

CLAIMS

WHAT IS CLAIMED IS:

1. A method, comprising:

- (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.

5. The method of claim 4, further comprising, prior to (a), conducting one or more chemical reactions or one or more enzymatic reactions on said polynucleotide to generate said double-stranded polynucleotide.

6. The method of claim 5, further comprising conducting a nucleic acid extension reaction using said polynucleotide.

7. The method of claim 5, further comprising contacting said polynucleotide with an entity having DNA methyltransferase activity.

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).

9. The method of claim 5, further comprising contacting said polynucleotide with an oxidizing agent.

10. The method of claim 9, wherein said oxidizing agent is a methylcytosine dioxygenase.

11. The method of claim 10, wherein said methylcytosine dioxygenase is a ten-eleven translocation (TET) enzyme.
12. The method of claim 5, further contacting said polynucleotide with an glycosylation agent.
13. The method of claim 5, wherein said polynucleotide comprises a methylated base.
14. The method of claim 13, further comprising conducting an oxidation reaction and a glycosylation reaction to generate said double-stranded polynucleotide.
15. The method of claim 14, wherein (c) comprises processing said sequencing data to identify said 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 95%.
17. The method of claim 5, wherein said polynucleotide comprises a methyl cytosine base or a hydroxymethyl cytosine base.
18. The method of claim 17, further comprising conducting a methyltransferase reaction, an oxidation reaction and a glycosylation reaction to generate said double-stranded polynucleotide.
19. The method of claim 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.
20. The method of claim 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 95%.
21. The method of claim 1, wherein said base is a 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 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%.
23. The method of claim 1, wherein said deaminase is an apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) enzyme or fragment thereof.
24. The method of claim 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 fragment thereof.
25. The method of claim 24, wherein said helicase is a UvrD helicase, *Geobacillus sterothermophilus* Bad protein, a PcrA helicase or fragment thereof.

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.