

CLAIMS

WHAT IS CLAIMED IS:

1. A method of determining whether an organism having cancer of a first tissue has a cancer located at a second tissue, the method comprising:
 - (a) obtaining cell-free DNA molecules from a first biological sample of the organism having cancer of a first tissue;
 - (b) performing an assay on the cell-free DNA molecules to determine a first methylation status of a target sequence in the cell-free DNA molecules, wherein the first methylation status of the target sequence indicates that a cell-free DNA molecule comprising the target sequence is from a second tissue of the organism, wherein the first tissue and the second tissue are different;
 - (c) determining an absolute amount of cell-free DNA molecules from the first biological sample comprising the target sequence with the first methylation status; and
 - (d) determining whether the organism has cancer at the second tissue based on the absolute amount.
2. The method of claim 1, wherein the methylation status comprises a methylation level.
3. The method of claim 1 or 2, wherein the assay comprises isolating the cell-free DNA molecule comprising the target sequence from the first biological sample.
4. The method of claim 3, wherein the assay comprises isolating the cell-free DNA molecule comprising the target sequence in an oil emulsion.
5. The method of any one of claims 1-4, wherein the assay comprises hybridizing the cell-free DNA molecule comprising the target sequence to a probe.
6. The method of claim 5, wherein the probe hybridizes to the target sequence.
7. The method of claim 6, wherein an affinity of hybridization of the probe to the target sequence depends on the first methylation status of the target sequence in the first biological sample.
8. The method of claim 7, wherein the probe hybridizes to the target sequence when a methylation site of the target sequence is methylated in the first biological sample.
9. The method of claim 7, wherein the probe hybridizes to the target sequence when a methylation site of the target sequence is unmethylated in the first biological sample.
10. The method of any one of claims 5-9, wherein the assay comprises detecting the hybridization of the probe to the target sequence.
11. The method of any one of claims 1-10, wherein the assay comprises amplifying the cell-free DNA molecule.
12. The method of claim 11, wherein the amplifying comprises use of a pair of primers.

13. The method of claim 12, wherein an affinity of hybridization of at least one primer of the pair of primers to the target sequence depends on the first methylation status of the target sequence.
14. The method of claim 13, wherein the at least one primer of the pair of primers hybridizes to the target sequence when a methylation site of the target sequence is methylated in the first biological sample.
15. The method of claim 13, wherein the at least one primer of the pair of primers hybridizes to the target sequence when a methylation site of the target sequence is unmethylated in the first biological sample.
16. The method of any one of claims 1-15, wherein the assay comprises bisulfite conversion of unmethylated cytosine residues in the cell-free DNA molecule to uracil.
17. The method of any one of claims 1-16, wherein the assay comprises performing methylation-aware sequencing of cell-free DNA molecules from the first biological sample.
18. The method of any one of claims 1-17, wherein the target sequence comprises at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 methylation sites.
19. The method of any one of claims 1-18, wherein the target sequence comprises at least 5 methylation sites.
20. The method of any one of claims 1-19, wherein the first methylation status comprises methylation density for individual sites within the target sequence, a distribution of methylated/unmethylated sites over a contiguous region within the target sequence, a pattern or level of methylation for each individual methylation site within the target sequence, or non-CpG methylation.
21. The method of any one of claims 1-20, wherein the target sequence comprises a higher methylation density in the first tissue than in the second tissue.
22. The method of claim 21, wherein the first methylation status comprises methylation density for individual sites within the target sequence, and the target sequence has a methylation density in the first tissue that is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%.
23. The method of claim 21, wherein the target sequence comprises a methylation density in the first tissue that is more than 50%.
24. The method of any one of claims 21-23, wherein the target sequence comprises a methylation density in the second tissue that is at most 50%, at most 40%, at most 30%, at most 20%, at most 10%, at most 5%, or 0%.
25. The method of any one of claims 21-24, wherein the target sequence comprises a methylation density in the second tissue that is less than 20%.

26. The method of any one of claims 1-17, wherein the target sequence comprises a lower methylation density in the first tissue than in the second tissue.
27. The method of claim 26, wherein the target sequence comprises a methylation density in the first tissue that is at most 50%, at most 40%, at most 30%, at most 20%, at most 10%, at most 5%, or 0%.
28. The method of claim 27, wherein the target sequence comprises a methylation density in the first tissue that is less than 50%.
29. The method of any one of claims 26-28, wherein the target sequence comprises a methylation density in the second tissue that is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100%.
30. The method of any one of claims 26-29, wherein the target sequence comprises a methylation density in the second tissue that is more than 80%.
31. The method of any one of claims 1-17, wherein the first tissue comprises liver tissue, and the target sequence comprises a polynucleotide sequence having at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 1.
32. The method of claim 31, wherein the first tissue comprises liver tissue, and wherein the assay comprises amplification using a primer comprising SEQ ID NO: 2, a primer comprising SEQ ID NO: 3, or both, or use of a detectably-labeled probe comprising SEQ ID NO: 4 for detection of the target sequence.
33. The method of claim 32, wherein the amplification further comprises use of a primer comprising SEQ ID NO: 5, a primer comprising SEQ ID NO: 6, or both, or use of a detectably-labeled probe comprising SEQ ID NO: 7 for detection of the target sequence.
34. The method of any one of claims 1-17, wherein the first tissue comprises colon tissue, and the target sequence comprises a polynucleotide sequence having at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 8.
35. The method of claim 34, wherein the first tissue comprises colon tissue, and wherein the amplification comprises use of a primer comprising SEQ ID NO: 9, a primer comprising SEQ ID NO: 10, or both, or use of a detectably-labeled probe comprising SEQ ID NO: 11 for detection of the target sequence.
36. The method of claim 35, wherein the methylation-specific amplification further comprises use of a primer comprising SEQ ID NO: 12, a primer comprising SEQ ID NO: 13, or both, or use of a detectably-labeled probe comprising SEQ ID NO: 14 for detection of the target sequence.
37. The method of any one of claims 1-36, wherein the cancer is selected from the group consisting of: bladder cancer, bone cancer, brain tumor, breast cancer, carcinoma of cervix,

colorectal cancer, esophageal cancer, gastrointestinal cancer, hematopoietic malignancy, head and neck squamous cell carcinoma, leukemia, liver cancer, lung cancer, lymphoma, myeloma, nasal cancer, nasopharyngeal cancer, oral cancer, oropharyngeal cancer, ovarian cancer, prostate cancer, sarcoma, stomach cancer, melanoma, and thyroid cancer.

38. The method of claim 37, wherein the cancer comprises hepatocellular carcinoma or colorectal carcinoma.

39. The method of any one of claims 1-38, further comprising determining a classification of the cancer in the second tissue.

40. The method of claim 39, wherein the determining the classification of cancer in the second tissue comprises assessing cell-free nucleic acid molecules from a second biological sample from the organism.

41. The method of claim 40, wherein the assessing comprises determining methylation profile, copy number variations, single polymorphism (SNP) profile, or fragmentation pattern of the cell-free nucleic acid molecules from the second biological sample.

42. The method of claim 40 or 41, wherein the assessing comprises determining an amount of cell-free nucleic acid molecules from the second biological sample that are from a pathogen.

43. The method of any one claims 40-42, wherein the second biological sample is same as the first biological sample.

44. The method of any one claims 40-42, wherein the second biological sample is different than the first biological sample.

45. A system configured to perform the method of any one of claims 1 to 44.

46. A non-transitory computer-readable medium comprising a series of instructions for controlling a computer system to perform the method of any one of claims 1 to 44.

47. A composition for determining an amount of cell-free DNA molecules from a biological sample that are from liver of an organism, comprising a pair of primers for amplification of a liver-specific marker based on methylation level of the liver-specific marker, wherein the liver-specific marker comprises a polynucleotide sequence having at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 1.

48. The composition of claim 47, wherein the pair of primers comprises a primer comprising SEQ ID NO: 2 and a primer comprising SEQ ID NO: 3.

49. The composition of claim 47 or 48, further comprising a detectably-labeled probe comprising SEQ ID NO: 4 for detection of the liver-specific marker.

50. The composition of any of claims 47-49, further comprising a primer comprising SEQ ID NO: 5 and a primer comprising SEQ ID NO: 6.

51. The composition of any of claims 47-50, further comprising a detectably-labeled probe comprising SEQ ID NO: 7 for detection of the liver-specific marker.
52. A composition for determining an amount of cell-free DNA molecules from a biological sample that are from colon of an organism, comprising a pair of primers for amplification of a colon-specific marker based on a methylation level of the colon-specific marker, wherein the colon-specific marker comprises a polynucleotide sequence having at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 8.
53. The composition of claim 52, wherein the pair of primers comprises a primer comprising SEQ ID NO: 9, and a primer comprising SEQ ID NO: 10.
54. The composition of claim 52 or 53, further comprising a detectably-labeled probe comprising SEQ ID NO: 11 for detection of the colon-specific marker.
55. The composition of any of claims 52-54, further comprising a primer comprising SEQ ID NO: 12 and a primer comprising SEQ ID NO: 13.
56. The composition of any of claims 52-55, further comprising a detectably-labeled probe comprising SEQ ID NO: 14 for detection of the colon-specific marker.