disorder to distinguish them from subjects that have a lower risk of developing colon cell proliferative disorder, or to identify subjects having a colon cell proliferative disorder precursor (such as intraductal papillary mucinous neoplasm (IPMN)) versus a non-IPMN, or to identify subjects that have a malignant IPMN versus a benign IPMN. Thus, these miRNAs can be used as an adjunctive tool to guide decisions regarding monitoring, treatment, and management of colon cell proliferative disorder.

[0005] Some examples of the present disclosure provide a machine learning model classifier trained on the miRNAs described herein that are differentially expressed in a sample of a subject having colon cell proliferative disorder, or having a high risk of developing colon cell proliferative disorder, for example, when the subject has a colorectal lesion. In an example, a method is provided for a blood-based minimally-invasive miRNA assay that can be used in a subject having a colorectal lesion to assess histologic severity. In another example, the miRNAs indicative of colon cell proliferative disorder are detected in cell-free samples from a subject, for example, bodily fluid samples from a subject, such as whole blood, plasma, or serum. As such, the present disclosure provides miRNAs that can be used to differentiate between the presence or absence of colon cell proliferative disorder, high-risk or low-risk colorectal lesions that warrant treatment such as, surgical resection, immunotherapy, radiation, or chemotherapy) and low-risk colorectal lesions that can be monitored. Monitoring and confirmation of the presence of colon cell proliferative disorder or lesions can be carried out, for example, by colonoscopy, ultrasound, MM, or CT scan.

In one aspect, the present disclosure refers to a method for determining a micro ribonucleic acid (miRNA) profile of a cell-free biological sample from a subject, comprising:

a) isolating ribonucleic acid (RNA) molecules from a cell-free biological sample;

b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules;

c) ligating adapters to the RNA molecules or to the cDNA molecules or derivatives thereof;

d) amplifying the cDNA molecules or derivatives thereof of c);

e) determining nucleic acid sequences of the amplified cDNA molecules or derivatives thereof; and

f) aligning the nucleic acid sequences to (i) a first reference human transcriptome of hairpin micro RNA (miRNA) sequences and (ii) (1) a reference human genome at genomic loci corresponding to a panel associated with colorectal cancer comprising at least one miRNA or (2) a second reference human transcriptome of mature miRNA sequences at the genomic loci corresponding to the panel associated with colorectal cancer comprising the at least one miRNA to produce aligned nucleic acid sequences, thereby producing the miRNA profile of the subject.

In another aspect, the present disclosure refers to a method, comprising:

a) isolating ribonucleic acid (RNA) molecules from a cell-free biological sample of a subject;

b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules;

c) ligating adapters to the RNA molecules or cDNA molecules or derivatives thereof;

d) amplifying the cDNA molecules or derivatives thereof of c);

e) determining nucleic acid sequences of the amplified cDNA molecules or derivatives thereof;

f) aligning the nucleic acid sequences to (i) a first reference human transcriptome of hairpin micro RNA (miRNA) sequences and (ii) (1) a reference human genome at genomic loci corresponding to a panel associated with colorectal cancer comprising at least one miRNA or (2) a second reference human transcriptome of mature miRNA sequences at the genomic loci corresponding to the panel associated with colorectal cancer comprising the at least one miRNA to produce aligned nucleic acid sequences, thereby producing a miRNA profile of the subject; and

g) computer processing the miRNA profile using a machine learning model trained to be capable of distinguishing between subjects without the colorectal cancer and subjects with the colorectal cancer to provide an output value associated with a presence or an absence of the colorectal cancer , thereby indicating the presence or the absence of the colorectal cancer in the subject.

[0006] In an example, the present disclosure provides a micro ribonucleic acid (miRNA) signature panel characteristic of a colon cell proliferative disorder, comprising: a pre-determined set of one or more, two or more, three or more, or four or more miRNAs selected from the group listed in **Tables 1-11**, wherein the four or more miRNAs are differentially expressed between a biological sample from a subject having the colon cell proliferative disorder or subtype thereof, and a biological sample from a subject without the colon cell proliferative disorder or subtype thereof.

[0007] In some examples, the miRNA signature panel is characteristic of advanced adenoma, and the signature panel comprises: a pre-determined set of miRNAs comprising: a) hsa-miR-1273a, hsa-miR-17-5p, hsa-miR-20a-3p, hsa-miR-20b-5p; b) hsa-miR-3065-5p, hsa-miR-4785, hsa-miR-5096, hsa-miR-5189-5p, or c) hsa-miR-545-3p, hsa-miR-570-3p, hsa-miR-624-3p, hsa-mir-1181, hsa-mir-6073, wherein the miRNAs are differentially expressed between a biological sample from a subject having advanced adenoma or subtype thereof, and a biological sample from a subject without advanced adenoma or subtype thereof.

[0014] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0015] In some examples, the colon cell proliferative disorder is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

[0016] In another example, the present disclosure provides a classifier capable of distinguishing a population of healthy subjects (e.g., subjects without colon cell proliferative disorder) from subjects with colon cell proliferative disorder, comprising: a) sets of measured values representative of differential miRNA expression in 6 or more, or 12 or more pre-selected miRNAs selected from the group listed in **Tables 1-11**, wherein the measured values are obtained from miRNA expression data from healthy subjects and subjects having a colon cell proliferative disorder, b) wherein the measured values are used to generate a set of features corresponding to properties of the differential miRNA expression, and wherein the set of features is computer processed using machine learning model (e.g., a statistical model), c) wherein the machine model provides a feature vector useful as a classifier capable of distinguishing a population of healthy subjects from subjects having a colon cell proliferative disorder.

[0017] In some examples, the pre-selected miRNAs comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0018] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0019] In some examples, the sets of measured values describe characteristics of the differential miRNA expression selected from the group consisting of: count or rate of observing fragments with different counts, raw miRNA abundance, miRNA abundance normalized to

housekeeping genes, miRNA abundance normalized to synthetic sequences, log normalized miRNA abundance, fragment length, fragment midpoint, read mapping position and read pile up along mature miRNAs or miRNA hairpins, and abundances of clusters of miRNAs.

[0020] In some examples, the machine learning model is trained using training data obtained from training biological samples, a first subset of the training biological samples identified as corresponding to a subject having a colon cell proliferative disorder and a second subset of the training biological samples identified corresponding to a subject as not having a colon cell proliferative disorder.

[0021] In some examples, the classifier is provided in a system for detecting a colon cell proliferative disorder comprising: a) a computer-readable medium comprising a classifier operable to classify the subjects based on a miRNA signature panel; and b) one or more processors for executing instructions stored on the computer-readable medium.

[0022] In some examples, the system comprises a classification circuit that is configured as a machine learning classifier selected from the group consisting of a deep learning classifier, a neural network classifier, a linear discriminant analysis (LDA) classifier, a quadratic discriminant analysis (QDA) classifier, a support vector machine (SVM) classifier, a random forest (RF) classifier, a linear kernel support vector machine classifier, a first or second order polynomial kernel support vector machine classifier, a ridge regression classifier, an elastic net algorithm classifier, a sequential minimal optimization algorithm classifier, a naive Bayes algorithm classifier, and principal component analysis classifier.

[0023] In another example, the present disclosure provides a method for determining a micro ribonucleic acid (miRNA) profile of a biological sample from a subject, comprising: a) isolating RNA molecules from the biological sample; b) ligating RNA adapters to the RNA molecules, before or after reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) amplifying the cDNA molecules; d) determining nucleic acid sequences of the cDNA molecules; e) aligning the nucleic acid sequences to a reference nucleic acid sequence for a panel of miRNAs selected from the group listed in **Tables 1-11**; and f) determining the miRNA profile of the subject based at least in part on the aligned nucleic acid sequences.

[0024] In some examples, the pre-selected miRNAs comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least

110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0025] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0026] In some examples, the method further comprises enriching or depleting the RNA molecules or the cDNA molecules.

[0027] In some examples, the reference nucleic acid sequence comprises genome, transcriptome, or custom transcriptome reference nucleic acid sequences.

[0028] In some examples, the method further comprises preparing a miRNA library before the amplifying.

[0029] In some examples, the adapter ligation comprises RNA adapter ligation, adapter blocking, adapter circularization and dimer removal before c).

[0030] In some examples, ligating the RNA adapters comprises performing adapter blocking, adapter circularization, and/or dimer removal.

[0031] In some examples, ligating the RNA adapters comprises performing 3′ RNA adapter ligation, 5′ RNA adapter ligation, reverse transcription with unique molecular identifier (UMI) assignment, and/or cDNA cleanup.

[0032] In another example, the present disclosure provides a method for determining a micro ribonucleic acid (miRNA) profile of a biological sample from a subject, comprising performing one or more of: 1) Extraction of RNA molecules from the biological sample followed by direct RNA counting, 2) Extraction of RNA molecules from the biological sample followed by A tailing, then reverse transcribing (RT) to cDNA with template switching, 3) Extraction of RNA molecules from the biological sample followed by A tailing, then reverse transcription polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR) or digital droplet PCR (ddPCR), 4) Extraction of RNA molecules from the biological sample followed by sequence-specific ligation, and then RT-PCR and qPCR or ddPCR, and 5) Extraction-free miRNA profiling of RNA molecules from the biological sample in absence of performing RNA isolation; and determining the miRNA profile of the biological sample from the subject.

[0033] In some examples, determining the miRNA profile comprises use of a reference nucleic acid sequence that is part of a human genome or human transcriptome database.

[0034] In some examples, determining the miRNA profile comprises generating a counts table of expressed miRNA.

[0035] In some examples, determining the miRNA profile comprises generating a counts table normalized based on expressed miRNA to identify differentially-abundant miRNA.

[0036] In some examples, the miRNA profile is associated with a colon cell proliferative disorder and provides classification of a subject as having a colon cell proliferative disorder or not having a colon cell proliferative disorder.

[0037] In some examples, the biological sample from the subject is selected from the group consisting of bodily fluid, stool, colonic effluent, urine, blood plasma, blood serum, whole blood, isolated blood cells, cells isolated from the blood, and combinations thereof.

[0038] In some examples, the method further comprises comparing the miRNA profile against a database of reference miRNA profiles from healthy subjects; and determining that the subject has an increased risk of having a colon cell proliferative disorder based at least in part on measuring a change of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% in miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0039] In some examples, the method further comprises comparing the miRNA profile against a database of reference miRNA profiles from healthy subjects; and determining that the subject has an increased risk of having a colon cell proliferative disorder based at least in part on measuring a change of at least 15% in miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0040] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0041] In some examples, the colon cell proliferative disorder is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

[0042] In some examples, the advanced adenoma comprises a tubular adenoma, a tubulovillous adenoma, a villous adenoma, an adenocarcinoma, or a hyperplastic polyp.

[0043] In another example, the present disclosure provides a method for detecting a presence or an absence of a colon cell proliferative disorder in a subject, comprising: a) isolating ribonucleic acid (RNA) molecules from the biological sample; b) ligating RNA adapters to the RNA molecules, before or after reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) amplifying the cDNA molecules; d) determining nucleic acid sequences of the cDNA molecules; e) aligning the nucleic acid sequences to a

30%, at least 35%, at least 40%, at least 45%, or at least 50% in the miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0052] In some examples, the method further comprises comparing the miRNA profile against a database of reference miRNA profiles from healthy subjects; and determining that the subject has an increased risk of having a colon cell proliferative disorder based at least in part on measuring a change of at least 15% in the miRNA expression of the miRNA profile relative to the reference miRNA profiles.

[0053] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0054] In some examples, the colon cell proliferative disorder is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

[0055] In another example, the present disclosure provides a method for determining a miRNA profile of a biological sample from a subject comprising: a) isolating ribonucleic acid (RNA) molecules from the biological sample; b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) ligating RNA adapters to the RNA molecules or the cDNA molecules; d) amplifying the cDNA molecules; e) determining nucleic acid sequences of the cDNA molecules; f) aligning the nucleic acid sequences to a reference nucleic acid sequence for a panel of miRNAs selected from the group listed in **Tables 1-11**; and g) determining the miRNA profile based at least in part on the aligned nucleic acid sequences.

[0056] In some examples, the pre-selected miRNAs comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0057] In some examples, the method further comprises administering a treatment to the subject based on the detected colon cell proliferative disorder. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0058] In another example, the present disclosure provides a method for detecting a presence or an absence of a colon cell proliferative disorder in a subject, comprising: a) isolating ribonucleic acid (RNA) molecules from the biological sample; b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules; c) ligating RNA adapters to the RNA molecules or the cDNA molecules; d) amplifying the cDNA molecules; e) determining nucleic acid sequences of the cDNA molecules; f) aligning the nucleic acid sequences to a reference nucleic acid sequence for a panel of miRNAs selected from the group listed in **Tables 1-11**; g) determining an miRNA profile based at least in part on the aligned nucleic acid sequences; h) computer processing the miRNA profile using a machine learning model trained to distinguish between subjects not having the colon cell proliferative disorder and subjects having the colon cell proliferative disorder; and i) outputting by the machine learning model a value associated with subjects having the colon cell proliferative disorder or with subjects not having the colon cell proliferative disorder, thereby detecting the presence or the absence of the colon cell proliferative disorder in the subject.

[0059] In some examples, the pre-selected miRNAs comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, or at least 250 miRNAs selected from the group listed in **Tables 1-11**.

[0060] In some examples, the colon cell proliferative disorder is selected from the group consisting of adenoma (adenomatous polyps), sessile serrated adenoma (SSA), advanced adenoma, colorectal dysplasia, colorectal adenoma, colorectal cancer, colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

[0061] In some examples, the method further comprises administering a treatment to the subject based on the detected colon cell proliferative disorder. In some examples, the treatment comprises chemotherapy, radiotherapy, immunotherapy, or surgery.

[0062] In another example, the present disclosure provides a method for monitoring minimal residual disease in a subject previously treated for a disease, comprising: determining a micro ribonucleic acid (miRNA) profile of a biological sample from the subject using a panel of miRNAs selected from the group listed in **Tables 1-11**, thereby generating a baseline miRNA state; determining a miRNA profile of a biological sample obtained from the subject at one or

CLAIMS

WHAT IS CLAIMED IS:

1. A method for determining a micro ribonucleic acid (miRNA) profile of a cell-free biological sample from a subject, comprising:

a) isolating ribonucleic acid (RNA) molecules from a cell-free biological sample;

b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules;

c) ligating adapters to the RNA molecules or to the cDNA molecules or derivatives thereof;

d) amplifying the cDNA molecules or derivatives thereof of c);

e) determining nucleic acid sequences of the amplified cDNA molecules or derivatives thereof; and

f) aligning the nucleic acid sequences to (i) a first reference human transcriptome of hairpin micro RNA (miRNA) sequences and (ii) (1) a reference human genome at genomic loci corresponding to a panel associated with colorectal cancer comprising at least one miRNA or (2) a second reference human transcriptome of mature miRNA sequences at the genomic loci corresponding to the panel associated with colorectal cancer comprising the at least one miRNA to produce aligned nucleic acid sequences, thereby producing the miRNA profile of the subject.

2. The method of claim 1, wherein the at least one miRNA is selected from the group listed in Tables 1-11.

3. The method of claim 2, wherein the at least one miRNA comprises at least 2 miRNAs selected from the group listed in Tables 1-11.

4. The method of claim 1, wherein the RNA molecules comprises cell-free RNA (cfRNA) molecules.

5. The method of claim 1, further comprising enriching or depleting the RNA molecules or the cDNA molecules.

6. The method of claim 1, further comprising preparing a miRNA library before the amplifying.

7. The method of claim 1, wherein the adapters comprise RNA adapters.

8. The method of claim 1, further comprising performing adapter blocking, adapter circularization, and dimer removal.

9. The method of claim 1, further comprising performing 3′ RNA adapter ligation, 5′ RNA adapter ligation, reverse transcription with unique molecular identifier (UMI) assignment, and cDNA cleanup.

10.. The method of claim 1, further comprising performing one or more of: 1) extracting the RNA molecules from the cell-free biological sample followed by performing direct RNA

counting, 2) extracting the RNA molecules from the cell-free biological sample followed by performing A tailing, then reverse transcription (RT) of the cDNA with template switching, 3) extracting the RNA molecules from the cell-free biological sample followed by performing A tailing, then performing reverse transcription polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR) or digital droplet PCR (ddPCR), 4) extracting the RNA molecules from the cell-free biological sample followed by performing sequence-specific ligation, and then performing RT-PCR and qPCR or ddPCR, or 5) performing extraction-free miRNA profiling of the RNA molecules from the cell-free biological sample in an absence of performing RNA isolation; and determining the miRNA profile of the cell-free biological sample from the subject.

11. The method of claim 10, wherein determining the miRNA profile further comprises generating a counts table of expressed miRNA.

12. The method of claim 10, wherein determining the miRNA profile further comprises generating a counts table normalized based on expressed miRNA to identify differentially-abundant miRNA.

13. The method of claim 10, wherein the miRNA profile is associated with the colorectal cancer and provides classification of the subject as having the colorectal cancer or not having the colorectal cancer.

14. The method of claim 1, wherein the cell-free biological sample comprises blood plasma or blood serum.

15. The method of claim 1, further comprising comparing the miRNA profile against a database of reference miRNA profiles from subjects without the colorectal cancer; and determining that the subject has an increased risk of having the colorectal cancer based at least in part on measuring a change of at least 15% in miRNA expression of the miRNA profile relative to the reference miRNA profiles.16. The method of claim 1, wherein the colorectal cancer is selected from the group consisting of colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

17. The method of claim 1, wherein the colorectal cancer is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

18. The method of claim 1, wherein the aligning in f) comprises aligning the nucleic acid sequences to (i) the first reference human transcriptome of hairpin miRNA sequences and (ii) the reference human genome at the genomic loci corresponding to the panel comprising the at least one miRNA.

19. The method of claim 1, wherein the aligning in f) comprises aligning the nucleic acid sequences to (i) the first reference human transcriptome of hairpin miRNA sequences and (ii) the second reference human transcriptome of mature miRNA sequences at the genomic loci corresponding to the panel comprising the at least one miRNA.

20. A method, comprising:

a) isolating ribonucleic acid (RNA) molecules from a cell-free biological sample of a subject;

b) reverse transcribing the RNA molecules to complementary deoxyribonucleic acid (cDNA) molecules;

c) ligating adapters to the RNA molecules or cDNA molecules or derivatives thereof;

d) amplifying the cDNA molecules or derivatives thereof of c);

e) determining nucleic acid sequences of the amplified cDNA molecules or derivatives thereof;

f) aligning the nucleic acid sequences to (i) a first reference human transcriptome of hairpin micro RNA (miRNA) sequences and (ii) (1) a reference human genome at genomic loci corresponding to a panel associated with colorectal cancer comprising at least one miRNA or (2) a second reference human transcriptome of mature miRNA sequences at the genomic loci corresponding to the panel associated with colorectal cancer comprising the at least one miRNA to produce aligned nucleic acid sequences, thereby producing a miRNA profile of the subject; and

g) computer processing the miRNA profile using a machine learning model trained to be capable of distinguishing between subjects without the colorectal cancer and subjects with the colorectal cancer to provide an output value associated with a presence or an absence of the colorectal cancer , thereby indicating the presence or the absence of the colorectal cancer in the subject.

21. The method of claim 20, wherein the at least one miRNA is selected from the group listed in Tables 1-11.

22. The method of claim 21, wherein the at least one miRNA comprises at least 2 miRNAs selected from the group listed in Tables 1-11.

23. The method of claim 20, wherein the RNA molecules comprise cell-free RNA (cfRNA) molecules.

24. The method of claim 20, further comprising enriching or depleting the RNA molecules or the cDNA molecules.

25. The method of claim 20, further comprising preparing a miRNA library before the amplifying.

26. The method of claim 20, wherein the adapters comprise RNA adapters.

27. The method of claim 20, further comprising performing adapter blocking, adapter circularization, and dimer removal.

28. The method of claim 20, further comprising performing 3' RNA adapter ligation, 5' RNA adapter ligation, reverse transcription with unique molecular identifier (UMI) assignment, and cDNA cleanup.

29. The method of claim 20, wherein producing the miRNA profile comprises generating a counts table of expressed miRNA.

30. The method of claim 20, wherein producing the miRNA profile comprises generating a counts table of expressed miRNA to identify differentially-abundant miRNA.

31. The method of claim 20, wherein the cell-free biological sample comprises blood plasma or blood serum.

32. The method of claim 20, further comprising comparing the miRNA profile against a database of reference miRNA profiles from subjects without the colorectal cancer; and determining that the subject has an increased risk of having the colorectal cancer based at least in part on measuring a change of at least 15% in an miRNA expression of the miRNA profile relative to the reference miRNA profiles.

33. The method of claim 20, wherein the colorectal cancer is selected from the group consisting of colon cancer, rectal cancer, colorectal carcinoma, colorectal adenocarcinoma, carcinoid tumors, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors (GISTs), lymphomas, and sarcomas.

34. The method of claim 20, wherein the colorectal cancer is selected from the group consisting of stage 1 colorectal cancer, stage 2 colorectal cancer, stage 3 colorectal cancer, and stage 4 colorectal cancer.

35. The method of claim 20, wherein the aligning in f) comprises aligning the nucleic acid sequences to (i) the first reference human transcriptome of hairpin miRNA sequences and (ii) the reference human genome at the genomic loci corresponding to the panel comprising the at least one miRNA.

36. The method of claim 20, wherein the aligning in f) comprises aligning the nucleic acid sequences to (i) the first reference human transcriptome of hairpin miRNA sequences and (ii) the second reference human transcriptome of mature miRNA sequences at the genomic loci corresponding to the panel comprising the at least one miRNA.