

CLAIMS

1. An in vitro method of screening for colorectal cancer and/or advanced adenoma in a
5 human subject, the method comprising:

- determining the methylation status of all DMR loci in a DMR set, wherein the DMR set comprises the loci of tables 2, 3 or 4, in a cell-free DNA sample of the human subject,
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- comparing the data obtained with reference values obtained from healthy individuals,
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- diagnosing colorectal cancer and/or advanced adenoma in the human subject if a hyper-methylation in one or more of the loci is detected, as compared to the reference sample.

2. The method of claim 1 wherein the methylation status is determined by methylation specific restriction enzyme quantitative polymerase chain reaction (MSRE-qPCR).
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3. The method of any one of claims 1 to 2 wherein the DMR loci are amplified by the oligonucleotide primer pairs provided in Table 5.

4. The method of any one of claims 1 to 3, wherein the DNA is isolated from blood,
25 plasma or stool of the human subject.

5. The method of any one of claims 1 to 4, wherein the human subject was asymptomatic for colorectal cancer at the time of screening.
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6. The method of any one of claims 1 to 5, wherein the human subject had been previously screened for colorectal cancer.

7. The method of claim 6, wherein the human subject had been screened for colorectal cancer within the last 10 years, within the last 5 years, within the last 4 years, within the last 3 years, within the last 2 years, or within the last year.
- 5 8. The method of claim 6 or 7, wherein a previous screen for colorectal cancer in the human subject had diagnosed the human subject as not having colorectal cancer.
9. The method of claim 8, wherein the previous screen for colorectal cancer that had diagnosed the human subject as not having colorectal cancer was within one year.
- 10 10. The method of claim 8 or 9, wherein the previous screen for colorectal cancer that had diagnosed the human subject as not having colorectal cancer was a colonoscopy.
11. The method of any one of claims 1 to 10, wherein the method includes diagnosis of
15 early stage colorectal cancer, optionally wherein the colorectal cancer is a stage 0, stage I, stage IIA, stage IIB, or stage IIC colorectal cancer.
12. The method of any one of claims 1 to 11, wherein the method includes diagnosis of early stage colorectal cancer, wherein the cancer has not metastasized.
- 20 13. The method of claim 1, wherein methylation status is determined using one or more methods selected from the group consisting of methylation sensitive restriction enzyme quantitative polymerase chain reaction (MSRE-qPCR), Methylation-Specific PCR, Methylation Specific Nuclease-assisted Minor-allele Enrichment PCR, and next-
25 generation sequencing.
14. A kit comprising:
(a) primer pairs for the detection of the methylation status of each of the DMR loci in one DMR set as defined in tables 2, 3 or 4 wherein the primers pairs are those of table 5,
30 the kit optionally further comprising:
(b) at least one methylation specific restriction enzyme and/or a bisulfite reagent
(c) a polymerase
15. The method of any one of claims 1 to 13, the diagnosis further comprising
35 determining the presence or absence of an oncogenic mutation.

16. The method of claim 15, wherein the oncogenic mutation is in a gene selected from the group of genes consisting of Kras, NRAS, PIK3CA, PTEN, TP53, BRAF, and APC gene.