

CLAIMS

WHAT IS CLAIMED IS:

~~1.~~ 1. A method, comprising:

~~—— (a) providing a forward polynucleotide and a cognate polynucleotide;~~

~~(b) determining a first identity of a first base at a locus of the forward polynucleotide and a second identity of a second base at or proximal to a corresponding locus of the cognate polynucleotide using sequencing; and~~

~~—— (c) using a computer comprising a processor, a memory, and instructions stored thereupon that, when executed, determine a value of a true base at a locus of an original polynucleotide corresponding to the locus of the forward polynucleotide based at least in part on the first identity of the first base and the second identity of the second base.~~

~~2. — The method of claim 1, wherein the second base is at the corresponding locus of the cognate polynucleotide.~~

~~3. — The method of claim 1, wherein the second base is proximal to the corresponding locus of the cognate polynucleotide.~~

~~4. — The method of any one of claims 1-3, wherein the forward polynucleotide and cognate polynucleotide are linked.~~

~~5. — The method of any one of claims 1-4, wherein at any time point prior to sequencing, the forward polynucleotide and the cognate polynucleotide are (i) covalently linked via a hairpin; (ii) linked as a double stranded polynucleotide via Watson-Crick base pairing; (iii) each coupled to a barcode; or (iv) any combination thereof.~~

~~6. — The method of claim 1, wherein the forward polynucleotide comprises a complementary deoxyribonucleic acid (cDNA) molecule or an amplicon thereof.~~

(a) generating a double-stranded polynucleotide comprising: (i) a first base that is a substrate for a deaminase that is unprotected, and (ii) a second base that is a substrate for a deaminase that is protected,

(b) contacting the double-stranded polynucleotide with a helicase and said deaminase to yield a nucleic acid molecule comprising a nucleic acid sequence comprising a deaminated base and said second base, wherein a base of said double-stranded polynucleotide is deaminated to yield said deaminated base, wherein said deaminated base is different from said second base;

(c) sequencing nucleotides of said nucleic acid sequence or a complement thereof to obtain sequencing data; and

(d) processing said sequencing data to identify and distinguish said first base and said second base.

2. The method of claim 1, wherein said nucleic acid molecule comprises sequences of both strands of said double-stranded polynucleotide, and said sequencing comprises sequencing said sequences of both strands.

3. The method of claim 1, wherein strands of said double-stranded polynucleotide are linked covalently via a hairpin.

4. The method of claim 1, further comprising, prior to (a), providing a polynucleotide and generating said double-stranded polynucleotide from said polynucleotide.

~~7.—5. The method of claim 1, further comprising contacting an RNA nucleotide with a reverse transcriptase, biologically active fragment thereof, or derivative thereof to generate the forward polynucleotide.~~

~~8.—The method of any one of claims 1-7, wherein the original polynucleotide comprises a deoxyribonucleic acid (DNA) polynucleotide isolated from a sample obtained from a subject.~~

~~9.—The method of any one of claim 1-8, wherein the original polynucleotide comprises a cell-free DNA (cfDNA) polynucleotide.~~

~~10.—The method of any one of claims 1-5 or 8-9, wherein the forward polynucleotide is the original polynucleotide or a portion thereof.~~

~~11.—The method of any one of claims 1-3 or 6, wherein the forward polynucleotide is an amplicon copy of the original polynucleotide.~~

~~12.—The method of any one of claims 1-11, wherein the value of the true base is determined to be a miscall if the first identity of the first base and the second identity of the second base, respectively, are determined to be any of the following combinations: adenine and adenine, adenine and cytosine, adenine and guanine, cytosine and adenine, cytosine and cytosine, cytosine and thymine, guanine and adenine, guanine and guanine, guanine and thymine, thymine and cytosine, thymine and guanine, or thymine and thymine.~~

~~13.—The method of any one of claims 1-12, wherein the value of the true base is adenine if the first identity of the first base and the second identity of the second base are determined to be adenine and thymine, respectively.~~

~~14.—The method of any one of claims 1-13, wherein the value of the true base is cytosine if the first identity of the first base and the second identity of the second base are determined to be cytosine and guanine, respectively.~~

~~15.—The method of any one of claims 1-14, wherein the value of the true base is guanine if the first identity of the first base and the second identity of the second base are determined to be guanine and cytosine, respectively.~~

~~16. The method of any one of claims 1-15, wherein the value of the true base is thymine if the first identity of the first base and the second identity of the second base are determined to be thymine and adenine, respectively.~~

~~17. The method of any one of claims 1-16, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 100.~~

~~18. The method of any one of claims 1-17, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 1,000.~~

~~19. The method of any one of claims 1-17, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 10,000.~~

~~20. The method of any one of claims 1-17, wherein the false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 100,000.~~

~~21. The method of any one of claims 1-17, wherein the false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 1,000,000.~~

~~22. The method of any one of claims 1-21, wherein determining the second identity of the second base comprises sequencing a read polynucleotide, wherein the read polynucleotide is a cognate amplicon of the cognate polynucleotide.~~

~~23. The method of any one of claims 1-22, wherein the value of the true base is determined before aligning data generated during the sequencing to a reference nucleic acid sequence.~~

~~24. The method of any one of claims 1-23, wherein a mutation having a frequency of no more than 0.1% in a population comprising a subject from which the sample was obtained is detected at the locus of the original polynucleotide with a sensitivity of at least 90% using a locus coverage of no more than 450 fold.~~

~~25. The method of any one of claim 1-24, further comprising, prior to (a), generating the forward polynucleotide and cognate polynucleotide by conducting one or more chemical reactions or one or more enzymatic reactions on the original~~said~~ polynucleotide ~~or derivative thereof~~to generate said double-stranded polynucleotide.~~

~~26. 6. The method of claim 25, further comprising, prior to (a), generating the forward polynucleotide and cognate polynucleotide by conducting a ~~deamination~~nucleic acid extension reaction using said polynucleotide.~~

~~27. — 7. The method of claim 26, wherein the deamination reaction is conducted with a deaminase.~~

~~28. — The method of claim 27, wherein the deaminase is APOBEC or a fragment thereof.~~

~~29. — The method of any one of claims 26–28, wherein the deamination reaction is conducted in a presence of a helicase or fragment thereof.~~

~~30. — A method, comprising:~~

~~—— (a) providing a forward polynucleotide and a cognate polynucleotide;~~

~~—— (b) contacting the forward polynucleotide and, optionally, the cognate polynucleotide with bisulfite;~~

~~—— (c) determining a first identity of a first base at a locus of the forward polynucleotide and a second identity of a second base at or proximal to a corresponding locus of the cognate polynucleotide using sequencing; and~~

~~—— (d) using a computer comprising a processor, a memory, and instructions stored thereupon that, when executed, determine a value of a true base at a locus of an original polynucleotide corresponding to the locus of the forward polynucleotide based at least in part on the identity of the first base and the identity of the second base.~~

~~31. — The method of claim 30, wherein the second base is at the corresponding locus of the cognate polynucleotide.~~

~~32. — The method of claim 30, wherein the second base is proximal to the corresponding locus of the cognate polynucleotide.~~

~~33. — The method of claim 30, further comprising contacting the forward polynucleotide and the cognate said polynucleotide with an entity having DNA methyltransferase activity.~~

~~34. — The method of claim 23, wherein the contacting the forward polynucleotide and the cognate polynucleotide with the entity having DNA methyltransferase activity is performed before the contacting the forward polynucleotide and, optionally, the cognate polynucleotide with bisulfite.~~

~~35. — The method of claim 23 or claim 34, wherein the entity having DNA methyltransferase activity is DNA (cytosine 5) methyltransferase 1 (DNMT1) or DNMT5.~~

~~36. — The method of any one of claims 30–35, wherein the forward polynucleotide comprises a 5-methyleytosine, a 5-hydroxymethyleytosine, or both.~~

~~37. — The method of any one of claims 30–36, wherein the forward polynucleotide and the cognate polynucleotide are linked by Watson-Crick basing pairing.~~

~~38. — The method of any one of claims 30–37, wherein the forward polynucleotide and the cognate polynucleotide are further linked by a hairpin, a barcode, or both.~~

~~39.—The method of any one of claims 33-38, wherein after the contacting with the entity having DNA methyltransferase activity, the cognate polynucleotide optionally comprises a 5-methylcytosine.~~

~~40.—The method of any one of claims 30-39, wherein the value of the true base is determined to be a miscall if the first identity of the first base and the second identity of the second base, respectively, is determined to be any of the following combinations: adenine and adenine, adenine and cytosine, adenine and guanine, cytosine and adenine, cytosine and cytosine, cytosine and thymine, guanine and adenine, guanine and guanine, guanine and cytosine, thymine and cytosine, or thymine and thymine.~~

~~41.—The method of any one of claims 30-40, wherein the value of the true base is adenine if the first identity of the first base and the second identity of the second bases are determined to be adenine and thymine, respectively.~~

~~42.—The method of any one of claims 30-41, wherein the value of the true base is cytosine if the first identity of the first base and the second identity of the second base are determined to be thymine and guanine, respectively.~~

~~43.—The method of any one of claims 30-42, wherein the value of the true base is guanine if the first identity of the first base and the second identity of the second bases are determined to be guanine and thymine, respectively.~~

~~44.—The method of any one of claims 30-43, wherein the value of the true base is thymine if the first identity of the first base and the second identity of the second base are determined to be thymine and adenine, respectively.~~

~~45.—The method of any one of claims 30-44, wherein the value of the true base is a methylated cytosine if the first identity of the first base and the second identity of the second base are determined to be cytosine and guanine, respectively.~~

~~46.—The method of any one of claims 30-45, wherein the value of the true base is determined before aligning data generated during the sequencing to a reference nucleic acid sequence.~~

~~47.—The method of any one of claims 30-46, further comprising prior to or after (b), conducting a chemical or enzymatic reaction using said forward polynucleotide and said cognate polynucleotide.~~

~~48.—A method, comprising:~~

~~(a) providing a forward polynucleotide and a cognate polynucleotide;~~

~~—(b) contacting the forward polynucleotide and, optionally, the cognate polynucleotide with an oxidizing agent;~~

~~—(c) determining a first identity of a first base at a locus of the forward polynucleotide and a second identity of a second base at or proximal to a corresponding locus of the cognate polynucleotide using sequencing; and~~

~~—(d) using a computer comprising a processor, a memory, and instructions stored thereupon that, when executed, determine a value of a true base at a locus of an original polynucleotide corresponding to the locus of the forward polynucleotide based at least in part on the identity of the first base and the identity of the second base.~~

~~49.—The method of claim 48, wherein the second base is at the corresponding locus of the cognate polynucleotide.~~

~~50.—The method of claim 48, wherein the second base is proximal to the corresponding locus of the cognate polynucleotide.~~

~~51.—The method of claim 48, wherein the oxidizing agent is a metal oxide.~~

~~52.—The method of claim 48, wherein the oxidizing agent is a ruthenate.~~

~~53.—The method of claim 52, wherein the oxidizing agent is potassium ruthenate.~~

~~54.—The method of claim 48, wherein the oxidizing agent is a methyleytosine dioxygenase.~~

~~55.—The method of claim 54, wherein the methyleytosine dioxygenase is a ten-eleven translocation (TET) enzyme, or a derivative thereof.~~

~~56.—The method of any one of claims 36-55, wherein the forward polynucleotide comprises a 5-methyleytosine, a 5-hydroxymethyleytosine, or both.~~

~~57.—The method of any one of claims 48-56, wherein the forward polynucleotide and the cognate polynucleotide are linked by Watson-Crick basing pairing.~~

~~58.—The method of any one of claims 48-57, wherein the forward polynucleotide and the cognate polynucleotide are further linked by a hairpin, a barcode, or both.~~

~~59.—The method of any one of claims 48-58, further comprising contacting the forward polynucleotide and the cognate polynucleotide with an entity having DNA-methyltransferase activity.~~

~~60.—The method of claim 59, wherein the~~8. The method of claim 7, wherein said entity having DNA methyltransferase activity is selected from DNA (cytosine-5)-methyltransferase 1 (DNMT1) or DNA (cytosine-5)-methyltransferase 5 (DNMT5).

~~61.—~~9. The method of claim ~~59 or claim 60, wherein the contacting the forward~~ polynucleotide and the cognate polynucleotide with an entity having DNA-methyltransferase activity is performed ~~after the contacting the forward polynucleotide and, optionally, the cognate polynucleotide with the oxidizing agent.~~

~~62. The method of any one of claims 48-61~~5, further comprising contacting ~~the forward polynucleotide and optionally the cognate~~said polynucleotide with ~~a deamination~~an oxidizing agent.

~~63.~~10. The method of claim ~~62~~9, wherein ~~the deamination agent is a deaminase.~~

~~64. The method of claim 63, wherein the deaminase is APOBEC, or a fragment thereof.~~

~~65. The method of claim 62, further comprising, contacting the forward polynucleotide and optionally the cognate polynucleotide with a helicase.~~

~~66. The method of claim 62, wherein the deamination agent is bisulfite.~~

~~67. The method of any one of claims 48-66, wherein the value of the true base is determined to be a miscall if the first identity of the first base and the second identity of the second base, respectively, are determined to be any of the following combinations: adenine and adenine, adenine and cytosine, adenine and guanine, cytosine and adenine, cytosine and cytosine, cytosine and thymine, guanine and adenine, guanine and guanine, guanine and cytosine, thymine and cytosine, or thymine and thymine.~~

~~68. The method of any one of claims 48-67, wherein the value of the true base is adenine if the first identity of the first base and the second identity of the second base are determined to be adenine and thymine, respectively.~~

~~69. The method of any one of claims 48-68, wherein the value of the true base is cytosine or 5-hydroxymethylcytosine (5hmC) if the first identity of the first base and the second identity of the second base are determined to be thymine and guanine, respectively.~~

~~70. The method of any one of claims 48-69, wherein the value of the true base is guanine if the first identity of the first base and the second identity of the second base are determined to be guanine and thymine, respectively.~~

~~71. The method of any one of claims 48-70, wherein the value of the true base is thymine if the first identity of the first base and the second identity of the second base are determined to be thymine and adenine, respectively.~~

~~72. The method of any one of claims 48-71, wherein the value of the true base is a 5-methylcytosine (5mC) if the first identity of the first base and the second identity of the second base are determined to be cytosine and guanine, respectively.~~

~~73. The method of any one of claims 48-72, wherein the value of the true base is determined before aligning data generated during the sequencing to a reference nucleic acid sequence.~~

~~74.—The method of any one of claims 48–73, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 100.~~

~~75.—The method of any one of claims 48–73, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 1,000.~~

~~76.—The method of any one of claims 48–73, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 10,000.~~

~~77.—The method of any one of claims 48–73, wherein the false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 100,000.~~

~~78.—The method of any one of claims 48–73, wherein the false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 1,000,000.~~

~~79.—The method of any one of claim 48–78, further comprising, prior to (a), generating the forward polynucleotide and cognate polynucleotide by conducting one or more chemical reactions or enzymatic reactions on the original polynucleotide or derivative thereof.~~

~~80.—A method, comprising:~~

~~(a) providing a forward polynucleotide and a cognate polynucleotide;~~

~~——(b) contacting the forward polynucleotide and, optionally, the cognate polynucleotide with an agent that specifically glycosylates 5-hydroxymethylcytosine (5hmC);~~

~~——(c) determining a first identity of a first base at a locus of the forward polynucleotide and a second identity of a second base at or proximal to a corresponding locus of the cognate polynucleotide using sequencing; and~~

~~——(d) using a computer comprising a processor, a memory, and instructions stored thereupon that, when executed, determine a value of a true base at a locus of an original polynucleotide corresponding to the locus of the forward polynucleotide based at least in part on the identity of the first base and the identity of the second base.~~

~~81.—The method of claim 80, wherein the second base is at the corresponding locus of the cognate polynucleotide.~~

~~82.—The method of claim 80, wherein the second base is proximal to the corresponding locus of the cognate polynucleotide.~~

~~83.—The method of claim 80, further comprising contacting the forward polynucleotide and the cognate polynucleotide with an entity having DNA methyltransferase activity.~~

~~84.—The method of claim 83, wherein the entity having DNA methyltransferase activity is selected from DNA (cytosine 5) methyltransferase 1 (DNMT1) or DNA (cytosine 5) methyltransferase 5 (DNMT5).~~

~~85.—The method of claim 83 or claim 84, wherein the contacting the forward polynucleotide and the cognate polynucleotide with the entity having DNA methyltransferase activity is performed after the contacting the forward polynucleotide and, optionally, the cognate polynucleotide with the agent that specifically glycosylates 5-hydroxymethylcytosine (5hmC).~~

~~86.—The method of any one of claims 80-85, further comprising contacting the forward polynucleotide and optionally the cognate polynucleotide with a deamination agent.~~

~~87.—The method of claim 86, wherein the deamination agent is a deaminase.~~

~~88.—The method of claim 87, wherein the deamination agent is APOBEC, or a fragment thereof.~~

~~89.—The method of claim 86, further comprising, contacting the forward polynucleotide and optionally the cognate polynucleotide with a helicase.~~

~~90.—The method of claim 86, wherein the deamination agent is bisulfite.~~

~~91.—The method of any one of claims 80-90, further comprising contacting the forward polynucleotide and optionally the cognate polynucleotide with an oxidizing agent.~~

~~92. The method of claim 91, wherein the said oxidizing agent is a methylcytosine dioxygenase.~~

~~93. 11. The method of claim ~~91~~10, wherein ~~the~~ said methylcytosine dioxygenase is a ten-eleven translocation (TET) enzyme, ~~or a derivative thereof.~~~~

~~94.—The method of any one of claims 91-93, wherein the contacting the forward polynucleotide and, optionally, the cognate polynucleotide with the oxidizing agent is performed after the contacting the forward polynucleotide and the cognate polynucleotide with the entity having DNA methyltransferase activity.~~

~~95.—The method of claim 94, wherein the entity having DNA methyltransferase activity is selected from DNA (cytosine 5) methyltransferase 1 (DNMT1) or DNA (cytosine 5) methyltransferase 5 (DNMT5).~~

~~96.—The method of any one of claims 80-95, further comprising contacting the forward polynucleotide and the cognate polynucleotide with the agent that specifically glycosylates 5-hydroxymethylcytosine (5hmC).~~

12. The method of claim 5, further contacting said polynucleotide with an glycosylation agent.

97. ~~13.~~ The method of claim 96, ~~wherein the agent that specifically glycosylates 5hmC is β -glucosyltransferase~~5, wherein said polynucleotide comprises a methylated base.

98. ~~14.~~ The method of claim 97, ~~wherein the contacting the forward polynucleotide and the cognate polynucleotide with the agent that specifically glycosylates 5hmC is performed after the contacting the forward polynucleotide and, optionally, the cognate polynucleotide with the oxidizing agent.~~

99. The method of any one of claims 80-98~~13~~13, further comprising ~~contacting the forward polynucleotide and, optionally, the cognate polynucleotide with~~conducting an oxidation reaction and a deaminase~~glycosylation reaction to generate said double-stranded polynucleotide.~~

100. ~~15.~~ The method of claim 99, ~~wherein the deaminase is selected from apolipoprotein B mRNA editing enzyme (APOBEC), a double-stranded DNA deaminase, or a fragment thereof.~~

101. ~~The method of claim 100, prior to said contacting the forward polynucleotide and, optionally, the cognate polynucleotide with APOBEC, treating the forward polynucleotide and the cognate polynucleotide so that one or more loci of the forward polynucleotide and the cognate polynucleotide are not linked.~~

102. ~~The method of claim 101, wherein the treating comprises separating the first polynucleotide or a portion thereof from the second polynucleotide or a portion thereof.~~

103. ~~The method of claim 102, wherein said separating~~14, wherein (c) ~~comprises contacting the forward polynucleotide and the cognate polynucleotide with a helicase.~~

104. ~~The method of claim 101 or claim 102, wherein said treating comprises contacting the forward polynucleotide and the cognate polynucleotide with a single-strand DNA-binding protein (SSB).~~

105. ~~The method of any one of claims 80-104, wherein the value of the true base is determined to be a miscall if the first identity of the first base and the second identity of the second base, respectively, is determined to be any of the following combinations: adenine and adenine, adenine and cytosine, adenine and guanine, cytosine and adenine, cytosine and cytosine, cytosine and thymine, guanine and adenine, guanine and guanine, thymine and cytosine, thymine and thymine, guanine and cytosine if not preceded by cytosine and guanine, cytosine and guanine if not followed by guanine and cytosine, respectively, or cytosine and guanine if not followed by guanine and thymine, respectively.~~

106. ~~The method of any one of claims 80-105, wherein the value of the true base is adenine if the first identity of the first base and the second identity of the second base are determined to be adenine and thymine, respectively.~~

~~107.—The method of any one of claims 80–106, wherein the value of the true base is cytosine if the first identity of the first base and the second identity of the second bases are determined to be thymine and guanine, respectively.~~

~~108.—The method of any one of claims 80–107, wherein the value of the true base is guanine if the first identity of the first base and the second identity of the second base are determined to be guanine and thymine, respectively.~~

~~109.—The method of any one of claims 80–108, wherein the value of the true base is thymine if the first identity of the first base and the second identity of the second base are determined to be thymine and adenine, respectively.~~

~~110.—The method of any one of claims 80–109, wherein the value of the true base is 5-methyleytosine (5mC) if the first identity of the first base and the second identity of the second base are determined to be cytosine and guanine, followed by guanine and cytosine, respectively, in a CpG context.~~

~~111.—The method of any one of claims 80–110, wherein the value of the true base is 5-hydroxymethyleytosine (5hmC) if the first identity of the first base and the second identity of the second base are determined to be cytosine and guanine, respectively, followed by guanine and thymine, respectively, in a CpG context.~~

~~112.—The method of any one of claims 80–111, wherein the value of the true base is determined before aligning data generated during the sequencing to a reference nucleic acid sequence.~~

~~113.—The method of any one of claims 91–95, further comprising contacting the forward polynucleotide and the cognate polynucleotide with a reducing agent.~~

~~114.—The method of claim 113, wherein said reducing agent is a borane or a derivative of a borane.~~

~~115.—The method of claim 113 or claim 114, wherein said reducing agent is selected from pyridine borane, 2-picoline borane (pic-borane), borane, diborane, tert-butylamine borane, ammonia borane, sodium borohydride (NaBH₄), sodium cyanoborohydride (NaBH₃CN), ethylenediamine borane, dimethylamine borane, sodium triacetoxymorohydride, morpholine borane, 4-methylmorpholine borane, trimethylamine borane, dicyclohexylamine borane, or lithium borohydride (LiBH₄), or a salt thereof.~~

~~116.—The method of claim 115, wherein said reducing agent is pyridine borane.~~

~~117.—The method of claim 113, wherein said reducing agent comprises lithium aluminum hydride, sodium amalgam, amalgam, sulfur dioxide, dithionate, thiosulfate, iodide, hydrogen~~

peroxide, hydrazine, diisobutylaluminum hydride, oxalic acid, carbon monoxide, cyanide, ascorbic acid, formic acid, dithiothreitol, beta-mercaptoethanol, or any combination thereof.

~~118.—The method of any one of claims 113–117, wherein the value of the true base is determined to be a miscall if the first identity of the first base and the second identity of the second base, respectively, is determined to be any of the following combinations: adenine and adenine, adenine and cytosine, adenine and guanine, cytosine and adenine, cytosine and cytosine, cytosine and thymine, guanine and adenine, guanine and guanine, thymine and cytosine, thymine and thymine, guanine and thymine if not preceded by thymine and guanine, thymine and guanine if not followed by guanine and cytosine, respectively, or thymine and guanine if not followed by guanine and thymine, respectively.~~

~~119.—The method of any one of claims 113–118, wherein the value of the true base is adenine if the first identity of the first base and the second identity of the second base are determined to be adenine and thymine, respectively.~~

~~120.—The method of any one of claims 113–119, wherein the value of the true base is cytosine if the first identity of the first base and the second identity of the second base are determined to be thymine and guanine, respectively.~~

~~121.—The method of any one of claims 113–120, wherein the value of the true base is guanine if the first identity of the first base and the second identity of the second base are determined to be guanine and thymine, respectively.~~

~~122.—The method of any one of claims 113–121, wherein the value of the true base is thymine if the first identity of the first base and the second identity of the second base are determined to be thymine and adenine, respectively.~~

~~123.—The method of any one of claims 113–122, wherein the value of the true base is 5-methyleytosine (5mC) if the first identity of the first base and the second identity of the second base are determined to be cytosine and guanine, followed by guanine and cytosine, respectively, in a CpG context.~~

~~124.—The method of any one of claims 113–123, wherein the value of the true base is 5-hydroxymethyleytosine (5hmC) if the first identity of the first base and the second identity of the second base are determined to be cytosine and guanine, respectively, followed by guanine and thymine, respectively, in a CpG context.~~

~~125.—The method of any one of claims 80–124, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 100.~~

~~126.—The method of any one of claims 80–124, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 1,000.~~

~~127.—The method of any one of claims 80–124, wherein a false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 10,000.~~

~~128.—The method of any one of claims 80–124, wherein the false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 100,000.~~

~~129.—The method of any one of claims 80–124, wherein the false positive rate for determining the identity of the true base at the locus of the original polynucleotide is no higher than 1 in 1,000,000.~~

~~130.—The method of any one of claim 80–129, further comprising, prior to (a), generating the forward polynucleotide and cognate polynucleotide by conducting one or more chemical reactions or enzymatic reactions on the original polynucleotide or derivative thereof.~~

~~131.—The method of any one of claims 80–130, wherein at any time point prior to sequencing, the forward polynucleotide and the cognate polynucleotide are (i) covalently linked via a hairpin; (ii) linked as a double-stranded polynucleotide via Watson-Crick base pairing; (iii) each coupled to a barcode; or (iv) any combination thereof.~~

~~132.—The method of any one of claims 1–131, further comprising diagnosing a condition in the subject based at least in part on the value of the true base variant at the locus.~~

~~133.—The method of claim 132, wherein the condition is a cancer.~~

~~134.—The method of claim 133, wherein the cancer is selected from a sarcoma, a glioma, an adenoma, leukemia, bladder cancer, breast cancer, colorectal cancer, endometrial cancer, kidney cancer, liver cancer, lung cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer, thyroid cancer.~~

~~135.—The method of claim 132, wherein the condition is a neurodegenerative condition.~~

~~136.—The method of claim 135, wherein the neurodegenerative condition is selected from Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, Parkinson's disease, spinocerebellar ataxia, spinal muscle atrophy, Lewy body dementia, or Huntington's disease.~~

~~137.—The method of any one of claims 1–136, wherein the sequencing comprises Maxam-Gilbert sequencing, Sanger sequencing, or high-throughput sequencing.~~

~~138.~~ The method of claim ~~137~~, wherein the high throughput sequencing comprises next generation sequencing or third generation sequencing.

~~139.~~ The method of claim ~~138~~, wherein the third generation sequencing is long read sequencing.

~~140.~~ A method, comprising:

~~—— (a) deaminating a cytosine base of a double stranded polynucleotide in a presence of a helicase to yield a deaminated cytosine base;~~

~~—— (b) sequencing at least a portion of said double stranded polynucleotide comprising said deaminated cytosine base or double stranded derivative thereof to obtain sequencing data; and~~

~~—— (c) processing said sequencing data to identify said ~~cytosine~~methylated base as methylated.~~

16. The method of claim 15, wherein (c) comprises processing said sequencing data to identify said methylated base as methylated with an accuracy of at least about ~~80~~95%.

~~141.~~ 17. The method of claim ~~140~~5, wherein said ~~sequencing polynucleotide~~ comprises sequencing at least a ~~portion of both strands of said double stranded polynucleotide or double stranded derivative thereof~~methyl cytosine base or a hydroxymethyl cytosine base.

~~142.~~ 18. The method of claim ~~141~~17, further comprising ~~processing said sequencing data~~conducting a methyltransferase reaction, an oxidation reaction and a glycosylation reaction to ~~identify said cytosine base with an accuracy of at least about 90%~~generate said double-stranded polynucleotide.

~~143.~~ 19. The method of claim ~~142~~, further comprising 18, wherein (c) comprises processing said sequencing data to identify said ~~cytosine base with an accuracy of at least about 95%~~methyl cytosine base as methyl cytosine or said hydroxymethyl cytosine base as hydroxymethyl cytosine.

~~144.~~ 20. The method of claim ~~143~~, further comprising 18, wherein (c) comprises processing said sequencing data to identify said methyl cytosine base as methyl cytosine or said hydroxymethyl cytosine base as hydroxymethyl cytosine with an accuracy of at least about ~~99~~95%.

~~145.~~ 21. The method of claim ~~140~~1, wherein said ~~deaminating base~~ is ~~performed with a deaminase~~cytosine base, a methyl cytosine base or a hydroxymethyl cytosine base, and wherein (c) comprises identifying said cytosine base, said methyl cytosine, or said hydroxymethyl cytosine as comprising cytosine.

22. The method of claim ~~145~~21, wherein (c) comprises processing said sequencing data to identify said cytosine base, said methyl cytosine, or said hydroxymethyl cytosine as comprising cytosine with an accuracy of at least about 95%.

~~146.~~ 23. The method of claim 1, wherein said deaminase is an apolipoprotein B mRNA editing ~~enzyme~~, catalytic polypeptide-like (APOBEC) enzyme, or ~~a~~ fragment thereof.

~~147.~~ 24. The method of claim ~~140~~1, wherein said helicase comprises an amino acid sequence that is at least 90% homologous to UvrD helicase, ~~-~~*Geobacillus sterothermophilus*-Bad protein, a PcrA helicase, or ~~a fragment thereof, or a~~ fragment thereof.

~~148.~~ 25. The method of claim ~~147~~24, wherein said helicase is a UvrD helicase, ~~-~~*Geobacillus sterothermophilus*-Bad protein, a PcrA helicase, or ~~a~~ fragment thereof.

~~149.—The method of claim 140, further comprising, prior to (b), subjecting said double-stranded polynucleotide comprising said deaminated cytosine base to one or more reactions to generate said double-stranded derivative thereof, wherein (c) comprises sequencing at least a portion of said double-stranded derivative thereof to obtain said sequencing data.~~

~~150.—The method of claim 140, further comprising, prior to (a), providing a sample double-stranded polynucleotide comprising a forward strand and a reverse strand.~~

~~151.—The method of claim 150, further comprising, prior to (a), separating said forward strand from said reverse strand.~~

~~152.—The method of claim 151, further comprising using said forward strand in a nucleic acid extension reaction to generate said double-stranded polynucleotide.~~

~~153.—The method of claim 152, wherein said deaminating is performed with a deaminase.~~

~~154.—The method of claim 153, wherein said deaminase is an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) enzyme, or a fragment thereof.~~

~~155.—The method of claim 154, wherein said helicase comprises an amino acid sequence that is at least 90% homologous to UvrD helicase, *Geobacillus sterothermophilus*-Bad protein, a PerA helicase, or a fragment thereof, or a fragment thereof.~~

~~156.—The method of claim 155, wherein said helicase is a UvrD helicase, *Geobacillus sterothermophilus*-Bad protein, a PerA helicase, or a fragment thereof.~~

~~157.—The method of claim 155, wherein said cytosine base is a methylcytosine base or a hydroxymethyl cytosine base.~~

~~158.—The method of claim 157, further comprising, prior to (b), subjecting said double-stranded polynucleotide comprising said deaminated cytosine base to one or more reactions to generate said double-stranded derivative thereof, wherein (c) comprises sequencing at least a portion of said double-stranded derivative thereof to obtain said sequencing data.~~

~~159.—The method of claim 158, wherein (c) comprises processing said sequencing data to identify said cytosine base as a cytosine base with an accuracy of at least about 90%.~~

~~160.—The method of claim 159, wherein (c) comprises processing said sequencing data to identify said cytosine base as a cytosine base with an accuracy of at least about 95%.~~

~~161.—The method of claim 160, wherein (c) comprises processing said sequencing data to identify said cytosine base as a cytosine base with an accuracy of at least about 99%.~~

~~162.—The method of claim 161, wherein said forward strand comprises a methylated cytosine base and said method further comprises using said forward strand in a nucleic acid extension reaction that generates a modified double stranded polynucleotide comprising (i) said forward strand comprising said methylated cytosine base and (ii) an additional reverse strand comprising said cytosine base.~~

~~163.—The method of claim 162, further comprising, prior to (a), converting said methylated cytosine base to glucosylated hydroxymethylcytosine~~

~~164.—The method of claim 163, wherein said methylated cytosine base is a methylcytosine base and said converting comprises subjecting said methylcytosine base to oxidation conditions to generate a hydroxymethylcytosine base and subjecting said hydroxymethylcytosine base to glucosylation conditions to generate said glucosylated hydroxymethylcytosine.~~

~~165.—The method of claim 163, wherein said methylated cytosine base is hydroxymethylcytosine and said converting comprises subjecting said hydroxymethylcytosine base to glucosylation conditions to generate said glucosylated hydroxymethylcytosine.~~

~~166.—The method of claim 163, wherein said deaminating is performed with a deaminase.~~

~~167.—The method of claim 166, wherein said deaminase is an apolipoprotein B mRNA editing enzyme, catalytic polypeptide like (APOBEC) enzyme, or a fragment thereof.~~

~~168.—The method of claim 167, wherein said helicase comprises an amino acid sequence that is at least 90% homologous to UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~169.—The method of claim 168, wherein said helicase is a UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~170.—The method of claim 169, further comprising, prior to (b), subjecting said double stranded polynucleotide comprising said deaminated cytosine base to one or more reactions to generate said double stranded derivative thereof, wherein (c) comprises sequencing at least a portion of said double stranded derivative thereof to obtain said sequencing data.~~

~~171.—The method of claim 170, further comprising processing said sequencing data to identify said methylated cytosine base as a methylated cytosine base with an accuracy of at least about 80%.~~

~~172.—The method of claim 171, further comprising processing said sequencing data to identify said methylated cytosine base as a methylated cytosine base with an accuracy of at least about 90%.~~

~~173.—The method of claim 172, further comprising processing said sequencing data to identify said methylated cytosine base as a methylated cytosine base with an accuracy of at least about 95%.~~

~~174.—The method of claim 173, further comprising processing said sequencing data to identify said methylated cytosine base as a methylated cytosine base with an accuracy of at least about 99%.~~

~~175.—A method, comprising:~~

~~——(a) deaminating a cytosine base of a double stranded polynucleotide, in a presence of a helicase, with a deaminase to yield a deaminated cytosine base;~~

~~——(b) sequencing at least a portion of said double stranded polynucleotide comprising said deaminated cytosine base or double stranded derivative thereof to obtain sequencing data; and~~

~~——(c) processing said sequencing data to identify said cytosine base.~~

~~176.—The method of claim 175, wherein said sequencing comprises sequencing at least a portion of both strands of said double stranded polynucleotide or double stranded derivative thereof.~~

~~177.—The method of claim 175, wherein said deaminase is an apolipoprotein B mRNA editing enzyme, catalytic polypeptide like (APOBEC) enzyme, or a fragment thereof.~~

~~178.—The method of claim 175, wherein said helicase comprises an amino acid sequence that is at least 90% homologous to UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~179.—The method of claim 178, wherein said helicase is a UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~180.—The method of claim 175, further comprising, prior to (a), providing a sample double stranded polynucleotide comprising a forward strand and a reverse strand.~~

~~181.—The method of claim 180, further comprising, prior to (a), separating said forward strand from said reverse strand.~~

~~182.—The method of claim 181, wherein said separating comprises subjecting said forward strand to a nucleic acid extension reaction that generates said double stranded polynucleotide.~~

~~183.—The method of claim 182, wherein said deaminase is an apolipoprotein B mRNA editing enzyme, catalytic polypeptide like (APOBEC) enzyme, or a fragment thereof.~~

~~184.—The method of claim 183, wherein said helicase comprises an amino acid sequence that is at least 90% homologous to UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~185.—The method of claim 184, wherein said helicase is a UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~186.—The method of claim 185, wherein said cytosine base is a methyleytosine base or a hydroxymethyl cytosine base.~~

~~187.—The method of claim 186, further comprising, prior to (b), subjecting said double-stranded polynucleotide comprising said deaminated cytosine base to one or more reactions to generate said double-stranded derivative thereof, wherein (c) comprises sequencing at least a portion of said double-stranded derivative thereof to obtain said sequencing data.~~

~~188.—The method of claim 181, wherein said forward strand comprises a methylated cytosine base and said separating comprises using said forward strand in a nucleic acid extension reaction that generates a modified double-stranded polynucleotide comprising (i) said forward strand comprising said methylated cytosine base and (ii) an additional reverse strand comprising said cytosine base.~~

~~189.—The method of claim 188, further comprising, prior to (a), converting said methylated cytosine base to glucosylated hydroxymethyleytosine~~

~~190.—The method of claim 189, wherein said methylated cytosine base is a methyleytosine base and said converting comprises subjecting said methyleytosine base to oxidation conditions to generate a hydroxymethyleytosine base and subjecting said hydroxymethyleytosine base to glucosylation conditions to generate said glucosylated hydroxymethyleytosine.~~

~~191.—The method of claim 189, wherein said methylated cytosine base is hydroxymethyleytosine and said converting comprises subjecting said hydroxymethyleytosine base to glucosylation conditions to generate said glucosylated hydroxymethyleytosine.~~

~~192.—The method of claim 189, wherein said deaminase is an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) enzyme, or a fragment thereof.~~

~~193.—The method of claim 192, wherein said helicase comprises an amino acid sequence that is at least 90% homologous to UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~194.—The method of claim 193, wherein said helicase is a UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~195.—The method of claim 194, further comprising, prior to (b), subjecting said double-stranded polynucleotide comprising said deaminated cytosine base to one or more reactions to generate said double-stranded derivative thereof, wherein (c) comprises sequencing at least a portion of said double-stranded derivative thereof to obtain said sequencing data.~~

~~196.—A kit, comprising:~~

~~——a deaminase;~~

~~——a helicase; and~~

~~——packaging and instructions therein to use said kit.~~

~~197.—The kit of claim 196, wherein said deaminase is an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) enzyme, or a fragment thereof.~~

~~198.—The kit of claim 196, further comprising a methyleytosine dioxygenase.~~

~~199.—The kit of claim 198, wherein said methyleytosine dioxygenase comprises a ten eleven translocation (TET) enzyme or fragment thereof.~~

~~200.—The kit of claim 196, further comprising a deoxyribonucleic acid (DNA) glucosyltransferase.~~

~~201.—The kit of claim 200, wherein said DNA glucosyltransferase comprises DNA beta-glucosyltransferase.~~

~~202.—The kit of claim 196, further comprise a DNA methyltransferase.~~

~~203.—The kit of claim 202, wherein said DNA methyltransferase comprises DNA methyltransferase 1 (DNMT1).~~

~~204.—The kit of claim 196, wherein said helicase comprises an amino acid sequence that is at least 90% homologous to UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~205.—The kit of claim 204, wherein said helicase is a UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PerA helicase, or a fragment thereof.~~

~~206.—A method, comprising:~~

~~——(a) contacting a polynucleotide comprising a base with one or more reagents that collectively transform said base to an altered base derived from said base, thereby generating a modified polynucleotide comprising said altered base; and~~

~~(b) sequencing at least a portion of said modified polynucleotide comprising said altered base or derivative thereof to obtain sequencing data with a coverage of no more than 10 fold; and~~

~~——(c) processing said sequencing data to identify said base with an accuracy of at least about 90%.~~

~~207.—The method of claim 206, wherein said modified polynucleotide or derivative thereof is a double-stranded polynucleotide.~~

~~208.—The method of claim 207, wherein said sequencing comprises sequencing at least a portion of both strands of said double-stranded polynucleotide.~~

~~209.—The method of claim 206, further comprising processing said sequencing data to identify said base with an accuracy of at least about 95%.~~

~~210.—The method of claim 209, further comprising processing said sequencing data to identify said base with an accuracy of at least about 99%.~~

~~211.—The method of claim 206, wherein said base is a cytosine base and (c) comprises processing said sequencing data to identify said cytosine base as cytosine with said accuracy of at least about 90%.~~

~~212.—The method of claim 211, wherein said cytosine base is a methylated cytosine base.~~

~~213.—The method of claim 212, further comprising processing said sequencing data to identify said methylated base as cytosine with an accuracy of at least about 95%.~~

~~214.—The method of claim 213, further comprising processing said sequencing data to identify said methylated base as cytosine with an accuracy of at least about 99%.~~

~~215.—The method of claim 206, wherein said one or more reagents comprise an oxidizing agent.~~

~~216.—The method of claim 206, wherein said one or more reagents comprise a DNA-glucosyltransferase.~~

~~217.—The method of claim 206, wherein said one or more reagents comprise a deaminase.~~

~~218.—The method of claim 206, wherein said one or more reagents comprise a helicase.~~

~~219.—The method of claim 206, wherein said one or more reagents comprise a DNA-methyltransferase.~~

~~220.—The method of claim 206, further comprising, prior to (a), providing a sample double-stranded polynucleotide comprising a forward strand comprising said polynucleotide and a reverse strand.~~

~~221.—The method of claim 220, further comprising, prior to (a), separating said forward strand from said reverse strand.~~

~~222.—The method of claim 221, wherein said separating comprises using said forward strand in a nucleic acid extension reaction that generates a double-stranded polynucleotide comprising said polynucleotide.~~

~~223.—The method of claim 222, wherein said methylated base is a methylated cytosine base.~~

~~224.—The method of claim 223, wherein said one or more reagents comprise a deaminase and a helicase.~~

~~225.—The method of claim 224, wherein said sequencing comprises sequencing at least a portion of both strands of said double stranded polynucleotide or double stranded derivative thereof.~~

~~226.—The method of claim 225, further comprising processing said sequencing data to identify said methylated cytosine base as cytosine with an accuracy of at least about 95%.~~

~~227.—The method of claim 226, further comprising processing said sequencing data to identify said methylated cytosine base as cytosine with an accuracy of at least about 99%.~~

~~228.—The method of claim 221, wherein said polynucleotide is derived from a population of polynucleotides, and wherein a base frequency of said methylated cytosine base in said population of polynucleotides is less than or equal to 50% at a given locus.~~

~~229.—The method of claim 223, wherein said methylated cytosine base comprises a methyleytosine base or a hydroxymethyleytosine base.~~

~~230.—The method of claim 229, wherein said methylated cytosine base comprises a methyleytosine base and said one or more reagents comprise an oxidating agent, a DNA glucosyltransferase, a deaminase and a helicase.~~

~~231.—The method of claim 229, wherein said methylated cytosine base comprises a hydroxymethyleytosine base and said one or more reagents comprise an oxidating agent, a DNA glucosyltransferase, a methyltransferase, a deaminase and a helicase.~~

~~232.—The method of claim 229, wherein said sequencing comprises sequencing at least a portion of both strands of said double stranded polynucleotide or double stranded derivative thereof.~~

~~233.—The method of claim 232, further comprising processing said sequencing data to identify said methylated cytosine base as methyleytosine or hydroxymethyleytosine with an accuracy of at least about 95%.~~

~~234.—The method of claim 233, further comprising processing said sequencing data to identify said methylated cytosine base as methyleytosine or hydroxymethyleytosine with an accuracy of at least about 99%.~~

~~235.—The method of claim 208, wherein said polynucleotide is a double stranded polynucleotide, with strands covalently linked via a hairpin.~~

~~236.—The method of claim 220, wherein said forward strand and said reverse strand are covalently linked via a hairpin.~~

26. The method of claim 1, further comprising using the sequencing data to diagnose a condition in a subject.

27. The method of claim 26, wherein the condition is a cancer or a neurodegenerative condition.