

(51) International Patent Classification:
G01N 33/48 (2006.01)(21) International Application Number:
PCT/US2010/046916(22) International Filing Date:
27 August 2010 (27.08.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
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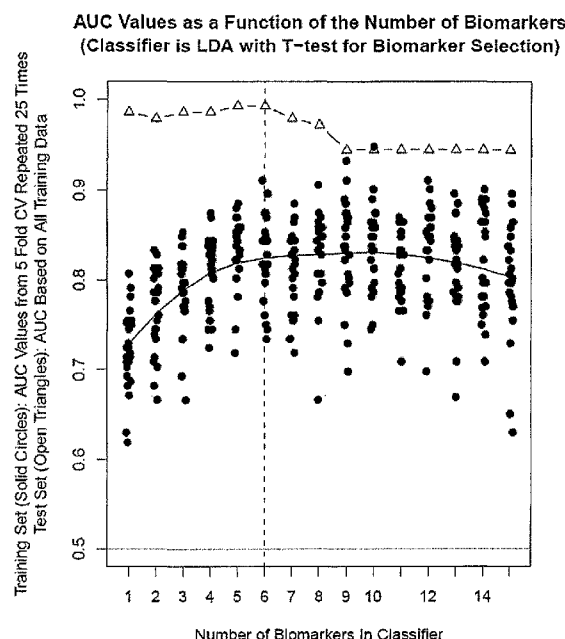
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

[Continued on next page]

(54) Title: MIRNA BIOMARKERS OF LUNG DISEASE

Figure 3



(57) Abstract: This application describes miRNAs that may be used as serum or plasma biomarkers for characterizing lung disease in a patient. These miRNA biomarkers may be used alone or in combination with other markers for the diagnosis, prognosis, or monitoring of diseases such as lung cancer. In one embodiment, a method for characterizing lung cancer in a patient with a lung tumor or lesion comprising the steps of: measuring the level of a miRNA in a serum sample, and determining reduced or elevated levels of the miRNA in the sample, thereby characterizing lung cancer in the patient.



(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK,

SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

MIRNA BIOMARKERS OF LUNG DISEASE

[001] This application claims priority to U.S. Provisional Application No. 61/237,972, filed August 28, 2009, which is incorporated by reference in its entirety.

[002] Work described in this application was partially funded by the Federal government under National Cancer Institution/NIH Grant No. 1R43CA141786-01. Accordingly, the Federal government may have certain rights in this invention.

[003] In the United States, lung cancer has the highest incidence and the highest mortality rate of all cancers (<http://seer.cancer.gov/statistics/>). Lung cancer is divided into two major classes: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), the former affecting 20% of patients and the latter 80%. NSCLC consists of three major subtypes: adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma, with adenocarcinoma and squamous cell carcinoma accounting for the vast majority (Sekido et al., *Biochim Biophys Acta*, 1378:F21-59; Forgacs et al., *Pathol Oncol Res*, 7:6-13 (2001)). An estimated 215,000 new cases of lung cancer and approximately 162,000 deaths from lung cancer occurred in the United States during 2008 (Jemal et al., *J Natl Cancer Inst*, 100:1672-94 (2008)). The high mortality rate of lung cancer has been attributed in part to the fact that more than 75% of lung cancer patients are diagnosed with regional or distant metastasis at first presentation, and in part to the lack of highly effective therapies for lung cancer. Although the current treatment options for lung cancer are limited, it has been shown that patients who are diagnosed at the earliest stage have a better chance of 5-yr survival (50%) than those in later stages (<10%) (see, e.g., <http://seer.cancer.gov/statistics>; Horner et al. (eds). SEER Cancer Statistics Review, 1975-2006, National Cancer Institute. Bethesda, MD). Consequently, extensive efforts have been directed to the detection of lung cancer at an early stage so that medical intervention may improve survival.

[004] Risk factors for lung cancer include age and tobacco consumption by smoking (first hand and second hand). Approximately 90% of all lung cancer cases occur in smokers. Additionally, significant differences exist in both incidence and mortality of lung cancer between males and females, suggesting

the possibility of inherent biological processes between the two sexes. These differences are among the most consistently reported significant risk factors in lung cancer (Visbal et al, *Ann Thorac Surg*, 78:209-15 (2004)).

[005] Several screening studies designed to detect lung cancer at an earlier stage have been undertaken. Many of these studies have used two major imaging technologies: chest X-ray and computed tomography (CT). A chest X-ray may reveal a mass in the lung that may be biopsied for verification and classification of lung cancer. Improvements in imaging technologies, most notably the development of spiral computed tomography (spiral CT), have enabled clinicians to detect smaller lung tumors in pre-symptomatic individuals. The Early Lung Cancer Action project (ELCAP), designed to screen symptom-free high-risk smokers using chest X-ray and low dose CT, showed that CT greatly improved the detection of smaller, potentially curable lesions (Henschke et al., *Lancet*, 354:99-105 (1999)). However, the major disadvantages were the rate of false positives and high cost (average \$600) that were difficult to justify as a screening paradigm. *Id.*; see also Henschke et al., *N Engl J Med*, 355:1763-71 (2006).

[006] These studies have generated an intense discussion within the scientific community centering on the design of the studies, particularly the lack of randomization, and the conclusions drawn with concerns of high cost and high false positive rate (Henschke et al., 1999; Gould et al., *N Engl J Med*, 356:743-747 (2007)). Between 25 and 60 percent of spiral CT scans of smokers and former smokers show benign abnormalities (Swensen et al., *Am J Respir Crit Care Med*, 165:508-13 (2002)). Imaging screening of asymptomatic patients does not distinguish benign from early malignant lesions. It has also been reported that repeated exposure to low dose CT scans may expose patients to potentially harmful levels of radiation (Brenner et al., *Radiology*, 231:440-5 (2004)).

[007] At present, there is still a need for clinically relevant markers for non-invasive detection of lung disease including cancer, monitoring response to therapy, or detecting lung cancer recurrence. It is also clear that such assays must be highly specific with reasonable sensitivity, and be readily available at a reasonable cost. Circulating biomarkers offer an alternative to imaging with the following advantages: 1) they are found in a minimally-invasive, easy to collect specimen type (blood or blood-derived fluids), 2) they can be monitored frequently over time in a subject to establish an accurate baseline, making it easy to detect

changes over time, 3) they can be provided at a reasonably low cost, 4) they may limit the number of patients undergoing repeated expensive and potentially harmful CT scans, and/or 5) unlike CT scans, biomarkers may potentially distinguish indolent from more aggressive lung lesions (see, e.g., Greenberg and Lee, *Opin Pulm Med*, 13:249-55 (2007)).

[008] Existing biomarker assays include several serum protein markers such as CEA (Okada et al., *Ann Thorac Surg*, 78:216-21 (2004)), CYFRA 21-1 (Schneider, *Adv Clin Chem*, 42:1-41 (2006)), CRP (Siemes et al., *J Clin Oncol*, 24:5216-22 (2006)), CA-125 (Schneider, 2006), and neuron-specific enolase and squamous cell carcinoma antigen (Siemes et al., 2006). Low sensitivity and specificity, with a significant number of false positive results due to benign pulmonary diseases have limited the application of these assays.

[009] Circulating nucleic acids such as DNA and mRNA have also been evaluated as possible diagnostic markers for lung cancer. These studies are based on the observations that circulating nucleic acids show differential expression that is suggestive of cancer. (See, e.g., Bremnes et al., *Lung Cancer*, 49:1-12 (2005); Johnson et al., *Cell*, 120:635-47 (2005); Yanaihara et al., *Cancer Cell*, 9:189-98 (2006); Chen et al., *Cell Res*, 18:997-1006 (2008); Fabbri et al., *Cancer J*, 14:1-6 (2008); Garofalo et al., *Oncogene*, 27:3845-55 (2008); Mitchell et al., *Proc Natl Acad Sci*, 105:10513-8 (2008); Schickel et al., *Oncogene*, 27:5959-74 (2008); Weiss et al., *Ann Oncol*, 19:1053-9 (2008); and Yu et al., *Cancer Cell*, 13:48-57 (2008).) The origin of free DNA in circulation is not completely understood, but they are thought to represent the stable remaining fraction from damaged (apoptotic, necrotic) tumor cells (Jahr et al., *Cancer Res*, 61:1659-65 (2001); Bianchi, *Placenta*, 25 Suppl A:S93-S101 (2004)).

[010] We herein describe methods for detecting, diagnosing, or monitoring lung disease by measuring miRNAs from serum or plasma.

[011] In some embodiments, the invention relates to the detection or monitoring of lung diseases such as small cell lung cancer or non-small cell lung cancer by detecting miRNAs from serum or plasma. The methods of the invention include detection of biomarkers that can be used to diagnose disease and/or evaluate the prognosis or aggressiveness of a lung disease. Further, the methods may be used to characterize the progression of a lung disease. In certain embodiments, the methods of the invention may be used to determine whether a

lung tumor or lesion in a patient is cancerous or benign. The patients tested using the methods of the invention may also be tested using other known methods in the art.

[012] In certain embodiments of the invention, the diagnosis or prognosis may be achieved by measuring the amount of a miRNA that is present in elevated or reduced levels in the serum or plasma of a subject with lung disease. In some instances, one serum or plasma miRNA may be detected (e.g., amplified and measured) to characterize lung disease, while in other embodiments, two or more miRNAs are detected from serum or plasma. Some embodiments include detecting a pair of miRNAs. In some instances, one miRNA in the pair is elevated in serum or plasma of patients with lung disease or lung cancer, and the other miRNA in the pair is reduced. In other circumstances, both miRNAs in the pair can be elevated or both reduced. In certain embodiments, non-miRNA biomarkers such as protein markers may also be measured. Some embodiments of the invention relate to diagnosis or prognosis of lung cancer, or determining the type of lung cancer in a patient. In some embodiments, the patient has previously been screened for lung disease.

[013] Additional embodiments of the invention are discussed throughout this application. Other objects, features, and advantages of the present invention will become apparent from the following detailed description. Any embodiment discussed with respect to one aspect of the invention applies to other aspects of the invention as well and vice versa. The embodiments in the Example section are understood to be embodiments of the invention that are applicable to all aspects of the invention.

[014] It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this application.

BRIEF DESCRIPTION OF THE DRAWINGS

[015] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings

in combination with the detailed description of specific embodiments presented herein.

[016] Figure 1 is a schematic outline of the study design for the mouse model for lung cancer. One hundred fifteen A/J mice were administered with an oral gavage of benzo(a)pyrene at the indicated dose at time (0) and at 10 weeks post the first gavage. Ten control mice received an oral gavage of 200 μ l cottonseed oil with no carcinogen at week/day 0. The number of mice sacrificed at each time point is shown below the arrows. The remaining mice were bled every two weeks until 18 weeks and every four weeks thereafter. At the end of 34 weeks, all remaining mice were sacrificed.

[017] Figure 2A and Figure 2B show temporal changes of miR-183 and miR-182 (Figure 2A) and miR-365 and miR-141 (Figure 2B) plasma miRNA expression in mice that developed lung tumors and in control mice.

[018] Figure 3 shows the distribution of area under the curve (AUC) values for the Linear Discriminate Analysis (LDA) classifier as a function of the number of biomarkers, as described in Example 5. This procedure determines the optimal number of biomarkers to use for the classifier. Solid black circles represent results from the training set; Open triangles represent results from the test set. The vertical dashed line is where performance levels off. The maximum mean AUC is where the number of features = 9.

[019] Figure 4 shows performance estimates of training and test data from the Linear Discriminate Analysis of Example 5. SENS, sensitivity; SPEC, specificity, NPV, negative predictive value; PPV, positive predictive value. Six differential miRNA pairs were used: miR-142-5p and miR181d; miR-142-3p and miR181d; miR-142-3p and miR-422a; miR-142-5p and miR-422a; miR-92 and miR-27b; and miR-24 and miR-27a.

[020] Figure 5 shows human precursor miRNA (pre-miRNA) sequences (SEQ ID NOS 1-323, respectively, in order of appearance), as provided by Release 13.0 of the miRBase::Sequences Database (<http://microrna.sanger.ac.uk>; Griffiths-Jones et al., Nucleic Acids Research, 2008, 36, Database Issue, D154-D158; Griffiths-Jones et al., Nucleic Acids Research, 2006, 34, Database Issue, D140-D144; Griffiths-Jones, Nucleic Acids Research, 2004, 32, Database Issue, D109-D111). The names of mature miRNAs from Tables 1-5 are also provided.

EXEMPLARY EMBODIMENTS

[021] In certain aspects, the methods of the invention provide assays for amplifying and measuring the amount of a miRNA in a serum or plasma sample, thereby characterizing a lung disease.

[022] To assist in understanding the present invention, certain terms are first defined. Additional definitions are provided throughout the application.

[023] As used herein, the term "microRNA" (miRNA or miR) includes human miRNAs, mature single stranded miRNAs, precursor miRNAs (pre-miR), and variants thereof, which may be naturally occurring. In some instances, the term "miRNA" also includes primary miRNA transcripts and duplex miRNAs. Unless otherwise noted, when used herein, the name of a specific miRNA refers to the mature miRNA of a precursor miRNA. For example, miR-122a refers to a mature miRNA sequence derived from pre-miR-122. The sequences for particular miRNAs, including human mature and precursor sequences, are reported in the miRBase::Sequences Database (<http://microrna.sanger.ac.uk> (version 15 released April 2010); Griffiths-Jones et al., *Nucleic Acids Research*, 2008, 36, Database Issue, D154-D158; Griffiths-Jones et al., *Nucleic Acids Research*, 2006, 34, Database Issue, D140-D144; Griffiths-Jones, *Nucleic Acids Research*, 2004, 32, Database Issue, D109-D111). For certain miRNAs, a single precursor contains more than one mature miRNA sequence. In other instances, multiple precursor miRNAs contain the same mature sequence. In some instances, mature miRNAs have been re-named based on new scientific consensus. For example, miR-213, as used herein, refers to a mature miRNA from pre-miR-181a-1, and is also called miR-181a*. Other miRNAs that have been re-named include miR-189 (also called miR-24*), which comes from pre-miR-24-1; miR-368 (also called miR-376c); and miR-422b (also called miR-378*). The skilled artisan will appreciate that scientific consensus regarding the precise nucleic acid sequence for a given miRNA, in particular for mature forms of the miRNAs, may change with time. MiRNAs detected by assays of this application include naturally occurring sequences for the miRNAs.

[024] The term "characterizing" is used herein to encompass detection and prognosis, and it includes detection of a miRNA for making diagnostic or prognostic determinations or predictions of disease. In some instances, the

characterization will identify whether a subject has a lung disease such as cancer, or will determine the disease state. Additionally, detection of a miRNA according to the methods herein includes measuring the amount of a miRNA that can be used to distinguish patients with lung cancer from patients having other lung diseases, or determine whether a patient with a lung tumor has cancer. In other circumstances, "characterizing" includes detection of a miRNA for determining the stage or aggressiveness of a disease state such as lung cancer, or determining an appropriate treatment method for lung disease.

[025] The use of the word "a", "an" or "the" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

I. SAMPLES

[026] Serum is typically the fluid, non-cellular portion of coagulated blood. Plasma is also a non-cellular blood sample, but unlike serum, plasma contains clotting factors. In some embodiments, serum or plasma samples are obtained from a human patient previously screened for lung disease using another diagnostic method. In various embodiments, serum or plasma samples are obtained from patients that have tested positive for a tumor or lung lesion. In certain embodiments, the patient has undergone imaging detection, e.g., by chest X-ray or CT scan. In other embodiments, the methods involve detection of miRNA in patients with a positive imaging result for lung disease. In some circumstances, samples are obtained from patients that have a lung tumor or lesion. The tumor or lesion may have been detected by chest X-ray or CT scan, or by other imaging or detection methods known in the art. Additional embodiments include measuring miRNA in samples from patients previously or currently undergoing treatment for a lung disease. In additional embodiments, the sample is from a patient suspected of having lung cancer or at risk of developing lung cancer. The volume of plasma or serum obtained and used for the assay may be varied depending upon clinical intent.

[027] One of skill in the art will recognize that many methods exist for obtaining and preparing serum samples. Generally, blood is drawn into a collection tube using standard methods and allowed to clot. The serum is then separated from the cellular portion of the coagulated blood. In some methods,

clotting activators such as silica particles are added to the blood collection tube. In other methods, the blood is not treated to facilitate clotting. Blood collection tubes are commercially available from many sources and in a variety of formats (e.g., Becton Dickinson Vacutainer[®] tubes--SST[™], glass serum tubes, or plastic serum tubes).

[028] In some methods, the blood is collected by venipuncture and processed within three hours after drawing to minimize hemolysis and minimize the release of miRNAs from intact cells in the blood. In some methods, blood is kept on ice until use. The blood may be fractionated by centrifugation to remove cellular components. In some embodiments, centrifugation to prepare serum can be at a speed of at least 500, 1000, 2000, 3000, 4000, or 5000 X G. In certain embodiments, the blood can be incubated for at least 10, 20, 30, 40, 50, 60, 90, 120, or 150 minutes to allow clotting. In other embodiments, the blood is incubated for at most 3 hours. When using plasma, the blood is not permitted to coagulate prior to separation of the cellular and acellular components. Serum or plasma can be frozen after separation from the cellular portion of blood until further assayed.

[029] Before analysis, RNA may be extracted from serum or plasma and purified using methods known in the art. Many methods are known for isolating total RNA, or for specifically extracting small RNAs, including miRNAs. The RNA may be extracted using commercially-available kits (e.g., Perfect RNA Total RNA Isolation Kit, Five Prime-Three Prime, Inc.; mirVana[™] kits, Ambion, Inc.). Alternatively, RNA extraction methods for the extraction of mammalian intracellular RNA or viral RNA may be adapted, either as published or with modification, for extraction of RNA from plasma and serum. RNA may be extracted from plasma or serum using silica particles, glass beads, or diatoms, as in the method or adaptations described in U.S. Publication No. 2008/0057502.

II. miRNA MARKERS FOR LUNG DISEASE

[030] Certain embodiments of the invention provide serum or plasma miRNAs as markers for lung disease. In some embodiments, miRNAs that are present at elevated levels in the serum and/or plasma of patients with lung disease are used as markers. In other embodiments, miRNAs that have reduced levels are used as markers. In some embodiments, more than one miRNA from serum or plasma will be used as markers. When more than one miRNA

biomarker is used, the miRNAs may all have elevated levels, all have reduced levels, or a mixture of miRNAs with elevated and reduced levels may be used.

[031] The terms “reduced levels” or “elevated levels” refer to the amount of a miRNA in a serum or plasma sample from a patient compared to the amount of the miRNA in serum or plasma from a cohort or cohorts that do not have the lung disease that the patient is being tested for. For instance, a miRNA that has reduced levels in the sera of lung cancer patients is present at lower amounts in lung cancer patient sera than in serum from a donor who does not have lung cancer (e.g., patients with benign tumors or normal patients). For certain miRNAs, elevated levels in a patient serum or plasma sample indicates presence or prognosis for a lung disease. Other miRNAs are present in reduced levels in patients with lung disease.

[032] Lung disease includes cancer and benign conditions. Lung cancer refers to malignant tumors of the lung, and can be classified as small cell or non-small cell lung cancer. In some embodiments, non-small cell lung cancer can be further characterized as adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma. In addition, cancers can be classified based on X-ray or CT scanning results, aggressiveness, pathology, and measurements of non-miRNA biomarkers, as well as other methods known in the art. In certain aspects, the lung cancer is classified by TNM principles (T-primary tumor, N-regional lymph nodes, M-distant metastasis) and/or stage 0, IA, IB, IIA, IIB, IIIA, IIIB or IV. (See, e.g., Lababede et al., *Chest*, 115:233-235 (1999).) In some embodiments, the methods described herein can be used to characterize a lung disease in a patient with at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sensitivity. The degree of sensitivity indicates the percentage of patients with a disease who are positively characterized as having the disease. In additional embodiments, the methods have at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% specificity (e.g., the percentage of non-diseased patients who are correctly characterized). The assay parameters can be adjusted to optimize for both sensitivity and specificity.

[033] In some cases, the level of the miRNA marker will be compared to a control to determine whether the level is reduced or elevated. The control may be an external control, such as a miRNA in a serum or plasma sample from a subject known to be free of lung disease. The external control may be a sample from a

normal (non-diseased) subject or from a patient with benign lung disease. In other circumstances, the external control may be a miRNA from a non-serum sample like a tissue sample or a known amount of a synthetic RNA. The external control may be a pooled, average, or individual sample; it may be the same or different miRNA as one being measured. An internal control is a marker from the same serum or plasma sample being tested, such as a miRNA control. See, e.g., US Publication No. US 2009/0075258, which is incorporated by reference in its entirety.

[034] Table 1 lists miRNAs that have elevated or reduced levels in serum from patients with lung disease. These miRNAs may be used in accordance with the invention. Some of the miRNAs are useful for characterizing lung cancer, including distinguishing the type of cancer and/or distinguishing cancer from benign lung disease. In addition, some miRNAs may be used to predict the aggressiveness or outcome of lung cancer.

Table 1. miRNAs with elevated or reduced levels in serum from patients with lung cancer. Levels are of miRNA in lung cancer patients compared to patients with benign tumors or lesions.

miRNA	Level	miRNA	Level	miRNA	Level
let-7a	reduced	miR-181a	elevated	miR-30b	reduced
let-7b	reduced	miR-181b	elevated	miR-30c	reduced
let-7c	reduced	miR-181d	elevated	miR-30d	elevated
let-7d	reduced	miR-185	reduced	miR-30e-3p	reduced
let-7e	reduced	miR-186	elevated	miR-30e-5p	reduced
let-7f	reduced	miR-18a	reduced	miR-320	elevated
let-7g	reduced	miR-190	reduced	miR-324-3p	elevated
let-7i	reduced	miR-191	elevated	miR-324-5p	elevated
miR-100	reduced	miR-192	reduced	miR-328	elevated
miR-101	reduced	miR-193b	elevated	miR-335	elevated
miR-103	elevated	miR-194	elevated	miR-339	reduced
miR-106a	reduced	miR-195	reduced	miR-340	reduced
miR-106b	reduced	miR-196b	reduced	miR-342	elevated
miR-10a	reduced	miR-197	elevated	miR-345	elevated
miR-10b	reduced	miR-199a*	reduced	miR-346	reduced
miR-125a	elevated	miR-19a	reduced	miR-361	elevated
miR-126	reduced	miR-19b	elevated	miR-365	elevated

miRNA	Level	miRNA	Level	miRNA	Level
miR-126*	reduced	miR-202	elevated	miR-374a	reduced
miR-130a	reduced	miR-204	elevated	miR-375	elevated
miR-130b	elevated	miR-205	reduced	miR-378	elevated
miR-132	elevated	miR-206	reduced	miR-382	elevated
miR-133a	elevated	miR-20a	reduced	miR-422a	elevated
miR-133b	reduced	miR-20b	elevated	miR-423	elevated
miR-134	elevated	miR-21	reduced	miR-432	reduced
miR-139	reduced	miR-210	elevated	miR-433	elevated
miR-140	reduced	miR-214	reduced	miR-483	elevated
miR-142-3p	reduced	miR-22	elevated	miR-485-3p	reduced
miR-142-5p	reduced	miR-221	elevated	miR-486-5p	elevated
miR-143	reduced	miR-222	elevated	miR-496	reduced
miR-145	elevated	miR-223	elevated	miR-497	reduced
miR-146a	elevated	miR-23a	elevated	miR-501	elevated
miR-146b	reduced	miR-24	elevated	miR-502	elevated
miR-148a	reduced	miR-25	elevated	miR-505	elevated
miR-150	elevated	miR-26a	reduced	miR-518b	elevated
miR-151	elevated	miR-26b	reduced	miR-525	reduced
miR-152	reduced	miR-27a	reduced	miR-566	elevated
miR-155	elevated	miR-27b	reduced	miR-584	elevated
miR-15a	reduced	miR-296	elevated	miR-605	elevated
miR-15b	reduced	miR-29a	reduced	miR-638	reduced
miR-16	reduced	miR-29c	reduced	miR-660	reduced
miR-17-5p	elevated	miR-301	elevated	miR-92	elevated
		miR-30a-5p	elevated	miR-93	elevated
		miR-98	reduced	miR-99a	elevated

[035] In certain embodiments, one serum miRNA is used to detect, diagnose, characterize, or monitor lung disease and/or lung cancer. In other embodiments, more than one miRNA is used as a marker. In additional embodiments, two or more miRNAs are used to characterize lung disease. In certain embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 miRNAs are detected in the methods of the invention. In certain methods, miRNAs that have reduced levels in serum or plasma from patients with lung disease are used as biomarkers.

In other embodiments, a miRNA with elevated levels in serum or plasma can be used as a biomarker. In certain embodiments, the patient has a lung tumor or lesion. In additional embodiments, the patient has previously been screened for lung disease.

[036] In some embodiments, a miRNA for diagnosing lung cancer is chosen from miR-375, miR-499, miR-22, miR-122a, miR-206, miR-103, miR-24, miR-26a, miR-498, miR-205, miR-222, and let-7c.

[037] In certain embodiments, a miRNA is chosen from let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, miR-106a, miR-106b, miR-125a, miR-126, miR-130b, miR-132, miR-133a, miR-133b, miR-140, miR-142-3p, miR-143, miR-146a, miR-150, miR-151, miR-155, miR-15a, miR-15b, miR-16, miR-181a, miR-181b, miR-181d, miR-186, miR-18a, miR-190, miR-191, miR-195, miR-197, miR-19b, miR-202, miR-206, miR-20a, miR-210, miR-214, miR-22, miR-221, miR-222, miR-223, miR-23a, miR-24, miR-25, miR-26a, miR-26b, miR-27b, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-3p, miR-320, miR-324-3p, miR-324-5p, miR-335, miR-342, miR-345, miR-346, miR-361, miR-365, miR-374, miR-378, miR-382, miR-422a, miR-432, miR-485-3p, miR-486, miR-496, miR-502, miR-584, miR-638, miR-660, miR-92, and miR-93.

[038] In additional embodiments, a miRNA is chosen from let-7f, let-7g, let-7i, miR-106b, miR-126, miR-126*, miR-140, miR-142-3p, miR-142-5p, miR-143, miR-145, miR-150, miR-15a, miR-15b, miR-181a, miR-181b, miR-181d, miR-202, miR-214, miR-27a, miR-27b, miR-30e-5p, miR-320, miR-324-3p, miR-340, miR-342, miR-345, miR-374, miR-378, miR-422a, miR-486, miR-518b, and miR-92.

[039] In other embodiments, at least one miRNA is chosen from let-7b, let-7c, let-7d, let-7e, miR-10a, miR-10b, miR-130b, miR-132, miR-133b, miR-139, miR-143, miR-152, miR-155, miR-15b, miR-17-5p, miR-193, miR-194, miR-195, miR-196b, miR-199a*, miR-19b, miR-202, miR-204, miR-205, miR-206, miR-20b, miR-21, miR-210, miR-214, miR-221, miR-27a, miR-27b, miR-296, miR-29a, miR-301, miR-324-3p, miR-324-5p, miR-339, miR-346, miR-365, miR-378, miR-422a, miR-432, miR-485-3p, miR-496, miR-497, miR-505, miR-518b, miR-525, miR-566, miR-605, miR-638, miR-660, and miR-93.

[040] In additional embodiments, a miRNA is chosen from miR-106a, miR-106b, miR-126*, miR-142-3p, miR-15b, miR-181c, miR-182, miR-26b, miR-30b, miR-30e-5p, miR-422b, let-7i, and let-7g.

[041] In further embodiments, a miRNA is chosen from miR-24, miR-92, miR-142-3p, miR-142-5p, miR-181d, miR-27a, miR-27b, miR-422a, miR-29b, miR-15a, miR-106b, miR-126, miR-140, and miR-202. In some embodiments, 2, 3, 4, 5, 6, 7, or 8 of these miRs can be used to distinguish patients with lung cancer from patients with benign lung tumors or lesions. In additional embodiments, 2, 3, 4, 5, 6, 7, or 8 miRNAs are chosen from miR-24, miR-92, miR-142-3p, miR-142-5p, miR-181d, miR-27a, miR-27b, and miR-422a.

[042] In certain embodiments, a miRNA for characterizing lung cancer vs. non-cancer samples is chosen from miR-15b, miR-182, miR-15a, miR-30b, miR-26b, miR-106b, let-7g, miR-142-3p, miR-301, miR-181c, miR-126, miR-346, miR-422b, and miR-92. Non-cancer samples include samples from subjects with benign lung tumors or lesions, or from normal subjects.

[043] Certain embodiments include a method for characterizing lung disease and/or lung cancer in a patient comprising the steps of measuring the level of a miRNA in a serum sample, wherein the miRNA is chosen from let-7b, let-7c, let-7d, let-7e, miR-10a, miR-10b, miR-130b, miR-132, miR-133b, miR-139, miR-143, miR-152, miR-155, miR-15b, miR-17-5p, miR-193, miR-194, miR-195, miR-196b, miR-199a*, miR-19b, miR-202, miR-204, miR-205, miR-206, miR-20b, miR-21, miR-210, miR-214, miR-221, miR-27a, miR-27b, miR-296, miR-29a, miR-301, miR-324-3p, miR-324-5p, miR-339, miR-346, miR-365, miR-378, miR-422a, miR-432, miR-485-3p, miR-496, miR-497, miR-505, miR-518b, miR-525, miR-566, miR-605, miR-638, miR-660, and miR-93; and determining reduced or elevated levels of the miRNA in the sample, thereby characterizing lung disease or lung cancer.

[044] Table 2 lists miRNAs that have elevated or reduced levels in plasma from patients with lung disease. These miRNAs may be used in accordance with the invention.

Table 2. miRNAs with elevated or reduced levels in plasma from patients with lung disease. Levels are of miRNA in lung cancer patients compared to patients without lung cancer.

miRNA	Level	miRNA	Level
let-7c	elevated	let-7a	reduced
miR-100	elevated	let-7d	reduced
miR-10a	elevated	let-7e	reduced
miR-10b	elevated	let-7g	reduced
miR-122a	elevated	let-7i	reduced
miR-125b	elevated	miR-1	reduced
miR-129	elevated	miR-103	reduced
miR-148a	elevated	miR-106a	reduced
miR-150	elevated	miR-125a	reduced
miR-17-5p	elevated	miR-130a	reduced
miR-183	elevated	miR-130b	reduced
miR-18a*	elevated	miR-133a	reduced
miR-18b	elevated	miR-145	reduced
miR-190	elevated	miR-148b	reduced
miR-192	elevated	miR-15a	reduced
miR-193a	elevated	miR-15b	reduced
miR-196b	elevated	miR-17-3p	reduced
miR-197	elevated	miR-181d	reduced
miR-19a	elevated	miR-18a	reduced
miR-19b	elevated	miR-196a	reduced
miR-200c	elevated	miR-198	reduced
miR-203	elevated	miR-199a	reduced
miR-206	elevated	miR-199a*	reduced
miR-20b	elevated	miR-212	reduced
miR-210	elevated	miR-22	reduced
miR-214	elevated	miR-221	reduced
miR-218	elevated	miR-23a	reduced
miR-296	elevated	miR-23b	reduced
miR-30a-3p	elevated	miR-26a	reduced
miR-31	elevated	miR-27a	reduced
miR-346	elevated	miR-27b	reduced
miR-34c	elevated	miR-29b	reduced
miR-375	elevated	miR-30b	reduced
miR-383	elevated	miR-30d	reduced
miR-422a	elevated	miR-30e-3p	reduced
miR-429	elevated	miR-320	reduced

miRNA	Level	miRNA	Level
miR-448	elevated	miR-323	reduced
miR-449	elevated	miR-326	reduced
miR-452	elevated	miR-331	reduced
miR-483	elevated	miR-335	reduced
miR-486	elevated	miR-339	reduced
miR-489	elevated	miR-374	reduced
miR-497	elevated	miR-377	reduced
miR-500	elevated	miR-379	reduced
miR-501	elevated	miR-410	reduced
miR-507	elevated	miR-423	reduced
miR-511	elevated	miR-433	reduced
miR-514	elevated	miR-485-3p	reduced
miR-516-3p	elevated	miR-485-5p	reduced
miR-520d	elevated	miR-487b	reduced
miR-527	elevated	miR-490	reduced
miR-7	elevated	miR-491	reduced
miR-92	elevated	miR-493	reduced
miR-93	elevated	miR-493-3p	reduced
miR-99a	elevated	miR-494	reduced
		miR-496	reduced
		miR-502	reduced
		miR-505	reduced
		miR-519d	reduced
		miR-539	reduced
		miR-542-3p	reduced
		miR-98	reduced

[045] In some embodiments, a single plasma miRNA may be used to characterize lung cancer. In other embodiments, one of the miRNAs from Table 2 may be used to characterize lung cancer, either alone or in combination with one or more additional miRNA markers.

[046] In certain embodiments, the methods distinguish lung cancer from benign lung disease. In some circumstances, at least one measured miRNA is elevated in the serum or plasma of lung cancer patients compared to patients with benign conditions or no disease. In some circumstances, at least one measured miRNA is reduced. In certain embodiments at least one measured miRNA is elevated and at least one miRNA is reduced. In other embodiments at least two elevated miRNAs or at least two reduced miRNAs are measured.

[047] In certain embodiments, one of the following miRNAs is used in combination with at least one other miRNA biomarker to determine whether a patient has lung cancer: let-7a, let-7b, let-7d, let-7f, let-7g, let-7i, miR-101, miR-106a, miR-106b, miR-125a, miR-126, miR-126*, miR-130b, miR-132, miR-133b, miR-140, miR-142-3p, miR-142-5p, miR-145, miR-146a, miR-146b, miR-148b, miR-150, miR-151, miR-15a, miR-15b, miR-181a, miR-181b, miR-181d, miR-185, miR-186, miR-190, miR-191, miR-193a, miR-199a*, miR-202, miR-210, miR-214, miR-222, miR-23a, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29b, miR-301, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-5p, miR-320, miR-324-3p, miR-326, miR-335, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-374, miR-375, miR-378, miR-422a, miR-422b, miR-425, miR-486, miR-496, miR-518b, miR-660, miR-7, miR-92, miR-93, miR-98, miR-99a, or miR-99b.

[048] In other embodiments, one of the following miRNAs is used in combination with at least one other miRNA biomarker to determine whether a patient has lung cancer or to distinguish lung cancer from benign lung disease: miR-422a; miR-29b; miR-92; miR-142-5p; miR-142-3p; miR-181d; miR-27b; miR-378; miR-27a; miR-30e-5p; miR-181a; miR-126; miR-342; miR-140; miR-15a; miR-324-3p; miR-374; miR-486; miR-518b; miR-106b; miR-145; miR-150; miR-191; miR-345; miR-126*; miR-148b; miR-214; miR-320; let-7g; let-7i; miR-146a; miR-15b; miR-185; miR-186; miR-23a; miR-24; miR-30a-5p; miR-340; miR-34a; miR-101; miR-132; miR-181b; miR-199a*; miR-202; miR-222; miR-422b; miR-660; miR-7; or miR-93.

[049] In other embodiments, one of the following miRNAs is used in combination with at least one other miRNA biomarker to determine whether a patient has lung cancer: let-7g, miR-106b, miR-126, miR-126*, miR-132, miR-140, miR-142-3p, miR-146a, miR-150, miR-15a, miR-15b, miR-181a, miR-181b, miR-181d, miR-214, miR-24, miR-30a-5p, miR-320, miR-342, miR-345, miR-374, miR-422a, miR-422b, miR-486, or miR-92.

[050] Some embodiments of the invention relate to amplifying and measuring at least a pair of miRNAs from serum. Table 3 includes pairs that may be used to characterize lung disease. These pairs may be used in combination with other lung disease biomarkers.

Table 3. miRNA pairs measured in serum samples.

miRNA pairs		
miR-202,miR-29b	miR-324-5p,miR-422a	miR-101,miR-92
miR-142-5p,miR-422a	miR-374,miR-422a	miR-126,miR-92
miR-24,miR-27a	miR-140,miR-345	miR-126*,miR-422a
miR-27b,miR-422a	miR-23a,miR-27a	miR-126*,miR-92
miR-140,miR-422a	miR-29b,miR-378	miR-150,miR-29a
miR-185,miR-93	miR-30e-5p,miR-422a	miR-15a,miR-92
miR-126,miR-181d	miR-30e-5p,miR-92	miR-30e-5p,miR-324-3p
miR-142-3p,miR-422a	miR-660,miR-92	miR-126,miR-378
miR-30e-5p,miR-345	miR-106b,miR-422a	miR-126,miR-422a
miR-29b,miR-422a	let-7g,miR-422a	miR-132,miR-29b
miR-26a,miR-422a	miR-148b,miR-92	miR-142-3p,miR-181a
miR-15a,miR-422a	miR-181b,miR-29b	miR-142-3p,miR-378
miR-146a,miR-27b	let-7f,miR-422a	miR-29b,miR-518b
miR-148b,miR-422a	miR-181a,miR-27b	miR-142-5p,miR-181d
let-7i,miR-422a	miR-29b,miR-324-3p	miR-193b,miR-29b
miR-340,miR-422a	miR-132,miR-30e-5p	miR-146b,miR-422a
miR-143,miR-150	miR-214,miR-422b	miR-15b,miR-92
miR-15a,miR-378	miR-140,miR-378	miR-142-5p,miR-23a
miR-15b,miR-422a	miR-29b,miR-30a-5p	miR-27a,miR-378
miR-27b,miR-92	let-7a,miR-422a	miR-197,miR-29b
miR-30e-3p,miR-422a	miR-142-5p,miR-191	miR-342,miR-374
miR-30b,miR-422a	miR-24,miR-27b	miR-422a,miR-487b
miR-142-3p,miR-92	miR-126*,miR-181d	miR-142-3p,miR-99b
miR-27a,miR-422a	miR-181d,miR-30e-5p	miR-29b,miR-320
miR-142-5p,miR-342	miR-142-3p,miR-191	miR-340,miR-378
miR-142-5p,miR-223	miR-181c,miR-29b	miR-29b,miR-486
miR-27b,miR-378	miR-181d,miR-27a	miR-23a,miR-422a
miR-186,miR-27a	miR-148b,miR-378	miR-222,miR-27b
miR-106b,miR-324-3p	miR-15a,miR-320	miR-142-5p,miR-24
miR-34a,miR-518b	miR-199a*,miR-422a	miR-148b,miR-181d
miR-27a,miR-92	miR-146a,miR-27a	miR-29b,miR-342
miR-30a-3p,miR-422a	miR-142-5p,miR-181a	miR-152,miR-422a
miR-181d,miR-29b	miR-15a,miR-486	miR-7,miR-92
miR-15b,miR-191	miR-27b,miR-342	miR-210,miR-422a
miR-192,miR-422a	miR-181a,miR-27a	let-7c,miR-422a
miR-422a,miR-576	let-7g,miR-342	miR-27b,miR-518b
miR-30c,miR-422a	miR-29b,miR-345	miR-145,miR-374

miRNA pairs		
miR-142-3p,miR-145	let-7e,miR-422a	miR-27a,miR-324-3p
miR-142-3p,miR-181d	miR-29b,miR-422b	miR-140,miR-186
miR-181d,miR-27b	miR-142-3p,miR-202	miR-185,miR-486
miR-142-5p,miR-186	miR-214,miR-422a	miR-422a,miR-496
miR-150,miR-29c	miR-142-5p,miR-145	miR-181a,miR-199a*
miR-200c,miR-422a	miR-422a,miR-497	miR-422a,miR-432
miR-185,miR-92	miR-29b,miR-433	miR-181d,miR-23b
miR-148a,miR-422a	miR-140,miR-92	miR-125b,miR-422a
miR-34a,miR-422a	miR-142-5p,miR-92	miR-145,miR-29b
miR-32,miR-422a	miR-142-3p,miR-181b	miR-142-5p,miR-146a
miR-214,miR-566	miR-30e-5p,miR-486	miR-126,miR-320
miR-206,miR-422a	miR-196b,miR-422a	miR-191,miR-374
miR-214,miR-518b	miR-222,miR-29b	miR-126,miR-145
miR-142-3p,miR-320	miR-142-3p,miR-518b	miR-150,miR-29b
miR-331,miR-422a	miR-29b,miR-30d	miR-133b,miR-422a
miR-26b,miR-422a	miR-29b,miR-361	miR-142-5p,miR-324-3p
miR-142-5p,miR-345	miR-342,miR-34a	miR-27b,miR-345
miR-27b,miR-324-3p	miR-106b,miR-181d	miR-374,miR-378
miR-30a-5p,miR-30e-5p	miR-340,miR-92	let-7b,miR-422a
miR-29b,miR-92	miR-486,miR-7	miR-346,miR-432*
miR-191,miR-27a	miR-21,miR-422a	miR-142-5p,miR-518b
miR-140,miR-181d	miR-106b,miR-92	miR-142-3p,miR-328
miR-422a,miR-660	let-7i,miR-92	miR-181a,miR-374
miR-126,miR-181a	miR-101,miR-486	let-7g,miR-181d
miR-126*,miR-378	miR-181a,miR-29b	miR-106b,miR-378
miR-15a,miR-181d	miR-142-5p,miR-150	miR-30a-5p,miR-422a
miR-224,miR-422a	miR-142-3p,miR-342	miR-339,miR-422a
miR-346,miR-92	miR-142-5p,miR-93	let-7i,miR-378
miR-146a,miR-374	miR-181a,miR-346	miR-146b,miR-374
miR-181d,miR-346	miR-15a,miR-425	miR-181b,miR-346
let-7f,miR-146a	let-7g,miR-146a	let-7d,miR-92
miR-106a,miR-15b	let-7a,miR-92	miR-146a,miR-15b
miR-106a, miR-422b	miR-126*, miR-26b	miR-106a, miR-26b
miR-15b, miR-30a-5p	let-7i, miR-26b	let-7g, miR-106a
miR-126*, miR-15b	miR-126*, miR-30b	miR-106a, miR-106b
miR-15b, miR-27b	miR-126, miR-15b	miR-126*, miR- 15a
miR-15b, miR-27a	miR-106b, miR-30a-5p	miR-15b, miR-30d
miR-15a, miR-30a-5p	miR-15a, miR-27b	miR-15b, miR-30b

miRNA pairs		
miR-126, miR-15a	miR-15a, miR-30d	miR-15b, miR-26b
miR-106b, miR-30d	miR-106b, miR-126*	miR-15a, miR-422a
miR-106a, miR-30a-5p	miR-106a, miR-30d	miR-15b, miR-301
miR-26b, miR-27b	miR-182, miR-27b	miR-106b, miR-27b

[051] In certain embodiments, the pair of miRNAs is chosen from miR-202 and miR-29b; miR-142-5p and miR-422a; miR-24 and miR-27a; miR-27b and miR-422a; miR-140 and miR-422a; miR-185 and miR-93; miR-126 and miR-181d; miR-142-3p and miR-422a; miR-30e-5p and miR-345; miR-29b and miR-422a; miR-324-5p and miR-422a; miR-374 and miR-422a; miR-140 and miR-345; miR-23a and miR-27a; miR-29b and miR-378; miR-30e-5p and miR-422a; miR-30e-5p and miR-92; miR-660 and miR-92; miR-106b and miR-422a; let-7g and miR-422a; miR-101 and miR-92; miR-126 and miR-92; miR-126* and miR-422a; miR-126* and miR-92; miR-150 and miR-29a; miR-15a and miR-92; miR-30e-5p and miR-324-3p; miR-126 and miR-378; miR-126 and miR-422a; miR-132 and miR-29b; miR-142-3p and miR-181a; miR-142-3p and miR-378; miR-148b and miR-92; miR-181b and miR-29b; miR-26a and miR-422a; miR-15a and miR-422a; miR-146a and miR-27b; miR-148b and miR-422a; let-7i and miR-422a; miR-340 and miR-422a; miR-143 and miR-150; miR-15a and miR-378; miR-15b and miR-422a; miR-27b and miR-92; miR-30e-3p and miR-422a; miR-30b and miR-422a; miR-142-3p and miR-92; miR-27a and miR-422a; miR-142-5p and miR-342; miR-142-5p and miR-223; miR-27b and miR-378; miR-186 and miR-27a; miR-106b and miR-324-3p; miR-34a and miR-518b; miR-27a and miR-92; miR-30a-3p and miR-422a; miR-181d and miR-29b; miR-15b and miR-191; miR-192 and miR-422a; miR-422a and miR-576; miR-30c and miR-422a; miR-142-3p and miR-145; miR-142-3p and miR-181d; miR-181d and miR-27b; miR-142-5p and miR-186; miR-150 and miR-29c; miR-200c and miR-422a; miR-185 and miR-92; miR-148a and miR-422a; miR-34a and miR-422a; miR-32 and miR-422a; miR-214 and miR-566; miR-206 and miR-422a; miR-214 and miR-518b; miR-142-3p and miR-320; miR-331 and miR-422a; miR-26b and miR-422a; miR-142-5p and miR-345; miR-27b and miR-324-3p; miR-30a-5p and miR-30e-5p; miR-29b and miR-92; miR-191 and miR-27a; miR-140 and miR-181d; miR-422a and miR-660; miR-126 and miR-181a; miR-126* and miR-378; miR-15a and miR-181d; let-7f and miR-422a; miR-

181a and miR-27b; miR-29b and miR-324-3p; miR-132 and miR-30e-5p; miR-214 and miR-422b; miR-140 and miR-378; miR-29b and miR-30a-5p; let-7a and miR-422a; miR-142-5p and miR-191; miR-24 and miR-27b; miR-126* and miR-181d; miR-181d and miR-30e-5p; miR-142-3p and miR-191; miR-181c and miR-29b; miR-181d and miR-27a; miR-148b and miR-378; miR-15a and miR-320; miR-199a* and miR-422a; miR-146a and miR-27a; miR-142-5p and miR-181a; miR-15a and miR-486; miR-27b and miR-342; miR-181a and miR-27a; let-7g and miR-342; miR-29b and miR-345; let-7e and miR-422a; miR-29b and miR-422b; miR-142-3p and miR-202; miR-214 and miR-422a; miR-142-5p and miR-145; miR-422a and miR-497; miR-29b and miR-433; miR-140 and miR-92; miR-142-5p and miR-92; miR-142-3p and miR-181b; miR-30e-5p and miR-486; miR-196b and miR-422a; miR-222 and miR-29b; miR-142-3p and miR-518b; miR-29b and miR-30d; miR-29b and miR-361; miR-342 and miR-34a; miR-106b and miR-181d; miR-340 and miR-92; miR-486 and miR-7; miR-21 and miR-422a; miR-106b and miR-92; let-7i and miR-92; miR-101 and miR-486; miR-181a and miR-29b; miR-142-5p and miR-150; miR-142-3p and miR-342; miR-29b and miR-518b; miR-142-5p and miR-181d; miR-193b and miR-29b; miR-146b and miR-422a; miR-15b and miR-92; miR-142-5p and miR-23a; miR-27a and miR-378; miR-197 and miR-29b; miR-342 and miR-374; miR-422a and miR-487b; miR-142-3p and miR-99b; miR-29b and miR-320; miR-340 and miR-378; miR-29b and miR-486; miR-23a and miR-422a; miR-222 and miR-27b; miR-142-5p and miR-24; miR-148b and miR-181d; miR-29b and miR-342; miR-152 and miR-422a; miR-7 and miR-92; miR-210 and miR-422a; let-7c and miR-422a; miR-27b and miR-518b; miR-145 and miR-374; miR-27a and miR-324-3p; miR-140 and miR-186; miR-185 and miR-486; miR-422a and miR-496; miR-181a and miR-199a*; miR-422a and miR-432; miR-181d and miR-23b; miR-125b and miR-422a; miR-145 and miR-29b; miR-142-5p and miR-146a; miR-126 and miR-320; miR-191 and miR-374; miR-126 and miR-145; miR-150 and miR-29b; miR-133b and miR-422a; miR-142-5p and miR-324-3p; miR-27b and miR-345; miR-374 and miR-378; let-7b and miR-422a; miR-346 and miR-432*; miR-142-5p and miR-518b; miR-142-3p and miR-328; miR-181a and miR-374; let-7g and miR-181d; miR-106b and miR-378; miR-30a-5p and miR-422a; miR-339 and miR-422a; let-7i and miR-378; miR-142-5p and miR-93; and miR-224 and miR-422a.

[052] In certain embodiments, the pair of miRNAs is chosen from let-7a and miR-181a; let-7a and miR-181d; let-7b and miR-150; let-7b and miR-181d; let-7b and miR-92; let-7c and miR-150; let-7c and miR-181d; let-7c and miR-92; let-7e and miR-378; let-7f and miR-181a; let-7f and miR-181d; let-7f and miR-342; let-7f and miR-92; let-7g and miR-150; let-7g and miR-181a; let-7g and miR-181d; let-7g and miR-342; let-7g and miR-92; let-7i and miR-486; let-7i and miR-92; miR-106b and miR-150; miR-106b and miR-181d; miR-106b and miR-92; miR-125a and miR-142-3p; miR-125a and miR-374; miR-126* and miR-181d; miR-126* and miR-30a-5p; miR-126* and miR-92; miR-126 and miR-146a; miR-126 and miR-150; miR-126 and miR-181a; miR-126 and miR-181d; miR-126 and miR-342; miR-126 and miR-92; miR-130b and miR-142-3p; miR-132 and miR-142-3p; miR-132 and miR-214; miR-140 and miR-150; miR-140 and miR-30a-5p; miR-140 and miR-345; miR-140 and miR-92; miR-142-3p and miR-146a; miR-142-3p and miR-150; miR-142-3p and miR-151; miR-142-3p and miR-181a; miR-142-3p and miR-181b; miR-142-3p and miR-181d; miR-142-3p and miR-186; miR-142-3p and miR-210; miR-142-3p and miR-22; miR-142-3p and miR-23a; miR-142-3p and miR-24; miR-142-3p and miR-30a-5p; miR-142-3p and miR-342; miR-142-3p and miR-345; miR-142-3p and miR-425; miR-142-3p and miR-486; miR-142-3p and miR-92; miR-142-3p and miR-99b; miR-142-5p and miR-30a-5p; miR-143 and miR-223; miR-143 and miR-486; miR-150 and miR-15b; miR-150 and miR-214; miR-150 and miR-29b; miR-150 and miR-374; miR-150 and miR-576; miR-15a and miR-181a; miR-15a and miR-181b; miR-15a and miR-181d; miR-15a and miR-210; miR-15a and miR-30a-5p; miR-15a and miR-342; miR-15a and miR-345; miR-15a and miR-486; miR-15a and miR-92; miR-15b and miR-17-5p; miR-15b and miR-181a; miR-15b and miR-181d; miR-15b and miR-24; miR-15b and miR-342; miR-15b and miR-92; miR-16 and miR-486; miR-16 and miR-92; miR-181a and miR-214; miR-181a and miR-26a; miR-181a and miR-26b; miR-181a and miR-30b; miR-181a and miR-30c; miR-181a and miR-374; miR-181a and miR-98; miR-181b and miR-214; miR-181b and miR-374; miR-181d and miR-214; miR-181d and miR-26b; miR-181d and miR-30b; miR-181d and miR-30c; miR-181d and miR-374; miR-181d and miR-432; miR-181d and miR-496; miR-181d and miR-638; miR-181d and miR-98; miR-193a and miR-422a; miR-195 and miR-486; miR-199a* and miR-92; miR-20a and miR-92; miR-214 and miR-342; miR-214 and miR-422b; miR-214 and miR-92; miR-24 and miR-374; miR-26a and

miR-342; miR-26a and miR-92; miR-26b and miR-342; miR-26b and miR-92; miR-27a and miR-30a-5p; miR-27b and miR-30d; miR-29b and miR-30a-5p; miR-30b and miR-342; miR-30b and miR-92; miR-30c and miR-342; miR-30c and miR-92; miR-320 and miR-98; miR-342 and miR-374; miR-346 and miR-422a; miR-346 and miR-518b; miR-346 and miR-566; miR-374 and miR-92; miR-422a and miR-496; miR-422a and miR-638; miR-422a and miR-98; miR-432 and miR-92; miR-496 and miR-92; and miR-7 and miR-92.

[053] In other embodiments, the pair of miRNAs is chosen from miR-142-5p and miR181d; miR-142-3p and miR181d; miR-142-3p and miR-422a; miR-142-5p and miR-422a; miR-92 and miR-27b; and miR-24 and miR-27a. In additional embodiments, the pair of miRNAs is chosen from miR-106a and miR-422b; miR-126* and miR-26b; miR-106a and miR-26b; miR-15b and miR-30a-5p; let-7a and miR-26b; let-7g and miR-106a; miR-126* and miR-15b; miR-126* and miR-30b; and miR-106a and miR-106b.

[054] In some embodiments, the pair is measured in a serum sample. Optionally, one or more additional miRNAs are measured.

[055] In additional embodiments, certain miRNA pairs may be used to characterize lung disease in female or male patients (Table 4). In certain embodiments, the methods detect sex-specific miRNA biomarkers.

Table 4: miRNA pairs for characterizing lung disease in female or male patients

miRNA Biomarker Pair (Females)	miRNA Biomarker Pair (Males)
miR-15a,miR-422a	miR-185,miR-93
miR-181d,miR-27b	miR-30e-5p,miR-433
miR-27b,miR-422a	miR-126,miR-378
miR-15b,miR-191	miR-342,miR-497
miR-181d,miR-29b	let-7f,miR-342
miR-142-3p,miR-422a	miR-142-5p,miR-422a
miR-23a,miR-27a	miR-145,miR-200c
miR-222,miR-27a	miR-148a,miR-19b
miR-126,miR-422a	miR-191,miR-340
miR-142-5p,miR-145	miR-200b,miR-422a
miR-142-3p,miR-145	miR-340,miR-378
miR-143,miR-223	

miRNA Biomarker Pair (Females)	miRNA Biomarker Pair (Males)
miR-324-5p,miR-422a	
miR-30e-5p,miR-422a	
miR-27a,miR-422a	
miR-126*,miR-222	
miR-140,miR-422a	
miR-101,miR-92	
miR-202,miR-29b	
miR-29b,miR-422a	
miR-30e-5p,miR-324-3p	
miR-181b,miR-29b	
miR-142-5p,miR-181a	
miR-126*,miR-422a	
miR-195,miR-93	
miR-140,miR-222	
miR-126,miR-181d	
miR-142-5p,miR-422a	
miR-24,miR-27a	
miR-27a,miR-361	
miR-346,miR-518b	
miR-222,miR-27b	
miR-15b,miR-181a	
miR-142-5p,miR-191	
miR-433,miR-487b	
miR-29b,miR-518b	
miR-106b,miR-422a	
miR-21,miR-422a	
miR-15b,miR-422a	
miR-181a,miR-27a	
miR-142-5p,miR-345	
miR-32,miR-422a	
miR-30e-5p,miR-92	
miR-148b,miR-422a	
miR-192,miR-422a	
let-7i,miR-422a	
miR-19b,miR-486	
miR-15a,miR-181d	
miR-142-3p,miR-26a	

miRNA Biomarker Pair (Females)	miRNA Biomarker Pair (Males)
miR-142-3p,miR-181a	
miR-29b,miR-378	
miR-15a,miR-181b	
miR-142-3p,miR-342	
miR-195,miR-20b	
miR-145,miR-27a	
miR-374,miR-422a	
miR-27b,miR-361	
miR-142-3p,miR-518b	
miR-140,miR-181d	
miR-27b,miR-326	
miR-139,miR-422a	
let-7g,miR-422a	
miR-140,miR-345	
miR-148b,miR-326	
miR-20a,miR-92	
miR-133b,miR-181b	
miR-132,miR-30e-5p	
miR-19b,miR-92	
miR-181a,miR-30c	
miR-197,miR-422a	
miR-142-5p,miR-222	
miR-148a,miR-422a	
let-7g,miR-181d	
miR-197,miR-29b	
miR-331,miR-422a	
miR-15a,miR-181a	
miR-339,miR-422a	
miR-181b,miR-215	
miR-145,miR-374	
miR-206,miR-518b	
miR-106b,miR-486	
miR-126*,miR-181a	
miR-422a,miR-576	
miR-17-5p,miR-422a	
miR-106b,miR-345	
miR-126,miR-342	

miRNA Biomarker Pair (Females)	miRNA Biomarker Pair (Males)
miR-139,miR-181d	
miR-140,miR-30a-5p	
miR-142-3p,miR-26b	
miR-142-3p,miR-361	
miR-19a,miR-92	
miR-222,miR-422a	
miR-296,miR-422a	
miR-29b,miR-382	

[056] Some embodiments of the invention relate to amplifying and measuring two or more miRNAs from plasma. One of the following miRNA plasma biomarkers may be used in combination with at least one other miRNA: miR-10b, miR-192, miR-206, miR-101, miR-205, miR-16, miR-151, miR-137, miR-215, miR-181a, miR-218, miR-126*, miR-125b, miR-326, miR-100, miR-31, miR-197, miR-222, miR-191, miR-200c, miR-186, miR-145, miR-155, miR-29c, let-7c, miR-181c, miR-125a, miR-134, miR-181d, let-7b, miR-127, miR-146a, miR-139, miR-152, miR-190, miR-30e-5p, miR-106b, miR-10a, miR-132, miR-148a, miR-213, miR-29a, miR-375, miR-133b, miR-15a, miR-107, miR-148b, miR-19a, miR-106a, miR-130a, miR-17-3p, miR-18a*, miR-195, miR-20b, miR-301, miR-339, miR-410, miR-188, miR-193a, let-7g, let-7i, miR-140, miR-181b, miR-25, miR-328, miR-133a, miR-150, miR-17-5p, miR-21, miR-214, miR-370, miR-383, miR-130b, miR-199a, miR-212, miR-221, miR-27b, miR-30e-3p, miR-338, miR-361, miR-141, miR-142-5p, miR-30a-3p, miR-30a-5p, miR-451, miR-142-3p, miR-146b, miR-15b, miR-18a, miR-210, miR-296, miR-323, miR-362, let-7a, miR-196b, miR-223, miR-29b, miR-324-5p, miR-376a, miR-379, miR-491, let-7d, miR-126, miR-182, miR-185, miR-204, miR-23a, miR-27a, miR-324-3p, miR-342, miR-34c, miR-382, miR-425, miR-432*, miR-103, miR-193b, miR-196a, miR-199a*, miR-199b, miR-28, miR-30d, miR-330, miR-423, miR-433, miR-485-5p, miR-20a, miR-23b, miR-26a, miR-30b, miR-30c, miR-320, miR-345, miR-422b, miR-335, miR-365, miR-486, miR-24, miR-26b, miR-331, miR-340, miR-34a, miR-374, miR-452, miR-483, miR-512-5p, let-7e, miR-32, miR-422a, miR-424, miR-432, miR-485-3p, miR-487b, miR-496, miR-505, miR-7, miR-202, miR-369-3p, miR-495, miR-502, miR-511, miR-516-3p, miR-517c, miR-92, miR-93, miR-99a, or miR-99b.

[057] Table 5 includes pairs of miRNAs that may be used to characterize lung cancer from plasma samples. Optionally, one or more additional miRNAs are measured.

Table 5. miRNA pairs measured in plasma samples

miRNA pairs		
let-7c:miR-326	miR-330:miR-375	miR-181a:miR-218
miR-326:miR-7	miR-134:miR-206	miR-151:miR-218
miR-206:miR-491	miR-432*:miR-491	miR-491:miR-512-5p
miR-339:miR-375	miR-181d:miR-375	miR-326:miR-375
miR-30a-3p:miR-326	miR-191:miR-200c	miR-200c:miR-326
miR-151:miR-206	miR-23a:miR-326	miR-20b:miR-30b
miR-10b:miR-30b	miR-125b:miR-146a	miR-491:miR-516-3p
miR-16,miR-487b	let-7i,miR-206	miR-192,miR-339
miR-326,miR-345	miR-200c,miR-339	miR-192,miR-196b
miR-140,miR-192	miR-16,miR-191	miR-7,miR-99b
miR-383,miR-491	miR-15a,miR-375	miR-213,miR-31
miR-339,miR-7	miR-17-5p,miR-30b	miR-16,miR-30b
miR-375,miR-99b	miR-206,miR-212	miR-137,miR-151
miR-199a,miR-375	miR-192,miR-30b	miR-218,miR-361
miR-206,miR-376a	miR-148b,miR-192	miR-190,miR-326
miR-375,miR-505	miR-16,miR-24	miR-204,miR-375
miR-200c,miR-301	miR-206,miR-320	miR-10b,miR-191
miR-451,miR-487b	miR-326,miR-34c	miR-125b,miR-30d
miR-218,miR-326	miR-191,miR-7	miR-126*,miR-375
miR-206,miR-213	miR-218,miR-410	miR-17-3p,miR-326
miR-206,miR-422b	miR-181a,miR-206	miR-31,miR-326
miR-192,miR-326	miR-206,miR-370	miR-151,miR-31
miR-205,miR-326	miR-222,miR-30a-3p	miR-125b,miR-326
miR-218,miR-491	miR-326,miR-423	miR-375,miR-433
miR-206,miR-361	miR-137,miR-491	miR-361,miR-432*
miR-206,miR-410	miR-192,miR-342	miR-142-5p,miR-375
miR-30a-3p,miR-99b	miR-206,miR-382	miR-126*,miR-192
miR-151,miR-383	miR-375,miR-423	miR-502,miR-512-5p
miR-218,miR-338	miR-17-5p,miR-487b	miR-326,miR-451
miR-212,miR-512-5p	miR-326,miR-511	miR-192,miR-324-3p
miR-375,miR-496	miR-155,miR-218	miR-200c,miR-222
		miR-191,miR-19b

[058] Other miRNAs and groups of miRNAs that can be used in the methods of the invention will be apparent from the Examples described herein.

III. METHODS TO MEASURE THE LEVEL OF A MIRNA

[059] Many methods of measuring the levels or amounts of miRNAs are contemplated. Any reliable, sensitive, and specific method can be used. In some embodiments, a miRNA is amplified prior to measurement. In other embodiments, the level of miRNA is measured during the amplification process. In still other methods, the miRNA is not amplified prior to measurement.

A. Amplification Reactions

[060] Many methods exist for amplifying miRNA nucleic acid sequences such as mature miRNAs, precursor miRNAs, and primary miRNAs. Suitable nucleic acid polymerization and amplification techniques include reverse transcription (RT), polymerase chain reaction (PCR), real-time PCR (quantitative PCR (q-PCR)), nucleic acid sequence-base amplification (NASBA), ligase chain reaction, multiplex ligatable probe amplification, invader technology (Third Wave), rolling circle amplification, in vitro transcription (IVT), strand displacement amplification, transcription-mediated amplification (TMA), RNA (Eberwine) amplification, and other methods that are known to persons skilled in the art. In certain embodiments, more than one amplification method is used, such as reverse transcription followed by real time quantitative PCR (qRT-PCR) (Chen et al., *Nucleic Acids Research*, 33(20):e179 (2005)).

[061] A typical PCR reaction includes multiple amplification steps, or cycles that selectively amplify target nucleic acid species: a denaturing step in which a target nucleic acid is denatured; an annealing step in which a set of PCR primers (forward and reverse primers) anneal to complementary DNA strands; and an elongation step in which a thermostable DNA polymerase elongates the primers. By repeating these steps multiple times, a DNA fragment is amplified to produce an amplicon, corresponding to the target DNA sequence. Typical PCR reactions include 20 or more cycles of denaturation, annealing, and elongation. In many cases, the annealing and elongation steps can be performed concurrently, in which case the cycle contains only two steps. Since mature miRNAs are single-stranded, a reverse transcription reaction (which produces a complementary cDNA sequence) may be performed prior to PCR reactions.

Reverse transcription reactions include the use of, e.g., a RNA-based DNA polymerase (reverse transcriptase) and a primer.

[062] In PCR and q-PCR methods, for example, a set of primers is used for each target sequence. In certain embodiments, the lengths of the primers depends on many factors, including, but not limited to, the desired hybridization temperature between the primers, the target nucleic acid sequence, and the complexity of the different target nucleic acid sequences to be amplified. In certain embodiments, a primer is about 15 to about 35 nucleotides in length. In other embodiments, a primer is equal to or fewer than 15, 20, 25, 30, or 35 nucleotides in length. In additional embodiments, a primer is at least 35 nucleotides in length.

[063] In a further aspect, a forward primer can comprise at least one sequence that anneals to a miRNA biomarker and alternatively can comprise an additional 5' non-complementary region. In another aspect, a reverse primer can be designed to anneal to the complement of a reverse transcribed miRNA. The reverse primer may be independent of the miRNA biomarker sequence, and multiple miRNA biomarkers may be amplified using the same reverse primer. Alternatively, a reverse primer may be specific for a miRNA biomarker.

[064] In some embodiments, two or more miRNAs are amplified in a single reaction volume. One aspect includes multiplex q-PCR, such as qRT-PCR, which enables simultaneous amplification and quantification of at least two miRNAs of interest in one reaction volume by using more than one pair of primers and/or more than one probe. The primer pairs comprise at least one amplification primer that uniquely binds each miRNA, and the probes are labeled such that they are distinguishable from one another, thus allowing simultaneous quantification of multiple miRNAs. Multiplex qRT-PCR has research and diagnostic uses, including but not limited to detection of miRNAs for diagnostic, prognostic, and therapeutic applications.

[065] The qRT-PCR reaction may further be combined with the reverse transcription reaction by including both a reverse transcriptase and a DNA-based thermostable DNA polymerase. When two polymerases are used, a "hot start" approach may be used to maximize assay performance (U.S. Patent Nos. 5,411,876 and 5,985,619). For example, the components for a reverse transcriptase reaction and a PCR reaction may be sequestered using one or more

thermoactivation methods or chemical alteration to improve polymerization efficiency (U.S. Patent Nos. 5,550,044, 5,413,924, and 6,403,341).

B. Detection of miRNAs

[066] In certain embodiments, labels, dyes, or labeled probes and/or primers are used to detect amplified or unamplified miRNAs. The skilled artisan will recognize which detection methods are appropriate based on the sensitivity of the detection method and the abundance of the target. Depending on the sensitivity of the detection method and the abundance of the target, amplification may or may not be required prior to detection. One skilled in the art will recognize the detection methods where miRNA amplification is preferred.

[067] A probe or primer may include Watson-Crick bases or modified bases. Modified bases include, but are not limited to, the AEGIS bases (from Eragen Biosciences), which have been described, e.g., in U.S. Patent Nos. 5,432,272, 5,965,364, and 6,001,983. In certain aspects, bases are joined by a natural phosphodiester bond or a different chemical linkage. Different chemical linkages include, but are not limited to, a peptide bond or a Locked Nucleic Acid (LNA) linkage, which is described, e.g., in U.S. Patent No. 7,060,809.

[068] In a further aspect, oligonucleotide probes or primers present in an amplification reaction are suitable for monitoring the amount of amplification product produced as a function of time. In certain aspects, probes having different single stranded versus double stranded character are used to detect the nucleic acid. Probes include, but are not limited to, the 5'-exonuclease assay (e.g., TaqMan™) probes (see U.S. Patent No. 5,538,848), stem-loop molecular beacons (see, e.g., U.S. Patent Nos. 6,103,476 and 5,925,517), stemless or linear beacons (see, e.g., WO 9921881, U.S. Patent Nos. 6,485,901 and 6,649,349), peptide nucleic acid (PNA) Molecular Beacons (see, e.g., U.S. Patent Nos. 6,355,421 and 6,593,091), linear PNA beacons (see, e.g. U.S. Patent No. 6,329,144), non-FRET probes (see, e.g., U.S. Patent No. 6,150,097), Sunrise™ /AmplifluorB™ probes (see, e.g., U.S. Patent No. 6,548,250), stem-loop and duplex Scorpion™ probes (see, e.g., U.S. Patent No. 6,589,743), bulge loop probes (see, e.g., U.S. Patent No. 6,590,091), pseudo knot probes (see, e.g., U.S. Patent No. 6,548,250), cyclicons (see, e.g., U.S. Patent No. 6,383,752), MGB Eclipse™ probe (Epoch Biosciences), hairpin probes (see, e.g., U.S. Patent No. 6,596,490), PNA light-up probes, antiprimer quench probes (Li et al., Clin. Chem.

53:624-633 (2006)), self-assembled nanoparticle probes, and ferrocene-modified probes described, for example, in U.S. Patent No. 6,485,901.

[069] In certain embodiments, one or more of the primers in an amplification reaction can include a label. In yet further embodiments, different probes or primers comprise detectable labels that are distinguishable from one another. In some embodiments a nucleic acid, such as the probe or primer, may be labeled with two or more distinguishable labels.

[070] In some aspects, a label is attached to one or more probes and has one or more of the following properties: (i) provides a detectable signal; (ii) interacts with a second label to modify the detectable signal provided by the second label, e.g., FRET (Fluorescent Resonance Energy Transfer); (iii) stabilizes hybridization, e.g., duplex formation; and (iv) provides a member of a binding complex or affinity set, e.g., affinity, antibody-antigen, ionic complexes, hapten-ligand (e.g., biotin-avidin). In still other aspects, use of labels can be accomplished using any one of a large number of known techniques employing known labels, linkages, linking groups, reagents, reaction conditions, and analysis and purification methods.

[071] MiRNAs can be detected by direct or indirect methods. In a direct detection method, one or more miRNAs are detected by a detectable label that is linked to a nucleic acid molecule. In such methods, the miRNAs may be labeled prior to binding to the probe. Therefore, binding is detected by screening for the labeled miRNA that is bound to the probe. The probe is optionally linked to a bead in the reaction volume.

[072] In certain embodiments, nucleic acids are detected by direct binding with a labeled probe, and the probe is subsequently detected. In one embodiment of the invention, the nucleic acids, such as amplified miRNAs, are detected using FlexMAP Microspheres (Luminex) conjugated with probes to capture the desired nucleic acids. Some methods may involve detection with polynucleotide probes modified with fluorescent labels or branched DNA (bDNA) detection, for example.

[073] In other embodiments, nucleic acids are detected by indirect detection methods. For example, a biotinylated probe may be combined with a streptavidin-conjugated dye to detect the bound nucleic acid. The streptavidin molecule binds a biotin label on amplified miRNA, and the bound miRNA is detected by detecting the dye molecule attached to the streptavidin molecule. In

one embodiment, the streptavidin-conjugated dye molecule comprises Phycolink® Streptavidin R-Phycoerythrin (PROzyme). Other conjugated dye molecules are known to persons skilled in the art.

[074] Labels include, but are not limited to: light-emitting, light-scattering, and light-absorbing compounds which generate or quench a detectable fluorescent, chemiluminescent, or bioluminescent signal (see, e.g., Kricka, L., *Nonisotopic DNA Probe Techniques*, Academic Press, San Diego (1992) and Garman A., *Non-Radioactive Labeling*, Academic Press (1997)). Fluorescent reporter dyes useful as labels include, but are not limited to, fluoresceins (see, e.g., U.S. Patent Nos. 5,188,934, 6,008,379, and 6,020,481), rhodamines (see, e.g., U.S. Patent Nos. 5,366,860, 5,847,162, 5,936,087, 6,051,719, and 6,191,278), benzophenoxazines (see, e.g., U.S. Patent No. 6,140,500), energy-transfer fluorescent dyes, comprising pairs of donors and acceptors (see, e.g., U.S. Patent Nos. 5,863,727; 5,800,996; and 5,945,526), and cyanines (see, e.g., WO 9745539), lissamine, phycoerythrin, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, FluorX (Amersham), Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5, 6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, SYPRO, TAMRA, Tetramethylrhodamine, and/or Texas Red, as well as any other fluorescent moiety capable of generating a detectable signal. Examples of fluorescein dyes include, but are not limited to, 6-carboxyfluorescein; 2',4',1,4,-tetrachlorofluorescein; and 2',4',5',7',1,4-hexachlorofluorescein. In certain aspects, the fluorescent label is selected from SYBR-Green, 6-carboxyfluorescein ("FAM"), TET, ROX, VICTM, and JOE. For example, in certain embodiments, labels are different fluorophores capable of emitting light at different, spectrally-resolvable wavelengths (e.g., 4-differently colored fluorophores); certain such labeled probes are known in the art and described above, and in U.S. Patent No. 6,140,054. A dual labeled fluorescent probe that includes a reporter fluorophore and a quencher fluorophore is used in some embodiments. It will be appreciated that pairs of fluorophores are chosen that have distinct emission spectra so that they can be easily distinguished.

[075] In still a further aspect, labels are hybridization-stabilizing moieties which serve to enhance, stabilize, or influence hybridization of duplexes, e.g., intercalators and intercalating dyes (including, but not limited to, ethidium bromide and SYBR-Green), minor-groove binders, and cross-linking functional groups (see, e.g., Blackburn et al., eds. "DNA and RNA Structure" in *Nucleic Acids in Chemistry and Biology* (1996)).

[076] In further aspects, methods relying on hybridization and/or ligation to quantify miRNAs may be used, including oligonucleotide ligation (OLA) methods and methods that allow a distinguishable probe that hybridizes to the target nucleic acid sequence to be separated from an unbound probe. As an example, HARP-like probes, as disclosed in U.S. Publication No. 2006/0078894 may be used to measure the quantity of miRNAs. In such methods, after hybridization between a probe and the targeted nucleic acid, the probe is modified to distinguish the hybridized probe from the unhybridized probe. Thereafter, the probe may be amplified and/or detected. In general, a probe inactivation region comprises a subset of nucleotides within the target hybridization region of the probe. To reduce or prevent amplification or detection of a HARP probe that is not hybridized to its target nucleic acid, and thus allow detection of the target nucleic acid, a post-hybridization probe inactivation step is carried out using an agent which is able to distinguish between a HARP probe that is hybridized to its targeted nucleic acid sequence and the corresponding unhybridized HARP probe. The agent is able to inactivate or modify the unhybridized HARP probe such that it cannot be amplified.

[077] In an additional embodiment of the method, a probe ligation reaction may be used to quantify miRNAs. In a Multiplex Ligation-dependent Probe Amplification (MLPA) technique (Schouten et al., *Nucleic Acids Research* 30:e57 (2002)), pairs of probes which hybridize immediately adjacent to each other on the target nucleic acid are ligated to each other only in the presence of the target nucleic acid. In some aspects, MLPA probes have flanking PCR primer binding sites. MLPA probes can only be amplified if they have been ligated, thus allowing for detection and quantification of miRNA biomarkers.

IV. EXAMPLES

[078] The following examples illustrate various embodiments of the invention and are not intended to limit the scope of the invention.

[079] The examples described herein include the use of qRT-PCR, which includes real-time monitoring of PCR products during the exponential phase instead of by an end-point measurement. The threshold cycle (C_t) measurements in the examples refer to the number of cycles it takes to reach a pre-defined point in the fluorescent signal.

[080] The examples also describe the use of Receiver Operator Characteristic (ROC) analysis. A Receiver Operator Characteristic curve is a graphical plot of the sensitivity vs. specificity for a binary classifier system as its discrimination threshold is varied. ROC analysis provides a tool to select possibly optimal models and to discard suboptimal ones independently from (and prior to specifying) the class distribution. Numerous examples of ROC analysis are present in the literature, including applications in selecting and applying biomarkers for the diagnosis of disease (Pepe et al., *Biometrics* 62:221-229 (2006); Dodd et al., *Biometrics* 59:614-623 (2003)). ROC analysis captures the continuum of sensitivity and specificity, but it can be summarized as a single quantity, i.e., the area under the curve (AUC) of the ROC. The AUC is closely related to the nonparametric Wilcoxon test and summarizes the separation of classes over all the thresholds. Advantages of the ROC technique include (1) it does not assume a parametric form of the class probability as required in the logistic regression method, (2) it is adaptable to outcome-dependent samplings, e.g. the case-control design, which are widely used in medical studies, and (3) it is relatively straightforward to assign different 'costs' to false positives and false negatives (Dodd 2003; Pepe 2006).

Example 1. Mouse Model for Early Detection of Lung Cancer

[081] The A/J mouse model was used to chemically induce lung tumors and monitor the miRNA expression profile in plasma. Benzo[a]pyrene (Sigma-Aldrich, St. Louis, MO, USA; cat. no. 48564) served as a carcinogen to chemically induce lung tumors in mice. A/J mice (67 male and 68 female) were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA; Stock Number 000646) and sent to Perry Scientific Inc. (San Diego, CA, USA), at the age of six

weeks. Perry Scientific performed all the animal related experiments including carcinogen administration, animal monitoring, blood withdrawal and plasma processing.

[082] To obtain baseline data, two weeks prior to carcinogen administration, 125 mice were bled via orbital sinus and 10 mice were sacrificed by isofluorane inhalation and bled out by cardiac puncture. On day 0, mice in the experimental group were dosed with carcinogen via oral gavage at a dose equivalent to 20 μ M in 200 μ l (250 mg/kg body weight) in cottonseed oil (Sigma-Aldrich, cat. no. C7767). Ten control mice (5 males and 5 females) received an oral gavage of 200 μ l cottonseed oil with no carcinogen. A second carcinogen dose (250 mg/kg body weight) was administered to the test group at 10 weeks post the first gavage, with the control animals receiving a second gavage of cottonseed oil as described above. Mice were sacrificed at specific time points (Figure 1), and the remaining mice were bled every two weeks (Figure 1). At termination, animals were weighed, bled via cardiac puncture, and lung lobes collected. Control mice were bled throughout the study and sacrificed at week 34 post-carcinogen administration.

[083] Blood samples were processed individually into plasma. For plasma preparation, blood samples were collected into BD Vacutainer® K2EDTA tubes (Becton, Dickinson and Company; Franklin Lakes, NJ, USA; cat. no. 367841). Blood was centrifuged at 2,000 x g for 10 minutes, after which the plasma layer was aspirated and put into a fresh tube and centrifuged at 2,500 x g for 10 minutes. The resulting plasma was immediately stabilized by the addition of 2X denaturing buffer from the *mirVana*™ miRNA Isolation Kit (Ambion Inc., Austin, TX, USA; cat no. AM1560) to achieve a final concentration of 1X. Plasma and buffer were mixed by vortexing and immediately frozen at -80 °C until shipment to Asuragen on dry ice. Cell pellets were also frozen at -80 °C and shipped to Asuragen with plasma samples.

[084] Plasma RNA was purified using the organic extraction of the *mirVana* PARIS™ Kit (Ambion, Inc.; Part No. AM1556), with the following modifications. Following the addition of acid phenol:chloroform and vortexing, samples were incubated on ice for 5 min then centrifuged at 13,000 x g for 10 min at 4 °C. The aqueous layer was removed, extracted with chloroform, and

centrifuged again. The aqueous layer was removed from the second extraction, and 3M NaOAc (1/10 volume), glycogen (5 mg/ml), and 100% ethanol (1.5 volume) were added to the samples. Lysate/ethanol mixtures were passed through a mirVana PARIS™ filter cartridge, and filters were washed once with 650 µl of Wash 1 buffer and twice with 650 µl of Wash 2/3 buffer. RNA was eluted with two aliquots of nuclease free water (50 µl) and stored at -80 °C.

[085] One half of the lung lobes from each mouse were fixed in 10% buffered formalin overnight, embedded in paraffin (FFPE), and processed for hematoxylin and eosin (H&E) stained slides. The other half were snap frozen at -80 °C. FFPE lung sections were step-sectioned at 5 mm intervals. The following sectioning guidelines were used: If no grossly visible tumors were present in either lung, three random 5 x 5 x 5 mm sections from each lung were taken for snap-freezing and for FFPE preparation. If grossly visible tumors were present, one 5 x 5 x 5 mm section of one tumor from each lung was snap-frozen and the other half was prepared for FFPE. If only one lung had tumors, random regions from the uninvolved lung were snap-frozen and FFPE prepared. For histopathology, the lung lobes preserved in formalin were sent to Pacific Pathology Inc. (San Diego, CA, USA) where they were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and prepared into slides.

[086] H&E-stained slides were sent to Asuragen and analyzed by a board certified pathologist and classified as normal, hyperplasia, adenoma, or adenocarcinoma of the lung. Table 6 shows the number of visible tumors observed in each sacrificed animals, and the pathological diagnosis in each of the lung lobes.

Table 6. Lung tumors and pathological diagnosis from experimental and control mice. SCC, squamous cell carcinoma.

Mouse Number	Sex	Pathology-Based Diagnosis Right Lobes	Pathology-Based Diagnosis Left Lobes	Number of Visible Tumors	Week of Termination
Experimental Animals					
8864	F	Normal	Normal	0	0
8865	F	Normal	Normal	0	0
8866	F	Normal	Normal	0	0
8867	F	Normal	Normal	0	0

Mouse Number	Sex	Pathology-Based Diagnosis Right Lobes	Pathology-Based Diagnosis Left Lobes	Number of Visible Tumors	Week of Termination
8868	F	Normal	Normal	0	0
8931	M	Normal	Normal	0	0
8932	M	Normal	Normal	0	0
8933	M	Normal	Normal	0	0
8934	M	Normal	Normal	0	0
8935	M	Normal	Normal	0	0
8858	F	Normal	Normal	0	2
8859	F	Normal	Adenoma	0	2
8860	F	Normal	Normal	0	2
8861	F	Normal	Normal	0	2
8862	F	Normal	Normal	0	2
8926	M	Normal	Normal	0	2
8927	M	Normal	Normal	0	2
8928	M	Normal	Normal	0	2
8929	M	Normal	Normal	0	2
8930	M	Normal	Normal	0	2
8853	F	Normal	Normal	0	4
8854	F	Normal	Normal	0	4
8855	F	Normal	Normal	0	4
8856	F	Normal	Normal	0	4
8857	F	Normal	Normal	0	4
8921	M	Normal	Normal	0	4
8922	M	Normal	Normal	0	4
8923	M	Normal	Normal	0	4
8924	M	Normal	Normal	0	4
8925	M	Normal	Normal	0	4
8509	M	Normal	Normal	0	6
8847	F	Normal	Normal	0	6
8848	F	Normal	Normal	0	6
8850	F	Normal	Normal	0	6
8851	F	Normal	Normal	0	6
8852	F	Normal	Normal	0	6
8917	M	Normal	Normal	0	6
8918	M	Normal	Normal	0	6
8919	M	Normal	Normal	0	6
8920	M	Normal	Normal	0	6
8507	M	Normal	Normal	0	8
8508	M	Normal	Normal	0	8
8841	F	Normal	Normal	0	8
8842	F	Normal	Normal	0	8

Mouse Number	Sex	Pathology-Based Diagnosis Right Lobes	Pathology-Based Diagnosis Left Lobes	Number of Visible Tumors	Week of Termination
8843	F	Normal	Normal	0	8
8845	F	Normal	Normal	0	8
8846	F	Normal	Normal	0	8
8912	M	Normal	Normal	0	8
8915	M	Normal	Normal	0	8
8941	M	Normal	Normal	0	8
8838	F	Normal	Normal	0	10
8839	F	Normal	Normal	0	10
8840	F	Normal	Normal	0	10
8907	M	Normal	Normal	0	10
8908	M	Normal	Normal	0	10
8909	M	Normal	Normal	0	10
8506	M	Normal	Normal	0	12
8833	F	Normal	Normal	0	12
8834	F	Normal	Normal	0	12
8835	F	Normal	Normal	0	12
8836	F	Normal	Normal	0	12
8837	F	Normal	Normal	0	12
8902	M	Normal	Normal	0	12
8903	M	Normal	Normal	0	12
8904	M	Normal	Normal	0	12
8940	M	Normal	Normal	0	12
8505	M	Normal	Normal	4	14
8828	F	Normal	Hyperplasia	0	14
8830	F	Normal	Normal		14
8831	F	Adenomas papillary (2)	Hyperplasia	3	14
8832	F	Hyperplasia	Adenocarcinoma	1	14
8899	M	Normal	Normal	1	14
8900	M	Normal	Normal	0	14
8816	F	Normal	Normal	0	16
8825	F	Adenocarcinoma in adenoma	Hyperplasia		16
8826	F	Hyperplasia	Adenoma	2	16
8827	F	Normal	Normal	2	16
8829	F	Normal	Normal	2	16
8895	M	Normal	Normal	2	16
8896	M	Normal	Normal	1	16
8897	M	Adenocarcinoma	Normal	5	16
8898	M	Adenoma	Normal	2	16

Mouse Number	Sex	Pathology-Based Diagnosis Right Lobes	Pathology-Based Diagnosis Left Lobes	Number of Visible Tumors	Week of Termination
8939	M	Normal	Normal	0	16
8822	F	Hyperplasia	Normal	2	18
8823	F	Adenocarcinoma in adenoma	Hyperplasia	1	18
8824	F	Adenoma	Hyperplasia	2	18
8890	M	Adenocarcinomas (2)	Hyperplasia	2	18
8892	M	Adenocarcinoma	Hyperplasia	4	18
8893	M	Adenocarcinomas (2)	Hyperplasia	8	18
8938	M	Normal	Normal	3	18
8808	F	Normal	Normal	0	22
8820	F	Adenomas papillary (2)	Hyperplasia	3	22
8821	F	Normal	Normal	2	22
8886	M	Adenocarcinoma	Hyperplasia	1	22
8887	M	Normal	Normal	0	22
8888	M	Adenoma	Hyperplasia	4	22
8889	M	Hyperplasia	Normal	1	22
8811	F	Normal	Normal	2	26
8812	F	Adenocarcinoma in adenoma	Normal	4	26
8813	F	Adenocarcinoma	Adenoma papillary	8	26
8814	F	Hyperplasia	Mucinous Adenocarcinoma	6	26
8885	M	Normal	Normal	1	26
8936	M	Adenocarcinoma in adenoma	Normal	4	26
8937	M	Adenocarcinoma in adenoma	Adenoma	2	26
8504	M	Adenoma, papillary	Normal	3	30
8806	F	Adenocarcinoma	Normal	6	30
8815	F	Hyperplasia	Adenoma	3	30
8819	F	Adenocarcinoma in adenoma	Adenoma	3	30
8874	M	Adenocarcinoma	Adenocarcinoma	11	30
8883	M	Adenocarcinoma	Adenocarcinoma	6	30
8884	M	Adenocarcinomas (2)	Normal	5	30
8805	F	Normal	Normal	0	34

Mouse Number	Sex	Pathology-Based Diagnosis Right Lobes	Pathology-Based Diagnosis Left Lobes	Number of Visible Tumors	Week of Termination
8807	F	Adenocarcinoma	Normal	2	34
8810	F	Hyperplasia	Adenocarcinoma papillary	6	34
8875	M	SCC	Adenoma	3	34
8878	M	Adenocarcinoma	Adenoma papillary	8	34
8880	M	Adenomas papillary (2)	Hyperplasia	6	34
Control Animals					
8870	M	Hyperplasia	Normal	0	34
8873	M	Normal	Normal	1	34
8502	M	Adenocarcinoma in adenoma	Normal	0	34
8501	F	Normal	Normal	0	34
8503	M	Normal	Normal	0	34
8801	F	Normal	Normal	0	34
8802	F	Normal	Normal	0	34
8803	F	Normal	Normal	0	34
8805	F	Normal	Normal	0	34

[087] miRNA expression was evaluated in plasma samples from mice with pathology-confirmed adenocarcinoma of the lung and from control mice that did not receive carcinogen. Control mice were age matched to the mice with adenocarcinoma. Expression levels of 329 miRNAs, were determined by qRT-PCR using TaqMan® MicroRNA Assays (Applied Biosystems) specific for each miRNA. A subset of the miRNAs (170) have identical mature sequences in both mice and humans and the remainder are mouse homologues of corresponding human miRNAs. Reverse transcription (RT) reaction components (Table 7) were assembled on ice prior to the addition of RNA template, and included ~1 ng plasma RNA per each 10 µl reaction. RT reactions were incubated in a 384-well GeneAmp® PCR System 9700 (Applied Biosystems) at 16 °C for 30 minutes, then at 42 °C for 30 minutes, then at 85 °C for 5 minutes. RT reactions were then frozen at -20 °C.

Table 7. Reverse transcription reaction components.

RT Reagent	Final Concentration
10X RT Buffer (Asuragen, Inc.)	1X
dNTPs (2.5 mM each) (GE Healthcare, Piscataway, NJ, USA; cat. no. 28-4065-53)	1 mM
5X TaqMan MicroRNA RT primers (Applied Biosystems, Inc.)	0.25X
RNasin plus RNase Inhibitor (40 U/mL) (Promega, Corp., Madison, WI, USA; cat. no. N251X)	0.4U/ μ l
Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT), (200U/ μ L) (Invitrogen Corp., Carlsbad, CA, USA; cat. no. 28025-021)	1U/ μ l
Mouse Plasma RNA	~1 ng

[088] PCR components (Table 8) were assembled on ice prior to the addition of cDNA (2 μ l) from the RT reaction. Reactions were incubated in an ABI PRISM™ 7900HT Fast Real-Time PCR system (Applied Biosystems) at 95 °C for 1 minute, then for 50 cycles at 95 °C for 5 seconds and 60 °C for 30 seconds. Results were analyzed with the 7900HT Fast Real-Time PCR system SDS V2.3 software (Applied Biosystems).

Table 8. PCR components.

qPCR Reagents	Final Concentration
MgCl ₂ (50mM)	5 mM
10X Platinum PCR Buffer, Minus MgCl ₂ (Invitrogen; cat. no. 53286)	1X
dNTPs (2.5mM each) (GE Healthcare; cat. no. 28-4065-53)	1 mM
20X TaqMan miRNA primer/probe mix (Applied Biosystems)	0.4X
50X ROX (Asuragen, Inc.)	1X
Platinum Taq DNA Polymerase (5U/μl) (Invitrogen, Corp.; cat. no. 10966-083)	0.033U/μl
cDNA from RT-reaction	2 μl

[089] Table 9 shows the average Ct data and ddCt values for cancer vs. control mice from the qRT-PCR experiment. miRNAs with p-values of 0.1 or less are shown.

Table 9. miRNA expression in plasma samples from mice with pathology-confirmed adenocarcinoma of the lung and from control mice that did not receive carcinogen.

	Normal	Normal	Cancer	Cancer	cancer-normal	t-test
miR-ID	Ave dCT	SD	Ave dCT	SD	ddCT	p-value
miR-124a	5.024	0.207	1.823	0.400	-3.201	0.009745
miR-141	-2.319	1.108	-4.446	1.887	-2.127	0.011532
miR-183	1.025	1.294	-1.054	1.848	-2.080	0.02066
miR-182	-2.074	1.340	-3.989	1.711	-1.915	0.020695
mmu-miR-718	5.556	0.220	3.416	0.997	-2.140	0.030195
miR-27b	-1.343	1.872	-3.049	0.996	-1.705	0.036353
miR-452	1.601	0.674	-0.313	1.708	-1.914	0.040553
miR-379	3.021	0.764	1.287	0.988	-1.735	0.041625
mmu-miR-429	-4.873	0.781	-6.663	1.970	-1.790	0.042703
mmu-miR-83	3.704	1.371	1.304	0.781	-2.400	0.043659

	Normal	Normal	Cancer	Cancer	cancer-normal	t-test
miR-433	2.504	1.310	0.867	1.784	-1.636	0.046172
mmu-miR-715	3.353	1.520	1.169	1.327	-2.185	0.050614
miR-200a	-4.099	1.544	-5.861	1.901	-1.762	0.052236
mmu-miR-694	-0.132	3.124	2.744	2.058	2.875	0.059031
miR-205	-3.726	1.365	-5.323	1.875	-1.597	0.060958
miR-423	-3.359	6.935	-12.157	11.792	-8.797	0.076461
mmu-miR-706	0.017	1.072	-1.309	1.650	-1.327	0.077327
miR-422b	-0.914	1.839	-2.576	1.930	-1.662	0.089258
miR-324-5p	0.864	1.571	-0.388	0.503	-1.252	0.089557
miR-200c	-5.662	1.485	-7.159	1.966	-1.497	0.09453
miR-378	-0.322	2.275	-1.960	0.738	-1.638	0.095018
miR-365	-3.013	1.678	-4.707	2.275	-1.694	0.098461
mmu-miR-542-5p	2.291	1.603	0.917	0.836	-1.374	0.099749

[090] In addition, a temporal study was performed to examine miRNA expression in animals that had been diagnosed with lung adenocarcinomas. Figure 2A and Figure 2B show the changes in expression of selected miRNAs over time, prior to the detection of tumors. This figure shows that differential expression of miRNAs can be detected as early as 10 weeks after carcinogen administration—at least 6 weeks prior to the detection of tumors. These results demonstrate that miRNA can be used to detect lung cancer at an early stage and are suitable for screening for lung cancer.

Example 2. qRT-PCR Identification of miRNA Biomarkers for the Detection of Lung Cancer

[091] The expression of 12 miRNAs were evaluated in serum samples from eight lung cancer patients and eight normal patients. Blood from lung cancer patients and normal donors was collected into BD Vacutainer® Plus Plastic Serum Tubes. All blood was collected at the time of diagnosis but prior to any medical intervention, such as tumor resection or treatment. Blood was centrifuged at 1,000 x g for 10 min and serum transferred to a fresh tube and immediately frozen at -80 °C. Serum RNA was extracted using the organic extraction of the mirVana PARIS™ Kit (Ambion, Inc., Austin, TX, USA; Part No.

AM1556), with the following modifications. Following the addition of acid phenol:chloroform and vortexing, samples were incubated on ice for 5 min then centrifuged at 13,000 x g for 10 min at 4 °C. The aqueous layer was removed, extracted with chloroform, and centrifuged again. The aqueous layer was removed from the second extraction, and 3M NaOAc (1/10 volume), glycogen (5mg/ml), and 100% ethanol (1.5 volume) were added to the samples.

Lysate/ethanol mixtures were passed through a mirVana PARIS™ filter cartridge, and filters were washed once with 650 µl of Wash 1 buffer and twice with 650 µl of Wash 2/3 buffer. RNA was eluted with two aliquots of nuclease free water (50 µl) and stored at -80 °C.

[092] miRNA expression levels were determined by qRT-PCR using TaqMan® MicroRNA Assays (Applied Biosystems; Foster City, CA, USA) specific for each miRNA. Reverse transcription (RT) reactions were performed using the TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA; cat. no. 4366597). Reaction components listed in Table 10 were assembled on ice prior to the addition of RNA template. All reaction components were as provided by the manufacturer (Applied Biosystems; Foster City, CA, USA) as a kit with multiple components. Serum RNA (1 ng total RNA per reaction) was added and mixed. RT reactions were incubated in a 384-well GeneAmp® PCR System 9700 (Applied Biosystems) at 16 °C for 30 minutes, then at 42 °C for 30 minutes, then at 85 °C for 5 minutes. RT reactions were then frozen at -20 °C.

Table 10. Reverse transcription reaction components.

RT Reagent	Final Concentration
10X RT Buffer	1X
dNTPs (100mM)	1 mM
5X TaqMan MicroRNA RT primers	0.25X
RNase Inhibitor (20U/µl)	0.26U/µl
Multiscribe MMLV-RT enzyme (50U/µl)	3.35U/µl
Human Serum RNA	~1 ng

[093] PCR components (Table 11) were assembled on ice prior to the addition of cDNA (4 μ l) from the RT reaction. Reactions were incubated in an ABI PRISM™ 7900HT Fast Real-Time PCR system (Applied Biosystems) at 95 °C for 1 minute, then for 50 cycles at 95 °C for 5 seconds and 60 °C for 30 seconds. Results were analyzed with the 7900HT Fast Real-Time PCR system SDS V2.3 software (Applied Biosystems).

Table 11. PCR components.

qPCR Reagents	Final Concentration
MgCl ₂ (50 mM) (Invitrogen, Corp.; cat. no. 52723)	5 mM
10X Platinum PCR Buffer, Minus Mg (Invitrogen Corp.; cat. no. 53286)	1X
dNTPs (2.5 mM each) (GE Healthcare; cat. no. 28-4065-53)	1 mM
20X TaqMan miRNA primer/probe mix (Applied Biosystems)	0.4X
50X ROX Internal Marker (Asuragen, Inc.)	1X
Platinum Taq DNA Polymerase (5U/ μ l) (Invitrogen, Corp.; cat. no. 10966-083)	0.033U/ μ l

[094] Table 12 provides average dCt values for cancer and normal samples for each miRNA. In addition, ddCt values represent the difference in expression between cancer and normal samples.

Table 12: Expression of miRNAs in serum from patients with and without lung cancer.

	Normal	Normal	Cancer	Cancer	Cancer-normal	ttest
miR-ID	Ave dCT	SD	Ave dCT	SD	ddCT	p-value
miR-375	2.595	1.470	-0.284	1.624	-2.878	0.002304
miR-499	9.492	2.276	13.093	1.348	3.600	0.022561
miR-22	-1.705	1.115	-2.715	0.787	-1.010	0.055164
miR-122a	2.547	1.088	3.925	1.642	1.378	0.067827
miR-206	1.039	1.870	2.816	1.781	1.777	0.071915
miR-103	-5.317	1.051	-4.264	1.195	1.053	0.08225
miR-24	-6.105	0.998	-6.920	1.020	-0.815	0.12848

	Normal	Normal	Cancer	Cancer	Cancer-normal	ttest
miR-26a	-7.577	1.080	-6.754	1.316	0.823	0.193305
miR-498	9.488	2.253	10.945	3.831	1.457	0.439918
miR-205	5.380	1.864	6.265	2.862	0.886	0.497533
miR-222	-4.169	1.014	-4.005	0.888	0.164	0.735768
let7c	-1.414	1.090	-1.300	0.688	0.114	0.805489

[095] In an expanded panel, the expression of 180 miRNAs were evaluated in serum samples from sixteen lung cancer patients and from twelve individuals with benign lung conditions (Table 13). In Table 13, Diff stands for differential status of the tumor; MOD stands for moderate; AC for adenocarcinoma; SCC for squamous cell carcinoma; R for right; RUL for right upper lobe; RML for right mid lobe; RLL for right lower lobe; LUL for left upper lobe; and LLL for left lower lobe.

Table 13. Histopathological and patient information for lung cancer and benign specimens.

AGE	SEX	Tumor Size (cm)	Diagnosis	Cell Type	Diff	Lobe
69	M	5.5	Cancer	SCC	MOD	LLL
69	M	3.0	Cancer	Adenocarcinoma	POOR	RUL
63	M	2.0	Cancer	Adenocarcinoma	POOR	RUL
60	M	3.3	Cancer	Adenocarcinoma	MOD/POOR	RUL
56	M	3.0	Cancer	SCC	MOD	RUL
64	F	2.5	Cancer	Adenocarcinoma	MOD	LUL
72	F	6.0	Cancer	SCC	MOD/POOR	LLL
73	F	7.5	Cancer	SCC	MOD	RLL
71	M	3.0	Cancer	SCC	WELL	LUL
72	M	2.8	Cancer	Adenocarcinoma	MOD	RLL
59	F	3.5 & 2.6	Cancer	Adenocarcinoma	POOR/WELL	RUL
69	M	3.8	Cancer	SCC	POOR	LUL
66	F	3.2	Cancer	Adenocarcinoma	POOR	RUL
66	F	3.5	Cancer	SCC	POOR	RUL
66	M	3.5	Cancer	SCC	WELL	LLL
73	F	5.5	Cancer	SCC	POOR	RLL

AGE	SEX	Tumor Size (cm)	Diagnosis	Cell Type	Diff	Lobe
71	M	NA	Benign	Infarct	NA	LLL Wedge
64	F	NA	Benign	Granuloma	NA	Wedge
59	F	NA	Benign	Lymphoplasmacytic	NA	LLL
60	M	NA	Benign	Necrotizing Granuloma	NA	RML
58	M	NA	Benign	Decortication	NA	LLL Decortication
60	F	NA	Benign	Granuloma		Wedge
73	F	NA	Benign	Granuloma	NA	RML
77	F	2.0	Benign	Hamartoma	NA	Wedge
69	F	2.3	Benign	Hamartoma	NA	LUL
71	F	NA	Benign	Hamartoma	NA	Wedge
56	M	0.5	Benign	No abnormalities identified	WELL	R
69	M	0.7 nodule	Benign	Granulomas	NA	RUL Wedge

[096] Blood from lung cancer patients and comparator donors was collected into BD Vacutainer® Plus Plastic Serum Tubes and processed as described above for the initial human samples.

[097] Table 14 provides differential expression data for miRNAs in the form of average dCt values for cancer and benign samples. In addition, ddCt values represent the difference in expression between cancer and benign samples.

Table 14. Expression of miRNAs in serum from patients with lung cancer or benign lung conditions.

miR-ID	Benign Ave dCT	Benign SD	Cancer Ave dCT	Cancer SD	Cancer - Benign ddCT	ttest P-value
miR-422a	2.6793	0.3602	1.0741	0.9172	-1.6052	5.10E-06
miR-27b	0.7835	0.4918	1.7571	0.5307	0.9735	4.94E-05
miR-181d	0.8962	0.6161	-0.1954	0.7101	-1.0916	2.40E-04
miR-126	-4.0254	0.3338	-3.2872	0.5391	0.7382	2.99E-04
miR-142-	-4.6372	0.3449	-3.6120	0.8137	1.0252	3.76E-04

miR-ID	Benign Ave dCT	Benign SD	Cancer Ave dCT	Cancer SD	Cancer - Benign ddCT	ttest P-value
3p						
miR-27a	-2.2922	0.6007	-1.3131	0.6512	0.9791	3.92E-04
miR-142- 5p	-0.4216	0.7273	0.7481	0.8222	1.1697	5.91E-04
miR-126*	-3.5995	0.2867	-3.0059	0.4729	0.5935	7.08E-04
miR-92	-6.8925	0.6114	-8.1185	0.9946	-1.2260	8.70E-04
miR-378	-1.3378	0.4795	-2.2688	0.7699	-0.9310	1.07E-03
miR-374	-1.0404	0.3363	-0.3476	0.6435	0.6928	2.25E-03
miR-15b	-1.3417	0.4185	-0.8038	0.4658	0.5379	4.02E-03
miR-30e- 5p	-2.5626	0.7111	-1.7810	0.6035	0.7816	4.15E-03
miR-140	-0.6399	0.5506	0.0188	0.5949	0.6587	6.01E-03
miR-181a	1.7785	0.9882	0.8296	0.6985	-0.9489	6.16E-03
miR-15a	1.7754	0.5195	2.4577	0.6611	0.6822	6.62E-03
miR-486	-6.1701	1.0226	-7.4543	1.2676	-1.2842	7.98E-03
miR-340	-1.0291	0.5294	-0.3092	0.7436	0.7199	8.45E-03
miR-518b	5.7505	1.1729	4.3957	1.3343	-1.3548	9.58E-03
miR-342	0.8380	0.5664	0.0741	0.8180	-0.7639	1.02E-02
miR-145	1.5007	0.7105	0.7670	0.6990	-0.7337	1.12E-02
let-7i	-1.8912	0.5005	-1.4026	0.5160	0.4886	1.86E-02
miR-150	-2.5234	0.8088	-3.7092	1.5066	-1.1858	2.06E-02
miR-320	-4.9158	0.7410	-5.7428	1.0027	-0.8270	2.37E-02
miR-345	0.2934	0.9660	-0.4029	0.5695	-0.6963	2.44E-02
miR-214	2.3882	1.1670	3.8445	1.9139	1.4563	2.94E-02
let-7f	0.2969	0.6481	0.9345	0.7784	0.6376	2.98E-02
miR-324- 3p	1.3654	0.6871	0.7783	0.6976	-0.5871	3.55E-02
miR-106b	-2.0518	0.7547	-1.5215	0.5235	0.5302	3.71E-02
miR-143	0.9101	1.0014	1.8296	1.2023	0.9195	4.13E-02
let-7g	-2.8322	0.3171	-2.3913	0.6574	0.4410	4.21E-02
miR-181b	1.1157	0.6983	0.4448	0.9198	-0.6708	4.48E-02
miR-202	1.3920	0.8357	0.6625	0.9352	-0.7295	4.51E-02
miR-191	-2.5784	0.6352	-2.9762	0.4085	-0.3978	5.42E-02
miR-199a*	-1.5205	0.5713	-1.0752	0.6451	0.4453	6.91E-02
miR-193b	2.3794	0.7481	1.5532	1.3659	-0.8262	7.02E-02
miR-496	4.3400	1.0930	5.3754	1.6993	1.0353	7.72E-02

miR-ID	Benign Ave dCT	Benign SD	Cancer Ave dCT	Cancer SD	Cancer - Benign ddCT	ttest P-value
miR-132	1.6586	1.1201	1.0688	0.6675	-0.5899	9.36E-02
miR-328	0.8189	0.8011	0.2829	0.8614	-0.5361	1.05E-01
miR-29a	-2.3257	0.6459	-1.6683	1.2372	0.6574	1.06E-01
miR-26a	-3.1762	0.4130	-2.8524	0.5688	0.3238	1.08E-01
miR-30b	-2.2309	0.5773	-1.9028	0.5152	0.3281	1.25E-01
miR-222	-1.9205	0.6562	-2.2670	0.4896	-0.3466	1.28E-01
miR-433	3.8847	1.0349	3.2175	1.1667	-0.6673	1.28E-01
miR-148a	-0.9623	0.8328	-0.5361	0.6067	0.4262	1.29E-01
miR-24	-3.1600	0.5440	-3.4913	0.5632	-0.3313	1.30E-01
miR-185	-0.4549	0.9216	0.1325	1.0365	0.5874	1.32E-01
let-7e	-2.6734	0.4214	-2.2989	0.7579	0.3745	1.36E-01
miR-29c	-0.5739	0.7037	-0.0001	1.1417	0.5737	1.38E-01
miR-566	2.4771	1.1749	1.7666	1.2689	-0.7104	1.42E-01
miR-432	2.3089	0.9280	2.9279	1.2706	0.6190	1.66E-01
let-7c	1.2401	0.5373	1.6456	0.8681	0.4055	1.67E-01
miR-26b	-3.6560	0.4932	-3.3775	0.5238	0.2785	1.71E-01
miR-186	-1.2855	0.6674	-1.6027	0.5389	-0.3172	1.76E-01
miR-30c	-2.3656	0.5170	-2.0711	0.5893	0.2945	1.80E-01
miR-21	-4.6211	0.6501	-4.2927	0.5931	0.3284	1.83E-01
miR-196b	3.5790	0.8693	4.1100	1.1353	0.5310	1.89E-01
miR-206	3.3545	1.2404	4.0606	1.5185	0.7062	2.00E-01
miR-133b	3.0461	1.0602	3.6031	1.1841	0.5570	2.09E-01
miR-101	-0.1229	1.0697	0.3882	1.0225	0.5111	2.11E-01
miR-30d	-2.3000	0.4638	-2.6151	0.7742	-0.3152	2.23E-01
miR-125a	0.9380	0.7768	0.5229	0.9426	-0.4151	2.26E-01
miR-423	2.3856	0.7807	1.9988	0.8556	-0.3869	2.30E-01
miR-98	2.3292	0.6178	2.7845	1.1810	0.4553	2.36E-01
miR-30a- 5p	-3.5098	0.4451	-3.7597	0.6011	-0.2499	2.37E-01
miR-382	3.0718	1.3008	2.5268	1.1063	-0.5449	2.42E-01
miR-146a	-3.3154	0.7922	-3.6180	0.5646	-0.3026	2.48E-01
miR-210	0.9449	0.6478	0.5916	0.8745	-0.3533	2.50E-01
miR-10b	1.8909	0.8881	2.3531	1.1545	0.4622	2.60E-01
miR-501	4.2875	1.8588	3.5968	1.3891	-0.6907	2.70E-01
miR-16	-9.0423	1.1022	-8.5257	1.3039	0.5165	2.79E-01
miR-195	-3.3951	1.2516	-2.8590	1.3747	0.5361	2.99E-01

miR-ID	Benign Ave dCT	Benign SD	Cancer Ave dCT	Cancer SD	Cancer - Benign ddCT	ttest P-value
miR-93	-3.5061	0.9429	-3.8992	1.0384	-0.3931	3.12E-01
let-7a	-1.5300	0.4402	-1.2614	0.8211	0.2686	3.15E-01
miR-497	2.2971	1.0066	2.6427	0.8125	0.3456	3.24E-01
let-7b	-2.3849	0.6326	-2.1077	0.7862	0.2772	3.26E-01
miR-130a	-2.1333	1.1347	-1.7624	0.8643	0.3709	3.35E-01
miR-346	2.9259	1.0873	3.5397	1.9410	0.6138	3.35E-01
miR-192	-0.1533	0.7412	0.1308	0.9346	0.2841	3.94E-01
miR-130b	-0.4571	0.8988	-0.7298	0.7741	-0.2727	3.97E-01
miR-133a	2.1153	1.5110	1.2847	3.0632	-0.8307	3.97E-01
miR-17-5p	-0.6945	0.6588	-0.9188	0.7430	-0.2243	4.15E-01
miR-20a	-4.6339	0.9997	-4.2700	1.2843	0.3640	4.29E-01
miR-483	2.9376	1.2951	2.5331	1.3943	-0.4045	4.41E-01
miR-660	-1.6504	0.5269	-1.4526	0.8660	0.1978	4.91E-01
miR-30e- 3p	3.9566	0.8947	4.1763	0.7757	0.2197	4.93E-01
miR-22	-0.4382	0.7316	-0.6420	0.8160	-0.2038	5.06E-01
miR-301	1.9104	0.7844	1.7216	0.7036	-0.1888	5.09E-01
miR-296	2.8720	0.3686	2.7212	0.8082	-0.1508	5.54E-01
let-7d	-0.2621	0.4927	-0.1031	0.8485	0.1591	5.68E-01
miR-335	0.6203	0.9959	0.4327	0.7237	-0.1876	5.68E-01
miR-99a	2.0535	0.9928	1.8082	1.2768	-0.2453	5.86E-01
miR-151	-0.0065	0.6454	-0.1485	0.7022	-0.1419	5.89E-01
miR-197	-0.2610	0.5401	-0.3910	0.7156	-0.1300	6.03E-01
miR-139	2.4268	0.8325	2.5865	0.8012	0.1598	6.12E-01
miR-638	0.5473	1.1720	0.7616	1.0756	0.2143	6.20E-01
miR-25	-2.6351	1.1161	-2.8617	1.2709	-0.2267	6.31E-01
miR-223	-7.0503	0.9827	-7.2432	1.0912	-0.1930	6.33E-01
miR-19b	-5.0280	0.7599	-5.1783	0.8950	-0.1504	6.43E-01
miR-134	3.4474	1.2533	3.2173	1.4493	-0.2300	6.64E-01
miR-525	5.3500	1.2998	5.5719	1.3734	0.2220	6.69E-01
miR-194	3.2777	0.9298	3.1344	0.8584	-0.1432	6.77E-01
miR-23a	0.1782	0.5417	0.0918	0.5748	-0.0865	6.90E-01
miR-584	2.1971	1.6012	2.0072	0.8763	-0.1899	6.91E-01
miR-10a	2.3596	0.8173	2.4861	0.9602	0.1266	7.16E-01
miR-605	3.1671	1.0273	2.9048	2.4778	-0.2622	7.34E-01
miR-152	1.2288	0.7870	1.3077	0.5498	0.0789	7.57E-01

miR-ID	Benign Ave dCT	Benign SD	Cancer Ave dCT	Cancer SD	Cancer - Benign ddCT	ttest P-value
miR-361	1.4327	0.7048	1.3446	0.9835	-0.0881	7.94E-01
miR-339	1.7024	0.6148	1.7631	0.6041	0.0607	7.96E-01
miR-18a	0.1604	0.5327	0.2132	0.7051	0.0528	8.30E-01
miR-365	1.4310	0.9063	1.3636	1.0200	-0.0674	8.58E-01
miR-100	2.5049	1.3365	2.5891	1.3062	0.0842	8.69E-01
miR-103	0.0781	0.9976	0.0383	0.7868	-0.0398	9.07E-01
miR-146b	-1.4331	0.5367	-1.4051	0.6798	0.0280	9.07E-01
miR-19a	-3.9132	0.7972	-3.8742	0.9759	0.0390	9.11E-01
miR-505	3.1605	1.3641	3.1189	0.9145	-0.0415	9.24E-01
miR-204	4.9078	1.2588	4.8708	1.1407	-0.0369	9.37E-01
miR-221	-1.9235	0.8297	-1.9439	0.8326	-0.0204	9.49E-01
miR-324- 5p	2.4533	0.4286	2.4384	0.8683	-0.0149	9.57E-01
miR-485- 3p	3.7173	1.7214	3.7477	1.3047	0.0303	9.58E-01
miR-155	1.7078	0.7514	1.7018	1.0942	-0.0061	9.87E-01
miR-20b	-1.2419	0.9574	-1.2497	1.4402	-0.0078	9.87E-01
miR-190	2.6134	0.7303	2.6156	0.9347	0.0022	9.95E-01
miR-502	3.8339	1.1577	3.8302	1.7387	-0.0038	9.95E-01
miR-106a	-3.8355	0.7495	-3.8341	0.9729	0.0014	9.97E-01

[098] Pairs of miRNA biomarkers were also evaluated for their abilities to distinguish serum from patients for these samples. Un-normalized qRT-PCR data were used to calculate dCt values for each miRNA pair that was evaluated. The dCt values of the various miRNA pairs were analyzed using Receiver Operator Characteristic (ROC) analysis to identify the diagnostic miRNA pairs for lung cancer. In this example, ROC curves were used to evaluate the capacities of individual and combinations of biomarker candidates to distinguish patient classes. The abundance(s) of biomarker candidates were measured, and the measurements were used to develop classifiers whose true positive and false positive rates were plotted in a ROC curve. The AUC was then calculated for each classifier. An ideal classifier has an ROC AUC value of 1, and classifiers were ranked based upon how close their ROC AUC is to 1.

[099] Table 15 includes miRNA pairs that distinguish patients with lung cancer from those with benign lung conditions. The miRNA pairs in Table 15 are listed in order of decreasing ROC AUC. Serum miRNA biomarker pairs for classifying patients with cancer vs. patients with benign lung conditions are presented. Mean values represent the average difference in Ct values between the two miRNAs in the pair. In Table 15, "Benign mean" represents the mean of data from 12 subjects with benign lung conditions; "Cancer mean" is the mean for data from 16 lung cancer patients; "Benign SD" is the standard deviation for data from patients with benign lung conditions; "Cancer SD" is the standard deviation for data from lung cancer patients; "Benign vs Cancer Assoc." refers to the p-value associated with the diagnosis of benign/lung cancer; ROC stands for receiver operating characteristic; and AUC is area under curve.

Table 15. microRNA biomarker pairs - benign lung and lung cancer patient sera.

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-202,miR-29b	2.33	-1.53	0.64	-3.86	1.09	0.99	5.74E-07
miR-142-5p,miR-422a	-2.77	-3.10	0.92	-0.33	1.31	0.98	1.34E-06
miR-24,miR-27a	1.31	-0.87	0.41	-2.18	0.56	0.98	2.71E-07
miR-27b,miR-422a	-2.57	-1.90	0.51	0.67	1.09	0.98	4.84E-08
miR-140,miR-422a	-2.26	-3.32	0.63	-1.06	1.10	0.97	9.36E-07
miR-185,miR-93	-0.98	3.05	0.39	4.03	0.48	0.97	4.03E-06
miR-126,miR-181d	-1.83	-4.92	0.63	-3.09	0.85	0.97	1.27E-06
miR-142-3p,miR-422a	-2.63	-7.32	0.56	-4.69	1.09	0.97	4.07E-08
miR-30e-5p,miR-345	-1.48	-2.86	0.81	-1.38	0.68	0.97	1.85E-05
miR-29b,miR-422a	-3.10	0.18	1.00	3.27	1.43	0.96	8.31E-07
miR-324-5p,miR-422a	-1.59	-0.23	0.58	1.37	0.81	0.96	4.37E-06
miR-374,miR-422a	-2.30	-3.72	0.56	-1.42	1.23	0.96	2.35E-06
miR-140,miR-345	-1.36	-0.93	0.75	0.42	0.78	0.96	3.97E-05
miR-23a,miR-27a	1.06	2.47	0.37	1.41	0.56	0.96	5.10E-06
miR-29b,miR-378	-2.42	4.30	1.00	6.72	1.21	0.96	6.22E-06
miR-30e-5p,miR-422a	-2.39	-5.24	0.85	-2.86	1.19	0.96	3.23E-06
miR-30e-5p,miR-92	-2.01	4.33	0.68	6.34	0.97	0.96	1.83E-06
miR-660,miR-92	-1.42	5.24	0.71	6.67	0.55	0.96	2.80E-06

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-106b,miR-422a	-2.13	-4.73	0.93	-2.60	0.95	0.96	2.91E-06
let-7g,miR-422a	-2.05	-5.51	0.46	-3.47	1.31	0.95	2.24E-05
miR-101,miR-92	-1.74	6.77	0.69	8.51	0.83	0.95	3.63E-06
miR-126,miR-92	-1.96	2.87	0.73	4.83	1.18	0.95	2.77E-05
miR-126*,miR-422a	-2.20	-6.28	0.42	-4.08	1.17	0.95	1.42E-06
miR-126*,miR-92	-1.82	3.29	0.59	5.11	1.11	0.95	2.37E-05
miR-150,miR-29a	1.84	-0.20	0.86	-2.04	0.80	0.95	3.74E-06
miR-15a,miR-92	-1.91	8.67	0.58	10.58	0.92	0.95	1.10E-06
miR-30e-5p,miR-324-3p	-1.37	-3.93	0.78	-2.56	0.58	0.95	1.33E-05
miR-126,miR-378	-1.67	-2.69	0.58	-1.02	0.92	0.95	9.56E-06
miR-126,miR-422a	-2.34	-6.70	0.49	-4.36	1.06	0.95	1.47E-07
miR-132,miR-29b	2.22	-1.20	1.23	-3.42	1.13	0.95	3.82E-05
miR-142-3p,miR-181a	-1.97	-6.42	0.85	-4.44	0.94	0.95	5.06E-06
miR-142-3p,miR-378	-1.96	-3.30	0.60	-1.34	1.06	0.95	5.05E-06
miR-148b,miR-92	-1.76	7.75	0.70	9.51	0.86	0.95	4.27E-06
miR-181b,miR-29b	2.31	-1.82	0.98	-4.13	1.16	0.95	7.59E-06
miR-26a,miR-422a	-1.93	-5.85	0.62	-3.93	1.08	0.95	8.71E-06
miR-15a,miR-422a	-2.29	-0.90	0.62	1.38	1.05	0.94	4.15E-07
miR-146a,miR-27b	1.24	-4.10	0.66	-5.34	0.45	0.94	3.44E-06
miR-148b,miR-422a	-2.02	-1.82	0.64	0.20	1.06	0.94	3.75E-06
let-7i,miR-422a	-2.09	-4.57	0.74	-2.48	1.07	0.94	3.99E-06
miR-340,miR-422a	-2.32	-3.71	0.70	-1.38	1.25	0.94	4.28E-06
miR-143,miR-150	-2.11	3.43	1.09	5.54	0.91	0.94	7.44E-06
miR-15a,miR-378	-1.61	3.12	0.70	4.73	0.82	0.94	9.88E-06
miR-15b,miR-422a	-2.14	-4.02	0.64	-1.88	1.25	0.94	1.13E-05
miR-27b,miR-92	-2.18	7.68	0.77	9.86	1.27	0.94	1.66E-05
miR-30e-3p,miR-422a	-1.83	1.28	0.83	3.10	1.08	0.94	4.76E-05
miR-30b,miR-422a	-1.93	-4.91	0.80	-2.98	1.20	0.94	5.37E-05
miR-142-3p,miR-92	-2.25	2.26	0.87	4.51	1.44	0.94	5.77E-05
miR-27a,miR-422a	-2.58	-4.97	0.68	-2.39	1.38	0.94	2.89E-06
miR-142-5p,miR-342	-1.93	-1.26	0.91	0.67	0.86	0.94	4.69E-06
miR-142-5p,miR-223	-1.36	6.63	0.48	7.99	0.71	0.94	5.23E-06
miR-27b,miR-378	-1.91	2.12	0.77	4.03	1.02	0.94	1.15E-05
miR-186,miR-27a	1.30	1.01	0.43	-0.29	0.75	0.94	1.35E-05

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-106b,miR-324-3p	-1.12	-3.42	0.73	-2.30	0.40	0.94	2.00E-05
miR-34a,miR-518b	-2.55	-3.39	1.40	-0.84	1.21	0.94	2.14E-05
miR-27a,miR-92	-2.20	4.60	0.74	6.80	1.42	0.94	4.48E-05
miR-30a-3p,miR-422a	-1.73	0.70	0.66	2.43	1.09	0.94	5.00E-05
miR-181d,miR-29b	2.55	-2.09	1.08	-4.63	0.95	0.93	5.26E-07
miR-15b,miR-191	-0.93	1.24	0.35	2.17	0.47	0.93	4.61E-06
miR-192,miR-422a	-1.89	-2.83	0.79	-0.94	1.09	0.93	2.82E-05
miR-422a,miR-576	2.39	-1.56	0.78	-3.95	1.64	0.93	8.46E-05
miR-30c,miR-422a	-1.90	-5.04	0.77	-3.14	1.25	0.93	8.81E-05
miR-142-3p,miR-145	-1.76	-6.14	0.57	-4.38	0.92	0.93	4.05E-06
miR-142-3p,miR-181d	-2.12	-5.53	0.75	-3.42	1.06	0.93	3.17E-06
miR-181d,miR-27b	2.09	0.11	0.84	-1.98	1.00	0.93	3.56E-06
miR-142-5p,miR-186	-1.49	0.86	0.65	2.35	0.77	0.93	1.10E-05
miR-150,miR-29c	1.76	-1.95	0.85	-3.71	0.88	0.93	1.55E-05
miR-200c,miR-422a	-2.20	-0.58	1.17	1.62	1.04	0.93	1.71E-05
miR-185,miR-92	-1.81	6.44	0.69	8.25	1.07	0.93	2.49E-05
miR-148a,miR-422a	-2.03	-3.64	0.93	-1.61	1.16	0.93	3.45E-05
miR-34a,miR-422a	-2.77	-0.32	1.74	2.46	1.23	0.93	3.79E-05
miR-32,miR-422a	-2.56	1.56	1.20	4.12	1.51	0.93	5.27E-05
miR-214,miR-566	-2.28	-0.09	1.19	2.19	1.29	0.93	5.85E-05
miR-206,miR-422a	-2.31	0.68	1.17	2.99	1.33	0.93	6.05E-05
miR-214,miR-518b	-2.84	-3.36	1.54	-0.52	1.65	0.93	8.68E-05
miR-142-3p,miR-320	-1.85	0.28	0.77	2.13	1.23	0.93	1.04E-04
miR-331,miR-422a	-1.61	-1.29	0.67	0.33	1.48	0.93	1.41E-04
miR-26b,miR-422a	-1.86	-6.33	0.76	-4.48	1.07	0.92	2.36E-05
miR-142-5p,miR-345	-1.86	-0.71	1.01	1.15	0.91	0.92	2.44E-05
miR-27b,miR-324-3p	-1.55	-0.58	0.80	0.97	0.86	0.92	5.10E-05
miR-30a-5p,miR-30e-5p	1.03	-0.95	0.52	-1.98	0.48	0.92	1.13E-05
miR-29b,miR-92	-2.78	9.83	1.08	12.60	1.56	0.92	1.64E-05
miR-191,miR-27a	1.38	-0.29	0.71	-1.66	0.67	0.92	1.72E-05
miR-140,miR-181d	-1.75	-1.54	0.74	0.22	1.04	0.92	3.67E-05
miR-422a,miR-660	1.80	4.33	0.57	2.53	1.15	0.92	3.75E-05
miR-126,miR-181a	-1.69	-5.80	1.08	-4.12	0.74	0.92	4.09E-05
miR-126*,miR-378	-1.52	-2.26	0.58	-0.74	0.97	0.92	5.24E-05

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-15a,miR-181d	-1.77	0.88	0.89	2.65	1.02	0.92	5.46E-05
let-7f,miR-422a	-2.24	-2.38	0.84	-0.14	1.44	0.92	5.53E-05
miR-181a,miR-27b	1.91	1.00	1.12	-0.92	1.01	0.92	6.84E-05
miR-29b,miR-324-3p	-2.12	1.59	1.14	3.70	1.24	0.92	8.87E-05
miR-132,miR-30e-5p	1.37	4.22	0.92	2.85	0.66	0.92	9.53E-05
miR-214,miR-422b	-2.22	0.40	0.91	2.61	1.48	0.92	1.07E-04
miR-140,miR-378	-1.59	0.70	0.75	2.29	1.03	0.92	1.17E-04
miR-29b,miR-30a-5p	-1.83	6.52	0.79	8.35	1.22	0.92	1.18E-04
let-7a,miR-422a	-1.87	-4.21	0.60	-2.34	1.57	0.92	1.70E-04
miR-142-5p,miR-191	-1.57	2.16	0.47	3.73	0.89	0.92	8.01E-06
miR-24,miR-27b	1.24	-3.94	0.45	-5.19	0.78	0.92	3.87E-05
miR-126*,miR-181d	-1.69	-4.50	0.67	-2.81	0.98	0.92	2.63E-05
miR-181d,miR-30e-5p	1.88	3.46	0.92	1.59	1.02	0.92	3.03E-05
miR-142-3p,miR-191	-1.42	-2.06	0.52	-0.64	0.87	0.92	3.24E-05
miR-181c,miR-29b	2.34	1.31	1.43	-1.03	1.09	0.92	3.99E-05
miR-181d,miR-27a	2.07	3.19	0.98	1.12	1.19	0.92	4.24E-05
miR-148b,miR-378	-1.46	2.14	0.69	3.61	0.84	0.92	4.39E-05
miR-15a,miR-320	-1.51	6.69	0.74	8.20	0.89	0.92	6.22E-05
miR-199a*,miR-422a	-2.05	-4.20	0.81	-2.15	1.32	0.92	6.35E-05
miR-146a,miR-27a	1.28	-1.02	0.92	-2.30	0.49	0.92	6.63E-05
miR-142-5p,miR-181a	-2.12	-2.20	1.02	-0.08	1.27	0.92	6.83E-05
miR-15a,miR-486	-1.97	7.95	0.94	9.91	1.19	0.92	6.85E-05
miR-27b,miR-342	-1.70	-0.06	0.81	1.64	1.03	0.92	7.00E-05
miR-181a,miR-27a	1.93	4.07	1.11	2.14	1.07	0.92	8.57E-05
let-7g,miR-342	-1.21	-3.67	0.45	-2.47	0.81	0.92	9.17E-05
miR-29b,miR-345	-2.02	2.90	0.98	4.92	1.27	0.92	1.00E-04
let-7e,miR-422a	-1.98	-5.35	0.65	-3.37	1.47	0.92	1.91E-04
miR-29b,miR-422b	-2.15	1.00	0.80	3.15	1.25	0.91	1.89E-05
miR-142-3p,miR-202	-1.70	-6.03	0.93	-4.33	0.82	0.91	2.31E-05
miR-214,miR-422a	-3.10	-0.29	1.16	2.81	1.86	0.91	2.84E-05
miR-142-5p,miR-145	-1.90	-1.92	0.65	-0.02	1.18	0.91	3.02E-05
miR-422a,miR-497	1.95	0.38	0.91	-1.57	1.11	0.91	3.66E-05
miR-29b,miR-433	-2.37	-0.95	1.31	1.42	1.20	0.91	3.72E-05
miR-140,miR-92	-1.89	6.25	0.75	8.14	1.21	0.91	6.62E-05

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-142-5p,miR-92	-2.40	6.47	0.93	8.87	1.55	0.91	6.67E-05
miR-142-3p,miR-181b	-1.70	-5.75	0.74	-4.06	1.07	0.91	7.16E-05
miR-30e-5p,miR-486	-2.07	3.61	1.02	5.67	1.24	0.91	7.27E-05
miR-196b,miR-422a	-2.13	0.90	0.95	3.04	1.37	0.91	9.10E-05
miR-222,miR-29b	1.83	-4.92	0.83	-6.75	1.18	0.91	1.01E-04
miR-142-3p,miR-518b	-2.38	-10.39	1.09	-8.01	1.55	0.91	1.13E-04
miR-29b,miR-30d	-1.93	5.34	0.85	7.27	1.30	0.91	1.37E-04
miR-29b,miR-361	-1.82	1.55	1.01	3.37	1.13	0.91	1.60E-04
miR-342,miR-34a	2.05	-1.52	1.40	-3.58	1.14	0.91	2.16E-04
miR-106b,miR-181d	-1.62	-2.95	1.31	-1.33	0.94	0.91	2.45E-04
miR-340,miR-92	-1.95	5.86	0.84	7.81	1.34	0.91	1.59E-04
miR-486,miR-7	1.41	-8.20	0.66	-9.61	0.96	0.91	1.82E-04
miR-21,miR-422a	-1.95	-7.30	0.73	-5.35	1.15	0.91	2.33E-05
miR-106b,miR-92	-1.76	4.84	0.84	6.60	0.95	0.91	2.59E-05
let-7i,miR-92	-1.71	5.00	0.71	6.72	0.99	0.91	2.73E-05
miR-101,miR-486	-1.80	6.05	0.89	7.84	0.96	0.91	3.00E-05
miR-181a,miR-29b	2.16	-1.41	1.11	-3.57	1.13	0.91	3.14E-05
miR-142-5p,miR-150	-2.36	2.10	1.05	4.46	1.35	0.91	3.42E-05
miR-142-3p,miR-342	-1.79	-5.48	0.47	-3.69	1.17	0.91	3.57E-05
miR-29b,miR-518b	-2.85	-2.74	1.36	0.11	1.71	0.91	6.39E-05
miR-142-5p,miR-181d	-2.26	-1.32	1.23	0.94	1.28	0.91	7.39E-05
miR-193b,miR-29b	2.23	-0.61	0.99	-2.84	1.42	0.91	8.62E-05
miR-146b,miR-422a	-1.63	-4.11	0.45	-2.48	1.17	0.91	9.89E-05
miR-15b,miR-92	-1.76	5.55	0.66	7.31	1.20	0.91	1.03E-04
miR-142-5p,miR-23a	-1.26	-0.60	0.81	0.66	0.65	0.91	1.10E-04
miR-27a,miR-378	-1.91	-0.95	0.86	0.95	1.24	0.91	1.11E-04
miR-197,miR-29b	1.78	-3.13	0.89	-4.91	1.31	0.91	2.93E-04
miR-342,miR-374	1.46	1.88	0.58	0.42	1.16	0.91	2.93E-04
miR-422a,miR-487b	1.83	-1.05	0.92	-2.88	1.32	0.91	2.93E-04
miR-142-3p,miR-99b	-1.45	-7.52	0.62	-6.08	1.25	0.91	2.93E-04
miR-29b,miR-320	-2.31	7.94	1.09	10.24	1.66	0.91	2.93E-04
miR-340,miR-378	-1.65	0.31	0.82	1.96	1.22	0.91	2.93E-04
miR-29b,miR-486	-2.80	9.10	1.25	11.90	1.89	0.90	1.40E-04
miR-23a,miR-422a	-1.52	-2.50	0.55	-0.98	1.17	0.90	3.13E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-222,miR-27b	1.24	-2.70	0.88	-3.95	0.89	0.90	3.20E-04
miR-142-5p,miR-24	-1.50	2.74	0.79	4.24	0.81	0.90	4.46E-05
miR-148b,miR-181d	-1.63	-0.09	0.83	1.54	0.93	0.90	5.60E-05
miR-29b,miR-342	-2.26	2.07	1.00	4.33	1.38	0.90	5.67E-05
miR-152,miR-422a	-1.68	-1.45	0.78	0.23	1.02	0.90	6.17E-05
miR-7,miR-92	-1.32	8.96	0.65	10.28	0.80	0.90	8.09E-05
miR-210,miR-422a	-1.25	-1.73	0.61	-0.48	0.80	0.90	1.26E-04
let-7c,miR-422a	-2.01	-1.44	0.80	0.57	1.38	0.90	1.27E-04
miR-27b,miR-518b	-2.37	-4.97	1.30	-2.60	1.49	0.90	1.66E-04
miR-145,miR-374	1.43	2.54	0.67	1.12	0.96	0.90	1.70E-04
miR-27a,miR-324-3p	-1.57	-3.66	0.73	-2.09	1.06	0.90	1.72E-04
miR-140,miR-186	-0.98	0.64	0.59	1.62	0.60	0.90	1.99E-04
miR-185,miR-486	-1.87	5.72	1.00	7.59	1.23	0.90	2.13E-04
miR-422a,miR-496	2.64	-1.66	1.16	-4.30	1.93	0.90	2.83E-04
miR-181a,miR-199a*	1.40	3.30	0.75	1.90	0.99	0.90	3.49E-04
miR-422a,miR-432	2.22	0.37	1.04	-1.85	1.70	0.90	3.51E-04
miR-181d,miR-23b	1.40	-2.13	0.50	-3.53	0.92	0.90	6.23E-05
miR-125b,miR-422a	-2.10	-0.93	1.09	1.17	1.30	0.90	1.23E-04
miR-145,miR-29b	2.08	-1.56	0.93	-3.63	1.39	0.90	1.38E-04
miR-142-5p,miR-146a	-1.47	2.90	1.12	4.37	0.71	0.90	2.51E-04
miR-126,miR-320	-1.57	0.89	0.84	2.46	1.10	0.90	3.57E-04
miR-191,miR-374	1.09	-1.54	0.53	-2.63	0.59	0.90	2.81E-05
miR-126,miR-145	-1.47	-5.53	0.95	-4.06	0.72	0.90	7.71E-05
miR-150,miR-29b	2.66	-5.54	1.25	-8.19	1.72	0.90	1.20E-04
miR-133b,miR-422a	-2.16	0.37	1.13	2.53	1.34	0.90	1.22E-04
miR-142-5p,miR-324-3p	-1.76	-1.79	0.94	-0.03	1.09	0.90	1.42E-04
miR-27b,miR-345	-1.69	0.49	1.14	2.18	0.88	0.90	1.50E-04
miR-374,miR-378	-1.62	0.30	0.60	1.92	1.17	0.90	1.73E-04
let-7b,miR-422a	-1.88	-5.06	0.82	-3.18	1.31	0.90	1.75E-04
miR-346,miR-432*	-1.63	-2.73	1.11	-1.09	0.91	0.90	2.34E-04
miR-142-5p,miR-518b	-2.52	-6.17	1.57	-3.65	1.54	0.90	2.37E-04
miR-142-3p,miR-328	-1.56	-5.46	0.71	-3.90	1.14	0.90	3.07E-04
miR-181a,miR-374	1.64	2.82	0.91	1.18	1.12	0.90	3.08E-04
let-7g,miR-181d	-1.53	-3.73	0.75	-2.20	1.10	0.90	3.15E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-106b,miR-378	-1.46	-0.71	1.09	0.75	0.81	0.90	3.79E-04
miR-30a-5p,miR-422a	-1.36	-6.19	0.55	-4.83	1.11	0.90	4.17E-04
miR-339,miR-422a	-1.67	-0.98	0.67	0.69	1.30	0.90	4.18E-04
let-7i,miR-378	-1.42	-0.55	0.87	0.87	0.96	0.90	4.18E-04
miR-142-5p,miR-93	-1.56	3.09	0.85	4.65	1.21	0.90	4.18E-04
miR-224,miR-422a	-1.44	0.66	0.73	2.10	1.27	0.90	4.18E-04
miR-30e-5p,miR-378	-1.71	-1.23	0.95	0.49	1.03	0.89	1.22E-04
miR-576,miR-92	-2.05	11.01	1.14	13.06	1.33	0.89	2.22E-04
miR-142-5p,miR-423	-1.56	-2.81	0.88	-1.25	1.19	0.89	4.54E-04
miR-320,miR-660	1.03	-3.27	0.53	-4.29	0.88	0.89	4.55E-04
miR-181a,miR-340	1.67	2.81	0.74	1.14	1.03	0.89	6.15E-05
miR-23a,miR-27b	1.05	-0.60	0.62	-1.65	0.59	0.89	1.12E-04
miR-30a-5p,miR-92	-0.98	3.38	0.56	4.36	0.58	0.89	1.30E-04
miR-27a,miR-345	-1.67	-2.59	0.96	-0.91	1.00	0.89	1.41E-04
miR-29b,miR-382	-2.17	-0.25	1.35	1.92	1.24	0.89	1.55E-04
miR-182,miR-422a	-1.65	1.67	1.10	3.32	0.92	0.89	1.94E-04
miR-132,miR-27a	1.57	3.95	1.04	2.38	0.89	0.89	2.18E-04
miR-374,miR-92	-1.92	5.85	0.79	7.77	1.39	0.89	2.19E-04
miR-145,miR-340	1.45	2.53	0.71	1.08	1.00	0.89	2.33E-04
miR-142-5p,miR-301	-1.36	-2.33	0.48	-0.97	1.03	0.89	2.53E-04
miR-185,miR-422a	-2.19	-3.13	1.04	-0.94	1.56	0.89	2.66E-04
miR-132,miR-27b	1.54	0.87	1.06	-0.66	0.87	0.89	2.73E-04
miR-126,miR-342	-1.50	-4.86	0.71	-3.36	1.08	0.89	2.91E-04
let-7i,miR-181d	-1.58	-2.79	1.05	-1.21	0.95	0.89	2.98E-04
miR-15b,miR-181d	-1.63	-2.24	0.96	-0.61	1.08	0.89	3.21E-04
miR-15b,miR-181a	-1.49	-3.12	0.91	-1.63	0.99	0.89	3.97E-04
miR-197,miR-422a	-1.47	-2.94	0.53	-1.46	1.18	0.89	4.47E-04
miR-142-3p,miR-486	-2.31	1.54	1.28	3.84	1.66	0.89	4.62E-04
miR-181a,miR-26a	1.27	4.95	0.74	3.68	0.97	0.89	4.97E-04
miR-130a,miR-320	-1.20	2.78	0.57	3.98	0.97	0.89	4.98E-04
miR-19a,miR-92	-1.26	2.98	0.47	4.24	1.10	0.89	4.98E-04
miR-143,miR-422a	-2.52	-1.77	1.16	0.76	1.68	0.89	1.39E-04
miR-191,miR-30b	0.73	-0.35	0.44	-1.07	0.45	0.89	2.37E-04
miR-126,miR-486	-2.02	2.15	1.13	4.17	1.39	0.89	3.39E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-422a,miR-98	2.06	0.35	0.77	-1.71	1.67	0.89	5.23E-04
miR-126*,miR-222	-0.94	-1.68	0.71	-0.74	0.70	0.89	5.41E-04
miR-191,miR-27b	1.36	-3.36	0.75	-4.72	0.71	0.89	4.19E-05
miR-181d,miR-340	1.81	1.93	0.86	0.11	1.07	0.89	5.66E-05
miR-151,miR-27b	1.09	-0.79	0.49	-1.88	0.71	0.89	9.90E-05
miR-29c,miR-422a	-2.18	-3.25	0.81	-1.07	1.52	0.89	1.33E-04
miR-21,miR-92	-1.57	2.27	0.68	3.84	1.07	0.89	1.48E-04
miR-29b,miR-326	-2.36	-2.27	1.32	0.09	1.45	0.89	1.56E-04
miR-142-5p,miR-378	-2.10	0.92	1.10	3.02	1.38	0.89	1.95E-04
miR-181a,miR-26b	1.20	5.43	0.61	4.24	0.80	0.89	2.06E-04
miR-145,miR-27b	1.68	0.72	1.05	-0.96	1.00	0.89	2.13E-04
miR-27b,miR-422b	-1.51	-1.21	0.89	0.30	0.95	0.89	2.17E-04
miR-186,miR-30e-5p	1.10	1.28	0.79	0.18	0.57	0.89	2.21E-04
miR-190,miR-422a	-1.61	-0.06	0.86	1.54	1.14	0.89	3.79E-04
miR-140,miR-222	-1.03	1.28	0.63	2.31	0.73	0.89	5.68E-04
miR-23b,miR-422a	-1.75	0.48	0.67	2.23	1.54	0.89	5.87E-04
miR-181d,miR-496	2.13	-3.44	1.14	-5.57	1.67	0.89	5.91E-04
miR-199a*,miR-92	-1.67	5.37	0.90	7.04	1.35	0.89	5.91E-04
miR-185,miR-324-3p	-1.17	-1.82	0.73	-0.64	0.87	0.89	5.92E-04
miR-222,miR-422a	-1.26	-4.60	0.78	-3.34	1.02	0.89	5.92E-04
miR-296,miR-422a	-1.45	0.19	0.37	1.65	1.35	0.89	5.92E-04
miR-142-5p,miR-222	-1.51	1.50	1.07	3.01	0.75	0.88	1.62E-04
miR-24,miR-374	1.02	-2.12	0.66	-3.14	0.69	0.88	5.41E-04
miR-125a,miR-142-3p	1.44	5.58	0.66	4.14	1.22	0.88	6.43E-04
miR-20a,miR-92	-1.61	2.26	0.72	3.86	1.32	0.88	6.43E-04
miR-27a,miR-93	-1.37	1.21	0.63	2.58	1.22	0.88	6.43E-04
miR-27a,miR-342	-1.74	-3.13	0.92	-1.39	1.05	0.88	1.07E-04
miR-145,miR-27a	1.71	3.79	0.98	2.08	1.06	0.88	1.78E-04
miR-29b,miR-328	-2.13	2.21	1.11	4.35	1.39	0.88	1.78E-04
miR-29a,miR-422a	-2.26	-5.00	0.77	-2.74	1.67	0.88	1.93E-04
miR-130a,miR-422a	-1.98	-4.81	1.10	-2.84	1.27	0.88	2.05E-04
miR-18a,miR-422a	-1.66	-2.52	0.63	-0.86	1.21	0.88	2.08E-04
miR-148b,miR-486	-1.82	7.07	1.14	8.89	1.11	0.88	2.39E-04
let-7f,miR-181d	-1.73	-0.60	0.86	1.13	1.20	0.88	2.49E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-214,miR-378	-2.44	3.73	1.08	6.16	1.75	0.88	2.52E-04
miR-126*,miR-181a	-1.54	-5.38	1.06	-3.84	0.90	0.88	2.98E-04
miR-142-3p,miR-26b	-0.76	-0.98	0.37	-0.22	0.55	0.88	3.26E-04
miR-126*,miR-24	-0.92	-0.44	0.52	0.49	0.63	0.88	3.42E-04
let-7f,miR-181a	-1.59	-1.48	0.70	0.11	1.19	0.88	3.44E-04
miR-150,miR-27a	2.17	-0.23	1.16	-2.40	1.52	0.88	3.58E-04
miR-181d,miR-199a*	1.54	2.42	0.75	0.88	1.12	0.88	3.63E-04
miR-142-3p,miR-345	-1.72	-4.93	1.19	-3.21	1.05	0.88	3.91E-04
miR-378,miR-497	1.28	-3.64	0.80	-4.91	0.84	0.88	3.98E-04
miR-181d,miR-497	1.44	-1.40	0.62	-2.84	1.11	0.88	4.16E-04
miR-32,miR-486	-2.52	10.43	1.32	12.94	1.84	0.88	4.60E-04
miR-191,miR-340	1.12	-1.55	0.59	-2.67	0.83	0.88	5.18E-04
miR-181d,miR-21	1.45	5.52	0.77	4.06	1.09	0.88	5.49E-04
miR-126,miR-202	-1.45	-5.42	0.86	-3.97	1.04	0.88	5.71E-04
miR-142-3p,miR-26a	-0.70	-1.46	0.17	-0.76	0.60	0.88	6.00E-04
miR-130b,miR-29b	1.92	-3.50	1.11	-5.41	1.40	0.88	6.01E-04
miR-148a,miR-181d	-1.52	-1.86	0.97	-0.34	1.09	0.88	7.00E-04
miR-15b,miR-24	-0.87	1.82	0.61	2.69	0.62	0.88	7.00E-04
miR-20a,miR-93	-0.75	-1.13	0.36	-0.37	0.75	0.88	7.00E-04
miR-142-5p,miR-193b	-2.00	-2.80	1.09	-0.81	1.61	0.88	7.02E-04
miR-148b,miR-30d	-0.90	3.19	0.55	4.09	0.70	0.88	7.02E-04
miR-346,miR-518b	-1.97	-2.83	1.17	-0.86	1.51	0.88	7.02E-04
miR-192,miR-92	-1.51	6.74	0.69	8.25	1.07	0.88	2.30E-04
miR-126*,miR-486	-1.88	2.57	1.01	4.45	1.27	0.88	2.70E-04
miR-194,miR-29b	1.64	0.30	1.13	-1.34	1.50	0.88	7.62E-04
miR-146a,miR-29b	1.78	-6.36	1.28	-8.14	0.84	0.88	1.39E-04
miR-181d,miR-374	1.79	1.94	0.79	0.15	1.25	0.88	1.93E-04
miR-378,miR-660	1.13	0.31	0.57	-0.82	0.77	0.88	2.23E-04
miR-29b,miR-566	-2.28	0.49	1.32	2.78	1.49	0.88	2.69E-04
miR-10a,miR-422a	-1.73	-0.32	0.86	1.41	1.22	0.88	2.80E-04
miR-148a,miR-345	-1.12	-1.26	0.73	-0.13	0.68	0.88	2.83E-04
miR-142-3p,miR-222	-1.33	-2.72	0.82	-1.39	0.85	0.88	3.12E-04
miR-101,miR-422a	-2.12	-2.80	1.06	-0.69	1.52	0.88	3.40E-04
miR-422a,miR-638	1.82	2.13	1.20	0.31	1.12	0.88	3.52E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-140,miR-150	-1.85	1.88	1.01	3.73	1.30	0.88	3.81E-04
miR-27b,miR-486	-2.24	6.96	1.22	9.19	1.60	0.88	4.24E-04
miR-27a,miR-486	-2.26	3.88	1.20	6.14	1.65	0.88	4.43E-04
miR-210,miR-29b	1.90	-2.04	1.03	-3.93	1.36	0.88	4.44E-04
miR-185,miR-25	-0.74	2.18	0.45	2.92	0.51	0.88	4.54E-04
miR-126,miR-181b	-1.41	-5.14	0.59	-3.73	1.10	0.88	4.54E-04
miR-142-5p,miR-486	-2.45	5.75	1.28	8.20	1.81	0.88	4.75E-04
miR-145,miR-199a*	1.18	3.02	0.63	1.84	0.87	0.88	5.03E-04
miR-139,miR-422a	-1.77	-0.25	0.94	1.51	1.30	0.88	5.08E-04
let-7i,miR-324-3p	-1.08	-3.26	0.69	-2.18	0.73	0.88	5.50E-04
miR-193b,miR-214	2.36	-0.01	1.20	-2.37	1.80	0.88	5.83E-04
miR-340,miR-342	-1.48	-1.87	0.86	-0.38	1.12	0.88	7.46E-04
miR-15a,miR-210	-1.04	0.83	0.59	1.87	0.80	0.88	8.13E-04
miR-130b,miR-422a	-1.33	-3.14	0.87	-1.80	1.04	0.88	8.29E-04
miR-27a,miR-30a-5p	-1.23	1.22	0.77	2.44	0.99	0.88	8.29E-04
miR-16,miR-422a	-2.12	-11.72	1.15	-9.60	1.84	0.88	8.30E-04
miR-19a,miR-422a	-1.64	-6.59	0.81	-4.95	1.42	0.88	8.30E-04
miR-32,miR-92	-2.29	11.04	1.43	13.33	1.72	0.88	8.30E-04
miR-34a,miR-422b	-1.92	0.37	1.60	2.29	1.16	0.88	8.30E-04
miR-15a,miR-181a	-1.63	0.00	1.14	1.63	0.98	0.87	3.99E-04
miR-27a,miR-301	-1.17	-4.20	0.54	-3.04	0.89	0.87	4.40E-04
miR-15b,miR-378	-1.47	0.00	0.81	1.46	1.08	0.87	5.26E-04
miR-34a,miR-566	-1.95	-0.12	1.34	1.83	1.31	0.87	6.89E-04
let-7f,miR-518b	-1.99	-5.45	1.12	-3.46	1.52	0.87	7.38E-04
miR-126*,miR-320	-1.42	1.32	0.82	2.74	1.08	0.87	7.97E-04
miR-222,miR-30e-5p	1.13	0.64	0.62	-0.48	0.70	0.87	1.61E-04
miR-433,miR-487b	1.41	0.21	0.76	-1.20	0.94	0.87	2.34E-04
miR-186,miR-29b	1.73	-4.36	1.05	-6.09	1.10	0.87	2.75E-04
miR-27b,miR-326	-1.54	-4.50	0.89	-2.97	1.01	0.87	2.79E-04
miR-181d,miR-214	2.71	-1.49	1.26	-4.20	2.00	0.87	3.53E-04
miR-17-5p,miR-27a	1.20	1.60	0.52	0.39	0.93	0.87	4.28E-04
let-7d,miR-422a	-1.76	-2.94	0.73	-1.18	1.39	0.87	4.84E-04
miR-27a,miR-518b	-2.33	-8.04	1.36	-5.71	1.68	0.87	5.44E-04
miR-27b,miR-320	-1.79	5.70	1.01	7.49	1.31	0.87	5.51E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-27b,miR-301	-1.17	-1.13	0.74	0.04	0.83	0.87	6.40E-04
miR-106b,miR-486	-1.81	4.12	1.11	5.93	1.31	0.87	6.68E-04
miR-22,miR-422a	-1.40	-3.12	0.82	-1.72	1.04	0.87	6.77E-04
miR-143,miR-181d	-2.01	0.01	1.45	2.03	1.32	0.87	7.39E-04
miR-181d,miR-576	2.13	-2.75	1.35	-4.88	1.75	0.87	9.79E-04
miR-142-5p,miR-30a-5p	-1.42	3.09	0.93	4.51	1.07	0.87	9.80E-04
miR-19b,miR-92	-1.08	1.86	0.53	2.94	1.02	0.87	9.80E-04
miR-214,miR-483	-1.93	-0.55	1.07	1.38	1.76	0.87	9.80E-04
miR-361,miR-422a	-1.52	-1.25	0.76	0.27	1.53	0.87	9.80E-04
miR-181a,miR-30b	1.28	4.01	0.71	2.73	0.78	0.87	1.44E-04
miR-486,miR-576	2.15	-10.17	1.36	-12.32	1.35	0.87	3.08E-04
miR-146a,miR-340	1.02	-2.29	0.70	-3.31	0.63	0.87	4.14E-04
miR-10b,miR-181a	-1.41	0.11	0.87	1.52	0.98	0.87	5.35E-04
miR-195,miR-92	-1.76	3.50	0.80	5.26	1.44	0.87	7.73E-04
miR-27a,miR-423	-1.36	-4.68	0.71	-3.31	1.16	0.87	1.06E-03
miR-32,miR-378	-1.98	5.56	1.14	7.54	1.60	0.87	1.06E-03
miR-140,miR-324-3p	-1.25	-2.01	0.89	-0.76	0.66	0.86	2.34E-04
miR-132,miR-140	1.25	2.30	1.07	1.05	0.46	0.86	2.73E-04
miR-125b,miR-145	-1.22	0.25	0.96	1.47	0.63	0.86	3.65E-04
let-7i,miR-191	-0.89	0.69	0.38	1.57	0.69	0.86	4.60E-04
let-7g,miR-150	-1.63	-0.31	0.68	1.32	1.30	0.86	5.51E-04
miR-132,miR-142-5p	1.76	2.08	1.40	0.32	0.97	0.86	5.54E-04
miR-142-3p,miR-24	-1.36	-1.48	0.72	-0.12	1.03	0.86	6.24E-04
miR-145,miR-15b	1.27	2.84	0.68	1.57	0.97	0.86	6.72E-04
miR-15b,miR-486	-1.82	4.83	1.11	6.65	1.34	0.86	7.28E-04
miR-340,miR-518b	-2.08	-6.78	1.14	-4.70	1.61	0.86	7.77E-04
miR-34a,miR-378	-2.16	3.70	1.62	5.86	1.39	0.86	8.17E-04
miR-10b,miR-422a	-2.07	-0.79	1.08	1.28	1.66	0.86	9.05E-04
miR-200c,miR-202	-1.35	0.72	0.52	2.07	1.18	0.86	1.07E-03
miR-126,miR-324-3p	-1.32	-5.39	0.74	-4.07	1.07	0.86	1.09E-03
miR-143,miR-186	-1.24	2.20	0.67	3.43	1.07	0.86	1.15E-03
miR-181d,miR-29a	1.75	3.22	1.03	1.47	1.45	0.86	1.15E-03
miR-125a,miR-126	1.15	4.96	0.77	3.81	0.92	0.86	1.16E-03
miR-126*,miR-422b	-1.19	-5.59	0.69	-4.40	0.98	0.86	1.16E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-146b,miR-27b	0.89	-2.22	0.51	-3.11	0.75	0.86	1.16E-03
miR-148b,miR-210	-0.97	-0.23	0.52	0.74	0.87	0.86	1.16E-03
miR-32,miR-320	-1.89	9.16	0.90	11.05	1.81	0.86	1.16E-03
miR-422a,miR-550	1.83	-1.65	1.21	-3.49	1.43	0.86	1.16E-03
miR-486,miR-660	1.48	-4.52	0.90	-6.00	0.90	0.86	2.10E-04
miR-142-3p,miR-422b	-1.62	-6.63	0.77	-5.01	1.17	0.86	3.09E-04
miR-30d,miR-92	-0.91	4.59	0.58	5.50	0.64	0.86	6.24E-04
miR-140,miR-30a-5p	-0.91	2.87	0.47	3.78	0.73	0.86	8.56E-04
miR-30e-5p,miR-422b	-1.45	-4.56	0.93	-3.10	1.08	0.86	8.94E-04
miR-142-3p,miR-193b	-1.85	-7.02	0.83	-5.17	1.60	0.86	1.20E-03
miR-24,miR-422a	-1.27	-5.84	0.61	-4.57	1.26	0.86	1.25E-03
miR-320,miR-340	1.55	-3.89	0.91	-5.43	1.40	0.86	1.25E-03
miR-140,miR-342	-1.42	-1.48	0.83	-0.06	0.90	0.86	2.27E-04
miR-126,miR-191	-1.14	-1.45	0.84	-0.31	0.60	0.86	3.02E-04
miR-126*,miR-191	-0.99	-1.02	0.76	-0.03	0.52	0.86	3.80E-04
miR-145,miR-26b	0.98	5.16	0.58	4.17	0.68	0.86	4.15E-04
miR-195,miR-486	-1.82	2.78	0.77	4.60	1.41	0.86	4.23E-04
miR-181a,miR-30c	1.25	4.15	0.75	2.90	0.85	0.86	4.38E-04
miR-145,miR-15a	1.42	-0.28	0.83	-1.69	1.00	0.86	4.89E-04
miR-145,miR-30c	1.03	3.87	0.57	2.84	0.75	0.86	5.02E-04
miR-125b,miR-181d	-1.58	0.85	1.19	2.44	0.94	0.86	5.61E-04
miR-10a,miR-181d	-1.22	1.46	0.86	2.68	0.77	0.86	5.67E-04
miR-181d,miR-200c	1.65	-1.24	1.01	-2.89	1.17	0.86	5.91E-04
let-7i,miR-181a	-1.44	-3.67	0.92	-2.23	1.02	0.86	6.99E-04
miR-194,miR-422a	-1.46	0.60	1.13	2.06	0.90	0.86	7.72E-04
miR-181c,miR-27b	1.63	3.48	0.91	1.85	1.26	0.86	7.80E-04
miR-30e-5p,miR-342	-1.55	-3.40	1.14	-1.86	1.02	0.86	8.35E-04
miR-142-3p,miR-425	-1.25	-6.75	0.93	-5.50	0.82	0.86	8.45E-04
let-7g,miR-191	-0.84	-0.25	0.58	0.59	0.58	0.86	8.47E-04
miR-142-5p,miR-181b	-1.84	-1.54	1.32	0.30	1.26	0.86	9.02E-04
miR-145,miR-26a	1.06	4.68	0.61	3.62	0.83	0.86	9.27E-04
miR-155,miR-422a	-1.60	-0.97	0.78	0.63	1.32	0.86	9.60E-04
miR-346,miR-422a	-2.22	0.25	1.09	2.47	1.84	0.86	9.85E-04
miR-22,miR-29b	1.73	-3.47	1.05	-5.21	1.34	0.86	1.02E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-148a,miR-222	-0.80	0.96	0.53	1.76	0.60	0.86	1.05E-03
miR-142-3p,miR-324-3p	-1.61	-6.00	0.87	-4.39	1.31	0.86	1.07E-03
miR-320,miR-374	1.52	-3.88	0.90	-5.40	1.20	0.86	1.09E-03
miR-296,miR-29b	1.67	-0.07	0.81	-1.74	1.41	0.86	1.09E-03
miR-15b,miR-342	-1.30	-2.18	0.67	-0.88	1.08	0.86	1.12E-03
miR-32,miR-342	-2.08	3.48	1.29	5.56	1.62	0.86	1.13E-03
let-7c,miR-92	-1.63	8.13	0.72	9.76	1.42	0.86	1.25E-03
miR-346,miR-566	-1.33	0.45	0.73	1.77	1.11	0.86	1.31E-03
miR-422a,miR-425	1.18	0.57	0.95	-0.61	0.78	0.86	1.33E-03
let-7f,miR-342	-1.40	-0.54	0.80	0.86	1.29	0.86	1.36E-03
miR-143,miR-223	-1.11	7.96	0.44	9.07	1.03	0.86	1.36E-03
miR-185,miR-186	-0.90	0.83	0.56	1.74	0.76	0.86	1.36E-03
miR-195,miR-422a	-2.14	-6.07	1.32	-3.93	1.82	0.86	1.36E-03
miR-16,miR-92	-1.74	-2.15	0.62	-0.41	1.47	0.86	7.20E-04
miR-142-5p,miR-422b	-1.76	-2.42	1.21	-0.66	1.20	0.86	7.25E-04
let-7b,miR-92	-1.50	4.51	0.74	6.01	1.20	0.86	7.65E-04
let-7e,miR-92	-1.60	4.22	0.87	5.82	1.44	0.86	1.47E-03
miR-27b,miR-296	-1.10	-2.09	0.44	-0.99	1.01	0.86	1.47E-03
miR-30d,miR-30e-5p	1.10	0.26	0.72	-0.83	0.64	0.85	2.38E-04
miR-125a,miR-29b	1.92	-1.94	0.92	-3.87	1.48	0.85	5.28E-04
miR-202,miR-27b	1.68	0.61	1.19	-1.07	1.06	0.85	5.50E-04
miR-143,miR-342	-1.68	0.07	1.12	1.76	1.12	0.85	5.52E-04
miR-140,miR-486	-1.94	5.53	1.09	7.47	1.43	0.85	5.57E-04
miR-202,miR-30e-5p	1.47	3.96	0.87	2.49	1.06	0.85	5.68E-04
miR-181b,miR-27b	1.60	0.33	0.87	-1.26	1.22	0.85	7.06E-04
miR-155,miR-29b	1.69	-1.11	1.17	-2.79	1.15	0.85	7.32E-04
let-7f,miR-191	-1.04	2.88	0.63	3.91	0.76	0.85	7.35E-04
miR-126,miR-518b	-2.09	-9.78	1.20	-7.68	1.58	0.85	7.38E-04
miR-150,miR-27b	2.13	-3.31	1.00	-5.44	1.73	0.85	7.68E-04
miR-191,miR-30c	0.69	-0.21	0.44	-0.91	0.52	0.85	9.28E-04
miR-196b,miR-342	-1.29	2.74	0.88	4.04	0.94	0.85	1.02E-03
miR-181d,miR-660	1.29	2.55	0.53	1.26	1.11	0.85	1.03E-03
miR-148a,miR-92	-1.65	5.93	0.88	7.58	1.36	0.85	1.11E-03
miR-193b,miR-34a	2.14	0.02	1.56	-2.12	1.50	0.85	1.11E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-496,miR-518b	-2.39	-1.41	1.42	0.98	1.94	0.85	1.32E-03
miR-23a,miR-29b	1.55	-2.78	0.96	-4.33	1.24	0.85	1.33E-03
miR-142-5p,miR-30d	-1.49	1.88	0.93	3.36	1.18	0.85	1.34E-03
let-7f,miR-92	-1.86	7.19	1.02	9.05	1.56	0.85	1.36E-03
miR-140,miR-145	-1.39	-2.14	0.89	-0.75	1.12	0.85	1.52E-03
miR-106a,miR-92	-1.23	3.06	0.66	4.28	1.10	0.85	1.59E-03
miR-17-5p,miR-422a	-1.38	-3.37	0.67	-1.99	1.41	0.85	1.59E-03
miR-210,miR-30e-5p	1.14	3.51	0.71	2.37	0.96	0.85	1.59E-03
miR-151,miR-422a	-1.46	-2.68	0.73	-1.22	1.29	0.85	1.59E-03
miR-193b,miR-30e-5p	1.61	4.94	1.05	3.34	1.50	0.85	1.59E-03
miR-21,miR-486	-1.62	1.55	1.10	3.17	1.36	0.85	1.59E-03
miR-221,miR-422a	-1.58	-4.60	0.85	-3.02	1.52	0.85	1.59E-03
miR-27b,miR-423	-1.34	-1.60	0.79	-0.27	1.22	0.85	1.59E-03
miR-21,miR-378	-1.30	-3.28	0.82	-1.98	1.08	0.85	1.72E-03
miR-106b,miR-150	-1.72	0.47	1.00	2.19	1.50	0.85	1.72E-03
miR-126,miR-150	-1.92	-1.50	0.84	0.42	1.80	0.85	1.72E-03
miR-186,miR-27b	1.25	-2.07	0.68	-3.32	0.82	0.85	2.24E-04
miR-145,miR-30b	1.06	3.73	0.64	2.67	0.70	0.85	3.39E-04
miR-150,miR-30e-5p	1.97	0.04	1.03	-1.93	1.40	0.85	3.44E-04
miR-126,miR-422b	-1.35	-6.02	0.62	-4.67	1.01	0.85	3.99E-04
miR-142-3p,miR-146a	-1.33	-1.32	0.81	0.01	0.97	0.85	7.42E-04
miR-29a,miR-342	-1.42	-3.16	0.93	-1.74	1.01	0.85	7.53E-04
miR-20a,miR-486	-1.67	1.54	0.98	3.21	1.27	0.85	7.84E-04
miR-140,miR-24	-0.99	2.52	0.60	3.51	0.74	0.85	8.03E-04
miR-15a,miR-191	-1.08	4.36	0.71	5.44	0.78	0.85	8.29E-04
miR-374,miR-518b	-2.05	-6.79	1.15	-4.74	1.62	0.85	9.49E-04
miR-21,miR-345	-1.07	-4.91	0.73	-3.85	0.76	0.85	9.58E-04
miR-139,miR-181d	-1.25	1.53	0.92	2.78	0.86	0.85	9.92E-04
miR-125b,miR-150	-1.68	4.27	1.04	5.95	1.30	0.85	1.08E-03
miR-126*,miR-342	-1.36	-4.44	0.76	-3.08	1.09	0.85	1.10E-03
let-7c,miR-181d	-1.50	0.34	1.01	1.84	1.11	0.85	1.10E-03
miR-193b,miR-27b	1.80	1.60	1.12	-0.21	1.41	0.85	1.16E-03
miR-30b,miR-92	-1.55	4.66	0.96	6.22	1.22	0.85	1.16E-03
miR-181a,miR-30e-5p	1.73	4.34	1.46	2.61	1.08	0.85	1.29E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-17-5p,miR-29b	1.70	-3.74	1.26	-5.44	1.22	0.85	1.30E-03
miR-140,miR-202	-1.38	-2.03	0.89	-0.66	1.08	0.85	1.36E-03
miR-142-3p,miR-301	-1.21	-6.55	0.76	-5.33	0.98	0.85	1.44E-03
miR-181b,miR-27a	1.65	3.41	1.08	1.76	1.33	0.85	1.67E-03
miR-142-5p,miR-181c	-1.75	-4.60	1.17	-2.85	1.43	0.85	1.86E-03
miR-148b,miR-320	-1.31	5.76	0.80	7.07	1.15	0.85	1.87E-03
let-7f,miR-145	-1.37	-1.20	0.81	0.17	1.22	0.85	1.87E-03
miR-126*,miR-296	-0.74	-6.47	0.29	-5.73	0.81	0.85	1.87E-03
miR-181d,miR-339	1.15	-0.81	0.76	-1.96	1.07	0.85	1.87E-03
miR-20a,miR-422a	-1.94	-7.31	0.98	-5.37	1.81	0.85	1.87E-03
miR-26a,miR-92	-1.55	3.72	0.91	5.27	1.37	0.85	1.87E-03
miR-432,miR-92	-1.85	9.20	1.20	11.05	1.55	0.85	1.87E-03
miR-487b,miR-92	-1.84	10.59	1.11	12.43	1.77	0.85	1.87E-03
let-7g,miR-92	-1.67	4.06	0.80	5.73	1.40	0.85	1.07E-03
miR-497,miR-518b	-1.70	-3.45	0.77	-1.75	1.46	0.85	1.13E-03
miR-301,miR-340	0.91	2.94	0.44	2.03	0.87	0.85	2.02E-03
miR-27b,miR-30a-5p	-1.23	4.29	0.74	5.52	0.90	0.84	6.98E-04
miR-34a,miR-433	-1.92	-1.52	1.36	0.40	1.28	0.84	7.42E-04
miR-17-5p,miR-27b	1.17	-1.48	0.58	-2.65	0.94	0.84	7.66E-04
miR-10b,miR-181d	-1.55	1.00	0.97	2.55	1.19	0.84	1.01E-03
miR-27a,miR-422b	-1.59	-4.29	1.06	-2.69	1.19	0.84	1.07E-03
miR-148b,miR-191	-0.94	3.43	0.73	4.37	0.63	0.84	1.13E-03
miR-181d,miR-34a	2.21	-1.47	1.58	-3.68	1.60	0.84	1.19E-03
miR-29b,miR-301	-1.69	1.25	1.17	2.94	1.25	0.84	1.19E-03
let-7e,miR-181d	-1.47	-3.57	0.82	-2.10	1.20	0.84	1.22E-03
miR-191,miR-26a	0.72	0.60	0.53	-0.13	0.52	0.84	1.30E-03
miR-142-5p,miR-328	-1.71	-1.24	1.14	0.47	1.32	0.84	1.38E-03
miR-143,miR-92	-2.15	7.80	1.23	9.95	1.82	0.84	1.61E-03
miR-142-5p,miR-197	-1.30	-0.16	0.92	1.14	1.00	0.84	1.64E-03
let-7c,miR-486	-1.69	7.41	0.96	9.10	1.47	0.84	1.85E-03
let-7c,miR-150	-1.59	3.76	0.97	5.36	1.35	0.84	1.90E-03
miR-27a,miR-30d	-1.29	0.01	0.71	1.30	1.15	0.84	1.98E-03
miR-30b,miR-378	-1.26	-0.89	0.84	0.37	1.04	0.84	2.02E-03
miR-181b,miR-200c	1.28	-0.97	0.79	-2.25	1.10	0.84	2.11E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-29c,miR-92	-1.80	6.32	0.78	8.12	1.69	0.84	2.13E-03
miR-30e-5p,miR-320	-1.61	2.35	1.24	3.96	1.23	0.84	2.14E-03
miR-186,miR-29c	0.89	-0.71	0.44	-1.60	0.83	0.84	2.18E-03
miR-193a,miR-29b	1.30	1.57	0.54	0.27	1.32	0.84	2.18E-03
miR-142-3p,miR-197	-1.16	-4.38	0.66	-3.22	1.19	0.84	2.18E-03
miR-27b,miR-361	-1.06	-0.65	0.74	0.41	0.99	0.84	2.18E-03
miR-28,miR-422a	-1.72	0.32	0.82	2.04	1.69	0.84	2.18E-03
let-7g,miR-378	-1.37	-1.49	0.59	-0.12	1.31	0.84	2.18E-03
miR-125b,miR-92	-1.72	8.64	1.28	10.36	1.47	0.84	2.18E-03
miR-140,miR-181b	-1.33	-1.76	0.97	-0.43	1.09	0.84	2.18E-03
miR-142-3p,miR-181c	-1.59	-8.81	0.89	-7.21	1.45	0.84	2.18E-03
miR-19a,miR-486	-1.32	2.26	0.76	3.58	1.18	0.84	2.18E-03
miR-26a,miR-342	-1.09	-4.01	0.58	-2.93	0.99	0.84	2.18E-03
miR-26b,miR-378	-1.18	-2.32	0.84	-1.14	1.08	0.84	2.18E-03
miR-30b,miR-342	-1.09	-3.07	0.66	-1.98	1.00	0.84	2.18E-03
miR-30d,miR-422a	-1.29	-4.98	0.56	-3.69	1.33	0.84	2.18E-03
miR-331,miR-92	-1.25	8.27	0.54	9.52	1.25	0.84	2.18E-03
miR-376a,miR-422a	-1.94	1.11	1.34	3.05	1.71	0.84	2.18E-03
miR-24,miR-29b	1.72	-6.30	1.00	-8.02	1.30	0.84	7.91E-04
miR-26a,miR-518b	-1.68	-8.93	1.04	-7.25	1.43	0.84	1.95E-03
miR-126,miR-99b	-1.13	-6.88	0.83	-5.75	0.97	0.84	2.35E-03
miR-142-5p,miR-202	-1.85	-1.81	1.36	0.04	1.08	0.84	4.28E-04
miR-27b,miR-433	-1.63	-3.10	1.20	-1.47	0.99	0.84	5.25E-04
let-7i,miR-486	-1.77	4.28	1.09	6.05	1.27	0.84	6.39E-04
miR-16,miR-486	-1.80	-2.87	0.88	-1.07	1.42	0.84	6.99E-04
miR-29b,miR-331	-1.70	1.58	1.02	3.28	1.27	0.84	7.68E-04
miR-15a,miR-202	-1.35	0.38	0.99	1.74	0.88	0.84	7.78E-04
miR-27b,miR-328	-1.53	-0.04	1.00	1.49	1.10	0.84	8.21E-04
miR-126,miR-24	-1.07	-0.87	0.63	0.20	0.81	0.84	8.50E-04
miR-328,miR-34a	1.83	-1.54	1.24	-3.38	1.31	0.84	8.93E-04
miR-142-3p,miR-566	-1.74	-7.11	1.18	-5.38	1.24	0.84	9.17E-04
miR-29b,miR-324-5p	-1.77	0.43	0.96	2.20	1.42	0.84	9.66E-04
miR-222,miR-27a	1.26	0.37	0.90	-0.89	0.92	0.84	1.17E-03
miR-142-5p,miR-326	-1.82	-5.73	1.44	-3.91	1.20	0.84	1.19E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-142-3p,miR-150	-2.21	-2.11	0.90	0.10	1.95	0.84	1.19E-03
miR-181d,miR-26b	1.34	4.55	0.97	3.22	0.96	0.84	1.22E-03
miR-27a,miR-328	-1.51	-3.11	1.08	-1.60	1.12	0.84	1.36E-03
miR-151,miR-27a	1.12	2.29	0.66	1.17	0.92	0.84	1.37E-03
miR-142-5p,miR-320	-2.00	4.49	1.23	6.49	1.61	0.84	1.39E-03
miR-214,miR-92	-2.78	9.28	1.53	12.06	2.35	0.84	1.45E-03
miR-29c,miR-342	-1.34	-1.41	0.99	-0.07	1.00	0.84	1.67E-03
miR-210,miR-27b	1.30	0.16	0.93	-1.14	1.00	0.84	1.69E-03
miR-181d,miR-29c	1.67	1.47	1.04	-0.20	1.40	0.84	1.87E-03
miR-126*,miR-186	-0.91	-2.31	0.69	-1.40	0.70	0.84	1.99E-03
miR-150,miR-214	2.68	-4.91	1.17	-7.59	2.50	0.84	2.06E-03
miR-214,miR-328	-2.03	1.57	1.54	3.60	1.56	0.84	2.07E-03
miR-214,miR-324-3p	-2.11	1.02	1.23	3.13	1.85	0.84	2.12E-03
miR-15a,miR-181b	-1.35	0.66	0.96	2.01	1.10	0.84	2.13E-03
miR-152,miR-181d	-1.17	0.33	0.85	1.50	0.95	0.84	2.39E-03
miR-32,miR-518b	-2.48	-1.43	1.48	1.05	2.21	0.84	2.46E-03
miR-342,miR-576	1.51	-3.51	1.01	-5.02	1.30	0.84	2.53E-03
miR-16,miR-93	-0.91	-5.54	0.72	-4.63	0.73	0.84	2.54E-03
miR-181d,miR-30a-3p	1.36	-2.34	0.82	-3.70	1.34	0.84	2.54E-03
miR-185,miR-345	-1.28	-0.75	0.93	0.54	1.07	0.84	2.54E-03
miR-378,miR-422a	-0.67	-4.02	0.35	-3.34	0.67	0.84	2.54E-03
let-7a,miR-518b	-1.62	-7.28	1.02	-5.66	1.55	0.84	2.55E-03
let-7f,miR-328	-1.17	-0.52	0.81	0.65	1.08	0.84	2.55E-03
miR-146a,miR-422a	-1.30	-5.99	0.77	-4.69	1.25	0.84	2.55E-03
miR-148a,miR-518b	-1.78	-6.71	1.34	-4.93	1.44	0.84	2.55E-03
miR-15a,miR-328	-1.22	0.96	0.85	2.18	1.04	0.84	2.55E-03
miR-197,miR-27a	1.11	2.03	0.71	0.92	1.04	0.84	2.55E-03
miR-27a,miR-361	-1.07	-3.73	0.70	-2.66	0.99	0.84	2.55E-03
miR-181d,miR-26a	1.42	4.07	0.76	2.66	1.10	0.84	7.63E-04
miR-126,miR-132	-1.33	-5.68	1.04	-4.36	0.90	0.84	1.25E-03
miR-374,miR-486	-1.98	5.13	1.26	7.11	1.54	0.84	1.26E-03
miR-224,miR-92	-1.18	10.05	0.64	11.23	1.10	0.84	2.75E-03
miR-143,miR-145	-1.65	-0.59	0.97	1.06	1.13	0.83	3.86E-04
miR-181d,miR-30b	1.42	3.13	0.97	1.71	0.95	0.83	6.41E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-340,miR-345	-1.42	-1.32	0.89	0.09	1.00	0.83	6.54E-04
miR-126,miR-345	-1.43	-4.32	1.09	-2.89	0.92	0.83	8.68E-04
miR-126*,miR-145	-1.33	-5.10	0.95	-3.77	0.91	0.83	9.06E-04
miR-126,miR-222	-1.06	-2.11	0.72	-1.05	0.79	0.83	1.16E-03
miR-29b,miR-99b	-1.85	0.05	0.96	1.90	1.56	0.83	1.24E-03
let-7a,miR-181d	-1.36	-2.43	0.69	-1.07	1.16	0.83	1.29E-03
let-7f,miR-378	-1.57	1.64	0.84	3.20	1.33	0.83	1.41E-03
miR-139,miR-29b	1.32	-0.58	0.94	-1.90	1.00	0.83	1.48E-03
miR-27a,miR-320	-1.81	2.62	1.14	4.43	1.47	0.83	1.60E-03
miR-130b,miR-142-3p	1.30	4.18	0.87	2.88	1.03	0.83	1.67E-03
miR-210,miR-92	-0.87	7.84	0.54	8.71	0.73	0.83	1.82E-03
miR-30c,miR-92	-1.52	4.53	0.96	6.05	1.27	0.83	1.83E-03
miR-32,miR-433	-1.79	0.23	1.24	2.01	1.44	0.83	1.92E-03
miR-340,miR-486	-2.00	5.14	1.28	7.15	1.68	0.83	1.97E-03
let-7i,miR-145	-1.22	-3.39	0.62	-2.17	1.11	0.83	2.00E-03
miR-148b,miR-181a	-1.15	-0.67	0.93	0.49	0.85	0.83	2.06E-03
let-7b,miR-324-3p	-0.86	-3.75	0.44	-2.89	0.80	0.83	2.41E-03
miR-29b,miR-423	-1.80	0.68	1.33	2.48	1.49	0.83	2.69E-03
miR-30c,miR-378	-1.23	-1.03	0.79	0.20	1.09	0.83	2.80E-03
miR-19b,miR-27a	1.13	-2.74	0.63	-3.86	1.06	0.83	2.96E-03
miR-126,miR-186	-1.06	-2.74	0.79	-1.69	0.90	0.83	2.97E-03
miR-214,miR-605	-1.70	-0.78	1.31	0.93	1.46	0.83	2.97E-03
miR-301,miR-422a	-1.42	-0.77	0.90	0.65	1.34	0.83	2.97E-03
miR-496,miR-92	-2.26	11.23	1.57	13.49	2.13	0.83	2.97E-03
miR-15a,miR-324-3p	-1.27	0.41	0.90	1.68	0.91	0.83	1.11E-03
miR-126*,miR-345	-1.29	-3.89	1.02	-2.60	0.85	0.83	1.15E-03
miR-200c,miR-518b	-1.93	-3.63	1.37	-1.70	1.41	0.83	1.25E-03
miR-26a,miR-378	-1.25	-1.84	0.65	-0.58	1.09	0.83	1.58E-03
miR-192,miR-324-3p	-0.87	-1.52	0.61	-0.65	0.68	0.83	1.62E-03
miR-142-3p,miR-210	-1.38	-5.58	0.85	-4.20	1.19	0.83	2.15E-03
miR-29a,miR-92	-1.88	4.57	0.77	6.45	1.79	0.83	2.18E-03
miR-125a,miR-374	1.11	1.98	0.63	0.87	1.14	0.83	3.19E-03
miR-126*,miR-181b	-1.26	-4.71	0.70	-3.45	1.25	0.83	3.20E-03
miR-26b,miR-342	-1.00	-4.49	0.74	-3.50	0.87	0.83	3.20E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-422a,miR-525	1.83	-2.67	1.28	-4.50	1.28	0.83	9.28E-04
miR-142-5p,miR-361	-1.26	-1.85	1.02	-0.60	0.80	0.83	1.14E-03
miR-140,miR-422b	-1.28	-2.64	0.82	-1.35	0.99	0.83	1.17E-03
miR-24,miR-340	1.05	-2.13	0.42	-3.18	0.93	0.83	1.17E-03
miR-145,miR-30e-5p	1.52	4.07	1.08	2.55	1.12	0.83	1.28E-03
miR-27b,miR-30d	-1.29	3.08	0.70	4.38	1.08	0.83	1.31E-03
miR-181b,miR-30e-5p	1.45	3.68	0.94	2.23	1.14	0.83	1.34E-03
miR-193a,miR-422a	-1.74	1.89	0.82	3.62	1.51	0.83	1.37E-03
miR-148b,miR-326	-1.24	-4.49	0.96	-3.24	0.88	0.83	1.47E-03
miR-151,miR-340	0.86	1.02	0.40	0.16	0.77	0.83	1.50E-03
miR-320,miR-34a	2.08	-7.28	1.46	-9.36	1.62	0.83	1.66E-03
miR-106b,miR-145	-1.26	-3.55	0.82	-2.29	1.03	0.83	1.67E-03
miR-199a*,miR-378	-1.38	-0.18	0.79	1.19	1.18	0.83	1.78E-03
miR-126*,miR-518b	-1.95	-9.35	1.27	-7.40	1.63	0.83	2.06E-03
miR-126,miR-146a	-1.04	-0.71	0.79	0.33	0.80	0.83	2.09E-03
miR-142-3p,miR-382	-1.57	-7.71	1.30	-6.14	1.13	0.83	2.15E-03
miR-181d,miR-185	1.68	1.35	1.33	-0.33	1.27	0.83	2.20E-03
miR-130a,miR-92	-1.60	4.76	1.30	6.36	1.19	0.83	2.32E-03
miR-15b,miR-324-3p	-1.12	-2.71	0.79	-1.58	0.94	0.83	2.44E-03
miR-106b,miR-191	-0.93	0.53	0.59	1.46	0.82	0.83	2.77E-03
miR-142-3p,miR-190	-1.02	-7.25	0.64	-6.23	0.92	0.83	2.92E-03
miR-126*,miR-150	-1.78	-1.08	0.89	0.70	1.72	0.83	3.14E-03
miR-145,miR-496	1.77	-2.84	1.17	-4.61	1.59	0.83	3.17E-03
miR-202,miR-374	1.39	2.43	0.94	1.04	1.26	0.83	3.39E-03
miR-27b,miR-93	-1.34	4.29	0.89	5.63	1.21	0.83	3.40E-03
miR-155,miR-27b	1.12	0.92	0.80	-0.20	0.99	0.83	3.44E-03
miR-143,miR-486	-2.20	7.08	1.42	9.29	2.04	0.83	3.45E-03
miR-148a,miR-378	-1.36	0.38	1.02	1.73	1.17	0.83	3.45E-03
miR-150,miR-374	1.88	-1.48	0.99	-3.36	1.83	0.83	3.45E-03
miR-181b,miR-374	1.36	2.16	0.67	0.79	1.36	0.83	3.45E-03
miR-222,miR-340	1.05	-0.89	0.67	-1.94	0.99	0.83	3.45E-03
miR-320,miR-576	1.68	-8.98	0.99	-10.65	1.59	0.83	3.45E-03
miR-126*,miR-324-3p	-1.18	-4.96	0.68	-3.78	0.96	0.83	1.19E-03
miR-143,miR-518b	-2.28	-4.84	1.74	-2.56	1.66	0.83	1.67E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-223,miR-27a	1.17	-4.76	0.64	-5.93	1.06	0.83	2.20E-03
miR-29b,miR-638	-1.39	2.34	1.08	3.73	1.09	0.83	2.52E-03
miR-26b,miR-518b	-1.61	-9.41	1.25	-7.80	1.32	0.83	3.04E-03
miR-186,miR-422a	-1.29	-3.96	0.74	-2.68	1.21	0.83	3.26E-03
miR-19b,miR-422a	-1.45	-7.71	0.75	-6.25	1.42	0.83	3.50E-03
miR-193b,miR-27a	1.80	4.67	1.15	2.87	1.75	0.83	3.70E-03
miR-181d,miR-30c	1.39	3.26	0.93	1.87	1.03	0.82	1.05E-03
miR-143,miR-181a	-1.87	-0.87	1.40	1.00	1.28	0.82	1.09E-03
miR-143,miR-345	-1.62	0.62	1.12	2.23	1.20	0.82	1.27E-03
miR-186,miR-29a	0.98	1.04	0.39	0.07	0.89	0.82	1.57E-03
miR-142-5p,miR-17-5p	-1.40	0.27	0.74	1.67	1.21	0.82	1.59E-03
miR-15a,miR-345	-1.38	1.48	1.17	2.86	0.90	0.82	1.62E-03
miR-30e-5p,miR-518b	-2.14	-8.31	1.64	-6.18	1.58	0.82	1.74E-03
miR-29b,miR-335	-1.56	2.64	1.05	4.20	1.26	0.82	1.81E-03
miR-202,miR-27a	1.68	3.69	1.28	2.01	1.26	0.82	1.87E-03
miR-193b,miR-200c	1.42	0.28	1.05	-1.14	1.11	0.82	1.98E-03
miR-145,miR-34a	1.81	-0.86	1.44	-2.67	1.34	0.82	2.03E-03
miR-148b,miR-30a-5p	-0.81	4.35	0.63	5.16	0.62	0.82	2.08E-03
miR-142-5p,miR-324-5p	-1.18	-2.87	0.83	-1.69	0.96	0.82	2.15E-03
miR-142-5p,miR-19b	-1.32	4.61	0.89	5.93	1.12	0.82	2.48E-03
miR-200c,miR-92	-1.82	8.99	1.40	10.81	1.44	0.82	2.53E-03
miR-142-3p,miR-30b	-0.70	-2.41	0.32	-1.71	0.67	0.82	2.54E-03
miR-378,miR-496	1.97	-5.68	1.12	-7.64	1.79	0.82	2.56E-03
miR-324-3p,miR-340	1.31	2.39	0.94	1.09	1.08	0.82	2.57E-03
miR-142-5p,miR-210	-1.52	-1.37	1.05	0.16	1.29	0.82	2.57E-03
miR-23a,miR-340	0.81	1.21	0.59	0.40	0.67	0.82	2.75E-03
miR-26b,miR-92	-1.48	3.24	0.91	4.72	1.34	0.82	2.83E-03
miR-150,miR-15b	1.72	-1.18	0.91	-2.91	1.63	0.82	2.84E-03
miR-422b,miR-576	1.68	-1.80	0.75	-3.48	1.66	0.82	3.08E-03
miR-155,miR-181d	-1.09	0.81	0.84	1.90	0.91	0.82	3.27E-03
miR-101,miR-324-3p	-1.10	-1.49	0.82	-0.39	0.96	0.82	3.68E-03
miR-181a,miR-98	1.41	-0.55	0.90	-1.96	1.31	0.82	3.72E-03
miR-142-3p,miR-22	-1.20	-4.20	0.81	-3.00	1.10	0.82	3.72E-03
miR-132,miR-214	2.21	-0.73	1.38	-2.94	2.09	0.82	3.86E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
let-7e,miR-486	-1.66	3.50	1.24	5.16	1.46	0.82	3.89E-03
miR-126,miR-328	-1.27	-4.84	0.95	-3.57	1.13	0.82	3.98E-03
miR-126,miR-30a-5p	-0.99	-0.52	0.59	0.47	0.98	0.82	3.99E-03
miR-106a,miR-422a	-1.61	-6.51	0.83	-4.91	1.64	0.82	4.00E-03
miR-210,miR-27a	1.33	3.24	0.88	1.91	1.30	0.82	4.00E-03
miR-30c,miR-342	-1.06	-3.20	0.60	-2.14	1.08	0.82	4.00E-03
miR-15b,miR-320	-1.37	3.57	0.91	4.94	1.12	0.82	1.98E-03
miR-15b,miR-17-5p	-0.76	-0.65	0.56	0.12	0.60	0.82	1.99E-03
miR-106b,miR-342	-1.29	-2.89	0.99	-1.60	1.01	0.82	2.23E-03
miR-15b,miR-518b	-1.89	-7.09	1.39	-5.20	1.52	0.82	2.30E-03
miR-200c,miR-378	-1.46	3.51	1.11	4.96	0.97	0.82	1.04E-03
miR-150,miR-34a	2.38	-4.89	1.81	-7.27	1.64	0.82	1.22E-03
miR-181d,miR-432	1.71	-1.41	1.03	-3.12	1.37	0.82	1.27E-03
miR-140,miR-181a	-1.61	-2.42	1.31	-0.81	1.06	0.82	1.30E-03
miR-142-3p,miR-433	-1.69	-8.52	1.00	-6.83	1.40	0.82	1.46E-03
miR-148b,miR-24	-0.84	4.01	0.47	4.85	0.75	0.82	2.12E-03
miR-132,miR-142-3p	1.61	6.29	1.34	4.68	1.17	0.82	2.22E-03
miR-133b,miR-145	-1.29	1.55	1.05	2.84	0.96	0.82	2.28E-03
miR-15a,miR-342	-1.45	0.94	0.76	2.38	1.33	0.82	2.32E-03
miR-324-3p,miR-374	1.28	2.41	0.68	1.13	1.17	0.82	2.35E-03
miR-200c,miR-422b	-1.27	0.04	0.84	1.31	1.08	0.82	2.36E-03
let-7g,miR-518b	-1.80	-8.58	1.23	-6.79	1.51	0.82	2.36E-03
miR-339,miR-92	-1.29	8.60	0.78	9.88	1.14	0.82	2.49E-03
miR-133b,miR-518b	-1.91	-2.70	1.38	-0.79	1.58	0.82	2.53E-03
let-7b,miR-486	-1.56	3.79	1.09	5.35	1.33	0.82	2.67E-03
miR-29a,miR-345	-1.35	-2.62	0.85	-1.27	1.20	0.82	2.70E-03
miR-133b,miR-181d	-1.65	2.15	1.18	3.80	1.40	0.82	2.90E-03
miR-142-5p,miR-335	-1.36	-1.04	1.09	0.31	1.07	0.82	2.91E-03
miR-192,miR-486	-1.57	6.02	0.95	7.59	1.43	0.82	2.92E-03
miR-185,miR-19b	-0.74	4.58	0.52	5.31	0.63	0.82	2.93E-03
let-7i,miR-320	-1.32	3.02	1.01	4.34	1.09	0.82	3.14E-03
miR-214,miR-320	-2.25	7.30	1.48	9.55	2.03	0.82	3.24E-03
miR-328,miR-374	1.23	1.86	0.86	0.63	1.08	0.82	3.24E-03
miR-191,miR-26b	0.60	1.08	0.40	0.48	0.54	0.82	3.52E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
let-7i,miR-181c	-1.20	-6.20	0.79	-5.00	1.10	0.82	3.59E-03
miR-29b,miR-93	-1.81	6.56	1.38	8.37	1.55	0.82	3.59E-03
miR-222,miR-374	1.04	-0.88	0.80	-1.92	0.89	0.82	3.66E-03
miR-126,miR-433	-1.40	-7.91	1.02	-6.51	1.27	0.82	4.19E-03
miR-142-3p,miR-151	-1.17	-4.63	0.60	-3.46	1.24	0.82	4.62E-03
miR-30a-3p,miR-378	-1.15	4.62	0.62	5.78	1.18	0.82	4.63E-03
miR-15a,miR-30a-5p	-0.93	5.29	0.65	6.22	0.81	0.82	3.03E-03
miR-410,miR-422a	-2.01	-0.02	1.36	2.00	1.82	0.82	3.53E-03
miR-142-3p,miR-30c	-0.73	-2.27	0.26	-1.54	0.68	0.81	1.61E-03
miR-140,miR-518b	-2.02	-6.39	1.26	-4.38	1.65	0.81	1.62E-03
miR-146b,miR-181d	-1.12	-2.33	0.66	-1.21	0.95	0.81	1.68E-03
miR-134,miR-29b	1.91	0.50	1.56	-1.41	1.34	0.81	1.79E-03
miR-142-5p,miR-194	-1.31	-3.70	0.92	-2.39	1.06	0.81	2.04E-03
miR-18a,miR-27b	0.93	-0.62	0.50	-1.55	0.83	0.81	2.07E-03
miR-142-3p,miR-186	-1.34	-3.35	0.90	-2.01	1.11	0.81	2.10E-03
miR-148b,miR-342	-1.37	-0.02	0.90	1.35	1.17	0.81	2.36E-03
let-7e,miR-518b	-1.73	-8.42	1.18	-6.69	1.47	0.81	2.46E-03
miR-142-5p,miR-339	-1.11	-2.12	0.88	-1.02	0.87	0.81	2.60E-03
miR-150,miR-576	1.76	-6.91	1.35	-8.66	1.41	0.81	2.61E-03
miR-181d,miR-98	1.55	-1.43	0.81	-2.98	1.45	0.81	2.67E-03
miR-145,miR-576	1.81	-2.18	1.32	-3.98	1.50	0.81	2.72E-03
let-7i,miR-342	-1.25	-2.73	0.84	-1.48	1.09	0.81	2.80E-03
miR-29c,miR-345	-1.27	-0.86	0.87	0.40	1.10	0.81	2.86E-03
let-7g,miR-181a	-1.39	-4.61	1.06	-3.22	1.14	0.81	2.91E-03
miR-181d,miR-192	1.38	1.05	1.12	-0.33	1.09	0.81	2.98E-03
miR-150,miR-32	2.52	-6.66	1.48	-9.18	2.34	0.81	3.05E-03
miR-143,miR-378	-1.85	2.25	1.35	4.10	1.59	0.81	3.17E-03
let-7g,miR-486	-1.73	3.34	1.24	5.06	1.51	0.81	3.39E-03
let-7f,miR-320	-1.47	5.21	0.90	6.68	1.37	0.81	3.40E-03
miR-15a,miR-422b	-1.30	-0.22	0.95	1.08	1.13	0.81	3.42E-03
miR-15a,miR-30d	-1.00	4.08	0.67	5.07	0.91	0.81	3.65E-03
miR-125b,miR-378	-1.42	3.09	1.09	4.51	1.22	0.81	3.71E-03
miR-142-3p,miR-23a	-1.11	-4.82	0.76	-3.71	1.02	0.81	3.94E-03
miR-29c,miR-324-3p	-1.16	-1.94	0.74	-0.78	1.10	0.81	4.09E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-29b,miR-339	-1.42	1.33	1.03	2.75	1.28	0.81	4.13E-03
let-7g,miR-324-3p	-1.03	-4.20	0.69	-3.17	0.98	0.81	4.63E-03
miR-19b,miR-27b	1.11	-5.81	0.73	-6.92	1.08	0.81	4.99E-03
let-7e,miR-378	-1.31	-1.34	0.72	-0.03	1.36	0.81	5.34E-03
miR-378,miR-432	1.55	-3.65	1.00	-5.20	1.54	0.81	5.35E-03
let-7b,miR-181d	-1.37	-3.28	1.14	-1.91	0.96	0.81	1.91E-03
miR-24,miR-30e-5p	1.11	-0.60	0.91	-1.71	0.85	0.81	2.65E-03
miR-365,miR-422a	-1.54	-1.25	1.07	0.29	1.32	0.81	2.80E-03
miR-320,miR-98	1.28	-7.24	0.71	-8.53	1.34	0.81	5.73E-03
miR-181d,miR-638	1.31	0.35	1.08	-0.96	0.87	0.81	1.44E-03
miR-15a,miR-518b	-2.04	-3.97	1.43	-1.94	1.57	0.81	1.58E-03
miR-106b,miR-181a	-1.48	-3.83	1.25	-2.35	1.02	0.81	1.93E-03
miR-181a,miR-576	1.83	-2.35	1.49	-4.18	1.35	0.81	2.20E-03
miR-199a*,miR-518b	-1.80	-7.27	1.07	-5.47	1.58	0.81	2.23E-03
miR-181a,miR-214	2.53	-0.61	1.86	-3.14	2.03	0.81	2.35E-03
let-7a,miR-181a	-1.22	-3.31	0.84	-2.09	1.02	0.81	2.37E-03
miR-125b,miR-342	-1.25	0.91	0.99	2.17	0.97	0.81	2.47E-03
miR-181b,miR-340	1.39	2.14	1.03	0.75	1.20	0.81	3.33E-03
miR-186,miR-340	1.04	-0.26	0.69	-1.29	0.95	0.81	3.57E-03
miR-422a,miR-7	1.63	0.55	1.07	-1.08	1.50	0.81	3.58E-03
miR-29b,miR-365	-1.51	1.55	1.00	3.06	1.38	0.81	3.58E-03
miR-142-3p,miR-30a-5p	-1.27	-1.13	0.70	0.15	1.24	0.81	3.71E-03
miR-30c,miR-518b	-1.65	-8.12	1.24	-6.47	1.44	0.81	3.74E-03
miR-150,miR-185	1.77	-2.07	1.17	-3.84	1.65	0.81	3.93E-03
let-7e,miR-320	-1.20	2.24	0.73	3.44	1.16	0.81	4.05E-03
let-7b,miR-150	-1.46	0.14	0.90	1.60	1.40	0.81	4.06E-03
miR-142-3p,miR-30d	-1.34	-2.34	0.68	-1.00	1.36	0.81	4.26E-03
miR-126,miR-193b	-1.56	-6.41	0.81	-4.84	1.60	0.81	4.80E-03
miR-202,miR-576	1.83	-2.26	1.07	-4.09	1.88	0.81	5.53E-03
miR-18a,miR-92	-1.28	7.05	0.73	8.33	1.18	0.80	2.81E-03
miR-214,miR-342	-2.19	1.55	1.04	3.74	2.14	0.80	3.05E-03
miR-126*,miR-30a-5p	-0.84	-0.09	0.53	0.75	0.80	0.80	3.88E-03
miR-133b,miR-378	-1.49	4.39	1.06	5.87	1.26	0.80	2.79E-03
miR-126*,miR-146a	-0.89	-0.28	0.72	0.61	0.70	0.80	2.81E-03

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Benign vs Cancer Assoc.
miR-422a,miR-422b	0.94	0.68	0.63	-0.25	0.83	0.80	2.99E-03
miR-30b,miR-518b	-1.68	-7.98	1.25	-6.30	1.43	0.80	3.22E-03
miR-148b,miR-181b	-1.27	-0.40	0.94	0.87	1.10	0.80	3.34E-03
miR-221,miR-27b	0.93	-2.71	0.72	-3.64	0.79	0.80	3.45E-03
miR-142-5p,miR-26b	-0.93	3.23	0.62	4.16	0.84	0.80	3.50E-03
miR-142-5p,miR-151	-1.31	-0.42	0.76	0.90	1.27	0.80	3.90E-03
let-7d,miR-142-3p	0.87	4.38	0.26	3.51	0.93	0.80	4.56E-03
miR-328,miR-340	1.26	1.85	0.82	0.59	1.24	0.80	5.31E-03
miR-296,miR-92	-1.08	9.76	0.64	10.84	1.10	0.80	5.60E-03
miR-140,miR-23a	-0.75	-0.82	0.38	-0.07	0.79	0.80	5.74E-03
miR-142-3p,miR-331	-0.99	-6.00	0.58	-5.01	1.02	0.80	5.84E-03
miR-181b,miR-214	2.25	-1.27	0.85	-3.52	2.12	0.80	1.92E-03
miR-326,miR-340	1.35	6.32	1.04	4.97	1.16	0.80	3.67E-03
let-7e,miR-191	-0.77	-0.10	0.60	0.68	0.62	0.80	2.59E-03
miR-30b,miR-320	-1.16	2.68	0.84	3.84	1.02	0.80	3.64E-03
miR-145,miR-214	2.31	-0.89	1.47	-3.20	2.17	0.80	3.85E-03
miR-140,miR-320	-1.49	4.27	1.04	5.76	1.37	0.80	4.15E-03
miR-496,miR-566	-1.75	1.86	1.17	3.61	1.66	0.80	4.64E-03
miR-23b,miR-92	-1.50	9.89	0.98	11.39	1.45	0.80	4.65E-03

[0100] The prevalence of serum biomarkers in paired analysis from Table 15 are shown in Table 16.

Table 16: Prevalence of serum biomarkers in pairs

miRNA	Paired Appearances	miRNA	Paired Appearances	miRNA	Paired Appearances
miR-422a	55	miR-181a	8	miR-145	5
miR-29b	25	miR-126	7	miR-150	5
miR-92	19	miR-342	7	miR-191	5
miR-142-5p	17	miR-140	6	miR-345	5
miR-142-3p	14	miR-15a	6	miR-126*	4
miR-181d	14	miR-324-3p	6	miR-148b	4
miR-27b	12	miR-374	6	miR-214	4
miR-378	12	miR-486	6	miR-320	4
miR-27a	11	miR-518b	6	let-7g	3
miR-30e-5p	9	miR-106b	5	let-7i	3

miRNA	Paired Appearances	miRNA	Paired Appearances	miRNA	Paired Appearances
miR-146a	3	miR-340	3	miR-222	2
miR-15b	3	miR-34a	3	miR-422b	2
miR-185	3	miR-101	2	miR-660	2
miR-186	3	miR-132	2	miR-7	2
miR-23a	3	miR-181b	2	miR-93	2
miR-24	3	miR-199a*	2		
miR-30a-5p	3	miR-202	2		

[0101] The data in Tables 15 and 16 show miRNAs and miRNA biomarker pairs that can distinguish patients with benign conditions of the lung from patients with lung cancer in serum samples, irrespective of patient sex or cancer type. These miRNAs are useful for diagnosis of lung cancer. The serum samples from cancer patients were from patients with early stage I through stage III lung cancer. These results show that miRNAs are suited to detect lung cancer at an early stage and are effective for screening patients and distinguishing patients with benign lung conditions from those with lung cancer.

Example 3. Differentially Expressed miRNAs in Serum from Male and Female Lung Cancer Patients

[0102] While the serum miRNAs in Tables 15 and 16 are able to distinguish patients with benign lung conditions from patients with lung cancers, further analysis of the data from Example 2 revealed that certain miRNAs pairs were better for distinguishing those patient groups within a specific sex. In particular, a number of miRNA pairs had an AUC ROC of 1.00 (Table 17). These pairs showed a significant improvement of the ROC AUC when distinguishing female patients with benign lung conditions from female lung cancer patients. Similarly, an improvement in AUC was also observed for specific miRNA pairs when distinguishing male patients with benign lung conditions from male patients with lung cancers (Table 18). The miRNAs and miRNA pairs in Tables 17 and 18 are useful for diagnosing lung cancer in female and male patients respectively. These miRNAs can be used in combinations of two or more.

Table 17. microRNAs pairs that are differentially expressed in serum of female patients with benign lung conditions as compared to female lung cancer patients.

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Female Benign vs Female Cancer Assoc.
miR-15a,miR-422a	-2.47	-0.93	0.69	1.53	0.25	1.00	1.26E-06
miR-181d,miR-27b	2.42	0.41	0.67	-2.01	0.39	1.00	2.81E-06
miR-27b,miR-422a	-2.83	-1.85	0.42	0.98	0.85	1.00	4.16E-06
miR-15b,miR-191	-1.27	1.22	0.35	2.49	0.25	1.00	4.79E-06
miR-181d,miR-29b	3.10	-1.87	0.63	-4.97	0.87	1.00	6.28E-06
miR-142-3p,miR-422a	-2.76	-7.16	0.58	-4.40	0.84	1.00	1.13E-05
miR-23a,miR-27a	1.30	2.53	0.40	1.24	0.27	1.00	1.22E-05
miR-222,miR-27a	1.94	0.69	0.54	-1.25	0.50	1.00	1.54E-05
miR-126,miR-422a	-2.50	-6.52	0.30	-4.03	0.92	1.00	1.91E-05
miR-142-5p,miR-145	-2.27	-2.07	0.59	0.20	0.67	1.00	2.04E-05
miR-142-3p,miR-145	-1.85	-6.12	0.64	-4.27	0.39	1.00	2.61E-05
miR-143,miR-223	-1.58	7.88	0.50	9.46	0.41	1.00	2.93E-05
miR-324-5p,miR-422a	-1.64	-0.12	0.59	1.53	0.33	1.00	3.27E-05
miR-30e-5p,miR-422a	-2.59	-5.10	0.83	-2.51	0.68	1.00	3.40E-05
miR-27a,miR-422a	-3.08	-4.90	0.86	-1.82	0.94	1.00	3.56E-05
miR-126*,miR-222	-1.39	-1.95	0.33	-0.56	0.48	1.00	4.02E-05
miR-140,miR-422a	-2.63	-3.25	0.70	-0.62	0.86	1.00	4.13E-05
miR-101,miR-92	-2.39	6.57	0.86	8.97	0.54	1.00	4.24E-05
miR-202,miR-29b	3.08	-1.42	0.55	-4.50	1.18	1.00	4.36E-05
miR-29b,miR-422a	-3.40	0.62	0.81	4.02	1.21	1.00	4.83E-05
miR-30e-5p,miR-324-3p	-1.38	-3.89	0.39	-2.51	0.46	1.00	5.23E-05
miR-181b,miR-29b	3.29	-1.41	0.56	-4.71	1.32	1.00	5.65E-05
miR-142-5p,miR-181a	-2.69	-2.70	1.05	-0.01	0.56	1.00	6.22E-05
miR-126*,miR-422a	-2.52	-6.16	0.37	-3.64	1.06	1.00	6.52E-05
miR-195,miR-93	-1.23	-0.12	0.48	1.11	0.27	1.00	7.27E-05
miR-140,miR-222	-1.49	0.96	0.54	2.46	0.41	1.00	7.76E-05
miR-126,miR-181d	-2.00	-5.08	0.47	-3.08	0.78	1.00	8.18E-05
miR-142-5p,miR-422a	-3.17	-3.11	1.24	0.07	0.74	1.00	8.18E-05
miR-24,miR-27a	1.45	-0.82	0.47	-2.27	0.48	1.00	9.51E-05
miR-27a,miR-361	-1.91	-3.91	0.58	-2.00	0.68	1.00	1.03E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Female Benign vs Female Cancer Assoc.
miR-346,miR-518b	-3.12	-3.23	1.35	-0.11	0.61	1.00	1.18E-04
miR-222,miR-27b	1.69	-2.36	0.17	-4.05	0.78	1.00	1.21E-04
miR-15b,miR-181a	-2.08	-3.58	0.87	-1.50	0.47	1.00	1.24E-04
miR-142-5p,miR-191	-1.88	2.10	0.57	3.97	0.69	1.00	1.25E-04
miR-433,miR-487b	1.86	0.03	0.80	-1.83	0.39	1.00	1.28E-04
miR-29b,miR-518b	-3.68	-3.01	1.17	0.67	1.34	1.00	1.40E-04
miR-106b,miR-422a	-2.78	-4.93	1.21	-2.15	0.61	1.00	1.50E-04
miR-21,miR-422a	-1.98	-7.01	0.73	-5.03	0.64	1.00	1.52E-04
miR-15b,miR-422a	-2.56	-3.99	0.77	-1.42	0.98	1.00	1.53E-04
miR-181a,miR-27a	2.60	4.49	1.22	1.90	0.35	1.00	1.58E-04
miR-142-5p,miR-345	-2.27	-1.00	0.82	1.27	0.75	1.00	1.61E-04
miR-32,miR-422a	-2.32	1.49	1.06	3.81	0.45	1.00	1.73E-04
miR-30e-5p,miR-92	-2.12	4.44	0.61	6.56	0.86	1.00	1.93E-04
miR-148b,miR-422a	-2.37	-1.65	0.91	0.73	0.77	1.00	2.02E-04
miR-192,miR-422a	-2.57	-2.91	0.89	-0.34	0.94	1.00	2.02E-04
let-7i,miR-422a	-2.32	-4.52	0.97	-2.20	0.66	1.00	2.10E-04
miR-19b,miR-486	-1.55	1.22	0.67	2.77	0.42	1.00	2.16E-04
miR-15a,miR-181d	-1.97	0.51	0.87	2.48	0.51	1.00	2.30E-04
miR-142-3p,miR-26a	-0.97	-1.55	0.16	-0.58	0.47	1.00	2.36E-04
miR-142-3p,miR-181a	-2.28	-6.76	1.00	-4.48	0.62	1.00	2.44E-04
miR-29b,miR-378	-2.72	4.28	0.58	7.00	1.34	1.00	3.51E-04
miR-15a,miR-181b	-1.93	0.29	0.95	2.22	0.42	1.00	3.58E-04
miR-142-3p,miR-342	-1.82	-5.31	0.53	-3.49	0.83	1.00	3.83E-04
miR-195,miR-20b	-0.89	-2.27	0.37	-1.39	0.32	1.00	4.08E-04
miR-145,miR-27a	2.17	3.86	0.99	1.69	0.67	1.00	4.11E-04
miR-374,miR-422a	-2.43	-3.55	0.58	-1.11	1.20	1.00	4.24E-04
miR-27b,miR-361	-1.64	-0.86	0.55	0.77	0.71	1.00	4.32E-04
miR-142-3p,miR-518b	-3.02	-10.77	1.19	-7.75	1.17	1.00	4.33E-04
miR-140,miR-181d	-2.13	-1.81	0.85	0.33	0.82	1.00	4.67E-04
miR-27b,miR-326	-2.07	-4.77	0.93	-2.70	0.67	1.00	4.73E-04
miR-139,miR-422a	-1.96	0.11	0.79	2.07	0.76	1.00	4.75E-04
let-7g,miR-422a	-2.29	-5.45	0.45	-3.16	1.21	1.00	5.12E-04
miR-140,miR-345	-1.73	-1.14	0.78	0.58	0.59	1.00	5.44E-04
miR-148b,miR-326	-1.80	-4.71	0.87	-2.91	0.59	1.00	7.05E-04

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Female Benign vs Female Cancer Assoc.
miR-20a,miR-92	-2.00	2.04	0.64	4.05	1.01	1.00	7.88E-04
miR-133b,miR-181b	-1.98	1.60	0.75	3.58	0.93	1.00	8.83E-04
miR-132,miR-30e-5p	1.50	4.32	0.72	2.82	0.56	1.00	9.62E-04
miR-19b,miR-92	-1.73	1.67	0.55	3.40	0.91	1.00	9.98E-04
miR-181a,miR-30c	1.67	4.48	0.84	2.81	0.59	1.00	1.02E-03
miR-197,miR-422a	-1.61	-2.91	0.70	-1.30	0.70	1.00	1.06E-03
miR-142-5p,miR-222	-1.98	1.10	1.07	3.08	0.59	1.00	1.06E-03
miR-148a,miR-422a	-1.90	-3.26	0.97	-1.36	0.66	1.00	1.07E-03
let-7g,miR-181d	-1.79	-4.01	0.66	-2.22	0.89	1.00	1.09E-03
miR-197,miR-29b	2.09	-3.24	0.50	-5.33	1.20	1.00	1.09E-03
miR-331,miR-422a	-2.05	-1.27	0.76	0.78	1.03	1.00	1.17E-03
miR-15a,miR-181a	-1.98	-0.53	1.10	1.46	0.59	1.00	1.22E-03
miR-339,miR-422a	-1.86	-0.80	0.70	1.06	0.94	1.00	1.24E-03
miR-181b,miR-215	2.65	-2.89	1.09	-5.54	1.29	1.00	1.33E-03
miR-145,miR-374	1.52	2.51	0.71	0.98	0.67	1.00	1.33E-03
miR-206,miR-518b	-3.45	-3.25	1.79	0.20	1.29	1.00	1.35E-03
miR-106b,miR-486	-2.13	4.17	1.00	6.29	0.94	1.00	1.47E-03
miR-126*,miR-181a	-2.04	-5.76	1.23	-3.72	0.49	1.00	1.53E-03
miR-422a,miR-576	2.97	-1.39	0.74	-4.35	1.78	1.00	1.54E-03
miR-17-5p,miR-422a	-1.93	-3.45	0.62	-1.52	1.09	1.00	1.56E-03
miR-106b,miR-345	-1.88	-2.82	1.11	-0.94	0.59	1.00	1.75E-03
miR-126,miR-342	-1.56	-4.68	0.82	-3.12	0.66	1.00	1.75E-03
miR-139,miR-181d	-1.46	1.56	0.90	3.02	0.50	1.00	1.75E-03
miR-140,miR-30a-5p	-1.19	2.79	0.55	3.98	0.79	1.00	1.75E-03
miR-142-3p,miR-26b	-0.87	-1.04	0.46	-0.17	0.36	1.00	1.75E-03
miR-142-3p,miR-361	-1.59	-6.17	1.14	-4.58	0.50	1.00	1.75E-03
miR-19a,miR-92	-1.47	2.86	0.48	4.33	0.97	1.00	1.75E-03
miR-222,miR-422a	-1.21	-4.21	0.43	-3.01	0.71	1.00	1.75E-03
miR-296,miR-422a	-1.64	0.25	0.27	1.88	1.45	1.00	1.75E-03
miR-29b,miR-382	-2.62	0.02	1.24	2.63	1.30	1.00	1.75E-03

Table 18. microRNA pairs that are differentially expressed in serum of male patients with benign lung conditions as compared to male lung cancer patients.

miRNA Biomarker Pair	Benign mean - Cancer mean	Benign mean	Benign SD	Cancer mean	Cancer SD	AUC ROC	Male Benign vs Male Cancer Assoc.
miR-185,miR-93	-1.02	3.02	0.52	4.04	0.35	1.00	8.52E-04
miR-30e-5p,miR-433	-1.46	-6.82	0.91	-5.36	0.41	1.00	1.16E-03
miR-126,miR-378	-1.74	-2.73	0.81	-0.99	0.80	1.00	2.19E-03
miR-342,miR-497	1.51	-0.74	0.16	-2.24	1.24	1.00	2.67E-03
let-7f,miR-342	-1.61	-1.09	0.22	0.52	1.47	1.00	2.70E-03
miR-142-5p,miR-422a	-2.46	-3.09	0.19	-0.63	1.61	1.00	2.70E-03
miR-145,miR-200c	1.86	-0.20	0.55	-2.06	1.03	1.00	2.70E-03
miR-148a,miR-19b	-1.60	3.30	0.99	4.90	0.65	1.00	2.70E-03
miR-191,miR-340	1.42	-1.26	0.39	-2.68	0.84	1.00	2.70E-03
miR-200b,miR-422a	-1.87	1.76	0.60	3.63	1.33	1.00	2.70E-03
miR-340,miR-378	-2.00	0.15	0.41	2.14	1.43	1.00	2.70E-03

Example 4. miRNA Markers That Distinguish Lung Cancer from Cancer-free Subjects With or Without Benign Conditions

[0103] In an additional study, serum miRNAs that distinguish lung cancer patients from cancer free human subjects with or without benign conditions are identified. Serum samples were obtained from patients with benign conditions, normal patients, and lung cancer patients (Table 19). All procedures and data analysis were performed as described above.

Table 19. Histopathological and patient information for lung cancer and benign specimens.

Sample ID	Age	Sex	Cancer Status	Diagnosis	Cell Type
1	59	F	Other	Benign	NA
2	60	F	Other	Benign	NA
3	64	F	Other	Benign	NA

Sample ID	Age	Sex	Cancer Status	Diagnosis	Cell Type
4	69	F	Other	Benign	NA
5	71	F	Other	Benign	NA
6	73	F	Other	Benign	NA
7	77	F	Other	Benign	NA
8	56	M	Other	Benign	NA
9	58	M	Other	Benign	NA
10	60	M	Other	Benign	NA
11	69	M	Other	Benign	NA
12	71	M	Other	Benign	NA
13	59	F	Cancer	Cancer	ADCA
14	64	F	Cancer	Cancer	ADCA
15	66	F	Cancer	Cancer	ADCA
15	66	F	Cancer	Cancer	ADCA
16	66	F	Cancer	Cancer	SCC
17	72	F	Cancer	Cancer	SCC
18	73	F	Cancer	Cancer	SCC
19	73	F	Cancer	Cancer	SCC
20	56	M	Cancer	Cancer	SCC
20	56	M	Cancer	Cancer	SCC
21	60	M	Cancer	Cancer	ADCA
21	60	M	Cancer	Cancer	ADCA
22	63	M	Cancer	Cancer	ADCA
23	66	M	Cancer	Cancer	SCC
24	69	M	Cancer	Cancer	ADCA
25	69	M	Cancer	Cancer	SCC
26	69	M	Cancer	Cancer	SCC
27	71	M	Cancer	Cancer	SCC
28	72	M	Cancer	Cancer	ADCA
29	79	M	Cancer	Cancer	ADCA
30	67	M	Cancer	Cancer	ADCA

Sample ID	Age	Sex	Cancer Status	Diagnosis	Cell Type
31	82	F	Cancer	Cancer	SCC
32	77	M	Cancer	Cancer	ADCA
33	41	M	Other	Normal	NA
34	40	F	Other	Normal	NA
35	66	F	Other	Normal	NA
36	65	F	Other	Normal	NA
37	20	F	Other	Normal	NA
38	41	M	Other	Normal	NA
39	37	M	Other	Normal	NA
40	50	M	Other	Normal	NA
41	47	F	Other	Normal	NA
42	50	F	Other	Normal	NA
43	51	F	Other	Normal	NA
44	70	F	Other	Normal	NA
45	55	F	Other	Normal	NA
46	69	M	Other	Normal	NA
47	61	M	Other	Normal	NA
48	60	M	Other	Normal	NA
49	58	M	Other	Normal	NA
50	53	M	Other	Normal	NA
51	75	M	Other	Normal	NA
52	55	M	Other	Normal	NA

[0104] Table 20 provides differential expression data for miRNAs in the form of average dCt values for cancer and other samples (other=benign and normal). Table 21 shows differentially expressed miRNA biomarker pairs that distinguish samples from lung cancer patients from normal patients and patients with benign tumors. Such miRNA and biomarker pairs can be used for screening and diagnosis of lung cancer.

Table 20. Expression of miRNAs in serum from patients with lung cancer or from patients with benign or normal conditions. Other=benign and normal cases.

miRNA	cancer dCT	Cancer SD	Other dCT	Other SD	Cancer - Other ddCT	ttest p-value
miR-15b	-0.523	0.624	-1.867	1.032	1.344	2.45E-09
miR-30a-5p	-3.139	0.571	-1.901	1.010	-1.239	3.11E-09
miR-346	4.384	1.995	1.228	2.523	3.156	3.98E-08
let-7g	-2.051	0.650	-3.095	0.948	1.044	1.47E-07
let-7a	-0.898	0.756	-2.176	1.131	1.278	5.14E-07
let-7f	0.878	0.944	-0.601	1.303	1.479	1.09E-06
miR-93	-7.708	0.932	-6.577	1.416	-1.131	1.59E-06
miR-214	3.494	1.727	1.205	2.038	2.289	1.92E-06
miR-15a	2.777	0.898	1.659	1.028	1.118	2.81E-06
let-7d	0.782	0.890	-0.266	0.969	1.049	4.26E-06
miR-142-3p	-3.320	0.843	-4.174	0.978	0.853	6.67E-06
miR-605	3.922	2.437	0.776	2.956	3.146	9.81E-06
miR-483	3.478	1.463	1.494	1.903	1.983	1.11E-05
miR-374	-0.243	0.834	-1.170	0.919	0.927	1.56E-05
miR-150	-3.282	1.435	-1.928	1.409	-1.354	1.59E-05
miR-486	-6.137	1.241	-4.782	1.564	-1.354	1.89E-05
miR-181d	0.507	1.026	1.643	1.140	-1.135	3.24E-05
miR-21	-3.900	0.608	-3.082	0.991	-0.817	7.43E-05
miR-320	-4.331	1.237	-3.075	1.461	-1.255	8.98E-05
miR-345	0.133	0.638	0.895	0.809	-0.762	9.71E-05
miR-30d	-1.569	1.155	-0.241	1.437	-1.328	1.02E-04
miR-145	1.626	1.094	0.029	1.717	1.597	1.37E-04
miR-365	1.679	1.098	2.738	1.175	-1.059	1.89E-04
miR-99a	3.577	1.230	2.575	1.118	1.001	2.29E-04
miR-23a	0.873	0.545	1.546	0.757	-0.672	2.41E-04
miR-133b	3.006	1.601	1.125	2.129	1.881	2.86E-04
miR-566	3.050	1.587	1.437	1.844	1.613	3.30E-04
miR-26b	-3.142	0.637	-3.772	0.878	0.629	3.50E-04
miR-22	0.286	0.976	1.237	1.089	-0.951	3.86E-04
miR-30c	-1.646	0.588	-2.129	0.652	0.483	6.45E-04
miR-190	2.962	1.061	2.004	1.171	0.958	7.39E-04
miR-485-3p	4.321	1.402	3.158	1.442	1.164	1.05E-03
miR-206	4.582	1.381	3.498	1.396	1.084	1.18E-03

miRNA	cancer dCT	Cancer SD	Other dCT	Other SD	Cancer - Other ddCT	ttest p-value
miR-181b	0.709	1.054	1.514	1.011	-0.804	1.24E-03
miR-19b	-4.720	0.805	-4.070	1.050	-0.650	1.52E-03
miR-148a	-0.268	0.761	0.443	0.917	-0.710	1.62E-03
let-7b	-1.755	0.708	-2.322	0.807	0.566	1.79E-03
miR-29a	-1.448	1.083	-0.491	1.275	-0.957	1.95E-03
miR-103	0.010	1.025	-0.952	1.274	0.961	2.05E-03
miR-30b	-1.462	0.546	-1.898	0.637	0.436	2.18E-03
miR-496	6.002	1.586	4.850	1.558	1.152	2.30E-03
miR-182	3.886	1.377	2.482	1.831	1.404	2.47E-03
miR-186	-0.683	0.639	-0.181	0.683	-0.502	2.47E-03
miR-99b	2.233	1.366	3.268	1.408	-1.035	2.82E-03
miR-125b	2.544	0.949	3.321	1.082	-0.777	2.95E-03
miR-26a	-3.017	0.883	-3.703	1.076	0.685	3.37E-03
miR-339	2.286	0.654	2.973	0.975	-0.686	3.42E-03
miR-342	0.460	0.887	1.055	0.843	-0.595	4.51E-03
miR-146a	-3.761	1.010	-3.126	1.055	-0.635	4.56E-03
miR-328	0.971	0.871	0.142	1.182	0.829	4.84E-03
miR-335	0.655	0.783	1.269	0.877	-0.613	4.94E-03
miR-106b	-1.435	0.656	-1.944	0.797	0.508	6.12E-03
miR-326	5.137	1.046	5.869	1.199	-0.732	6.28E-03
miR-17-5p	-0.410	0.910	-0.955	0.830	0.545	6.80E-03
miR-29c	-0.087	1.212	0.875	1.407	-0.961	6.85E-03
miR-133a	2.968	2.760	4.572	2.392	-1.605	7.58E-03
miR-146b	-1.593	1.008	-1.052	0.868	-0.542	8.03E-03
miR-181a	1.149	0.818	1.705	0.854	-0.556	8.94E-03
miR-10b	2.422	1.089	3.088	1.046	-0.666	9.43E-03
miR-19a	-2.693	1.102	-1.781	1.478	-0.912	1.02E-02
miR-130b	-0.325	0.766	0.163	0.768	-0.488	1.03E-02
miR-410	3.440	1.646	2.508	1.434	0.932	1.07E-02
miR-126	-3.241	0.733	-3.653	0.830	0.411	1.17E-02
miR-422a	2.734	1.662	3.572	1.377	-0.839	1.43E-02
miR-425	1.878	0.795	2.375	0.812	-0.497	1.49E-02
miR-151	0.360	0.773	0.797	0.717	-0.436	1.52E-02
miR-20a	-3.836	1.304	-4.484	1.271	0.648	1.72E-02
miR-660	-0.907	0.835	-0.409	0.849	-0.498	1.76E-02
miR-497	3.057	0.925	3.687	1.097	-0.630	1.92E-02

miRNA	cancer dCT	Cancer SD	Other dCT	Other SD	Cancer - Other ddCT	ttest p-value
miR-100	2.862	1.415	3.688	1.429	-0.826	1.98E-02
miR-194	3.243	0.960	2.533	1.236	0.710	2.10E-02
miR-30e-5p	-0.729	1.007	0.056	1.367	-0.785	2.15E-02
miR-210	1.024	0.850	1.462	0.773	-0.438	2.41E-02
miR-423	2.172	0.924	1.547	1.119	0.625	2.59E-02
miR-204	5.281	1.609	4.442	1.566	0.838	2.92E-02
miR-9*	2.947	1.060	3.492	1.027	-0.545	3.09E-02
miR-25	-2.165	1.097	-1.600	1.111	-0.565	3.40E-02
miR-301	2.140	0.717	1.711	0.824	0.430	3.56E-02
miR-502	4.651	1.659	5.422	1.511	-0.772	3.70E-02
miR-518b	4.924	1.447	3.973	1.855	0.951	4.13E-02
miR-125a	0.901	1.248	1.533	1.226	-0.631	4.33E-02
miR-223	-5.788	1.381	-5.053	1.652	-0.735	4.44E-02
miR-134	3.501	1.357	2.828	1.340	0.674	4.72E-02
miR-132	1.518	0.796	1.957	0.894	-0.439	5.03E-02
miR-24	-3.178	0.614	-2.922	0.708	-0.257	6.34E-02
miR-361	1.789	0.961	2.155	0.796	-0.366	6.61E-02
miR-28	3.299	0.980	2.869	0.949	0.431	6.67E-02
miR-185	1.585	1.359	2.330	1.626	-0.745	7.50E-02
miR-432	3.290	1.405	2.728	1.224	0.562	7.60E-02
miR-192	0.357	0.940	0.725	0.828	-0.368	7.93E-02
let-7c	1.936	0.836	1.595	0.774	0.341	7.93E-02
miR-296	4.006	1.424	3.484	1.208	0.522	7.99E-02
miR-152	2.617	1.223	3.180	1.315	-0.563	8.79E-02
miR-139	3.347	0.883	3.737	0.963	-0.390	9.01E-02
miR-191	-1.679	1.115	-1.199	1.230	-0.480	1.16E-01
miR-30e-3p	4.375	1.159	3.924	1.219	0.451	1.27E-01
miR-30a-3p	3.592	1.065	3.996	1.087	-0.404	1.28E-01
miR-32	3.558	2.615	2.639	2.302	0.919	1.29E-01
miR-16	-7.960	1.397	-8.410	1.700	0.450	1.50E-01
miR-10a	3.135	1.137	3.562	1.214	-0.427	1.57E-01
miR-505	3.804	0.899	3.471	1.002	0.332	1.73E-01
miR-143	3.697	1.939	3.076	1.787	0.620	1.82E-01
miR-148b	1.275	0.877	1.522	0.717	-0.247	1.82E-01
miR-324-5p	3.015	0.871	3.256	0.779	-0.241	1.95E-01
miR-222	-1.220	0.858	-0.993	0.743	-0.227	2.09E-01

miRNA	cancer dCT	Cancer SD	Other dCT	Other SD	Cancer - Other ddCT	ttest p-value
miR-200c	2.177	2.198	2.817	2.051	-0.640	2.26E-01
miR-324-3p	2.038	1.273	2.413	1.213	-0.375	2.28E-01
miR-126*	-2.694	0.451	-2.813	0.555	0.119	2.33E-01
miR-638	2.384	1.822	1.926	1.546	0.458	2.51E-01
miR-181c	4.089	1.069	3.769	1.175	0.319	2.69E-01
miR-7	2.534	1.084	2.255	1.024	0.279	2.89E-01
miR-378	1.215	3.252	1.976	2.796	-0.760	2.91E-01
let-7e	1.287	3.313	0.605	2.787	0.682	3.37E-01
miR-340	2.165	2.654	2.808	2.604	-0.643	3.51E-01
miR-140	0.181	0.659	0.064	0.580	0.118	4.27E-01
miR-382	3.241	1.348	3.495	1.295	-0.254	4.35E-01
miR-422b	1.176	1.174	0.932	1.293	0.244	4.65E-01
miR-98	5.914	0.927	6.143	1.263	-0.229	4.77E-01
miR-155	1.620	1.136	1.778	0.950	-0.158	5.16E-01
miR-197	0.073	0.927	0.215	0.872	-0.142	5.23E-01
miR-23b	3.618	1.178	3.797	1.124	-0.179	5.25E-01
miR-106a	-2.070	1.499	-2.262	1.314	0.192	5.44E-01
miR-18a	1.848	1.364	1.668	1.160	0.180	5.46E-01
miR-34a	3.868	1.378	4.067	1.386	-0.199	5.69E-01
miR-195	-1.630	1.559	-1.825	1.430	0.195	5.81E-01
miR-202	2.694	2.197	2.941	1.719	-0.247	5.81E-01
miR-20b	-0.425	1.325	-0.575	1.142	0.150	6.01E-01
miR-376a	4.192	1.482	4.363	1.353	-0.172	6.15E-01
miR-142-5p	0.923	0.932	1.036	0.948	-0.114	6.41E-01
miR-92	4.110	3.501	4.401	2.527	-0.291	6.62E-01
miR-127	3.641	1.726	3.819	1.636	-0.178	6.73E-01
miR-27a	-0.307	0.859	-0.182	1.170	-0.126	6.74E-01
miR-199a*	-0.915	0.770	-0.977	0.656	0.062	6.96E-01
miR-550	4.637	1.079	4.559	1.015	0.078	7.43E-01
miR-101	-0.181	1.511	-0.133	1.343	-0.048	8.89E-01
let-7i	-0.730	0.653	-0.709	0.754	-0.020	9.14E-01
miR-501	4.581	1.674	4.548	1.542	0.033	9.32E-01
miR-130a	-1.039	0.940	-1.020	0.944	-0.019	9.36E-01
miR-27b	2.845	1.229	2.820	1.405	0.025	9.45E-01
miR-584	3.214	1.501	3.226	1.529	-0.012	9.76E-01
miR-433	3.993	1.531	3.992	1.332	0.001	9.97E-01

miRNA	cancer dCT	Cancer SD	Other dCT	Other SD	Cancer - Other ddCT	ttest p-value
miR-221	-1.894	1.070	-1.894	1.014	-0.001	9.98E-01

Table 21. Differentially expressed miRNA biomarker pairs that distinguish lung cancer patients from normal patients with no lung tumors and patients with benign lung tumors. Other=benign and normal cases.

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-15a,miR-92	2.31	10.55	1.17	8.24	0.77	0.96	1.83E-13
miR-15b,miR-92	2.47	7.22	1.25	4.75	0.93	0.95	8.99E-13
miR-214,miR-422b	2.09	2.40	1.18	0.31	0.72	0.95	4.61E-12
miR-15b,miR-24	1.59	2.67	0.63	1.09	0.77	0.95	7.90E-13
miR-146a,miR-15b	-1.99	-3.27	1.09	-1.29	0.93	0.94	6.21E-11
let-7g,miR-150	2.41	1.28	1.23	-1.14	1.04	0.93	5.66E-12
miR-142-3p,miR-92	2.07	4.46	1.28	2.39	0.74	0.93	5.38E-11
miR-346,miR-422b	2.94	3.28	2.00	0.34	0.84	0.93	1.48E-10
let-7a,miR-92	2.42	6.85	1.35	4.43	1.00	0.93	2.10E-11
let-7f,miR-146a	2.15	4.69	1.02	2.54	1.10	0.92	2.23E-11
miR-106b,miR-92	1.65	6.30	0.91	4.66	0.71	0.92	2.70E-11
let-7g,miR-342	1.65	-2.48	1.00	-4.13	0.92	0.92	3.57E-09
let-7a,miR-181a	1.82	-2.03	1.00	-3.85	0.81	0.92	3.34E-11
let-7d,miR-92	2.27	8.57	1.47	6.30	0.77	0.92	1.14E-10
miR-15b,miR-181a	1.87	-1.66	1.11	-3.53	0.82	0.92	1.50E-10
let-7f,miR-92	2.64	8.64	1.46	6.00	1.24	0.92	9.94E-11
let-7g,miR-92	2.18	5.69	1.28	3.52	0.87	0.92	4.53E-11
let-7b,miR-92	1.72	5.99	1.04	4.27	0.70	0.92	1.05E-10
let-7g,miR-146a	1.70	1.75	0.93	0.05	0.88	0.91	2.78E-10
miR-142-3p,miR-181a	1.47	-4.42	0.89	-5.89	0.79	0.91	2.36E-09
miR-181d,miR-346	-4.22	-3.91	2.07	0.31	2.29	0.91	1.11E-10
miR-126,miR-92	1.59	4.52	1.06	2.92	0.77	0.91	3.38E-09
miR-150,miR-15b	-2.70	-2.80	1.68	-0.10	1.25	0.91	6.51E-10
let-7f,miR-181a	2.04	-0.24	1.27	-2.28	0.86	0.91	2.84E-10
miR-15b,miR-342	1.94	-0.95	1.26	-2.89	1.04	0.91	6.17E-09
miR-7,miR-92	1.39	10.26	0.83	8.88	0.64	0.91	2.65E-10
miR-181b,miR-346	-3.93	-3.72	2.27	0.21	1.95	0.91	4.07E-10
miR-181b,miR-214	-3.06	-2.82	1.76	0.24	1.56	0.91	5.08E-10

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-15a,miR-425	1.57	0.87	1.10	-0.71	0.87	0.90	2.72E-08
miR-146a,miR-374	-1.61	-3.57	1.09	-1.96	0.80	0.90	6.42E-09
miR-146b,miR-374	-1.49	-1.38	1.09	0.11	0.76	0.90	3.15E-08
miR-181a,miR-346	-3.69	-3.28	2.29	0.41	1.78	0.90	9.09E-10
miR-150,miR-214	-3.66	-6.84	2.25	-3.19	1.83	0.90	1.34E-09
miR-214,miR-92	3.46	11.29	2.00	7.84	1.95	0.90	2.12E-09
miR-142-3p,miR-30a-5p	2.03	-0.18	1.17	-2.21	1.01	0.90	4.42E-10
miR-346,miR-92	4.29	12.16	2.62	7.87	2.15	0.90	1.13E-09
miR-374,miR-92	2.09	7.51	1.40	5.42	0.88	0.90	1.16E-09
miR-346,miR-422a	3.97	1.70	2.12	-2.27	2.22	0.90	6.06E-10
miR-150,miR-346	-4.53	-7.75	2.83	-3.22	2.16	0.89	9.72E-10
miR-15b,miR-335	1.85	-1.20	0.73	-3.05	1.29	0.89	1.80E-09
miR-342,miR-346	-3.76	-3.99	2.40	-0.23	1.87	0.89	2.05E-09
miR-146a,miR-15a	-1.83	-6.60	1.37	-4.78	0.86	0.89	2.54E-08
miR-181d,miR-214	-3.34	-2.99	1.94	0.34	1.91	0.89	2.74E-09
let-7b,miR-150	1.95	1.57	1.26	-0.38	1.00	0.89	3.83E-09
miR-190,miR-92	2.06	10.69	1.23	8.63	1.15	0.89	2.93E-09
miR-181d,miR-98	-2.11	-3.08	1.59	-0.97	0.92	0.89	1.44E-08
let-7a,miR-150	2.66	2.44	1.76	-0.22	1.32	0.89	4.15E-09
miR-214,miR-425	2.81	1.70	1.44	-1.11	1.76	0.89	2.46E-09
miR-142-3p,miR-181d	2.00	-3.79	1.32	-5.79	1.35	0.89	9.09E-08
miR-214,miR-342	2.89	3.08	1.88	0.19	1.57	0.89	7.89E-09
miR-142-3p,miR-425	1.37	-5.19	0.91	-6.56	0.73	0.89	7.70E-09
let-7g,miR-181d	2.11	-2.56	1.35	-4.67	1.41	0.89	7.42E-08
miR-30c,miR-92	1.67	6.11	1.10	4.45	0.89	0.89	7.84E-09
let-7a,miR-181d	2.35	-1.40	1.53	-3.75	1.68	0.89	1.18E-07
miR-130b,miR-15a	-1.56	-3.10	1.04	-1.55	0.91	0.88	2.52E-08
miR-142-3p,miR-345	1.57	-3.45	1.05	-5.02	0.86	0.88	1.42E-08
miR-15a,miR-181d	2.23	2.30	1.46	0.06	1.58	0.88	1.22E-07
let-7f,miR-150	2.87	4.22	1.90	1.35	1.49	0.88	6.69E-09
miR-15b,miR-345	1.97	-0.69	1.01	-2.66	1.21	0.88	1.56E-09
miR-346,miR-518b	2.13	-0.60	1.64	-2.72	0.89	0.88	1.99E-08
miR-24,miR-374	-1.21	-2.97	0.77	-1.76	0.69	0.88	1.22E-08
let-7f,miR-342	2.11	0.47	1.42	-1.64	1.19	0.88	1.96E-08
let-7g,miR-345	1.68	-2.22	0.97	-3.90	1.13	0.88	2.61E-08
miR-15a,miR-181a	1.71	1.67	1.23	-0.04	0.90	0.88	3.07E-08
miR-15a,miR-30a-5p	2.27	5.91	0.97	3.64	1.44	0.88	4.23E-10
let-7d,miR-486	2.41	6.96	1.31	4.55	1.52	0.88	5.15E-09
miR-146a,miR-214	-2.91	-7.28	1.56	-4.37	1.81	0.88	3.27E-09

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-15a,miR-345	1.81	2.64	1.07	0.83	1.04	0.88	4.06E-09
miR-15b,miR-93	1.46	3.22	1.06	1.76	0.72	0.88	2.05E-08
miR-15b,miR-181d	2.40	-1.03	1.52	-3.43	1.59	0.88	5.80E-08
miR-146b,miR-214	-2.86	-5.16	1.48	-2.31	1.76	0.88	2.02E-09
miR-15a,miR-210	1.52	1.76	1.12	0.24	0.89	0.88	1.06E-07
miR-142-3p,miR-99b	1.36	-6.25	1.23	-7.60	1.05	0.88	2.18E-07
miR-346,miR-486	4.43	10.55	2.60	6.12	2.78	0.88	1.18E-08
let-7a,miR-342	1.89	-1.32	1.27	-3.21	1.03	0.88	1.33E-08
miR-181a,miR-214	-2.80	-2.36	1.79	0.44	1.67	0.88	1.72E-08
let-7f,miR-24	1.75	4.09	0.98	2.34	1.05	0.87	3.00E-09
let-7g,miR-24	1.29	1.14	0.85	-0.15	0.70	0.87	7.88E-09
miR-214,miR-375	4.29	0.81	2.70	-3.48	2.67	0.87	2.30E-08
let-7g,miR-181b	1.81	-2.75	1.30	-4.56	1.10	0.87	1.03E-07
miR-142-3p,miR-146a	1.59	0.51	1.15	-1.07	0.68	0.87	6.05E-09
miR-15b,miR-326	2.00	-5.65	1.09	-7.65	1.40	0.87	2.43E-08
miR-130b,miR-142-3p	-1.32	2.99	0.95	4.30	0.65	0.87	1.86E-08
let-7g,miR-181a	1.58	-3.19	1.16	-4.76	0.80	0.87	3.08E-08
miR-335,miR-374	-1.47	0.91	0.76	2.38	1.03	0.87	1.71E-08
miR-130b,miR-15b	-1.72	0.23	1.00	1.95	1.15	0.87	2.62E-08
let-7d,miR-150	2.51	4.15	2.02	1.65	1.13	0.87	6.84E-08
miR-181d,miR-193a	-2.40	-4.89	1.42	-2.48	1.58	0.87	2.23E-08
miR-142-3p,miR-342	1.54	-3.71	1.13	-5.25	0.77	0.87	2.53E-08
miR-15b,miR-30a-5p	2.43	2.58	0.88	0.15	1.70	0.87	9.93E-10
let-7i,miR-30a-5p	1.16	2.40	0.70	1.24	0.83	0.87	8.47E-08
let-7f,miR-181d	2.56	0.39	1.69	-2.18	1.72	0.87	1.05E-07
miR-151,miR-15b	-1.75	0.87	0.95	2.62	1.30	0.87	6.22E-08
miR-17-5p,miR-92	1.59	7.28	1.23	5.69	0.85	0.87	9.94E-08
miR-106b,miR-150	1.88	1.89	1.35	0.00	1.04	0.87	3.93E-08
miR-125a,miR-214	-2.87	-2.62	1.85	0.25	1.89	0.87	6.22E-08
miR-132,miR-15b	-1.80	2.01	0.98	3.80	1.26	0.87	2.71E-08
miR-320,miR-346	-4.27	-8.72	2.21	-4.44	2.94	0.87	1.16E-08
miR-146a,miR-26a	-1.36	-0.78	0.79	0.57	0.90	0.87	2.52E-08
miR-132,miR-214	-2.74	-2.03	1.91	0.72	1.63	0.87	5.53E-08
miR-181d,miR-496	-2.25	-5.45	1.78	-3.20	1.33	0.86	3.26E-07
miR-15b,miR-19b	1.99	4.23	1.07	2.24	1.31	0.86	8.91E-09
miR-20a,miR-92	1.73	3.88	1.54	2.15	0.84	0.86	5.61E-07
miR-301,miR-92	1.54	9.86	1.13	8.32	0.90	0.86	9.60E-08
miR-143,miR-223	1.48	9.70	1.09	8.22	1.02	0.86	5.36E-07
miR-320,miR-496	-2.43	-10.38	1.64	-7.95	1.79	0.86	4.11E-07

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-142-3p,miR-150	2.30	0.04	1.85	-2.26	0.78	0.86	1.68E-08
miR-26b,miR-92	1.78	4.60	1.26	2.82	1.09	0.86	1.01E-07
miR-15a,miR-326	1.85	-2.32	1.39	-4.17	1.14	0.86	2.28E-07
miR-181a,miR-190	-1.46	-1.81	1.14	-0.35	0.93	0.86	4.85E-07
miR-126*,miR-30a-5p	1.27	0.42	0.79	-0.85	0.86	0.86	6.46E-08
miR-29b,miR-375	2.56	1.87	2.41	-0.69	1.57	0.86	7.76E-07
miR-486,miR-98	-2.26	-9.73	1.69	-7.48	1.35	0.86	1.60E-07
miR-150,miR-374	-2.32	-3.10	1.87	-0.77	1.12	0.86	9.90E-08
miR-155,miR-214	-2.48	-1.96	1.81	0.52	1.41	0.86	7.40E-08
miR-181b,miR-496	-2.07	-5.37	1.51	-3.30	1.18	0.86	7.05E-08
miR-150,miR-30c	-1.90	-1.70	1.62	0.21	0.89	0.86	2.24E-07
miR-30b,miR-92	1.62	6.30	1.11	4.68	1.05	0.86	1.10E-07
miR-142-3p,miR-181b	1.70	-3.98	1.24	-5.68	1.13	0.86	2.83E-07
let-7g,miR-30a-5p	2.14	1.05	0.91	-1.09	1.57	0.86	8.39E-09
miR-103,miR-92	2.02	7.72	1.39	5.69	1.31	0.86	1.15E-07
miR-181b,miR-26b	-1.41	3.85	1.12	5.26	0.84	0.86	3.73E-07
miR-181d,miR-30b	-1.55	1.96	1.19	3.50	0.98	0.86	4.14E-07
miR-422a,miR-566	-2.48	-0.37	1.37	2.11	1.85	0.86	7.14E-08
let-7f,miR-181b	2.27	0.20	1.61	-2.07	1.40	0.86	1.03E-07
miR-422a,miR-605	-4.03	-1.30	2.39	2.73	2.81	0.85	5.42E-08
miR-15b,miR-210	1.69	-1.57	1.23	-3.25	1.12	0.85	3.32E-07
miR-126,miR-150	1.83	0.10	1.62	-1.73	0.76	0.85	2.76E-07
let-7d,miR-181d	2.20	0.32	1.57	-1.88	1.48	0.85	2.83E-07
miR-181d,miR-26b	-1.70	3.66	1.19	5.36	1.09	0.85	1.23E-07
let-7f,miR-326	2.20	-4.23	1.37	-6.43	1.47	0.85	5.55E-08
let-7f,miR-345	2.14	0.73	1.33	-1.41	1.49	0.85	8.61E-08
let-7d,miR-181a	1.67	-0.31	1.45	-1.98	0.73	0.85	2.09E-07
miR-214,miR-99b	2.68	0.53	1.87	-2.15	1.80	0.85	2.18E-07
miR-106a,miR-486	1.50	4.07	1.16	2.57	0.93	0.85	3.34E-07
miR-214,miR-365	3.34	1.89	2.00	-1.45	2.42	0.85	1.04E-07
miR-15b,miR-181b	2.10	-1.22	1.51	-3.33	1.31	0.85	1.39E-07
miR-214,miR-326	2.90	-1.65	1.98	-4.55	1.88	0.85	1.10E-07
miR-140,miR-150	1.59	3.54	1.27	1.96	0.82	0.85	1.37E-07
miR-181d,miR-432	-1.78	-2.87	1.57	-1.09	0.87	0.85	4.64E-07
miR-15a,miR-335	1.68	2.12	1.07	0.44	1.19	0.85	1.42E-07
miR-140,miR-30a-5p	1.31	3.32	0.86	2.01	0.89	0.85	1.09E-07
miR-15a,miR-181b	1.94	2.11	1.43	0.17	1.33	0.85	4.93E-07
miR-26a,miR-92	1.84	4.73	1.33	2.89	1.16	0.85	1.77E-07
miR-142-3p,miR-24	1.18	-0.09	0.99	-1.27	0.58	0.85	2.10E-07

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-151,miR-374	-1.37	0.58	1.08	1.95	0.98	0.85	1.43E-06
miR-181d,miR-374	-2.02	0.74	1.66	2.76	1.45	0.85	1.43E-06
miR-142-3p,miR-22	1.71	-3.63	1.20	-5.34	1.14	0.85	2.25E-07
miR-320,miR-98	-2.13	-7.88	1.45	-5.75	1.43	0.85	1.51E-07
miR-150,miR-15a	-2.54	-6.13	1.97	-3.59	1.15	0.85	3.28E-08
miR-125a,miR-98	-1.61	-2.67	1.49	-1.06	1.00	0.85	1.53E-06
miR-486,miR-496	-2.62	-12.25	1.95	-9.63	1.77	0.85	4.71E-07
miR-142-3p,miR-146b	1.47	-1.67	1.18	-3.14	0.67	0.85	6.87E-08
miR-194,miR-92	1.86	10.98	1.30	9.12	1.34	0.85	4.70E-07
miR-98,miR-99b	1.55	0.69	1.37	-0.86	0.84	0.85	8.26E-07
miR-193a,miR-92	2.34	12.98	1.63	10.64	1.70	0.85	4.79E-07
miR-142-3p,miR-486	2.20	2.85	1.76	0.65	1.35	0.85	4.71E-07
miR-132,miR-15a	-1.63	-1.32	1.10	0.31	1.17	0.85	3.08E-07
miR-126,miR-146a	1.11	0.57	0.82	-0.54	0.62	0.85	7.03E-08
miR-181d,miR-206	-2.14	-4.07	1.48	-1.93	1.56	0.85	4.55E-07
miR-142-3p,miR-99a	1.96	-5.49	1.40	-7.45	1.30	0.85	2.32E-07
miR-181a,miR-30c	-1.07	2.76	0.79	3.83	0.60	0.85	8.83E-08
miR-15a,miR-486	2.44	8.94	1.68	6.50	1.57	0.85	1.04E-07
miR-210,miR-214	-2.63	-2.47	1.92	0.17	1.70	0.85	2.29E-07
miR-181a,miR-374	-1.49	1.37	1.23	2.86	0.79	0.85	2.69E-07
miR-30a-5p,miR-374	-2.05	-2.87	1.17	-0.82	1.41	0.85	2.94E-08
miR-133b,miR-92	3.05	10.78	1.91	7.73	2.31	0.85	2.91E-07
miR-214,miR-99a	3.38	1.38	2.22	-2.00	2.41	0.85	2.06E-07
miR-15a,miR-24	1.42	6.00	1.16	4.58	0.79	0.84	2.95E-07
let-7f,miR-335	2.01	0.21	0.92	-1.80	1.56	0.84	3.63E-08
miR-15b,miR-99b	1.79	-3.49	1.28	-5.28	1.37	0.84	1.11E-06
let-7c,miR-181d	1.51	1.47	1.17	-0.04	1.01	0.84	7.45E-07
let-7a,miR-125a	1.87	-1.78	1.36	-3.65	1.61	0.84	2.36E-06
miR-342,miR-374	-1.56	0.66	1.37	2.22	0.79	0.84	5.64E-07
miR-374,miR-425	1.37	-2.16	1.03	-3.53	0.83	0.84	1.93E-07
let-7g,miR-132	1.50	-3.54	0.88	-5.04	1.12	0.84	1.19E-07
let-7f,miR-125a	2.08	0.00	1.59	-2.08	1.70	0.84	2.61E-06
miR-214,miR-345	2.97	3.40	1.86	0.42	2.05	0.84	8.39E-08
miR-126,miR-181d	1.52	-3.74	1.39	-5.26	1.34	0.84	2.61E-06
let-7d,miR-320	2.25	5.12	1.24	2.87	1.65	0.84	5.38E-08
let-7f,miR-221	1.48	2.80	0.94	1.31	1.07	0.84	1.72E-07
miR-142-3p,miR-186	1.40	-2.59	1.14	-3.99	0.84	0.84	5.42E-07
miR-422a,miR-496	-2.30	-3.54	1.85	-1.24	1.14	0.84	1.14E-07
miR-197,miR-214	-2.38	-3.43	1.85	-1.05	1.65	0.84	9.62E-07

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-181a,miR-26b	-1.14	4.32	0.97	5.46	0.59	0.84	3.96E-07
miR-193a,miR-422a	2.24	2.77	1.70	0.53	1.47	0.84	4.19E-07
miR-15a,miR-342	1.78	2.38	1.40	0.60	1.08	0.84	3.58E-07
miR-92,miR-98	-2.03	-11.15	1.74	-9.12	1.09	0.84	4.90E-07
miR-181d,miR-505	-1.43	-3.26	1.19	-1.83	1.19	0.84	2.97E-06
miR-181a,miR-26a	-1.24	4.15	1.03	5.39	0.66	0.84	3.65E-07
miR-133b,miR-150	3.28	6.36	2.14	3.08	2.40	0.84	2.67E-07
miR-126*,miR-92	1.31	5.06	1.02	3.76	0.93	0.84	1.39E-06
miR-142-3p,miR-210	1.28	-4.33	1.07	-5.61	0.73	0.84	5.10E-07
miR-181d,miR-30c	-1.60	2.14	1.34	3.73	1.19	0.84	3.50E-06
miR-214,miR-24	2.56	6.74	1.81	4.17	1.75	0.84	2.78E-07
miR-181a,miR-30b	-1.02	2.58	0.77	3.60	0.72	0.84	9.02E-07
miR-24,miR-30c	-0.78	-1.57	0.59	-0.78	0.59	0.84	1.72E-06
miR-410,miR-92	2.07	11.20	1.82	9.13	1.24	0.84	1.47E-06
miR-142-3p,miR-335	1.44	-3.96	1.07	-5.41	0.96	0.84	3.63E-07
miR-130b,miR-374	-1.34	-0.07	1.07	1.27	0.92	0.84	1.16E-06
miR-15b,miR-301	0.93	-2.64	0.65	-3.57	0.69	0.84	6.30E-07
miR-142-3p,miR-19b	1.59	1.47	1.36	-0.12	0.97	0.84	1.25E-06
miR-181b,miR-206	-1.85	-3.88	1.47	-2.03	1.31	0.84	1.55E-06
miR-150,miR-30b	-1.85	-1.88	1.66	-0.03	1.00	0.83	1.11E-06
miR-30c,miR-342	1.14	-2.06	1.02	-3.20	0.62	0.83	1.14E-06
miR-496,miR-99a	2.27	3.81	1.64	1.54	1.71	0.83	1.07E-06
miR-142-3p,miR-23a	1.53	-4.17	1.06	-5.70	1.14	0.83	6.18E-07
miR-181a,miR-98	-1.53	-2.40	1.42	-0.87	0.82	0.83	1.67E-06
miR-550,miR-92	1.41	12.45	1.15	11.04	1.00	0.83	2.02E-06
let-7c,miR-92	1.58	9.72	1.28	8.14	1.05	0.83	1.16E-06
miR-206,miR-92	2.21	12.32	1.78	10.11	1.43	0.83	7.83E-07
miR-142-5p,miR-30a-5p	1.06	4.05	1.04	2.99	0.66	0.83	4.83E-06
miR-150,miR-206	-2.45	-7.91	2.09	-5.46	1.17	0.83	2.57E-07
let-7d,miR-342	1.74	0.40	1.54	-1.35	0.95	0.83	9.01E-07
miR-214,miR-423	1.77	1.42	1.43	-0.35	1.22	0.83	1.45E-06
miR-150,miR-26a	-2.07	-0.31	1.80	1.76	1.22	0.83	1.17E-06
miR-150,miR-193a	-2.56	-8.56	2.16	-6.00	1.69	0.83	1.72E-06
miR-181d,miR-30e-3p	-1.45	-3.82	1.24	-2.38	1.02	0.83	3.50E-06
miR-103,miR-181a	1.42	-1.16	1.14	-2.59	0.95	0.83	9.87E-07
miR-150,miR-26b	-1.98	-0.15	1.75	1.83	1.08	0.83	8.95E-07
miR-125a,miR-15b	-1.94	1.40	1.52	3.33	1.65	0.83	5.85E-06
miR-142-3p,miR-324-5p	1.15	-6.29	1.19	-7.44	0.70	0.83	5.85E-06
miR-27b,miR-30d	1.26	4.38	1.02	3.11	0.76	0.83	4.81E-07

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-214,miR-335	2.84	2.87	1.82	0.04	2.29	0.83	8.02E-07
miR-142-3p,miR-151	1.35	-3.63	1.20	-4.98	0.85	0.83	2.35E-06
let-7c,miR-150	1.81	5.30	1.48	3.49	1.16	0.83	9.35E-07
miR-151,miR-15a	-1.59	-2.46	1.19	-0.87	1.22	0.83	1.87E-06
miR-496,miR-92	2.29	13.67	1.79	11.38	1.70	0.83	1.88E-06
miR-150,miR-98	-2.37	-6.87	2.28	-4.50	1.05	0.83	1.40E-06
miR-26b,miR-342	1.24	-3.58	1.06	-4.82	0.83	0.83	2.11E-06
miR-320,miR-638	-1.81	-6.77	1.39	-4.96	1.33	0.83	1.60E-06
miR-29b,miR-30a-5p	1.44	7.79	1.35	6.35	0.87	0.83	4.08E-06
let-7b,miR-181d	1.64	-2.26	1.25	-3.91	1.43	0.82	7.09E-06
miR-346,miR-566	1.49	1.33	1.39	-0.16	0.88	0.82	3.34E-06
miR-106b,miR-19b	1.17	3.31	0.75	2.14	1.03	0.82	2.46E-06
let-7g,miR-93	1.17	1.69	1.04	0.52	0.74	0.82	2.64E-06
miR-133b,miR-422b	1.60	1.80	1.36	0.20	0.99	0.82	1.28E-06
miR-326,miR-374	-1.60	5.36	1.20	6.96	1.22	0.82	1.80E-06
miR-326,miR-496	-1.96	-0.95	1.70	1.01	1.32	0.82	2.86E-06
miR-422a,miR-98	-1.82	-0.85	1.85	0.97	1.07	0.82	9.09E-06
miR-181b,miR-374	-1.72	0.93	1.55	2.65	1.15	0.82	4.21E-06
miR-106b,miR-345	1.15	-1.61	0.79	-2.76	1.02	0.82	3.99E-06
miR-193a,miR-486	2.56	11.44	1.86	8.88	2.02	0.82	1.89E-06
miR-496,miR-99b	1.71	3.12	1.29	1.41	1.25	0.82	1.14E-06
miR-150,miR-7	-1.65	-5.87	1.42	-4.22	1.19	0.82	4.37E-06
miR-422a,miR-638	-1.50	0.25	1.35	1.75	1.02	0.82	4.43E-06
miR-346,miR-483	1.31	0.99	1.26	-0.32	0.67	0.82	2.39E-06
miR-181b,miR-505	-1.17	-3.11	1.20	-1.93	0.92	0.82	1.06E-05
miR-151,miR-214	-2.71	-3.17	1.95	-0.47	2.03	0.82	9.45E-07
miR-181d,miR-190	-1.99	-2.44	1.48	-0.45	1.77	0.82	8.13E-06
miR-16,miR-92	1.64	-0.20	1.75	-1.84	0.72	0.82	6.96E-06
miR-150,miR-496	-2.59	-9.32	2.43	-6.73	1.29	0.82	1.46E-06
miR-140,miR-92	1.35	7.96	1.18	6.61	0.85	0.82	1.89E-06
miR-181d,miR-638	-1.73	-1.96	1.48	-0.23	1.18	0.82	2.71E-06
let-7c,miR-30a-5p	1.54	5.08	1.06	3.54	1.29	0.82	2.34E-06
miR-125a,miR-26b	-1.23	4.03	1.19	5.27	1.20	0.82	1.28E-05
miR-125a,miR-496	-1.83	-5.12	1.45	-3.30	1.49	0.82	5.42E-06
let-7g,miR-151	1.46	-2.40	1.19	-3.86	1.22	0.82	8.95E-06
miR-374,miR-99b	1.40	-3.20	1.32	-4.60	1.05	0.82	1.36E-05
miR-126*,miR-181d	1.24	-3.19	1.24	-4.43	0.90	0.82	1.36E-05
miR-126,miR-181a	0.99	-4.36	0.79	-5.36	0.87	0.82	1.09E-05
miR-18a,miR-486	1.51	8.00	1.33	6.49	1.04	0.82	3.70E-06

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-199a*,miR-92	1.34	6.88	1.27	5.55	0.77	0.81	3.58E-06
miR-214,miR-361	2.58	1.73	2.03	-0.85	1.95	0.81	2.41E-06
miR-190,miR-326	1.70	-2.18	1.43	-3.88	1.43	0.81	1.21E-05
miR-181d,miR-28	-1.43	-2.76	1.31	-1.33	1.11	0.81	1.39E-05
miR-150,miR-505	-1.76	-7.12	1.68	-5.36	1.27	0.81	1.35E-05
miR-150,miR-29b	-1.78	-8.06	1.69	-6.28	1.23	0.81	9.87E-06
miR-345,miR-374	-1.59	0.40	1.24	1.99	1.21	0.81	2.42E-06
let-7b,miR-345	1.22	-1.92	0.98	-3.14	0.96	0.81	4.65E-06
miR-30b,miR-99b	1.05	-4.41	0.93	-5.47	0.93	0.81	1.73E-05
miR-125a,miR-374	-1.56	1.11	1.48	2.66	1.35	0.81	1.73E-05
miR-486,miR-638	-1.93	-8.60	1.65	-6.67	1.47	0.81	6.21E-06
let-7i,miR-92	1.20	7.04	1.08	5.84	0.98	0.81	1.69E-05
miR-15a,miR-361	1.43	0.98	1.22	-0.45	1.08	0.81	5.69E-06
miR-15b,miR-197	1.44	-0.60	1.33	-2.03	1.35	0.81	1.95E-05
miR-296,miR-486	1.82	10.15	1.39	8.32	1.27	0.81	8.33E-07
miR-181d,miR-410	-2.03	-2.95	2.05	-0.92	1.62	0.81	1.96E-05
miR-432,miR-92	1.80	11.07	1.52	9.27	1.43	0.81	7.88E-06
miR-125a,miR-142-3p	-1.49	4.20	1.42	5.69	1.36	0.81	2.01E-05
miR-15a,miR-324-5p	1.39	-0.20	1.24	-1.59	1.04	0.81	8.48E-06
miR-181d,miR-296	-1.62	-3.51	1.56	-1.89	0.96	0.81	5.33E-06
miR-195,miR-486	1.60	4.56	1.44	2.96	0.89	0.81	1.28E-06
miR-143,miR-486	2.11	9.98	1.87	7.86	1.59	0.81	7.80E-06
miR-26b,miR-99b	1.14	-6.12	1.08	-7.26	0.89	0.81	2.14E-05
miR-181d,miR-204	-1.93	-4.79	1.57	-2.85	1.58	0.81	6.80E-06
miR-30b,miR-342	1.09	-1.88	1.01	-2.97	0.72	0.81	5.72E-06
miR-342,miR-98	-1.72	-3.17	1.75	-1.45	0.74	0.81	3.20E-06
miR-15b,miR-17-5p	0.88	-0.06	0.86	-0.94	0.57	0.81	1.08E-05
miR-27a,miR-30a-5p	1.07	2.83	1.05	1.76	1.09	0.81	2.34E-05
miR-126,miR-146b	0.99	-1.62	0.92	-2.61	0.61	0.81	3.43E-06
miR-150,miR-576	-1.53	-8.60	1.37	-7.07	1.09	0.81	6.06E-06
miR-26a,miR-342	1.31	-3.44	1.17	-4.75	0.84	0.81	3.22E-06
let-7i,miR-486	1.34	5.43	1.23	4.09	0.95	0.81	7.84E-06
miR-140,miR-345	0.85	0.05	0.86	-0.81	0.72	0.81	2.48E-05
miR-181b,miR-26a	-1.47	3.71	1.32	5.18	1.05	0.81	6.81E-06
miR-20b,miR-486	1.43	5.70	1.33	4.27	0.96	0.81	6.13E-06
miR-130b,miR-496	-1.91	-6.59	1.63	-4.68	1.52	0.81	8.73E-06
miR-106b,miR-181d	1.58	-1.95	1.36	-3.53	1.66	0.80	2.80E-05
miR-150,miR-296	-1.92	-7.34	1.85	-5.42	1.13	0.80	4.78E-06
miR-150,miR-550	-1.61	-8.03	1.56	-6.42	1.08	0.80	9.82E-06

miRNA Biomarker Pair	Cancer mean - Other mean	Cancer mean	Cancer SD	Other mean	Other SD	AUC ROC	Other vs Cancer Assoc.
miR-214,miR-382	2.41	0.18	2.21	-2.23	1.91	0.80	1.68E-05
miR-143,miR-320	1.95	8.14	1.76	6.18	1.37	0.80	5.87E-06
let-7e,miR-378	1.38	0.02	1.24	-1.36	1.01	0.80	7.54E-06
miR-30c,miR-99b	1.09	-4.59	1.05	-5.69	0.93	0.80	3.24E-05
miR-361,miR-374	-1.21	2.05	1.26	3.27	0.99	0.80	3.34E-05
miR-181a,miR-193a	-1.77	-4.15	1.48	-2.38	1.53	0.80	1.48E-05
miR-206,miR-486	2.35	10.71	2.15	8.36	1.74	0.80	1.01E-05
miR-326,miR-410	-1.62	1.65	1.33	3.26	1.24	0.80	4.30E-06
miR-24,miR-26b	-0.87	-0.03	0.76	0.84	0.70	0.80	1.19E-05
miR-142-3p,miR-326	1.33	-8.41	1.37	-9.74	1.93	0.80	3.75E-05
miR-181b,miR-30b	-1.25	2.15	1.18	3.40	0.78	0.80	5.09E-06
miR-214,miR-93	2.44	7.28	2.22	4.84	1.86	0.80	1.17E-05
miR-132,miR-142-3p	-1.39	4.77	1.24	6.16	1.02	0.80	7.03E-06
miR-16,miR-486	1.77	-1.81	1.73	-3.58	1.23	0.80	1.33E-05
miR-126,miR-342	1.06	-3.66	1.02	-4.72	0.72	0.80	9.88E-06
miR-181a,miR-496	-1.77	-4.87	1.53	-3.10	1.52	0.80	1.75E-05
miR-28,miR-92	1.56	11.03	1.37	9.47	1.29	0.80	1.54E-05
miR-30c,miR-345	1.17	-1.80	0.87	-2.97	1.10	0.80	1.34E-05
miR-505,miR-92	1.53	11.54	1.30	10.01	1.30	0.80	1.50E-05
let-7b,miR-342	1.19	-2.18	0.97	-3.37	1.02	0.80	1.10E-05
let-7c,miR-181b	1.21	1.28	1.17	0.07	0.85	0.80	1.32E-05

[0105] Table 22 shows miRNAs that can be used in combination with other miRNA biomarkers to characterize lung disease, as well as the prevalence of those biomarkers in pairs from Table 21.

Table 22: Prevalence of serum biomarkers in paired analysis of cancer vs. (normal and benign) patients.

miRNA	Paired Appearances	miRNA	Paired Appearances	miRNA	Paired Appearances
miR-92	29	miR-181a	15	miR-342	9
miR-214	22	let-7f	13	miR-24	8
miR-142-3p	21	miR-374	13	miR-30a-5p	8
miR-181d	21	let-7g	11	miR-486	7
miR-15b	20	miR-346	11	let-7a	6
miR-15a	17	miR-181b	10	let-7d	6
miR-150	16	miR-146a	9	miR-345	6

miRNA	Paired Appearances	miRNA	Paired Appearances	miRNA	Paired Appearances
miR-98	6	miR-320	4	miR-106b	2
miR-30c	5	miR-326	4	miR-126*	2
miR-335	5	miR-425	4	miR-133b	2
miR-422a	5	miR-496	4	miR-140	2
miR-125a	4	miR-99b	4	miR-151	2
miR-126	4	miR-146b	3	miR-190	2
miR-130b	4	miR-193a	3	miR-301	2
miR-132	4	miR-26a	3	miR-375	2
miR-210	4	miR-30b	3	miR-422b	2
miR-26b	4	let-7b	2	miR-99a	2

Example 5. Lung Cancer Classifier Development

[0106] An initial training set of 14 miRNAs were selected from pairs in Table 15 to further verify their diagnostic potential. These miRNAs were shown above to have diagnostic potential for distinguishing patients with benign lung conditions from patients with lung cancer, as evidenced by the high AUC ROC scores for miRNA biomarker pairs that include these miRNAs. From a group of 20 benign lung samples and 34 lung cancer samples (Table 23), a set of training samples (12 benign, 16 lung cancer) was chosen from patients that were age and sex matched. qRT-PCR was performed as described above in Example 2.

Table 23. Histopathological and patient information for lung cancer and benign specimens.

Sample ID	Age	Sex	Cell Type	AJCC Stage	Smoking History	Diagnosis
1	59	F	Lymphoplasmacytic	NA	no	Benign
2	60	F	Granuloma	NA	yes	Benign
3	64	F	Granuloma	NA	yes	Benign
4	69	F	Hamartoma	NA	yes	Benign
5	71	F	Granuloma	NA	yes	Benign
6	73	F	Granuloma	NA	yes	Benign
7	77	F	Hamartoma	NA	yes	Benign
8	56	M	No Malignancy	NA	yes	Benign

Sample ID	Age	Sex	Cell Type	AJCC Stage	Smoking History	Diagnosis
10	60	M	Necrotizing Granuloma	NA	yes	Benign
11	69	M	Granulomas	NA	yes	Benign
12	71	M	Infarct	NA	yes	Benign
13	59	F	ADCA	IB	yes	LC
14	64	F	ADCA	IA	yes	LC
15	66	F	ADCA	IB	yes	LC
16	66	F	SCC	IIB	yes	LC
17	72	F	SCC	IB	yes	LC
18	73	F	SCC	IIB	yes	LC
19	73	F	SCC	IIIA	yes	LC
20	56	M	SCC	IIB	yes	LC
21	60	M	ADCA	IB	yes	LC
22	63	M	ADCA	IIIA	yes	LC
23	66	M	SCC	IB	yes	LC
24	69	M	ADCA	IIIA	yes	LC
25	69	M	SCC	IB	yes	LC
26	69	M	SCC	IIB	yes	LC
27	71	M	SCC	IIB	yes	LC
28	72	M	ADCA	IB	yes	LC
29	79	M	ADCA	IIIA	yes	LC
30	67	M	ADCA	IA	yes	LC
31	82	F	SCC	IIB	yes	LC
32	77	M	ADCA	IIIA	yes	LC
53	70	F	SCC	IB	yes	LC
54	49	M	Granuloma	NA	yes	Benign
55	75	F	Large	IIIA	yes	LC
56	80	M	ADCA	IB	yes	LC
57	35	F	Granuloma	NA	no	Benign
58	NA	F	Granuloma	NA	yes	Benign

Sample ID	Age	Sex	Cell Type	AJCC Stage	Smoking History	Diagnosis
59	52	M	Granuloma	NA	no	Benign
60	74	M	SCC	IIB	yes	LC
61	65	F	Adenosquamous	IIB	yes	LC
62	77	M	SCC	IIB	yes	LC
63	51	M	Granuloma	NA	yes	Benign
64	55	M	NSCLC	IB	yes	LC
65	47	F	Harmatoma	NA	yes	Benign
66	80	F	Spindle Cell ADCA	IIIA	yes	LC
67	45	F	Granuloma	NA	yes	Benign
68	54	F	NSCLC	IB	yes	LC
69	46	F	Pleomorphic Carcinoma	IB	yes	LC
70	68	M	Large	IB	yes	LC
71	87	M	SCC	IB	yes	LC
72	87	M	SCC	IB	yes	LC
73	80	M	ADCA	IA	yes	LC
74	40	F	Hamartoma	NA	yes	Benign

[0107] To verify a subset of the 14 miRNAs in the training set, a separate test sample set was selected from Table 23 that was composed of 8 benign and 18 LuCa samples. miRNA biomarker selection and classifier evaluation was generated by performing 25 repetitions of 5-fold cross validation on the training samples, and measuring the AUC ROC values as a function of the number of miRNA biomarkers (features). A range of biomarkers was used in order to determine the optimal number of features for classification. Five-fold cross-validation is a process where the training set is subdivided into an 80% sample set and a 20% sample set, and feature selection and classifier training is performed on the 80% training samples. Performance is measured by classifying the remaining 20% samples not used for feature selection and classifier training. This

process was repeated 5 times so that all samples were in the 20% test set once and only once. One iteration of 5-fold cross-validation produced a single AUC value. Five-fold cross-validation was repeated 25 times in order to achieve a better sampling of combinations of 80% and 20% sets, and to identify an empirical distribution of AUC values for a given number of biomarkers in the classifier. Top biomarkers according to a simple Welch's t-test were used as inputs into a Linear Discriminate Analysis (LDA) classifier for training. Biomarkers were defined as pairs of miRNAs (differential expression of two miRNAs), where the expression value of one miRNA is subtracted from the expression value of another miRNA. All possible pairs were investigated. Figure 3 shows the distribution of AUC values for the LDA classifier as a function of the number of biomarkers. This procedure determines the optimal number of biomarkers to use for the classifier, and performance expectations moving forward. A classifier of 6 differentially expressed miRNA pairs (miR-142-5p and miR181d; miR-142-3p and miR181d; miR-142-3p and miR-422a; miR-142-5p and miR-422a; miR-92 and miR-27b; miR-24 and miR-27a) was identified as the optimal classifier for diagnosis of lung cancer in this experiment. This classifier is marked by the vertical dotted line in Figure 3. A cutoff for the LDA classifier was set so that the continuous valued classification probabilities were converted into calls diagnosing patients as having lung cancer (classifier-positive) or benign (classifier-negative) tumors. For simplicity, a cutpoint of 0.5 was chosen as the threshold. This value produced a near maximum of sensitivity and specificity. Performance estimates of training and test data using a cutoff of 0.5 of the LDA classifier trained on six biomarker pairs is provided in Figure 4. The sensitivity (SENS), specificity (SPEC), negative predictive value (NPV) and positive predictive value (PPV) are as indicated.

[0108] Any of the six pairs identified as part of the classifier in this experiment may be used to diagnose lung cancer. Predictive accuracy may increase with the use of more than one pair from the optimal classifier set.

Example 6. qRT-PCR for evaluation of microRNA expression in human lung cancer patient plasma RNA

[0109] To identify miRNA biomarkers for lung cancer in human plasma, qRT-PCR was used to compare levels of miRNAs present in the plasma of patients with lung cancer (n=14) to the levels in patents without lung cancer

(n=12). Patient information and lung cancer pathology information are shown in Table 24.

Table 24. Human Lung Cancer and Normal Patient Information and Tumor Pathology. TNM stage (Sobin and Wittekind, 2002)

Age	Sex	Tumor Location	Cell & Tumor Characteristics	TNM Stage	AJCC Stage
66	M	upper lobe left lung	ADCA	NA	NA
48	M	lower lobe left lung	BA	NA	NA
72	M	upper lobe right lung	SCC	T3NxM0	NA
66	M	left lung	SCC	T3NxM0	NA
54	M	lower lobe right lung	SCC	NA	IIIB
69	M	upper lobe left lung	UC	NA	IIIA
52	M	lower lobe left lung	SCC	NA	IIIA
54	F	left lung	ADCA	NA	IV
49	F	left lung	SCC	T4N1M0	III
67	M	upper lobe right lung	SCC	T3N1M0	III
59	F	right lung	SCC	T3N2M0	III
51	M	left lung	SCC	T4N2M0	III
44	M	right lung	SCC	T4NXM0	III
52	M	upper lobe left lung	SCC	T3N1M0	III
Normal Patients					
Age	Sex	Tumor Location	Cell Characteristics	TNM Stage	AJCC Stage
39	F	-	Normal	-	-

44	M	-	Normal	-	-
58	F	-	Normal	-	-
59	F	-	Normal	-	-
61	M	-	Normal	-	-
52	M	-	Normal	-	-
59	M	-	Normal	-	-
57	F	-	Normal	-	-
59	F	-	Normal	-	-
56	M	-	Normal	-	-
52	F	-	Normal	-	-
62	F	-	Normal	-	-

[0110] For plasma preparation, whole blood (10 ml) was collected from each donor into a BD Vacutainer® K2EDTA tube (Becton, Dickinson and Company; Franklin Lakes, NJ, USA). Tubes were inverted 8-10 times immediately after blood collection and then centrifuged for 10 minutes at 2,000 x g within two hours of collection. Plasma was transferred to a new tube using a serological pipette and frozen at -80 °C until it was subjected to RNA isolation.

[0111] Plasma RNA was purified using the organic extraction of the mirVana PARIS™ Kit (Part No. AM1556; Applied Biosystems/Ambion; Austin, TX, USA), with the following modifications. After thawing plasma on ice, an equal volume of 2X denaturing solution from the mirVana PARIS™ Kit was added and the mixture was incubated on ice for five minutes. An equal volume of acid phenol:chloroform was added, then the mixture was vortexed for one minute, and incubated on ice for five minutes. Tubes were centrifuged at 13,000 x g for 15 minutes at 4 °C, and the aqueous phase was removed to a fresh tube. Glycogen (5 mg/ml), 3M NaOAc (1/10 volume), and 100% ethanol (1.5 volume) were added to the samples. Samples were mixed by inversion and incubated at room temperature. Lysate/ethanol mixtures were passed through a mirVana PARIS™ filter cartridge, and filters were washed once with 650 µl of Wash 1 buffer and twice with 650 µl of Wash 2/3 buffer. RNA was eluted with nuclease free water (50 µl, heated to 95 °C), by centrifugation at 10,000 x g for 1 minute.

[0112] qRT-PCR reactions were performed using TaqMan® MicroRNA Assays (Applied Biosystems; Foster City, CA, USA) specific for each individual miRNA. Reverse transcription reactions were assembled on ice prior to the addition of RNA template (Table 25). Next, 0.25 µl of plasma RNA template per reaction was added and mixed. RT reactions were incubated in a 384-well GeneAmp® PCR System 9700 (Applied Biosystems) at 16 °C for 30 minutes, then at 42 °C for 30 minutes, then at 85 °C for 5 minutes, and then were frozen in a -20 °C freezer.

Table 25. Reverse transcription reaction components.

Component	µl per 10µl rxn	Final Concentration
Nuclease-free water	5.85	-
10X RT Buffer (Asuragen, Inc.)	1.0	1X
dNTP mix (100 mM), (Ambion, Inc.; Austin, TX USA)	0.1	1 mM each
1.25 X RT Primer (Applied Biosystems, Inc., Foster City, CA, USA)	2.0	0.25X
RNasin® Ribonuclease Inhibitor (20 U/µl, Promega, Corp. Madison, WI, USA; cat. no. N2115)	0.13	0.26 U/µl
Multiscribe™ Recombinant Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT) (50 U/µl, Applied Biosystems, Inc., Foster City, CA, USA; part no. 4311235)	0.67	3.35 U/µl
Human Plasma RNA	0.25	-

[0113] PCR components were assembled on ice prior to the addition of the cDNA from the RT reactions (Table 26). PCRs were incubated in an ABI PRISM™ 7900HT Fast Real-Time PCR system (Applied Biosystems) at 95 °C for 1 minute, then for 50 cycles at 95 °C for 5 seconds, and then at 60 °C for 30 seconds. Initial data analysis was done using the 7500 Fast System SDS V2.3 software (Applied Biosystems).

Table 26. Real-time PCR components.

Component	μl per 15μl rxn	Final Concentration
Nuclease-free water	6.1	-
MgCl ₂ (50 mM)	1.5	5 mM
10X Platinum PCR Buffer, Minus Mg (Invitrogen; cat.no. 53286)	1.5	1X
dNTP mix (2.5 mM each) (Ambion, Inc.; Austin, TX USA)	1.5	0.25 mM each
3X TaqMan Assay Buffer	2.0	0.4X
50X ROX Internal marker	0.3	1X
Platinum® Taq DNA Polymerase (5U/μl) (Invitrogen Corp., Carlsbad, CA, USA; SKU#, 10966-083)	0.1	0.033U/μl
cDNA from RT reaction	2.0	

[0114] The qRT-PCR data were initially assessed for outliers. All miRNAs in a given sample with raw Ct values of 50 were eliminated from further analysis. All data from samples with fewer than 150 miRNAs that had raw Ct values less than 50 were eliminated. The average raw Ct for 50 miRNAs that were detected in each plasma sample was calculated for each individual sample. The average Ct for a given sample was subtracted from the raw Ct values for each miRNA in the corresponding sample to produce a dCt for each miRNA that was detected. These normalized measures were used to identify miRNAs that were present at significantly different levels in the plasma samples from normal donors and lung cancer patients.

[0115] The average dCt values for each miRNA in the normal donor and lung cancer patient samples were calculated. The average dCt values for the lung cancer patient samples were subtracted from the average dCt values for the normal donors to determine the variance in the levels of the various miRNAs between the two patient sets. The student t-test was then used to evaluate the capacity of the various miRNAs to distinguish the plasmas of lung cancer patients and normal donors.

[0116] Table 27 provides differential expression data for miRNAs in the form of average dCt values for cancer and normal samples. In addition, ddCt values represent the difference in expression between cancer and normal samples.

Table 27. miRNA Biomarkers Differentially Expressed Between Plasma Samples of Lung Cancer Patients and Plasma Samples of Normal Patients.

miRNA	Cancer Average dCt	Cancer SD	Normal Average dCt	Normal SD	ddCt (Cancer - Normal)	t-test
miR-499	14.92	2.91	20.32	1.75	-5.40	9.72E-03
miR-498	15.86	3.34	20.61	3.92	-4.75	7.34E-03
miR-205	6.94	2.61	11.69	2.60	-4.75	2.73E-04
miR-122a	9.05	1.47	13.44	3.32	-4.39	2.20E-03
miR-519c	12.89	18.06	16.50	5.51	-3.61	6.26E-01
miR-515-5p	15.34	5.81	18.90	6.53	-3.56	3.97E-01
miR-215	7.22	2.05	10.52	1.85	-3.30	2.36E-04
miR-449	11.00	2.12	14.02	3.43	-3.02	5.19E-02
miR-200a*	11.13	3.52	13.92	4.29	-2.80	8.63E-02
miR-375	5.91	2.96	8.68	0.91	-2.78	4.27E-03
miR-515-3p	11.23	2.15	14.00	2.25	-2.77	5.46E-02
miR-206	8.08	1.86	10.84	2.82	-2.76	9.56E-03
miR-448	16.67	2.38	19.41	4.33	-2.74	1.11E-01
miR-520d	12.17	4.82	14.81	5.14	-2.65	2.51E-01
miR-7	6.35	1.20	8.99	1.85	-2.65	7.93E-04
miR-129	12.94	1.93	15.48	2.58	-2.54	1.10E-02
miR-218	9.78	1.57	12.22	2.96	-2.44	2.08E-02
miR-192	2.79	0.98	5.17	2.12	-2.38	2.79E-03
miR-302a*	13.71	2.80	15.99	4.94	-2.27	2.14E-01
miR-451	-3.87	1.07	-1.64	1.66	-2.23	8.05E-04
miR-517*	16.16	4.45	18.27	4.16	-2.11	3.63E-01
miR-95	9.36	1.38	11.46	2.27	-2.10	3.19E-02
miR-520h	13.40	4.22	15.48	6.16	-2.08	4.39E-01
miR-525	9.01	2.08	11.07	4.14	-2.06	1.38E-01
miR-367	16.65	2.60	18.65	2.02	-2.00	4.60E-01
miR-124a	14.03	6.20	16.01	4.05	-1.98	6.14E-01
miR-16	-5.75	1.12	-3.84	1.81	-1.91	5.26E-03
miR-9*	9.48	1.24	11.38	3.72	-1.90	1.35E-01
miR-31	8.14	1.22	10.02	2.12	-1.87	1.51E-02
miR-136	14.00	2.43	15.80	3.08	-1.81	1.78E-01
miR-32	4.58	1.60	6.34	4.03	-1.76	1.96E-01

miRNA	Cancer Average dCt	Cancer SD	Normal Average dCt	Normal SD	ddCt (Cancer - Normal)	t-test
miR-200a	9.04	1.83	10.80	1.92	-1.76	3.61E-02
miR-512-5p	11.10	1.00	12.84	3.23	-1.75	9.53E-02
miR-125b	5.99	0.95	7.73	1.46	-1.74	3.67E-03
miR-514	15.39	3.68	17.12	2.55	-1.73	2.23E-01
miR-30e-5p	1.54	1.10	3.21	3.04	-1.67	9.36E-02
miR-483	8.17	2.23	9.84	3.68	-1.66	1.90E-01
miR-383	9.65	1.07	11.28	3.06	-1.63	1.01E-01
miR-216	11.93	4.12	13.52	3.84	-1.59	3.27E-01
miR-195	0.82	1.27	2.41	2.57	-1.59	6.89E-02
miR-137	10.44	1.48	12.03	2.63	-1.59	8.01E-02
miR-138	10.95	2.24	12.53	2.96	-1.59	1.54E-01
miR-18b	10.90	1.61	12.44	2.89	-1.54	1.26E-01
miR-501	8.64	3.37	10.16	2.16	-1.51	3.32E-01
miR-101	1.01	1.10	2.49	0.97	-1.48	1.64E-03
miR-373*	11.57	2.36	13.04	3.78	-1.48	2.58E-01
miR-183	10.45	2.07	11.92	1.47	-1.47	6.55E-02
miR-365	6.16	1.15	7.60	2.13	-1.44	7.52E-02
miR-141	7.46	2.24	8.89	1.86	-1.43	8.79E-02
miR-193a	8.84	1.17	10.27	1.80	-1.43	2.98E-02
miR-182*	13.84	3.72	15.25	5.66	-1.41	6.75E-01
miR-96	9.46	2.57	10.76	1.81	-1.30	2.15E-01
miR-453	11.17	2.33	12.41	4.18	-1.24	3.75E-01
miR-10b	5.29	2.50	6.53	1.54	-1.24	1.37E-01
miR-486	-3.17	1.13	-1.94	1.88	-1.23	6.36E-02
miR-29c	0.92	0.88	2.15	0.73	-1.22	7.09E-04
miR-217	15.57	4.55	16.72	3.56	-1.15	6.68E-01
miR-497	7.92	1.99	9.06	1.71	-1.14	1.95E-01
miR-346	12.04	2.82	13.17	2.40	-1.13	3.55E-01
miR-489	12.77	2.64	13.88	1.93	-1.12	2.26E-01
miR-429	10.14	3.78	11.18	2.95	-1.04	4.69E-01
miR-200c	5.05	1.39	6.05	0.60	-1.00	2.56E-02
miR-197	-0.44	1.09	0.52	2.67	-0.96	2.64E-01
miR-185	5.80	1.87	6.74	2.43	-0.95	2.91E-01
miR-296	7.12	1.00	8.05	0.89	-0.93	1.91E-02
miR-214	5.36	1.21	6.29	3.10	-0.93	3.45E-01
miR-299-3p	12.86	3.54	13.76	3.65	-0.90	6.14E-01
miR-29a	0.90	1.03	1.78	0.82	-0.88	2.32E-02
miR-520a*	16.86	4.44	17.73	4.79	-0.87	7.99E-01
miR-526a	14.96	2.77	15.82	5.53	-0.86	7.32E-01
miR-507	15.97	3.63	16.83	4.64	-0.85	6.12E-01

miRNA	Cancer Average dCt	Cancer SD	Normal Average dCt	Normal SD	ddCt (Cancer - Normal)	t-test
miR-432*	10.52	1.19	11.35	2.83	-0.83	3.77E-01
miR-128a	9.94	0.81	10.77	0.91	-0.83	1.02E-01
miR-193b	9.82	1.68	10.63	2.24	-0.82	3.12E-01
miR-372	13.70	2.33	14.51	3.70	-0.81	5.80E-01
miR-516-3p	7.97	1.33	8.78	2.77	-0.81	3.70E-01
miR-378	8.98	1.43	9.76	0.71	-0.79	1.39E-01
miR-520d*	14.84	2.95	15.62	5.39	-0.78	7.48E-01
miR-100	6.13	0.94	6.90	1.53	-0.76	1.50E-01
miR-184	13.58	3.37	14.32	3.39	-0.75	5.94E-01
miR-20b	1.68	1.07	2.41	1.49	-0.74	1.83E-01
miR-188	6.45	1.25	7.19	3.10	-0.74	4.54E-01
miR-150	1.22	1.15	1.94	1.08	-0.72	1.11E-01
miR-19a	-0.82	0.83	-0.10	1.45	-0.71	1.50E-01
miR-25	-0.33	1.19	0.38	0.44	-0.71	5.58E-02
miR-19b	-2.17	0.73	-1.47	1.17	-0.70	8.97E-02
miR-92	-4.42	1.03	-3.78	2.21	-0.64	3.73E-01
miR-376a*	9.47	1.78	10.08	1.35	-0.60	4.21E-01
miR-135b	12.67	3.43	13.22	2.27	-0.55	7.05E-01
miR-202	7.26	2.04	7.80	3.64	-0.54	6.55E-01
miR-452	7.16	1.51	7.68	2.21	-0.53	4.95E-01
miR-10a	5.83	1.03	6.34	1.48	-0.51	3.32E-01
miR-93	-0.50	0.62	-0.03	0.26	-0.46	2.02E-02
let-7f	2.49	1.80	2.95	3.35	-0.46	6.78E-01
miR-148a	3.19	0.63	3.64	0.92	-0.45	1.69E-01
miR-203	11.63	4.32	12.07	3.30	-0.45	7.69E-01
miR-299-5p	10.67	1.50	11.10	2.82	-0.44	6.67E-01
miR-20a	-1.34	1.00	-0.93	1.68	-0.41	4.64E-01
miR-182	5.87	2.36	6.28	1.17	-0.41	5.80E-01
miR-520c	12.30	1.81	12.69	2.42	-0.38	6.84E-01
miR-511	9.88	2.11	10.26	1.72	-0.38	6.19E-01
miR-510	13.51	2.81	13.84	2.54	-0.33	7.54E-01
let-7c	3.31	0.97	3.64	0.96	-0.33	3.91E-01
miR-18a*	6.06	1.15	6.39	1.32	-0.33	5.17E-01
miR-139	5.96	0.74	6.27	0.56	-0.31	2.41E-01
miR-204	8.90	0.92	9.19	1.09	-0.30	4.69E-01
miR-527	13.03	3.34	13.32	5.13	-0.29	8.89E-01
miR-190	5.55	1.41	5.82	0.92	-0.27	5.60E-01
miR-99a	6.83	1.37	7.10	1.91	-0.27	6.90E-01
miR-30a-3p	5.81	0.59	6.07	1.18	-0.26	5.07E-01
miR-518d	10.54	1.76	10.78	3.73	-0.25	8.38E-01

miRNA	Cancer Average dCt	Cancer SD	Normal Average dCt	Normal SD	ddCt (Cancer - Normal)	t-test
miR-17-5p	0.76	0.67	1.00	1.22	-0.24	5.59E-01
miR-517a	15.36	2.77	15.58	3.38	-0.22	9.06E-01
miR-210	3.64	0.95	3.85	1.99	-0.21	7.45E-01
miR-520a	15.16	2.80	15.35	4.92	-0.20	9.13E-01
miR-500	9.79	2.41	9.98	1.05	-0.19	8.33E-01
miR-422a	6.48	1.31	6.64	2.63	-0.16	8.50E-01
miR-208	17.02	2.56	17.16	2.34	-0.15	9.07E-01
miR-302a	16.05	5.66	16.18	4.20	-0.13	9.69E-01
miR-520g	14.64	4.07	14.75	4.42	-0.12	9.56E-01
miR-196b	6.83	1.13	6.95	1.56	-0.11	8.44E-01
miR-526b*	15.90	3.56	16.01	4.78	-0.11	9.50E-01
miR-142-3p	-1.22	0.68	-1.15	1.53	-0.07	8.81E-01
miR-199b	8.18	1.48	8.24	1.63	-0.05	9.33E-01
miR-34c	6.72	0.70	6.76	1.18	-0.04	9.14E-01
miR-517c	10.66	3.19	10.68	2.12	-0.01	9.91E-01
let-7b	0.52	1.61	0.53	1.10	-0.01	9.83E-01
miR-329	10.61	1.42	10.60	2.16	0.01	9.95E-01
miR-381	10.99	1.65	10.98	2.78	0.01	9.91E-01
miR-106b	0.39	0.69	0.38	1.15	0.01	9.69E-01
miR-424	8.65	1.22	8.62	0.84	0.03	9.42E-01
miR-148b	3.79	0.60	3.74	1.73	0.05	9.32E-01
miR-126	0.28	1.13	0.23	1.43	0.05	9.28E-01
miR-132	4.66	0.82	4.61	1.00	0.05	8.86E-01
let-7a	1.49	1.01	1.43	1.77	0.06	9.24E-01
miR-345	4.05	0.50	3.99	0.93	0.06	8.41E-01
miR-374	1.95	0.87	1.88	0.85	0.07	8.35E-01
miR-450	10.64	2.91	10.56	0.71	0.07	9.48E-01
miR-325	19.66	1.84	19.58	4.95	0.08	9.69E-01
miR-17-3p	6.86	0.83	6.77	1.49	0.09	8.53E-01
let-7i	0.86	1.02	0.76	2.26	0.09	8.96E-01
miR-181b	2.68	0.72	2.57	1.76	0.11	8.39E-01
miR-30a-5p	-0.25	0.85	-0.37	1.28	0.11	7.95E-01
miR-18a	6.16	0.84	6.04	0.99	0.12	7.44E-01
miR-106a	2.33	0.67	2.20	0.87	0.13	6.89E-01
miR-146b	1.05	0.51	0.92	0.97	0.13	6.80E-01
miR-147	18.12	3.46	17.97	3.45	0.15	9.32E-01
miR-103	1.82	0.85	1.68	1.53	0.15	7.69E-01
miR-30d	1.15	0.66	1.00	0.55	0.15	5.24E-01
miR-422b	3.51	0.94	3.34	2.86	0.17	8.44E-01
miR-135a	8.57	0.90	8.40	0.66	0.18	6.18E-01

miRNA	Cancer Average dCt	Cancer SD	Normal Average dCt	Normal SD	ddCt (Cancer - Normal)	t-test
miR-196a	7.99	1.57	7.81	1.63	0.18	7.74E-01
miR-335	5.14	1.40	4.94	2.42	0.20	8.00E-01
miR-21	-1.38	0.83	-1.62	1.13	0.23	5.57E-01
miR-23a	1.42	0.49	1.18	0.34	0.24	1.49E-01
miR-130b	1.75	0.97	1.50	0.95	0.25	5.20E-01
let-7d	3.78	1.40	3.53	1.28	0.25	6.40E-01
miR-495	7.86	0.75	7.60	1.01	0.25	5.03E-01
miR-26b	-0.46	1.24	-0.71	1.60	0.26	6.55E-01
miR-15b	1.33	1.27	1.04	1.61	0.29	6.16E-01
miR-130a	1.54	1.30	1.19	0.85	0.35	4.25E-01
let-7e	7.16	2.12	6.82	1.95	0.35	6.75E-01
miR-30c	1.32	0.88	0.97	1.85	0.35	5.60E-01
miR-34b	17.45	3.79	17.08	2.65	0.36	8.59E-01
miR-377	12.83	3.23	12.46	2.43	0.37	7.57E-01
miR-140	3.21	0.98	2.85	0.87	0.37	3.18E-01
miR-520b	17.47	1.67	17.10	2.22	0.37	7.38E-01
let-7g	0.06	0.86	-0.31	0.93	0.38	2.97E-01
miR-542-3p	11.03	2.43	10.65	0.81	0.38	5.88E-01
miR-98	5.72	1.04	5.33	1.55	0.39	4.82E-01
miR-502	7.79	0.82	7.39	3.28	0.40	6.90E-01
miR-199a*	1.46	0.68	1.06	0.65	0.40	1.45E-01
miR-503	12.38	2.88	11.98	2.20	0.41	7.23E-01
miR-23b	3.86	0.63	3.44	0.61	0.43	9.05E-02
miR-320	0.90	1.29	0.47	2.35	0.43	5.77E-01
miR-212	8.16	1.12	7.68	3.05	0.48	6.16E-01
miR-518c*	11.99	3.66	11.49	4.28	0.50	7.62E-01
miR-26a	-1.95	0.77	-2.45	0.70	0.51	9.24E-02
miR-152	4.37	0.54	3.86	0.70	0.52	5.05E-02
miR-142-5p	2.40	0.86	1.89	0.79	0.52	1.23E-01
miR-154*	10.20	1.53	9.67	1.19	0.54	4.24E-01
miR-505	4.37	0.60	3.82	0.50	0.56	1.67E-02
miR-105	14.01	4.11	13.45	3.41	0.56	7.54E-01
miR-223	-2.23	0.60	-2.80	0.91	0.57	8.13E-02
miR-432	4.51	1.22	3.93	1.05	0.58	2.02E-01
miR-324-3p	4.32	0.63	3.69	0.87	0.64	4.85E-02
miR-24	-0.27	0.95	-0.91	1.27	0.64	1.70E-01
miR-28	5.44	0.86	4.80	0.93	0.64	8.36E-02
miR-149	13.18	3.27	12.49	1.73	0.69	5.19E-01
miR-219	12.63	3.46	11.93	2.22	0.70	5.73E-01
miR-433	5.73	1.05	5.03	1.23	0.70	1.36E-01

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miR-221	-0.28	0.70	-1.00	0.61	0.71	1.06E-02
miR-146a	-0.57	0.63	-1.31	0.52	0.74	3.13E-03
miR-133b	4.68	0.77	3.94	1.17	0.75	7.52E-02
miR-181d	2.19	0.65	1.43	1.34	0.76	9.40E-02
miR-224	8.03	1.59	7.25	0.91	0.78	1.60E-01
miR-181c	4.54	0.62	3.74	0.65	0.80	4.14E-03
miR-485-5p	8.02	1.05	7.22	1.45	0.80	1.28E-01
miR-423	3.83	0.52	3.01	0.99	0.81	2.10E-02
miR-376b	10.41	3.28	9.60	2.69	0.82	5.88E-01
miR-504	19.43	1.91	18.60	2.06	0.83	4.61E-01
miR-29b	5.46	1.80	4.63	1.73	0.83	2.42E-01
miR-1	9.49	3.81	8.65	4.25	0.84	6.15E-01
miR-425	4.92	0.67	4.08	1.23	0.84	4.98E-02
miR-15a	4.65	0.96	3.80	1.19	0.85	6.09E-02
miR-107	7.16	1.11	6.30	1.18	0.85	7.14E-02
miR-133a	7.24	1.34	6.38	0.89	0.86	6.48E-02
miR-382	4.69	1.32	3.83	1.96	0.86	2.13E-01
miR-508	14.81	3.67	13.95	3.85	0.86	5.98E-01
miR-324-5p	5.01	0.95	4.14	0.90	0.86	2.60E-02
miR-493-3p	8.94	1.71	8.07	1.98	0.87	2.48E-01
miR-376a	4.01	1.41	3.14	2.40	0.87	2.84E-01
miR-27a	2.75	1.51	1.87	0.48	0.88	5.53E-02
miR-361	4.39	1.31	3.50	2.49	0.89	2.84E-01
miR-342	3.18	0.65	2.28	0.61	0.90	1.32E-03
miR-496	8.29	1.45	7.39	1.38	0.90	1.17E-01
miR-199a	7.65	1.23	6.73	0.66	0.92	2.44E-02
miR-30b	0.74	0.95	-0.20	0.86	0.94	1.41E-02
miR-509	10.99	2.32	10.04	4.06	0.95	4.85E-01
miR-518c	15.52	3.23	14.57	4.89	0.96	6.15E-01
miR-211	12.00	3.08	11.04	1.44	0.96	3.44E-01
miR-125a	4.10	0.70	3.10	0.87	1.00	4.54E-03
miR-186	1.05	0.58	0.05	0.98	1.00	6.49E-03
miR-30e-3p	6.03	1.04	5.01	1.43	1.02	5.33E-02
miR-155	4.43	0.81	3.40	1.57	1.03	5.71E-02
miR-369-3p	10.05	2.75	9.02	1.04	1.03	2.30E-01
miR-380-3p	12.73	3.70	11.69	1.09	1.03	4.40E-01
miR-338	6.79	1.62	5.74	2.90	1.04	2.85E-01
miR-340	7.19	1.35	6.14	1.76	1.05	1.41E-01
miR-323	7.40	0.94	6.35	1.24	1.05	2.63E-02
miR-379	7.43	1.40	6.36	1.64	1.07	9.03E-02

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miR-523	18.31	3.96	17.23	4.04	1.07	6.07E-01
miR-494	9.67	2.01	8.60	1.36	1.07	1.30E-01
miR-488	19.32	2.87	18.24	4.26	1.08	6.58E-01
miR-127	3.79	1.01	2.69	0.92	1.09	8.18E-03
miR-362	7.75	1.81	6.65	1.72	1.10	1.25E-01
miR-380-5p	13.64	3.87	12.53	1.05	1.11	4.85E-01
miR-330	9.99	1.07	8.87	0.90	1.12	7.91E-03
miR-485-3p	6.78	2.34	5.64	1.14	1.14	1.22E-01
miR-331	5.30	1.42	4.15	1.47	1.15	6.07E-02
miR-525*	14.27	2.93	13.11	4.19	1.16	4.31E-01
miR-181a	3.11	0.79	1.95	1.75	1.16	5.19E-02
miR-518a	14.18	3.31	13.01	1.66	1.17	5.60E-01
miR-27b	3.70	1.38	2.54	0.65	1.17	1.12E-02
miR-518e	12.68	3.06	11.52	4.33	1.17	4.69E-01
miR-328	4.46	0.72	3.28	1.14	1.18	6.03E-03
miR-301	4.31	0.89	3.13	0.74	1.18	1.11E-03
miR-198	12.16	2.90	10.95	3.57	1.20	3.60E-01
miR-145	3.96	0.93	2.76	1.53	1.21	2.88E-02
miR-412	15.14	2.67	13.93	4.58	1.22	5.36E-01
miR-213	8.89	0.92	7.65	1.69	1.24	3.91E-02
miR-33	11.04	3.12	9.78	1.64	1.26	2.34E-01
miR-191	0.70	0.70	-0.56	0.43	1.26	1.12E-05
miR-520e	15.89	2.25	14.62	3.13	1.26	3.72E-01
miR-153	13.74	4.11	12.47	3.04	1.27	4.48E-01
miR-143	10.70	4.07	9.42	0.87	1.28	2.71E-01
miR-519d	10.08	2.32	8.80	3.83	1.28	3.30E-01
miR-99b	5.41	0.78	4.12	1.13	1.28	3.61E-03
miR-134	4.75	1.57	3.44	2.56	1.30	1.44E-01
miR-154	10.45	2.99	9.13	1.36	1.32	1.86E-01
miR-518b	11.66	2.69	10.32	3.41	1.35	2.88E-01
miR-410	4.99	1.38	3.63	2.02	1.36	6.32E-02
miR-339	5.00	0.99	3.61	0.65	1.38	3.07E-04
miR-151	2.06	0.85	0.67	2.01	1.39	4.25E-02
miR-34a	9.63	2.42	8.16	4.89	1.47	3.59E-01
miR-187	11.03	3.50	9.47	1.39	1.56	1.44E-01
miR-518f	16.54	3.42	14.96	4.52	1.59	3.44E-01
miR-302b*	16.36	4.66	14.77	10.39	1.60	8.65E-01
miR-222	1.40	0.80	-0.26	1.26	1.66	9.65E-04
miR-491	6.15	1.30	4.47	2.74	1.68	7.14E-02
miR-302d	17.55	3.40	15.83	3.69	1.72	3.64E-01

miRNA	Cancer Average dCt	Cancer SD	Normal Average dCt	Normal SD	ddCt (Cancer - Normal)	t-test
miR-370	8.23	2.20	6.49	1.59	1.74	2.85E-02
miR-368	12.18	3.72	10.42	2.36	1.75	2.66E-01
miR-490	12.44	2.92	10.69	3.28	1.75	1.67E-01
miR-189	16.98	4.15	15.22	4.24	1.76	4.19E-01
miR-526b	18.30	4.33	16.52	3.99	1.78	4.25E-01
miR-539	7.73	2.58	5.93	1.09	1.81	3.51E-02
miR-493	10.90	3.44	9.10	1.50	1.81	1.20E-01
miR-487b	7.48	3.19	5.64	1.79	1.85	7.84E-02
miR-126*	0.06	3.20	-1.89	1.08	1.95	4.74E-02
miR-371	17.55	3.02	15.56	3.01	2.00	1.52E-01
miR-369-5p	11.37	4.74	9.29	0.69	2.08	3.84E-01
miR-455	14.46	3.74	12.38	2.70	2.08	1.66E-01
miR-337	15.71	4.66	13.59	1.81	2.12	3.79E-01
miR-128b	14.50	3.21	12.30	1.98	2.21	4.63E-02
miR-326	7.41	0.88	5.16	1.36	2.25	1.09E-04
miR-373	16.08	4.49	13.83	3.98	2.25	2.17E-01
miR-487a	11.12	4.01	8.85	1.94	2.27	1.27E-01
miR-452*	12.84	4.18	10.47	1.24	2.37	1.59E-01
miR-9	11.88	4.01	9.44	0.82	2.44	7.45E-02
miR-409-5p	12.34	4.03	9.87	1.51	2.47	1.99E-01
miR-519e	12.44	2.93	9.89	3.03	2.56	4.33E-02
miR-542-5p	15.61	4.73	12.74	1.91	2.87	1.18E-01
miR-517b	17.60	4.35	14.57	5.50	3.03	3.87E-01
miR-520f	16.59	4.22	13.21	4.64	3.38	9.69E-02
miR-22	8.62	2.78	5.17	2.12	3.44	1.55E-03

Example 7. QRT-PCR for Evaluation of MicroRNA Expression in Human Lung Cancer Patient Plasma RNA

[0117] A second set of plasma samples, isolated from a distinct set of lung cancer and normal patients (Table 28), was used to perform an additional comparison of miRNAs expressed in lung cancer and normal patients. In Table 28, TNM (tumor, node, metastasis) stage is described in Sobin and Wittekind, New Jersey: John Wiley & Sons, 2002. The following abbreviations are used in Table 28: adenocarcinoma (ADCA); bronchoalveolar (BA), squamous cell carcinoma (SCC), and not available (NA).

Table 28. Human Lung Cancer and Normal Patient Information and Tumor Pathology. .

Lung Cancer Patients			
Age	Sex	Cell & Tumor Characteristics	TNM Stage
70	F	upper lobe of left lung, ADCA	NA
66	M	upper lobe of left lung, ADCA	NA
48	M	lower lobe of left lung, BA	NA
69	M	Lower lobe of left lung, SCC	NA
66	F	Upper lobe of right lung, SCC	NA
72	M	Upper lobe of right lung, SCC	T3NxM0
66	M	Left lung, SCC	T3NxM0
46	M	Left lung, SCC	T3NxM0
62	M	Right lung, SCC	T2NxM0
Normal Patients			
Age	Sex	Cell & Tumor Characteristics	TNM Stage
40	F	Normal	-
41	F	Normal	-
59	F	Normal	-
56	M	Normal	-
52	F	Normal	-
37	M	Normal	-
34	M	Normal	-

[0118] To assess the expression of miRNAs, real-time RT-PCR detection with TaqMan® Micro RNA Assays (Applied Biosystems, Foster City, CA, USA) was used to screen RNA isolated from seven normal human plasma and nine lung cancer patient plasma samples.

[0119] Plasma RNA was isolated as described above in Example 6. qRT-PCR reactions were performed using TaqMan® MicroRNA Assays (Applied Biosystems; Foster City, CA, USA) specific for each individual miRNA. Reverse transcription reactions were assembled on ice prior to the addition of RNA

template (Table 29). Next, 0.25 μ l of plasma RNA template per reaction was added and mixed. RT reactions were incubated in a 384-well GeneAmp® PCR System 9700 (Applied Biosystems) at 16 °C for 30 minutes, then at 42 °C for 30 minutes, then at 85 °C for 5 minutes, and then were frozen in a -20 °C freezer.

Table 29. Reverse transcription reaction components.

Component	μ l per 10 μ l rxn	Final Concentration
Nuclease-free water	5.6	
10X Reverse Transcription Buffer (Ambion, Inc., Austin, TX, USA)	100	1X
dNTP mix (2.5mM each) (Ambion, Inc. Austin, TX, USA)	1.0	0.25 mM each
1.25X RT Primer (Applied Biosystems)	2.0	0.25 X
RNasin® Ribonuclease Inhibitor (40 U/ μ l, Promega, Corp. Madison, WI, USA; cat. no. N2115)	0.1	0.4 U/ μ l
Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT) (200 U/ μ l) (Invitrogen)	0.05	1 U/ μ l
Human Plasma RNA	0.25	-

[0120] PCR components were assembled on ice prior to the addition of the cDNA from the RT reactions (Table 30). PCRs were incubated in an ABI PRISM™ 7900HT Fast Real-Time PCR system (Applied Biosystems) at 95 °C for 1 minute, then for 50 cycles at 95 °C for 5 seconds and then at 60 °C for 30 seconds. Initial data analysis was done using the 7500 Fast System SDS V2.3 software (Applied Biosystems).

Table 30. Real-time PCR components

Component	μ l per 15 μ l rxn	Final Concentration
Nuclease-free water	6.1	-

MgCl ₂ (50mM)	1.5	5 mM
10X Platinum PCR Buffer, Minus Mg (Invitrogen Corp., Carlsbad, CA, USA)	1.5	1X
dNTP mix (2.5 mM each) (Ambion)	1.5	0.25 mM each
3X TaqMan Assay (Applied Biosystems)	2.0	0.4X
50X ROX Internal marker	0.3	1X
Platinum® Taq DNA Polymerase (5U/μl) (Invitrogen)	0.1	0.033 U/μl
cDNA from RT reaction	2.0	

[0121] The qRT-PCR data were initially assessed for outliers. All miRNAs in a given sample with raw Ct values of 50 were eliminated from further analysis. All data from samples with fewer than 150 miRNAs that had raw Ct values less than 50 were eliminated. The average raw Ct for 50 miRNAs that were detected in each plasma sample was calculated for each individual sample. The average Ct for a given sample was subtracted from the raw Ct values for each miRNA in the corresponding sample to produce a dCt for each miRNA that was detected. These normalized measures were used to identify miRNAs that were present at significantly different levels in the plasma samples from normal donors and lung cancer patients.

[0122] The average dCt values for each miRNA in the normal donor and lung cancer patient samples were calculated. The average dCt values for the lung cancer patient samples were subtracted from the average dCt values for the normal donors to determine the variance in the levels of the various miRNAs between the two patient sets. The student t-test was then used to evaluate the capacity of the various miRNAs to distinguish the plasmas of lung cancer patients and normal donors.

[0123] Table 31 provides dCt values for normal and cancer patients, as well as ddCt values for cancer vs normal samples for each miRNA.

Table 31. miRNA Biomarkers Differentially Expressed Between Plasma Samples of Lung Cancer Patients and Plasma Samples of Normal Patients.

miRNA	Normal Average	Normal SD	Cancer Average	Cancer SD	Cancer - Normal	p-value Cancer - Normal
miR-369-5p	6.85	0.42	10.19	1.67	-6.85	2.10E-01
miR-218	12.91	5.50	6.75	1.67	-6.16	1.02E-01
miR-489	12.17	0.91	6.84	0.53	-5.34	1.43E-03
miR-455	14.66	1.48	9.71	4.67	-4.96	1.54E-01
miR-511	9.32	2.05	4.74	0.49	-4.58	7.65E-03
miR-507	15.24	3.54	11.04	1.82	-4.20	4.08E-02
miR-516-3p	10.45	3.30	6.57	2.14	-3.88	4.48E-02
miR-448	16.11	3.36	12.34	2.25	-3.77	6.14E-02
miR-483	8.13	2.26	4.42	1.88	-3.71	1.34E-02
miR-122a	9.90	2.79	6.41	4.58	-3.49	2.21E-01
miR-383	10.16	2.98	6.94	1.41	-3.22	5.05E-02
miR-206	8.53	2.95	5.36	2.41	-3.18	7.67E-02
miR-34c	12.19	2.27	9.07	4.52	-3.11	1.55E-01
miR-375	5.04	1.54	2.14	2.43	-2.90	2.90E-02
miR-514	13.85	3.16	11.10	2.79	-2.75	1.51E-01
miR-452*	7.01	1.39	4.32	0.44	-2.70	1.27E-02
miR-302b*	15.04	5.63	12.35	1.98	-2.70	4.04E-01
miR-527	12.54	3.69	10.04	1.43	-2.50	3.25E-01
miR-340	2.28	0.47	4.64	0.86	-2.28	1.35E-01
miR-134	1.76	0.89	-0.34	0.72	-2.10	3.11E-03
miR-429	6.95	0.83	4.96	1.57	-1.99	2.06E-02
miR-31	6.99	1.52	5.01	1.96	-1.98	9.24E-02
miR-200c	2.49	1.02	0.51	0.37	-1.98	1.47E-03
miR-33	9.07	2.12	7.17	2.10	-1.90	1.85E-01
miR-152	1.53	1.25	-0.30	0.37	-1.83	1.03E-02
miR-518b	8.88	2.90	7.06	2.03	-1.82	2.57E-01
miR-452	7.83	1.23	6.13	1.72	-1.70	8.72E-02
miR-146a	-4.76	1.25	-6.39	0.44	-1.63	2.02E-02
miR-155	-0.43	1.13	-2.02	0.10	-1.59	2.32E-02
miR-10b	4.69	1.64	3.11	0.93	-1.58	1.15E-01
miR-34a	10.13	2.33	8.56	2.13	-1.56	2.63E-01
miR-210	2.13	0.79	0.60	1.52	-1.52	4.55E-02
miR-213	6.79	0.84	5.30	1.29	-1.49	7.16E-02
miR-346	9.17	2.33	7.72	4.58	-1.45	4.86E-01
miR-362	3.67	0.66	2.30	0.81	-1.37	8.91E-03
miR-214	5.14	2.14	3.79	1.63	-1.34	2.68E-01

miRNA	Normal Average	Normal SD	Cancer Average	Cancer SD	Cancer - Normal	p-value Cancer - Normal
miR-422a	5.79	0.87	4.49	1.28	-1.31	5.97E-02
miR-181a	2.43	1.16	1.20	0.82	-1.23	6.97E-02
miR-127	1.77	1.96	0.55	1.59	-1.22	2.78E-01
miR-520d	14.69	3.20	13.48	3.92	-1.21	6.49E-01
miR-135a	6.23	0.86	5.02	0.62	-1.21	6.96E-02
miR-191	-3.94	1.09	-5.15	0.46	-1.21	4.41E-02
miR-432	0.02	1.02	-1.18	1.11	-1.20	9.43E-02
miR-449	7.47	1.38	6.27	2.05	-1.20	3.26E-01
miR-328	0.58	0.80	-0.60	0.49	-1.19	1.52E-02
miR-486	-5.54	2.54	-6.70	0.92	-1.16	3.55E-01
miR-149	10.20	3.16	9.07	2.96	-1.13	6.00E-01
miR-150	-2.32	1.89	-3.44	1.25	-1.12	2.77E-01
miR-197	-0.34	0.93	-1.46	0.81	-1.12	5.62E-02
miR-146b	-2.33	0.62	-3.37	0.77	-1.04	2.67E-02
miR-30a-3p	3.14	0.69	2.11	1.16	-1.03	8.18E-02
miR-181b	1.52	0.45	0.50	1.15	-1.02	5.56E-02
miR-425	1.78	0.70	0.79	0.67	-1.00	3.33E-02
miR-376a	2.69	1.08	1.70	0.60	-0.99	9.58E-02
miR-382	2.21	1.45	1.29	0.69	-0.91	2.23E-01
miR-223	-7.86	1.23	-8.73	0.72	-0.87	1.91E-01
miR-99b	2.34	1.27	1.48	0.78	-0.86	2.55E-01
miR-28	1.11	1.32	0.29	0.34	-0.82	2.10E-01
miR-497	5.55	0.77	4.74	0.71	-0.82	1.28E-01
miR-142-5p	0.49	2.86	-0.32	0.72	-0.81	5.54E-01
miR-186	-1.82	0.85	-2.60	0.64	-0.78	1.16E-01
miR-369-3p	8.03	1.05	7.27	1.98	-0.76	4.60E-01
miR-92	-6.18	1.79	-6.94	1.09	-0.76	4.67E-01
miR-224	3.33	0.97	2.58	1.24	-0.75	2.87E-01
miR-100	4.75	0.33	4.00	1.20	-0.75	1.44E-01
miR-106b	-1.13	0.59	-1.85	0.90	-0.72	1.21E-01
miR-183	6.87	2.37	6.16	1.37	-0.71	5.62E-01
miR-24	-5.08	1.13	-5.77	1.13	-0.70	3.17E-01
miR-203	8.21	0.74	7.51	2.59	-0.69	5.42E-01
miR-99a	5.24	0.93	4.56	0.49	-0.68	2.16E-01
miR-7	4.02	1.35	3.38	1.46	-0.64	4.79E-01
miR-17-5p	-1.80	0.63	-2.42	0.79	-0.62	1.60E-01
miR-342	-0.72	0.82	-1.34	0.43	-0.62	1.58E-01
miR-129	13.07	2.15	12.45	1.91	-0.62	6.98E-01
miR-330	5.38	0.79	4.78	1.60	-0.59	4.10E-01

miRNA	Normal Average	Normal SD	Cancer Average	Cancer SD	Cancer - Normal	p-value Cancer - Normal
miR-324-3p	0.45	1.02	-0.14	0.31	-0.59	2.41E-01
miR-301	0.88	0.46	0.29	0.74	-0.58	1.43E-01
miR-132	1.77	0.61	1.20	0.97	-0.57	2.38E-01
miR-211	8.81	1.68	8.25	2.49	-0.56	6.63E-01
miR-30a-5p	-2.45	0.36	-2.98	0.32	-0.54	2.34E-02
miR-18b	9.78	0.54	9.25	3.12	-0.53	7.09E-01
miR-324-5p	2.09	1.01	1.56	0.85	-0.53	3.65E-01
let-7c	-0.48	1.01	-1.00	0.41	-0.52	3.04E-01
miR-190	3.86	0.80	3.36	0.40	-0.51	2.23E-01
miR-196b	2.60	1.05	2.11	0.73	-0.50	3.82E-01
miR-133b	1.01	1.20	0.52	0.53	-0.49	4.21E-01
miR-181c	5.55	0.88	5.09	0.60	-0.47	3.29E-01
miR-501	5.00	1.36	4.53	1.84	-0.47	6.40E-01
miR-370	3.92	0.84	3.47	0.88	-0.45	3.89E-01
miR-19b	-5.57	1.30	-6.01	1.38	-0.44	5.86E-01
miR-10a	3.84	0.62	3.41	1.53	-0.44	5.10E-01
miR-222	-2.92	0.90	-3.34	0.82	-0.42	4.27E-01
miR-26b	-4.90	0.72	-5.24	0.51	-0.34	3.97E-01
miR-345	0.92	1.01	0.60	0.78	-0.33	5.90E-01
miR-19a	-3.23	1.52	-3.53	1.57	-0.30	7.49E-01
miR-192	0.86	1.43	0.61	0.99	-0.25	7.46E-01
miR-148a	2.33	0.50	2.09	0.41	-0.24	4.32E-01
miR-296	4.41	1.53	4.17	0.62	-0.24	7.51E-01
miR-193a	9.83	2.32	9.62	4.57	-0.21	9.19E-01
miR-21	-2.95	0.50	-3.12	0.07	-0.18	4.58E-01
miR-422b	3.22	0.91	3.07	1.32	-0.14	8.25E-01
miR-18a*	3.09	1.61	2.96	1.66	-0.14	8.89E-01
miR-30c	-3.19	0.47	-3.32	1.17	-0.13	7.89E-01
miR-126*	-3.91	1.02	-4.02	0.52	-0.12	8.18E-01
miR-125b	3.64	0.79	3.53	1.16	-0.11	8.52E-01
miR-105	12.26	1.93	12.16	5.18	-0.11	9.64E-01
miR-140	-1.34	0.99	-1.44	0.90	-0.10	8.62E-01
miR-107	3.93	0.64	3.85	0.86	-0.08	8.62E-01
miR-500	7.31	2.33	7.23	3.62	-0.08	9.65E-01
miR-93	-4.61	0.86	-4.68	0.53	-0.07	8.88E-01
miR-20b	-1.78	1.20	-1.85	0.85	-0.07	9.15E-01
miR-126	-4.72	0.86	-4.78	0.89	-0.06	9.03E-01
miR-151	-0.51	0.79	-0.54	2.53	-0.03	9.75E-01
miR-361	3.00	1.22	2.97	1.41	-0.02	9.75E-01

miRNA	Normal Average	Normal SD	Cancer Average	Cancer SD	Cancer - Normal	p-value Cancer - Normal
miR-30d	-1.94	0.82	-1.93	0.31	0.01	9.87E-01
miR-320	-2.50	1.07	-2.49	1.09	0.02	9.81E-01
miR-202	8.25	3.09	8.27	3.94	0.02	9.92E-01
miR-20a	-5.91	1.13	-5.88	1.36	0.03	9.64E-01
miR-26a	-6.16	0.87	-6.12	0.72	0.04	9.38E-01
miR-98	1.66	1.15	1.73	0.64	0.06	9.24E-01
miR-18a	2.64	0.70	2.74	0.46	0.09	8.00E-01
miR-188	7.41	1.66	7.51	1.88	0.10	9.28E-01
miR-505	2.80	0.39	2.92	1.00	0.11	8.00E-01
miR-30e-5p	1.32	1.47	1.48	0.98	0.16	8.37E-01
miR-194	4.30	0.93	4.47	1.36	0.17	8.07E-01
miR-365	3.91	1.26	4.09	1.29	0.18	8.31E-01
miR-423	2.31	1.08	2.55	0.66	0.24	6.72E-01
miR-331	-0.10	0.70	0.15	1.85	0.25	7.48E-01
miR-30e-3p	1.76	0.63	2.02	1.26	0.25	6.55E-01
miR-130b	0.91	0.77	1.16	0.46	0.25	5.72E-01
miR-29c	0.90	1.10	1.16	0.74	0.26	6.58E-01
miR-433	2.89	1.05	3.19	2.46	0.30	7.78E-01
miR-145	1.87	0.73	2.17	2.45	0.30	7.63E-01
miR-142-3p	-4.16	0.78	-3.85	0.87	0.31	5.37E-01
miR-374	-2.07	0.51	-1.76	1.15	0.31	5.33E-01
miR-9*	5.91	0.33	6.23	0.52	0.31	2.74E-01
miR-25	-0.93	1.05	-0.58	0.54	0.35	5.12E-01
miR-410	3.42	2.92	3.78	2.10	0.36	8.18E-01
let-7f	-1.12	0.55	-0.73	0.97	0.39	4.00E-01
miR-485-3p	2.40	1.40	2.82	1.59	0.43	6.33E-01
miR-485-5p	4.80	0.82	5.24	0.99	0.44	4.51E-01
let-7a	-2.14	0.91	-1.65	0.71	0.49	3.39E-01
miR-29a	-0.12	0.68	0.38	0.78	0.50	2.68E-01
miR-453	13.41	3.43	13.93	2.98	0.53	8.04E-01
miR-30b	-3.88	0.51	-3.35	1.07	0.53	2.74E-01
miR-125a	0.10	1.19	0.65	0.45	0.55	3.53E-01
miR-432*	10.92	1.99	11.48	5.95	0.56	8.47E-01
miR-103	-1.67	0.97	-1.08	0.23	0.59	2.71E-01
miR-16	-9.07	1.52	-8.44	1.42	0.63	4.82E-01
miR-379	6.37	3.12	7.01	4.44	0.64	7.83E-01
miR-215	6.65	1.41	7.30	6.01	0.65	7.84E-01
miR-22	2.52	0.76	3.19	0.57	0.67	1.30E-01
miR-335	0.72	0.68	1.46	0.91	0.74	1.35E-01

miRNA	Normal Average	Normal SD	Cancer Average	Cancer SD	Cancer - Normal	p-value Cancer - Normal
miR-490	9.31	1.44	10.05	1.56	0.74	4.34E-01
miR-181d	0.68	1.25	1.47	1.84	0.79	3.97E-01
miR-15a	0.92	0.78	1.72	0.60	0.80	8.48E-02
miR-185	4.09	1.53	4.90	0.58	0.81	2.90E-01
miR-27b	1.86	1.51	2.79	0.74	0.93	2.36E-01
miR-339	1.73	0.61	2.66	1.03	0.93	7.71E-02
miR-23a	1.66	0.75	2.60	0.84	0.95	6.77E-02
miR-15b	-1.55	0.50	-0.60	1.04	0.95	5.97E-02
miR-493-3p	6.38	1.09	7.35	2.74	0.97	4.11E-01
miR-148b	2.29	0.96	3.27	0.56	0.98	9.89E-02
let-7i	-0.94	0.97	0.08	1.19	1.03	1.31E-01
miR-377	11.85	2.75	12.88	2.85	1.03	5.44E-01
let-7g	-2.13	0.52	-1.07	0.78	1.06	1.76E-02
miR-27a	0.11	1.17	1.18	0.85	1.08	1.10E-01
miR-130a	-0.14	1.58	0.96	1.22	1.10	2.26E-01
miR-182	2.05	2.15	3.15	1.19	1.10	3.77E-01
miR-196a	5.16	1.43	6.29	1.66	1.13	2.33E-01
miR-326	5.04	0.75	6.19	1.43	1.15	9.74E-02
let-7d	0.36	1.07	1.55	0.68	1.19	5.48E-02
miR-323	5.47	1.14	6.70	1.09	1.23	1.15E-01
let-7b	-3.10	1.27	-1.85	3.05	1.25	3.48E-01
miR-23b	2.86	1.15	4.12	1.02	1.26	7.88E-02
miR-106a	-1.31	0.69	-0.04	0.68	1.27	1.60E-02
let-7e	5.75	0.69	7.02	2.16	1.27	1.73E-01
miR-451	-4.08	2.18	-2.78	1.15	1.31	2.54E-01
miR-212	5.96	1.34	7.27	3.00	1.31	3.27E-01
miR-101	0.47	1.34	1.86	0.38	1.39	7.90E-02
miR-496	7.36	0.64	8.77	3.00	1.41	2.47E-01
miR-17-3p	5.27	0.87	6.69	4.64	1.42	4.40E-01
miR-221	-2.94	1.90	-1.51	1.24	1.43	1.73E-01
miR-1	3.09	2.05	4.64	4.56	1.55	4.39E-01
miR-491	4.46	0.49	6.05	5.13	1.59	4.64E-01
miR-32	1.95	1.13	3.55	1.47	1.60	7.20E-02
miR-502	6.90	1.61	8.66	2.23	1.76	1.41E-01
miR-136	12.12	2.23	13.90	1.98	1.78	2.19E-01
miR-199a*	-2.10	0.91	-0.14	1.13	1.96	7.48E-03
miR-204	5.99	1.29	8.02	2.36	2.03	8.27E-02
miR-539	1.85	0.62	3.93	1.95	2.08	2.29E-02
miR-198	9.75	2.43	11.97	1.74	2.22	2.07E-01

miRNA	Normal Average	Normal SD	Cancer Average	Cancer SD	Cancer - Normal	p-value Cancer - Normal
miR-29b	4.50	0.61	6.79	4.32	2.28	1.86E-01
miR-139	4.52	1.27	6.81	4.87	2.30	2.91E-01
miR-199a	4.90	1.50	7.44	2.03	2.54	3.11E-02
miR-195	-1.55	1.46	1.00	1.84	2.55	2.27E-02
miR-378	5.92	0.88	8.54	4.88	2.62	1.84E-01
miR-133a	4.04	1.21	6.77	3.37	2.73	9.58E-02
miR-493	6.03	0.50	8.91	3.06	2.88	4.81E-02
miR-199b	7.40	3.00	10.32	4.75	2.92	2.64E-01
miR-487b	2.88	1.44	5.84	2.27	2.95	3.48E-02
miR-518d	12.26	2.54	15.45	4.24	3.19	1.71E-01
miR-494	5.25	0.65	9.43	4.10	4.18	3.49E-02
miR-519d	10.41	3.51	15.51	2.22	5.10	5.16E-02
miR-542-3p	9.78	1.61	17.13	0.94	7.35	3.14E-03

Example 8. Combinations of miRNAs that Distinguish Plasma from Lung Cancer Patients and Plasma from Normal Patients

[0124] The un-normalized qRT-PCR data, generated as described in Example 6, were used to calculate dCt values for each pair of miRNAs that was quantified. The dCt results for the various miRNA pairs in the lung cancer and normal donor plasma samples were analyzed using Receiver-Operator Characteristic (ROC) analysis to determine which miRNA pairs have the capacity to distinguish plasma samples from lung cancer patients and those from normal patients. One miRNA pair (let-7c:miR-326) accurately classified the 14 lung cancer patient samples and 12 normal donor samples that were analyzed (Table 32). Twenty additional miRNA pairs accurately classified all but one of the 26 samples (ROC AUC >0.98) and 221 miRNA pairs had an ROC AUC score of at least 0.93 (Table 32). 166 independent miRNAs were included at least once in the 221 miRNA pairs of Table 32 (Table 33). Numerous miRNAs were used in multiple biomarkers pairs, indicating the strength of their variable levels in the plasmas of lung cancer patients and normal donors.

Table 32. Biomarker pairs that can be used to identify plasma from lung cancer patients.

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
let-7c,miR-326	-2.581	-4.107	1.227	-1.526	0.977	1.000	3.76E-06
miR-326,miR-7	3.787	1.066	1.573	-2.721	1.292	0.994	5.72E-07
miR-206,miR-491	-4.433	1.933	2.352	6.366	0.706	0.994	5.58E-06
miR-339,miR-375	4.387	-0.555	2.34	-4.942	1.279	0.994	5.77E-06
miR-30a-3p,miR-326	-2.505	-1.602	0.847	0.903	0.825	0.988	8.26E-08
miR-151,miR-206	4.145	-6.025	1.923	-10.17	1.055	0.988	8.06E-07
miR-10b,miR-30b	-2.478	4.175	1.094	6.654	0.711	0.988	5.06E-07
miR-330,miR-375	3.556	4.056	1.129	0.5	1.444	0.988	8.51E-07
miR-134,miR-206	4.061	-3.335	2.143	-7.395	1.012	0.988	4.50E-06
miR-432*,miR-491	-2.755	4.087	1.122	6.842	1.093	0.988	1.66E-06
miR-181d,miR-375	3.773	-3.266	1.845	-7.039	1.514	0.988	6.74E-06
miR-191,miR-200c	2.258	-4.345	1.38	-6.603	0.576	0.988	2.71E-05
miR-23a,miR-326	-2.005	-5.99	0.67	-3.985	1.377	0.988	3.27E-04
miR-181a,miR-218	3.525	-6.162	1.367	-9.687	1.232	0.982	3.87E-07
miR-151,miR-218	3.852	-7.152	1.693	-11.004	1.214	0.982	6.72E-07
miR-491,miR-512-5p	3.294	-4.693	1.361	-7.987	1.384	0.982	3.08E-06
miR-326,miR-375	4.862	1.86	2.118	-3.003	1.751	0.982	1.27E-06
miR-200c,miR-326	-3.247	-2.363	1.432	0.884	1.301	0.982	3.03E-06
miR-20b,miR-30b	-1.7	0.935	0.791	2.635	0.818	0.982	1.88E-05
miR-491,miR-516-3p	2.483	-1.823	1.497	-4.306	0.743	0.982	2.50E-05
miR-125b,miR-146a	-2.414	6.68	1.271	9.094	1.453	0.982	1.87E-04
miR-16,miR-487b	-3.219	-12.463	1.565	-9.244	0.902	0.976	1.70E-06
miR-326,miR-345	2.189	3.358	1.025	1.17	0.62	0.976	1.06E-06
miR-140,miR-192	2.354	0.428	1.127	-1.926	0.75	0.976	1.88E-06
miR-383,miR-491	-3.309	3.498	1.557	6.808	1.042	0.976	1.47E-06
miR-339,miR-7	3.405	-1.35	1.636	-4.756	1.201	0.976	2.90E-06
miR-375,miR-99b	-4.127	0.126	2.101	4.253	1.365	0.976	4.27E-06
miR-199a,miR-375	3.998	2.022	2.221	-1.976	1.361	0.976	1.22E-05
miR-206,miR-376a	-3.632	4.069	2.158	7.701	1.13	0.976	2.22E-05
miR-375,miR-505	-3.565	1.066	2.078	4.63	1.299	0.976	2.40E-05
miR-200c,miR-301	-2.181	0.736	1.467	2.917	0.943	0.976	1.44E-04
miR-451,miR-487b	-3.483	-10.526	1.419	-7.043	1.167	0.970	4.25E-07
miR-218,miR-326	-4.775	1.758	2.029	6.533	1.644	0.970	7.52E-07

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-206,miR-213	-3.739	-0.548	1.432	3.191	1.495	0.970	1.28E-06
miR-206,miR-422b	-2.932	4.567	1.453	7.499	0.656	0.970	1.94E-06
miR-192,miR-326	-3.958	-4.625	1.422	-0.667	1.79	0.970	4.07E-06
miR-205,miR-326	-5.331	-0.247	2.68	5.084	1.383	0.970	2.42E-06
miR-218,miR-491	-4	3.237	1.991	7.237	1.131	0.970	2.33E-06
miR-206,miR-361	-3.644	3.694	1.846	7.338	0.941	0.970	2.68E-06
miR-206,miR-410	-4.117	3.088	2.074	7.205	1.201	0.970	2.83E-06
miR-30a-3p,miR-99b	-1.539	0.404	0.676	1.943	0.541	0.970	1.17E-06
miR-151,miR-383	3.021	-7.591	1.125	-10.612	1.322	0.970	3.05E-06
miR-218,miR-338	-3.544	2.533	1.997	6.076	0.871	0.970	1.03E-05
miR-212,miR-512-5p	2.338	-2.562	0.818	-4.899	1.165	0.970	1.20E-05
miR-375,miR-496	-3.513	-2.507	1.622	1.006	1.35	0.970	3.20E-06
miR-125b,miR-326	-3.819	-1.454	1.395	2.364	1.993	0.970	2.14E-05
miR-375,miR-433	-3.899	-0.297	2.138	3.602	1.456	0.970	1.38E-05
miR-361,miR-432*	2.177	-5.657	0.981	-7.833	0.996	0.970	1.04E-05
miR-142-5p,miR-375	3.709	-3.011	1.817	-6.72	1.587	0.970	1.03E-05
miR-126*,miR-192	3.618	-2.726	3.7	-6.344	1.554	0.970	3.71E-04
miR-192,miR-339	-3.212	-2.209	1.267	1.003	1.199	0.964	7.76E-07
miR-192,miR-196b	-1.673	-3.784	0.693	-2.111	0.672	0.964	2.03E-06
miR-7,miR-99b	-3.219	0.94	1.324	4.16	1.244	0.964	1.39E-06
miR-213,miR-31	2.851	0.485	1.097	-2.366	1.357	0.964	8.52E-06
miR-16,miR-30b	-2.854	-6.488	1.137	-3.634	1.28	0.964	5.02E-06
miR-137,miR-151	-2.98	8.385	1.354	11.365	1.186	0.964	3.56E-06
miR-218,miR-361	-3.403	4.699	1.854	8.102	1.133	0.964	9.35E-06
miR-190,miR-326	-2.875	-2.216	1.149	0.659	1.439	0.964	1.60E-05
miR-204,miR-375	3.036	2.9	1.546	-0.136	1.093	0.964	5.75E-06
miR-10b,miR-191	-2.706	4.092	0.827	6.797	1.602	0.964	7.67E-05
let-7i,miR-206	2.852	-7.226	1.658	-10.078	0.941	0.964	1.89E-05
miR-200c,miR-339	-2.38	0.054	1.598	2.433	0.562	0.964	7.64E-05
miR-16,miR-191	-3.172	-6.452	0.997	-3.28	2.171	0.964	3.14E-04
miR-15a,miR-375	3.933	-0.809	2.627	-4.741	1.514	0.964	1.04E-04
miR-17-5p,miR-30b	-1.179	0.021	0.599	1.2	0.724	0.964	1.98E-04
miR-206,miR-212	-3.234	-0.074	1.284	3.161	0.855	0.958	9.84E-08
miR-192,miR-30b	-2.691	2.046	1.119	4.738	0.968	0.958	8.39E-07
miR-148b,miR-192	2.441	1.001	1.169	-1.44	0.8	0.958	2.08E-06
miR-16,miR-24	-2.548	-5.478	1.194	-2.93	0.86	0.958	1.84E-06

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-206,miR-320	-3.19	7.179	1.448	10.369	1.082	0.958	1.33E-06
miR-326,miR-34c	2.292	0.694	1.193	-1.598	0.666	0.958	4.24E-06
miR-191,miR-7	3.012	-5.642	1.176	-8.654	1.338	0.958	4.22E-06
miR-218,miR-410	-3.67	4.367	1.98	8.037	0.979	0.958	6.12E-06
miR-181a,miR-206	3.917	-4.968	1.895	-8.886	1.362	0.958	2.92E-06
miR-206,miR-370	-3.847	0.507	1.493	4.354	1.829	0.958	8.63E-06
miR-222,miR-30a-3p	1.917	-4.407	0.844	-6.324	0.879	0.958	9.47E-06
miR-326,miR-423	1.435	3.585	0.787	2.15	0.45	0.958	8.83E-06
miR-137,miR-491	-3.268	4.292	1.736	7.56	1.117	0.958	7.53E-06
miR-192,miR-342	-2.906	-0.395	1.167	2.511	1.525	0.958	2.65E-05
miR-206,miR-382	-3.617	3.394	1.975	7.011	1.325	0.958	1.25E-05
miR-375,miR-423	-3.732	1.792	2.137	5.525	1.133	0.958	1.41E-05
miR-17-5p,miR-487b	-1.765	-5.972	0.75	-4.206	0.929	0.958	3.09E-05
miR-326,miR-511	3.331	-1.766	1.725	-5.097	1.433	0.958	1.60E-05
miR-155,miR-218	3.407	-4.95	1.869	-8.357	1.487	0.958	2.72E-05
miR-502,miR-512-5p	2.078	-3.048	0.794	-5.126	1.254	0.958	1.02E-04
miR-326,miR-451	4.483	11.283	1.583	6.801	2.924	0.958	2.66E-04
miR-192,miR-324-3p	-2.747	-1.537	0.913	1.211	1.871	0.958	3.03E-04
miR-200c,miR-222	-2.659	3.647	1.719	6.306	1.268	0.958	1.44E-04
miR-191,miR-19b	1.962	2.879	0.738	0.916	1.513	0.958	9.08E-04
miR-125b,miR-30d	-1.858	4.974	0.794	6.832	1.528	0.958	1.60E-03
miR-126*,miR-375	4.818	-5.343	4.264	-10.161	1.649	0.958	1.12E-03
miR-17-3p,miR-326	-2.159	-0.548	1.082	1.611	0.426	0.952	2.36E-06
miR-31,miR-326	-4.124	0.729	1.784	4.853	1.56	0.952	1.69E-06
miR-151,miR-31	3.261	-6.084	1.107	-9.346	1.535	0.952	5.98E-06
miR-192,miR-487b	-3.021	-3.844	1.462	-0.824	1.091	0.952	3.49E-06
miR-21,miR-326	-2.015	-8.794	1.076	-6.779	0.525	0.952	5.34E-06
miR-30a-5p,miR-326	-2.135	-7.665	1.208	-5.53	0.488	0.952	1.07E-05
miR-323,miR-375	3.637	1.676	1.891	-1.962	1.188	0.952	5.25E-06
miR-212,miR-383	2.111	-1.492	1.042	-3.603	0.83	0.952	6.46E-06
miR-152,miR-29c	1.741	3.447	0.87	1.707	0.667	0.952	6.28E-06
miR-206,miR-338	-3.801	1.295	2.033	5.095	1.204	0.952	6.79E-06
miR-218,miR-222	-3.952	7.905	1.771	11.856	1.9	0.952	1.57E-05
miR-192,miR-221	-2.621	3.067	1.408	5.689	0.997	0.952	1.21E-05
miR-132,miR-326	-2.197	-2.749	1.28	-0.552	0.603	0.952	1.60E-05
miR-375,miR-485-5p	-3.762	-2.511	1.824	1.251	1.579	0.952	8.36E-06

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-197,miR-491	-2.634	-6.586	1.654	-3.951	0.759	0.952	3.87E-05
miR-132,miR-375	3.015	-0.961	1.801	-3.975	1.139	0.952	3.37E-05
miR-21,miR-375	3.281	-6.749	1.711	-10.03	1.547	0.952	3.00E-05
miR-181c,miR-200c	1.796	-0.508	1.234	-2.304	0.667	0.952	1.28E-04
miR-30a-3p,miR-375	2.73	0.305	1.903	-2.425	1.032	0.952	1.51E-04
miR-16,miR-20a	-1.497	-4.405	1.106	-2.908	0.53	0.952	2.39E-04
miR-191,miR-93	1.725	1.202	0.903	-0.523	0.551	0.946	5.38E-06
miR-181a,miR-210	1.367	-0.524	0.594	-1.891	0.61	0.946	6.88E-06
miR-210,miR-218	2.356	-5.643	1.168	-7.998	0.935	0.946	7.11E-06
miR-410,miR-432*	2.392	-5.266	1.175	-7.658	0.97	0.946	7.44E-06
miR-191,miR-451	3.494	4.575	1.087	1.082	2.035	0.946	6.46E-05
miR-181c,miR-192	2.86	1.754	1.253	-1.106	1.432	0.946	2.10E-05
miR-134,miR-137	2.895	-5.694	1.532	-8.59	1.216	0.946	1.66E-05
miR-186,miR-29c	2.228	0.124	1.076	-2.104	1.171	0.946	4.64E-05
miR-146a,miR-192	2.468	-3.358	1.432	-5.826	1.126	0.946	5.23E-05
miR-181a,miR-29c	2.384	2.189	0.914	-0.195	1.856	0.946	9.90E-04
miR-100,miR-326	-3.014	-1.279	1.351	1.735	1.035	0.946	1.25E-06
miR-218,miR-422b	-2.534	5.705	1.053	8.238	1.145	0.946	6.35E-06
miR-192,miR-223	-2.369	5.019	0.972	7.388	1.024	0.946	3.92E-06
miR-30b,miR-451	3.176	4.612	1.301	1.436	1.403	0.946	4.81E-06
miR-31,miR-410	-3.234	3.147	1.637	6.381	1.198	0.946	6.09E-06
miR-106b,miR-326	-2.235	-7.022	1.197	-4.787	0.743	0.946	7.70E-06
miR-29c,miR-326	-3.474	-6.488	1.435	-3.013	1.686	0.946	1.27E-05
miR-10b,miR-487b	-2.797	-1.88	1.302	0.917	1.22	0.946	8.40E-06
miR-10b,miR-28	-2.3	-0.739	1.226	1.561	0.788	0.946	7.86E-06
miR-205,miR-331	-5.304	1.401	3.03	6.705	1.702	0.946	1.48E-05
let-7c,miR-222	-1.993	1.903	1.136	3.896	0.738	0.946	2.00E-05
miR-218,miR-29b	-2.835	4.347	1.61	7.182	1.007	0.946	1.71E-05
miR-31,miR-362	-2.627	0.743	1.423	3.37	1.125	0.946	2.24E-05
miR-106b,miR-186	-0.988	-0.658	0.548	0.33	0.429	0.946	2.89E-05
miR-215,miR-342	-3.483	4.038	2.027	7.522	1.365	0.946	2.90E-05
miR-192,miR-505	-2.494	-1.587	1.236	0.907	1.301	0.946	4.84E-05
miR-125b,miR-30e-3p	-2.56	0.175	1.35	2.735	1.244	0.946	3.94E-05
miR-375,miR-491	-4.588	-0.668	2.409	3.92	2.334	0.946	5.27E-05
miR-324-5p,miR-375	3.852	-0.507	2.448	-4.36	1.44	0.946	6.05E-05
miR-101,miR-326	-3.63	-6.399	1.624	-2.768	2.237	0.946	1.53E-04

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-29c,miR-99b	-2.508	-4.481	1.264	-1.973	1.444	0.946	1.15E-04
miR-375,miR-379	-3.982	-2.067	2.425	1.915	1.784	0.946	7.08E-05
miR-206,miR-326	-5.008	0.67	2.306	5.677	1.724	0.940	1.67E-06
miR-181a,miR-31	3.034	-5.027	1.277	-8.061	1.332	0.940	5.11E-06
miR-10a,miR-222	-2.167	4.428	1.018	6.594	0.809	0.940	3.13E-06
miR-218,miR-376a	-3.22	5.238	1.627	8.459	0.999	0.940	3.32E-06
miR-145,miR-218	3.629	-5.391	1.582	-9.02	1.532	0.940	4.30E-06
miR-24,miR-451	2.87	3.601	1.31	0.731	1.126	0.940	3.36E-06
miR-375,miR-485-3p	-3.365	-0.624	1.221	2.741	1.579	0.940	6.38E-06
miR-218,miR-99b	-3.663	3.97	1.668	7.633	1.624	0.940	8.40E-06
miR-326,miR-486	3.481	10.585	1.765	7.104	1.296	0.940	6.30E-06
miR-137,miR-181a	-2.752	7.328	1.273	10.08	1.21	0.940	8.55E-06
let-7c,miR-99b	-1.615	-2.1	0.74	-0.485	0.77	0.940	1.62E-05
miR-155,miR-31	2.902	-3.715	1.284	-6.617	1.473	0.940	2.47E-05
miR-192,miR-28	-2.482	-2.65	1.412	-0.168	0.932	0.940	2.03E-05
miR-15b,miR-16	2.205	7.077	1.373	4.872	0.629	0.940	3.50E-05
miR-197,miR-410	-2.319	-5.43	1.288	-3.112	0.883	0.940	1.68E-05
miR-134,miR-218	3.661	-4.613	2.302	-8.275	1.111	0.940	4.05E-05
miR-100,miR-181a	-1.924	3.02	0.886	4.943	1.015	0.940	4.01E-05
miR-101,miR-191	-2.697	0.309	1.415	3.006	1.265	0.940	3.00E-05
miR-19a,miR-24	-1.35	-0.546	0.796	0.804	0.512	0.940	3.00E-05
miR-29a,miR-326	-3.128	-6.507	1.551	-3.379	1.528	0.940	2.89E-05
let-7i,miR-151	-1.293	-1.201	0.745	0.092	0.498	0.940	2.49E-05
miR-151,miR-197	2.346	2.493	1.179	0.147	1.148	0.940	3.14E-05
miR-383,miR-410	-2.994	4.654	1.535	7.647	1.457	0.940	3.37E-05
miR-146b,miR-192	2.059	-1.737	1.197	-3.795	0.911	0.940	4.61E-05
miR-215,miR-324-3p	-3.278	2.896	1.712	6.174	1.654	0.940	4.84E-05
miR-361,miR-383	2.52	-5.26	1.328	-7.78	1.254	0.940	4.62E-05
miR-192,miR-328	-3.071	-1.678	1.334	1.393	1.842	0.940	1.14E-04
miR-213,miR-215	3.466	1.635	2.076	-1.831	1.549	0.940	6.12E-05
miR-21,miR-222	-1.427	-2.784	0.916	-1.358	0.617	0.940	9.66E-05
miR-196b,miR-326	-1.71	-0.879	1.088	0.831	0.754	0.940	9.57E-05
miR-200c,miR-342	-1.898	1.868	1.236	3.765	0.816	0.940	1.08E-04
miR-133a,miR-375	3.537	1.484	2.141	-2.053	1.794	0.940	1.20E-04
miR-342,miR-451	3.133	7.053	1.352	3.92	2.104	0.940	3.12E-04
miR-205,miR-362	-3.553	-0.331	2.377	3.222	1.574	0.940	1.47E-04

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-186,miR-200c	2.001	-4.001	1.403	-6.002	0.877	0.940	2.13E-04
miR-107,miR-375	3.558	1.632	2.537	-1.926	1.628	0.940	2.72E-04
miR-200c,miR-328	-2.178	0.584	1.518	2.762	1.185	0.940	4.09E-04
miR-19b,miR-326	-2.951	-9.587	1.206	-6.635	2.482	0.940	1.84E-03
miR-16,miR-331	-2.948	-11.052	1.597	-8.104	0.861	0.935	6.96E-06
miR-223,miR-451	2.801	1.638	0.975	-1.163	1.402	0.935	1.26E-05
miR-31,miR-99b	-3.157	2.735	1.402	5.893	1.388	0.935	6.80E-06
miR-375,miR-487b	-3.972	-0.895	1.313	3.076	2.14	0.935	2.81E-05
miR-186,miR-205	4.5	-5.765	2.4	-10.265	1.736	0.935	1.20E-05
miR-206,miR-485-5p	-3.558	0.058	1.661	3.616	1.615	0.935	1.17E-05
miR-222,miR-31	3.536	-6.738	1.694	-10.274	1.586	0.935	1.24E-05
miR-151,miR-92	2.026	6.479	1.107	4.454	0.743	0.935	1.26E-05
miR-10a,miR-328	-1.686	1.366	0.789	3.051	0.835	0.935	2.45E-05
miR-195,miR-487b	-2.601	-5.908	1.358	-3.307	1.115	0.935	1.67E-05
miR-191,miR-29c	2.485	-0.22	1.431	-2.705	0.879	0.935	1.96E-05
miR-205,miR-99b	-4.371	1.446	2.398	5.817	1.755	0.935	1.84E-05
miR-206,miR-222	-4.419	6.679	2.341	11.099	1.927	0.935	2.07E-05
miR-181d,miR-206	3.513	-5.896	1.726	-9.409	1.651	0.935	2.05E-05
miR-218,miR-370	-3.394	1.72	1.799	5.113	1.461	0.935	1.93E-05
miR-218,miR-493-3p	-2.778	0.871	1.528	3.649	1.126	0.935	1.95E-05
miR-137,miR-410	-2.952	5.448	1.531	8.4	1.279	0.935	1.69E-05
miR-195,miR-331	-2.462	-4.488	1.411	-2.026	1.03	0.935	3.22E-05
miR-192,miR-301	-3.203	-1.527	1.557	1.676	1.584	0.935	2.88E-05
miR-339,miR-451	3.615	8.867	1.329	5.252	2.141	0.935	8.19E-05
miR-19b,miR-451	1.531	1.697	0.544	0.165	0.93	0.935	1.03E-04
miR-19a,miR-451	1.52	3.055	0.686	1.535	0.798	0.935	3.59E-05
miR-326,miR-512-5p	4.016	-3.435	1.559	-7.451	2.353	0.935	7.74E-05
miR-196b,miR-375	3.076	0.915	1.564	-2.161	1.465	0.935	2.76E-05
miR-181d,miR-31	2.63	-5.955	1.059	-8.585	1.562	0.935	9.28E-05
miR-192,miR-222	-3.205	1.384	1.586	4.589	1.66	0.935	4.56E-05
miR-192,miR-199a*	-2.229	1.356	1.29	3.585	0.922	0.935	3.35E-05
miR-326,miR-93	2.714	7.909	1.289	5.195	1.551	0.935	8.99E-05
miR-140,miR-16	2.281	8.962	1.26	6.682	1.151	0.935	6.63E-05
miR-218,miR-485-5p	-3.228	1.313	1.954	4.541	1.459	0.935	6.98E-05
miR-20b,miR-24	-1.444	1.945	0.93	3.39	0.586	0.935	8.30E-05
miR-212,miR-218	2.772	-1.331	1.693	-4.103	1.284	0.935	8.33E-05

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-326,miR-452	2.775	0.254	1.849	-2.521	1.06	0.935	9.99E-05
miR-155,miR-375	3.651	-0.926	2.112	-4.577	2.093	0.935	1.92E-04
miR-16,miR-326	-4.161	-13.16	1.529	-8.999	3.11	0.935	6.99E-04
miR-10b,miR-140	-1.946	1.556	1.151	3.502	1.143	0.935	2.49E-04
miR-30e-5p,miR-326	-3.92	-5.871	1.737	-1.951	2.924	0.935	7.73E-04
miR-106b,miR-151	-1.372	-1.666	0.644	-0.294	1.029	0.935	8.49E-04
miR-19a,miR-326	-2.963	-8.228	1.155	-5.265	2.766	0.935	3.73E-03
miR-142-5p,miR-326	-1.734	-5.011	0.588	-3.277	1.806	0.935	7.19E-03
miR-192,miR-23b	-2.347	-1.078	1.249	1.269	0.812	0.929	7.97E-06
miR-206,miR-502	-3.155	0.295	1.473	3.45	1.348	0.929	7.32E-06
miR-31,miR-491	-3.549	1.992	1.585	5.541	1.728	0.929	1.73E-05
miR-218,miR-30e-3p	-3.216	3.476	1.363	6.692	1.647	0.929	2.35E-05
miR-140,miR-451	2.602	7.086	1.09	4.483	1.326	0.929	2.17E-05
miR-100,miR-222	-2.426	4.731	1.35	7.156	0.899	0.929	1.57E-05
miR-101,miR-223	-1.999	3.246	1.154	5.245	0.802	0.929	2.90E-05
miR-142-5p,miR-192	2.449	-0.385	1.386	-2.834	0.997	0.929	2.58E-05
miR-139,miR-326	-2.501	-1.393	1.065	1.108	1.365	0.929	4.43E-05
miR-146a,miR-29c	1.968	-1.496	1.171	-3.463	0.734	0.929	3.13E-05
miR-192,miR-26a	-2.301	4.736	1.342	7.037	0.878	0.929	2.73E-05
miR-107,miR-192	2.499	4.37	1.293	1.871	1.162	0.929	2.60E-05
miR-181b,miR-326	-2.137	-4.73	1.212	-2.593	0.92	0.929	3.31E-05
miR-134,miR-432*	2.471	-5.438	1.402	-7.909	1.143	0.929	4.75E-05
miR-205,miR-491	-3.969	0.959	2.2	4.928	2.007	0.929	6.83E-05
miR-213,miR-486	2.406	11.996	1.486	9.59	1.033	0.929	6.77E-05
miR-137,miR-376a	-2.466	6.429	1.378	8.895	1.249	0.929	7.22E-05
miR-31,miR-423	-2.688	4.314	1.33	7.002	1.571	0.929	0.000122073
miR-324-5p,miR-7	3.016	-1.338	1.805	-4.355	1.419	0.929	7.64E-05
miR-486,miR-99b	-2.515	-8.578	1.503	-6.063	1.237	0.929	9.41E-05
miR-218,miR-320	-2.867	8.191	1.85	11.059	1.253	0.929	0.000104295
miR-200c,miR-330	-1.822	-4.65	1.246	-2.828	0.706	0.929	0.000130877
miR-326,miR-505	1.692	3.039	0.894	1.347	0.984	0.929	0.000146787
miR-146a,miR-190	1.507	-5.628	0.904	-7.135	0.771	0.929	0.000118807
miR-193a,miR-205	2.861	1.862	2.048	-0.998	1.07	0.929	0.000189991
miR-181a,miR-30e-5p	2.829	1.572	1.16	-1.257	2.96	0.929	0.007701382
miR-101,miR-126*	-3.391	0.952	3.488	4.343	1.882	0.929	0.005004755
miR-125a,miR-192	2.927	1.308	1.296	-1.618	1.468	0.929	2.21E-05

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-181d,miR-218	3.27	-7.032	1.533	-10.302	1.608	0.929	2.33E-05
miR-152,miR-192	2.554	1.585	1.188	-0.969	1.229	0.929	1.87E-05
miR-151,miR-181b	1.275	-0.625	0.593	-1.9	0.71	0.929	6.69E-05
miR-151,miR-210	1.595	-1.581	0.889	-3.176	0.717	0.929	3.60E-05
miR-125a,miR-375	3.797	-1.21	1.887	-5.007	2.063	0.929	6.81E-05
miR-152,miR-375	3.688	-0.839	2.167	-4.527	1.849	0.929	9.32E-05
miR-148b,miR-375	3.548	-1.445	2.338	-4.994	1.958	0.929	0.000309212
miR-126*,miR-218	4.372	-9.198	3.315	-13.57	2.107	0.929	0.000499998
miR-10a,miR-375	2.646	0.396	1.888	-2.25	1.407	0.929	0.000437429
miR-10a,miR-326	-2.755	-1.582	1.273	1.173	1.089	0.923	3.89E-06
miR-206,miR-422a	-2.597	1.603	1.579	4.2	0.517	0.923	2.60E-05
miR-29c,miR-342	-2.125	-2.257	0.993	-0.132	1.043	0.923	2.25E-05
miR-181a,miR-383	2.794	-6.534	1.032	-9.328	1.574	0.923	4.92E-05
miR-383,miR-422b	-1.808	6.133	0.704	7.941	0.999	0.923	4.27E-05
miR-31,miR-370	-3.194	0.335	1.751	3.529	1.445	0.923	3.27E-05
miR-215,miR-326	-4.157	-0.192	2.214	3.964	1.874	0.923	2.60E-05
miR-31,miR-485-5p	-2.674	0.117	1.188	2.791	1.412	0.923	3.61E-05
miR-301,miR-375	4.116	-0.969	2.065	-5.085	2.058	0.923	3.67E-05
miR-181c,miR-375	3.806	-0.799	1.882	-4.605	1.943	0.923	4.03E-05
miR-31,miR-328	-3.054	3.676	1.547	6.731	1.591	0.923	5.26E-05
miR-181c,miR-29c	2.023	3.617	1.155	1.593	0.871	0.923	3.51E-05
miR-15b,miR-451	2.526	5.2	1.44	2.674	1.152	0.923	4.57E-05
miR-151,miR-486	2.619	5.229	1.453	2.611	1.164	0.923	3.27E-05
miR-328,miR-375	3.944	-0.83	2.069	-4.774	2.001	0.923	5.15E-05
miR-190,miR-191	-1.848	4.53	1.03	6.378	0.876	0.923	4.81E-05
miR-101,miR-27a	-2.24	-1.737	1.432	0.503	0.869	0.923	6.95E-05
miR-145,miR-206	3.964	-4.119	2.347	-8.082	1.759	0.923	5.43E-05
miR-218,miR-423	-3.125	5.534	1.432	8.659	1.84	0.923	0.000106511
miR-222,miR-29c	2.886	0.478	1.409	-2.408	1.619	0.923	8.32E-05
miR-192,miR-99b	-3.208	-2.619	1.437	0.589	1.892	0.923	0.000103222
miR-151,miR-516-3p	2.195	-5.916	1.207	-8.111	1.069	0.923	5.14E-05
miR-30d,miR-326	-2.095	-6.262	1.048	-4.167	1.152	0.923	7.70E-05
miR-330,miR-7	2.752	3.542	1.489	0.79	1.385	0.923	5.79E-05
miR-222,miR-486	2.893	4.575	1.712	1.682	1.277	0.923	5.27E-05
miR-127,miR-375	4.026	-1.329	2.107	-5.355	2.136	0.923	7.05E-05
miR-342,miR-375	3.749	-2.063	1.733	-5.812	2.143	0.923	8.35E-05

miRNA Biomarker Pair	Cancer - Normal	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC. ROC	Normal vs Cancer Assoc
miR-101,miR-152	-1.93	-3.358	1.118	-1.429	0.96	0.923	8.18E-05
miR-101,miR-27b	-2.523	-2.69	1.365	-0.168	1.345	0.923	8.59E-05
miR-218,miR-433	-3.174	3.518	1.711	6.691	1.747	0.923	0.000105844
miR-192,miR-330	-2.827	-7.014	1.517	-4.187	1.513	0.923	8.44E-05
miR-10b,miR-340	-2.212	-2.485	1.287	-0.273	1.109	0.923	8.74E-05
miR-145,miR-375	3.794	-1.357	1.909	-5.151	2.155	0.923	0.000102562
miR-206,miR-328	-3.938	3.617	2.349	7.556	1.945	0.923	9.47E-05
miR-206,miR-362	-3.489	0.705	2.113	4.194	1.582	0.923	7.12E-05
miR-192,miR-423	-2.756	-1.04	1.21	1.716	1.82	0.923	0.000275531
miR-151,miR-452	1.912	-5.102	1.386	-7.014	0.545	0.923	0.000173117
miR-148a,miR-148b	-0.854	-0.6	0.471	0.254	0.489	0.923	0.00015307
miR-215,miR-323	-3.348	0.04	2.186	3.387	1.463	0.923	0.000115248
miR-34c,miR-375	3.116	1.559	2.075	-1.556	1.288	0.923	0.000117925
miR-324-3p,miR-375	3.594	-0.985	2.145	-4.579	1.931	0.923	0.000151045
miR-335,miR-451	2.786	9.015	1.809	6.229	1.381	0.923	0.000174237
miR-125b,miR-342	-2.362	2.865	1.159	5.227	1.575	0.923	0.000356556
miR-190,miR-99b	-1.829	-0.13	0.914	1.699	1.205	0.923	0.000335029
miR-145,miR-192	3.033	1.177	1.511	-1.856	2.059	0.923	0.00042416
miR-10b,miR-342	-2.489	1.61	1.25	4.099	1.669	0.923	0.00038973
miR-222,miR-375	4.413	-3.601	2.351	-8.014	2.929	0.923	0.000411581
miR-27b,miR-375	4.227	-1.437	2.894	-5.663	2.24	0.923	0.000328816
miR-423,miR-486	2.046	6.999	1.224	4.954	1.273	0.923	0.000378623
miR-199b,miR-215	3.254	1.169	2.444	-2.085	1.495	0.923	0.000415598
miR-151,miR-375	4.072	-2.997	2.144	-7.069	2.945	0.923	0.000763553
miR-181d,miR-486	1.987	5.359	1.326	3.372	1.245	0.923	0.000628771
miR-191,miR-375	4.016	-4.431	2.375	-8.447	2.932	0.923	0.001046642
miR-146a,miR-200c	1.74	-5.62	1.544	-7.361	0.591	0.923	0.001134329
miR-23a,miR-375	3.246	-3.679	1.948	-6.926	2.409	0.923	0.001202938
miR-140,miR-375	3.331	-2.242	2.407	-5.572	2.026	0.923	0.000807039
miR-213,miR-29c	2.389	7.889	1.137	5.5	1.986	0.923	0.001863408
miR-18a,miR-326	-2.062	-1.256	0.943	0.806	1.935	0.923	0.00412067
let-7b,miR-99b	-1.295	-4.889	1.828	-3.594	0.388	0.923	0.021340617

Table 33. Biomarkers that can be used in combination to identify plasma from lung cancer patients, and the prevalence of each miRNA.

Biomarker	No. Used	Biomarker	No. Used	Biomarker	No. Used
miR-10b	65	miR-410	15	miR-425	7
miR-192	62	miR-188	14	miR-432*	7
miR-206	61	miR-193a	14	miR-103	6
miR-101	58	let-7g	13	miR-193b	6
miR-205	52	let-7i	13	miR-196a	6
miR-16	50	miR-140	13	miR-199a*	6
miR-151	44	miR-181b	13	miR-199b	6
miR-137	43	miR-25	13	miR-28	6
miR-215	43	miR-328	13	miR-30d	6
miR-181a	42	miR-133a	12	miR-330	6
miR-218	42	miR-150	12	miR-423	6
miR-126*	41	miR-17-5p	12	miR-433	6
miR-125b	39	miR-21	12	miR-485-5p	6
miR-326	39	miR-214	12	miR-20a	5
miR-100	38	miR-370	12	miR-23b	5
miR-31	36	miR-383	12	miR-26a	5
miR-197	35	miR-130b	11	miR-30b	5
miR-222	34	miR-199a	11	miR-30c	5
miR-191	32	miR-212	11	miR-320	5
miR-200c	32	miR-221	11	miR-345	5
miR-186	31	miR-27b	11	miR-422b	5
miR-145	30	miR-30e-3p	11	miR-335	4
miR-155	30	miR-338	11	miR-365	4
miR-29c	30	miR-361	11	miR-486	4
let-7c	28	miR-141	10	miR-24	3
miR-181c	27	miR-142-5p	10	miR-26b	3
miR-125a	26	miR-30a-3p	10	miR-331	3
miR-134	25	miR-30a-5p	10	miR-340	3
miR-181d	25	miR-451	10	miR-34a	3
let-7b	24	miR-142-3p	9	miR-374	3
miR-127	24	miR-146b	9	miR-452	3
miR-146a	24	miR-15b	9	miR-483	3
miR-139	23	miR-18a	9	miR-512-5p	3
miR-152	23	miR-210	9	let-7e	2
miR-190	23	miR-296	9	miR-32	2
miR-30e-5p	23	miR-323	9	miR-422a	2
miR-106b	22	miR-362	9	miR-424	2
miR-10a	22	let-7a	8	miR-432	2

Biomarker	No. Used	Biomarker	No. Used	Biomarker	No. Used
miR-132	21	miR-196b	8	miR-485-3p	2
miR-148a	21	miR-223	8	miR-487b	2
miR-213	21	miR-29b	8	miR-496	2
miR-29a	21	miR-324-5p	8	miR-505	2
miR-375	21	miR-376a	8	miR-7	2
miR-133b	19	miR-379	8	miR-202	1
miR-15a	18	miR-491	8	miR-369-3p	1
miR-107	17	let-7d	7	miR-495	1
miR-148b	17	miR-126	7	miR-502	1
miR-19a	17	miR-182	7	miR-511	1
miR-106a	15	miR-185	7	miR-516-3p	1
miR-130a	15	miR-204	7	miR-517c	1
miR-17-3p	15	miR-23a	7	miR-92	1
miR-18a*	15	miR-27a	7	miR-93	1
miR-195	15	miR-324-3p	7	miR-99a	1
miR-20b	15	miR-342	7	miR-99b	1
miR-301	15	miR-34c	7		
miR-339	15	miR-382	7		

Example 9. QRT-PCR for Evaluation of MicroRNA Expression in Human Lung Cancer Patient Serum RNA

[0125] To further assess the selectivity and sensitivity of certain miRNAs and combinations of miRNA, an additional set of serum samples, isolated from a distinct set of lung cancer patients and normal subjects (Table 34), was used to compare miRNAs expressed in serum from lung cancer patients and normal subjects. Histopathological analyses of lung lobe sections from cancer patients and normal subjects were performed by a board certified pathologist to determine a pathological diagnosis.

Table 34. Histopathological and patient information for serum samples. ADCA, adenocarcinoma; SCCA, squamous cell carcinoma.

Sample ID	Sex	Diagnosis	Cell type
1	M	Cancer	ADCA
2	M	Cancer	ADCA
3	M	Cancer	ADCA
4	M	Cancer	SCCA

5	M	Cancer	SCCA
6	F	Cancer	ADCA
7	F	Cancer	SCCA
8	M	Cancer	SCCA
9	M	Cancer	ADCA
10	F	Cancer	ADCA
11	M	Cancer	ADCA
12	F	Cancer	ADCA
13	F	Cancer	ADCA
14	M	Cancer	ADCA
15	F	Cancer	SCCA
16	F	Cancer	ADCA
17	M	Cancer	SCCA
18	M	Cancer	ADCA
19	M	Cancer	ADCA
20	M	Cancer	SCCA
21	F	Cancer	ADCA
22	F	Cancer	ADCA
23	F	Cancer	ADCA
24	F	Cancer	SCCA
25	F	Cancer	SCCA
26	M	Cancer	SCCA
27	M	Cancer	ADCA
28	M	Cancer	ADCA
29	M	Cancer	SCCA
30	M	Cancer	ADCA
31	F	Normal	Normal
32	F	Normal	Normal
33	F	Normal	Normal
34	M	Normal	Normal
35	F	Normal	Normal
36	M	Normal	Normal
37	M	Normal	Normal
38	M	Normal	Normal
39	M	Normal	Normal
40	M	Normal	Normal
41	M	Normal	Normal
42	M	Normal	Normal
43	M	Normal	Normal

44	F	Normal	Normal
45	M	Normal	Normal
46	F	Normal	Normal
47	F	Normal	Normal
48	F	Normal	Normal
49	F	Normal	Normal
50	M	Normal	Normal

[0126] First, the expression of 180 miRNAs in serum RNA was evaluated from the 30 lung cancer patients and 20 normal subjects (Table 34). Blood collection, serum RNA extraction, and qRT-PCR were performed as described in Example 2 above. Table 35 shows the mean dCt and ddCt values following PCR amplification of miRNAs from the serum samples. dCt values were calculated as the difference between the Ct of a specific miRNA and the mean Ct for the top 50 expressed miRNAs as a normalizer. The miRNAs are arranged in increasing t-test p-value.

Table 35. Expression of miRNAs in serum from patients having lung cancer and from normal subjects.

miRNA ID	Cancer mean dCt	Cancer SD	Normal mean dCt	Normal SD	Cancer – Normal ddCt	ttest p-value
miR-15b	0.86	0.36	-1.47	0.42	2.33	5.19E-25
miR-340	1.22	0.77	5.44	0.93	-4.22	6.49E-21
let-7e	-0.72	0.93	3.50	1.01	-4.22	6.11E-20
miR-103	1.78	0.58	-0.79	0.60	2.57	7.76E-20
let-7f	2.19	0.79	-0.52	0.38	2.71	6.24E-19
miR-605	8.11	2.13	0.34	1.70	7.77	3.58E-18
miR-346	7.64	2.02	1.27	1.27	6.37	1.66E-16
miR-214	6.95	1.75	1.38	1.32	5.57	3.52E-16
miR-378	0.74	0.66	4.36	1.31	-3.63	3.58E-16
miR-182	6.21	1.19	2.34	0.88	3.87	6.00E-16
miR-190	4.88	0.79	2.42	0.64	2.46	1.46E-15
miR-422b	3.23	0.58	1.01	0.84	2.22	8.17E-15
miR-185	1.31	1.24	4.78	0.80	-3.47	3.32E-14
miR-133b	5.18	1.59	0.63	1.30	4.55	3.33E-14

miR-26a	-1.26	0.73	-3.17	0.48	1.91	9.23E-14
miR-142-3p	-1.29	0.76	-3.27	0.50	1.98	1.09E-13
let-7a	0.28	0.86	-1.93	0.57	2.21	2.08E-13
miR-30e-5p	-0.31	0.76	2.29	1.00	-2.60	1.63E-12
miR-145	2.52	0.72	-0.09	1.25	2.61	1.90E-12
miR-106a	-2.64	0.95	-0.69	0.22	-1.95	6.28E-12
miR-30c	-0.20	0.45	-1.20	0.34	1.00	3.61E-11
miR-15a	3.93	0.65	2.37	0.65	1.56	6.88E-11
miR-374	0.99	0.67	-0.53	0.59	1.53	7.83E-11
miR-483	6.70	2.16	1.98	1.72	4.72	1.17E-10
let-7g	-1.19	0.56	-2.49	0.55	1.31	1.34E-10
miR-23a	1.45	0.63	3.16	0.83	-1.71	2.48E-10
miR-26b	-1.19	0.69	-2.96	0.84	1.77	3.34E-10
miR-143	2.10	1.22	4.93	1.30	-2.83	4.12E-10
miR-133a	3.55	1.64	6.80	1.12	-3.25	5.67E-10
miR-22	1.25	0.86	3.32	1.04	-2.07	6.67E-10
miR-18c	6.22	0.86	4.09	1.11	2.13	8.03E-10
miR-320	-2.99	0.85	-1.24	0.72	-1.75	1.13E-09
miR-134	5.75	1.29	3.22	0.84	2.53	1.24E-09
miR-193b	3.97	1.30	6.97	1.19	-3.00	1.29E-09
miR-18a	1.52	0.83	3.90	1.42	-2.38	1.33E-09
miR-126	-1.65	0.54	-2.92	0.63	1.27	1.64E-09
miR-223	-5.87	1.30	-1.41	2.61	-4.45	2.11E-09
miR-204	7.43	1.45	4.85	0.99	2.58	2.07E-08
miR-195	-2.13	1.14	-0.11	0.88	-2.02	2.11E-08
miR-19a	-2.61	1.03	0.43	2.08	-3.04	2.86E-08
miR-200c	6.01	1.37	2.92	1.27	3.09	3.22E-08
miR-152	3.23	0.74	5.15	1.34	-1.91	8.04E-08
miR-566	5.71	1.59	2.35	2.00	3.36	1.12E-07
miR-30a-5p	-1.39	0.61	-0.41	0.42	-0.97	1.13E-07
miR-410	6.06	2.14	3.01	0.66	3.04	1.64E-07
miR-146a	-1.25	0.70	-2.34	0.54	1.10	2.94E-07
miR-485-3p	5.75	1.47	3.76	0.71	1.99	1.03E-06
miR-328	2.44	0.66	0.90	1.30	1.53	1.37E-06
miR-30d	-0.33	0.74	1.58	1.61	-1.91	1.60E-06
let-7d	1.55	0.89	0.33	0.58	1.23	1.89E-06
miR-27b	2.59	0.77	4.30	1.35	-1.72	2.32E-06
miR-155	3.79	0.97	2.49	0.65	1.31	3.15E-06
miR-432	6.23	2.00	3.54	1.00	2.69	4.44E-06

miR-301	3.23	0.68	2.20	0.74	1.03	5.96E-06
miR-28	5.03	1.04	3.53	0.93	1.50	8.10E-06
miR-502	5.08	1.49	6.97	0.70	-1.89	8.46E-06
miR-487b	5.90	1.32	4.04	1.20	1.87	8.86E-06
miR-191	-1.02	0.55	0.12	1.00	-1.14	1.03E-05
miR-29a	0.03	0.82	1.59	1.45	-1.56	1.26E-05
miR-193a	6.44	1.12	4.34	1.67	2.10	1.75E-05
miR-486	-4.64	1.27	-2.80	1.33	-1.84	1.91E-05
miR-186	0.31	0.88	1.31	0.37	-0.99	1.93E-05
miR-221	-0.20	0.80	-1.27	0.81	1.07	3.08E-05
miR-505	5.85	1.08	4.47	0.93	1.38	4.43E-05
miR-181a	3.61	0.76	2.75	0.50	0.86	4.82E-05
miR-550	6.58	0.93	5.53	0.58	1.06	5.94E-05
miR-21	-2.15	0.42	-1.60	0.52	-0.56	1.28E-04
let-7i	-0.11	0.57	0.80	0.95	-0.90	1.45E-04
miR-496	7.89	1.83	5.81	1.44	2.08	1.54E-04
miR-192	1.18	0.97	2.17	0.57	-0.99	1.58E-04
miR-30e-3p	-9.07	13.86	4.59	1.37	-13.66	3.22E-04
miR-101	1.64	0.93	0.55	1.10	1.09	4.95E-04
miR-222	-0.42	0.35	0.11	0.65	-0.52	5.66E-04
miR-206	5.76	1.59	4.25	1.19	1.52	6.69E-04
miR-516-3p	5.66	2.70	3.31	1.24	2.35	6.76E-04
miR-34a	3.01	2.87	5.43	1.08	-2.42	7.69E-04
miR-365	2.88	1.22	4.01	0.88	-1.13	8.64E-04
miR-17-5p	0.32	0.68	-0.27	0.38	0.59	9.61E-04
miR-98	4.62	1.06	3.55	0.95	1.07	1.00E-03
miR-339	3.67	0.96	4.74	1.01	-1.07	1.25E-03
miR-433	6.13	1.50	4.66	1.14	1.47	1.34E-03
miR-338	5.53	1.12	6.56	0.83	-1.03	1.46E-03
miR-20b	-0.39	1.13	0.89	1.57	-1.28	1.60E-03
miR-125b	4.03	1.29	5.10	0.84	-1.08	2.11E-03
miR-150	0.07	1.23	-0.91	0.69	0.98	2.20E-03
miR-19b	-3.47	0.94	-2.62	0.82	-0.84	2.35E-03
miR-148a	1.29	0.56	1.85	0.68	-0.56	2.65E-03
miR-27a	0.61	1.07	1.48	0.64	-0.87	3.08E-03
miR-324-5p	4.08	0.86	4.82	0.76	-0.74	3.73E-03
miR-148b	3.13	0.53	2.71	0.41	0.42	4.73E-03
miR-18b	3.11	0.80	2.35	0.96	0.76	4.88E-03
miR-525	6.48	2.16	4.92	1.12	1.56	5.42E-03

miR-130b	1.69	0.66	1.18	0.51	0.51	5.49E-03
miR-106b	-0.55	0.74	-1.13	0.63	0.58	6.17E-03
miR-342	2.52	0.68	1.96	0.70	0.56	6.88E-03
miR-126*	-1.91	0.46	-1.55	0.41	-0.36	7.08E-03
miR-199a*	0.56	1.04	-0.10	0.41	0.66	1.00E-02
miR-224	5.02	1.19	6.03	1.35	-1.01	1.03E-02
miR-25	-0.99	1.06	-0.31	0.67	-0.68	1.34E-02
miR-331	3.34	0.62	4.22	1.64	-0.88	1.55E-02
let-7b	-2.11	0.87	-1.57	0.51	-0.54	1.58E-02
miR-140	0.69	0.75	1.17	0.50	-0.48	1.61E-02
miR-142-5p	2.06	0.70	2.56	0.68	-0.50	1.68E-02
miR-296	4.39	1.06	5.20	1.25	-0.80	2.15E-02
miR-382	5.43	1.64	4.33	1.39	1.09	2.29E-02
miR-210	3.00	0.86	2.46	0.74	0.55	2.35E-02
miR-146b	0.47	0.74	0.04	0.47	0.43	2.54E-02
miR-130a	-0.18	0.85	0.67	1.71	-0.85	2.78E-02
miR-30b	-0.24	0.47	-0.70	0.98	0.46	3.31E-02
miR-423	-3.16	11.84	2.09	1.32	-5.25	5.50E-02
miR-345	1.63	0.76	2.04	0.69	-0.41	5.59E-02
miR-638	2.79	1.53	3.57	1.16	-0.78	6.91E-02
miR-422a	4.35	0.81	4.86	1.14	-0.51	7.14E-02
miR-99a	5.42	1.38	4.71	1.33	0.72	7.36E-02
miR-335	2.72	0.87	2.26	0.84	0.45	7.49E-02
miR-425	3.69	0.69	3.38	0.40	0.31	7.61E-02
miR-326	7.62	0.83	7.15	0.99	0.46	9.43E-02
miR-16	-7.89	1.09	-7.39	0.98	-0.50	1.03E-01
miR-93	-2.67	0.84	-3.06	0.76	0.38	1.09E-01
miR-7	3.86	1.34	3.29	1.00	0.57	1.17E-01
miR-29c	1.63	0.80	2.01	0.58	-0.39	1.24E-01
miR-197	1.46	1.02	0.97	1.03	0.49	1.30E-01
miR-196a	6.93	1.59	6.32	0.77	0.61	1.34E-01
miR-99b	5.22	1.04	4.76	1.07	0.46	1.43E-01
miR-100	5.71	1.29	5.12	1.46	0.59	1.53E-01
miR-376a	6.17	1.40	5.55	1.41	0.62	1.55E-01
miR-375	5.57	1.53	6.23	1.12	-0.66	1.58E-01
let-7c	2.30	0.82	2.64	0.94	-0.34	1.84E-01
miR-24	-1.88	0.57	-2.05	0.26	0.17	2.27E-01
miR-132	3.11	0.70	2.82	1.01	0.29	2.48E-01
miR-660	0.78	0.80	1.05	0.75	-0.27	2.71E-01

miR-127	5.26	1.68	4.75	1.41	0.51	2.75E-01
miR-10b	4.88	1.23	4.54	0.85	0.34	2.89E-01
miR-139	4.84	1.06	5.14	1.01	-0.31	3.32E-01
miR-18d	2.43	0.69	2.66	1.18	-0.23	3.97E-01
miR-497	4.88	1.15	5.19	1.21	-0.31	4.00E-01
miR-361	3.25	0.74	3.10	0.47	0.15	4.52E-01
miR-1	5.58	1.86	5.15	1.83	0.43	4.54E-01
miR-10a	4.86	1.31	4.58	1.33	0.28	4.96E-01
miR-584	4.39	1.15	4.16	1.36	0.23	5.51E-01
miR-324-3p	3.34	1.02	3.54	1.55	-0.20	6.11E-01
miR-151	1.86	0.75	1.96	0.67	-0.10	6.44E-01
miR-30a-3p	5.24	1.02	5.09	1.29	0.15	6.56E-01
miR-202	4.66	1.69	4.45	1.36	0.21	6.58E-01
miR-194	1.98	8.64	2.79	0.90	-0.81	6.85E-01
miR-501	5.23	1.58	5.40	1.09	-0.17	6.94E-01
miR-23b	4.90	0.94	5.00	1.59	-0.10	7.88E-01
miR-125a	2.86	0.92	2.76	1.73	0.10	7.93E-01
miR-20a	-3.56	0.99	-3.50	0.79	-0.06	8.34E-01
miR-92	-5.77	0.94	-5.74	0.74	-0.02	9.22E-01

[0127] Based on analysis of differentially expressed miRNA pairs, 27 miRNAs were selected for further validation in serum samples from an additional 55 lung cancer patients and from 75 cancer-free, presumed normal individuals (Table 36).

Table 36. Lung cancer patient, tumor pathology, and normal subject information. TNM Stage (Sobin and Wittekind, 2002); AJCC Stage (Greene, 2002); ADCA, adenocarcinoma; ADCA/BA, adenocarcinoma of the bronchoalveolar; SCCA, squamous cell carcinoma; NA, not applicable.

Specimen ID	Age	Sex	Cell Type	TNM Stage	AJCC Stage	Smoking History
1	80	M	Normal	NA	NA	Yes
2	66	M	Normal	NA	NA	Yes
3	78	M	Normal	NA	NA	No
4	70	M	Normal	NA	NA	Yes
5	38	M	Normal	NA	NA	No
6	66	M	Normal	NA	NA	Yes

7	67	F	Normal	NA	NA	No
8	62	M	Normal	NA	NA	Yes
9	63	F	Normal	NA	NA	Yes
10	69	M	Normal	NA	NA	Yes
11	82	M	Normal	NA	NA	Yes
12	55	F	Normal	NA	NA	No
13	55	M	Normal	NA	NA	Yes
14	79	M	Normal	NA	NA	Yes
15	71	F	Normal	NA	NA	No
16	62	F	Normal	NA	NA	No
17	79	M	Normal	NA	NA	Yes
18	77	M	Normal	NA	NA	Yes
19	78	M	Normal	NA	NA	Yes
20	69	M	Normal	NA	NA	Yes
21	62	M	Normal	NA	NA	Yes
22	55	F	Normal	NA	NA	Yes
23	56	M	Normal	NA	NA	Yes
24	55	F	Normal	NA	NA	Yes
25	79	M	Normal	NA	NA	Yes
26	77	M	Normal	NA	NA	Yes
27	79	M	Normal	NA	NA	Yes
28	55	F	Normal	NA	NA	Yes
29	51	M	Normal	NA	NA	Yes
30	56	M	Normal	NA	NA	Yes
31	78	F	Normal	NA	NA	No
32	52	M	Normal	NA	NA	Yes
33	75	M	Normal	NA	NA	Yes
34	68	F	Normal	NA	NA	Yes
35	57	M	Normal	NA	NA	No
36	67	M	Normal	NA	NA	Yes
37	85	M	Normal	NA	NA	Yes
38	76	M	Normal	NA	NA	Yes
39	53	M	Normal	NA	NA	Yes
40	51	M	Normal	NA	NA	Yes
41	65	M	Normal	NA	NA	Yes
42	78	F	Normal	NA	NA	Yes
43	83	M	Normal	NA	NA	Yes
44	72	M	Normal	NA	NA	Yes
45	61	F	Normal	NA	NA	Yes

46	69	M	Normal	NA	NA	No
47	55	F	Normal	NA	NA	Yes
48	51	M	Normal	NA	NA	Yes
49	56	F	Normal	NA	NA	Yes
50	70	M	Normal	NA	NA	Yes
51	57	M	Normal	NA	NA	Yes
52	63	M	Normal	NA	NA	Yes
53	73	M	Normal	NA	NA	Yes
54	70	M	Normal	NA	NA	Yes
55	75	F	Normal	NA	NA	Yes
56	73	F	Normal	NA	NA	Yes
57	57	F	Normal	NA	NA	Yes
58	78	M	Normal	NA	NA	Yes
59	63	M	Normal	NA	NA	Yes
60	74	M	Normal	NA	NA	Yes
61	79	M	Normal	NA	NA	Yes
62	55	M	Normal	NA	NA	Yes
63	84	F	Normal	NA	NA	Yes
64	54	F	Normal	NA	NA	Yes
65	56	F	Normal	NA	NA	Yes
66	82	M	Normal	NA	NA	Yes
67	64	F	Normal	NA	NA	Yes
68	77	M	Normal	NA	NA	Yes
69	51	F	Normal	NA	NA	Yes
70	37	M	Normal	NA	NA	Yes
71	54	M	Normal	NA	NA	Yes
72	68	F	Normal	NA	NA	Yes
73	54	M	Normal	NA	NA	Yes
74	62	F	Normal	NA	NA	Yes
75	54	F	Normal	NA	NA	No
76	77	M	ADCA	T1N1	IIA	Yes
77	77	M	SCCA	T2N0	IB	Yes
78	84	M	SCCA	T2N0	IB	Yes
79	52	M	SCCA	TN2	IIIA	Yes
80	49	F	ADCA	T1N1	IIA	Yes
81	60	M	ADCA	T2NOMO	IB	Yes
82	66	F	ADCA/BA	T1N0	IA	Yes
83	69	M	SCCA	T2N1MO	IIB	Yes
84	68	M	ADCA	N2	IIIA	Yes

85	56	M	SCCA	T2N1M0	IIB	Yes
86	85	M	ADCA	T2N0	IB	Yes
87	68	F	SCCA	T2N1M0	IIB	Yes
88	66	M	SCCA	T2N0M0	IB	Yes
89	61	M	SCCA	T2N1	IIB	Yes
90	58	M	ADCA	M1	IV	Yes
91	64	F	ADCA	T1N0M0	IA	Yes
92	72	F	SCCA	T2N0	IB	Yes
93	67	M	ADCA	T1Nx	IA	Yes
94	63	F	ADCA	T1N0	IA	Yes
95	80	M	ADCA	T2N0	IB	Yes
96	63	F	ADCA	T1NOMO	IA	Yes
97	48	F	ADCA/BA	T1N0M0	IA	Yes
98	70	F	SCCA	T1N0M0	IA	Yes
99	71	M	SCCA	T3N0	IIB	Yes
100	73	F	SCCA	T3N0M0	IIB	Yes
101	72	M	ADCA/BA	T2N0	IB	Yes
102	74	M	SCCA	T2N0	IB	Yes
103	51	F	ADCA	T1N0	IA	Yes
104	83	M	SCCA	T1N0	IA	Yes
105	66	M	ADCA	T2N1M0	IIB	Yes
106	65	F	SCCA	T2N0	IB	Yes
107	74	F	ADCA	T1N0M0	IA	Yes
108	77	M	SCCA	T2N1M0	IIB	Yes
109	82	F	SCCA	T2N0	IB	Yes
110	67	M	ADCA	T1N0	IA	Yes
111	61	M	ADCA	TN2	IIIA	Yes
112	82	F	SCCA	T2N1M0	IIB	Yes
113	58	M	SCCA	T1N0M1	IV	Yes
114	78	M	ADCA	T1NOMO	IA	Yes
115	77	M	ADCA	T1N2	IIIA	Yes
116	72	M	ADCA	T2N0	IB	Yes
117	59	F	ADCA	T2N0	IB	Yes
118	72	M	SCCA	T1NOMO	IA	Yes
119	71	F	ADCA	T2N1	IIB	Yes
120	72	F	SCCA	T2NO	IB	Yes
121	73	F	ADCA	T2N2M0	IIIA	Yes
122	69	M	SCCA	T2N0	IB	Yes
123	66	F	SCCA	T2N1M0	IIB	Yes

124	55	M	ADCA	T4	IIIB	Yes
125	68	F	ADCA/BA	T1N0M0	IA	Yes
126	64	F	ADCA	T2NOMO	IB	Yes
127	77	M	SCCA	T1N0	IA	Yes
128	76	F	ADCA	N2	IIIA	Yes
129	60	F	ADCA	T1N0	IA	Yes
130	66	M	SCCA	T2N0M0	IB	Yes

[0128] The 27 miRNAs were evaluated for their ability to distinguish lung cancer patient samples from normal subject samples shown in Table 36. Table 37 shows the mean Ct values and standard deviation for each miRNA for the cancer and the normal group. The dCt values represent the difference of the Ct values between the cancer and the normal group. The miRNAs are arranged in increasing t-test p-value. Twenty-four of the 27 miRNAs were differentially expressed at a high statistical significance level, having t-test p-values of less than 0.001. These twenty-four miRNAs can be used for diagnosing lung cancer.

Table 37. Expression of miRNAs in serum from patients having lung cancer or from normal subjects.

miRNA ID	Normal mean Ct	Normal SD	Cancer mean Ct	Cancer SD	Cancer-Normal dCt	ttest p-value
miR-15b	27.90	1.52	30.80	1.41	2.91	1.78E-20
miR-182	32.99	1.72	35.75	1.26	2.76	4.19E-17
miR-15a	31.21	1.68	33.90	1.52	2.68	5.00E-16
miR-30b	27.20	1.27	29.07	1.23	1.87	7.33E-14
let-7i	27.32	1.56	29.38	1.13	2.06	1.01E-13
miR-26b	26.12	1.51	28.10	1.10	1.98	1.38E-13
miR-106b	27.63	1.70	29.84	1.30	2.21	3.94E-13
let-7g	26.69	1.54	28.61	1.02	1.92	6.04E-13
miR-106a	25.45	1.59	27.51	1.48	2.05	9.07E-12
miR-142-3p	25.50	1.64	27.41	1.25	1.90	4.43E-11
miR-301	31.56	1.36	33.29	1.46	1.73	1.98E-10
miR-150	27.65	0.97	28.92	1.18	1.28	4.28E-10
miR-181c	34.36	1.41	36.01	1.41	1.65	1.52E-09
miR-25	27.31	1.98	29.23	1.62	1.92	3.44E-08
miR-324-3p	31.65	2.19	33.48	1.69	1.83	8.72E-07

miR-126	26.29	1.41	27.45	1.37	1.16	6.02E-06
miR-27a	28.64	1.64	29.97	1.59	1.33	8.30E-06
miR-126*	26.46	1.25	27.37	1.14	0.92	3.56E-05
miR-660	25.55	3.36	27.78	3.26	2.23	2.43E-04
miR-346	35.86	1.61	36.94	1.59	1.08	3.60E-04
miR-422a	32.34	1.71	33.48	1.57	1.13	3.81E-04
miR-30d	27.92	1.72	29.02	1.66	1.10	3.83E-04
miR-30a-5p	27.06	1.75	28.12	1.64	1.06	6.27E-04
miR-422b	32.47	2.02	33.57	1.45	1.10	8.28E-04
miR-30e-5p	28.41	2.61	29.70	1.70	1.30	1.65E-03
miR-27b	31.89	1.48	32.59	1.33	0.70	6.25E-03
miR-92	25.84	3.71	25.47	3.05	-0.37	5.46E-01

[0129] Receiver Operator Characteristic (ROC) analysis was used to identify the miRNA pairs having the capacity to distinguish serum samples of lung cancer patients from those of normal patients. Select differentially-expressed pairs that distinguished normal patients from cancer patients are shown in Table 38 and are arranged in decreasing AUC ranging from 0.99 to 0.74.

Table 38. miRNA biomarker pairs that can be used to identify serum from lung cancer patients.

miRNA Biomarker Pair	Cancer mean – Normal mean	Cancer mean	Cancer SD	Normal mean	Normal SD	AUC ROC	ttest p-value
miR-126*, miR-15b	-1.99	-3.43	0.89	-1.44	0.67	0.99	6.21E-25
miR-15b, miR-27b	2.21	-1.78	0.86	-3.99	0.95	0.98	7.97E-27
miR-126, miR-15b	-1.74	-3.35	1.12	-1.61	0.76	0.97	3.30E-16
miR-126*, miR-15a	-1.80	-6.55	0.81	-4.76	0.87	0.94	1.49E-22
miR-15b, miR-27a	1.57	0.83	0.93	-0.74	0.74	0.94	1.47E-17
miR-106b, miR-30a-5p	1.15	1.72	0.56	0.57	0.55	0.93	2.58E-21
miR-15b, miR-30a-5p	1.84	2.68	1.10	0.84	0.68	0.93	6.13E-18
miR-15b, miR-30d	1.81	1.79	1.08	-0.02	0.69	0.93	6.96E-18
miR-15a, 3 miR-30a-5p	1.64	5.79	0.84	4.15	0.83	0.92	8.12E-20
miR-15a, miR-27b	2.00	1.33	0.90	-0.68	1.13	0.92	8.07E-21
miR-15b, miR-30b	1.03	1.73	0.76	0.70	0.71	0.92	2.58E-12
miR-126*, miR-26b	-1.07	-0.73	0.52	0.34	0.64	0.92	7.36E-19
miR-126, miR-15a	-1.56	-6.48	1.10	-4.93	0.92	0.92	1.32E-13
miR-15a, miR-30d	1.62	4.91	0.87	3.29	0.85	0.91	1.04E-18

miR-15b, miR-26b	0.92	2.71	0.79	1.78	0.41	0.91	1.33E-11
miR-106b, miR-30d	1.12	0.83	0.61	-0.29	0.60	0.91	2.69E-18
miR-106b, miR-126*	1.30	2.47	0.63	1.18	0.72	0.91	8.20E-20
miR-15a, miR-422a	1.56	0.46	0.99	-1.10	0.80	0.91	4.86E-16
miR-106a, miR-30a-5p	0.99	-0.62	0.58	-1.60	0.44	0.91	8.79E-18
miR-106a, miR-30d	0.96	-1.51	0.57	-2.47	0.45	0.91	2.49E-17
miR-15b, miR-301	1.18	-2.48	0.85	-3.66	0.54	0.91	3.59E-14
miR-26b, miR-27b	1.28	-4.49	0.56	-5.77	0.87	0.90	4.08E-18
miR-182, miR-27b	2.14	3.19	1.07	1.05	1.35	0.90	6.45E-18
miR-106b, miR-27b	1.51	-2.74	0.72	-4.25	1.03	0.90	2.49E-17
miR-142-3p, miR-27b	1.20	-5.18	0.58	-6.38	0.94	0.89	3.90E-15
miR-106a, miR-126*	1.14	0.13	0.62	-1.00	0.66	0.89	1.28E-17
let-7i, miR-126*	1.14	2.01	0.63	0.87	0.79	0.89	8.05E-16
miR-27b, miR-30b	-1.17	3.51	0.58	4.69	1.02	0.89	1.67E-13
miR-126*, miR-182	-1.85	-8.32	1.02	-6.47	1.15	0.88	7.45E-17
miR-15a, miR-27a	1.40	3.97	0.84	2.57	0.87	0.88	1.13E-15
miR-106b, miR-126	1.05	2.39	0.89	1.35	0.79	0.88	2.41E-10
miR-142-3p, miR-15b	-1.00	-3.40	0.81	-2.39	0.70	0.88	3.57E-11
let-7i, miR-27b	1.36	-3.21	0.70	-4.57	0.99	0.88	1.07E-15
miR-126*, miR-30b	-0.96	-1.70	0.62	-0.75	0.71	0.88	3.01E-13
miR-150, miR-15b	-1.63	-1.88	1.18	-0.25	0.94	0.87	2.68E-13
miR-15b, miR-422a	1.68	-2.71	1.40	-4.40	0.73	0.87	5.28E-12
miR-182, miR-422a	1.67	2.31	1.22	0.64	1.04	0.86	6.23E-13
miR-182, miR-30a-5p	1.71	7.63	1.20	5.92	1.10	0.86	2.75E-13
miR-15b, miR-422b	1.79	-2.77	1.52	-4.55	0.96	0.86	2.69E-11
miR-182, miR-30d	1.70	6.72	1.20	5.02	1.05	0.86	1.78E-13
miR-126, miR-26b	-0.82	-0.65	0.84	0.17	0.76	0.86	9.36E-08
miR-126, miR-182	-1.62	-8.29	1.21	-6.67	1.27	0.85	2.19E-11
let-7g, miR-126*	1.00	1.23	0.65	0.24	0.86	0.85	8.28E-12
miR-15a, miR-422b	1.59	0.35	1.33	-1.24	1.02	0.85	4.27E-11
miR-27b, miR-301	-1.03	-0.70	0.66	0.33	0.88	0.85	6.93E-12
miR-126, miR-30b	-0.71	-1.62	0.80	-0.92	0.82	0.85	3.08E-06
miR-Let-7g, miR-15b	-0.99	-2.20	0.91	-1.21	0.68	0.85	7.70E-10
miR-126*, miR-142-3p	-0.98	-0.03	0.73	0.95	0.85	0.84	7.76E-11
miR-106a, miR-27b	1.35	-5.08	0.79	-6.43	1.15	0.84	8.96E-13
miR-25, miR-30a-5p	0.85	1.10	0.71	0.25	0.63	0.84	1.37E-10
miR-15a, miR-30e-5p	1.42	4.23	1.01	2.81	1.97	0.83	4.57E-07
miR-106b, miR-422a	1.02	-3.63	0.93	-4.65	0.65	0.83	3.79E-10
miR-182, miR-422b	1.71	2.18	1.37	0.47	1.30	0.83	8.37E-11
miR-106b, miR-27a	0.88	-0.13	0.66	-1.01	0.72	0.83	4.90E-11
let-7i, miR-15b	-0.84	-1.42	0.80	-0.58	0.55	0.83	1.23E-09
miR-126, miR-142-3p	-0.74	0.05	0.96	0.78	0.85	0.83	1.50E-05
miR-Let-7g, miR-27b	1.21	-3.98	0.82	-5.20	1.08	0.82	2.59E-11

miR-30a-5p, miR-324-3p	-0.77	-5.36	0.55	-4.59	0.75	0.82	5.20E-10
miR-150, miR-182	-1.50	-6.84	1.22	-5.34	1.09	0.82	7.73E-11
miR-182, miR-27a	1.51	5.82	1.14	4.31	1.26	0.82	8.04E-11
miR-150, miR-15a	-1.45	-5.01	1.20	-3.56	1.14	0.81	2.31E-10
miR-15b, miR-30e-5p	1.61	1.10	1.25	-0.51	1.89	0.81	4.18E-08
miR-15b, miR-181c	1.18	-5.28	1.11	-6.46	0.88	0.81	2.59E-09
miR-106b, miR-422b	1.09	-3.73	1.05	-4.82	0.89	0.81	1.03E-08
miR-25, miR-30d	0.82	0.21	0.76	-0.61	0.63	0.80	2.41E-09
miR-15b, miR-92	3.30	5.34	2.87	2.04	3.38	0.80	1.98E-08
miR-126*, miR-301	-0.81	-5.91	0.80	-5.10	0.57	0.80	6.31E-09
miR-106a, miR-126	0.89	0.06	0.90	-0.83	0.89	0.80	1.44E-07
miR-15a, miR-301	0.99	0.64	0.98	-0.35	0.77	0.79	1.09E-08
miR-106a, miR-15b	-0.85	-3.30	0.99	-2.44	0.59	0.79	1.75E-07
miR-106b, miR-30e-5p	0.92	0.14	0.70	-0.77	1.82	0.79	1.34E-04
miR-30d, miR-324-3p	-0.74	-4.46	0.67	-3.73	0.81	0.79	1.00E-07
miR-15a, miR-92	3.03	8.43	2.93	5.41	3.39	0.79	2.74E-07
let-7i, miR-126	0.90	1.93	1.00	1.04	0.97	0.79	1.30E-06
miR-142-3p, miR-15a	-0.82	-6.53	0.84	-5.71	0.70	0.79	4.66E-08
miR-181c, miR-27b	1.00	3.47	0.89	2.48	0.88	0.79	5.18E-09
miR-106a, miR-422a	0.90	-5.94	1.00	-6.83	0.69	0.78	1.35E-07
miR-106b, miR-92	2.60	4.38	2.93	1.78	3.40	0.78	7.78E-06
let-7i, miR-30d	0.97	0.37	1.03	-0.60	0.82	0.78	9.84E-08
miR-26b, miR-30d	0.89	-0.92	0.94	-1.80	0.77	0.78	1.06E-07
miR-182, miR-30e-5p	1.55	6.08	1.22	4.53	2.07	0.78	4.58E-07
miR-26b, miR-30a-5p	0.92	-0.02	0.95	-0.94	0.75	0.78	4.19E-08
miR-182, miR-92	3.07	10.21	3.02	7.15	3.41	0.78	3.08E-07
let-7i, miR-30a-5p	1.00	1.26	1.06	0.26	0.82	0.77	7.13E-08
miR-182, miR-301	1.12	2.51	1.20	1.39	1.01	0.77	1.69E-07
miR-15b,3 miR-46	1.79	-6.11	2.03	-7.89	1.58	0.77	4.01E-07
miR-106a, miR-92	2.41	2.04	2.87	-0.37	3.43	0.77	2.71E-05
miR-27a, miR-27b	0.63	-2.62	0.52	-3.25	0.71	0.77	3.97E-08
miR-15a, miR-346	1.69	-3.02	1.97	-4.71	1.47	0.77	5.42E-07
miR-15a, miR-324-3p	0.90	0.47	0.85	-0.44	1.00	0.77	1.49E-07
miR-106b, miR-15b	-0.69	-0.96	0.87	-0.27	0.44	0.77	6.62E-07
miR-15b, miR-324-3p	1.07	-2.68	1.11	-3.75	0.96	0.76	7.93E-08
miR-126*, miR-181c	-0.75	-8.65	0.87	-7.91	0.71	0.76	1.01E-06
let-7g, miR-126	0.75	1.16	0.92	0.41	1.01	0.76	2.14E-05
miR-15a, miR-181c	1.00	-2.15	0.97	-3.15	1.02	0.76	9.88E-08
miR-142-3p, miR-27a	0.57	-2.57	0.68	-3.14	0.68	0.75	5.81E-06
miR-106b, miR-150	0.94	0.92	0.82	-0.02	1.11	0.75	1.68E-07
miR-182, miR-346	1.67	-1.27	1.82	-2.94	1.66	0.75	4.51E-07
miR-126*, miR-25	-1.00	-1.85	1.03	-0.85	1.07	0.75	4.15E-07
miR-26b, miR-27a	0.65	-1.87	0.75	-2.52	0.78	0.75	4.13E-06

miR-25, miR-92	2.29	3.76	2.85	1.47	3.48	0.75	6.64E-05
miR-15a, miR-26b	0.72	5.82	0.84	5.10	0.67	0.74	7.99E-07
let-7g, miR-182	-0.88	-7.11	1.06	-6.23	0.99	0.74	4.23E-06
miR-15a, miR-30b	0.84	4.85	0.87	4.01	0.95	0.74	8.58E-07
let-7i, miR-92	2.44	3.92	2.98	1.47	3.30	0.74	2.17E-05
miR-106a, miR-27a	0.72	-2.47	0.73	-3.19	0.91	0.74	1.75E-06
miR-142-3p, miR-182	-0.92	-8.40	0.97	-7.48	1.11	0.74	1.67E-06
miR-182, miR-30b	0.89	6.69	0.98	5.80	0.98	0.74	1.27E-06
miR-324-3p, miR-422a	0.68	-0.02	0.94	-0.70	0.87	0.74	5.00E-05
let-7i, miR-182	-0.80	-6.40	1.03	-5.59	0.90	0.74	1.03E-05
miR-182, miR-26b	0.79	7.63	0.91	6.84	0.94	0.74	4.74E-06
let-7g, miR-30a-5p	0.85	0.49	1.07	-0.37	0.98	0.74	8.75E-06

[0130] In addition to the differential paired analysis of miRNA (Table 38), several miRNA biomarker based classifier models that discriminated lung-cancer from normal (cancer free) subjects were evaluated. Classifier models were designed with data obtained from the samples described in Table 34. When applied to the unrelated samples shown in Table 36, the models demonstrated good performance in discriminating lung cancer patients from normal subjects.

[0131] Specifically, the simple Welch's t-test and Relief (Kira and Rendel, Proc 10th Natl Conf on Artificial Intelligence, MIT Press, pp. 129-134 (1992)) methods were used to select features (i.e., miRNAs) as inputs, and the Linear Discriminant Analysis (LDA) and Linear Support vector machines (LSVM) were used as the classifiers during training (Burges, *Data Mining and Drug Discovery* 2:121-167 (1998)). Table 39 shows representative examples of classifier models that distinguished lung cancer from cancer free subjects in both training and test sets.

Table 39. Performance of classification models and feature selection in training set and test set of normal subjects and lung cancer patients.

Name	Classifier	Feature Selection Method	Number of Features (miRNAs)	Training Set AUC	Test Set AUC
LSVM Train r+TTest+4	LSVM Train	TTest	4	1.000	0.919
LSVM Train +TTest+5	LSVM Train	TTest	5	1.000	0.890
LSVM Train +TTest+6	LSVM Train	TTest	6	1.000	0.879

LSVM Train +TTest+7	LSVM Train	TTest	7	1.000	0.831
LSVM Train +Relief+4	LSVM Train	Relief	4	0.996	0.958
LSVM Train +Relief+5	LSVM Train	Relief	5	0.997	0.958
LSVM Train +Relief+6	LSVM Train	Relief	6	0.997	0.933
LSVM Train +Relief+7	LSVM Train	Relief	7	0.997	0.931
LDA Train+TTest+4	LDA Train	TTest	4	1.000	0.886
LDA Train+TTest+5	LDA Train	TTest	5	1.000	0.875
LDA Train+TTest+6	LDA Train	TTest	6	0.998	0.816
LDA Train+TTest+7	LDA Train	TTest	7	0.998	0.785
LDA Train+Relief+4	LDA Train	Relief	4	0.996	0.939
LDA Train+Relief+5	LDA Train	Relief	5	0.997	0.908
LDA Train+Relief+6	LDA Train	Relief	6	0.996	0.908
LDA Train+Relief+7	LDA Train	Relief	7	0.996	0.917

[0132] The model's performance was estimated on training data by performing 25 repetitions of 5-fold cross validation on the training samples and measuring the ROC AUC values as previously described in Example 5. The frequency of appearance of miRNA pairs in each of the classification models is shown in Table 40.

Table 40. Prevalence of paired biomarkers in classifier generation. The classification models are as indicated.

LSVM Train+TTest+7		LSVM Train+Relief+7		LDA Train+TTest+7		LDA Train +Relief+7	
miRNA Pair	Pair Frequency	miRNA Pair	Pair Frequency	miRNA Pair	Pair Frequency	miRNA Pair	Pair Frequency
106a,15b	125	106a,26b	125	106a,15b	125	106a,26b	125
106a,422b	125	126*,26b	125	106a,422b	125	126*,26b	125
15b,30a-5p	125	let-7i,26b	115	15b,30a-5p	125	let-7i,26b	115
126*,15b	101	126*,15b	104	126*,15b	101	126*,15b	104
106a,26b	95	106a,15b	101	106a,26b	95	106a,15b	101
let-7g,106a	79	126*,30b	50	let-7g,106a	79	126*,30b	50
106a,106b	55	26b,30a-5p	49	106a,106b	55	26b,30a-5p	49
181c,30e-5p	45	106a,422b	38	181c,30e-5p	45	106a,422b	38
106a,142-3p	40	30d,30e-5p	27	106a,142-3p	40	30d,30e-5p	27
106a,182	20	26b,30e-5p	25	106a,182	20	26b,30e-5p	25
182,30a-5p	9	422a,422b	16	182,30a-5p	9	422a,422b	16
106a,15a	8	126,15b	14	106a,15a	8	126,15b	14
142-3p,30a-5p	6	15b,30a-5p	12	142-3p,30a-5p	6	15b,30a-5p	12

126*,26b	5	106a,30b	10	126*,26b	5	106a,30b	10
15b,30e-5p	5	26b,27b	10	15b,30e-5p	5	26b,27b	10
182,30e-5p	5	106a,15a	9	182,30e-5p	5	106a,15a	9
15b,25	4	let-7i,15b	9	15b,25	4	let-7i,15b	9
126,30e-5p	3	26b,27a	6	126,30e-5p	3	26b,27a	6
126*,422b	3	30e-5p,422b	6	126*,422b	3	30e-5p,422b	6
142-3p,27b	3	106a,106b	4	142-3p,27b	3	106a,106b	4
181c,27b	3	15b,25	4	181c,27b	3	15b,25	4
26b,27a	3	15b,30e-5p	3	26b,27a	3	15b,30e-5p	3
422a,422b	3	106a,324-3p	2	422a,422b	3	106a,324-3p	2
30a-5p,422b	2	181c,30e-5p	2	30a-5p,422b	2	181c,30e-5p	2
106a,126	1	let-7i,422b	2	106a,126	1	let-7i,422b	2
106a,181c	1	106a,182	1	106a,181c	1	106a,182	1
106a,324-3p	1	126*,422b	1	106a,324-3p	1	126*,422b	1
		15b,27b	1			15b,27b	1
		25,26b	1			25,26b	1
		30b,30e-5p	1			30b,30e-5p	1
		let-7i,15a	1			let-7i,15a	1
		let-7i,30b	1			let-7i,30b	1

[0133] The unique miRNAs that were most frequently associated with the classifier models from Table 40 and that distinguished lung cancer from cancer free subjects are shown in Table 41. These miRNAs can be used to distinguish patients with lung cancer from cancer free subjects, and therefore can be used in the characterization or diagnosis of lung cancer.

Table 41: miRNAs that can be used to distinguish patients with lung cancer from cancer free subjects

LSVM Train+TTest+7	LSVM Train+Relief+7	LDA Train+TTest+7	LDA Train+Relief+7
106a	106a	106a	106a
106b		106b	
126*	126*	126*	126*
142-3p		142-3p	

15b	15b	15b	15b
181c		181c	
182		182	
26b	26b	26b	26b
30a-5p	30a-5p	30a-5p	30a-5p
	30b		30b
	30d		30d
30e-5p	30e-5p	30e-5p	30e-5p
422b	422b	422b	422b
	let-7i		let-7i
let-7g		let-7g	

[0134] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

[0135] All references cited herein are incorporated herein by reference in their entirety. To the extent publications and patents or patent applications incorporated by reference contradict the invention contained in the specification, the specification will supersede any contradictory material.

What is claimed is:

1. A method for characterizing lung cancer in a patient with a lung tumor or lesion comprising the steps of:

a. measuring the level of a miRNA in a serum sample, wherein the miRNA is chosen from let-7b, let-7c, let-7d, let-7e, miR-10a, miR-10b, miR-130b, miR-132, miR-133b, miR-139, miR-143, miR-152, miR-155, miR-15b, miR-17-5p, miR-193, miR-194, miR-195, miR-196b, miR-199a*, miR-19b, miR-202, miR-204, miR-205, miR-206, miR-20b, miR-21, miR-210, miR-214, miR-221, miR-27a, miR-27b, miR-296, miR-29a, miR-301, miR-324-3p, miR-324-5p, miR-339, miR-346, miR-365, miR-378, miR-422a, miR-432, miR-485-3p, miR-496, miR-497, miR-505, miR-518b, miR-525, miR-566, miR-605, miR-638, miR-660, and miR-93; and

b. determining reduced or elevated levels of the miRNA in the sample, thereby characterizing lung cancer in the patient.

2. The method of claim 1, wherein the lung cancer is small cell or non-small cell lung cancer.

3. The method of claim 1, wherein the patient has previously been screened for lung disease.

4. The method of claim 1, wherein the patient is suspected of having lung cancer or is at risk of developing lung cancer.

5. The method of claim 3, wherein the screening was by CT scan or chest x-ray.

6. The method of claim 1, further comprising amplifying the miRNA.

7. The method of claim 6, wherein the amplification is by quantitative reverse transcriptase polymerase chain reaction.

8. The method of claim 1, further comprising amplifying, measuring, and determining reduced or elevated levels of a second miRNA.

9. A method for characterizing lung disease in a patient comprising the steps of:

a. measuring the level of a first miRNA and a second miRNA in a serum sample, wherein the first miRNA is chosen from let-7a, let-7b, let-7d, let-7f, let-7g, let-7i, miR-101, miR-106a, miR-106b, miR-125a, miR-126, miR-126*, miR-130b, miR-132, miR-133b, miR-140, miR-142-3p, miR-142-5p, miR-145, miR-146a, miR-146b, miR-148b, miR-150, miR-151, miR-15a,

miR-15b, miR-181a, miR-181b, miR-181d, miR-185, miR-186, miR-190, miR-191, miR-193a, miR-199a*, miR-202, miR-210, miR-214, miR-222, miR-23a, miR-24, miR-26a, miR-26b, miR-27a, miR-27b, miR-29b, miR-301, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-5p, miR-320, miR-324-3p, miR-326, miR-335, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-374, miR-375, miR-378, miR-422a, miR-422b, miR-425, miR-486, miR-496, miR-518b, miR-660, miR-7, miR-92, miR-93, miR-98, miR-99a, and miR-99b; and

b. detecting reduced or elevated levels of the first and second miRNAs in the sample, thereby characterizing lung disease in the patient.

10. The method of claim 9, wherein the first miRNA is chosen from let-7g, miR-106b, miR-126, miR-126*, miR-132, miR-140, miR-142-3p, miR-146a, miR-150, miR-15a, miR-15b, miR-181a, miR-181b, miR-181d, miR-214, miR-24, miR-30a-5p, miR-320, miR-342, miR-345, miR-374, miR-422a, miR-422b, miR-486, and miR-92.

11. The method of claim 9, wherein the first and second miRNAs are a pair chosen from Table 3.

12. The method of claim 9, wherein the lung disease is cancer.

13. The method of claim 9, wherein the patient has a lung tumor or lesion.

14. The method of claim 9, wherein the patient has previously been screened for lung disease.

15. The method of claim 12, wherein the cancer is small cell or non-small cell lung cancer.

16. The method of claim 9, further comprising amplifying the first miRNA and the second miRNA.

17. The method of claim 16, wherein the amplification is by quantitative reverse transcriptase polymerase chain reaction.

18. The method of claim 9, wherein the patient is male.

19. The method of claim 9, wherein the patient is female.

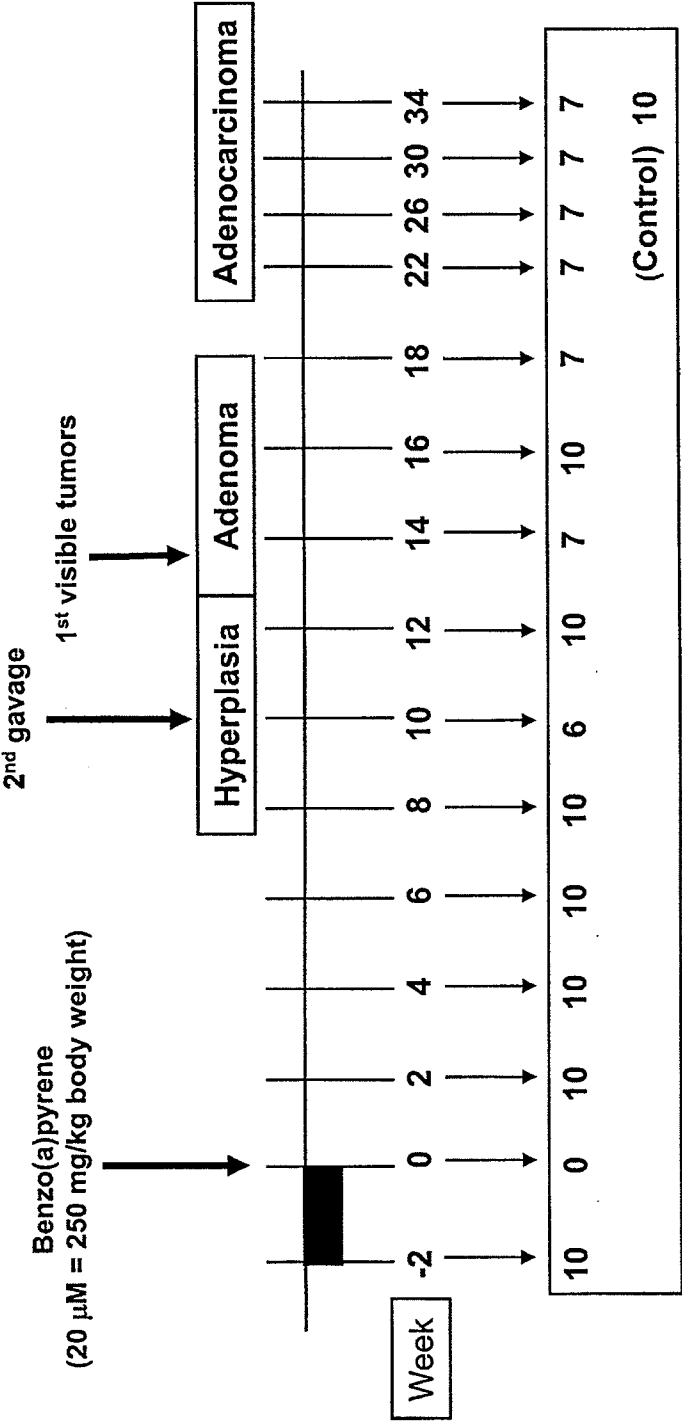


Figure 1

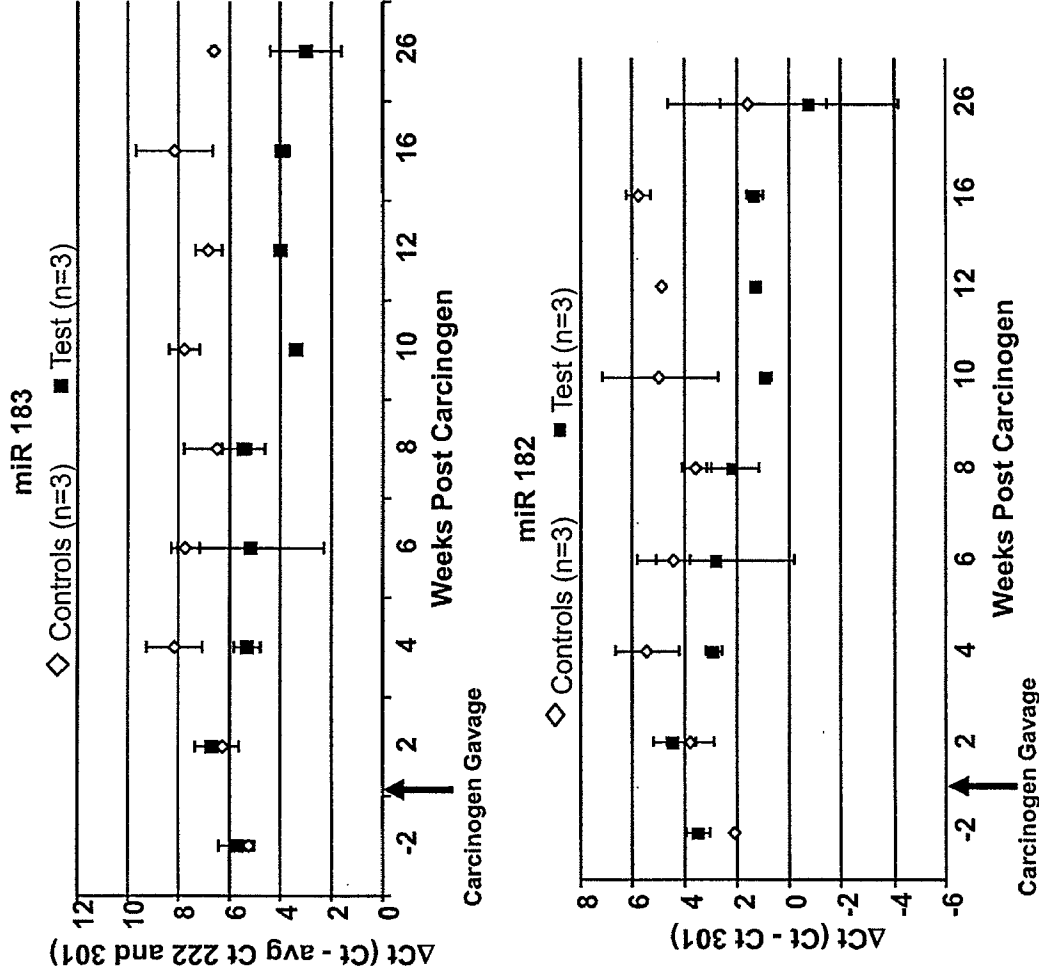


Figure 2A

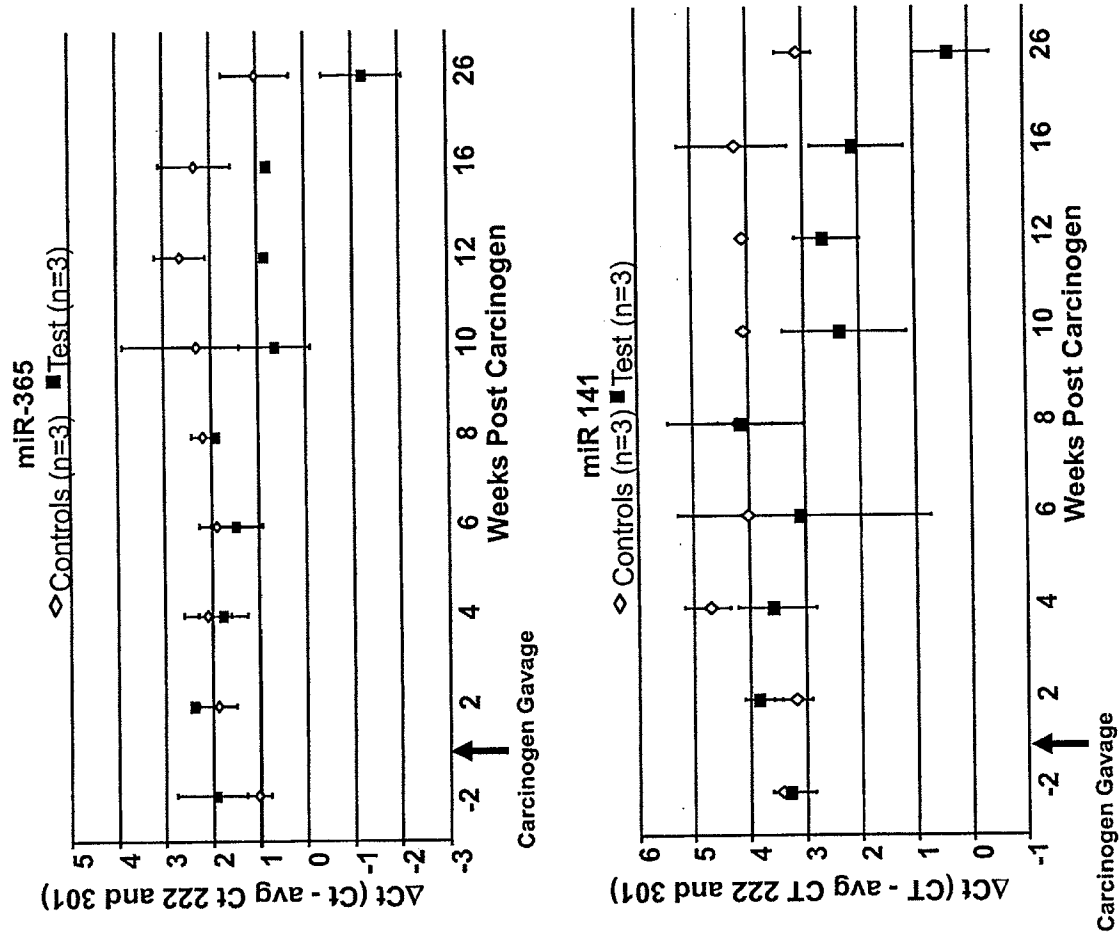


Figure 2B

AUC Values as a Function of the Number of Biomarkers
(Classifier is LDA with T-test for Biomarker Selection)

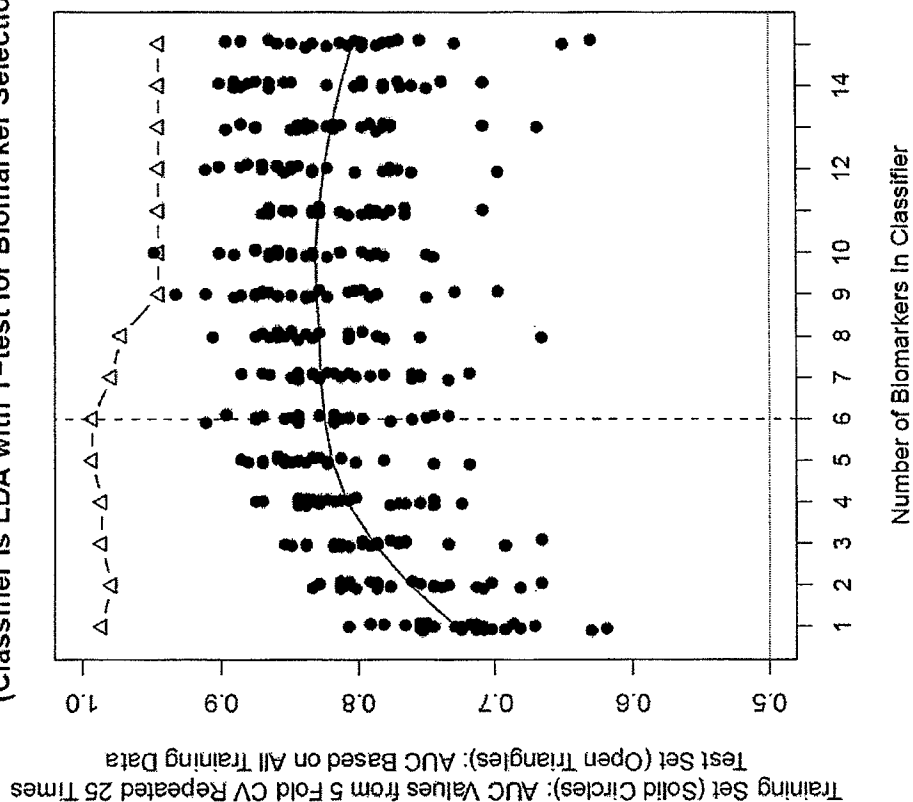


Figure 3

Training Set Results (Based on Median CV Probability)				Test Set Results (Based on ALL Training Data)			
Metric	Est	lower	upper	Metric	Est	lower	upper
SENS	.75	.49	.93	SENS	1	.72	1
SPEC	.88	.67	.98	SPEC	.94	.77	1
NPV	0.82	0.55	0.96	NPV	0.89	0.6	0.99
PPV	0.82	0.61	0.95	PPV	1	0.85	1

	Actual Benign	Actual LuCa
Predicted Benign	8	1
Predicted Lung Cancer	0	17

	Actual Benign	Actual LuCa
Predicted Benign	9	2
Predicted Lung Cancer	3	14

Figure 4

pre-miRNA	Sequence	Mature miRNA
let-7a-1 (SEQ ID NO:1)	UGGGAUGAGGUAGUAGGUUGUAUAGUUUAGGGUCACACCCACCACUGGGA GAUAACUAUACAACUACUGUCUUUCCUA	let-7a
let-7a-2 (SEQ ID NO:2)	AGGUUGAGGUAGUAGGUUGUAUAGUUUAGAAUUAUCAAGGGAGAUAAU GUACAGCCUCCUAGCUUCCU	let-7a
let-7a-3 (SEQ ID NO:3)	GGGUGAGGUAGUAGGUUGUAUAGUUUGGGGCUCUGCCCUGCUAUGGGAUAA CUAUACAAUCUACUGUCUUUCCU	let-7a
let-7b (SEQ ID NO:4)	CGGGUGAGGUAGUAGGUUGUGUGGUUUCAGGGCAGUGAUGUUGCCCCUCG GAAGAUAAUUAUACAACCUACUGCCUCCUCCUG	let-7b
let-7c (SEQ ID NO:5)	GCAUCCGGGUUGAGGUAGUAGGUUGUAUGGUUUAGAGUUACACCCUGGGAG UUAACUGUACAACCUUCUAGCUUCCUUGGAGC	let-7c
let-7d (SEQ ID NO:6)	CCUAGGAAGAGGUAGUAGGUUGCAUAGUUUAGGGCAGGGAUUUUGCCCAC AAGGAGGUAAUUAUACGACCUGCUGCCUUCUAGG	let-7d
let-7e (SEQ ID NO:7)	CCCGGGCUGAGGUAGGAGGUUGUAUAGUUGAGGAGGACACCCAAGGAGAU ACUAUACGGCCUCCUAGCUUCCCCAGG	let-7e
let-7f-1 (SEQ ID NO:8)	UCAGAGUGAGGUAGUAGAUUGUAUAGUUGUGGGGUAGUGAUUUUACCCUGU UCAGGAGAUAAUUAUACAACUUAUUGCCUCCCCUGA	let-7f
let-7f-2 (SEQ ID NO:9)	UGUGGGAUGAGGUAGUAGAUUGUAUAGUUUAGGGUCAUACCCCAUCUUGG AGAUAAUUAUACAGUCUACUGUCUUUCCACG	let-7f
let-7g (SEQ ID NO:10)	AGGCUGAGGUAGUAGUUUGUACAGUUUGAGGGUCUAUGAUACCACCCGGUA CAGGAGAUAAUUAUACAGGCCACUGCCUUGCCA	let-7g
let-7i (SEQ ID NO:11)	CUGGCUGAGGUAGUAGUUUGUGCUGUUGGUCGGGUUGUGACAUUGCCCGCU GUGGAGAUAAUUAUACGCAAGCUACUGCCUUGCUA	let-7i
miR-1-1 (SEQ ID NO:12)	UGGGAAACAUAUCUUCUUUAUUGCCCAUAUGGACCUGCUAAGCUAUGGAAU GUAAAGAAGUAUGUAUCUCA	miR-1
miR-1-2 (SEQ ID NO:13)	ACCUACUCAGAGUACAUAUCUUCUUUAUGUACCCAUAUGAACAUACAAUGCU AUGGAAUGUAAAGAAGUAUGUAUUUUUGGUAGGC	miR-1
miR-100 (SEQ ID NO:14)	CCUGUUGCCACAAACCCGUAGAUCGGAACUUGUGGUAAUAGUCCGCACAAG CUUGUAUCUAUAGGUUAGUGUCUGUUAGG	miR-100
miR-101-1 (SEQ ID NO:15)	UGCCCUGGCUCAGUUUAUCACAGUGCUGAUGCUGUCUAUUCUAAAGGUACAG UACUGUGAUAAUGAAGGAUGGCA	miR-101
miR-101-2 (SEQ ID NO:16)	ACUGUCCUUUUUCGGUUAUCAUGGUACCGAUGCUGUAUAUCUGAAAGGUAC AGUACUGUGAUAAUGAAGAAUGGUGGU	miR-101
miR-103-1 (SEQ ID NO:17)	UACUGCCCUCGGCUUCUUUACAGUGCUGCCUUGUUGCAUAUGGAUCAAGCA GCAUUGUACAGGGCUAUGAAGGCAUUG	miR-103
miR-103-2 (SEQ ID NO:18)	UUGUGCUUUCAGCUUCUUUACAGUGCUGCCUUGUAGCAUUCAGGUCAAGCA GCAUUGUACAGGGCUAUGAAGAAACCA	miR-103

FIG. 5A

pre-miRNA	Sequence	Mature miRNA
miR-105-1 (SEQ ID NO:19)	UGUGCAUCGUGGUCAAAGCUCAGACUCCUGUGGUGGCUGCUAUGCACCA CGGAUGUUUGAGCAUGUGCUACGGUGUCUA	miR-105
miR-105-2 (SEQ ID NO:20)	UGUGCAUCGUGGUCAAAGCUCAGACUCCUGUGGUGGCUGCUAUGCACCA CGGAUGUUUGAGCAUGUGCUAUGGUGUCUA	miR-105
miR-106a (SEQ ID NO:21)	CCUUGGCCAUGUAAAAGUGCUUACAGUGCAGGUAGCUUUUUGAGAUCUACU GCAAUGUAAGCACUUCUACAUUACCAUGG	miR-106a
miR-106b (SEQ ID NO:22)	CCUGCCGGGGCUAAAGUGCUGACAGUGCAGAUAGUGGUCCUCUCCGUGCUA CCGCACUGUGGGUACUUGCUGCUCCAGCAGG	miR-106b
miR-107 (SEQ ID NO:23)	CUCUCUGCUUUCAGCUUCUUUACAGUGUUGCCUUGUGGCAUGGAGUUAAG CAGCAUUGUACAGGGCUAUCAAAGCACAGA	miR-107
miR-10a (SEQ ID NO:24)	GAUCUGUCUGUCUUCUGUAUAUACCCUGUAGAUCGAAUUUGUGUAAGGAA UUUUGUGGUCACAAAUUCGUAUCUAGGGGAAUAUGUAGUUGACAUAAACAC UCCGCUCU	miR-10a
miR-10b (SEQ ID NO:25)	CCAGAGGUUGUAACGUUGUCUAUAUAUACCCUGUAGAACCGAAUUUGUGUG GUAUCCGUUAUAGUCACAGAUUCGAUUCUAGGGGAAUAUAUGGUCGAUGCAA AAACUUCA	miR-10b
miR-122 (SEQ ID NO:26)	CCUUAGCAGAGCUGUGGAGUGUGACAAUGGUGUUUGUGUCUAAACUAUCAA ACGCCAUUAUCACACUAAAAGCUACUGCUAGGC	miR-122a (also called miR-122)
miR-124-1 (SEQ ID NO:27)	AGGCCUCUCUCUCCGUGUUCACAGCGGACCUUGAUUUAAAUGUCCAUAACA UUAAGGCACGCGGUGAAUGCCAAGAAUGGGGCGUG	miR-124a (also called miR-124)
miR-124-2 (SEQ ID NO:28)	AUCAAGAUUAGAGGCUCUGCUCUCCGUGUUCACAGCGGACCUUGAUUUAAU GUCAUACAAUUAAGGCACGCGGUGAAUGCCAAGAGCGGAGCCUACGGCUGC ACUUGAA	miR-124a (also called miR-124)
miR-124-3 (SEQ ID NO:29)	UGAGGGCCCCUCUGCGUGUUCACAGCGGACCUUGAUUUAAUGUCUAUACAA UUAAGGCACGCGGUGAAUGCCAAGAGAGGCGCCUCC	miR-124a (also called miR-124)
miR-125a (SEQ ID NO:30)	UGCCAGUCUCUAGGUCCUGAGACCCUUAACCUUGUGAGGACAUCCAGGGU CACAGGUGAGGUUCUUGGGAGCCUGGCGUCUGGCC	miR-125a
miR-125b-1 (SEQ ID NO:31)	UGCGCUCCUCUCAGUCCUGAGACCCUAACUUGUGAUGUUUACCGUUUAAA UCCACGGGUUAGGCUCUUGGGAGCUGCGAGUCGUGCU	miR-125b
miR-125b-2 (SEQ ID NO:32)	ACCAGACUUUCCUAGUCCUGAGACCCUAACUUGUGAGGUUUUUAGUAA CAUCACAAGUCAGGCUCUUGGGACCUAGGCGGAGGGGA	miR-125b
miR-126 (SEQ ID NO:33)	CGCUGGCGACGGGACAUUAUUACUUUUGGUACGCGCUGUGACACUCAAAC UCGUACCGUGAGUAAUAAUGCGCCGUCCACGGCA	miR-126; miR- 126*
miR-127 (SEQ ID NO:34)	UGUGAUCACUGUCUCCAGCCUGCUGAAGCUCAGAGGGCUCUGAUUCAGAAA GAUCAUCGGAUCCGUCUGAGCUUGGCUGGUCGGAAGUCUCAUCAUC	miR-127

FIG. 5B

pre-miRNA	Sequence	Mature miRNA
miR-128-1 (SEQ ID NO:35)	UGAGCUGUUGGAUUCGGGGCCGUAGCACUGUCUGAGAGGUUUACAUUUCUC ACAGUGAACCGGUCUCUUUUUCAGCUGCUUC	miR-128a
miR-128-2 (SEQ ID NO:36)	UGUGCAGUGGGAAGGGGGGCCGAUACACUGUACGAGAGUGAGUAGCAGGUC UCACAGUGAACCGGUCUCUUUUCCUACUGUGUC	miR-128b
miR-129-1 (SEQ ID NO:37)	GGAUCUUUUUGCGGUCUGGGCUUGCUGUCCUCUCAACAGUAGUCAGGAAG CCCUUACCCCAAAAAGUAUCU	miR-129
miR-129-2 (SEQ ID NO:38)	UGCCCUUCGCGAAUCUUUUUGCGGUCUGGGCUUGCUGUACAUAACUCAUA GCCGGAAGCCCUUACCCCAAAAAGCAUUUGCGGAGGGCG	miR-129
miR-130a (SEQ ID NO:39)	UGCUGCUGGCCAGAGCUCUUUUACAUAUGUGCUACUGUCUGCACCUGUCAC UAGCAGUGCAAUGUAAAAGGGCAUUGGCCGUGUAGUG	miR-130a
miR-130b (SEQ ID NO:40)	GGCCUGCCCGACACUCUUUCCUGUUGCACUACUAUAGGCCGCGUGGGAAGC AGUGCAAUGAUGAAAGGGCAUCGGUCAGGUC	miR-130b
miR-132 (SEQ ID NO:41)	CCGCCCCCGCGUCUCCAGGGCAACCGUGGCUUUCGAUUGUUACUGUGGGAA CUGGAGGUAAACAGUCUACAGCCAUGGUCGCCCGCAGCACGCCACGCGC	miR-132
miR-133a-1 (SEQ ID NO:42)	ACAAUGC UUUGCUAGAGCUGGUAAAAUGGAACCAAUCGCCUCUCAAUGG AUUUGGUCCCCUUAACACAGCUGUAGCUAUGCAUUGA	miR-133a
miR-133a-2 (SEQ ID NO:43)	GGGAGCCAAAUGC UUUGCUAGAGCUGGUAAAAUGGAACCAAUCGACUGUC CAAUGGAUUUGGUCCCCUUAACACAGCUGUAGCUGUGCAUUGAUGGCGCCG	miR-133a
miR-133b (SEQ ID NO:44)	CCUCAGAAGAAAGAUGCCCCUGCUCUGGCUCAAACGGAACCAAGUCC GUCUUCUGAGAGGUUUGGUCCCCUUAACACAGCUACAGCAGGGCUGGCAA UGCCAGUCCUUGGAGA	miR-133b
miR-134 (SEQ ID NO:45)	CAGGGUGUGUGACUGGUUGACCAGAGGGGCAUGCACUGUGUUCACCCUGUG GGCCACCUAGUCACCAACCCUC	miR-134
miR-135a-1 (SEQ ID NO:46)	AGGCCUCGCGUUCUCUAUGGCUUUUUAUCCUAUGUGAUUCUACUGCUCA CUCAUAUAGGGAUUGGAGCCGUGGCGCACGGCGGGGACA	miR-135a
miR-135a-2 (SEQ ID NO:47)	AGAUAUUUACUCUCUAGUGCUUUUUGGCUUUUUAUCCUAUGUGAUAGUAA UAAAGUCUAUGUAGGGAUGGAAGCCAUGAAAUAUUGUGAAAAUCA	miR-135a
miR-135b (SEQ ID NO:48)	CACUCUGCUGUGGCCUAUGGCUUUUAUCCUAUGUGAUUGCUGUCCCAA CUCAUGUAGGGCUAAAAGCCAUGGGCUACAGUGAGGGGCGAGCUCC	miR-135b
miR-136 (SEQ ID NO:49)	UGAGCCUCGAGGACUCCAUUUGUUUGAUGAUGGAUUCUUAUGCUCCA CAUCGUCUCAAUGAGUCUUCAGAGGGUUCU	miR-136
miR-137 (SEQ ID NO:50)	GGUCCUCUGACUCUCUUCGGUGACGGGAUUCUUGGGUGGAUAAUACGGAU UACGUUGUUAUUGCUUAAGAAUACGCGUAGUCGAGGAGAGUACCAGCGGCA	miR-137

FIG. 5C

pre-miRNA	Sequence	Mature miRNA
miR-138-1 (SEQ ID NO:51)	CCCUGGCAUGGUGUGGUGGGGCGAGCUGGUGUUGUGAAUCAGGCCGUUGCCA AUCAGAGAACGGCUACUUCACAACACCAGGGCCACACCACACUACAGG	miR-138
miR-138-2 (SEQ ID NO:52)	CGUUGCUGCAGCUGGUGUUGUGAAUCAGGCCGACGAGCAGCGCAUCCUCUU ACCCGGCUAUUUCACGACACCAGGGUUGCAUCA	miR-138
miR-139 (SEQ ID NO:53)	GUGUAUUCUACAGUGCACGUGUCUCCAGUGUGGCUCGGAGGCUGGAGACGC GGCCUGUUGGAGUAAC	miR-139
miR-140 (SEQ ID NO:54)	UGUGUCUCUCUCUGUGUCCUGGCCAGUGGUUUUACCCUAUGGUAGGUUACGU CAUGCUGUUCUACCACAGGGUAGAACCACGGACAGGAUACGGGGGCACC	miR-140
miR-141 (SEQ ID NO:55)	CGGCCGGCCUGGGUCCAUCUCCAGUACAGUGUUGGAUGGUCUAAUUGUG AAGCUCUAAACACUGUCUGGUAAAGAUGGCUCGCCGGUGGGUUC	miR-141
miR-142 (SEQ ID NO:56)	GACAGUGCAGUCACCCAUAAAGUAGAAAGCACUACUAAACAGCACUGGAGGG UGUAGUGUUUCCUACUUUAUGGAUGAGUGUACUGUG	miR-142-3p; miR-142-5p
miR-143 (SEQ ID NO:57)	GCGCAGCGCCUGUCUCCAGCCUGAGGUGCAGUGCUGCAUCUCUGGUCAG UUGGGAGUCUGAGAUGAAGCACUGUAGCUCAGGAAGAGAGAAGUUGUUCUG CAGC	miR-143
miR-145 (SEQ ID NO:58)	CACCUUGUCCUCACGGUCCAGUUUUCAGGAAUCCCUUAGAUGCUAAGAU GGGGAUCCUGGAAAUACUGUUCUUGAGGUCAUGGUU	miR-145
miR-146a (SEQ ID NO:59)	CCGAUGUGUAUCCUCAGCUUUGAGAACUGAAUCCAUGGGUUGUGUCAGUG UCAGACCUCUGAAAUUCAGUUCUUCAGCUGGGAAUUCUCUGUCAUCGU	miR-146a
miR-146b (SEQ ID NO:60)	CCUGGCACUGAGAACUGAAUCCAUAGGCUGUGAGCUCUAGCAAUGCCUG UGGACUCAGUUCUGGUGCCCGG	miR-146b
miR-147 (SEQ ID NO:61)	AAUCUAAAGACAACAUUUCUGCACACACACCAGACUAUGGAAGCCAGUGUG UGGAAAUGCUUCUGCUAGAUU	miR-147
miR-147b (SEQ ID NO:62)	UAUAAAUCUAGUGGAAACAUUUCUGCACAAACUAGAUCUGGACACCAGUG UGCGGAAAUGCUUCUGCUACAUUUUUAGG	miR-147b
miR-148a (SEQ ID NO:63)	GAGGCAAAGUUCUGAGACACUCCGACUCUGAGUAUGAUAGAAGUCAGUGCA CUACAGAACUUUGUCUC	miR-148a
miR-148b (SEQ ID NO:64)	CAAGCACGAUUAGCAUUUGAGGUGAAGUUCUGUUAUACACUCAGGCUGUGG CUCUCUGAAAGUCAGUGCAUCACAGAACUUUGUCUCGAAAGCUUUCUA	miR-148b
miR-149 (SEQ ID NO:65)	GCCGGCGCCGAGCUCUGGCUCGUGUCUUCACUCCCGUGCUUGUCCGAGG AGGGAGGGAGGGACGGGGGCUGUGCUGGGGCAGCUGGA	miR-149
miR-150 (SEQ ID NO:66)	CUCCCCAUGGCCUGUCUCCCAACCCUUGUACCAGUGCUGGGCUCAGACCC UGGUACAGGCCUGGGGACAGGGACCUGGGGAC	miR-150

FIG. 5D

pre-miRNA	Sequence	Mature miRNA
miR-151 (SEQ ID NO:67)	UUUCCUGCCCUCGAGGAGCUCACAGUCUAGUAUGUCUCAUCCCCUACUAGA CUGAAGCUCCUUGAGGACAGGGAUGGUCAUACUCACCUC	miR-151
miR-152 (SEQ ID NO:68)	UGUCCCCCCCCGGCCAGGUUCUGUGAUACACUCCGACUCGGGCUCUGGAGC AGUCAGUGCAUGACAGAACUUGGGCCCGGAAGGACC	miR-152
miR-153-1 (SEQ ID NO:69)	CUCACAGCUGCCAGUGUCAUUUUUGUGAUCUGCAGCUAGUAUUCUCACUCC AGUUGCAUAGUCACAAAAGUGAUCAUUGGCAGGUGUGGC	miR-153
miR-153-2 (SEQ ID NO:70)	AGCGGUGGCCAGUGUCAUUUUUGUGAUGUUGCAGCUAGUAAUAUGAGCCCA GUUGCAUAGUCACAAAAGUGAUCAUUGGAAACUGUG	miR-153
miR-154 (SEQ ID NO:71)	GUGGUACUUGAAGAUAGGUUAUCCGUGUUGCCUUCGUUUUUUUGUGACGA AUCAUACACGGUUGACCUAUUUUUUCAGUACCAA	miR-154; miR- 154*
miR-155 (SEQ ID NO:72)	CUGUUAUUGCUAAUCGUGAUAGGGGUUUUUGCCUCCAACUGACUCCUACAU AUUAGCAUUAACAG	miR-155
miR-15a (SEQ ID NO:73)	CCUUGGAGUAAAGUAGCAGCACAUAAUGGUUUGUGGAUUUUGAAAAGGUGC AGGCCAUAUUGUGCUGCCUCAAAAUAACAAGG	miR-15a
miR-15b (SEQ ID NO:74)	UUGAGGCCUUAAGUACUGUAGCAGCACAUCAUGGUUUACAUGCUCACAGUC AAGAUGCGAAUCAUUAUUUGCUGCUCUAGAAAUUUAAGGAAAUUCAU	miR-15b
miR-16-1 (SEQ ID NO:75)	GUCAGCAGUGCCUAGCAGCACGUAAAUAUUGGCGUUAAGAUUCUAAAAUU AUCUCCAGUAUUAACUGUGCUGCUGAAGUAAGGUUGAC	miR-16
miR-16-2 (SEQ ID NO:76)	GUUCCACUCUAGCAGCACGUAAAUAUUGGCGUAGUGAAAUUAUAUUAAC ACCAUAUUAACUGUGCUGCUUUAGUGUGAC	miR-16
miR-17 (SEQ ID NO:77)	GUCAGAAUAUUGUCAAGUGCUUACAGUGCAGGUAGUGAUUUGUGCAUCUA CUGCAGUGAAGGCACUUGUAGCAUUAUGGUGAC	miR-17-3p; miR- 17-5p
miR-181a-1 (SEQ ID NO:78)	UGAGUUUUGAGGUUGCUUCAGUGAACAUUCAAACGUGUCGGUGAGUUUGGA AUUAAAAUCAAAACCAUCGACCGUUGAUUGUACCCUAUGGCUAACCAUCAU CUACUCCA	miR-181a; miR- 213 (also called miR-181a*)
miR-181a-2 (SEQ ID NO:79)	AGAAGGGCUAUCAGGCCAGCCUUCAGAGGACUCCAAGGAACAUAUCAAACGCU GUCGGUGAGUUUGGAUUUGAAAAAACACUGACCGUUGACUGUACCUUGG GGUCCUUA	miR-181a
miR-181b-1 (SEQ ID NO:80)	CCUGUGCAGAGAUUUAUUUUUAAAAGGUCACAAUCAACAUUCAUUGCUGUC GGUGGGUUGAACUGUGUGGACAAGCUCACUGAACAAUGAAUGCAACUGUGG CCCCGCUU	miR-181b
miR-181b-2 (SEQ ID NO:81)	CUGAUGGCUGCACUCAACAUUCAUUGCUGUCGGUGGGUUUGAGUCUGAAUC AACUCACUGAUCAAUGAAUGCAAACUGCGGACCAACA	miR-181b
miR-181c (SEQ ID NO:82)	CGGAAAAUUUGCCAAGGGUUUGGGGAACAUAUCAAACUGUCGGUGAGUUUG GGCAGCUCAGGCAAACCAUCGACCGUUGAGUGGACCCUGAGGCCUGGAAUU GCCAUCCU	miR-181c

FIG. 5E

pre-miRNA	Sequence	Mature miRNA
miR-181d (SEQ ID NO:83)	GUCCCCUCCCCUAGGCCACAGCCGAGGUCACAAUCAUUAUUGUUGUC GGUGGGUUGUGAGGACUGAGGCCAGCCACCGGGGAUGAAUGUCACUGU GGCUGGGCCAGACACGGCUUAAGGGGAUUGGGGAC	miR-181d
miR-182 (SEQ ID NO:84)	GAGCUGCUUGCCUCCCCCGUUUUUGGCAAUGGUAGAACUCACACUGGUGA GGUAAACAGGAUCCGGUGGUUCUAGACUUGCCAACUAUGGGGCGAGGACUCA GCCGGCAC	miR-182; miR-182*
miR-183 (SEQ ID NO:85)	CCGCAGAGUGUGACUCCUGUUCUGUGUAUGGCACUGGUAGAAUUCACUGUG AACAGUCUCAGUCAGUGAAUUACCGAAGGGCCAUAACACAGAGCAGAGACAG AUCCACGA	miR-183
miR-184 (SEQ ID NO:86)	CCAGUCACGUCCCCUUAUCACUUUCCAGCCCAGCUUUGUGACUGUAAGUG UUGGACGGAGAACUGAUAAAGGGUAGGUGAUUGA	miR-184
miR-185 (SEQ ID NO:87)	AGGGGGCGAGGGAUUGGAGAGAAAGGCAGUCCUGAUUGGUCCCCUCCCCAG GGCUGGGCUUCCUCUGGUCCUCCCCUCCCA	miR-185
miR-186 (SEQ ID NO:88)	UGCUGUAACUUUCCAAAGAAUUCUCCUUUUGGGCUUUCUGGUUUUAUUUU AAGCCCAAAGGUGAAUUUUUGGGAAGUUUGAGCU	miR-186
miR-187 (SEQ ID NO:89)	GGUCGGGCUACCAUGACACAGUGUGAGACCUCGGGCUACAACACAGGACC CGGGCGCUGCUCUGACCCUCGUGUCUUGUGUUGCAGCCGGAGGGACGCAG GUCCGCA	miR-187
miR-188 (SEQ ID NO:90)	UGCUCUCCUCUCUCACAUCCCUUGCAUGGUGGAGGGUGAGCUUUCUGAAAAC CCCUCCACAUGCAGGGUUUGCAGGAUGGCGAGCC	miR-188
miR-18a (SEQ ID NO:91)	UGUUCUAAGGUGCAUCUAGUGCAGAUAGUGAAGUAGAUUAGCAUCUACUGC CCUAAGUGCUCCUUCUGGCA	miR-18a; miR-18a*
miR-18b (SEQ ID NO:92)	UGUGUUAAGGUGCAUCUAGUGCAGUUAGUGAAGCAGCUUAGAAUCUACUGC CCUAAAUGCCCCUUCUGGCA	miR-18b
miR-190 (SEQ ID NO:93)	UGCAGGCCUCUGUGUGAUAGUUUGAUUAUUAUAGGUUGUUAUUUAUCCAA CUAUUAUCAAACAUAUUCUACAGUGUCUUGCC	miR-190
miR-191 (SEQ ID NO:94)	CGGCUGGACAGCGGGCAACGGAAUCCCAAAGCAGCUGUUGUCUCCAGAGC AUUCCAGCUGCGCUUGGAUUUCGUCCCCUGCUCUCCUGCCU	miR-191
miR-192 (SEQ ID NO:95)	GCCGAGACCCGAGUGCACAGGGCUCUGACCUAUGAAUUGACAGCCAGUGCUC UCGUCUCCCCUCUGGCUGCCAAUCCAUAAGGUCACAGGUAUGUUCGCCUCA AUGCCAGC	miR-192
miR-193a (SEQ ID NO:96)	CGAGGAUGGGAGCUGAGGGCUGGGUCUUUGCGGGCGAGAUGAGGGUGUCGG AUCAACUGGCCUACAAAGUCCAGUUCUCGGCCCCCG	miR-193a
miR-193b (SEQ ID NO:97)	GUGGUCUCAGAAUCGGGGUUUUGAGGGCGAGAUGAGUUUAUGUUUUUAUCCA ACUGGCCCUCAAAGUCCCGUUUUGGGGUCAU	miR-193b
miR-194-1 (SEQ ID NO:98)	AUGGUGUUAUCAAGUGUAACAGCAACUCCAUGUGGACUGUGUACCAAUUUC CAGUGGAGAUGCUGUUACUUUGAUGGUUACCAA	miR-194

FIG. 5F

pre-miRNA	Sequence	Mature miRNA
miR-194-2 (SEQ ID NO:99)	UGGUUCCCGCCCCUGUAAACAGCAACUCCAUGUGGAAGUGCCCACUGGUUC CAGUGGGGCGUGUGUUAUCUGGGGCGAGGGCCAG	miR-194
miR-195 (SEQ ID NO:100)	AGCUUCCCGGGCUCUAGCAGCACAGAAAUAUUGGCACAGGGAAGCGAGUCU GCCAAUAUUGGCUGUGCUGCUCCAGGCAGGGUGGUG	miR-195
miR-196a-1 (SEQ ID NO:101)	GUGAAUUAGGUAGUUUCAUGUUGUUGGGCCUGGGUUUCUGAACACAACAAC AUUAAACCACCCGAUUCAC	miR-196a
miR-196a-2 (SEQ ID NO:102)	UGCUCGCUCAGCUGAUCUGGGCUUAGGUAGUUUCAUGUUGUUGGGAUUGA GUUUUGAACUCGGCAACAAGAAACUGCCUGAGUUACAUCAGUCGGUUUUCG UCGAGGGC	miR-196a
miR-196b (SEQ ID NO:103)	ACUGGUCGGUGAUUUAGGUAGUUUCCUGUUGUUGGGAUCCACCUUUCUCUC GACAGCACGACACUGCCUUAUUAUUCAGUUG	miR-196b
miR-197 (SEQ ID NO:104)	GGCUGUGCCGGUAGAGAGGGCAGUGGGAGGUAAGAGCUCUUCACCCUUA CCACCUUUCUCCACCCAGCAUGGCC	miR-197
miR-198 (SEQ ID NO:105)	UCAUUGGUCCAGAGGGGAGAUAGGUUCCUGUGAUUUUCCUUCUUCUUAU AGAAUAAAUGA	miR-198
miR-199a-1 (SEQ ID NO:106)	GCCAAACCAGUGUUCAGACUACCUGUUCAGGAGGCUCUCAUUGUGUACAGU AGUCUGCACAUUGGUUAGGC	miR-199a (also called miR-199-5p); miR-199a* (also called miR-199-3p)
miR-199a-2 (SEQ ID NO:107)	AGGAAGCUUCUGGAGAUCCUGCUCCGUCGCCCCAGUGUUCAGACUACCUGU UCAGGACAAUGCCGUUGUACAGUAGUCUGCACAUUGGUUAGACUGGGCAAG GGAGAGCA	miR-199a (also called miR-199-5p); miR-199a* (also called miR-199-3p)
miR-199b (SEQ ID NO:108)	CCAGAGGACACCUCCACUCCGUCUACCCAGUGUUUAGACUAUCUGUUCAGG ACUCCCAAUUGUACAGUAGUCUGCACAUUGGUUAGGCUGGGCUGGGUUAG ACCCUCGG	miR-199b
miR-19a (SEQ ID NO:109)	GCAGUCCUCUGUUAGUUUUGCAUAGUUGCACUACAAGAAGAAUGUAGUUGU GCAAAUCUAUGCAAAACUGAUGGUGGCCUGC	miR-19a
miR-19b-1 (SEQ ID NO:110)	CACUGUUCUAUGGUUAGUUUUGCAGGUUUGCAUCCAGCUGUGUGAUUUCU GCUGUGCAAAUCCAUGCAAAACUGACUGUGGUAGUG	miR-19b
miR-19b-2 (SEQ ID NO:111)	ACAUUGCUACUACAAUAGUUUUGCAGGUUUGCAUUCAGCGUAUAUAUG UAUAUGUGGCUGUGCAAAUCCAUGCAAAACUGAUUGUGAUAAUGU	miR-19b
miR-200a (SEQ ID NO:112)	CCGGGCCCCUGUGAGCAUCUUAACGGACAGUGCUGGAUUUCCAGCUUGAC UCUAAACACUGUCUGGUACGAUGUCAAAGGUGACCCGC	miR-200a; miR-200a*
miR-200b (SEQ ID NO:113)	CCAGCUCGGGCAGCCGUGGCCAUUUACUGGGCAGCAUUGGAUGGAGUCAG GUCUCUAAUACUGCCUGGUAAUGAUGACGGCGGAGCCUGCACG	miR-200b

FIG. 5G

pre-miRNA	Sequence	Mature miRNA
miR-200c (SEQ ID NO:114)	CCCUCGUCUUACCCAGCAGUGUUUGGGUGCGGUUGGGAGUCUCUAAUACUG CCGGGUAAUGAUGGAGG	miR-200c
miR-202 (SEQ ID NO:115)	CGCCUCAGAGCCGCCCCGCCGUCCUUUUUCCUAUGCAUUAUACUUCUUUGAG GAUCUGGCCUAAAAGAGGUUAUAGGGCAUGGGAAAACGGGGCGGUCGGGUCCU CCCCAGCG	miR-202
miR-203 (SEQ ID NO:116)	GUGUUGGGGACUCGCGCGCUGGGUCCAGUGGUUCUUAACAGUUCAACAGUU CUGUAGCGCAAUUGUGAAAUGUUUAGGACCACUAGACCCGGCGGGCGCGGC GACAGCGA	miR-203
miR-204 (SEQ ID NO:117)	GGCUACAGUCUUUCUUAUGUGACUCGUGGACUCCCUUUGUCAUCCUAUG CCUGAGAAUAUAUGAAGGAGGCUGGGAAGGCAAAGGGACGUCAAUUGUCA UCACUGGC	miR-204
miR-205 (SEQ ID NO:118)	AAAGAUCCUCAGACAAUCCAUGUGCUUCUCUUGUCCUUAUUCACCCGGAG UCUGUCUCAUACCCAACCAGAUUUCAGUGGAGUGAAGUUCAGGAGGCAUGG AGCUGACA	miR-205
miR-206 (SEQ ID NO:119)	UGC U U C C G A G G C C A C A U G C U U C U U A U A U C C C C A U A U G G A U U A C U U U G C U A U G G A A U G U A A G G A A G U G U G G U U U C G G C A A G U G	miR-206
miR-208a (SEQ ID NO:120)	UGACGGGCGAGCUUUUGGCCCGGGUUAUACCUGAUGCUCACGUUAUAGACG AGCAAAAAGCUUGUUGUCA	miR-208 (also called miR-208a)
miR-20a (SEQ ID NO:121)	GUAGCACUAAAGUGCUUAUAGUGCAGGUAGUGUUUAGUUAUCUACUGCAU AUGAGCACUUAAGUACUGC	miR-20a
miR-20b (SEQ ID NO:122)	AGUACCAAAGUGCUCUAGUGCAGGUAGUUUUGGCAUGACUCUACUGUAGU AUGGGCACUUCAGUACU	miR-20b
miR-21 (SEQ ID NO:123)	UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACAC CAGUCGAUGGGCUGUCUGACA	miR-21
miR-210 (SEQ ID NO:124)	ACCCGGCAGUGCCUCCAGGCGCAGGGCAGCCCCUGCCACCGCACACUGCG CUGCCCCAGACCCACUGUGCGUGUGACAGCGGCUGAUCUGUGCCUGGGCAG CGCGACCC	miR-210
miR-211 (SEQ ID NO:125)	UCACCUGGCCAUGUGACUUGUGGGCUUCCCUUUGUCAUCCUUCGCCUAGGG CUCUGAGCAGGGCAGGGACAGCAAAGGGGUGCUCAGUUGUCACUUCCACA GCACGGAG	miR-211
miR-212 (SEQ ID NO:126)	CGGGGCACCCCGCCCGGACAGCGCGCCGGCACC UUGGCUCUAGACUGCUUA CUGCCCGGGCCGCCUCAGUAAACAGUCUCCAGUCACGGCCACCGACGCCUG GCCCGCC	miR-212
miR-214 (SEQ ID NO:127)	GGCCUGGCUGGACAGAGUUGUCAUGUGUCUGCCUGUCUACACUUGCUGUGC AGAACAUCCGCUCACCUGUACAGCAGGCACAGACAGGCAGUCACAUGACAA CCAGCCU	miR-214
miR-215 (SEQ ID NO:128)	AUCAUUCAGAAAUGGUUAUACAGGAAAAUGACCUAUGAAUUGACAGACAAUA UAGCUGAGUUUGUCUGUCAUUUCUUUAGGCCAAUAUUCUGUAUGACUGUGC UACUUCAA	miR-215

FIG. 5H

pre-miRNA	Sequence	Mature miRNA
miR-216a (SEQ ID NO:129)	GAUGGCUGUGAGUUGGCUUAAUCUCAGCUGGCAACUGUGAGAUGUUCAUAC AAUCCUCACAGUGGUCUCUGGGAUUAUGCUAAACAGAGCAAUUUCCUAGC CCUCACGA	miR-216 (also called miR-216a)
miR-217 (SEQ ID NO:130)	AGUAUAAUUAUACAUAUAGUUUUUGAUGUCGCAGAUACUGCAUCAGGAACUG AUUGGAUAAGAAUCAGUCACCAUCAGUCCUAAUGCAUUGCCUUCAGCAUC UAAACAAG	miR-217
miR-218-1 (SEQ ID NO:131)	GUGAUAAUGUAGCGAGAUUUUCUGUUGUGCUUGAUCUAACCAUGUGGUUGC GAGGUAUGAGUAAAACAUGGUUCCGUAAGCACCAUGGAACGUCACGCAGC UUUCUACA	miR-218
miR-218-2 (SEQ ID NO:132)	GACCAGUCGCGUGCGGGGCUUCCUUGUGCUUGAUCUAACCAUGUGGUGGA ACGAUGGAAACGGAACAUGGUUCUGUCAAGCACCGCGGAAAGCACCGUGCU CUCCUGCA	miR-218
miR-219-1 (SEQ ID NO:133)	CCGCCCCGGGCCGCGGCCUCCUGAUUGUCCAAACGCAAUUCUCGAGUCUAUG GCUCGCGCCGAGAGUUGAGUCUGGACGUCCGAGCCGCCGCCCCAAACCU CGAGCGGG	miR-219
miR-219-2 (SEQ ID NO:134)	ACUCAGGGGCUUCGCCACUGAUUGUCCAAACGCAAUUCUUGUACGAGUCUG CGGCCAACCGAGAAUUGUGGCUGGACAUCUGUGGCUGAGCUCCGGG	miR-219
miR-22 (SEQ ID NO:135)	GGCUGAGCCGCGAGUAGUUCUUCAGUGGCAAGCUUUAUGUCCUGACCCAGCU AAAGCUGCCAGUUGAAGAACUGUUGCCUCUGCC	miR-22
miR-221 (SEQ ID NO:136)	UGAACAUCCAGGUCUGGGGCAUGAACCUGGCAUACAAUGUAGAUUUCUGUG UUCGUUAGGCAACAGCUACAUUGUCUGCUGGGUUCAGGCUACCUGGAAAC AUGUUCUC	miR-221
miR-222 (SEQ ID NO:137)	GCUGCUGGAAGGUGUAGGUACCCUCAUUGGCUCAGUAGCCAGUGUAGAUC UGUCUUUCGUAAUCAGCAGCUACAUCUGGCUACUGGGUCUCUGAUGGCAUC UUCUAGCU	miR-222
miR-223 (SEQ ID NO:138)	CCUGGCCUCCUGCAGUGCCACGCUCCGUGUAUUUGACAAGCUGAGUUGGAC ACUCCAUGUGGUAGAGUGUCAGUUUGUCAAAUACCCCAAGUGCGGCACAUG CUUACCAG	miR-223
miR-224 (SEQ ID NO:139)	GGGCUUUAAGUCACUAGUGGUUCCGUUUAGUAGAUGAUUGUGCAUUGUUU CAAAUUGGUGCCUAGUGACUACAAAGCCC	miR-224
miR-23a (SEQ ID NO:140)	GGCCGGCUGGGGUUCCUGGGGAUGGGAUUUGCUUCCUGUCACAAAUACA UGCCAGGGAUUUCCAACCGACC	miR-23a
miR-23b (SEQ ID NO:141)	CUCAGGUGCUCUGGCUGCUUGGGUUCCUGGCAUGCUGAUUUGUGACUUAAG AUUAAAAUCACAUUGCCAGGGAUUACCACGCAACCACGACCUUGGC	miR-23b
miR-24-1 (SEQ ID NO:142)	CUCCGGUGCCUACUGAGCUGAUUACAGUUCUCAUUUACACACUGGCUCAG UUCAGCAGGAACAGGAG	miR-24; miR-189 (also called miR- 24*)
miR-24-2 (SEQ ID NO:143)	CUCUGCCUCCCGUGCCUACUGAGCUGAAACACAGUUGGUUUGUGUACACUG GCUCAGUUCAGCAGGAACAGGG	miR-24

FIG. 5I

pre-miRNA	Sequence	Mature miRNA
miR-25 (SEQ ID NO:144)	GGCCAGUGUUGAGAGGCGGAGACUUGGGCAAUUGCUGGACGCUGCCCUGGG CAUUGCACUUGUCUCGGUCUGACAGUGCCGGCC	miR-25
miR-26a-1 (SEQ ID NO:145)	GUGGCCUCGUUCAAGUAAUCCAGGAUAGGCUGUGCAGGUCCCAAUGGGCCU AUUCUUGGUUACUUGCACGGGGACGC	miR-26a
miR-26a-2 (SEQ ID NO:146)	GGCUGUGGCUGGAUUCAAGUAAUCCAGGAUAGGCUGUUUCCAUCUGUGAGG CCUAUUCUUGAUUACUUGUUUCUGGAGGCAGCU	miR-26a
miR-26b (SEQ ID NO:147)	CCGGGACCCAGUUCAAGUAAUCCAGGAUAGGUUGUGUGCUGUCCAGCCUGU UCUCCAUAUACUUGGCUCGGGGACCGG	miR-26b
miR-27a (SEQ ID NO:148)	CUGAGGAGCAGGGCUUAGCUGCUUGUGAGCAGGGUCCACACCAAGUCGUGU UCACAGUGGCUAAGUUCGCCCCCAG	miR-27a
miR-27b (SEQ ID NO:149)	ACCUCUCUAACAAGGUGCAGAGCUUAGCUGAUUGGUGAACAGUGAUUGGUU UCCGCUUUGUUCACAGUGGCUAAGUUCUGCACCUGAAGAGAAGGUG	miR-27b
miR-28 (SEQ ID NO:150)	GGUCCUUGCCCUC AAGGAGCUCACAGUCUAUUGAGUUACCUUUCUGACUUU CCCACUAGAUUGUGAGCUCCUGGAGGGCAGGCACU	miR-28
miR-296 (SEQ ID NO:151)	AGGACCCUUCAGAGGGCCCCCCCCUCAAUCCUGUUGUGCCUAAUUCAGAGG GUUGGGUGGAGGCUCUCCUGAAGGGCUCU	miR-296
miR-299 (SEQ ID NO:152)	AAGAAAUGGUUUACCGUCCCAUAUACAUUUUGAAUAUGUAUGUGGGGAUGGU AAACCGCUUCUU	miR-299-3p; miR-299-5p
miR-29a (SEQ ID NO:153)	AUGACUGAUUUUUUGGUGUUCAGAGUCAAUAAUAAUUUUCUAGCACCAUC UGAAAUCGGUUAU	miR-29a
miR-29b-1 (SEQ ID NO:154)	CUUCAGGAAGCUGGUUUCAUAUGGUGGUUUAGAUUUAAAUAGUGAUUGUCU AGCACCAUUUGAAAUCAGUGUUCUUGGGG	miR-29b
miR-29b-2 (SEQ ID NO:155)	CUUCUGGAAGCUGGUUUCACAUUGGUGGCUUAGAUUUUUCCAUCUUUGUAUC UAGCACCAUUUGAAAUCAGUGUUUUAGGAG	miR-29b
miR-29c (SEQ ID NO:156)	AUCUCUUACACAGGCUGACCGAUUUCUCCUGGUGUUCAGAGUCUGUUUUUG UCUAGCACCAUUUGAAAUCGGUUAUGAUGUAGGGGGA	miR-29c
miR-301a (SEQ ID NO:157)	ACUGCUAACGAAUGCUCUGACUUUAUUGCACUACUGUACUUUACAGCUAGC AGUGCAAUAGUAUUGUCAAGCAUCUGAAAGCAGG	miR-301 (also called miR-301a)
miR-302a (SEQ ID NO:158)	CCACCACUAAACGUGGAUGUACUUGCUUUGAAACUAAAGAAGUAAGUGCU UCCAUGUUUUGGUGAUGG	miR-302a; miR-302a*
miR-302b (SEQ ID NO:159)	GCUCCCUCAACUUUAACAUGGAAGUGCUUUCUGUGACUUUAAAAGUAAGU GCUUCCAUGUUUUAGUAGGAGU	miR-302b; miR-302b*

FIG. 5J

pre-miRNA	Sequence	Mature miRNA
miR-302d (SEQ ID NO:160)	CCUCUACUUUAACAUGGAGGCACUUGCUGUGACAUGACAAAAUAAGUGCU UCCAUGUUUGAGUGUGG	miR-302d; miR-302d*
miR-30a (SEQ ID NO:161)	GCGACUGUAAACAUCCUCGACUGGAAGCUGUGAAGCCACAGAUGGGCUUUC AGUCGGAUGUUUGCAGCUGC	miR-30a-3p; miR-30a-5p
miR-30b (SEQ ID NO:162)	ACCAAGUUUCAGUUAUGUAAACAUCUACACUCAGCUGUAAUACAUGGAU UGGCUGGGAGGUGGAUGUUUACUUCAGCUGACUUGGA	miR-30b
miR-30c-1 (SEQ ID NO:163)	ACCAUGCUGUAGUGUGUGUAAACAUCUACACUCUCAGCUGUGAGCUCAAG GUGGCUGGGAGAGGGUUGUUUACUCCUUCUGCCAUGGA	miR-30c
miR-30c-2 (SEQ ID NO:164)	AGAUACUGUAAACAUCUACACUCUCAGCUGUGGAAAGUAAGAAAGCUGGG AGAAGGCUGUUUACUCUUUCU	miR-30c
miR-30d (SEQ ID NO:165)	GUUGUUGUAAACAUCUCCCGACUGGAAGCUGUAAGACACAGCUAAGCUUUA GUCAGAUGUUUGCUGCUAC	miR-30d
miR-30e (SEQ ID NO:166)	GGGCAGUCUUUGCUACUGUAAACAUCUUGACUGGAAGCUGUAAGGUGUUC AGAGGAGCUUUCAGUCGGAUGUUUACAGCGGCAGGCUGCCA	miR-30e-3p; miR-30e-5p
miR-31 (SEQ ID NO:167)	GGAGAGGAGGCAAGAUGCUGGCAUAGCUGUUGAACUGGGAACCUGCUAUGC CAACAUAUUGCCAUCUUUCC	miR-31
miR-32 (SEQ ID NO:168)	GGAGAUUUGCACAUAUAAGUUGCAUGUUGUCACGGCCUCAUGCAAUU UAGUGUGUGUGAUUUUUC	miR-32
miR-320a (SEQ ID NO:169)	GCUUCGCUCCCCUCCGCCUUCUCUCCCCGGUUCUCCCCGAGUCGGGAAAA GCUGGGUUGAGAGGGCGAAAAAGGAUGAGGU	miR-320 (also called miR-320a)
miR-323 (SEQ ID NO:170)	UUGGUACUUGGAGAGAGGUGGUCGUGGCGCGUUCGCUUUAUUUAUGGCGC ACAUUACACGGUCGACCUCUUGCAGUAUCUAAUC	miR-323
miR-324 (SEQ ID NO:171)	CUGACUAUGCCUCCCCGCAUCCCCUAGGGCAUUGGUGUAAAGCUGGAGACC CACUGCCCCAGGUGCUGCUGGGGUUGUAGUC	miR-324-3p; miR-324-5p
miR-325 (SEQ ID NO:172)	AUACAGUGCUUGGUUCCUAGUAGGUGUCCAGUAAGUGUUUGUGACAUAAUU UGUUUAUUGAGGACCUCUAUCAAUCAAGCACUGUGCUAGGCUCUGG	miR-325
miR-326 (SEQ ID NO:173)	CUCAUCUGUCUGUUGGGCUGGAGGCAGGGCCUUGUGAAGGCGGGUGGUGC UCAGAUCCGCUUGGGCCUUCUCCAGCCCCGAGGCGGAUUA	miR-326
miR-328 (SEQ ID NO:174)	UGGAGUGGGGGGCGAGGAGGGGCUAGGGAGAAAGUGCAUACAGCCCCUGG CCCUCUCUGCCCUUCCGUCCCCUG	miR-328
miR-329-1 (SEQ ID NO:175)	GGUACCUGAAGAGAGGUUUUCUGGGUUUCUGUUUCUUUAUGAGGACGAAA CACACCUGGUUAACCUCUUUCCAGUAUC	miR-329
miR-329-2 (SEQ ID NO:176)	GUGGUACCUGAAGAGAGGUUUUCUGGGUUUCUGUUUCUUUAUUGAGGACGA AACACACCUGGUUAACCUCUUUCCAGUAUCAA	miR-329

FIG. 5K

pre-miRNA	Sequence	Mature miRNA
miR-33a (SEQ ID NO:177)	CUGUGGUGCAUUGUAGUUGCAUUGCAUGUUCUGGUGGUACCCAUGCAAUGU UUCCACAGUGCAUCACAG	miR-33 (also called miR-33a)
miR-330 (SEQ ID NO:178)	CUUUGGCGAUCACUGCCUCUCUGGGCCUGUGUCUUAGGCUCUGCAAGAUA ACCGAGCAAAGCACACGGCCUGCAGAGAGGCAGCGCUCUGCCC	miR-330
miR-331 (SEQ ID NO:179)	GAGUUUGGUUUUGUUUGGUUUUGUUCUAGGUUAGGUCCCAGGGAUCCCAGA UCAAACCAGGCCCCUGGGCCUAUCCUAGAACCAACCUAAGCUC	miR-331
miR-335 (SEQ ID NO:180)	UGUUUUGAGCGGGGGUCAAGAGCAAUAACGAAAAAUGUUUGUCAUAAACCG UUUUUCAUUAUUGCUCCUGACCUCUCUCAUUUGCUAUUAUCA	miR-335
miR-337 (SEQ ID NO:181)	GUAGUCAGUAGUUGGGGGUGGGGAACGGCUUCAUACAGGAGUUGAUGCACA GUUAUCCAGCUCUUAUAUGAUGCCUUUCUUAUCCCCUCAA	miR-337
miR-338 (SEQ ID NO:182)	UCUCCAACAAUAUCCUGGUGCUGAGUGAUGACUCAGGCGACUCCAGCAUCA GUGAUUUUGUUGAAGA	miR-338
miR-339 (SEQ ID NO:183)	CGGGGCGGGCGCUCUCCUGUCCUCCAGGAGCUCACGUGUGCCUGCCUGUG AGCGCCUCGACGACAGAGCCGGCGCCUGCCCCAGUGUCUGCGC	miR-339
miR-340 (SEQ ID NO:184)	UUGUACCUGGUGUGAUUAUAAAGCAAUGAGACUGAUUGUCAUAUGUCGUUU GUGGGAUCCGUCUCAGUUACUUUAUAGCCAUACCUGGUUAUCUUA	miR-340
miR-342 (SEQ ID NO:185)	GAAACUGGGCUCAAGGUGAGGGGUGCUAUCUGUGAUUGAGGGACAUGGUUA AUGGAAUUGUCUCACACAGAAUACGCACCCGUCACCUUGGCCUACUUA	miR-342
miR-345 (SEQ ID NO:186)	ACCCAAACCCUAGGUCUGCUGACUCCUAGUCCAGGGCUCGUGAUGGCUGGU GGGCCUGAACGAGGGGUCUGGAGGCCUGGGUUUGAAUAUCGACAGC	miR-345
miR-346 (SEQ ID NO:187)	GGUCUCUGUGUUGGGCGUCUGUCUGCCCGCAUGCCUGCCUCUCUGUUGCUC UGAAGGAGGCAGGGGCGUGGCCUGCAGCUGCCUGGGCAGAGCGG	miR-346
miR-34a (SEQ ID NO:188)	GGCCAGCUGUGAGUGUUUCUUUGGCAGUGUCUUAGCUGGUUGUUGUGAGCA AUAGUAAGGAAGCAAUCAGCAAGUAUACUGCCCUAGAAGUGCUGCACGUUG UGGGGCC	miR-34a
miR-34b (SEQ ID NO:189)	GUGCUCGGUUUGUAGGCAGUGUCAUUAGCUGAUUGUACUGUGGUGGUACA AUCACUAACUCCACUGCCAUAACAAGGCAC	miR-34b
miR-34c (SEQ ID NO:190)	AGUCUAGUUACUAGGCAGUGUAGUUAGCUGAUUGCUAAUAGUACCAAUCAC UAACCACACGGCCAGGUAAAAGAUU	miR-34c
miR-361 (SEQ ID NO:191)	GGAGCUUAUCAGAAUCUCCAGGGGUACUUUAUAAUUUCAAAGUCCCCCA GGUGUGAUUCUGAUUUUGCUUC	miR-361
miR-362 (SEQ ID NO:192)	CUUGAAUCCUUGGAACCUAGGUGUGAGUGCUAUUUCAGUGCAACACACCUA UUCAAGGAUUCAAA	miR-362

FIG. 5L

pre-miRNA	Sequence	Mature miRNA
miR-365-1 (SEQ ID NO:193)	ACCGCAGGGAAAAUGAGGGACUUUUGGGGGCAGAUGUGUUUCCAUCCACU AUCAUAAUGCCCCUAAAAUCCUUAUUGCUCUUGCA	miR-365
miR-365-2 (SEQ ID NO:194)	AGAGUGUUCAAGGACAGCAAGAAAAUGAGGGACUUUCAGGGGCAGCUGUG UUUUCUGACUCAGUCAUAAUGCCCCUAAAAUCCUUAUUGUUCUUGCAGUG UGCAUCGGG	miR-365
miR-367 (SEQ ID NO:195)	CCAUUACUGUUGCUAAUAUGCAACUCUGUUGAAUAUAAAUUGGAAUUGCAC UUUAGCAAUGGUGAUGG	miR-367
miR-369 (SEQ ID NO:196)	UUGAAGGGGAGAUCCGACCGUGUUAUAUUCGCUUUAUUGACUUCGAAUAAUAC AUGGUUGAUCUUUUCUCAG	miR-369-3p; miR- 369-5p
miR-370 (SEQ ID NO:197)	AGACAGAGAAGCCAGGUCACGUCUCUGCAGUUAACAGCUCACGAGUGCCU GCUGGGGUGGAACCGUCUGUCU	miR-370
miR-371 (SEQ ID NO:198)	GUGGCACUCAAAACUGUGGGGGCACUUUCUGCUCUCUGGUGAAAGUGCCGCC AUCUUUUGAGUGUUAC	miR-371
miR-372 (SEQ ID NO:199)	GUGGGCCUCAAAUGUGGAGCACUAUUCUGAUGUCCAAGUGGAAAGUGCUGC GACAUUUGAGCGUCAC	miR-372
miR-373 (SEQ ID NO:200)	GGGAUACUCAAAAUGGGGGCGCUUCCUUUUUGUCUGUACUGGGAAGUGCU UCGAUUUUGGGGUGUCCC	miR-373; miR- 373*
miR-374a (SEQ ID NO:201)	UACAUCGGCCAUAUAUAAUACAACCUGAUAGUGUUAUAGCACUUAUCAGAU UGUAUUGUAAUUGUCUGUGUA	miR-374 (also called miR-374a)
miR-375 (SEQ ID NO:202)	CCCCGCGACGAGCCCCUCGCACAAACCGGACCUGAGCGUUUUGUUCGUUCG GCUCGCGUGAGGC	miR-375
miR-376a-1 (SEQ ID NO:203)	UAAAAGGUAGAUAUCCUUCUAUGAGUACAUUAUUUAUGAUUAAUCAUAGA GGAAAUCCACGUUUUC	miR-376a; miR- 376a*
miR-376a-2 (SEQ ID NO:204)	GGUAUUUAAAAGGUAGAUUUUCCUUCUAUGGUUACGUGUUUGAUGGUUAAU CAUAGAGGAAAAUCCAGUUUUCAGUAUC	miR-376a
miR-376b (SEQ ID NO:205)	CAGUCCUUCUUUGGUUUUAAAACGUGGAUAUCCUUCUAUGUUUACGUGA UUCUGGUUAAUCAUAGAGGAAAAUCCAUGUUUUCAGUAUCAAUGCUG	miR-376b
miR-376c (SEQ ID NO:206)	AAAAGGUGGAUAUCCUUCUAUGUUUAUGUUUAUUUAUGGUUAAACAUAGAG GAAAUCCACGUUUU	miR-368 (also called miR-376c)
miR-377 (SEQ ID NO:207)	UUGAGCAGAGGUUGCCCUUGGUGAAUUCGCUUUAUUUAUGUUGAAUCACAC AAAGGCAACUUUUGUUUG	miR-377
miR-378 (SEQ ID NO:208)	AGGGCUCCUGACUCCAGGUCCUGUGUGUUACCUAGAAUAGCACUGGACUU GGAGUCAGAAGGCCU	miR-378; miR- 422b (also called miR-378*)

FIG. 5M

pre-miRNA	Sequence	Mature miRNA
miR-379 (SEQ ID NO:209)	AGAGAUGGUAGACUAUGGAACGUAGGCGUUAUGAUUUCUGACCUAUGUAAC AUGGUCCACUAACUCU	miR-379
miR-380 (SEQ ID NO:210)	AAGAUGGUUGACCAUAGAACAUGCGCUAUCUCUGUGUCGUAUGUAAUAUGG UCCACAUCUU	miR-380 (also called miR-380- 3p); miR-380* (also called miR- 380-5p)
miR-381 (SEQ ID NO:211)	UACUUAAGCGAGGUUGCCCUUUGUAUAUUCGGUUUAUUGACAUGGAAUAU ACAAGGGCAAGCUCUCUGUGAGUA	miR-381
miR-382 (SEQ ID NO:212)	UACUUGAAGAGAAGUUGUUCGUGGUGGAUUCGCUUUACUUAUGACGAAUCA UUCACGGACAACACUUUUUUCAGUA	miR-382
miR-383 (SEQ ID NO:213)	CUCCUCAGAUCAAGGUGAUUGUGGCUUUGGGUGGAUAUUAUAGCCAC AGCACUGCCUGGUCAGAAAGAG	miR-383
miR-409 (SEQ ID NO:214)	UGGUACUCGGGGAGAGGUUACCCGAGCAACUUUGCAUCUGGACGACGAAUG UUGCUCGGUGAACCCCUUUUCGGUAUCA	miR-409-5p
miR-410 (SEQ ID NO:215)	GGUACCUGAGAAGAGGUUGUCUGUGAUGAGUUCGCUUUUAUUAUGACGAA UAUAACACAGAUGGCCUGUUUUCAGUACC	miR-410
miR-412 (SEQ ID NO:216)	CUGGGGUACGGGAUGGAUGGUCGACCAGUUGGAAAGUAAUUGUUUCUAAU GUACUUCACCUGGUCCACUAGCCGUCCGUAUCCGUCGAG	miR-412
miR-422a (SEQ ID NO:217)	GAGAGAAGCACUGGACUUAGGGUCAGAAGGCCUGAGUCUCUCUGCUGCAGA UGGGCUCUCUGUCCUGAGCCAAGCUUUGUCCUCCUGG	miR-422a
miR-423 (SEQ ID NO:218)	AUAAAGGAAGUAGGCGAGGGGCAGAGAGCGAGACUUUUCUAUUUCCAA AAGCUCGGUCUGAGGCCCCUCAGUCUUGCUCCUAACCCGCGC	miR-423
miR-424 (SEQ ID NO:219)	CGAGGGGAUACAGCAGCAAUUAUGUUUUGAAGUGUUCUAAAUGGUUCAA ACGUGAGGCGCUGCUAUACCCCUUGGUGGGAAGGUAGAAGGUGGGG	miR-424
miR-425 (SEQ ID NO:220)	GAAAGCGCUUUGGAAUGACACGAUCACUCCGUUGAGUGGGCACCCGAGAA GCCAUCGGGAUGUCGUGUCCGCCAGUGCUCUUUC	miR-425
miR-429 (SEQ ID NO:221)	CGCCGGCCGAUGGGCGUCUUACCAGACAUGGUUAGACCUGGCCCUUGUCU AAUACUGUCUGGUAAAACCGUCCAUCGCGUC	miR-429
miR-432 (SEQ ID NO:222)	UGACUCCUCCAGGUCUUGGAGUAGGUCAUUGGGUGGAUCCUCUAUUUCCUU ACGUGGGCCACUGGAUGGCUCUCCAUGUCUUGGAGUAGAUA	miR-432; miR- 432*
miR-433 (SEQ ID NO:223)	CCGGGGAGAAGUACGGUGAGCCUGUCAUUAUUCAGAGAGGCUGAUAUCCUCU GUGUUGAGAAGGAUCAUGAUGGGCUCCUGGUGUUCUCCAGG	miR-433
miR-448 (SEQ ID NO:224)	GCCGGGAGGUUGAACAUCUGCAUAGUGCGCCAGGAAAUCCCUAUUUCAU AUAAGAGGGGGCUGGCUGGUUGCAUUGUAGGAUGUCCCAUCCAGCCC ACUUCGUA	miR-448

FIG. 5N

pre-miRNA	Sequence	Mature miRNA
miR-449a (SEQ ID NO:225)	CUGUGUGUGAUGAGCUGGCAGUGUAUUGUUAGCUGGUUGAAUAUGUGAAUG GCAUCGGCUAACAUGCAACUGCUGUCUUAUUGCAUAUACA	miR-449 (also called miR-449a)
miR-450a-1 (SEQ ID NO:226)	AAACGAUACUAAACUGUUUUUGCGAUGUGUCCUAAUAUGCACUAUAAAUA UAUUGGGAACAUUUUGCAUGUAUAGUUUUGUAUCAAUUAUA	miR-450 (also called miR-450a)
miR-450a-2 (SEQ ID NO:227)	CCAAAGAAAGAUGCUGAAACUAUUUUUGCGAUGUGUCCUAAUAUGUAAUAU AAAUGUAUUGGGGACAUUUUGCAUUCAUAGUUUUGUAUCAAUAAUAUGG	miR-450 (also called miR-450a)
miR-451 (SEQ ID NO:228)	CUUGGGAAUGGCAAGGAAACCGUUACCAUACUGAGUUUAGUAUUGGUAAU GGUUCUCUUGCUAUACCCAGA	miR-451
miR-452 (SEQ ID NO:229)	GCUAAGCACUUACAACUGUUUGCAGAGGAAACUGAGACUUUGUAACUAUGU CUCAGUCUCAUCUGCAAAGAAGUAAGUGCUUUGC	miR-452; miR- 452*
miR-453 (SEQ ID NO:230)	GCAGGAAUGCUGCGAGCAGUGCCACCUCUAGGUACUCGGAGGGAGGUUGUC CGUGGUGAGUUCGCAUUAUUUAAUGAUGC	miR-453
miR-455 (SEQ ID NO:231)	UCCCGUGGCGUGAGGGUAUGUGCCUUUGGACUACAUCGUGGAAGCCAGCACC AUGCAGUCCAUGGGCAUUAUACAUUGCCUCAAGGCCUAUGUCAUC	miR-455
miR-483 (SEQ ID NO:232)	GAGGGGGAAGACGGGAGGAAAGAAGGGAGUGGUUCCAUCACGCCUCCUCAC UCCUCUCCUCCCGUCUUCUCCUCUC	miR-483
miR-485 (SEQ ID NO:233)	ACUUGGAGAGAGGCUGGCCGUGAUGAAUUCGAUUAUCAAAGCGAGUCAUA CACGGCUCUCCUCUCUUUUAGU	miR-485-3p; miR- 485-5p
miR-486 (SEQ ID NO:234)	GCAUCCUGUACUGAGCUGCCCCGAGGCCCUUCAUGCUGCCCAGCUCGGGGC AGCUCAGUACAGGAUAC	miR-486
miR-487a (SEQ ID NO:235)	GGUACUUGAAGAGUGGUUAUCCUGCUGUGUUCGCUUAAUUUAUGACGAAU CAUACAGGGACAUCAGUUUUUCAGUAUC	miR-487a
miR-487b (SEQ ID NO:236)	UUGGUACUUGGAGAGUGGUUAUCCUGUCCUGUUCGUUUUGCUCAUGUCGA AUCGUACAGGGUCAUCCACUUUUUCAGUAUCAA	miR-487b
miR-488 (SEQ ID NO:237)	GAGAAUCAUCUCUCCAGAUAAUGGCACUCUCAACAAGUUUCCAAAUUGU UUGAAAGGCUAUUUCUUGGUCAGAUACUCUC	miR-488
miR-489 (SEQ ID NO:238)	GUGGCAGCUUGGUGGUCGUAUGUGUGACGCCAUUUACUUGAACCUUUAGGA GUGACAUCACAUUACGGCAGCUAAACUGCUAC	miR-489
miR-490 (SEQ ID NO:239)	UGGAGGCCUUGCUGGUUUUGGAAAGUUAUUGUUCGACACCAUGGAUCUCCA GGUGGGUCAAGUUUAGAGAUGCACCAACCUGGAGGACUCCAUGCUGUUGAG CUGUUCACAAGCAGCGGACACUCCA	miR-490
miR-491 (SEQ ID NO:240)	UUGACUUAGCUGGGUAGUGGGGAACCCUCCAUGAGGAGUAGAACACUCCU UAUGCAAGAUUCCCUUCUACCUGGCUGGGUUGG	miR-491

FIG. 50

pre-miRNA	Sequence	Mature miRNA
miR-493 (SEQ ID NO:241)	CUGGCCUCCAGGGCUUUGUACAUGGUAGGCUUUCAUUCAUUCGUUUGCACA UUCGGUGAAGGUCUACUGUGUGCCAGGCCUGUGCCAG	miR-493 (also called miR-493- 3p); miR-493* (also called miR- 493-5p)
miR-494 (SEQ ID NO:242)	GAUACUCGAAGGAGAGGUUGUCCGUGUUGUCUUCUCUUUAUUUAUGAUGAA ACAUACACGGGAAACCUCUUUUUUAGUAUC	miR-494
miR-495 (SEQ ID NO:243)	UGGUACCUGAAAAGAAGUUGCCCAUGUUAUUUUCGCUUUAUUGUGACGAA ACAAACAUGGUGCACUUCUUUUUCGGUAUCA	miR-495
miR-496 (SEQ ID NO:244)	CCCAAGUCAGGUACUCGAAUGGAGGUUGUCCAUGGUGUGUUCAUUUUAUUU AUGAUGAGUAUUACAUGGCCAAUCUCCUUUCGGUACUCAAUUCUUCUUGGG	miR-496
miR-497 (SEQ ID NO:245)	CCACCCCGUCCUGUCUCCCGCCCCAGCAGCACACUGUGGUUUGUACGGCAC UGUGGCCACGUCCAAACCACACUGUGGUGUUAAGAGCGAGGGUGGGGAGGC ACCGCCGAGG	miR-497
miR-498 (SEQ ID NO:246)	AACCCUCCUUGGGGAAGUGAAGCUCAGGCUGUGAUUUAAGCCAGGGGGCGU UUUUCUAUAACUGGAUGAAAAGCACCUCAGAGCUUGAAGCUCACAGUUUG AGAGCAAUCGUCUAAGGAAGUU	miR-498
miR-499 (SEQ ID NO:247)	GCCCUGUCCCCUGUGCCUUGGGCGGGCGGCUGUUAAGACUUGCAGUGAUGU UUAACUCCUCUCCACGUGAACAUACAGCAAGUCUGUGCUGCUUCCCGUCC CUACGCGUCCUGGGCAGGGU	miR-499
miR-500 (SEQ ID NO:248)	GCUCUUUUUCUCUAAUCCUUGCUACCUGGGUGAGAGUGCUGUCUGAAUGCA AUGCACCUGGGCAAGGAUUCUGAGAGCGAGAGC	miR-500
miR-501 (SEQ ID NO:249)	GCUCUCCUUCUCUAAUCCUUGUCCUGGGUGAGAGUGCUUUCUGAAUGCA AUGCACCCGGGCAAGGAUUCUGAGAGGGUGAGC	miR-501
miR-502 (SEQ ID NO:250)	UGCUCUUUUUCUCUAAUCCUUGCUAUCUGGGUGCUAGUGCUGGCUCAAUGC AAUGCACCUGGGCAAGGAUUCAGAGAGGGGGAGCU	miR-502
miR-503 (SEQ ID NO:251)	UGCCCUAGCAGCGGGAACAGUUCUGCAGUGAGCGAUCGGUGCUCUGGGGUA UUGUUUCCGUGCCAGGGUA	miR-503
miR-504 (SEQ ID NO:252)	GCUGCUGUUGGGAGACCCUGGUCUGCACUCUAUCUGUAUUCUACUGAAGG GAGUGCAGGGCAGGGUUUCCCAUACAGAGGGC	miR-504
miR-505 (SEQ ID NO:253)	GAUGCACCCAGUGGGGGAGCCAGGAAGUAUUGAUGUUUCUGCCAGUUUAGC GUCAACACUUGCUGGUUUCCUCUCUGGAGCAUC	miR-505
miR-506 (SEQ ID NO:254)	GCCACCACCAUCAGCCAUACUAUGUGUAGUGCCUUAUUCAGGAAGGUGUUA CUUAAUAGAUUAAUUAUUUGUAAGGCACCCUUCUGAGUAGAGUAAUGUGCAA CAUGGACAACAUUUGUGGUGGC	miR-506
miR-507 (SEQ ID NO:255)	GUGCUGUGUGUAGUGCUUCACUUAAGAAGUGCCAUGCAUGUGUCUAGAAA UAUGUUUUGCACCUUUUGGAGUGAAUUAUGCACAACAGAUAC	miR-507

FIG. 5P

pre-miRNA	Sequence	Mature miRNA
miR-508 (SEQ ID NO:256)	CCACCUUCAGCUGAGUGUGCCCUACUCCAGAGGGCGUCACUCAUGUAA ACUAAAACAUGAUUGUAGCCUUUUGGAGUAGAGUAAUACACAUCACGUAAC GCAUAAUUUGGUGG	miR-508
miR-509-1 (SEQ ID NO:257)	CAUGCUGUGUGUGGUACCCUACUGCAGACAGUGGCAAUCAUGUAUAAUUA AAUAGAUUGGUACGUCUGUGGGUAGAGUACUGCAUGACACAUG	miR-509
miR-509-2 (SEQ ID NO:258)	CAUGCUGUGUGUGGUACCCUACUGCAGACAGUGGCAAUCAUGUAUAAUUA AAUAGAUUGGUACGUCUGUGGGUAGAGUACUGCAUGACAC	miR-509
miR-509-3 (SEQ ID NO:259)	GUGGUACCCUACUGCAGACGUGGCAAUCAUGUAUAAUUAUAAAAUGAUUGGU ACGUCUGUGGGUAGAGUACUGCAU	miR-509
miR-510 (SEQ ID NO:260)	GUGGUGUCCUACUCAGGAGAGUGGCAAUCACAUGUAUAAUAGGUGUGAUUGA AACCUCUAAAGAGUGGAGUAAAC	miR-510
miR-511-1 (SEQ ID NO:261)	CAAUAGACACCCAUUCGUGUCUUUUGCUCUGCAGUCAGUAAAUAUUUUUUUG UGAAUGUGUAGCAAAGACAGAAUGGUGGUCCAUG	miR-511
miR-511-2 (SEQ ID NO:262)	CAAUAGACACCCAUUCGUGUCUUUUGCUCUGCAGUCAGUAAAUAUUUUUUUG UGAAUGUGUAGCAAAGACAGAAUGGUGGUCCAUG	miR-511
miR-512-1 (SEQ ID NO:263)	UCUCAGUCUGUGGCACUCAGCCUUGAGGGCACUUUCUGGUGCCAGAAUGAA AGUGCUGUCAUAGCUGAGGUCCAUGACUGAGG	miR-512-5p
miR-512-2 (SEQ ID NO:264)	GGUACUUCUCAGUCUGUGGCACUCAGCCUUGAGGGCACUUUCUGGUGCCAG AAUGAAAGUGCUGUCAUAGCUGAGGUCCAUGACUGAGGCGAGCACC	miR-512-5p
miR-514-1 (SEQ ID NO:265)	AACAUGUUGUCUGUGGUACCCUACUCUGGAGAGUGACAAUCAUGUAUAAUU AAUUUGAUUGACACUUCUGUGAGUAGAGUAACGCAUGACACGUACG	miR-514
miR-514-2 (SEQ ID NO:266)	GUUGUCUGUGGUACCCUACUCUGGAGAGUGACAAUCAUGUAUAACUAAAUU UGAUUGACACUUCUGUGAGUAGAGUAACGCAUGACAC	miR-514
miR-514-3 (SEQ ID NO:267)	GUUGUCUGUGGUACCCUACUCUGGAGAGUGACAAUCAUGUAUAACUAAAUU UGAUUGACACUUCUGUGAGUAGAGUAACGCAUGACAC	miR-514
miR-515-1 (SEQ ID NO:268)	UCUCAUGCAGUCAUUCUCCAAAAGAAAGCACUUUCUGUUGUCUGAAAGCAG AGUGCCUUCUUUUGGAGCGUACUGUUUGAGA	miR-515-3p; miR-515-5p
miR-515-2 (SEQ ID NO:269)	UCUCAUGCAGUCAUUCUCCAAAAGAAAGCACUUUCUGUUGUCUGAAAGCAG AGUGCCUUCUUUUGGAGCGUACUGUUUGAGA	miR-515-3p; miR-515-5p
miR-516a-1 (SEQ ID NO:270)	UCUCAGGCUGUGACCUUCUCGAGGAAAGAAGCACUUUCUGUUGUCUGAAAG AAAAGAAAGUGCUUCCUUUCAGAGGGUACGGUUUGAGA	miR-516-3p (also called miR-516a-3p)
miR-516a-2 (SEQ ID NO:271)	UCUCAGGUUGUGACCUUCUCGAGGAAAGAAGCACUUUCUGUUGUCUGAAAG AAAAGAAAGUGCUUCCUUUCAGAGGGUACGGUUUGAGA	miR-516-3p (also called miR-516a-3p)

FIG. 5Q

pre-miRNA	Sequence	Mature miRNA
miR-517a (SEQ ID NO:272)	UCUCAGGCAGUGACCCUCUAGAUGGAAGCACUGUCUGUUGUAUAAAAGAAA AGAUCGUGCAUCCCUUUAGAGUGUUACUGUUUGAGA	miR-517a; miR- 517*
miR-517b (SEQ ID NO:273)	GUGACCCUCUAGAUGGAAGCACUGUCUGUUGUCUAAGAAAAGAUCGUGCAU CCCUUUAGAGUGUUAC	miR-517b; miR- 517*
miR-517c (SEQ ID NO:274)	GAAGAUCUCAGGCAGUGACCCUCUAGAUGGAAGCACUGUCUGUUGUCUAAG AAAAGAUCGUGCAUCCCUUUAGAGUGUUACUGUUUGAGAAAAUC	miR-517c; miR- 517*
mir-518a-1 (SEQ ID NO:275)	UCUCAAGCUGUGACUGCAAAGGGAAGCCCUUUCUGUUGUCUGAAAGAAGAG AAAGCGCUUCCCUUUGCUGGAUUACGGUUUGAGA	mir-518a
mir-518a-2 (SEQ ID NO:276)	UCUCAAGCUGUGGGUCUGCAAAGGGAAGCCCUUUCUGUUGUCUAAAAGAAG AGAAAGCGCUUCCCUUUGCUGGAUUACGGUUUGAGA	mir-518a
miR-518b (SEQ ID NO:277)	UCAUGCUGUGGCCCUCCAGAGGGAAGCGCUUUCUGUUGUCUGAAAGAAAAC AAAGCGCUCCCUUUAGAGGUUUACGGUUUGA	miR-518b
miR-518c (SEQ ID NO:278)	GCGAGAAGAUCUCAUGCUGUGACUCUCUGGAGGGAAGCACUUUCUGUUGUC UGAAAGAAAACAAAGCGCUUCUCUUUAGAGUGUUACGGUUUGAGAAAAGC	miR-518c; miR- 518c*
miR-518d (SEQ ID NO:279)	UCCCAUGCUGUGACCCUCUAGAGGGAAGCACUUUCUGUUGUCUGAAAGAAA CCAAAGCGCUUCCCUUUGGAGCGUUACGGUUUGAGA	miR-518d
miR-518e (SEQ ID NO:280)	UCUCAGGCUGUGACCCUCUAGAGGGAAGCGCUUUCUGUUGGCUAAAAGAAA AGAAAGCGCUUCCCUUCAGAGUGUUAACGCUUUGAGA	miR-518e
miR-518f (SEQ ID NO:281)	UCUCAUGCUGUGACCCUCUAGAGGGAAGCACUUUCUCUUGUCUAAAAGAAA AGAAAGCGCUUCUCUUUAGAGGAUUACUCUUUGAGA	miR-518f
miR-519c (SEQ ID NO:282)	UCUCAGCCUGUGACCCUCUAGAGGGAAGCGCUUUCUGUUGUCUGAAAGAAA AGAAAGUGCAUCUUUUUAGAGGAUUACAGUUUGAGA	miR-519c
miR-519d (SEQ ID NO:283)	UCCCAUGCUGUGACCCUCCAAAGGGAAGCGCUUUCUGUUUGUUUCUCUUA AACAAAGUGCCUCCCUUUAGAGUGUUACCGUUUGGGA	miR-519d
miR-519e (SEQ ID NO:284)	UCUCAUGCAGUCAUUCUCCAAAAGGGAGCACUUUCUGUUUGAAAGAAAACA AAGUGCCUCCCUUUAGAGUGUUACUGUUUGAGA	miR-519e
miR-520a (SEQ ID NO:285)	CUCAGGCUGUGACCCUCCAGAGGGAAGUACUUUCUGUUGUCUGAGAGAAAA GAAAGUGCUUCCCUUUGGACUGUUUCGGUUUGAG	miR-520a; miR- 520a*
miR-520b (SEQ ID NO:286)	CCCUCUACAGGGAAGCGCUUUCUGUUGUCUGAAAGAAAAGAAAGUGCUUCC UUUUAGAGGG	miR-520b
miR-520c (SEQ ID NO:287)	UCUCAGGCUGUCGUCCUCUAGAGGGAAGCACUUUCUGUUGUCUGAAAGAAA AGAAAGUGCUUCCCUUUUAGAGGGUUACCGUUUGAGA	miR-510c

FIG. 5R

pre-miRNA	Sequence	Mature miRNA
miR-520d (SEQ ID NO:288)	UCUCAAGCUGUGAGUCUACAAAGGGAAGCCCUUUCUGUUGUCUAAAAGAAA AGAAAGUGCUUCUCUUUGGUGGGUUACGGUUUGAGA	miR-520d; miR- 520d*
miR-520e (SEQ ID NO:289)	UCUCCUGCUGUGACCCUCAAGAUGGAAGCAGUUUCUGUUGUCUGAAAGGAA AGAAAGUGCUUCCUUUUUGAGGGUUACUGUUUGAGA	miR-520e
miR-520f (SEQ ID NO:290)	UCUCAGGCUGUGACCCUCUAAAAGGGAAGCGCUUUCUGUGGUCAGAAAGAAA AGCAAGUGCUUCCUUUUAGAGGGUUACCGUUUGGGA	miR-520f
miR-520g (SEQ ID NO:291)	UCCCAUGCUGUGACCCUCUAGAGGAAGCACUUUCUGUUUGUUGUCUGAGAA AAAACAAAGUGCUUCCUUUAGAGUGUUACCGUUUGGGA	miR-520g
miR-520h (SEQ ID NO:292)	UCCCAUGCUGUGACCCUCUAGAGGAAGCACUUUCUGUUUGUUGUCUGAGAA AAAACAAAGUGCUUCCUUUAGAGUUACUGUUUGGGA	miR-520h
miR-523 (SEQ ID NO:293)	UCUCAUGCUGUGACCCUCUAGAGGGAAGCGCUUUCUGUUGUCUGAAAGAAA AGAACGCGCUUCCCUAUAGAGGGUUACCCUUUGAGA	miR-523
miR-525 (SEQ ID NO:294)	CUCAAGCUGUGACUCUCCAGAGGGAUGCACUUUCUCUUAUGUGAAAAA GAAGGCGCUUCCCUUAGAGCGUUACGGUUUGGG	miR-525; miR- 525*
miR-526a-1 (SEQ ID NO:295)	CUCAGGCUGUGACCCUCUAGAGGGAAGCACUUUCUGUUGCUUGAAAGAAGA GAAAGCGCUUCCUUUAGAGGAUUACUCUUUGAG	miR-526a
miR-526a-2 (SEQ ID NO:296)	GUGACCCUCUAGAGGGAAGCACUUUCUGUUGAAAGAAAAGAACAUGCAUCC UUUCAGAGGGUUAC	miR-526a
miR-526b (SEQ ID NO:297)	UCAGGCUGUGACCCUCUAGAGGGAAGCACUUUCUGUUGUCUGAAAGAAGAG AAAGUGCUUCCUUUUAGAGGCUUACUGUCUGA	miR-526b; miR- 526b*
miR-527 (SEQ ID NO:298)	UCUCAAGCUGUGACUGCAAAGGGAAGCCCUUUCUGUUGUCUAAAAGAAAAG AAAGUGCUUCCCUUUGGUGAAUUACGGUUUGAGA	miR-527
miR-539 (SEQ ID NO:299)	AUACUUGAGGAGAAAUAUCCUUGGUGUGUUCGCUUUUAUUUAUGAUGAAUC AUACAAGGACAAUUCUUUUUGAGUAU	miR-539
miR-542 (SEQ ID NO:300)	CAGAUCUCAGACAUCUCGGGGAUCAUCAUGUCACGAGAUACCAGUGUGCAC UUGUGACAGAUUGAUAAACUGAAAGGUCUGGGAGCCACUCAUCUUCA	miR-542-3p; miR- 542-5p
miR-550-1 (SEQ ID NO:301)	UGAUGC UUUGCUGGCUGGUGCAGUGCCUGAGGGAGUAAGAGCCCGUUGUU GUAAGAUAGUGUCUACUCCCUAGGCACAUCCCAACAAGUCUCU	miR-550
miR-550-2 (SEQ ID NO:302)	UGAUGC UUUGCUGGCUGGUGCAGUGCCUGAGGGAGUAAGAGCCCGUUGUU GUCAGAUAGUGUCUACUCCCUAGGCACAUCCAGCGAGUCUCU	miR-550
miR-566 (SEQ ID NO:303)	GCUAGGCGUGGUGGCGGGCGCCUGUGAUCCCAACUACUCAGGAGGCUGGGG CAGCAGAAUCGCUUGAACCCGGGAGGCGAAGGUUGCAGUGAGC	miR-566

FIG. 5S

pre-miRNA	Sequence	Mature miRNA
miR-576 (SEQ ID NO:304)	UACAAUCCAACGAGGAUUCUAAUUUCUCCACGUCUUUGGUAUAAGGUUUG GCAAAGAUGUGGAAAAAUUGGAAUCCUCAUUCGAUUGGUUAUAACCA	miR-576
miR-584 (SEQ ID NO:305)	UAGGGUGACCAGCCAUAUUGGUUUGCCUGGGACUGAGGAUUUGCUGGGAU AUGUCAGUUCAGGCCAACAGGCUGGUUGGUCUCCCUGAAGCAAC	miR-584
miR-605 (SEQ ID NO:306)	GCCCUAGCUUGGUUCUAAAUCCCAUGGUGCCUUCUCCUUGGGAAAAACAGA GAAGGCACUAUGAGAUUUAGAAUCAAGUUAGG	miR-605
miR-618 (SEQ ID NO:307)	CUCUUGUUCACAGCCAAACUCUACUUGUCCUUCUGAGUGUAAUUACGUACA UGCAGUAGCUCAGGAGACAAGCAGGUUUACCCUGUGGAUGAGUCUGA	miR-618
miR-638 (SEQ ID NO:308)	GUGAGCGGGCGCGGCAGGGAUCGCGGGCGGGUGGCGGCCUAGGGCGCGGAG GGCGGACCGGGAUUGGCGCGCCGUGCGCCGCCGCGUAACUGCGGCGCU	miR-638
miR-660 (SEQ ID NO:309)	CUGCUCUUCUCCCAUACCCAUUGCAUAUCGGAGUUGUGAAUUCUAAAAC ACCUCUUGUGUGCAUGGAUUACAGGAGGGUGAGCCUUGUCAUCGUG	miR-660
miR-7-1 (SEQ ID NO:310)	UUGGAUGUUGGCCUAGUUCUGUGUGGAAGACUAGUGAUUUUGUUGUUUUA GAUAAUAAAUCGACAACAAAUCACAGUCUGCCAUAUGGCACAGGCCAUGC CUCUACAG	miR-7
miR-7-2 (SEQ ID NO:311)	CUGGAUACAGAGUGGACCGCUGGCCCAUCUGGAAGACUAGUGAUUUUGU UGUUGUCUACUGCGCUCAACAACAAUCCAGUCUACCUAUUGGUGCCAG CCAUCGCA	miR-7
miR-7-3 (SEQ ID NO:312)	AGAUUAGAGUGGCUGUGGUCUAGUGCUGUGUGGAAGACUAGUGAUUUUGU GUUCUGAUGUACUACGACAACAAGUCACAGCCGCCUCAUAGCGCAGACUC CCUUCGAC	miR-7
miR-9-1 (SEQ ID NO:313)	CGGGGUUGGUUGUUAUCUUUGGUUAUCUAGCUGUAUGAGUGGUGUGGAGUC UUCAUAAAGCUAGAUAAACGAAAGUAAAAUAACCCCA	miR-9; miR-9*
miR-9-2 (SEQ ID NO:314)	GGAAGCGAGUUGUUAUCUUUGGUUAUCUAGCUGUAUGAGUGUAUUGGUCUU CAUAAAGCUAGAUAAACGAAAGUAAAAUCCUUCA	miR-9; miR-9*
miR-9-3 (SEQ ID NO:315)	GGAGGCCCGUUUCUCUCUUUGGUUAUCUAGCUGUAUGAGUGCCACAGAGCC GUCAUAAAGCUAGAUAAACGAAAGUAGAAUUGAUUCUA	miR-9; miR-9*
miR-92a-1 (SEQ ID NO:316)	CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUGUGUUUCUGUAUGGUAAU GCACUUGUCCCGGCCUGUUGAGUUUGG	miR-92 (also called miR-92a)
miR-92a-2 (SEQ ID NO:317)	UCAUCCUGGGUGGGGAUUUGUUGCAUUACUUGUGUUCUAUAUAAAGUAUU GCACUUGUCCCGGCCUGUGGAAGA	miR-92 (also called miR-92a)
miR-93 (SEQ ID NO:318)	CUGGGGGCUCAAAGUGCUGUUCGUGCAGGUAGUGUGAUUACCAACCUAC UGCUGAGCUAGCACUCCCGAGCCCCCGG	miR-93

FIG. 5T

pre-miRNA	Sequence	Mature miRNA
miR-95 (SEQ ID NO:319)	AACACAGUGGGCACUCAUAAAUGUCUGUUGAAUUGAAAUGCGUUACAUUC AACGGGUAAUUUAUUGAGCACCCACUCUGUG	miR-95
miR-96 (SEQ ID NO:320)	UGGCCGAUUUUUGGCACUAGCACAUUUUUGCUUGUGUCUCUCCGCUCUGAGC AAUCAUGUGCAGUGCCAAUAUGGGAAA	miR-96
miR-98 (SEQ ID NO:321)	AGGAUUCUGCUCAUGCCAGGGUGAGGUAGUAAGUUGUAUUGUUGUGGGGUA GGGAUAUUAGGCCCCAAUUAGAAGUAACUAUACAACUUACUACUUUCCCU GGUGUGUGGCAUAUUC	miR-98
miR-99a (SEQ ID NO:322)	CCCAUUGGCAUAAACCCGUAGAUCCGAUCUUGUGGUGAAGUGGACCGCACA AGCUCGCUUCUAUGGGUCUGUGUCAGUGUG	miR-99a
miR-99b (SEQ ID NO:323)	GGCACCACCCGUAGAACCGACCUUGCGGGGCCUUCGCCGCACACAAGCUC GUGUCUGUGGGUCCGUGUC	miR-99b

FIG. 5U

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/046916

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/48 (2010.01)

USPC - 436/63

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - G01N 33/48 (2010.01)

USPC - 436/63, 64, 811, 813

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0215865 A1 (PLASTERK et al) 27 August 2009 (27.08.2009) entire document	1, 3-14, 16-19
-		-----
Y		2, 15
Y	US 6,242,204 B1 (TORCZYNSKI et al) 05 June 2001 (05.06.2001) entire document	2, 15

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Date of the actual completion of the international search

08 October 2010

Date of mailing of the international search report

18 OCT 2010

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