comprising:
8 for each nucleic acid molecule of the plurality of nucleic acid molecules:
9 hybridizing a first compound comprising one or more nucleotides to the
10 first portion of the first strand, the first compound attached to a first end of the second
11 strand to form an elongated second strand with a first end comprising the first compound,
12 the first compound having a first end not contacting the second strand, the one or more
13 nucleotides being either all methylated or all unmethylated,
14 separating the first strand from the elongated second strand,
15 determining a first methylation status for each of one or more first sites of
16 the elongated second strand, the one or more first sites at the first end of the elongated
17 second strand;
18 calculating a first methylation level using the first methylation statuses for the
19 plurality of elongated second strands at the one or more first sites;
20 calculating a jagged end value using the first methylation level, wherein the
21 jagged end value is proportional to an average length of the first strands that overhang the second
22 strands.

1 61. The method of claim 60, wherein the one or more nucleotides are all
2 methylated, and the one or more nucleotides are cytosines.

1 62. The method of claim 61, wherein the one or more nucleotides are adjacent
2 to an adenine, a cytosine, or a thymine.

1 63. The method of claim 61, wherein the one or more nucleotides are adjacent
2 to a guanine.

1 64. The method of claim 60, wherein the plurality of nucleic acid molecules
2 have sizes within a size range.

1 65. The method of claim 64, further comprising:
2 estimating a fractional concentration of clinically-relevant DNA using the jagged
3 end value.

1 66. The method of claim 64, wherein:
2 the plurality of nucleic acid molecules is a first plurality of nucleic acid molecules,
3 and
4 the biological sample includes a second plurality of nucleic acid molecules,
5 the method further comprising:
6 determining sizes of the nucleic acid molecules of the second plurality of
7 nucleic acid molecules,
8 filtering the second plurality of nucleic acid molecules to nucleic acid
9 molecules having sizes within the size range to obtain the first plurality of nucleic acid
10 molecules.

1 67. The method of claim 60, wherein the first end of the elongated second
2 strand is the 3' end of the elongated second strand.

1 68. The method of claim 60, further comprising:
2 for each control nucleic acid molecules of a plurality of control nucleic acid
3 molecules, each control nucleic acid molecule of the plurality of control nucleic acid molecules
4 being double-stranded with a first strand having a first portion at an end and a second strand,
5 wherein the first portion of the control nucleic acid molecule of the plurality of control nucleic
6 acid molecules has no complementary portion from the second strand of the control nucleic acid
7 molecule, is not hybridized to the second strand of the control nucleic acid molecule, and is at a
8 first end of the first strand, and wherein the length of the first portion of the first strand of the
9 control nucleic acid molecule is known:

10 hybridizing the first compound to the first portion of the control nucleic
11 acid molecule, the first compound attached to a first end of the second strand of the
12 control nucleic acid molecule to form an elongated second strand of the control nucleic
13 acid molecule with a first end comprising the first compound, the first compound having
14 a first end not contacting the second strand of the control nucleic acid molecule,
15 separating the first strand from the elongated second strand, and
16 determining a third methylation status for each of one or more first sites of
17 the elongated second strand of the control nucleic acid molecule, the one or more first
18 sites at the first end of the elongated second strand of the control nucleic acid molecule,
19 wherein:
20 calculating the first methylation level uses the third methylation status,
21 and
22 the plurality of control nucleic acid molecules are characterized by a
23 known quantity,
24 the method further comprising:
25 comparing the jagged end value to a reference value, the reference value
26 obtained without hybridizing the first compounds to the plurality of control nucleic acid
27 molecules, and
28 calculating a quantity of jagged ends using the comparison of the jagged
29 end value to the reference value and using the known quantity of the plurality of control
30 nucleic acid molecules.

1 69. The method of claim 60, wherein:
2 the one or more nucleotides are all unmethylated,
3 the method further comprising:
4 for each nucleic acid molecule of the plurality of nucleic acid molecules:
5 determining a second methylation status for each of one or more
6 second sites of the elongated second strand, the one or more second sites at a
7 second end of the elongated second strand,
8 calculating a second methylation level using the second methylation
9 statuses for the plurality of elongated second strands at the one or more second sites,
10 wherein:

11 calculating the jagged end value using the second methylation level.

1 70. The method of claim 69, wherein the one or more second sites comprise
2 the outermost 30 sites at the second end of the elongated second strand.

1 71. The method of claim 69, wherein:
2 the first methylation level is a mean of the first methylation statuses,
3 the second methylation level is a mean of the second methylation statuses, and
4 calculating the jagged end value is by calculating a difference between the first
5 methylation level and the second methylation level and dividing the difference by the first
6 methylation level.

1 72. A method of analyzing a biological sample obtained from an individual,
2 the biological sample including a plurality of nucleic acid molecules, the plurality of nucleic acid
3 molecules being cell-free, each nucleic acid molecule of the plurality of nucleic acid molecules
4 being double-stranded with a first strand having a first portion at an end and a second strand,
5 wherein the first portion of the first strand of a first subset of the plurality of nucleic acid
6 molecules has no complementary portion from the second strand, is not hybridized to the second
7 strand, and is at a first end of the first strand, the method comprising:

8 for each nucleic acid molecule of the first subset of the plurality of nucleic acid
9 molecules:

10 hybridizing a first compound comprising one or more nucleotides to the
11 first portion of the first strand, the first compound attached to a first end of the second
12 strand to form an elongated second strand with a first end comprising the first compound,
13 the first compound having a first end not contacting the second strand, the first compound
14 comprising one or more nucleotides that are methylated cytosines,
15 converting the one or more nucleotides that are unmethylated cytosines to
16 thymines,
17 separating the first strand from the elongated second strand,
18 determining a first location, wherein the first location has a thymine in the
19 second strand nearest the first end of the elongated second strand,

20 determining a second location, wherein the second location has a
21 methylated cytosine in the first compound nearest the thymine, and
22 determining a distance from the first end of the elongated second strand
23 using at least one selected from the first location and the second location;
24 calculating a jagged end value using the distances for the first subset of the
25 plurality of nucleic acid molecules.

1 73. The method of claim 72, further comprising:
2 for each nucleic acid molecule of a second subset of the plurality of nucleic acid
3 molecules, wherein a first portion of each nucleic acid molecule of the second subset of the
4 plurality of nucleic acid molecules has a complementary portion from the second strand, is
5 hybridized to the second strand, and is at a first end of the first strand:
6 converting unmethylated cytosines in the nucleic acid molecule to
7 thymines,
8 determining that a thymine is at an end of the second strand and that the
9 second strand is not elongated, and
10 identifying the nucleic acid molecule as not having a jagged end.

1 74. The method of claim 73, further comprising:
2 for each nucleic acid molecule of the second subset of the plurality of nucleic acid
3 molecules:
4 determining a distance of the thymine to the end of the second strand,
5 wherein calculating the jagged end value uses the distances for the second subset
6 of the plurality of nucleic acid molecules.

1 75. The method of any one of claims 34-67 and 69-74, further comprising:
2 determining a jagged end value using nucleic acid molecules from the biological
3 sample and a known quantity of a plurality of control nucleic acid molecules, wherein an
4 overhang length of each of the control nucleic acid molecules is known;
5 comparing the jagged end value to a reference value, the reference value obtained
6 without hybridizing the first compounds to the plurality of control nucleic acid molecules, and

7 calculating a quantity of jagged ends using the comparison of the jagged end
8 value to the reference value and using the known quantity of the plurality of control nucleic acid
9 molecules.

1 76. A computer program product comprising instructions that when executed
2 control a computing system to perform the method of any one of the above method claims.

1 77. A computer readable storage medium comprising the computer program
2 product of claim 76.

1 78. A computing system comprising the computer program product of claim
2 76.

1 79. A data packet or packets comprising computer program code that when
2 executed by a processor implements the method of any one of the above method claims.