US 20100035244A1

(54) **DIAGNOSTIC FOR LUNG DISORDERS USING CLASS PREDICTION**

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(57) **ABSTRACT**

The present invention provides methods for diagnosis and prognosis of lung cancer using expression analysis of one or more groups of genes, and a combination of expression analysis with bronchoscopy. The methods of the invention provide far superior detection accuracy for lung cancer when compared to any other currently available method for lung cancer diagnostic or prognosis. The invention also provides methods of diagnosis and prognosis of other lung diseases, particularly in individuals who are exposed to air pollutants, such as cigarette or cigar smoke, smog, asbestos and the like air contaminants or pollutants.

Table 1: 96 gene Group

GenBank ID	Gene Name	Direction of expression in lung Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
NM_003335	UBE1L	down	1316_at
NM_000918	P4HB	up	200654_at
NM_006430.1	CCT4	up	200877_at
NM_001416.1	EIF4A1	up	201530_x_at
NM_004090	DUSP3	up	201537_s_at
NM_006406.1	PRDX4	up	201923_at
NM_003001.2	SDHC	up	202004_x_at
NM_001319	CSNK1G2	down	202573_at
NM_006545.1	TUSC4	up	203246_s_at
NM_021145.1	DMTF1	down	203301_s_at
NM_002437.1	MPV17	up	203466_at
NM_006286	TFDP2	up	203588_s_at
NM_001003698 /// NM_001003699 /// NM_002955	RREB1	down	203704_s_at
NM_001123 /// NM_006721	ADK	up	204119_s_at
NM_024824	FLJ11806	up	204216_s_at
NM_004935.1	CDK5	up	204247_s_at
NM_002853.1	RAD1	down	204461_x_at
NM_019067.1	FLJ10613	down	205010_at
NM_024917.1	CXorf34	down	205238_at
NM_020979.1	APS	down	205367_at
NM_005597.1	NFIC	down	206929_s_at
NM_007031.1	HSF2BP	down	207020_at
NM_009590.1	AOC2	down	207064_s_at
NM_020217.1	DKFZp547I014	down	207283_at
NM_025026.1	FLJ14107	down	207287_at
NM_014709.1	USP34	down	207365_x_at
NM_014896.1	KIAA0894	down	207436_x_at
AF010144	--	down	207953_at
NM_005374.1	MPP2	down	207984_s_at
NM_001696	ATP6V1E1	up	208678_at
NM_005494 /// NM_058246	DNAJB6	up	209015_s_at
NM_006534 /// NM_181659	NCOA3	down	209061_at
NM_006368	CREB3	up	209432_s_at
NM_002268 /// NM_032771	KPNA4	up	209653_at
NM_014033	DKFZP586A0522	down	209703_x_at
NM_016138	COQ7	down	209746_s_at
NM_007048 /// NM_194441	BTN3A1	down	209770_at
NM_006694	JTB	up	210434_x_at
NM_000051 /// NM_138292 /// NM_138293	ATM	down	210858_x_at
NM_000410 /// NM_139002 /// NM_139003 /// NM_139004 /// NM_139005 /// NM_139006 /// NM_139007 /// NM_139008 /// NM_139009 /// NM_139010 /// NM_139011	HFE	down	211328_x_at

Table 1: 96 gene Group			
GenBank ID	Gene Name	Direction of expression in lung Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
NM_003335	UBE1L	down	1316_at
NM_000918	P4HB	up	200654_at
NM_006430.1	CCT4	up	200877_at
NM_001416.1	EIF4A1	up	201530_x_at
NM_004090	DUSP3	up	201537_s_at
NM_006406.1	PRDX4	up	201923_at
NM_003001.2	SDHC	up	202004_x_at
NM_001319	CSNK1G2	down	202573_at
NM_006545.1	TUSC4	up	203246_s_at
NM_021145.1	DMTF1	down	203301_s_at
NM_002437.1	MPV17	up	203466_at
NM_006286	TFDP2	up	203588_s_at
NM_001003698 /// NM_001003699 /// NM_002955	RREB1	down	203704_s_at
NM_001123 /// NM_006721	ADK	up	204119_s_at
NM_024824	FLJ11806	up	204216_s_at
NM_004935.1	CDK5	up	204247_s_at
NM_002853.1	RAD1	down	204461_x_at
NM_019067.1	FLJ10613	down	205010_at
NM_024917.1	CXorf34	down	205238_at
NM_020979.1	APS	down	205367_at
NM_005597.1	NFIC	down	206929_s_at
NM_007031.1	HSF2BP	down	207020_at
NM_009590.1	AOC2	down	207064_s_at

Figure 1

NM_020217.1	DKFZp547I014	down	207283_at
NM_025026.1	FLJ14107	down	207287_at
NM_014709.1	USP34	down	207365_x_at
NM_014896.1	KIAA0894	down	207436_x_at
AF010144	---	down	207953_at
NM_005374.1	MPP2	down	207984_s_at
NM_001696	ATP6V1E1	up	208678_at
NM_005494 /// NM_058246	DNAJB6	up	209015_s_at
NM_006534 /// NM_181659	NCOA3	down	209061_at
NM_006368	CREB3	up	209432_s_at
NM_002268 /// NM_032771	KPNA4	up	209653_at
NM_014033	DKFZP586A0522	down	209703_x_at
NM_016138	COQ7	down	209746_s_at
NM_007048 /// NM_194441	BTN3A1	down	209770_at
NM_006694	JTB	up	210434_x_at
NM_000051 /// NM_138292 /// NM_138293	ATM	down	210858_x_at
NM_000410 /// NM_139002 /// NM_139003 /// NM_139004 /// NM_139005 /// NM_139006 /// NM_139007 /// NM_139008 /// NM_139009 /// NM_139010 /// NM_139011	HFE	down	211328_x_at

Figure 1 continued

NM_004691	ATP6V0D1	up	212041_at
NM_012070 /// NM_139321 /// NM_139322	ATRN	down	212517_at
NM_006095	ATP8A1	down	213106_at
AI632181	---	down	213212_x_at
AW024467	---	down	213919_at
NM_021814	ELOVL5	down	214153_at
NM_005547.1	IVL	down	214599_at
NM_203458	N2N	down	214722_at
NM_015547 /// NM_147161	THEA	down	214763_at
AB007958.1	KIAA0792	down	214833_at
NM_207488	FLJ42393	down	214902_x_at
NM_005809 /// NM_181737 /// NM_181738	PRDX2	down	215067_x_at
NM_016248 /// NM_144490	AKAP11	down	215336_at
AK022213.1	FLJ12151	down	215373_x_at
NM_005708	GPC6	down	215387_x_at
NM_207102	FBXW12	down	215600_x_at
AK023895	---	down	215609_at
NM_144606 /// NM_144997	FLCN	down	215645_at
NM_018530	GSDML	down	215659_at
AK021474	---	down	215892_at
U43604.1	---	down	216012_at
AU147017	---	down	216110_x_at
AF222691.1	LNX1	down	216187_x_at
NM_015116	LRCH1	down	216745_x_at

Figure 1 continued

NM_001005375 /// NM_001005785 /// NM_001005786 /// NM_004081 /// NM_020363 /// NM_020364 /// NM_020420	DAZ2	down	216922_x_at
AC004692	---	down	217313_at
NM_001014	RPS10	down	217336_at
NM_000585 /// NM_172174 /// NM_172175	IL15	down	217371_s_at
NM_054020 /// NM_172095 /// NM_172096 /// NM_172097	CATSPER2	down	217588_at
BE466926	---	down	217671_at
NM_018011	FLJ10154	down	218067_s_at
NM_024077	SECISBP2	down	218265_at
NM_012394	PFDN2	up	218336_at
NM_019011 /// NM_207111 /// NM_207116	TRIAD3	down	218425_at
NM_017646	TRIT1	down	218617_at
NM_021800	DNAJC12	up	218976_at
NM_016049	C14orf122	up	219203_at
NM_014395	DAPP1	down	219290_x_at
NM_014336	AIPL1	down	219977_at
NM_018097	C15orf25	down	220071_x_at
NM_019014	POLR1B	down	220113_x_at
NM_024804	FLJ12606	down	220215_at
NM_018260	FLJ10891	down	220242_x_at
NM_018118	MCM3APAS	down	220459_at

Figure 1 continued

NM_014128		down	220856_x_at
NM_024084	MGC3196	down	220934_s_at
NM_005294	GPR21	down	221294_at
AF077053	PGK1	down	221616_s_at
NM_138387	G6PC3	up	221759_at
NM_024531	GPR172A	up	222155_s_at
NM_000693	ALDH1A3	down	222168_at
NM_018509	PRO1855	up	222231_s_at
NM_033128	SCIN	down	222272_x_at
NM_020706	SFRS15	down	222310_at
AI523613	---	down	222358_x_at
NM_014884	SFRS14	down	64371_at

Figure 1 continued

Table 2: 84 Gene Group				
GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
NM_030757.1	MKRN4	makorin, ring finger protein, 4 /// makorin, ring finger protein, 4	down	208082_x_at
R83000	BTF3	basic transcription factor 3	down	214800_x_at
AK021571.1	MUC20	mucin 20	down	215208_x_at
NM_014182.1	ORMDL2	ORM1-like 2 (S. cerevisiae)	up	218556_at
NM_17932.1	FLJ20700	hypothetical protein FLJ20700	down	207730_x_at
U85430.1	NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	down	210556_at
AI683552	---	---	down	217679_x_at
BC002642.1	CTSS	cathepsin S	down	202901_x_at
AW024467	RIPX	rap2 interacting protein x	down	213939_s_at
NM_030972.1	MGC5384	hypothetical protein MGC5384 /// hypothetical protein MGC5384	down	208137_x_at

Figure 2

Table 2: 84 Gene Group				
GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
BC021135.1	INADL	InaD-like protein	down	214705_at
AL161952.1	GLUL	glutamate-ammonia ligase (glutamine synthase)	down	215001_s_at
AK026565.1	FLJ10534	hypothetical protein FLJ10534	down	218155_x_at
AK023783.1	---	Homo sapiens cDNA FLJ13721 fis, clone PLACE2000450.	down	215604_x_at
BF218804	AFURS1	ATPase family homolog up-regulated in senescence cells	down	212297_at
NM_001281.1	CKAP1	cytoskeleton associated protein 1	up	201804_x_at
NM_024006.1	IMAGE3455200	hypothetical protein IMAGE3455200	up	217949_s_at
AK023843.1	PGF	placental growth factor, vascular endothelial growth factor-related protein	down	215179_x_at
BC001602.1	CFLAR	CASP8 and FADD-like apoptosis regulator	down	211316_x_at

Figure 2 continued

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
BC034707.1	---	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_060312.1 (H.sapiens) hypothetical protein FLJ20489 [Homo sapiens]	down	217653_x_at
BC064619.1	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	down	266_s_at
AY280502.1	EPHB6	EphB6	down	204718_at
BC059387.1	MYO1A	myosin IA	down	211916_s_at
	---	Homo sapiens transcribed sequences	down	215032_at
AF135421.1	GMPPB	GDP-mannose pyrophosphorylase B	up	219920_s_at
BC061522.1	MGC70907	similar to MGC9515 protein	down	211996_s_at
L76200.1	GUK1	guanylate kinase 1	up	200075_s_at
U50532.1	CG005	hypothetical protein from BCRA2 region	down	214753_at
BC006547.2	EEF2	eukaryotic translation elongation factor 2	down	204102_s_at
BC008797.2	FVT1	follicular lymphoma variant translocation 1	down	202419_at

Table 2: 84 Gene Group

Figure 2 continued

Table 2: 84 Gene Group

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
BC000807.1	ZNF160	zinc finger protein 160	down	214715_x_at
AL080112.1	---	---	down	216859_x_at
BC033718.1 /// BC046176.1 /// BC038443.1	C21orf106	chromosome 21 open reading frame 106	down	215529_x_at
NM_000346.1	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	up	202936_s_at
BC008710.1	SUI1	putative translation initiation factor	up	212130_x_at
Hs.288575 (UNIGENE ID)	---	Homo sapiens cDNA FLJ14090 fis, clone MAMMA1000264.	down	215204_at
AF020591.1	AF020591	zinc finger protein	down	218735_s_at
BC000423.2	ATP6V0B	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit c" /// ATPase, H+ transporting, lysosomal 21kDa, V0 subunit c"	up	200078_s_at
BC002503.2	SAT	spermidine/spermine N1-acetyltransferase	down	203455_s_at
BC008710.1	SUI1	putative translation initiation factor	up	212227_x_at

Figure 2 continued

Table 2: 84 Gene Group				
GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
	---	Homo sapiens transcribed sequences	down	222282_at
BC009185.2	DCLRE1C	DNA cross-link repair 1C (PSO2 homolog, S. cerevisiae)	down	219678_x_at
Hs.528304 (UNIGENE ID)	ADAM28	a disintegrin and metalloproteinase domain 28	down	208268_at
U50532.1	CG005	hypothetical protein from BCRA2 region	down	221899_at
BC013923.2	SOX2	SRY (sex determining region Y)-box 2	down	213721_at
BC031091	ODAG	ocular development-associated gene	down	214718_at
NM_007062	PWP1	nuclear phosphoprotein similar to S. cerevisiae PWP1	up	201608_s_at
Hs.249591 (Unigene ID)	FLJ20686	hypothetical protein FLJ20686	down	205684_s_at
BC075839.1 /// BC073760.1	KRT8	keratin 8	up	209008_x_at
BC072436.1 /// BC004560.2	HYOU1	hypoxia up-regulated 1	up	200825_s_at
BC001016.2	NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa	up	218160_at

Figure 2 continued

Table 2: 84 Gene Group

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
Hs.286261 (Unigene ID)	FLJ20195	hypothetical protein FLJ20195	down	57739_at
AF348514.1	---	Homo sapiens fetal thymus prothymosin alpha mRNA, complete cds	down	211921_x_at
BC005023.1	CGI-128	CGI-128 protein	up	218074_at
BC066337.1 /// BC058736.1 /// BC050555.1	KTN1	kinectin 1 (kinesin receptor)	down	200914_x_at
	---	---	down	216384_x_at
Hs.216623 (Unigene ID)	ATP8B1	ATPase, Class I, type 8B, member 1	down	214594_x_at
BC072400.1	THOC2	THO complex 2	down	222122_s_at
BC041073.1	PRKX	protein kinase, X-linked	down	204060_s_at
U43965.1	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	down	215314_at
	---	---	down	208238_x_at
BC021258.2	TRIM5	tripartite motif-containing 5	down	210705_s_at
BC016057.1	USH1C	Usher syndrome 1C (autosomal recessive, severe)	down	211184_s_at
BC016713.1 /// BC014535.1 /// AF237771.1	PARVA	parvin, alpha	down	215418_at

Figure 2 continued

Table 2: 84 Gene Group

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
BC000360.2	EIF4EL3	eukaryotic translation initiation factor 4E-like 3	up	209393_s_at
BC007455.2	SH3GLB1	SH3-domain GRB2-like endophilin B1	up	210101_x_at
BC000701.2	KIAA0676	KIAA0676 protein	down	212052_s_at
BC010067.2	CHC1	chromosome condensation 1	down	215011_at
BC023528.2 /// BC047680.1	C14orf87	chromosome 14 open reading frame 87	up	221932_s_at
BC064957.1	KIAA0102	KIAA0102 gene product	up	201239_s_at
Hs.156701 (Unigene ID)	---	Homo sapiens cDNA FLJ14253 fis, clone OVARC1001376.	down	215553_x_at
BC030619.2	KIAA0779	KIAA0779 protein	down	213351_s_at
BC008710.1	SUI1	putative translation initiation factor	up	202021_x_at
U43965.1	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	down	209442_x_at
BC066329.1	SDHC	succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa	up	210131_x_at

Figure 2 continued

Table 2: 84 Gene Group

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
Hs.438867 (Unigene ID)	---	Homo sapiens transcribed sequence with weak similarity to protein ref:NP_060312.1. (H.sapiens) hypothetical protein FLJ20489 [Homo sapiens]	down	217713_x_at
BC035025.2 /// BC050330.1	ALMS1	Alstrom syndrome 1	down	214707_x_at
BC023976.2	PDAP2	PDGFA associated protein 2	up	203272_s_at
BC074852.2 /// BC074851.2	PRKY	protein kinase, Y-linked	down	206279_at
Hs.445885 (Unigene ID)	KIAA1217	Homo sapiens cDNA FLJ12005 fis, clone HEMBB1001565.	down	214912_at
BC008591.2 /// BC050440.1 /// BC048096.1	KIAA0100	KIAA0100 gene product	up	201729_s_at
AF365931.1	ZNF264	zinc finger protein 264	down	205917_at

Figure 2 continued

Table 2: 84 Gene Group				
GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
AF257099.1	PTMA	prothymosin, alpha (gene sequence 28)	down	200772_x_at
BC028912.1	DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	up	202842_s_at

Figure 2 continued

Table 3: 50 Gene Group			
GenBank ID	Gene Name	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
NM_007062.1	PWP1	up in cancer	201608_s_at
NM_001281.1	CKAP1	up in cancer	201804_x_at
BC000120.1		up in cancer	202355_s_at
NM_014255.1	TMEM4	up in cancer	202857_at
BC002642.1	CTSS	up in cancer	202901_x_at
NM_000346.1	SOX9	up in cancer	202936_s_at
NM_006545.1	NPR2L	up in cancer	203246_s_at
BG034328		up in cancer	203588_s_at
NM_021822.1	APOBEC3G	up in cancer	204205_at
NM_021069.1	ARGBP2	up in cancer	204288_s_at
NM_019067.1	FLJ10613	up in cancer	205010_at
NM_017925.1	FLJ20686	up in cancer	205684_s_at
NM_017932.1	FLJ20700	up in cancer	207730_x_at
NM_030757.1	MKRN4	up in cancer	208082_x_at
NM_030972.1	MGC5384	up in cancer	208137_x_at
AF126181.1	BCG1	up in cancer	208682_s_at
U93240.1		up in cancer	209653_at
U90552.1		up in cancer	209770_at
AF151056.1		up in cancer	210434_x_at
U85430.1	NFATC3	up in cancer	210556_at
U51007.1		up in cancer	211609_x_at
BC005969.1		up in cancer	211759_x_at
NM_002271.1		up in cancer	211954_s_at
AL566172		up in cancer	212041_at
AB014576.1	KIAA0676	up in cancer	212052_s_at
BF218804	AFURS1	down in cancer	212297_at

Figure 3

Table 3: 50 Gene Group			
GenBank ID	Gene Name	Direction of Expression in Cancer	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
AK022494.1		down in cancer	212932_at
AA114843		down in cancer	213884_s_at
BE467941		down in cancer	214153_at
NM_003541.1	HIST1H4K	down in cancer	214463_x_at
R83000	BTF3	down in cancer	214800_x_at
AL161952.1	GLUL	down in cancer	215001_s_at
AK023843.1	PGF	down in cancer	215179_x_at
AK021571.1	MUC20	down in cancer	215208_x_at
AK023783.1	---	down in cancer	215604_x_at
AU147182		down in cancer	215620_at
AL080112.1	---	down in cancer	216859_x_at
AW971983		down in cancer	217588_at
AI683552	---	down in cancer	217679_x_at
NM_024006.1	IMAGE3455200	down in cancer	217949_s_at
AK026565.1	FLJ10534	down in cancer	218155_x_at
NM_014182.1	ORMDL2	down in cancer	218556_at
NM_021800.1	DNAJC12	down in cancer	218976_at
NM_016049.1	CGI-112	down in cancer	219203_at
NM_019023.1	PRMT7	down in cancer	219408_at
NM_021971.1	GMPPB	down in cancer	219920_s_at
NM_014128.1	---	down in cancer	220856_x_at
AK025651.1		down in cancer	221648_s_at
AA133341	C14orf87	down in cancer	221932_s_at
AF198444.1		down in cancer	222168_at

Figure 3 continued

Table 4: 36 Gene Group			
GenBank ID	Gene Name	Gene Description	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
NM_007062.1	PWP1	nuclear phosphoprotein similar to S. cerevisiae PWP1	201608_s_at
NM_001281.1	CKAP1	cytoskeleton associated protein 1	201804_x_at
BC002642.1	CTSS	cathepsin S	202901_x_at
NM_000346.1	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	202936_s_at
NM_006545.1	NPR2L	homologous to yeast nitrogen permease (candidate tumor suppressor)	203246_s_at
BG034328		transcription factor Dp-2 (E2F dimerization partner 2)	203588_s_at
NM_019067.1	FLJ10613	hypothetical protein FLJ10613	205010_at
NM_017925.1	FLJ20686	hypothetical protein FLJ20686	205684_s_at
NM_017932.1	FLJ20700	hypothetical protein FLJ20700	207730_x_at
NM_030757.1	MKRN4	makorin, ring finger protein, 4 /// makorin, ring finger protein, 4	208082_x_at
NM_030972.1	MGC5384	hypothetical protein MGC5384	208137_x_at

Figure 4

Table 4: 36 Gene Group

GenBank ID	Gene Name	Gene Description	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
NM_002268 /// NM_032771	KPNA4	karyopherin alpha 4 (importin alpha 3)	209653_at
NM_007048 /// NM_194441	BTN3A1	butyrophilin, subfamily 3, member A1	209770_at
NM_006694	JBT	jumping translocation breakpoint	210434_x_at
U85430.1	NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	210556_at
NM_004691	ATP6V0D1	ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d isoform 1	212041_at
AB014576.1	KIAA0676	KIAA0676 protein	212052_s_at
BF218804	AFURS1	ATPase family homolog up-regulated in senescence cells	212297_at
BE467941		EVOVL family member 5, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	214153_at
R83000	BTF3	basic transcription factor 3	214800_x_at
AL161952.1	GLUL	glutamate-ammonia ligase (glutamine synthase)	215001_s_at
AK023843.1	PGF	placental growth factor, vascular endothelial growth factor-related protein	215179_x_at
AK021571.1	MUC20	mucin 20	215208_x_at

Figure 4 continued

GenBank ID	Gene Name	Gene Description	Exemplary probe: Affymetrix Id in the Human Genome U133 chip
AK023783.1	---	Homo sapiens cDNA FLJ13721 fis, clone PLACE2000450.	215604_x_at
AL080112.1	---	---	216859_x_at
AW971983		cation, sperm associated 2	217588_at
AI683552	---	---	217679_x_at
NM_024006.1	IMAGE3455200	hypothetical protein IMAGE3455200	217949_s_at
AK026565.1	FLJ10534	hypothetical protein FLJ10534	218155_x_at
NM_014182.1	ORMDL2	ORM1-like 2 (S. cerevisiae)	218556_at
NM_021800.1	DNAJC12	J Domain containing protein 1	218976_at
NM_016049.1	CGI-112	comparative gene identification transcript 112	219203_at
NM_021971.1	GMPPB	GDP-mannose pyrophosphorylase B	219920_s_at
NM_014128.1	---	---	220856_x_at
AA133341	C14orf87	chromosome 14 open reading frame 87	221932_s_at
AF198444.1		Homo sapiens 10q21 mRNA sequence	222168_at

Table 4: 36 Gene Group (heading row above table)

Figure 4 continued

Figure 5
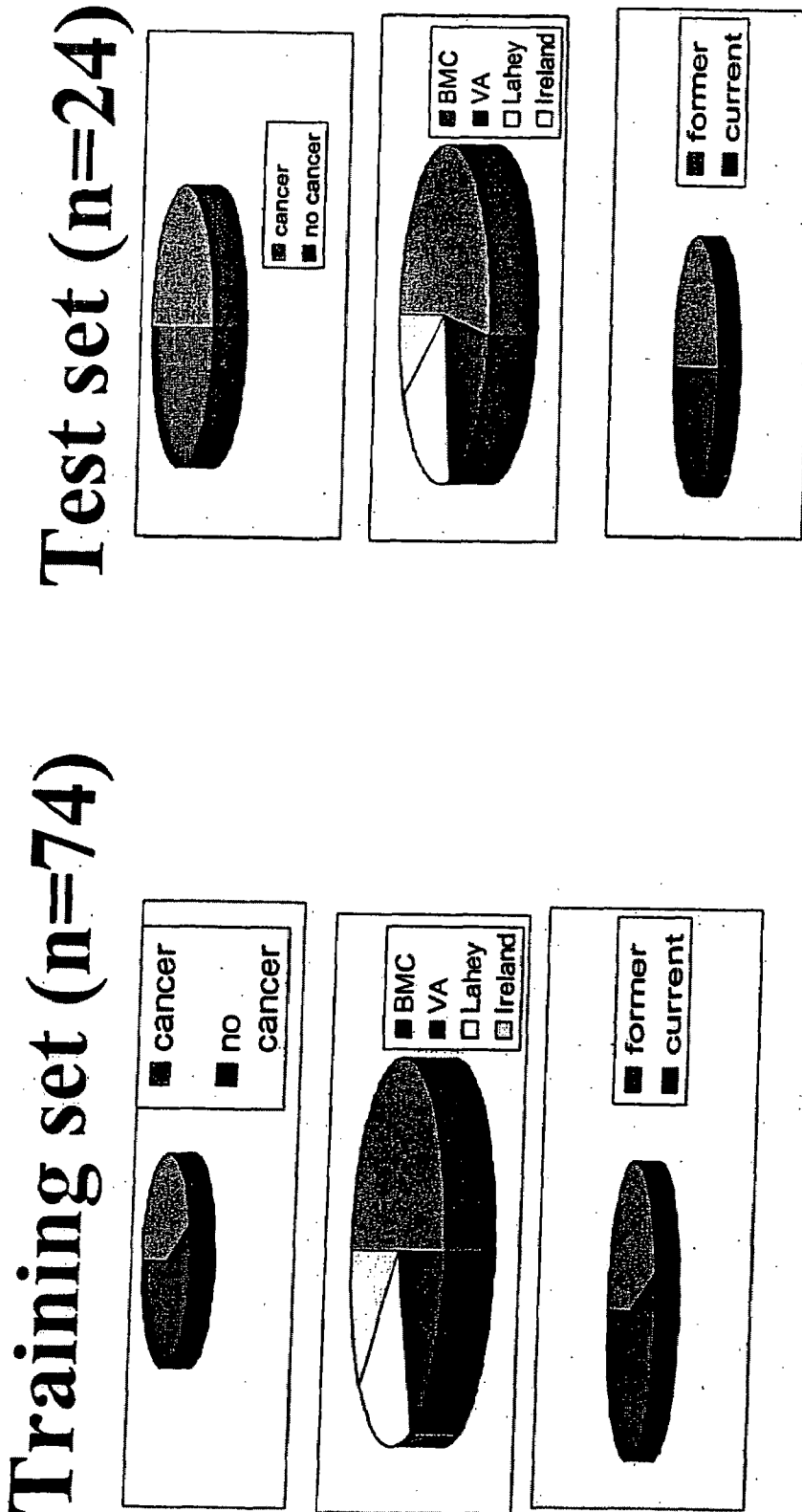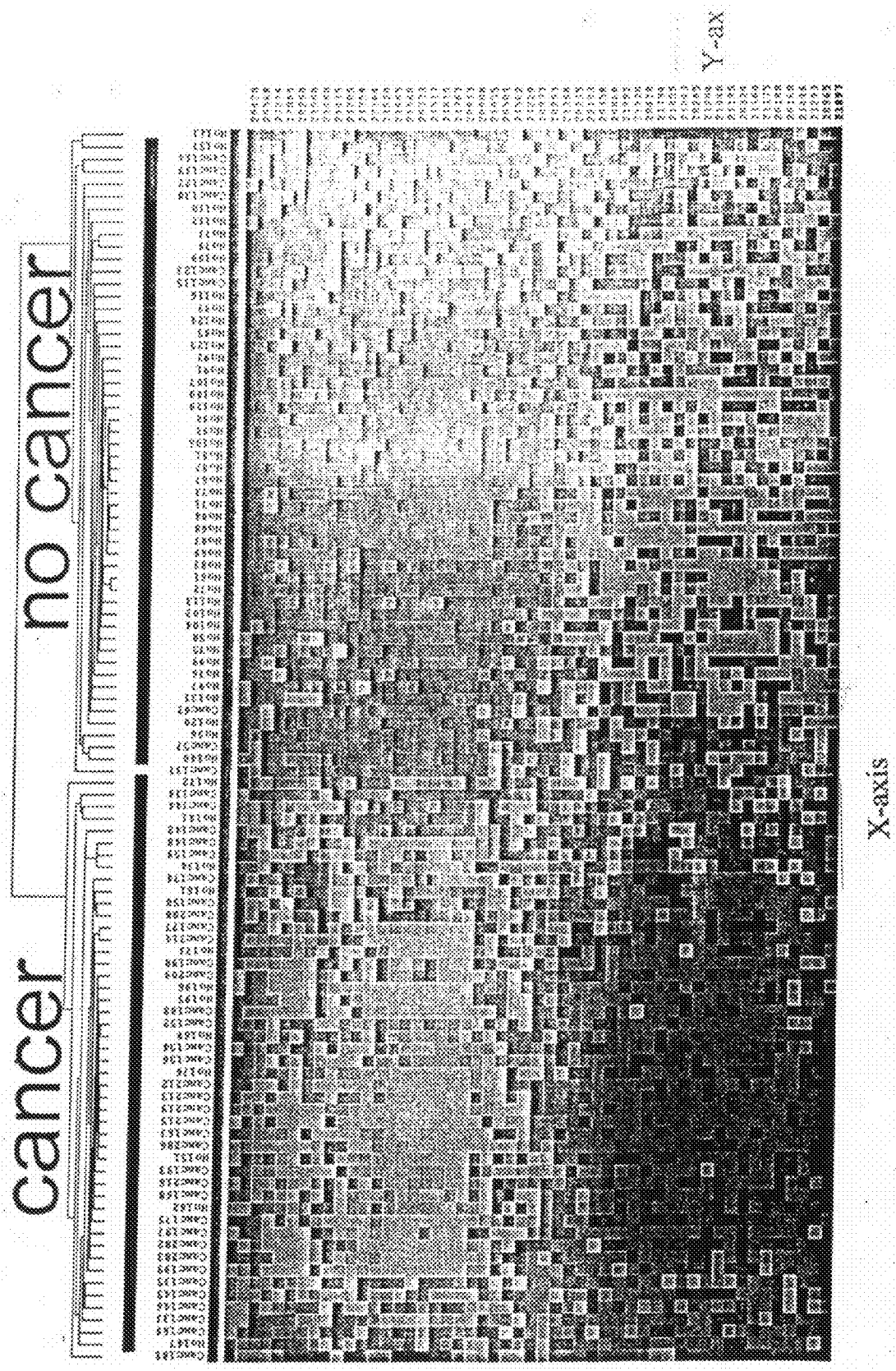
Figure 6
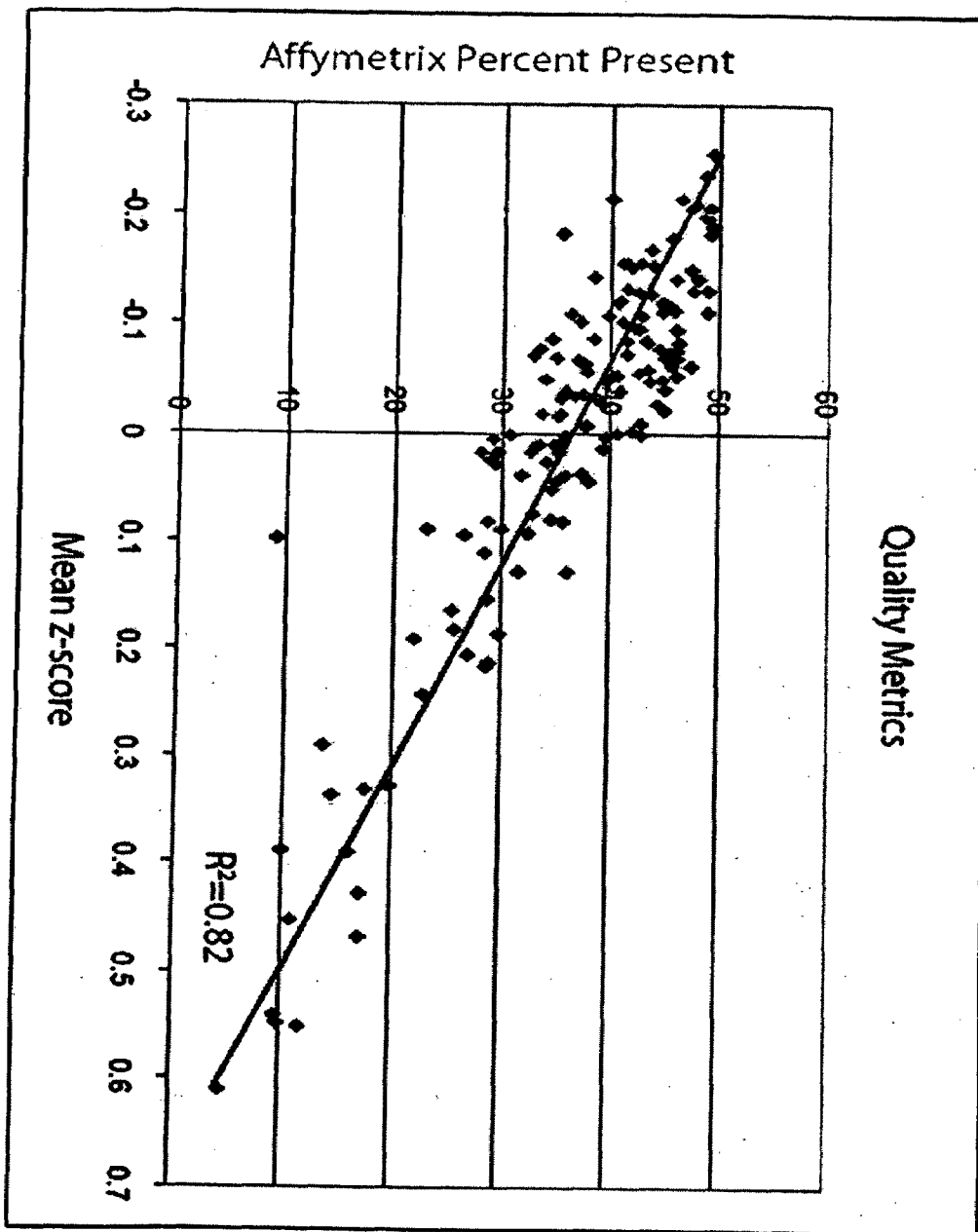
Figure 7

Histogram of 1000 separate Actual and Random runs
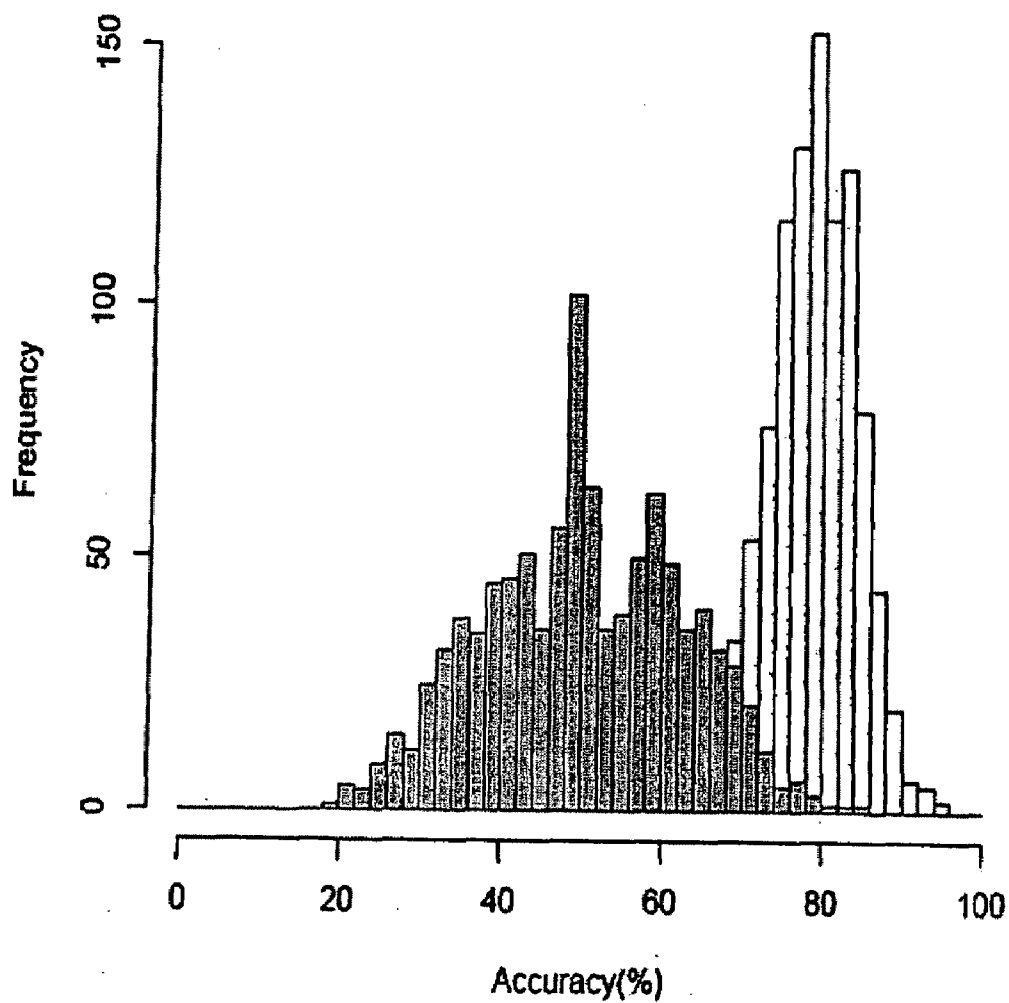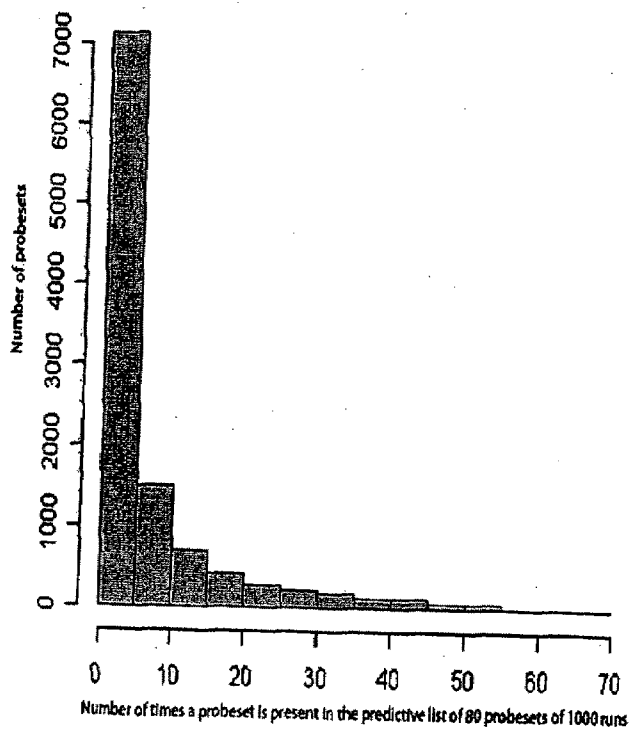
Figure 8
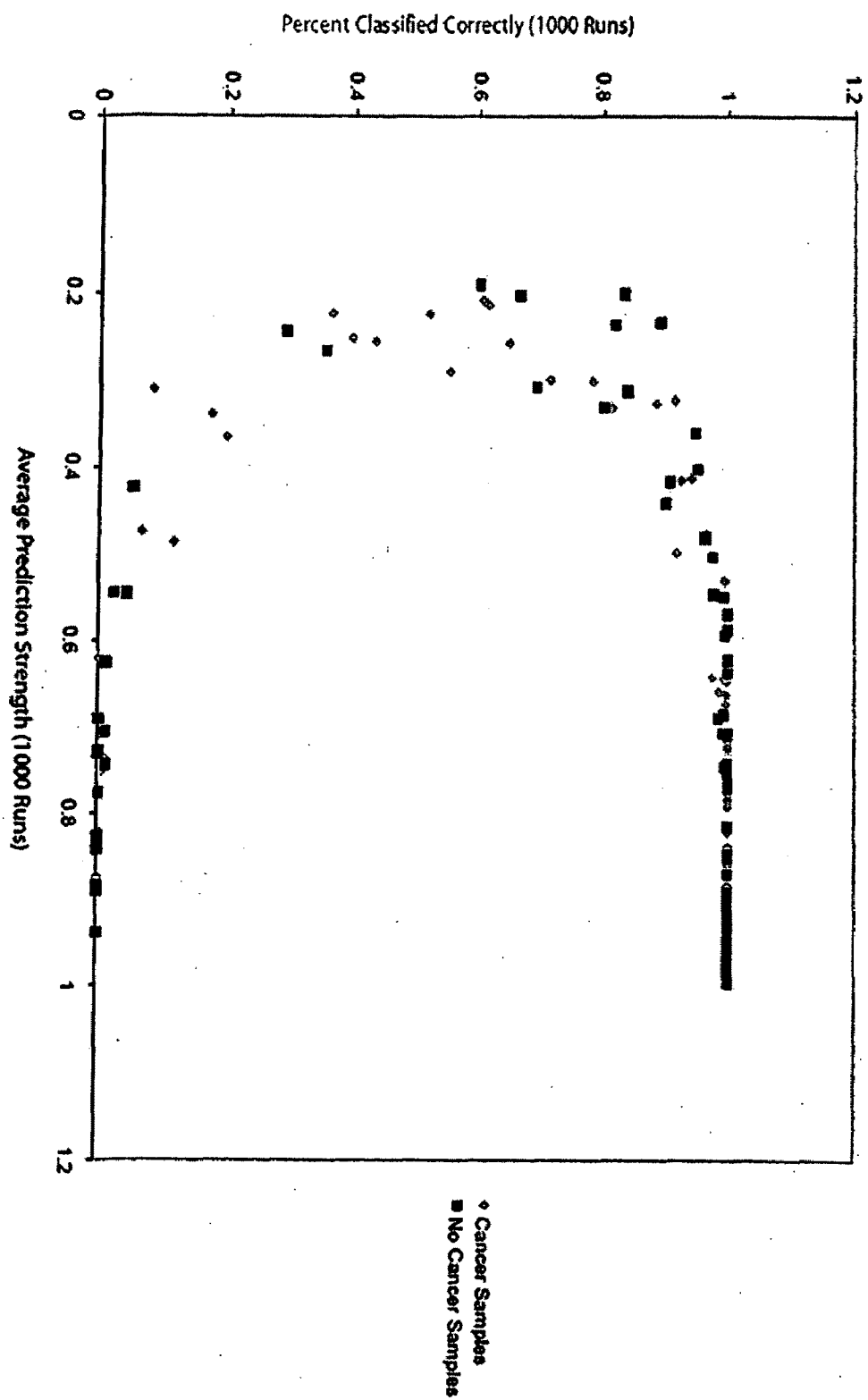
Figure 9
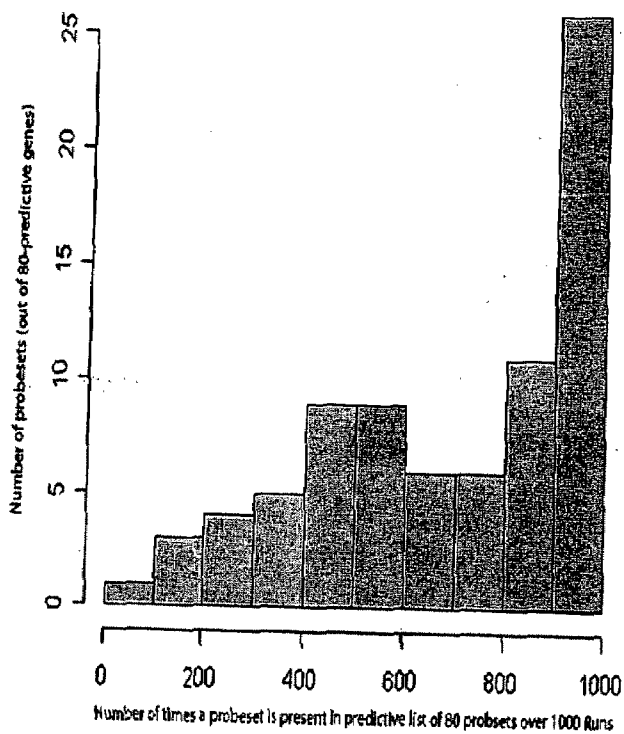
Figure 10A

Figure 10B

Figure 11

cancer type
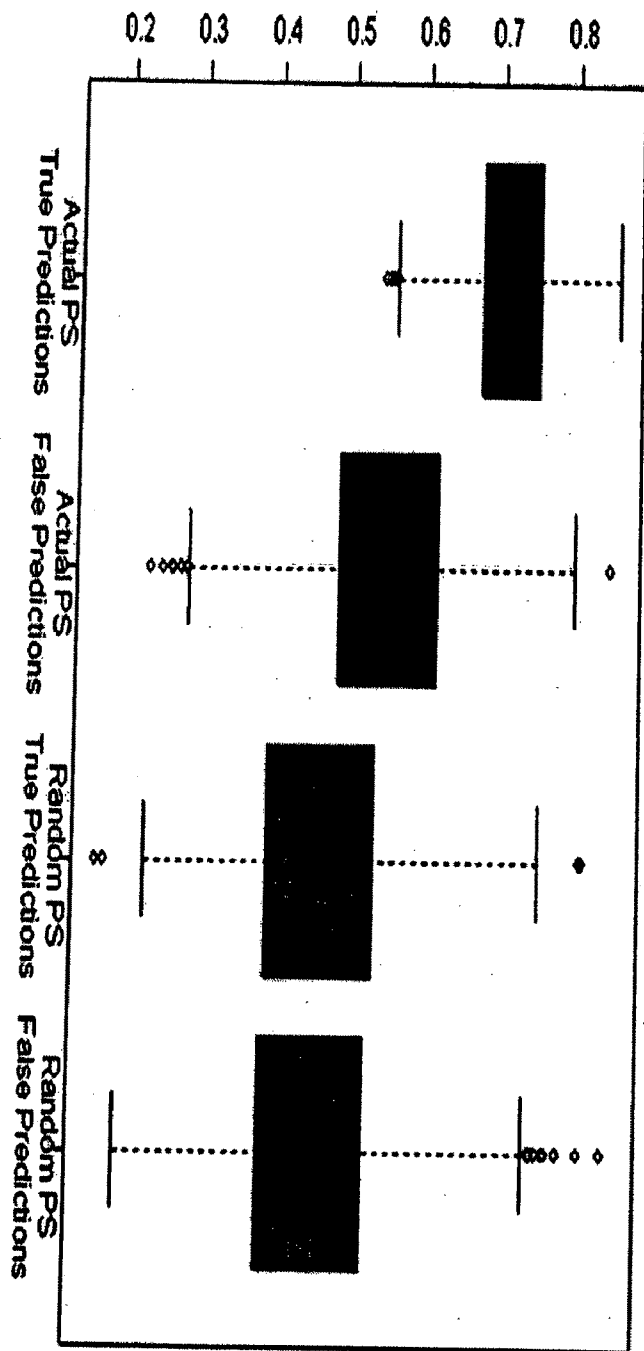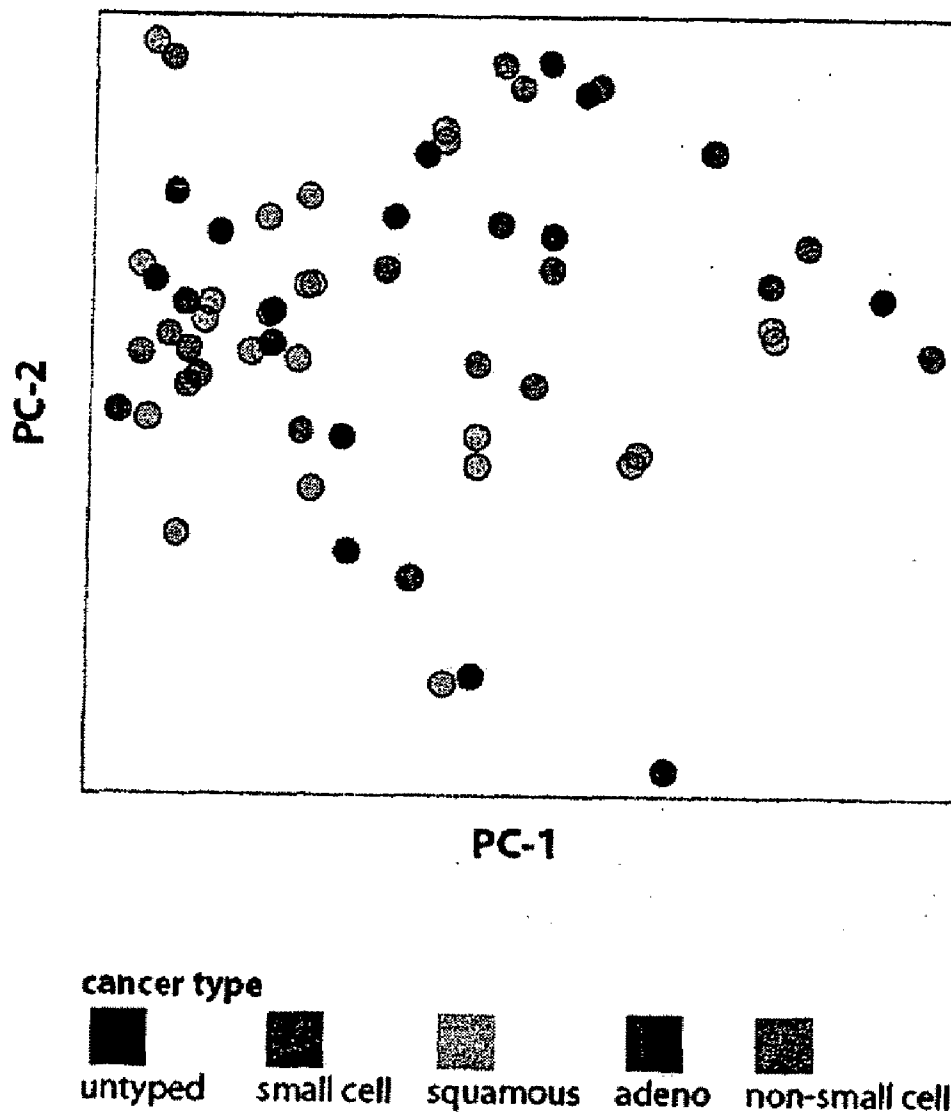
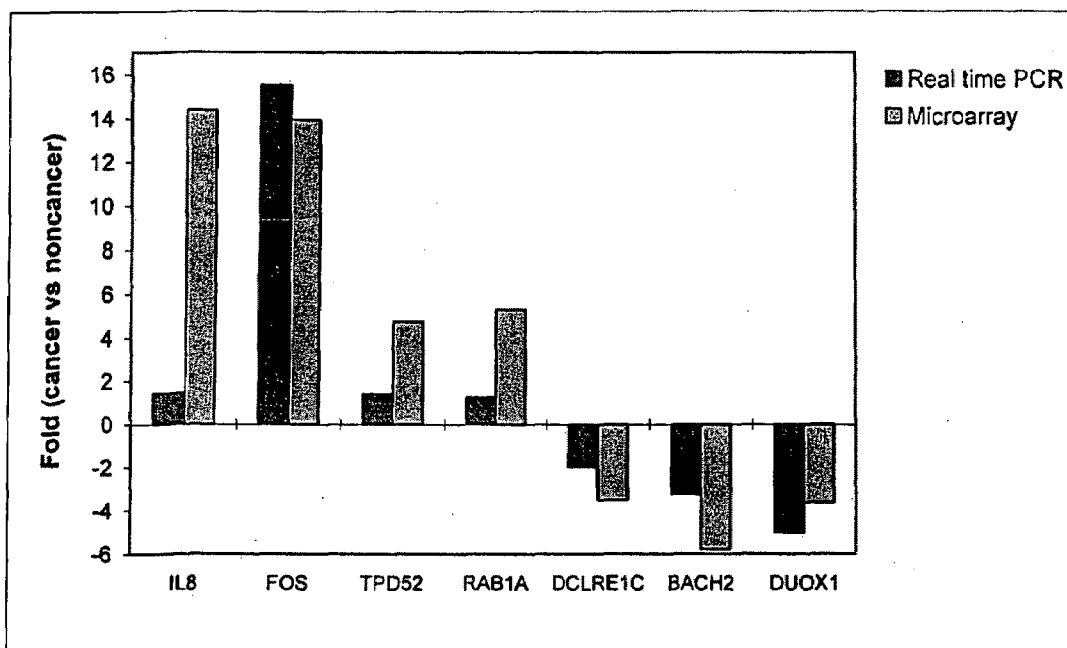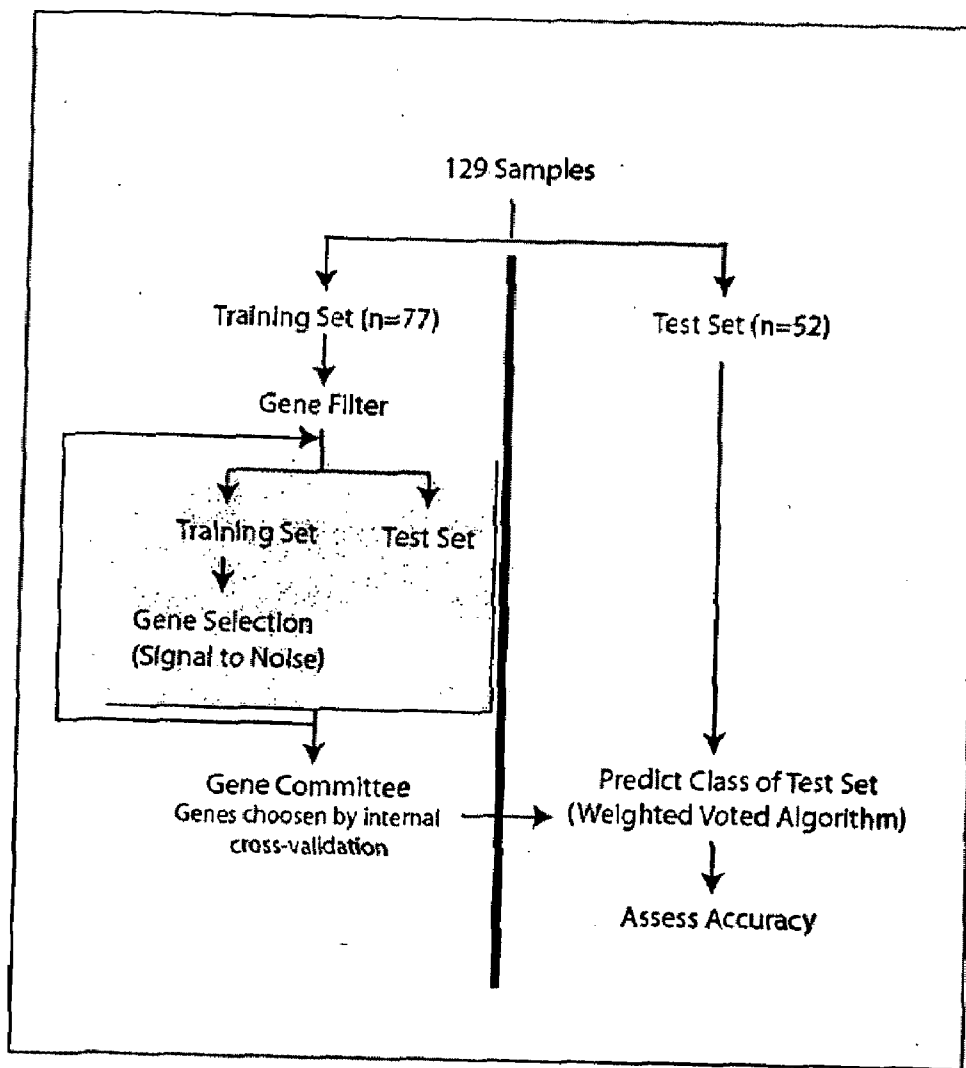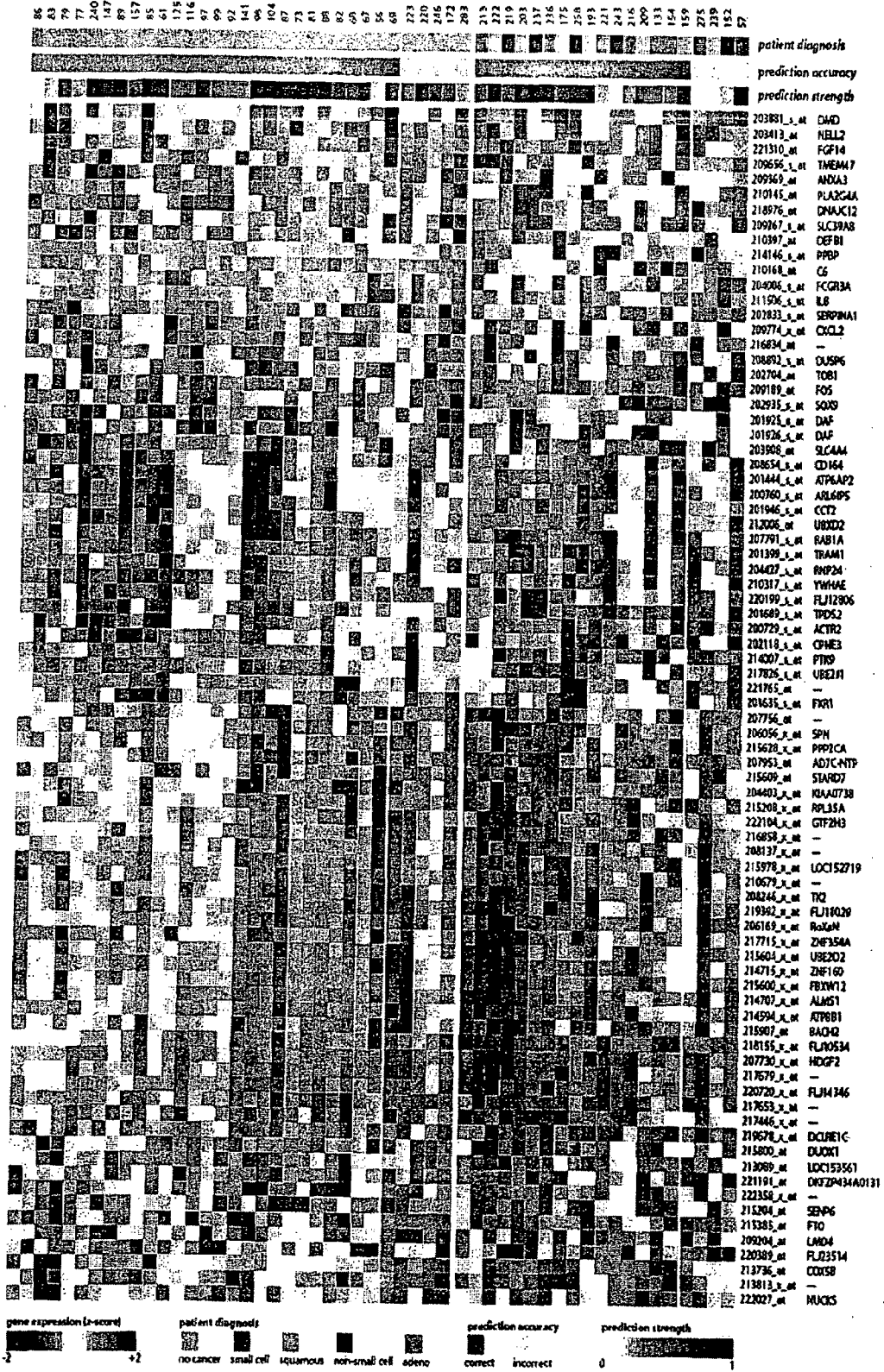untyped   small cell   squamous   adeno   non-small cell

Figure 12

Figure 13

Figure 14

Figure 15

Figure 16
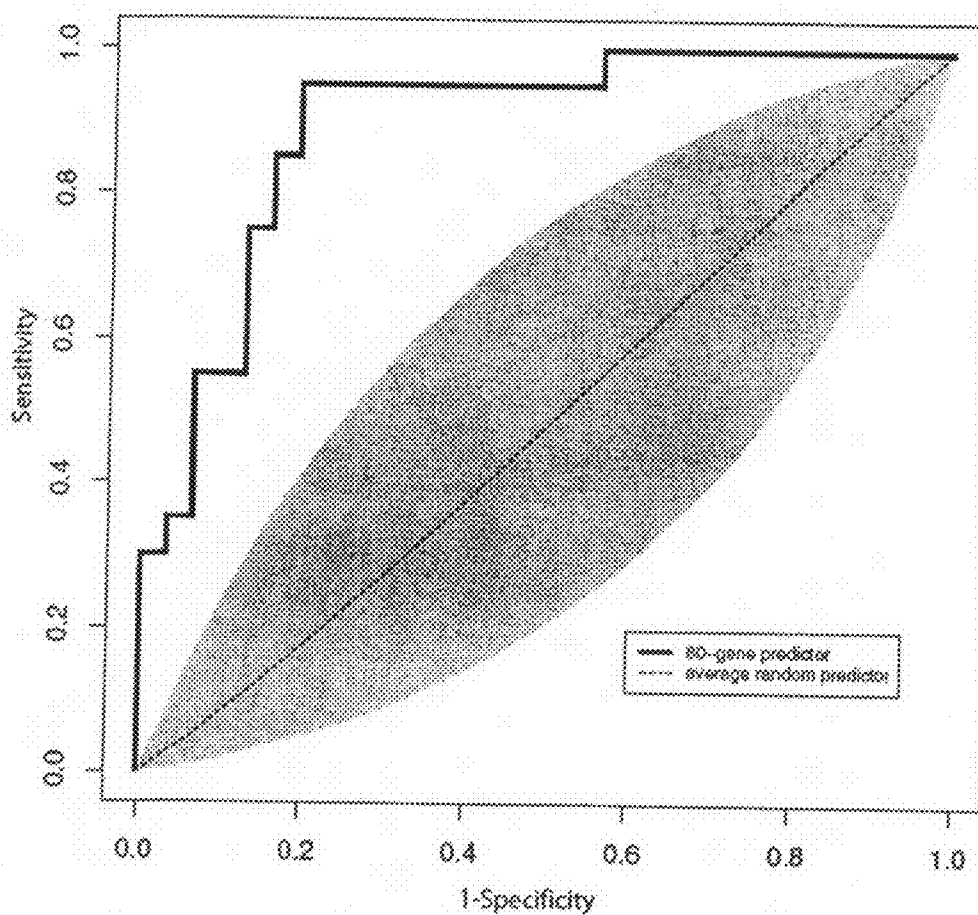
Figure 17

Figure 18
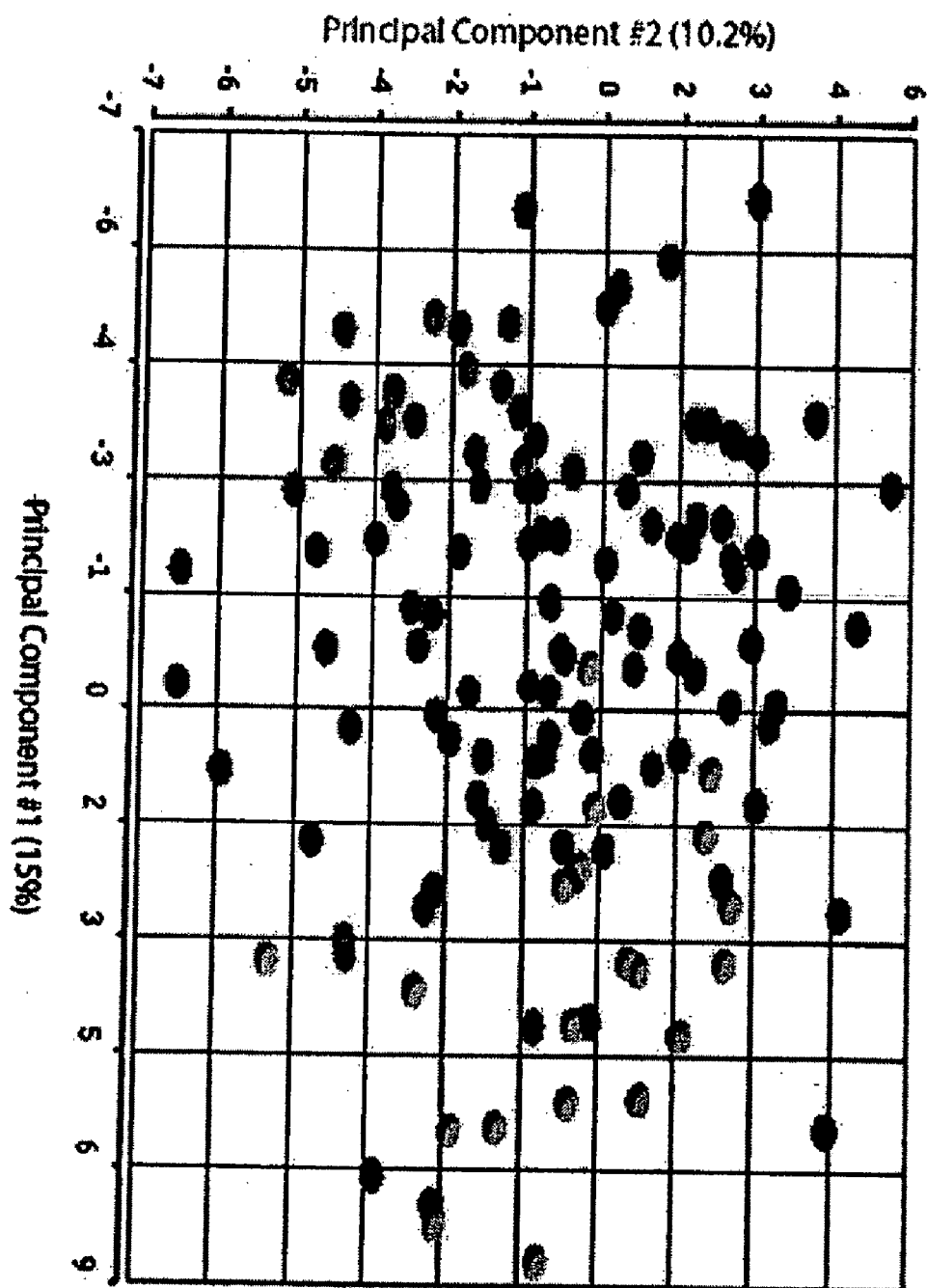
Figure 19

19A

stage 1

stage 2

stage 3

stage 4

19B

adeno

squamous

small cell
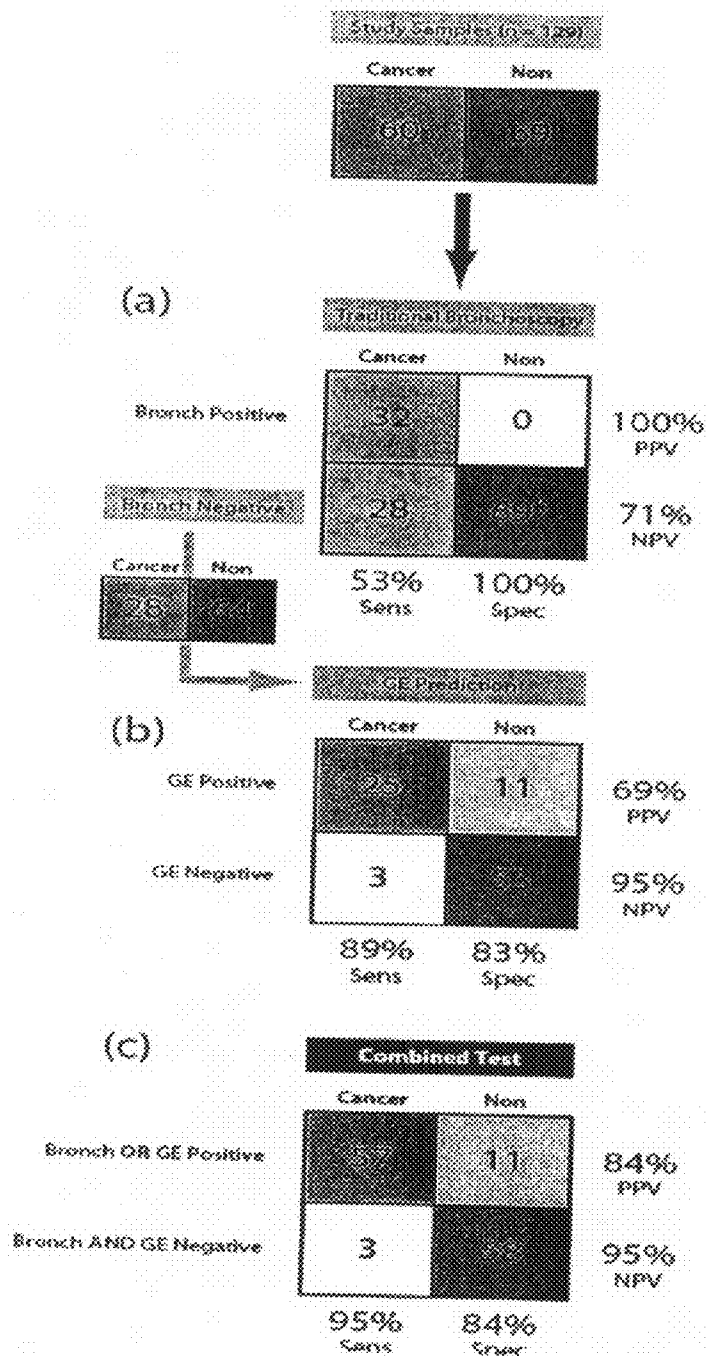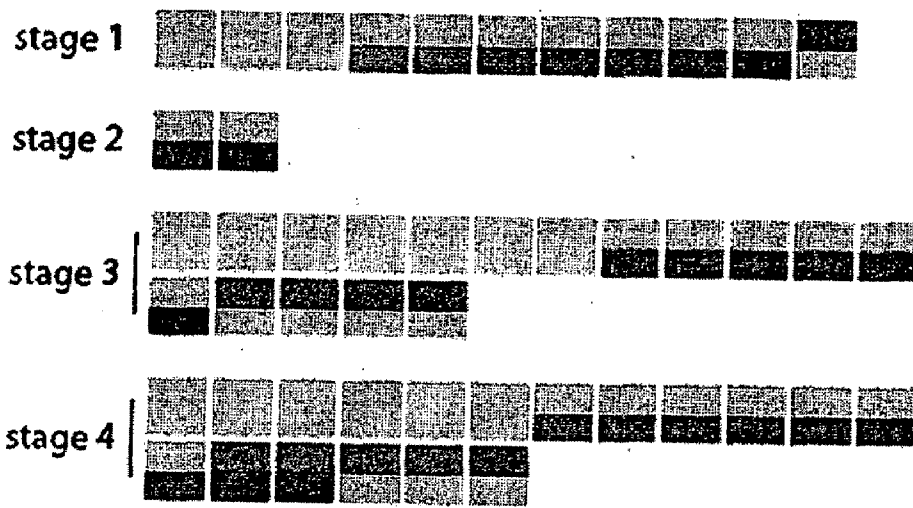
correct
class prediction error
bronchoscopy diagnosis error

DIAGNOSTIC FOR LUNG DISORDERS USING CLASS PREDICTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit under 35 U.S.C. 119(e) from the provisional application Ser. No. 60/671,243, filed on Apr. 14, 2005, the content of which is herein incorporated by reference in its entirety.
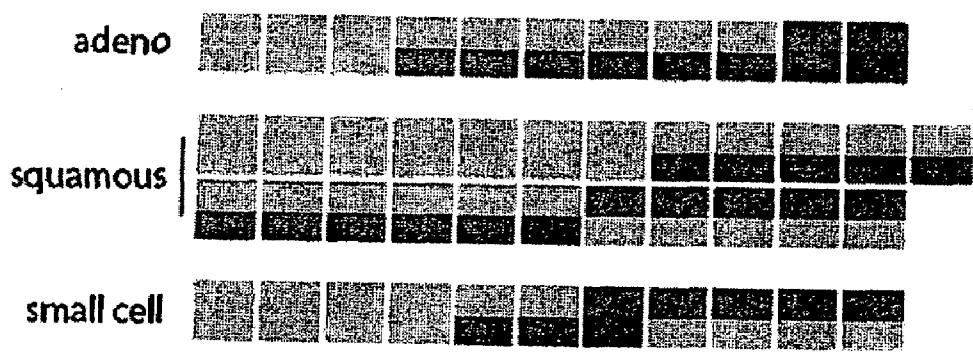
BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention
[0003] The present invention is directed to diagnostic and prognostic methods by using analysis of gene group expression patterns in a subject. More specifically, the invention is directed to diagnostic and prognostic methods for detecting lung diseases, particularly lung cancer in subjects, preferably humans that have been exposed to air pollutants.
[0004] 2. Background
[0005] Lung disorders represent a serious health problem in the modern society. For example, lung cancer claims more than 150,000 lives every year in the United States, exceeding the combined mortality from breast, prostate and colorectal cancers. Cigarette smoking is the most predominant cause of lung cancer. Presently, 25% of the U.S. population smokes, but only 10% to 15% of heavy smokers develop lung cancer. There are also other disorders associated with smoking such as emphysema. There are also health questions arising from people exposed to smokers, for example, second hand smoke. Former smokers remain at risk for developing such disorders including cancer and now constitute a large reservoir of new lung cancer cases. In addition to cigarette smoke, exposure to other air pollutants such as asbestos, and smog, pose a serious lung disease risk to individuals who have been exposed to such pollutants.
[0006] Approximately 85% of all subjects with lung cancer die within three years of diagnosis. Unfortunately survival rates have not changed substantially of the past several decades. This is largely because there are no effective methods for identifying smokers who are at highest risk for developing lung cancer and no effective tools for early diagnosis.
[0007] The methods that are currently employed to diagnose lung cancer include chest X-ray analysis, bronchoscopy or sputum cytological analysis, computer tomographic analysis of the chest, and positron electron tomographic (PET) analysis. However, none of these methods provide a combination of both sensitivity and specificity needed for an optimal diagnostic test.
[0008] Classification of human lung cancer by gene expression profiling has been described in several recent publications (M. Garber, "Diversity of gene expression in adenocarcinoma of the lung," PNAS, 98(24): 13784-13789 (2001); A. Bhattacharjee, "Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses," PNAS, 98(24):13790-13795 (2001)), but no specific gene set is used as a classifier to diagnose lung cancer in bronchial epithelial tissue samples.
[0009] Moreover, while it appears that a subset of smokers are more susceptible to, for example, the carcinogenic effects of cigarette smoke and are more likely to develop lung cancer, the particular risk factors, and particularly genetic risk factors, for individuals have gone largely unidentified. Same applies to lung cancer associated with, for example, asbestos exposure.
[0010] Therefore, there exists a great need to develop sensitive diagnostic methods that can be used for early diagnosis and prognosis of lung diseases, particularly in individuals who are at risk of developing lung diseases, particularly individuals who are exposed to air pollutants such as cigarette/cigar smoke, asbestos and other toxic air pollutants.

SUMMARY OF THE INVENTION

[0011] The present invention provides compositions and methods for diagnosis and prognosis of lung diseases which provides a diagnostic test that is both very sensitive and specific.
[0012] We have found a group of gene transcripts that we can use individually and in groups or subsets for enhanced diagnosis for lung diseases, such as lung cancer, using gene expression analysis. We provide detailed guidance on the increase and/or decrease of expression of these genes for diagnosis and prognosis of lung diseases, such as lung cancer.
[0013] One example of the gene transcript groups useful in the diagnostic/prognostic tests of the invention are set forth in Table 6. We have found that taking groups of at least 20 of the Table 6 genes provides a much greater diagnostic capability than chance alone.
[0014] Preferably one would use more than 20 of these gene transcript, for example about 20-100 and any combination between, for example, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, and so on. Our preferred groups are the groups of 96 (Table 1), 84 (Table 2), 50 (Table 3), 36 Cable 4), 80 (Table 5), 535 (Table 6) and 20 (Table 7). In some instances, we have found that one can enhance the accuracy of the diagnosis by adding certain additional genes to any of these specific groups. When one uses these groups, the genes in the group are compared to a control or a control group. The control groups can be non-smokers, smokers, or former smokers. Preferably, one compares the gene transcripts or their expression product in the biological sample of an individual against a similar group, except that the members of the control groups do not have the lung disorder, such as emphysema or lung cancer. For example, comparing can be performed in the biological sample from a smoker against a control group of smokers who do not have lung cancer. When one compares the transcripts or expression products against the control for increased expression or decreased expression, which depends upon the particular gene and is set forth in the tables—not all the genes surveyed will show an increase or decrease. However, at least 50% of the genes surveyed must provide the described pattern. Greater reliability if obtained as the percent approaches 100%. Thus, in one embodiment, one wants at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the genes surveyed to show the altered pattern indicative of lung disease, such as lung cancer, as set forth in the tables, infra.
[0015] In one embodiment, the invention provides a group of genes the expression of which is altered in individuals who are at risk of developing lung diseases, such as lung cancer, because of the exposure to air pollutants. The invention also provides groups of genes the expression of which is consistently altered as a group in individuals who are at risk of developing lung diseases because of the exposure to air pollutants.

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[0016] The present invention provides gene groups the expression pattern or profile of which can be used in methods to diagnose lung diseases, such as lung cancer and even the type of lung cancer, in more than 60%, preferably more than 65%, still more preferably at least about 70%, still more preferably about 75%, or still more preferably about 80%-95% accuracy from a sample taken from airways of an individual screened for a lung disease, such as lung cancer.

[0017] In one embodiment, the invention provides a method of diagnosing a lung disease such as lung cancer using a combination of bronchoscopy and the analysis of gene expression pattern of the gene groups as described in the present invention.

[0018] Accordingly, the invention provides gene groups that can be used in diagnosis and prognosis of lung diseases. Particularly, the invention provides groups of genes the expression profile of which provides a diagnostic and or prognostic test to determine lung disease in an individual exposed to air pollutants. For example, the invention provides groups of genes the expression profile of which can distinguish individuals with lung cancer from individuals without lung cancer.

[0019] In one embodiment, the invention provides an early asymptomatic screening system for lung cancer by using the analysis of the disclosed gene expression profiles. Such screening can be performed, for example, in similar age groups as colonoscopy for screening colon cancer. Because early detection in lung cancer is crucial for efficient treatment, the gene expression analysis system of the present invention provides a vastly improved method to detect tumor cells that cannot yet be discovered by any other means currently available.

[0020] The probes that can be used to measure expression of the gene groups of the invention can be nucleic acid probes capable of hybridizing to the individual gene/transcript sequences identified in the present invention, or antibodies targeting the proteins encoded by the individual gene group gene products of the invention. The probes are preferably immobilized on a surface, such as a gene or protein chip so as to allow diagnosis and prognosis of lung diseases in an individual.

[0021] In one embodiment, the invention provides a group of genes that can be used as individual predictors of lung disease. These genes were identified using probabilities with a 1-test analysis and show differential expression in smokers as opposed to non-smokers. The group of genes comprise ranging from 1 to 96, and all combinations in between, for example 5, 10, 15, 20, 25, 30, for example at least 36, at least about, 40, 45, 50, 60, 70, 80, 90, or 96 gene transcripts, selected from the group consisting of genes identified by the following GenBank sequence identification numbers (the identification numbers for each gene are separated by ";" while the alternative GenBank ID numbers are separated by "///"): NM_003335; NM_000918; NM_006430.1; NM_001416.1; NM_004090; NM_006406.1; NM_003001.2; NM_001319; NM_006545.1; NM_021145.1; NM_002437.1; NM_006286; NM_001003698///NM_001003699///NM_002955; NM_001123///NM_006721; NM_024824; NM_004935. 1; NM_002853.1; NM_019067.1; NM_024917.1; NM_020979.1; NM_005597.1; NM_007031.1; NM_009590.1; NM_020217.1; NM_025026.1; NM_014709.1; NM_014896.1; AF010144; NM_005374. 1; NM_001696; NM_005494///NM_058246;

NM_006534///NM_181659; NM_006368; NM_002268///NM_032771; NM_014033; NM_016138; NM_007048///NM_194441; NM_006694; NM_000051///NM_138292///NM_138293; NM_000410///NM_139002///NM_139003///NM_ 139004///NM_139005///NM_139006///NM_139007/// NM_139008///NM_139009///NM_139010///NM_ 139011; NM_004691; NM_012070///NM_139321/// NM_139322; NM_006095; AI632181; AW024467; NM_021814; NM_005547.1; NM_203458; NM_015547///NM_147161; AB007958.1; NM_207488; NM_005809///NM_181737///NM_181738; NM_0162481///NM_144490; AK022213.1; NM_005708; NM_207102; AK023895; NM_144606/NM_144997; NM_018530; AK021474; U43604.1; AU147017; AF222691.1; NM_015116; NM_001005375///NM_ 001005785///NM_001005786///NM_004081///NM_ 020363///NM_020364///NM_020420; AC004692; NM_001014; NM_000585///NM_172174///NM_172175; NM_054020///NM_172095///NM_172096///NM_ 172097; BE466926; NM_018011; NM_024077; NM_012394; NM_019011///NM_207111///NM_207116; NM_017646; NM_021800; NM_016049; NM_014395; NM_014336; NM_018097; NM_019014; NM_024804; NM_018260; NM_018118; NM_014128; NM_024084; NM_005294; AF077053; NM_138387; NM_024531; NM_000693; NM_018509; NM_033128; NM_020706; AI523613; and NM_014884, the expression profile of which can be used to diagnose lung disease, for example lung cancer, in lung cell sample from a smoker, when the expression pattern is compared to the expression pattern of the same group of genes in a smoker who does not have or is not at risk of developing lung cancer.

[0022] In another embodiment, the gene/transcript analysis comprises a group of about 10-20, 20-30, 3040, 40-50, 50-60, 60-70, 70-80, 80, 80-90, 90-100, 100-120, 120-140, 140-150, 1150-160, 160-170, 170-180, 180-190, 190-200, 200-210, 210-220, 220-230, 230-240, 240-250, 250-260, 260-270, 270-280, 280-290, 290-300, 300-310, 310-320, 320-330, 330-340, 340-350, 350-360, 360-370, 370-380, 380-390, 390-400, 400-410, 410-420, 420-430, 430-440, 440-450, 450-460, 460-470, 470-480, 480-490, 490-500, 500-510, 510-520, 520-530, and up to about 535 genes selected from the group consisting of genes or transcripts as shown in the Table 6.

[0023] In one embodiment, the genes are selected from the group consisting of genes or transcripts as shown in Table 5.

[0024] In another embodiment, the genes are selected from the genes or transcripts as shown in Table 7.

[0025] In one embodiment, the transcript analysis gene group comprises a group of individual genes the change of expression of which is predictive of a lung disease either alone or as a group, the gene transcripts selected from the group consisting of NM_007062.1; NM_001281.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; NM_002268///NM_ 032771; NM_007048///NM_194441; NM_006694; U85430.1; NM_004691; AB014576.1; BF218804; BE467941; R83000; AL161952.1; AK023843.1; AK021571. 1; AK023783.1; AL080112.1; AW971983; AI683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_021971.1; NM_014128.1; AA133341; AF198444.1.

[0026] In one embodiment, the gene group comprises a probe set capable of specifically hybridizing to at least all of the 36 gene products. Gene product can be mRNA which can be recognized by an oligonucleotide or modified oligonucleotide probe, or protein, in which case the probe can be, for example an antibody specific to that protein or an antigenic epitope of the protein.

[0027] In yet another embodiment, the invention provides a gene group, wherein the expression pattern of the group of genes provides diagnostic for a lung disease. The gene group comprises gene transcripts encoded by a gene group consisting of at least for example 5, 10, 15, 20, 25, 30, preferably at least 36, still more preferably 40, still more preferably 45, and still more preferably 46, 47, 48, 49, or all 50 of the genes selected from the group consisting of and identified by their GenBank identification numbers: NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U 93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; AB014576.1; BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; AI683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF198444.1. In one preferred embodiment, one can use at least 20 of the 36 genes that overlap with the individual predictors and, for example, 5-9 of the non-overlapping genes and combinations thereof.

[0028] In another embodiment, the invention provides a group of about 30-180, preferably, a group of about 36-150 genes, still more preferably a group of about 36-100, and still more preferably a group of about 36-50 genes, the expression profile of which is diagnostic of lung cancer in individuals who smoke.

[0029] In one embodiment, the invention provides a group of genes the expression of which is decreased in an individual having lung cancer. In one embodiment, the group of genes comprises at least 5-10, 10-15, 15-20, 20-25 genes selected from the group consisting of NM_000918; NM_006430.1; NM_001416.1; NM_004090; NM_006406.1; NM_003001.2; NM_006545.1; NM_002437.1; NM_006286; NM_001123///NM_006721; NM_024824; NM_004935.1; NM_001696; NM_005494///NM_058246; NM_006368; NM_002268///NM_032771; NM_006694; NM_004691; NM_012394; NM_021800; NM_016049; NM_138387; NM_024531; and NM_018509. One or more other genes can be added to the analysis mixtures in addition to these genes.

[0030] In another embodiment, the group of genes comprises genes selected from the group consisting of NM_014182.1; NM_001281.1; NM_024006.1; AF135421.1; L76200.1; NM_000346.1; BC008710.1; BC000423.2; BC008710.1; NM_007062; BC075839.1///BC073760.1; BC072436.1///BC004560.2; BC001016.2; BC005023.1; BC000360.2; BC007455.2; BC023528.2///BC047680.1; BC064957.1; BC008710.1; BC066329.1; BC023976.2; BC008591.2///BC050440.1///BC048096.1; and BC028912.1.

In yet another embodiment, the group of genes comprises genes selected from the group consisting of NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1, BC002642.1, NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; and AB014576.1.

[0031] In one embodiment, the invention provides a group of genes the expression of which is increased in an individual having lung cancer. In one embodiment, the group of genes comprises genes selected from the group consisting of NM_003335; NM_001319; NM_021145.1; NM_001003698///NM_001003699///; NM_002955; NM_002853.1; NM_019067.1; NM_024917.1; NM_020979.1; NM_005597.1; NM_007031.1; NM_009590.1; NM_020217.1; NM_025026.1; NM_014709.1; NM_014896.1; AF010144; NM_005374.1; NM_006534///NM_181659; NM_014033; NM_016138; NM_007048///NM_194441; NM_000051///NM_138292///NM_138293; NM_000410///NM_139002///NM_139003///NM_139004///NM_139005///NM_139006///NM_139007///NM_139008///NM_139009///NM_139010///NM_139011; NM_012070///NM_139321/NM_139322; NM_006095; A1632181; AW024467; NM_021814; NM_005547.1; NM_203458; NM_015547///NM_147161; AB007958.1; NM_207488; NM_005809///NM_181737///NM_181738; NM_016248///NM_144490; AK022213.1; NM_005708; NM_207102; AK023895; NM_144606///NM_144997; NM_018530; AK021474; U43604.1; AU147017; AF222691.1; NM_015116; NM_001005375///NM_001005785///NM_001005786///NM_004081///NM_020363///NM_020364///NM_020420; AC004692; NM_001014; NM_000585///NM_172174///NM_172175; NM_054020///NM_172095///NM_172096///NM_172097; BE466926; NM_018011; NM_024077; NM_019011///NM_207111///NM_207116; NM_017646; NM_014395; NM_014336; NM_018097; NM_019014; NM_024804; NM_018260; NM_018118; NM_014128; NM_024084; NM_005294; AF077053; NM_000693; NM_033128; NM_020706; AI523613; and NM_014884.

[0032] In one embodiment, the group of genes comprises genes selected from the group consisting of NM_030757.1; R83000; AK021571.1; NM_17932.1; U85430.1; AI683552; BC002642.1; AW024467; NM_030972.1; BC021135.1; AL161952.1; AK026565.1; AK023783.1; BF218804; AK023843.1; BC001602.1; BC034707.1; BC064619.1; AY280502.1; BC059387.1; BC061522.1; U50532.1; BC006547.2; BC008797.2; BC000807.1; AL080112.1; BC033718.1///BC046176.1///; BC038443.1; Hs.288575 (UNIGENE ID); AF020591.1; BC002503.2; BC009185.2; Hs.528304 (UNIGENE ID); U50532.1; BC013923.2; BC031091; Hs.249591 (Unigene ID); Hs.286261 (Unigene ID); AF348514.1; BC066337.1///BC058736.1///BC050555.1; Hs.216623 (Unigene ID); BC072400.1; BC041073.1; U43965.1; BC021258.2; BC016057.1; BC016713.1///BC014535.1///AF237771.1; BC000701.2; BC010067.2; Hs.156701 (Unigene ID); BC030619.2; U43965.1; Hs.438867 (Unigene ID); BC035025.2///BC050330.1; BC074852.2///BC074851.2; Hs.445885 (Unigene ID); AF365931.1; and AF257099.1.

[0033] In one embodiment, the group of genes comprises genes selected from the group consisting of BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; AI683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF198444.1.

[0034] In another embodiment, the invention provides a method for diagnosing a lung disease comprising obtaining a nucleic acid sample from lung, airways or mouth of an individual exposed to an air pollutant, analyzing the gene transcript levels of one or more gene groups provided by the present invention in the sample, and comparing the expression pattern of the gene group in the sample to an expression pattern of the same gene group in an individual, who is exposed to similar air pollutant but not having lung disease, such as lung cancer or emphysema, wherein the difference in the expression pattern is indicative of the test individual having or being at high risk of developing a lung disease. The decreased expression of one or more of the genes, preferably all of the genes including the genes listed on Tables 14 as "down" when compared to a control, and/or increased expression of one or more genes, preferably all of the genes listed on Tables 14 as "up" when compared to an individual exposed to similar air pollutants who does not have a lung disease, is indicative of the person having a lung disease or being at high risk of developing a lung disease, preferably lung cancer, in the near future and needing frequent follow ups to allow early treatment of the disease.

[0035] In one preferred embodiment, the lung disease is lung cancer. In one embodiment, the air pollutant is cigarette smoke.

[0036] Alternatively, the diagnosis can separate the individuals, such as smokers, who are at lesser risk of developing lung diseases, such as lung cancer by analyzing the expression pattern of the gene groups of the invention provides a method of excluding individuals from invasive and frequent follow ups.

[0037] Accordingly, the invention provides methods for prognosis, diagnosis and therapy designs for lung diseases comprising obtaining an airway sample from an individual who smokes and analyzing expression profile of the gene groups of the present invention, wherein an expression pattern of the gene group that deviates from that in a healthy age, race, and gender matched smoker, is indicative of an increased risk of developing a lung disease. Tables 1-4 indicate the expression pattern differences as either being down or up as compared to a control, which is an individual exposed to similar airway pollutant but not affected with a lung disease.

[0038] The invention also provides methods for prognosis, diagnosis and therapy designs for lung diseases comprising obtaining an airway sample from a non-smoker individual and analyzing expression profile of the gene groups of the present invention, wherein an expression pattern of the gene group that deviates from that in a healthy age, race, and gender matched smoker, is indicative of an increased risk of developing a lung disease.

[0039] In one embodiment, the analysis is performed from a biological sample obtained from bronchial airways.

[0040] In one embodiment, the analysis is performed from a biological sample obtained from buccal mucosa.

[0041] In one embodiment, the analysis is performed using nucleic acids, preferably RNA, in the biological sample.

[0042] In one embodiment, the analysis is performed analyzing the amount of proteins encoded by the genes of the gene groups of the invention present in the sample.

[0043] In one embodiment the analysis is performed using DNA by analyzing the gene expression regulatory regions of the groups of genes of the present invention using nucleic acid polymorphisms, such as single nucleic acid polymorphisms or SNPs, wherein polymorphisms known to be associated with increased or decreased expression are used to indicate increased or decreased gene expression in the individual. For example, methylation patterns of the regulatory regions of these genes can be analyzed.

[0044] In one embodiment, the present invention provides a minimally invasive sample procurement method for obtaining airway epithelial cell RNA that can be analyzed by expression profiling of the groups of genes, for example, by array-based gene expression profiling. These methods can be used to diagnose individuals who are already affected with a lung disease, such as lung cancer, or who are at high risk of developing lung disease, such as lung cancer, as a consequence of being exposed to air pollutants. These methods can also be used to identify further patterns of gene expression that are diagnostic of lung disorders/diseases, for example, cancer or emphysema, and to identify subjects at risk for developing lung disorders.

[0045] The invention further provides a gene group microarray consisting of one or more of the gene groups provided by the invention, specifically intended for the diagnosis or prediction of lung disorders or determining susceptibility of an individual to lung disorders.

[0046] In one embodiment, the invention relates to a method of diagnosing a disease or disorder of the lung comprising obtaining a sample, nucleic acid or protein sample, from an individual to be diagnosed; and determining the expression of group of identified genes in said sample, wherein changed expression of such gene compared to the expression pattern of the same gene in a healthy individual with similar life style and environment is indicative of the individual having a disease of the lung.

[0047] In one embodiment, the invention relates to a method of diagnosing a disease or disorder of the lung comprising obtaining at least two samples, nucleic acid or protein samples, in at least one time interval from an individual to be diagnosed; and determining the expression of the group of identified genes in said sample, wherein changed expression of at least about for example 5, 10, 15, 20, 25, 30, preferably at least about 36, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180 of such genes in the sample taken later in time compared to the sample taken earlier in time is diagnostic of a lung disease.

[0048] In one embodiment, the disease of the lung is selected from the group consisting of asthma, chronic bronchitis, emphysema, primary pulmonary hypertension, acute respiratory distress syndrome, hypersensitivity pneumonitis, eosinophilic pneumonia, persistent fungal infection, pulmonary fibrosis, systemic sclerosis, idiopathic pulmonary hemosiderosis, pulmonary alveolar proteinosis, and lung cancer, such as adenocarcinoma, squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and benign neoplasm of the lung (e.g., bronchial adenomas and hamartomas).

[0049] In a particular embodiment, the nucleic acid sample is RNA.

[0050] In a preferred embodiment, the nucleic acid sample is obtained from an airway epithelial cell. In one embodiment, the airway epithelial cell is obtained from a bronchoscopy or buccal mucosal scraping.

[0051] In one embodiment, individual to be diagnosed is an individual who has been exposed to tobacco smoke, an individual who has smoked, or an individual who currently smokes.

[0052] The invention also provides an array, for example, a microarray for diagnosis of a disease of the lung having immobilized thereon a plurality of oligonucleotides which hybridize specifically to genes of the gene groups which are differentially expressed in airways exposed to air pollutants, such as cigarette smoke, and have or are at high risk of developing lung disease, as compared to those individuals who are exposed to similar air pollutants and airways which are not exposed to such pollutants. In one embodiment, the oligonucleotides hybridize specifically to one allelic form of one or more genes which are differentially expressed for a disease of the lung. In a particular embodiment, the differentially expressed genes are selected from the group consisting of the genes shown in tables 1-4; preferably the group of genes comprises genes selected from the Table 3. In one preferred embodiment, the group of genes comprises the group of at least 20 genes selected from Table 3 and additional 5-10 genes selected from Tables 1 and 2. In one preferred embodiment, at least about 10 genes are selected from Table 4.

BRIEF DESCRIPTION OF FIGURES

[0053] FIG. 1 shows Table 1, which sets forth a listing a group of 96 genes, their expression profile in lung cancer as compared to an individual not having lung cancer but being exposed to similar environmental stress, i.e. air pollutant, in this example, cigarette smoke. These genes were identified using Student's t-test.

[0054] FIG. 2 shows Table 2, listing a group of 84 genes, their expression profile in lung cancer as compared to an individual not having lung cancer but being exposed to similar environmental stress, i.e. air pollutant, in this example, cigarette smoke. These genes were identified using Student's t-test.

[0055] FIG. 3 shows Table 3, listing a group of 50 genes, and their expression profile in lung cancer as compared using a class-prediction model to an individual not having lung cancer but being exposed to similar environmental stress, i.e. air pollutant, in this example, cigarette smoke.

[0056] FIG. 4 shows Table 4, listing a group of 36 genes, their expression profile in lung cancer as compared to an individual not having lung cancer but being exposed to similar environmental stress, i.e. air pollutant, in this example, cigarette smoke. This group of genes is a combination of predictive genes identified using both Student's t-test and class-prediction model.

[0057] FIG. 5 shows an example of the results using class prediction model as obtained in Example 1. Training set included 74 samples, and the test set 24 samples. The mean age for the training set was 55 years, and the mean pack years smoked by the training set was 38. The mean age for the test set was 56 years, and the mean pack years smoked by the test set was 41.

[0058] FIG. 6 shows an example of the 50 gene class prediction model obtained in Example 1. Each square represents expression of one transcript. The transcript can be identified

by the probe identifier on the y-axis according to the Affymetrix Human Genome Gene chip U133 probe numbers (see Appendix). The individual samples are identified on the x-axis. The samples are shown in this figure as individuals with lung cancer ("cancer") and individuals without lung cancer ("no cancer"). The gene expression is shown as higher in darker squares and lower in lighter squares. One can clearly see the differences between the gene expression of these 50 genes in these two groups just by visually observing the pattern of lighter and darker squares.

[0059] FIG. 7 shows a comparison of sample-quality metrics. The graph plots the Affymetrix MAS 5.0 percent present (y-axis) versus the z-score derived filter (x-axis). The two metrics have a correlation (R2) of 0.82.

[0060] FIG. 8 shows distribution of accuracies for real vs. random 1000 runs. Histogram comparing test set class prediction accuracies of 1000 "sample randomized" classifiers generated by randomly assigning samples into training and test sets with true class labels (unshaded) versus 1000 "sample and class randomized" classifiers where the training set class labels were randomized following sample assignment to the training or test set (shaded).

[0061] FIG. 9 shows classification accuracy as a function of the average prediction strength over the 1000 runs of the algorithm with different training/test sets.

[0062] FIG. 10A shows the number of times each of the 80-predictive probe sets from the actual biomarker was present in the predictive lists of 80 probe sets derived from 1000 runs of the algorithm.

[0063] FIG. 10B shows the Number of times a probe set was present in the predictive lists of 80 probe sets derived from 1000 random runs of the algorithm described in Supplemental Table 7.

[0064] FIG. 11 shows Boxplot of the Prediction Strength values of the test set sample predictions made by the Weighted Voting algorithm across the 1000 runs with different training and test sets. The black boxplots (first two boxes from the left) are derived from the actual training and test set data with correct sample labels, the grey boxplots (last two boxes on the right) are derived from the test set predictions based on training sets with randomized sample labels.

[0065] FIG. 12 shows homogeneity of gene expression in large airway samples from smokers with lung cancer of varying cell types. Principal Component Analysis (PCA) was performed on the gene-expression measurements for the 80 genes in our predictor and all of the airway epithelium samples from patients with lung cancer. Gene expression measurements were Z(0,1) normalized prior to PCA. The graph shows the sample loadings for the first two principal components which together account for 58% of the variation among samples from smokers with cancer. There is no apparent separation of the samples with regard to lung tumor subtype.

[0066] FIG. 13 shows real time RT-PCR and microarray data for selected genes distinguishing smokers with and without cancer. Fold change for each gene is shown as the ratio of average expression level of cancer group (n=3) to the average expression of non-cancer group (n=3). Four genes (IL8, FOS, TPD52, and RAB1A) were found to be up-regulated in cancer group on both microarray and RT-PCR platforms; three genes (DCLRE1C, BACH2, and DUOX1) were found to be down-regulated in cancer group on both platforms.

[0067] FIG. 14 shows the class prediction methodology used. 129 samples (69 from patients without cancer; 60 from

patients with lung cancer) were separated into a training (n=77) and a test set (n=52). The most frequently chosen 40 up- and 40 down-regulated genes from internal cross validation on the training set were selected for the final gene committee. The weighted voted algorithm using this committee of 80 genes was then used to predict the class of the test set samples.

[0068] FIG. 15 shows hierarchical clustering of class-predictor genes. Z-score-normalized gene-expression measurements of the eighty class-predictor genes in the 52 test-set samples are shown in a false-color scale and organized from top to bottom by hierarchical clustering. The Affymetrix U133A probeset ID and HUGO symbol are given to the right of each gene. The test-set samples are organized from left to right first by whether the patient had a clinical diagnosis of cancer. Within these two groups, the samples are organized by the accuracy of the class-predictor diagnosis (samples classified incorrectly are on the right shown in dark green). 43/52 (83%) test samples are classified correctly. The sample ID is given at the top of each column. The prediction strength of each of the diagnoses made by the class-prediction algorithm is indicated in a false-color scale immediately below the prediction accuracy. Prediction strength is a measure of the level of diagnostic confidence and varies on a continuous scale from 0 to 1 where 1 indicates a high degree of confidence.

[0069] FIG. 16 shows a Comparison of Receiver Operating Characteristic (ROC) curves. Sensitivity (y-axis) and 1-Specificity (x-axis) were calculated at various prediction strength thresholds where a prediction of no cancer was assigned a negative prediction strength value and a prediction of cancer was assigned a positive prediction strength value. The solid black line represents the ROC curve for the airway gene expression classifier. The dotted black line represents the average ROC curve for 1000 classifiers derived by randomizing the training set class labels ("class randomized"). The upper and lower lines of the gray shaded region represent the average ROC curves for the top and bottom half of random biomarkers (based on area under the curve). There is a significant difference between the area under the curve of the actual classifier and the random classifiers (p=0.004; empiric p-value based on permutation)

[0070] FIG. 17 shows the Principal Component Analysis (PCA) of biomarker gene expression in lung tissue samples. The 80 biomarker probesets were mapped to 64 probesets in the Bhattacharjee et al. HGU95Av2 microarray dataset of lung cancer and normal lung tissue. The PCA is a representation of the overall variation in expression of the 64 biomarker probesets. The normal lung samples (NL) are represented in green, the adenocarcinomas (AD) in red, the small cells (SC) in blue, and the squamous (SQ) lung cancer samples in yellow. The normal lung samples separate from the lung cancer samples along the first principal component (empirically derived p-value=0.023, see supplemental methods).

[0071] FIGS. 18A-18C show data obtained in this study. FIG. 18A shows bronchoscopy results for the 129 patients in the study. Only 32 of the 60 patients that had a final diagnosis of cancer had bronchoscopies that were diagnostic of lung cancer. The remaining 97 samples had bronchoscopies that were negative for lung cancer including 5 that had a definitive alternate benign diagnosis. This resulted in 92 patients with non-diagnostic bronchoscopy that required further tests and/or clinical follow-up. FIG. 18B shows biomarker prediction results. 36 of the 92 patients with non-diagnostic broncho-

scopies exhibited a gene expression profile that was positive for lung cancer. This resulted in 25 of 28 cancer patients with non-diagnostic bronchoscopies being predicted to have cancer. FIG. 18C shows combined test results. In a combined test where a positive test result from either bronchoscopy or gene expression is considered indicative of lung cancer a sensitivity of 95% (57 of 60 cancer patients) with only a 16% false positive rate (11 of 69 non-cancer patients) is achieved. The shading of each contingency table is reflective of the overall fraction of each sample type in each quadrant.

[0072] FIGS. 19A-19B show a comparison of bronchoscopy and biomarker prediction by A) cancer stage or B) cancer subtype. Each square symbolizes one patient sample. The upper half represents the biomarker prediction accuracy and the lower half represents the bronchoscopy accuracy. Not all cancer samples are represented in this figure. FIG. 19A includes only Non Small Cell cancer samples that could be staged using the TMN system (48 of the 60 total cancer samples). FIG. 19B includes samples that could be histologically classified as Adenocarcinoma, Squamous Cell Carcinoma and Small Cell Carcinoma (45 of the 60 total cancer samples).

DETAILED DESCRIPTION OF THE INVENTION

[0073] The present invention is directed to gene/transcript groups and methods of using the expression profile of these gene/transcript groups in diagnosis and prognosis of lung diseases.

[0074] We provide a method that significantly increases the diagnostic accuracy of lung diseases, such as lung cancer. When one combines the gene expression analysis of the present invention with bronchoscopy, the diagnosis of lung cancer is dramatically better by detecting the cancer in an earlier stage than any other available method to date, and by providing far fewer false negatives and/or false positives than any other available method.

[0075] We have found a group of gene transcripts that we can use individually and in groups or subsets for enhanced diagnosis for lung diseases, such as lung cancer, using gene expression analysis. We provide detailed guidance on the increase and/or decrease of expression of these genes for diagnosis and prognosis of lung diseases, such as lung cancer.

[0076] One example of the gene transcript groups useful in the diagnostic/prognostic tests of the invention is set forth in Table 6. We have found that taking any group that has at least 20 of the Table 6 genes provides a much greater diagnostic capability than chance alone.

[0077] Preferably one would use more than 20 of these gene transcript, for example about 20-100 and any combination between, for example, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, and so on. Our preferred groups are the groups of 96 (Table 1), 84 (Table 2), 50 (Table 3), 36 (Table 4), 80 (Table 5), 535 (Table 6) and 20 (Table 7). In some instances, we have found that one can enhance the accuracy of the diagnosis by adding additional genes to any of these specific groups.

[0078] Naturally, following the teachings of the present invention, one may also include one or more of the genes and/or transcripts presented in Tables 1-7 into a kit or a system for a multicancer screening kit. For example, any one or more genes and or transcripts from Table 7 may be added as a lung cancer marker for a gene expression analysis.

[0079] When one uses these groups, the genes in the group are compared to a control or a control group. The control groups can be non-smokers, smokers, or former smokers.

Preferably, one compares the gene transcripts or their expression product in the biological sample of an individual against a similar group, except that the members of the control groups do not have the lung disorder, such as emphysema or lung cancer. For example, comparing can be performed in the biological sample from a smoker against a control group of smokers who do not have lung cancer. When one compares the transcripts or expression products against the control for increased expression or decreased expression, which depends upon the particular gene and is set forth in the tables—not all the genes surveyed will show an increase or decrease. However, at least 50% of the genes surveyed must provide the described pattern. Greater reliability if obtained as the percent approaches 100%. Thus, in one embodiment, one wants at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% of the genes surveyed to show the altered pattern indicative of lung disease, such as lung cancer, as set forth in the tables as shown below.

[0080] The presently described gene expression profile can also be used to screen for individuals who are susceptible for lung cancer. For example, a smoker, who is over a certain age, for example over 40 years old, or a smoker who has smoked, for example, a certain number of years, may wish to be screened for lung cancer. The gene expression analysis as described herein can provide an accurate very early diagnosis for lung cancer. This is particularly useful in diagnosis of lung cancer, because the earlier the cancer is detected, the better the survival rate is.

[0081] For example, when we analyzed the gene expression results, we found, that if one applies a less stringent threshold, the group of 80 genes as presented in Table 5 are part of the most frequently chosen genes across 1000 statistical test runs (see Examples below for more details regarding the statistical testing). Using random data, we have shown that no random gene shows up more than 67 times out of 1000. Using such a cutoff, the 535 genes of Table 6 in our data show up more than 67 times out of 1000. All the 80 genes in Table 5 form a subset of the 535 genes. Table 7 shows the top 20 genes which are subset of the 535 list. The direction of change in expression is shown using signal to noise ratio. A negative number in Tables 5, 6, and 7 means that expression of this gene or transcript is up in lung cancer samples. Positive number in Table 5, 6, and 7, indicates that the expression of this gene or transcript is down in lung cancer.

[0082] Accordingly, any combination of the genes and/or transcripts of Table 6 can be used. In one embodiment, any combination of at least 5-10, 10-20, 20-30, 3040, 40-50, 50-60, 60-70, 70-80, 80, 80-90, 90-100, 100-120, 120-140, 140-150, 150-160, 160-170, 170-180, 180-190, 190-200, 200-210, 210-220, 220-230, 230-240, 240-250, 250-260, 260-270, 270-280, 280-290, 290-300, 300-310, 310-320, 320-330, 330-340, 340-350, 350-360, 360-370, 370-380, 380-390, 390-400, 400-410, 410-420, 420-430, 430-440, 440-450, 450-460, 460-470, 470-480, 480-490, 490-500, 500-510, 510-520, 520-530, and up to about 535 genes selected from the group consisting of genes or transcripts as shown in the Table 6.

[0083] Table 7 provides 20 of the most frequently variably expressed genes in lung cancer when compared to samples without cancer. Accordingly, in one embodiment, any combination of about 3-5, 5-10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or all 20 genes and/or transcripts of Table 7, or any subcombination thereof are used.

[0084] In one embodiment, the invention provides a gene group the expression profile of which is useful in diagnosing lung diseases and which comprises probes that hybridize ranging from 1 to 96 and all combinations in between for example 5, 10, 15, 20, 25, 30, 35, at least about 36, at least to 40, at lest to 50, at least to 60, to at least 70, to at least 80, to at least 90, or all of the following 96 gene sequences: NM_003335; NM_00918; NM_006430.1; NM_001416.1; NM_004090; NM_006406.1; NM_003001.2; NM_001319; NM_006545.1; NM_021145.1; NM_002437.1; NM_006286; NM_001003698///NM_0010036991///NM_002955; NM_001123///NM_006721; NM_024824; NM_004935.1; NM_002853.1; NM_019067.1; NM_024917.1; NM_020979.1; NM_005597.1; NM_007031.1; NM_009590.1; NM_020217.1; NM_025026.1; NM_014709.1; NM_014896.1; AF010144; NM_005374.1; NM_001696; NM_0054941///NM_058246; NM_006534///NM_181659; NM_006368; NM_0022681///NM_032771; NM_014033; NM_016138; NM_007048///NM_194441; NM_006694; NM_000051///NM_138292///NM_138293; N_000410///NM_139002///NM_139003///NM_139004///NM_139005///NM_139006///NM_139007///NM_139008///NM_139009///NM_139010///NM_139011; NM_004691; NM_012070///NM_139321///NM_139322; NM_006095; A1632181; AW024467; NM_021814; NM_005547.1; NM_203458; NM_015547///NM_147161; AB007958.1; NM_207488; NM_005809/NM_181737///NM_181738; NM_016248///NM_144490; AK022213.1; NM_005708; NM_207102; AK023895; NM_144606///NM_144997; NM_018530; AK021474; U43604.1; AU147017; AF222691.1; NM_015116; NM_001005375///NM_001005785///NM_001005786///NM_004081///NM_020363///NM_020364///NM_020420; AC004692; NM_001014; NM_000585///NM_172174///NM_172175; NM_054020///NM_172095///NM_172096///NM_172097; BE466926; NM_018011; NM_024077; NM_012394; NM_019011///NM_207111///NM_207116; NM_017646; NM_021800; NM_016049; NM_014395; NM_014336; NM_018097; NM_019014; NM_024804; NM_018260; NM_018118; NM_014128; NM_024084; NM_005294; AF077053; NM_138387; NM_024531; NM_000693; NM_018509; NM_033128; NM_020706; AI523613; and NM_014884

[0085] In one embodiment, the invention provides a gene group the expression profile of which is useful in diagnosing lung diseases and comprises probes that hybridize to at least, for example, 5, 10, 15, 20, 25, 30, 35, at least about 36, at least to 40, at, least to 50, at least to 60, to at least 70, to at least 80, to all of the following 84 gene sequences: NM_030757.1; R83000; AK021571.1; NM_014182.1; NM_17932.1; U85430.1; AI683552; BC002642.1; AW024467; NM_030972.1; BC021135.1; AL161952.1; AK026565.1; AK023783.1; BF218804; NM_001281.1; NM_024006.1; AK023843.1; BC001602.1; BC034707.1; BC064619.1; AY280502.1; BC059387.1; AF135421.1; BC061522.1; L76200.1; U50532.1; BC006547.2; BC008797.2; BC000807.1; AL080112.1; BC033718.1///BC046176.1///BC038443.1; NM_000346.1; BC008710.1; Hs.288575 (UNIGENE ID); AF020591.1; BC000423.2; BC002503.2; BC008710.1; BC009185.2; Hs.528304 (UNIGENE ID); U50532.1; BC013923.2; BC031091; NM_007062; Hs.249591 (Unigene ID); BC075839.1///BC073760.1; BC072436.1///BC004560.2; BC00116.2; Hs.286261 (Uni-

gene ID); AF348514.1; BC005023.1; BC066337.1///BC058736.1///BC050555.1; Hs.216623 (Unigene ID); BC072400.1; BC041073.1; U43965.1; BC021258.2; BC016057.1; BC016713.1///BC014535.1///AF237771.1; BC000360.2; BC007455.2; BC000701.2; BC010067.2; BC023528.2///BC047680.1; BC064957.1;Hs.156701 (Unigene ID); BC030619.2; BC008710.1; U43965.1; BC066329.1; Hs.438867 (Unigene ID); BC035025.2///BC050330.1; BC023976.2; BC074852.2///BC074851.2; Hs.445885 (Unigene ID); BC008591.2///BC050440.1///; BC048096.1; AF365931.1; AF257099.1; and BC028912.1.

[0086] In one embodiment, the invention provides a gene group the expression profile of which is useful in diagnosing lung diseases and comprises probes that hybridize to at least, for example 5, 10, 15, 20, 25, 30, preferably at least about 36, still more preferably at least to 40, still more preferably at lest to 45, still more preferably all of the following 50 gene sequences, although it can include any and all members, for example, 20, 21, 22, up to and including 36: NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; AB014576.1; BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; A1683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF198444.1. In one preferred embodiment, one can use at least 20-30, 3040, of the 50 genes that overlap with the individual predictor genes identified in the analysis using the t-test, and, for example, 5-9 of the non-overlapping genes, identified using the t-test analysis as individual predictor genes, and combinations thereof.

[0087] In one embodiment, the invention provides a gene group the expression profile of which is useful in diagnosing lung diseases and comprises probes that hybridize to at least for example 5, 10, 15, 20, preferably at least about 25, still more preferably at least to 30, still more preferably all of the following 36 gene sequences: NM_007062.1; NM_001281.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; NM_002268///NM_032771; NM_007048///NM_194441; NM_006694; U85430.1; NM_004691; AB014576.1; BF218804; BE467941; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AL080112.1; AW971983; AM683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_021971.1; NM_014128.1; AA133341; and AF198444.1. In one preferred embodiment, one can use at least 20 of the 36 genes that overlap with the individual predictors and, for example, 5-9 of the non-overlapping genes, and combinations thereof.

[0088] The expression of the gene groups in an individual sample can be analyzed using any probe specific to the nucleic acid sequences or protein product sequences encoded by the gene group members. For example, in one embodiment, a probe set useful in the methods of the present invention is selected from the nucleic acid probes of between 10-15, 15-20, 20-180, preferably between 30-180, still more

preferably between 36-96, still more preferably between 36-84, still more preferably between 36-50 probes, included in the Affymetrix Inc. gene chip of the Human Genome U133 Set and identified as probe ID Nos: 208082_x_at, 214800_x_at, 215208 x_at, 218556_at, 207730_x_at 210556_at, 217679_x_at, 202901_x_at 213939_s_at, 208137_x_at, 214705_at, 215001_s_at, 218155_x_at, 215604_x_at, 212297_at, 201804_x_at, 217949_s_at, 215179_x_at, 211316_x_at, 217653_x_at, 266_s_at, 204718_at, 211916_s_at, 215032 at, 219920_s_at 211996 s_at, 200075_s_at, 214753_at, 204102_s_at, 202419_at; 214715_x_at, 216859_x_at, 215529_x_at, 202936_s_at, 212130_x_at, 215204_at, 218735_s_at, 200078_s_at, 203455_s_at, 212227_x_at, 222282_at, 219678_x_at, 208268_at, 221899_at, 213721_at, 214718_at, 201608_s_at, 205684_s_at, 209008_x_at, 200825_s_at 218160_at, 57739_at, 211921_x_at, 218074_at, 200914_x_at, 216384_x_at, 214594_x_at, 222122s_5 at 204060_s_at, 215314_at, 208238_x_at, 210705_s_at, 211184_s_at, 215418_at, 209393_s_at 210101_x_at, 212052_s_at, 215011_at, 221932_s_at, 201239_s_at, 215553_x_at, 213351_sat, 202021_x_at, 209442_x_at, 210131_x_at, 217713_x_at, 214707_x_at, 203272_s_at, 206279_at, 214912_at, 201729_s_at, 205917_at, 200772_x_at, 202842_s_at, 203588_s_at, 209703_x_at, 217313_at, 217588_at, 214153_at, 222155_s_at, 203704_s_at, 220934_s_at, 206929_s_at, 220459_at, 215645_at 217336_at, 203301_s_at, 207283_at, 222168_at, 222272_x_at, 219290_x_at, 204119_s_at, 215387_x_at, 222358_x_at 205010_at 1316 at 216187_x_at, 208678_at, 222310 at, 210434_x_at, 220242_x_at, 207287_at 207953_at, 209015_s_at, 221759_at, 220856_x_at, 200654_at, 220071_x_at, 216745_x_at, 218976_at, 214833_at, 202004_x_at, 209653_at, 210858_x_at, 212041_at, 221294_at, 207020_at, 204461_x_at, 205367_at, 219203_at, 215067_x_at, 212517_at, 220215_at, 201923_at, 215609_at, 207984_s_at, 215373_x_at, 216110_x_at, 215600_x_at, 216922_x_at, 215892_at, 201530_x_at, 217371__at, 222231_s_at, 218265_at, 201537_s_at, 221616_s_at, 213106_at, 215336_at, 209770_at, 209061_at, 202573_at, 207064_S_at, 64371_at, 219977_at, 218617_at, 214902_x_at, 207436_x_at, 215659_at, 204216_s_at, 214763_at, 200877_at, 218425_at, 203246_s_at, 203466_at, 204247_s_at, 216012_at, 211328 x_at, 218336_at, 209746_s_at, 214722_at, 214599_at, 220113_x_at, 213212_x_at, 217671_at, 207365_x_at, 218067_s_at, 205238_at, 209432_s_at, and 213919_at. In one preferred embodiment, one can use at least, for example, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 110, 120, 130, 140, 150, 160, or 170 of the 180 genes that overlap with the individual predictors genes and, for example, 5-9 of the non-overlapping genes and combinations thereof.

[0089] Sequences for the Affymetrix probes are provided in the Appendix to the specification, all the pages of which are herein incorporated by reference in their entirety.

[0090] One can analyze the expression data to identify expression patters associated with any lung disease that is caused by exposure to air pollutants, such as cigarette smoke, asbestos or any other lung disease. For example, the analysis can be performed as follows. One first scans a gene chip or mixture of beads comprising probes that are hybridized with a study group samples. For example, one can use samples of non-smokers and smokers, non-asbestos exposed individuals and asbestos-exposed individuals, non-smog exposed individuals and smog-exposed individuals, smokers without a lung disease and smokers with lung disease, to obtain the

differentially expressed gene groups between individuals with no lung disease and individuals with lung disease. One must, of course select appropriate groups, wherein only one air pollutant can be selected as a variable. So, for example, one can compare non-smokers exposed to asbestos but not smog and non-smokers not exposed to asbestos or smog.

[0091] The obtained expression analysis, such as microarray or microbead raw data consists of signal strength and detection p-value. One normalizes or scales the data, and filters the poor quality chips/bead sets based on images of the expression data, control probes, and histograms. One also filters contaminated specimens which contain non-epithelial cells. Lastly, one filters the genes of importance using detection p-value. This results in identification of transcripts present in normal airways (normal airway transcriptome). Variability and multiple regression analysis can be used. This also results in identification of effects of smoking on airway epithelial cell transcription. For this analysis, one can use T-test and Pearson correlation analysis. One can also identify a group or a set of transcripts that are differentially expressed in samples with lung disease, such as lung cancer and samples without cancer. This analysis was performed using class prediction models.

[0092] For analysis of the data, one can use, for example, a weighted voting method. The weighted voting method ranks,

and gives a weight "p" to all genes by the signal to noise ration of gene expression between two classes: $P = \text{mean}_{(class\ 1)} - \text{mean}_{(class\ 2)}/\text{Sd}_{(class\ 1)} = \text{sd}_{(class\ 2)}$. Committees of variable sizes of the top ranked genes are used to evaluate test samples, but genes with more significant p-values can be more heavily weighed. Each committee genes in test sample votes for one class or the other, based on bow close that gene expression level is to the class 1 mean or the class 2 mean. $V_{(gene\ A)} = P_{(gene\ A)}$, i.e. level of expression in test sample less the average of the mean expression values in the two classes. Votes for each class are tallied and the winning class is determined along with prediction strength as $PS = V_{win} - V_{lose}/V_{win} + V_{lose}$. Finally, the accuracy can be validated using cross-validation +/− independent samples.

[0093] Table 1 shows 96 genes that were identified as a group distinguishing smokers with cancer from smokers without cancer. The difference in expression is indicated at the column on the right as either "down", which indicates that the expression of that particular transcript was lower in smokers with cancer than in smokers without cancer, and "up", which indicates that the expression of that particular transcript was higher in smokers with cancer than smokers without cancer. In one embodiment, the exemplary probes shown in the column "Affymetrix Id in the Human Genome U133 chip" can be used. Sequences for the Affymetrix probes are provided in the Appendix.

TABLE 1

96 Gene Group

Affymetrix Id	GenBank ID	Gene Description	Gene Name	Direction in Cancer
1316_at	NM_003335	ubiquitin-activated enzyme E1-like	UBE1L	down
200654_at	NM_000918	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)	P4HB	up
200877_at	NM_006430.1	chaperonin containing TCP1, subunit 4 (delta)	CCT4	up
201530_x_at	NM_001416.1	eukaryotic translation factor 4A, isoform 1	EIF4A1	up
201537_s_at	NM_004090	dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)	DUSP3	up
201923_at	NM_006406.1	peroxiredoxin 4	PRDX4	up
202004_x_at	NM_003001.2	succinate dehydrogenase complex, subunit C, integral membrane protein, 15 kDa	SDHC	up
202573_at	NM_001319	casein kinase 1, gamma 2	CSNK1G2	down
203246_s_at	NM_006545.1	tumor suppressor candidate 4	TUSC4	up
203301_s_at	NM_021145.1	cyclin D binding myb-like transcription factor 1	DMTF1	down
203466_at	NM_002437.1	MpV17 transgene, murine homolog, glomerusclerosis	MPV17	up
203588_s_at	NM_006286	transcription factor Dp-2 (E2F dimerization partner 2)	TFDP2	up
203704_s_at	NM_001003698 /// NM_001003699 /// NM_002955	ras responsive element binding protein 1	RREB1	down

TABLE 1-continued

<u>96 Gene Group</u>

Affymetrix Id	GenBank ID	Gene Description	Gene Name	Direction in Cancer
204119_s_at	NM_001123 /// NM_006721	adenosine kinase	ADK	up
204216_s_at	NM_024824	nuclear protein UKp68	FLJ11806	up
204247_s_at	NM_004935.1	cyclin-dependent kinase 5	CDK5	up
204461_x_at	NM_002853.1	RAD1 homolog	RAD1	down
205010_at	NM_019067.1	hypothetical protein FLJ10613	FLJ10613	down
205238_at	NM_024917.1	chromosome X open reading frame 34	CXorf34	down
205367_at	NM_020979.1	adaptor protein with pleckstrin homology and src homology 2 domains	APS	down
206929_s_at	NM_005597.1	nuclear factor I/c (CCAAT-binding transcription factor)	NFIC	down
207020_at	NM_007031.1	heat shock transcription factor 2 binding protein	HSF2BP	down
207064_s_at	NM_009590.1	amine oxidase, copper containing 2 (retina-specific)	AOC2	down
207283_at	NM_020217.1	hypothetical protein DKFZp547I014	DKFZp547I014	down
207287_at	NM_025026.1	hypothetical protein FLJ14107	FLJ14107	down
207365_x_at	NM_014709.1	ubiquitin specific protease 34	USP34	down
207436_x_at	NM_014896.1	KIAA0894 protein	KIAA0894	down
207953_at	AF010144	—	—	down
207984_s_at	NM_005374.1	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member2	MPP2	down
208678_at	NM_001696	ATPase, H+ transporting, lysosomal 31 kDa, V1 subunit E, isoform 1	ATP6V1E1	up
209015_s_at	NM_005494 /// NM_058246	DnaJ (Hsp40) homolog, subfamily B, member 6	DNAJB6	up
209061_at	NM_006534 /// NM_181659	nuclear receptor coactivator 3	NCOA3	down
209432_s_at	NM_006368	cAMP responsive element binding protein 3	CREB3	up
209653_at	NM_002268 /// NM_032771	karyopherin alpha 4 (importin alpha 3)	KPNA4	up
209703_x_at	NM_014033	DKFZP586A0522 protein	DKFZP586A0522	down
209746_s_at	NM_016138	coenzyme Q7 homolog, ubiquinone	COQ7	down
209770_at	NM_007048 /// NM_194441	butyrophilin, subfamily 3, member A1	BTN3A1	down
210434_x_at	NM_006694	jumping translocation breakpoint	JTB	up
210858_x_at	NM_000051 /// NM_138292 /// NM_138293	ataxia telangiectasia mutated (includes complementation groups A, C, and D	ATM	down
211328_x_at	NM_000410 /// NM_139002 /// NM_139003 /// NM_139004 /// NM_139005 /// NM_139006 /// NM_139007 /// NM_139008 /// NM_139009 /// NM_139010 /// NM_139011	hemochromatosis	HFE	down
212041_at	NM_004691	ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d isoform 1	ATP6V0D1	up

TABLE 1-continued

<u>96 Gene Group</u>

Affymetrix Id	GenBank ID	Gene Description	Gene Name	Direction in Cancer
212517_at	NM_012070 /// NM_139321 /// NM_139322	attractin	ATRN	down
213106_at	NM_006095	ATPase, aminophospholipid transporter (APLT), Class I, type 8A, member 1	ATP8A1	down
213212_x_at	AI632181	Similar to FLJ40113 protein	—	down
213919_at	AW024467	—	—	down
214153_at	NM_021814	ELOVL family member 5, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	ELOVL5	down
214599_at	NM_005547.1	involucrin	IVL	down
214722_at	NM_203458	similar to NOTCH2 protein	N2N	down
214763_at	NM_015547 /// NM_147161	thiosterase, adipose associated	THEA	down
214833_at	AB007958.1	KIAA0792 gene product	KIAA0792	down
214902_x_at	NM_207488	FLJ42393 protein	FLJ42393	down
215067_x_at	NM_005809 /// NM_181737 /// NM_181738	peroxiredoxin 2	PRDX2	down
215336_at	NM_016248 /// NM_144490	A kinase (PRKA) anchor protein	AKAP11	down
215373_x_at	AK022213.1	hypothetical protein FLJ12151	FLJ12151	down
215387_x_at	NM_005708	Glypican 6	GPC6	down
215600_x_at	NM_207102	F-box and WD-40 domain protein 12	FBXW12	down
215609_at	AK023895	—	—	down
215645_at	NM_144606 /// NM_144997	Hypothetical protein MGC13008	FLCN	down
215659_at	NM_018530	Gasdermin-like	GSDML	down
215892_at	AK021474	—	—	down
216012_at	U43604.1	human unidentified mRNA, partial sequence	—	down
216110_x_at	AU147017	—	—	down
216187_x_at	AF222691.1	*Homo sapiens* Alu repeat	LNX1	down
216745_x_at	NM_015116	Leucine-rich repeats and calponin homology (CH) domain containing 1	LRCH1	down
216922_x_at	NM_001005375 /// NM_001005785 /// NM_001005786 /// NM_004081 /// NM_020363 /// NM_020364 /// NM_020420	deleted in azoospermia	DAZ2	down
217313_at	AC004692	—	—	down
217336_at	NM_001014	ribosomal protein S10	RPS10	down
217371_s_at	NM_000585 /// NM_172174 /// NM_172175	interleukin 15	IL15	down
217588_at	NM_054020 /// NM_172095 /// NM_172096 /// NM_172097	cation channel, sperm associated 2	CATSPER2	down

TABLE 1-continued

96 Gene Group

Affymetrix Id	GenBank ID	Gene Description	Gene Name	Direction in Cancer
217671_at	BE466926	—	—	down
218067_s_at	NM_018011	hypothetical protein FLJ10154	FLJ10154	down
218265_at	NM_024077	SECIS binding protein 2	SECISBP2	down
218336_at	NM_012394	prefoldin 2	PFDN2	up
218425_at	NM_019011 /// NM_207111 /// NM_207116	TRIAD3 protein	TRIAD3	down
218617_at	NM_017646	tRNA isopentenyltransferase 1	TRIT1	down
218976_at	NM_021800	DnaJ (Hsp40) homolog, subfamily C, member 12	DNAJC12	up
219203_at	NM_016049	chromosome 14 open reading frame 122	C14orf122	up
219290_x_at	NM_014395	dual adaptor of phosphotyrosine and 3-phosphoinositides	DAPP1	down
219977_at	NM_014336	aryl hydrocarbon receptor interacting protein-like 1	AIPL1	down
220071_x_at	NM_018097	chromosome 15 open reading frame 25	C15orf25	down
220113_x_at	NM_019014	polymerase (RNA) I polypeptide B, 128 kDa	POLR1B	down
220215_at	NM_024804	hypothetical protein FLJ12606	FLJ12606	down
220242_x_at	NM_018260	hypothetical protein FLJ10891	FLJ10891	down
220459_at	NM_018118	MCM3 minichromosome maintenace deficient 3 (s. cerevisiae) associated protein, antisense	MCM3APAS	down
220856_x_at	NM_014128	—		down
220934_s_at	NM_024084	hypothetical protein MGC3196	MGC3196	down
221294_at	NM_005294	G protein-coupled receptor 21	GPR21	down
221616_s_at	AF077053	Phosphoglycerate kinase 1	PGK1	down
221759_at	NM_138387	glucose-6-phosphatase catalytic subunit-related	G6PC3	up
222155_s_at	NM_024531	G protein-coupled receptor 172A	GPR172A	up
222168_at	NM_000693	Aldehyde dehydrogenase 1 family, member A3	ALDH1A3	down
222231_s_at	NM_018509	hypothetical protein PRO1855	PRO1855	up
222272_x_at	NM_033128	scinderin	SCIN	down
222310_at	NM_020706	splicing factor, arginine/serine-rich 15	SERS15	down
222358_x_at	AI523613	—	—	down
64371_at	NM_014884	splicing factor, arginine/serine-rich 14	SFRS14	down

[0094] Table 2 shows one preferred 84 gene group that was identified as a group distinguishing smokers with cancer from smokers without cancer. The difference in expression is indicated at the column on the right as either "down", which indicates that the expression of that particular transcript was lower in smokers with cancer than in smokers without cancer,

and "up", which indicates that the expression of that particular transcript was higher in smokers with cancer than smokers without cancer. These genes were identified using traditional Student's t-test analysis.

[0095] In one embodiment, the exemplary probes shown in the column "Affymetrix Id in the Human Genome U133 chip" can be used in the expression analysis.

TABLE 2

84 Gene Group

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction in Cancer	Affymetrix ID
NM_030757.1	MKRN4	makorin, ring finger protein, 4 /// makorin, ring finger protein, 4	down	208082_x_at
R83000	BTF3	basic transcription factor 3	down	214800_x_at
AK021571.1	MUC20	mucin 20	down	215208_x_at
NM_014182.1	ORMDL2	ORM1-like 2 (*S. cerevisiae*)	up	218556_at
NM_17932.1	FLJ20700	hypothetical protein FLJ20700	down	207730_x_at
U85430.1	NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	down	210556_at
AI683552	—	—	down	217679_x_at
BC002642.1	CTSS	cathepsin S	down	202901_x_at
AW024467	RIPX	rap2 interacting protein x	down	213939_s_at
NM_030972.1	MGC5384	hypothetical protein MGC5384 /// hypothetical protein MGC5384	down	208137_x_at
BC021135.1	INADL	InaD-like protein	down	214705_at
AL161952.1	GLUL	glutamate-ammonia ligase (glutamine synthase)	down	215001_s_at
AK026565.1	FLJ10534	hypothetical protein FLJ10534	down	218155_x_at
AK023783.1	—	*Homo sapiens* cDNA FLJ13721 fis, clone PLACE2000450.	down	215604_x_at
BF218804	AFURS1	ATPase family homolog up-regulated in senescence cells	down	212297_at
NM_001281.1	CKAP1	cytoskeleton associated protein 1	up	201804_x_at
NM_024006.1	IMAGE3455200	hypothetical protein IMAGE3455200	up	217949_s_at
AK023843.1	PGF	placental growth factor, vascular endothelial growth factor-related protein	down	215179_x_at
BC001602.1	CFLAR	CASP8 and FADD-like apoptosis regulator	down	211316_x_at
BC034707.1	—	*Homo sapiens* transcribed sequence with weak similarity to protein ref: NP_060312.1 (*H. sapiens*) hypothetical protein FLJ20489 [*Homo sapiens*]	down	217653_x_at
BC064619.1	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	down	266_s_at
AY280502.1	EPHB6	EphB6	down	204718_at
BC059387.1	MYO1A	myosin IA	down	211916_s_at
	—	*Homo sapiens* transcribed sequences	down	215032_at
AF135421.1	GMPPB	GDP-mannose pyrophosphorylase B	up	219920_s_at
BC061522.1	MGC70907	similar to MGC9515 protein	down	211996_s_at
L76200.1	GUK1	guanylate kinase 1	up	200075_s_at
U50532.1	CG005	hypothetical protein from BCRA2 region	down	214753_at
BC006547.2	EEF2	eukaryotic translation elongation factor 2	down	204102_s_at
BC008797.2	FVT1	follicular lymphoma variant translocation 1	down	202419_at
BC000807.1	ZNF160	zinc finger protein 160	down	214715_x_at

TABLE 2-continued

<u>84 Gene Group</u>

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction in Cancer	Affymetrix ID
AL080112.1	—	—	down	216859_x_at
BC033718.1 /// BC046176.1 /// BC038443.1	C21orf106	chromosome 21 open reading frame 106	down	215529_x_at
NM_000346.1	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	up	202936_s_at
BC008710.1	SUI1	putative translation initiation factor	up	212130_x_at
Hs.288575 (UNIGENE ID)	—	*Homo sapiens* cDNA FLJ14090 fis, clone MAMMA1000264.	down	215204_at
AF020591.1	AF020591	zinc finger protein	down	218735_s_at
BC000423.2	ATP6V0B	ATPase, H+ transporting, lysosomal 21 kDa, V0 subunit c" /// ATPase, H+ transporting, lysosomal 21 kDa, V0 subunit c"	up	200078_s_at
BC002503.2	SAT	spermidine/spermine N1-acetyltransferase	down	203455_s_at
BC008710.1	SUI1	putative translation initiation factor	up	212227_x_at
	—	*Homo sapiens* transcribed sequences	down	222282_at
BC009185.2	DCLRE1C	DNA cross-link repair 1C (PSO2 homolog, *S. cerevisiae*)	down	219678_x_at
Hs.528304 (UNIGENE ID)	ADAM28	a disintegrin and metalloproteinase domain 28	down	208268_at
U50532.1	CG005	hypothetical protein from BCRA2 region	down	221899_at
BC013923.2	SOX2	SRY (sex determining region Y)-box 2	down	213721_at
BC031091	ODAG	ocular development-associated gene	down	214718_at
NM_007062	PWP1	nuclear phosphoprotein similar to *S. cerevisiae* PWP1	up	201608_s_at
Hs.249591 (Unigene ID)	FLJ20686	hypothetical protein FLJ20686	down	205684_s_at
BC075839.1 /// BC073760.1	KRT8	keratin 8	up	209008_x_at
BC072436.1 /// BC004560.2	HYOU1	hypoxia up-regulated 1	up	200825_s_at
BC001016.2	NDUFA8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19 kDa	up	218160_at
Hs.286261 (Unigene ID)	FLJ20195	hypothetical protein FLJ20195	down	57739_at
AF348514.1	—	*Homo sapiens* fetal thymus prothymosin alpha mRNA, complete cds	down	211921_x_at
BC005023.1	CGI-128	CGI-128 protein	up	218074_at
BC066337.1 /// BC058736.1 /// BC050555.1	KTN1	kinectin 1 (kinesin receptor)	down	200914_x_at
	—	—	down	216384_x_at
Hs.216623 (Unigene ID)	ATP8B1	ATPase, Class I, type 8B, member 1	down	214594_x_at
BC072400.1	THOC2	THO complex 2	down	222122_s_at
BC041073.1	PRKX	protein kinase, X-linked	down	204060_s_at
U43965.1	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	down	215314_at
	—	—	down	208238_x_at
BC021258.2	TRIM5	tripartite motif-containing 5	down	210705_s_at

TABLE 2-continued

84 Gene Group

GenBank ID (unless otherwise mentioned)	Gene Name	Description	Direction in Cancer	Affymetrix ID
BC016057.1	USH1C	Usher syndrome 1C (autosomal recessive, severe)	down	211184_s_at
BC016713.1 /// BC014535.1 /// AF237771.1	PARVA	parvin, alpha	down	215418_at
BC000360.2	EIF4EL3	eukaryotic translation initiation factor 4E-like 3	up	209393_s_at
BC007455.2	SH3GLB1	SH3-domain GRB2-like endophilin B1	up	210101_x_at
BC000701.2	KIAA0676	KIAA0676 protein	down	212052_s_at
BC010067.2	CHC1	chromosome condensation 1	down	215011_at
BC023528.2 /// BC047680.1	C14orf87	chromosome 14 open reading frame 87	up	221932_s_at
BC064957.1	KIAA0102	KIAA0102 gene product	up	201239_s_at
Hs.156701 (Unigene ID)	—	Homo sapiens cDNA FLJ14253 fis, clone OVARC1001376.	down	215553_x_at
BC030619.2	KIAA0779	KIAA0779 protein	down	213351_s_at
BC008710.1	SUI1	putative translation initiation factor	up	202021_x_at
U43965.1	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	down	209442_x_at
BC066329.1	SDHC	succinate dehydrogenase complex, subunit C, integral membrane protein, 15 kDa	up	210131_x_at
Hs.438867 (Unigene ID)	—	Homo sapiens transcribed sequence with weak similarity to protein ref: NP_060312.1 (H. sapiens) hypothetical protein FLJ20489 [Homo sapiens]	down	217713_x_at
BC035025.2 /// BC050330.1	ALMS1	Alstrom syndrome 1	down	214707_x_at
BC023976.2	PDAP2	PDGFA associated protein 2	up	203272_s_at
BC074852.2 /// BC074851.2	PRKY	protein kinase, Y-linked	down	206279_at
Hs.445885 (Unigene ID)	KIAA1217	Homo sapiens cDNA FLJ12005 fis, clone HEMBB1001565.	down	214912_at
BC008591.2 /// BC050440.1 /// BC048096.1	KIAA0100	KIAA0100 gene product	up	201729_s_at
AF365931.1	ZNF264	zinc finger protein 264	down	205917_at
AF257099.1	PTMA	prothymosin, alpha (gene sequence 28)	down	200772_x_at
BC028912.1	DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	up	202842_s_at

[0096] Table 3 shows one preferred 50 gene group that was identified as a group distinguishing smokers with cancer from smokers without cancer. The difference in expression is indicated at the column on the right as either "down", which indicates that the expression of that particular transcript was lower in smokers with cancer than in smokers without cancer, and "up", which indicates that the expression of that particular transcript was higher in smokers with cancer than smokers without cancer.

[0097] This gene group was identified using the GenePattern server from the Broad Institute, which includes the Weighted Voting algorithm. The default settings, i.e., the signal to noise ratio and no gene filtering, were used.

[0098] In one embodiment, the exemplary probes shown in the column "Affymetrix Id in the Human Genome U133 chip" can be used in the expression analysis.

TABLE 3

50 Gene Group

GenBank ID	Gene Name	Direction in Cancer	Affymetrix Id in the Human Genome U133 chip
NM__007062.1	PWP1	up in cancer	201608__s__at
NM__001281.1	CKAP1	up in cancer	201804__x__at
BC000120.1		up in cancer	202355__s__at
NM__014255.1	TMEM4	up in cancer	202857__at
BC002642.1	CTSS	up in cancer	202901__x__at
NM__000346.1	SOX9	up in cancer	202936__s__at
NM__006545.1	NPR2L	up in cancer	203246__s__at
BG034328		up in cancer	203588__s__at
NM__021822.1	APOBEC3G	up in cancer	204205__at
NM__021069.1	ARGBP2	up in cancer	204288__s__at
NM__019067.1	FLJ10613	up in cancer	205010__at
NM__017925.1	FLJ20686	up in cancer	205684__s__at
NM__017932.1	FLJ20700	up in cancer	207730__x__at
NM__030757.1	MKRN4	up in cancer	208082__x__at
NM__030972.1	MGC5384	up in cancer	208137__x__at
AF126181.1	BCG1	up in cancer	208682__x__at
U93240.1		up in cancer	209653__at
U90552.1		up in cancer	209770__at
AF151056.1		up in cancer	210434__x__at
U85430.1	NFATC3	up in cancer	210556__at
U51007.1		up in cancer	211609__x__at
BC005969.1		up in cancer	211759__x__at
NM__002271.1		up in cancer	211954__s__at
AL566172		up in cancer	212041__at
AB014576.1	KIAA0676	up in cancer	212052__s__at
BF218804	AFURS1	down in cancer	212297__at
AK022494.1		down in cancer	212932__at
AA114843		down in cancer	213884__s__at
BE467941		down in cancer	214153__at
NM__003541.1	HIST1H4K	down in cancer	214463__x__at
R83000	BTF3	down in cancer	214800__x__at
AL161952.1	GLUL	down in cancer	215001__s__at

TABLE 3-continued

50 Gene Group

GenBank ID	Gene Name	Direction in Cancer	Affymetrix Id in the Human Genome U133 chip
AK023843.1	PGF	down in cancer	215179__x__at
AK021571.1	MUC20	down in cancer	215208__x__at
AK023783.1	—	down in cancer	215604__x__at
AU147182		down in cancer	215620__at
AL080112.1	—	down in cancer	216859__x__at
AW971983		down in cancer	217588__at
AI683552	—	down in cancer	217679__x__at
NM__024006.1	IMAGE3455200	down in cancer	217949__s__at
AK026565.1	FLJ10534	down in cancer	218155__x__at
NM__014182.1	ORMDL2	down in cancer	218556__at
NM__021800.1	DNAJC12	down in cancer	218976__at
NM__016049.1	CGI-112	down in cancer	219203__at
NM__019023.1	PRMT7	down in cancer	219408__at
NM__021971.1	GMPPB	down in cancer	219920__s__at
NM__014128.1	—	down in cancer	220856__x__at
AK025651.1		down in cancer	221648__s__at
AA133341	C14orf87	down in cancer	221932__s__at
AF198444.1		down in cancer	222168__at

[0099] Table 4 shows one preferred 36 gene group that was identified as a group distinguishing smokers with cancer from smokers without cancer. The difference in expression is indicated at the column on the right as either "down", which indicates that the expression of that particular transcript was lower in smokers with cancer than in smokers without cancer, and "up", which indicates that the expression of that particular transcript was higher in smokers with cancer than smokers without cancer.

[0100] In one embodiment, the exemplary probes shown in the column "Affymetrix Id in the Human Genome U133 chip" can be used in the expression analysis.

TABLE 4

36 Gene Group

GenBank ID	Gene Name	Gene Description	Affy ID
NM__007062.1	PWP1	nuclear phosphoprotein similar to S. cerevisiae PWP1	201608__s__at
NM__001281.1	CKAP1	cytoskeleton associated protein 1	201804__x__at
BC002642.1	CTSS	cathepsin S	202901__x__at
NM__000346.1	SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	202936__s__at
NM__006545.1	NPR2L	homologous to yeast nitrogen permease (candidate tumor suppressor)	203246__s__at
BG034328		transcription factor Dp-2 (E2F dimerization partner 2)	203588__s__at
NM__019067.1	FLJ10613	hypothetical protein FLJ10613	205010__at
NM__017925.1	FLJ20686	hypothetical protein FLJ20686	205684__s__at
NM__017932.1	FLJ20700	hypothetical protein FLJ20700	207730__x__at
NM__030757.1	MKRN4	makorin, ring finger protein, 4 /// makorin, ring finger protein, 4	208082__x__at
NM__030972.1	MGC5384	hypothetical protein MGC5384	208137__x__at

TABLE 4-continued

36 Gene Group

GenBank ID	Gene Name	Gene Description	Affy ID
NM_002268 /// NM_032771	KPNA4	karyopherin alpha 4 (importin alpha 3)	209653_at
NM_007048 /// NM_194441	BTN3A1	butyrophilin, subfamily 3, member A1	209770_at
NM_006694	JBT	jumping translocation breakpoint	210434_x_at
U85430.1	NFATC3	nuclear factor of activated T-cells, cytoplamic, calcineurin-dependent 3	210556_at
NM_004691	ATP6V0D1	ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d isoform 1	212041_at
AB014576.1	KIAA0676	KIAA0676 protein	212052_s_at
BF218804	AFURS1	ATPase family homolog up-regulated in senescence cells	212297_at
BE467941		EVOVL family member 5, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	214153_at
R83000	BTF3	basic transcription factor 3	214800_x_at
AL161952.1	GLUL	glutamate-ammonia ligase (glutamine synthase)	215001_s_at
AK023843.1	PGF	placental growth factor, vascular endothelial growth factor-related protein	215179_x_at
AK021571.1	MUC20	mucin 20	215208_x_at
AK023783.1	—	Homo sapiens cDNA FLJ13721 fis, clone PLACE2000450.	215604_x_at
AL080112.1	—	—	216859_x_at
AW971983		cation, sperm associated 2	217588_at
AI683552	—	—	217679_x_at
NM_024006.1	IMAGE3455200	hypothetical protein IMAGE3455200	217949_s_at
AK026565.1	FLJ10534	hypothetical protein FLJ10534	218155_x_at
NM_014182.1	ORMDL2	ORM1-like 2 (S. cerevisiae)	218556_at
NM_021800.1	DNAJC12	J Domain containing protein 1	218976_at
NM_016049.1	CGI-112	comparative gene identification transcript 112	219203_at
NM_021971.1	GMPPB	GDP-mannose pyrophosphorylase B	219920_s_at
NM_014128.1	—	—	220856_x_at
AA133341	C14orf87	chromosome 14 open reading frame 87	221932_s_at
AF198444.1		Homo sapiens 10q21 mRNA sequence	222168_at

[0101] In one embodiment, the gene group of the present invention comprises at least, for example, 5, 10, 15, 20, 25, 30, more preferably at least 36, still more preferably at least about 40, still more preferably at least about 50, still more preferably at least about 60, still more preferably at least about 70, still more preferably at least about 80, still more preferably at least about 86, still more preferably at least about 90, still more preferably at least about 96 of the genes as shown in Tables 14.

[0102] In one preferred embodiment, the gene group comprises 36-180 genes selected from the group consisting of the genes listed in Tables 1-4.

[0103] In one embodiment, the invention provides group of genes the expression of which is lower in individuals with cancer.

[0104] Accordingly, in one embodiment, the invention provides of a group of genes useful in diagnosing lung diseases, wherein the expression of the group of genes is lower in individuals exposed to air pollutants with cancer as compared to individuals exposed to the same air pollutant who do not have cancer, the group comprising probes that hybridize at least 5, preferably at least about 5-10, still more preferably at least about 10-20, still more preferably at least about 20-30, still more preferably at least about 3040, still more preferably at least about 40-50, still more preferably at least about 50-60, still more preferably at least about 60-70, still more preferably about 72 genes consisting of transcripts (transcripts are identified using their GenBank ID or Unigene ID numbers and the corresponding gene names appear in Table 1): NM_003335; NM_001319; NM_021145.1;

NM__001003698///NM__001003699 µl; NM__002955; NM__002853.1; NM__019067.1; NM__024917.1; NM__020979.1; NM__005597.1; NM__007031.1; NM__009590.1; NM__020217.1; NM__025026.1; NM__014709.1; NM__014896.1; AF010144; NM__005374. 1; NM__006534///NM__81659; NM__014033; NM__016138; NM__007048///NM__194441; NM__000051///NM__ 138292///NM__138293; NM__000410///NM__139002/// NM__139003///NM__139004///NM__139005///NM__ 1390061///NM__139007 µl NM__139008///NM__139009/// NM__139010///NM__139011; NM__012070///NM__ 139321///NM__139322; NM__006095; AJ632181; AW024467; NM__021814; NM__005547.1; NM__203458; NM__015547///NM__147161; AB007958.1; NM__207488; NM__005809///NM__181737///NM__181738; NM__016248///NM__144490; AK022213.1; NM__005708; NM__207102; AK023895; NM__144606///NM__144997; NM__018530; AK021474; U43604.1; AU147011; AF222691.1; NM__015116; NM__001005375/NM__ 001005785///NM__001005786///NM__0040811///NM__ 020363///NM__020364///NM__020420; AC004692; NM__001014; NM__000585/NM__172174///NM__72175; NM__054020///NM__172095///NM__172096///NM__ 172097; BE466926; NM__018011; NM__024077; NM__019011///NM__207111///NM__207116; NM__017646; NM__014395; NM__014336; NM__018097; NM__019014; NM__024804; NM__018260; NM__018118; NM__014128; NM__024084; NM__005294; AF077053; NM__000693; NM__033128; NM__020706; AM523613; and NM__014884.

[0105] In another embodiment, the invention provides of a group of genes useful in diagnosing lung diseases wherein the expression of the group of genes is lower in individuals exposed to air pollutants with cancer as compared to individuals exposed to the same air pollutant who do not have cancer, the group comprising probes that hybridize at least 5, preferably at least about 5-10, still more preferably at least about 10-20, still more preferably at least about 20-30, still more preferably at least about 30-40, still more preferably at least about 40-50, still more preferably at least about 50-60, still more preferably about 63 genes consisting of transcripts (transcripts are identified using their GenBank ID or Unigene ID numbers and the corresponding gene names appear in Table 2): NM__030757.1; R83000; AK021571.1; NM__17932.1; U85430.1; A1683552; BC002642.1; AW024467; NM__030972.1; BC021135.1; AL161952.1; AK026565.1; AK023783.1; BF218804; AK023843.1; BC001602.1; BC034707.1; BC064619.1; AY280502.1; BC059387.1; BC061522.1; U50532.1; BC006547.2; BC008797.2; BC000807.1; AL080112.1; BC033718.1/// BC046176.1; BC038443.1; Hs.288575 (UNIGENE ID); AF020591.1; BC002503.2; BC009185.2; Hs.528304 (UNI-GENE ID); U50532.1; BC013923.2; BC031091; Hs.249591 (Unigene ID); Hs.286261 (Unigene ID); AF348514.1; BC066337.1///BC058736.1///BC050555.1; Hs.216623 (Unigene ID); BC072400.1; BC041073.1; U43965.1; BC021258.2; BC016057.1; BC016713.1///BC014535.1/// AF237771.1; BC000701.2; BC010067.2; Hs.156701 (Unigene ID); BC030619.2; U43965.1; Hs.438867 (Unigene ID); BC035025.2///BC050330.1; BC074852.2///BC074851.2; Hs.445885 (Unigene ID); AF365931.1; and AF257099.1

[0106] In another embodiment, the invention provides of a group of genes useful in diagnosing lung diseases wherein the expression of the group of genes is lower in individuals exposed to air pollutants with cancer as compared to indi-

viduals exposed to the same air pollutant who do not have cancer, the group comprising probes that hybridize at least 5, preferably at least about 5-10, still more preferably at least about 10-20, still more preferably at least about 20-25, still more preferably about 25 genes consisting of transcripts (transcripts are identified using their GenBank ID or Unigene ID numbers and the corresponding gene names appear in Table 3): BF218804; AK022494.1; AA114843; BE467941; NM__003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; AI683552; NM__024006.1; AK026565.1; NM__014182.1; NM__021800.1; NM__016049.1; NM__019023.1; NM__021971.1; NM__014128.1; AK025651.1; AA133341; and AF198444.1.

[0107] In another embodiment, the invention provides of a group of genes useful in diagnosing lung diseases wherein the expression of the group of genes is higher in individuals exposed to air pollutants with cancer as compared to individuals exposed to the same air pollutant who do not have cancer, the group comprising probes that hybridize at least to 5, preferably at least about 5-10, still more preferably at least about 10-20, still more preferably at least about 20-25, still more preferably about 25 genes consisting of transcripts (transcripts are identified using their GenBank ID or Unigene ID numbers and the corresponding gene names appear in Table 1): NM__000918; NM__006430.1; NM__001416.1; NM__004090; NM__006406.1; NM__003001.2; NM__006545.1; NM__002437.1; NM__006286; NM__001123///NM__006721; NM__024824; NM__004935. 1; NM__001696; NM__005494///NM__058246; NM__006368; NM__002268///NM__032771; NM__006694; NM__004691; NM__012394; NM__021800; NM__016049; NM__138387; NM__024531; and NM__018509.

[0108] In another embodiment, the invention provides of a group of genes useful in diagnosing lung diseases wherein the expression of the group of genes is higher in individuals exposed to air pollutants with cancer as compared to individuals exposed to the same air pollutant who do not have cancer, the group comprising probes that hybridize at least to 5, preferably at least about 5-10, still more preferably at least about 10-20, still more preferably at least about 20-23, still more preferably about 23 genes consisting of transcripts (transcripts are identified using their GenBank ID or Unigene ID numbers and the corresponding gene names appear in Table 2): NM__014182.1; NM__001281.1; NM__024006.1; AF135421.1; L76200.1; NM__000346.1; BC008710.1; BC000423.2; BC008710.1; NM__007062; BC075839.1/// BC073760.1; BC072436.1///BC004560.2; BC001016.2; BC005023.1; BC000360.2; BC007455.2; BC023528.2/// BC047680.1; BC064957.1; BC008710.1; BC066329.1; BC023976.2; BC008591.2///BC050440.1///BC048096.1; and BC028912.1.

[0109] In another embodiment, the invention provides of a group of genes useful in diagnosing lung diseases wherein the expression of the group of genes is higher in individuals exposed to air pollutants with cancer as compared to individuals exposed to the same air pollutant who do not have cancer, the group comprising probes that hybridize at least to 5, preferably at least about 5-10, still more preferably at least about 10-20, still more preferably at least about 20-25, still more preferably about 25 genes consisting of transcripts (transcripts are identified using their GenBank ID or Unigene ID numbers and the corresponding gene names appear in Table 3): NM__007062.1; NM__001281.1; BC000120.1;

NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; and AB014576.1.

[0110] In one embodiment, the invention provides a method of diagnosing lung disease comprising the steps of measuring the expression profile of a gene group in an individual suspected of being affected or being at high risk of a lung disease (i.e. test individual), and comparing the expression profile (i.e. control profile) to an expression profile of an individual without the lung disease who has also been exposed to similar air pollutant than the test individual (i.e. control individual), wherein differences in the expression of genes when compared between the afore mentioned test individual and control individual of at least 10, more preferably at least 20, still more preferably at least 30, still more preferably at least 36, still more preferably between 36-180, still more preferably between 36-96, still more preferably between 36-84, still more preferably between 36-50, is indicative of the test individual being affected with a lung disease. Groups of about 36 genes as shown in table 4, about 50 genes as shown in table 3, about 84 genes as shown in table 2 and about 96 genes as shown in table 1 are preferred. The different gene groups can also be combined, so that the test individual can be screened for all, three, two, or just one group as shown in tables 1-4.

[0111] For example, if the expression profile of a test individual exposed to cigarette smoke is compared to the expression profile of the 50 genes shown in table 3, using the Affymetrix inc probe set on a gene chip as shown in table 3, the expression profile that is similar to the one shown in FIG. 10 for the individuals with cancer, is indicative that the test individual has cancer. Alternatively, if the expression profile is more like the expression profile of the individuals who do not have cancer in FIG. 10, the test individual likely is not affected with lung cancer.

[0112] The group of 50 genes was identified using the GenePattern server from the Broad Institute, which includes the Weighted Voting algorithm. The default settings, i.e., the signal to noise ratio and no gene filtering, were used. GenePattern is available through the World Wide Wed at location broad.mit.edu/cancer/software/genepattern. This program allows analysis of data in groups rather than as individual genes. Thus, in one preferred embodiment, the expression of substantially all 50 genes of Table 3, are analyzed together. The expression profile of lower that normal expression of genes selected from the group consisting of BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; AM683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF198444.1, and the gene expression profile of higher than normal expression of genes selected from the group consisting of NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; and AB014576.1, is indicative

of the individual having or being at high risk of developing lung disease, such as lung cancer. In one preferred embodiment, the expression pattern of all the genes in the Table 3 is analyzed. In one embodiment, in addition to analyzing the group of predictor genes of Table 3, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10-15, 15-20, 20-30, or more of the individual predictor genes identified using the t-test analysis are analyzed. Any combination of, for example, 5-10 or more of the group predictor genes and 5-10, or more of the individual genes can also be used.

[0113] The term "expression profile" as used herein, refers to the amount of the gene product of each of the analyzed individual genes in the sample. The "expression profile" is like a signature expression map, like the one shown for each individual in FIG. 10, on the Y-axis.

[0114] The term "lung disease", as used herein, refers to disorders including, but hot limited to, asthma, chronic bronchitis, emphysema, bronchiectasis, primary pulmonary hypertension and acute respiratory distress syndrome. The methods described herein may also be used to diagnose or treat lung disorders that involve the immune system including, hypersensitivity pneumonitis, eosinophilic pneumonias, and persistent fungal infections, pulmonary fibrosis, systemic sclerosis, idiopathic pulmonary hemosiderosis, pulmonary alveolar proteinosis, cancers of the lung such as adenocarcinoma, squamous cell carcinoma, small cell and large cell carcinomas, and benign neoplasm of the lung including bronchial adenomas and hamartomas. In one preferred embodiment, the lung disease is lung cancer.

[0115] The biological samples useful according to the present invention include, but are not limited to tissue samples, cell samples, and excretion samples, such as sputum or saliva, of the airways. The samples useful for the analysis methods according to the present invention can be taken from the mouth, the bronchial airways, and the lungs.

[0116] The term "air pollutants", as used herein, refers to any air impurities or environmental airway stress inducing agents, such as cigarette smoke, cigar smoke, smog, asbestos, and other air pollutants that have suspected or proven association to lung diseases.

[0117] The term "individual", as used herein, preferably refers to human. However, the methods are not limited to humans, and a skilled artisan can use the diagnostic/prognostic gene groupings of the present invention in, for example, laboratory test animals, preferably animals that have lungs, such as non-human primates, murine species, including, but not limited to rats and mice, dogs, sheep, pig, guinea pigs, and other model animals.

[0118] The phrase "altered expression" as used herein, refers to either increased or decreased expression in an individual exposed to air pollutant, such as a smoker, with cancer when compared to an expression pattern of the lung cells from an individual exposed to similar air pollutant, such as smoker, who does not have cancer. Tables 1 and 2 show the preferred expression pattern changes of the invention. The terms "up" and "down" in the tables refer to the amount of expression in a smoker with cancer to the amount of expression in a smoker without cancer. Similar expression pattern changes are likely associated with development of cancer in individuals who have been exposed to other airway pollutants.

[0119] In one embodiment, the group of genes the expression of which is analyzed in diagnosis and/or prognosis of lung cancer are selected from the group of 80 genes as shown in Table 5. Any combination of genes can be selected from the 80 genes. In one embodiment, the combination of 20 genes shown in Table 7 is selected. In one embodiment, a combination of genes from Table 6 is selected.

TABLE 5

Group of 80 genes for prognostic and diagnostic testing of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
200729_s_at	ACTR2	736	−0.22284
200760_s_at	ARL6IP5	483	−0.21221
201399_s_at	TRAM1	611	−0.21328
201444_s_at	ATP6AP2	527	−0.21487
201635_s_at	FXR1	458	−0.2162
201689_s_at	TPD52	565	−0.22292
201925_s_at	DAF	717	−0.25875
201926_s_at	DAF	591	−0.23228
201946_s_at	CCT2	954	−0.24592
202118_s_at	CPNE3	334	−0.21273
202704_at	TOB1	943	−0.25724
202833_s_at	SERPINA1	576	−0.20583
202935_s_at	SOX9	750	−0.25574
203413_at	NELL2	629	−0.23576
203881_s_at	DMD	850	−0.24341
203908_at	SLC4A4	887	−0.23167
204006_s_at	FCGR3A /// FCGR3B	207	−0.20071
204403_x_at	KIAA0738	923	0.167772
204427_s_at	RNP24	725	−0.2366
206056_x_at	SPN	976	0.196398
206169_x_at	RoXaN	984	0.259637
207730_x_at	HDGF2	969	0.169108
207756_at	—	855	0.161708
207791_s_at	RAB1A	823	−0.21704
207953_at	AD7C-NTP	1000	0.218433
208137_x_at	—	996	0.191938
208246_x_at	TK2	982	0.179058
208654_s_at	CD164	388	−0.21228
208892_s_at	DUSP6	878	−0.25023
209189_at	FOS	935	−0.27446
209204_at	LMO4	78	0.158674
209267_s_at	SLC39A8	228	−0.24231
209369_at	ANXA3	384	−0.19972
209656_s_at	TMEM47	456	−0.23033
209774_x_at	CXCL2	404	−0.2117
210145_at	PLA2G4A	475	−0.26146
210168_at	C6	458	−0.24157
210317_s_at	YWHAE	803	−0.29542
210397_at	DEFB1	176	−0.22512
210679_x_at	—	970	0.181718
211506_s_at	IL8	270	−0.3105
212006_at	UBXD2	802	−0.22094
213089_at	LOC153561	649	0.164097
213736_at	COX5B	505	0.155243
213813_x_at	—	789	0.178643
214007_s_at	PTK9	480	−0.21285
214146_s_at	PPBP	593	−0.24265
214594_x_at	ATP8B1	962	0.284039
214707_x_at	ALMS1	750	0.164047
214715_x_at	ZNF160	996	0.198532
215204_at	SENP6	211	0.169986
215208_x_at	RPL35A	999	0.228485
215385_at	FTO	164	0.187634
215600_x_at	FBXW12	960	0.17329
215604_x_at	UBE2D2	998	0.224878
215609_at	STARD7	940	0.191953
215628_x_at	PPP2CA	829	0.16391
215800_at	DUOX1	412	0.160036
215907_at	BACH2	987	0.178338
215978_x_at	LOC152719	645	0.163399
216834_at	—	633	−0.25508
216858_x_at	—	997	0.232969
217446_x_at	—	942	0.182612
217653_x_at	—	976	0.270552

TABLE 5-continued

Group of 80 genes for prognostic and diagnostic testing of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
217679_x_at	—	987	0.265918
217715_x_at	ZNF354A	995	0.223881
217826_s_at	UBE2J1	812	−0.23003
218155_x_at	FLJ10534	998	0.186425
218976_at	DNAJC12	486	−0.22866
219392_x_at	FLJ11029	867	0.169113
219678_x_at	DCLRE1C	877	0.169975
220199_s_at	FLJ12806	378	−0.20713
220389_at	FLJ23514	102	0.239341
220720_x_at	FLJ14346	989	0.17976
221191_at	DKFZP434A0131	616	0.185412
221310_at	FGF14	511	−0.19965
221765_at	—	319	−0.25025
222027_at	NUCKS	547	0.171954
222104_x_at	GTF2H3	981	0.186025
222358_x_at	—	564	0.194048

TABLE 6

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
200729_s_at	ACTR2	736	−0.22284
200760_s_at	ARL6IP5	483	−0.21221
201399_s_at	TRAM1	611	−0.21328
201444_s_at	ATP6AP2	527	−0.21487
201635_s_at	FXR1	458	−0.2162
201689_s_at	TPD52	565	−0.22292
201925_s_at	DAF	717	−0.25875
201926_s_at	DAF	591	−0.23228
201946_s_at	CCT2	954	−0.24592
202118_s_at	CPNE3	334	−0.21273
202704_at	TOB1	943	−0.25724
202833_s_at	SERPINAI	576	−0.20583
202935_s_at	SOX9	750	−0.25574
203413_at	NELL2	629	−0.23576
203881_s_at	DMD	850	−0.24341
203908_at	SLC4A4	887	−0.23167
204006_s_at	FCGR3A /// FCGR3B	207	−0.20071
204403_x_at	KIAA0738	923	0.167772
204427_s_at	RNP24	725	−0.2366
206056_x_at	SPN	976	0.196398
206169_x_at	RoXaN	984	0.259637
207730_x_at	HDGF2	969	0.169108
207756_at	—	855	0.161708
207791_s_at	RAB1A	823	−0.21704
207953_at	AD7C-NTP	1000	0.218433
208137_x_at	—	996	0.191938
208246_x_at	TK2	982	0.179058
208654_s_at	CD164	388	−0.21228
208892_s_at	DUSP6	878	−0.25023
209189_at	FOS	935	−0.27446
209204_at	LMO4	78	0.158674

TABLE 6-continued

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
209267_s_at	SLC39A8	228	−0.24231
209369_at	ANXA3	384	−0.19972
209656_s_at	TMEM47	456	−0.23033
209774_x_at	CXCL2	404	−0.2117
210145_at	PLA2G4A	475	−0.26146
210168_at	C6	458	−0.24157
210317_s_at	YWHAE	803	−0.29542
210397_at	DEFB1	176	−0.22512
210679_x_at	—	970	0.181718
211506_s_at	IL8	270	−0.3105
212006_at	UBXD2	802	−0.22094
213089_at	LOC153561	649	0.164097
213736_at	COX5B	505	0.155243
213813_x_at	—	789	0.178643
214007_s_at	PTK9	480	−0.21285
214146_s_at	PPBP	593	−0.24265
214594_x_at	ATP8B1	962	0.284039
214707_x_at	ALMS1	750	0.164047
214715_x_at	ZNF160	996	0.198532
215204_at	SENP6	211	0.169986
215208_x_at	RPL35A	999	0.228485
215385_at	FTO	164	0.187634
215600_x_at	FBXW12	960	0.17329
215604_x_at	UBE2D2	998	0.224878
215609_at	STARD7	940	0.191953
215628_x_at	PPP2CA	829	0.16391
215800_at	DUOX1	412	0.160036
215907_at	BACH2	987	0.178338
215978_x_at	LOC152719	645	0.163399
216834_at	—	633	−0.25508
216858_x_at	—	997	0.232969
217446_x_at	—	942	0.182612
217653_x_at	—	976	0.270552
217679_x_at	—	987	0.265918
217715_x_at	ZNF354A	995	0.223881
217826_s_at	UBE2J1	812	−0.23003
218155_x_at	FLJ10534	998	0.186425
218976_at	DNAJC12	486	−0.22866
219392_x_at	FLJ11029	867	0.169113
219678_x_at	DCLRE1C	877	0.169975
220199_s_at	FLJ12806	378	−0.20713
220389_at	FLJ23514	102	0.239341
220720_x_at	FLJ14346	989	0.17976
221191_at	DKFZP434A0131	616	0.185412
221310_at	FGF14	511	−0.19965
221765_at	—	319	−0.25025
222027_at	NUCKS	547	0.171954
222104_x_at	GTF2H3	981	0.186025
222358_x_at	—	564	0.194048
202113_s_at	SNX2	841	−0.20503
207133_x_at	ALPK1	781	0.155812
218989_x_at	SLC30A5	765	−0.198
200751_s_at	HNRPC	759	−0.19243
220796_x_at	SLC35E1	691	0.158199
209362_at	SURB7	690	−0.18777
216248_s_at	NR4A2	678	−0.19796
203138_at	HAT1	669	−0.18115
221428_s_at	TBL1XR1	665	−0.19331
218172_s_at	DERL1	665	−0.16341
215861_at	FLJ14031	651	0.156927
209288_s_at	CDC42EP3	638	−0.20146
214001_x_at	RPS10	634	0.151006
209116_x_at	HBB	626	−0.12237
215595_x_at	GCNT2	625	0.136319
208891_at	DUSP6	617	−0.17282

TABLE 6-continued

<u>Group of 535 genes useful in prognosis or diagnosis of lung cancer.</u>

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
215067_x_at	PRDX2	616	0.160582
202918_s_at	PREI3	614	−0.17003
211985_s_at	CALM1	614	−0.20103
212019_at	RSL1D1	601	0.152717
216187_x_at	KNS2	591	0.14297
215066_at	PTPRF	587	0.143323
212192_at	KCTD12	581	−0.17535
217586_x_at	—	577	0.147487
203582_s_at	RAB4A	567	−0.18289
220113_x_at	POLR1B	563	0.15764
217232_x_at	HBB	561	−0.11398
201041_s_at	DUSP1	560	−0.18661
211450_s_at	MSH6	544	−0.15597
202648_at	RPS19	533	0.150087
202936_s_at	SOX9	533	−0.17714
204426_at	RNP24	526	−0.18959
206392_s_at	RARRES1	517	−0.18328
208750_s_at	ARF1	515	−0.19797
202089_s_at	SLC39A6	512	−0.19904
211297_s_at	CDK7	510	−0.15992
215373_x_at	FLJ12151	509	0.146742
213679_at	FLJ13946	492	−0.10963
201694_s_at	EGR1	490	−0.19478
209142_s_at	UBE2G1	487	−0.18055
217706_at	LOC220074	483	0.11787
212991_at	FBXO9	476	0.148288
201289_at	CYR61	465	−0.19925
206548_at	FLJ23556	465	0.141583
202593_s_at	MIR16	462	−0.17042
202932_at	YES1	461	−0.17637
220575_at	FLJ11800	461	0.116435
217713_x_at	DKFZP566N034	452	0.145994
211953_s_at	RANBP5	447	−0.17838
203827_at	WIPI49	447	−0.17767
221997_s_at	MRPL52	444	0.132649
217662_x_at	BCAP29	434	0.116886
218519_at	SLC35A5	428	−0.15495
214833_at	KIAA0792	428	0.132943
201339_s_at	SCP2	426	−0.18605
203799_at	CD302	422	−0.16798
211090_s_at	PRPF4B	421	−0.1838
220071_x_at	C15orf25	420	0.138308
203946_s_at	ARG2	415	−0.14964
213544_at	ING1L	415	0.137052
209908_s_at	—	414	0.131346
201688_s_at	TPD52	410	−0.18965
215587_x_at	BTBD14B	410	0.139952
201699_at	PSMC6	409	−0.13784
214902_x_at	FLJ42393	409	0.140198
214041_x_at	RPL37A	402	0.106746
203987_at	FZD6	392	−0.19252
211696_x_at	HBB	392	−0.09508
218025_s_at	PECI	389	−0.18002
215852_x_at	KIAA0889	382	0.12243
209458_x_at	HBA1 /// HBA2	380	−0.09796
219410_at	TMEM45A	379	−0.22387
215375_x_at	—	379	0.148377
206302_s_at	NUDT4	376	−0.18873
208783_s_at	MCP	372	−0.15076
211374_x_at	—	364	0.131101
220352_x_at	MGC4278	364	0.152722
216609_at	TXN	363	0.15162
201942_s_at	CPD	363	−0.1889
202672_s_at	ATF3	361	−0.12935

TABLE 6-continued

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
204959_at	MNDA	359	−0.21676
211996_s_at	KIAA0220	358	0.144358
222035_s_at	PAPOLA	353	−0.14487
208808_s_at	HMGB2	349	−0.15222
203711_s_at	HIBCH	347	−0.13214
215179_x_at	PGF	347	0.146279
213562_s_at	SQLE	345	−0.14669
203765_at	GCA	340	−0.1798
214414_x_at	HBA2	336	−0.08492
217497_at	ECGF1	336	0.123255
220924_s_at	SLC38A2	333	−0.17315
218139_s_at	C14orf108	332	−0.15021
201096_s_at	ARF4	330	−0.18887
220361_at	FLJ12476	325	−0.15452
202169_s_at	AASDHPPT	323	−0.15787
202527_s_at	SMAD4	322	−0.18399
202166_s_at	PPP1R2	320	−0.16402
204634_at	NEK4	319	−0.15511
215504_x_at	—	319	0.145981
202388_at	RGS2	315	−0.14894
215553_x_at	WDR45	315	0.137586
200598_s_at	TRA1	314	−0.19349
202435_s_at	CYP1B1	313	0.056937
216206_x_at	MAP2K7	313	0.10383
212582_at	OSBPL8	313	−0.17843
216509_x_at	MLLT10	312	0.123961
200908_s_at	RPLP2	308	0.136645
215108_x_at	TNRC9	306	−0.1439
213872_at	C6orf62	302	−0.19548
214395_x_at	EEF1D	302	0.128234
222156_x_at	CCPG1	301	−0.14725
201426_s_at	VIM	301	−0.17461
221972_s_at	Cab45	299	−0.1511
219957_at	—	298	0.130796
215123_at	—	295	0.125434
212515_s_at	DDX3X	295	−0.14634
203357_s_at	CAPN7	295	−0.17109
211711_s_at	PTEN	295	−0.12636
206165_s_at	CLCA2	293	−0.17699
213959_s_at	KIAA1005	289	−0.16592
215083_at	PSPC1	289	0.147348
219630_at	PDZK1IP1	287	−0.15086
204018_x_at	HBA1 /// HBA2	286	−0.08689
208671_at	TDE2	286	−0.17839
203427_at	ASF1A	286	−0.14737
215281_x_at	POGZ	286	0.142825
205749_at	CYP1A1	285	0.107118
212585_at	OSBPL8	282	−0.13924
211745_x_at	HBA1 /// HBA2	281	−0.08437
208078_s_at	SNF1LK	278	−0.14395
218041_x_at	SLC38A2	276	−0.17003
212588_at	PTPRC	270	−0.1725
212397_at	RDX	270	−0.15613
208268_at	ADAM28	269	0.114996
207194_s_at	ICAM4	269	0.127304
222252_x_at	—	269	0.132241
217414_x_at	HBA2	266	−0.08974
207078_at	MED6	261	0.1232
215268_at	KIAA0754	261	0.13669
221387_at	GPR147	261	0.128737
201337_s_at	VAMP3	259	−0.17284
220218_at	C9orf68	259	0.125851
222356_at	TBL1Y	259	0.126765

TABLE 6-continued

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
208579_x_at	H2BFS	258	−0.16608
219161_s_at	CKLF	257	−0.12288
202917_s_at	S100A8	256	−0.19869
204455_at	DST	255	−0.13072
211672_s_at	ARPC4	254	−0.17791
201132_at	HNRPH2	254	−0.12817
218313_s_at	GALNT7	253	−0.179
218930_s_at	FLJ11273	251	−0.15878
219166_at	C14orf104	250	−0.14237
212805_at	KIAA0367	248	−0.16649
201551_s_at	LAMP1	247	−0.18035
202599_s_at	NRIP1	247	−0.16226
203403_s_at	RNF6	247	−0.14976
214261_s_at	ADH6	242	−0.1414
202033_s_at	RB1CC1	240	−0.18105
203896_s_at	PLCB4	237	−0.20318
209703_x_at	DKFZP586A0522	234	0.140153
211699_x_at	HBA1 /// HBA2	232	−0.08369
210764_s_at	CYR61	231	−0.13139
206391_at	RARRES1	230	−0.16931
201312_s_at	SH3BGRL	225	−0.12265
200798_x_at	MCL1	221	−0.13113
214912_at	—	221	0.116262
204621_s_at	NR4A2	217	−0.10896
217761_at	MTCBP-1	217	−0.17558
205830_at	CLGN	216	−0.14737
218438_s_at	MED28	214	−0.14649
207475_at	FABP2	214	0.097003
208621_s_at	VIL2	213	−0.19678
202436_s_at	CYP1B1	212	0.042216
202539_s_at	HMGCR	210	−0.15429
210830_s_at	PON2	209	−0.17184
211906_s_at	SERPINB4	207	−0.14728
202241_at	TRIB1	207	−0.10706
203594_at	RTCD1	207	−0.13823
215863_at	TFR2	207	0.095157
221992_at	LOC283970	206	0.126744
221872_at	RARRES1	205	−0.11496
219564_at	KCNJ16	205	−0.13908
201329_s_at	ETS2	205	−0.14994
214188_at	HIS1	203	0.1257
201667_at	GJA1	199	−0.13848
201464_x_at	JUN	199	−0.09858
215409_at	LOC254531	197	0.094182
202583_s_at	RANBP9	197	−0.13902
215594_at	—	197	0.101007
214326_x_at	JUND	196	−0.1702
217140_s_at	VDAC1	196	−0.14682
215599_at	SMA4	195	0.133438
209896_s_at	PTPN11	195	−0.16258
204846_at	CP	195	−0.14378
222303_at	—	193	−0.10841
218218_at	DIP13B	193	−0.12136
211015_s_at	HSPA4	192	−0.13489
208666_s_at	ST13	191	−0.13361
203191_at	ABCB6	190	0.096808
202731_at	PDCD4	190	−0.1545
209027_s_at	ABI1	190	−0.15472
205979_at	SCGB2A1	189	−0.15091
216351_x_at	DAZ1 /// DAZ3 /// DAZ2 /// DAZ4	189	0.106368

TABLE 6-continued

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
220240_s_at	C13orf11	188	−0.16959
204482_at	CLDN5	187	0.094134
217234_s_at	VIL2	186	−0.16035
214350_at	SNTB2	186	0.095723
201693_s_at	EGR1	184	−0.10732
212328_at	KIAA1102	182	−0.12113
220168_at	CASC1	181	−0.1105
203628_at	IGF1R	180	0.067575
204622_x_at	NR4A2	180	−0.11482
213246_at	C14orf109	180	−0.16143
218728_s_at	HSPC163	180	−0.13248
214753_at	PFAAP5	179	0.130184
206336_at	CXCL6	178	−0.05634
201445_at	CNN3	178	−0.12375
209886_s_at	SMAD6	176	0.079296
213376_at	ZBTB1	176	−0.17777
213887_s_at	POLR2E	175	−0.16392
204783_at	MLF1	174	−0.13409
218824_at	FLJ10781	173	0.1394
212417_at	SCAMP1	173	−0.17052
202437_s_at	CYP1B1	171	0.033438
217528_at	CLCA2	169	−0.14179
218170_at	ISOC1	169	−0.14064
206278_at	PTAFR	167	0.087096
201939_at	PLK2	167	−0.11049
200907_s_at	KIAA0992	166	−0.18323
207480_s_at	MEIS2	166	−0.15232
201417_at	SOX4	162	−0.09617
213826_s_at	—	160	0.097313
214953_s_at	APP	159	−0.1645
204897_at	PTGER4	159	−0.08152
201711_x_at	RANBP2	158	−0.17192
202457_s_at	PPP3CA	158	−0.18821
206683_at	ZNF165	158	−0.08848
214581_x_at	TNFRSF21	156	−0.14624
203392_s_at	CTBP1	155	−0.16161
212720_at	PAPOLA	155	−0.14809
207758_at	PPM1F	155	0.090007
220995_at	STXBP6	155	0.106749
213831_at	HLA-DQA1	154	0.193368
212044_s_at	—	153	0.098889
202434_s_at	CYP1B1	153	0.049744
206166_s_at	CLCA2	153	−0.1343
218343_s_at	GTF3C3	153	−0.13066
202557_at	STCH	152	−0.14894
201133_s_at	PJA2	152	−0.18481
213605_s_at	MGC22265	151	0.130895
210947_s_at	MSH3	151	−0.12595
208310_s_at	C7orf28A /// C7orf28B	151	−0.15523
209307_at	—	150	−0.1667
215387_x_at	GPC6	148	0.114691
213705_at	MAT2A	147	0.104855
213979_s_at	—	146	0.121562
212731_at	LOC157567	146	−0.1214
210117_at	SPAG1	146	−0.11236
200641_s_at	YWHAZ	145	−0.14071
210701_at	CFDP1	145	0.151664
217152_at	NCOR1	145	0.130891
204224_s_at	GCH1	144	−0.14574
202028_s_at	—	144	0.094276
201735_s_at	CLCN3	144	−0.1434
208447_s_at	PRPS1	143	−0.14933
220926_s_at	C1orf22	142	−0.17477
211505_s_at	STAU	142	−0.11618

TABLE 6-continued

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
221684_s_at	NYX	142	0.102298
206906_at	ICAM5	141	0.076813
213228_at	PDE8B	140	−0.13728
217202_s_at	GLUL	139	−0.15489
211713_x_at	KIAA0101	138	0.108672
215012_at	ZNF451	138	0.13269
200806_s_at	HSPD1	137	−0.14811
201466_s_at	JUN	135	−0.0667
211564_s_at	PDLIM4	134	−0.12756
207850_at	CXCL3	133	−0.17973
221841_s_at	KLF4	133	−0.1415
200605_s_at	PRKAR1A	132	−0.15642
221198_at	SCT	132	0.08221
201772_at	AZIN1	131	−0.16639
205009_at	TFF1	130	−0.17578
205542_at	STEAP1	129	−0.08498
218195_at	C6orf211	129	−0.14497
213642_at	—	128	0.079657
212891_s_at	GADD45GIP1	128	−0.09272
202798_at	SEC24B	127	−0.12621
222207_x_at	—	127	0.10783
202638_s_at	ICAM1	126	0.070364
200730_s_at	PTP4A1	126	−0.15289
219355_at	FLJ10178	126	−0.13407
220266_s_at	KLF4	126	−0.15324
201259_s_at	SYPL	124	−0.16643
209649_at	STAM2	124	−0.1696
220094_s_at	C6orf79	123	−0.12214
221751_at	PANK3	123	−0.1723
200008_s_at	GDI2	123	−0.15852
205078_at	PIGF	121	−0.13747
218842_at	FLJ21908	121	−0.08903
202536_at	CHMP2B	121	−0.14745
220184_at	NANOG	119	0.098142
201117_s_at	CPE	118	−0.20025
219787_s_at	ECT2	117	−0.14278
206628_at	SLC5A1	117	−0.12838
204007_at	FCGR3B	116	−0.15337
209446_s_at	—	116	0.100508
211612_s_at	IL13RA1	115	−0.17266
220992_s_at	C1orf25	115	−0.11026
221899_at	PFAAP5	115	0.11698
221719_s_at	LZTS1	115	0.093494
201473_at	JUNB	114	−0.10249
221193_s_at	ZCCHC10	112	−0.08003
215659_at	GSDML	112	0.118288
205157_s_at	KRT17	111	−0.14232
201001_s_at	UBE2V1 /// Kua-UEV	111	−0.16786
216789_at	—	111	0.105386
205506_at	VIL1	111	0.097452
204875_s_at	GMDS	110	−0.12995
207191_s_at	ISLR	110	0.100627
202779_s_at	UBE2S	109	−0.11364
210370_s_at	LY9	109	0.096323
202842_s_at	DNAJB9	108	−0.15326
201082_s_at	DCTN1	107	−0.10104
215588_x_at	RIOK3	107	0.135837
211076_x_at	DRPLA	107	0.102743
210230_at	—	106	0.115001
206544_x_at	SMARCA2	106	−0.12099
208852_s_at	CANX	105	−0.14776
215405_at	MYO1E	105	0.086393
208653_s_at	CD164	104	−0.09185
206355_at	GNAL	103	0.1027

TABLE 6-continued

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
210793_s_at	NUP98	103	−0.13244
215070_x_at	RABGAP1	103	0.125029
203007_x_at	LYPLA1	102	−0.17961
203841_x_at	MAPRE3	102	−0.13389
206759_at	FCER2	102	0.081733
202232_s_at	GA17	102	−0.11373
215892_at	—	102	0.13866
214359_s_at	HSPCB	101	−0.12276
215810_x_at	DST	101	0.098963
208937_s_at	ID1	100	−0.06552
213664_at	SLC1A1	100	−0.12654
219338_s_at	FLJ20156	100	−0.10332
206595_at	CST6	99	−0.10059
207300_s_at	F7	99	0.082445
213792_s_at	INSR	98	0.137962
209674_at	CRY1	98	−0.13818
40665_at	FMO3	97	−0.05976
217975_at	WBP5	97	−0.12698
210296_s_at	PXMP3	97	−0.13537
215483_at	AKAP9	95	0.125966
212633_at	KIAA0776	95	−0.16778
206164_at	CLCA2	94	−0.13117
216813_at	—	94	0.089023
208925_at	C3orf4	94	−0.1721
219469_at	DNCH2	94	−0.12003
206016_at	CXorf37	93	−0.11569
216745_x_at	LRCH1	93	0.117149
212999_x_at	HLA-DQB1	92	0.110258
216859_x_at	—	92	0.116351
201636_at	—	92	−0.13501
204272_at	LGALS4	92	0.110391
215454_x_at	SFTPC	91	0.064918
215972_at	—	91	0.097654
220593_s_at	FLJ20753	91	0.095702
222009_at	CGI-14	91	0.070949
207115_x_at	MBTD1	91	0.107883
216922_x_at	DAZ1 /// DAZ3 /// DAZ2 /// DAZ4	91	0.086888
217626_at	AKR1C1 /// AKR1C2	90	0.036545
211429_s_at	SERPINA1	90	−0.11406
209662_at	CETN3	90	−0.10879
201629_s_at	ACP1	90	−0.14441
201236_s_at	BTG2	89	−0.09435
217137_x_at	—	89	0.070954
212476_at	CENTB2	89	−0.1077
218545_at	FLJ11088	89	−0.12452
208857_s_at	PCMT1	89	−0.14704
221931_s_at	SEH1L	88	−0.11491
215046_at	FLJ23861	88	−0.14667
220222_at	PRO1905	88	0.081524
209737_at	AIP1	87	−0.07696
203949_at	MPO	87	0.113273
219290_x_at	DAPP1	87	0.111366
205116_at	LAMA2	86	0.05845
222316_at	VDP	86	0.091505
203574_at	NFIL3	86	−0.14335
207820_at	ADH1A	86	0.104444
203751_x_at	JUND	85	−0.14118
202930_s_at	SUCLA2	85	−0.14884
215404_x_at	FGFR1	85	0.119684
216266_s_at	ARFGEF1	85	−0.12432
212806_at	KIAA0367	85	−0.13259

TABLE 6-continued

Group of 535 genes useful in prognosis or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
219253__at	—	83	−0.14094
214605__x__at	GPR1	83	0.114443
205403__at	IL1R2	82	−0.19721
222282__at	PAPD4	82	0.128004
214129__at	PDE4DIP	82	−0.13913
209259__s__at	CSPG6	82	−0.12618
216900__s__at	CHRNA4	82	0.105518
221943__x__at	RPL38	80	0.086719
215386__at	AUTS2	80	0.129921
201990__s__at	CREBL2	80	−0.13645
220145__at	FLJ21159	79	−0.16097
221173__at	USH1C	79	0.109348
214900__at	ZKSCAN1	79	0.075517
203290__at	HLA-DQA1	78	−0.20756
215382__x__at	TPSAB1	78	−0.09041
201631__s__at	IER3	78	−0.12038
212188__at	KCTD12	77	−0.14672
220428__at	CD207	77	0.101238
215349__at	—	77	0.10172
213928__s__at	HRB	77	0.092136
221228__s__at	—	77	0.0859
202069__s__at	IDH3A	76	−0.14747
208554__at	POU4F3	76	0.107529
209504__s__at	PLEKHB1	76	−0.13125
212989__at	TMEM23	75	−0.11012
216197__at	ATF7IP	75	0.115016
204748__at	PTGS2	74	−0.15194
205221__at	HGD	74	0.096171
214705__at	INADL	74	0.102919
213939__s__at	RIPX	74	0.091175
203691__at	PI3	73	−0.14375
220532__s__at	LR8	73	−0.11682
209829__at	C6orf32	73	−0.08982
206515__at	CYP4F3	72	0.104171
218541__s__at	C8orf4	72	−0.09551
210732__s__at	LGALS8	72	−0.13683
202643__s__at	TNFAIP3	72	−0.16699
218963__s__at	KRT23	72	−0.10915
213304__at	KIAA0423	72	−0.12256
202768__at	FOSB	71	−0.06289
205623__at	ALDH3A1	71	0.045457
206488__s__at	CD36	71	−0.15899
204319__s__at	RGS10	71	−0.10107
217811__at	SELT	71	−0.16162
202746__at	ITM2A	70	−0.06424
221127__s__at	RIG	70	0.110593
209821__at	C9orf26	70	−0.07383
220957__at	CTAGE1	70	0.092986
215577__at	UBE2E1	70	0.10305
214731__at	DKFZp547A023	70	0.102821
210512__s__at	VEGF	69	−0.11804
205267__at	POU2AF1	69	0.101353
216202__s__at	SPTLC2	69	−0.11908
220477__s__at	C20orf30	69	−0.16221
205863__at	S100A12	68	−0.10353
215780__s__at	SET /// LOC389168	68	−0.10381
218197__s__at	OXR1	68	−0.14424
203077__s__at	SMAD2	68	−0.11242
222339__x__at	—	68	0.121585
200698__at	KDELR2	68	−0.15907
210540__s__at	B4GALT4	67	−0.13556
217725__x__at	PAI-RBP1	67	−0.14956
217082__at	—	67	0.086098

TABLE 7

Group of 20 genes useful in prognosis and/or diagnosis of lung cancer.

Affymetrix probe ID No. that can be used to identify the gene/ nucleic acid sequence in the next column	Gene symbol	Number of runs the gene is indicated in cancer samples as differentially expressed out of 1000 test runs	Signal to noise in a cancer sample. Negative values indicate increase of expression in lung cancer, positive values indicate decrease of expression in lung cancer.
207953_at	AD7C-NTP	1000	0.218433
215208_x_at	RPL35A	999	0.228485
215604_x_at	UBE2D2	998	0.224878
218155_x_at	FLJ10534	998	0.186425
216858_x_at	—	997	0.232969
208137_x_at	—	996	0.191938
214715_x_at	ZNF160	996	0.198532
217715_x_at	ZNF354A	995	0.223881
220720_x_at	FLJ14346	989	0.17976
215907_at	BACH2	987	0.178338
217679_x_at	—	987	0.265918
206169_x_at	RoXaN	984	0.259637
208246_x_at	TK2	982	0.179058
222104_x_at	GTF2H3	981	0.186025
206056_x_at	SPN	976	0.196398
217653_x_at	—	976	0.270552
210679_x_at	—	970	0.181718
207730_x_at	HDGF2	969	0.169108
214594_x_at	ATP8B1	962	0.284039

[0120] One can use the above tables to correlate or compare the expression of the transcript to the expression of the gene product. Increased expression of the transcript as shown in the table corresponds to increased expression of the gene product. Similarly, decreased expression of the transcript as shown in the table corresponds to decreased expression of the gene product

[0121] The analysis of the gene expression of one or more genes and/or transcripts of the groups or their subgroups of the present invention can be performed using any gene expression method known to one skilled in the art. Such methods include, but are not limited to expression analysis using nucleic acid chips (e.g. Affymetrix chips) and quantitative RT-PCR based methods using, for example real-time detection of the transcripts. Analysis of transcript levels according to the present invention can be made using total or messenger RNA or proteins encoded by the genes identified in the diagnostic gene groups of the present invention as a starting material. In the preferred embodiment the analysis is an immunohistochemical analysis with an antibody directed against proteins comprising at least about 10-20, 20-30, preferably at least 36, at least 36-50, 50, about 50-60, 60-70, 70-80, 80-90, 96, 100-180, 180-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-535 proteins encoded by the genes and/or transcripts as shown in Tables 1-7.

[0122] The methods of analyzing transcript levels of the gene groups in an individual include Northern-blot hybridization, ribonuclease protection assay, and reverse transcriptase polymerase chain reaction (RT-PCR) based methods. The different RT-PCR based techniques are the most suitable quantification method for diagnostic purposes of the present invention, because they are very sensitive and thus require only a small sample size which is desirable for a diagnostic test. A number of quantitative RT-PCR based methods have been described and are useful in measuring the amount of transcripts according to the present invention. These methods include RNA quantification using PCR and complementary DNA (cDNA) arrays (Shalon et al., Genome Research 6(7):639-45, 1996; Bernard et al., Nucleic Acids Research 24(8):1435-42, 1996), real competitive PCR using a MALDI-TOF Mass spectrometry based approach (Ding et al, PNAS, 100: 3059-64, 2003), solid-phase mini-sequencing technique, which is based upon a primer extension reaction (U.S. Pat. No. 6,013,431, Suomalainen et al. Mol. Biotechnol. June; 15(2):123-31, 2000), ion-pair high-performance liquid chromatography (Doris et al. J. Chromatogr. A May 8; 806(1):47-60, 1998), and 5' nuclease assay or real-time RT-PCR (Holland et al. Proc Natl Acad Sci USA 88: 7276-7280, 1991).

[0123] Methods using RT-PCR and internal standards differing by length or restriction endonuclease site from the desired target sequence allowing comparison of the standard with the target using gel electrophoretic separation methods followed by densitometric quantification of the target have also been developed and can be used to detect the amount of the transcripts according to the present invention (see, e.g., U.S. Pat. Nos. 5,876,978; 5,643,765; and 5,639,606.

[0124] The samples are preferably obtained from bronchial airways using, for example, endoscopic cytobrush in connection with a fiber optic bronchoscopy. In one embodiment, the cells are obtained from the individual's mouth buccal cells, using, for example, a scraping of the buccal mucosa.

[0125] In one preferred embodiment, the invention provides a prognostic and/or diagnostic immunohistochemical approach, such as a dip-stick analysis, to determine risk of developing lung disease. Antibodies against proteins, or antigenic epitopes thereof, that are encoded by the group of genes of the present invention, are either commercially available or can be produced using methods well know to one skilled in the art.

[0126] The invention contemplates either one dipstick capable of detecting all the diagnostically important gene products or alternatively, a series of dipsticks capable of

detecting the amount proteins of a smaller sub-group of diagnostic proteins of the present invention.

[0127] Antibodies can be prepared by means well known in the art. The term "antibodies" is meant to include monoclonal antibodies, polyclonal antibodies and antibodies prepared by recombinant nucleic acid techniques that are selectively reactive with a desired antigen. Antibodies against the proteins encoded by any of the genes in the diagnostic gene groups of the present invention are either known or can be easily produced using the methods well known in the art. Internet sites such as Biocompare through the World Wide Web at "biocompare.com/abmatrix.asp?antibody=y" provide a useful tool to anyone skilled in the art to locate existing antibodies against any of the proteins provided according to the present invention.

[0128] Antibodies against the diagnostic proteins according to the present invention can be used in standard techniques such as Western blotting or immunohistochemistry to quantify the level of expression of the proteins of the diagnostic airway proteome. This is quantified according to the expression of the gene transcript, i.e. the increased expression of transcript corresponds to increased expression of the gene product, i.e. protein. Similarly decreased expression of the transcript corresponds to decreased expression of the gene product or protein. Detailed guidance of the increase or decrease of expression of preferred transcripts in lung disease, particularly lung cancer, is set forth in the tables. For example, Tables 5 and 6 describe a group of genes the expression of which is altered in lung cancer.

[0129] Immunohistochemical applications include assays, wherein increased presence of the protein can be assessed, for example, from a saliva or sputum sample.

[0130] The immunohistochemical assays according to the present invention can be performed using methods utilizing solid supports. The solid support can be a any phase used in performing immunoassays, including dipsticks, membranes, absorptive pads, beads, microtiter wells, test tubes, and the like. Preferred are test devices which may be conveniently used by the testing personnel or the patient for self-testing, having minimal or no previous training. Such preferred test devices include dipsticks, membrane assay systems as described in U.S. Pat. No. 4,632,901. The preparation and use of such conventional test systems is well described in the patent, medical, and scientific literature. If a stick is used, the anti-protein antibody is bound to one end of the stick such that the end with the antibody can be dipped into the solutions as described below for the detection of the protein. Alternatively, the samples can be applied onto the antibody-coated dipstick or membrane by pipette or dropper or the like.

[0131] The antibody against proteins encoded by the diagnostic airway transcriptome (the "protein") can be of any isotype, such as IgA, IgG or IgM, Fab fragments, or the like. The antibody may be a monoclonal or polyclonal and produced by methods as generally described, for example, in Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, incorporated herein by reference. The antibody can be applied to the solid support by direct or indirect means. Indirect bonding allows maximum exposure of the protein binding sites to the assay solutions since the sites are not themselves used for binding to the support. Preferably, polyclonal antibodies are used since polyclonal antibodies can recognize different epitopes of the protein thereby enhancing the sensitivity of the assay.

[0132] The solid support is preferably non-specifically blocked after binding the protein antibodies to the solid support. Non-specific blocking of surrounding areas can be with whole or derivatized bovine serum albumin, or albumin from other animals, whole animal serum, casein, non-fat milk, and the like.

[0133] The sample is applied onto the solid support with bound protein-specific antibody such that the protein will be bound to the solid support through said antibodies. Excess and unbound components of the sample are removed and the solid support is preferably washed so the antibody-antigen complexes are retained on the solid support. The solid support may be washed with a washing solution which may contain a detergent such as Tween-20, Tween-80 or sodium dodecyl sulfate.

[0134] After the protein has been allowed to bind to the solid support, a second antibody which reacts with protein is applied. The second antibody may be labeled, preferably with a visible label. The labels may be soluble or particulate and may include dyed immunoglobulin binding substances, simple dyes or dye polymers, dyed latex beads, dye-containing liposomes, dyed cells or organisms, or metallic, organic, inorganic, or dye solids. The labels may be bound to the protein antibodies by a variety of means that are well known in the art. In some embodiments of the present invention, the labels may be enzymes that can be coupled to a signal producing system. Examples of visible labels include alkaline phosphatase, beta-galactosidase, horseradish peroxidase, and biotin. Many enzyme-chromogen or enzyme-substrate-chromogen combinations are known and used for enzyme-linked assays. Dye labels also encompass radioactive labels and fluorescent dyes.

[0135] Simultaneously with the sample, corresponding steps may be carried out with a known amount or amounts of the protein and such a step can be the standard for the assay. A sample from a healthy individual exposed to a similar air pollutant such as cigarette smoke, can be used to create a standard for any and all of the diagnostic gene group encoded proteins.

[0136] The solid support is washed again to remove unbound labeled antibody and the labeled antibody is visualized and quantified. The accumulation of label will generally be assessed visually. This visual detection may allow for detection of different colors, for example, red color, yellow color, brown color, or green color, depending on label used. Accumulated label may also be detected by optical detection devices such as reflectance analyzers, video image analyzers and the like. The visible intensity of accumulated label could correlate with the concentration of protein in the sample. The correlation between the visible intensity of accumulated label and the amount of the protein may be made by comparison of the visible intensity to a set of reference standards. Preferably, the standards have been assayed in the same way as the unknown sample, and more preferably alongside the sample, either on the same or on a different solid support.

[0137] The concentration of standards to be used can range from about 1 mg of protein per liter of solution, up to about 50 mg of protein per liter of solution. Preferably, two or more different concentrations of an airway gene group encoded proteins are used so that quantification of the unknown by comparison of intensity of color is more accurate.

[0138] For example, the present invention provides a method for detecting risk of developing lung cancer in a subject exposed to cigarette smoke comprising measuring the

transcription profile of the proteins encoded by one or more groups of genes of the invention in a biological sample of the subject. Preferably at least about 30, still more preferably at least about 36, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or about 180 of the proteins encoded by the airway transcriptome in a biological sample of the subject are analyzed. The method comprises binding an antibody against each protein encoded by the gene in the gene group (the "protein") to a solid support chosen from the group consisting of dip-stick and membrane; incubating the solid support in the presence of the sample to be analyzed under conditions where antibody-antigen complexes form; incubating the support with an anti-protein antibody conjugated to a detectable moiety which produces a signal; visually detecting said signal, wherein said signal is proportional to the amount of protein in said sample; and comparing the signal in said sample to a standard, wherein a difference in the amount of the protein in the sample compared to said standard of the same group of proteins, is indicative of diagnosis of or an increased risk of developing lung cancer. The standard levels are measured to indicate expression levels in an airway exposed to cigarette smoke where no cancer has been detected.

[0139] The assay reagents, pipettes/dropper, and test tubes may be provided in the form of a kit. Accordingly, the invention further provides a test kit for visual detection of the proteins encoded by the airway gene groups, wherein detection of a level that differs from a pattern in a control individual is considered indicative of an increased risk of developing lung disease in the subject. The test kit comprises one or more solutions containing a known concentration of one or more proteins encoded by the airway transcriptome (the "protein") to serve as a standard; a solution of a anti-protein antibody bound to an enzyme; a chromogen which changes color or shade by the action of the enzyme; a solid support chosen from the group consisting of dip-stick and membrane carrying on the surface thereof an antibody to the protein. Instructions including the up or down regulation of the each of the genes in the groups as provided by the Tables 1 and 2 are included with the kit.

[0140] The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Genome Analysis: A Laboratory Manual Series (Vols. I-IV), Using Antibodies: A Laboratory Manual, Cells: A Laboratory Manual, PCR Primer: A Laboratory Manual, and Molecular Cloning: A Laboratory Manual (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) Biochemistry (4th Ed.) Freeman, New York, Gait, "*Oligonucleotide Synthesis: A Practical Approach*" 1984, IRL Press, London, Nelson and Cox (2000), Lehninger, *Principles of Biochemistry* 3rd Ed., W.H. Freeman Pub., New York, N.Y. and Berg et al. (2002) *Biochemistry, 5th* Ed., W.H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

[0141] The methods of the present invention can employ solid substrates, including arrays in some preferred embodiments. Methods and techniques applicable to polymer (including protein) array synthesis have been described in U.S. Ser. No. 09/536,841, WO 00/58516, U.S. Pat. Nos. 5,143,854, 5,242,974, 5,252,743, 5,324,633, 5,384,261, 5,405,783, 5,424,186, 5,451,683, 5,482,867, 5,491,074, 5,527,681, 5,550,215, 5,571,639, 5,578,832, 5,593,839, 5,599,695, 5,624,711, 5,631,734, 5,795,716, 5,831,070, 5,837,832, 5,856,101, 5,858,659, 5,936,324, 5,968,740, 5,974,164, 5,981,185, 5,981,956, 6,025,601, 6,033,860, 6,040,193, 6,090,555, 6,136,269, 6,269,846 and 6,428,752, in PCT Applications Nos. PCT/US99/00730 (International Publication Number WO 99/36760) and PCT/US01/04285, which are all incorporated herein by reference in their entirety for all purposes.

[0142] Patents that describe synthesis techniques in specific embodiments include U.S. Pat. Nos. 5,412,087, 6,147,205, 6,262,216, 6,310,189, 5,889,165, and 5,959,098. Nucleic acid arrays are described in many of the above patents, but the same techniques are applied to polypeptide and protein arrays.

[0143] Nucleic acid arrays that are useful in the present invention include, but are not limited to those that are commercially available from Affymetrix (Santa Clara, Calif.) under the brand name GeneChip7. Example arrays are shown on the website at affymetrix.com.

[0144] Examples of gene expression monitoring, and profiling methods that are useful in the methods of the present invention are shown in U.S. Pat. Nos. 5,800,992, 6,013,449, 6,020,135, 6,033,860, 6,040,138, 6,177,248 and 6,309,822. Other examples of uses are embodied in U.S. Pat. Nos. 5,871,928, 5,902,723, 6,045,996, 5,541,061, and 6,197,506.

[0145] The present invention also contemplates sample preparation methods in certain preferred embodiments. Prior to or concurrent with expression analysis, the nucleic acid 5 ample may be amplified by a variety of mechanisms, some of which may employ PCR. See, e.g., *PCR Technology: Principles and Applications for DNA Amplification* (Ed. H. A. Erlich, Freeman Press, NY, N.Y., 1992); *PCR Protocols: A Guide to Methods and Applications* (Eds. Innis, et al., Academic Press, San Diego, Calif., 1990); Mattila et al., *Nucleic Acids Res.* 19, 4967 (1991); Eckert et al., *PCR Methods and Applications* 1, 17 (1991); PCR (Eds. McPherson et al., IRL Press, Oxford); and U.S. Pat. Nos. 4,683,202, 4,683,195, 4,800,1594,965,188, and 5,333,675, and each of which is incorporated herein by reference in their entireties for all purposes. The sample may be amplified on the array. See, for example, U.S. Pat. No. 6,300,070 and U.S. patent application Ser. No. 09/513,300, which are incorporated herein by reference.

[0146] Other suitable amplification methods include the ligase chain reaction (LCR) (e.g., Wu and Wallace, *Genomics* 4, 560 (1989), Landegren et al., *Science* 241, 1077 (1988) and Barringer et al. *Gene* 89:117 (1990)), transcription amplification (Kwoh et al., *Proc. Natl. Acad. Sci. USA* 86, 1173 (1989) and WO88/10315), self-sustained sequence replication (Guatelli et al., *Proc. Nat. Acad. Sci. USA,* 87, 1874 (1990) and WO90/06995), selective amplification of target polynucleotide sequences (U.S. Pat. No. 6,410,276), consensus sequence primed polymerase chain reaction (CP-PCR) (U.S. Pat. No. 4,437,975), arbitrarily primed polymerase chain reaction (AP-PCR) (U.S. Pat. No. 5,413,909, 5,861,245) and nucleic acid based sequence amplification

(NABSA). (U.S. Pat. Nos. 5,409,818, 5,554,517, and 6,063,603). Other amplification methods that may be used are described in, U.S. Pat. Nos. 5,242,794, 5,494,810, 4,988,617 and in U.S. Ser. No. 09/854,317, each of which is incorporated herein by reference.

[0147] Additional methods of sample preparation and techniques for reducing the complexity of a nucleic sample are described, for example, in Dong et al., *Genome Research* 11, 1418 (2001), in U.S. Pat. Nos. 6,361,947, 6,391,592 and U.S. patent application Ser. Nos. 09/916,135, 09/920,491, 09/910,292, and 10/013,598.

[0148] Methods for conducting polynucleotide hybridization assays have been well developed in the art. Hybridization assay procedures and conditions will vary depending on the application and are selected in accordance with the general binding methods known including those referred to in: Maniatis et al. Molecular Cloning: A Laboratory Manual (2nd Ed. Cold Spring Harbor, N.Y., 1989); Berger and Kimmel *Methods in Enzymology, Vol.* 152, *Guide to Molecular Cloning Techniques* (Academic Press, Inc., San Diego, Calif., 1987); Young and Davism, *P.N.A.S,* 80: 1194 (1983). Methods and apparatus for carrying out repeated and controlled hybridization reactions have been described, for example, in U.S. Pat. Nos. 5,871,928, 5,874,219, 6,045,996 and 6,386,749, 6,391,623 each of which are incorporated herein by reference

[0149] The present invention also contemplates signal detection of hybridization between the sample and the probe in certain embodiments. See, for example, U.S. Pat. Nos. 5,143,854, 5,578,832; 5,631,734; 5,834,758; 5,936,324; 5,981,956; 6,025,601; 6,141,096; 6,185,030; 6,201,639; 6,218,803; and 6,225,625, in provisional U.S. Patent application 60/364,731 and in PCT Application PCT/US99/06097 (published as WO99/47964).

[0150] Examples of methods and apparatus for signal detection and processing of intensity data are disclosed in, for example, U.S. Pat. Nos. 5,143,854, 5,547,839, 5,578,832, 5,631,734, 5,800,992, 5,834,758; 5,856,092, 5,902,723, 5,936,324, 5,981,956, 6,025,601, 6,090,555, 6,141,096, 6,185,030, 6,201,639; 6,218,803; and 6,225,625, in U.S. Patent application 60/364,731 and in PCT Application PCT/US99/06097 (published as WO99/47964).

[0151] The practice of the present invention may also employ conventional biology methods, software and systems. Computer software products of the invention typically include computer readable medium having computer-executable instructions for performing the logic steps of the method of the invention. Suitable computer readable medium include floppy disk, CD-ROM/DVD/DVD-ROM, hard-disk drive, flash memory, ROM/RAM, magnetic tapes and etc. The computer executable instructions may be written in a suitable computer language or combination of several languages. Basic computational biology methods are described in, e.g. Setubal and Meidanis et al., *Introduction to Computational Biology Methods* (PWS Publishing Company, Boston, 1997); Salzberg, Searles, Kasif, (Ed.), *Computational Methods in Molecular Biology*, (Elsevier, Amsterdam, 1998); Rashidi and Buehler, *Bioinformatics Basics: Application in Biological Science and Medicine* (CRC Press, London, 2000) and Ouelette and Bzevanis *Bioinformatics: A Practical Guide for Analysis of Gene and Proteins* (Wiley & Sons, Inc., 2nd ed., 2001).

[0152] The present invention also makes use of various computer program products and software for a variety of purposes, such as probe design, management of data, analysis, and instrument operation. See, for example, U.S. Pat. Nos. 5,593,839, 5,795,716, 5,733,729, 5,974,164, 6,066,454, 6,090,555, 6,185,561, 6,188,783, 6,223,127, 6,229,911 and 6,308,170.

[0153] Additionally, the present invention may have embodiments that include methods for providing gene expression profile information over networks such as the Internet as shown in, for example, U.S. patent application Ser. Nos. 10/063,559, 60/349,546, 60/376,003, 60/394,574, 60/403,381.

[0154] Throughout this specification, various aspects of this invention are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 10-20 should be considered to have specifically disclosed sub-ranges such as from 10-13, from 10-14, from 10-15, from 11-14, from 11-16, etc., as well as individual numbers within that range, for example, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20. This applies regardless of the breadth of the range. In addition, the fractional ranges are also included in the exemplified amounts that are described. Therefore, for example, a range of 1-3 includes fractions such as 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, etc. This applies particularly to the amount of increase or decrease of expression of any particular gene or transcript.

[0155] The present invention has many preferred embodiments and relies on many patents, applications and other references for details known to those of the art. Therefore, when a patent, application, or other reference is cited or repeated throughout the specification, it should be understood that it is incorporated by reference in its entirety for all purposes as well as for the proposition that is recited.

EXAMPLES

Example 1

[0156] In this study, we used three study groups: 1) normal non-smokers (n=23); 2) smokers without cancer (active v. former smokers) (n=52); 3) smokers with suspect cancer (n=98: 45 cancer, 53 no cancer).

[0157] We obtained epithelial nucleic acids (RNA/DNA) from epithelial cells in mouth and airway (bronchoscopy). We also obtained nucleic acids from blood to provide one control.

[0158] We analyzed gene expression using RNA and U133A Affymetrix array that represents transcripts from about 22,500 genes.

[0159] The microarray data analysis was performed as follows. We first scanned the Affymetrix chips that had been hybridized with the study group samples. The obtained microarray raw data consisted of signal strength and detection p-value. We normalized or scaled the data, and filtered the poor quality chips based on images, control probes, and histograms according to standard Affymetrix instructions. We also filtered contaminated specimens which contained non-epithelial cells. Lastly, the genes of importance were filtered using detection p-value. This resulted in identification of transcripts present in normal airways (normal airway transcriptome), with variability and multiple regression analysis. This also resulted in identification of effects of smoking on

airway epithelial cell transcription. For this, we used T-test and Pearson correlation analysis. We also identified a group or a set of transcripts that were differentially expressed in samples with lung cancer and samples without cancer. This analysis was performed using class prediction models.

[0160] We used weighted voting method. The weighted voting method ranks, and gives a weight "p" to all genes by the signal to noise ration of gene expression between two classes: $P = mean_{(class\ 1)} - mean_{(class\ 2)} / sd_{(class\ 1)} = sd_{(class\ 2)}$. Committees of variable sizes of the top ranked genes were used to evaluate test samples, but genes with more significant p-values were more heavily weighed. Each committee genes in test sample votes for one class or the other, based on how close that gene expression level is to the class 1 mean or the class 2 mean. $V_{(gene\ A)} = P_{(gene\ A)}$, i.e. level of expression in test sample less the average of the mean expression values in the two classes. Votes for each class were tallied and the winning class was determined along with prediction strength as $PS = V_{win} - V_{lose} / V_{win} + V_{lose}$. Finally, the accuracy was validated using cross-validation +/– independent samples.

[0161] FIG. 8 shows diagrams of the class prediction model analysis used in the Example 1.

[0162] The results of the weighted voting method for a 50 gene group analysis (50 gene committee) were as follows. Cross-validation (n=74) resulted in accuracy of 81%, with sensitivity of 76% and specificity of 85%. In an independent dataset (n=24) the accuracy was 88%, with sensitivity of 75% and specificity of 100%.

[0163] We note that with sensitivity to bronchoscopy alone only 18/45 (40%) of cancers were diagnosed at the time of bronchoscopy using brushings, washings, biopsy or Wang.

[0164] We performed a gene expression analysis of the human genome using isolated nucleic acid samples comprising lung cell transcripts from individuals. The chip used was the Human Genome U133 Set. We used Microarray Suite 5.0 software to analyze raw data from the chip (i.e. to convert the image file into numerical data). Both the chip and the software are proprietary materials from Affymetrix. Bronchoscopy was performed to obtain nucleic acid samples from 98 smoker individuals.

[0165] We performed a Student's t-test using gene expression analysis of 45 smokers with lung cancer and 53 smokers without lung cancer. We identified several groups of genes that showed significant variation in their expression between smokers with cancer and smokers without cancer. We further identified at least three groups of genes that, when their expression was analyzed in combination, the results allowed us to significantly increase diagnostic power in identifying cancer carrying smokers from smokers without cancer.

[0166] The predictor groups of genes were identified using the GenePattern server from the Broad Institute, which includes the Weighted Voting algorithm. The default settings, i.e., the signal to noise ratio and no gene filtering, were used. GenePattern is available at World Wide Web from broad.mit. edu/cancer/software/genepattern. This program allows analysis of data in groups rather than as individual genes.

[0167] Table 1 shows the top 96 genes from our analysis with different expression patterns in smokers with cancer and smokers without cancer.

[0168] Table 2 shows the 84 genes that were also identified in our previous screens as individual predictors of lung cancer.

[0169] Table 4 shows a novel group of 36 genes the expression of which was different between the smokers with cancer and smokers without cancer.

[0170] Table 3 shows a group of 50 genes that we identified as most predictive of development of cancer in smokers. That is, that when the expression of these genes was analyzed and reflected the pattern (expression down or up) as shown in Table 3, we could identify the individuals who will develop cancer based on this combined expression profile of these genes. When used in combination, the expression analysis of these 50 genes was predictive of a smoker developing lung cancer in over 70% of the samples. Accuracy of diagnosis of lung cancer in our sample was 80-85% on cross-validation and independent dataset (accuracy includes both the sensitivity and specificity). The sensitivity (percent of cancer cases correctly diagnosed) was approximately 75% as compared to sensitivity of 40% using standard bronchoscopy technique. (Specificity is percent of non-cancer cases correctly diagnosed).

[0171] These data show the dramatic increase of diagnostic power that can be reached using the expression profiling of the gene groups as identified in the present study.

Example 2

[0172] We report here a gene expression profile, derived from histologically normal large airway epithelial cells of current and former smokers with clinical suspicion of lung cancer that is highly sensitive and specific for the diagnosis of lung cancer. This airway signature is effective in diagnosing lung cancer at an early and potentially resectable stage. When combined with results from bronchoscopy (i.e. washings, brushings, and biopsies of the affected area), the expression profile is diagnostic of lung cancer in 95% of cases. We further show that the airway epithelial field of injury involves a number of genes that are differentially expressed in lung cancer tissue, providing potential information about pathways that may be involved in the genesis of lung cancer.

[0173] Patient Population: We obtained airway brushings from current and former smokers (n—208) undergoing fiber optic bronchoscopy as a diagnostic study for clinical suspicion of lung cancer between January 2003 and May 2005. Patients were recruited from 4 medical centers:

[0174] Boston University Medical Center, Boston, Mass.; Boston Veterans Administration, West Roxbury, Mass.; Lahey Clinic, Burlington, Mass.; and Trinity College, Dublin, Ireland. Exclusion criteria included never smokers, cigar smokers and patients on a mechanical ventilator at the time of their bronchoscopy. Each subject was followed clinically, post-bronchoscopy, until a final diagnosis of lung cancer or an alternate benign diagnosis was made. Subjects were classified as having lung cancer if their bronchoscopy studies (brushing, bronchoalveolar lavage or endobronchial biopsy) or a subsequent lung biopsy (transthoracic biopsy or surgical lung biopsy) yielded tumor cells on pathology/cytology. Subjects were classified with an alternative benign diagnosis if the bronchoscopy or subsequent lung biopsy yielded a non-lung cancer diagnosis or if their radiographic abnormality resolved on follow up chest imaging. The study was approved by the Institutional Review Boards of all 4 medical centers and all participants provided written informed consent.

[0175] Airway epithelial cell collection: Following completion of the standard diagnostic bronchoscopy studies, bronchial airway epithelial cells were obtained from the "uninvolved" right mainstem bronchus with an endoscopic

cytobrush (Cellebrity Endoscopic Cytobrush, Boston Scientific, Boston, Mass.). If a suspicious lesion (endobronchial or submucosal) was seen in the right mainstem bronchus, cells were then obtained from the uninvolved left mainstem bronchus. The brushes were immediately placed in TRIzol reagent (Invitrogen, Carlsbad, Calif.) after removal from the bronchoscope and kept at −80° C. until RNA isolation was performed. RNA was extracted from the brushes using TRIzol Reagent (Invitrogen) as per the manufacturer protocol, with a yield of 8-15 μg of RNA per patient. Integrity of the RNA was confirmed by denaturing gel electrophoresis. Epithelial cell content and morphology of representative bronchial brushing samples was quantified by cytocentrifugation (ThermoShandon Cytospin, Pittsburgh, Pa.) of the cell pellet and staining with a cytokeratin antibody (Signet, Dedham Mass.). These samples were reviewed by a pathologist who was blinded to the diagnosis of the patient.

[0176] Microarray data acquisition and preprocessing: 6-8 μg of total RNA was processed, labeled, and hybridized to Affymetrix HG-U133A GeneChips containing approximately 22,215 human transcripts as described previously (17). We obtained sufficient quantity of high quality RNA for microarray studies from 152 of the 208 samples. The quantity of RNA obtained improved during the course of the study so that 90% of brushings yielded sufficient high quality RNA during the latter half of the study. Log-normalized probe-level data was obtained from CEL files using the Robust Multichip Average (RMA) algorithm(18). A z-score filter was employed to filter out arrays of poor quality (see supplement for details), leaving 129 samples with a final diagnosis available for analysis.

[0177] Microarray Data Analysis: Class Prediction

[0178] To develop and test a gene expression predictor capable of distinguishing smokers with and without lung cancer, 60% of samples (n=77) representing a spectrum of clinical risk for lung cancer and approximately equal numbers of cancer and no cancer subjects were randomly assigned to a training set (see Supplement). Using the training set samples, the 22,215 probesets were filtered via ANCOVA using pack-years as the covariate; probesets with a p-value greater than 0.05 for the difference between the two groups were excluded. This training-set gene filter was employed to control for the potential confounding effect of cumulative tobacco exposure, which differed between subjects with and without cancer (see Table 1a).

	Cancer	NonCancer
Samples	60	69
Age **	64.1 +/− 9.0	49.8 +/− 15.2
Smoking Status	51.7% F, 48..3% C	37.7% F, 62..3% C
Gender	80% M, 20% F	73.9% M, 26.1% F
PackYears **	57.4 +/− 25..6	29.4 +/− 27..3
Age Started	15.2 +/− 4.2	16.7 +/− 6.8
Smoking intensity (PPD): Currents *	1.3 +/− 0.45	0.9 +/− 0.5
Months Quit: Formers	113 +/− 118	158 +/− 159

* Two classes statistically different: $p < 0.05$
** Two classes statistically different: $p < 0.001$

[0179] Table 1a shows demographic features and characteristics of the two patient classes being studied. Statistical differences between the two patient classes and associated p

values were calculated using T-tests, Chi-square tests and Fisher's exact tests where appropriate.

[0180] Gene selection was conducted through internal cross-validation within the training set using the weighted voting algorithm(19). The internal cross-validation was repeated 50 times, and the top 40 up- and top 40 down-regulated probesets in cancer most frequently chosen during internal cross-validation runs were selected as the final gene committee of 80 features (see sections, infra, for details regarding the algorithm and the number of genes selected for the committee).

[0181] The accuracy, sensitivity, and specificity of the biomarker were assessed on the independent test set of 52 samples. This was accomplished by using the weighted vote algorithm to predict the class of each test set sample based on the gene expression of the 80 probesets and the probe set weights derived from the 77 samples in the training set. To assess the performance of our classifier, we first created 1000 predictors from the training set where we randomized the training set class labels. We evaluated the performance of these "class-randomized" classifiers for predicting the sample class of the test set samples and compared these to our classifier using ROC analysis. To assess whether the performance of our gene expression profile depends on the specific training and test sets from which it was derived and tested, we next created 500 new training and test sets with our 129 samples and derived new "sample-randomized" classifiers from each of these training sets which were then tested on the corresponding test set. To assess the specificity of our classifier genes, we next created 500 classifiers each composed of 80 randomly selected genes. We then tested the ability of these "gene-randomized" classifiers to predict the class of samples in the test set. To evaluate the robustness of our class prediction algorithm and data preprocessing, we also used these specific 80 genes to generate predictive models with an alternate class prediction algorithm (Prediction Analysis of Microarrays (PAM)(20)) and with MAS 5.0 generated expression data instead of RMA. Finally, the performance of our predictor was compared to the diagnostic yield of bronchoscopy.

[0182] Quantitative PCR Validation: Real time PCR (QRT-PCR) was used to confirm the differential expression of a select number of genes in our predictor. Primer sequences were designed with Primer Express software (Applied Biosystems, Foster City, Calif.). Forty cycles of amplification, data acquisition, and data analysis were carried out in an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster City, Calif.). All real time PCR experiments were carried out in triplicate on each sample (see sections infra).

[0183] Linking to lung cancer tissue microarray data: The 80-gene lung cancer biomarker derived from airway epithelium gene expression was evaluated for its ability to distinguish between normal and cancerous lung tissue using an Affymetrix HGU95Av2 dataset published by Bhattacharjee et al(21) that we processed using RMA. By mapping Unigene identifiers, 64 HGU95Av2 probesets were identified that measure the expression of genes that corresponded to the 80 probesets in our airway classifier. This resulted in a partial airway epithelium signature that was then used to classify tumor and normal samples from the dataset. In addition, PCA analysis of the lung tissue samples was performed using the expression of these 64 probesets.

[0184] To further assess the statistical significance of the relationship between datasets, Gene Set Enrichment Analysis

(22) was performed to determine if the 64 biomarker genes are non-randomly distributed within the HGU95Av2 probesets ordered by differential expression between normal and tumor tissue. Finally, a two-tailed Fisher Exact Test was used to test if the proportion of biomarker genes among the genes differentially expressed between normal and tumor lung tissue is different from the overall proportion of differentially expressed genes (see sections, infra).

[0185] Statistical Analysis: RMA was performed in Bio-Conductor. The upstream gene filtering by ANCOVA, and the implementation of the weighted voted algorithm and internal cross validation used to generate the data were executed through an R script we wrote for this purpose. The PAM algorithm was carried out using the 'pamr' library in R. All other statistical analyses including Student's T-Tests, Fisher's exact tests, ROC curves and PCA were performed using the R statistical package.

[0186] Study Population and Epithelial samples: 129 subjects that had microarrays passing the quality control filter described above were included in the class prediction analysis (see Supplemental FIG. 1). Demographic data on these subjects, including 60 smokers with primary lung cancer and 69 smokers without lung cancer is presented in Table 1. Cell type and stage information for all cancer patients is shown in Supplemental Table 1. Bronchial brushings yielded 90% epithelial cells, as determined by cytokeratin staining, with the majority being ciliated cells with normal bronchial airway morphology. No dysplastic or cancer cells were seen on any representative brushings obtained from smokers with or without cancer.

[0187] Class Prediction analysis: Comparison of demographic features for 77 subjects in the training set vs. the 52 samples in the test set is shown in Supplemental Table 2. An 80 gene class prediction committee capable of distinguishing smokers with and without cancer was built on the training set of 77 samples and tested on the independent sample set (FIG. 14). The accuracy, sensitivity and specificity of this model was 83% (43/52), 80% (16/20) and 84% (27/32) respectively. When samples predicted with a low degree of confidence (as defined by a Prediction Strength metric <0.3; see Supplement for details) were considered non-diagnostic, the overall accuracy of the model on the remaining 43 samples in the test set increased to 88% (93% sensitivity, 86% specificity). Hierarchical clustering of the 80 genes selected for the diagnostic biomarker in the test set samples is shown in FIG. 15. Principal Component Analysis of all cancer samples according to the expression of these 80 genes did not reveal grouping by cell type (FIG. 10). The accuracy of this 80-gene classifier was similar when microarray data was preprocessed in MAS 5.0 and when the PAM class prediction algorithm was used (see Supplemental Table 3).

[0188] The 80-gene predictor's accuracy, sensitivity and specificity on the 52 sample test set was significantly better than the performance of classifiers derived from randomizing the class labels of the training set (p=0.004; empiric p-value for random classifier AUC>true classifier AUC; FIG. 16). The performance of the classifier was not dependent on the particular composition of the training and test set on which it was derived and tested: 500 training and test sets (derived from the 129 samples) resulted in classifiers with similar accuracy as the classifier derived from our training set (FIG. 11). Finally, we demonstrated that the classifier is better able to distinguish the two sample classes than 500 classifiers derived by randomly selecting genes (see FIG. 12).

[0189] Real time PCR: Differential expression of select genes in our diagnostic airway profile was confirmed by real time PCR (see FIG. 13).

[0190] Linking to lung cancer tissue: Our airway biomarker was also able to correctly classify lung cancer tissue from normal lung tissue with 98% accuracy. Principal Component Analysis demonstrated separation of non-cancerous samples from cancerous samples in the Bhattacharjee dataset according to the expression of our airway signature (see FIG. 17). Furthermore, our class prediction genes were statistically overrepresented among genes differentially expressed between cancer vs. no cancer in the Bhattacharjee dataset by Fisher exact test (p<0.05) and Gene Enrichment Analysis (FDR<0.25, see Supplement for details).

[0191] Synergy with Bronchoscopy: Bronchoscopy was diagnostic (via endoscopic brushing, washings or biopsy of the affected region) in 32/60 (53%) of lung cancer patients and 5/69 non-cancer patients. Among non-diagnostic bronchoscopies (n=92), our class prediction model had an accuracy of 85% with 89% sensitivity and 83% specificity. Combining bronchoscopy with our gene expression signature resulted in a 95% diagnostic sensitivity (57/60) across all cancer subjects. Given the approximate 50% disease prevalence in our cohort, a negative bronchoscopy and negative gene expression signature for lung cancer resulted in a 95% negative predictive value (NPV) for disease (FIG. 18). In patients with a negative bronchoscopy, the positive predictive value of our gene expression profile for lung cancer was approximately 70% (FIG. 18).

[0192] Stage and cell type subgroup analysis: The diagnostic yield of our airway gene expression signature vs. bronchoscopy according to stage and cell type of the lung cancer samples is shown in FIG. 19.

[0193] Lung cancer is the leading cause of death from cancer in the United States, in part because of the lack of sensitive and specific diagnostic tools that are useful in early-stage disease. With approximately 90 million former and current smokers in the U.S., physicians increasingly encounter smokers with clinical suspicion for lung cancer on the basis of an abnormal radiographic imaging study and/or respiratory symptoms. Flexible bronchoscopy represents a relatively noninvasive initial diagnostic test to employ in this setting. This study was undertaken in order to develop a gene expression-based diagnostic, that when combined with flexible bronchoscopy, would provide a sensitive and specific one-step procedure for the diagnosis of lung cancer. Based on the concept that cigarette smoking creates a respiratory tract "field defect", we examined the possibility that profiles of gene expression in relatively easily accessible large airway epithelial cells would serve as an indicator of the amount and type of cellular injury induced by smoking and might provide a diagnostic tool in smokers who were being evaluated for the possibility of lung cancer.

[0194] We have previously shown that smoking induces a number of metabolizing and anti-oxidant genes, induces expression of several putative oncogenes and suppresses expression of several potential tumor suppressor genes in large airway epithelial cells(17). We show here that the pattern of airway gene expression in smokers with lung cancer differs from smokers without lung cancer, and the expression profile of these genes in histologically normal bronchial epithelial cells can be used as a sensitive and specific predictor of the presence of lung cancer. We found that the expression signature was particularly useful in early stage disease where

bronchoscopy was most often negative and where most problems with diagnosis occur. Furthermore, combining the airway gene expression signature with bronchoscopy results in a highly sensitive diagnostic approach capable of identifying 95% of lung cancer cases.

[0195] Given the unique challenges to developing biomarkers for disease using DNA microarrays(23), we employed a rigorous computational approach in the evaluation of our dataset. The gene expression biomarker reported in this paper was derived from a training set of samples obtained from smokers with suspicion of lung cancer and was tested on an independent set of samples obtained from four tertiary medical centers in the US and Ireland. The robust nature of this approach was confirmed by randomly assigning samples into separate training and test sets and demonstrating a similar overall accuracy (FIG. 11). In addition, the performance of our biomarker was significantly better than biomarkers obtained via randomization of class labels in the training set (FIG. 16) or via random 80 gene committees (FIG. 8). Finally, the performance of our 80-gene profile remained unchanged when microarray data was preprocessed via a different algorithm or when a second class prediction algorithm was employed.

[0196] In terms of limitations, our study was not designed to assess performance as a function of disease stage or subtype. Our gene expression predictor, however, does appear robust in early stage disease compared with bronchoscopy (see FIG. 19). Our profile was able to discriminate between cancer and no cancer across all subtypes of lung cancer (see FIG. 10). 80% of the cancers in our dataset were NSCLC and our biomarker was thus trained primarily on events associated with that cell type. However, given the high yield for bronchoscopy alone in the diagnosis of small cell lung cancer, this does not limit the sensitivity and negative predictive value of the combined bronchoscopy and gene expression signature approach. A large-scale clinical trial is needed to validate our signature across larger numbers of patients and establish its efficacy in early stage disease as well as its ability to discriminate between subtypes of lung cancer.

[0197] In addition to serving as a diagnostic biomarker, profiling airway gene expression across smokers with and without lung cancer can also provide insight into the nature of the "field of injury" reported in smokers and potential pathways implicated in lung carcinogenesis. Previous studies have demonstrated allelic loss and methylation of tumor suppressor genes in histologically normal bronchial epithelial cells from smokers with and without lung cancer(12; 13; 15). Whether these changes are random mutational effects or are directly related to lung cancer has been unclear. The finding that our airway gene signature was capable of distinguishing lung cancer tissue from normal lung (FIG. 4) suggests that the airway biomarker is, at least in part, reflective of changes occurring in the cancerous tissue and may provide insights into lung cancer biology.

[0198] Among the 80 genes in our diagnostic signature, a number of genes associated with the RAS oncogene pathway, including Rab 1a and FOS, are up regulated in the airway of smokers with lung cancer. Rab proteins represent a family of at least 60 different Ras-like GTPases that have crucial roles in vesicle trafficking, signal transduction, and receptor recycling, and dysregulation of RAB gene expression has been implicated in tumorigenesis(24). A recent study by Shimada et al. (25) found a high prevalence of Rab1A-overexpression in head and neck squamous cell carcinomas and also in pre-

malignant tongue lesions, suggesting that it may be an early marker of smoking-related respiratory tract carcinogenesis.

[0199] In addition to these RAS pathway genes, the classifier contained several pro-inflammatory genes, including Interleukin-8 (IL-8) and beta-defensin 1 that were up regulated in smokers with lung cancer. IL-8, originally discovered as a chemotactic factor for leukocytes, has been shown to contribute to human cancer progression through its mitogenic and angiogenic properties(26; 27). Beta defensins, antimicrobial agents expressed in lung epithelial cells, have recently found to be elevated in the serum of patients with lung cancer as compared to healthy smokers or patients with pneumonia (28). Higher levels of these mediators of chronic inflammation in response to tobacco exposure may result in increased oxidative stress and contribute to tumor promotion and progression in the lung(29; 30)

[0200] A number of key antioxidant defense genes were found to be decreased in airway epithelial cells of subjects with lung cancer, including BACH2 and dual oxidase 1, along with a DNA repair enzyme, DNA repair protein 1C. BACH-2, a transcription factor, promotes cell apoptosis in response to high levels of oxidative-stress(31). We have previously found that a subset of healthy smokers respond differently to tobacco smoke, failing to induce a set of detoxification enzymes in their normal airway epithelium, and that these individuals may be predisposed to its carcinogenic effects (17). Taken together, these data suggest that a component of the airway "field defect" may reflect whether a given smoker is appropriately increasing expression of protective genes in response to the toxin. This inappropriate response may reflect a genetic susceptibility to lung cancer or alternatively, epigenetic silencing or deletion of that gene by the carcinogen.

[0201] In summary, our study has identified an airway gene expression biomarker that has the potential to directly impact the diagnostic evaluation of smokers with suspect lung cancer. These patients usually undergo fiberoptic bronchoscopy as their initial diagnostic test. Gene expression profiling can be performed on normal-appearing airway epithelial cells obtained in a simple, non-invasive fashion at the time of the bronchoscopy, prolonging the procedure by only 3-5 minutes, without adding significant risks. Our data strongly suggests that combining results from bronchoscopy with the gene expression biomarker substantially improves the diagnostic sensitivity for lung cancer (from 53% to 95%). In a setting of 50% disease prevalence, a negative bronchoscopy and negative gene expression signature for lung cancer results in a 95% negative predictive value (NPV), allowing these patients to be followed non-aggressively with repeat imaging studies. For patients with a negative bronchoscopy and positive gene expression signature, the positive predictive value is ~70%, and these patients would likely require further invasive testing (i.e. transthoracic needle biopsy or open lung biopsy) to confirm the presumptive lung cancer diagnosis. However, this represents a substantial reduction in the numbers of patients requiring further invasive diagnostic testing compared to using bronchoscopy alone. In our study, 92/129 patients were bronchoscopy negative and would have required further diagnostic work up. However, the negative predictive gene expression profile in 56 of these 92 negative bronchoscopy subjects would leave only 36 subjects who would require further evaluation (see FIG. 18).

[0202] The cross-sectional design of our study limits interpretation of the false positive rate for our signature. Given that the field of injury may represent whether a smoker is appro-

priately responding to the toxin, derangements in gene expression could precede the development of lung cancer or indicate a predisposition to the disease. Long-term follow-up of the false positive cases is needed (via longitudinal study) to assess whether they represent smokers who are at higher risk for developing lung cancer in the future. If this proves to be true, our signature could serve as a screening tool for lung cancer among healthy smokers and have the potential to identify candidates for chemoprophylaxis trials.

Study Patients and Sample Collection

[0203] A. Primary sample set: We recruited current and former smokers undergoing flexible bronchoscopy for clinical suspicion of lung cancer at four tertiary medical centers. All subjects were older than 21 years of age and had no contraindications to flexible bronchoscopy including hemodynamic instability, severe obstructive airway disease, unstable cardiac or pulmonary disease (i.e. unstable angina, congestive heart failure, respiratory failure) inability to protect airway or altered level of consciousness and inability to provide informed consent. Never smokers and subjects who only smoked cigars were excluded from the study. For each consented subject, we collected data regarding their age, gender, race, and a detailed smoking history including age started, age quit, and cumulative tobacco exposure. Former smokers were defined as patients who had not smoked a cigarette for at least one month prior to entering our study. All subjects were followed, post-bronchoscopy, until a final diagnosis of lung cancer or an alternative diagnosis was made (mean follow-up time=52 days). For those patients diagnosed with lung cancer, the stage and cell type of their tumor was recorded. The clinical data collected from each subject in this study can be accessed in a relational database at http://pulm.bumc.bu.edu/CancerDx/. The stage and cell type of the 60 cancer samples used to train and test the class prediction model is shown in Supplemental Table 1 below.

Cell Type	
NSCLC	48
Squamous Cell	23
Adenocarcinoma	11
Large Cell	4
Not classified	10
Small Cell	11
Unknown	1

Stage NSCLC staging	
IA	2
IB	9
IIA	2
IIB	0
IIIA	9
IIIB	9
IV	17

[0204] Supplemental Table 1 above shows cell type and staging information for 60 lung cancer patients in the 129 primary sample set used to build and test the class prediction model. Staging information limited to the 48 non-small cell samples.

[0205] The demographic features of the samples in training and test shown are shown in Supplemental Table 2 below. The Table shows patient demographics for the primary dataset (n=129) according to training and test set status. Statistical differences between the two patient classes and associated p values were calculated using T-tests, Chi-square tests and Fisher's exact tests where appropriate. PPD=packs per day, F=former smokers, C=current smokers, M=male, F=female.

	Training set	Test set
Samples	77	52
Age	59.3 +/− 13.1	52.1 +/− 15.6
Smoking Status	41.6% F, 58.4% C	48.1% F, 51.9% C
Gender*	83.1% M, 16.9% F	67.3% M, 32.7% F
PackYears	45.6 +/− 31	37.7 +/− 27.8
Age Started	16.2 +/− 6.3	15.8 +/− 5.3
Smoking intensity (PPD): Currents	1.1 +/− 0.53	1 +/− 0.5
Months Quit: Formers	128 +/− 139	139+/141

*Two classes statistically different: $p < 0.05$

[0206] While our study recruited patients whose indication for bronchoscopy included a suspicion for lung cancer, each patient's clinical pre-test probability for disease varied. In order to ensure that our class prediction model was trained on samples representing a spectrum of lung cancer risk, three independent pulmonary clinicians, blinded to the final diagnoses, evaluated each patient's clinical history (including age, smoking status, cumulative tobacco exposure, co-morbidities, symptoms/signs and radiographic findings) and assigned a pre-bronchoscopy probability for lung cancer. Each patient was classified into one of three risk groups: low (<10% probability of lung cancer), medium (10-50% probability of lung cancer) and high (>50% probability of lung cancer). The final risk assignment for each patient was decided by the majority opinion.

Prospective Sample Set:

[0207] After completion of the primary study, a second set of samples was collected from smokers undergoing flexible bronchoscopy for clinical suspicion of lung cancer at 5 medical centers (St. Elizabeth's Hospital in Boston, Mass. was added to the 4 institutions used for the primary dataset). Inclusion and exclusion criteria were identical to the primary sample set. Forty additional subjects were included in this second validation set. Thirty-five subjects had microarrays that passed our quality-control filter. Demographic data on these subjects, including 18 smokers with primary lung cancer and 17 smokers without lung cancer, is presented in Supplemental Table 3. There was no statistical difference in age or cumulative tobacco exposure between case and controls in this prospective cohort (as opposed to the primary dataset; see Table 1a).

[0208] Supplemental Table 3 below shows patient demographics for the prospective validation set (n=35) by cancer status. Statistical differences between the two patient classes and associated p values were calculated using T-tests, Chisquare tests and Fisher's exact tests where appropriate. PPD=packs per day, F=former smokers, C=current smokers, M=male, F=female.

	Cancer	No Cancer
Samples	18	17
Age	66.1 +/− 11.4	62.2 +/− 11.1
Smoking Status	66.7% F, 33.3% C	52.9% F, 47.1% C
Gender*	66.6% M, 33.3% F	70.6% M, 29.4% F
PackYears	46.7 +/− 28.8	60 +/− 44.3
Age Started	16.4 +/− 7.3	14.2 +/− 3.8
Smoking intensity (PPD): Currents	1.1 +/− 0.44	1.2 +/− 0.9
Months Quit: Formers	153 +/− 135	93 +/− 147

*Two classes statistically different: $p < 0.05$

Airway Epithelial Cell Collection:

[0209] Bronchial airway epithelial cells were obtained from the subjects described above via flexible bronchoscopy. Following local anesthesia with 2% topical lidocaine to the oropharynx, flexible bronchoscopy was performed via the mouth or nose. Following completion of the standard diagnostic bronchoscopy studies (i.e. bronchoalveolar lavage, brushing and endo/transbronchial biopsy of the affected region), brushings were obtained via three endoscopic cytobrushes from the right mainstem bronchus. The cytobrush was rubbed over the surface of the airway several times and then retracted from the bronchoscope so that epithelial cells could be placed immediately in TRIzol solution and kept at −80° C. until RNA isolation was performed.

[0210] Given that these patients were undergoing bronchoscopy for clinical indications, the risks from our study were minimal, with less than a 5% risk of a small amount of bleeding from these additional brushings. The clinical bronchoscopy was prolonged by approximately 34 minutes in order to obtain the research samples. All participating subjects were recruited by IRB-approved protocols for informed consent, and participation in the study did not affect subsequent treatment. Patient samples were given identification numbers in order to protect patient privacy.

Microarray Data Acquisition and Preprocessing

[0211] Microarray data acquisition: 6-8 μg of total RNA from bronchial epithelial cells were converted into double-stranded cDNA with SuperScript II reverse transcriptase (Invitrogen) using an oligo-dT primer containing a T7 RNA polymerase promoter (Genset, Boulder, Colo.). The ENZO Bioarray RNA transcript labeling kit (Enzo Life Sciences, Inc, Farmingdale, N.Y.) was used for in vitro transcription of the purified double stranded cDNA. The biotin-labeled cRNA was then purified using the RNeasy kit (Qiagen) and fragmented into fragments of approximately 200 base pairs by alkaline treatment. Each cRNA sample was then hybridized overnight onto the Affymetrix HG-U133A array followed by a washing and staining protocol. Confocal laser scanning (Agilent) was then performed to detect the streptavidin-labeled fluor.

[0212] Preprocessing of array data via RMA: The Robust Multichip Average (RMA) algorithm was used for background adjustment, normalization, and probe-level summarization of the microarray samples in this study (Irizarry R A, et al., Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 2003; 31(4):e15.). RMA expression measures were computed using the R statistical package and the just RMA function in the Affymetrix Bioconductor package. A total of 296 CEL files from airway epithelial samples included in this study as well as those previously processed in our lab were analyzed using RMA. RMA was chosen for probe-level analysis instead of Microarray Suite 5.0 because it maximized the correlation coefficients observed between 7 pairs of technical replicates (Supplemental Table 4).

SUPPLEMENTAL TABLE 4

Pearson Correlation Coefficients (22,215 probe-sets)			
	Affy	log2Affy	RMA
Average	0.972	0.903	0.985
SD	0.017	0.029	0.009
Median	0.978	0.912	0.987

[0213] Supplemental Table 4 shows the Average Pearson Correlations between 7 pairs of replicate samples where probe-set gene expression values were determined using Microarray Suite 5.0 (Affy), logged data from Microarray Suite 5.0 (log 2 Affy), and RMA. RMA maximizes the correlation between replicate samples.

[0214] Sample filter: To filter out arrays of poor quality, each probeset on the array was z-score normalized to have a mean of zero and a standard deviation of 1 across all 152 samples. These normalized gene-expression values were averaged across all probe-sets for each sample. The assumption explicit in this analysis is that poor-quality samples will have probeset intensities that consistently trend higher or lower across all samples and thus have an average z-score that differs from zero. This average z-score metric correlates with Affymetrix MAS 5.0 quality metrics such as percent present (FIG. 7) and GAPDH 3'/5' ratio. Microarrays that had an average z-score with a value greater than 0.129 (~15% of the 152 samples) were filtered out. The resulting sample set consisted of 60 smokers with cancer and 69 smokers without cancer.

[0215] Prospective validation test set: CEL files for the additional 40 samples were added to the collection of airway epithelial CEL files described above, and the entire set was analyzed using RMA to derive expression values for the new samples. Microarrays that had an average z-score with a value greater than 0.129 (5 of the 40 samples) were filtered out. Class prediction of the 35 remaining prospective samples was conducted using the vote weights for the 80-predictive probesets derived from the training set of 77 samples using expression values computed in the section above.

Microarray Data Analysis

[0216] Class Prediction Algorithm: The 129-sample set (60 cancer samples, 69 no cancer samples) was used to develop a class-prediction algorithm capable of distinguishing between the two classes. One potentially confounding difference between the two groups is a difference in cumulative tobacco-smoke exposure as measured by pack-years. To insure that the genes chosen for their ability to distinguish patients with and without cancer in the training set were not simply distinguishing this difference in tobacco smoke exposure, the pack-years each patient smoked was included as a covariate in the training set ANCOVA gene filter.

[0217] In addition, there are differences in the pre-bronchoscopy clinical risk for lung cancer among the 129 patients. Three physicians reviewed each patient's clinical data (in-

cluding demographics, smoking histories, and radiographic findings) and divided the patients into three groups: high, medium, and low pre-bronchoscopy risk for lung cancer (as described above). In order to control for differences in pre-bronchoscopy risk for lung cancer between the patients with and without a final diagnosis of lung cancer, the training set was constructed with roughly equal numbers of cancer and no cancer samples from a spectrum of lung cancer risk.

[0218] The weighted voting algorithm (Golub T R, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999; 286 (5439):531-537) was implemented as the class prediction method, with several modifications to the gene-selection methodology. Genes that varied between smokers with and without cancer in the training set samples after adjusting for tobacco-smoke exposure ($p<0.05$) were identified using an ANCOVA with pack-years as the covariate. Further gene selection was performed using the signal to noise metric and internal cross-validation where the 40 most consistently up-

the algorithm where either: 1) different training/tests sets were used; 2) the cancer status of the training set of 77 samples were randomized; or 3) the genes in the classifier were randomly chosen (see randomization section below for details).

[0220] Randomization: The accuracy, sensitivity, specificity, and area under the ROC curve (using the signed prediction strength as a continuous cancer predictor) for the 80-probeset predictor (above) were compared to 1000 runs of the algorithm using three different types of randomization. First, the class labels of the training set of 77 samples were permuted and the algorithm, including gene selection, was re-run 1000 times (referred to in Supplemental Table 5 as Random 1).

[0221] Supplemental Table 5 below shows results of a comparison between the actual classifier and random runs (explained above). Accur=Accuracy, Sens=Sensitivity, Spec=Specificity, AUC area under the curve, and sd=standard deviation. All p-value are empirically derived.

SUPPLEMENTAL TABLE 5

	Accur	sd(Accur)	p-value	Sens	sd(Sens)	p-value	Spec	sd(Spec)	p-value	AUC	sd(AUC)	p-value
Actual Classifier	0.827			0.8			0.844			0.897		
Random 1	0.491	0.171	0.018	0.487	0.219	0.114	0.493	0.185	0.015	0.487	0.223	0.004
Random 2	0.495	0.252	0.078	0.496	0.249	0.173	0.495	0.263	0.073	0.495	0.309	0.008
Random 3	0.495	0.193	0.021	0.491	0.268	0.217	0.498	0.17	0.006	0.492	0.264	0.007

and the 40 most consistently down-regulated probesets were identified. The internal cross validation involved leaving 30% of the training samples out of each round of cross-validation, and selecting genes based on the remaining 70% of the samples. The final gene committee consisted of eighty probesets that were identified as being most frequently up-regulated or down-regulated across 50 rounds of internal cross-validation. The parameters of this gene-selection algorithm were chosen to maximize the average accuracy, sensitivity and specificity obtained from fifty runs. This algorithm was implemented in R and yields results that are comparable to the original implementation of the weighted-voted algorithm in GenePattern when a specific training, test, and gene set are given as input.

[0219] After determination of the optimal gene-selection parameters, the algorithm was run using a training set of 77 samples to arrive at a final set of genes capable of distinguishing between smokers with and without lung cancer. The accuracy, sensitivity and specificity of this classifier were tested against 52 samples that were not included in the training set. The performance of this classifier in predicting the class of each test-set sample was assessed by comparing it to runs of

[0222] The second randomization used the 80 genes in the original predictor but permuted the class labels of the training set samples over 1000 runs to randomize the gene weights used in the classification step of the algorithm (referred to in Supplemental Table 5 as Random 2).

[0223] In both of these randomization methods, the class labels were permuted such that half of the training set samples was labeled correctly. The third randomization method involved randomly selecting 80 probesets for each of 1000 random classifiers (referred to in Supplemental Table 5 as Random 3).

[0224] The p-value for each metric and randomization method shown indicate the percentage of 1000 runs using that randomization method that exceeded or was equal to the performance of the actual classifier.

[0225] In addition to the above analyses, the actual classifier was compared to 1000 runs of the algorithm where different training/test sets were chosen but the correct sample labels were retained. Empirically derived p-values were also computed to compare the actual classifier to the 1000 runs of the algorithm (see Supplemental Table 6). These data indicate that the actual classifier was derived using a representative training and test set.

SUPPLEMENTAL TABLE 6

	Accur	sd(Accur)	p-value	Sens	sd(Sens)	p-value	Spec	sd(Spec)	p-value	AUC	sd(AUC)	p-value
Actual Classifier	0.827			0.8			0.844			0.897		
1000 Runs	0.784	0.054	0.283	0.719	0.104	0.245	0.83	0.06	0.407	0.836	0.053	0.108

[0226] Supplemental Table 6 above shows a comparison of actual classifier to 1000 runs of the algorithm with different training/test sets.

[0227] Finally, these 1000 runs of the algorithm were also compared to 1000 runs were the class labels of different training sets were randomized in the same way as described above. Empirically derived p-values were computed to compare 1000 runs to 1000 random runs (Supplemental Table 7).

without lung cancer, we examined the effect of different class-prediction and data preprocessing algorithms. We tested the 80-probesets in our classifier to generate predictive models using the Prediction Analysis of Microarrays (PAM) algorithm (Tibshirani R, et al., Diagnosis of multiple cancer types by shrunken centroids of gene expression. Proc Natl Acad Sci USA 2002; 99(10):6567-6572), and we also tested the ability of the WV algorithm to use probeset level data that

SUPPLEMENTAL TABLE 7

	Accur	sd(Accur)	p-value	Sens	sd(Sens)	p-value	Spec	sd(Spec)	p-value	AUC	sd(AUC)	p-value
1000 Runs	0.784	0.054		0.719	0.104		0.83	0.06		0.836	0.053	
1000 Random Runs	0.504	0.126	0.002	0.501	0.154	0.025	0.506	0.154	0.003	0.507	0.157	0.001

[0228] Supplemental Table 7 above shows comparison of runs of the algorithm using different training/test sets to runs where the class labels of the training sets were randomized (1000 runs were conducted).

[0229] The distribution of the prediction accuracies summarized in Supplemental Tables 6 and 7 is shown in FIG. 8.

[0230] Characteristics of the 1000 additional runs of the algorithm: The number of times a sample in the test set was classified correctly and its average prediction strength was computed across the 1000 runs of the algorithm. The average prediction strength when a sample was classified correctly was 0.54 for cancers and 0.61 for no cancers, and the average prediction strength when a sample was misclassified was 0.31 for cancer and 0.37 for no cancers. The slightly higher prediction strength for smokers without cancer is reflective of the fact that predictors have a slightly higher specificity on average. Supplemental FIG. 3 shows that samples that are consistently classified correctly or classified incorrectly are classified with higher confidence higher average prediction strength). Interestingly, 64% of the samples that are consistently classified incorrectly (incorrect greater than 95% of the time, n=22 samples) are samples from smokers that do not currently have a final diagnosis of cancer. This significantly higher false-positive rate might potentially reflect the ability of the biomarker to predict future cancer occurrence or might indicate that a subset of smokers with a cancer-predisposing gene-expression phenotype are protected from developing cancer through some unknown mechanism.

[0231] In order to further assess the stability of the biomarker gene committee, the number of times the 80-predictive probesets used in the biomarker were selected in each of the 1000 runs (Supplemental Table 6) was examined. (See FIG. 10A) The majority of the 80-biomarker probesets were chosen frequently over the 1000 runs (37 probesets were present in over 800 runs, and 58 of the probesets were present in over half of the runs). For purposes of comparison, when the cancer status of the training set samples are randomized over 1000 runs (Supplemental Table 7), the most frequently selected probeset is chosen 66 times, and the average is 7.3 times. (See FIG. 10B).

[0232] Comparison of RMA vs. MAS 5.0 and weighted voting vs. PAM: To evaluate the robustness of our ability to use airway gene expression to classify smokers with and

had been derived using the MAS 5.0 algorithm instead of RMA. The accuracy of the classifier was similar when microarray data was preprocessed in MAS 5.0 and when the PAM class prediction algorithm was used (see Supplemental Table 8)

SUPPLEMENTAL TABLE 8

	Accuracy	Sensitivity	Specificity
WV - RMA data	82.69%	80%	84.38%
PAM - RMA data	86.54%	90%	84.38%
WV - MAS5 data	82.69%	80%	84.38%
PAM - MAS5 data	86.54%	95%	81.25%

[0233] Supplemental Table 8 shows a comparison of accuracy, sensitivity and specificity for our 80 probeset classifier on the 52 sample test set using alternative microarray data preprocessing algorithms and class prediction algorithms.

[0234] Prediction strength: The Weighted voting algorithm predicts a sample's class by summing the votes each gene on the class prediction committee gives to one class versus the other. The level of confidence with which a prediction is made is captured by the Prediction Strength (PS) and is calculated as follows:

$$PS = \frac{V_{winning} - V_{losing}}{V_{winning} + V_{losing}}$$

[0235] $V_{winning}$ refers to the total gene committee votes for the winning class and V_{losing} refers to the total gene committee votes for the losing class. Since $V_{winning}$ is always greater than V_{losing}, PS confidence varies from 0 (arbitrary) to 1 (complete confidence) for any given sample.

[0236] In our test set, the average PS for our gene profile's correct predictions (43/52 test samples) is 0.73 (+/-0.27), while the average PS for the incorrect predictions (9/52 test samples) is much lower: 0.49 (+/-0.33; p<z; Student T-Test). This result shows that, on average, the Weighted Voting algorithm is more confident when it is making a correct prediction than when it is making an incorrect prediction. This result holds across 1000 different training/test set pairs (FIG. 11):

[0237] Cancer cell type: To determine if the tumor cell subtype affects the expression of genes that distinguish airway epithelium from smokers with and without lung cancer, Principal Component Analysis (PCA) was performed on the gene-expression measurements for the 80 probesets in our predictor and all of the airway epithelium samples from patients with lung cancer (FIG. 12). Gene expression measurements were Z(0,1) normalized prior to PCA. There is no apparent separation of the samples with regard to cancer subtype.

Link to Lung Cancer Tissue Microarray Dataset

[0238] Preprocessing of Bhattacharjee data: The 254 CEL files from HgU95Av2 arrays used by Bhattacharjee et al. (Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci USA 2001; 98(24):13790-13795) were downloaded from the MIT Broad Institute's database available through internet (broad.mit.edu/mpr/lung). RMA-derived expression measurements were computed using these CEL files as described above. Technical replicates were filtered by choosing one at random to represent each patient. In addition, arrays from carcinoid samples and patients who were indicated to have never smoked were excluded, leaving 151 samples. The z-score quality filter described above was applied to this data set resulting in 128 samples for further analysis (88 adenocarcinomas, 3 small cell, 20 squamous, and 17 normal lung samples).

[0239] Probesets were mapped between the HGU133A array and HGU95Av2 array using Chip Comparer at the Duke University's database available through the world wide web at tenero.duhs.duke.edu/genearray/perl/chip/chipcomparer. pl. 64 probesets on the HGU95Av2 array mapped to the 80-predictive probesets. The 64 probesets on the HGU95Av2 correspond to 48 out of the 80 predictive probesets (32/80 predictive probesets have no clear corresponding probe on the HGU95Av2 array).

the 128 samples and 1000 randomly chosen sets of 64 probesets. The mean difference between normal and malignant samples was calculated based on the projected values for principal component 1 for the actual 64 probesets and for each of the 1000 random sets of 64 probesets. The mean difference between normal and malignant from the 1000 random gene sets was used to generate a null distribution. The observed difference between the normal and malignant samples using the biomarker probesets was greater than the difference observed using randomly selected genes (p=0.026 for mean difference and p=0.034 for median difference).

[0241] The second analysis involved using the weighted voted algorithm to predict the class of 108 samples in the Bhattacharjee dataset using the 64 probesets and a training set of 10 randomly chosen normal tissues and 10 randomly chosen tumor tissues. The samples were classified with 89.8% accuracy, 89.1% sensitivity, and 100% specificity (see Supplemental Table 9 below, Single Run). To examine the significance of these results, the weighted voted algorithm was re-run using two types of data randomization. First, the class labels of the training set of 20 samples were permuted and the algorithm, including gene selection, was re-run 1000 times (referred to in Supplemental Table 9 as Random 1). The second randomization involved permuting the class labels of the training set of 20 samples and re-running the algorithm 1000 times keeping the list of 64-probesets constant (referred to in Supplemental Table 9 as Random 2). In the above two types of randomization, the class labels were permuted such that half the samples were correctly labeled. The p-value for each metric and randomization method shown indicate the percentage of 1000 runs using that randomization method that exceeded or were equal to the performance of the actual classifier. Genes that distinguish between large airway epithelial cells from smokers with and without cancer are significantly better able to distinguish lung cancer tissue from normal lung tissue than any random run where the class labels of the training set are randomized.

SUPPLEMENTAL TABLE 9

	Accur	sd(Accur)	p-value	Sens	sd(Sens)	p-value	Spec	sd(Spec)	p-value	AUC	sd(AUC)	p-value
Single Run	0.898			0.891			1			0.984		
Random 1	0.486	0.218	0.007	0.486	0.217	0.008	0.484	0.352	0.131	0.481	0.324	0.005
Random 2	0.498	0.206	0.009	0.499	0.201	0.011	0.494	0.344	0.114	0.494	0.324	0.014

[0240] Analyses of Bhattacharjee dataset: In order to explore the expression of genes that we identified as distinguishing large airway epithelial cells from smokers with and without lung cancer in lung tumors profiled by Bhattacharjee, two different analyses were performed. Principal component analysis was used to organize the 128 Bhattacharjee samples according to the expression of the 64 mapped probesets. Principal component analysis was conducted in R using the package prcomp on the z-score normalized 128 samples by 64 probeset matrix. The normal and malignant samples in the Bhattacharjee dataset appear to separate along principal component 1 (see FIG. 17). To assess the significance of this result, the principal component analysis was repeated using

[0242] Supplemental Table 9 above shows results of a comparison between the predictions of the Bhattacharjee samples using the 64 probesets that map to a subset of the 80-predictive probesets and random runs (explained above). Accur=Accuracy, Sens=Sensitivity, Spec=Specificity, AUC=area under the curve, and sd=standard deviation.

[0243] Real Time PCR: Quantitative RT-PCR analysis was used to confirm the differential expression of a seven genes from our classifier. Primer sequences for the candidate genes and a housekeeping gene, the 18S ribosomal subunit, were designed with PRIMER EXPRESS® software (Applied Biosystems) (see Supplemental Table 10).

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SUPPLEMENTAL TABLE 10

Candidate and housekeeping gene primers for real time PCR assay

Gene Symbol	Affy ID	Ensembl ID	Name	Forward Primer	Reverse Primer
BACH2	215907_a t	ENSG000001 12182	BTB and CNC homology 1, basic leucine zipper transcription factor 2	TGGCAAAACCGCATCTCT AC (SEQ ID No. 1)	ACCACCATGCCCAGCTAA (SEQ ID No. 2)
DCLRE1 C	219678_x _at	ENSG000001 52457	DNA cross-link repair 1C	GCACTTTGAGGTGGGCAA T (SEQ ID No. 3)	CCAGGCTGGTGTCGAACTC (SEQ ID No. 4)
DUOX1	215800_a t	ENSG000001 37857	dual oxidase 1	GAGAGAAAGCAAAGGAG TGAACTT (SEQ ID No. 5)	CATGTGAGTCTGAAATTACAGCATT (SEQ ID No. 6)
FOS	209189_a t	ENSG000001 70345	v-fos FBJ murine osteosarcoma viral oncogene homolog	AGATGTAGCAAAACGCAT GGA (SEQ ID No. 7)	CTCTGAAGTGTCACTGGGAACA (SEQ ID No. 8)
IL8	211506_s _at	ENSG000001 69429	interleukin 8	GCTAAAGAACTTAGATGT CAGTGCAT (SEQ ID No. 9)	GGTGGAAAGGTTTGGAGTATGTC (SEQ ID No. 10)
RAB1A	207791_s _at	ENSG000001 38069	RAB1A, member RAS oncogene family	GGAGCCCATGGCATCATA (SEQ ID No. 11)	TTGAAGGACTCCTGATCTGTCA (SEQ ID No. 12)

[0244] Primer sequences for five other housekeeping genes (HPRT1, SDHA, YWHAZ, GAPDH, and TBP) were adopted from Vandesompele et al. (Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002; 3(7)). RNA samples (1 µg of the RNA used in the microarray experiment) were treated with DNAfree (Ambion, Austin, Tex.), according to the manufacturer's protocol, to remove contaminating genomic DNA. Total RNA was reverse-transcribed using random hexamers (Applied Biosystems) and Super-Script II reverse transcriptase (Invitrogen). The resulting first-strand cDNA was diluted with nuclease-free water (Ambion) to 5 ng/µl. PCR amplification mixtures (25 µl) contained 10 ng template cDNA, 12.5 µl of 2×SYBR Green PCR master mix (Applied Biosystems) and 300 nM forward and reverse primers. Forty cycles of amplification and data acquisition were carried out in an Applied Biosystems 7500 Real Time PCR System. Threshold determinations were automatically performed by Sequence Detection Software (version 1.2.3). (Applied Biosystems) for each reaction. All real-time PCR experiments were carried out in triplicate on each sample (6 samples total; 3 smokers with lung cancer and 3 smokers without lung cancer).

[0245] Data analysis was performed using the geNorm tool (Id.). Three genes (YWHAZ, GAPDH, and TBP) were determined to be the most stable housekeeping genes and were used to normalize all samples. Data from the QRT-PCR for 7 genes along with the microarray results for these genes is shown in FIG. 13.

REFERENCES

[0246] (1) Parkin D M, et al., CA Cancer J Clin 2005; 55(2):74-108.

[0247] (2) Shields P G. Ann Oncol 1999; 10 Suppl 5:7-11.

[0248] (3) Hirsch F R, et al., Clin Cancer Res 2001; 7(1): 5-22.

[0249] (4) Jett J R. Clin Cancer Res 2005; 11(13 Pt 2):4988s-4992s.

[0250] (5) Macredmond R, et al., Thorax 2006; 61(1):54-56.

[0251] (6) Postmus P E. Chest 2005; 128(1):16-18.

[0252] (7) Mazzone P, et al., Clin Chest Med 2002; 23(1): 137-58, ix.

[0253] (8) Schreiber G, and McCrory D C. Chest 2003; 123(1 Suppl):115S-128S.

[0254] (9) Janssen-Heijnen M L, et al., Epidemiology 2001; 12(2):256-258.

[0255] (10) Salomaa E R, et al., Chest 2005; 128(4):2282-2288.

[0256] (11) Auerbach 0, et al., Arch Environ Health 1970; 21(6):754-768.

[0257] (12) Powell C A, et al., Clin Cancer Res 1999; 5(8): 2025-2034.

[0258] (13) Wistuba I I, et al., J Natl Cancer Inst 1997; 89(18):1366-1373.

[0259] (14) Franklin W A, et al., J Clin Invest 1997; 100(8): 2133-2137.

[0260] (15) Guo M, et al., Clin Cancer Res 2004; 10(15): 5131-5136.

[0261] (16) Miyazu Y M, et al., Cancer Res 2005; 65(21): 9623-9627.

[0262] (17) Spira A, et al., Proc Natl Acad Sci USA 2004; 101(27):10143-10148.

[0263] (18) Bolstad B M, et al., Bioinformatics 2003; 19(2):185-193.

[0264] (19) Golub T R, et al, Science 1999; 286(5439):531-537.

[0265] (20) Tibshirani R, et al., Proc Natl Acad Sci USA 2002; 99(10):6567-6572.

[0266] (21) Bhattacharjee A, et al., Proc Natl Acad Sci USA 2001; 98(24):13790-13795.

[0267] (22) Subramanian A, et al., Proc Nati Acad Sci USA 2005; 102(43):15545-15550.

[0268] (23) Simon R, et al., J Natl Cancer Inst 2003; 95(1):14-18.

[0269] (24) Cheng K W, et al., Cancer Res 2005; 65(7):2516-2519.

[0270] (25) Shimada K, et al., Br J Cancer 2005; 92(10):1915-1921.

[0271] (26) Xie K. Cytokine Growth Factor Rev 2001, 12(4):375-391.

[0272] (27) Campa D, et al., Cancer Epidemiol Biomarkers Prev 2005; 14(10):2457-2458.

[0273] (28) Arimura Y, et al., Anticancer Res. 24, 4051-4057. 2004.

[0274] (29) Coussens L M, and Werb Z. Nature 2002; 420 (6917):860-867.

[0275] (30) Godschalk R, et al., Carcinogenesis 2002; 23(12):2081-2086.

[0276] (31) Kamio T, et al., Blood 2003; 102(9):3317-3322

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20100035244A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

We claim:

1. A method of diagnosing lung cancer in an individual comprising the steps of:

a) measuring a biological sample comprising lung epithelial tissue from the individual for the expression of at least 20 gene transcripts from Table 6;

b) comparing the expression of the at least 20 gene transcripts to a control sample of those transcripts from individuals without cancer,

wherein increased expression of the gene transcripts as indicated by a negative score in the last column of Table 6 and/or decreased expression of the gene transcripts as indicated by a positive score in the last column of Table 6 is indicative of the individual having lung cancer.

2. The method of claim 1, wherein at least 40 gene transcripts are measured.

3. The method of claim 1, wherein at least 60 gene transcripts are measured.

4. The method of claim 1, wherein at least 70 gene transcripts are measured.

5. The method of claim 1, wherein the gene transcript measured is set forth in Table 5.

6. The method of claim 1, wherein the gene transcript measured is set forth in Table 7.

7. The method of claim 1, wherein the gene transcript measured is set forth in Table 1 wherein the measurement of the gene transcript relative to the control uses the third column of Table 1 setting forth direction of expression in lung cancer to determine if the individual has lung cancer.

8. The method of claim 7, wherein the transcript measured is at least Table 3.

9. The method of claim 7, wherein the transcript used is at least the transcripts set forth in Table 4.

10. A method of diagnosing a lung disease in an individual exposed to air pollutant comprising the steps of

a) measuring expression profile of a gene group in the sample from a test individual; and

b) comparing the expression profile of the test individual to an expression profile of a first control individual exposed to similar air pollutant who does not have the lung disease and the expression profile of a second control individual exposed to similar air pollutants who has the lung disease,

wherein similarity of the expression profile of the test individual with the expression profile of the first control rather than that of the second control is indicative of the test individual being not affected with the lung disease and wherein similarity of the expression profile of the test individual with the expression profile of the second control individual rather than that of the first control individual is indicative of the test individual being affected or at high risk of developing the lung disease.

11. The method of claim 7, wherein the gene group comprises at least 30, sequences of genes selected from the group consisting with GenBank identification Nos. NM_003335; NM_000918; NM_006430.1; NM_001416.1; NM_004090; NM_006406.1; NM_003001.2; NM_001319; NM_006545.1; NM_021145.1; NM_002437.1; NM_006286; NM_001003698///NM_001003699///NM_002955; NM_001123///NM_006721; NM_024824; NM_004935.1; NM_002853.1; NM_019067.1; NM_024917.1; NM_020979.1; NM_005597.1; NM_007031.1; NM_009590.1; NM_020217.1; NM_025026.1; NM_014709.1; NM_014896.1; AF010144; NM_005374.1; NM_001696; NM_005494///NM_058246; NM_006534///NM_181659; NM_006368; NM_002268///NM_032771; NM_014033; NM_016138; NM_007048///NM_194441; NM_006694, NM_000051///NM_138292///NM_138293; NM_000410///NM_139002///NM_139003///NM_139004///NM_139005///NM_139006///NM_139007///NM_139008///NM_139009///NM_139010///NM_139011; NM_004691; NM_012070///NM_139321///NM_139322; NM_006095; AI632181; AW024467; NM_021814; NM_005547.1; NM_203458; NM_015547///NM_147161; AB007958.1; NM_207488; NM_005809///NM_181737///NM_181738; NM_016248///NM_144490; AK022213.1; NM_005708;

NM_207102; AK023895; NM_144606///NM_144997; NM_018530; AK021474; U43604.1; AU147017; AF222691.1; NM_015116; NM_001005375///NM_001005785///NM_001005786///NM_004081///NM_020363///NM_020364///NM_020420; AC004692; NM_001014; NM_000585///NM_172174///NM_172175; NM_054020///NM_172095///NM_172096///NM_172097; BE466926; NM_018011; NM_024077; NM_012394; NM_019011///NM_207111///NM_207116; NM_017646; NM_021800; NM_016049; NM_014395; NM_014336; NM_018097; NM_019014; NM_024804; NM_018260; NM_018118; NM_014128; NM_024084; NM_005294; AF077053; NM_138387; NM_024531; NM_000693; NM_018509; NM_033128; NM_020706; A1523613; and NM_014884.

12. The method of claim 1, wherein the gene group comprises sequences of genes selected from the group consisting of genes with GenBank identification Nos. NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; AB014576.1; BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; AI683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF198444.1.

13. The method of claim 1, wherein the gene group comprises sequences of genes selected from the group consisting of genes with GenBank identification Nos. NM_007062.1; NM_001281.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; NM_002268///NM_032771; NM_007048///NM_194441; NM_006694; U85430.1; NM_004691; AB014576.1; BF218804; BE467941; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AL080112.1; AW971983; AM683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_021971.1; NM_014128.1; AA133341; and AF198444.1

14. The method of claim 1, wherein the group comprises sequences of genes selected from the group consisting of genes with GenBank or Unigene identification Nos. NM_030757.1; R83000; AK021571.1; NM_014182.1; NM_17932.1; U85430.1; A1683552; BC002642.1; AW024467; NM_030972.1; BC021135.1; AL161952.1; AK026565.1; AK023783.1; BF218804; NM_001281.1; NM_024006.1; AK023843.1; BC001602.1; BC034707.1; BC064619.1; AY280502.1; BC059387.1; AF135421.1; BC061522.1; L76200.1; U50532.1; BC006547.2; BC008797.2; BC000807.1; AL080112.1; BC033718.1; BC046176.1///BC038443.1; NM_000346.1; BC008710.1; Hs.288575 (UNIGENE ID); AF020591.1; BC000423.2; BC002503.2; BC008710.1; BC009185.2; Hs.528304(UNIGENE ID); U50532.1; BC013923.2; BC031091; NM_007062; Hs.249591 Unigene ID); BC075839.1/// BC073760.1; BC072436.1///BC004560.2; BC001016.2; Hs.286261 (Unigene ID); AF348514.1; BC005023.1; BC066337.1///BC058736.1///BC050555.1; Hs.216623

(Unigene D); BC072400.1; BC041073.1; U43965.1; BC021258.2; BC016057.1; BC016713.1///BC014535.1/// AF237771.1; BC000360.2; BC007455.2; BC000701.2; BC010067.2; BC023528.2///BC047680.1; BC064957.1; Hs.156701 (Unigene ID); BC030619.2; BC008710.1; U43965.1; BC066329.1; Hs.438867 (Unigene ID); BC035025.2///BC050330.1; BC023976.2; BC074852.2 μl BC074851.2; Hs.445885 (Unigene ID); BC008591.2/// BC050440.1 μl; BC048096.1; AF365931.1; AF257099.1; and BC028912.1.

15. The method of claim 1, wherein the group comprises sequences of genes selected from the group consisting of genes with GenBank or Unigene identification Nos: NM_003335; NM_001319; NM_021145.1; NM_001003698///NM_001003699///; NM_002955; NM_002853.1; NM_019067.1; NM_024917.1; NM_020979.1; NM_005597.1; NM_007031.1; NM_009590.1; NM_020217.1; NM_025026.1; NM_014709.1; NM_014896.1; AF010144; NM_005374.1; NM_006534///NM_181659; NM_014033; NM_016138; NM_007048///NM_194441; NM_000051///NM_138292/NM_138293; NM_000410/// NM_139002///NM_139003///NM_139004///NM_139005///NM_139006///NM_139007///NM_139008/// NM_139009///NM_139010///NM_139011; NM_012070///NM_139321///NM_139322; NM_006095; AI632181; AW024467; NM_021814; NM_005547.1; NM_203458; NM_015547///NM_147161; AB007958.1; NM_207488; NM_005809///NM_181737///NM_181738; NM_016248///NM_144490; AK022213.1; NM_005708; NM_207102; AK023895; NM_144606 μl NM_144997; NM_018530; AK021474; U43604.1; AU147017; AF222691.1; NM_015116; NM_001005375///NM_001005785///NM_001005786///NM_00408///NM_020363///NM_020364///NM_020420; AC004692; NM_001014; NM_000585///NM_172174///NM_172175; NM_054020///NM_172095///NM_172096 μl NM_172097; BE466926; NM_018011; NM_024077; NM_019011///NM_207111///NM_207116; NM_017646; NM_014395; NM_014336; NM_018097; NM_019014; NM_024804; NM_018260; NM_018118; NM_014128; NM_024084; NM_005294; AF077053; NM_000693; NM_033128; NM_020706; AI523613; and NM_014884, and wherein decrease in expression of at least 5 of these genes is indicative of the individual being affected with a lung disease.

16. The method of claim 1, wherein the group comprises sequences of genes selected from the group consisting of genes with GenBank or Unigene identification Nos NM_030757.1; R83000; AK021571.1; NM_17932.1; U85430.1; A1683552; BC002642.1; AW024467; NM_030972.1; BC021135.1; AL161952.1; AK026565.1; AK023783.1; BF218804; AK023843.1; BC001602.1; BC034707.1; BC064619.1; AY280502.1; BC059387.1; BC061522.1; U50532.1; BC006547.2; BC008797.2; BC000807.1; AL080112.1; BC033718.1///BC046176.1///; BC038443.1; Hs.288575 (UNIGENE ID); AF020591.1; BC002503.2; BC009185.2; Hs.528304 (UNIGENE ID); U50532.1; BC013923.2; BC031091; Hs.249591 (Unigene ID); Hs.286261 (Unigene ID); AF348514.1; BC066337.1/// BC058736.1///BC050555.1; Hs.216623 (Unigene ID); BC072400.1; BC041073.1; U43965.1; BC021258.2; BC016057.1; BC016713.1///BC014535.1///AF23771.1; BC000701.2; BC010067.2; Hs.156701 (Unigene ID);

BC030619.2; U43965.1; Hs.438867 (Unigene ID); BC035025.2///BC050330.1; BC074852.2///BC074851.2; Hs.445885 (Unigene ID); AF365931.1; and AF257099.1, and wherein decrease in expression of at least 5 of these genes is indicative of the individual being affected with a lung disease.

17. The method of claim **1**, wherein the group comprises sequences of genes selected from the group consisting of genes with GenBank or Unigene identification Nos BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; AI683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF198444.1, and wherein decrease in expression of at least 5 of these genes is indicative of the individual being affected with a lung disease.

18. The method of claim **1**, wherein the group comprises sequences of genes selected from the group consisting of genes with GenBank or Unigene identification Nos NM_000918; NM_006430.1; NM_001416.1; NM_004090; NM_006406.1; NM_003001.2; NM_006545.1; NM_002437.1; NM_006286; NM_001123///NM_006721; NM_024824; NM_004935.1; NM_001696; NM_005494///NM_058246; NM_006368; NM_002268///NM_032771; NM_006694; NM_004691; NM_012394; NM_021800; NM_016049; NM_138387; NM_024531; and NM_018509, and wherein increase in expression of at least 5 of these genes is indicative of the individual being affected with a lung disease.

19. The method of claim **1**, wherein the group comprises sequences of genes selected from the group consisting of genes with GenBank or Unigene identification Nos NM_014182.1; NM_001281.1; NM_024006.1; AF135421.1; L76200.1; NM_000346.1; BC008710.1; BC000423.2; BC008710.1; NM_007062; BC075839.1///BC073760.1; BC072436.1///BC004560.2; BC001016.2; BC005023.1; BC000360.2; BC007455.2; BC023528.2///BC047680.1; BC064957.1; BC008710.1; BC066329.1; BC023976.2; BC008591.2///BC050440.1///BC048096.1; and BC028912.1, and wherein increase in expression of at least 5 of these genes is indicative of the individual being affected with a lung disease.

20. The method of claims **4-7**, wherein the group comprises sequences of genes selected from the group consisting of genes with GenBank or Unigene identification Nos NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; and AB014576.1, and wherein increase in expression of at least 5 of these genes is indicative of the individual being affected with a lung disease.

21. The method of claim **1**, wherein the group comprises 5-9 sequences of genes selected from group 1 and group 2, group 1 consisting of genes with GenBank or Unigene identification Nos. NM_003335; NM_001319; NM_021145.1; NM_001003698///NM_001003699///NM_002955; NM_002853.1; NM_019067.1; NM_024917.1; NM_020979.1; NM_005597.1; NM_007031.1; NM_009590.1; NM_020217.1; NM_025026.1;

NM_014709.1; NM_014896.1; AF010144; NM_005374.1; NM_006534///NM_181659; NM_014033; NM_016138; NM_007048///NM_194441; NM_000051///NM_138292///NM_138293; NM_000410///NM_139002///NM_139003///NM_139004///NM_139005///NM_139006///NM_139007///NM_139008///NM_139009///NM_139010///NM_139011; NM_012070///NM_139321///NM_139322; NM_006095; A1632181; AW024467; NM_021814; NM_005547.1; NM_203458; NM_015547///NM_147161; AB007958.1; NM_207488; NM_005809///NM_181737///NM_181738; NM_016248///NM_144490; AK022213.1; NM_005708; NM_207102; AK023895; NM_144606///NM_144997; NM_018530; AK021474; U43604.1; AU147017; AF222691.1; NM_015116; NM_001005375///NM_001005785///NM_001005786///NM_004081///NM_020363///NM_020364///NM_020420; AC004692; NM_001014; NM_000585///NM_172174///NM_172175; NM_054020///NM_172095///NM_172096///NM_172097; BE466926; NM_018011; NM_024077; NM_019011///NM_207111///NM_207116; NM_017646; NM_014395; NM_014336; NM_018097; NM_019014; NM_024804; NM_018260; NM_018118; NM_014128; NM_024084; NM_005294; AF077053; NM_000693; NM_033128; NM_020706; AI523613; and NM_014884, and group 2 consisting of genes with GenBank or Unigene identification Nos. NM_000918; NM_006430.1; NM_001416.1; NM_004090; NM_006406.1; NM_003001.2; NM_006545.1; NM_002437.1; NM_006286; NM_001123///NM_006721; NM_024824; NM_004935.1; NM_001696; NM_005494///NM_058246; NM_006368; NM_002268 µl NM_032771; NM_006694; NM_004691; NM_012394; NM_021800; NM_016049; NM_138387; NM_024531; and NM_018509, and a group of at least 20 genes selected from group 3 and group 4, group 3 consisting of genes with GenBank or Unigene identification Nos BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; A1683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF198444.1 and group 4 consisting of genes with GenBank or Unigene identification Nos. NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069.1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; and AB014576.1.

22. The method of claim **20**, wherein decrease in the expression of any one of the group 0.1 genes and increase in the expression of any one of the group 2 genes, and decrease of the group 3 genes and increase of the group 4 genes is indicative of the individual being affected with a lung disease.

23. The method of claim **1**, wherein the group comprises 5-9 sequences of genes selected from group 5 and group 6, group 5 consisting of genes with GenBank or Unigene identification Nos. NM_030757.1; R83000; AK021571.1; NM_17932.1; U85430.1; AI683552; BC002642.1; AW024467; NM_030972.1; BC021135.1; AL161952.1; AK026565.1; AK023783.1; BF218804; AK023843.1;

BC001602.1; BC034707.1; BC064619.1; AY280502.1; BC059387.1; BC061522.1; U50532.1; BC006547.2; BC008797.2; BC000807.1; AL080112.1; BC033718.1/// BC046176.1///; BC038443.1; Hs.288575 (UNIGENE ID); AF020591.1; BC002503.2; BC009185.2; Hs.528304(UNIGENE ID); U50532.1; BC013923.2; BC031091; Hs.249591 (Unigene ID); Hs.286261 (Unigene ID); AF348514.1; BC066337.1///BC058736.1///BC050555.1; Hs.216623 (Unigene ID); BC072400.1; BC041073.1; U43965.1; BC021258.2; BC016057.1; BC016713.1///BC014535.1/// AF237771.1; BC000701.2; BC010067.2; Hs.156701 (Unigene ID); BC030619.2; U43965.1; Hs.438867 (Unigene ID); BC035025.2///BC050330.1; BC074852.2///BC074851.2; Hs.445885 (Unigene ID); AF365931.1; and AF257099.1, and group 6 consisting of genes with GenBank or Unigene identification Nos. NM_014182.1; NM_001281.1; NM_024606.1; AF135421.1; L76200.1; NM_000346.1; BC008710.1; BC000423.2; BC008710.1; NM_007062; BC075839.1///BC073760.1; BC072436.1///BC004560.2; BC010016.2; BC005023.1; BC000360.2; BC007455.2; BC023528.2///BC047680.1; BC064957.1; BC008710.1; BC066329.1; BC023976.2; BC008591.2 μl BC050440.1///; BC048096.1; and BC028912.1, and a group of at least 20 genes selected from group 3 and group 4, group 3 consisting of genes with GenBank or Unigene identification Nos BF218804; AK022494.1; AA114843; BE467941; NM_003541.1; R83000; AL161952.1; AK023843.1; AK021571.1; AK023783.1; AU147182; AL080112.1; AW971983; A1683552; NM_024006.1; AK026565.1; NM_014182.1; NM_021800.1; NM_016049.1; NM_019023.1; NM_021971.1; NM_014128.1; AK025651.1; AA133341; and AF 198444.1 and group 4 consisting of genes with GenBank or Unigene identification Nos. NM_007062.1; NM_001281.1; BC000120.1; NM_014255.1; BC002642.1; NM_000346.1; NM_006545.1; BG034328; NM_021822.1; NM_021069. 1; NM_019067.1; NM_017925.1; NM_017932.1; NM_030757.1; NM_030972.1; AF126181.1; U93240.1; U90552.1; AF151056.1; U85430.1; U51007.1; BC005969.1; NM_002271.1; AL566172; and AB014576.1, wherein decrease in the expression of the group 5 genes and increase in the expression of the group 6 genes, and decrease the group 3 genes and increase of the group 4 genes is indicative of the individual being affected with a lung disease.

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