CLAIMS

1. A panel of isolated polynucleotides comprising, consisting of, or consisting essentially of at least two isolated polynucleotides, each of said isolated polynucleotides having a polynucleotide sequence of any of Target 1 to Target 1849 listed in Table 1, or a complementary or substantially complementary sequence thereof.

2. The panel of claim 1, which comprises, consists of, or consists essentially of at least 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, more of the isolated polynucleotides, or the isolated polynucleotides corresponding to all of Target 1 to Target 1849 listed in Table 1.

3. The panel of claim 1 or 2, which comprises at least 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, more of the isolated polynucleotides, or the isolated polynucleotides corresponding to all of Target 1 to Target 1849 listed in Table 1.

4. The panel of claim 1 or 2, which consists of at least 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, more of the isolated polynucleotides, or the isolated polynucleotides corresponding to all Target 1 to Target 1849 listed in Table 1.

5. The panel of claim 1 or 2, which consists essentially of at least 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, or more of the isolated polynucleotides, or the isolated polynucleotides corresponding to all of Target 1 to Target 1849 listed in Table 1.

~~6. The panel of any one of claims 1-5, wherein the isolated polynucleotides are DNA molecules, RNA molecules, or a combination thereof.~~

~~7. The panel of any one of claims 1-6, wherein the isolated polynucleotides are immobilized on a substrate.~~

~~8. The panel of claim 7, wherein the substrate comprises a solid surface, a porous surface, or a combination thereof.~~

~~9. The panel of claim 7 or 8, wherein the substrate is a part of a bead, a tube, a microtiter plate, a membrane, a gel, or a glass slide.~~

~~10. The panel of any one of claims 7-9, wherein the isolated polynucleotide molecules are immobilized spatially apart from each other on a substrate.~~

~~11. A kit or system, which comprises a panel of any one of claims 1-10.~~

~~12. The kit or system of claim 11, which is configured for assessing methylation status of at least two of Target 1 to Target 1849 listed in Table 1.~~

~~13. The kit or system of claim 12, which is configured for assessing methylation status of at least 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, or all of Target 1 to Target 1849 listed in Table 1.~~

~~14. The kit or system of any one of claims 11-13, wherein the isolated polynucleotides are configured as control polynucleotides.~~

~~15. The kit or system of claim 14, wherein the isolated polynucleotides have a level of concentration from about 1 femtomolar to about 1 millimolar.~~

~~1~~6. A kit or system, which comprises reagents for assessing methylation status of at least two of Target 1 to Target 1849 listed in Table 1.

~~1~~7. The kit or system of claim ~~1~~6, which comprises reagents for assessing methylation status of at least 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, or all of Target 1 to Target 1849 listed in Table 1.

~~1~~8. The kit or system of claim ~~1~~6 or ~~1~~7, wherein the reagents comprise, consist essentially of, or consist of a probe or primer configured for hybridizing with each of the targets whose methylation status is to be assessed.

~~1~~9. The kit or system of claim ~~1~~8, wherein the reagents comprise, consist essentially of, or consist of a single probe or primer configured for hybridizing with each of the targets whose methylation status is to be assessed.

~~20~~10. The kit or system of claim ~~1~~8, wherein the reagents comprise, consist essentially of, or consist of multiple probes or primers configured for hybridizing with each of the targets whose methylation status is to be assessed.

~~21. The kit or system of any one of claims 18-20, wherein the one or more primers comprise, consist essentially of, or consist of a sequence set forth in any of SEQ ID NO:1- SEQ ID NO:17504, a complementary or substantially complementary sequence thereof, or any combination thereof.~~

~~22. The kit or system of any one of claims 16-21, which further comprises a common primer for amplifying each of the targets whose methylation status is to be assessed.~~

~~23. The kit or system of claim 22, wherein the common primer comprises, consists essentially of, or consists of a sequence set forth in SEQ ID NO. 17505, or a complementary or substantially complementary sequence thereof.~~

~~24. The kit or system of any one of claims 16-23, which further comprises an agent for isolating the targets from a sample.~~

25. The kit or system of any one of claims 16-24, which further comprises a reagent for preparing a library of the targets.

26. The kit or system of claim 25, wherein the reagent for preparing a library of the targets comprises an enzyme, *e.g.*, a ligase or a single-stranded DNA (ssDNA) ligase.

27. The kit or system of claim 26, wherein the ssDNA ligase is T4 RNA ligase I, thermostable 5' App DNA/RNA ligase, T4 RNA ligase 2, truncated T4 RNA ligase 2, *e.g.*, T4 RNA ligase 2 Truncated, T4 RNA ligase2 Truncated K227Q, T4 RNA ligase2 Truncated KQ, or T4 DNA ligase, a Thermus bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and CircLigase II™), or an archaebacterium RNA ligase such as Methanobacterium thermoautotrophicum RNA ligase 1

28. The kit or system of any one of claims 16-27, which further comprises a reagent for amplifying the targets or a library of the targets.

29. The kit or system of claim 28, wherein the reagent for amplifying the targets or a library of the targets comprises an enzyme, e.g., an enzyme to be used in a polynucleotide amplification reaction selected from the group consisting of polymerase chain reaction (PCR), strand displacement amplification (SDA), transcription mediated amplification (TMA), ligase chain reaction (LCR), nucleic acid sequence based amplification (NASBA), primer extension, rolling circle amplification (RCA), self sustained sequence replication (3SR), and loop-mediated isothermal amplification (LAMP).

30. The kit or system of any one of claims 16-29, which further comprises an agent for purifying the targets, a library of the targets, amplified targets or a library of amplified targets.

31. The kit or system of any one of claims 16-30, which further comprises a reagent for assessing methylation status of the targets.

32. The kit or system of claim 31, wherein the reagent for assessing methylation status of the targets is a reagent to be used in a polynucleotide methylation, *e.g.*, DNA methylation, detecting method selected from the group consisting of mass spectrometry, methylation-specific PCR (MSP), bisulphite sequencing, the HpaII tiny fragment Enrichment by ligation-mediated PCR assay (HELP Assay), GlaI hydrolysis and ligation adapter dependent PCR assay (GLAD-PCR assay), restriction landmark genomic scanning (RLGS), methylated DNA immunoprecipitation (MeDIP or mDIP), pyrosequencing, molecular break light assay for DNA adenine methyltransferase activity, methyl sensitive Southern blotting and high resolution Melt (HRM) analysis.

33. The kit or system of claim 31 or 32, wherein the reagent for assessing methylation status of the targets is a chemical agent, e.g., bisulfite or sodium bisulfite.

34. The kit or system of claim 31 or 32, wherein the reagent for assessing methylation status of the targets is a biological agent, *e.g.*, a polypeptide or an enzyme.

35. The kit or system of claim 34, wherein the enzyme is a methylation-sensitive restriction enzyme (MSRE).

36. The kit or system of claim 35, wherein the MSRE selectively cleaves at a residue when it is unmethylated.

37. The kit or system of claim 35, wherein the MSRE selectively cleaves at the residue when it is methylated.

38. The kit or system of any one of claims 35-38, wherein the MSRE is selected from the group consisting of HpaII, SalI, SalI-HF®, ScrFI, BbeI, NotI, SmaI, XmaI, MboI, BstBI, ClaI, MluI, NaeI, NarI, PvuI, SacII, HhaI, and a combination thereof.

~~39. The kit or system of claim 34, wherein the enzyme is a polynucleotide polymerase.~~

~~40. The kit or system of claim 39, wherein the polynucleotide polymerase is configured to be used in PCR.~~

~~41. The kit or system of claim 40, wherein the polynucleotide polymerase is a DNA polymerase, e.g., a DNA polymerase without a 3' to 5' exonuclease activity.~~

~~42. The kit or system of any one of claims 16-41, which further comprises a panel of any one of claims 1-10.~~

~~43. The kit or system of any one of claims 16-42, which further comprises a reference sample and/or information of a control locus.~~

~~44. The kit or system of any one of claims 16-43, which further comprises separate containers, e.g., vials, for one or more components and/or instructions for using the kit or system.~~

~~45. The kit or system of any one of claims 16-44, which further comprises a computer readable medium containing executable instructions for obtaining a methylation metric of a sample based on the methylation status assessment.~~

~~46. The kit or system of claim 45, wherein the computer readable medium is configured for obtaining a methylation metric in the form of average methylation frequency, methylation haplotype load, unmethylation haplotype load, percent discordant reads, or a combination thereof.~~

~~47. The kit or system of any one of claims 16-46, which is configured for assessing cancer or neoplasia in a subject.~~

~~48. The kit or system of claim 47, which is configured for assessing lung cancer or colorectal cancer in a subject.~~

~~49. The kit or system of claim 47, which is configured for pan-cancer analysis or profiling in a subject.~~

~~50~~11. A method for assessing cancer or neoplasia in a subject, which method comprises:

a) providing a sample from a subject containing at least two target polynucleotides of said subject, said at least two target polynucleotides having polynucleotide sequences of at least two of Target 1 to Target 1849 listed in Table 1, or a complementary or substantially complementary sequence thereof;

b) assessing methylation status of said at least two target polynucleotides; and

c) assessing cancer or neoplasia in said subject based on assessment of methylation status of said at least two target polynucleotides.

~~51~~12. The method of claim ~~50~~11, wherein the sample comprises circulating cell-free or tumor DNA (ctDNA).

~~52~~13. The method of claim ~~50-~~11 or ~~51~~12, wherein the sample is a blood, serum, plasma, or body fluid sample, or any combination thereof.

~~53. The method of any one of claims 50-52, wherein the subject is a mammal.~~

~~54. The method of claim 53, wherein the mammal is a non-human mammal, *e.g.*, a pet, a farm animal, a companion animal or an experimental animal.~~

~~55. The method of claim 53, wherein the mammal is a human.~~

~~56. The method of any one of claims 50-55, which comprises assessing methylation status of target polynucleotides having polynucleotide sequences of at least 10, 50, 100, 200,~~

300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, or all of Target 1 to Target 1849 listed in Table 1, or a complementary or substantially complementary sequence thereof.

57. The method of any one of claims 50-56, wherein the methylation status of target polynucleotides is assessed using a probe or primer configured for hybridizing with each of the target polynucleotides.

58. The method of any one of claim 50-57 wherein the methylation status of a target polynucleotide is assessed using a single probe or primer configured for hybridizing with the target polynucleotide.

59. The method of any one of claim 50-57 wherein the methylation status of a target polynucleotide is assessed using multiple probes or primers configured for hybridizing with the target polynucleotide.

60. The method of any one of claim 50-59 wherein the one or more primers comprise, consist essentially of, or consist of a sequence set forth in any of SEQ ID NO:1- SEQ ID NO:17504, a complementary or substantially complementary sequence thereof, or any combination thereof.

61. The method of any one of claim 50-60 which further comprises using a common primer for amplifying each of the target polynucleotide whose methylation status is to be assessed.

62. The method of claim 61, wherein the common primer comprises, consists essentially of, or consists of a sequence set forth in SEQ ID NO:17505, a complementary or substantially complementary sequence thereof.

63. The method of any one of claim 50-62 which further comprises isolating the target polynucleotides from a sample.

64. The method of any one of claim 50-63 which further comprises preparing a library of the target polynucleotides.

65. The method of claim 64, wherein the library of the target polynucleotides is prepared using an enzyme, e.g., a ligase or a single-stranded DNA (ssDNA) ligase.

66. The method of claim 65, wherein the ssDNA ligase is T4 RNA ligase I, thermostable 5' App DNA/RNA ligase, T4 RNA ligase 2, truncated T4 RNA ligase 2, *e.g.*, T4 RNA ligase 2 Truncated, T4 RNA ligase2 Truncated K227Q, T4 RNA ligase2 Truncated KQ, or T4 DNA ligase, a Thermus bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and CircLigase II™), or an archaebacterium RNA ligase such as Methanobacterium thermoautotrophicum RNA ligase 1.

67. The method of any one of claim 50-66 which further comprises amplifying the target polynucleotides.

68. The method of claim 67, wherein the target polynucleotides are amplified using a procedure selected from the group consisting of polymerase chain reaction (PCR), strand displacement amplification (SDA), transcription mediated amplification (TMA), ligase chain reaction (LCR), nucleic acid sequence based amplification (NASBA), primer extension, rolling circle amplification (RCA), self sustained sequence replication (3SR), and loop-mediated isothermal amplification (LAMP).

69. The method of any one of claim 50-68 which further comprises purifying the target polynucleotides, a library of the target polynucleotides, amplified target polynucleotides or a library of amplified target polynucleotides.

70. The method of any one of claim 50-69, wherein the methylation status of target polynucleotides is assessed using mass spectrometry, methylation-specific PCR (MSP), methylation-sensitive sequencing, e.g., bisulphite sequencing, the HpaII tiny fragment

~~Enrichment by ligation-mediated PCR assay (HELP Assay), GlaI hydrolysis and ligation adapter dependent PCR assay (GLAD-PCR assay), restriction landmark genomic scanning (RLGS), methylated DNA immunoprecipitation (MeDIP or mDIP), pyrosequencing, molecular break light assay for DNA adenine methyltransferase activity, methyl sensitive Southern blotting or high resolution Melt (HRM) analysis.~~

~~71. The method of claim 70, wherein assessing methylation status of target polynucleotides comprises using a chemical agent, e.g., bisulfite or sodium bisulfite.~~

~~72. The method of claim 70, wherein assessing methylation status of target polynucleotides comprises using a biological agent, *e.g.*, a polypeptide or an enzyme.~~

~~73. The method of claim 72, wherein the enzyme is a methylation-sensitive restriction enzyme (MSRE).~~

~~74. The method of claim 73, wherein the MSRE selectively cleaves at a residue when it is unmethylated.~~

~~75. The method of claim 73, wherein the MSRE selectively cleaves at the residue when it is methylated.~~

~~76. The method of claim 73-75, wherein the MSRE is selected from the group consisting of HpaII, SalI, SalI-HF®, ScrFI, BbeI, NotI, SmaI, XmaI, MboI, BstBI, ClaI, MluI, NaeI, NarI, PvuI, SacII, HhaI, and a combination thereof.~~

~~77. The method of claim 72, wherein the enzyme is a polynucleotide polymerase.~~

~~78. The method of claim 77, wherein the polynucleotide polymerase is configured to be used in PCR.~~

79. The method of claim 78, wherein the polynucleotide polymerase is a DNA polymerase, e.g., a DNA polymerase without a 3' to 5' exonuclease activity.

80. The method of any one of claim 50-79, wherein the methylation status of target polynucleotides is assessed using methylation-sensitive sequencing, *e.g.*, bisulphite sequencing.

81. The method of claim 80, wherein the methylation-sensitive sequencing is conducted with a format selected from the group consisting of Maxam-Gilbert sequencing, a chain-termination method, shotgun sequencing, bridge PCR, single-molecule real-time sequencing, ion semiconductor (ion torrent sequencing), sequencing by synthesis, sequencing by ligation (SOLiD sequencing), chain termination (Sanger sequencing), massively parallel signature sequencing (MPSS), polony sequencing, 454 pyrosequencing, Illumina (Solexa) sequencing, DNA nanoball sequencing, heliscope single molecule sequencing, single molecule real time (SMRT) sequencing, nanopore DNA sequencing, tunnelling currents DNA sequencing, sequencing by hybridization, sequencing with mass spectrometry, microfluidic Sanger sequencing, a microscopy-based technique, RNAP sequencing, and in vitro virus high-throughput sequencing.

82. The method of claim 80 or 81, which further comprises, prior to the methylation-sensitive sequencing, *e.g.*, bisulphite sequencing, obtaining a library of linear, single-stranded ligation products, each of the linear, single-stranded ligation products comprises of a linear, single-stranded target polynucleotide linked to an adaptor comprising a unique molecular identifier (UMI) sequence that earmarks the single-stranded target polynucleotide to which the adaptor is ligated.

83. The method of claim 82, wherein the sequencing reads from the target polynucleotides are first adapter trimmed to remove any adapter sequence originating from the library construction process to obtain trimmed sequencing reads.

84. The method of claim 83, wherein the trimmed sequencing reads are mapped to a reference genome, e.g., a human reference genome, using an alignment program to obtain an aligned read file.

85. The method of claim 84, wherein the aligned read file is grouped into sets corresponding to each target region from Target 1 to Target 1849 listed in Table 1, or a complementary sequence or substantially complementary thereof, that can be used for methylation status assessment.

86. The method of any one of claim 50-85, wherein methylation status of each of the target polynucleotides is assessed.

87. The method of claim 86, wherein the methylation status of each of the target polynucleotides is assessed to obtain a methylation metric, *e.g.*, in the form of average methylation frequency, methylation haplotype load, unmethylation haplotype load, percent discordant reads, or a combination thereof.

88. The method of claim 87, wherein methylation status of a sample is assessed using the methylation metrics from each of the target polynucleotides.

89. The method of claim 88, wherein the methylation metric for each of the target polynucleotides is compared to a threshold or reference value to assess methylation status of a sample.

90. The method of claim 88 or 89, wherein a numerical methylation matrix is computed using the methylation metrics from each of the target polynucleotides to assess methylation status of a sample.

91. The method of claim 90, wherein the numerical methylation matrix from a sample comprises a single numerical number or value.

92. The method of claim 90, wherein the numerical methylation matrix from a sample comprises multiple numerical numbers or values.

93. The method of any one of claim 90-92, wherein the numerical methylation matrix is computed using a classification algorithm.

94. The method of claim 93, wherein the classification algorithm is linear discriminant analysis, logistic regression, naïve bayes classification, perceptron classification, quadratic classification, k-nearest neighbors, boosting, decision tree, random forest, neural network, learning vector quantization, or support vector machines.

95. The method of any one of claim 90-94, wherein the methylation matrix is compared to a threshold or reference value to assess methylation status of a sample.

96. The method of any one of claim 87-95, wherein the methylation metric for each of the target polynucleotides is obtained using a computer.

97. The method of any one of claim 90-96, wherein the methylation matrix of a sample is obtained using a computer based on the methylation metrics from each of the target polynucleotides.

98. The method of any of claims 50-97, wherein methylation status of at least two target polynucleotides from multiple samples, *e.g.*, multiple samples from multiple subjects, are assessed sequentially or simultaneously.

99. The method of any of claims 50-98, which is used for diagnosis, prognosis, stratification, risk assessment, or treatment monitoring of cancer or neoplasia in a subject.

100. The method of claim 99, wherein the cancer is a lymphoma, a leukemia, a brain cancer, a multiple myeloma, a pancreatic cancer, a liver cancer, a stomach cancer, a breast

~~cancer, a kidney cancer, a lung cancer, a colorectal cancer, a colon cancer, a prostate cancer, an ovarian cancer, a cervical cancer, a skin cancer, an esophagus cancer, or a head and neck cancer.~~

~~101. The method of claim 99, which is used for diagnosis, prognosis, stratification, risk assessment, or treatment monitoring of lung cancer in a subject.~~

~~102. The method of claim 101, wherein the lung cancer is non-small-cell lung carcinoma or small-cell lung carcinoma.~~

~~103. The method of claim 102, wherein the non-small-cell lung carcinoma is adenocarcinoma of the lung (also known as pulmonary adenocarcinoma), squamous-cell carcinoma (SCC) of the lung or large-cell carcinoma (LCC).~~

~~104. The method of claim 99, which is used for diagnosis, prognosis, stratification, risk assessment, or treatment monitoring of colorectal cancer in a subject.~~

~~105. The method of claim 99, which is used for diagnosis, prognosis, stratification, risk assessment, or treatment monitoring of pan-cancer analysis or profiling in a subject.~~

~~106. The method of any of claims 50-105, wherein cancer or neoplasia in multiple subjects are assessed sequentially or simultaneously.~~

~~107. The method of any one of claim 50-106, which has sensitivity of at least 10%.~~

~~108. The method of any one of claim 50-107, which has specificity of at least 10%.~~

~~109. The method of any one of claim 50-108, which further comprises treating a subject or altering treatment of a subject based on the assessment of cancer or neoplasia in the subject.~~

~~110. The method of claim 109, which comprises treating a human patient or altering treatment of a human patient based on the assessment of cancer or neoplasia in the human patient.~~

~~111. The method of claim 109 or 110, wherein the treatment is chemotherapy, radiotherapy, immunotherapy cell therapy, surgery, treatment with a drug, *e.g.*, a small molecule drug or a large molecule drug such an antibody drug.~~