

Claims:

1. ~~— A method for identifying 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC) in a DNA sample comprising the steps of:~~
 - a. ~~— providing a DNA sample comprising target DNA having 5mC and/or 5hmC;~~
 - b. ~~— modifying the DNA sample comprising the steps of:~~
 - i. ~~— converting the 5mC and 5hmC in the DNA sample to 5-carboxyleytosine (5caC) and/or 5-formyleytosine (5fC); and~~
 - ii. ~~— converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide a modified DNA sample comprising modified target DNA;~~
 - c. ~~— cleaving the modified target DNA;~~
 - d. ~~— adding adapter DNA molecules to the cleaved modified target DNA; and~~
 - e. ~~— detecting the sequence of the modified target DNA;~~

~~wherein the presence of a cleavage site provides the location of either a 5mC or 5hmC in the target DNA.~~
2. ~~— A method for identifying 5mC in a DNA sample comprising the steps of:~~
 - a. ~~— providing a DNA sample comprising target DNA having 5mC;~~
 - b. ~~— modifying the DNA sample comprising the steps of:~~
 - i. ~~— adding a blocking group to the 5hmC in the DNA sample;~~
 - ii. ~~— converting the 5mC in the DNA sample to 5caC and/or 5fC; and~~
 - iii. ~~— converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide a modified DNA sample comprising modified target DNA;~~
 - c. ~~— cleaving the modified target DNA;~~
 - d. ~~— adding adapter DNA molecules to the cleaved modified target DNA; and~~
 - e. ~~— detecting the sequence of the modified target DNA;~~

~~wherein the presence of a cleavage site provides the location of a 5mC in the target DNA.~~

3. ~~— A method for identifying 5mC and identifying 5hmC in a DNA sample comprising the steps of:~~
 - a. ~~— identifying 5-methylcytosine (5mC) in the DNA sample comprising the steps of:~~
 - i. ~~— providing a first DNA sample comprising target DNA having 5mC and/or 5hmC;~~
 - ii. ~~— modifying the DNA in the first sample comprising the steps of:~~
 1. ~~— adding a blocking group to the 5-hydroxymethylcytosine (5hmC) in the first DNA sample;~~
 2. ~~— converting the 5mC in the first DNA sample to 5caC and/or 5fC; and~~
 3. ~~— converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide a modified first DNA sample comprising modified target DNA;~~
 - iii. ~~— cleaving the modified target DNA;~~
 - iv. ~~— adding adapter DNA molecules to the cleaved modified target DNA; and~~
 - v. ~~— detecting the sequence of the modified target DNA; wherein the presence of a cleavage site provides the location of a 5mC in the target DNA.~~
 - b. ~~— identifying 5mC or 5hmC in the DNA sample comprising the steps of:~~
 - i. ~~— providing a second DNA sample comprising the target DNA;~~
 - ii. ~~— modifying the DNA in the second sample comprising the steps of:~~
 1. ~~— converting the 5mC and 5hmC in the second DNA sample to 5caC and/or 5fC; and~~
 2. ~~— converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide a modified second DNA sample comprising modified target DNA;~~

- iii. ~~cleaving the modified target DNA;~~
 - iv. ~~adding adapter DNA molecules to the cleaved modified target DNA;~~
 - ~~and~~
 - v. ~~detecting the sequence of the modified target DNA from the second sample; wherein the presence of a cleavage site provides the location of either a 5mC or 5hmC in the target DNA; and~~
 - e. ~~comparing the results of steps (a) and (b), wherein a cleavage site present in step (b) but not in step (a) provides the location of 5hmC in the target DNA.~~
4. ~~The method according to claims 2 or 3, wherein the blocking group added to the 5hmC is a sugar.~~
 5. ~~The method of claim 4, wherein the sugar is glucose or a modified glucose.~~
 6. ~~The method of claim 5, wherein the blocking group is added to the 5hmC by contacting the DNA sample with uridine diphosphate (UDP) linked to a sugar in the presence of a glucosyltransferase enzyme.~~
 7. ~~The method of claim 6, wherein the glucosyltransferase enzyme is selected from the group consisting of T4 bacteriophage β -glucosyltransferase (β GT), T4 bacteriophage α -glucosyltransferase (α GT), and derivatives and analogs thereof.~~
 8. ~~The method according to any one of claims 1 to 7, wherein the step of converting the 5mC in the DNA sample to 5caC and/or 5fC and/or the step of converting the 5mC and 5hmC in the DNA sample to 5caC and/or 5fC comprises contacting the DNA sample with a ten-eleven translocation (TET) enzyme.~~
 9. ~~The method of claim 8, wherein the TET enzyme is selected from the group consisting of human TET1, TET2, and TET3; murine Tet1, Tet2, and Tet3; *Naegleria* TET (NgTET); *Coprinopsis cinerea* (CcTET) and derivatives or analogues thereof.~~
 10. ~~The method of claim 9, wherein the TET enzyme is NgTET, murine TET2, human TET1, or derivatives thereof.~~
 11. ~~A method for identifying 5caC or 5fC in a DNA sample comprising the steps of:~~
 - a. ~~providing a DNA sample comprising target DNA having 5caC and/or 5fC;~~

- b. ~~converting the 5caC and 5fC to dihydrouracil (DHU) to provide a modified DNA sample comprising modified target DNA;~~
 - e. ~~cleaving the modified target DNA;~~
 - d. ~~adding adapter DNA molecules to the cleaved modified target DNA; and~~
 - e. ~~detecting the sequence of the modified target DNA;~~
- wherein the presence of a cleavage site provides the location of either a 5caC or 5fC in the target DNA.

12. ~~A method for identifying 5caC in a DNA sample comprising the steps of:~~

- a. ~~providing a DNA sample comprising target DNA having 5caC;~~
 - b. ~~adding a blocking group to the 5fC in the DNA sample~~
 - e. ~~converting the 5caC to dihydrouracil (DHU) to provide a modified DNA sample comprising modified target DNA;~~
 - d. ~~cleaving the modified target DNA;~~
 - e. ~~adding adapter DNA molecules to the cleaved modified target DNA; and~~
 - f. ~~detecting the sequence of the modified target DNA;~~
- wherein the presence of a cleavage site provides the location of a 5caC in the target DNA.

13. ~~The method of claim 12, wherein the step of adding a blocking group to the 5fC comprises contacting the DNA sample with an aldehyde reactive compound selected from hydroxylamine derivatives, hydrazine derivatives, and hydrazide derivatives.~~

14. ~~The method of claim 13, wherein the hydroxylamine derivative is O-ethylhydroxylamine.~~

15. ~~A method for identifying 5fC in a DNA sample comprising the steps of:~~

- a. ~~providing a DNA sample comprising target DNA having 5fC;~~
- b. ~~adding a blocking group to the 5caC in the DNA sample;~~
- e. ~~converting the 5fC to dihydrouracil (DHU) to provide a modified DNA sample comprising modified target DNA;~~

- d. ~~cleaving the modified target DNA;~~
 - e. ~~adding adapter DNA molecules to the cleaved modified target DNA; and~~
 - f. ~~detecting the sequence of the modified target DNA;~~
- ~~wherein the presence of a cleavage site provides the location of a 5fC in the target DNA.~~

16. ~~The method of claim 15, wherein the step of adding a blocking group to 5caC comprises contacting the DNA sample with a carboxylic acid derivatization reagent, and an amine, hydrazine or hydroxylamine compound.~~

17. ~~The method of claim 16, wherein the step of adding a blocking group to 5caC comprises contacting the DNA sample with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and with ethylamine.~~

18. ~~A method for identifying 5hmC in a DNA sample comprising:~~

- a. ~~providing a DNA sample comprising target DNA having 5hmC;~~
- b. ~~modifying the DNA in the sample comprising the steps of:~~
 - i. ~~converting the 5hmC in the DNA sample to 5caC and/or 5fC; and~~
 - ii. ~~converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide a modified DNA sample comprising modified target DNA;~~
- e. ~~cleaving the modified target DNA;~~
- d. ~~adding adapter DNA molecules to the cleaved modified target DNA; and~~
- e. ~~detecting the sequence of the modified target DNA;~~

~~wherein the presence of a cleavage site provides the location of 5hmC in the target DNA.~~

19. ~~The method of claim 17, wherein the step of converting the 5hmC to 5caC and/or 5fC comprises contacting the DNA sample with an oxidizing agent.~~

20. ~~The method of claim 19, wherein the oxidizing agent is potassium perruthenate, Cu(II)/TEMPO, potassium ruthenate, or manganese oxide.~~

21. ~~The method according to any one of claims 1 to 20, wherein the DNA sample is whole genomic DNA.~~

- ~~22. The method according to any one of claims 1 to 20, further comprising size selection of the cleaved modified target DNA.~~
- ~~23. The method according to any one of claims 1 to 20, wherein the method further comprises the step of amplifying the copy number of the modified target DNA.~~
- ~~24. The method of claim 23, wherein the step of amplifying the copy number of the modified target DNA comprises performing the polymerase chain reaction (PCR) or primer extension.~~
- ~~25. The method according to any one of claims 1 to 20, wherein the step of converting the 5caC and/or 5fC to DHU comprises contacting the DNA sample with a reducing agent.~~
- ~~26. The method of claim 25, wherein the reducing agent is selected from the group consisting of pyridine borane, 2 picoline borane (pic-BH₃), borane, sodium borohydride, sodium cyanoborohydride, and sodium triacetoxyborohydride.~~
- ~~27. The method of claim 26, wherein the reducing agent is pyridine borane or 2 picoline borane.~~
- ~~28. The method according to any one of claims 1 to 27, wherein the step of cleaving the modified target DNA comprises contacting the modified target DNA with one or more endonucleases.~~
- ~~29. The method of claim 28, wherein the one or more endonucleases are selected from one or more of the group consisting of Apurinic/apyrimidinic Endonuclease 1 (APE 1), Uracil DNA glycosylase (UDG), Endonuclease III, *Tma* Endonuclease III, *Tth* Endonuclease IV, Endonuclease V, Endonuclease VIII, Formamidopyrimidine DNA Glycosylase (Fpg), and hNEIL1.~~
- ~~30. The method of claim 28, wherein the endonuclease comprises Uracil Specific Excision Reagent (USER).~~
- ~~31. The method according to any one of claims 1 to 30, wherein the step of detecting the sequence of the modified target DNA comprises a next generation sequencing method.~~
- ~~32.1. A method for cleaving a modified target DNA, the method comprising:~~
converting 5caC and/or 5fC in a target DNA to dihydrouracil (DHU) to provide a

modified target DNA; and

contacting the modified target DNA comprising one or more DHU with one or more endonucleases that cleave the modified target DNA at, or adjacent to, the one or more DHU; wherein ~~the one or more DHU in the modified target DNA are derived from one or more of 5mC, 5hmC, 5caC, and 5fC.~~

~~33. The method of claim 32, wherein the one or more DHU in the modified target DNA are derived from 5mC and/or 5hmC.~~

~~34. The method of claim 32, wherein the one or more DHU in the modified target DNA are derived from 5caC and/or 5fC.~~

~~35.2. The method of claim 331, wherein the method further comprises, prior to the contacting-converting step, modifying a target DNA comprising 5mC and/or 5hmC comprising the steps of: (i) converting the 5mC and/or 5hmC in the target DNA to 5-carboxylcytosine (5caC) and/or 5-formylcytosine (5fC); and (ii) converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide the modified target DNA.~~

~~36.3. The method of claim 332, wherein the method further comprises, prior to the contacting-coverting the 5mC and/or 5hmC step, modifying a the target DNA comprising 5mC and 5hmC comprising the steps of: (i) adding a blocking group to the 5hmC in the target DNA; (ii) converting the 5mC in the target DNA to 5-carboxyleytosine (5caC) and/or 5-formyleytosine (5fC); and (iii) converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide the modified target DNA.~~

~~37.4. The method of claim 363, wherein the blocking group added to the 5hmC is a sugar.~~

~~38. The method of claim 37, wherein the sugar is glucose or a modified glucose.~~

~~39. The method of claim 37, wherein the blocking group is added to the 5hmC by contacting the target DNA with uridine diphosphate (UDP) linked to a sugar in the presence of a glucosyltransferase enzyme.~~

~~40. The method of claim 39, wherein the glucosyltransferase enzyme is selected from the group consisting of T4 bacteriophage β -glucosyltransferase (β GT), T4 bacteriophage α -glucosyltransferase (α GT), and derivatives and analogs thereof.~~

~~41. The method of claim 34, wherein the method further comprises, prior to the contacting step, modifying a target DNA comprising 5caC and/or 5fC comprising the~~

~~step of converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide the modified target DNA.~~

42. ~~The method of claim 34, wherein the method further comprises, prior to the contacting step, modifying a target DNA comprising 5caC and/or 5fC comprising the steps of: (i) adding a blocking group to the 5fC in the target DNA; and (ii) converting the 5caC to dihydrouracil (DHU) to provide the modified target DNA.~~

43. ~~The method of claim 42, wherein the step of adding a blocking group to the 5fC comprises contacting the target DNA with an aldehyde reactive compound selected from hydroxylamine derivatives, hydrazine derivatives, and hydrazide derivatives.~~

44. ~~The method of claim 43, wherein the hydroxylamine derivative is O-ethylhydroxylamine.~~

45. ~~The method of claim 34, wherein the method further comprises, prior to the contacting step, modifying a target DNA comprising 5caC and/or 5fC comprising the steps of: (i) adding a blocking group to the 5caC in the target DNA; and (ii) converting the 5fC to dihydrouracil (DHU) to provide the modified target DNA.~~

46. ~~The method of claim 45, wherein the step of adding a blocking group to 5caC comprises contacting the target DNA with a carboxylic acid derivatization reagent, and an amine, hydrazine or hydroxylamine compound.~~

47. ~~The method of claim 46, wherein the step of adding a blocking group to 5caC comprises contacting the target DNA with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and with ethylamine.~~

48.5. ~~The method according to any one of claims 35-2 to 474, wherein the step of converting the 5mC and/or 5hmC in the target DNA to 5caC and/or 5fC, or the step of converting the 5mC in the target DNA to 5caC and/or 5fC comprises contacting the target DNA with a ten eleven translocation (TET) enzyme.~~

49.6. ~~The method of claim 548, wherein the TET enzyme is selected from the group consisting of human TET1, TET2, and TET3; murine Tet1, Tet2, and Tet3; *Naegleria* TET (NgTET); *Coprinopsis cinerea* (CcTET) and derivatives or analogues thereof.~~

50.7. ~~The method of claim 496, wherein the TET enzyme is NgTET, murine TET2, human TET1, or derivatives thereof.~~

- ~~51.~~—The method of claim ~~33~~, wherein the method further comprises, prior to the contacting step, modifying a target DNA comprising 5mC and/or 5hmC comprising the steps of: (i) converting the 5mC in the target DNA to 5-carboxyleytosine (5caC) and/or 5-formyleytosine (5fC); and (ii) converting the 5caC and/or 5fC to dihydrouracil (DHU) to provide the modified target DNA.
- ~~52.8.~~ The method of claim ~~51~~2 to 4, wherein the step of converting the 5hmC to 5caC and/or 5fC comprises contacting the target DNA with an oxidizing agent.
- ~~53.9.~~ The method of claim ~~52~~8, wherein the oxidizing agent is potassium perruthenate, Cu(II)/TEMPO, potassium ruthenate, or manganese oxide.
- ~~54.10.~~ The method according to any one of claims ~~35~~1 to ~~53~~9, wherein the step of converting the 5caC and/or 5fC to DHU comprises contacting the target DNA with a reducing agent.
- ~~55.11.~~ The method of claim ~~54~~10, wherein the reducing agent is selected from the group consisting of pyridine borane, 2-picoline borane (pic-BH₃), borane, sodium borohydride, sodium cyanoborohydride, and sodium triacetoxymorohydride.
- ~~56.~~—The method of claim ~~55~~, wherein the reducing agent is pyridine borane or 2-picoline borane.
- ~~57.12.~~ The method according to any one of claims ~~32~~1 to ~~56~~11, wherein the one or more endonucleases are selected from one or more of the group consisting of Apurinic/aprimidinic Endonuclease 1 (APE 1), Uracil DNA glycosylase (UDG), Endonuclease III, *Tma* Endonuclease III, *Tth* Endonuclease IV, Endonuclease V, Endonuclease VIII, Formamidopyrimidine DNA Glycosylase (Fpg), and hNEIL1.
- ~~58.13.~~ The method of claim ~~57~~12, wherein the endonuclease comprises Uracil-Specific Excision Reagent (USER).
14. The method of any one of claims 1 to 13, further comprising the step of adding adapter DNA molecules to the cleaved modified target DNA.
15. The method of any one of claims 1 to 14, further comprising detecting the sequence of the cleaved modified target DNA, wherein the presence of a cleavage site provides the location of either a 5caC or 5fC in the target DNA.