CLAIMS

What is claimed is:

1. A method of determining cell type or cell states comprising:

(a)(i) providing or having been provided a sample comprising DNA and generating a methylation profile for the DNA in the sample; or (ii) providing or having been provided a methylation profile of the DNA in the sample, wherein the methylation profile comprises co-associated CpG methylation patterns and/or methylation haplotype blocks (MHBs) (tightly coupled CpG sites) of the DNA; and

(b) detecting cell type or cell state comprising:

(i) counting co-associated CpG methylation patterns in the DNA , wherein co-associated CpG methylation patterns comprises two or more CpGs in the DNA; or

(ii) counting MHBs;

(c) assigning the DNA to a cell type or cell state based on reference CpG values or reference MHB values, wherein reference CpG values or reference MHB values are determined from reference cell types or reference cell states; and

(d) counting DNA molecules assigned to each reference CpG value or reference MHB value, wherein each reference CpG value or reference MHB value corresponds to a cell type or a cell state.

2. The method of claim 1, further comprising counting known *single* CpG methylation profiles to increase sensitivity.

~~3. The method of claim 1, wherein the sample is a blood sample.~~

3~~4~~. The method of claim 1, wherein reference values are differentially methylated CpGs derived from DNA originating from known cell types and known cell states, optionally of bacterial, viral, fungal, or eukaryotic parasitic origin.

4~~5~~. The method of claim 1, wherein the sample is a blood sample, plasma, urine, saliva, stool, tissue, or biopsy sample.

~~6. The method of claim 1, wherein the sample comprises a bodily fluid.~~

~~7. The method of claim 6, wherein the bodily fluid is selected from whole blood, plasma, urine, saliva, or stool.~~

~~8. The method of claim 1, wherein the sample does not comprise a solid tissue biopsy.~~

5~~9~~. The method of claim 1, wherein the DNA is cell-free DNA and/or a rar cell type circulating DNA and is plasma-derived.

6~~10~~. The method of claim 1, further comprising determining cell state-specific signatures by the method of claim 1 or providing or having been provided cell state-specific signatures of the sample.

~~11. The method of claim 1, wherein the DNA is cell-free and a rare cell type circulating DNA.~~

7~~12~~. The method of claim 1, wherein:

(a) the sample comprises cell-free DNA (cfDNA); and

(b) the sample is collected from a tumor microenvironment comprising tumor infiltrating leukocytes.

~~13. The method of claim 12, wherein the tumor microenvironment comprises tumor infiltrating leukocytes.~~

8~~14~~. The method of claim 1, wherein the DNA is cell-free tumor ctDNA.

~~15. The method of claim 1, wherein the subject has been administered immunotherapy prior to providing a sample.~~

9~~16~~. The method of claim 1, wherein the cell state measured is from DNA from a circulating, cell-free tumor infiltrating leukocyte (TIL) and, optionally, the sample is a sample from a tumor microenvironment (TME).

10~~17~~. The method of claim 9 ~~16~~, comprising:

profiling TILs according to methylation signatures; and

determining the proportions of distinct TIL subsets from a cell type-specific methylation profile identified in the cell-free DNA.

11~~18~~. The method of claim 1, wherein DNA is classified as originating from a normal leukocyte cell, a tumor-associated cell, or a tumor infiltrating leukocyte.

~~19. The method of claim 1, comprising administering a cancer treatment to the subject (e.g., immunotherapy, chemotherapy, radiation) and measuring cell type and cell state in a sample as an indication of treatment response.~~

~~20. The method of claim 1, wherein if ctilDNA levels are decreased compared to ctilDNA levels in a responder to immunotherapy, the subject is determined to be at risk for being a non-responder to immunotherapy.~~

~~21. The method of claim 1, wherein~~

~~the sample comprises cell-free DNA (cfDNA); and~~

~~the sample is blood from a subject having, suspected of having, or at risk for having sepsis.~~

~~22. The method of claim 1, wherein the sample comprises a nucleic acid mixture comprising DNA or RNA, or any combination thereof.~~

1223. The method of claim 1, wherein the methylation profile for the DNA in the sample is generated using microarrays or bisulfite sequencing.

~~24. The method of claim 1, wherein the sample is a blood sample from a subject having, suspected of having, or at risk for having sepsis.~~

~~25. The method of claim 24, wherein exhausted lymphocyte cell states are measured.~~

~~26. The method of claim 24, wherein exhausted T cells are measured.~~

~~27. The method of claim 24, wherein organ-specific cell states or organ-specific cell types are measured.~~

~~28. The method of claim 24, wherein the DNA originates from an organ, a damaged organ, a T cell, exhausted T cells, an immune cell, a microbe, septic tissue, or a secondary infection site.~~

~~29. The method of claim 24, wherein if cfDNA analysis detects DNA originating from a microbial pathogen, the subject is diagnosed with an infection or sepsis.~~

~~30. The method of claim 24, wherein if cfDNA analysis detects reduced cfDNA originating from a microbial pathogen compared to the cfDNA originating from a microbial pathogen, and the subject is administered a treatment (e.g., antibiotic), the subject is determined to be responding to treatment.~~

~~31. The method of claim 24, wherein if cfDNA analysis detects reduced cfDNA from a microbial pathogen compared to the cfDNA analysis measured at an earlier time, it is determined that the subject is responding to a treatment or an infection is improving.~~

~~32. The method of claim 24, wherein if cfDNA analysis detects elevated cfDNA from an organ tissue, an infection source is determined to be the organ tissue with elevated detected cfDNA.~~

~~33. The method of claim 24, wherein if cfDNA analysis detects elevated cfDNA from an organ tissue suspected of being damaged compared to a control, the organ is determined to be damaged.~~

~~34. The method of claim 24, wherein if cfDNA analysis detects reduced cfDNA from a damaged organ tissue compared to the cfDNA analysis measured at an earlier time, it is determined that the organ damage is improving.~~

~~35. The method of claim 24, wherein if cfDNA analysis detects elevated cfDNA from an organ tissue suspected of being damaged compared to a control, the organ is determined to be damaged.~~

~~36. The method of claim 24, wherein if cfDNA analysis detects elevated cfDNA from multiple organ systems compared to a control, the subject is determined to be at risk for multi-organ failure.~~

~~37. The method of claim 24, wherein if cfDNA analysis detects elevated cfDNA from exhausted T cells or an opportunistic pathogen compared to a control, the subject is determined to be at risk for a secondary infection.~~

13~~38~~. A computer-aided method for detecting at least one abundance of at least one cell identity in a biological sample, the sample comprising DNA, the method comprising:

providing a plurality of reads, each read comprising a sequence of the DNA and associated methylation status;

providing a CpG library comprising a plurality of entries, each entry comprising a CpG site and a corresponding cell identity, each CpG site comprising

a co-associated CpG site, and each corresponding cell identity comprising a cell type or a cell state;

transforming, using a computing device, the plurality of reads into a plurality of read assignments according to at least one assignment rule, each read assignment comprising one of a cell identity, a cell-related identity, and an unrelated identity; and

transforming, using the computing device, the plurality of read assignments into the at least one abundance, each abundance corresponding to one cell identity, each abundance comprising a total number of read assignments comprising the one cell identity.

14~~39~~. The computer-aided method of claim 13~~38~~, wherein the at least one assignment rule comprises at least one of:

transforming, using the computing device, the read into the cell-related identity if the read comprises no more than one CpG site from the plurality of entries of the CpG library;

transforming, using the computing device, the read into the cell identity if the read comprises at least two CpG sites from the plurality of entries of the CpG library with the same corresponding cell identity; and

transforming, using the computing device, the read into the unrelated identity if the read does not comprise any CpG site from the plurality of entries of the CpG library.

15~~40~~. The computer-aided method of any one of claims 13-14~~ 38-39~~, further comprising transforming, using the computing device, each abundance into at least one of a relative abundance and an absolute abundance, wherein:

each relative abundance comprises the abundance of one cell identity normalized by the total of all abundances of all cell identities; and

each absolute abundance comprises the abundance of one cell identity normalized by a sum of the abundance and the total number of read assignments.

~~41. The computer-aided method of any one of claims 38-40, wherein the DNA comprises cell-free DNA.~~

16~~42~~. The computer-aided method of any one of claims 13-15 ~~38-41~~, wherein providing the plurality of reads further comprises performing bisulfite sequencing or microarray methylation profiling on the DNA.

~~43. The computer-aided method of any one of claims 38-42, wherein each CpG site is differentially methylated within cells of one cell identity and each co-associated CpG site comprises a sequence position proximal to at least one additional CpG site with the same corresponding cell identity.~~

~~44. The computer-aided method of any one of claims 38-43, wherein providing the CpG library further comprises:~~

~~providing DNA corresponding to one cell identity;~~

~~performing bisulfite sequencing or microarray methylation profiling on the plurality of isolated DNA to obtain a plurality of isolated reads, each isolated read comprising an isolated sequence of an isolated DNA and associated methylation status;~~

~~performing differential methylated region analysis on the plurality of isolated reads to identify a plurality of candidate CpG sites; and~~

~~assigning a candidate CpG site as an entry of the CpG library for the one cell identity if the candidate CpG site comprises a sequence position proximal to at least one additional candidate CpG site.~~

~~45. The computer-aided method of any one of claims 38-44, wherein the biological sample comprises a bodily fluid.~~

~~46. The computer-aided method of any one of claims 38-45, wherein the bodily fluid is selected from whole blood, plasma, urine, saliva, or stool.~~

~~47. The computer-aided method of any one of claims 38-46, wherein the biological sample does not comprise a solid tissue biopsy.~~

1748. A computing device configured to detect at least one abundance of at least one cell identity in a biological sample, the sample comprising DNA, the computing device comprising at least one processor and a non-volatile computer-readable media, the non-volatile computer-readable media containing instructions executable on the at least one processor to:

receive a plurality of reads, each read comprising a sequence of the DNA and associated methylation status;

provide a CpG library comprising a plurality of entries, each entry comprising a CpG site and a corresponding cell identity, each CpG site comprising a co-associated CpG site, and each corresponding cell identity comprising a cell type or a cell state;

transform the plurality of reads into a plurality of read assignments according to at least one assignment rule, each read assignment comprising one of a cell identity, a cell-related identity, and an unrelated identity; and

transform the plurality of read assignments into the at least one abundance, each abundance corresponding to one cell identity, each abundance comprising a total number of read assignments comprising the one cell identity.

1849. The computing device of claim 1748, wherein the at least one assignment rule comprises at least one of:

transforming, using the computing device, the read into the cell-related identity if the read comprises no more than one CpG site from the plurality of entries of the CpG library;

transforming, using the computing device, the read into the cell identity if the read comprises at least two CpG sites from the plurality of entries of the CpG library with the same corresponding cell identity; and

transforming, using the computing device, the read into the unrelated identity if the read does not comprise any CpG site from the plurality of entries of the CpG library.

19~~50~~. The computing device of any one of claims 17-18 ~~48-49~~, wherein the non-volatile computer-readable media further contains instructions executable on the at least one processor to transform each abundance into at least one of a relative abundance and an absolute abundance, wherein:

each relative abundance comprises the abundance of one cell identity normalized by the total of all abundances of all cell identities; and

each absolute abundance comprises the abundance of one cell identity normalized by a sum of the abundance and the total number of read assignments.

20~~51~~. The computing device of any one of claims 17-19 ~~48-50~~, wherein each CpG site is differentially methylated within cells of one cell identity and each co-associated CpG site comprises a sequence position proximal to at least one additional CpG site with the same corresponding cell identity.

~~52. The computing device of any one of claims 48-51, wherein the DNA comprises cell-free DNA.~~

~~53. The computing device of any one of claims 48-52, wherein the biological sample comprises a bodily fluid.~~

~~54. The computing device of any one of claims 48-53, wherein the bodily fluid is selected from whole blood, plasma, urine, saliva, or stool.~~

~~55. The computing device of any one of claims 48-54, wherein the biological sample does not comprise a solid tissue biopsy.~~

~~56. A computer-aided method for detecting at least one abundance of at least one cell identity in a biological sample, the sample comprising DNA, the method comprising:~~

~~providing a plurality of reads, each read comprising a sequence of the DNA and associated methylation status;~~

providing a Methylation Haplotype Block (MHB) library comprising a plurality of entries, each entry comprising an MHB and a corresponding cell identity, each MHB comprising at least two co-associated CpG sites, and each corresponding cell identity comprising a cell type or a cell state;

transforming, using a computing device, the plurality of reads into a plurality of read assignments according to at least one assignment rule, each read assignment comprising one of a cell identity, a cell-related identity, and an unrelated identity; and

transforming, using the computing device, the plurality of read assignments into the at least one abundance, each abundance corresponding to one cell identity, each abundance comprising a total number of read assignments comprising the one cell identity.

57. The computer-aided method of claim 56, wherein the at least one assignment rule comprises transforming, using the computing device, the read into the cell identity if the read comprises at least one MHB from the plurality of entries of the MHB library with the corresponding cell identity.

58. The computer-aided method of any one of claims 56-57, further comprising transforming, using the computing device, each abundance into a relative abundance, wherein each relative abundance comprises the abundance of one cell identity normalized by the total of all abundances of all cell identities.

59. The computer-aided method of any one of claims 56-58, wherein the DNA comprises cell-free DNA.

60. The computer-aided method of any one of claims 56-59, wherein providing the plurality of reads further comprises performing bisulfite sequencing or microarray methylation profiling, on the DNA.

61. The computer-aided method of any one of claims 56-60, wherein each MHB site comprises at least two differentially methylated CpG sites proximal to each other within cells of one cell identity.

62. The computer-aided method of any one of claims 56-61, wherein providing the MHB library further comprises:

providing a plurality of isolated DNA corresponding to one cell identity;

performing bisulfite sequencing or microarray methylation profiling on the plurality of isolated DNA to obtain a plurality of isolated reads, each isolated read comprising an isolated sequence of isolated DNA and associated methylation status;

performing differential methylated region analysis on the plurality of isolated reads to identify a plurality of candidate CpG sites; and

assigning each sequence including at least two candidate CpG sites proximal to each other as an MHB corresponding to the one cell identity in the MHB library for the one cell identity.

63. The computer-aided method of any one of claims 56-62, wherein the biological sample comprises a bodily fluid.

64. The method of any one of claims 56-63, wherein the bodily fluid is selected from whole blood, plasma, urine, saliva, or stool.

65. The method of any one of claims 56-64, wherein the biological sample does not comprise a solid tissue biopsy.

66. A computing device configured to detect at least one abundance of at least one cell identity in a biological sample, the sample comprising DNA, the computing device comprising at least one processor and a non-volatile computer-readable media, the non-volatile computer-readable media containing instructions executable on the at least one processor to:

receive a plurality of reads, each read comprising a sequence of the DNA and associated methylation status;

receive a Methylation Haplotype Block (MHB) library comprising a plurality of entries, each entry comprising an MHB and a corresponding cell identity, each MHB comprising at least two co-associated CpG sites, and each corresponding cell identity comprising a cell type or a cell state;

transform, using a computing device, the plurality of reads into a plurality of read assignments according to at least one assignment rule, each read assignment comprising one of a cell identity, a cell-related identity, and an unrelated identity; and

transform, using the computing device, the plurality of read assignments into the at least one abundance, each abundance corresponding to one cell identity, each abundance comprising a total number of read assignments comprising the one cell identity.

67. The computing device of claim 66, wherein the at least one assignment rule comprises transforming, using the computing device, the read into the cell identity if the read comprises at least one MHB from the plurality of entries of the MHB library with the corresponding cell identity.

68. The computing device of any one of claims 66-67, wherein the non-volatile computer-readable media further contains instructions executable on the at least one processor to transform each abundance into a relative abundance, wherein each relative abundance comprises the abundance of one cell identity normalized by the total of all abundances of all cell identities.

69. The computing device of any one of claims 66-68, wherein each MHB site comprises at least two differentially methylated CpG sites proximal to each other within cells of one cell identity.

70. The computer-aided method of any one of claims 66-69, wherein the DNA comprises cell-free DNA.

~~71. The computing device of any one of claims 66-70, wherein the biological sample comprises a bodily fluid.~~

~~72. The computing device of any one of claims 66-71, wherein the bodily fluid is selected from whole blood, plasma, urine, saliva, or stool.~~

~~73. The computing device of any one of claims 66-72, wherein the biological sample does not comprise a solid tissue biopsy.~~

~~74. A computer-aided method for detecting at least one abundance of at least two cell identities in a biological sample, the sample comprising DNA, the method comprising:~~

~~providing a plurality of reads, each read comprising a sequence of the DNA and associated methylation status;~~

~~providing a signature matrix comprising at least two pluralities of differentially methylated CpG sites, each portion corresponding to each cell identity of the at least two cell identities; and~~

~~deconvolving, using a computing device, the plurality of reads into at least two relative abundances, each relative abundance comprising a portion of one cell identity within the biological sample.~~

~~75. The computer-aided method of claim 74, wherein the DNA comprises cell-free DNA.~~

~~76. A computing device configured to detect at least one abundance of at least two cell identities in a biological sample, the sample comprising a plurality of DNA, the computing device comprising at least one processor and a non-volatile computer-readable media, the non-volatile computer-readable media containing instructions executable on the at least one processor to:~~

~~receive a plurality of reads, each read comprising a sequence of the DNA and associated methylation status;~~

~~receive a signature matrix comprising at least two pluralities of differentially methylated CpG sites, each portion corresponding to each cell identity of the at least two cell identities; and~~

~~deconvolve the plurality of reads into at least two relative abundances, each relative abundance comprising a portion of one cell identity within the biological sample.~~

~~77. The computer-aided method of claim 76, wherein the DNA comprises cell-free DNA.~~