CLAIMS

1. A method for profiling genetic and epigenetic characteristics of a cell-free DNA (cfDNA) sample from a subject, the method comprising:

(a) subjecting the cell-free DNA sample to digestion with at least one methylation-sensitive restriction endonuclease, to obtain restriction endonuclease-treated DNA in which methylated sites are intact and unmethylated sites are cut;

(b) preparing a sequencing library from the restriction endonuclease-treated DNA while preserving the sequence information at the ends of the DNA molecules, wherein preparing the sequencing library comprises ligating sequencing adapters to DNA molecules in the restriction endonuclease-treated DNA, wherein each adapter is capable of ligation to both the digested and undigested DNA molecules;

(c) sequencing the sequencing library by a high-throughput sequencing method to provide sequencing data; and

(d) determining from the sequencing data a methylation value for at least one restriction locus,

wherein an amount of cell-free DNA comprising 3000 haploid equivalents is sufficient for the method, and wherein the cell-free DNA sample is not subjected to amplification prior to library preparation.

2. The method of claim 1, wherein step (d) further comprises determining from the sequencing data at least one additional genetic or epigenetic characteristic of the cell-free DNA sample selected from DNA mutation, copy number variation and nucleosome positioning, wherein determining the methylation value and the at least one additional genetic or epigenetic characteristic of the cell-free DNA sample is carried out based on the same sequencing data.

3. The method of claim 1 or claim 2, wherein an amount of cell-free DNA comprising 6,000 haploid equivalents is sufficient for the method.

4. The method of any one of claims 1 – 3, wherein the cell-free DNA is plasma cell-free DNA, and wherein the amount of the cell-free DNA is an amount obtained from 9-10 ml of blood.

5. The method of any one of claims 1 – 4, wherein:

i) the amount of cell-free DNA is between 10-200 ng; and/or

ii) the amount of cell-free DNA is between 20-100 ng.

6. The method of any one of claims 1 – 5, wherein the at least one methylation-sensitive restriction endonuclease produces non-blunt ends, and the method further comprises subjecting the restriction endonuclease-treated DNA to end repair prior to the ligation of sequencing adapters, to obtain DNA molecules with blunt ends.

7. The method of any one of claims 1 – 6, wherein the high-throughput sequencing is:

i) whole genome high-throughput sequencing; and/or

ii) target-specific high-throughput sequencing.

8. The method of any one of claims 1 – 7, wherein determining a methylation value for at least one restriction locus comprises:

(i) selecting at least one restriction locus and determining the number of sequence reads covering a predefined genomic region of at least 50 bps in length that contains said restriction locus; and

(ii) calculating a methylation value for the at least one restriction locus based on the read count determined in step (i) and a reference read count.

9. The method of claim 8, wherein step (i) comprises determining the number of sequence reads covering a predefined genomic region of at least 100 bps in length that contains said restriction locus.

10. The method of any one of claims 1 – 9, wherein the at least one restriction locus is a plurality of restriction loci.

11. The method of any one of claims 1 – 10, wherein the at least one methylation-sensitive restriction endonuclease is a plurality of methylation-sensitive restriction endonucleases, and wherein the digestion with the plurality of methylation-sensitive restriction endonucleases is a simultaneous digestion.

12. The method of claim 11, wherein:

i) the plurality of methylation-sensitive restriction endonucleases comprises HinP1I; or

ii) the plurality of methylation-sensitive restriction endonucleases comprises AciI; or

iii) the digestion is carried out using HinP1I and AciI.

13. The method of any one of claims 1 – 12, wherein the step of subjecting the cell-free DNA sample to digestion with at least one methylation-sensitive restriction endonuclease further comprises determining digestion efficacy, and proceeding to preparing a sequencing library if the digestion efficacy is above a predefined threshold.

14. A method for detecting cancer-related genetic and epigenetic changes in a cell-free DNA sample (cfDNA) from a subject, the method comprising: profiling methylation and optionally at least one additional genetic and epigenetic characteristics of the cfDNA sample according to any one of claims 1-13, to obtain a genetic and epigenetic profile of the cfDNA sample; and comparing the genetic and epigenetic profile of the cfDNA sample to one or more reference genetic and epigenetic profile selected from a cancer profile and a non-cancer profile, to detect cancer-associated genetic and epigenetic changes in the cfDNA sample.