

have to be identified before cancer can be detected. Thus, only screening for well-known point mutations is possible for screening, or the specific point mutations for a patient must be identified through previous invasive biopsied for a previously detector tumor.

SUMMARY

5 [0006] Examples can analyze cell-free DNA for disease detection (e.g., cancer) of a particular organ. Some examples may use an organ-associated sample that is from a particular organ or passes through the particular organ, as may occur, for example, in urine, saliva, blood, and stool samples. The analysis may be performed in various ways.

10 [0006a] In one aspect, the present disclosure refers to a method of analyzing a biological sample of an organism, the biological sample being a urine sample, the biological sample including a mixture of cell-free DNA molecules from a plurality of tissues types, the method comprising:

identifying N genomic sites, N being an integer greater than or equal to 10;

for each of M tissue types:

15 obtaining N tissue-specific methylation levels at the N genomic sites, N being greater than or equal to M, wherein the tissue-specific methylation levels form a matrix A of dimensions N by M, wherein one of the M tissue types is a first diseased tissue type corresponding to a first disease of a first organ, wherein the first diseased tissue type is cancerous urothelium cells, and wherein a second tissue type of the M tissue types is normal urothelium;

20 analyzing, by a computer system, a plurality of cell-free DNA molecules from the biological sample, the plurality of cell-free DNA molecules being at least 1,000 cell-free DNA molecules, wherein analyzing a cell-free DNA molecule includes:

identifying a location of the cell-free DNA molecule in a reference genome corresponding to the organism;

25 identifying a set of the plurality of cell-free DNA molecules that are each located at any one of the N genomic sites,

measuring N mixture methylation levels at the N genomic sites using the set of the plurality of cell-free DNA molecules;

30 determining, by the computer system, a first fractional contribution of the first diseased tissue type in the mixture using the N mixture methylation levels and the N tissue-specific methylation levels of each of the M tissue types; and

using the first fractional contribution of the first diseased tissue type to determine a level of the first disease for the first organ in the organism.

[0006b] In another aspect, the present disclosure refers to a computer product comprising a computer readable medium storing a plurality of instructions for controlling a computer system to perform an operation of any of the methods of the present disclosure.

[0006c] In another aspect, the present disclosure refers to a system comprising:

- 5 the computer product of the present disclosure; and
 one or more processors for executing instructions stored on the computer readable medium.

[0006d] In another aspect, the present disclosure refers to a system comprising means for performing any of the methods as disclosed herein.

- 10 **[0006e]** In another aspect, the present disclosure refers to a system configured to perform any of the methods as disclosed herein.

[0006f] In another aspect, the present disclosure refers to a system comprising modules that respectively perform the steps of any of the methods as disclosed herein.

- [0007]** In some examples, methylation levels of cell-free DNA can be measured in a sample.
15 Tissue-specific methylation patterns can be used to determine fractional contributions from different tissue types. One tissue type can be a diseased tissue type of a particular disease of a particular organ. The fractional contribution of the diseased tissue type can be used to determine a level (classification) of the particular disease in the sample. As an example, methylation patterns can be used determine a percentage of cancerous bladder lining cells, and the percentage
20 can be used to determine a level of cancer.

- [0008]** In other examples, sizes of organ-associated cell-free DNA can be measured. In an example using a urine sample voided from the bladder, a size profile can be measured of cell-free DNA fragments that naturally occur in the urine. A statistical measure of the size profile can indicate that the cell-free DNA fragments are collectively longer than expected for subjects with
25 healthy bladder tissue. The indication of longer fragments can be used to identify bladder cancer in the subject. In another example using a urine sample, the urine sample can be retrieved from the renal pelvis. A determination about whether the kidney is inflamed can be made based on the statistical measure indicating that the cell-free DNA fragments are collectively longer than expected for subjects with a non-inflamed kidney.

[0009] In other examples, two different samples can be analyzed to determine whether a particular organ has cancer. Cell-free DNA in a blood sample and organ-associated sample (e.g., urine, saliva, or a stool sample) can both be analyzed to identify chromosomal regions exhibiting a copy number aberration. If the blood sample does not indicate cancer and the organ-associated
5 sample does, then the subject can be identified as having cancer in an organ associated with the

WHAT IS CLAIMED IS:

1 1. A method of analyzing a biological sample of an organism, the biological
2 sample being a urine sample, the biological sample including a mixture of cell-free DNA
3 molecules from a plurality of tissues types, the method comprising:
4 identifying N genomic sites, N being an integer greater than or equal to 10;
5 for each of M tissue types:
6 obtaining N tissue-specific methylation levels at the N genomic sites, N being
7 greater than or equal to M, wherein the tissue-specific methylation levels form a matrix A of
8 dimensions N by M, wherein one of the M tissue types is a first diseased tissue type
9 corresponding to a first disease of a first organ, wherein the first diseased tissue type is
10 cancerous urothelium cells, and wherein a second tissue type of the M tissue types is normal
11 urothelium;
12 analyzing, by a computer system, a plurality of cell-free DNA molecules from the
13 biological sample, the plurality of cell-free DNA molecules being at least 1,000 cell-free DNA
14 molecules, wherein analyzing a cell-free DNA molecule includes:
15 identifying a location of the cell-free DNA molecule in a reference genome
16 corresponding to the organism;
17 identifying a set of the plurality of cell-free DNA molecules that are each located
18 at any one of the N genomic sites,
19 measuring N mixture methylation levels at the N genomic sites using the set of
20 the plurality of cell-free DNA molecules;
21 determining, by the computer system, a first fractional contribution of the first
22 diseased tissue type in the mixture using the N mixture methylation levels and the N tissue-
23 specific methylation levels of each of the M tissue types; and
24 using the first fractional contribution of the first diseased tissue type to determine
25 a level of the first disease for the first organ in the organism.

1 2. The method of claim 1, wherein a third tissue type of the M tissue types is
2 a second diseased tissue type corresponding to a second disease of the first organ, the method
3 further comprising:

4 determining, by the computer system, a second fractional contribution of the
5 second diseased tissue type in the mixture using the N mixture methylation levels and the N
6 tissue-specific methylation levels of each of the M tissue types; and
7 using the second fractional contribution of the second diseased tissue type to
8 determine a level of the second disease for the first organ in the organism.

1 3. The method of claim 1, wherein a third tissue type of the M tissue types is
2 a second diseased tissue type corresponding to a second disease of a second organ, the method
3 further comprising:

4 determining, by the computer system, a second fractional contribution of the
5 second diseased tissue type in the mixture using the N mixture methylation levels and the N
6 tissue-specific methylation levels of each of the M tissue types; and
7 using the second fractional contribution of the second diseased tissue type to
8 determine a level of the second disease for the second organ in the organism.

1 4. The method of claim 1, wherein the first organ is a bladder.

2
3 5. The method of claim 4, wherein the first disease is bladder cancer.

1 6. The method of claim 1, wherein the first disease is cancer.

2
3 7. The method of claim 6, wherein the level of the first disease is that cancer
4 exists.

1 8. The method of claim 1, further comprising:
2 preparing the urine sample by adding ethylenediaminetetraacetic acid (EDTA).

1 9. The method of claim 1, wherein the N genomic sites comprise sites that
2 have less than a threshold amount of inter-individual variation.

1 10. The method of claim 1, wherein the M tissue types comprise kidney,
2 urothelium, neutrophils, B-cells, and T-cells.

1 11. The method of claim 1, wherein the M tissue types comprise a small
2 intestine.

3 12. The method of claim 1, wherein the N genomic sites comprise sites
4 common to a methylome of normal urothelium and a tumor methylome of cancerous urothelium
5 cells.

1 13. The method of claim 1, wherein the N genomic sites comprise sites in
2 regions that occur in differentially methylated regions that occur in a specified percentage of
3 tumor urothelial biopsies.

4 14. The method of claim 1, wherein using the first fractional contribution of
5 the first diseased tissue type to determine the level of the first diseased for the first organ in the
6 organism comprises comparing the first fractional contribution to a cutoff value.

7 15. A computer product comprising a computer readable medium storing a
8 plurality of instructions for controlling a computer system to perform an operation of any of the
9 methods above.

1 16. A system comprising:
2 the computer product of claim 15; and
3 one or more processors for executing instructions stored on the computer readable
4 medium.

1 17. A system comprising means for performing any of the methods above.

1 18. A system configured to perform any of the above methods.

1 19. A system comprising modules that respectively perform the steps of any
2 of the above methods.