i. adding a blocking group to the 5-hydroxymethylcytosine (5hmC) in the nucleic acid sample;
ii. converting the 5mC in the nucleic acid sample to 5-carboxylcytosine (5caC) and/or 5-formylcytosine (5fC); and
iii. converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide a modified nucleic acid sample comprising a modified target nucleic acid; and

c. detecting the sequence of the modified target nucleic acid; wherein a cytosine (C) to thymine (T) transition in the sequence of the modified target nucleic acid compared to the target nucleic acid provides the location of a 5mC in the target nucleic acid.

[0012] In examples of the method for identifying 5mC in a target nucleic acid, the percentages of the T at each transition location provide a quantitative level of 5mC at each location in the target nucleic acid. In examples, the nucleic acid is DNA. In other examples, nucleic acid is RNA.

[0013] In another example, the present disclosure provides a method for identifying 5mC or 5hmC in a target nucleic acid comprising the steps of:

a. providing a nucleic acid sample comprising the target nucleic acid;
b. modifying the nucleic acid comprising the steps of:
i. converting the 5mC and 5hmC in the nucleic acid sample to 5-carboxylcytosine (5caC) and/or 5fC; and
ii. converting the 5caC and/or 5fC to DHU to provide a modified nucleic acid sample comprising a modified target nucleic acid; and
c. detecting the sequence of the modified target nucleic acid; wherein a cytosine (C) to thymine (T) transition in the sequence of the modified target nucleic acid compared to the target nucleic acid provides the location of either a 5mC or 5hmC in the target nucleic acid.

[0013a] In one aspect, the present disclosure refers to a method for identifying 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC) in a target nucleic acid comprising the steps of: providing a nucleic acid sample comprising the target nucleic acid; modifying the target nucleic acid comprising the steps of: converting the 5mC and 5hmC in the nucleic acid sample to 5-carboxylcytosine (5caC) and/or 5-formylcytosine (5fC) by contacting the nucleic acid sample with a ten-eleven translocation (TET) enzyme so that one or more 5caC or 5fC residues are generated; and converting the 5caC and/or 5fC to dihydrouracil (DHU) by treating the target nucleic acid with a borane reducing agent that reduces 5caC and/or 5fC to DHU to provide a modified nucleic acid sample comprising a modified target nucleic acid; and
detecting the sequence of the modified target nucleic acid, wherein the detecting step comprises detecting the presence of DHU in the sequence, or converting the DHU to thymine (T) by replication with a polymerase and detecting the presence of the thymine (T) in the sequence; wherein a cytosine (C) to thymine (T) transition or a cytosine (C) to DHU transition in the sequence of the modified target nucleic acid compared to the target nucleic acid provides the location of either a 5mC or 5hmC in the target nucleic acid.

[0014] In examples of the method for identifying 5mC or 5hmC, the percentages of the T at each transition location provide a quantitative level of 5mC or 5hmC at each location in the target nucleic acid. In examples, the nucleic acid is DNA. In other examples, nucleic acid is RNA.

[0015] In another example, the disclosure provides a method for identifying 5mC and identifying 5hmC in a target nucleic acid comprising:

a. identifying 5mC in the target nucleic acid comprising the steps of:
i. providing a first nucleic acid sample comprising the target nucleic acid;

d. optionally amplifying the copy number of the modified target nucleic acid; and
e. determining the sequence of the modified target nucleic acid; wherein a cytosine (C) to thymine (T) transition in the sequence of the modified target nucleic acid compared to the target nucleic acid provides the location of a 5caC in the target nucleic acid.

[0022] In examples of the method for identifying 5caC in a target nucleic acid, the percentages of the T at each transition location provide a quantitative level for 5caC at each location in the target nucleic acid. In examples, the nucleic acid is DNA. In other examples, nucleic acid is RNA.

[0023] In examples of the disclosure, adding a blocking group to the 5fC in the nucleic acid sample comprises contacting the nucleic acid with an aldehyde reactive compound including, for example, hydroxylamine derivatives (such as O-ethylhydroxylamine), hydrazine derivatives, and hydrazide derivatives.

[0024] In another example, the disclosure provides a method for identifying 5fC in a target nucleic acid comprising the steps of:

a. providing a nucleic acid sample comprising the target nucleic acid;
b. adding a blocking group to the 5caC in the nucleic acid sample
c. converting the 5fC to DHU to provide a modified nucleic acid sample comprising a modified target nucleic acid;
d. optionally amplifying the copy number of the modified target nucleic acid;
e. detecting the sequence of the modified target nucleic acid; wherein a cytosine (C) to thymine (T) transition in the sequence of the modified target nucleic acid compared to the target nucleic acid provides the location of a 5fC in the target nucleic acid.

[0025] In examples of the method for identifying 5fC in a target nucleic acid, the percentages of the T at each transition location provide a quantitative level for 5fC at each location in the target nucleic acid. In examples, the nucleic acid (sample and target) is DNA. In other examples, nucleic acid (sample and target) is RNA.

[0025a] In one aspect, the present disclosure refers to a method for chemically modifying a nucleic acid sample comprising the steps of: providing a nucleic acid sample comprising 5-carboxylcytosine (5caC) and/or 5-formylcytosine (5fC); and converting the 5caC and/or 5fC to dihydrouracil (DHU) by treating the nucleic acid with a borane reducing agent that reduces 5caC and/or 5fC to DHU to provide a modified nucleic acid sample comprising a modified nucleic acid.

[0026] In examples, the step of adding a blocking group to the 5caC in the nucleic acid sample comprises contacting the nucleic acid sample with a carboxylic acid derivatization reagent, including, for example, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and (ii) an amine (such as ethylamine), hydrazine, or hydroxylamine compound.

Claims:

1. A method for identifying 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC) in a target nucleic acid comprising the steps of:

providing a nucleic acid sample comprising the target nucleic acid;

modifying the target nucleic acid comprising the steps of:

converting the 5mC and 5hmC in the nucleic acid sample to 5-carboxylcytosine (5caC) and/or 5-formylcytosine (5fC) by contacting the nucleic acid sample with a ten-eleven translocation (TET) enzyme so that one or more 5caC or 5fC residues are generated; and

converting the 5caC and/or 5fC to dihydrouracil (DHU) by treating the target nucleic acid with a borane reducing agent that reduces 5caC and/or 5fC to DHU to provide a modified nucleic acid sample comprising a modified target nucleic acid; and

detecting the sequence of the modified target nucleic acid, wherein the detecting step comprises detecting the presence of DHU in the sequence, or converting the DHU to thymine (T) by replication with a polymerase and detecting the presence of the thymine (T) in the sequence;

wherein a cytosine (C) to thymine (T) transition or a cytosine (C) to DHU transition in the sequence of the modified target nucleic acid compared to the target nucleic acid provides the location of either a 5mC or 5hmC in the target nucleic acid.

2. The method of claim 1, wherein the borane reducing agent is 2-picoline borane.

3. The method of claim 1 or claim 2, wherein the step of detecting the sequence of the modified target nucleic acid comprises one or more of chain termination sequencing, microarray, high-throughput sequencing, and restriction enzyme analysis.

4. The method of any one of claims 1-3, wherein the TET enzyme is selected from the group consisting of human TET1, TET2, and TET3; murine TET1, TET2, and TET3; *Naegleria* TET (NgTET); and *Coprinopsis cinerea* TET (CcTET).

5. The method of any one of claims 1-4, further comprising a step of blocking one or more modified cytosines.

6. The method of claim 5, wherein the step of blocking comprises adding a sugar to a 5hmC.

7. The method of any one of claims 1-6, wherein the method further comprises a step of amplifying the copy number of one or more nucleic acid sequences.

8. A method for chemically modifying a nucleic acid sample comprising the steps of:

providing a nucleic acid sample comprising 5-carboxylcytosine (5caC) and/or 5-formylcytosine (5fC); and

converting the 5caC and/or 5fC to dihydrouracil (DHU) by treating the nucleic acid with a borane reducing agent that reduces 5caC and/or 5fC to DHU to provide a modified nucleic acid sample comprising a modified nucleic acid.

9. The method of claim 8, wherein the borane reducing agent is selected from the group consisting of 2-picoline borane (pic-BH3), borane, sodium borohydride, sodium cyanoborohydride, and sodium triacetoxyborohydride.

10. The method of claim 8, wherein the borane reducing agent is 2-picoline borane.

11. The method of any one of claims 8-10, further comprising the step of detecting the sequence of the modified nucleic acid.

12. The method of claim 11, wherein the step of detecting the sequence of the modified nucleic acid comprises one or more of chain termination sequencing, microarray, high-throughput sequencing, and restriction enzyme analysis.

13. The method of claim 11 or claim 12, wherein the sequencing step provides a quantitative level of one or more cytosine modifications in the nucleic acid sample.

14. The method of any one of claims 8-13, wherein the method further comprises a step of amplifying the copy number of one or more nucleic acid sequences.

15. The method of any one of claims 1-14, wherein the nucleic acid sample is a DNA sample.