

SUMMARY

[0007a] In one ~~aspect~~example, there is ~~provided~~disclosed a method of analyzing a biological sample of an organism, the biological sample including nucleic acid molecules originating from normal cells and potentially from cells associated with cancer, wherein the biological sample is harvested from a process to obtain nucleic acid molecules that are cell-free in the biological sample, the method comprising: analyzing a plurality of DNA molecules from the biological sample, wherein analyzing a DNA molecule includes: determining a location of the DNA molecule in a genome of the organism; determining whether the DNA molecule is methylated at one or more sites; for each of a plurality of sites: determining a respective number of DNA molecules that are methylated at the site; calculating a first methylation level based on the respective numbers of DNA molecules methylated at the plurality of sites; comparing the first methylation level to a first cutoff value; determining a first classification of a level of cancer based on the comparison.

[0007b] In another aspect, there is provided a method of determining a first methylation profile from a biological sample of an organism, the biological sample including cell-free DNA comprising a mixture of cell-free DNA molecules originating from a first tissue and from a second tissue, the method comprising: obtaining a second methylation profile corresponding to DNA molecules of the second tissue, the second methylation profile providing a second methylation density at each of a plurality of loci in a genome of the organism, the second methylation density at a particular locus corresponding to a proportion of DNA molecules of the second tissue that are methylated; determining a cell-free methylation profile from the cell-free DNA molecules of the mixture, the cell-free methylation profile providing a mixture a methylation density at each of the plurality of loci; determining a percentage of the cell-free DNA molecules in the mixture that are from the first tissue; and determining the first methylation profile of the first tissue by: for each of the plurality of loci: calculating a differential parameter that includes a difference between the second methylation density of the second methylation profile and the mixture methylation density of the cell-free methylation profile, the difference being scaled by the percentage.

[0007c] In another aspect, there is provided a method of determining a first methylation profile from a biological sample of an organism, the biological sample including cell-free DNA comprising a mixture of cell-free DNA originating from a first tissue and from a second tissue, the method comprising: analyzing a plurality of DNA molecules from the biological sample, wherein analyzing a DNA molecule includes: determining a location of the DNA molecule in a genome of the organism; determining a genotype of the DNA molecule; determining whether the

DNA fragments, where reference values for the size parameter can be used to determine methylation levels. In one aspect, the present invention refers to a method of analyzing a biological sample of an organism, the biological sample including nucleic acid molecules originating from normal cells and potentially from cells associated with cancer, wherein the biological sample is harvested from a process to obtain nucleic acid molecules that are cell-free in the biological sample, the method comprising analyzing a plurality of DNA molecules from the biological sample, wherein analyzing a DNA molecule includes determining a location of the DNA molecule in a genome of the organism; and determining whether the DNA molecule is methylated at one or more sites; for each of a plurality of sites: determining a respective number of DNA molecules that are methylated at the site; calculating a first methylation level based on the respective numbers of DNA molecules methylated at the plurality of sites; comparing the first methylation level to a first cutoff value; and determining a first classification of a level of cancer based on the comparison.

[0009] Additionally, a methylation level can be used to determine a level of cancer. In the context of cancer, the measurement of the methylomic changes in plasma can allow one to detect the cancer (e.g. for screening purposes), for monitoring (e.g. to detect response following anti-cancer treatment; and to detect cancer relapse) and for prognostication (e.g. for measuring the load of cancer cells in the body or for staging purposes or for assessing the chance of death from disease or disease progression or metastatic processes).

[0010] A better understanding of the nature and advantages of embodiments of the present invention may be gained with reference to the following detailed description and the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1A shows a table 100 of sequencing results for maternal blood, placenta, and maternal plasma according to embodiments of the present invention.

[0012] FIG. 1B shows methylation density in 1-Mb windows of sequenced samples according to embodiments of the present invention.

[0013] FIGS. 2A-2C show plots of the beta-values against the methylation indices: (A) Maternal blood cells, (B) Chorionic villus sample, (C) Term placental tissue.

WHAT IS CLAIMED IS:

1. A method of analyzing a biological sample of an organism, the biological sample including nucleic acid molecules originating from normal cells and potentially from cells associated with cancer, wherein the biological sample is harvested from a process to obtain nucleic acid molecules that are cell-free in the biological sample, the method comprising:

analyzing a plurality of DNA molecules from the biological sample, wherein analyzing a DNA molecule includes:

determining a location of the DNA molecule in a genome of the organism;

and

determining whether the DNA molecule is methylated at one or more sites;
for each of a plurality of sites:

determining a respective number of DNA molecules that are methylated at the site;

calculating a first methylation level based on the respective numbers of DNA molecules methylated at the plurality of sites;

comparing the first methylation level to a first cutoff value; and

determining a first classification of a level of cancer based on the comparison.

2. The method of claim 1, wherein determining whether the DNA molecule is methylated at one or more sites includes:

performing methylation-aware sequencing.

3. The method of claim 2, wherein performing methylation-aware sequencing includes:

treating the DNA molecules with sodium bisulfite; and

performing sequencing of the treated DNA molecules.

4. The method of claim 3, wherein the treatment of the cell-free DNA molecules with sodium bisulfite is part of Tet-assisted bisulfite conversion or oxidative bisulfite sequencing for the detection of 5-hydroxymethylcytosine.

5. The method of claim 2, wherein the respective numbers of DNA molecules for each region are determined by aligning sequence reads obtained from the methylation-aware sequencing.

17. The method of claim 15, wherein the clusters include a first cluster corresponding to the first type of cancer, a second cluster corresponding to the second type of cancer, and a third cluster corresponding to absence of cancer.

18. The method of claim 15, wherein the clustering of the other organisms is further based on respective normalized values of a second set of regions for the other organisms, the second set of regions correspond to regions that are determined to exhibit a deletion or an amplification, wherein the respective normalized value for a region is determined from a respective number of DNA molecules from the region, the method further comprising:

for each of the second set of regions:

determining a respective number of DNA molecules as being from the region;

and

calculating a respective normalized value from the respective number; and

comparing the respective normalized values of the second set of regions for the organism to the respective normalized values of the other organisms as part of determining which cluster the organism belongs.

19. The method of claim 18, wherein the clustering of the other organisms is further based on respective methylation densities of hypermethylated CpG islands, the method further comprising:

for each of the hypermethylated CpG islands:

determining a respective methylation density; and

comparing the respective methylation densities of the hypermethylated CpG islands for the organism to the methylation densities of the other organisms as part of determining which cluster the organism belongs.

20. The method of claim 13, further comprising:

for each of the second plurality of regions:

computing a respective difference between the region methylation level and the respective region cutoff value; and

calculating a respective probability corresponding to the respective difference;

wherein determining the second amount of regions includes:

calculating a cumulative score including the respective probabilities.

21. The method of claim 20, wherein calculating the cumulative score includes: