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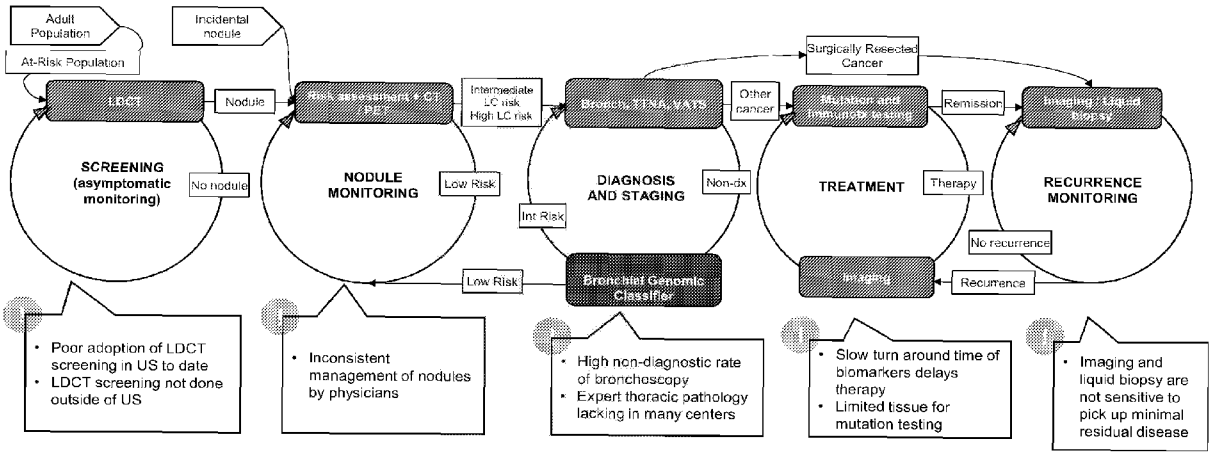

FIG. 3

(57) Abstract: Provided herein are methods, systems, and kits for improving the current clinical pathway of care for lung conditions using genomic classifiers at various decision points within the existing pathway to minimize unnecessary invasive procedures, enhance early detection and disease recurrence, and monitor efficacy of interventive therapies for prevention or disease reversal.

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METHODS AND SYSTEMS FOR IDENTIFYING OR MONITORING LUNG DISEASE

CROSS-REFERENCE

[1] This application claims priority to U.S. provisional application 62/514,595 filed on June 2, 2017 and U.S. provisional application 62/546,936 filed on August 17, 2017, each of which is entirely incorporated herein by reference.

BACKGROUND

[2] There are methods currently available for detecting lung conditions, such as lung cancer. Such current clinical pathway of care for lung conditions suffer from a high rate of unnecessary invasive procedures, an inability to detect early lung conditions, or assess subject risk for developing a lung condition.

SUMMARY

[3] The present disclosure provides methods and systems for determining whether a subject has or is at risk of having a lung condition, such as, for example, lung cancer. Methods of the present disclosure may permit a subject to be screened or monitored for a progression or regression of the lung condition, in some cases using a sample non-invasively obtained from the subject (e.g., a nasal tissue sample). This may advantageously be used to screen for subjects that as asymptomatic for the lung condition, but who may otherwise be at risk of developing the lung condition (e.g., subjects exposed to cigarette smoke or air pollution), or to monitor subjects that have or are suspected of having the lung condition.

[4] An aspect of the present disclosure provides a method for screening a subject for a lung condition, the method comprising (a) assaying epithelial tissue from a first sample obtained from a subject that has been (1) computer analyzed for a presence of one or more risk factors for developing the lung condition and (2) identified with the presence of the one or more risk factors, to identify a presence or absence of one or more biomarkers associated with a risk of developing the lung condition in the first sample; and; and (b) upon identifying the presence or absence of the one or more biomarkers, (i) directing an electronic imaging scan of a lung region of the subject to be obtained, which lung region is suspected of having the lung condition, or (ii) assaying other epithelial tissue from a second sample of the subject. In some embodiments, the method further comprises, prior to (b), receiving a request to assay the first sample comprising the epithelial tissue of the subject.

[5] In some embodiments, the electronic imaging scan is a low-dose computerized tomography (LDCT) scan or magnetic resonance imaging (MRI). In some embodiments, the LDCT scan provides a radiation exposure to the subject of less than about 5 millisieverts (mSv).

[6] In some embodiments, the lung condition is lung cancer, chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), or any combination thereof. In some embodiments, the lung condition is a lung cancer and the lung cancer comprises: a non-small cell lung cancer; an adenocarcinoma; a squamous cell carcinoma; a large cell carcinoma; a small cell lung cancer; or any combination thereof.

[7] In some embodiments, the first sample or the second sample is obtained by a bronchoscopy. In some embodiments, the first sample or the second sample is obtained by fine needle aspiration. In some embodiments, the first sample or the second sample comprises a mucous epithelial tissue, a nasal epithelial tissue, a lung epithelial tissue, or any combination thereof. In some embodiments, the first sample or the second sample comprises epithelial tissue obtained along an airway of the subject.

[8] In some embodiments, a portion of the first sample or the second sample is subjected to cytological testing that identifies the sample as ambiguous or suspicious. In some embodiments, upon identifying the first sample or the second sample as ambiguous or suspicious, performing (b) on a second portion of the sample, which second portion comprises the epithelial tissue.

[9] In some embodiments, the second sample is different from the first sample. In some embodiments, the second sample is a different sample type from the first sample. In some embodiments, the first sample is obtained from the subject at a first time point and the second sample is obtained from the subject at a second time point, and the second time point is after the first time point. In some embodiments, the second time point is within about 1-2 years of the first time point.

[10] In some embodiments, (a) comprises comparing the presence or absence of the one or more biomarkers to a reference set of one or more biomarkers. In some embodiments, the subject is in need of a treatment for the lung condition. In some embodiments, the subject is suspected of having an increased risk for developing a lung condition. In some embodiments, the subject is asymptomatic with respect to the lung condition. In some embodiments, the subject has not previously received the electronic imaging scan. In some embodiments, the subject has not previously received a definitive diagnosis.

[11] In some embodiments, the one or more risk factors comprise: smoking; exposure to environmental smoke; exposure to radon; exposure to air pollution; exposure to radiation; exposure to an industrial substance; inherited or environmentally-acquired gene mutations; a subject's age; a subject having a secondary health condition; or any combination thereof. In some embodiments, the subject has two or more risk factors.

[12] In some embodiments, the one or more biomarkers comprise at least five biomarkers. In some embodiments, the one or more biomarkers comprise one or more of: a gene or fragment thereof; a sequence variant; a fusion; a mitochondrial transcript; an epigenetic modification; a copy number variation; a loss of heterozygosity (LOH); or any combination thereof. In some embodiments, the presence or absence of the one or more biomarkers comprises a level of expression.

[13] In some embodiments, the method identifies whether the subject is at an increased risk for developing the lung condition. In some embodiments, the identifying of (b) comprises employing a trained algorithm. In some embodiments, the trained algorithm is trained by a training set comprising epithelial cells obtained from an airway of an individual. In some embodiments, the trained algorithm is trained by a training set comprising samples benign for the lung condition and samples malignant for the lung condition. In some embodiments, the trained algorithm is trained by a training set comprising samples obtained from subjects having one or more risk factors.

[14] In some embodiments, the method further comprises, prior to (a), computer analyzing the subject to identify the presence of said one or more risk factors in the subject for developing the lung condition.

[15] Another aspect of the present disclosure provides a method for monitoring a subject having or suspected of having a lung condition. The method comprises (a) assaying a first sample comprising epithelial tissue obtained from a subject suspected of having the lung condition to identify a presence or an absence of one or more biomarkers associated with the lung condition, wherein the subject has previously received a positive indication of a presence of one or more lung nodules; and (b) upon identifying the presence or absence of the one or more biomarkers, (i) obtaining a second sample from the subject or (ii) directing the subject to obtain an electronic imaging scan of a lung region of the subject based on a result from (a).

[16] In some embodiments, the positive indication is previously identified by an electronic imaging scan. In some embodiments, the electronic imaging scan is a low-dose computerized tomography (LDCT) scan or magnetic resonance imaging (MRI). In some embodiments, the LDCT scan provides a radiation exposure to the subject of less than about 5 millisieverts (mSv).

[17] In some embodiments, the one or more lung nodules is at least two nodules. In some embodiments, the obtaining the second sample from the subject comprises performing a bronchoscopy, a transthoracic needle aspiration (TTNA), or a video-assisted thorascopic surgery (VATS) on the subject. In some embodiments, the obtaining the second sample from the subject comprises performing a tissue biopsy.

[18] In some embodiments, the presence or absence of the one or more biomarkers identifies the subject as high-risk or as low-risk of having the lung condition. In some embodiments, (b) further comprises recommending (i) or (ii) depending on an assessed risk.

[19] In some embodiments, the lung condition is lung cancer, chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), or any combination thereof. In some embodiments, the lung condition is a lung cancer and the lung cancer comprises: a non-small cell lung cancer; an adenocarcinoma; a squamous cell carcinoma; a large cell carcinoma; a small cell lung cancer; or any combination thereof.

[20] In some embodiments, the first sample or the second sample is obtained by a bronchoscopy. In some embodiments, the first sample or the second sample is obtained by fine needle aspiration. In some embodiments, the first sample or the second sample comprises a mucous epithelial tissue, a nasal epithelial tissue, a lung epithelial tissue, or any combination thereof. In some embodiments, the first sample or the second sample comprises epithelial tissue obtained along an airway of the subject.

[21] In some embodiments, the second sample is different from the first sample. In some embodiments, the second sample is a different sample type from the first sample. In some embodiments, the second sample is obtained from the subject at a time period later in time than the first sample is obtained from the subject. In some embodiments, the time period is from about 1 year to about 2 years.

[22] In some embodiments, (b) comprises comparing the presence or absence of the one or more biomarkers to a reference set of one or more biomarkers. In some embodiments, the subject is a subject in need of a treatment for the lung condition. In some embodiments, the subject is suspected of having an increased risk for developing a lung condition. In some embodiments, the subject is asymptomatic for the lung condition. In some embodiments, the subject has not previously received a definitive diagnosis.

[23] In some embodiments, the one or more biomarkers comprise at least five biomarkers. In some embodiments, the one or more biomarkers comprise one or more of: a gene or fragment thereof; a sequence variant; a fusion; a mitochondrial transcript; an epigenetic modification; a copy number variation; a loss of heterozygosity (LOH); or any combination thereof. In some embodiments, the presence or absence of the one or more biomarkers comprises a level of expression.

[24] In some embodiments, the method identifies whether the subject is at an increased risk of having the lung condition. In some embodiments, the identifying of (a) comprises employing a trained algorithm. In some embodiments, the trained algorithm is trained by a training set

comprising epithelial cells obtained from an airway of an individual. In some embodiments, the trained algorithm is trained by a training set comprising samples benign for the lung condition and samples malignant for the lung condition. In some embodiments, the trained algorithm is trained by a training set comprising samples obtained from subjects having one or more risk factors. In some embodiments, the method further comprises analyzing a blood sample from the subject, performing an electronic imaging scan on the subject, or a combination thereof.

[25] In some embodiments, the second sample is a sample of epithelial, and wherein subsequent to (b), the sample of epithelial tissue is assayed for a presence or absence of one or more additional biomarkers. In some embodiments, the one or more additional biomarkers are the one or more biomarkers.

[26] Another aspect of the present disclosure provides a method for monitoring a subject having or suspected of having a lung condition wherein the subject has previously received a recommendation to complete an interventive therapy for preventing or reversing the lung condition. The method comprises (a) subsequent to the subject completing at least a portion of the interventive therapy for the lung condition, assaying a first sample comprising epithelial tissue obtained from the subject to generate genetic data; (b) processing the genetic data to identify a presence or absence of one or more biomarkers associated with the lung condition; and (c) computer generating a report comprising a recommendation that a second sample be obtained from the subject.

[27] Another aspect of the present disclosure provides a method. The method comprises (a) assaying a first sample comprising epithelial tissue obtained from a subject and identifying a presence or absence of one or more biomarkers, wherein the subject has previously received a recommendation to complete an interventive therapy for preventing or reversing a lung condition; and (b) upon completing at least a portion of the interventive therapy for the lung condition, obtaining a second sample from the subject and repeating (a) with the second sample.

[28] In some embodiments, the method identifies subject compliance to the interventive therapy. In some embodiments, the method identifies efficacy of the interventive therapy to preventing or reversing the lung condition. In some embodiments, the interventive therapy comprises administering a pharmaceutical composition to the subject. In some embodiments, the pharmaceutical composition comprises a chemotherapeutic. In some embodiments, the interventive therapy comprises an exercise regime, a dietary regime, a reduction or omission of smoking, or any combination thereof.

[29] In some embodiments, the lung condition is lung cancer, chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), or any combination thereof. In some

embodiments, the lung condition is a lung cancer and the lung cancer comprises: a non-small cell lung cancer; an adenocarcinoma; a squamous cell carcinoma; a large cell carcinoma; a small cell lung cancer; or any combination thereof.

[30] In some embodiments, the first sample or the second sample is obtained by a bronchoscopy. In some embodiments, the first sample or the second sample is obtained by fine needle aspiration. In some embodiments, the first sample or the second sample comprises a mucous epithelial tissue, a nasal epithelial tissue, a lung epithelial tissue, or any combination thereof. In some embodiments, the first sample or the second sample comprises epithelial tissue obtained along an airway of the subject.

[31] In some embodiments, the second sample is different from the first sample. In some embodiments, the second sample is a different sample type from the first sample. In some embodiments, the second sample is obtained from the subject at a time period later in time than the first sample is obtained from the subject. In some embodiments, the time period is from about 1 year to about 2 years.

[32] In some embodiments, (a) comprises comparing the presence or absence of the one or more biomarkers to a reference set of one or more biomarkers. In some embodiments, the subject is a subject in need of a treatment for the lung condition. In some embodiments, the subject is suspected of having an increased risk for developing a lung condition. In some embodiments, the subject is asymptomatic with respect to the lung condition. In some embodiments, the subject has not previously received a definitive diagnosis.

[33] In some embodiments, the one or more biomarkers comprise at least five biomarkers. In some embodiments, the one or more biomarkers comprise one or more of: a gene or fragment thereof; a sequence variant; a fusion; a mitochondrial transcript; an epigenetic modification; a copy number variation; a loss of heterozygosity (LOH); or any combination thereof. In some embodiments, the presence or absence of the one or more biomarkers comprises a level of expression.

[34] In some embodiments, the identifying of (a) comprises employing a trained algorithm. In some embodiments, the trained algorithm is trained by a training set comprising epithelial cells obtained from an airway of an individual. In some embodiments, the trained algorithm is trained by a training set comprising samples benign for the lung condition and samples malignant for the lung condition. In some embodiments, the trained algorithm is trained by a training set comprising samples obtained from subjects having one or more risk factors. In some embodiments, the method further comprises analyzing a blood sample from the subject, performing an electronic imaging scan on the subject, or a combination thereof.

[35] In some embodiments, (b) comprises processing the genetic data to identify an expression level corresponding to each of the one or more biomarkers. In some embodiments, (b) comprises processing the genetic data to identify at least one genetic aberration in the one or more biomarkers.

[36] Another aspect of the present disclosure provides a method for monitoring the subject for a lung condition. The method comprises (a) assaying a first sample comprising epithelial tissue obtained from a subject and identifying a presence or absence of one or more biomarkers, wherein the subject has previously initiated a treatment for a lung condition; and (b) upon receiving a confirmation of remission, obtaining a second sample from the subject and repeating (a) with the second sample.

[37] In some embodiments, the method identifies early stage lung condition recurrence through non-invasive monitoring. In some embodiments, the lung condition is lung cancer, chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), or any combination thereof. In some embodiments, the lung condition is a lung cancer and the lung cancer comprises: a non-small cell lung cancer; an adenocarcinoma; a squamous cell carcinoma; a large cell carcinoma; a small cell lung cancer; or any combination thereof.

[38] In some embodiments, the first sample or the second sample is obtained by a bronchoscopy. In some embodiments, the first sample or the second sample is obtained by fine needle aspiration. In some embodiments, the first sample or the second sample comprises a mucous epithelial tissue, a nasal epithelial tissue, a lung epithelial tissue, or any combination thereof. In some embodiments, the first sample or the second sample comprises epithelial tissue obtained along an airway of the subject.

[39] In some embodiments, the second sample is different from the first sample. In some embodiments, the second sample is a different sample type from the first sample. In some embodiments, the second sample is obtained from the subject at a time period later in time than the first sample is obtained from the subject. In some embodiments, the time period is from about 1 year to about 2 years.

[40] In some embodiments, (a) comprises comparing the presence or absence of the one or more biomarkers to a reference set of one or more biomarkers. In some embodiments, the subject is a subject in need of a treatment for the lung condition. In some embodiments, the subject is suspected of having an increased risk for a recurrence of the lung condition. In some embodiments, the subject is asymptomatic with respect to the lung condition.

[41] In some embodiments, the one or more biomarkers comprise at least five biomarkers. In some embodiments, the one or more biomarkers comprise one or more of: a gene or fragment

thereof; a sequence variant; a fusion; a mitochondrial transcript; an epigenetic modification; a copy number variation; a loss of heterozygosity (LOH); or any combination thereof. In some embodiments, the presence or absence of the one or more biomarkers comprises a level of expression.

[42] In some embodiments, the identifying of (a) comprises employing a trained algorithm. In some embodiments, the trained algorithm is trained by a training set comprising epithelial cells obtained from an airway of an individual. In some embodiments, the trained algorithm is trained by a training set comprising samples benign for the lung condition and samples malignant for the lung condition. In some embodiments, the trained algorithm is trained by a training set comprising samples obtained from subjects having one or more risk factors. In some embodiments, the method further comprises analyzing a blood sample from the subject, performing an electronic imaging scan on the subject, or a combination thereof. Another aspect of the present disclosure provides a method for monitoring a subject having or suspected of having a lung condition. The method comprises (a) assaying a first sample comprising epithelial tissue obtained from a subject suspected of having the lung condition to identify a presence or absence of one or more biomarkers associated with the lung condition, wherein the subject has previously received a negative indication of a presence of a lung nodule; and (b) upon identifying the presence or absence of the one or more biomarkers, (i) obtaining a second sample from the subject or (ii) directing the subject to obtain an electronic imaging scan of a lung region of the subject based on a result from (a). In some embodiments, the method further comprises, prior to (a), computer analyzing the subject for a presence of one or more risk factors for developing the lung condition, and identifying the subject with the presence of the one or more risk factors.

[43] Another aspect of the present disclosure provides a system for screening a subject for a lung condition. The system comprises one or more computer databases comprising health or physiological data of a subject; and one or more computer processors that are individually or collectively programmed to (i) analyze the health or physiological data for a presence of one or more risk factors for the subject developing the lung condition, and (2) upon identifying the one or more risk factors, generate a recommendation that epithelial tissue from a sample of the subject be assayed for one or more biomarkers associated with a risk of developing the lung condition.

[44] Another aspect of the present disclosure provides a system for screening a subject for a lung condition. The system comprises one or more computer databases comprising (i) a first data set comprising data indicative of a presence of one or more risk factors for the subject

developing the lung condition, and (ii) a second data set comprising data indicative of a presence or absence of one or more biomarkers in epithelial tissue in a sample of the subject, which one or more biomarkers are associated with a risk of developing the lung condition; and one or more computer processors that are individually or collectively programmed to (i) analyzing the first data set to identify the presence of the one or more risk factors, (ii) analyzing the second data set to identify the presence or absence of the one or more biomarkers, and (iii) upon identifying the presence or absence of the one or more biomarkers, generate a report that (1) directs an electronic imaging scan of a lung region of the subject to be obtained, which lung region is suspected of exhibiting the lung condition, or (2) directs other epithelial tissue from a second sample of the subject to be assayed.

[45] Another aspect of the present disclosure provides a system for monitoring a subject having or suspected of having a lung condition. The system comprises one or more computer databases comprising a data set comprising data indicative of a presence or absence of one or more biomarkers in epithelial tissue in a first sample of the subject, which one or more biomarkers are associated with the lung condition; and one or more computer processors that are individually or collectively programmed to (i) determine that the subject has previously received a positive indication of a presence of one or more lung nodules, (ii) subsequent to (i), process the data set to identify the presence or absence of the one or more biomarkers, and (iii) upon identifying the presence or absence of the one or more biomarkers, generate a report that (1) directs a second sample to be obtained from the subject, or (2) directs another electronic imaging scan of a lung region of the subject to be obtained.

[46] Another aspect of the present disclosure provides a system for monitoring a subject having or suspected of having a lung condition wherein the subject has previously received a recommendation to complete an interventive therapy for preventing or reversing the lung condition. The system comprises one or more computer databases comprising a data set comprising genetic data; and one or more computer processors that are individually or collectively programmed to (i) subsequent to the subject completing at least a portion of the interventive therapy for the lung condition, process the genetic data to identify a presence or absence of one or more biomarkers associated with the lung condition, and (iii) generate a report comprising a recommendation that a second sample be obtained from the subject.

[47] Another aspect of the present disclosure provides a non-transitory computer readable medium comprising machine-executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[48] Another aspect of the present disclosure provides a computer system comprising one or more computer processors and memory coupled thereto. The memory comprises a non-transitory computer-readable medium comprising machine-executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[49] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[50] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[51] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "figure" and "FIG." herein), of which:

[52] **FIG. 1** shows a diagram highlighting the clinical challenges of lung cancer diagnosis.

[53] **FIG. 2** shows the benefit of integrating methods that include genomic classifier analysis into the clinical pathway of care for lung cancer.

[54] **FIG. 3** shows an improved clinical decisions pathway which includes a genomic classifier analysis.

[55] **FIG. 4** shows the benefit of integrating methods that include genomic classifier analysis into the clinical pathway of care with a 47% reduction in procedure recommendations.

[56] **FIG. 5** shows the benefit of integrating methods that include genomic classifier analysis into the clinical pathway of care for idiopathic pulmonary fibrosis (IPF).

[57] **FIG. 6** shows a positive change in treatment decision by integrating genomic classifier analysis into the clinical pathway of care to differentiate usual interstitial pneumonia (UIP) from other interstitial lung disease (ILD) pathologies.

[58] **FIG. 7** shows the etiologic field of injury shares common pathways.

[59] **FIG. 8** shows an example of the difference between field of cancerization and the field of injury in a subject.

[60] **FIG. 9** shows a molecular view of the field of injury and field of cancerization.

[61] **FIG. 10** shows a standard clinical pathway of care for lung cancer improved by inclusion of a genomic classifier analysis (Bronchial Genomic Classifier).

[62] **FIG. 11a-b** shows an improved clinical pathway of care for lung cancer by inclusion of multiple genomic classifier analysis (Bronchial Genomic Classifier; Nasa-Detect; Nasa-Risk Stratifier; Nasa-Protect Monitoring; Nasa-Recurrence).

[63] **FIG. 12** shows test characteristics of the Nasa-Detect classifier.

[64] **FIG. 13** shows test characteristics of the Nasa-Risk Stratifier classifier.

[65] **FIG. 14** shows test characteristics of the Nasa-Protect classifier.

[66] **FIG. 15** shows test characteristics of the Nasa-Recurrence classifier.

[67] **FIG. 16** shows evaluation of genomics in practice and prevention.

[68] **FIG. 17** shows an example of the samples characteristics and sample types used in the methods described herein.

[69] **FIG. 18** shows different subject cohorts with nasal / bronchial brushing samples.

[70] **FIG. 19** shows examples of training samples used to train a genomic classifier, such as the Nasa-Detect classifier.

[71] **FIG. 20** shows examples of training samples used to train a genomic classifier, such as the Nasa-Risk Stratifier classifier.

[72] **FIG. 21** shows types of biomarkers and the technology platforms used to detect different types of biomarkers.

[73] **FIG. 22** shows an example of RNA sequencing for genomic classifiers.

[74] **FIG. 23** shows an example of RNA sequencing.

[75] **FIG. 24** shows a flow diagram of a training and validation of a genomic classifier comprising a trained algorithm.

[76] **FIG. 25** shows an example of the diverse cytological and histological subtypes employed in training sets used to train a genomic classifier.

[77] **FIG. 26** shows a computer control system that may be programmed or otherwise configured to implement methods provided herein.

[78] **FIG. 27** shows challenges and solutions in machine learning applications.

[79] **FIG. 28** shows an analysis pipeline in the development and evaluation of a molecular genomic classifier to predict usual interstitial pneumonia (UIP) pattern in ILD patients.

[80] **FIG. 29** shows gene selection using DESeq2 and a classifier using a volcano plot to show 151 genes selected by DESeq2 (adjusted p-value < 0.05 and fold change > 2) and 190 predictive genes in a classifier, with 32 common between two sets of genes.

[81] **FIG. 30** shows gene selection using DESeq2 and a classifier using a principal component analysis (PCA) plot of all transbronchial biopsies (TBB) samples using only DESeq2 selected genes showing that these genes may not be sufficient to separate UIP samples (circle) from non-UIP samples (cross).

[82] **FIG. 31** shows gene selection using DESeq2 and a classifier using a PCA plot of all TBB samples using classifier genes illustrating that TBB samples can be classified into UIP (circle) and non-UIP (cross) samples using these genes.

[83] **FIG. 32** shows a comparison between *in silico* and *in vitro* mixing within a patient. **FIG. 32** shows a scatterplot of *in silico* and *in vitro* mixing comparison scored by an ensemble classifier with an R-squared value of 0.99.

[84] **FIG. 33** shows a comparison between *in silico* and *in vitro* mixing within a patient. **FIG. 33** shows a scatterplot of *in silico* and *in vitro* mixing comparison scored by a penalized logistic regression classifier with an R-squared value of 0.98.

[85] **FIG. 34** shows classification scores of Ensemble Model. Different gray coloring distinguishes samples with histopathology UIP, non-UIP, and non-diagnostic. Circle, up-pointing triangle, square and down-pointing triangle indicate *in silico* mixed sample, upper, middle and lower lobe samples respectively.

[86] **FIG. 35** shows classification scores of Penalized Logistic Regression Model from leave one patient out cross validation. Different gray coloring distinguishes samples with histopathology UIP, non-UIP, and non-diagnostic. Circle, up-pointing triangle, square and down-pointing triangle indicate *in silico* mixed sample, upper, middle and lower lobe samples respectively.

[87] **FIG. 36A-B** shows receiver operating characteristic (ROC) curves from leave-one patient-out cross validation (LOPO CV) and validation on independent test set (Testing). The asteroid on each ROC curve corresponds to the prospectively defined decision boundary of each proposed model.

[88] **FIG. 37** shows classification performance from leave-one patient-out cross validation and validation on independent test set.

[89] **FIG. 38** shows a heatmap of correlation matrix showing intra- and inter-patient heterogeneity in 6-representative patient data with multiple samples.

[90] **FIG. 39** shows a PCA plot using genes selected by comparing a non-UIP subtype and UIP samples. The first two principal components in PCA of all training samples using significantly differentially expressed genes comparing UIP samples (circle) and respiratory bronchiolitis (RB).

[91] **FIG. 40** shows a PCA plot using genes selected by comparing a non-UIP subtype and UIP samples. The first two principal components in PCA of all training samples using significantly differentially expressed genes comparing UIP samples (circle) and bronchiolitis.

[92] **FIG. 41** shows a PCA plot using genes selected by comparing a non-UIP subtype and UIP samples. The first two principal components in PCA of all training samples using significantly differentially expressed genes comparing UIP samples (circle) and hypersensitivity pneumonia (HP).

[93] **FIG. 42** shows a PCA plot using genes selected by comparing a non-UIP subtype and UIP samples. The first two principal components in PCA of all training samples using significantly differentially expressed genes comparing UIP samples (circle) and non-specific interstitial pneumonia (NSIP).

[94] **FIG. 43** shows a PCA plot using genes selected by comparing a non-UIP subtype and UIP samples. The first two principal components in PCA of all training samples using significantly differentially expressed genes comparing UIP samples (circle) and (organizing pneumonia (OP).

[95] **FIG. 44** shows a PCA plot using genes selected by comparing a non-UIP subtype and UIP samples. The first two principal components in PCA of all training samples using significantly differentially expressed genes comparing UIP samples (circle) and sarcoidosis.

[96] **FIG. 45** shows variability in gene expressions. The darker upper gray dots indicate genes removed from the training classification.

[97] **FIG. 46A-B** show threshold vs. sensitivity / specificity in *in silico* mixed samples using the training set in an Ensemble Model (**FIG. 46A**) and in a penalized logistic regression model (**FIG. 46B**).

[98] **FIG. 47A-C** show score variability simulation for the ensemble model. The final threshold of score variability, 0.90, may be defined by specificity (dotted vertical line) in **FIG.**

47A. The individual threshold of score variability for sensitivity (1.80) and flip-rate (1.15) may be indicated by a dotted vertical line in **FIG. 47B** and **FIG. 47C**.

[99] **FIG. 48A-C** show score variability simulation for the penalized logistic regression model. The final threshold of score variability, 0.48, may be defined by specificity (vertical line) indicated in **FIG. 48A**. The individual threshold of score variability for sensitivity (0.78) and flip-rate (0.68) are indicated by gray vertical lines in **FIG. 48B** and **FIG. 48C**.

DETAILED DESCRIPTION

[100] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[101] The term "cancer," as used herein, generally refers to a condition of abnormal cell growth. The cancer may include a solid tumor or circulating cancer cells. The cancer may metastasize. The cancer may be a tissue-specific cancer. The cancer may be a lung cancer. The cancer may be malignant or benign.

[102] The term "lung cancer," as used herein, generally refers to a cancer or tumor of a lung or lung-associated tissue. For example, a lung cancer may comprise a non-small cell lung cancer, a small cell lung cancer, a lung carcinoid tumor, or any combination thereof. A non-small cell lung cancer may comprise an adenocarcinoma, a squamous cell carcinoma, a large cell carcinoma, or any combination thereof. A lung carcinoid tumor may comprise a bronchial carcinoid. A lung cancer may comprise a cancer of a lung tissue, such as a bronchiole, an epithelial cell, a smooth muscle cell, an alveoli, or any combination thereof. A lung cancer may comprise a cancer of a trachea, a bronchius, a bronchiole, a terminal bronchiole, or any combination thereof. A lung cancer may comprise a cancer of a basal cell, a goblet cell, a ciliated cell, a neuroendocrine cell, a fibroblast cell, a macrophage cell, a Clara cell, or any combination thereof

[103] The term "disease or condition," as used herein, generally refers to an abnormal or pathological condition. A disease or condition may be a lung disease or lung condition. A lung disease or condition may include a lung cancer, interstitial lung disease (ILD), chronic obstructive pulmonary disease (COPD), chronic bronchitis, cystic fibrosis, asthma, emphysema, pneumonia, tuberculosis, pulmonary edema, acute respiratory distress syndrome, or pneumoconiosis. Types of ILD may include idiopathic pulmonary fibrosis, non-specific

interstitial pneumonia, desquamative interstitial pneumonia, respiratory bronchiolitis, acute interstitial pneumonia, lymphoid interstitial pneumonia, or cryptogenic organizing pneumonia.

[104] The term "interstitial lung disease" (ILD), as used herein, generally refers to a disease of the interstitial lung tissue. An ILD may comprise an interstitial pneumonia, an idiopathic pulmonary fibrosis, a nonspecific interstitial pneumonitis, a hypersensitivity pneumonitis, a crytogenic organizing pneumonia (COP), an acute interstitial pneumonitis, a desquamative interstitial pneumonitis; a sarcoidosis, an asbestosis, or any combination thereof.

[105] Low-dose computerized tomography (CT) scan (LDCT) generally refers to an imaging procedure that reduces radiation exposure to a subject. For example, a radiation exposure from a LDCT may be less than about 1.5 millisievert (mSv). A radiation exposure from a LDCT may be less than about: 5 mSv, 4 mSv, 3 mSv, 2 mSv, 1 mSv, 0.5 mSv, 0.1 mSv or less. A radiation exposure from a LDCT may be from about 1.0 mSv to about 2.0 mSv. A radiation exposure from an LDCT may be from about 0.5 mSv to about 1.5 mSv. A radiation exposure from an LDCT may be from about 1.0 mSv to about 4.0 mSv. A radiation exposure from an LDCT may be from about 1.0 mSv to about 3.0 mSv. A tube current setting for a LDCT may be less than about: 40 milliampere*seconds (mAs), 35 mAs, 30 mAs, 25 mAs, 20 mAs, 15 mAs, 10 mAs, 5 mAs, 1mAs or less and still yield sufficient image quality. A tube current setting for a LDCT may be from about 20 mAs to about 40 mAs. A tube current setting from a LDCT may be from about 20 mAs to about 50 mAs. A tube current setting from a LDCT may be from about 20 mAs to about 80 mAs. A tube current setting from a LDCT may be from about 20 mAs to about 100 mAs.

[106] A radiation exposure from a median dose CT scan may be greater than or equal to about 1 mSv, 5 mSv, 6 mSv, 7 mSv, 8 mSv, 9 mSv, 10 mSv, 15 mSv or more. A radiation exposure from a median dose CT scan may be about 8 mSv. A radiation exposure from a median dose CT scan may be from about 7 mSv to about 10 mSv. A radiation exposure from a median dose CT scan may be from about 1 mSv to about 10 mSv. A radiation exposure from a median dose CT scan may be from about 5 mSv to about 10 mSv. A radiation exposure from a median dose CT scan may be from about 1 mSv to about 5 mSv. A tube current setting for a median dose CT scan may be greater than or equal to about: 100 mAs, 125 mAs, 150 mAs, 175 mAs, 200 mAs, 225 mAs, 250 mAs, 300 mAs, 350 mAs, 400 mAs, 500 mAs or more. A tube current setting for a median dose CT scan may be from about 200 mAs to about 250 mAs. A tube current setting for a median dose CT scan may be from about 150 mAs to about 250 mAs. A tube current setting for a median dose CT scan may be from about 100 mAs to about 300 mAs. A tube current setting for a median dose CT scan may be from about 100 mAs to about 200 mAs. A tube

current setting for a median dose CT scan may be from about 150 mAs to about 300 mAs. A tube current setting for a median dose CT scan may be from about 150 mAs to about 400 mAs.

[107] The term “homology,” as used herein, generally refers to calculations of "homology" or “percent homology” between two or more nucleotide or amino acid sequences that can be determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first sequence). The nucleotides at corresponding positions may then be compared, and the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100). For example, if a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent homology between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. In some embodiments, the length of a sequence aligned for comparison purposes is at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 95%, of the length of the reference sequence. In some cases, a sequence homology may be from about 70% to 100%. In some cases, a sequence homology may be from about 80% to 100%. In some cases, a sequence homology may be from about 90% to 100%. In some cases, a sequence homology may be from about 95% to 100%. In some cases, a sequence homology may be from about 70% to 99%. In some cases, a sequence homology may be from about 80% to 99%. In some cases, a sequence homology may be from about 90% to 99%. In some cases, a sequence homology may be from about 95% to 99%. A BLAST® search may determine homology between two sequences. The two sequences can be genes, nucleotides sequences, protein sequences, peptide sequences, amino acid sequences, or fragments thereof. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A non-limiting example of such a mathematical algorithm is described in Karlin, S. and Altschul, S., Proc. Natl. Acad. Sci. USA, 90- 5873-5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0), as described in Altschul, S. *et al.*, Nucleic Acids Res., 25:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, any relevant parameters of the respective programs (e.g., NBLAST) can be used. For example, parameters for sequence comparison can be set at score=

100, word length= 12, or can be varied (e.g., W=5 or W=20). Other examples include the algorithm of Myers and Miller, CABIOS (1989), ADVANCE, ADAM, BLAT, and FASTA. In another embodiment, the percent identity between two amino acid sequences can be accomplished using, for example, the GAP program in the GCG software package (Accelrys, Cambridge, UK).

[108] The term "fragment," as used herein, generally refers to a portion of a sequence, such as a subset that may be shorter than a full length sequence. A fragment may be a portion of a gene. A fragment may be a portion of a peptide or protein. A fragment may be a portion of an amino acid sequence. A fragment may be a portion of an oligonucleotide sequence. A fragment may be less than about: 20, 30, 40, or 50 amino acids in length. A fragment may be less than about: 20, 30, 40, or 50 nucleotides in length. A fragment may be from about 10 amino acids to about 50 amino acids in length. A fragment may be from about 10 amino acids to about 40 amino acids in length. A fragment may be from about 10 amino acids to about 30 amino acids in length. A fragment may be from about 10 amino acids to about 20 amino acids in length. A fragment may be from about 20 amino acids to about 50 amino acids in length. A fragment may be from about 30 amino acids to about 50 amino acids in length. A fragment may be from about 40 amino acids to about 50 amino acids in length. A fragment may be from about 10 nucleotides to about 50 nucleotides in length. A fragment may be from about 10 nucleotides to about 40 nucleotides in length. A fragment may be from about 10 nucleotides to about 30 nucleotides in length. A fragment may be from about 10 nucleotides to about 20 nucleotides in length. A fragment may be from about 20 nucleotides to about 50 nucleotides in length. A fragment may be from about 30 nucleotides to about 50 nucleotides in length. A fragment may be from about 40 nucleotides to about 50 nucleotides in length.

[109] The term "subject," as used herein, generally refers to any individual that has, may have, or may be suspected of having a disease condition (e.g., lung disease). The subject may be an animal. The animal can be a mammal, such as a human, non-human primate, a rodent such as a mouse or rat, a dog, a cat, pig, sheep, or rabbit. Animals can be fish, reptiles, or others. Animals can be neonatal, infant, adolescent, or adult animals. The subject may be a living organism. The subject may be a human. Humans can be greater than or equal to 1, 2, 5, 10, 20, 30, 40, 50, 60, 65, 70, 75, 80 or more years of age. A human may be from about 18 to about 90 years of age. A human may be from about 18 to about 30 years of age. A human may be from about 30 to about 50 years of age. A human may be from about 50 to about 90 years of age. The subject may have one or more risk factors of a condition and be asymptomatic. The subject may be asymptomatic of a condition. The subject may have one or more risk factors for a condition. The subject may

be symptomatic for a condition. The subject may be symptomatic for a condition and have one or more risk factors of the condition. The subject may have or be suspected of having a disease, such as a cancer or a tumor. The subject may be a patient being treated for a disease, such as a cancer patient, a tumor patient, or a cancer and tumor patient. The subject may be predisposed to a risk of developing a disease such as a cancer or a tumor. The subject may be in remission from a disease, such as a cancer or a tumor. The subject may not have a cancer, may not have a tumor, or may not have a cancer or a tumor. The subject may be healthy.

[110] The term "tissue sample," as used herein, generally refers to any tissue sample of a subject. A tissue sample may comprise cells obtained from a portion of an airway, such as epithelial cells obtained from a portion of an airway. A tissue sample may be a nasal tissue, a bronchial tissue, a lung tissue, an esophagus tissue, a larynx tissue, an oral tissue or any combination thereof. A tissue sample may be a sample suspected or confirmed of having a disease or condition such as a cancer or a tumor. A tissue sample may be a sample removed from a subject, such as a tissue brushing, a swabbing, a tissue biopsy, an excised tissue, a fine needle aspirate, a tissue washing, a cytology specimen, a bronchoscopy, or any combination thereof. A tissue sample may be an ambiguous or suspicious sample, such as a sample obtained by fine needle aspiration, a bronchoscopy, or other small volume sample collection method. A tissue sample may be an intact region of a patient's body receiving cancer therapy, such as radiation. A tissue sample may be a tumor in a patient's body. A tissue sample may comprise cancerous cells, tumor cells, non-cancerous cells, or a combination thereof. A tissue may comprise invasive cells, non-invasive cells, or a combination thereof. A tissue sample may be a nasal tissue, a trachea tissue, a lung tissue, a pharynx tissue, a larynx tissue, a bronchus tissue, a pleura tissue, an alveoli tissue, breast tissue, bladder tissue, kidney tissue, liver tissue, colon tissue, thyroid tissue, cervical tissue, prostate tissue, heart tissue, muscle tissue, pancreas tissue, anal tissue, bile duct tissue, a bone tissue, uterine tissue, ovarian tissue, endometrial tissue, vaginal tissue, vulvar tissue, stomach tissue, ocular tissue, sinus tissue, penile tissue, salivary gland tissue, gut tissue, gallbladder tissue, gastrointestinal tissue, bladder tissue, brain tissue, spinal tissue, a blood sample, or any combination thereof.

[111] The term "increased risk" in the context of developing or having a lung condition, as used herein, generally refers to an increased risk or probability associated with the occurrence of a lung condition in a subject. An increased risk of developing a lung condition can include a first occurrence of the condition in a subject or can include subsequent occurrences, such as a second, third, fourth, or subsequent occurrence. An increased risk of developing a lung condition can include a) a risk of developing the condition for a first time, b) a risk of relapse or of developing

the condition again, c) a risk of developing the condition in the future, d) a risk of being predisposed to developing the condition in the subject's lifetime, or e) a risk of being predisposed to developing the condition as an infant, adolescent, or adult. An increased risk of a lung condition occurrence or recurrence can include a risk of the condition (such as cancer) becoming metastatic. An increased risk of tumor or cancer occurrence or recurrence can include a risk of occurrence of a stage I cancer, a stage II cancer, a stage III cancer, or a stage IV cancer. Risk of tumor or cancer occurrence or recurrence can include a risk for a blood cancer, tissue cancer (e.g., a tumor), or a cancer becoming metastatic to one or more organ sites from other sites.

[112] The term "an effectiveness of a interventive therapy or treatment regime," as used herein, generally refers to an assessment or determination about whether an interventive therapy or treatment regime has achieved the results it may be intended to achieve. For example, an effectiveness of a treatment regime, such as administration of an anti-cancer drug, may be an assessment of the anti-cancer drug to reduce a tumor or cancer cell invasiveness, to kill cancer or tumor cells, or to eliminate a cancer or tumor in a subject, to reverse the progression of the disease, or to prevent the disease from developing. A treatment regime may include a surgery (i.e., surgical resection), a nutrition regime, a physical activity, radiation, chemotherapy, cell transplantation, blood fusion, or others. An interventive therapy may include administering to a subject: a pharmaceutical composition, an exercise regime, a dietary regime, a reduction or omission of one or more risk factors (such as smoking or second hand smoke exposure), or any combination thereof.

[113] As shown in **FIG. 1**, greater than about 225,000 new cases of lung cancer may be diagnosed per year. About 90% of subjects newly diagnoses with lung cancer may be subjects having a prior history of smoking. Lung cancer causes about 160,000 deaths per year. Developing new methods, systems, and kits, such as those described herein, may improve early detection of lung cancer or an increased risk of developing lung cancer, wherein early detection may be a key improvement for reducing overall mortality. Further, current clinical standards of care make it difficult to accurately diagnose lung cancer without the need for invasive, high-risk, costly invasive procedures, such as surgery or lung biopsy. Approximately 40% of subjects undergoing an invasive lung biopsy as part of a current clinical standard of care do not have cancer. Therefore, new methods, systems, and kits, such as those described herein, may also reduce the number of unnecessary invasive procedures (carrying associated risks and extra costs) while improving early detection and highly accurate diagnosis of lung cancer.

[114] As shown in **FIG. 2**, integrating genomic classifiers at different decision points within current clinical standards of care can reduce the number of unnecessary invasive procedures and identify subjects having low risk for lung cancer. For example, about 1.8 million to 2 million cases of incidental lung nodules may be detected by imaging scans in the US annually. The current clinical standard of care dictates these subjects, having nodules detected by imaging scan, then receive an invasive bronchoscopy to further evaluate whether lung nodules may be indicative of a presence of lung cancer. About 140,000 subjects (or about: 60-70% of the 350,000 subjects having a bronchoscopy) may receive an ambiguous or suspicious result. Current clinical standard of care dictates that bronchoscopies having an ambiguous or suspicious result, then receive a diagnostic surgery to determine a histopathological truth. However, about 70-80% of those subjects having an ambiguous or suspicious result may have lung tissue that may be histopathologically benign. Therefore, new methods, systems, and kits, such as those described herein, can improve the current clinical standard of care such that an ambiguous or suspicious result will be followed by analysis on one or more genomic classifiers to identify subjects having a low risk of lung cancer from those subjects having an increased risk or high risk of lung cancer. And, those subjects having an increased risk or high risk of lung cancer will be subjected to the invasive diagnostic surgery – thereby avoiding an unnecessary invasive procedure on a low-risk population.

[115] **FIG. 3** shows a current clinical standard of care with the addition/improvement of a bronchial genomic classifier as described herein. From a generic adult population, those individuals identified as at-risk for lung cancer may receive an imaging scan, such as a low dose CT scan. If no nodules may be identified, another imaging scan may be obtained at a later time point. If a nodule may be identified, a subject may receive a risk assessment, a CT scan, a PET scan, magnetic resonance imaging (MRI) scan, an X-ray, or any combination thereof. Currently, there is poor adoption of low dose CT scanning in the United States. If a risk assessment, a CT scan, a PET scan, an MRI scan, an X-ray, or any combination thereof identifies the subject as having an low risk of lung cancer, then another risk assessment, another CT scan, another PET scan, another MRI scan, another X-ray, or any combination thereof may be performed at a later time point. If a risk assessment, a CT scan, a PET scan, an MRI scan, an X-ray, or any combination thereof identifies the subject as having an intermediate or high risk of lung cancer, a subject may receive a bronchoscopy, a transthoracic needle aspiration (TTNA), a video-assisted thoracic-scopic surgery (VATS), any method to obtain an airway tissue sample, or any combination thereof. If the airway sample obtained is identified as ambiguous or suspicious, a bronchial genomic classifier may be run to identify the risk of lung cancer. If the bronchial

genomic classifier identifies the sample as a low risk, then another risk assessment, another CT scan, another PET scan, another MRI scan, another X-ray, or any combination thereof may be performed. If the bronchial genomic classifier identifies a sample as intermediate risk, then another bronchoscopy, another transthoracic needle aspiration (TTNA), another video-assisted thoracic-scopic surgery (VATS), another method to obtain an airway tissue sample, or any combination thereof may be performed. A bronchoscopy sample may ambiguous or suspicious. A high percentage of bronchoscopy samples may be ambiguous or suspicious. Therefore, adding a bronchial genomic classifier to the current clinical standard of care may significantly reduce the number of ambiguous or suspicious results. If a subject is identified as having a lung cancer, the subject may treated for the lung cancer and may be monitored for recurrence of lung cancer by imaging, liquid biopsy, or a combination thereof. However, these current methods of imaging and liquid biopsy to identify disease recurrence suffer from low sensitivity and minimal ability to identify residual disease.

[116] As shown in **FIG. 4**, addition of a bronchial genomic classifier to the clinical standard of care of lung cancer may significantly improve subject management and may have of positive impact. For example, prior to the addition of a bronchial genomic classifier, about 37% or more of intermediate to low risk subjects may be subjected to an invasive procedure. In contrast, by the addition of a bronchial genomic classifier to the clinical standard of care, there may be a reduction of about 47% or more in the number of invasive procedures performed on intermediate to low risk subjects.

[117] As shown in **FIG. 5**, addition of a genomic classifier to the clinical standard of care of idiopathic pulmonary fibrosis (IPF) may significantly reduce the number of unnecessary invasive procedures. For example, about 200,000 subjects in the US and Europe may be evaluated for a suspected presence of IPF and may receive a diagnostic high-resolution computed tomography (HRCT). Of those 200,000 subjects, about 150,000 subjects (or 70-75%) may receive an ambiguous or suspicious result from the HRCT. Those subjects having an ambiguous or suspicious result, may receive a diagnostic surgery to identify a histopathological truth (a presence or absence of IPF). However, implementation of a genomic classifier as described herein, may identify a presence or an absence of a classic interstitial pneumonia pattern (UIP) (a pattern for IPF). In the case of an identification of a presence of classic UIP, a subject may then receive a diagnostic surgery or treatment. In the case of an identification of an absence of classic UIP, a subject may not receive an invasive procedure.

[118] **FIG. 6** shows a graph of percent decrease in the number of biopsies and highlights the clinical utility of employing a genomic classifier in differentiating UIP from other ILD

pathologies. For example, introduction of a genomic classifier may have a strong clinical impact on improving management approaches for ILD. A significant decrease in the number of invasive biopsies may be observed by the inclusion of a genomic classifier in differentiating UIP from other ILD pathologies.

[119] As shown in **FIG. 7**, the etiologic field of injury may share common pathways. For example, etiologic exposures and chronic airway injury may modify a tissue microenvironment, such as an airway epithelial environment. An altered microenvironment may result in one or more molecular aberrations and activation of one or more repair pathways. Phenotype may be determined by intrinsic host response to an injury. COPD, ILD, asthma or any combination thereof may reflect a host response that may increase risk for a lung cancer. Biomarker analysis from airway epithelium may represent significant opportunities to identify the continuum of change.

[120] As shown in **FIG. 8**, there may be more than one field, such as a field of cancerization and a field of injury. A field of injury may include genomic alterations associated with a presence of a lung cancer that may be found in cells throughout the respiratory track. A field of cancerization may include tumor-specific genomic alterations that may be present in the surrounding airways, such as proximal a tumor source. There may be interplay between a field of injury and a field of cancerization. For example, molecular alternations found in the upper airway may or may not be related to the field of injury, the field of cancerization, or a combination thereof. An at-risk molecular signature may be implemented for any lung condition, such as a lung cancer, ILD, COPD, asthma, or others.

[121] **FIG. 9** shows a molecular view of the field of injury and field of cancerization concepts. Injury may include smoking or environmental exposures. Injury signatures (such as altered RNA expression) and disease signatures (such as additional mutations, transcriptional dysregulation, and others) may be outlined for lung conditions such as cancer, fibrosis, and emphysema.

[122] **FIG. 10** shows a similar pathway to **FIG. 3** showing the current state of clinical decisions improved by the addition of a single bronchial genomic classifier. However, the current state of clinical care may benefit from the addition of other genomic classifiers at other decision points within the clinical care pathway.

[123] **FIG. 11a** and **FIG. 11b** show addition of various genomic classifiers at specific decision points within the current clinical standard of care that improve early detection and minimize unnecessary invasive procedures. For example, an at-risk population may be identified within a generic population. An at-risk population may include subjects having an increased risk of developing or having a lung condition (such as lung cancer). An at-risk population may be

identified by identifying a presence of one or more risk factors associated with the lung condition. Subjects may be given a questionnaire that may assess the presence of the one or more risk factors. Subjects may be prompted by a medical professional to provide answers to questions that may assess the presence of the one or more risk factors. A sample (such as a non-invasive sample, such as a nasal brushing) may be obtained from subjects that may be identified as at-risk for the lung condition. Data obtained from the sample (such as for example expression levels or sequence variant data) may be input to a genomic classifier (such as a Nasa-DETECT classifier). The genomic classifier may identify the sample as positive or negative. A subject receiving a positive result may receive an imaging scan (such as a low-dose CT scan) to scan for lung nodules. A subject receiving a negative result may have another sample obtained at a later time point, the data from which may be input to the genomic classifier.

[124] Subjects having a confirmed presence of a lung nodule based on an imaging scan (such as a low-dose CT scan), may have a sample obtained. Data from the sample (such as expression levels or sequence variant data) may be input to a genomic classifier (such as a Nasa-RISK classifier). The genomic classifier may identify the sample as high risk or low risk for a lung condition (such as lung cancer). A subject receiving a high risk result from the classifier may receive an invasive procedure (such as a bronchoscopy, a TTNA, or a VATS) to confirm a presence or an absence of the lung condition. A subject receiving a low risk result from the classifier may receive another imaging scan to scan for the presence of a nodule followed by inputting data from another sample into the genomic classifier at a later time point.

[125] Subjects having a low risk of a lung condition as identified by a genomic classifier (such as the Nasa-RISK Stratifier classifier or the Bronchial Genomic Classifier) may receive an interventive therapy to slow or reversal disease progression or prevent occurrence of a lung condition. A sample from a subject may be obtained following at least completion of a portion of the interventive therapy. Data from the sample (such as expression levels or sequence variant data) may be input to a genomic classifier (such as a Nasa-PROTECT Monitoring classifier). The genomic classifier may identify the efficacy of the interventive therapy, a subject compliance, a disease reversal or lung condition prevention, or a combination thereof.

[126] Subjects having a curative treatment such as a surgically resected cancer or a therapy regime (such as administration of a pharmaceutical composition), may have a sample obtained following the curative treatment. Data from the sample (such as expression levels or sequence variant data) may be input to a genomic classifier (such as a Nasa-RECURRENCE classifier). The genomic classifier may provide early detection of a lung condition recurrence.

[127] **FIG. 12** shows characteristics of a Nasa-DETECT classifier. This classifier may detect lung injury in at-risk populations. This classifier may (i) optimize an imaging screening funnel; (ii) may augment an imaging scan with a more specific initial screening tool; (iii) may enhance early detection of subjects whom may benefit from interventive therapy; or (iv) any combination thereof. Subjects evaluated by this classifier may be previously determined to be at risk for lung cancer. A positive result from this classifier may include a recommendation for a follow-up investigation with an imaging scan (such as a LDCT) and an absence of nodules by the LDCT may indicate the subject as a candidate for interventive therapy. A negative result from this classifier may include monitoring again with this classifier at a later time point.

[128] **FIG. 13** shows characteristics of a Nasa-RISK Stratifier classifier. This classifier may stratify nodule risk. This classifier may minimize the number of indeterminate pulmonary nodules. This classifier may accelerate biopsy in those subjects who may need a biopsy while avoiding an invasive biopsy in those subjects that do not need one. Subjects evaluated by this classifier may include subjects having an identified pulmonary lesion. A low risk result from this classifier may include surveillance or an indication of the subject as a candidate for an interventive therapy. An intermediate result from this classifier may include a use of clinical judgement. A high risk result from this classifier may include a subject receiving a biopsy. This classifier may be developed on a Next-Generation Sequencing (NGS) platform. This classifier may include sequencing information, radiological features, or a combination thereof.

[129] **FIG. 14** shows characteristics of a Nasa-PROTECT classifier. This classifier may be a companion diagnostic to monitor lung injury reversal. This classifier may identify subject compliance with a given treatment or therapy. This classifier may identify subjects that may be benefiting from a recommended treatment or therapy. Subjects evaluated by this classifier may include Nasa-DETECT positive and nodule negative subject populations. Subjects evaluated by this classifier may include nodule positive and low risk by Nasa-RISK Stratifier classifier.

[130] **FIG. 15** shows characteristics of Nasa-RECURRENCE classifier. This classifier may be a non-invasive monitoring method to test for recurrence among subject having received a curative surgical resection or curative treatment regime. This classifier may identify emergence or reemergence of early stage disease. This classifier may comprise high sensitivity to identify recurrence. Subjects evaluated by this classifier may include subjects having a lung cancer surgically resected for cure or receiving a curative treatment regime.

[131] **FIG. 16** shows the ACCE evaluation process for genetic testing. The four main criteria in evaluations a genetic test include **A**nalytic validity, **C**linical validity, **C**linical utility, and **E**thical implications.

[132] **FIG. 17** shows examples of (i) types samples used to train and to validate genomic classifiers and (ii) types of samples input into a genomic classifier for identification. Samples may include samples obtained from: a subject having a pre-existing benign lung disease; a subject having chronic pulmonary infections; a subject having a suppressed immune system; a subject having an increased hereditary risk of developing a lung condition; a non-smoker having environmental exposure; or any combination thereof. Samples may be obtained from a plurality of different countries. Subpopulations from cohorts may drive specific classifier development and validation. Classifiers may be developed for specific population, types of exposures, or combinations thereof. For example, classifiers may be developed for environmental pollution in China or for a genetic predisposition to a lung condition. A genomic classifier may be developed to screen for a lung condition, to diagnose a lung condition, to evaluate a treatment for a lung condition, to monitor a subject's condition, or any combination thereof. Samples may be collected annually from a subject. Samples obtained annually may include nasal brushing, a blood sample, an imaging scan, or combinations thereof.

[133] **FIG. 18** shows cohorts with nasal or bronchial brushing samples. Each cohort may be identified (AEGIS, DECAMP1, LTP2, DECAMP2, and Lahey). The number of subjects enrolled and the position in the current standard of care may be identified (at bronchoscopy, post imaging scan, or at screening) and indicated for each sample cohort. Inclusion criteria may be indicated, including age of subject and smoking history. Types of samples (nasal brush, bronchial brush, blood, imaging scan) and follow-up duration (12 months, 24 months, 48 months) may also be indicated for each sample cohort.

[134] **FIG. 19** shows examples of training samples used to train and validate a classifier (such as a Nasa-DETECT classifier). Cohorts DECAMP2 and Lahey may be employed for training of this classifier. Samples may include nasal brushing, blood samples, or a combination thereof. Additional data may be collected from each subject providing a sample including: whether the subject may be a former or current smoker; time since discontinuation of smoking; presence of co-morbidities; a family history of lung conditions; a pre-bronchial risk; or any combination thereof. Training samples used to train and validate a classifier may be greater than about: 100 samples, 200 samples, 300 samples, 400 samples, 500 samples, 600 samples, 700 samples, 800 samples, 900 samples, 1000 samples, 1100 samples, 1200 samples, 1300 samples, 1400 samples, 1500 samples, 1600 samples, 1700 samples, 1800 samples, 1900 samples, 2000 samples, or more (for example 1950 samples obtained from different subjects). In some cases, training samples may comprise from about 100 samples to about 200 samples. In some cases, training samples may comprise from about 100 samples to about 300 samples. In some cases, training samples

may comprise from about 100 samples to about 400 samples. In some cases, training samples may comprise from about 100 samples to about 500 samples. In some cases, training samples may comprise from about 100 samples to about 600 samples. In some cases, training samples may comprise from about 100 samples to about 700 samples. In some cases, training samples may comprise from about 100 samples to about 800 samples. In some cases, training samples may comprise from about 100 samples to about 900 samples. In some cases, training samples may comprise from about 100 samples to about 1000 samples. In some cases, training samples may comprise from about 100 samples to about 1500 samples. In some cases, training samples may comprise from about 100 samples to about 2000 samples. In some cases, training samples may comprise from about 100 samples to about 3000 samples. In some cases, training samples may comprise from about 100 samples to about 4000 samples. In some cases, training samples may comprise from about 100 samples to about 5000 samples. Subjects providing a sample may be smokers, non-smokers with exposure risk, or health subjects without a smoking history or exposure risk.

[135] **FIG. 20** shows examples of training samples used to train and validate a classifier (such as a Nasa-RISK Stratifier classifier. Cohorts AEGIS and DECAMP1 may be employed for training of this classifier. Samples may include nasal brushing, bronchial brushing, blood sample, or any combination thereof. Additional data may be collected from each subject providing a sample including: whether the subject may be a former or current smoker; time since discontinuation of smoking; presence of co-morbidities; a pre-bronchial risk; or any combination thereof. Training samples used to train and to validate a classifier may be greater than about: 100 samples, 200 samples, 300 samples, 400 samples, 500 samples, 600 samples, 700 samples, 800 samples, 900 samples, 1000 samples, 1100 samples, 1200 samples, 1300 samples, 1400 samples, 1500 samples, 1600 samples, 1700 samples, 1800 samples, 1900 samples, 2000 samples, 2100 samples, 2200 samples, 2300 samples, 2400 samples, 2500 samples, 2600 samples, 2700 samples, 2800 samples 2900 samples, 3000 samples, or more (for example 2350 samples obtained from different subjects). In some cases, training samples may comprise from about 100 samples to about 200 samples. In some cases, training samples may comprise from about 100 samples to about 300 samples. In some cases, training samples may comprise from about 100 samples to about 400 samples. In some cases, training samples may comprise from about 100 samples to about 500 samples. In some cases, training samples may comprise from about 100 samples to about 600 samples. In some cases, training samples may comprise from about 100 samples to about 700 samples. In some cases, training samples may comprise from about 100 samples to about 800 samples. In some cases, training samples may comprise from about 100

samples to about 900 samples. In some cases, training samples may comprise from about 100 samples to about 1000 samples. In some cases, training samples may comprise from about 100 samples to about 1500 samples. In some cases, training samples may comprise from about 100 samples to about 2000 samples. In some cases, training samples may comprise from about 100 samples to about 3000 samples. In some cases, training samples may comprise from about 100 samples to about 4000 samples. In some cases, training samples may comprise from about 100 samples to about 5000 samples. Subjects providing a sample may be smokers or non-smokers.

[136] **FIG. 21** shows biomarkers and the technology employed to detect their presence or absence. For example, genomic biomarkers (including mutations and imbalance) may be detected by next-generation sequencing (NGS), microarrays, fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR), or any combination thereof. Epigenetic biomarkers (such as DNA methylation, such as 5-hydroxymethylated cytosine, 5-methylated cytosine, 5-carboxymethylated cytosine, or 5-formylated cytosine) may be detected by NGS, microarrays, PCR, mass spectrometry (MS), or any combination thereof. Transcriptomic biomarkers (such as RNA expression levels) may be detected by NGS, microarrays, PCR, or any combination thereof. Proteomic biomarkers (such as a presence of a protein) may be detected by protein arrays, immunohistochemical staining (IHC), or a combination thereof.

[137] **FIG. 22** shows RNA sequencing for a genomic classifier and thyroid FNA analysis of the genomic classifier. **FIG. 23** shows an example of RNA sequencing of gene A, gene B, and gene C. Transcription into RNA may be followed by: (i) detecting one or more expression levels (such as counts of each transcript); (ii) detecting one or more variants (such as a sequence of each transcript); (iii) detecting a number of chromosome copies (such as loss of heterozygosity (LOH)); or (iv) any combination thereof.

[138] **FIG. 24** shows a flow diagram of a trained algorithm as described herein. For example, an algorithm may receive one or more types of sequencing data from a sample. Data received into an algorithm may be normalized. Feature extraction or feature selection may occur along with supervised machine learning. One or more clinical covariates may be added to the algorithm. One or more training labels may be added to the algorithm. One or more locks may be incorporated into the algorithm. Analytical validation may be confirmed. Clinical validation may be confirmed. A genomic classifier may be launched.

[139] **FIG. 25** shows an example of a training set rich in Bethesda cytology and histology subtypes. For example, **FIG. 25** shows 507 samples of a total 634 samples in a training set that have both Bethesda cytology and histology subtypes. A training set may span all biological categories.

Accuracy, specificity and sensitivity

[140] A method as described herein may (i) determine a presence or an absence of a condition, such as a lung cancer or (ii) classify a tissue as benign or malignant, such methods may provide a specificity of diagnosis that may be greater than about 70%. In some embodiments, the specificity may be at least about: 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more. In some cases, the specificity may be from about 70% to about 99%. In some cases, the specificity may be from about 80% to about 99%. In some cases, the specificity may be from about 85% to about 99%. In some cases, the specificity may be from about 90% to about 99%. In some cases, the specificity may be from about 95% to about 99%. In some cases, the specificity may be from about 70% to about 95%. In some cases, the specificity may be from about 80% to about 95%. In some cases, the specificity may be from about 85% to about 95%. In some cases, the specificity may be from about 90% to about 95%. In some cases, the specificity may be from about 70% to 100%. In some cases, the specificity may be from about 80% to 100%. In some cases, the specificity may be from about 85% to 100%. In some cases, the specificity may be from about 90% to 100%. In some cases, the specificity may be from about 90% to 100%.

[141] A method as described herein may (i) determine a presence or an absence of a condition, such as a lung cancer or (ii) classify a tissue as benign or malignant, such methods may provide a sensitivity of diagnosis that may be greater than about 70%. In some embodiments, the sensitivity may be at least about: 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more. In some cases, the sensitivity may be from about 70% to about 99%. In some cases, the sensitivity may be from about 80% to about 99%. In some cases, the sensitivity may be from about 85% to about 99%. In some cases, the sensitivity may be from about 90% to about 99%. In some cases, the sensitivity may be from about 95% to about 99%. In some cases, the sensitivity may be from about 70% to about 95%. In some cases, the sensitivity may be from about 80% to about 95%. In some cases, the sensitivity may be from about 85% to about 95%. In some cases, the sensitivity may be from about 90% to about 95%. In some cases, the sensitivity may be from about 70% to 100%. In some cases, the sensitivity may be from about 80% to 100%. In some cases, the sensitivity may be from about 85% to 100%. In some cases, the sensitivity may be from about 90% to 100%. In some cases, the sensitivity may be from about 90% to 100%.

[142] A method as described herein may (i) determine a presence or an absence of a condition, such as a lung cancer or (ii) classify a tissue as benign or malignant, such methods may provide a sensitivity of diagnosis that may be greater than about 70% and a specificity that may be greater

than about 70%. The sensitivity may be greater than about 70% and the specificity may be greater than about 80%. The sensitivity may be greater than about 70% and the specificity may be greater than about 90%. The sensitivity may be greater than about 70% and the specificity may be greater than about 95%. The sensitivity may be greater than about 80% and the specificity may be greater than about 70%. The sensitivity may be greater than about 80% and the specificity may be greater than about 80%. The sensitivity may be greater than about 80% and the specificity may be greater than about 90%. The sensitivity may be greater than about 80% and the specificity may be greater than about 95%. The sensitivity may be greater than about 90% and the specificity may be greater than about 70%. The sensitivity may be greater than about 90% and the specificity may be greater than about 80%. The sensitivity may be greater than about 90% and the specificity may be greater than about 90%. The sensitivity may be greater than about 90% and the specificity may be greater than about 95%. The sensitivity may be greater than about 95% and the specificity may be greater than about 70%. The sensitivity may be greater than about 95% and the specificity may be greater than about 80%. The sensitivity may be greater than about 95% and the specificity may be greater than about 90%. The sensitivity may be greater than about 95% and the specificity may be greater than about 75%.

[143] A method as described herein may (i) determine a presence of a condition, such as a lung cancer or (ii) classify a tissue as benign or malignant, such method may provide a negative predictive value (NPV) that may be greater than or equal to about 95%. The NPV may be at least about: 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5% or more. In some cases, the NPV may be from about 95% to about 99%. In some cases, the NPV may be from about 96% to about 99%. In some cases, the NPV may be from about 97% to about 99%. In some cases, the NPV may be from about 98% to about 99%. In some cases, the NPV may be from about 95% to 100%. In some cases, the NPV may be from about 96% to 100%. In some cases, the NPV may be from about 97% to 100%. In some cases, the NPV may be from about 98% to 100%.

[144] In some embodiments, the nominal specificity is greater than or equal to about 50%. In some embodiments, the nominal specificity is greater than or equal to about 60%. In some embodiments, the nominal specificity is greater than or equal to about 70%. In some embodiments, the nominal negative predictive value (NPV) is greater than or equal to about 95%. In some embodiments, the NPV is at least about: 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5% (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100%) and the specificity (or

positive predictive value (PPV)) is at least about: 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, or 99.5% (e.g., 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100%). In some cases the NPV is at least about 95%, and the specificity is at least about 50%. In some cases the NPV is at least about 95% and the specificity is at least about 70%. In some cases the NPV is at least about 95% and the specificity is at least about 75%. In some cases the NPV is at least about 95% and the specificity is at least about 80%.

[145] Sensitivity may refer to TP/(TP+FN), where TP is true positive and FN is false negative. Number of Continued Indeterminate results divided by the total number of malignant results based on adjudicated histopathology diagnosis. Specificity typically refers to TN/(TN+FP), where TN is true negative and FP is false positive. The number of benign results divided by the total number of benign results based on adjudicated histopathology diagnosis. Positive Predictive Value (PPV): TP/(TP+FP); Negative Predictive Value (NPV): TN/(TN+FN).

[146] The present methods and compositions also relate to the use of biomarker panels for purposes of identification, classification, diagnosis, or to otherwise characterize a biological sample. A panel may identify one or more of the following: a field of injury; a field of cancerization; a presence of a condition (such as ILD, COPD, or lung cancer); an increased risk of developing a condition; a presence of a disease recurrence; a reversal of a disease; a prevention of a disease; or any combination thereof. The methods and compositions may also use groups of biomarker panels. Often the pattern of levels of gene expression of biomarkers in a panel (also known as a signature such as an injury signature or a cancerization signature) may be determined and then may be used to evaluate the signature of the same panel of biomarkers in a biological sample, such as by a measure of similarity between the sample signature and the reference signature. In some embodiments, the method involves measuring (or obtaining) the levels of two or more gene expression products that may be within a biomarker panel and/or within a classification panel. For example, in some embodiments, a biomarker panel or a classification panel may contain at least about: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 33, 35, 38, 40, 43, 45, 48, 50, 53, 58, 63, 65, 68, 100, 120, 140, 142, 145, 147, 150, 152, 157, 160, 162, 167, 175, 180, 185, 190, 195, 200, or 300 biomarkers. In some embodiments, a biomarker panel or a classification panel contains no greater than or equal to about: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 33, 35, 38, 40, 43, 45, 48, 50, 53, 58, 63, 65, 68, 100, 120, 140, 142, 145, 147, 150, 152, 157, 160, 162, 167, 175, 180, 185, 190, 195, 200, or 300 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 1 to about 500 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 1 to about

400 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 1 to about 300 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 1 to about 200 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 1 to about 100 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 1 to about 500 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 100 to about 500 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 200 to about 500 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 300 to about 500 biomarkers. In some embodiments, a biomarker panel or a classification panel contains from about 400 to about 500 biomarkers. In some embodiments, a classification panel contains at least about: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 different biomarker panels. In other embodiments, a classification panel contains no greater than or equal to about: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 different biomarker panels. A biomarker panel may comprise a panel of genes that may identify an injury signature, confirm a presence of an interstitial pneumonia pattern (UIP), identify a risk of developing a disease, identify a risk of disease recurrence, monitor a disease progression, or any combination thereof.

[147] One or more risk factors that may increase a risk or likelihood of developing lung cancer may including smoking, exposure to environmental smoke (such as secondhand smoke), exposure to radon, exposure to industrial substances (such as asbestos, arsenic, diesel exhaust, mustard gas, uranium, beryllium, vinyl chloride, nickel chromates, coal products, chloromethyl ethers, gasoline), inherited or environmentally-acquired gene mutations, tuberculosis, exposure to air pollution, exposure to radiation (such as previous radiation therapy), a subject's age, having a secondary condition (such as chronic obstructive pulmonary disease (COPD)), interstitial lung disease (ILD), asthma, or others), consumption of a dietary supplement (such as beta carotene) or any combination thereof. A risk factor that may increase a risk or a likelihood of developing a lung cancer may comprise cigarette smoking, cigar smoking, pipe smoking, or any combination thereof.

[148] A subject having one risk factor may identify the subject as an at-risk individual. A subject having two risk factors may identify the subject as an at-risk individual. A subject having three risk factors may identify the subject as an at-risk individual. Individual risk factors may not be weighted equally. The presence of a single risk factor, such as smoking, may identify the subject as an at-risk individual. The presence of a single risk factor, such as having a particular genetic mutation, may not be sufficient alone but needed in combination with other risk factors to identify the subject as an at-risk individual.

[149] A subject may be given a questionnaire (written or computerized) to provide answers to one or more questions that assess the presence of one or more risk factors. A medical professional may request answers to one or more questions directly from a subject to assess the presence of one or more risk factors. A non-invasive sample may be provided by a subject to assess a presence of one or more risk factors. A previous medical history of a subject may be provided to assess a presence of one or more risk factors. A medical professional may retain health or physiological data of a subject, which may comprise, for example, a medical history of the subject.

[150] An inconclusive diagnosis can lead to unnecessary surgery, delayed diagnosis, delayed treatment, or any combination thereof. In the current clinical pathway, from 15-70% of diagnosis may be uncertain or inconclusive. In the case of an inconclusive diagnosis, diagnostic surgery may be recommended. A portion of those subjects recommended for surgery, due to an inconclusive diagnosis, may be benign. Development of genomic classifiers that can diagnosis or classify a sample with high sensitivity and specificity may be needed.

[151] Currently there may be about 225,000 new cases of lung cancer each year. In about 90% of these new cases, the subject may be identified as a smoker during at least a portion of their life. About 40% of subjects that undergo an invasive biopsy do not have cancer. Further, early detection may also be important to reducing mortality. However, current standards of care require invasive procedures to diagnose.

[152] Lung tissue, such as peripheral lung nodules may be difficult to obtain a biopsy and can yield high rates of inconclusive or non-diagnostic bronchoscopies. Therefore, alternative options for diagnosing lung cancer may be desired.

[153] Smoking may alter gene expression of epithelial cells throughout an airway including epithelial cells of the nose, mouth, oral cavity, nasal cavity, pharynx, larynx, trachea, lung, bronchus, alveolus, or any combination thereof.

[154] Isolating epithelial cells from a portion of an airway and assaying for a gene signature or panel of biomarkers in the isolated epithelial cells may determine a risk of developing cancer or confirm a presence of cancer or classifying a lung tissue as benign or malignant. Such assaying may be performed, for example, using nucleic acid amplification (e.g., PCR), array hybridization or sequencing. Such sequencing may be massively parallel sequencing (e.g., Illumina, Pacific Biosciences of California, or Oxford Nanopore). Sequencing may provide sequencing reads, which may be used to identify genetic (or genomic) aberrations (e.g., copy number variation, single nucleotide polymorphism, single nucleotide variant, insertion or deletion, etc.) and an expression level corresponding to a gene or expression levels corresponding to genes. This may

advantageously provide information relating to genetic aberrations in a genome of the subject together with information relating to a level of expression of a transcript messenger ribonucleic acid molecule (mRNA) from the same sample.

[155] An isolated epithelial cell may be isolated from a section of an airway that may be distant from the site of a cancer or a tumor. For example, an isolated epithelial cell may be a nasal epithelial cell or an oral epithelial cell and a gene signature of expression level of a panel of biomarkers obtained from the isolated nasal epithelial cell may predict a risk of developing cancer or confirm a presence of cancer in a bronchial tissue or in a peripheral lung nodule. Tumor-specific genomic alternations may be present in the surrounding airway tissues. Genomic alterations associated with the presence of a cancer may be found in cells throughout an airway.

[156] Subtypes of interstitial lung disease (ILD) may be difficult to differentiate and to diagnosis with clinical certainty. Many subjects having ILD, such as about 42%, report at least one year delay from initial symptoms to receiving a confirmed diagnosis. Misdiagnosis may be common. At least 55% of subjects having ILD report at least one misdiagnosis.

[157] About 200,000 subjects in the US and Europe suspected of ILD may be evaluated each year. About 25-30% of subjects receiving a high-resolution CT scan show a presence of UIP. About 70-75% (about 150,000) subjects receive an uncertain or inconclusive diagnosis following high-resolution CT scan. These subjects receiving an inconclusive diagnosis may be recommended for diagnostic surgery.

[158] There may be a need to develop a genomic classifier using gene signatures (such as class UIP pattern for IPF) to improve diagnostic accuracy and reduce the number of subjects receiving diagnostic surgery.

[159] The methods described herein provide a genomic classifier to identify the presence of an ILD (such as IPF) by assaying for a biomarker panel (such as a classic UIP pattern) in a sample obtained from a subject suspected of having the ILD. The method may have at least about 88% specificity and at least about 67% sensitivity. For subjects having a positive UIP pattern identified by a genomic classifier, the percent of subjects having a subsequent diagnostic biopsy decreased from about 59% without use of the genomic classifier to about 29% with use of the genomic classifier.

[160] High resolution computed tomography (HRCT) criteria for a classic UIP pattern may include at least four of: a subpleural basal predominance, a reticular abnormality, a honeycombing with or without traction bronchiectasis, and an absence of features listed as inconsistent with UIP pattern. A possible UIP pattern may include three of the following: subpleural basal predominance, a reticular abnormality, an absence of features listing as

inconsistent with UIP pattern. Indications that may be inconsistent with a classic UIP pattern include any of the following: upper or mid-lung predominance, peribronchvascular predominance, extensive ground glass abnormality, profuse micronodules, discrete cysts, diffuse mosaic attenuation or air-trapping, consolidation of bronchopulmonary segments or lobes.

[161] A subject (such as a subject at a low risk for developing a lung cancer) may receive a bronchoscopy, a transthoracic needle aspiration (TTNA), a video-assisted thoracic-scopic surgery (VATS) or other method to obtain an airway tissue sample, such as a lung tissue sample. If the bronchoscopy may be inconclusive or non-diagnostic, a classifier (such as a Bronchial Genomic Classifier) may be applied to identify and classify the airway tissue sample and avoid a further invasive procedure.

[162] A subject may receive a biopsy, such as a transbronchial biopsy. A classifier (such as a Genomic Classifier) may be applied to one or more expression levels obtained from the biopsy to detect a presence or an absence of one or more genes of a panel of genes or a gene expression pattern (such as the classic IPF "UIP pattern"). A classifier may identify a presence or an absence of an ILD, such as IPF, in the biopsy.

[163] For subjects who may be at an increased risk of developing lung cancer (based on one or more risk factors) as compared to the general population, a classifier (such as a Nasa-Detect classifier) may be employed to determine a presence or an absence of an "injury" signature in a subject that may be an early detection method for lung cancer diagnosis. A classifier (such as a Nasa-Detect classifier) may be applied to one or more expression levels assayed in a sample obtained from a subject to detect a presence or an absence of one or more genes of a panel of genes or a gene expression pattern. The panel of genes may comprise a signature of "injury" that may predispose a subject to develop a lung cancer or may be an early indicator of a presence of the disease. This classifier may be utilized to identify subjects that may be potential candidates for interventive therapy or injury reversal. If the classifier (such as the Nasa-Detect classifier) reports a negative result, that the subject does not have a presence or an altered expression of one or more genes of the "injury" panel, the classifier may be re-run on a second sample obtained from the subject at a later time point to monitor changes in gene expression. If the classifier (such as the Nasa-Detect classifier) reports a positive result, that the subject does have a presence or an altered expression of one or more genes of the "injury" panel, then a subject may receive a low-dose CT scan (LDCT).

[164] A classifier may be trained to detect "injury" in "at-risk" populations of subjects. A positive result may include a recommendation for a follow-up investigation with a LDCT. A negative result may include a recommendation for monitoring with a second classifier (such as

Nasa-Detect classifier) at a recurring time interval, such as about: every 0.5 year, every 1 year, every 1.5 years, every 2 years, every 2.5 years, every 3 years, every 3.5 years, every 4 years, every 4.5 years, or every 5 years, or longer. In some cases, a recurring time interval may be from about 0.5 year to about 3 years. In some cases, a recurring time interval may be from about 1 years to about 3 years. In some cases, a recurring time interval may be from about 2 years to about 3 years. In some cases, a recurring time interval may be from about 0.5 year to about 2 years. In some cases, a recurring time interval may be from about 0.5 year to about 1.5 years. A classifier trained to detect "injury" in "at-risk" populations may (i) optimize the subset of subjects that may be screened by an LDCT, (ii) augment LDCT screening with a specific screening tool, (iii) detect subjects that may benefit from interventive therapy, or any combination thereof.

[165] A subject may receive a low-dose CT scan to determine a presence or absence of one or more lung nodules. If the LDCT shows an absence of lung nodules, (i) the classifier (such as the Nasa-Detect classifier) may be re-run on a second sample obtained from the subject at a later time point to monitor changes in gene expression of the one or more genes of the "injury" panel or (ii) the subject may be recommended for receiving an interventive therapy. If the LDCT shows a presence of one or more lung nodules, a classifier (such as a Nasa-Risk Stratifier classifier) may be applied to one or more expression levels assayed in a sample run obtained from a subject.

[166] A subject recommended from interventive therapy (such as a subject with an absence of lung nodules as measured by LDCT), may receive one or more drug therapies. Following administering of one or more drug therapies, a sample may be obtained from the subject, assayed for one or more expression levels and run on a classifier (such as a Nasa-Protect Monitoring classifier). The classifier (such as the Nasa-Protect Monitoring classifier) may be trained to monitor changes of a particular set of biomarkers and to make a recommendation of whether to continue a particular drug regime. A result of the classifier (such as the Nasa-Protect Monitoring classifier) may be to recommend ceasing a drug therapy, switching to a different drug therapy, switching to a different non-drug therapy, maintaining a current therapy, or any combination thereof. A classifier (such as a Nasa-Protect Monitoring classifier) may be utilized as a companion diagnostic to monitor a reversal of a field of injury that may halt progression of a cancer, such as lung cancer.

[167] A classifier (such as a Nasa-Protect classifier) may be trained as a companion diagnostic to monitor lung injury reversal. A classifier may be trained to identify a subset of subjects that may be benefiting from a particular treatment or drug regime.

[168] When a LDCT yields a presence of one or more lung nodules, a sample may be obtained from a subject. The sample may be assayed for one or more expression levels and the one or more expression levels input into a classifier (such as a Nasa-Risk Stratifier classifier). A classifier (such as a Nasa-Risk Stratifier classifier) may be run prior to a bronchoscopy or other invasive procedure. A classifier (such as a Nasa-Risk Stratifier classifier) may identify a subject at low-risk for developing lung cancer, at high-risk for developing lung cancer, at low-risk of having lung cancer, or at high-risk of having lung cancer. When a result of the classifier (such as the Nasa-Risk Stratifier classifier) yields a low-risk result, another LDCT may be performed on the subject at a later point in time. When a result of the classifier (such as the Nasa-Risk Stratifier classifier) yields a high-risk result, then the subject may receive a bronchoscopy, a transthoracic needle aspiration (TTNA), a video-assisted thoracic-scopic surgery (VATS), or another invasive procedure. A classifier (such as a Nasa-Risk Stratifier classifier) may shift the course of next steps for a subject into two different categories (such as a subject with high-risk and a subject with low-risk). This shift in the course of next steps may improve early detection of cancer with a lower false positive.

[169] A classifier (such as a Nasa-Risk Stratifier classifier) may be trained to stratify a risk of a presence of nodules, such as nodules detected by LDCT, to better inform next clinical steps. A classifier may include radiological selection features. A classifier may be developed on an Next-generation sequencing (NGS) platform. A classifier yielding a low-risk result, may include a recommendation of continued surveillance or monitoring of a subject or include a recommendation of a subject as a potential candidate for interventive therapy. A classifier yielding a high-risk result, may include a recommendation to proceed with a surgical biopsy. A classifier may accelerate surgical biopsy in those subjects that need further testing and avoid surgical biopsy in those subjects that do not. A classifier may minimize the number of indeterminate pulmonary nodules. A subject population for a classifier may include subjects having confirmed presence of pulmonary lesions, such as by LDCT.

[170] In some cases, a bronchoscopy or other invasive procedure (such as TTNA or VATS) may yield a positive cancer diagnosis. In some cases, a bronchoscopy may yield a non-diagnostic result. In these cases, when a bronchoscopy may yield a non-diagnostic result, a sample may be obtained from the subject, assayed for one or more expression levels, and the expression levels may be input into a classifier (such as a Bronchial Genomic Classifier). If a classifier (such as a Bronchial Genomic Classifier) returns a result of intermediate risk, a subject may receive a second bronchoscopy or invasive procedure. If a classifier (such as a Bronchial Genomic Classifier) returns a result of low-risk, a subject may receive an interventive therapy or a second

LDCT. In some cases, a bronchoscopy may yield a cancerous or malignant result. A subject receiving a cancerous or malignant result from a bronchoscopy or other invasive procedure may have the affected tissue surgically resected. If the affected tissue can be surgically resected, a sample may be obtained from a subject, assayed for one or more expression levels, and the expression levels may be input into a classifier (such as a Nasa-Recurrence classifier). After a cancer, such as an early stage cancer, may be detected and resected, a classifier (such as a Nasa-Recurrence classifier) may predict early recurrence through monitoring. If a result of a classifier (such as a Nasa-Recurrence classifier) may indicate no risk of recurrence than a second sample from the subject may be obtained at a later point in time, assayed for one or more expression levels, and the expression levels run through the classifier (such as the Nasa-Recurrence classifier). If a result of a classifier (such as a Nasa-Recurrence classifier) may indicate a risk of recurrence, a sample may be obtained from a subject and mutation testing, immune toxicology testing, or a combination thereof may be performed on the sample. Based on a result of the mutation or immunotx testing, a therapy may be recommended to a subject following by therapy monitoring and a second mutation or immunotx testing.

[171] A classifier (such as a Nasa-Recurrence classifier) may be trained to non-invasively monitor subjects for a recurrence of cancer. A classifier may be trained to monitor subject that underwent curative surgical resection of a tumor for a recurrence of the tumor or cancer. In some cases, a classifier may indicate recurrence is detected or no recurrence is detected. A subject population may include subjects having received surgical resection to cure a lung cancer. A classifier may identify recurrence of disease in early stages.

[172] If an affected tissue identified as cancerous or malignant cannot be surgically resected, a sample may be obtained from a subject and mutation or immunotx testing may be performing on the sample.

Samples

[173] One or more samples may be obtained from a subject. One or more samples may be a same type of sample, such as one or more biopsies. One or more samples obtained from a subject may be different types of samples, such as a biopsy and a fine needle aspiration.

[174] A type of sample may include a blood sample, a tissue sample, or an image sample. A sample may comprise cell-free DNA. A blood sample may comprise cell-free DNA. A blood sample may comprise blood cells. A blood sample may comprise serum or plasma. A tissue sample may be obtained by surgical biopsy, surgical resection, needle aspiration, fine needle aspiration, a tissue swabbing, a tissue brushing or any combination thereof. A tissue sample may comprise epithelial cells, blood cells or a combination thereof. A tissue sample may comprise

cancerous cells, non-cancerous cells, or a combination thereof. An image sample may be obtained by a bronchoscopy, a CT scan (such as a low-dose CT scan), a VATS, or a TTNA, or any combination thereof.

[175] A sample may be an isolated and purified sample. A sample may be a freshly isolated sample. Cells from a freshly isolated sample may be isolated and cultures. A sample may comprise one or more cells. An isolated sample may comprise a heterogeneous mixture of cells. A sample may be purified to comprise a homogeneous mixture of cells. A sample may comprise about: 100 cells, 1,000 cells, 5,000 cells, 10,000 cells, 20,000 cells, 30,000 cells, 40,000 cells, 50,000 cells, 60,000 cells, 70,000 cells, 80,000 cells, 90,000 cells, 100,000 cells, 150,000 cells, 200,000 cells, 250,000 cells, 300,000 cells, 350,000 cells, 400,000 cells, 450,000 cells, 500,000 cells, 550,000 cells, 600,000 cells, 650,000 cells, 700,000 cells, 750,000 cells, 800,000 cells, 850,000 cells, 900,000 cells, 950,000 cells, or more. A sample may comprise from about 30,000 cells to about 1,000,000 cells. A sample may comprise from about 20,000 cells to about 50,000 cells. A sample may comprise from about 100,000 cells to about 400,000 cells. A sample may comprise from about 400,000 cells to about 800,000 cells.

[176] A sample may comprise epithelial cells. A sample may comprise blood cells. A sample may comprise nasal tissue, oral tissue (gum tissue, cheek tissue, tongue tissue, or others), pharynx tissue, larynx tissue, trachea tissue, bronchi tissue, lung tissue, or any combination thereof.

[177] A classifier may be trained with one or more training samples. A classifier may be trained with one or more different types of training samples. Different training sample types may comprise a surgical biopsy, a tissue resection, a needle aspiration, a fine needle aspiration, a blood sample, a cell-free DNA sample, an image or imaging data (such as a CT scan), or any combination thereof. A classifier may be trained with at least two different types of training samples, such as a surgical biopsy and a fine needle aspiration. A classifier may be trained with at least three different types of training samples, such as a surgical biopsy, fine needle aspiration, and blood sample. A classifier may be trained with at least three different types of training samples, such as a surgical biopsy, fine needle aspiration, and an image obtained from a CT scan. A classifier may be trained with at least four different types of training samples, such as a surgical biopsy, fine needle aspiration, a blood sample, and an image obtained from a CT scan.

[178] Training samples may be obtained from one or more subjects. Subject may include subjects having a different country of birth. Subject may include subject having a different place of residence. Training samples may represent at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 different countries of birth. Training samples may represent at least

about 3 different countries of birth. Training samples may represent at least about 5 different countries of birth. Training samples may represent at least about 10 different countries of birth. Training samples may represent from about 2 to about 10 different countries of birth. Training samples may represent from about 3 to about 15 different countries of birth. Training samples may represent from about 2 to about 20 different countries of birth. Training samples may represent at least about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 different countries of residence. Training samples may represent at least about 3 different countries of residence. Training samples may represent at least about 5 different countries of residence. Training samples may represent at least about 10 different countries of residence. Training samples may represent from about 2 to about 10 different countries of residence. Training samples may represent from about 3 to about 15 different countries of residence. Training samples may represent from about 2 to about 20 different countries of residence.

[179] Training samples may comprise one or more samples obtained from a subject suspected of having a condition (such as lung cancer), a subject having a confirmed diagnosis of a condition (such as lung cancer), a subject having a pre-existing condition (such as a benign lung disease), a subject having lung nodules identified on a LDCT, a subject that may be a non-smoker, a subject that may be a non-smoker with environmental exposure to smoking, a current smoker, a previous smoker, a subject having smoked at least about: 1, 10, 20, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 2,000, 3,000, 4,000, 5,000, 10,000, 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000, 19,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000 or more cigarettes or cigars or e-cigarettes in their lifetime, a subject having an increased hereditary risk of developing a condition (such as lung cancer), a subject having a suppressed immune system, a subject having chronic pulmonary infections, or any combination thereof. In some cases, a subject may have smoked from about 1 to about 10 cigarettes, cigars, e-cigarettes in their lifetime. In some cases, a subject may have smoked from about 1 to about 100 cigarettes, cigars, e-cigarettes in their lifetime. In some cases, a subject may have smoked from about 1 to about 1000 cigarettes, cigars, e-cigarettes in their lifetime. In some cases, a subject may have smoked from about 1000 to about 10,000 cigarettes, cigars, e-cigarettes in their lifetime. In some cases, a subject may have smoked from about 10,000 to about 50,000 cigarettes, cigars, e-cigarettes in their lifetime. In some cases, a subject may have smoked from about 10,000 to about 100,000 cigarettes, cigars, e-cigarettes in their lifetime.

[180] A smoker may be an individual having at least about: 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500 cigarettes, cigars, or e-cigarettes in their lifetime. A smoker may

be an individual having at least about 100 cigarettes, cigars, or e-cigarettes in their lifetime. A smoker may be an individual having at least about 500 cigarettes, cigars, or e-cigarettes in their lifetime. A smoker may be an individual having had greater than about: 5, 10, 20, 30, 40, or 50 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had greater than about 5 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had greater than about 10 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had greater than about 20 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had greater than about 30 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had from about 1 pack to about 12 packs (or more) of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had from about 10 packs to about 25 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had from about 25 packs to about 50 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had from about 1 pack to about 50 packs of cigarettes, cigars, e-cigarettes per year. A smoker may be an individual having had from about 10 packs to about 50 packs of cigarettes, cigars, e-cigarettes per year.

[181] Training samples may comprise one or more samples obtained from a smoker having received a positive diagnosis of a condition (such as lung cancer), a smoker having received a negative diagnosis of a condition (such as lung cancer), a smoker not having previously received a diagnosis, a non-smoker with environmental exposure having received a positive diagnosis of a condition (such as lung cancer), a non-smoker with environmental exposure having received a negative diagnosis of a condition (such as lung cancer), a non-smoker with environmental exposure not having previously received a diagnosis, a non-smoker having received a positive diagnosis of a condition (such as lung cancer), a non-smoker having received a negative diagnosis of a condition (such as lung cancer), a non-smoker not having previously received a diagnosis, or any combination thereof.

[182] One or more types of genomic information may be obtained from a sample, such as a training sample or a validation sample. For example, a sample may be assayed for an expression level of one or more genes (such as genes of a biomarker panel). A sample may be assayed for a presence of an absence of one or more genes. A sample may be assayed for an expression level, a count or number of reads, a sequence variant, a fusion, a loss of heterozygosity (LOH), a mitochondrial transcript, one or more of any of these, or any combination thereof.

[183] A sample may be collected from the same subject more than one time. For example, a first sample may be collected from a subject and a second sample may be collected about 1 year after the first sample has been collected. Samples may be collected from the same subject daily,

multiple times a week, bi-weekly, weekly, bi-monthly, monthly, bi-yearly, yearly, every two years, every three years, every four years, or every five years. In some examples, a first sample is collected at a given point in time and at least a second sample is collected within a time period of 1 day, 2 days, 3 days ,4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years or more with respect to the given point in time. Results from the second sample may be compared to results of the first sample to monitor a disease progression in the subject, an efficacy of a prescribed treatment or therapy, or a change in a risk of developing a condition, or any combination thereof.

[184] A classifier may be trained to spot one or more features. A feature may relate to a condition (such as a lung cancer), a tissue type (such as a lung tissue), a population (such as subjects of a similar genetic makeup), an exposure risk (such as an environmental pollution or exposure to cigarette or cigar smoke), an injury profile, or any combination thereof. A classifier may be part of a screening assay, a diagnostic assay, a treatment regime, a monitoring regime, or any combination thereof.

[185] The present disclosure provides methods for storing a sample for a period of time, such as seconds, minutes, hours, days, weeks, months, years or longer, after the sample has been obtained and before the sample is analyzed by one or more methods of the present disclosure. In some cases, the sample obtained from a subject may be subdivided prior to the step of storage or further analysis such that different portions of the sample may be subject to different downstream methods or processes including but not limited to storage, cytological analysis, adequacy tests, nucleic acid extraction, molecular profiling or a combination thereof.

[186] In some cases, a portion of the sample may be stored while another portion of the sample may be further manipulated. Such manipulations may include but may not be limited to molecular profiling; cytological staining; nucleic acid (RNA or DNA) extraction, detection, or quantification; gene expression product (RNA or Protein) extraction, detection, or quantification; fixation; and examination. The sample may be fixed prior to or during storage by any method known to the art such as using glutaraldehyde, formaldehyde, or methanol. In other cases, the sample is obtained and stored and subdivided after the step of storage for further analysis such that different portions of the sample may be subject to different downstream methods or processes including but not limited to storage, cytological analysis, adequacy tests, nucleic acid extraction, molecular profiling or a combination thereof. In some cases, samples may be obtained and analyzed by, for example cytological analysis, and the resulting sample material is further analyzed by one or more molecular profiling methods provided herein. In such cases, the samples may be stored between the steps of cytological analysis and the steps of molecular

profiling. Samples may be stored upon acquisition to facilitate transport, or to wait for the results of other analyses. In another embodiment, samples may be stored while awaiting instructions from a physician or other medical professional.

[187] Cytological assays mark the current diagnostic standard for many types of suspected tumors including for example thyroid tumors or nodules. In some embodiments of the present disclosure, samples that assay as negative, indeterminate, diagnostic, or non-diagnostic may be subjected to subsequent assays to obtain more information. In the present disclosure, these subsequent assays may comprise molecular profiling of genomic DNA, RNA, mRNA expression product levels, miRNA levels, gene expression product levels or gene expression product alternative splicing. In some embodiments of the present disclosure, molecular profiling refers to the determination of the number (e.g., copy number) and/or type of genomic DNA in a biological sample. In some cases, the number and/or type may further be compared to a control sample or a sample considered normal. In some embodiments, genomic DNA can be analyzed for copy number variation, such as an increase (amplification) or decrease in copy number, or variants, such as insertions, deletions, truncations and the like. Molecular profiling may be performed on the same sample, a portion of the same sample, or a new sample may be acquired using any of the methods described herein. The molecular profiling company may request additional sample by directly contacting the individual or through an intermediary such as a physician, third party testing center or laboratory, or a medical professional. In some cases, samples may be assayed using methods and compositions of the molecular profiling business in combination with some or all cytological staining or other diagnostic methods. In other cases, samples may be directly assayed using the methods and compositions of the molecular profiling business without the previous use of routine cytological staining or other diagnostic methods. In some cases the results of molecular profiling alone or in combination with cytology or other assays may enable those skilled in the art to diagnose or suggest treatment for the subject. In some cases, molecular profiling may be used alone or in combination with cytology to monitor tumors or suspected tumors over time for malignant changes.

[188] The molecular profiling methods of the present disclosure provide for extracting and analyzing protein or nucleic acid (RNA or DNA) from one or more samples from a subject. In some cases, nucleic acid is extracted from the entire sample obtained. In other cases, nucleic acid is extracted from a portion of the sample obtained. In some cases, the portion of the sample not subjected to nucleic acid extraction may be analyzed by cytological examination or immuno-histochemistry. In some cases, multiple samples may be obtained from locations in close proximity to one another in a subject. For example, two different samples may be obtained from

two different locations that are located at most about 500 millimeters (mm), 400 mm, 300 mm, 200 mm, 100 mm, 90 mm, 80 mm, 70 mm, 60 mm, 50 mm, 40 mm, 30 mm, 20 mm, 10 mm, 9 mm, 8 mm, 7 mm, 6 mm, 5 mm, 4 mm, 3 mm, 2 mm, 1 mm or less apart. In some cases multiple samples (e.g., obtained from proximate locations) may be analyzed by different methods. For example, a first sample may be analyzed by cytological examination or immuno-histochemistry, and a second sample may be analyzed via molecular profiling.

[189] In some embodiments, the methods of the present disclosure comprise extracting nucleic acid molecules (e.g., DNA, RNA) from a tissue sample from a subject and generating a nucleic acid sequencing library. For example, a nucleic acid library may be generated by amplifying cDNA generated from isolated RNA by reverse transcription (RT-PCR). In some cases cDNA may be amplified by polymerase chain reaction (PCR).

Classifiers

[190] Intensity values for a sample can be analyzed using feature selection techniques including filter techniques which assess the relevance of features by looking at the intrinsic properties of the data, wrapper methods which embed the model hypothesis within a feature subset search, and embedded techniques in which the search for an optimal set of features may be built into a classifier algorithm.

[191] Filter techniques useful in the methods of the present disclosure include (1) parametric methods such as the use of two sample t-tests, ANOVA analyses, Bayesian frameworks, and Gamma distribution models (2) model free methods such as the use of Wilcoxon rank sum tests, between-within class sum of squares tests, rank products methods, random permutation methods, or TNoM which involves setting a threshold point for fold-change differences in expression between two datasets and then detecting the threshold point in each gene that minimizes the number of misclassifications (3) and multivariate methods such as bivariate methods, correlation based feature selection methods (CFS), minimum redundancy maximum relevance methods (MRMR), Markov blanket filter methods, and uncorrelated shrunken centroid methods. Wrapper methods useful in the methods of the present disclosure include sequential search methods, genetic algorithms, and estimation of distribution algorithms. Embedded methods useful in the methods of the present disclosure include random forest algorithms, weight vector of support vector machine algorithms, and weights of logistic regression algorithms. Bioinformatics, 2007 Oct. 1; 23(19):2507-17 provides an overview of the relative merits of the filter techniques provided above for the analysis of intensity data.

[192] Selected features may then be classified using a classifier algorithm. Illustrative algorithms include but may not be limited to methods that reduce the number of variables such as

principal component analysis algorithms, partial least squares methods, and independent component analysis algorithms. Illustrative algorithms further include but may not be limited to methods that handle large numbers of variables directly such as statistical methods and methods based on machine learning techniques. Statistical methods include penalized logistic regression, prediction analysis of microarrays (PAM), methods based on shrunken centroids, support vector machine analysis, and regularized linear discriminant analysis. Machine learning techniques include bagging procedures, boosting procedures, random forest algorithms, and combinations thereof. Cancer Inform, 2008; 6: 77-97 provides an overview of the classification techniques provided above for the analysis of microarray intensity data.

[193] The subject methods and algorithms enable: 1) gene expression analysis of samples containing low amount and/or low quality of nucleic acid; 2) a significant reduction of false positives and false negatives, 3) a determination of the underlying genetic, metabolic, or signaling pathways responsible for the resulting pathology, 4) the ability to assign a statistical probability to the accuracy of a diagnosis, a risk of developing a condition, a monitoring of changes in a condition, an effectiveness of an interventive therapy, or combinations thereof, 5) the ability to resolve ambiguous results, and 6) the ability to distinguish between lung conditions or sub-types of lung conditions.

[194] In some embodiments, the methods of the present disclosure provide for an upfront method of determining the cellular make-up of a particular biological sample so that the resulting molecular profiling signatures can be calibrated against the dilution effect due to the presence of other cell and/or tissue types. In one aspect, this upfront method may be an algorithm that uses a combination of known cell and/or tissue specific gene expression patterns as an upfront mini-classifier for each component of the sample. This algorithm utilizes this molecular fingerprint to pre-classify the samples according to their composition and then apply a correction/normalization factor. This data may in some cases then feed in to a final classification algorithm which may incorporate that information to aid in the final diagnosis.

[195] Raw gene expression level and alternative splicing data may in some cases be improved through the application of algorithms designed to normalize and or improve the reliability of the data. In some embodiments of the present disclosure the data analysis requires a computer or other device, machine or apparatus for application of the various algorithms described herein due to the large number of individual data points that may be processed. A "machine learning algorithm" refers to a computational-based prediction methodology, also known to persons skilled in the art as a "classifier", employed for characterizing a gene expression profile. The signals corresponding to certain expression levels, which may be obtained by, e.g., microarray-

based hybridization assays, may be typically subjected to the algorithm in order to classify the expression profile. Supervised learning generally involves "training" a classifier to recognize the distinctions among classes and then "testing" the accuracy of the classifier on an independent test set. For new, unknown samples the classifier can be used to predict the class in which the samples belong.

[196] In some cases, the robust multi-array Average (RMA) method may be used to normalize the raw data. The RMA method begins by computing background-corrected intensities for each matched cell on a number of microarrays. The background corrected values may be restricted to positive values as described by Irizarry et al. *Biostatistics* 2003 Apr. 4 (2): 249-64. After background correction, the base-2 logarithm of each background corrected matched-cell intensity may be then obtained. The back-ground corrected, log-transformed, matched intensity on each microarray may be then normalized using the quantile normalization method in which for each input array and each probe expression value, the array percentile probe value may be replaced with the average of all array percentile points, this method may be more completely described by Bolstad *et al. Bioinformatics* 2003. Following quantile normalization, the normalized data may then be fit to a linear model to obtain an expression measure for each probe on each microarray. Tukey's median polish algorithm (Tukey, J. W., *Exploratory Data Analysis.* 1977) may then be used to determine the log-scale expression level for the normalized probe set data.

[197] Data may further be filtered to remove data that may be considered suspect. In some embodiments, data deriving from microarray probes that have fewer than about: 1, 2, 3, 4, 5, 6, 7 or 8 guanosine+cytosine nucleotides may be considered to be unreliable due to their aberrant hybridization propensity or secondary structure issues. A microarray probe having greater than or equal to about 4 guanosine+cytosine nucleotides may be considered unreliable. A microarray probe having greater than or equal to about 6 guanosine+cytosine nucleotides may be considered unreliable. A microarray probe having greater than or equal to about 8 guanosine+cytosine nucleotides may be considered unreliable. A microarray probe having from about 4 guanosine+cytosine nucleotides to about 8 guanosine+cytosine nucleotides may be considered unreliable. Similarly, data deriving from microarray probes that have greater than or equal to about: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 guanosine+cytosine nucleotides may be considered unreliable due to their aberrant hybridization propensity or secondary structure issues. A microarray probe having greater than or equal to about 10 guanosine+cytosine nucleotides may be unreliable. A microarray probe having greater than or equal to about 15 guanosine+cytosine nucleotides may be unreliable. A microarray probe having greater than or equal to about 20 guanosine+cytosine nucleotides may be unreliable. A

microarray probe having greater than or equal to about 25 guanosine+cytosine nucleotides may be unreliable. A microarray probe having from about 8 guanosine+cytosine nucleotides to about 30 guanosine+cytosine nucleotides may be unreliable. A microarray probe having from about 10 guanosine+cytosine nucleotides to about 30 guanosine+cytosine nucleotides may be unreliable. A microarray probe having from about 12 guanosine+cytosine nucleotides to about 30 guanosine+cytosine nucleotides may be unreliable. A microarray probe having from about 15 guanosine+cytosine nucleotides to about 30 guanosine+cytosine nucleotides may be unreliable.

[198] In some cases, unreliable probe sets may be selected for exclusion from data analysis by ranking probe-set reliability against a series of reference datasets. For example, RefSeq or Ensembl (EMBL) may be considered very high quality reference datasets. Data from probe sets matching RefSeq or Ensembl sequences may in some cases be specifically included in microarray analysis experiments due to their expected high reliability. Similarly data from probe-sets matching less reliable reference datasets may be excluded from further analysis, or considered on a case by case basis for inclusion. In some cases, the Ensembl high throughput cDNA and/or mRNA reference datasets may be used to determine the probe-set reliability separately or together. In other cases, probe-set reliability may be ranked. For example, probes and/or probe-sets that match perfectly to all reference datasets may be ranked as most reliable (1). Furthermore, probes and/or probe-sets that match two out of three reference datasets may be ranked as next most reliable (2), probes and/or probe-sets that match one out of three reference datasets may be ranked next (3) and probes and/or probe sets that match no reference datasets may be ranked last (4). Probes and or probe-sets may then be included or excluded from analysis based on their ranking. For example, one may choose to include data from category 1, 2, 3, and 4 probe-sets; category 1, 2, and 3 probe-sets; category 1 and 2 probe-sets; or category 1 probe-sets for further analysis. In another example, probe-sets may be ranked by the number of base pair mismatches to reference dataset entries. It is understood that there may be many methods understood in the art for assessing the reliability of a given probe and/or probe-set for molecular profiling and the methods of the present disclosure encompass any of these methods and combinations thereof.

[199] Methods of data analysis of gene expression levels or of alternative splicing may further include the use of a feature selection algorithm as provided herein. In some embodiments of the present disclosure, feature selection is provided by use of the LIMMA software package (Smyth, G. K. (2005). Limma: linear models for microarray data. In: *Bioinformatics and Computational Biology Solutions using R and Bioconductor*, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, pages 397-420).

[200] Methods of data analysis of gene expression levels and or of alternative splicing may further include the use of a pre-classifier algorithm. For example, an algorithm may use a cell-specific molecular fingerprint to pre-classify the samples according to their composition and then apply a correction/normalization factor. This data/information may then be fed in to a final classification algorithm which may incorporate that information to aid in the final diagnosis or prognosis, or monitoring evaluation.

[201] Methods of data analysis of gene expression levels and or of alternative splicing may further include the use of a classifier algorithm as provided herein. In some embodiments of the present disclosure a support vector machine (SVM) algorithm, a random forest algorithm, or a combination thereof is provided for classification of microarray data. In some embodiments, identified markers that distinguish samples (e.g., benign vs. malignant, normal vs. malignant, low risk vs. high risk) or distinguish types (e.g., ILD vs. lung cancer) may selected based on statistical significance. In some cases, the statistical significance selection is performed after applying a Benjamini Hochberg correction for false discovery rate (FDR).

[202] In some cases, the classifier algorithm may be supplemented with a meta-analysis approach such as that described by Fishel and Kaufman *et al.* 2007 *Bioinformatics* 23(13): 1599-606. In some cases, the classifier algorithm may be supplemented with a meta-analysis approach such as a repeatability analysis. In some cases, the repeatability analysis selects markers that appear in at least one predictive expression product marker set.

[203] In some cases, the results of feature selection and classification may be ranked using a Bayesian post-analysis method. For example, microarray data may be extracted, normalized, and summarized using methods known in the art such as the methods provided herein. The data may then be subjected to a feature selection step such as any feature selection methods known in the art such as the methods provided herein including but not limited to the feature selection methods provided in LIMMA. The data may then be subjected to a classification step such as any of the classification methods known in the art such as the use of any of the algorithms or methods provided herein including but not limited to the use of SVM or random forest algorithms. The results of the classifier algorithm may then be ranked by according to a posterior probability function. For example, the posterior probability function may be derived from examining known molecular profiling results, such as published results, to derive prior probabilities from type I and type II error rates of assigning a marker to a category (e.g., ILD, COPD, lung cancer etc.). These error rates may be calculated based on reported sample size for each study using an estimated fold change value (e.g., 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.2, 2.4, 2.5, 3, 4, 5, 6, 7, 8, 9, 10 or more). A fold change value may be about: 0.5, 0.8, 1.0, 1.1,

1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, or 10.0. A fold change value may be from about 0.5 to about 10.0. A fold change value may be from about 0.5 to about 1.0. A fold change value may be from about 0.5 to about 5.0. A fold change value may be from about 2.0 to about 8.0. A fold change value may be from about 2.0 to about 6.0. A fold change value may be from about 6.0 to about 10.0. A fold change value may be from about 5.0 to about 10.0. A fold change value may be from about 8.0 to about 10.0. These prior probabilities may then be combined with a molecular profiling dataset of the present disclosure to estimate the posterior probability of differential gene expression. Finally, the posterior probability estimates may be combined with a second dataset of the present disclosure to formulate the final posterior probabilities of differential expression. Additional methods for deriving and applying posterior probabilities to the analysis of microarray data may be known in the art and have been described for example in Smyth, G. K. 2004 *Stat. Appl. Genet. Mol. Biol.* 3: Article 3. In some cases, the posterior probabilities may be used to rank the markers provided by the classifier algorithm. In some cases, markers may be ranked according to their posterior probabilities and those that pass a chosen threshold may be chosen as markers whose differential expression is indicative of or diagnostic for samples that may be for example benign, malignant, normal, low risk, high risk, or condition type (ILD, COPD, lung cancer). Illustrative threshold values include prior probabilities of at least about: 0.7, 0.75, 0.8, 0.85, 0.9, 0.925, 0.95, 0.975, 0.98, 0.985, 0.99, 0.995 or higher. A probability may be at least about 0.7. A probability may be at least about 0.75. A probability may be at least about 0.8. A probability may be at least about 0.85. A probability may be at least about 0.9. A probability may be at least about 0.95. A probability may be at least about 0.99. A probability may be from about 0.75 to about 0.995. A probability may be from about 0.80 to about 0.995. A probability may be from about 0.85 to about 0.995. A probability may be from about 0.9 to about 0.995. A probability may be from about 0.85 to about 0.95. A probability may be from about 0.8 to about 0.95. A probability may be from about 0.75 to about 0.95.

[204] A statistical evaluation of the results of the molecular profiling may provide a quantitative value or values indicative of one or more of the following: the likelihood of diagnostic accuracy, the likelihood of cancer, disease or condition, the likelihood of a particular cancer, disease or condition, the likelihood of the success of a particular therapeutic intervention. Thus a physician, who may not be likely to be trained in genetics or molecular biology, need not understand the raw data. Rather, the data may be presented directly to the physician in its most useful form to guide patient care. The results of the molecular profiling can be statistically evaluated using a number of methods known to the art including, but not limited to: the students

T test, the two sided T test, pearson rank sum analysis, hidden markov model analysis, analysis of q-q plots, principal component analysis, one way ANOVA, two way ANOVA, LIMMA and the like.

[205] In some embodiments of the present disclosure, results may be classified using a trained algorithm. Trained algorithms of the present disclosure include algorithms that have been developed using a reference set of known malignant, benign, and normal samples. Training samples may comprise FNA samples, surgical biopsy samples, bronchoscope samples, or any combination thereof. Algorithms suitable for categorization of samples include but may not be limited to k-nearest neighbor algorithms, concept vector algorithms, naive bayesian algorithms, neural network algorithms, hidden markov model algorithms, genetic algorithms, and mutual information feature selection algorithms or any combination thereof. In some cases, trained algorithms of the present disclosure may incorporate data other than gene expression or alternative splicing data such as but not limited to DNA polymorphism data, sequencing data, scoring or diagnosis by cytologists or pathologists of the present disclosure, information provided by the pre-classifier algorithm of the present disclosure, or information about the medical history of the subject of the present disclosure.

[206] Classifiers used early in the sequential analysis may be used to either rule-in or rule-out a sample as benign or suspicious or a sample as low-risk or high-risk or samples having ILD from samples not having ILD. In some embodiments, such sequential analysis ends with the application of a "main" classifier to data from samples that have not been ruled out by the preceding classifiers, wherein the main classifier may be obtained from data analysis of gene expression levels in multiple types of tissue and wherein the main classifier may be capable of designating the sample as benign or suspicious (or malignant).

[207] In the next step of the example classification process, a first comparison may be made between the gene expression level(s) of the sample and the first set of biomarkers or first classifier. If the result of this first comparison is a match, the classification process ends with a result, such as designating the sample as low risk or high risk for developing a lung condition or for identifying samples having ILD vs. lung cancer. If the result of the comparison is not a match, the gene expression level(s) of the sample may be compared in a second round of comparison to a second set of biomarkers or second classifier. If the result of this second comparison is a match, the classification process ends with a result, such as (a) reporting a diagnosis to a subject with a lung condition, (b) reporting a risk of developing a lung condition, (c) reporting an effectiveness of an interventive therapy, (d) recommending a follow-on procedure such as an imaging scan, another sample acquisition, a bronchoscopy, a biopsy, a

surgical resection, a pharmaceutical composition. If the result of the comparison is not a match, the process continues in a similar stepwise process of comparisons until a match is found, or until all sets of biomarkers or classifiers included in the classification process may be used as a basis of comparison. In some embodiments, the final comparison in the classification process is between the gene expression level(s) of the sample and a main classifier, as described herein.

[208] In some cases, a method may employ more than one machine learning algorithm. For example, a method may employ about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 machine learning algorithms or more. In some cases, a method may employ at least about 4 machine learning algorithms. In some cases, a method may employ at least about 5 machine learning algorithms. In some cases, a method may employ at least about 6 machine learning algorithms. In some cases, a method may employ at least about 7 machine learning algorithms. In some cases, a method may employ at least about 8 machine learning algorithms. In some cases, a method may employ at least about 9 machine learning algorithms. In some cases, a method may employ at least about 10 machine learning algorithms. In some cases, a method may employ from about 4 machine learning algorithms to about 10 machine learning algorithms. In some cases, a method may employ from about 6 machine learning algorithms to about 10 machine learning algorithms. In some cases, a method may employ from about 4 machine learning algorithms to about 8 machine learning algorithms. In some cases, a method may employ from about 4 machine learning algorithms to about 15 machine learning algorithms. A method may employ more than one machine learning algorithm in a sequential manner. In some cases, a method may employ a mixture of machine learning algorithms and fusion calling algorithms. For example, a method may employ at least one machine learning algorithm and at least one fusion calling algorithm. In some cases, a method may employ at least 5 machine learning algorithms and at least one fusion calling algorithm. In some cases, a method may employ at least 7 machine learning algorithms and at least one fusion calling algorithm.

[209] The present methods and systems may identify a presence or an absence of one or more biomarkers in a sample. For example, biomarkers may comprise biomarkers from Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 1, Table 2, or a combination thereof. In some cases, biomarkers may comprise biomarkers from Table 1, Table 2, Table 3, or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 4, Table 5, Table 6, Table 7, or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 8, Table 9, Table 10, or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 11, Table 12, Table 13, or any combination thereof. In some cases, biomarkers may

comprise biomarkers from Table 1 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 2 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 3 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 4 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 5 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 6 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 7 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 8 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 9 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 10 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 11 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 12 or any combination thereof. In some cases, biomarkers may comprise biomarkers from Table 13 or any combination thereof.

[210] A presence or an absence or a differential expression of one or more biomarkers may be indicative of a presence of one or more risk factors for developing a condition, such as a lung cancer, IPF, ILD, COPD, or any combination thereof. A presence or an absence or a differential expression of one or more biomarkers may identify an effectiveness of an inventive therapy for preventing or reversing a condition (such as a lung cancer, IPF, ILD, COPD). A presence or an absence or a differential expression of one or more biomarkers may identify a risk or a presence of remission of a condition (such as a lung cancer, IPF, ILD, COPD) in a subject. A presence or an absence or a differential expression of one or more biomarkers may distinguish a smoker with condition from a smoker without a condition (such as lung cancer, IPF, ILD, COPD). A presence or an absence or a differential expression of one or more biomarkers may identify a diagnosis of a condition (such as lung cancer, IPF, ILD, COPD), a prognosis of a condition (such as lung cancer, IPF, ILD, COPD), or a combination thereof. A presence or an absence or a differential expression of one or more biomarkers may identify a field of injury. A presence or an absence or a differential expression of one or more biomarkers may identify a relationship between expression profiles of a first cell type or a first cell obtained from a first location and a second cell type or a second cell obtained from a second location. For example, a presence or an absence or a differential expression of one or more biomarkers in a nasal tissue may be indicative of a presence of a condition (such as lung cancer, IPF, ILD, COPD) in a bronchial tissue.

[211] **Table 1** – Examples of biomarkers that may be up-regulated in IPF

Mmp7/MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)
Pla2g2a/PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)
Lcn2/LCN2	Lipocalin 2
Cthrc1/CTHRC1	Collagen triple helix repeat containing 1
C6/C6	Complement component 6
Ctse/CTSE	Cathepsin E
Dclk1/DCLK1	Double cortin-like kinase 1
Anln/ANLN	Anillin, actin binding protein
Kcnn4/KCNN4	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
Aspn/ASPN	Asporin
Pkib/PKIB	Protein kinase (cAMP-dependent, catalytic) inhibitor β
Fhl2/FHL2	Four and a half LIM domains 2
Mnd1/MND1	Meiotic nuclear divisions 1 homolog (*Saccharomyces cerevisiae*)
Mycn/MYCN	V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
Calca/CALCA	Calcitonin-related polypeptide α
Slc2a5/SLC2A5	Solute carrier family 2 (facilitated glucose/fructose transporter), member 5
Fkbp11/FKBP11	FK506 binding protein 11, 19 kD
Gdf15/GDF15	Growth differentiation factor 15
Gal/GAL	Galanin prepropeptide
Top2a/TOP2A	Topoisomerase (DNA) II α, 170 kD
Tmem213/TMEM213	Transmembrane protein 213
Podnl1/PODNL1	Podocan-like 1
Pln/PLN	Phospholamban
Mia/MIA	Melanoma inhibitory activity
Bik/BIK	BCL2-interacting killer (apoptosis inducing)
Col1a2/COL1A2	Collagen, type I, α 2
Ccnb2/CCNB2	Cyclin B2
MGC105649/C15orf48	Chromosome 15 open reading frame 48
Ptges/PTGES	Prostaglandin E synthase
Ctsk/CTSK	Cathepsin K
Nuf2/NUF2	NUF2, NDC80 kinetochore complex component, homolog (*S. cerevisiae*)

Bub1b/BUB1B	Budding uninhibited by benzimidazoles 1 homolog β (yeast)
Fap/FAP	Fibroblast activation protein, α
Col5a1/COL5A1	Collagen, type V, α 1
Fkbp10/FKBP10	FK506 binding protein 10, 65 kD
Uchl1/UCHL1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
Pla2g7/PLA2G7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
Spc25/SPC25	SPC25, NDC80 kinetochore complex component, homolog (*S. cerevisiae*)
Mlf1ip/MLF1IP	MLF1 interacting protein
Sel1l3/SEL1L3	sel-1 suppressor of lin-12-like 3 (*Caenorhabditis elegans*)
Foxm1/FOXM1	Forkhead box M1

[212] **Table 2** – Examples of biomarkers that may be down regulated in IPF

Esm1/ESM1	Endothelial cell-specific molecule 1
Tmem100/TMEM100	Transmembrane protein 100
Stxbp6/STXBP6	Syntaxin binding protein 6 (amisyn)
Gcom1/GCOM1	GRINL1A complex locus 1
Hpgd/HPGD	Hydroxyprostaglandin dehydrogenase 15-(NAD)
Vegfa/VEGFA	Vascular endothelial growth factor A
Mme/MME	Membrane metallo-endopeptidase
Emp2/EMP2	Epithelial membrane protein 2
Slc1a1/SLC1A1	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1
Clic5/CLIC5	Chloride intracellular channel 5
Ptprr/PTPRR	Protein tyrosine phosphatase, receptor type, R
Anxa3/ANXA3	Annexin A3
Lrrn3/LRRN3	Leucine rich repeat neuronal 3
Rapgef5/RAPGEF5	Rap guanine nucleotide exchange factor (GEF) 5
Olfml2a/OLFML2A	Olfactomedin-like 2A
Sgef/ARHGEF26	Rho guanine nucleotide exchange factor (GEF) 26
Sdpr/SDPR	Serum deprivation response
Adrb2/ADRB2	Adrenoceptor β 2, surface
Ramp2/RAMP2	Receptor (G protein–coupled) activity modifying protein 2
Ccdc68/CCDC68	Coiled-coil domain containing 68
RGD1306437/C13orf1	NA

Cav2/CAV2	Caveolin 2
Npr3/NPR3	Natriuretic peptide receptor C/guanylate cyclase C
Tal1/TAL1	T cell acute lymphocytic leukemia 1
Lifr/LIFR	Leukemia inhibitory factor receptor α
Prkce/PRKCE	Protein kinase C, ε
Cav1/CAV1	Caveolin 1, caveolae protein, 22 kD
RGD1311307/C6orf145	NA
Nebl/NEBL	Nebulette
Nedd9/NEDD9	Neural precursor cell expressed, developmentally down-regulated 9
S1pr5/S1PR5	Sphingosine-1-phosphate receptor 5
Afap1l1/AFAP1L1	Actin filament associated protein 1-like 1
Thbd/THBD	Thrombomodulin
Pard6b/PARD6B	Par-6 partitioning defective 6 homolog β (*C. elegans*)
Radil/RADIL	Ras association and DIL domains
Dnase2b/DNASE2B	DeoxyRNase II β
LOC691221/C5orf4	Chromosome 5 open reading frame 4
Sh3bp5/SH3BP5	SH3-domain binding protein 5 (BTK-associated)
Fgg/FGG	Fibrinogen γ chain
Epb4.1l5/EPB41L5	Erythrocyte membrane protein band 4.1-like 5
Tspan12/TSPAN12	Tetraspanin 12
Slc4a1/SLC4A1	Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)
Zfp365/ZNF365	Zinc finger protein 365
Phactr1/PHACTR1	Phosphatase and actin regulator 1
Gpd1/GPD1	Glycerol-3-phosphate dehydrogenase 1 (soluble)
Veph1/VEPH1	Ventricular zone expressed PH domain homolog 1 (zebrafish)
Selenbp1/SELENBP1	Selenium binding protein 1

[213] **Table 3** – Examples of biomarkers that may be differentially expressed in COPD

Gene
PCDH7
CCDC81
CEACAM5
PTPRH
C12orf36
B3GNT6

Gene
PLAG1
PDE7B
CACHD1
EPB41L2
FRMD4A
PRKCE
SULF1
TLE1
FAM114A1
ELF5
SGCE
SEC14L3
GPR155
ITGA9
PTGFR
ISLR
SLC5A7
ZNF483
DPYSL3
TNS3
FMNL2
GALE
CNTN3
HSD17B13
PTPRM
HLF
PROS1
PLA2G4A
KAL1
TCN1
DPP4
GPR98
KCNA1
CABLES1
PEG10

Gene
PPP1R9A
POLA2
C17orf37
ABCC4
CA8
CYP2A13
SETBP1
ANKS1B
CHP
THSD4
MPDU1
CD109
STK32A
HHLA2
AMMECR1
NPAS3
GXYLT2
KLF12
CA12
C21orf121
SH3BP4
FABP6
GUCY1B3
FUT3
STX10
FTO
CNTN4
ATP8A1
GMDS
ZNF671
WBP5
MYO5B
FLRT3
SCGB1A1
SCNN1G

Gene
CFTR
LOC339524
THSD7A
CACNB4
DQX1
GLI3
NFAT5
RUNX1T1
SNTB1
C16orf89
PRKD1
ANXA6
YIPF1
ATP10B
HK2
ABHD2
DNAH5
GGT7
FBN1
PRSS12
TMPRSS4
AMIGO2
TMEM54
CAPRIN2

[214] **Table 4** – Examples of biomarkers that may distinguish smokers with lung cancer from smokers without lung cancer.

Affymetrix ID	GenBank ID	Gene Name
1316_at	NM_003335	UBE1L
200654_at	NM_000918	P4HB
200877_at	NM_006430.1	CCT4
201530_x_at	NM_001416.1	EIF4A1
201537_s_at	NM_004090	DUSP3
201923_at	NM_006406.1	PRDX4
202004_x_at	NM_003001.2	SDHC

Affymetrix ID	GenBank ID	Gene Name
202573_at	NM_001319	CSNK1G2
203246_s_at	NM_006545.1	TUSC4
203301_s_at	NM_021145.1	DMTF1
203466_at	NM_002437.1	MPV17
203588_s_at	NM_006286	TFDP2
203704_s_at	NM_001003698 ///	RREB1
	NM_001003699 ///	
	NM_002955	
204119_s_at	NM_001123 ///	ADK
	NM_006721	
204216_s_at	NM_024824	FLJ11806
204247_s_at	NM_004935.1	CDK5
204461_x_at	NM_002853.1	RAD1
205010_at	NM_019067.1	FLJ10613
205238_at	NM_024917.1	CXorf34
205367_at	NM_020979.1	APS
206929_s_at	NM_005597.1	NFIC
207020_at	NM_007031.1	HSF2BP
207064_s_at	NM_009590.1	AOC2
207283_at	NM_020217.1	DKFZp547I014
207287_at	NM_025026.1	FLJ14107
207365_x_at	NM_014709.1	USP34
207436_x_at	NM_014896.1	KIAA0894
207953_at	AF010144	—
207984_s_at	NM_005374.1	MPP2
208678_at	NM_001696	ATP6V1E1
209015_s_at	NM_005494 ///	DNAJB6
	NM_058246	
209061_at	NM_006534 ///	NCOA3
	NM_181659	
209432_s_at	NM_006368	CREB3
209653_at	NM_002268 ///	KPNA4
	NM_032771	
209703_x_at	NM_014033	DKFZP586A0522
209746_s_at	NM_016138	COQ7

Affymetrix ID	GenBank ID	Gene Name
209770_at	NM_007048 ///	BTN3A1
	NM_194441	
210434_x_at	NM_006694	JTB
210858_x_at	NM_000051 ///	ATM
	NM_138292 ///	
	NM_138293	
211328_x_at	NM_000410 ///	HFE
	NM_139002 ///	
	NM_139003 ///	
	NM_139004 ///	
	NM_139005 ///	
	NM_139006 ///	
	NM_139007 ///	
	NM_139008 ///	
	NM_139009 ///	
	NM_139010 ///	
	NM_139011	
212041_at	NM_004691	ATP6V0D1
212517_at	NM_012070 ///	ATRN
	NM_139321 ///	
	NM_139322	
213106_at	NM_006095	ATP8A1
213212_x_at	AI632181	—
213919_at	AW024467	—
214153_at	NM_021814	ELOVL5
214599_at	NM_005547.1	IVL
214722_at	NM_203458	N2N
214763_at	NM_015547 ///	THEA
	NM_147161	
214833_at	AB007958.1	KIAA0792
214902_x_at	NM_207488	FLJ42393
215067_x_at	NM_005809 ///	PRDX2
	NM_181737 ///	
	NM_181738	
215336_at	NM_016248 ///	AKAP11

Affymetrix ID	GenBank ID	Gene Name
	NM_144490	
215373_x_at	AK022213.1	FLJ12151
215387_x_at	NM_005708	GPC6
215600_x_at	NM_207102	FBXW12
215609_at	AK023895	—
215645_at	NM_144606 ///	FLCN
	NM_144997	
215659_at	NM_018530	GSDML
215892_at	AK021474	—
216012_at	U43604.1	—
216110_x_at	AU147017	—
216187_x_at	AF222691.1	LNX1
216745_x_at	NM_015116	LRCH1
216922_x_at	NM_001005375 ///	DAZ2
	NM_001005785 ///	
	NM_001005786 ///	
	NM_004081 ///	
	NM_020363 ///	
	NM_020364 ///	
	NM_020420	
217313_at	AC004692	—
217336_at	NM_001014	RPS10
217371_s_at	NM_000585 ///	IL15
	NM_172174 ///	
	NM_172175	
217588_at	NM_054020 ///	CATSPER2
	NM_172095 ///	
	NM_172096 ///	
	NM_172097	
217671_at	BE466926	—
218067_s_at	NM_018011	FLJ10154
218265_at	NM_024077	SECISBP2
218336_at	NM_012394	PFDN2
218425_at	NM_019011 ///	TRIAD3
	NM_207111 ///	

Affymetrix ID	GenBank ID	Gene Name
	NM_207116	
218617_at	NM_017646	TRIT1
218976_at	NM_021800	DNAJC12
219203_at	NM_016049	C14orf122
219290_x_at	NM_014395	DAPP1
219977_at	NM_014336	AIPL1
220071_x_at	NM_018097	C15orf25
220113_x_at	NM_019014	POLR1B
220215_at	NM_024804	FLJ12606
220242_x_at	NM_018260	FLJ10891
220459_at	NM_018118	MCM3APAS
220856_x_at	NM_014128	
220934_s_at	NM_024084	MGC3196
221294_at	NM_005294	GPR21
221616_s_at	AF077053	PGK1
221759_at	NM_138387	G6PC3
222155_s_at	NM_024531	GPR172A
222168_at	NM_000693	ALDH1A3
222231_s_at	NM_018509	PRO1855
222272_x_at	NM_033128	SCIN
222310_at	NM_020706	SFRS15
222358_x_at	AI523613	—
64371_at	NM_014884	SFRS14

[215] **Table 5** – Examples of biomarkers that may distinguish smokers with cancer from smokers without cancer.

GenBank ID	Gene Name	Affymetrix ID
NM_030757.1	MKRN4	208082_x_at
R83000	BTF3	214800_x_at
AK021571.1	MUC20	215208_x_at
NM_014182.1	ORMDL2	218556_at
NM_17932.1	FLJ20700	207730_x_at
U85430.1	NFATC3	210556_at
AI683552	—	217679_x_at
BC002642.1	CTSS	202901_x_at

GenBank ID	Gene Name	Affymetrix ID
AW024467	RIPX	213939_s_at
NM_030972.1	MGC5384	208137_x_at
BC021135.1	INADL	214705_at
AL161952.1	GLUL	215001_s_at
AK026565.1	FLJ10534	218155_x_at
AK023783.1	—	215604_x_at
BF218804	AFURS1	212297_at
NM_001281.1	CKAP1	201804_x_at
NM_024006.1	IMAGE3455200	217949_s_at
AK023843.1	PGF	215179_x_at
BC001602.1	CFLAR	211316_x_at
BC034707.1	—	217653_x_at
BC064619.1	CD24	266_s_at
AY280502.1	EPHB6	204718_at
BC059387.1	MYO1A	211916_s_at
	—	215032_at
AF135421.1	GMPPB	219920_s_at
BC061522.1	MGC70907	211996_s_at
L76200.1	GUK1	200075_s_at
U50532.1	CG005	214753_at
BC006547.2	EEF2	204102_s_at
BC008797.2	FVT1	202419_at
BC000807.1	ZNF160	214715_x_at
AL080112.1	—	216859_x_at
BC033718.1 ///	C21orf106	215529_x_at
BC046176.1 ///		
BC038443.1		
NM_000346.1	SOX9	202936_s_at
BC008710.1	SUI1	212130_x_at
Hs.288575	—	215204_at
(Unigene ID)		
AF020591.1	AF020591	218735_s_at
BC000423.2	ATP6V0B	200078_s_at
BC002503.2	SAT	203455_s_at
BC008710.1	SUI1	212227_x_at

GenBank ID	Gene Name	Affymetrix ID
	—	222282_at
BC009185.2	DCLRE1C	219678_x_at
Hs.528304	ADAM28	208268_at
(UNIGENE ID)		
U50532.1	CG005	221899_at
BC013923.2	SOX2	213721_at
BC031091	ODAG	214718_at
NM_007062	PWP1	201608_s_at
Hs.249591	FLJ20686	205684_s_at
(Unigene ID)		
BC075839.1 ///	KRT8	209008_x_at
BC073760.1		
BC072436.1 ///	HYOU1	200825_s_at
BC004560.2		
BC001016.2	NDUFA8	218160_at
Hs.286261	FLJ20195	57739_at
(Unigene ID)		
AF348514.1	—	211921_x_at
BC005023.1	CGI-128	218074_at
BC066337.1 ///	KTN1	200914_x_at
BC058736.1 ///		
BC050555.1		
	—	216384_x_at
Hs.216623	ATP8B1	214594_x_at
(Unigene ID)		
BC072400.1	THOC2	222122_s_at
BC041073.1	PRKX	204060_s_at
U43965.1	ANK3	215314_at
	—	208238_x_at
BC021258.2	TRIM5	210705_s_at
BC016057.1	USH1C	211184_s_at
BC016713.1 ///	PARVA	215418_at
BC014535.1 ///		
AF237771.1		
BC000360.2	EIF4EL3	209393_s_at

GenBank ID	Gene Name	Affymetrix ID
BC007455.2	SH3GLB1	210101_x_at
BC000701.2	KIAA0676	212052_s_at
BC010067.2	CHC1	215011_at
BC023528.2 ///	C14orf87	221932_s_at
BC047680.1		
BC064957.1	KIAA0102	201239_s_at
Hs.156701	—	215553_x_at
(Unigene ID)		
BC030619.2	KIAA0779	213351_s_at
BC008710.1	SUI1	202021_x_at
U43965.1	ANK3	209442_x_at
BC066329.1	SDHC	210131_x_at
Hs.438867	—	217713_x_at
(Unigene ID)		
BC035025.2 ///	ALMS1	214707_x_at
BC050330.1		
BC023976.2	PDAP2	203272_s_at
BC074852.2 ///	PRKY	206279_at
BC074851.2		
Hs.445885	KIAA1217	214912_at
(Unigene ID)		
BC008591.2 ///	KIAA0100	201729_s_at
BC050440.1 ///		
BC048096.1		
AF365931.1	ZNF264	205917_at
AF257099.1	PTMA	200772_x_at
BC028912.1	DNAJB9	202842_s_at

[216] **Table 6** – Examples of biomarkers that may distinguish smokers with lung cancer from smokers without lung cancer.

GenBank ID	Gene Name	Affymetrix ID
NM_007062.1	PWP1	201608_s_at
NM_001281.1	CKAP1	201804_x_at
BC000120.1		202355_s_at
NM_014255.1	TMEM4	202857_at

GenBank ID	Gene Name	Affymetrix ID
BC002642.1	CTSS	202901_x_at
NM_000346.1	SOX9	202936_s_at
NM_006545.1	NPR2L	203246_s_at
BG034328		203588_s_at
NM_021822.1	APOBEC3G	204205_at
NM_021069.1	ARGBP2	204288_s_at
NM_019067.1	FLJ10613	205010_at
NM_017925.1	FLJ20686	205684_s_at
NM_017932.1	FLJ20700	207730_x_at
NM_030757.1	MKRN4	208082_x_at
NM_030972.1	MGC5384	208137_x_at
AF126181.1	BCG1	208682_s_at
U93240.1		209653_at
U90552.1		209770_at
AF151056.1		210434_x_at
U85430.1	NFATC3	210556_at
U51007.1		211609_x_at
BC005969.1		211759_x_at
NM_002271.1		211954_s_at
AL566172		212041_at
AB014576.1	KIAA0676	212052_s_at
BF218804	AFURS1	212297_at
AK022494.1		212932_at
AA114843		213884_s_at
BE467941		214153_at
NM_003541.1	HIST1H4K	214463_x_at
R83000	BTF3	214800_x_at
AL161952.1	GLUL	215001_s_at
AK023843.1	PGF	215179_x_at
AK021571.1	MUC20	215208_x_at
AK023783.1	—	215604_x_at
AU147182		215620_at
AL080112.1	—	216859_x_at
AW971983		217588_at
AI683552	—	217679_x_at

GenBank ID	Gene Name	Affymetrix ID
NM_024006.1	IMAGE3455200	217949_s_at
AK026565.1	FLJ10534	218155_x_at
NM_014182.1	ORMDL2	218556_at
NM_021800.1	DNAJC12	218976_at
NM_016049.1	CGI-112	219203_at
NM_019023.1	PRMT7	219408_at
NM_021971.1	GMPPB	219920_s_at
NM_014128.1	—	220856_x_at
AK025651.1		221648_s_at
AA133341	C14orf87	221932_s_at
AF198444.1		222168_at

[217] **Table 7** – Examples of biomarkers that may distinguish smokers having lung cancer from smokers without lung cancer.

GenBank ID	Gene Name	Affymetrix ID
NM_007062.1	PWP1	201608_s_at
NM_001281.1	CKAP1	201804_x_at
BC002642.1	CTSS	202901_x_at
NM_000346.1	SOX9	202936_s_at
NM_006545.1	NPR2L	203246_s_at
BG034328		203588_s_at
NM_019067.1	FLJ10613	205010_at
NM_017925.1	FLJ20686	205684_s_at
NM_017932.1	FLJ20700	207730_x_at
NM_030757.1	MKRN4	208082_x_at
NM_030972.1	MGC5384	208137_x_at
NM_002268 /// NM_032771	KPNA4	209653_at
NM_007048 /// NM_194441	BTN3A1	209770_at
NM_006694	JBT	210434_x_at
U85430.1	NFATC3	210556_at
NM_004691	ATP6V0D1	212041_at
AB014576.1	KIAA0676	212052_s_at
BF218804	AFURS1	212297_at

GenBank ID	Gene Name	Affymetrix ID
BE467941		214153_at
R83000	BTF3	214800_x_at
AL161952.1	GLUL	215001_s_at
AK023843.1	PGF	215179_x_at
AK021571.1	MUC20	215208_x_at
AK023783.1	—	215604_x_at
AL080112.1	—	216859_x_at
AW971983		217588_at
AI683552	—	217679_x_at
NM_024006.1	IMAGE3455200	217949_s_at
AK026565.1	FLJ10534	218155_x_at
NM_014182.1	ORMDL2	218556_at
NM_021800.1	DNAJC12	218976_at
NM_016049.1	CGI-112	219203_at
NM_021971.1	GMPPB	219920_s_at
NM_014128.1	—	220856_x_at
AA133341	C14orf87	221932_s_at
AF198444.1		222168_at

[218] **Table 8** – Examples of biomarkers that may identify a diagnosis or a prognosis of lung cancer.

Affymetrix ID	Gene symbol (HUGO ID)
200729_s_at	ACTR2
200760_s_at	ARL6IP5
201399_s_at	TRAM1
201444_s_at	ATP6AP2
201635_s_at	FXR1
201689_s_at	TPD52
201925_s_at	DAF
201926_s_at	DAF
201946_s_at	CCT2
202118_s_at	CPNE3
202704_at	TOB1
202833_s_at	SERPINA1
202935_s_at	SOX9

Affymetrix ID	Gene symbol (HUGO ID)
203413_at	NELL2
203881_s_at	DMD
203908_at	SLC4A4
204006_s_at	FCGR3A /// FCGR3B
204403_x_at	KIAA0738
204427_s_at	RNP24
206056_x_at	SPN
206169_x_at	RoXaN
207730_x_at	HDGF2
207756_at	—
207791_s_at	RAB1A
207953_at	AD7C-NTP
208137_x_at	—
208246_x_at	TK2
208654_s_at	CD164
208892_s_at	DUSP6
209189_at	FOS
209204_at	LMO4
209267_s_at	SLC39A8
209369_at	ANXA3
209656_s_at	TMEM47
209774_x_at	CXCL2
210145_at	PLA2G4A
210168_at	C6
210317_s_at	YWHAE
210397_at	DEFB1
210679_x_at	—
211506_s_at	IL8
212006_at	UBXD2
213089_at	LOC153561
213736_at	COX5B
213813_x_at	—
214007_s_at	PTK9
214146_s_at	PPBP
214594_x_at	ATP8B1

Affymetrix ID	Gene symbol (HUGO ID)
214707_x_at	ALMS1
214715_x_at	ZNF160
215204_at	SENP6
215208_x_at	RPL35A
215385_at	FTO
215600_x_at	FBXW12
215604_x_at	UBE2D2
215609_at	STARD7
215628_x_at	PPP2CA
215800_at	DUOX1
215907_at	BACH2
215978_x_at	LOC152719
216834_at	—
216858_x_at	—
217446_x_at	—
217653_x_at	—
217679_x_at	—
217715_x_at	ZNF354A
217826_s_at	UBE2J1
218155_x_at	FLJ10534
218976_at	DNAJC12
219392_x_at	FLJ11029
219678_x_at	DCLRE1C
220199_s_at	FLJ12806
220389_at	FLJ23514
220720_x_at	FLJ14346
221191_at	DKFZP434A0131
221310_at	FGF14
221765_at	—
222027_at	NUCKS
222104_x_at	GTF2H3
222358_x_at	—

[219] **Table 9** – Examples of biomarkers that may identify a diagnosis or a prognosis of lung cancer.

Affymetrix ID	(HUGO ID)
200729_s_at	ACTR2
200760_s_at	ARL6IP5
201399_s_at	TRAM1
201444_s_at	ATP6AP2
201635_s_at	FXR1
201689_s_at	TPD52
201925_s_at	DAF
201926_s_at	DAF
201946_s_at	CCT2
202118_s_at	CPNE3
202704_at	TOB1
202833_s_at	SERPINA1
202935_s_at	SOX9
203413_at	NELL2
203881_s_at	DMD
203908_at	SLC4A4
204006_s_at	FCGR3A /// FCGR3B
204403_x_at	KIAA0738
204427_s_at	RNP24
206056_x_at	SPN
206169_x_at	RoXaN
207730_x_at	HDGF2
207756_at	—
207791_s_at	RAB1A
207953_at	AD7C-NTP
208137_x_at	—
208246_x_at	TK2
208654_s_at	CD164
208892_s_at	DUSP6
209189_at	FOS
209204_at	LMO4
209267_s_at	SLC39A8
209369_at	ANXA3

Affymetrix ID	(HUGO ID)
209656_s_at	TMEM47
209774_x_at	CXCL2
210145_at	PLA2G4A
210168_at	C6
210317_s_at	YWHAE
210397_at	DEFB1
210679_x_at	—
211506_s_at	IL8
212006_at	UBXD2
213089_at	LOC153561
213736_at	COX5B
213813_x_at	—
214007_s_at	PTK9
214146_s_at	PPBP
214594_x_at	ATP8B1
214707_x_at	ALMS1
214715_x_at	ZNF160
215204_at	SENP6
215208_x_at	RPL35A
215385_at	FTO
215600_x_at	FBXW12
215604_x_at	UBE2D2
215609_at	STARD7
215628_x_at	PPP2CA
215800_at	DUOX1
215907_at	BACH2
215978_x_at	LOC152719
216834_at	—
216858_x_at	—
217446_x_at	—
217653_x_at	—
217679_x_at	—
217715_x_at	ZNF354A
217826_s_at	UBE2J1
218155_x_at	FLJ10534

Affymetrix ID	(HUGO ID)
218976_at	DNAJC12
219392_x_at	FLJ11029
219678_x_at	DCLRE1C
220199_s_at	FLJ12806
220389_at	FLJ23514
220720_x_at	FLJ14346
221191_at	DKFZP434A0131
221310_at	FGF14
221765_at	—
222027_at	NUCKS
222104_x_at	GTF2H3
222358_x_at	—
202113_s_at	SNX2
207133_x_at	ALPK1
218989_x_at	SLC30A5
200751_s_at	HNRPC
220796_x_at	SLC35E1
209362_at	SURB7
216248_s_at	NR4A2
203138_at	HAT1
221428_s_at	TBL1XR1
218172_s_at	DERL1
215861_at	FLJ14031
209288_s_at	CDC42EP3
214001_x_at	RPS10
209116_x_at	HBB
215595_x_at	GCNT2
208891_at	DUSP6
215067_x_at	PRDX2
202918_s_at	PREI3
211985_s_at	CALM1
212019_at	RSL1D1
216187_x_at	KNS2
215066_at	PTPRF
212192_at	KCTD12

Affymetrix ID	(HUGO ID)
217586_x_at	—
203582_s_at	RAB4A
220113_x_at	POLR1B
217232_x_at	HBB
201041_s_at	DUSP1
211450_s_at	MSH6
202648_at	RPS19
202936_s_at	SOX9
204426_at	RNP24
206392_s_at	RARRES1
208750_s_at	ARF1
202089_s_at	SLC39A6
211297_s_at	CDK7
215373_x_at	FLJ12151
213679_at	FLJ13946
201694_s_at	EGR1
209142_s_at	UBE2G1
217706_at	LOC220074
212991_at	FBXO9
201289_at	CYR61
206548_at	FLJ23556
202593_s_at	MIR16
202932_at	YES1
220575_at	FLJ11800
217713_x_at	DKFZP566N034
211953_s_at	RANBP5
203827_at	WIPI49
221997_s_at	MRPL52
217662_x_at	BCAP29
218519_at	SLC35A5
214833_at	KIAA0792
201339_s_at	SCP2
203799_at	CD302
211090_s_at	PRPF4B
220071_x_at	C15orf25

Affymetrix ID	(HUGO ID)
203946_s_at	ARG2
213544_at	ING1L
209908_s_at	—
201688_s_at	TPD52
215587_x_at	BTBD14B
201699_at	PSMC6
214902_x_at	FLJ42393
214041_x_at	RPL37A
203987_at	FZD6
211696_x_at	HBB
218025_s_at	PECI
215852_x_at	KIAA0889
209458_x_at	HBA1 /// HBA2
219410_at	TMEM45A
215375_x_at	—
206302_s_at	NUDT4
208783_s_at	MCP
211374_x_at	—
220352_x_at	MGC4278
216609_at	TXN
201942_s_at	CPD
202672_s_at	ATF3
204959_at	MNDA
211996_s_at	KIAA0220
222035_s_at	PAPOLA
208808_s_at	HMGB2
203711_s_at	HIBCH
215179_x_at	PGF
213562_s_at	SQLE
203765_at	GCA
214414_x_at	HBA2
217497_at	ECGF1
220924_s_at	SLC38A2
218139_s_at	C14orf108
201096_s_at	ARF4

Affymetrix ID	(HUGO ID)
220361_at	FLJ12476
202169_s_at	AASDHPPT
202527_s_at	SMAD4
202166_s_at	PPP1R2
204634_at	NEK4
215504_x_at	—
202388_at	RGS2
215553_x_at	WDR45
200598_s_at	TRA1
202435_s_at	CYP1B1
216206_x_at	MAP2K7
212582_at	OSBPL8
216509_x_at	MLLT10
200908_s_at	RPLP2
215108_x_at	TNRC9
213872_at	C6orf62
214395_x_at	EEF1D
222156_x_at	CCPG1
201426_s_at	VIM
221972_s_at	Cab45
219957_at	—
215123_at	—
212515_s_at	DDX3X
203357_s_at	CAPN7
211711_s_at	PTEN
206165_s_at	CLCA2
213959_s_at	KIAA1005
215083_at	PSPC1
219630_at	PDZK1IP1
204018_x_at	HBA1 /// HBA2
208671_at	TDE2
203427_at	ASF1A
215281_x_at	POGZ
205749_at	CYP1A1
212585_at	OSBPL8

Affymetrix ID	(HUGO ID)
211745_x_at	HBA1 /// HBA2
208078_s_at	SNF1LK
218041_x_at	SLC38A2
212588_at	PTPRC
212397_at	RDX
208268_at	ADAM28
207194_s_at	ICAM4
222252_x_at	—
217414_x_at	HBA2
207078_at	MED6
215268_at	KIAA0754
221387_at	GPR147
201337_s_at	VAMP3
220218_at	C9orf68
222356_at	TBL1Y
208579_x_at	H2BFS
219161_s_at	CKLF
202917_s_at	S100A8
204455_at	DST
211672_s_at	ARPC4
201132_at	HNRPH2
218313_s_at	GALNT7
218930_s_at	FLJ11273
219166_at	C14orf104
212805_at	KIAA0367
201551_s_at	LAMP1
202599_s_at	NRIP1
203403_s_at	RNF6
214261_s_at	ADH6
202033_s_at	RB1CC1
203896_s_at	PLCB4
209703_x_at	DKFZP586A0522
211699_x_at	HBA1 /// HBA2
210764_s_at	CYR61
206391_at	RARRES1

Affymetrix ID	(HUGO ID)
201312_s_at	SH3BGRL
200798_x_at	MCL1
214912_at	—
204621_s_at	NR4A2
217761_at	MTCBP-1
205830_at	CLGN
218438_s_at	MED28
207475_at	FABP2
208621_s_at	VIL2
202436_s_at	CYP1B1
202539_s_at	HMGCR
210830_s_at	PON2
211906_s_at	SERPINB4
202241_at	TRIB1
203594_at	RTCD1
215863_at	TFR2
221992_at	LOC283970
221872_at	RARRES1
219564_at	KCNJ16
201329_s_at	ETS2
214188_at	HIS1
201667_at	GJA1
201464_x_at	JUN
215409_at	LOC254531
202583_s_at	RANBP9
215594_at	—
214326_x_at	JUND
217140_s_at	VDAC1
215599_at	SMA4
209896_s_at	PTPN11
204846_at	CP
222303_at	—
218218_at	DIP13B
211015_s_at	HSPA4
208666_s_at	ST13

Affymetrix ID	(HUGO ID)
203191_at	ABCB6
202731_at	PDCD4
209027_s_at	ABI1
205979_at	SCGB2A1
216351_x_at	DAZ1 /// DAZ3 ///
	DAZ2 /// DAZ4
220240_s_at	C13orf11
204482_at	CLDN5
217234_s_at	VIL2
214350_at	SNTB2
201693_s_at	EGR1
212328_at	KIAA1102
220168_at	CASC1
203628_at	IGF1R
204622_x_at	NR4A2
213246_at	C14orf109
218728_s_at	HSPC163
214753_at	PFAAP5
206336_at	CXCL6
201445_at	CNN3
209886_s_at	SMAD6
213376_at	ZBTB1
213887_s_at	POLR2E
204783_at	MLF1
218824_at	FLJ10781
212417_at	SCAMP1
202437_s_at	CYP1B1
217528_at	CLCA2
218170_at	ISOC1
206278_at	PTAFR
201939_at	PLK2
200907_s_at	KIAA0992
207480_s_at	MEIS2
201417_at	SOX4
213826_s_at	—

Affymetrix ID	(HUGO ID)
214953_s_at	APP
204897_at	PTGER4
201711_x_at	RANBP2
202457_s_at	PPP3CA
206683_at	ZNF165
214581_x_at	TNFRSF21
203392_s_at	CTBP1
212720_at	PAPOLA
207758_at	PPM1F
220995_at	STXBP6
213831_at	HLA-DQA1
212044_s_at	—
202434_s_at	CYP1B1
206166_s_at	CLCA2
218343_s_at	GTF3C3
202557_at	STCH
201133_s_at	PJA2
213605_s_at	MGC22265
210947_s_at	MSH3
208310_s_at	C7orf28A /// C7orf28B
209307_at	—
215387_x_at	GPC6
213705_at	MAT2A
213979_s_at	—
212731_at	LOC157567
210117_at	SPAG1
200641_s_at	YWHAZ
210701_at	CFDP1
217152_at	NCOR1
204224_s_at	GCH1
202028_s_at	—
201735_s_at	CLCN3
208447_s_at	PRPS1
220926_s_at	C1orf22
211505_s_at	STAU

Affymetrix ID	(HUGO ID)
221684_s_at	NYX
206906_at	ICAM5
213228_at	PDE8B
217202_s_at	GLUL
211713_x_at	KIAA0101
215012_at	ZNF451
200806_s_at	HSPD1
201466_s_at	JUN
211564_s_at	PDLIM4
207850_at	CXCL3
221841_s_at	KLF4
200605_s_at	PRKAR1A
221198_at	SCT
201772_at	AZIN1
205009_at	TFF1
205542_at	STEAP1
218195_at	C6orf211
213642_at	—
212891_s_at	GADD45GIP1
202798_at	SEC24B
222207_x_at	—
202638_s_at	ICAM1
200730_s_at	PTP4A1
219355_at	FLJ10178
220266_s_at	KLF4
201259_s_at	SYPL
209649_at	STAM2
220094_s_at	C6orf79
221751_at	PANK3
200008_s_at	GDI2
205078_at	PIGF
218842_at	FLJ21908
202536_at	CHMP2B
220184_at	NANOG
201117_s_at	CPE

Affymetrix ID	(HUGO ID)
219787_s_at	ECT2
206628_at	SLC5A1
204007_at	FCGR3B
209446_s_at	—
211612_s_at	IL13RA1
220992_s_at	C1orf25
221899_at	PFAAP5
221719_s_at	LZTS1
201473_at	JUNB
221193_s_at	ZCCHC10
215659_at	GSDML
205157_s_at	KRT17
201001_s_at	UBE2V1 /// Kua-UEV
216789_at	—
205506_at	VIL1
204875_s_at	GMDS
207191_s_at	ISLR
202779_s_at	UBE2S
210370_s_at	LY9
202842_s_at	DNAJB9
201082_s_at	DCTN1
215588_x_at	RIOK3
211076_x_at	DRPLA
210230_at	—
206544_x_at	SMARCA2
208852_s_at	CANX
215405_at	MYO1E
208653_s_at	CD164
206355_at	GNAL
210793_s_at	NUP98
215070_x_at	RABGAP1
203007_x_at	LYPLA1
203841_x_at	MAPRE3
206759_at	FCER2
202232_s_at	GA17

Affymetrix ID	(HUGO ID)
215892_at	—
214359_s_at	HSPCB
215810_x_at	DST
208937_s_at	ID1
213664_at	SLC1A1
219338_s_at	FLJ20156
206595_at	CST6
207300_s_at	F7
213792_s_at	INSR
209674_at	CRY1
40665_at	FMO3
217975_at	WBP5
210296_s_at	PXMP3
215483_at	AKAP9
212633_at	KIAA0776
206164_at	CLCA2
216813_at	—
208925_at	C3orf4
219469_at	DNCH2
206016_at	CXorf37
216745_x_at	LRCH1
212999_x_at	HLA-DQB1
216859_x_at	—
201636_at	—
204272_at	LGALS4
215454_x_at	SFTPC
215972_at	—
220593_s_at	FLJ20753
222009_at	CGI-14
207115_x_at	MBTD1
216922_x_at	DAZ1 /// DAZ3 ///
	DAZ2 /// DAZ4
217626_at	AKR1C1 /// AKR1C2
211429_s_at	SERPINA1
209662_at	CETN3

Affymetrix ID	(HUGO ID)
201629_s_at	ACP1
201236_s_at	BTG2
217137_x_at	—
212476_at	CENTB2
218545_at	FLJ11088
208857_s_at	PCMT1
221931_s_at	SEH1L
215046_at	FLJ23861
220222_at	PRO1905
209737_at	AIP1
203949_at	MPO
219290_x_at	DAPP1
205116_at	LAMA2
222316_at	VDP
203574_at	NFIL3
207820_at	ADH1A
203751_x_at	JUND
202930_s_at	SUCLA2
215404_x_at	FGFR1
216266_s_at	ARFGEF1
212806_at	KIAA0367
219253_at	—
214605_x_at	GPR1
205403_at	IL1R2
222282_at	PAPD4
214129_at	PDE4DIP
209259_s_at	CSPG6
216900_s_at	CHRNA4
221943_x_at	RPL38
215386_at	AUTS2
201990_s_at	CREBL2
220145_at	FLJ21159
221173_at	USH1C
214900_at	ZKSCAN1
203290_at	HLA-DQA1

Affymetrix ID	(HUGO ID)
215382_x_at	TPSAB1
201631_s_at	IER3
212188_at	KCTD12
220428_at	CD207
215349_at	—
213928_s_at	HRB
221228_s_at	—
202069_s_at	IDH3A
208554_at	POU4F3
209504_s_at	PLEKHB1
212989_at	TMEM23
216197_at	ATF7IP
204748_at	PTGS2
205221_at	HGD
214705_at	INADL
213939_s_at	RIPX
203691_at	PI3
220532_s_at	LR8
209829_at	C6orf32
206515_at	CYP4F3
218541_s_at	C8orf4
210732_s_at	LGALS8
202643_s_at	TNFAIP3
218963_s_at	KRT23
213304_at	KIAA0423
202768_at	FOSB
205623_at	ALDH3A1
206488_s_at	CD36
204319_s_at	RGS10
217811_at	SELT
202746_at	ITM2A
221127_s_at	RIG
209821_at	C9orf26
220957_at	CTAGE1
215577_at	UBE2E1

Affymetrix ID	(HUGO ID)
214731_at	DKFZp547A023
210512_s_at	VEGF
205267_at	POU2AF1
216202_s_at	SPTLC2
220477_s_at	C20orf30
205863_at	S100A12
215780_s_at	SET /// LOC389168
218197_s_at	OXR1
203077_s_at	SMAD2
222339_x_at	—
200698_at	KDELR2
210540_s_at	B4GALT4
217725_x_at	PAI-RBP1
217082_at	—

[220] **Table 10** – Examples of biomarkers that may identify a diagnosis or prognosis of lung cancer.

Affymetrix ID	HUGO ID
207953_at	AD7C-NTP
215208_x_at	RPL35A
215604_x_at	UBE2D2
218155_x_at	FLJ10534
216858_x_at	—
208137_x_at	—
214715_x_at	ZNF160
217715_x_at	ZNF354A
220720_x_at	FLJ14346
215907_at	BACH2
217679_x_at	—
206169_x_at	RoXaN
208246_x_at	TK2
222104_x_at	GTF2H3
206056_x_at	SPN
217653_x_at	—
210679_x_at	—

Affymetrix ID	HUGO ID
207730_x_at	HDGF2
214594_x_at	ATP8B1

[221] **Table 11** – Examples of biomarkers that may identify a relationship between expression profiles of epithelial cells in the bronchus and upper airways in response to smoke.

AffyID	GeneName (HUGO ID)
202437_s_at	CYP1B1
206561_s_at	AKR1B10
202436_s_at	CYP1B1
205749_at	CYP1A1
202435_s_at	CYP1B1
201884_at	CEACAM5
205623_at	ALDH3A1
217626_at	—
209921_at	SLC7A11
209699_x_at	AKR1C2
201467_s_at	NQO1
201468_s_at	NQO1
202831_at	GPX2
214303_x_at	MUC5AC
211653_x_at	AKR1C2
214385_s_at	MUC5AC
216594_x_at	AKR1C1
205328_at	CLDN10
209160_at	AKR1C3
210519_s_at	NQO1
217678_at	SLC7A11
205221_at	HGD /// LOC642252
204151_x_at	AKR1C1
207469_s_at	PIR
206153_at	CYP4F11
205513_at	TCN1
209386_at	TM4SF1
209351_at	KRT14
204059_s_at	ME1

AffyID	GeneName (HUGO ID)
209213_at	CBR1
210505_at	ADH7
214404_x_at	SPDEF
204058_at	ME1
218002_s_at	CXCL14
205499_at	SRPX2
210065_s_at	UPK1B
204341_at	TRIM16 /// TRIM16L ///
	LOC653524
221841_s_at	KLF4
208864_s_at	TXN
208699_x_at	TKT
210397_at	DEFB1
204971_at	CSTA
211657_at	CEACAM6
201463_s_at	TALDO1
214164_x_at	CA12
203925_at	GCLM
201118_at	PGD
201266_at	TXNRD1
203757_s_at	CEACAM6
202923_s_at	GCLC
214858_at	GPC1
205009_at	TFF1
219928_s_at	CABYR
203963_at	CA12
210064_s_at	UPK1B
219956_at	GALNT6
208700_s_at	TKT
203824_at	TSPAN8
207126_x_at	UGT1A10 /// UGT1A8 /// UGT1A7 ///
	UGT1A6 /// UGT1A
213441_x_at	SPDEF
207430_s_at	MSMB

AffyID	GeneName (HUGO ID)
209369_at	ANXA3
217187_at	MUC5AC
209101_at	CTGF
212221_x_at	IDS
215867_x_at	CA12
214211_at	FTH1
217755_at	HN1
201431_s_at	DPYSL3
204875_s_at	GMDS
215125_s_at	UGT1A10 /// UGT1A8 /// UGT1A7 ///
	UGT1A6 /// UGT1A
63825_at	ABHD2
202922_at	GCLC
218313_s_at	GALNT7
210297_s_at	MSMB
209448_at	HTATIP2
204532_x_at	UGT1A10 /// UGT1A8 /// UGT1A7 ///
	UGT1A6 /// UGT1A
200872_at	S100A10
216351_x_at	DAZ1 /// DAZ3 /// DAZ2 /// DAZ4
212223_at	IDS
208680_at	PRDX1
206515_at	CYP4F3
208596_s_at	UGT1A10 /// UGT1A8 /// UGT1A7 ///
	UGT1A6 /// UGT1A
209173_at	AGR2
204351_at	S100P
202785_at	NDUFA7
204970_s_at	MAFG
222016_s_at	ZNF323
200615_s_at	AP2B1

AffyID	GeneName (HUGO ID)
206094_x_at	UGT1A6
209706_at	NKX3-1
217977_at	SEPX1
201487_at	CTSC
219508_at	GCNT3
204237_at	GULP1
213455_at	LOC283677
213624_at	SMPDL3A
206770_s_at	SLC35A3
217975_at	WBP5
201263_at	TARS
218696_at	EIF2AK3
212560_at	C11orf32
218885_s_at	GALNT12
212326_at	VPS13D
217955_at	BCL2L13
203126_at	IMPA2
214106_s_at	GMDS
209309_at	AZGP1
205112_at	PLCE1
215363_x_at	FOLH1
206302_s_at	NUDT4 /// NUDT4P1
200916_at	TAGLN2
205042_at	GNE
217979_at	TSPAN13
203397_s_at	GALNT3
209786_at	HMGN4
211733_x_at	SCP2
207222_at	PLA2G10
204235_s_at	GULP1
205726_at	DIAPH2
203911_at	RAP1GAP
200748_s_at	FTH1
212449_s_at	LYPLA1
213059_at	CREB3L1

AffyID	GeneName (HUGO ID)
201272_at	AKR1B1
208731_at	RAB2
205979_at	SCGB2A1
212805_at	KIAA0367
202804_at	ABCC1
218095_s_at	TPARL
205566_at	ABHD2
209114_at	TSPAN1
202481_at	DHRS3
202805_s_at	ABCC1
219117_s_at	FKBP11
213172_at	TTC9
202554_s_at	GSTM3
218677_at	S100A14
203306_s_at	SLC35A1
204076_at	ENTPD4
200654_at	P4HB
204500_s_at	AGTPBP1
208918_s_at	NADK
221485_at	B4GALT5
221511_x_at	CCPG1
200733_s_at	PTP4A1
217901_at	DSG2
202769_at	CCNG2
202119_s_at	CPNE3
200945_s_at	SEC31L1
200924_s_at	SLC3A2
208736_at	ARPC3
221556_at	CDC14B
221041_s_at	SLC17A5
215071_s_at	HIST1H2AC
209682_at	CBLB
209806_at	HIST1H2BK
204485_s_at	TOM1L1
201666_at	TIMP1

AffyID	GeneName (HUGO ID)
203192_at	ABCB6
202722_s_at	GFPT1
213135_at	TIAM1
203509_at	SORL1
214620_x_at	PAM
208919_s_at	NADK
212724_at	RND3
212160_at	XPOT
212812_at	SERINC5
200696_s_at	GSN
217845_x_at	HIGD1A
208612_at	PDIA3
219288_at	C3orf14
201923_at	PRDX4
211960_s_at	RAB7
64942_at	GPR153
201659_s_at	ARL1
202439_s_at	IDS
209249_s_at	GHITM
218723_s_at	RGC32
200087_s_at	TMED2
209694_at	PTS
202320_at	GTF3C1
201193_at	IDH1
212233_at	—
213891_s_at	—
203041_s_at	LAMP2
202666_s_at	ACTL6A
200863_s_at	RAB11A
203663_s_at	COX5A
211404_s_at	APLP2
201745_at	PTK9
217823_s_at	UBE2J1
202286_s_at	TACSTD2
212296_at	PSMD14

AffyID	GeneName (HUGO ID)
211048_s_at	PDIA4
214429_at	MTMR6
219429_at	FA2H
212181_s_at	NUDT4
222116_s_at	TBC1D16
221689_s_at	PIGP
209479_at	CCDC28A
218434_s_at	AACS
214665_s_at	CHP
202085_at	TJP2
217992_s_at	EFHD2
203162_s_at	KATNB1
205406_s_at	SPA17
203476_at	TPBG
201724_s_at	GALNT1
200599_s_at	HSP90B1
200929_at	TMED10
200642_at	SOD1
208946_s_at	BECN1
202562_s_at	C14orf1
201098_at	COPB2
221253_s_at	TXNDC5
201004_at	SSR4
203221_at	TLE1
201588_at	TXNL1
218684_at	LRRC8D
208799_at	PSMB5
201471_s_at	SQSTM1
204034_at	ETHE1
208689_s_at	RPN2
212665_at	TIPARP
200625_s_at	CAP1
213220_at	LOC92482
200709_at	FKBP1A
203279_at	EDEM1

AffyID	GeneName (HUGO ID)
200068_s_at	CANX
200620_at	TMEM59
200075_s_at	GUK1
209679_s_at	LOC57228
210715_s_at	SPINT2
209020_at	C20orf111
208091_s_at	ECOP
200048_s_at	JTB
218194_at	REXO2
209103_s_at	UFD1L
208718_at	DDX17
219241_x_at	SSH3
216210_x_at	TRIOBP
50277_at	GGA1
218023_s_at	FAM53C
32540_at	PPP3CC
43511_s_at	—
212001_at	SFRS14
208637_x_at	ACTN1
201997_s_at	SPEN
205073_at	CYP2J2
40837_at	TLE2
204447_at	ProSAPiP1
204604_at	PFTK1
210273_at	PCDH7
208614_s_at	FLNB
206510_at	SIX2
200675_at	CD81
219228_at	ZNF331
209426_s_at	AMACR
204000_at	GNB5
221742_at	CUGBP1
208883_at	EDD1
210166_at	TLR5
211026_s_at	MGLL

AffyID	GeneName (HUGO ID)
220446_s_at	CHST4
207636_at	SERPINI2
212226_s_at	PPAP2B
210347_s_at	BCL11A
218424_s_at	STEAP3
204287_at	SYNGR1
205489_at	CRYM
36129_at	RUTBC1
215418_at	PARVA
213029_at	NFIB
221016_s_at	TCF7L1
209737_at	MAGI2
220389_at	CCDC81
213622_at	COL9A2
204740_at	CNKSR1
212126_at	—
207760_s_at	NCOR2
205258_at	INHBB
213169_at	—
33760_at	PEX14
220968_s_at	TSPAN9
221792_at	RAB6B
205752_s_at	GSTM5
218974_at	FLJ10159
221748_s_at	TNS1
212185_x_at	MT2A
209500_x_at	TNFSF13 /// TNFSF12-TNFSF13
215445_x_at	1-Mar
220625_s_at	ELF5
32137_at	JAG2
219747_at	FLJ23191
201397_at	PHGDH
207913_at	CYP2F1
217853_at	TNS3

AffyID	GeneName (HUGO ID)
1598_g_at	GAS6
203799_at	CD302
203329_at	PTPRM
208712_at	CCND1
210314_x_at	TNFSF13 /// TNFSF12-TNFSF13
213217_at	ADCY2
200953_s_at	CCND2
204326_x_at	MT1X
213488_at	SNED1
213505_s_at	SFRS14
200982_s_at	ANXA6
211732_x_at	HNMT
202587_s_at	AK1
396_f_at	EPOR
200878_at	EPAS1
213228_at	PDE8B
215785_s_at	CYFIP2
213601_at	SLIT1
37953_s_at	ACCN2
205206_at	KAL1
212859_x_at	MT1E
217165_x_at	MT1F
204754_at	HLF
218225_at	SITPEC
209784_s_at	JAG2
211538_s_at	HSPA2
211456_x_at	LOC650610
204734_at	KRT15
201563_at	SORD
202746_at	ITM2A
218025_s_at	PECI
203914_x_at	HPGD
200884_at	CKB
204753_s_at	HLF

AffyID	GeneName (HUGO ID)
207718_x_at	CYP2A6 /// CYP2A7 /// CYP2A7P1 /// CYP2A13
218820_at	C14orf132
204745_x_at	MT1G
204379_s_at	FGFR3
207808_s_at	PROS1
207547_s_at	FAM107A
208581_x_at	MT1X
205384_at	FXYD1
213629_x_at	MT1F
823_at	CX3CL1
203687_at	CX3CL1
211295_x_at	CYP2A6
204755_x_at	HLF
209897_s_at	SLIT2
40093_at	BCAM
211726_s_at	FMO2
206461_x_at	MT1H
219250_s_at	FLRT3
210524_x_at	—
220798_x_at	PRG2
219410_at	TMEM45A
205680_at	MMP10
217767_at	C3 /// LOC653879
220562_at	CYP2W1
210445_at	FABP6
205725_at	SCGB1A1
213432_at	MUC5B /// LOC649768
209074_s_at	FAM107A
216346_at	SEC14L3

[222] **Table 12** – Examples of biomarkers that may be differentially expressed in bronchial epithelial genes among genes highly changed in a nasal epithelium in response to smoking.

AffxID	Hugo ID
203369_x_at	—
218434_s_at	AACS
205566_at	ABHD2
217687_at	ADCY2
210505_at	ADH7
205623_at	ALDH3A1
200615_s_at	AP2B1
214875_x_at	APLP2
212724_at	ARHE
201659_s_at	ARL1
208736_at	ARPC3
213624_at	ASM3A
209309_at	AZGP1
217188_s_at	C14orf1
200620_at	C1orf8
200068_s_at	CANX
213798_s_at	CAP1
200951_s_at	CCND2
202769_at	CCNG2
201884_at	CEACAM5
203757_s_at	CEACAM6
214665_s_at	CHP
205328_at	CLDN10
203663_s_at	COX5A
202119_s_at	CPNE3
221156_x_at	CPR8
201487_at	CTSC
205749_at	CYP1A1
207913_at	CYP2F1
206153_at	CYP4F11
206514_s_at	CYP4F3
216351_x_at	DAZ4
203799_at	DCL-1

AffxID	Hugo ID
212665_at	DKFZP434J214
201430_s_at	DPYSL3
211048_s_at	ERP70
219118_at	FKBP11
214119_s_at	FKBP1A
208918_s_at	FLJ13052
217487_x_at	FOLH1
200748_s_at	FTH1
201723_s_at	GALNT1
218885_s_at	GALNT12
203397_s_at	GALNT3
218313_s_at	GALNT7
203925_at	GCLM
219508_at	GCNT3
202722_s_at	GFPT1
204875_s_at	GMDS
205042_at	GNE
208612_at	GRP58
214040_s_at	GSN
214307_at	HGD
209806_at	HIST1H2BK
202579_x_at	HMGN4
207180_s_at	HTATIP2
206342_x_at	IDS
203126_at	IMPA2
210927_x_at	JTB
203163_at	KATNB1
204017_at	KDELR3
213174_at	KIAA0227
212806_at	KIAA0367
210616_s_at	KIAA0905
221841_s_at	KLF4
203041_s_at	LAMP2
213455_at	LOC92689
218684_at	LRRC5

AffxID	Hugo ID
204059_s_at	ME1
207430_s_at	MSMB
210472_at	MT1G
213432_at	MUC5B
211498_s_at	NKX3-1
201467_s_at	NQO1
206303_s_at	NUDT4
213498_at	OASIS
200656_s_at	P4HB
213441_x_at	PDEF
207469_s_at	PIR
207222_at	PLA2G10
209697_at	PPP3CC
201923_at	PRDX4
200863_s_at	RAB11A
208734_x_at	RAB2
203911_at	RAP1GA1
218723_s_at	RGC32
200087_s_at	RNP24
200872_at	S100A10
205979_at	SCGB2A1
202481_at	SDR1
217977_at	SEPX1
221041_s_at	SLC17A5
203306_s_at	SLC35A1
207528_s_at	SLC7A11
202287_s_at	TACSTD2
210978_s_at	TAGLN2
205513_at	TCN1
201666_at	TIMP1
208699_x_at	TKT
217979_at	TM4SF13
203824_at	TM4SF3
200929_at	TMP21
221253_s_at	TXNDC5

AffxID	Hugo ID
217825_s_at	UBE2J1
215125_s_at	UGT1A10
210064_s_at	UPK1B
202437_s_at	CYP1B1

[223] **Table 13** – Examples of biomarkers

AFFYID	Gene Name (HUGO ID)
213693_s_at	MUC1
211695_x_at	MUC1
207847_s_at	MUC1
208405_s_at	CD164
220196_at	MUC16
217109_at	MUC4
217110_s_at	MUC4
204895_x_at	MUC4
214385_s_at	MUC5AC
1494_f_at	CYP2A6
210272_at	CYP2B7P1
206754_s_at	CYP2B7P1
210096_at	CYP4B1
208928_at	POR
207913_at	CYP2F1
220636_at	DNAI2
201999_s_at	DYNLT1
205186_at	DNALI1
220125_at	DNAI1
210345_s_at	DNAH9
214222_at	DNAH7
211684_s_at	DYNC1I2
211928_at	DYNC1H1
200703_at	DYNLL1
217918_at	DYNLRB1
217917_s_at	DYNLRB1
209009_at	ESD
204418_x_at	GSTM2

AFFYID	Gene Name (HUGO ID)
215333_x_at	GSTM1
217751_at	GSTK1
203924_at	GSTA1
201106_at	GPX4
200736_s_at	GPX1
204168_at	MGST2
200824_at	GSTP1
211630_s_at	GSS
201470_at	GSTO1
201650_at	KRT19
209016_s_at	KRT7
209008_x_at	KRT8
201596_x_at	KRT18
210633_x_at	KRT10
207023_x_at	KRT10
212236_x_at	KRT17
201820_at	KRT5
204734_at	KRT15
203151_at	MAP1A
200713_s_at	MAPRE1
204398_s_at	EML2
40016_g_at	MAST4
208634_s_at	MACF1
205623_at	ALDH3A1
212224_at	ALDH1A1
205640_at	ALDH3B1
211004_s_at	ALDH3B1
202054_s_at	ALDH3A2
205208_at	ALDH1L1
201612_at	ALDH9A1
201425_at	ALDH2
201090_x_at	K-ALPHA-1
202154_x_at	TUBB3
202477_s_at	TUBGCP2
203667_at	TBCA

AFFYID	Gene Name (HUGO ID)
204141_at	TUBB2A
207490_at	TUBA4
208977_x_at	TUBB2C
209118_s_at	TUBA3
209251_x_at	TUBA6
211058_x_at	K-ALPHA-1
211072_x_at	K-ALPHA-1
211714_x_at	TUBB
211750_x_at	TUBA6
212242_at	TUBA1
212320_at	TUBB
212639_x_at	K-ALPHA-1
213266_at	76P
213476_x_at	TUBB3
213646_x_at	K-ALPHA-1
213726_x_at	TUBB2C

[224] **Table 14** – shows sample distribution.

Representative histopathology types	Training set		Test set
	# samples	# patients	# patients
Usual Interstitial pneumonia (UIP)	136	34	11
Difficult UIP	40	11	7
Favor UIP	22	5	4
UIP (lower lobe) + Nonspecific interstitial pneumonia (NSIP) (upper lobe)	5	1	
Difficult UIP (lower lobe) + NSIP (upper lobe)	4	1	
UIP (lower lobe) + Pulmonary hypertension (upper lobe)	5	1	
Favor HP (lower lobe) + Difficult UIP (upper lobe)			1
UIP Total	212 (60%)	53 (59%)	23 (47%)
Respiratory bronchiolitis (RB); Smoking-related interstitial fibrosis	26	7	7
Hypersensitivity pneumonitis; Favor HP	19	4	4
Sarcoidosis	17	5	4
NSIP; Cellular NSIP; Favor NSIP	18	5	3
Diffuse alveolar damage; DAD with hemosiderosis	2	1	2
Amyloid or light chain deposition			1

Representative histopathology types	Training set		Test set
	# samples	# patients	# patients
Bronchiolitis	12	3	1
Eosinophilic pneumonia (EP)	5	1	1
Exogenous lipid pneumonia			1
Organizing alveolar hemorrhage			1
Organizing pneumonia (OP)	29	7	
Pneumocystis pneumonia (PP)	4	1	
Emphysema	10	3	
Non-UIP Total	142 (40%)	37 (41%)	26 (53%)
Total	354	90	49

[225] **Table 15**

	Up-regulated genes	Down-regulated genes	Total number of differentially expressed genes	# genes overlapping with those from all non-UIP samples
All non-UIP (N = 147)	55	96	151	151 (100%)
Bronchiolitis (N = 10)	41	34	75	6 (8%)
HP (N = 13)	32	53	85	14 (16%)
NSIP (N =12)	37	49	86	13 (15%)
OP (N = 23)	1	15	16	31 (52%)
RB (N = 16)	549	152	701	64 (9%)
Sarcoidosis (N = 11)	448	726	1174	93 (8%)

[226] **Table 15** shows the number of significantly expressed genes (p-adjusted < 0.05, fold change > 2) between each non-UIP subtype and UIP samples (n= 212). The number of differentially expressed genes overlapping with those between UIP and non-UIP samples is summarized in the third column.

[227] **Table 16**

Classifier	Between-run	Intra-run (Residual)	Inter-run (Total)
Ensemble	0.28 (4.0%)	0.37 (5.3%)	0.46 (6.5%)
Penalized logistic regression	0.10 (2.6%)	0.19 (4.9%)	0.22 (5.6%)

[228] **Table 16** shows an estimation of variability of scores from the two classifiers using linear mixed effect models. The percentage (%) may be the ratio of estimated variability to the range between %5 and 95% quantiles in classification scores.

[229] Classifier described herein may diagnosis a condition, such as IPF or lung cancer, while avoiding an invasive procedure. One disadvantage of an unsupervised clustering analysis may be an inability to (a) distinguish a malignant tissue from a benign tissue, (b) distinguish a UIP pattern from a non-UIP pattern, (c) distinguish a sample having a particular expression pattern from another sample that may not have the particular expression pattern or (d) any combination thereof because of (i) a small sample size, (ii) disease heterogeneity (for example heterogeneity in a non-UIP pattern disease subtype), (iii) pooling and batch effects of different samples, or (iv) any combination thereof. A trained machine learning algorithm may overcome these disadvantages. Methods described herein may eliminate the need for an invasive procedure and provide a non-invasive prognostic tool, diagnostic tool, or a combination thereof with high clinical accuracy despite the limitation of a small sample size, disease heterogeneity, or pooling and batch effects of different samples. In some cases, RNA-seq data may be input into the machine learning algorithm. Heterogeneity may occur within samples obtained from the same subject. For example, histopathology features may not be uniform across a tissue (such as a lung tissue) and gene expression profiles may vary depending on a location from which a sample is obtained. Heterogeneity may occur within a disease. For example, a presence of a non-UIP pattern may comprise more than one disease subtype such as a collection of heterogeneous diseases.

[230] In some cases, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more samples may be collected from a subject and separately analyzed. In some cases, 2 samples may be collected from a subject and separately analyzed. In some cases, 3 samples may be collected from a subject and separately analyzed. In some cases, 4 samples may be collected from a subject and separately analyzed. In some cases, 5 samples may be collected from a subject and separately analyzed. In some cases, 6 samples may be collected from a subject and separately analyzed. In some cases, 7 samples may be collected from a subject and separately analyzed. In some cases, 8 samples may be collected from a subject and separately analyzed. In some cases, 9 samples may be collected from a subject and separately analyzed. In some cases, 10 samples may be collected from a subject and separately analyzed. In some cases, from 1 to 10 samples may be collected form a subject and separately analyzed. In some cases, from 1 to 5 samples may be collected form a subject and separately analyzed. In some cases, from 1 to 20 samples may be collected form a subject and separately analyzed.

[231] A classifier, such as a locked classifier, may yield a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof in an independent test set as compared to a validation set (that may be used to validate the classifier). A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over at least about 5 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over at least about 10 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over at least about 50 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over at least about 100 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over at least about 500 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over at least about 1000 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over from about 1 to about 10 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over from about 1 to about 100 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over from about 1 to about 500 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over from about 1 to about 1000 independent test samples. A classifier may maintain a substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over from about 1 to about 5000 independent test samples. Independent test samples may be obtained from a subject.

[232] To maintain substantially similar accuracy, NPV, PPV, sensitivity, specificity, or any combination thereof over a plurality of independent test samples, batch effects may be removed. Removal of biomarkers yielding high variability across samples may be removed from selection features of a classifier or from downstream analysis. Biomarkers highly sensitive to batch effects may be removed from downstream analysis or removed from feature selection. A classifier may not substantially vary performance (such as accuracy, NPV, PPV, sensitivity, or specificity) over a plurality of independent sample runs.

[233] The methods may include identifying subjects having heterogeneity within a plurality of samples obtained from a subject. For example, the methods may include identifying a subject

having a sample assigned a non-UIP pattern and another sample from the same subject assigned a UIP-pattern. Heterogeneity in samples from the same subject may be observed in histopathologic diagnosis, gene expression, or a combination thereof. For example, UIP and non-UIP pattern diseases may be heterogeneous. Biomarkers that may distinguish or diagnose a non-UIP pattern disease may not be applicable to distinguishing or diagnosing another non-UIP pattern disease. A new set of biomarkers may be developed for each disease, disease sub-type, UIP pattern, or non-UIP pattern disease. Biomarkers that may distinguish or diagnose a presence of a non-UIP pattern disease may be applicable to distinguishing or diagnosis another non-UIP pattern disease.

[234] Samples in the training set may comprise a plurality of conditions (such as diseases or disease subtypes). Samples in an independent test set may comprise a plurality of conditions (such as disease or disease subtypes). Samples in an independent test set may comprise a least one disease or disease subtype that is different from the samples in the training set. Samples in the training set may comprise a least one disease or disease subtype that is different from the samples in the independent test set. Samples in the independent test set may comprise at least two additional diseases or disease subtypes than the samples in the training set. For example, the at least two additional diseases or disease subtypes may be amyloid or light chain deposition, exogenous lipid pneumonia, and organizing alveolar hemorrhage, or any combination thereof. One or more new diseases or disease subtypes may emerge from an independent test set that may not be included in a training set. Samples in the training set may comprise at least two additional diseases or disease subtype than the samples in the independent test set.

[235] The methods may include evaluating classifier performance with *in silico* samples. *In silico* samples may simulate mixing of *in vitro* samples in an independent test set, particularly when a sample size may be small. *In silico* samples may also aid in determining decision boundaries of a classifier, optimal number of samples required to achieve optimal classifier performance, or a combination thereof. The methods may be applicable to pooled samples, for example, when a small sample size may be present.

[236] A small sample size may be samples obtained from less than 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10, or 5 different subjects. A small sample size may be a plurality of samples obtained from about 50 to about 100 different subjects. A small sample size may be a plurality of samples obtained from about 1 to about 50 different subjects. A small sample size may be a plurality of samples obtained from about 1 to about 100 different subjects. A small sample size may be a plurality of samples obtained from about 1 to about 200 different subjects. A small sample size may be a plurality of samples obtained from about 1 to about 10 different subjects. A

small sample size may be a plurality of samples obtained from about 1 to about 5 different subjects. A small sample size may be a plurality of samples obtained from about 1 to about 2 different subjects. A small sample size may be a plurality of samples obtained from about 1 to about 15 different subjects. A small sample size may be a plurality of samples obtained from about 1 to about 8 different subjects. A small sample size may be a plurality of samples obtained from about 5 to about 50 different subjects. A small sample size may be a plurality of samples obtained from about 5 to about 100 different subjects. A small sample size may comprise a small sample size of independent test samples or training samples. A small sample size may be indicative of a limited access to subjects – such as subjects having a rare subtype of a disease. A small sample size may be expanded by including replicates of a single sample, such as 1, 2, 3, 4, 5, or more replicates of a single sample. A small sample size may be expanded by including from about 1 to about 2 replicates of a single sample. A small sample size may be expanded by including from about 1 to about 3 replicates of a single sample. A small sample size may be expanded by including from about 1 to about 4 replicates of a single sample. A small sample size may be expanded by including from about 1 to about 5 replicates of a single sample. A small sample size may be expanded by including from about 1 to about 10 replicates of a single sample. A small sample size may be expanded by including from about 1 to about 15 replicates of a single sample. A small sample size may be expanded by including from about 1 to about 20 replicates of a single sample.

Examples

[237] Example 1

[238] Background - To accurately diagnose Idiopathic Pulmonary Fibrosis (IPF) while avoiding invasive procedures, a classifier may be developed using RNA-seq data that identifies histopathologic pattern of usual interstitial pneumonia (UIP), a hallmark characteristic of IPF. This approach may challenge encountered in the development of a classifier, including sample size, heterogeneity, and batch effects, while applying machine learning to genomic data in clinical settings.

[239] Methods - Exome-enriched RNA sequencing may be performed on 354 individual transbronchial biopsies (TBBs) from 90 patients to use in the training algorithms. Pooled TBB samples composed of 3-5 individual TBBs from 49 additional patients as an independent validation may be sequenced. Unsupervised clustering and differentially expressed gene analysis may be performed to characterize disease heterogeneity and to select genomic features that may distinguish between UIP from non-UIP. To overcome the small sample size and potential disease heterogeneity, machine learning algorithms may be trained using multiple samples per patient.

Simulated *in silico* mixed samples to mimic pooled samples of the test set may be evaluated. The machine learning algorithm may be validated on the test set, and its robustness may be further evaluated using technical replicates across multiple batches.

[240] Results – Unsupervised clustering and differential gene expression analyses may show high heterogeneity within patients, particularly among the non-UIP group. The developed classifiers, using penalized logistic regression model and ensemble models may classify histopathologic UIP with a receiver-operator characteristic area under the curve (AUC) of about 0.9 in cross-validation, when multiple samples may be tested per patient. A decision boundary may be defined to optimize specificity at ≥ 85% using TBB pools that may be simulated *in silico* from the individual training set samples. The penalized logistic regression model may show greater reproducibility across technical replicates, and may be chosen as the final model. The final model may show sensitivity of 70% and specificity of 88% in the independent test set, using samples that may be pooled in the laboratory prior to molecular testing.

[241] Conclusions - Overcoming challenges of sample size, disease and sampling heterogeneity, pooling and batch effects, a method as described here may provide a highly accurate and robust classifier for the identification of UIP, leveraging machine learning and RNA-seq.

[242] Introduction - Interstitial lung disease (ILD) consists of a variety of diseases affecting the pulmonary interstitium with similar clinical presentation; idiopathic pulmonary fibrosis (IPF) may be the most common ILD with the worst prognosis. The cause of IPF remains largely unknown making accurate and timely diagnoses challenging. An accurate diagnosis for IPF often entails multidisciplinary evaluation of clinical, radiologic and histopathologic features [Flaherty *et al*, 2004 and Travis *et al*, 2013, which are entirely incorporated herein by reference], and patients frequently suffer an uncertain and lengthy process. In particular, determining the presence of usual interstitial pneumonia (UIP), a hallmark characteristic of IPF, often requires histopathology via invasive surgery that may not be an option for sick or elderly patients. Furthermore, the quality of the histopathology reading may be highly variable across clinics [Flaherty *et al*, 2007, which is entirely incorporated herein by reference]. Thus, a consistent, accurate, non-invasive diagnosis tool to distinguish UIP from non-UIP without the need for surgery may be critical to reduce the suffering of patients and to enable physicians to reach confident clinical diagnoses faster and make better treatment decisions.

[243] To build this new diagnostic tool, exome-enriched RNA sequencing data may be utilized from transbronchial biopsy samples (TBBs) collected via bronchoscopy, a less invasive procedure compared to surgery. Several studies have revealed that genomic information in

transcriptomic data may be indicative of phenotypic variation such as cancer and other chronic disease [Tuch *et al* 2010, Twine *et al* 2011, which are entirely incorporated herein by reference]; and that complex traits may be driven by large number of genes spread across the whole genome including ones with no apparent relevance to disease [Boyle *et al*, 2017, which is entirely incorporated herein by reference]. More importantly, the feasibility of identifying UIP using transcriptomic data has been established [Pankratz *et al*, 2017, which is entirely incorporated herein by reference]. The methods and systems as described herein provide analytical solutions to such problems.

[244] Machine learning methods have been extensively applied to solve biomedical problems, and have deepened our understanding of diseases such as breast cancer [Sorlie *et al.*, which is entirely incorporated herein by reference], and glioblastoma [Brennan *et al.*, which is entirely incorporated herein by reference], by allowing researchers to construct biological pathways, identify clinically relevant diseases and better predict disease risk. However, recent advances in machine learning may be often designed for large data sets such as medical imaging data and social media data. Yet, clinical studies, including this one, often have limited sample sizes due to the challenges in accruing patients. The issue may be more pronounced in the present example since many patients may be too sick to allow biopsy samples; among the ones collected, a substantial proportion yielded non-diagnostic results, rendering them unsuitable for supervised learning. In addition, the non-UIP category may not be one disease, but a collection of heterogeneous diseases. This, coupled with the small sample size, may indicate that small numbers of samples may be available in each non-UIP disease category, making the classification even more challenging. Another unique feature of this example may be heterogeneity within a patient. Histopathology features may not be uniform across the entire lung and genomic signatures vary depending on the location of the biopsy sample [Kim *et al*, which is entirely incorporated herein by reference]. To better understand such heterogeneity, multiple samples (up to 5) per patient may be collected and sequenced separately for patients in the training set. This data set may represent both a challenge and an opportunity, which may be described in details in later sections.

[245] Because a classifier may serve as the foundation for a diagnostic product, there may be two additional requirements. First, for cost-effectiveness, only one sequencing run per patient may be commercially viable and the independent test set may need to reflect this reality. Analytically bridging individual samples in the training set and pooled samples in the test set may become a necessity. Secondly, it may be important that a final locked classifier not only performs well on the independent test set, but may also maintain performance for all incoming

future samples. Therefore, developing a classifier that may be highly robust to foreseeable batch effects in the future may become critically important.

[246] In the following sections, some of the challenges with quantitative analysis may be illustrated, practical solutions to overcome those challenges may be described, evidence of improvement may be shown, and limitations of these approaches may be discussed.

[247] Materials and Methods

[248] Study design

[249] Patients under medical evaluation for ILD that may be 18 years of age or older and may be undergoing a planned, clinically indicated lung biopsy procedure to obtain a histopathology diagnosis may be eligible for enrollment in a multi-center sample collection study (BRonchial sAmple collection for a noVel gEnomic test; BRAVE) [Pankratz *et al*]. Patients for whom a bronchoscopy procedure may not be indicated, not recommended or difficult may not be eligible for participation in the study. Patients may be groups based on the type of biopsy being performed for pathology: BRAVE-1 patients may undergo surgical lung biopsy (SLB), BRAVE-2 patients may undergo TBB for pathology, and BRAVE-3 patients may undergo cryobiopsy. The study may be approved by institutional review boards at each institution and all patients may be provided informed consent prior to their participation.

[250] During study accrual, 201 BRAVE patients may be prospectively divided into a group of 113 considered for use in training (enrolled December 2012 to July 2015) and 88 may be used in validation (enrolled August 2014 and May 2016). The training group may ultimately yield 90 patients with usable RNA sequence data and reference standard pathology truth labels that may be used to train and cross-validate the models. The validation group may yield 49 patients that met prospective test set inclusion criteria related to sample handling, sample adequacy, and the determination of reference standard truth labels. All clinical information related to the test set, to include reference labels and associated pathology may be blinded to the algorithm development team until after the classifier parameters may be finalized, locked, and the test set may be prospectively scored.

[251] Total RNA may be extracted and input into TruSeq RNA Access Library Prep procedure (Illumina, San Diego, CA) to enrich for expressed exonic sequences, and sequenced on the NextSeq 500 instruments with a NextSeq v2 chemistry 150 cycle kit (Illumina, San Diego, CA). For the training set, RNA sequencing data may be generated separately for each of 354 individual TBB samples from 90 patients and eight additional TBB samples may be chosen for quality control and sequenced repeatedly over eight different batches, which may be referred to as sentinels. For the independent test set, total RNA extracted from available TBB samples for

each patient may be mixed by equal mass and sequenced using the same procedure as that for the training set but at a later time on a different batch. Therefore, for the training set, there may be up to 5 sequencing data per patient, one corresponding to an individual TBB sample; in contrast, for the test set, there may be 1 sequencing data per patient, since all TBB samples and the corresponding RNA material derived from the same test patient may be pooled together prior to sequencing which may be representative of how a commercial samples may be run.

[252] Pathology reviews and label assignment

[253] Histopathology diagnoses may be determined centrally by a consensus of three expert pathologists using biopsies and slides collected specifically for pathology, following processes described [Pankratz *et al* and Kim *et al*]. The central pathology diagnoses may be determined separately for each lung lobe samples for pathology. A reference standard label may then be determined for each patient from the lobe-level diagnoses according to the following rules. If any lob may be diagnosed as any UIP subtype, e.g., classic UIP (all features of UIP may be present), difficult UIP (less than all features of classic UIP may be well represented), favor UIP (fibrosing interstitial process with UIP leading the differential), or any combination of these, then 'UIP' may be assigned as the reference label for that patient. If any lung lobe may be diagnosed with a 'non-UIP' pathology condition [Pankratz *et al*] and any other lobe may be non-diagnostic or may be diagnosed with unclassifiable fibrosis, then 'non-UIP' may be assigned as the patient level reference label. When all lobes may be diagnostic for unclassifiable fibrosis (e.g., chronic interstitial fibrosis, not otherwise classified or 'CIF, NOC') or may be non-diagnostic, then no reference label may be assigned and the patient may be excluded. This patient-level reference label process may be identical between training and testing sets, however individual TBB samples in the training set may be directly inherited sample level reference labels from the lung lobe of origin, in addition to the reference label determined at the patient level.

[254] Molecular testing, sequencing pipeline, and data QC

[255] Up to five TBB samples may be sampled from each patient by bronchoscopy. Typically, two upper lobe and three lower lobe samples may be collected during the clinically indicated diagnostic procedure. TBB samples for molecular testing may be placed into a nucleic acid preservative and may be stored at 4°C for up to 18 days, prior to and during shipment to the development laboratory, followed by frozen storage. Total RNA may be extracted, may be quantitated, may be pooled by patient where appropriate, and 15ng input into the TruSeq RNA Access Library Prep procedure (Illumina, San Diego, CA), which may enrich for the coding transcriptome using multiple rounds of amplification and hybridization to probes specific to exonic sequences. Libraries which met in-process yield criteria may be sequenced on NextSeq

500 instruments (2 x 75bp paired-end reads) using the High Output kit (Illumina, San Diego, CA). Raw sequencing (FASTQ) files may be aligned to the Human Reference assembly 37 (Genome Reference Consortium) using the STAR RNA-seq aligner software [Dobin *et al*, which is entirely incorporated herein by reference]. Raw read counts for 63,677 Ensembl annotated gene-level features may be summarized using HTSeq [Anders *et al*, 2015, which is entirely incorporated herein by reference]. Data quality metrics may be generated using RNA-SeQC [DeLuca *et al*, which is entirely incorporated herein by reference]. Library sequence data which met minimum criteria for total reads, mapped unique reads, mean per-base coverage, base duplication rate, the percentage of bases aligned to coding regions, the base mismatch rate, and uniformity of coverage within genes may be accepted for use in downstream analysis.

[256] Normalization

[257] Sequence data may be filtered to exclude any features that may not be targeted for enrichment by the library assay, resulting in 26,268 genes. For the training set, expression count data for 26,268 Ensembl genes may be normalized by *sizefactor* estimated with the median-of-ratio method and transformed to approximately log2 by variance-stabilizing transformation (VST) using a parametric method, which may be a closed-form expression (DESeq2 package) [Love *et al*, 2014, which is entirely incorporated herein by reference]. The vector of geometric approaches and VST from the training set may be frozen and separately reapplied to the independent test set for the normalization to mimic future clinical patterns.

[258] For algorithm training and development, RNA sequence data may be generated separately for each of 354 individual TBB samples from 90 patients. Eight additional TBB samples ('sentinels') may be replicated in each of eight processing runs, from total RNA through to sequence data, to monitor for batch effects. For validation, total RNA may be extracted from a minimum of three and a maximum of five TBBs per patient may be mixed by equal mass within each patient prior to library preparation and sequencing. Patients in the training set thus may contribute up to five sequence libraries to training, whereas patients in the test set may be represented by a single sequenced library, analogous to the planned testing of clinical samples.

[259] Differential expression analysis

[260] Whether differentially expressed genes found using a standard pipeline [Anders *et al.*, 2013, which is entirely incorporated herein by reference], may be used directly to classify UIP from non-UIP samples may be explored. Differentially expressed genes may be identified using DESeq2, a Bioconductor R package [Love *et al.* 2014]. Raw gene-level expression counts of the training set may be used to perform the differential analysis. A cutoff of p-value < 0.05 after multiple-testing adjustment and fold change > 2 may be used to select differentially expressed

genes. Within the training set, pairwise differential analyses may be performed between all non-UIP and UIP samples, and between UIP samples and each non-UIP disease with more than 10 samples available, including bronchiolitis (N = 10), hypersensitivity pneumonitis (HP) (N = 13), nonspecific interstitial pneumonia (NSIP) (N =12), organizing pneumonia (OP) (N = 23), respiratory bronchiolitis (RB) (N = 16), and sarcoidosis (N = 11). Principal component analysis (PCA) plots of all the training samples may be generated using differentially expressed genes identified above.

[261] Gene expression correlation heatmap

[262] The correlations r^2 values of samples in 6 representative patients may be computed using their VST gene expression, and a heatmap of the correlation matrix with patient order preserved may be plotted to visualize intra- and inter-patient heterogeneity in gene expression. The 6 patients may be selected to represent the full spectrum of with-in patient heterogeneity including two non-UIP and two UIP patients with the same or similar labels between upper and lower lobes, as well as one UIP and one non-UIP patients each having different labels at upper versus lower lobes. The heatmap may be generated using the heatmap.2 function of the gplots R package.

[263] Classifier development

[264] The development and evaluation of a classifier may be summarized in **FIG. 28**. A goal may be to build a robust binary classifier may be built on TBB samples to provide accurate and reproducible UIP/non-UIP predictions, and to meet the clinical need to reduce invasive procedures for ILD patients. A high specificity test (specificity > 85%) may be designed to ensure a high positive predictive value. When the test may predict UIP, that result may be associated with high confidence.

[265] Feature filtering for classifier development

[266] First, features that may not be biologically meaningful or less informative may be removed due to low expression level without variation among samples may be filtered. Genes annotated in Ensembl as pseudogenes, ribosomal RNAs, individual exons in T-cell receptor or Immunoglobulin genes and non-informative and low expressed genes may be excluded with raw counts expression level < 5 for the entire training set or expressed with count > 0 for less than 5% of samples in the training set.

[267] Genes with highly variable expression in the same sample that maybe processed in multiple batched may be excluded, as this may suggest sensitivity to technical, rather than biological factors. To identify such genes, a linear mixed effect model may be fitted on the sentinel TBB samples processed across multiple assay plates. This model may be fitted for each

gene separately where g_{ij} may be the gene expression of sample j and batch i, μ may be the average gene expression

$$g_{ij} = \mu + \beta sample_{ij} + batch_i + e_{ij} \quad (1)$$

[268] for the entire set, $sample_{ij}$ may be a fixed effect of biologically different samples, and $batch_i$ may be the batch-specific random effect. The total variation may be used to identify highly variable genes; the top 5% of genes by this measure may be excluded (**FIG. 39-44**). As a result, 17,601 Ensembl genes may remain as candidates for the downstream analysis.

[269] *In silico* mixing within patient

[270] The classifiers may be trained and optimized on individual TBB samples to maximize sampling diversity and the information content available during the feature selection and weighting process. Multiple TBB samples may be pooled at the post-extraction stage, as RNA, and the pooled RNA may be processed in a single reaction through library prep, sequencing and classification [Pankratz *et al*]. Whether a classifier developed on individual samples may achieve high performance on pooled samples may be evaluated. A method may be developed to simulate pooled samples "*in silico*" from individual sample data. First, raw read counts may be normalized by *sizefactor* computed using geometric approaches across genes within the entire training set. The normalized count C_{ij} for sample $i = 1, \ldots, n$ and gene $j = 1, \ldots, m$ may be computed by

$$C_{ij} = K_{ij} / S_j$$

where $s_j = \text{median}_i \frac{k_{ij}}{(\prod_{q=1}^{m} k_{iq})^{1/m}}$ and K_{ij} may be the raw count for sample i and gene j. Then, for each training patient $p = 1, \ldots, P$, *in silico* mixed count K^p_{ij} may be defined by

$$K^p_{ij} = \frac{1}{n_p} \sum_{i \in I(p)} C_{ij}$$

where $I(p)$ may be the index set of individual sample i that may belong to patient p. The frozen variance stabilizing transformation (VST) in the training set may applied to K^p_{ij}.

[271] Training classifiers

[272] As the test may be intended to recognize and call a reference label defined by pathology, the reference label may be defined to be the response variable in classifier training [Tuch *et al*], and the exome-enriched, filtered and normalized RNA sequence data as the predictive features. Multiple classification models may be evaluated, to include random forest, support vector machine (SVM), gradient boosting, neural network and penalized logistic regression [Dobson *et al*, which is entirely incorporated herein by reference]. Each classifier may be evaluated based on 5-fold cross-validation and leave-one-patient-out cross-validation (LOPO CV) [Friedman *et al*,

which is entirely incorporated herein by reference]. Ensemble models may also be examined by combining individual machine learning methods via weighted average of scores of individual models.

[273] To minimize overfitting, during training and evaluation, each cross-validation fold may be stratified such that all data from a single patient may be either included or held out from a given fold. Hyper-parameter tuning may be performed within each cross-validation split in a nested-cross validation manner [Krstajic D *et al*, 2014, which is entirely incorporated herein by reference]. A random search and one standard error rule [Hastie, Tibshirani and Friedman, 2009, which is entirely incorporated herein by reference] may be chosen for selection of best parameters from inner CV to further minimize potential overfitting. Ultimately, hyper-parameter tuning may be repeated on the full training set to define the parameters for in the final locked classifier. The pipeline of training various machine learning algorithms may be automated and performed using R packages: DESeq2, hclust, cv.glmnet, caret and caretEnsemble.

[274] Best practices for a fully independent validation may require that all classifier parameters, including the test decision boundary may be prospectively defined. This therefore may be done using only the training set data. Since the test set may classify pooled TBBs at the patient-level, the proposed *in silico* mixing model may be used to simulate the distribution of patient-level scores within the training set. Within-patient mixtures may be simulated 100 times at each LOPO CV-fold, with gene-level technical variability added to the VST expressions. The gene-level technical variability may be estimated using the mixed effect model. Equation (1) on the TBB samples may be replicated across multiple processing batches. The final decision boundary may be chosen to optimize specificity (> 0.85) without severely compromising sensitivity (≥ 0.65). Performance may be estimated using patient-level LOPO CV scores from replicated *in silico* mixing simulation. To be conservative for specificity, a criterion for averaged specificity of greater than 90% to choose a final decision boundary. For decision boundaries with similar estimated performances in simulation, the decision boundary with highest specificity may be chosen, **FIG. 46A-B**.

[275] Evaluate batch effect and monitoring scheme for future samples

[276] To ensure the extensibility of classification performance to a future, unseen clinical patient population, it may be crucial to ensure there may be no severe technical factor, referred as batch effects that may cause globe shifts, rotations, compressions, or expansions of score distributions over time. To quantify batch effects in existing data and to evaluate the robustness of the candidate classifiers to observable batch effects, the scored nine different TBB samples, triplicated within each batch and processed across three different processing batches, and used

linear mixed effect model to evaluate variability of scores for each classifier. The model that may be more robust against batch effect, as indicated by low score variability in linear mixed models, may be chosen as the final model for independent validation. To monitor batch effects, UIP and non-UIP control samples may be processed in each new processing batch. To capture a potential batch effect, scores of these replicated control samples may be compared and whether estimated score variability remains smaller than the pre-specified threshold, σ_{sv}, may be determined in training using the *in silico* patient-level LOPO CV scores.

[277] Independent validation

[278] A final candidate classifier may be prospectively validated on a blinded, independent test set of TBB samples from 49 patients. Classification scores on the test set may be derived using the locked algorithm and may be compared against the pre-set decision boundary to give the binary prediction of UIP vs. non-UIP calls: classification score above the decision boundary may be called UIP, equal or below the decision boundary may be called non-UIP. The continuous classification scores may be compared against the histopathology labels to construct the ROC and calculate the AUC. The binary classification predictions may be compared against the histopathology labels to calculate the binary classification performance such as sensitivity and specificity.

[279] Score variability simulation

[280] In a clinical setting, it may be important to monitor if classification scores of future clinical samples remain stable and may not be affected by potential technical factors. To do this, the limit of score variability that the classifier can tolerate may need to be addressed prospectively. Under the assumption that the LOPO CV scores can represent the distribution of classification scores in the targeted population, a simulation may be performed for sensitivity, specificity and flip-rate between UIP and non-UIP calls. As a first step, a simulated noise may be added to *in silico* patient-level LOPO CV scores, where a noise may be simulated as $e \sim N(O, \sigma^2)$, and σ^2 may be 0, 0.01, …,10. Then, sensitivity, specificity and flip-rate may be computed using scores with the simulated noise. The simulation may be replicated 1,000 times. Using 1,000 sets of simulated scores, individual thresholds, σ_{spec}, σ_{sens} and σ_{flip} may be defined as the maximum of standard deviation, σ, of a noise where the estimated (averaged) specificity > 0.9, sensitivity > 0.65, and flip-rate < 0.15, respectively. The final threshold for classification score variability may be defined as

$$\sigma_{sv} = \min(\sigma_{spec}, \sigma_{sens}, \sigma_{flip})$$

[281] The thresholds for the ensemble model may be 0.9, 1.8, and 1.15 for specificity, sensitivity, and flip-rate, respectively and the final threshold may be $\sigma^{E}_{sv} = 0.9$ (**FIG. 48A-C**). The thresholds for the penalized regression model may be 0.48, 0.78 and 0.68 for specificity, sensitivity, and flip-rate, respectively and the final threshold may be $\sigma^{PL}_{sv} = 0.48$.

[282] Results

[283] Distribution of ILD diseases

[284] **Table 14** summarizes a distribution of patients for ILD diseases within UIP and non-UIP groups. Among collected patients, the prevalence of patients with UIP pattern may be higher in the training set (59%) than in the test set (47%) with p-value of 0.27. Three patients in the training set and one patient in the test set may have potential heterogeneity within patient: one lobe may be assigned as one of several non-UIP diseases (nonspecific interstitial pneumonia, pulmonary hypertension, or favor hypersensitivity pneumonitis), while the other lobe may be assigned a UIP pattern, driving the final patient-level label as UIP.

[285] The non-UIP group may include a diversity of heterogeneous diseases that may be commonly encountered in clinical practice. Due to the small sample size, several diseases may have one or two patients. Three new diseases—amyloid or light chain deposition, exogenous lipid pneumonia, and organizing alveolar hemorrhage—may be present in the test set, which may not exist in the training set.

[286] Intra-patient heterogeneity

[287] Heterogeneity in samples from the same patient may be observed in both histopathologic diagnosis and gene expression. Three such patients with diseases across UIP and non-UIP groups, may pose a computational challenge for patient-level diagnostic classification. The correlation matrix of samples from six patients may also reveal prominent intra- and inter-patient variability in expression profiles (**FIG. 38**). **FIG. 38** shows two non-UIP patients with the same labels across different lobes and similar gene expression pattern (patients 1 and 2 in **FIG. 38**), two UIP patients with the same or similar labels and highly correlated expression profiles (patients 5 and 6 in **FIG. 38**), as well as one UIP and one non-UIP patient with dissimilar labels and heterogeneous expression (patients 3 and 4 in **FIG. 38**), providing a representative visualization of the full spectrum of heterogeneity that may be observed within and across patients.

[288] DE analysis between UIP and non-UIP

[289] It may first be investigated whether differentially expressed genes found by DESeq2 between UIP and non-UIP may be predictive of the two diagnostic classes. 151 significantly differentially expressed genes may be identified between UIP and non-UIP (adjusted $p < 0.05$,

fold change >2), with 55 up-regulated and 96 down-regulated genes in UIP (**FIG. 29, Table 15**). However, using these differentially expressed genes alone it may be challenging to separate the two classes perfectly, as shown by the PCA plot (**FIG. 30**). In contrast, PCA spanned by the 190 classifier genes may separate the two classes much better (**FIG. 31**).

[290] Heterogeneity in patients of non-UIP diseases

[291] Heterogeneity may be observed in gene expression of non-UIP samples, consisting of more than a dozen clinically defined diseases. Genes may be identified that may be significantly different (adjusted $p < 0.05$, fold change >2) between UIP samples and each non-UIP disease subtype with a sample size greater than 10 (**Table 15**). The higher the number of differentially expressed genes, the more dissimilar the non-UIP disease subtype may be from UIP. A comparison of the list of differential genes in each non-UIP subtype with that from all non-UIP samples may show that the number of overlapping genes may be highly dependent on the number of differential genes identified in the individual non-UIP subtype, indicating that some non-UIP diseases may have more dominant effects on the overall differential genes found between all non-UIP and UIP samples (**Table 15**). Moreover, there may be few overlapping differential genes among those identified in individual non-UIP diseases. For example, 172 genes may be common between 1174 differential genes in Sarcoidosis and 701 in RB, and 6 common genes may be found among differential genes from sarcoidosis, RB and NSIP. There may be no common genes among differential genes from bronchiolitis, NSIP and HP. This may suggest distinct molecular expression patterns within diseases in non-UIP samples.

[292] The PCA plot using the differentially expressed genes between a non-UIP subtype and UIP samples may show that the specific non-UIP disease subtype may tend to be well-separated from UIP samples for diseases such as RB and HP (**FIG. 39** and **FIG. 41**), but other non-UIP samples may be interspersed with UIP samples (**FIG. 40** and **FIG. 43**). This may demonstrate that differential genes derived from one non-UIP subtype may not be generalizable to other non-UIP diseases.

[293] Comparison between *in silico* mixing and *in vitro* pooling within patient

[294] *In silico* mixed samples within each patient may be used to model *in vitro* pooled samples for evaluation within the training set. To ensure *in silico* mixed and *in vitro* pooled samples may be reasonably matched, the pooled samples of 11 patients may be sequenced and compared with *in silico* mixed samples. The average r-squared based on expression level of 26,268 genes for the pairs of *in silico* mixed and *in vitro* pooled samples may be 0.99 (SD = 0.003), which may indicate that the simulated expression level of *in silico* mixed samples may be well-matched with

that of in vitro pooled samples, considering the average r-squared values may be 0.98 (SD = 0.008) for technical replicates and 0.94 (0.04) for biological replicates.

[295] The classification scores of *in silico* and *in vitro* mixed samples by two candidate classifiers, the ensemble and penalized logistic regression models (described below) may also be compared in a scatterplot (**FIG. 32** and **FIG. 33**). The number of replicates for each *in vitro* pooled sample may range from 3 to 5, so the mean score of the multiple replicates may be used. The classification scores of *in silico* mixed samples may be highly correlated with those of *in vitro* pooled samples with Pearson's correlation of 0.99 for both classifiers (**FIG. 32** and **FIG. 33**). The points may fall right around the line of X = Y with no obvious shift or rotation.

[296] Cross-validation performance on the training set

[297] Multiple methods of feature selection and machine learning algorithms on training set of 354 TBB samples from 90 patients may be evaluated. As an initial attempt, individual methods and ensemble models may be evaluated separately based on 5-fold CV and cross-validated AUC (cvAUC) as estimated using the mean of the empirical AUC of each fold. Overall, the linear models such as the penalized regression model (cvAUC=0.89) may outperform non-linear tree-based models, such as random forest (cvAUC=0.83) and gradient boosting (cvAUC=0.84). The cvAUC of a neural network classifier may be under 0.8. The best performance may be achieved by (1) the ensemble model of SVMs with linear and radial kernels, and (2) penalized logistic regression; both of which have cvAUC = 0.89. However, with the heterogeneity among diseases and the small samples size, CV performance on all models may be found to vary significantly depending on the split.

[298] In LOPO CV, the patient-level performance may be evaluated by using 100 replicates of *in silico* mixed samples for each patient within LOPO CV folds. The computed classification scores of individual samples and averaged scores of *in silico* mixed samples may be shown in **FIG. 34** and **FIG. 35**. Overall, the patient-level performance may be slightly higher compared to the sample-level performance. Based on combined scores across LOPO CV folds, the ensemble model and the penalized logistic regression model may achieve the best performance with an AUC of 0.9 [0.87-0.93] and 0.87 [0.83-0.91] at sample-level and 0.93 [0.88-0.98] and 0.91 [0.85-0.97] at *in silico* mixing patient-level, respectively (**FIG. 36A**).

[299] Robustness of classifiers

[300] The estimated score variability may be 0.46 and 0.22 for the ensemble model and the penalized logistic regression model, respectively (**Table 16**). Both may be less than 0.9 and 0.48, the pre-specified thresholds of acceptable score variability (**FIG. 47A-C** and **FIG. 48A-C**). Considering the score range of the ensemble classifier may be wider than the penalized logistic

regression classifier, the proportion of the variability to the range of 5% and 95 % quantiles of scores may be compared. Overall, the penalized logistic regression classifier may have less variability in scores than the ensemble model. This may imply that the penalized logistic regression may be more robust to the technical (reagent/laboratory) batch effects and may offer more consistent scores for technical replicates. (**Table 16**). With high cross-validation performance and robustness, the penalized logistic regression model may be chosen as our final candidate model for the independent validation.

[301] Independent validation performance

[302] Using the locked penalized logistic classifier with a pre-specified decision boundary, 0.87, the validation performance may be evaluated based on the independent test set of *in vitro* mixed samples. The final classifier may achieve specificity 0.88 [0.70-0.98] and sensitivity 0.70 [0.47-0.87] with AUC 0.87 [0.76-0.98] (**FIG. 36B** and **FIG. 37**). The point estimate of the validation performance may be lower than *in silico* patient-level training CV performance, but with p-values, 0.6, 0.7, and 1 for AUC, sensitivity and specificity, respectively, indicating negligible difference.

[303] Discussion

[304] In this study, accurate and robust classification may be achievable even when critical challenges exist. By leveraging appropriate statistical methodologies, machine learning approaches, and RNA sequencing technology, a meaningful diagnostic test may be provided to improve the care of patients with interstitial lung diseases.

[305] Machine learning, particularly deep learning, may have experienced revolutionary progress in the last few years. Empowered with these recently developed and highly sophisticated tools, classification performance may be dramatically improved in many applications [Lecun *et al*, which is entirely incorporated herein by reference]. However, most of these tools may require readily available and high-confidence labels as well as large sample size: the magnitude of the performance improvement may be directly and positively related with the number of samples with high-quality labels [Gu *et al* and Sun *et al*, which are entirely incorporated herein by reference]. In this project, like many other clinical studies based on patient samples, the sample size may be limited: for example, 90 patients in the training set (**Table 14**). On top of that, the non-UIP group may not be one physiologically homogenous disease, but rather a collection of many types of diseases, each with its own distinct biology, several of which may have only one or two patients in the training set [Libbrecht *et al*, which is entirely incorporated herein by reference] (**Table 14**). Not surprisingly, these various types of non-UIP diseases may be not only physiologically distinct, but may be also different at the

molecular and genomic level. The training samples may be utilized to identify common features across non-UIP diseases in respect to differentiating from the UIP group may be tried but none emerged (**Table 15**, **FIG. 38**). Furthermore, three or more disease types (Amyloid or light chain deposition, Exogenous lipid pneumonia, and Organizing alveolar hemorrhage) may present in the test set and may not be encountered in the training set (**Table 14**). A change in UIP proportions may also be observed between training (59%) and testing (47%). The last two factors may help explain the slightly lower performance in the test set as compared to the cross-validation performance of the training set. Recent advances in machine learning that leverage large sample size may not be applicable in this situation. In some case, a focus may be on more traditional linear models or tree-based models. It may also explain among candidates, why linear models may outperform non-linear tree-based models because a sample size in individual non-UIP disease groups may be too small to power any interaction the tree-model may be trying to capture.

[306] To directly address the small training size, up to 5 distinct TBB samples within the same patient may be run from RNA extraction through sequencing to successfully expand the 90 patient set to encompass 354 samples (**Table 14**). This, in concept, may be similar to the data augmentation idea, but instead of simulating or extrapolating the augmented data, sequencing data may be generated from real experiments on multiple TBB samples from the same patient. The goal may be to provide additional information to enhance classification performance. Special caution may be taken to use patient as the smallest unit when defining the cross-validation fold and evaluating performance. This may prevent patients with more samples from having higher weight, or samples from the same patient straddling on both side of model building and model evaluation, causing over-fitting. A nested cross-validation may also be applied as well as the one SD (standard deviation) rule for model selection and parameter optimization to correctly factor-in the high variability on performance due to small sample size and to aggressively trim down the model complexity to guard against overfitting.

[307] While running multiple TBB samples per patient in the training set may help with the sample size limitation, it may create a new problem. In the commercial setting, it may be economically viable only if it may be limited to test one sequencing run per patient. To achieve that, RNA material from multiple TBB samples within one patient may need to be pooled together before sequencing. However, whether a classifier trained on individual TBB samples may be applicable to pooled TBB samples may become a critical question that may need to be addressed before setting off the validation experiment. To answer this question, a series of in-silico mixing simulations may be performed to mimic patient-level *in-vitro* pools of the test set.

This approach may also be the fundamental building block for defining the prospective decision boundary of the classifier as well as the optimal number of TBBs required to achieve the best classification performance [Pankratz *et al*]. The simulated in-silico data may agree well with the experimental in-vitro data (**FIG. 32** and **33**) giving confidence in using this approach to extrapolate expected performance to pooled samples and proceed with the validation experiments with the pooled setting. This *in silico* approach may work well in this example since samples pooled together may be of the same type (TBB) and from the same patient, thus have similar characteristics such as the rate of duplicated reads or the total number of reads. However, it may be tricky to extend the proposed *in-silico* mixing model to mix samples of different characteristics or qualities, for example UIP vs non-UIP samples or TBB mixed with different type of samples such as blood. In those cases, samples with substantially higher total number of reads may tend to dominate the expressions of combined samples violating the basic assumptions of the mixed model proposed here. More sophisticated methodology may be required to accurately model such complex procedures and biological interaction.

[308] A successful validation that may meet the required clinical performance (**FIG. 36A-B** and **FIG. 37**) may be the first step towards a useful commercial product aiming to improve patient care. Equally important, but often overlooked, may be the importance of providing consistent and reliable performance for the future patient stream. This may require proactive anticipation to address any potential batch effects of sequencing data from incoming patients that may cause systematic changes in classification scores and result in false clinical predictions. This important issue may be tackled starting from the upstream feature selection (**FIG. 39-44**) where genes that may be highly sensitive to batch effects may be removed from any downstream analysis. Furthermore, additional experimental data may be generated for 10 distinct TBB samples in three different batches; none of the batches may be used in generating training samples. This experiment may be leveraged to directly evaluate each candidate model's robustness against unseen batches and may help select the final model. However, experimental data may evaluate a finite number of batches. Thus, to anticipate unforeseen changes, a monitoring scheme may be developed based on control samples run in each of the commercial plate/batch to detect any unexpected potential changes. If such unexpected changes may occur, a normalization method that may directly addresses batch correction may be necessary to map new scores to the space of validation classification scores.

[309] Conclusions

[310] Limited sample size and high heterogeneity within the non-UIP class may be two major classification challenges faced in this example and which may commonly exist in clinical

studies. In addition, a successful commercial product may need to perform economically and consistently for all future incoming samples, which may require the underlying classification model to be applicable to pooled samples and highly robust against assay variability. It may be feasible to achieve highly accurate and robust classification despite these difficulties. The methodologies may have proven to be successful in this example and may be applicable to other clinical scenarios facing similar difficulties.

[311] Example 2 – Molecular Profiling and Cytological Examination

[312] An individual is symptomatic for lung cancer. The individual consults her primary care physician who examines the individual and refers her to an endocrinologist. The endocrinologist obtains a sample via bronchoscopy, and sends the sample to a cytological testing laboratory. The cytological testing laboratory performs routine cytological testing on a portion of the bronchoscopy, the results of which are suspicious or ambiguous (i.e., indeterminate). The cytological testing laboratory suggests to the endocrinologist that the remaining sample may be suitable for molecular profiling, and the endocrinologist agrees.

[313] The remaining sample is analyzed using the methods and compositions herein. The results of the molecular profiling analysis suggest a high probability of early stage lung cancer. The results further suggest that molecular profiling analysis combined with patient data. The endocrinologist reviews the results and prescribes the recommended therapy.

[314] The cytological testing laboratory bills the endocrinologist for routine cytological tests and for the molecular profiling. The endocrinologist remits payment to the cytological testing laboratory and bills the individual's insurance provider for all products and services rendered. The cytological testing laboratory passes on payment for molecular profiling to the molecular profiling business and withholds a small differential.

[315] Example 3

[316] A subject is at-risk for lung cancer due to exposure to second-hand smoke. The subject is asymptomatic for lung cancer. A medical professional obtains a nasal tissue sample from the subject. A molecular classifier as described herein analyzes the nasal tissue sample. Based on a presence or absence of a plurality of biomarkers, a medical professional recommends the subject to receive a low-dose CT scan or recommends analyzing another nasal tissue sample 1 year later using the molecule classifier.

[317] Example 4

[318] A subject has previously received confirmation of a presence of a lung nodule. A medical professional obtains a nasal tissue sample from the subject. A molecular classifier as described herein analyzes the nasal tissue sample. Based on a presence or absence of a plurality of

biomarkers, a medical professional recommends the subject to receive a bronchoscopy or recommends analyzing another nasal tissue sample 1 year later using the molecular classifier.

[319] Example 5

[320] A subject is currently receiving an interventive therapy. A medical professional obtains a nasal tissue sample from the subject. A molecular classifier as described herein analyzes the nasal tissue sample. Based on a presence or absence of a plurality of biomarkers, a medical professional recommends the subject continue the interventive therapy or stop the interventive therapy and begin a different interventive therapy.

[321] Example 6

[322] A subject has previously received a surgical resection of a malignant tumor. A medical professional obtains a nasal tissue sample from the subject. A molecular classifier as described herein analyzes the nasal tissue sample. Based on a presence or absence of a plurality of biomarkers, a medical professional recommends a treatment regime for the subject or recommends analyzing another nasal tissue sample 1 year later using the molecular classifier.

Computer control systems

[323] The present disclosure provides computer control systems that are programmed to implement methods of the disclosure. **FIG. 26** shows a computer system **2601** that is programmed or otherwise configured to implement the methods provided herein. The computer system **2601** can regulate various aspects of diagnosing a lung condition in a subject, predicting a risk of developing a lung condition in a subject, predicting an efficacy of treatment in a subject having a lung condition, or combinations thereof of the present disclosure, such as, for example, (i) comparing one or more biomarkers of a sample to a reference set of biomarkers, (ii) training an algorithm to develop a classifier, (iii) applying a classifier to make a diagnosis, a prediction, or a recommendation based on a sample input, or (iv) any combination thereof. The computer system **2601** can be an electronic device of a user or a computer system that is remotely located with respect to the electronic device. The electronic device can be a mobile electronic device.

[324] The computer system **2601** includes a central processing unit (CPU, also "processor" and "computer processor" herein) **2605**, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system **2601** also includes memory or memory location **2610** (e.g., random-access memory, read-only memory, flash memory), electronic storage unit **2615** (e.g., hard disk), communication interface **2620** (e.g., network adapter) for communicating with one or more other systems, and peripheral devices **2625**, such as cache, other memory, data storage and/or electronic display adapters. The memory **2610**, storage unit **2615**, interface **2620** and peripheral devices **2625** are in communication with the

CPU **2605** through a communication bus (solid lines), such as a motherboard. The storage unit **2615** can be a data storage unit (or data repository) for storing data. The computer system **2601** can be operatively coupled to a computer network ("network") **2630** with the aid of the communication interface **2620**. The network **2630** can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network **2630** in some cases is a telecommunication and/or data network. The network **2630** can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network **2630**, in some cases with the aid of the computer system **2601**, can implement a peer-to-peer network, which may enable devices coupled to the computer system **2601** to behave as a client or a server.

[325] The CPU **2605** can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory **2610**. The instructions can be directed to the CPU **2605**, which can subsequently program or otherwise configure the CPU **2605** to implement methods of the present disclosure. Examples of operations performed by the CPU **2605** can include fetch, decode, execute, and writeback.

[326] The CPU **2605** can be part of a circuit, such as an integrated circuit. One or more other components of the system **2601** can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[327] The storage unit **2615** can store files, such as drivers, libraries and saved programs. The storage unit **2615** can store user data, e.g., user preferences and user programs. The computer system **2601** in some cases can include one or more additional data storage units that are external to the computer system **2601**, such as located on a remote server that is in communication with the computer system **2601** through an intranet or the Internet.

[328] The computer system **2601** can communicate with one or more remote computer systems through the network **2630**. For instance, the computer system **2601** can communicate with a remote computer system of a user (e.g., service provider). Examples of remote computer systems include personal computers (e.g., portable PC), slate or tablet PC's (e.g., Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (e.g., Apple® iPhone, Android-enabled device, Blackberry®), or personal digital assistants. The user can access the computer system **2601** via the network **2630**.

[329] Methods as described herein can be implemented by way of machine (e.g., computer processor) executable code stored on an electronic storage location of the computer system **2601**, such as, for example, on the memory **2610** or electronic storage unit **2615**. The machine

executable or machine readable code can be provided in the form of software. During use, the code can be executed by the processor **2605**. In some cases, the code can be retrieved from the storage unit **2615** and stored on the memory **2610** for ready access by the processor **2605**. In some situations, the electronic storage unit **2615** can be precluded, and machine-executable instructions are stored on memory **2610**.

[330] The code can be pre-compiled and configured for use with a machine having a processer adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

[331] Aspects of the systems and methods provided herein, such as the computer system **2601**, can be embodied in programming. Various aspects of the technology may be thought of as "products" or "articles of manufacture" typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible "storage" media, terms such as computer or machine "readable medium" refer to any medium that participates in providing instructions to a processor for execution.

[332] Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include

dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

[333] The computer system **2601** can include or be in communication with an electronic display **2635** that comprises a user interface (UI) **2640** for providing, for example, an output or readout of the classifier or trained algorithm. Examples of UI's include, without limitation, a graphical user interface (GUI) and web-based user interface.

[334] Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the central processing unit **2605**. The algorithm can, for example, (i) determine a presence or one or more biomarkers in a sample compared to a reference set of biomarkers.

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[356] Libbrecht MW, Noble WS: Machine learning applications in genetics and genomics. Nat Rev Genet 2015, 16:321-332.

[357] Wong SC, Gatt A, Stamatescu V, McDonnell MD: Understanding data augmentation for classification: when to warp? In. IEEE; 2016: 1-6; arXiv:1609.08764.

[358] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for screening a subject for a lung condition, comprising:

(a) assaying epithelial tissue from a first sample obtained from a subject that has been (1) computer analyzed for a presence of one or more risk factors for developing said lung condition and (2) identified with said presence of said one or more risk factors, to identify a presence or absence of one or more biomarkers associated with a risk of developing said lung condition in said first sample; and

(b) upon identifying said presence or absence of said one or more biomarkers, (i) directing an electronic imaging scan of a lung region of said subject to be obtained, which lung region is suspected of exhibiting said lung condition, or (ii) assaying other epithelial tissue from a second sample of said subject.

2. The method of claim 1, further comprising, prior to (b), receiving a request to assay said first sample comprising said epithelial tissue of said subject.

3. The method of claim 1, wherein said electronic imaging scan is a low-dose computerized tomography (LDCT) scan or magnetic resonance imaging (MRI).

4. The method of claim 3, wherein said LDCT scan provides a radiation exposure to said subject of less than about 5 millisieverts (mSv).

5. The method of claim 1, wherein said lung condition is lung cancer, chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), or any combination thereof.

6. The method of claim 1, wherein said lung condition is a lung cancer, and wherein said lung cancer comprises: a non-small cell lung cancer; an adenocarcinoma; a squamous cell carcinoma; a large cell carcinoma; a small cell lung cancer; or any combination thereof.

7. The method of claim 1, wherein said first sample or said second sample is obtained by a bronchoscopy.

8. The method of claim 1, wherein said first sample or said second sample is obtained by fine needle aspiration.

9. The method of claim 1, wherein said first sample or said second sample comprises a mucous epithelial tissue, a nasal epithelial tissue, a lung epithelial tissue, or any combination thereof.

10. The method of claim 1, wherein said first sample or said second sample comprises epithelial tissue obtained along an airway of said subject.

11. The method of claim 1, wherein a portion of said first sample or said second sample is subjected to cytological testing that identifies said first sample or said second sample as ambiguous or suspicious.

12. The method of claim 11, wherein upon identifying said first sample or said second sample as ambiguous or suspicious, performing (b) on a second portion of said sample, which second portion comprises said epithelial tissue.

13. The method of claim 1, wherein said second sample is different from said first sample.

14. The method of claim 1, wherein said second sample is a different sample type from said first sample.

15. The method of claim 1, wherein said first sample is obtained from said subject at a first time point and said second sample is obtained from said subject at a second time point, wherein said second time point is after said first time point.

16. The method of claim 15, wherein said second time point is within about 1-2 years of said first time point.

17. The method of claim 1, wherein (a) comprises comparing said presence or absence of said one or more biomarkers to a reference set of one or more biomarkers.

18. The method of claim 1, wherein said subject is in need of a treatment for said lung condition.

19. The method of claim 1, wherein said subject is suspected of having an increased risk for developing a lung condition.

20. The method of claim 1, wherein said subject is asymptomatic with respect to said lung condition.

21. The method of claim 1, wherein said subject has not previously received said electronic imaging scan.

22. The method of claim 1, wherein said subject has not previously received a definitive diagnosis.

23. The method of claim 1, wherein said one or more risk factors comprise: smoking; exposure to environmental smoke; exposure to radon; exposure to air pollution; exposure to radiation; exposure to an industrial substance; inherited or environmentally-acquired gene mutations; a subject's age; a subject having a secondary health condition; or any combination thereof.

24. The method of claim 1, wherein said subject has two or more risk factors.

25. The method of claim 1, wherein said one or more biomarkers comprise at least five biomarkers.

26. The method of claim 1, wherein said one or more biomarkers comprise one or more of: a gene or fragment thereof; a sequence variant; a fusion; a mitochondrial transcript; an epigenetic modification; a copy number variation; a loss of heterozygosity (LOH); or any combination thereof.

27. The method of claim 1, wherein said presence or absence of said one or more biomarkers comprises a level of expression.

28. The method of claim 1, wherein said method identifies whether said subject is at an increased risk for developing said lung condition.

29. The method of claim 1, wherein said identifying of (b) comprises employing a trained algorithm.

30. The method of claim 29, wherein said trained algorithm is trained by a training set comprising epithelial cells obtained from an airway of an individual.

31. The method of claim 29, wherein said trained algorithm is trained by a training set comprising samples benign for said lung condition and samples malignant for said lung condition.

32. The method of claim 29, wherein said trained algorithm is trained by a training set comprising samples obtained from subjects having one or more risk factors.

33. The method of claim 1, further comprising, prior to (a), computer analyzing said subject to identify said presence of said one or more risk factors in said subject for developing said lung condition.

34. A method for monitoring a subject having or suspected of having a lung condition, comprising:

(a) assaying a first sample comprising epithelial tissue obtained from a subject suspected of having said lung condition to identify a presence or absence of one or more biomarkers associated with said lung condition, wherein said subject has previously received a positive indication of a presence of one or more lung nodules; and

(b) upon identifying said presence or absence of said one or more biomarkers, (i) obtaining a second sample from said subject or (ii) directing said subject to obtain an electronic imaging scan of a lung region of said subject based on a result from (a).

35. The method of claim 34, wherein said positive indication is previously identified by an electronic imaging scan.

36. The method of claim 34, wherein said one or more lung nodules is at least two nodules.

37. The method of claim 34, wherein said obtaining said second sample from said subject comprises performing a bronchoscopy, a transthoracic needle aspiration (TTNA), or a video-assisted thorascopic surgery (VATS) on said subject.

38. The method of claim 34, wherein said obtaining said second sample from said subject comprises performing a tissue biopsy.

39. The method of claim 34, wherein said presence or absence of said one or more biomarkers identifies said subject as high-risk or as low-risk of having said lung condition.

40. The method of claim 39, wherein (b) further comprises recommending (i) or (ii) depending on an assessed risk.

41. The method of claim 34, further comprising analyzing a blood sample from said subject, performing an electronic imaging scan on said subject, or a combination thereof.

42. The method of claim 34, wherein said second sample is a sample of epithelial, and wherein subsequent to (b), said sample of epithelial tissue is assayed for a presence or absence of one or more additional biomarkers.

43. The method of claim 42, wherein said one or more additional biomarkers are said one or more biomarkers.

44. A method for monitoring a subject having or suspected of having a lung condition wherein said subject has previously received a recommendation to complete an interventive therapy for preventing or reversing said lung condition, said method comprising:

(a) subsequent to said subject completing at least a portion of said interventive therapy for said lung condition, assaying a first sample comprising epithelial tissue obtained from said subject to generate genetic data;

(b) processing said genetic data to identify a presence or absence of one or more biomarkers associated with said lung condition; and

(c) computer generating a report comprising a recommendation that a second sample be obtained from said subject.

45. The method of claim 44, wherein said method identifies subject compliance to said interventive therapy.

46. The method of claim 44, wherein said method identifies efficacy of said interventive therapy to preventing or reversing said lung condition.

47. The method of claim 44, wherein said interventive therapy comprises administering a pharmaceutical composition to said subject.

48. The method of claim 47, wherein said pharmaceutical composition comprises a chemotherapeutic.

49. The method of claim 44, wherein said interventive therapy comprises an exercise regime, a dietary regime, a reduction or omission of smoking, or any combination thereof.

50. The method of claim 44, wherein (b) comprises processing said genetic data to identify an expression level corresponding to each of said one or more biomarkers.

51. A method for monitoring said subject for a lung condition, comprising:

(a) assaying a first sample comprising epithelial tissue obtained from a subject and identifying a presence or absence of one or more biomarkers, wherein said subject has previously initiated a treatment for a lung condition; and

(b) upon receiving a confirmation of remission, obtaining a second sample from said subject and repeating (a) with said second sample.

52. The method of claim 44 or 51, wherein (b) comprises processing said genetic data to identify at least one genetic aberration in said one or more biomarkers.

53. The method of claim 51, wherein said method identifies early stage lung condition recurrence through non-invasive monitoring.

54. The method of claim 51, wherein said subject is suspected of having an increased risk for a recurrence of said lung condition.

55. A method for monitoring a subject having or suspected of having a lung condition, comprising:

(a) assaying a first sample comprising epithelial tissue obtained from a subject suspected of having said lung condition to identify a presence or absence of one or more biomarkers associated with said lung condition, wherein said subject has previously received a negative indication of a presence of a lung nodule; and

(b) upon identifying said presence or absence of said one or more biomarkers, (i) obtaining a second sample from said subject or (ii) directing said subject to obtain an electronic imaging scan of a lung region of said subject based on a result from (a).

56. The method of claim 55, further comprising, prior to (a), computer analyzing said subject for a presence of one or more risk factors for developing said lung condition, and identifying said subject with said presence of said one or more risk factors.

57. A system for screening a subject for a lung condition, comprising:

one or more computer databases comprising health or physiological data of a subject; and

one or more computer processors that are individually or collectively programmed to (i) analyze said health or physiological data for a presence of one or more risk factors for said subject developing said lung condition, and (2) upon identifying said one or more risk factors,

generate a recommendation that epithelial tissue from a sample of said subject be assayed for one or more biomarkers associated with a risk of developing said lung condition.

58. A system for screening a subject for a lung condition, comprising:

one or more computer databases comprising (i) a first data set comprising data indicative of a presence of one or more risk factors for said subject developing said lung condition, and (ii) a second data set comprising data indicative of a presence or absence of one or more biomarkers in epithelial tissue in a sample of said subject, which one or more biomarkers are associated with a risk of developing said lung condition; and

one or more computer processors that are individually or collectively programmed to (i) analyzing said first data set to identify said presence of said one or more risk factors, (ii) analyzing said second data set to identify said presence or absence of said one or more biomarkers, and (iii) upon identifying said presence or absence of said one or more biomarkers, generate a report that (1) directs an electronic imaging scan of a lung region of said subject to be obtained, which lung region is suspected of exhibiting said lung condition, or (2) directs other epithelial tissue from a second sample of said subject to be assayed.

59. A system for monitoring a subject having or suspected of having a lung condition, comprising:

one or more computer databases comprising a data set comprising data indicative of a presence or absence of one or more biomarkers in epithelial tissue in a first sample of said subject, which one or more biomarkers are associated with said lung condition; and

one or more computer processors that are individually or collectively programmed to (i) determine that said subject has previously received a positive indication of a presence of one or more lung nodules, (ii) subsequent to (i), process said data set to identify said presence or absence of said one or more biomarkers, and (iii) upon identifying said presence or absence of said one or more biomarkers, generate a report that (1) directs a second sample to be obtained from said subject, or (2) directs another electronic imaging scan of a lung region of said subject to be obtained.

60. A system for monitoring a subject having or suspected of having a lung condition wherein said subject has previously received a recommendation to complete an interventive therapy for preventing or reversing said lung condition, comprising:

one or more computer databases comprising a data set comprising genetic data; and

one or more computer processors that are individually or collectively programmed to (i) subsequent to said subject completing at least a portion of said interventive therapy for said lung condition, process said genetic data to identify a presence or absence of one or more biomarkers

associated with said lung condition, and (iii) generate a report comprising a recommendation that a second sample be obtained from said subject.

~225K
New Cases per Year
(90% are Smokers)
~160K
Deaths per Year
EARLY DETECTION
is Key to Reducing Mortality
But Lung Cancer is Hard to
Diagnose without Invasive,
Risky and Costly Surgeries
40%
of Patients Undergoing
Invasive Biopsy Do Not
Have Cancer

FIG. 1

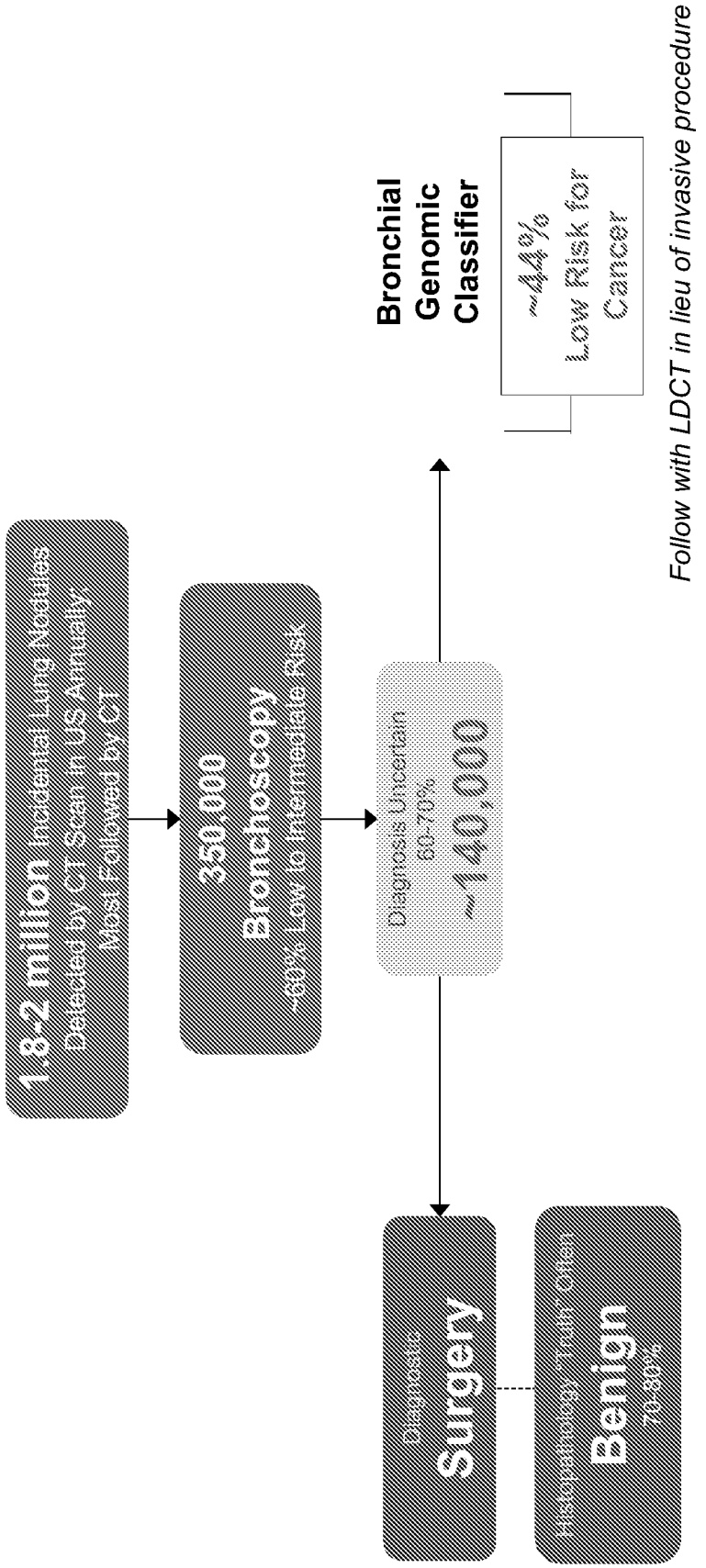

1.8.2 million Incidental Lung Nodules
Detected by CT Scan in US Annually
Most Followed by CT
350,000
Bronchoscopy
~60% Low to Intermediate Risk
Diagnosis Uncertain
60-70%
~140,000
Bronchial
Genomic
Classifier
~44%
Low Risk for
Cancer
Follow with LDCT in lieu of invasive procedure
Diagnostic
Surgery
Benign
70-80%

FIG. 2

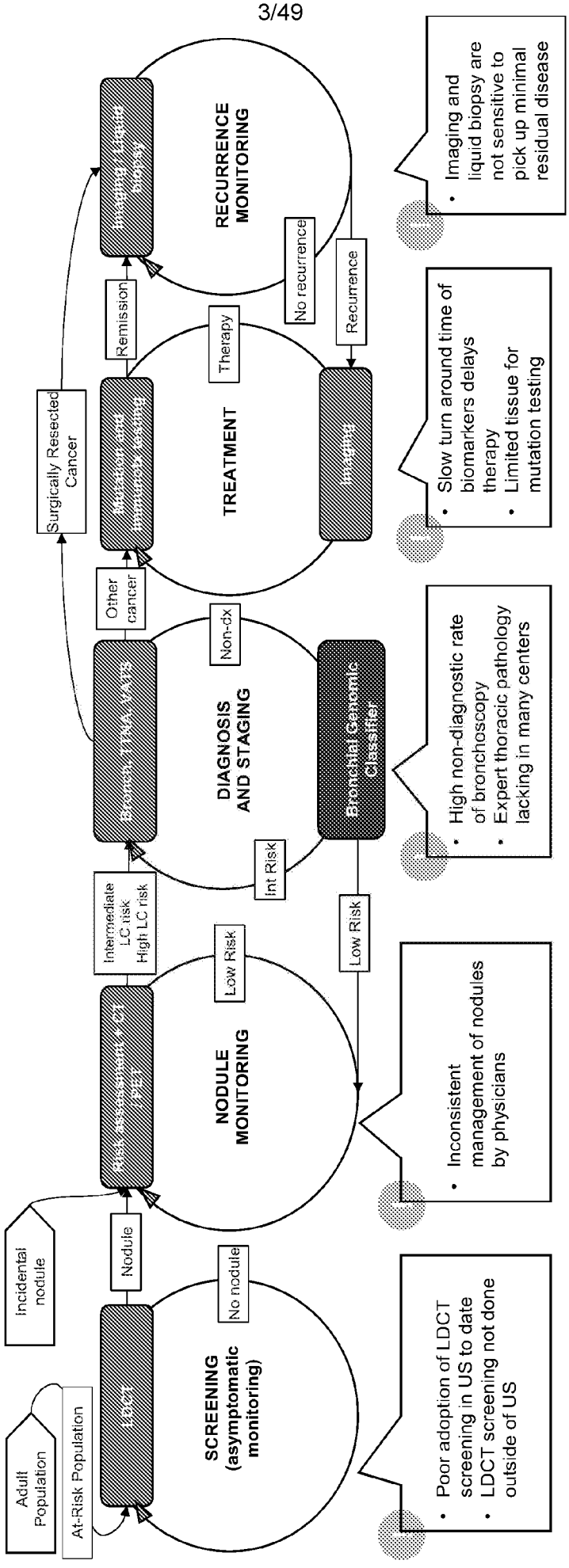

Adult Population
At-Risk Population
Incidental nodule
LDCT
Nodule
No nodule
SCREENING (asymptomatic monitoring)
Low Risk
Intermediate LC risk
High LC risk
NODULE MONITORING
Int Risk
Low Risk
Non-dx
Other cancer
DIAGNOSIS AND STAGING
Therapy
Remission
Recurrence
No recurrence
TREATMENT
RECURRENCE MONITORING
Surgically Resected Cancer
Poor adoption of LDCT screening in US to date
LDCT screening not done outside of US
Inconsistent management of nodules by physicians
High non-diagnostic rate of bronchoscopy
Expert thoracic pathology lacking in many centers
Slow turn around time of biomarkers delays therapy
Limited tissue for mutation testing
Imaging and liquid biopsy are not sensitive to pick up minimal residual disease

FIG. 3

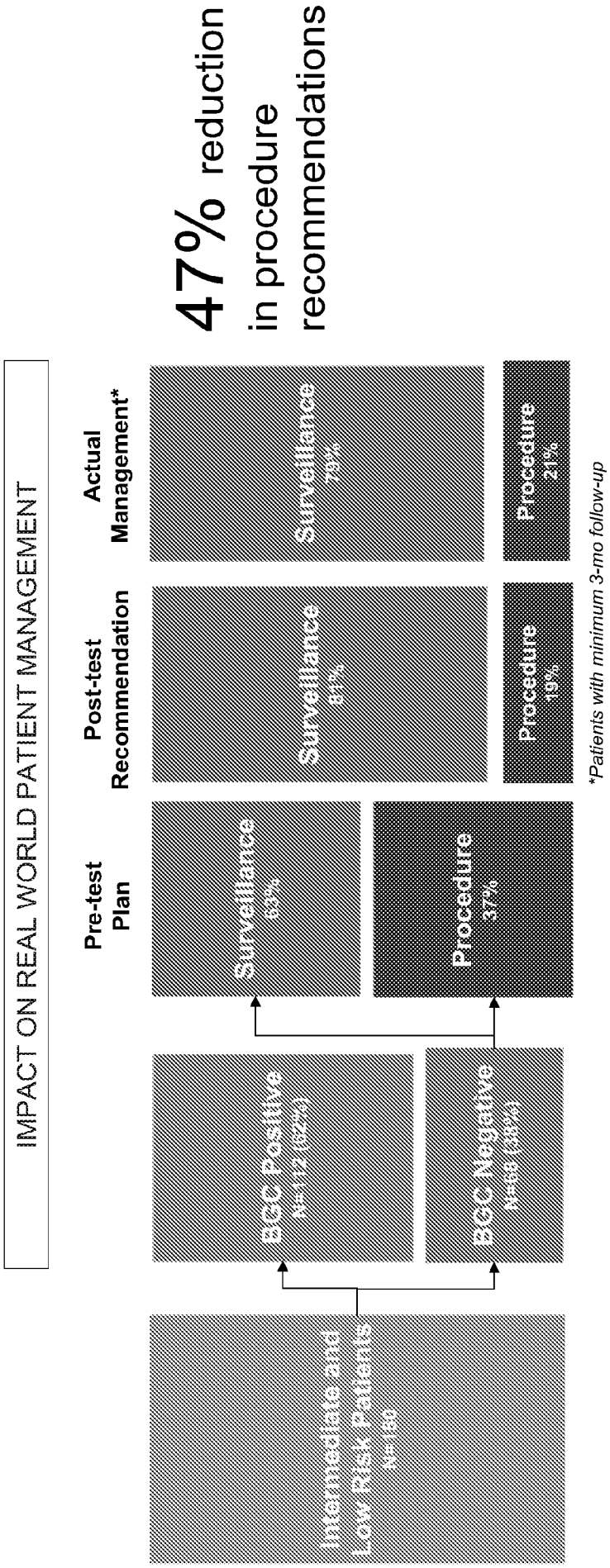

47% reduction in procedure recommendations
IMPACT ON REAL WORLD PATIENT MANAGEMENT
Pre-test Plan
Post-test Recommendation
Actual Management*
Intermediate and Low Risk Patients N=150
BGC Positive N=112 (82%)
BGC Negative N=38 (18%)
Surveillance 63%
Procedure 37%
Surveillance 81%
Procedure 19%
Surveillance 79%
Procedure 21%
*Patients with minimum 3-mo follow-up

FIG. 4

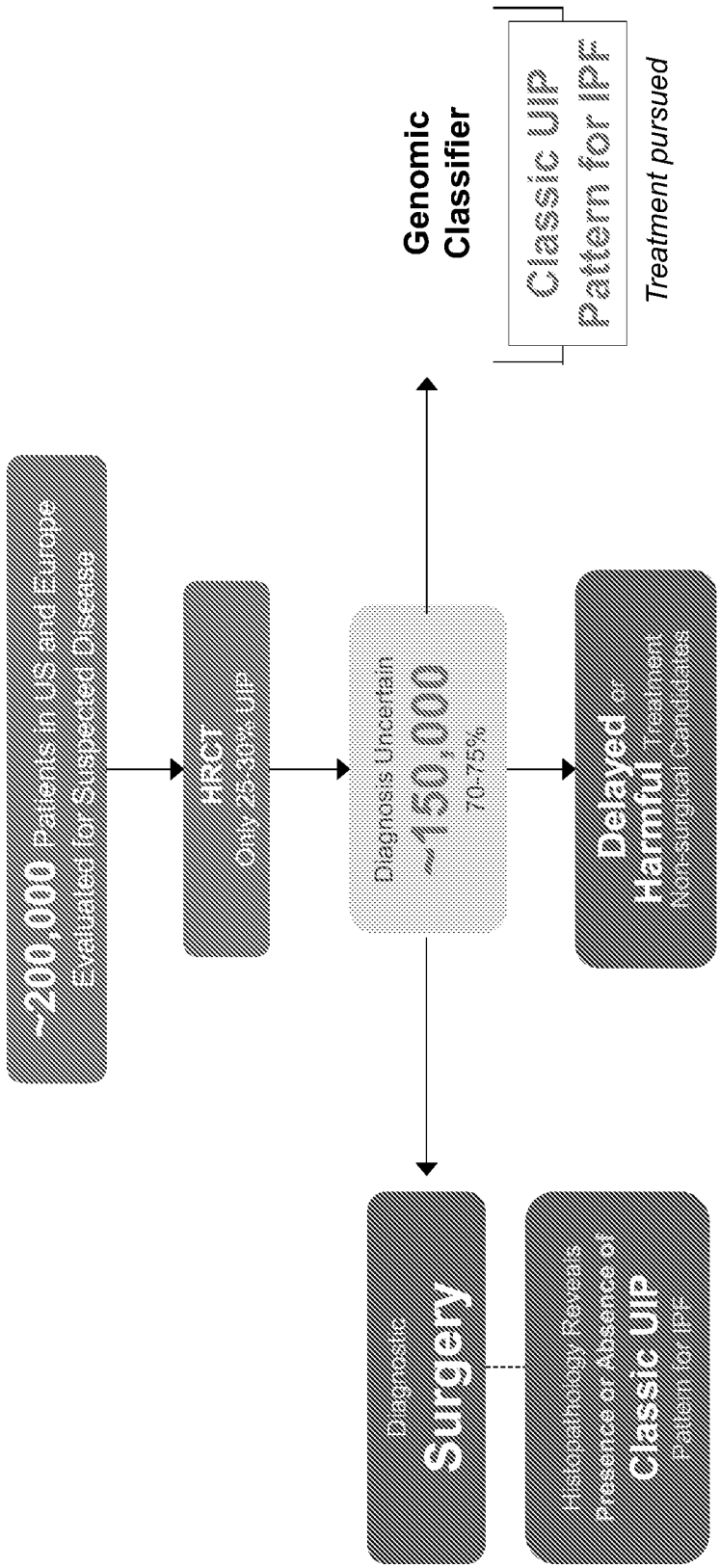

~200,000 Patients in US and Europe
Evaluated for Suspected Disease
HRCT
Only 25-30% UIP
Diagnosis Uncertain
~160,000
70-75%
Delayed or Harmful Treatment
Non-Fibrotic Candidates
Genomic Classifier
Classic UIP Pattern for IPF
Treatment pursued
Diagnostic Surgery
Histopathology Reveals Presence or Absence of Classic UIP Pattern for IPF

FIG. 5

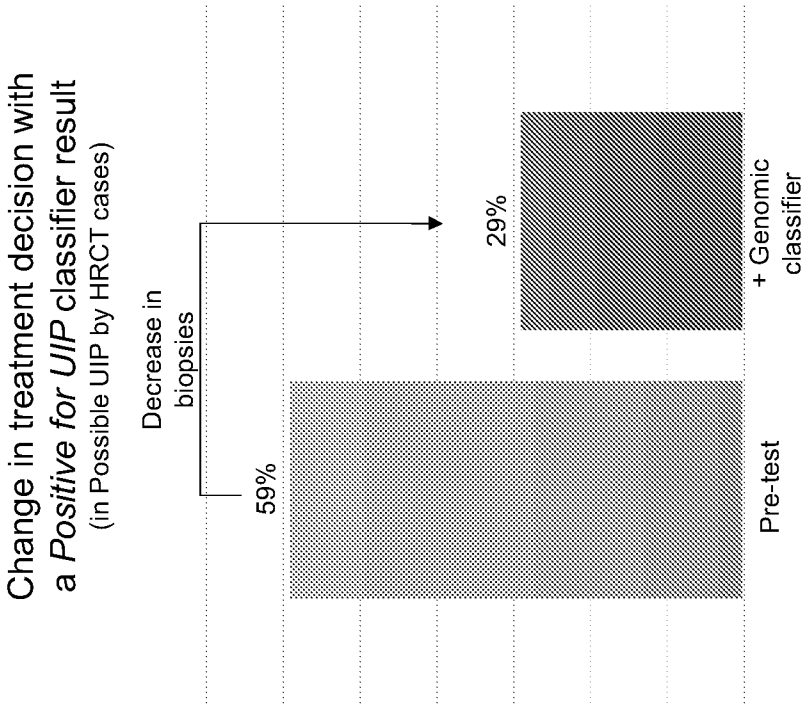

Change in treatment decision with a *Positive for UIP* classifier result
(in Possible UIP by HRCT cases)
Decrease in biopsies
59%
29%
Pre-test
+ Genomic classifier

FIG. 6

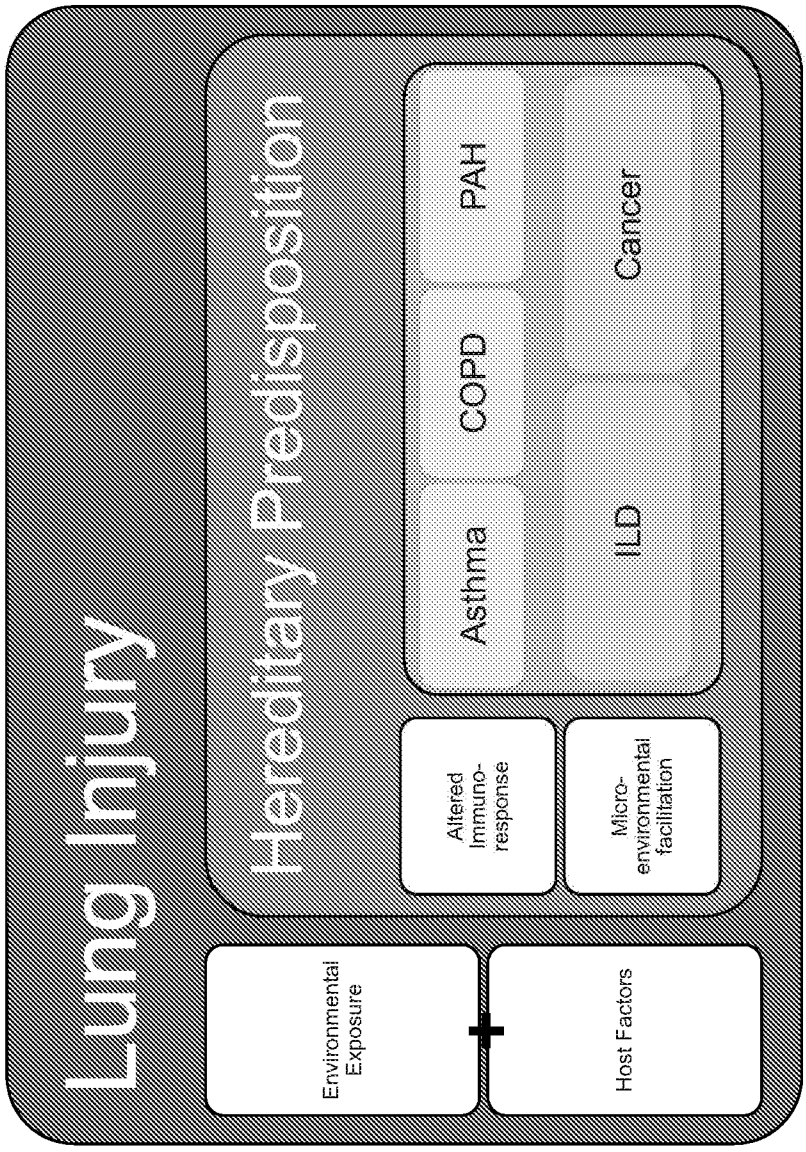

Lung Injury
Environmental Exposure
+
Host Factors
Hereditary Predisposition
Altered Immuno-response
Micro-environmental facilitation
Asthma
COPD
PAH
ILD
Cancer

FIG. 7

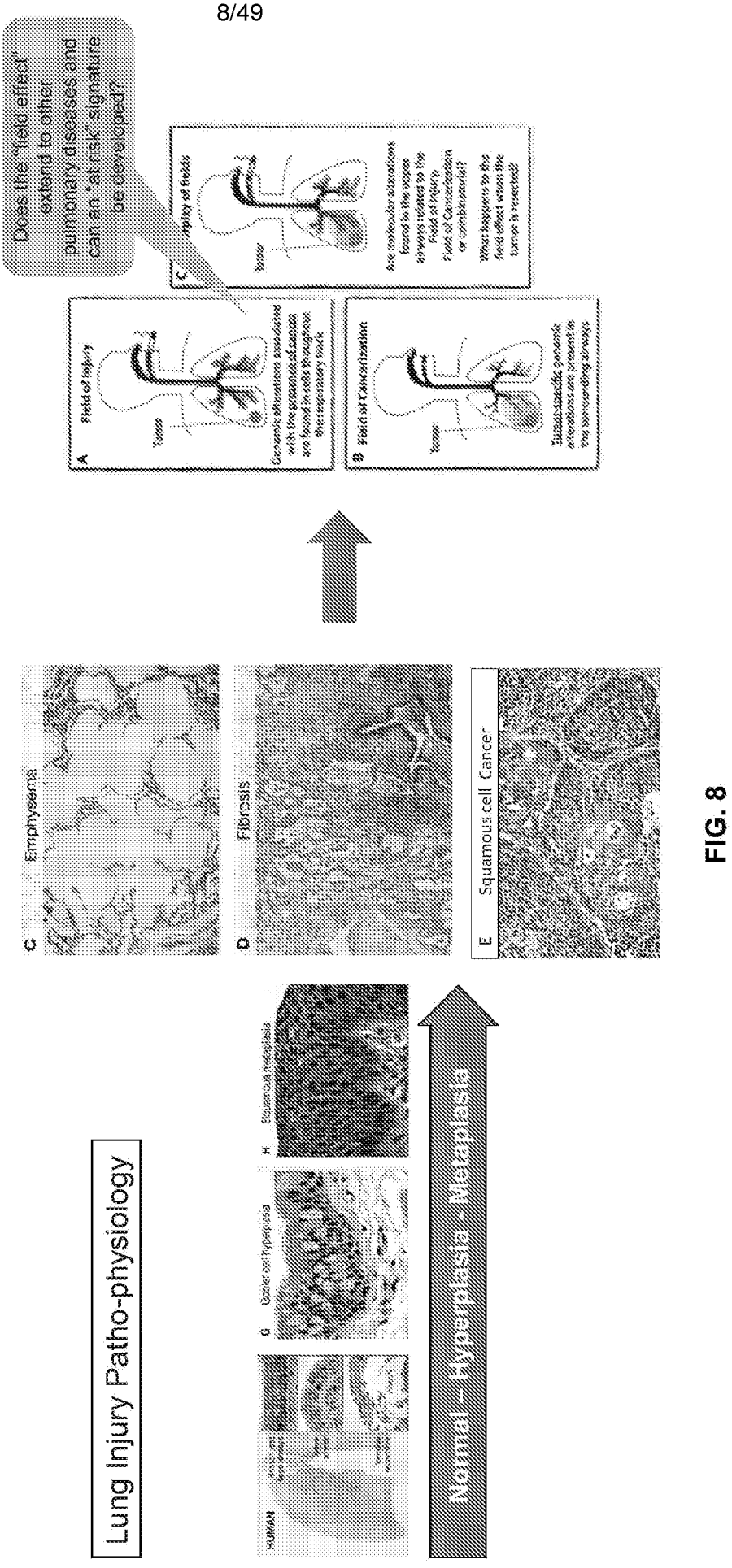

Does the "field effect" extend to other pulmonary deases and can an "at risk" signature be developed?
A Field of Injury
Tumor
Genomic alterations associated with the presence of cancer are found in cells throughout the respiratory track
B Field of Cancerization
Tumor
Tumor specific genomic alterations are present in the surrounding airways
C Emphysema
D Fibrosis
E Squamous cell Cancer
Lung Injury Patho-physiology
HUMAN
G Goblet cell hyperplasia
H Squamous metaplasia
Normal – Hyperplasia - Metaplasia

FIG. 8

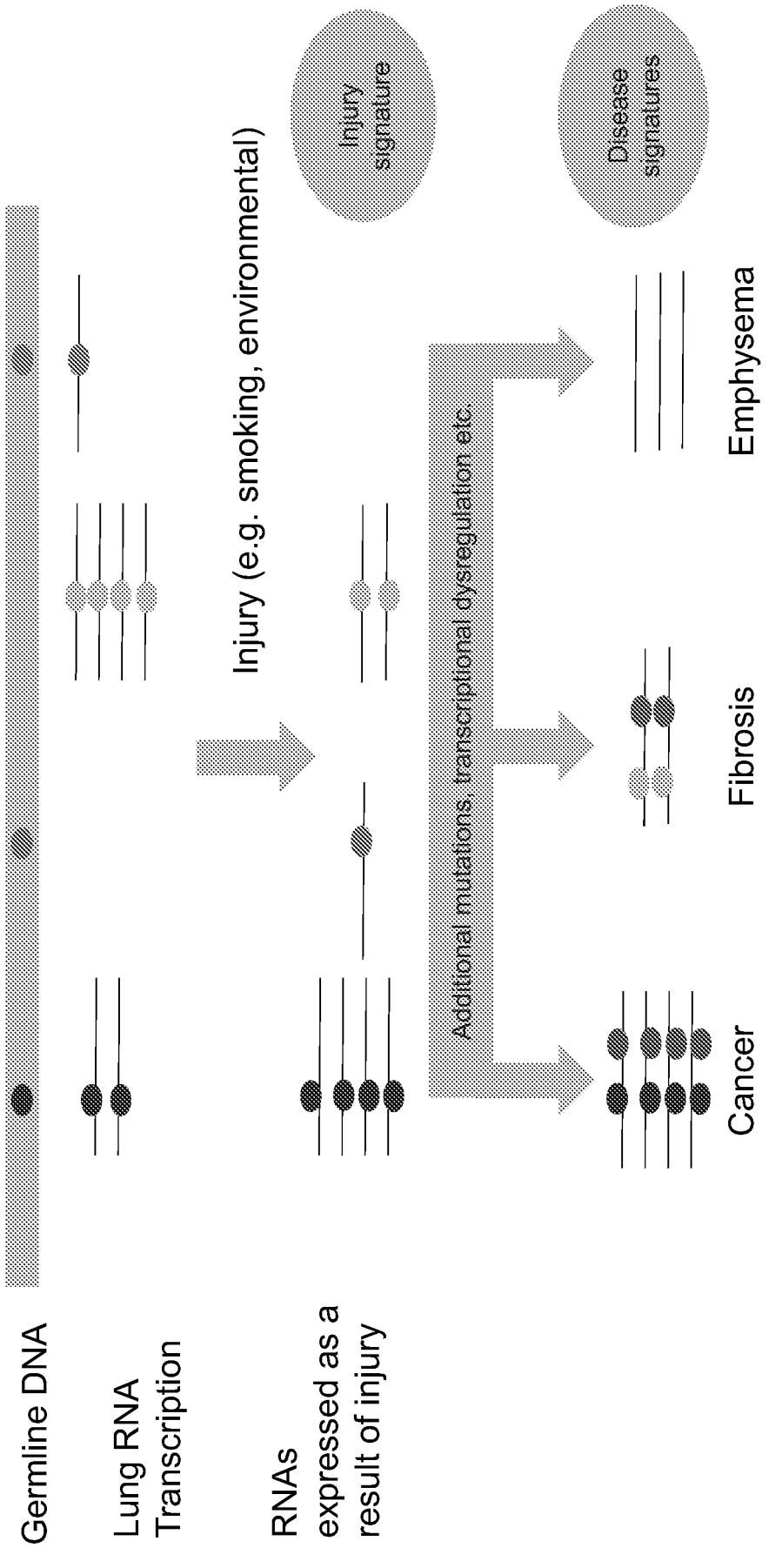

Germline DNA
Lung RNA Transcription
RNAs expressed as a result of injury
Injury (e.g. smoking, environmental)
Injury signature
Additional mutations, transcriptional dysregulation etc
Disease signatures
Emphysema
Fibrosis
Cancer

FIG. 9

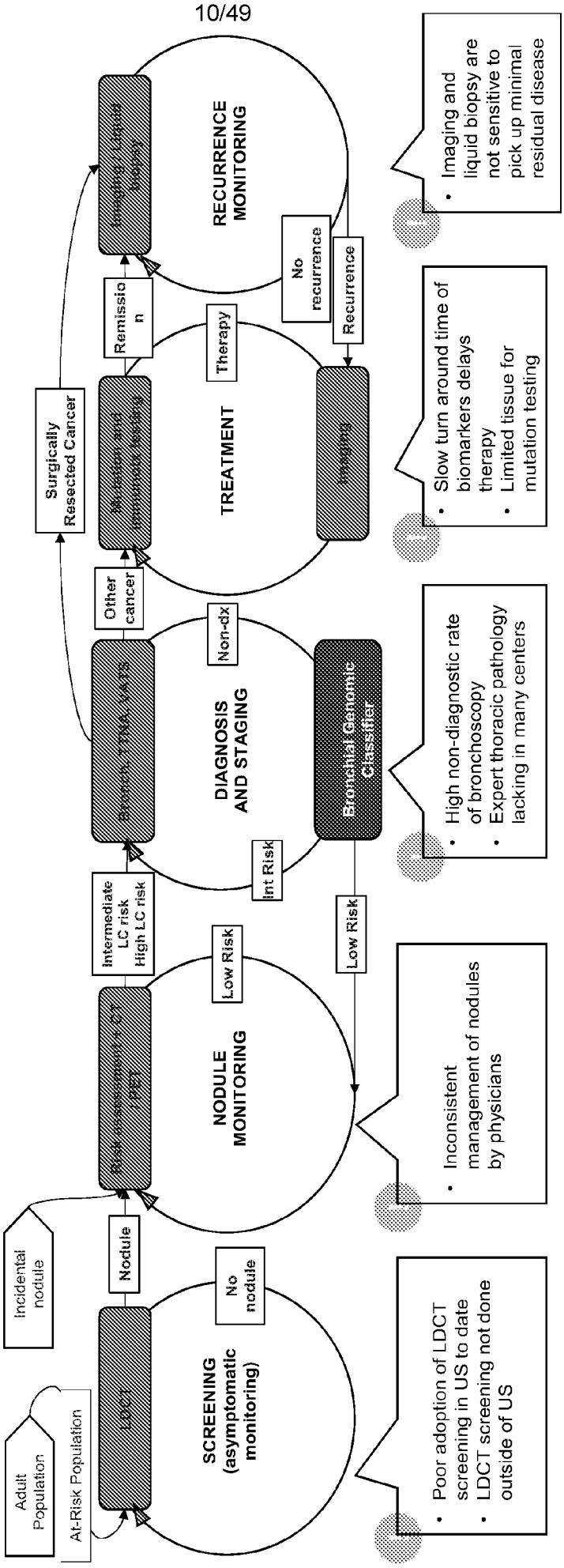

Adult Population
At-Risk Population
LDCT
SCREENING (asymptomatic monitoring)
No nodule
Nodule
Incidental nodule
Risk assessment / CT / PET
NODULE MONITORING
Low Risk
Intermediate LC risk
High LC risk
Low Risk
Int Risk
Bronch, TTNA, VATS
DIAGNOSIS AND STAGING
Non-dx
Bronchial Genomic Classifier
Other cancer
Surgically Resected Cancer
Mutation and immunoscr testing
TREATMENT
Therapy
Imaging
Remission
Imaging / Liquid biopsy
RECURRENCE MONITORING
No recurrence
Recurrence
• Poor adoption of LDCT screening in US to date
• LDCT screening not done outside of US
• Inconsistent management of nodules by physicians
• High non-diagnostic rate of bronchoscopy
• Expert thoracic pathology lacking in many centers
• Slow turn around time of biomarkers delays therapy
• Limited tissue for mutation testing
• Imaging and liquid biopsy are not sensitive to pick up minimal residual disease

FIG. 10

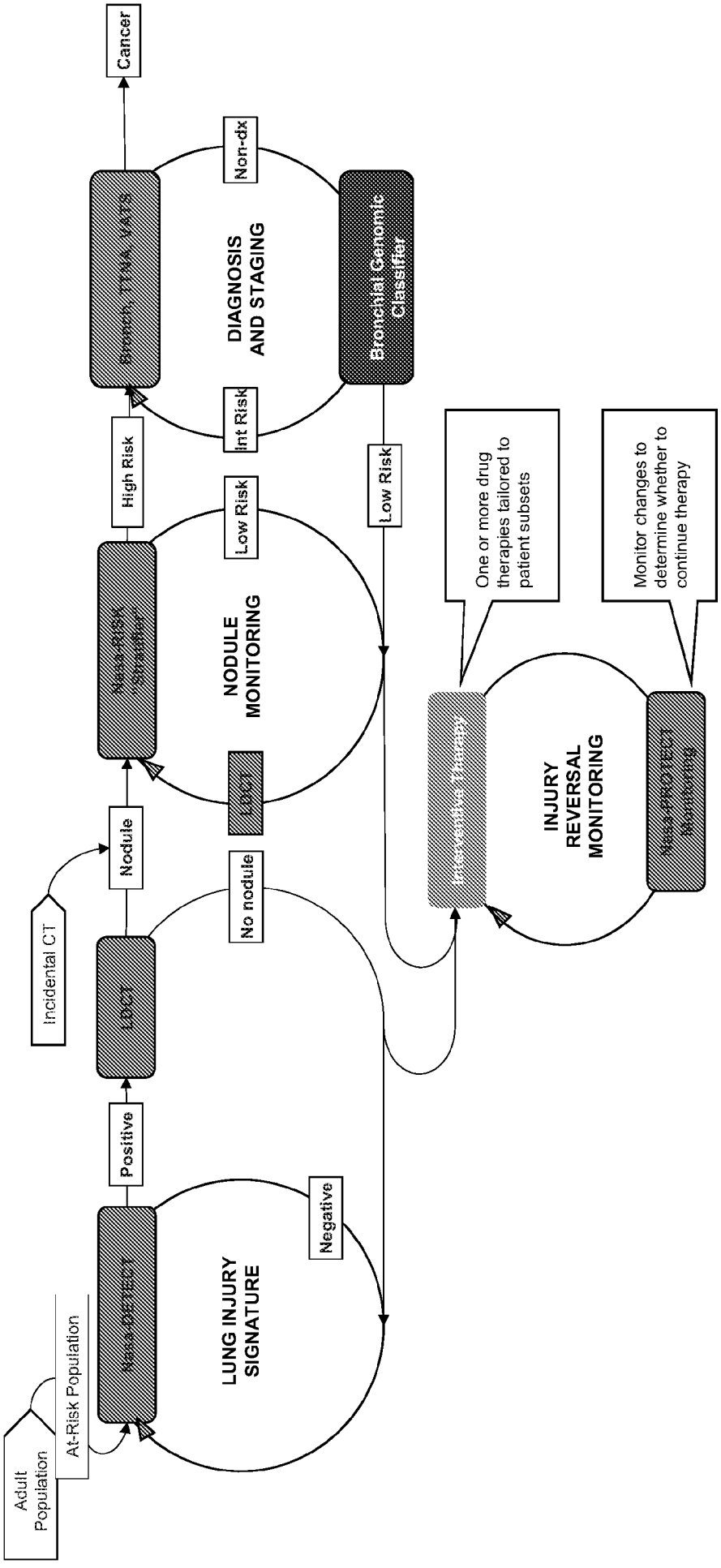

Adult Population
At-Risk Population
Nasa DETECT
Positive
Negative
LUNG INJURY SIGNATURE
LDCT
Incidental CT
Nodule
No nodule
Nasa RISK Stratifier
LDCT
Low Risk
High Risk
NODULE MONITORING
Bronch, TTNA, VATS
Non-dx
Int Risk
DIAGNOSIS AND STAGING
Cancer
Bronchial Genomic Classifier
Low Risk
Intervention Therapy
One or more drug therapies tailored to patient subsets
Nasa PROTECT Monitoring
Monitor changes to determine whether to continue therapy
INJURY REVERSAL MONITORING

FIG. 11a

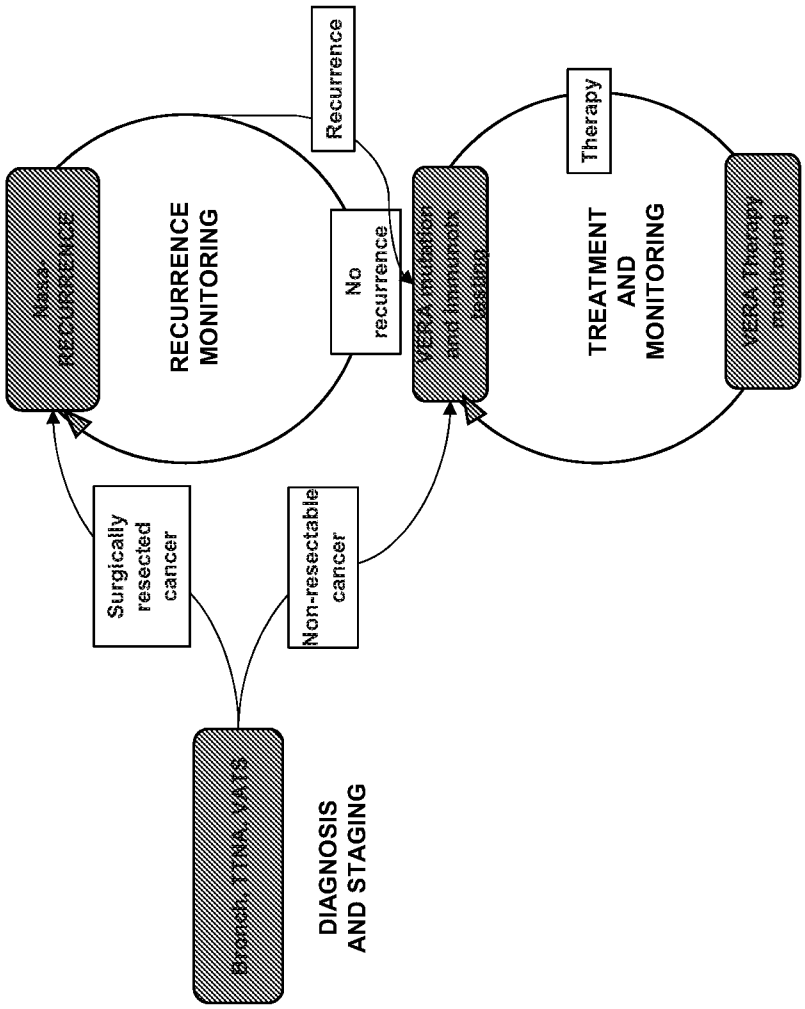
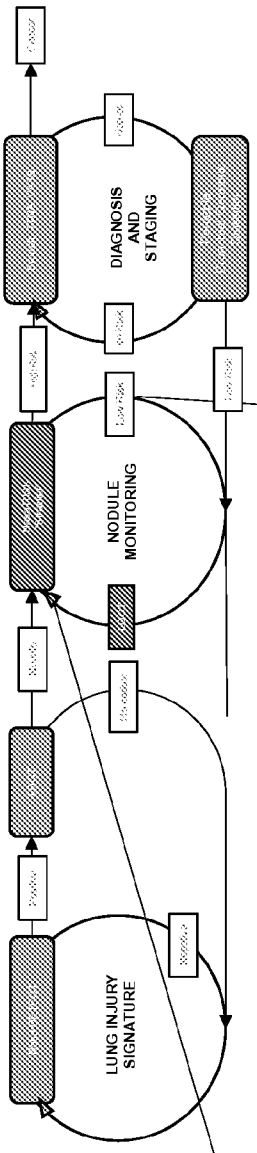

DIAGNOSIS AND STAGING
Branch, TTNA, VATS
Surgically resected cancer
Non-resectable cancer
RECURRENCE MONITORING
Vera RECURRENCE
Recurrence
No recurrence
TREATMENT AND MONITORING
VERA initiation and immune testing
Therapy
VERA Therapy monitoring

FIG. 11b

Parameter	Characteristic
Description	Detecting "lung injury" in "at-risk" populations
Test results	• Positive: Recommend follow-up investigation with LDCT; nodule negative by LDCT would be candidate for interventive therapy • Negative: Monitor with Nasa-DETECT at some interval (2 years?)
Utility	• Optimize the LDCT lung cancer screening funnel • Augment LDCT with a more specific initial screening tool • Detect patients who could benefit from interventive therapy
Performance requirements	• Sensitive for "injury," would need to correlate to earlier detection of disease
Study test population	• Patients determined to be at risk for lung cancer (heritable or acquired)

Challenges:
- Large trial size and long duration to capture enough events for low prevalence test population
- High performance requirements
- Keeping cost per test low on a scalable platform
- Going through FDA / CMS dual review process
- Managing global potential

FIG. 12

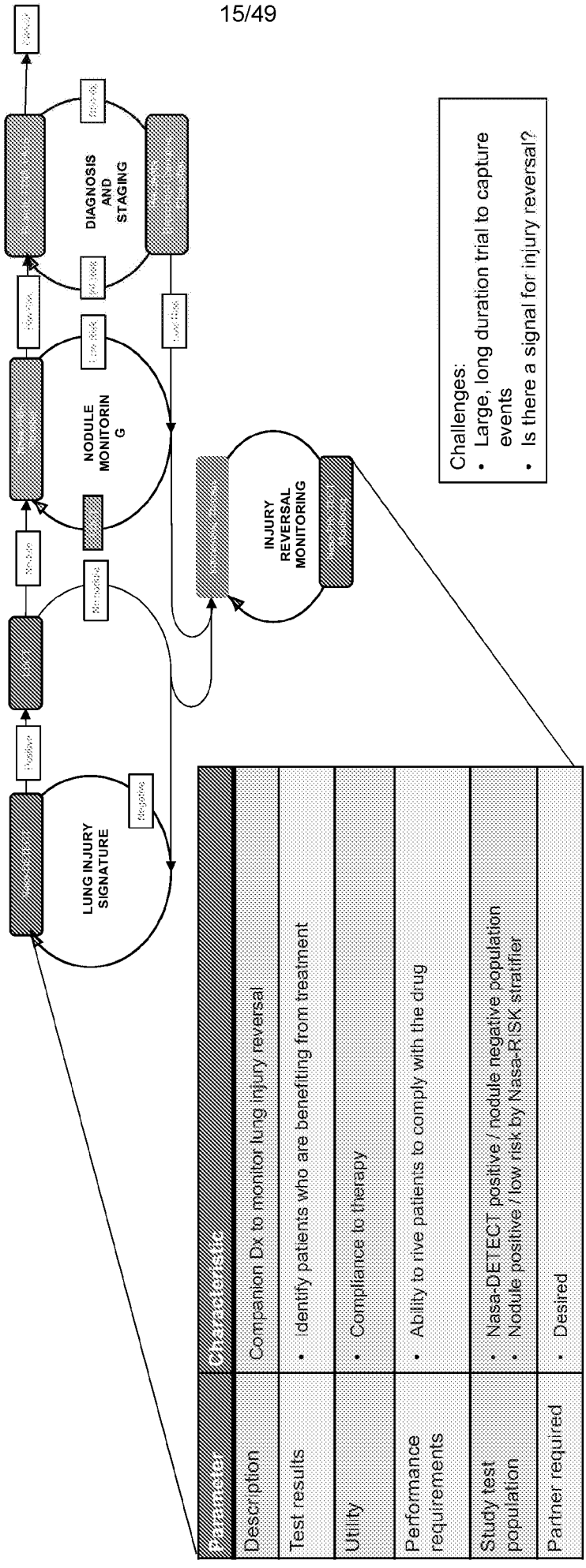


Parameter	Characteristic
Description	Nodule risk stratification to guide next steps
Test results	• Low risk: Surveillance or candidate for interventive therapy • Indeterminate: Use clinical judgment • High risk: Proceed to biopsy
Utility	• Minimize indeterminate pulmonary nodules • Accelerate biopsy in those who need while avoiding in those that do not
Performance requirements	• "3-state classifier" ▪ High SENS to move patients to low risk of cancer ▪ High SPEC to move patients to high risk of cancer
Study test population	• Patients with pulmonary lesion

Opportunities:
- Classifier developed on NGS platform and could include radiological features

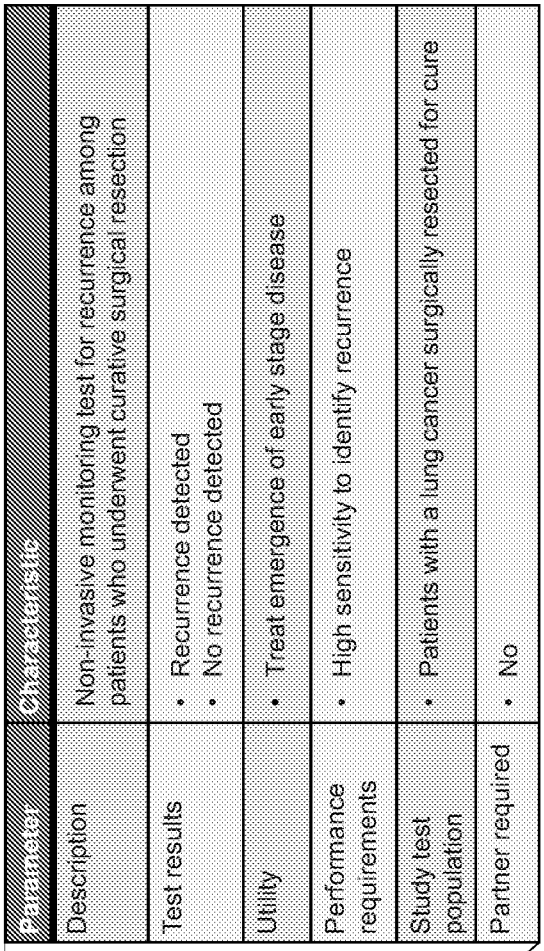
Challenges:
- Improving on clinical and radiologic factors in a clinically meaningful way
- Competitive (Oncocyte, Indi)
- Demonstrating utility may be difficult

FIG. 13



Parameter	Characteristic
Description	Companion Dx to monitor lung injury reversal
Test results	• Identify patients who are benefiting from treatment
Utility	• Compliance to therapy
Performance requirements	• Ability to rive patients to comply with the drug
Study test population	• Nasa-DETECT positive / nodule negative population • Nodule positive / low risk by Nasa-RISK stratifier
Partner required	• Desired

FIG. 14



Parameter	Characteristic
Description	Non-invasive monitoring test for recurrence among patients who underwent curative surgical resection
Test results	• Recurrence detected • No recurrence detected
Utility	• Treat emergence of early stage disease
Performance requirements	• High sensitivity to identify recurrence
Study test population	• Patients with a lung cancer surgically resected for cure
Partner required	• No

Challenges:
- Dependent on whether marker is field of injury or field cancerization (50 person NCI study currently investigating)
- Could be competitive with liquid biopsy

FIG. 15

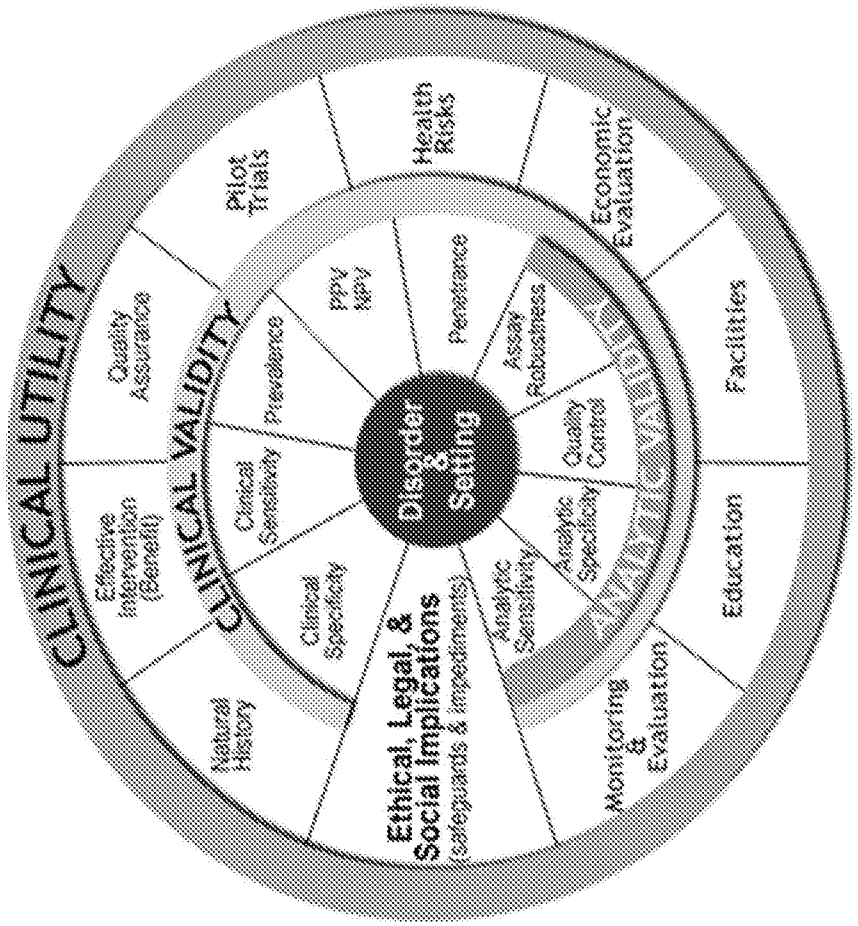

CLINICAL UTILITY
Pilot Trials
Health Risks
Economic Evaluation
Facilities
Education
Monitoring & Evaluation
Ethical, Legal, & Social Implications
(safeguards & impediments)
Natural History
Effective Intervention (Benefit)
Quality Assurance
CLINICAL VALIDITY
Clinical Specificity
Clinical Sensitivity
Prevalence
PPV NPV
Prevalence
Assay Robustness
Quality Control
Analytic Specificity
Analytic Sensitivity
ANALYTIC VALIDITY
Disorder & Setting

FIG. 16

Broad enrollment creates a rich biorepository
Enroll from multiple countries
Subpopulations from cohorts drive specific classifier development and validation
Potential for classifiers for specific populations and exposures (e.g. environmental pollution in China, genetic predisposition)
Screen
Diagnose
Treat
Monitor
Product 1
Product 2
Product 3
Product 4
Data collected annually:
Nasal brushing (+ blood, CT imaging)
Clinical follow up
Therapies, imaging, pathology reports, final diagnoses
Samples

FIG. 17

Cohort	N Planned (current)	Position in clinical flow	Prevalence (estimated)	Clinical sites	Inclusion criteria	Nasal brush	Biopsy-at brush	Blood	CT	Follow-up duration (months)
AEGIS	2000	At bronchoscopy	75%	• 28 sites, mostly academic	• Clinically indicated bronchoscopy for lung cancer dx • Age >18 • Ever smoker (>100 cigs in life)	✓	✓	✓		12
DECAMP1	600 (350)	At bronchoscopy	30-35%	• 11 military and VA hospitals • 3 academic	• Clinically indicated bronchoscopy for lung cancer dx • 7-30 mm pulmonary nodule • Current / former smoker (>20 PY) • Age >45 yrs	✓	✓	✓	✓	24
LTP2 (NCI EDRN)	200 (100)	Post CT	20-25%	• 4 sites (BU, UCLA, Vanderbilt, NYU)	• Diagnostic work up for IPN • 7-23mm pulmonary nodule • Current/former smoker >30 pack-yrs • Age > 50 yrs	✓		✓	✓	24
DECAMP2	800 (450)	At screening	1.5% annual incidence	• 11 military and VA sites • 3 academic	• Age 50-79 • Current/former smoker (20 pack yrs.) • COPD or first degree relative with lung cancer	✓ (0, 12, 24 months)	✓ (0,24 months)	✓ (0,12,24 months)	✓ (annual)	48+
Laney	3000 (1500)	At screening	1% annual incidence	• Laney / affiliate	• Enrolled in Laney screening program • Meet NCCN 2 criteria for screening	✓			✓	48+

Current VCYT access to sample

No current VCYT access to sample

FIG. 18

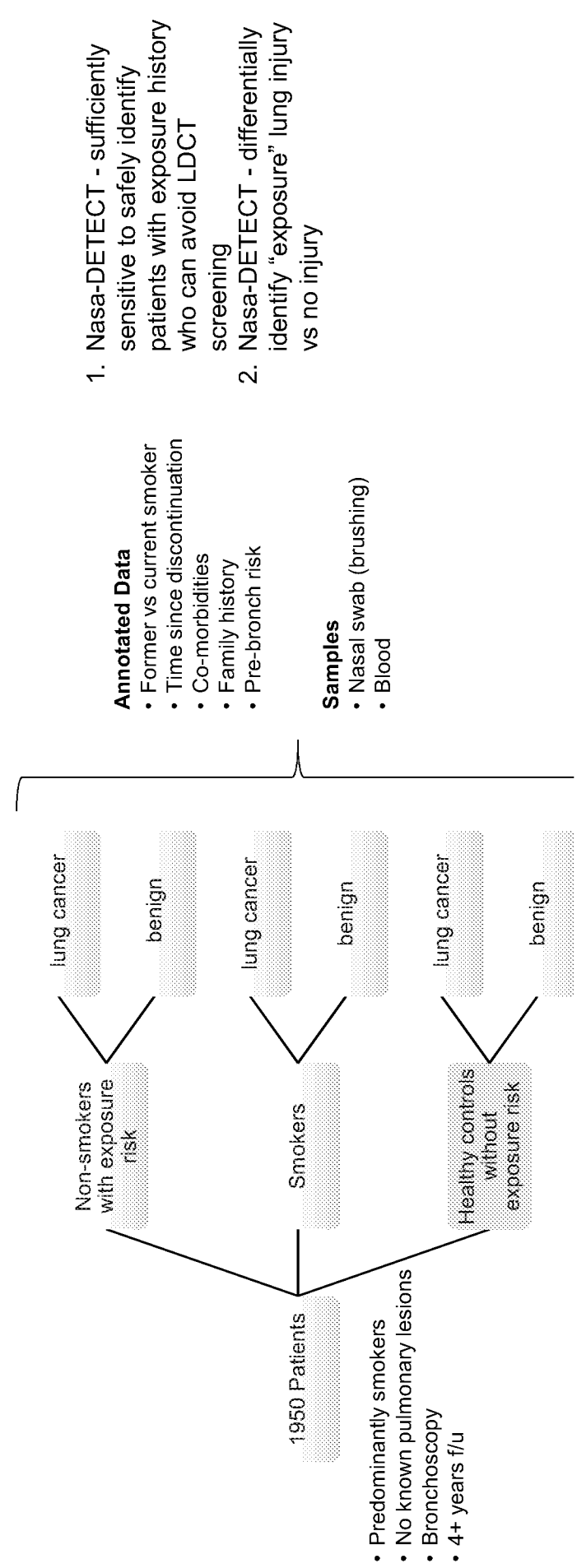

1. Nasa-DETECT - sufficiently sensitive to safely identify patients with exposure history who can avoid LDCT screening
2. Nasa-DETECT - differentially identify "exposure" lung injury vs no injury
Annotated Data
• Former vs current smoker
• Time since discontinuation
• Co-morbidities
• Family history
• Pre-bronch risk
Samples
• Nasal swab (brushing)
• Blood
1950 Patients
• Predominantly smokers
• No known pulmonary lesions
• Bronchoscopy
• 4+ years f/u
Non-smokers with exposure risk
Smokers
Healthy controls without exposure risk
lung cancer
benign
lung cancer
benign
lung cancer
benign

FIG. 19

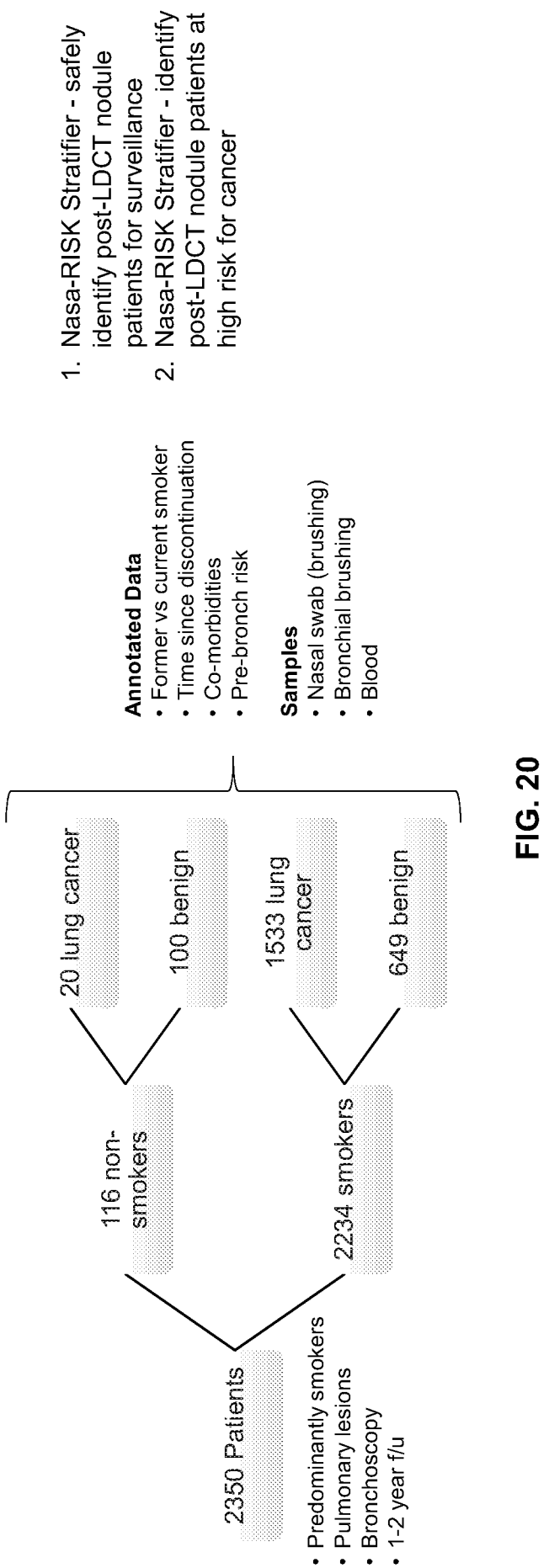

1. Nasa-RISK Stratifier - safely identify post-LDCT nodule patients for surveillance
2. Nasa-RISK Stratifier - identify post-LDCT nodule patients at high risk for cancer
Annotated Data
• Former vs current smoker
• Time since discontinuation
• Co-morbidities
• Pre-bronch risk
Samples
• Nasal swab (brushing)
• Bronchial brushing
• Blood
20 lung cancer
100 benign
1533 lung cancer
649 benign
116 non-smokers
2234 smokers
2350 Patients
• Predominantly smokers
• Pulmonary lesions
• Bronchoscopy
• 1-2 year f/u

FIG. 20

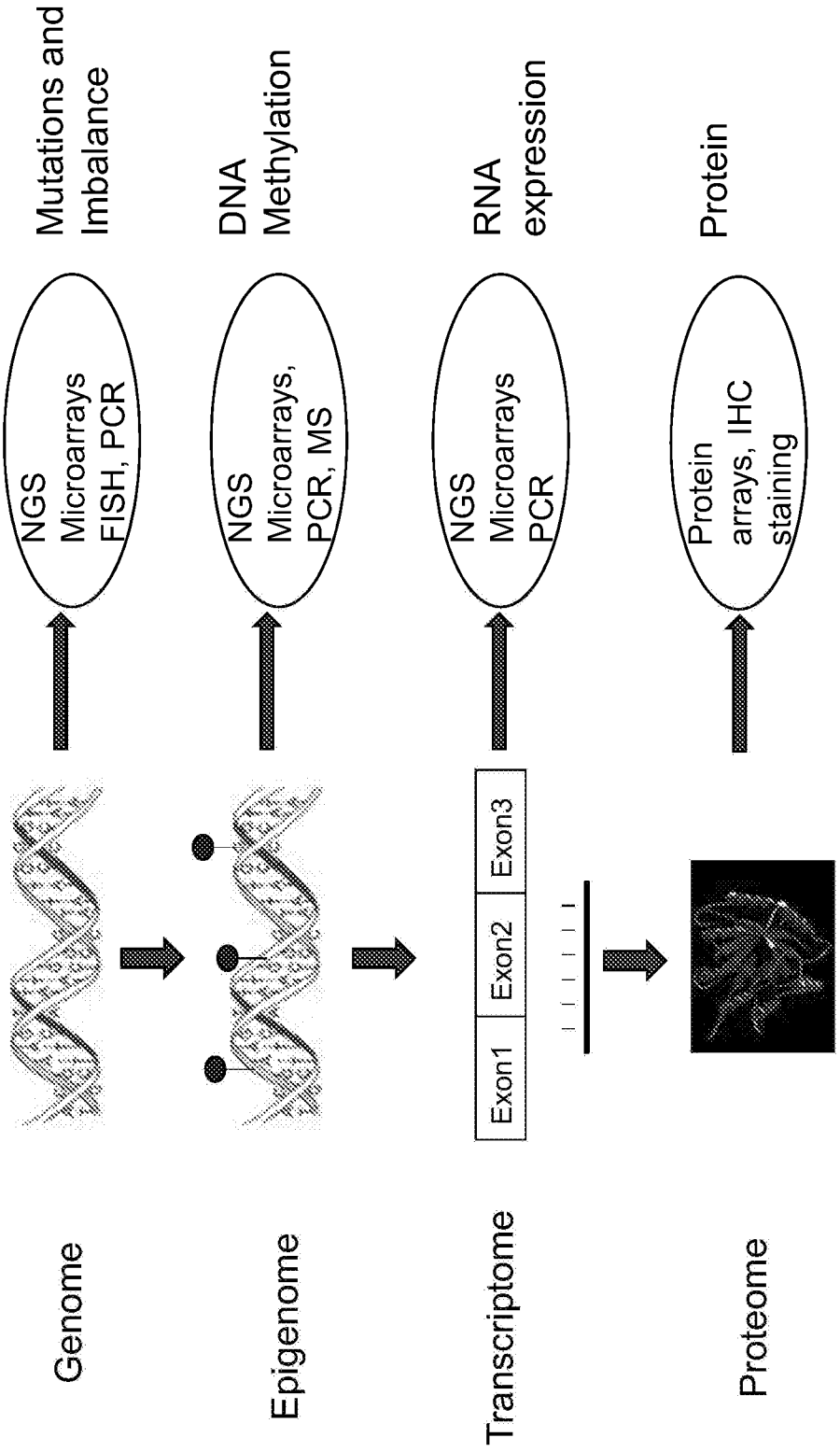

Genome
Epigenome
Transcriptome
Proteome
Exon1
Exon2
Exon3
NGS
Microarrays
FISH, PCR
NGS
Microarrays,
PCR, MS
NGS
Microarrays
PCR
Protein
arrays, IHC
staining
Mutations and
Imbalance
DNA
Methylation
RNA
expression
Protein

FIG. 21

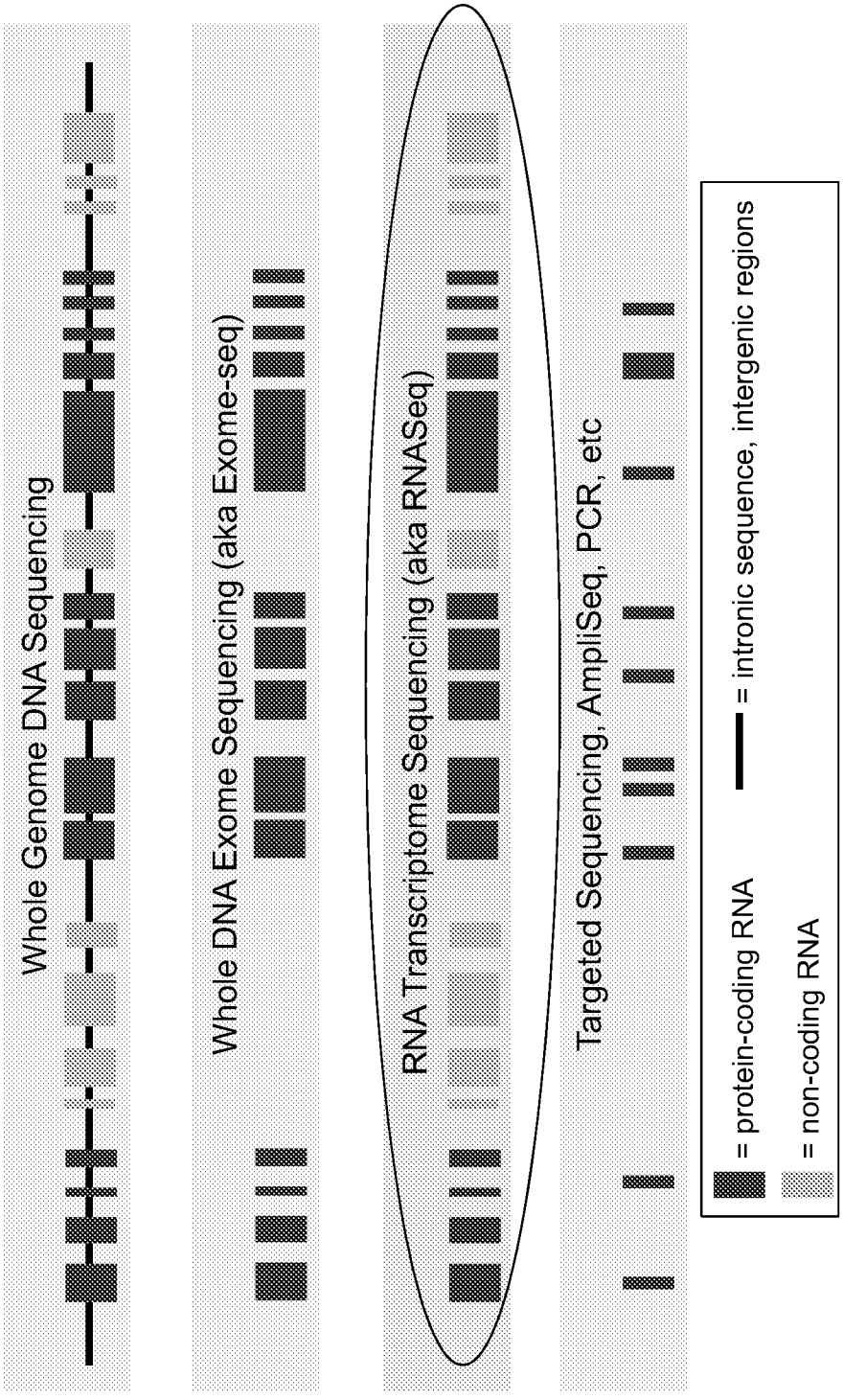

Whole Genome DNA Sequencing
Whole DNA Exome Sequencing (aka Exome-seq)
RNA Transcriptome Sequencing (aka RNASeq)
Targeted Sequencing, AmpliSeq, PCR, etc
= protein-coding RNA
= intronic sequence, intergenic regions
= non-coding RNA

FIG. 22

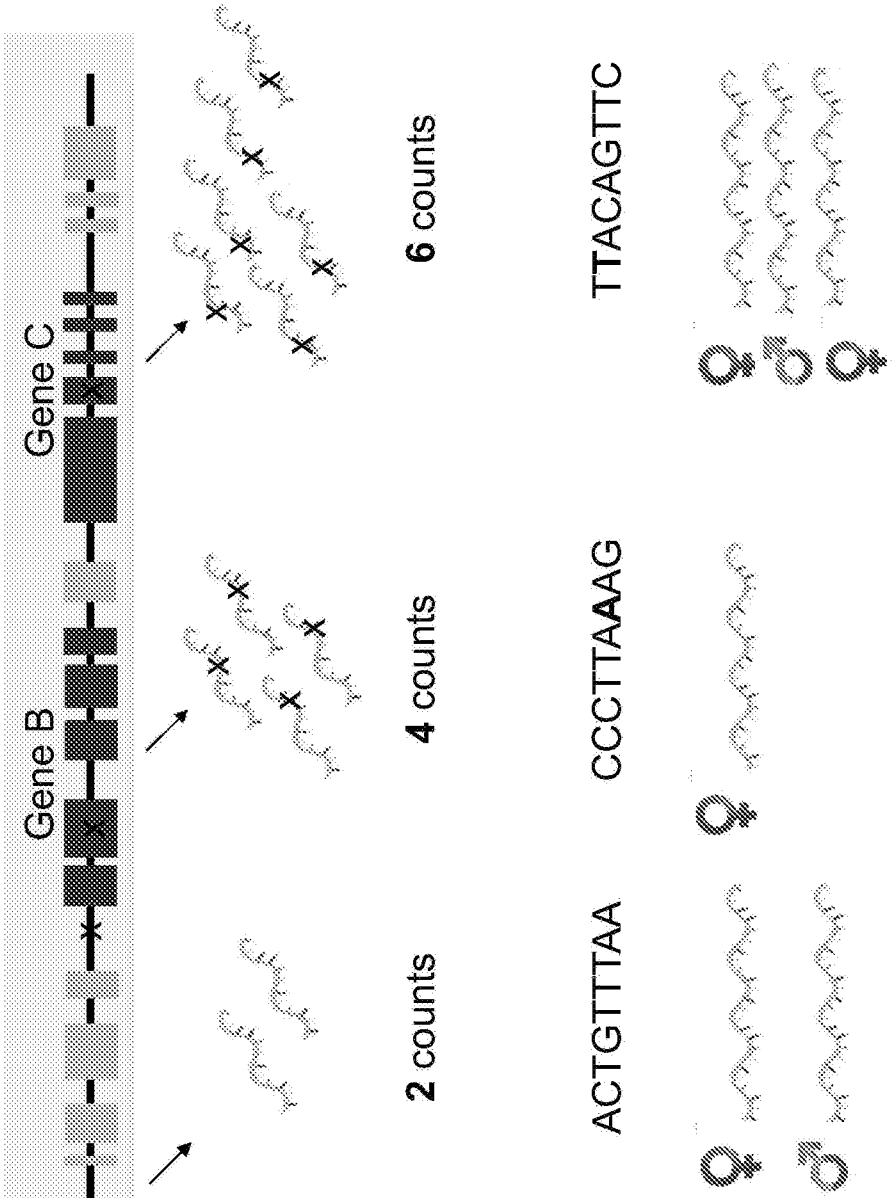

Gene A
Gene B
Gene C
Transcription into RNA
Expression Level ie **counts** of each transcript
2 counts
4 counts
6 counts
Variants ie **sequence** of each transcript
ACTGTTTAA
CCCTTAAAG
TTACAGTTC
How many **Chromosome Copies** ie LOH

FIG. 23

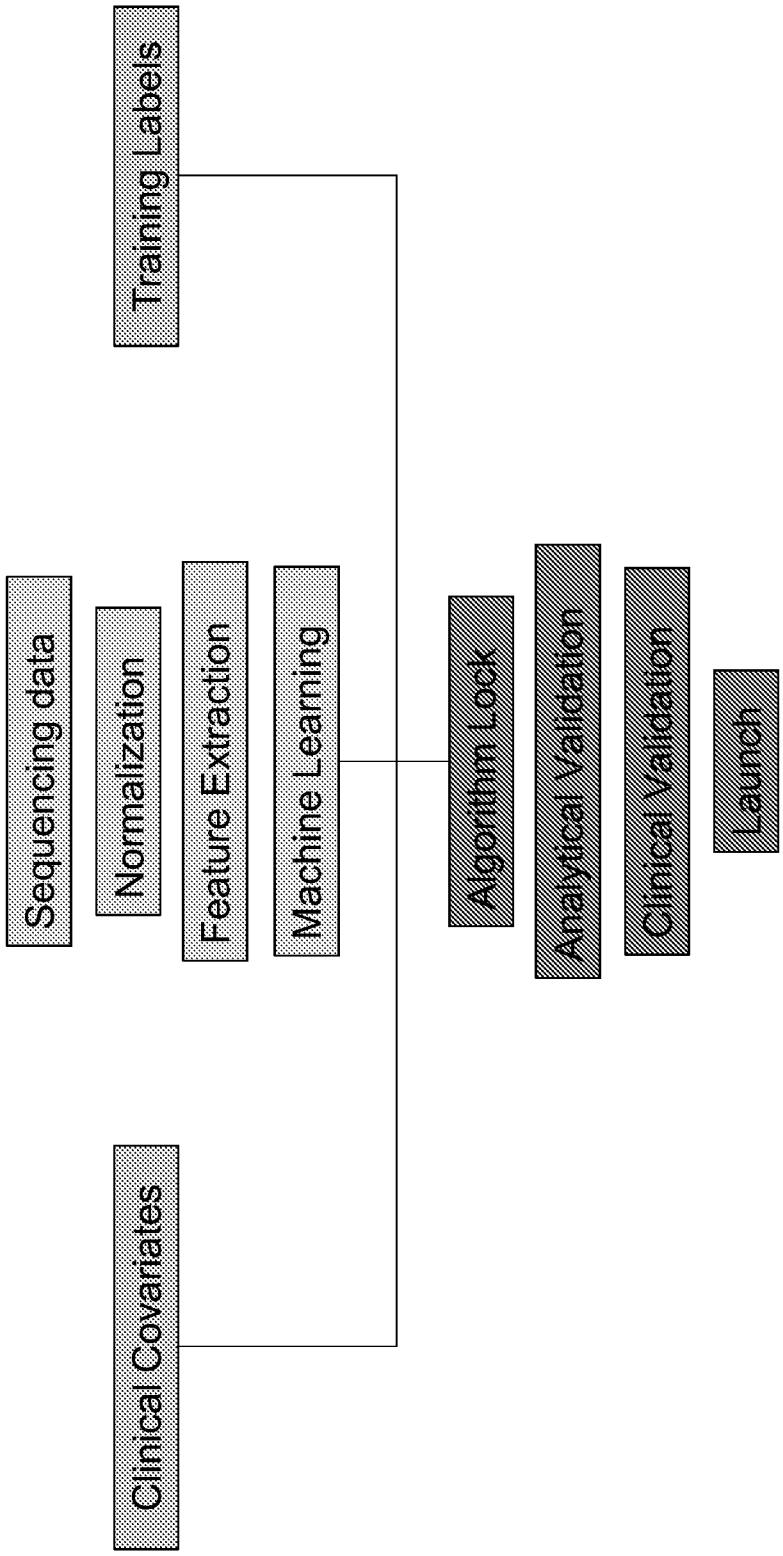

Training Labels
Sequencing data
Normalization
Feature Extraction
Machine Learning
Clinical Covariates
Algorithm Lock
Analytical Validation
Clinical Validation
Launch

FIG. 24

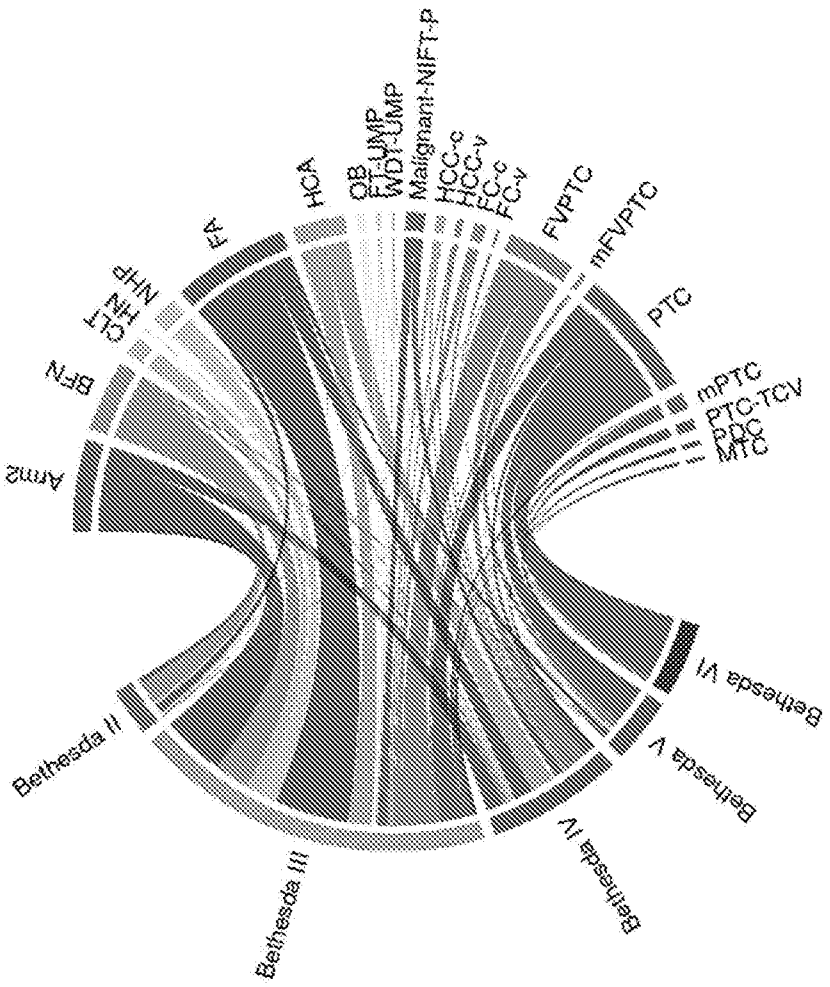


FIG. 25

*This plot depicts the subset of N=507 samples out of a total N=634 in training set for which we have both Bethesda category and histology subtype

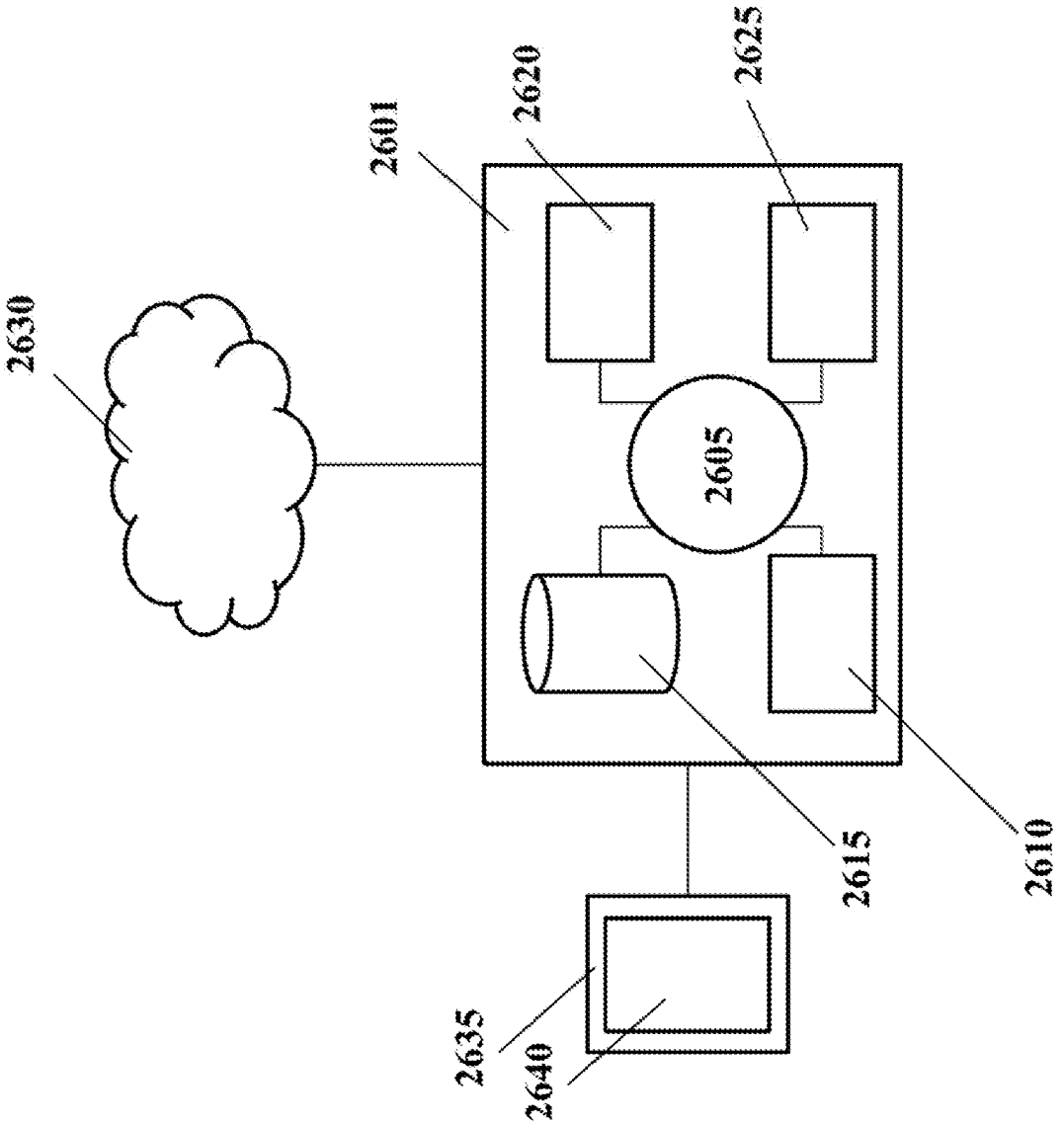


FIG. 26

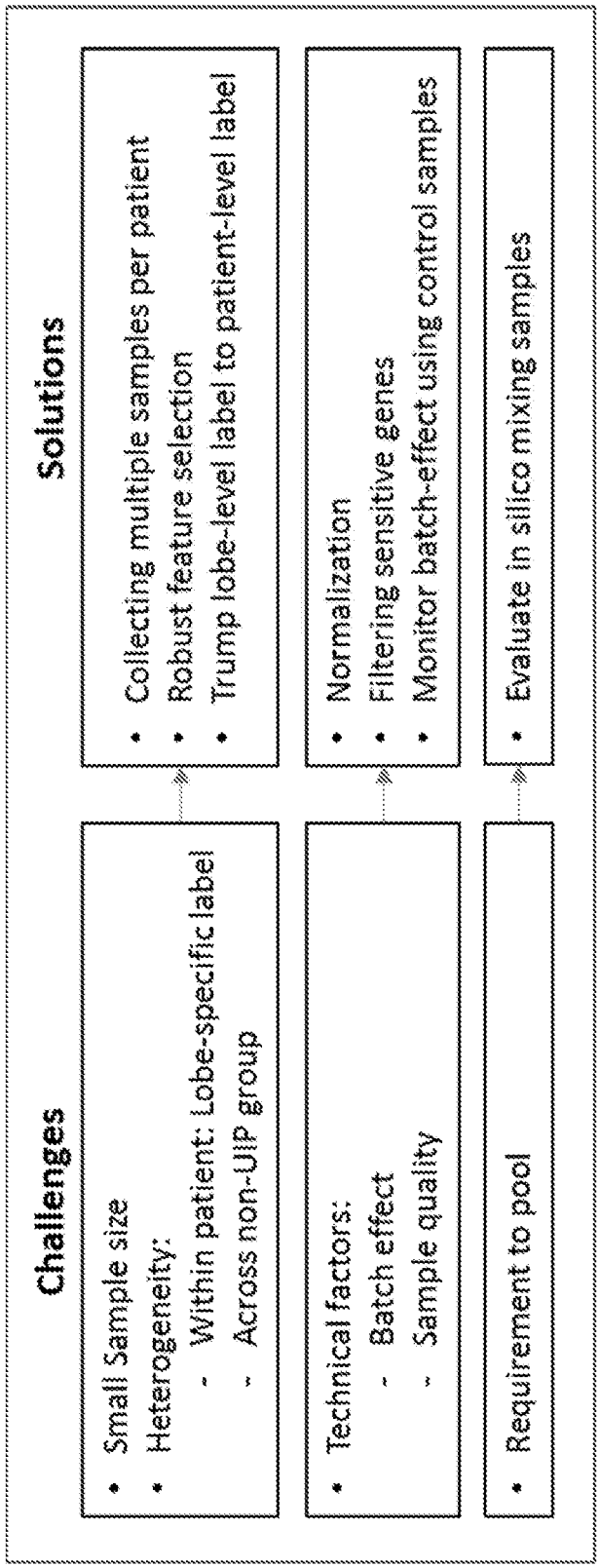

Challenges
Solutions
Small Sample size
Heterogeneity:
- Within patient: Lobe-specific label
- Across non-UIP group
Collecting multiple samples per patient
Robust feature selection
Trump lobe-level label to patient-level label
Technical factors:
- Batch effect
- Sample quality
Normalization
Filtering sensitive genes
Monitor batch-effect using control samples
Requirement to pool
Evaluate in silico mixing samples

FIG. 27

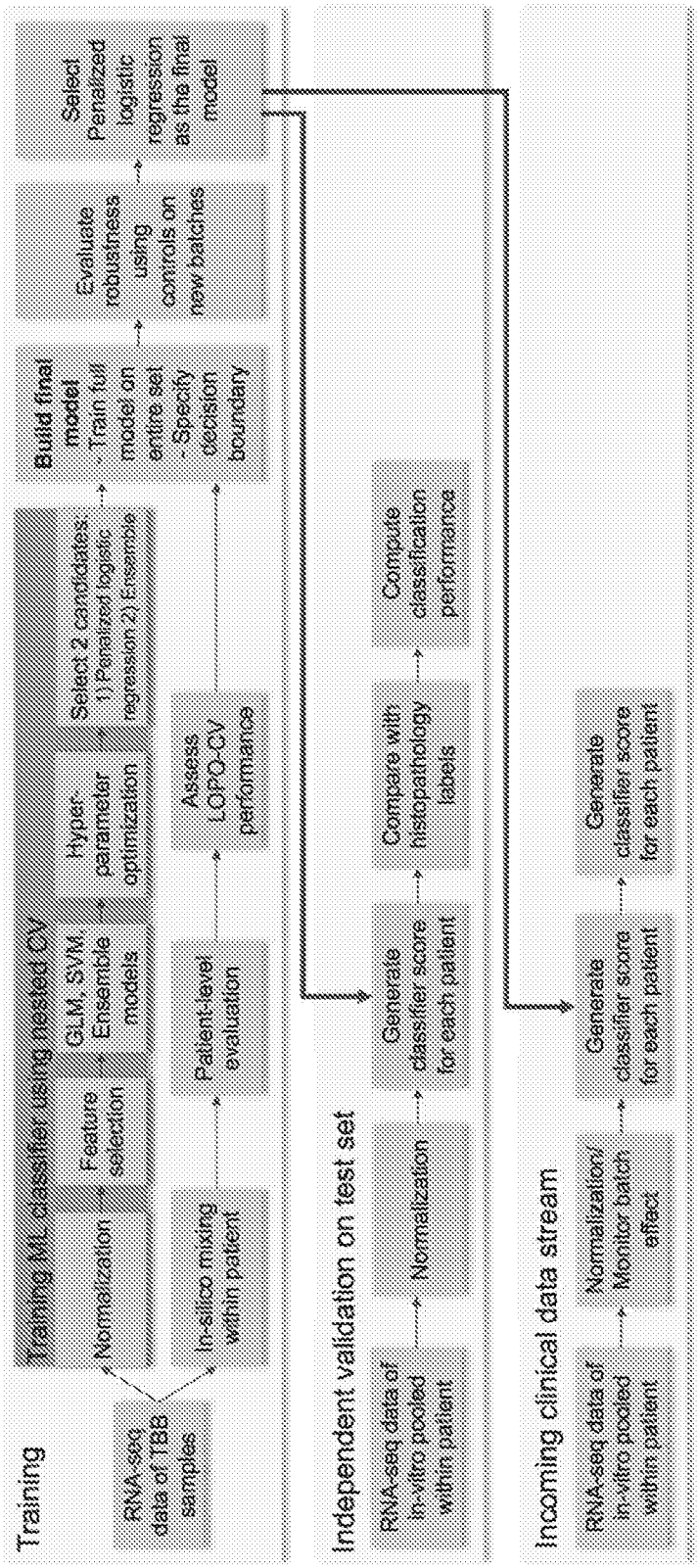

Training
RNA-seq data of TBB samples
Normalization
In-silico mixing within patient
Training ML classifier using nested CV
Feature selection
GLM, SVM, Ensemble models
Hyper-parameter optimization
Select 2 candidates: 1) Penalized logistic regression 2) Ensemble
Patient-level evaluation
Assess LOPO-CV performance
Build final model - Train full model on entire set - Specify decision boundary
Evaluate robustness using controls on new batches
Select Penalized logistic regression as the final model
Independent validation on test set
RNA-seq data of in-vitro pooled within patient
Normalization
Generate classifier score for each patient
Compare with histopathology labels
Compute classification performance
Incoming clinical data stream
RNA-seq data of in-vitro pooled within patient
Normalization/ Monitor batch effect
Generate classifier score for each patient
Generate classifier score for each patient

FIG. 28

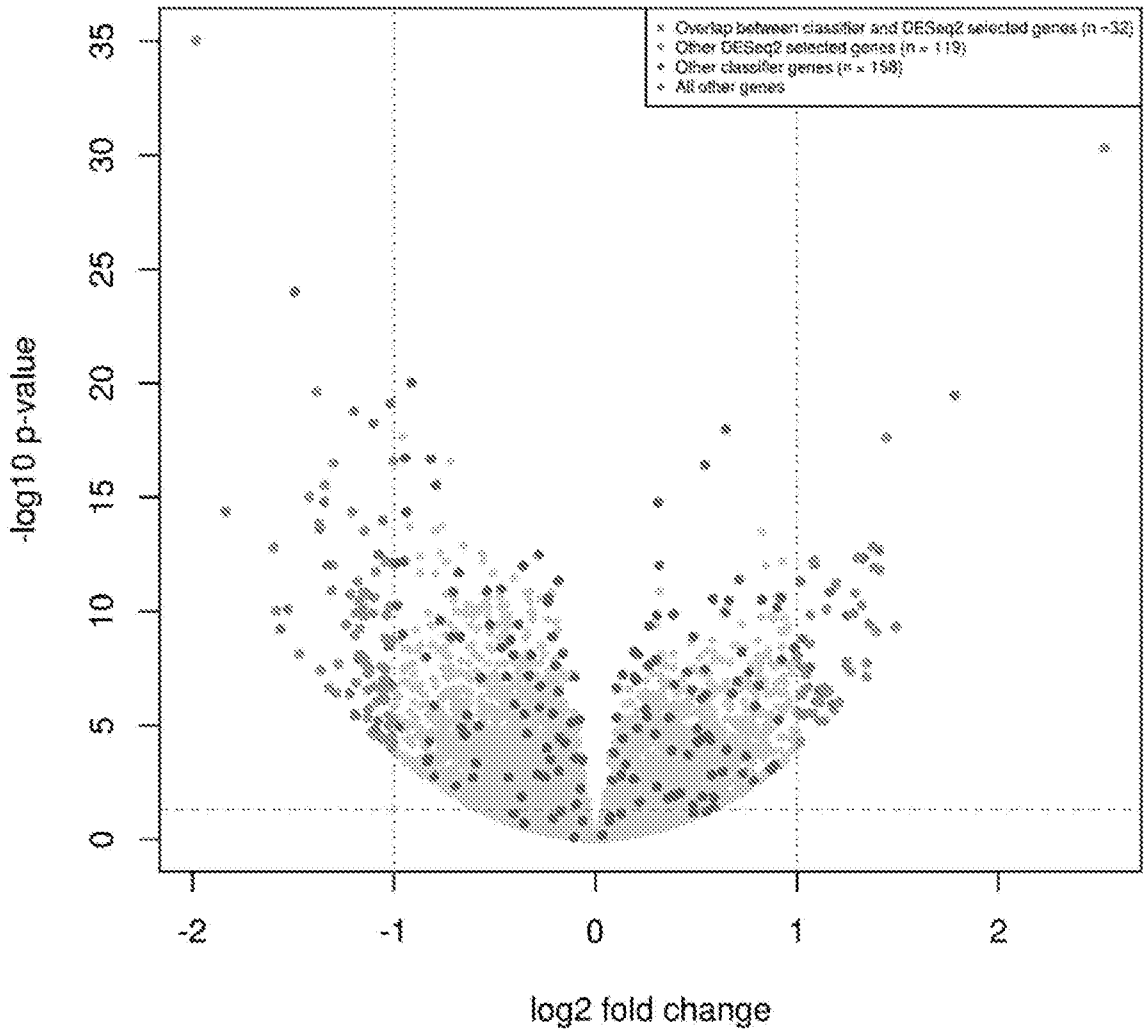

Overlap between classifier and DESeq2 selected genes (n = 32)
Other DESeq2 selected genes (n = 119)
Other classifier genes (n = 158)
All other genes
-log10 p-value
0
5
10
15
20
25
30
35
-2
-1
0
1
2
log2 fold change

FIG. 29

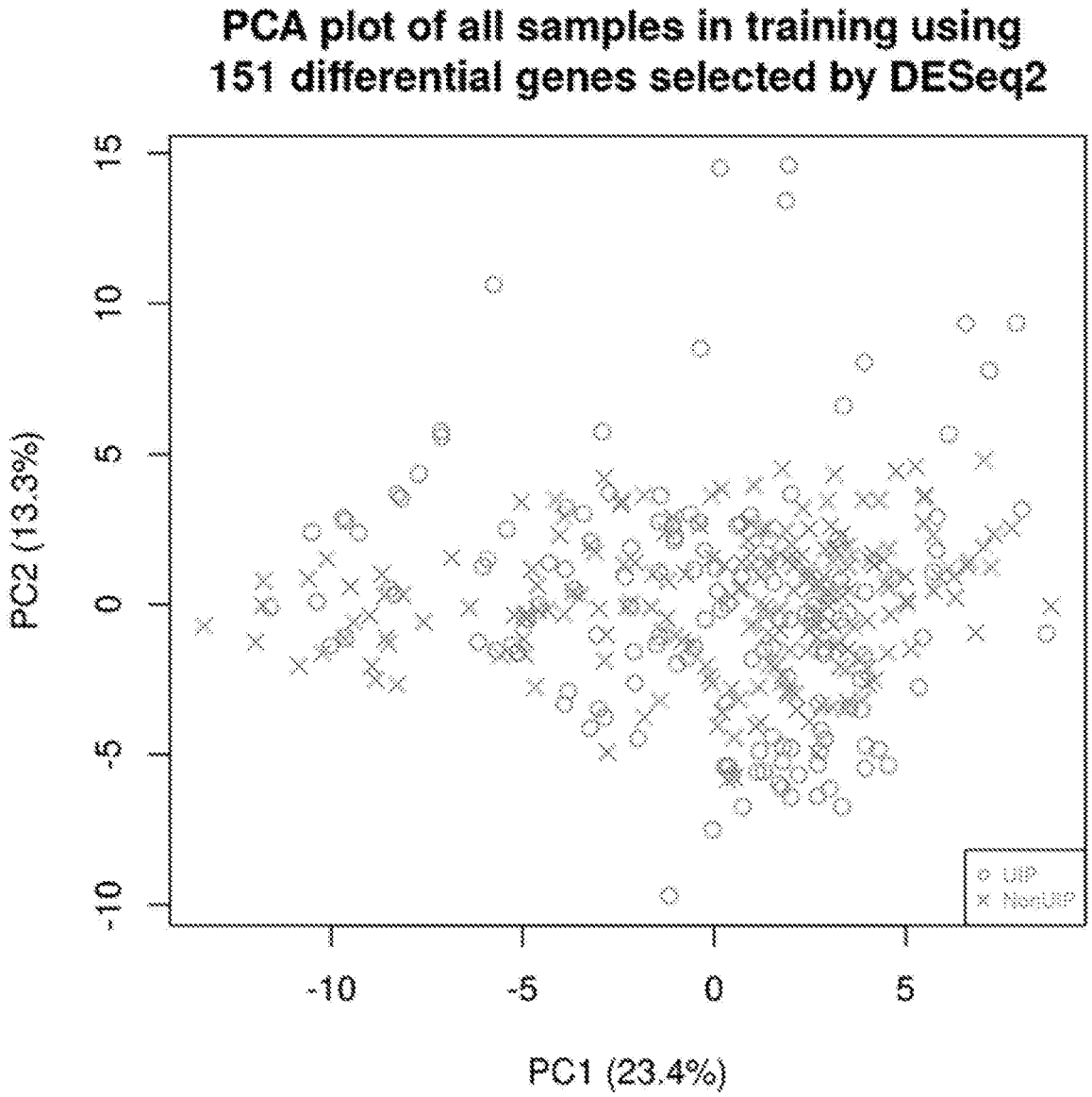

PCA plot of all samples in training using
151 differential genes selected by DESeq2
PC2 (13.3%)
PC1 (23.4%)
15
10
5
0
-5
-10
-10
-5
0
5
UIP
NonUIP

FIG. 30

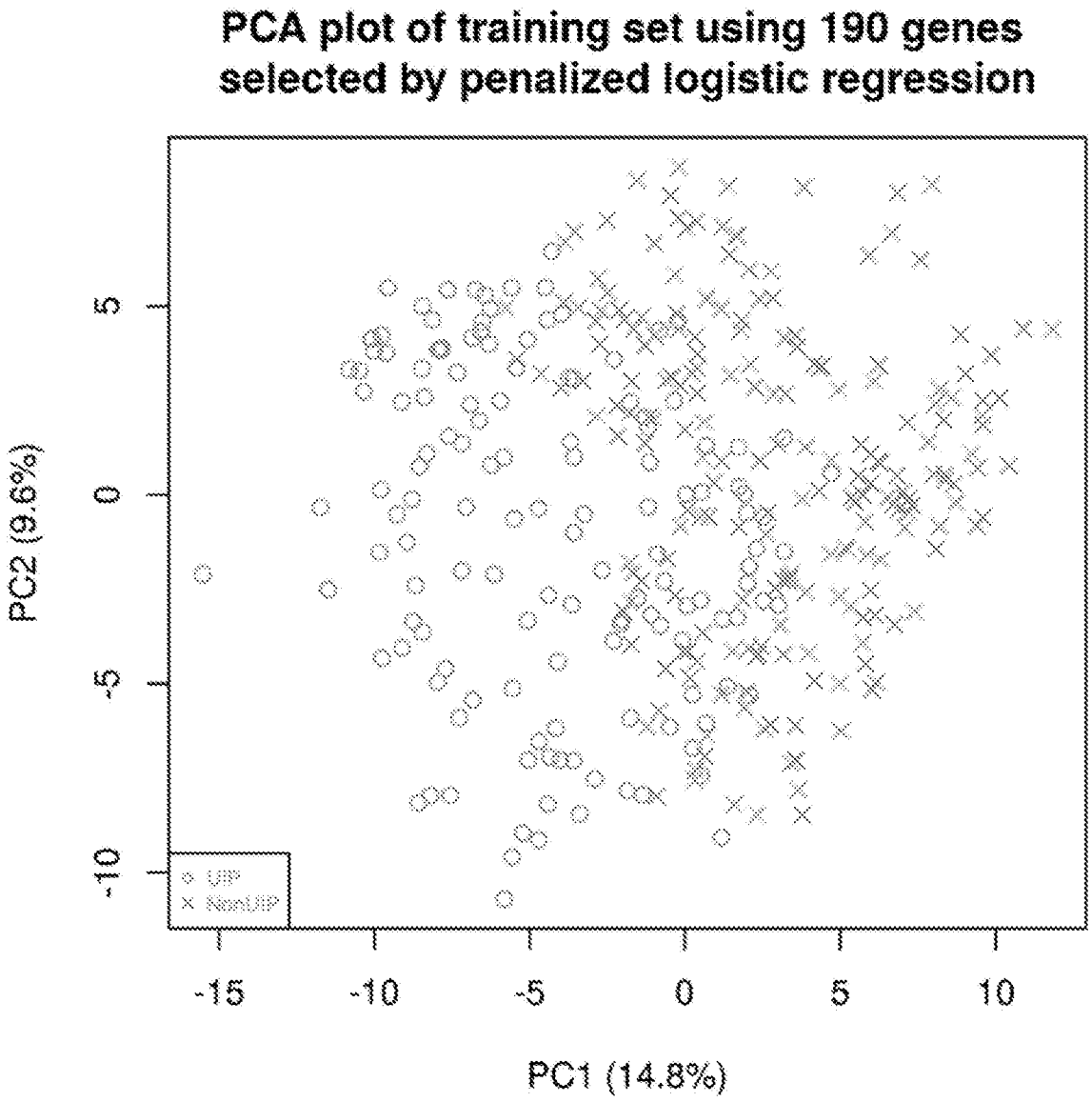

PCA plot of training set using 190 genes selected by penalized logistic regression
PC2 (9.6%)
PC1 (14.8%)
5
0
-5
-10
-15
-10
-5
0
5
10
UIP
NonUIP

FIG. 31

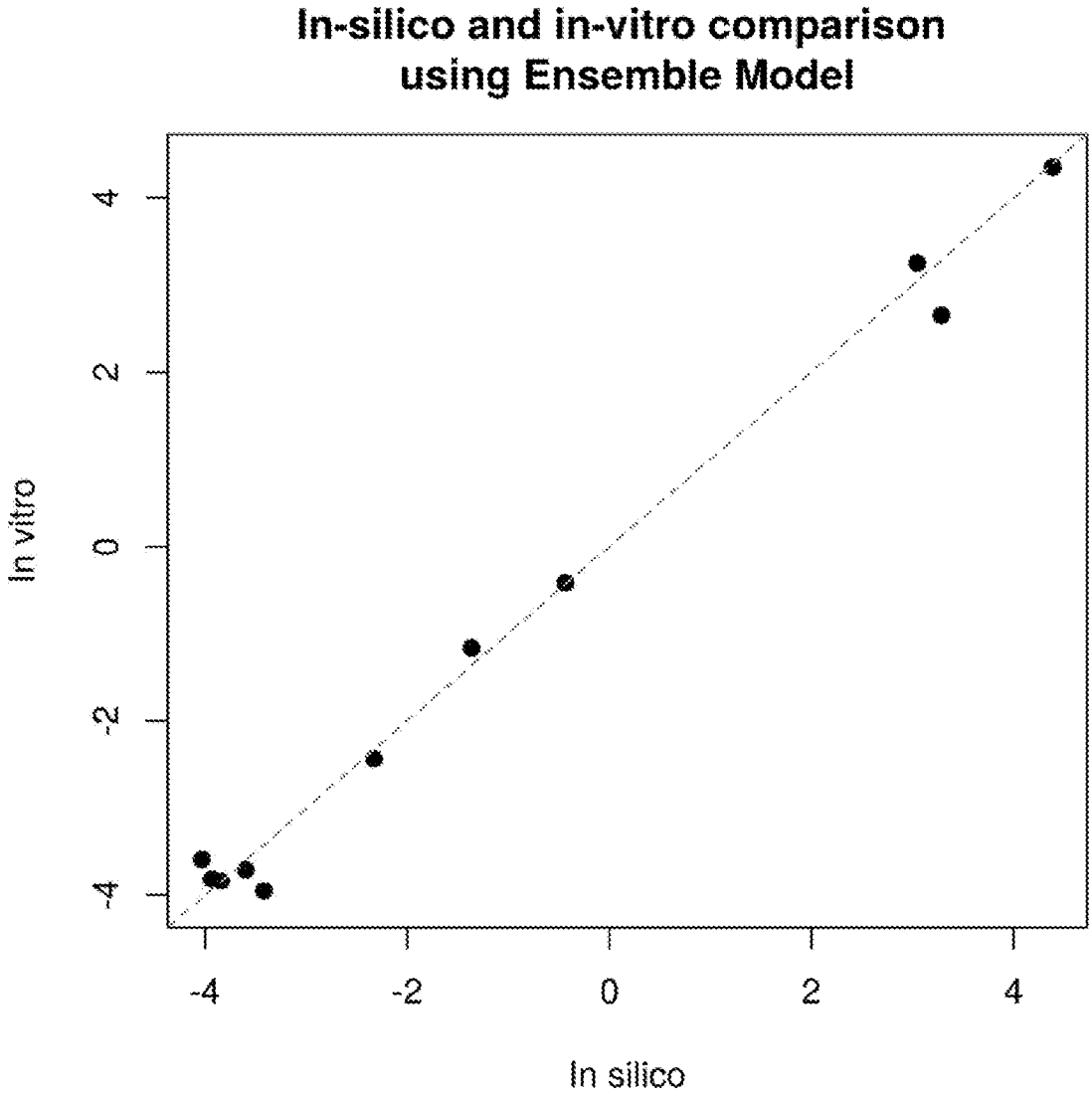

In-silico and in-vitro comparison
using Ensemble Model
In vitro
In silico
4
2
0
-2
-4
-4
-2
0
2
4

FIG. 32

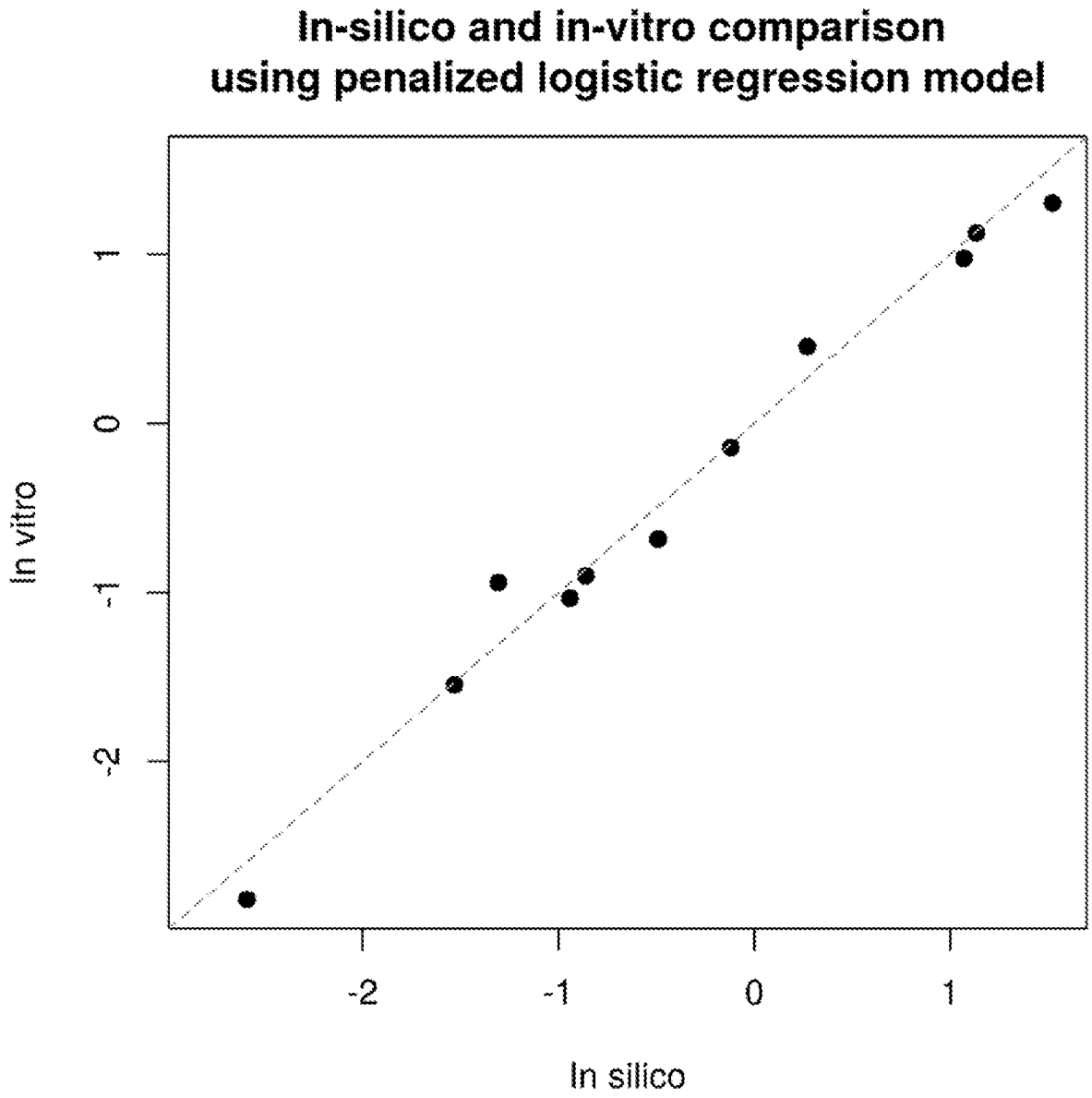

In-silico and in-vitro comparison
using penalized logistic regression model
In vitro
In silico
-2
-1
0
1
-2
-1
0
1

FIG. 33

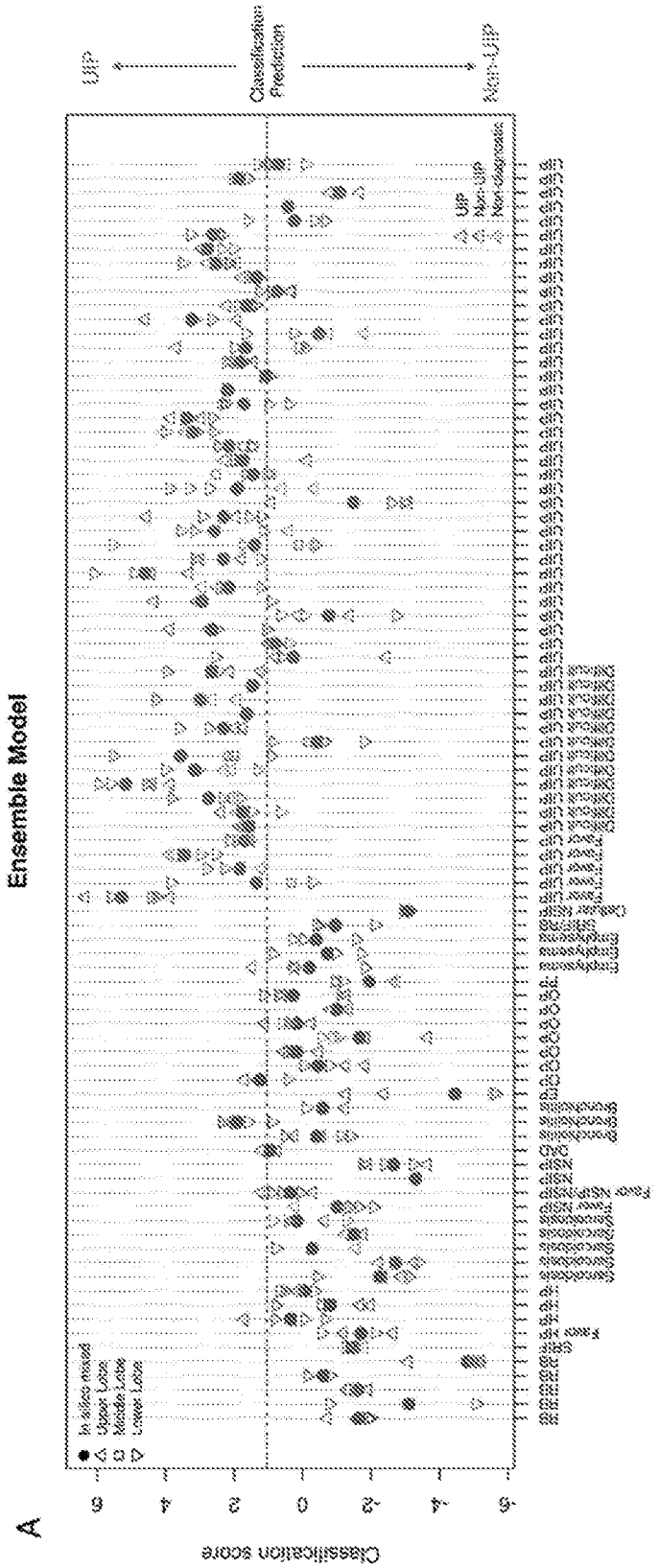

A
Ensemble Model
Classification score
6
4
2
0
-2
-4
-6
In silico model
Upper Lobe
Middle Lobe
Lower Lobe
UIP
Non-UIP
Non-diagnostic
Classification Prediction
UIP
Non-UIP

FIG. 34

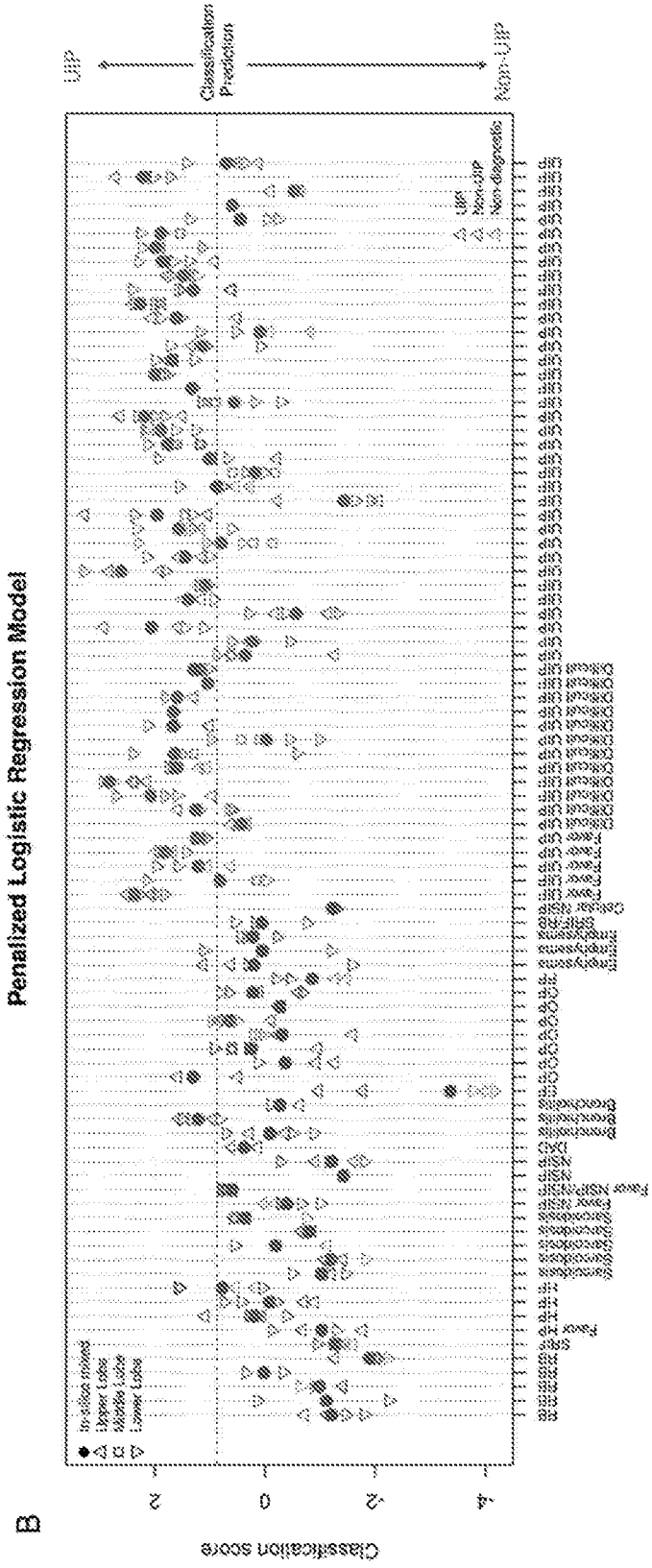

Penalized Logistic Regression Model
B
Classification score
UIP
Classification Prediction
Non-UIP

FIG. 35

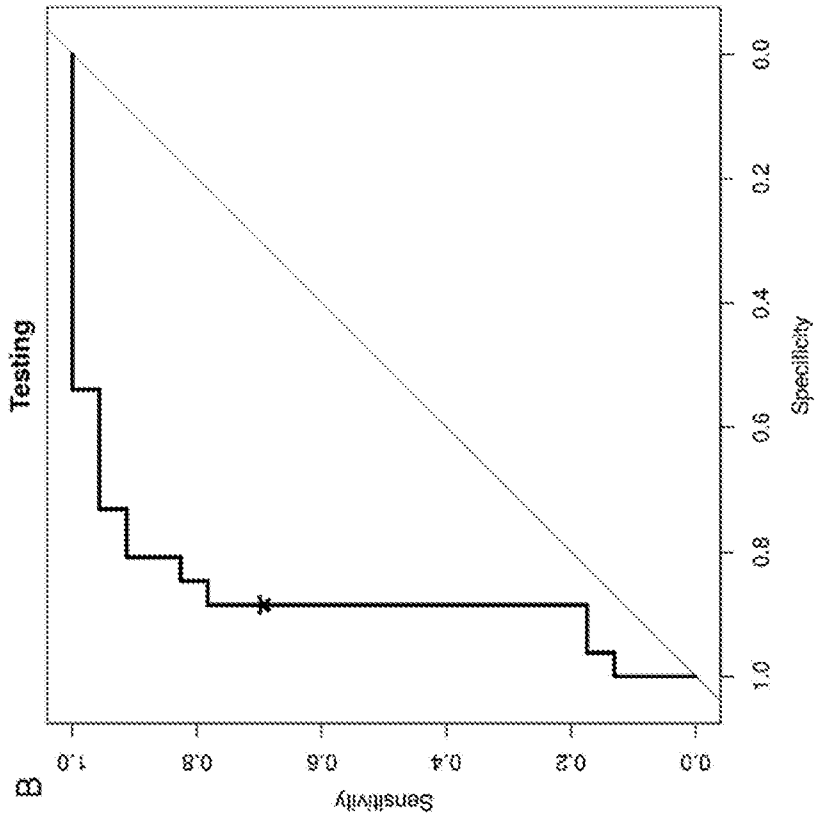


FIG. 36B

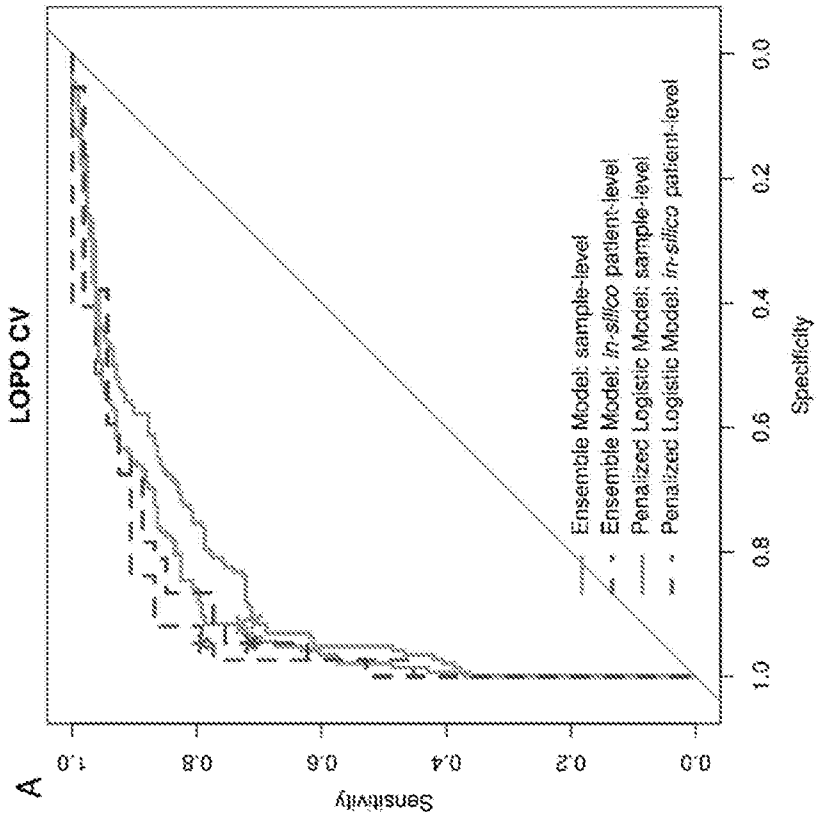


FIG. 36A

C	LOPO CV				Independent Testing
	Ensemble Model		Penalized Logistic Model		Penalized Logistic Model
	Sample-level	*In-silico* mixing	Sample-level	*In-silico* mixing	*In-vitro* mixing
AUC	0.90 [0.87-0.93]	0.93 [0.88-0.98]	0.87 [0.83-0.91]	0.91 [0.85-0.97]	0.87 [0.76-0.98]
Specificity	0.92 [0.86-0.96]	0.95 [0.82-0.99]	0.91 [0.85-0.95]	0.95 [0.82-0.99]	0.88 [0.70-0.98]
Sensitivity	0.73 [0.67-0.79]	0.79 [0.66-0.89]	0.71 [0.64-0.77]	0.72 [0.58-0.83]	0.70 [0.47-0.87]

FIG. 37

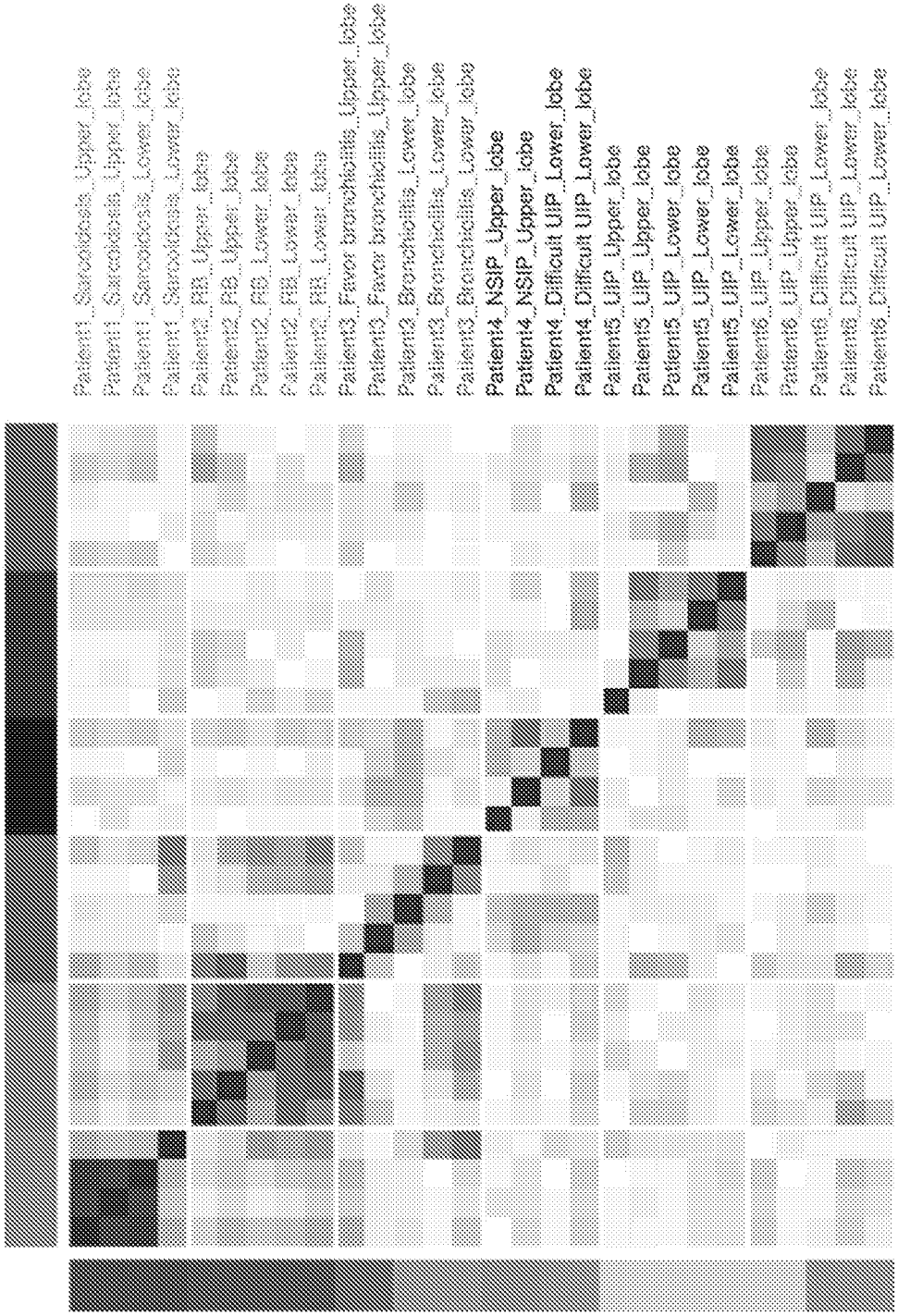

Patient1_Sarcoidosis_Upper_lobe
Patient1_Sarcoidosis_Upper_lobe
Patient1_Sarcoidosis_Lower_lobe
Patient1_Sarcoidosis_Lower_lobe
Patient2_RB_Upper_lobe
Patient2_RB_Upper_lobe
Patient2_RB_Lower_lobe
Patient2_RB_Lower_lobe
Patient2_RB_Lower_lobe
Patient3_Fever bronchiolitis_Upper_lobe
Patient3_Fever bronchiolitis_Upper_lobe
Patient3_Bronchiolitis_Lower_lobe
Patient3_Bronchiolitis_Lower_lobe
Patient3_Bronchiolitis_Lower_lobe
Patient4_NSIP_Upper_lobe
Patient4_NSIP_Upper_lobe
Patient4_Difficult UIP_Lower_lobe
Patient4_Difficult UIP_Lower_lobe
Patient5_UIP_Upper_lobe
Patient5_UIP_Upper_lobe
Patient5_UIP_Lower_lobe
Patient5_UIP_Lower_lobe
Patient5_UIP_Lower_lobe
Patient6_UIP_Upper_lobe
Patient6_UIP_Upper_lobe
Patient6_Difficult UIP_Lower_lobe
Patient6_Difficult UIP_Lower_lobe
Patient6_Difficult UIP_Lower_lobe

FIG. 38

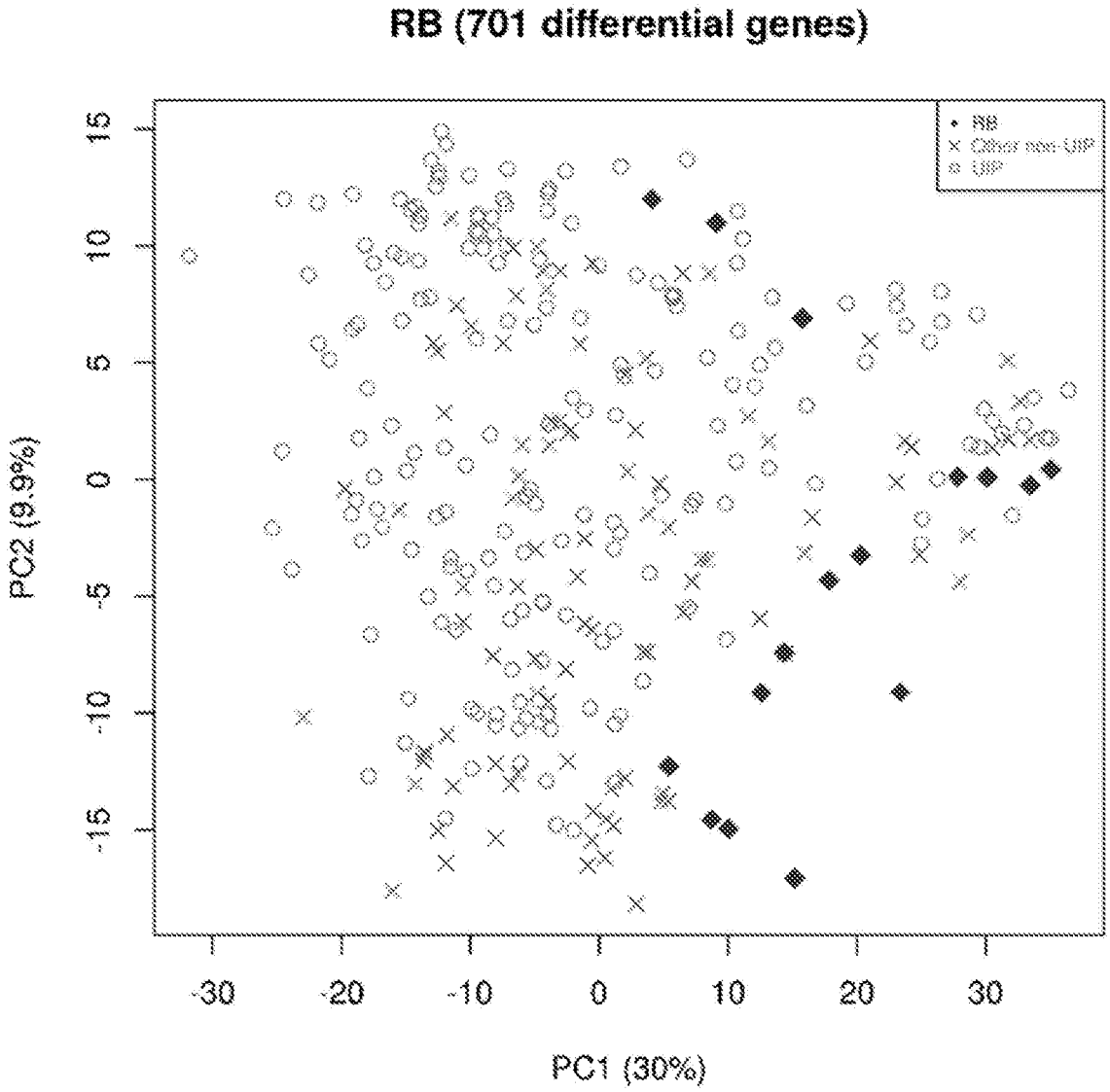

RB (701 differential genes)
RB
Other non-UIP
UIP
PC2 (9.9%)
15
10
5
0
-5
-10
-15
-30
-20
-10
0
10
20
30
PC1 (30%)

FIG. 39

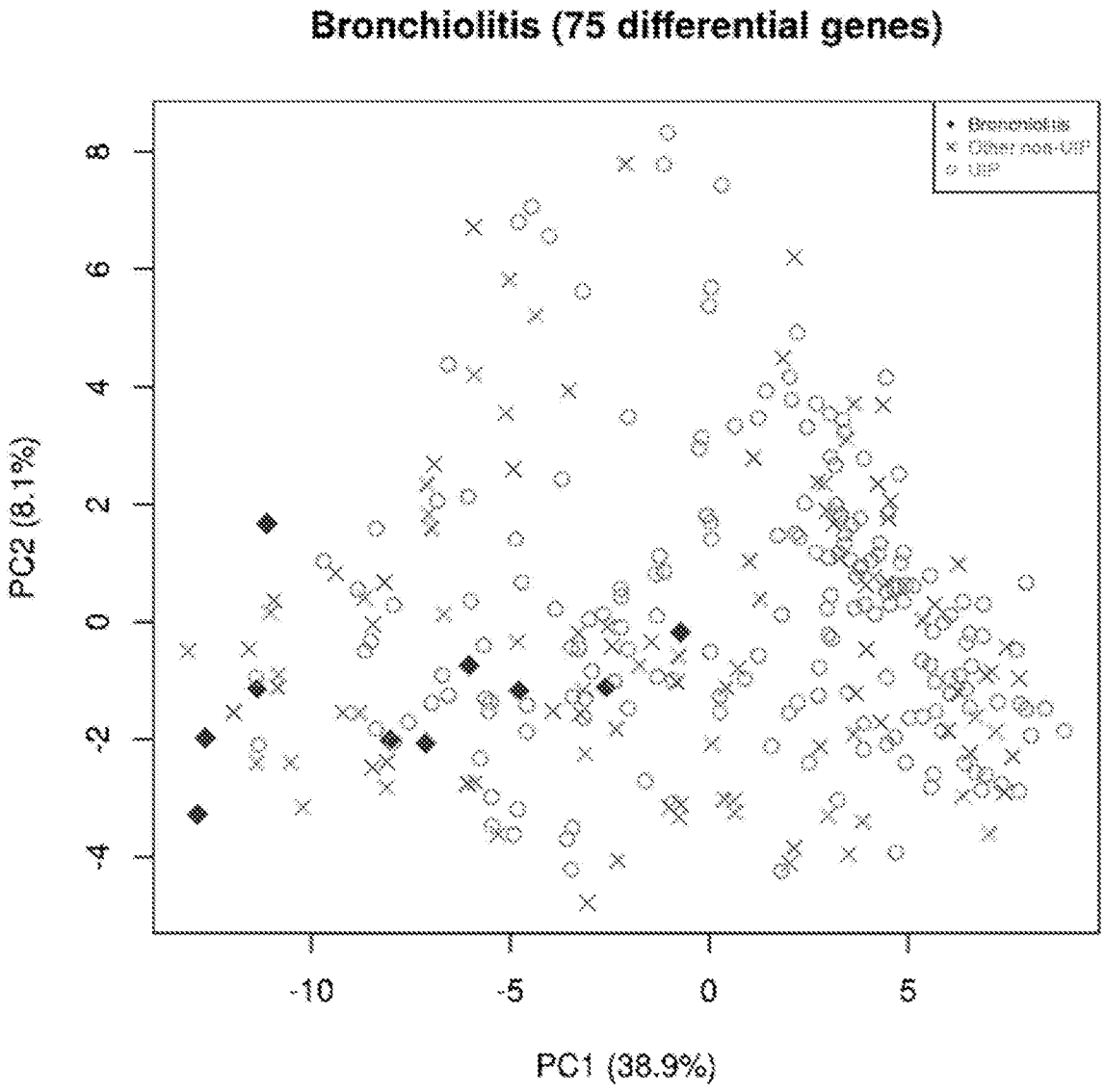

Bronchiolitis (75 differential genes)
Bronchiolitis
Other non-URI
URI
PC2 (8.1%)
8
6
4
2
0
-2
-4
-10
-5
0
5
PC1 (38.9%)

FIG. 40

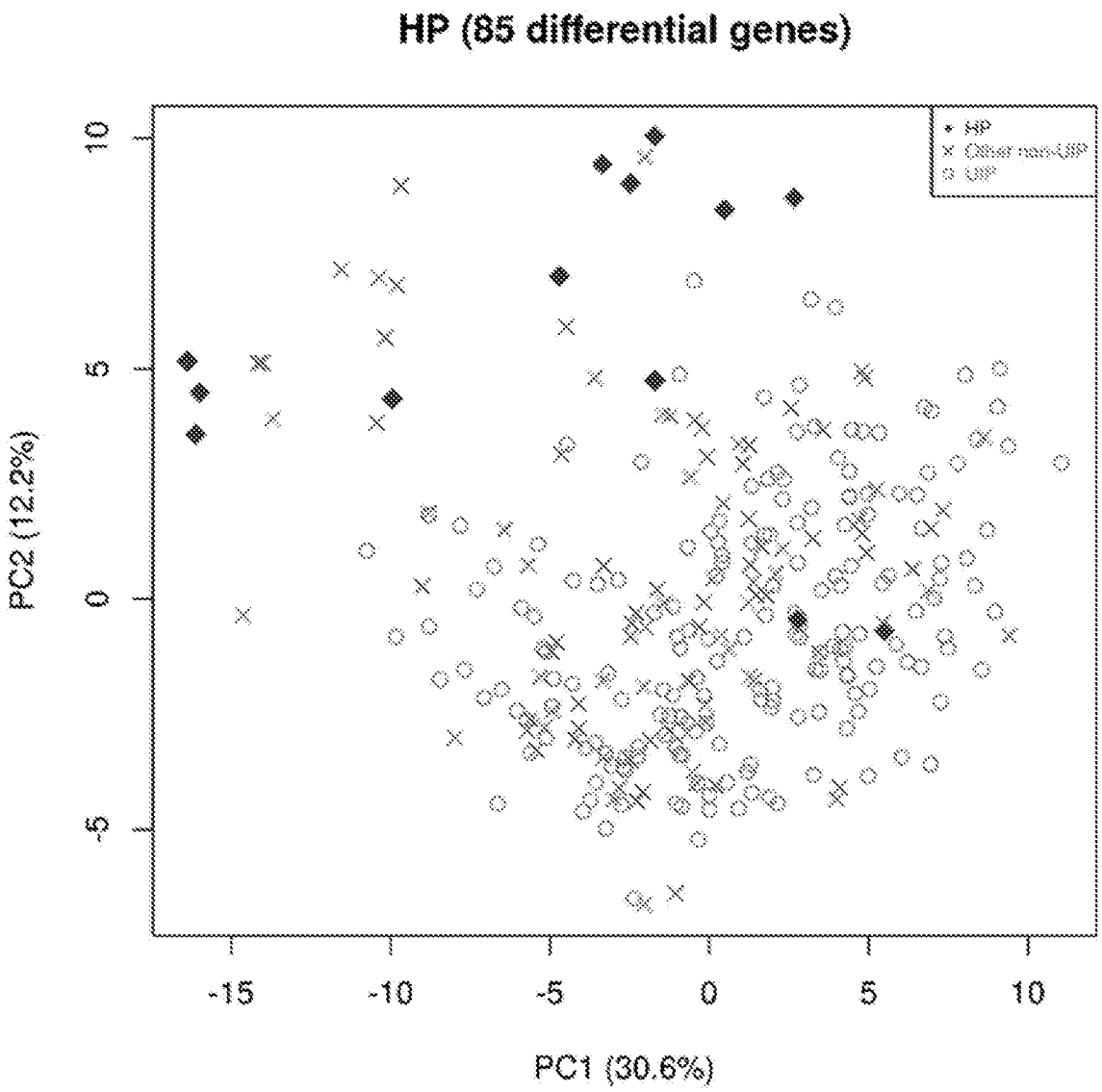

HP (85 differential genes)
HP
Other non-UIP
UIP
PC2 (12.2%)
10
5
0
-5
-15
-10
-5
0
5
10
PC1 (30.6%)

FIG. 41

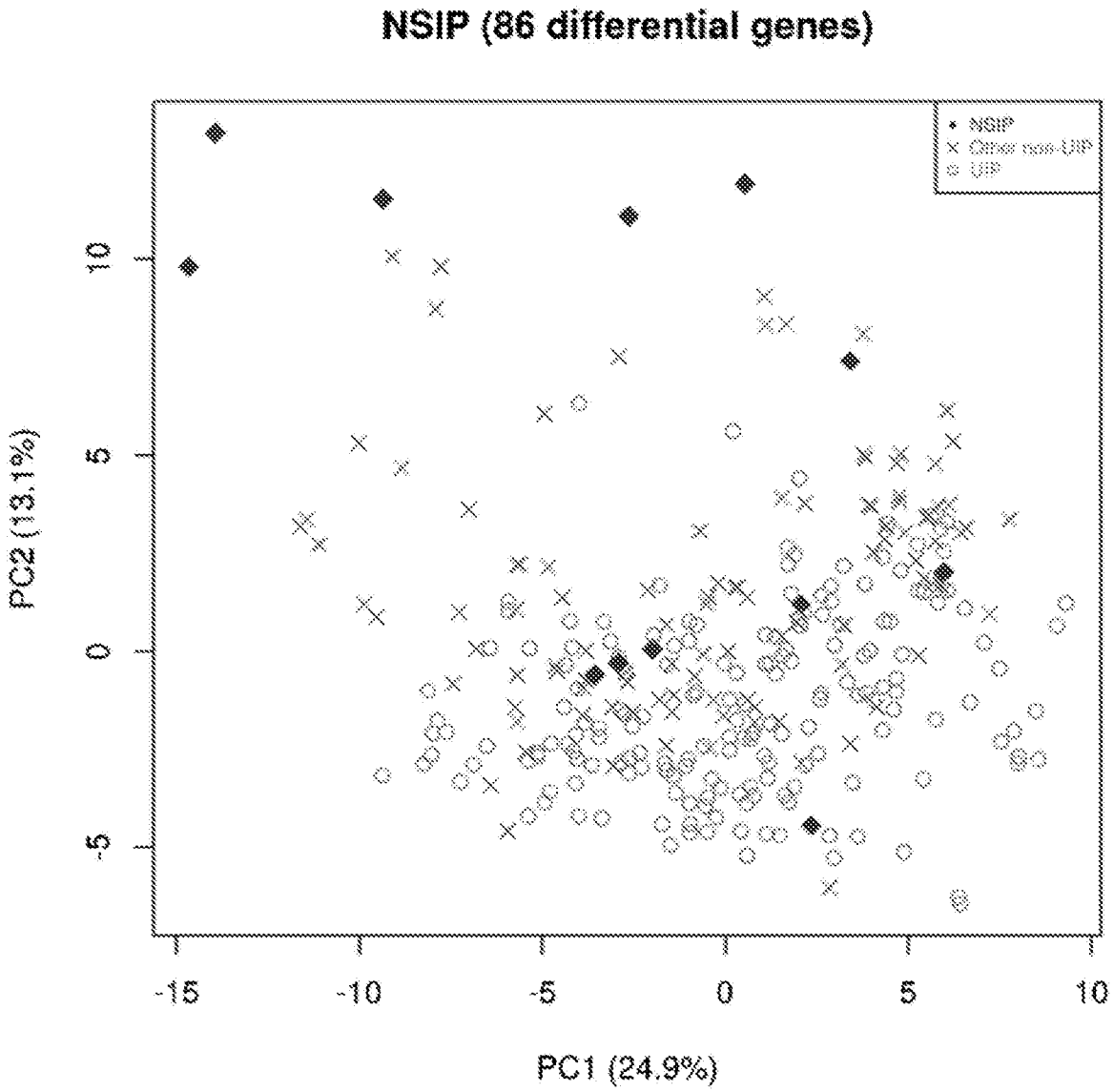

NSIP (86 differential genes)
NSIP
Other non-UIP
UIP
PC2 (13.1%)
10
5
0
-5
-15
-10
-5
0
5
10
PC1 (24.9%)

FIG. 42

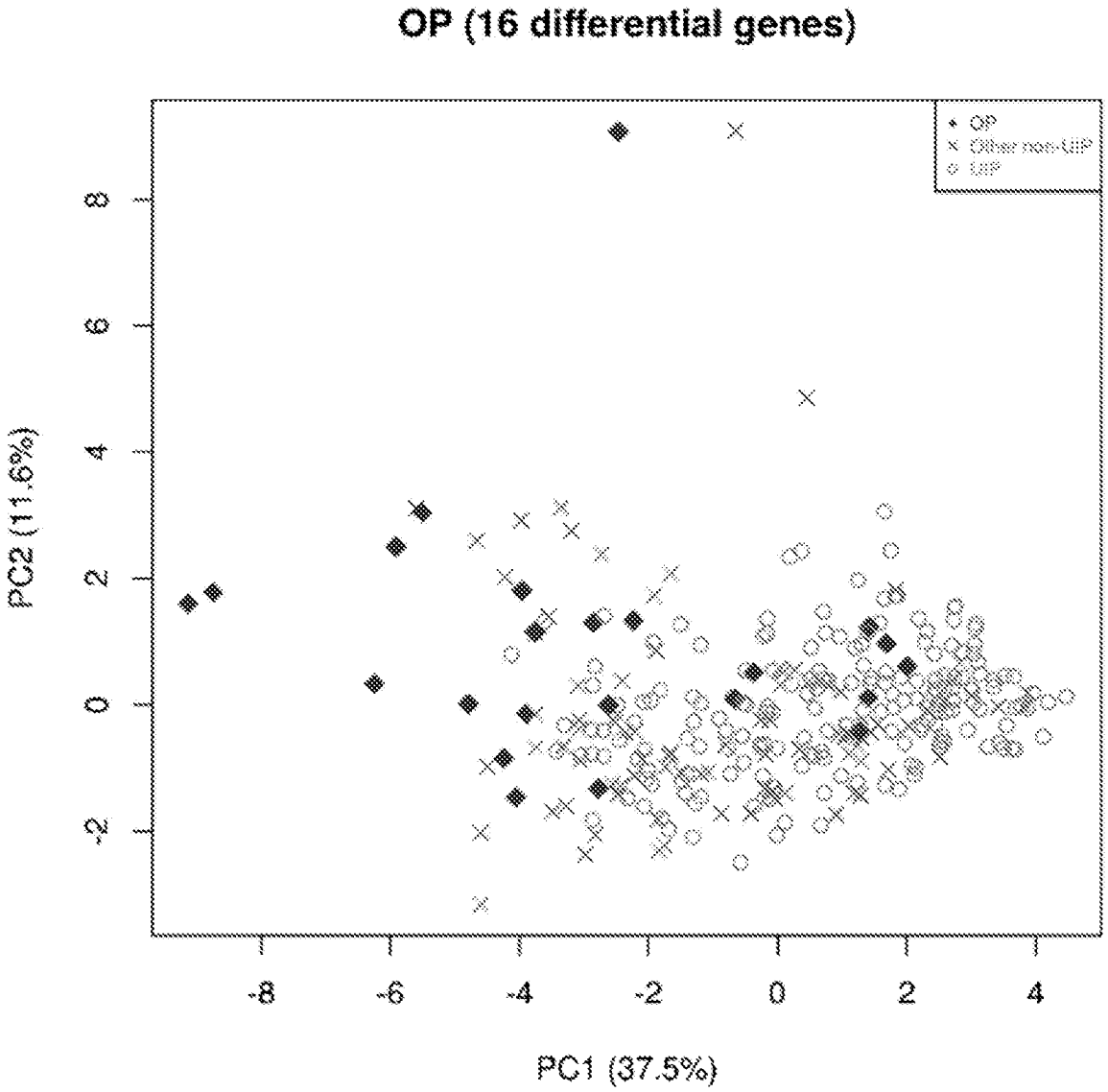

OP (16 differential genes)
OP
Other non-UIP
UIP
PC2 (11.6%)
PC1 (37.5%)
-8
-6
-4
-2
0
2
4
-2
0
2
4
6
8

FIG. 43

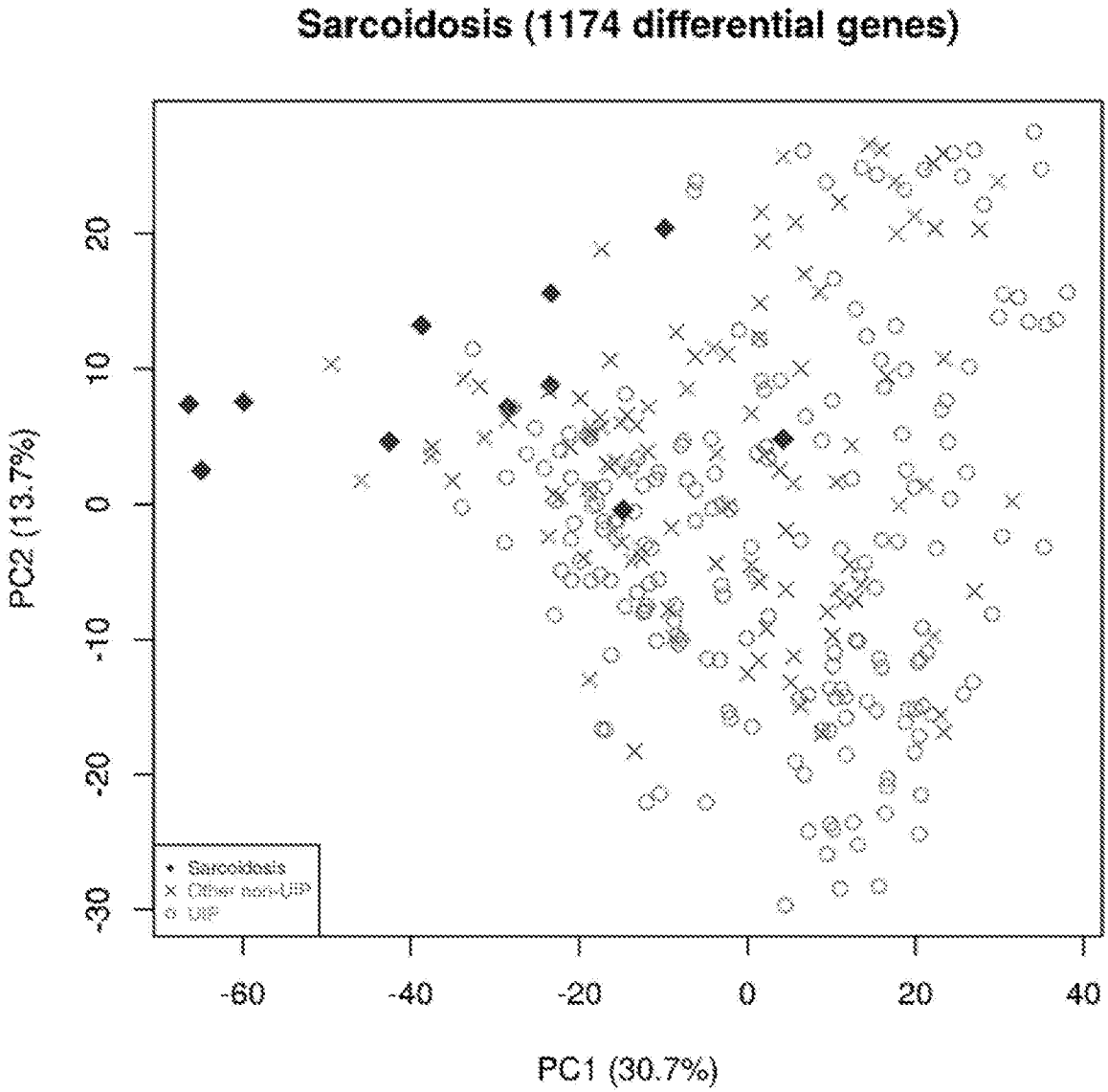

Sarcoidosis (1174 differential genes)
PC2 (13.7%)
PC1 (30.7%)
20
10
0
-10
-20
-30
-60
-40
-20
0
20
40
Sarcoidosis
Other non-UIP
UIP

FIG. 44

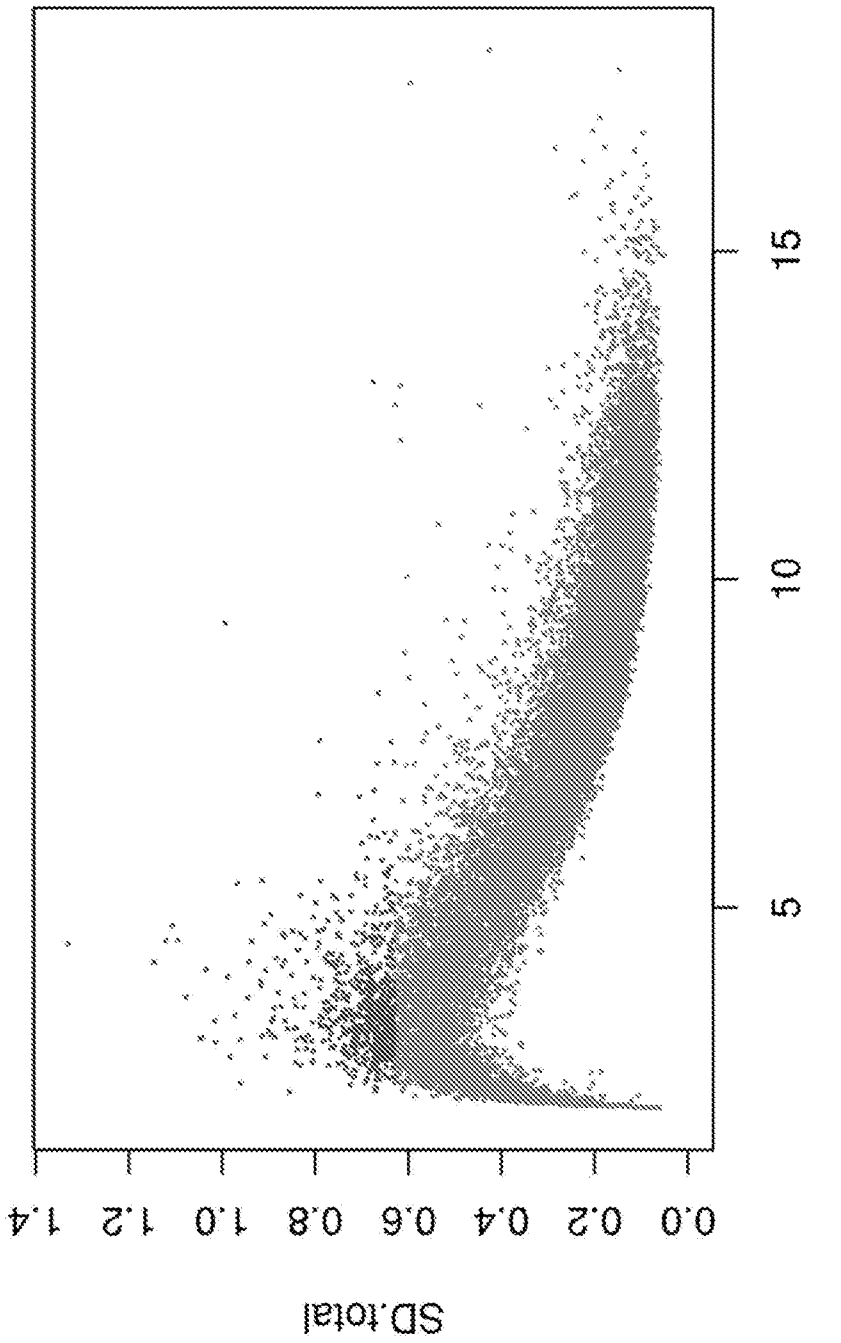


FIG. 45

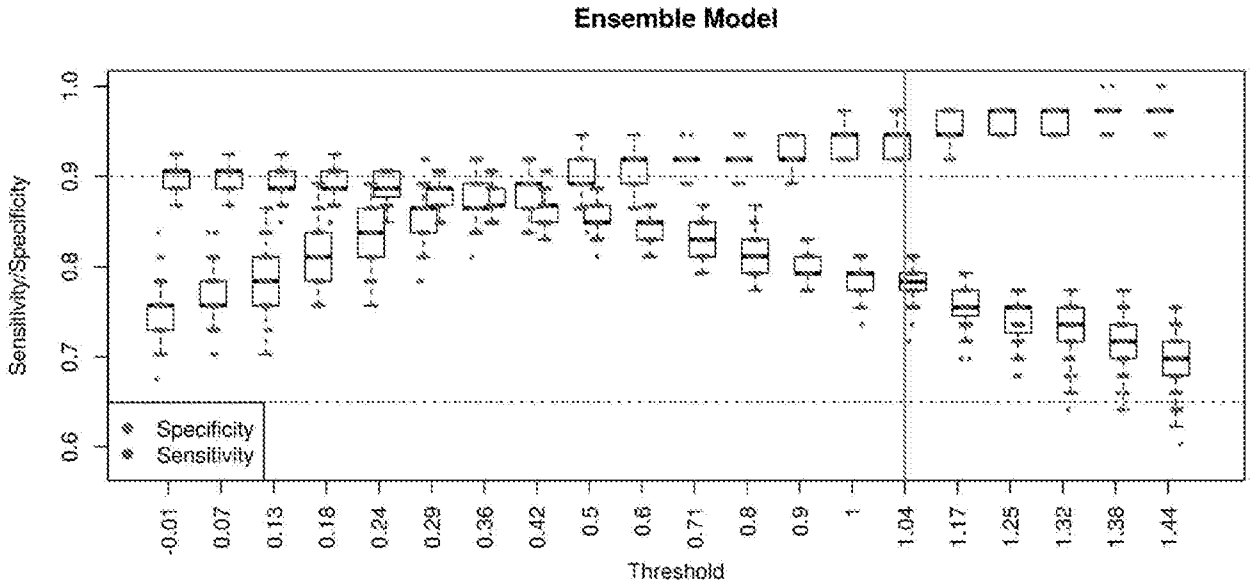


FIG. 46A

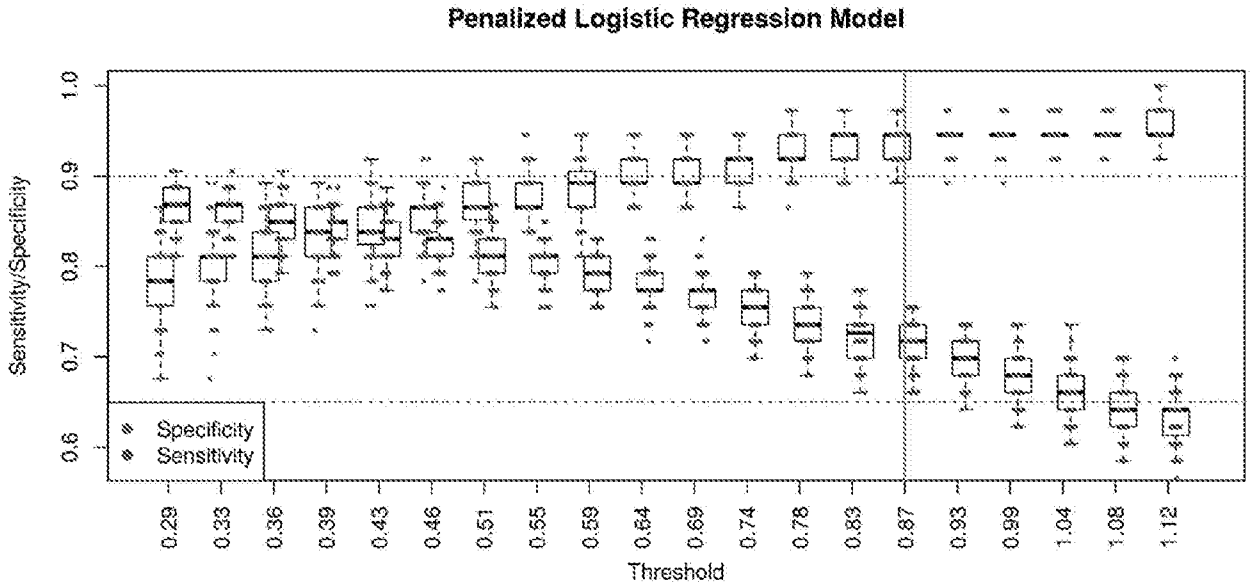


FIG. 46B

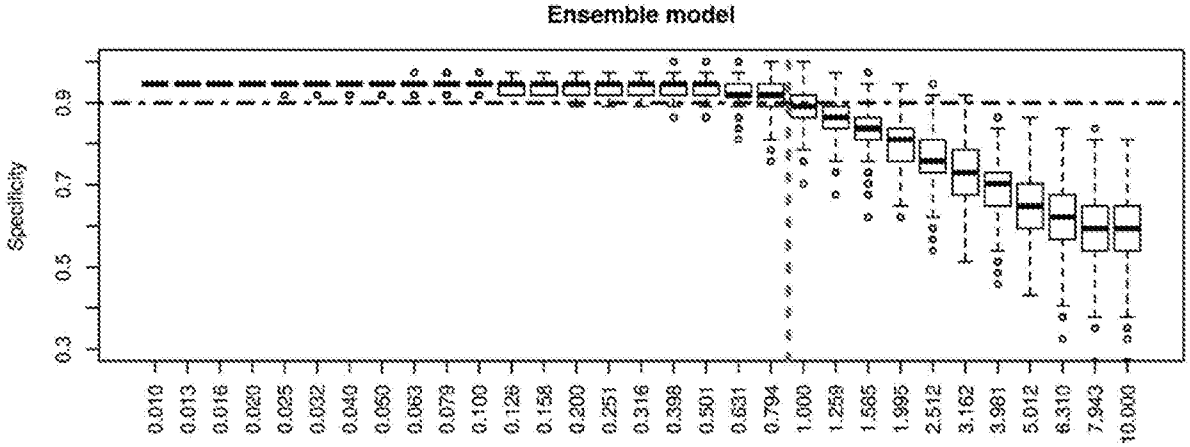


FIG. 47A

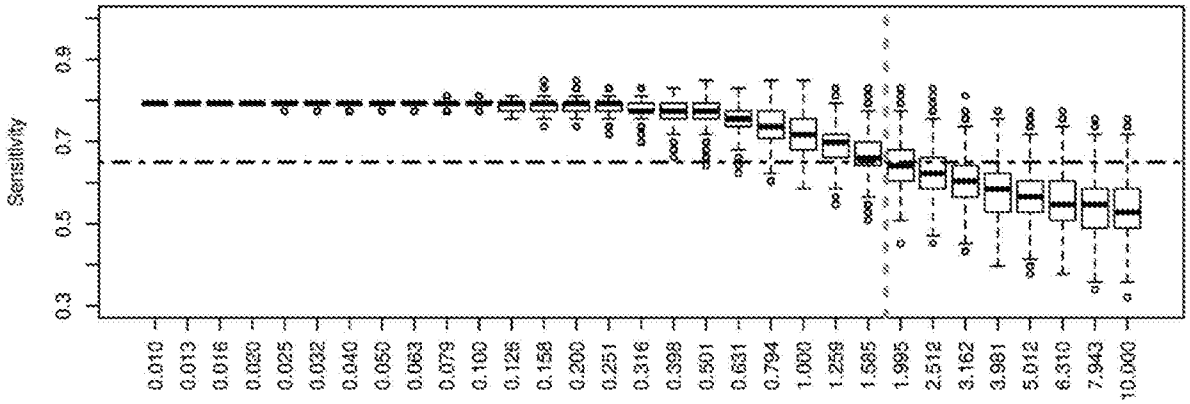


FIG. 47B

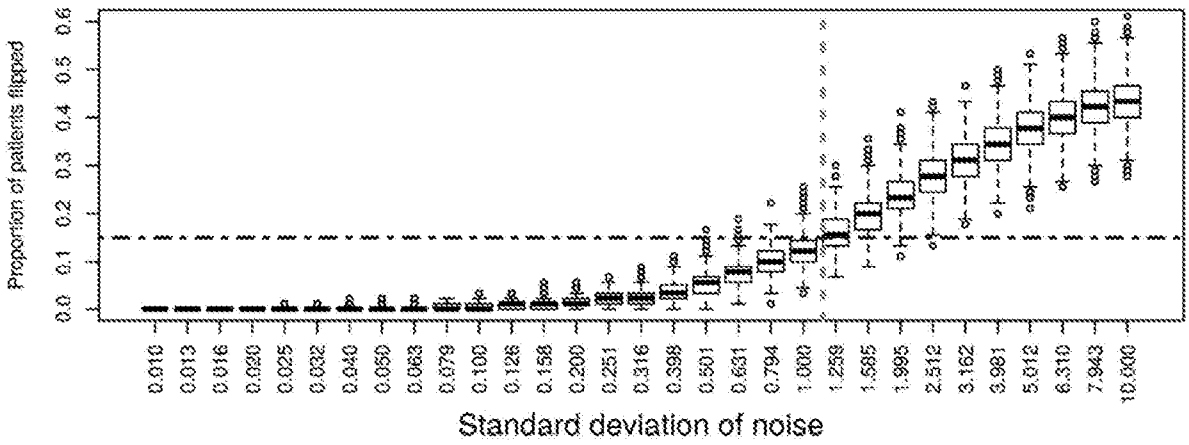


FIG. 47C

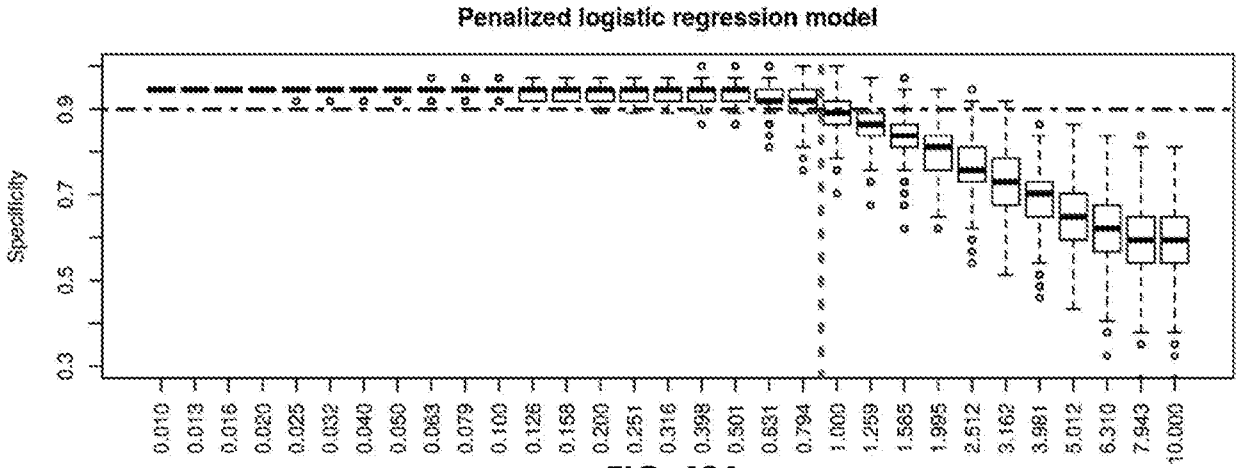


FIG. 48A

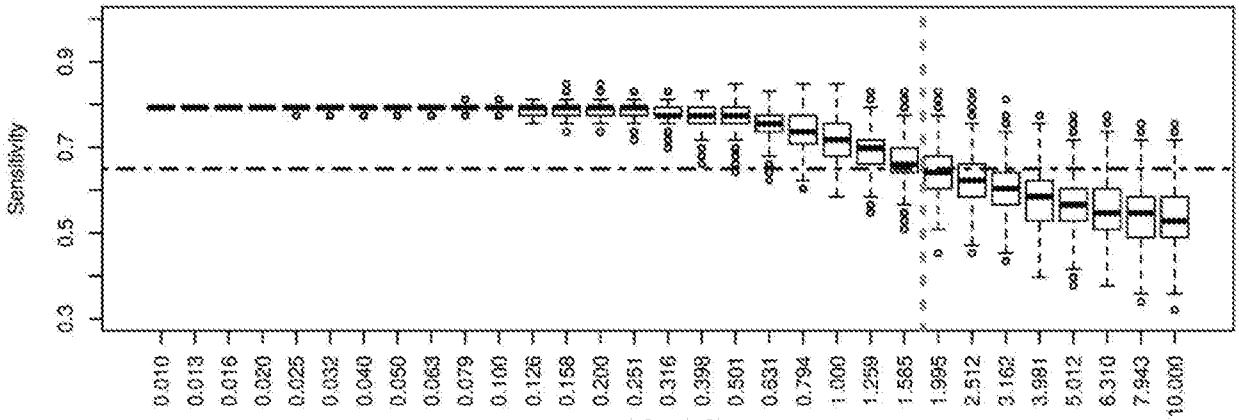


FIG. 48B

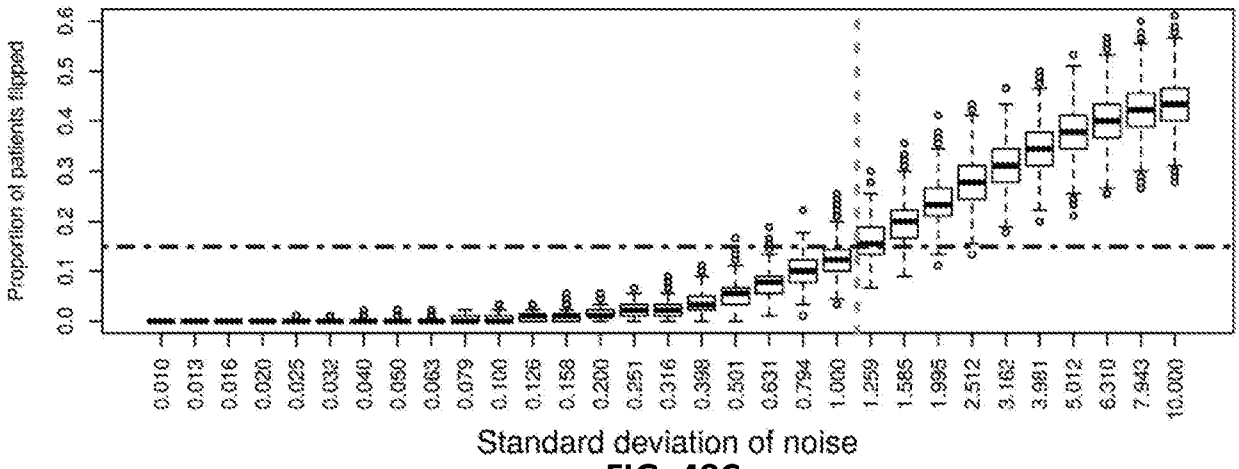


FIG. 48C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/035702

A. CLASSIFICATION OF SUBJECT MATTER

A61B 5/00(2006.01)i, A61B 5/055(2006.01)i

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)
A61B 5/00; A61N 5/10; A61B 5/08; G06F 19/00; G06F 19/18; A61M 11/00; C12Q 1/68; A61B 5/055

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: lung, biomarker, sample, genetic, processor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015-0337385 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPT. OF HEALTH & HUMAN SERVICES) 26 November 2015 See paragraphs [10]-[178] and figures 1-19.	1-60
A	US 2017-0127976 A1 (MICHAEL PHILLIPS) 11 May 2017 See paragraphs [39]-[66] and figures 1-8A.	1-60
A	US 2015-0088430 A1 (ALLEGRO DIAGNOSTICS CORP.) 26 March 2015 See paragraphs [4]-[30] and figures 1-4.	1-60
A	KR 10-2016-0079086 A (MEDIAL RESEARCH LTD.) 05 July 2016 See claims 1-10 and figures 1-2.	1-60
A	JP 2015-519966 A (KONINKLIJKE PHILIPS N.V.) 16 July 2015 See claims 1-10 and figures 1-3.	1-60

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2018 (11.09.2018)	**12 September 2018 (12.09.2018)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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