CLAIMS

What is claimed is:

1. A method of determining the methylation status of a target nucleic acid molecule in a sample, wherein the method comprises the steps of:
(a) bisulfite treatment of the sample for converting the unmethylated cytosine bases in the target nucleic acid molecule to uracil;
(b) amplifying at least part of the converted target nucleic acid molecule, said part comprising a target region the methylation status of which is to be analyzed, in a polymerase chain reaction (PCR), using a pair of methylation-specific primers under conditions allowing such amplification to generate PCR amplification products (amplicons);
(c) contacting the amplicons with a methylation-specific plasmonic nanoprobe comprising a plasmonic nanoparticle and a non-ionic oligonucleotide analog probe covalently coupled thereto, the oligonucleotide analog probe comprising a base sequence that is complementary to the target region, under conditions that allow the oligonucleotide analog probe and the amplicons comprising the target region to hybridize to each other, wherein the probe generates a detectable signal if hybridized to the target nucleic acid molecule that is distinguishable from the signal of the unhybridized probe;
(d) determining the methylation status of the target nucleic acid molecule based on the determination of the melting temperature T_m of the hybrid of the nanoprobe and the target nucleic acid.

2. The method according to claim 1, wherein the methylation status of one or more CpG islands within the target nucleic acid molecule is determined.

3. The method according to claim 1 or 2, wherein asymmetric PCR (aPCR) is used in step (b) of the method.

4. The method according to any one of claims 1-3, wherein at least one of the methylation-specific primers hybridizes to the converted target nucleic acid molecule at a region comprising one or more CpG islands, such that the primer pair preferably hybridizes to the converted methylated nucleic acid molecule over the converted unmethylated molecule during the PCR.

5. The method according to any one of claims 1-4, wherein a blocker sequence is added during the PCR to minimize the amplification of the converted unmethylated nucleic acid molecule.

6. The method according to any one of claims 1-5, wherein the plasmonic nanoparticle used in step (c) of the method is a plasmonic gold nanoparticle.

7. The method according to any one of claims 1-6, wherein the non-ionic oligonucleotide analog probe used in step (c) of the method is a morpholino oligonucleotide probe.

8. The method according to any one of claims 1-7, wherein the detectable signal in step (c) of the method is the color of the assay solution that is indicative of whether the probe is hybridized to the amplicons or not.

9. The method according to any one of claims 1-8, wherein the melting temperature in step (d) of the method is indicated by a color change caused by nanoprobe dissociation and subsequent aggregation.

10. The method according to any one of claims 1-9, wherein the method further comprises isolating genomic DNA from the sample prior to step (a) of the method.

11. The method according to any one of claims 1-10, wherein the method further comprises using a nucleic acid molecule comprising the unmethylated target nucleotide sequence as a negative control, and/or using a nucleic acid molecule comprising the methylated target nucleotide sequence as a positive control.

12. The method according to any one of claims 1-11, wherein the target nucleic acid molecule is the human Septin 9 (SEPT9) gene promoter, the methylation-specific primers have the nucleic acid sequences 5'-ATTAGTTATTATGTCGGATTTCGC-3' (SEQ ID NO: 1) and 5'-CAACACGTCCGCGACCG-3' (SEQ ID NO: 2), the blocker has the nucleic acid sequence 5'-GTTATTATGTTGGATTTTGTGGTTAATGTGTAG-3' and is labelled with C3 spacer at the 3' end (SEQ ID NO: 3), and the oligonucleotide analog probe used is a morpholino oligonucleotide having the nucleic acid sequence 5'-CAACTACGCGTTAACCGCGATTTTT-3' (SEQ ID NO: 4), preferably wherein the method is used to determine the methylation status of the human Septin 9 (SEPT9) gene promoter, preferably in the cell-free DNA (cfDNA) fragments circulating in the blood released from colorectal cancer cells.

13. Use of a kit in the method according to any one of claims 1-12 for determining the methylation status of a target nucleic acid molecule in a sample, preferably of one or more CpG islands within

said target nucleic acid molecule, wherein the kit comprises a pair of methylation-specific primers and a methylation-specific plasmonic nanoprobe as defined in any one of claims 1-12.

14. The use according to claim 13, wherein the kit further comprises a blocker sequence for use in the method according to any one of claims 1-12.

15. The use according to claim 13 or 14, wherein: (i.) the kit further comprises bisulfite or a salt thereof, and/or (ii.) the target nucleic acid molecule is the human SEPT9 gene promoter, and the kit comprises a pair of methylation-specific primers having the nucleic acid sequences 5'-ATTAGTTATTATGTCGGATTTCGC-3' (SEQ ID NO: 1) and 5'-CAACACGTCCGCGACCG-3' (SEQ ID NO: 2), and a plasmonic gold nanoparticle functionalized with a morpholino oligonucleotide having the nucleic acid sequence 5'-CAACTACGCGTTAACCGCGATTTTT-3' (SEQ ID NO: 4), preferably wherein the kit further comprises a blocker having the nucleic acid sequence 5'-GTTATTATGTTGGATTTTGTGGTTAATGTGTAG-3' and is labelled with C3 spacer at the 3' end (SEQ ID NO: 3).