FIG. 1A

100

	Maternal blood (1st trimester)		CVS		Term placenta		Maternal plasma (1st trimester)		Maternal plasma (3rd trimester)		Maternal plasma (post-delivery)	
	watson	crick	watson	crick	watson	crick	watson	crick	watson	crick	watson	crick
read counts	547522712	547522712	422873853	422873853	637537170	637537170	4567226854	4567226854	1289337674	1289337674	1277615263	1277615263
mappability (%)	45.08	44.39	44.22	43.83	46.70	44.40	29.82	29.48	35.81	35.39	37.86	37.40
mappable reads	246808492	243029086	186985998	185346026	297735873	283050215	1361958504	1346252254	461659066	456249836	483697803	477778078
guous rate (%)[a]	6.72	6.83	5.84	5.89	5.26	5.54	6.35	6.43	5.86	5.93	5.98	6.05
cation rate (%)[b]	6.60	5.86	9.52	9.27	32.94	30.39	64.66	64.41	25.70	25.18	41.01	40.56
e genome depth after val of ambiguous and	11.29	11.19	8.36	8.31	9.43	9.25	25.40	25.29	16.95	16.85	14.08	14.00
ıambda conversion rate (%)[c]	99.98	99.98	99.93	99.94	99.95	99.96	99.96	99.97	99.96	99.96	99.96	99.96
Genomic C seq M count[d]	268498966	265662985	190603545	188460370	226378366	223722865	640697054	635295175	456206420	453569856	397672185	395159659
Genomic C seq U count	4484594136	4449649557	4232382985	4201356735	4968445023	4908568234	1.1699E+10	1.1641E+10	8576357118	8538105685	6896711473	6865335640
Genomic C coverage (%)	66.45	66.39	62.66	62.55	67.49	67.40	80.48	80.46	69.84	69.76	67.68	67.61
Genomic C seq depth	8.14	8.07	7.57	7.51	8.89	8.78	21.12	21.01	15.46	15.39	12.48	12.43
Genomic C methyltation density (%	5.65	5.63	4.31	4.29	4.36	4.36	5.19	5.17	5.05	5.04	5.45	5.44
CpG seq M count	265203963	262504347	183242316	181245307	213757505	211325313	627866879	622753476	443226176	440725023	389810638	387417582
CpG seq U count	104163657	103975011	145623817	148498340	147248261	146617702	310000675	308133594	206357402	205477045	142858625	142271154
CpG coverage (%)	85.17	85.12	81.02	80.92	84.23	84.15	92.05	92.02	86.13	86.06	84.93	84.86
CpG seq depth	13.12	13.01	11.68	11.71	12.82	12.71	33.30	33.05	23.06	22.94	18.91	18.81
CpG methyltation density (%)	71.80	71.63	55.72	54.97	59.21	59.04	66.95	66.90	68.23	68.20	73.18	73.14
NonCpG seq M count	3279043	3147047	7286715	7162226	12502145	12301418	12818574	12532920	12979169	12843942	7859475	7740641
NonCpG seq U count	4302393851	4285094041	3979826287	3971182968	4560807406	4537834784	1.1356E+10	1.1308E+10	8367572669	8330532598	6749182596	6719112682
NonCpG coverage (%)	0.13	0.13	0.12	0.12	0.13	0.13	0.16	0.16	0.14	0.14	0.13	0.13
NonCpG seq depth	1.52	1.52	1.41	1.41	1.62	1.61	4.02	4.00	2.96	2.95	2.39	2.38
NonCpG methylation density (%)	0.08	0.07	0.18	0.18	0.27	0.27	0.11	0.11	0.15	0.15	0.12	0.12
CHG seq M count	1029734	1000287	2100977	2071015	3223342	3176751	3766562	3691190	3625562	3592617	2347392	2314323
CHG seq U count	1345439602	1341393096	1217982855	1216609371	1362682765	1357568840	3416559203	3404225988	2519517702	2509439947	2036338055	2028059603
CHG coverage (%)	77.90	77.82	73.26	73.13	77.50	77.38	88.16	88.12	79.68	79.59	77.84	77.75
CHG seq depth	10.89	10.85	9.86	9.85	11.04	11.00	27.65	27.55	20.40	20.32	16.48	16.42
CHG methylation density (%)	0.08	0.07	0.17	0.17	0.24	0.23	0.11	0.11	0.14	0.14	0.12	0.11
CHH seq M count	2249309	2146760	5185738	5091211	9278803	9124667	9052012	8841730	9353607	9251325	5512083	5426318
CHH seq U count	2956954249	2943700945	2761843432	2754573597	3198124641	3180265944	7939543368	7903584596	5848054967	5821092651	4712844541	4691053079
CHH coverage (%)	61.96	61.90	58.43	58.32	63.54	63.45	77.53	77.52	65.96	65.89	63.65	63.58
CHH seq depth	6.84	6.81	6.40	6.38	7.42	7.38	18.38	18.30	13.55	13.48	10.91	10.86
CHH methylation density (%)	0.08	0.07	0.19	0.18	0.29	0.29	0.11	0.11	0.16	0.16	0.12	0.12

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	Adult male blood		Adult female blood		Adult male plasma		Adult female plasma	
	Watson	Crick	Watson	Crick	Watson	Crick	Watson	Crick
Raw read counts	196544792	196544792	197387755	197387755	179688600	179688600	141199439	141199439
Total mappability (%)	43.52	42.97	44.94	44.35	22.47	22.29	18.98	18.79
Total mappable reads	85526774	84462496	88699242	87542953	40380938	40048964	26795699	26537762
Ambiguous rate (%)[a]	6.05	6.13	6.29	6.37	6.09	6.14	6.29	6.35
Duplication rate (%)[b]	1.99	1.73	1.88	1.53	7.89	7.64	11.71	11.47
Whole genome depth after removal of ambiguous and	4.13	4.09	4.28	4.24	1.83	1.82	1.16	1.15
lambda conversion rate (%)[c]	99.96	99.97	99.97	99.97	99.96	99.97	99.96	99.97
Genomic C seq M count[d]	127154246	125487793	124983462	123238608	55049489	54630306	37403328	37048480
Genomic C seq U count	2047971831	2026731827	2058153525	2039985644	938186429	933377753	598496591	594974253
Genomic C coverage (%)	51.66	51.55	55.17	55.06	43.76	43.66	38.39	38.28
Genomic C seq depth	3.72	3.68	3.74	3.70	1.70	1.69	1.09	1.08
Genomic C methyltation density (%	5.85	5.83	5.72	5.70	5.54	5.53	5.88	5.86
CpG seq M count	125161042	123578850	123020928	121374309	54155037	53756413	36788819	36453865
CpG seq U count	49564325	49936003	47562383	47812716	21930864	21830555	13971713	13867179
CpG coverage (%)	73.37	73.25	76.24	76.12	63.93	63.80	57.87	57.77
CpG seq depth	6.20	6.16	6.06	6.01	2.70	2.68	1.80	1.79
CpG methyltation density (%)	71.63	71.22	72.12	71.74	71.18	71.12	72.48	72.44
NonCpG seq M count	1979410	1899750	1951160	1856642	894139	873671	614025	594289
NonCpG seq U count	1960312392	1950307695	1976031265	1966232257	915375235	910910490	583173346	580050464
NonCpG coverage (%)	0.10	0.10	0.11	0.11	0.08	0.08	0.07	0.07
NonCpG seq depth	0.69	0.69	0.70	0.70	0.32	0.32	0.21	0.21
NonCpG methylation density (%)	0.10	0.10	0.10	0.09	0.10	0.10	0.11	0.10
CHG seq M count	618974	598717	594336	573203	279945	272311	191120	186135
CHG seq U count	612387336	610126996	607312325	605093927	283916067	282826050	180374803	179612404
CHG coverage (%)	63.24	63.11	66.75	66.62	54.69	54.56	48.57	48.45
CHG seq depth	4.96	4.94	4.91	4.90	2.30	2.29	1.46	1.45
CHG methylation density (%)	0.10	0.10	0.10	0.09	0.10	0.10	0.11	0.10
CHH seq M count	1360436	1301033	1356824	1283439	614194	601360	422905	408154
CHH seq U count	1347925056	1340180699	1368718940	1361138330	631459168	628084440	402798543	400438060
CHH coverage (%)	46.93	46.83	50.48	50.38	39.32	39.22	34.20	34.11
CHH seq depth	3.12	3.10	3.17	3.15	1.46	1.45	0.93	0.93
CHH methylation density (%)	0.10	0.10	0.10	0.09	0.10	0.10	0.10	0.10

FIG. 1A (Cont.)

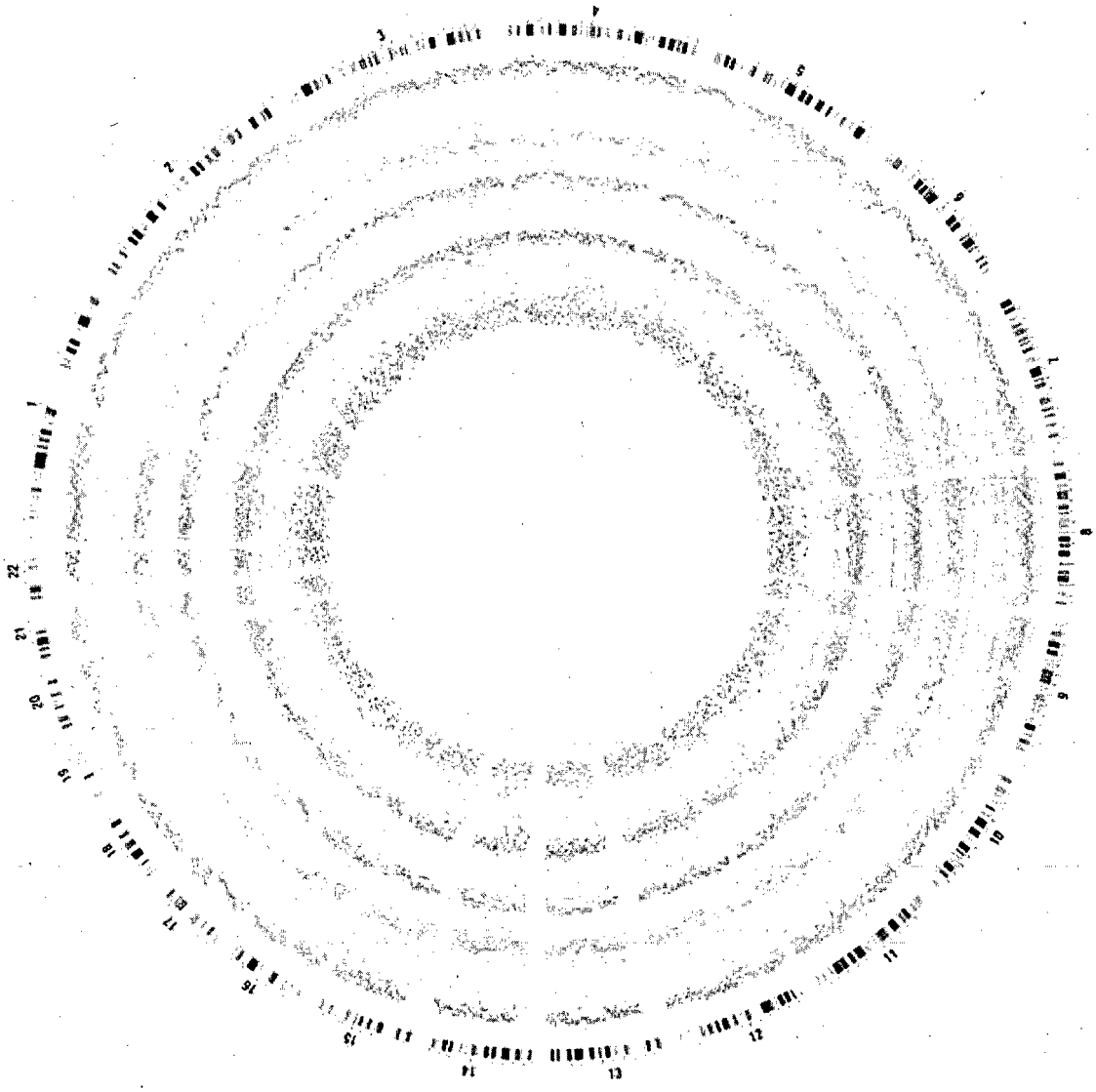


FIG. 1B

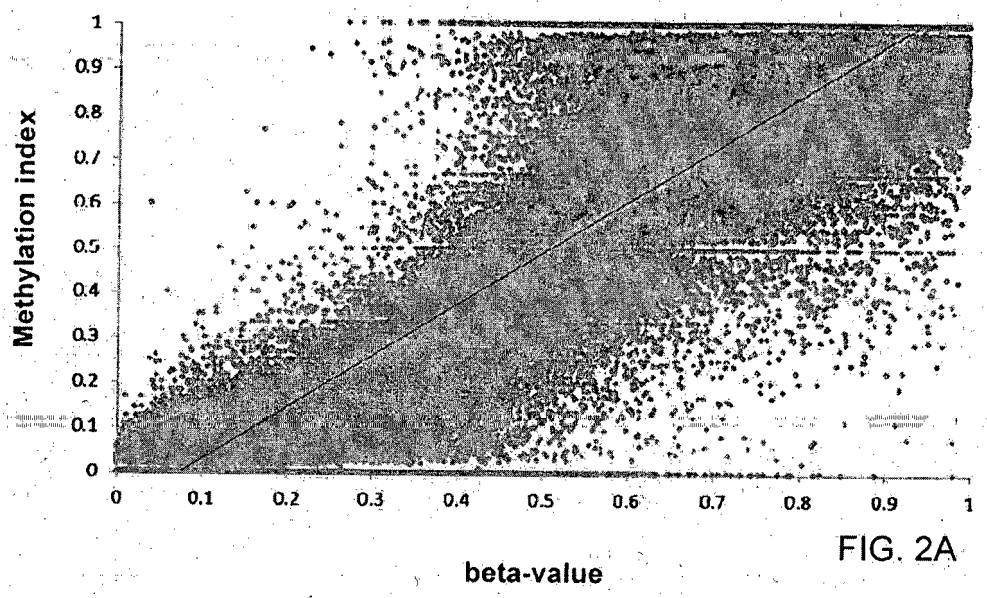


FIG. 2A

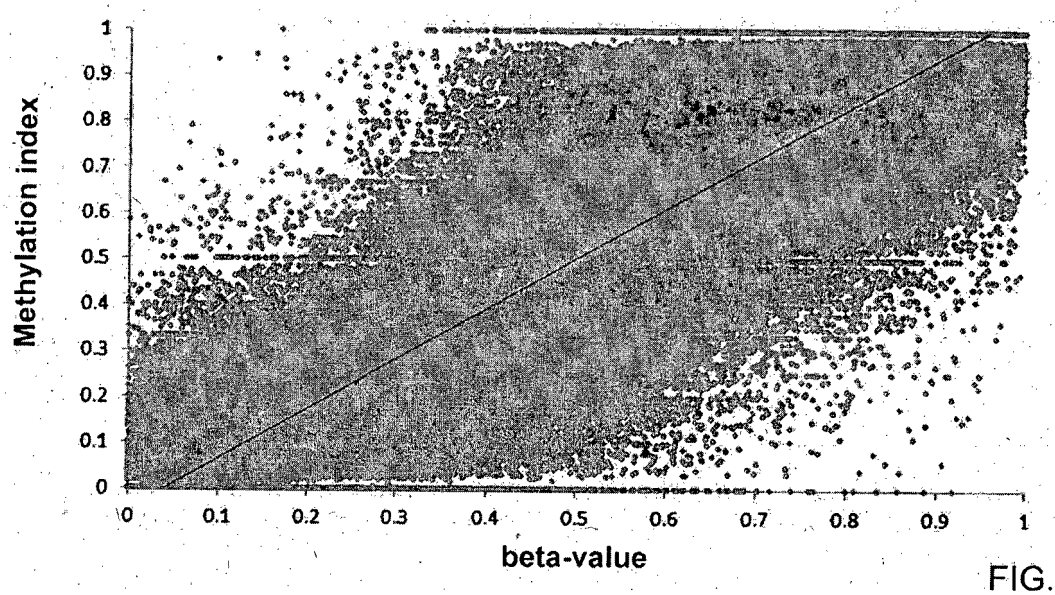


FIG. 2B

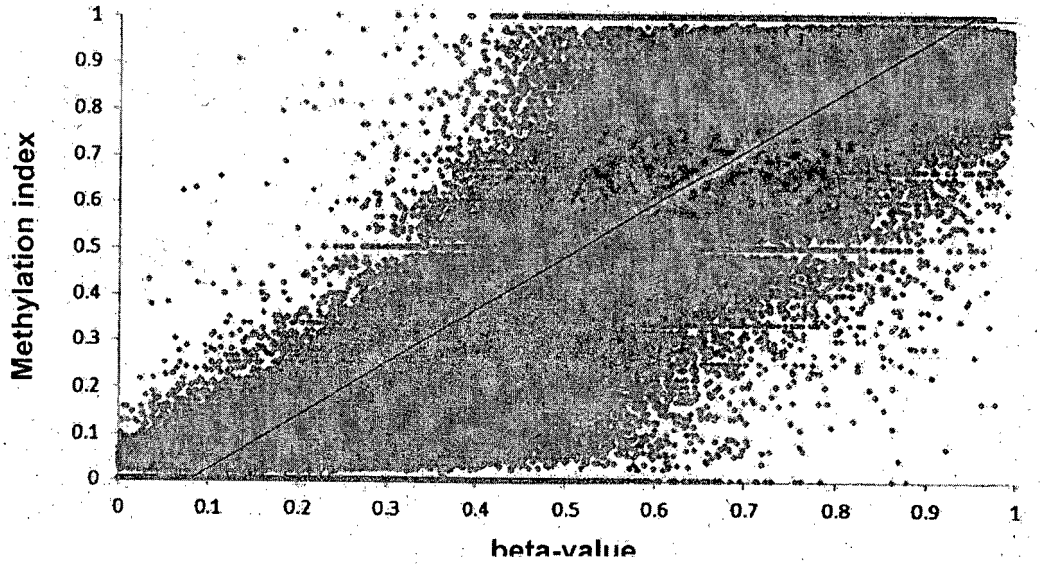


FIG. 2C

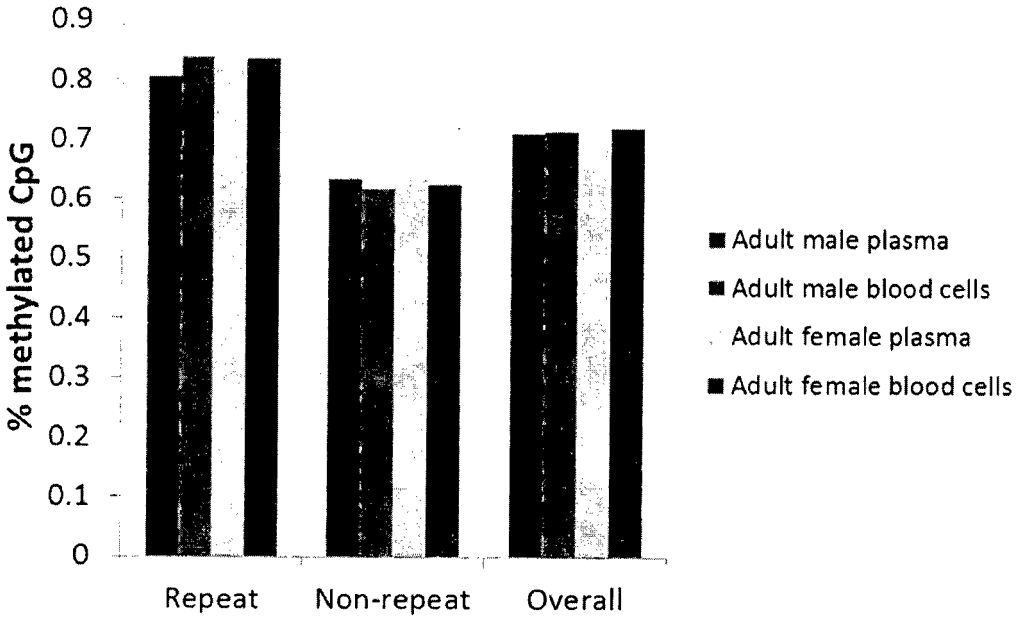

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
% methylated CpG
Repeat
Non-repeat
Overall
Adult male plasma
Adult male blood cells
Adult female plasma
Adult female blood cells

FIG. 3A

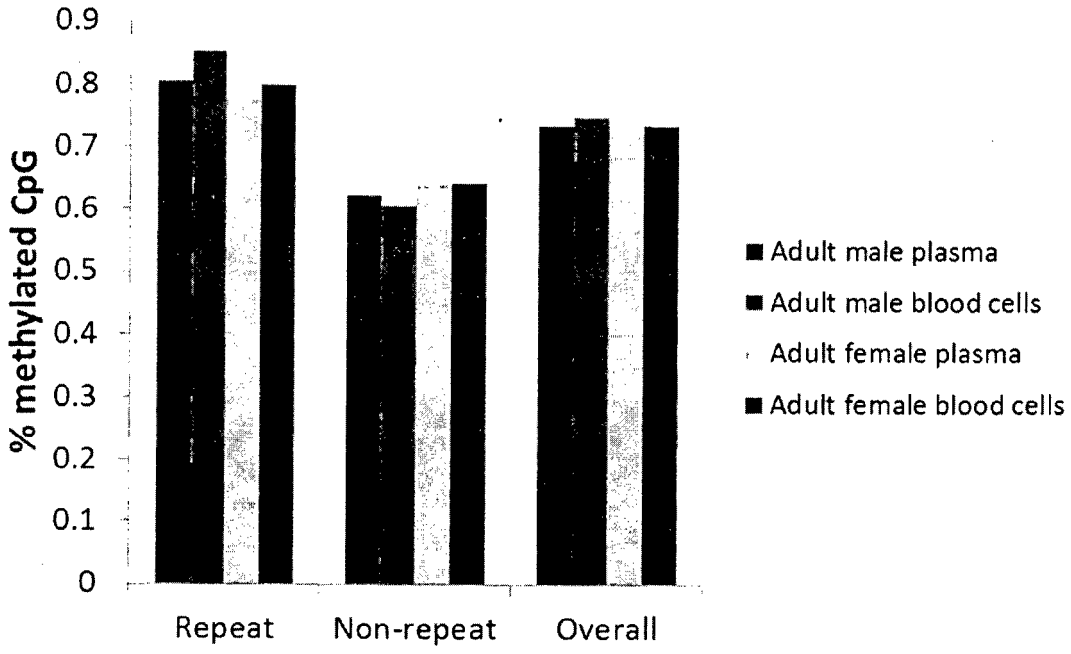

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
% methylated CpG
Repeat
Non-repeat
Overall
Adult male plasma
Adult male blood cells
Adult female plasma
Adult female blood cells

FIG. 3B

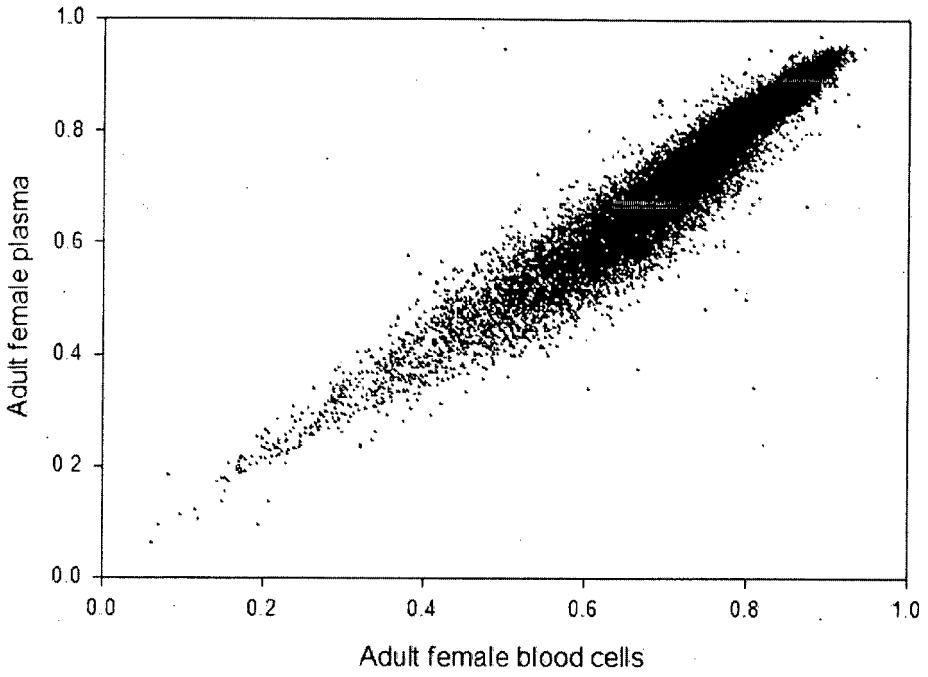

1.0
0.8
0.6
0.4
0.2
0.0
Adult female plasma
0.0
0.2
0.4
0.6
0.8
1.0
Adult female blood cells

FIG. 4A

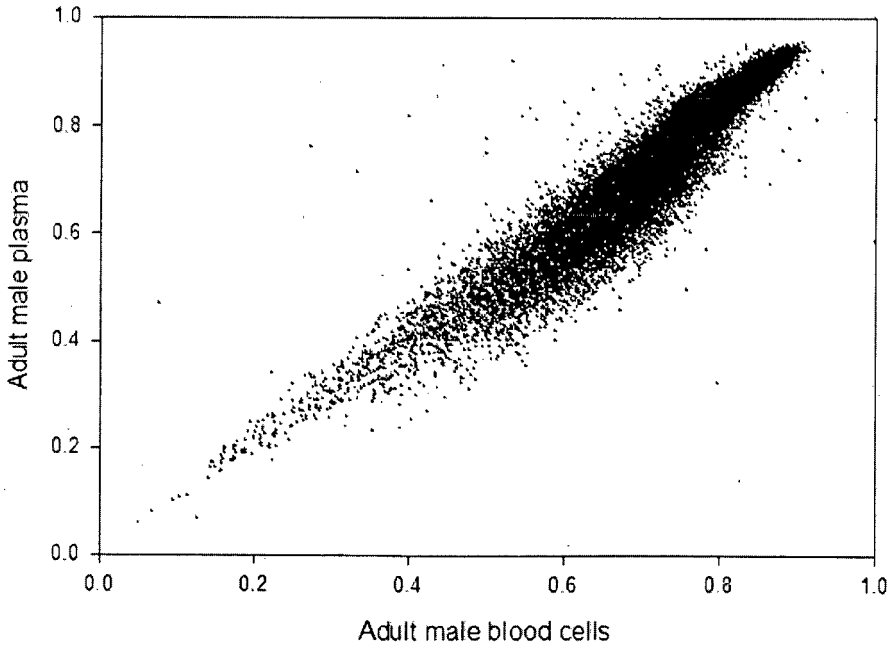

1.0
0.8
0.6
0.4
0.2
0.0
Adult male plasma
0.0
0.2
0.4
0.6
0.8
1.0
Adult male blood cells

FIG. 4B

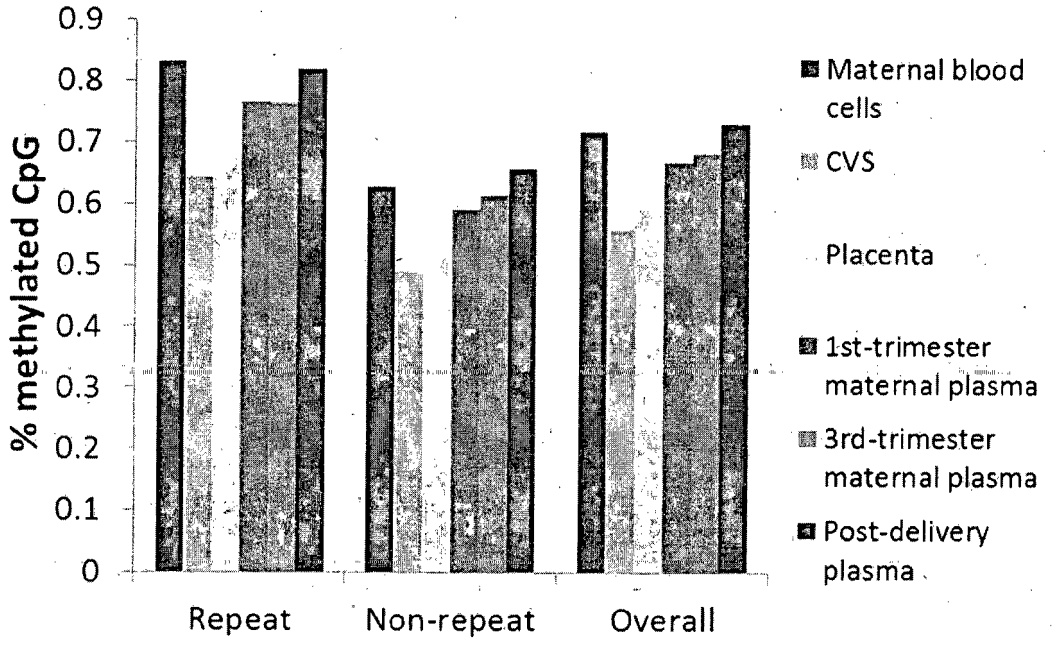

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
% methylated CpG
Repeat
Non-repeat
Overall
Maternal blood cells
CVS
Placenta
1st-trimester maternal plasma
3rd-trimester maternal plasma
Post-delivery plasma

FIG. 5A

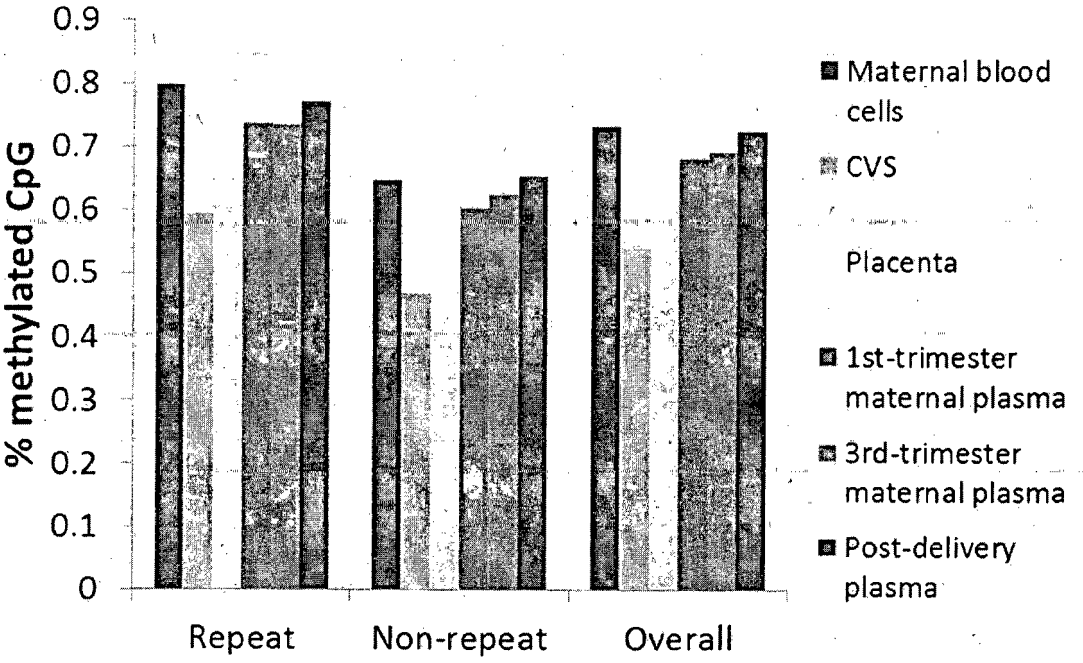

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
% methylated CpG
Repeat
Non-repeat
Overall
Maternal blood cells
CVS
Placenta
1st-trimester maternal plasma
3rd-trimester maternal plasma
Post-delivery plasma

FIG. 5B

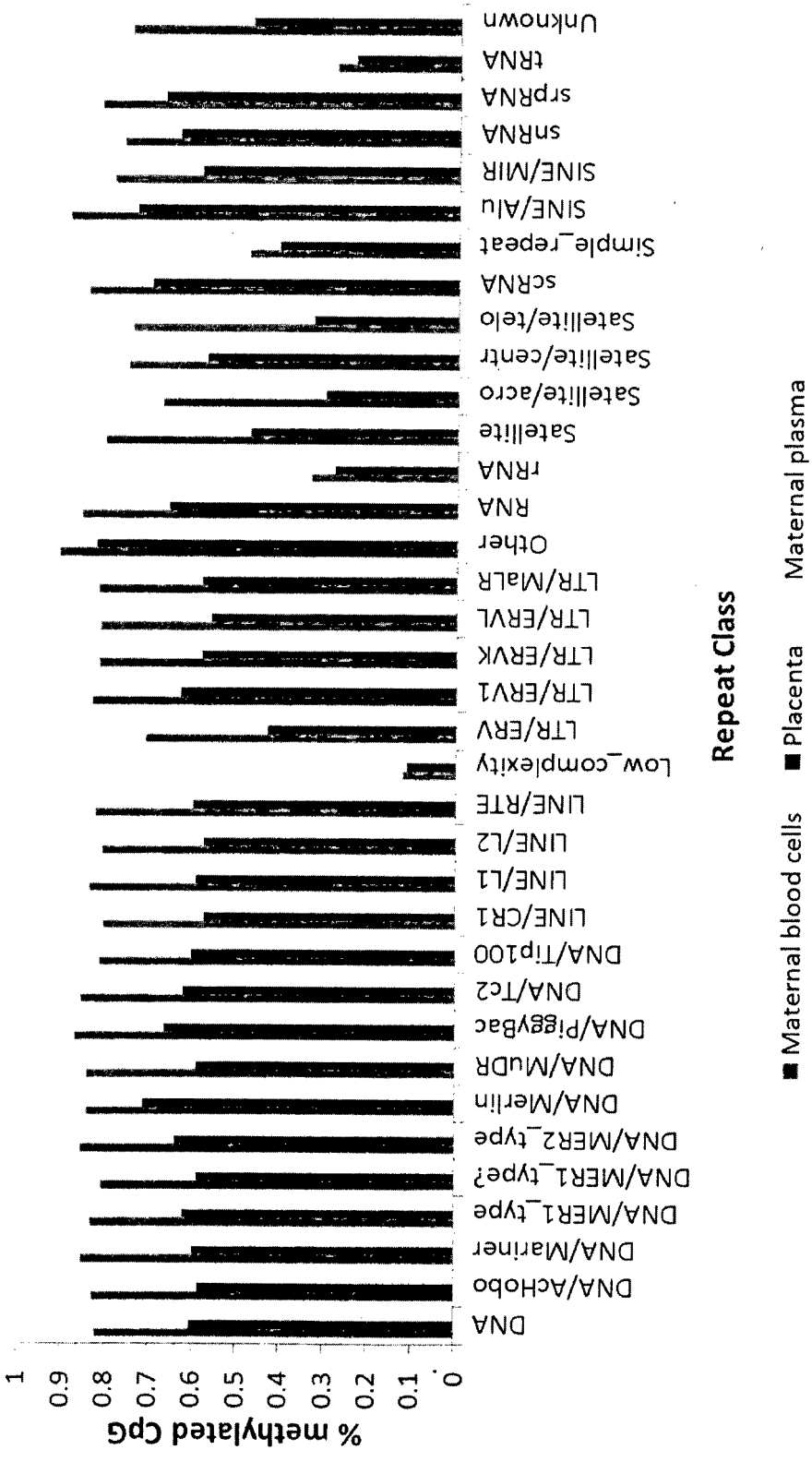

% methylated CpG
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
DNA
DNA/AcHobo
DNA/Mariner
DNA/MER1_type
DNA/MER1_type?
DNA/MER2_type
DNA/Merlin
DNA/MuDR
DNA/PiggyBac
DNA/Tc2
DNA/Tip100
LINE/CR1
LINE/L1
LINE/L2
LINE/RTE
low_complexity
LTR/ERV
LTR/ERV1
LTR/ERVK
LTR/ERVL
LTR/MaLR
Other
RNA
rRNA
Satellite
Satellite/acro
Satellite/centr
Satellite/telo
scRNA
Simple_repeat
SINE/Alu
SINE/MIR
snRNA
srpRNA
tRNA
Unknown
Repeat Class
Maternal blood cells
Placenta
Maternal plasma

FIG. 6

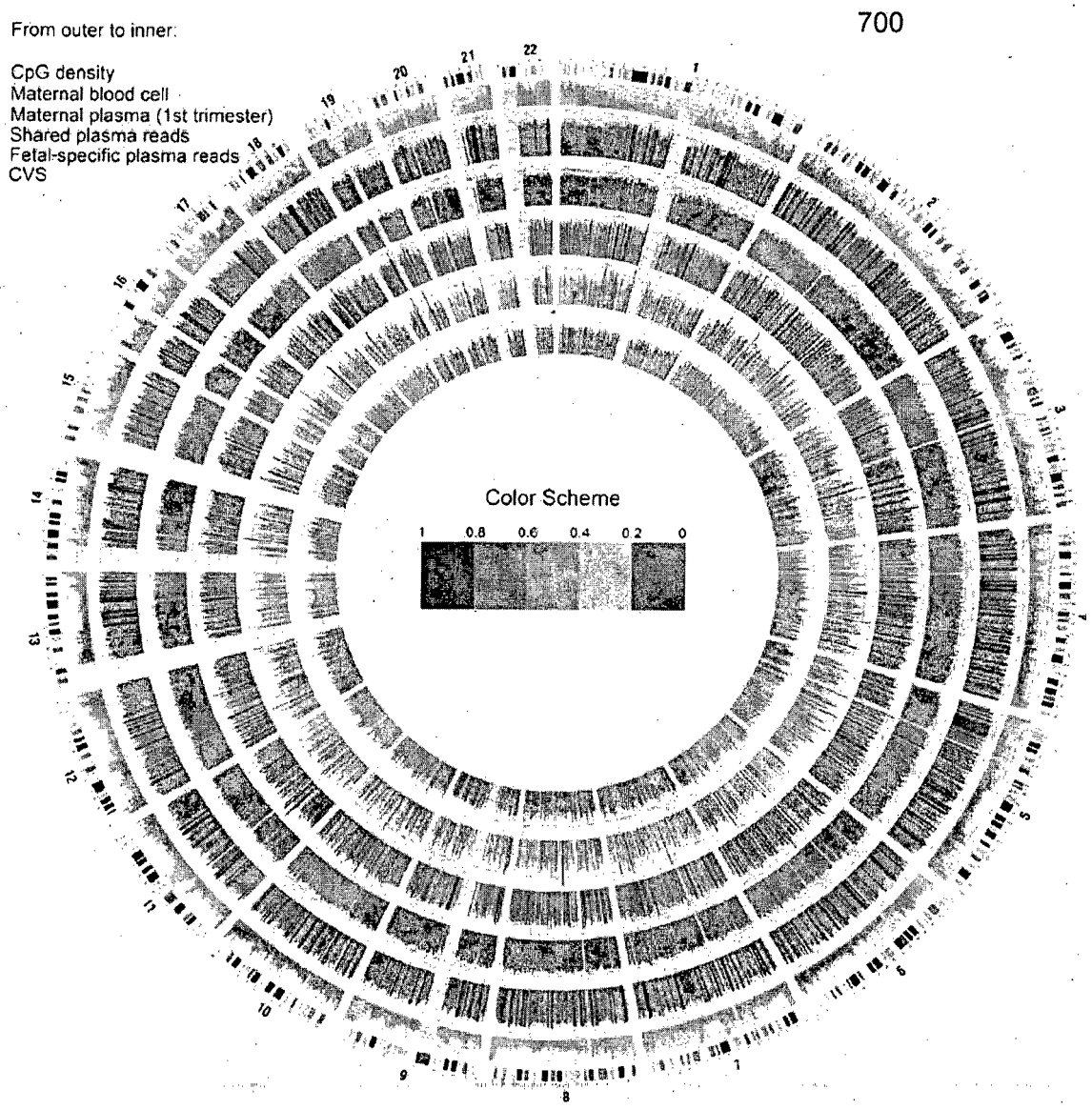

From outer to inner:
CpG density
Maternal blood cell
Maternal plasma (1st trimester)
Shared plasma reads
Fetal-specific plasma reads
CVS
700
Color Scheme
1
0.8
0.6
0.4
0.2
0

FIG. 7A

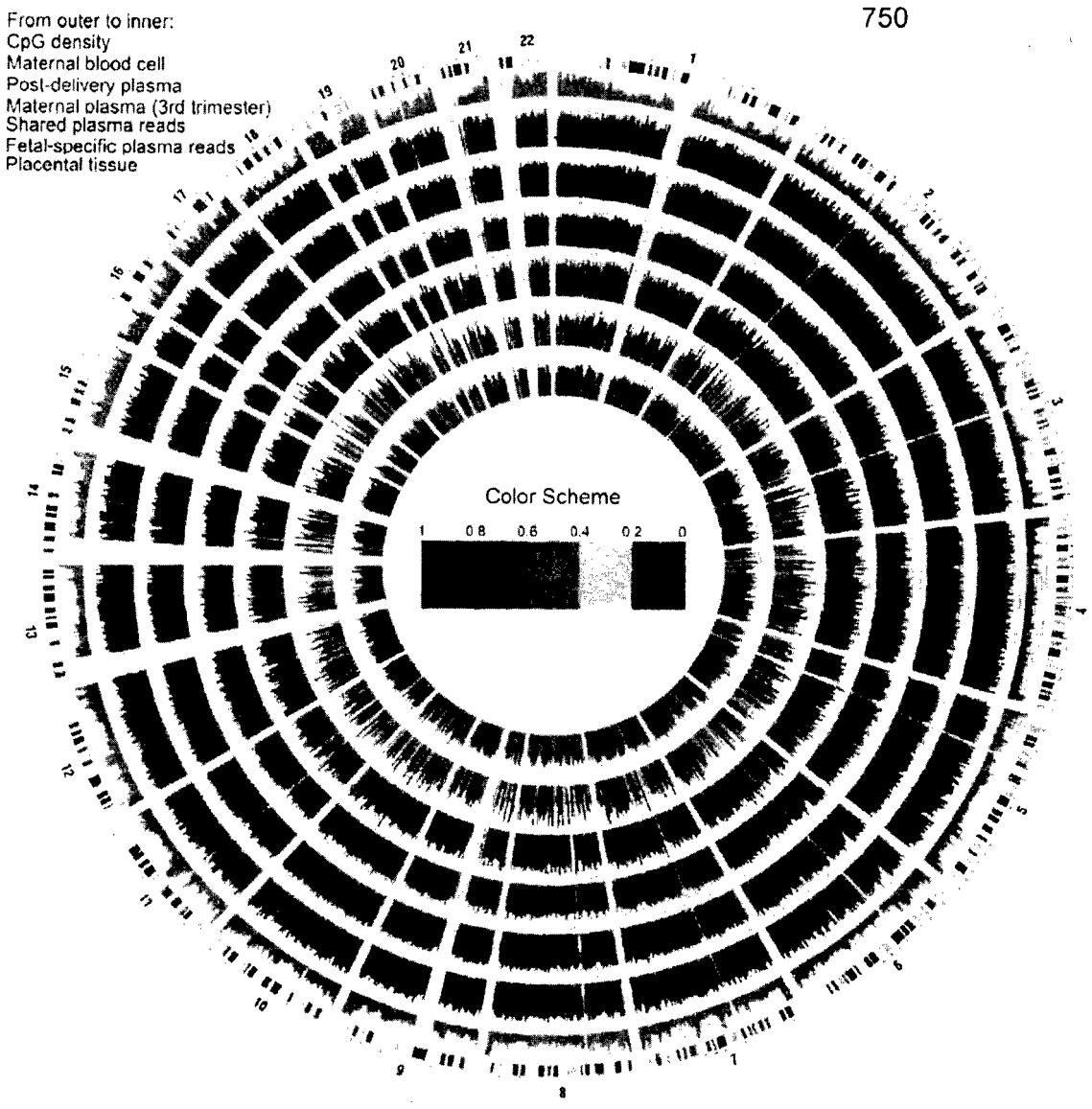

From outer to inner:
CpG density
Maternal blood cell
Post-delivery plasma
Maternal plasma (3rd trimester)
Shared plasma reads
Fetal-specific plasma reads
Placental tissue
750
Color Scheme
1
0.8
0.6
0.4
0.2
0

FIG. 7B

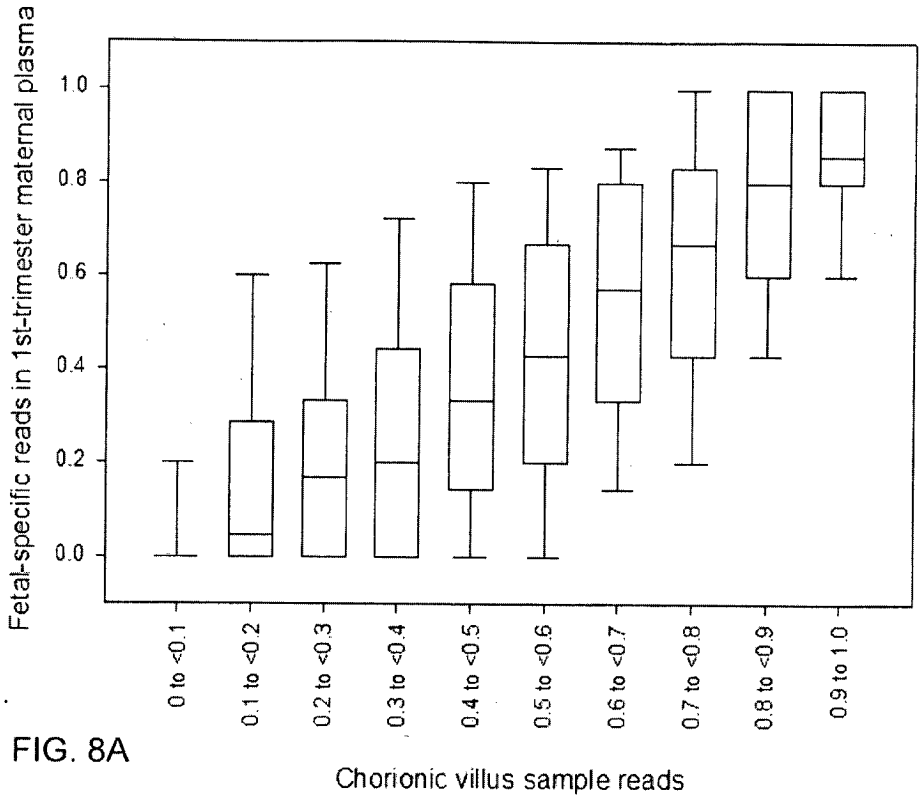

Fetal-specific reads in 1st-trimester maternal plasma
1.0
0.8
0.6
0.4
0.2
0.0
0 to <0.1
0.1 to <0.2
0.2 to <0.3
0.3 to <0.4
0.4 to <0.5
0.5 to <0.6
0.6 to <0.7
0.7 to <0.8
0.8 to <0.9
0.9 to 1.0
Chorionic villus sample reads

FIG. 8A

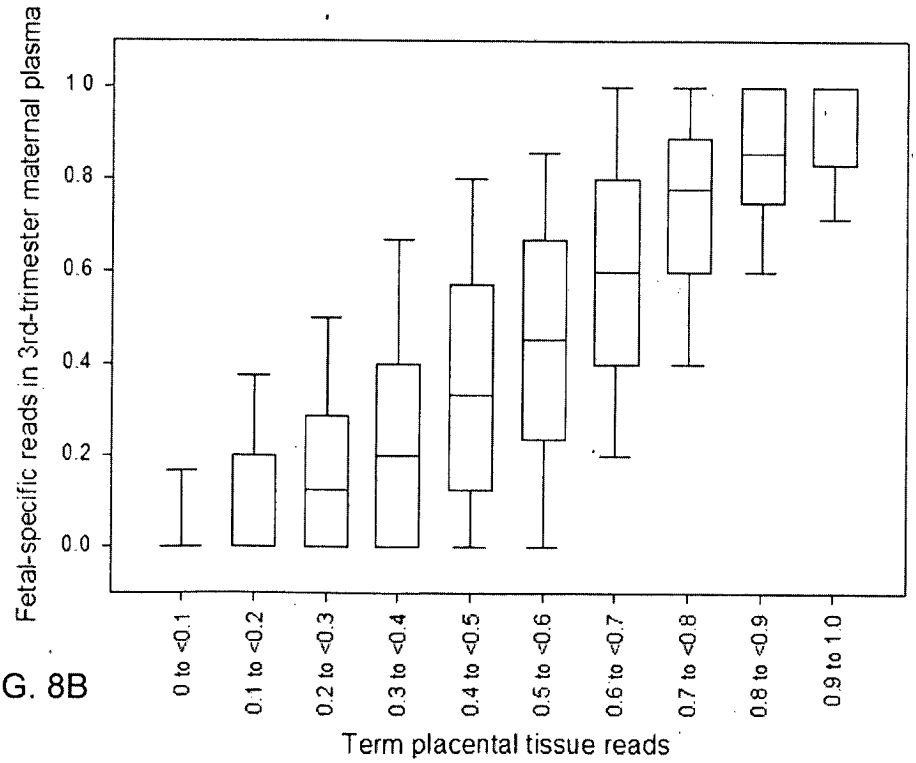

Fetal-specific reads in 3rd-trimester maternal plasma
1.0
0.8
0.6
0.4
0.2
0.0
0 to <0.1
0.1 to <0.2
0.2 to <0.3
0.3 to <0.4
0.4 to <0.5
0.5 to <0.6
0.6 to <0.7
0.7 to <0.8
0.8 to <0.9
0.9 to 1.0
Term placental tissue reads

FIG. 8B

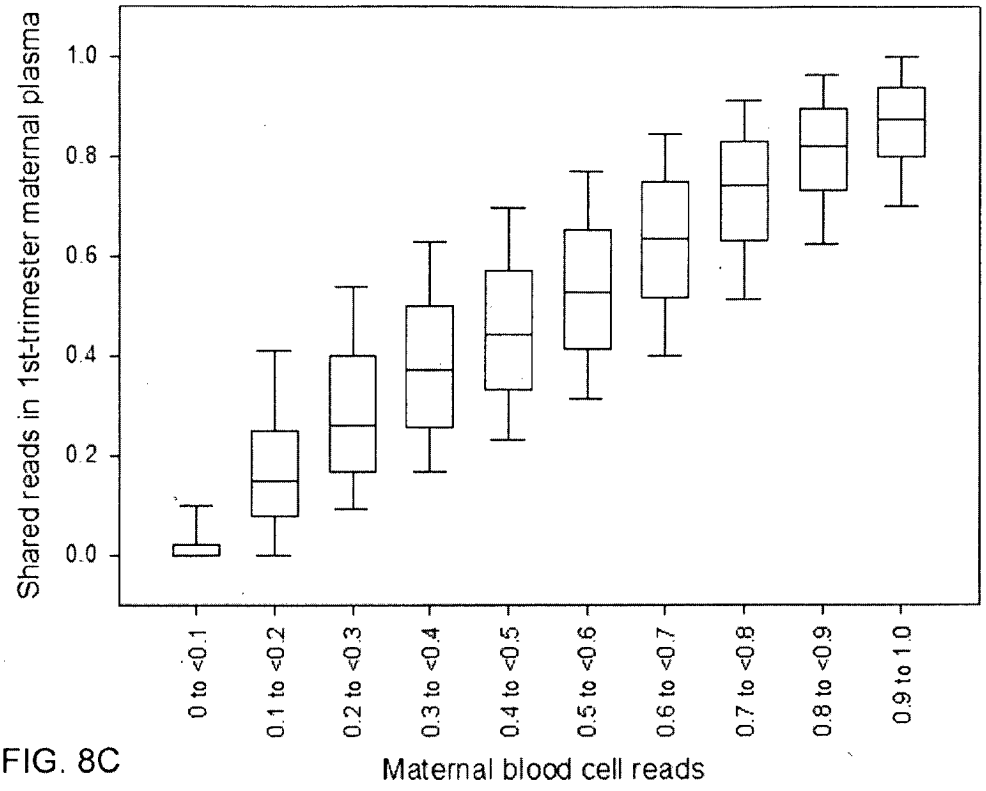

Shared reads in 1st-trimester maternal plasma
1.0
0.8
0.6
0.4
0.2
0.0
0 to <0.1
0.1 to <0.2
0.2 to <0.3
0.3 to <0.4
0.4 to <0.5
0.5 to <0.6
0.6 to <0.7
0.7 to <0.8
0.8 to <0.9
0.9 to 1.0
Maternal blood cell reads

FIG. 8C

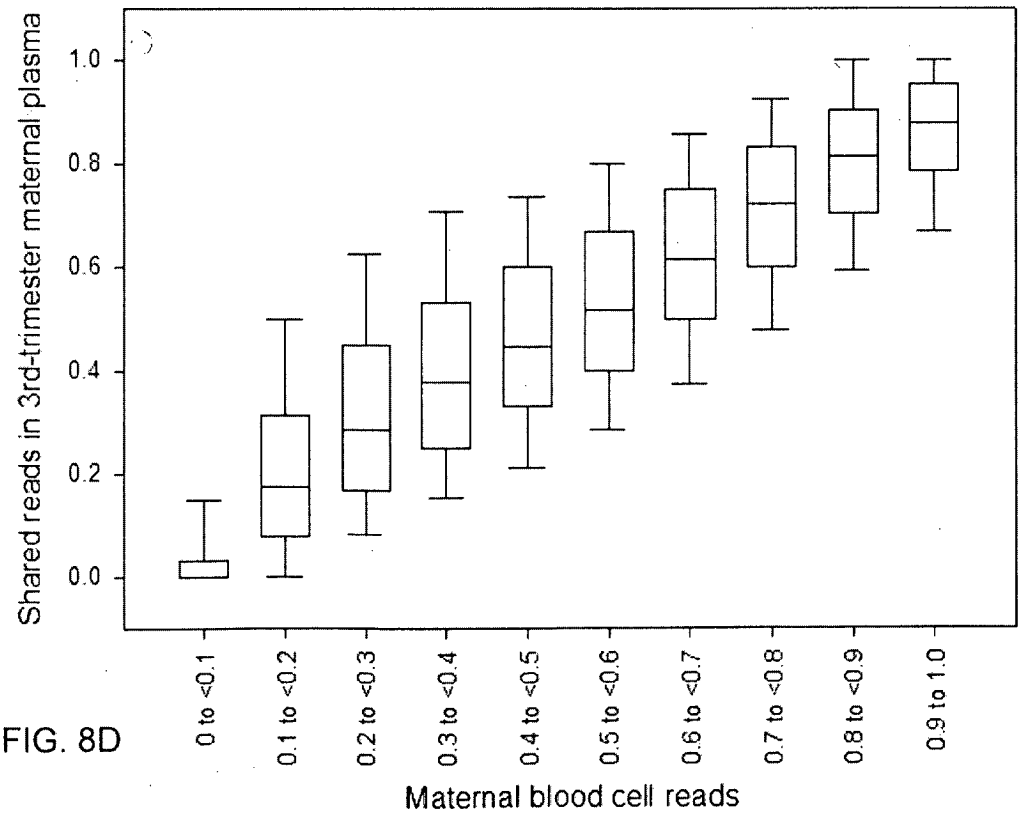

Shared reads in 3rd-trimester maternal plasma
1.0
0.8
0.6
0.4
0.2
0.0
0 to <0.1
0.1 to <0.2
0.2 to <0.3
0.3 to <0.4
0.4 to <0.5
0.5 to <0.6
0.6 to <0.7
0.7 to <0.8
0.8 to <0.9
0.9 to 1.0
Maternal blood cell reads

FIG. 8D

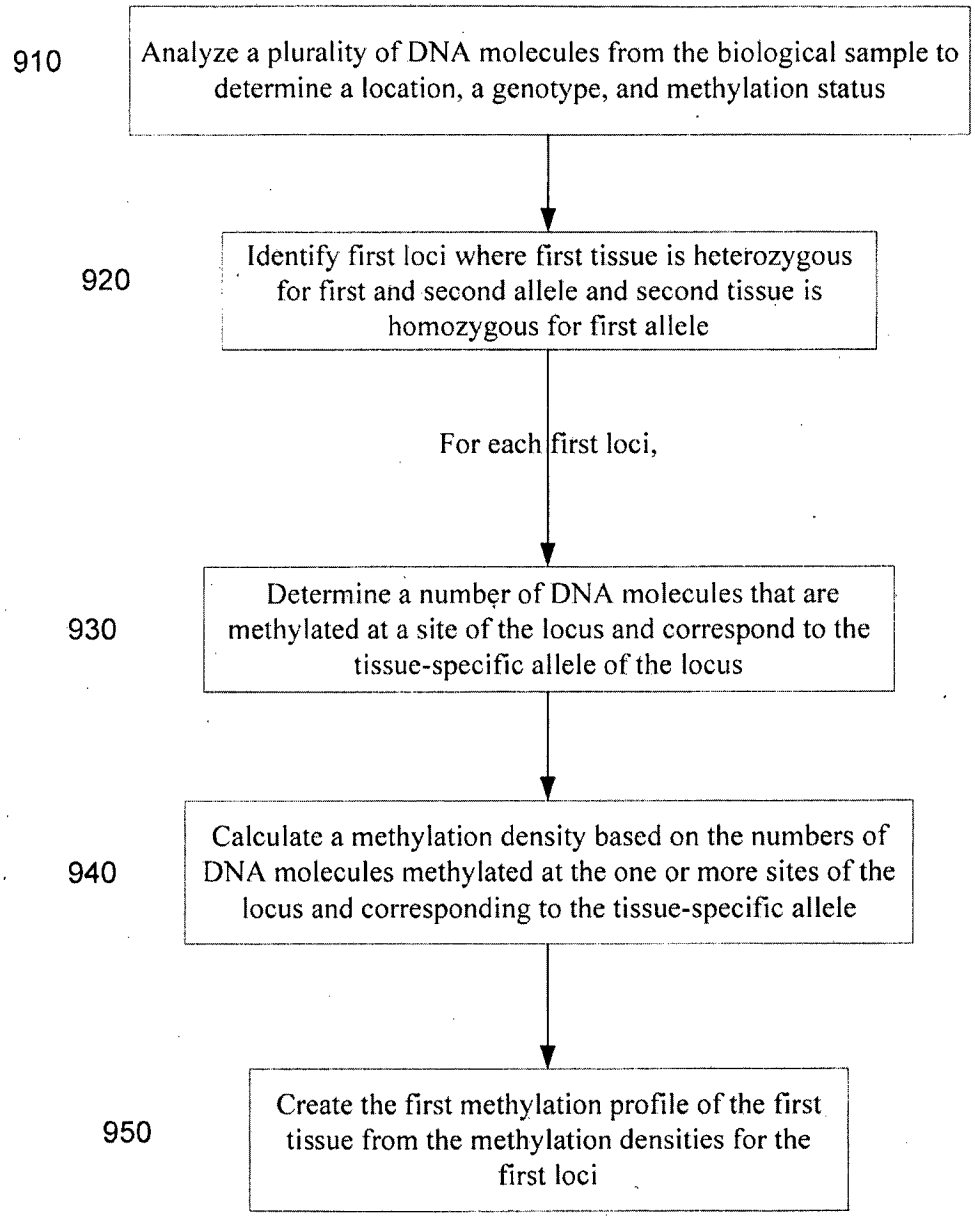

910
Analyze a plurality of DNA molecules from the biological sample to determine a location, a genotype, and methylation status
920
Identify first loci where first tissue is heterozygous for first and second allele and second tissue is homozygous for first allele
For each first loci,
930
Determine a number of DNA molecules that are methylated at a site of the locus and correspond to the tissue-specific allele of the locus
940
Calculate a methylation density based on the numbers of DNA molecules methylated at the one or more sites of the locus and corresponding to the tissue-specific allele
950
Create the first methylation profile of the first tissue from the methylation densities for the first loci

FIG. 9

900

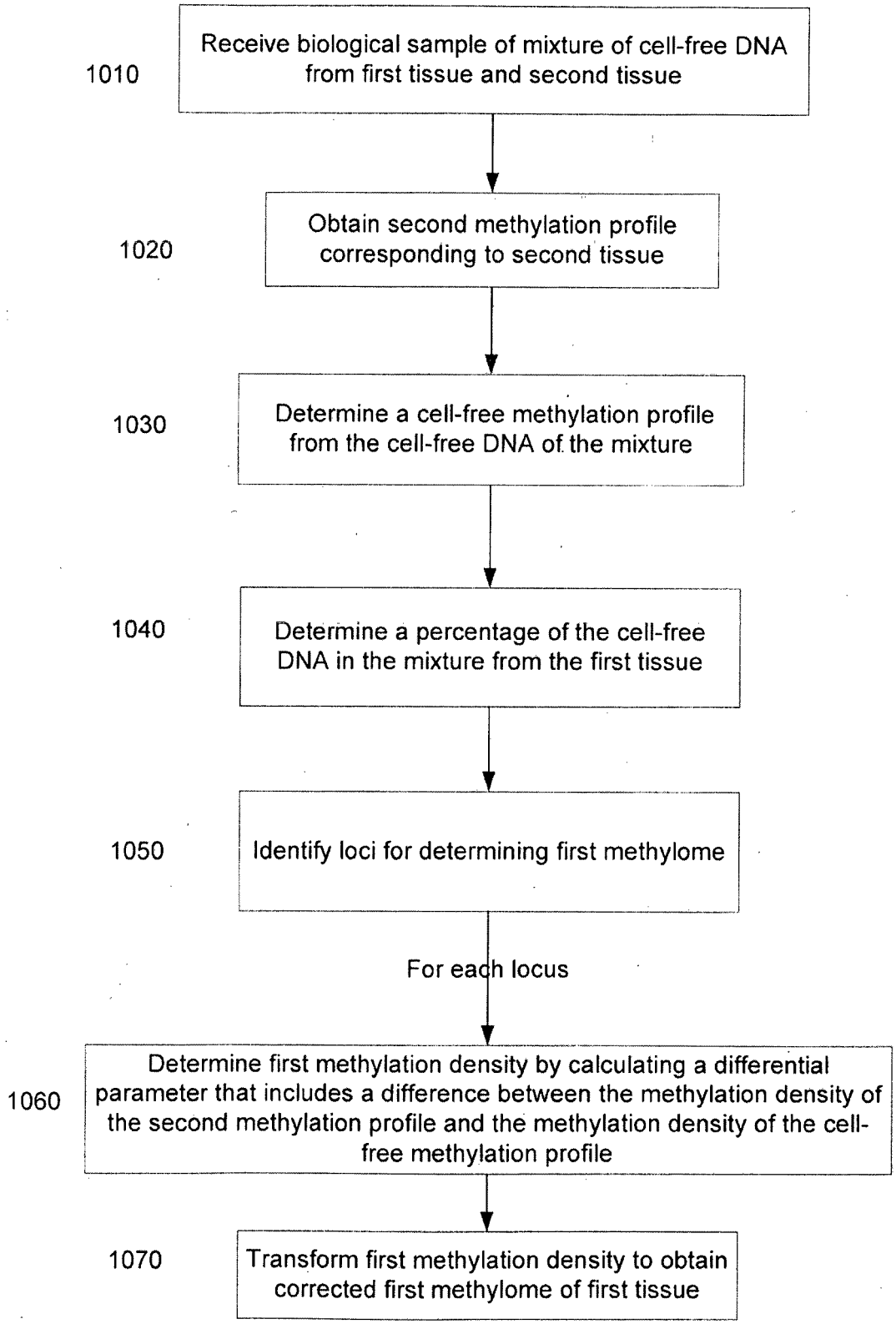
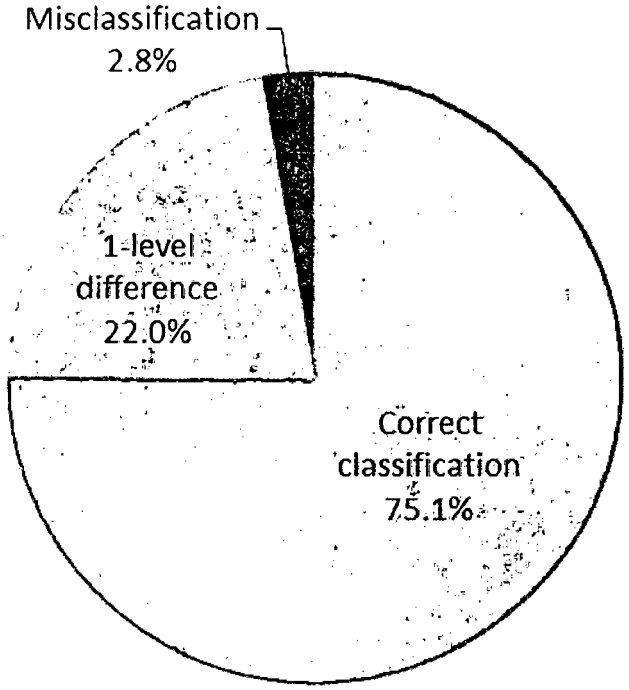

1010
Receive biological sample of mixture of cell-free DNA from first tissue and second tissue
1020
Obtain second methylation profile corresponding to second tissue
1030
Determine a cell-free methylation profile from the cell-free DNA of the mixture
1040
Determine a percentage of the cell-free DNA in the mixture from the first tissue
1050
Identify loci for determining first methylome
For each locus
1060
Determine first methylation density by calculating a differential parameter that includes a difference between the methylation density of the second methylation profile and the methylation density of the cell-free methylation profile
1070
Transform first methylation density to obtain corrected first methylome of first tissue

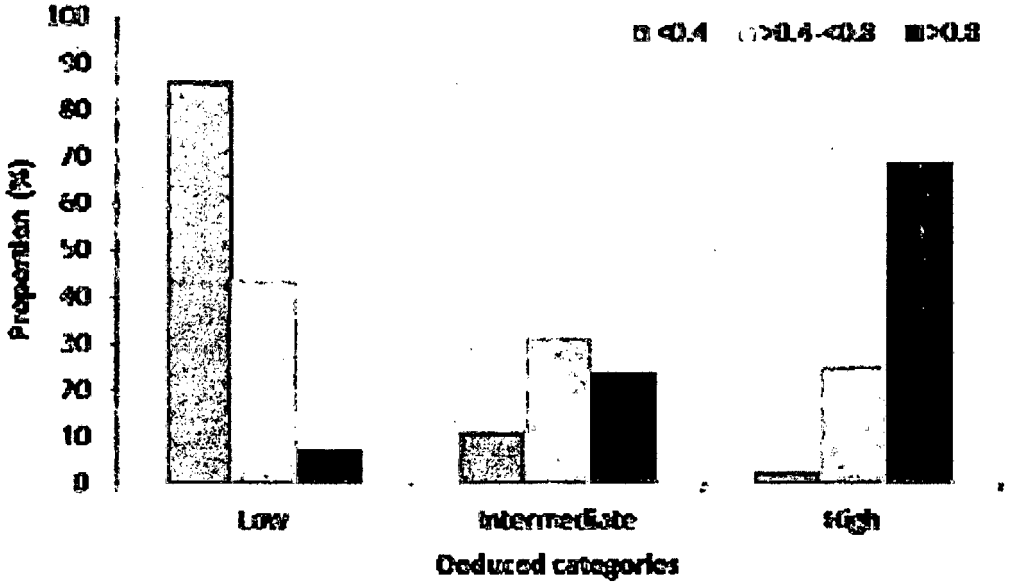
FIG. 10

1000



1100

FIG. 11A



1150

FIG. 11B

No.	Gene locus	Genomic location	Methylation density (sequencing data)				Deduction			Methylation status in literatures	
			Maternal blood *(BKG)*	Placenta	Maternal plasma *(MP)*	Deduced values *(D)*[1]	Methylation predictive score *(MPS)*[2]	Deduced categories[3]	Placental methylation level[4]	Placental status[5]	References
1	RASSF1A	chr3:50352938-50353234	0.000	0.637	0.067	0.506	1.060	High	Inter	Hypermethylated	(1)
2	CGI009	chr21:25856489-25856756	0.001	0.432	0.050	0.374	0.849	High	Inter	Hypermethylated	(2)
3	CGI028	chr21:33321942-33322182	0.035	0.331	0.040	0.073	0.367	Low	Low	Hypermethylated	(2)
4	CGI051	chr21:36995232-36995509	0.043	0.303	0.046	0.072	0.365	Low	Low	Hypermethylated	(2)
5	CGI071	chr21:41140466-41140898	0.031	0.531	0.046	0.146	0.483	Inter	Inter	Hypermethylated	(2)
6	CGI113	chr21:44953820-44953972	0.013	0.547	0.051	0.306	0.739	Inter	Inter	Hypermethylated	(2)
7	CGI137	chr21:46249872-46250196	0.940	0.413	0.820	0.030	0.299	Low	Inter	Hypomethylated	(2)
8	CGI149	chr21:46911575-46912533	0.004	0.440	0.030	0.202	0.573	Inter	Inter	Hypermethylated	(2)
9	HLCS	chr21:37274945-37275031	0.038	0.576	0.059	0.203	0.575	Inter	Inter	Hypermethylated	(2)
10	PDE9A	chr21:42979270-42979525	0.950	0.303	0.823	-0.018	0.222	Low	Low	Hypomethylated	(2)
11	-	chr21:36589647-36589995	0.952	0.149	0.827	0.007	0.262	Low	Low	Hypomethylated	Unpublished
12	SERPINB5	chr18:59294739-59295038	0.936	0.530	0.856	0.329	0.777	Inter	Inter	Hypomethylated	(3)
13	VAPA	chr18:10022563-10023186	0.036	0.752	0.082	0.382	0.861	High	Inter	Hypermethylated	(4)
14	CABLES1	chr18:19035954-19036069	0.335	0.801	0.491	1.519	2.680	High	High	Hypermethylated	(4)
15	B4GALT6	chr18:27486040-27486932	0.113	0.827	0.331	1.764	3.072	High	High	Hypermethylated	(4)

1200

FIG. 12A

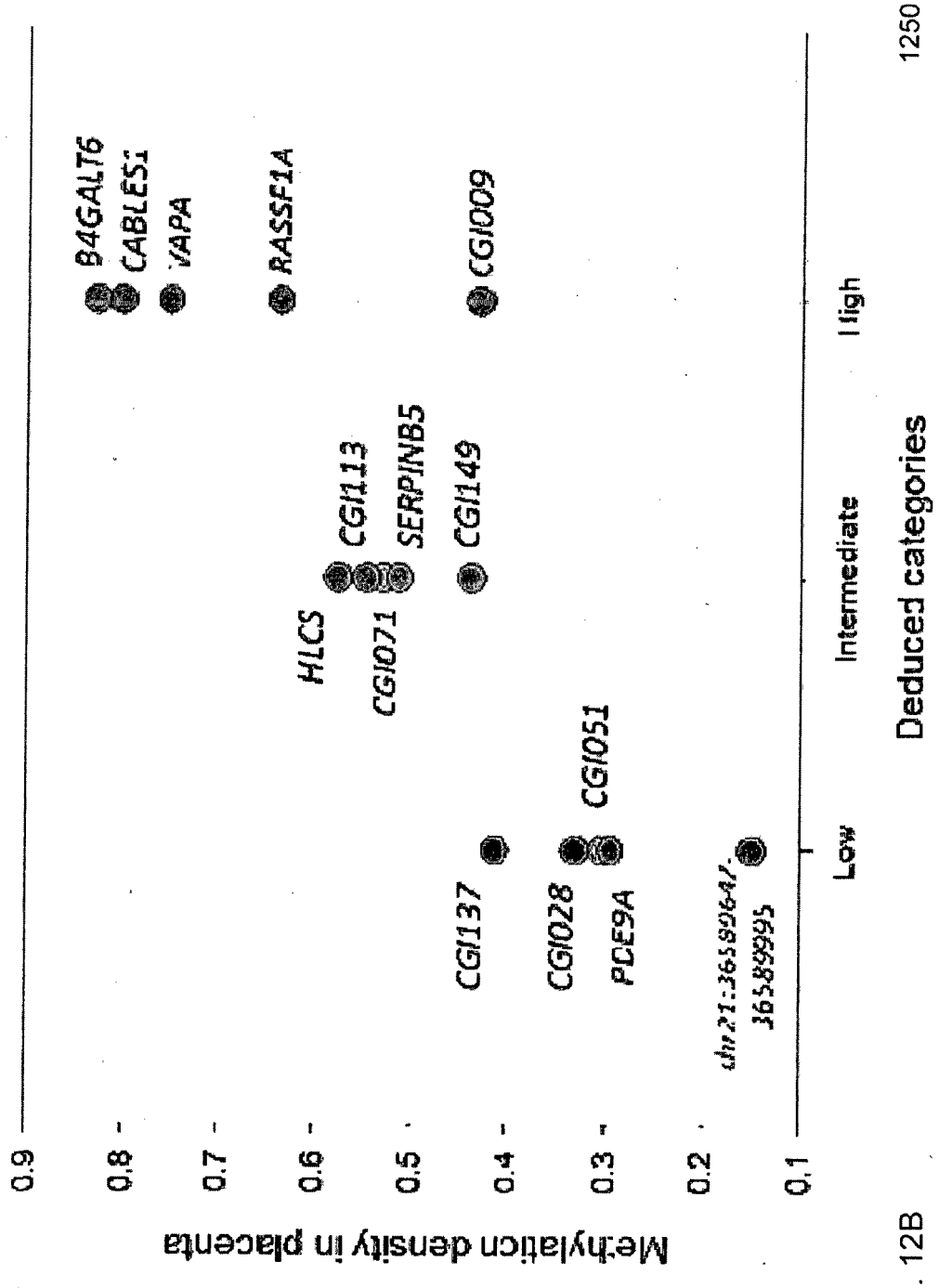
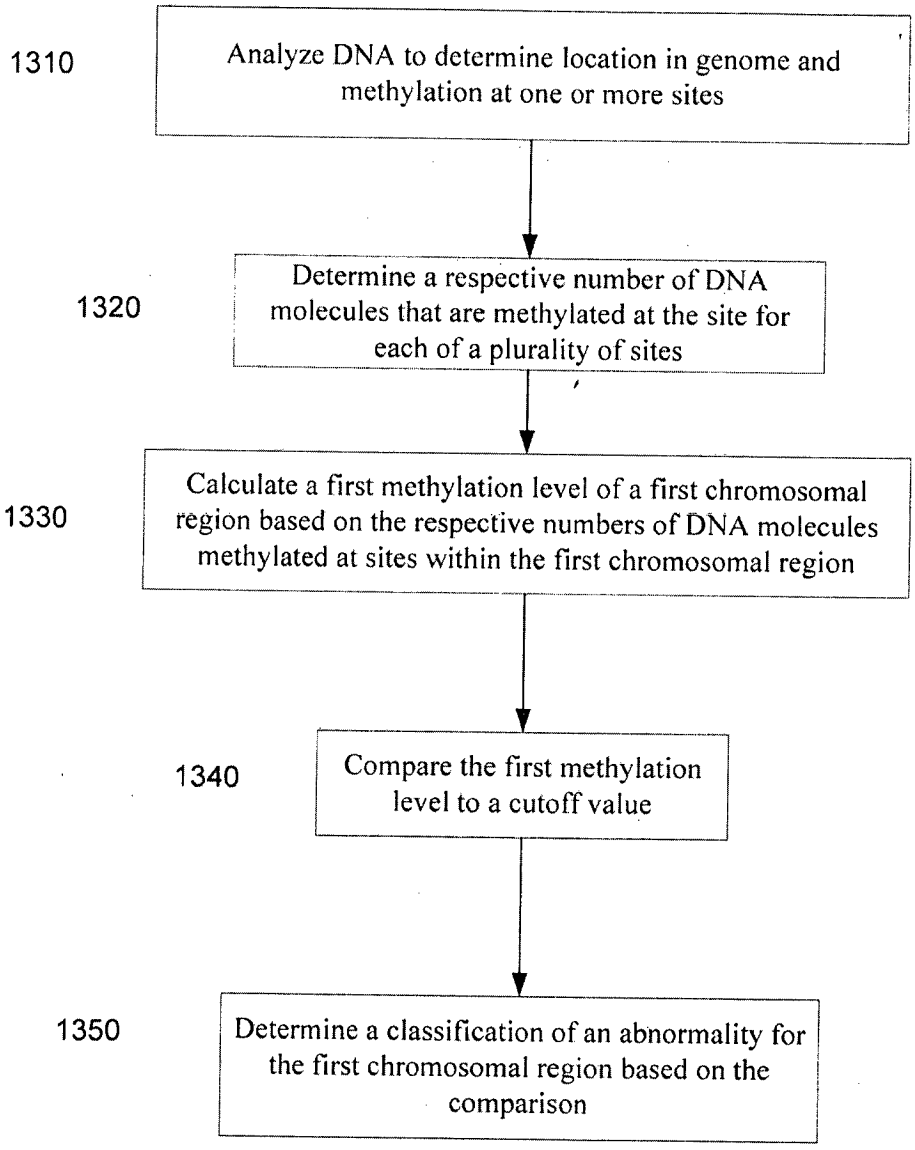

Methylation density in placenta
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
B4GALT6
CABLES1
VAPA
RASSF1A
CGI009
CGI113
SERPINB5
HLCS
CGI071
CGI149
CGI137
CGI028
CGI051
PDE9A
chr21:36589647-36589995
Low
Intermediate
High
Deduced categories
1250

FIG. 12B


1310
Analyze DNA to determine location in genome and methylation at one or more sites
1320
Determine a respective number of DNA molecules that are methylated at the site for each of a plurality of sites
1330
Calculate a first methylation level of a first chromosomal region based on the respective numbers of DNA molecules methylated at sites within the first chromosomal region
1340
Compare the first methylation level to a cutoff value
1350
Determine a classification of an abnormality for the first chromosomal region based on the comparison

1300

FIG. 13

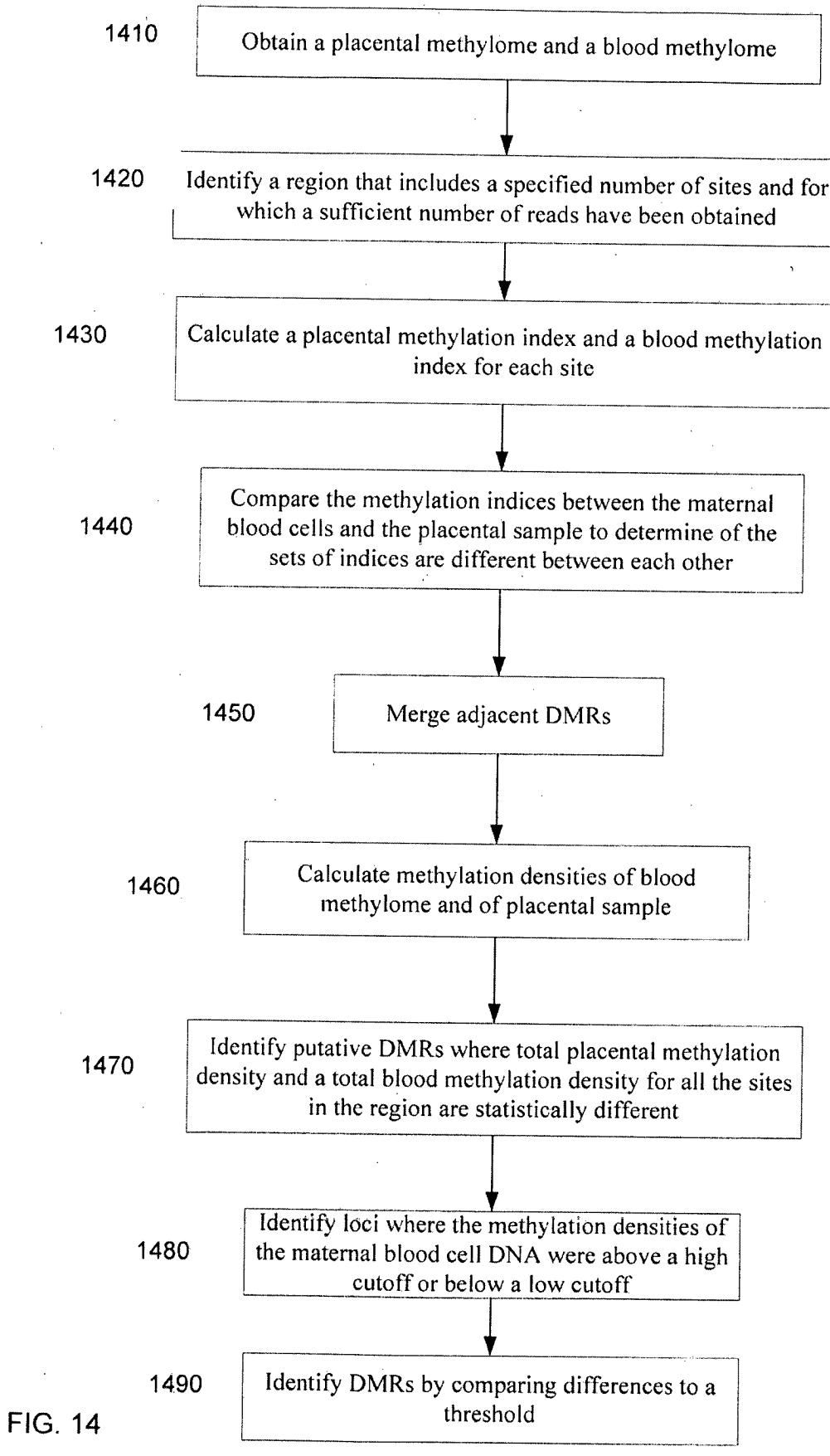

1410
Obtain a placental methylome and a blood methylome
1420
Identify a region that includes a specified number of sites and for which a sufficient number of reads have been obtained
1430
Calculate a placental methylation index and a blood methylation index for each site
1440
Compare the methylation indices between the maternal blood cells and the placental sample to determine of the sets of indices are different between each other
1450
Merge adjacent DMRs
1460
Calculate methylation densities of blood methylome and of placental sample
1470
Identify putative DMRs where total placental methylation density and a total blood methylation density for all the sites in the region are statistically different
1480
Identify loci where the methylation densities of the maternal blood cell DNA were above a high cutoff or below a low cutoff
1490
Identify DMRs by comparing differences to a threshold
1400

FIG. 14

					BS-Seq[c]			DMR[d]								
No.[a]	Gene locus	Genomic location[b]	Size[a]	Placental status[b]	Maternal blood cells	CVS	Meth diff	Chr	Start	End	Size	Maternal blood cells	CVS	p value	Meth diff	Class[e]
1	RASSF1A	chr3:50352938-50353234	297	Hypermethylated	0.00	63.73	63.73	chr3	50351000	50354000	3000	6.26	66.68	0.00E+00	60.42	TP
2	CGI009	chr21:25856489-25856756	268	Hypermethylated	0.08	43.20	43.13	chr21	25855500	25857500	2000	4.97	48.70	0.00E+00	43.73	TP
3	CGI028	chr21:33321942-33322182	241	Hypermethylated	3.51	33.10	29.59	chr21	33316400	33323900	7500	4.52	23.85	0.00E+00	19.33	FN
4	CGI051	chr21:36995232-36995509	278	Hypermethylated	4.26	30.33	26.07	chr21	36992800	37003900	11100	3.12	21.91	0.00E+00	18.79	FN
5	CGI071	chr21:41140466-41140898	433	Hypermethylated	3.09	53.06	49.97	chr21	41140000	41141000	1000	3.40	52.00	0.00E+00	48.60	TP
6	CGI113	chr21:44953820-44953972	153	Hypermethylated	1.27	54.69	53.42	chr21	44948500	44954700	6200	7.18	57.23	0.00E+00	50.05	TP
7	CGI137	chr21:46249872-46250196	325	Hypomethylated	94.00	41.34	52.65	chr21	46227800	46250400	22600	89.74	52.67	0.00E+00	37.07	TP
8	CGI149	chr21:46911575-46912533	959	Hypermethylated	0.40	43.95	43.56	chr21	46911200	46912700	1500	5.31	46.17	0.00E+00	40.86	TP
9	HLCS	chr21:37274945-37275031	87	Hypermethylated	3.77	57.61	53.85	chr21	37274200	37275200	1000	7.33	54.24	0.00E+00	46.91	TP
10	PDE9A	chr21:42979270-42979525	256	Hypomethylated	95.01	30.33	64.68	chr21	42978100	43004800	26700	89.97	51.44	0.00E+00	38.53	TP
11	-	chr21:36589647-36589995	349	Hypomethylated	95.17	14.89	80.28	chr21	36589200	36590200	1000	94.49	19.27	0.00E+00	75.22	TP
12	SERPINB5	chr18:59294739-59295038	300	Hypomethylated	93.64	53.00	40.64	chr18	59294800	59296300	1500	88.47	40.76	3.33E-27	47.71	TP
13	VAPA	chr18:10022563-10023187	625	Hypermethylated	3.63	75.17	71.54	chr18	10021800	10023300	1500	24.08	80.33	0.00E+00	56.25	TP
14	CABLES1	chr18:19035954-19036070	117	Hypermethylated	33.47	80.09	46.62	-	-	-	-	-	-	-	-	FN
15	B4GALT6	chr18:27486040-27486933	894	Hypermethylated	11.34	82.75	71.41	chr18	27486100	27487100	1000	11.34	82.75	9.6408E-86	71.41	TP
16	CASP8	chr2:201830342-201831885	1544	Hypermethylated	0.52	47.54	47.03	chr2	201830200	201832200	2000	3.77	49.13	4.22E-42	45.36	TP
17	RARB	chr3:25444840-25445090	251	Hypermethylated	2.28	26.93	24.65	chr3	25443900	25445400	1500	3.44	22.97	9.65E-50	19.53	FN
18	APC	chr5:112100999-112101943	945	Hypermethylated	0.71	45.78	45.06	chr5	112100300	112101800	1500	3.59	48.09	0.00E+00	44.50	TP
19	DAB2IP_ori	chr9:121541221-121541460	240	Hypomethylated	84.87	52.63	32.24	chr9	121540400	121541500	1100	85.21	36.86	5.2605E-10	48.35	TP
20	DAB2IP_ex	chr9:123128261-123129077	817	Hypomethylated	95.09	32.99	62.10	chr9	123127700	123129200	1500	90.72	50.61	1.4706E-19	40.11	TP
21	THY1	chr11:118798952-118799382	431	Hypermethylated	0.88	39.32	38.44	chr11	118797600	118799600	2000	2.25	27.28	0.00E+00	25.03	TP
22	PTPN6	chr12:6930211-6933772	3562	Hypermethylated	1.52	79.80	78.29	chr12	6930000	6934000	4000	4.26	80.13	0.00E+00	75.87	TP
23	PYCARD_ex	chr16:31121808-31122146	339	Hypermethylated	4.90	46.51	41.61	-	-	-	-	-	-	-	-	FN
24	chr18A	chr18:55090284-55090606	323	Hypermethylated	6.14	31.79	25.65	chr18	55089700	55094200	4500	4.78	22.91	0.00E+00	18.13	FN
25	chr21A	chr21:39279723-39280004	282	Hypermethylated	12.80	64.02	51.21	chr21	39279300	39279800	500	4.74	62.07	7.17E-62	57.33	TP
26	chr21B	chr21:44161027-44161371	345	Hypermethylated	0.86	83.90	83.04	chr21	44160700	44161700	1000	11.06	77.32	0.00E+00	66.26	TP
27	chr21C	chr21:33320544-33320829	286	Hypermethylated	5.49	37.92	32.43	chr21	33316400	33323900	7500	4.52	23.85	0.00E+00	19.33	FN
28	chr21D	chr21:42189223-42189683	461	Hypermethylated	13.02	85.84	72.82	chr21	42188700	42189700	1000	7.78	81.67	3.62E-77	73.89	TP
29	chr21EI	chr21:42355712-42355908	197	Hypermethylated	0.00	57.85	57.85	chr21	42355000	42357500	2500	5.21	62.23	0.00E+00	57.02	TP
30	chr21EII	chr21:42357215-42357341	127	Hypermethylated	3.57	48.25	44.68	chr21	42355000	42357500	2500	5.21	62.23	0.00E+00	57.02	TP
31	chr21H	chr21:32268803-32268943	141	Hypermethylated	0.49	78.84	78.35	chr21	32268300	32269300	1000	20.49	85.25	4.9563E-34	64.76	TP
32	chr21I	chr21:44079235-44079535	301	Hypermethylated	1.23	67.44	66.21	chr21	44079400	44080400	1000	15.19	68.06	4.9E-59	52.87	TP
33	SERPINB5_P	chr18:59294811-	465	Hypomethylated	91.96	39.71	52.25	chr18	59294800	59296300	1500	88.47	40.76	3.3294E-	47.71	TP

FIG. 15A

1500

		BS-Seq			DMR[c]								
No.[a]	Gene locus	Maternal blood cells	Placenta	Meth diff[b]	Chr	Start	End	Size	Maternal blood cells	Placenta	p value	Meth diff	Class[d]
1	RASSF1A	0.00	60.84	60.84	chr3	50352100	50354100	2000	2.16	62.21	0.00E+00	60.05	TP
2	CGI009	0.08	20.50	20.43	chr21	25855600	25857100	1500	2.04	24.37	0.00E+00	22.33	TP
3	CGI028	3.51	17.38	13.86	-	-	-	-	-	-	-	-	TN
4	CGI051	4.26	13.93	9.67	-	-	-	-	-	-	-	-	TN
5	CGI071	3.09	24.85	21.76	chr21	41139900	41141400	1500	6.83	29.28	0.00E+00	22.45	TP
6	CGI113	1.27	40.24	38.97	chr21	44948500	44954700	6200	7.18	45.00	0.00E+00	37.82	TP
7	CGI137	94.00	37.10	56.90	chr21	46248400	46250400	2000	92.28	43.85	1.66E-76	48.43	TP
8	CGI149	0.40	22.19	21.79	chr21	46911200	46912700	1500	5.31	25.73	0.00E+00	20.42	TP
9	HLCS	3.77	58.49	54.72	chr21	37274200	37275200	1000	7.33	56.83	0.00E+00	49.50	TP
10	PDE9A	95.01	32.41	62.60	chr21	42978900	42990100	11200	90.89	44.38	0.00E+00	46.51	TP
11	-	95.17	19.92	75.24	chr21	36589200	36590200	1000	94.49	22.66	0.00E+00	71.83	TP
12	SERPINB5	93.64	28.06	65.57	chr18	59294500	59295500	1000	92.11	36.53	3.85E-31	55.58	TP
13	VAPA	3.63	73.88	70.25	chr18	10022100	10023100	1000	11.67	75.76	0.00E+00	64.09	TP
14	CABLES1	33.47	81.40	47.93	-	-	-	-	-	-	-	-	FN
15	B4GALT6	11.34	88.57	77.23	chr18	27485800	27486800	1000	28.97	90.00	4.3779E-59	61.03	TP
16	CASP8	0.52	56.27	55.75	chr2	201830200	201832200	2000	3.77	57.60	1.33E-52	53.83	TP
17	RARB	2.28	38.18	35.90	chr3	25444000	25445000	1000	2.36	24.42	8.00E-54	22.06	TP
18	APC	0.71	34.89	34.18	chr5	112100700	112101700	1000	0.38	32.97	0.00E+00	32.59	TP
19	DAB2IP_ori	84.87	40.28	44.60	chr9	121540400	121541400	1000	84.79	30.63	1.7338E-14	54.16	TP
20	DAB2IP_ex	95.09	16.14	78.95	chr9	123128600	123129100	500	95.14	17.42	9.6247E-47	77.72	TP
21	THY1	0.88	22.28	21.40	chr11	118797800	118799300	1500	1.22	20.50	0.00E+00	19.28	FN
22	PTPN6	1.52	81.33	79.82	chr12	6930000	6934000	4000	4.26	81.58	0.00E+00	77.32	TP
23	PYCARD_ex	4.90	48.73	43.83	chr16	31121700	31122200	500	4.85	32.87	1.1158E-34	28.02	TP
24	chr18A	6.14	16.25	10.10	-	-	-	-	-	-	-	-	TN
25	chr21A	12.80	74.65	61.85	chr21	39278300	39280300	2000	9.96	75.07	0.00E+00	65.11	TP
26	chr21B	0.86	81.56	80.70	chr21	44160700	44161700	1000	11.06	78.6	0.00E+00	67.54	TP
27	chr21C	5.49	14.91	9.42	-	-	-	-	-	-	-	-	TN
28	chr21D	13.02	88.71	75.70	chr21	42188700	42189700	1000	7.78	84.42	3.5806E-77	76.64	TP
29	chr21EI	0.00	73.21	73.21	chr21	42355300	42357800	2500	4.66	79.19	0.00E+00	74.53	TP
30	chr21EII	3.57	84.00	80.43	chr21	42355300	42357800	2500	4.66	79.19	0.00E+00	74.53	TP
31	chr21H	0.49	77.49	77.00	chr21	32268400	32268900	500	18.4	83.85	2.1972E-30	65.45	TP
32	chr21I	1.23	58.62	57.39	chr21	44079300	44080300	1000	12.16	72.55	2.3559E-60	60.39	TP
33	SERPINB5_P	91.96	28.61	63.35	chr18	59294500	59295500	1000	92.11	36.53	3.85E-31	55.58	TP

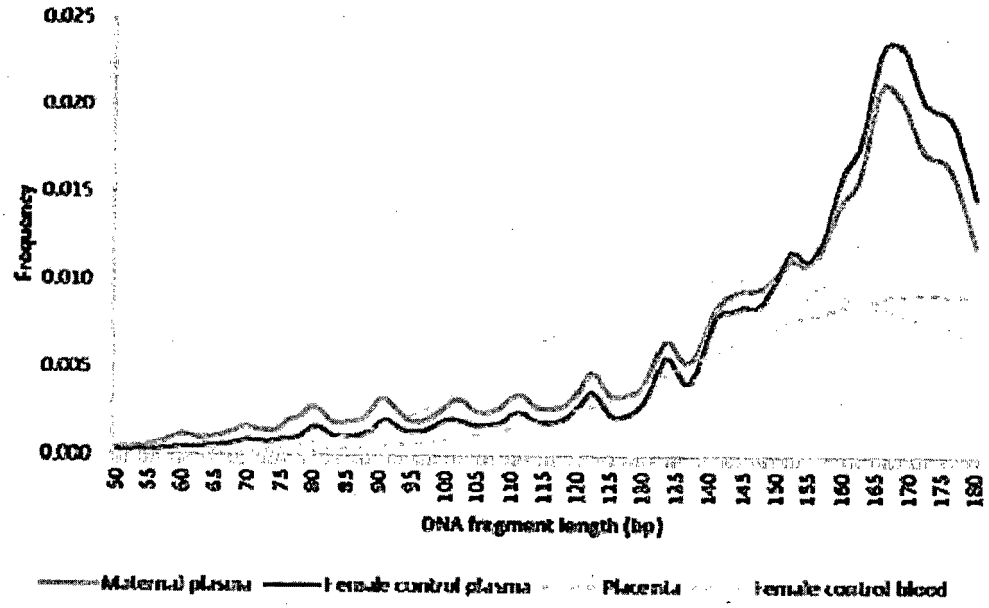
FIG. 15B

1550

	First trimester maternal plasma		Third trimester maternal plasma	
	Hypermethylated[a]	Hypomethylated[b]	Hypermethylated[a]	Hypomethylated[b]
No. predicted loci	3081	44455	1746	14930
No. loci with methylation densities > 40% in the placental tissue[c] data	1,678	N/A	1,525	N/A
No. loci with methylation densities < 60% in the placental tissue[c] data	N/A	23468	N/A	13475
No. of loci overlapped with DMRs mined from the placental tissue[c] and maternal blood cell data	1457	21812	1279	12677

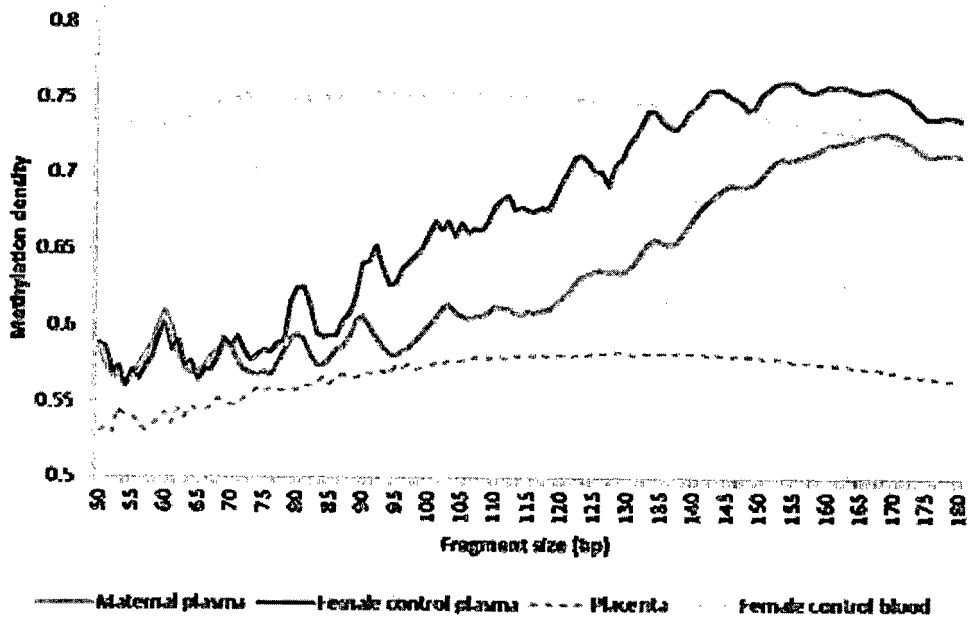
FIG. 16

1600



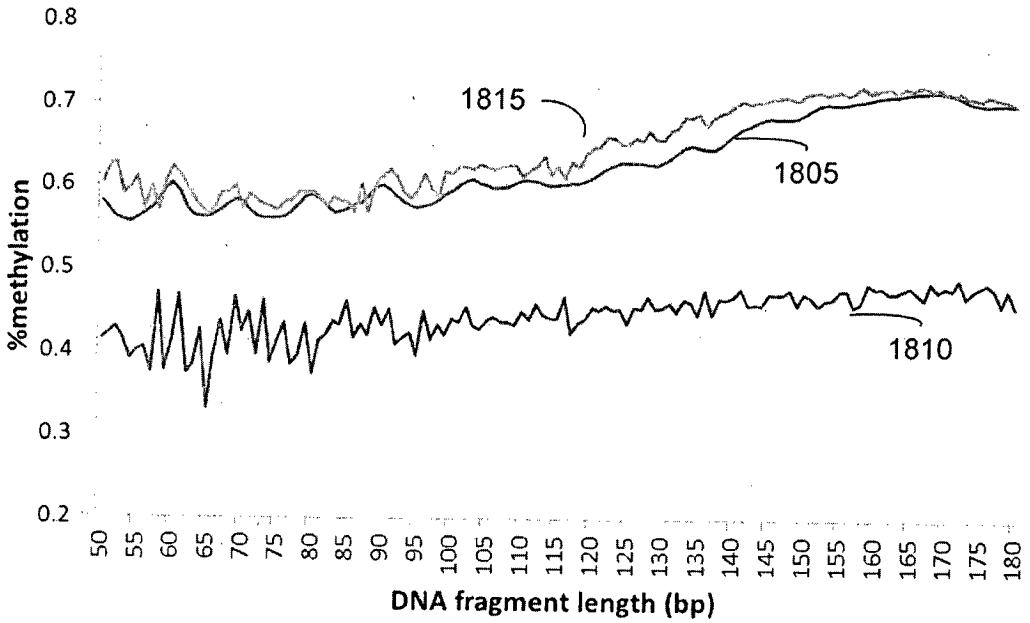
1700

FIG. 17A



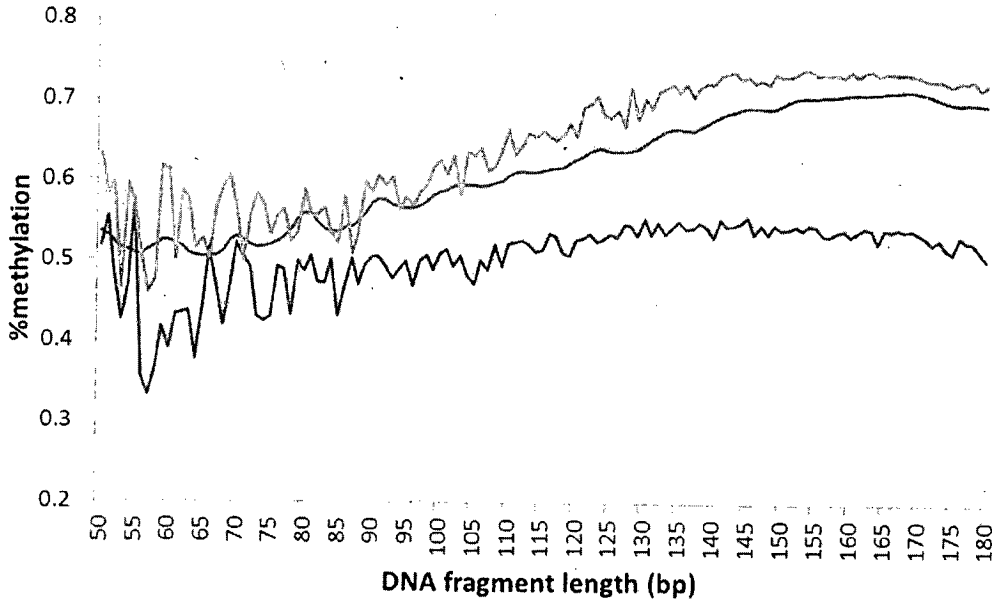
1750

FIG. 17B



1800

FIG. 18A



1850

FIG. 18B

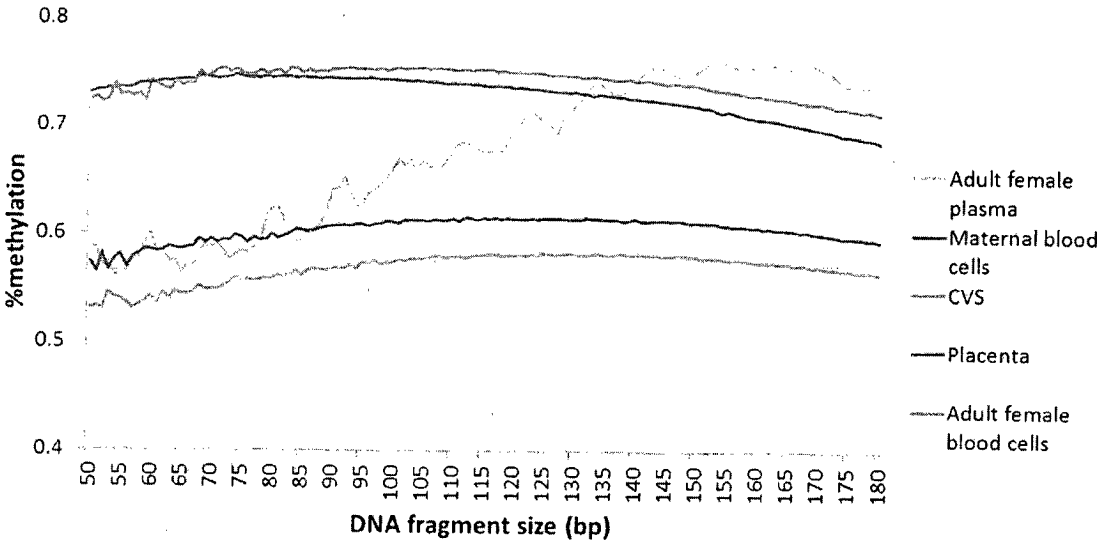
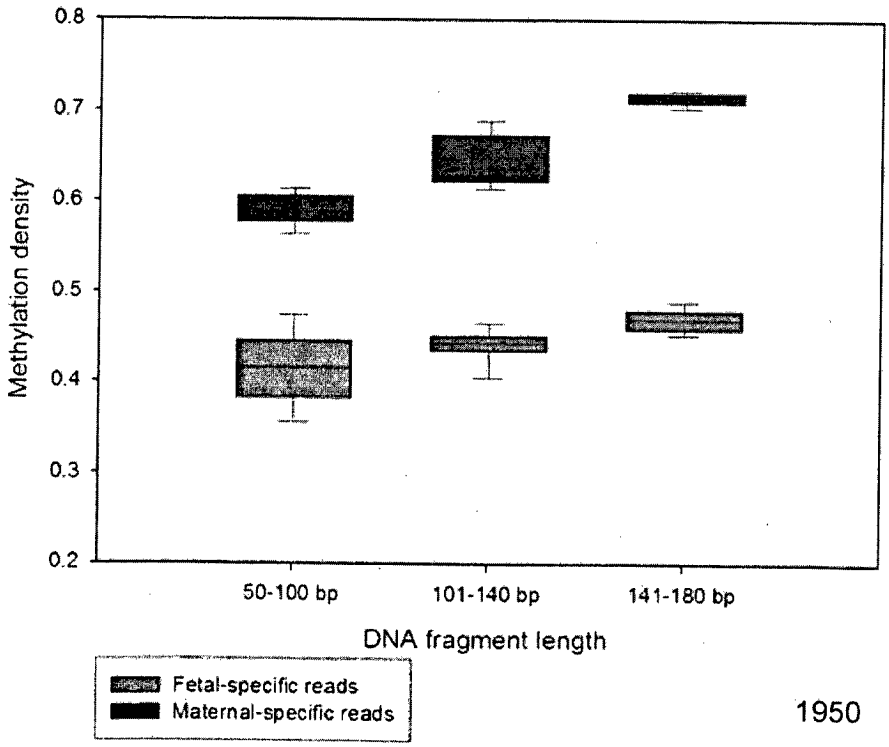


FIG. 19A

1900



1950

FIG. 19B

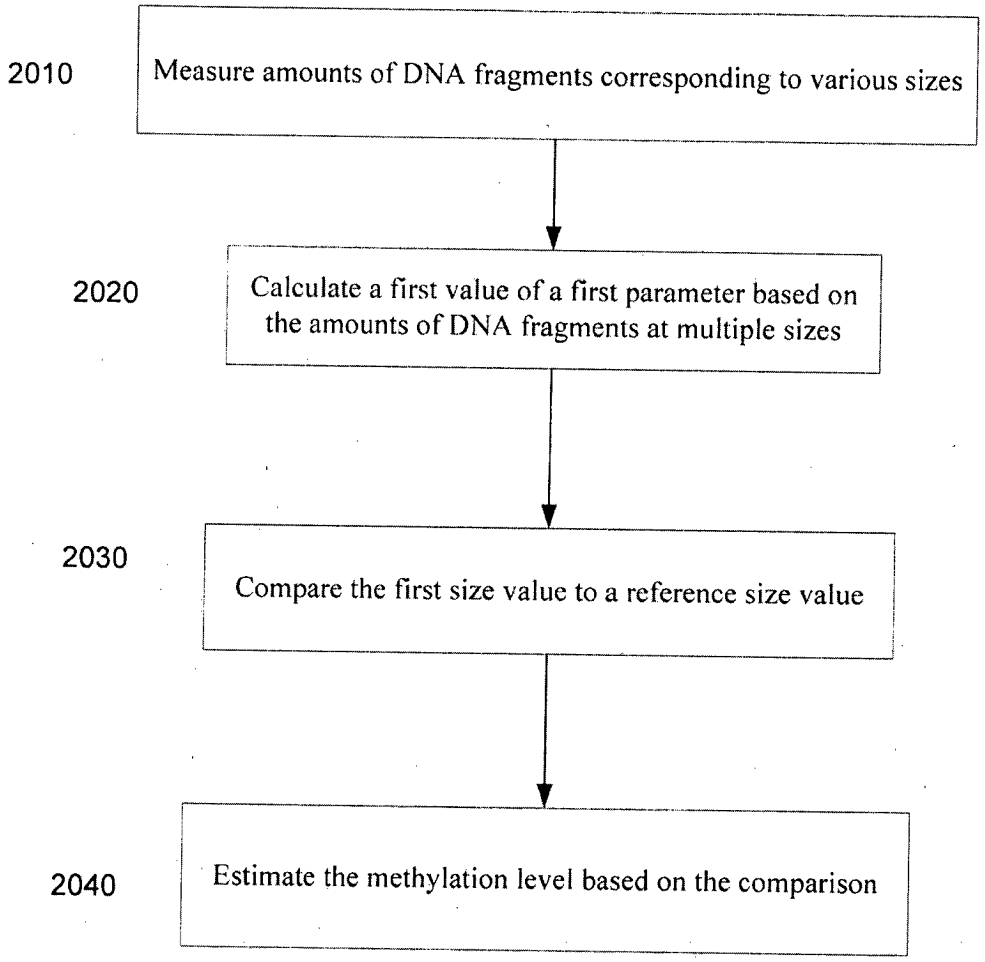

2010
Measure amounts of DNA fragments corresponding to various sizes
2020
Calculate a first value of a first parameter based on the amounts of DNA fragments at multiple sizes
2030
Compare the first size value to a reference size value
2040
Estimate the methylation level based on the comparison

FIG. 20

2000

	HCC patient			
Chromosome	Buffy coat	Non-tumoral liver tissue	Tumor tissue	Pre-operative plasma
1	65.4%	65.7%	44.1%	60.2%
2	68.3%	68.2%	42.0%	58.6%
3	68.2%	68.5%	44.6%	60.9%
4	68.9%	67.7%	37.8%	56.9%
5	67.4%	66.7%	37.8%	56.9%
6	67.4%	67.7%	43.2%	59.9%
7	69.1%	68.8%	43.0%	60.0%
8	69.1%	68.0%	40.4%	60.0%
9	66.4%	66.2%	44.9%	60.6%
10	68.9%	68.2%	40.8%	58.5%
11	65.5%	65.6%	41.6%	58.5%
12	67.6%	67.4%	45.9%	62.0%
13	69.8%	68.8%	40.6%	60.2%
14	66.4%	66.2%	41.4%	59.2%
15	66.1%	66.8%	44.6%	61.0%
16	68.9%	67.6%	45.6%	60.7%
17	64.0%	64.9%	45.8%	60.2%
18	69.9%	69.1%	42.9%	60.0%
19	62.3%	62.7%	47.5%	61.0%
20	66.0%	65.3%	39.1%	57.8%
21	61.8%	61.1%	39.9%	57.7%
22	67.9%	67.6%	49.1%	63.8%
All autosomes	67.1%	66.9%	42.9%	59.7%

FIG. 21A

2100

Summary	Raw read count	Mapped read count	non-duplicate fragments (Autosomes only)	Proportion of reads usable %	Depth per strand	CpG coverage %	Depth on CpG sites with reads covered per strand
Control 1	159554637	56323206	41653660	26%	1.12	54.86%	3.56
Control 2	118056735	49232079	45741385	39%	1.23	60.26%	3.24
Control 3	181913746	75753907	68081010	37%	1.82	65.74%	4.37
Control 4	142782870	50195168	42370350	30%	1.13	59.15%	3.14
C06	262763161	197344994	88266088	33%	2.11	64.31%	3.65
Buffy coat	579329822	439663895	375814248	65%	10.07	78.49%	21.67
Non-cancer liver tissue	549773334	417736311	355154964	65%	9.51	84.89%	18.21
Tumor tissue	568004640	487494291	176985129	31%	4.74	83.48%	8.32
Pre-operative plasma	796183998	637735722	474751039	60%	12.72	87.18%	18.69
Post-operative plasma	921833848	624851729	550104801	60%	14.73	89.94%	24.81

FIG. 21B

2150

Chr	Plasma DNA methylation density					
	HCC Patient		Control 1	Control 2	Control 3	Control 4
	Pre-operative	Post-operative				
1	60.2%	69.6%	70.0%	70.3%	70.1%	71.3%
2	58.6%	71.9%	72.2%	72.4%	72.3%	73.5%
3	60.9%	72.3%	72.7%	73.1%	72.9%	73.9%
4	56.9%	71.8%	71.9%	72.2%	72.2%	73.4%
5	56.9%	70.9%	71.0%	71.3%	71.3%	72.6%
6	59.9%	71.2%	71.6%	71.9%	71.7%	72.8%
7	60.0%	72.2%	72.8%	72.8%	72.5%	73.9%
8	60.0%	72.0%	72.6%	72.6%	72.4%	73.7%
9	60.6%	70.4%	71.0%	71.1%	71.0%	72.2%
10	58.5%	71.7%	72.0%	72.1%	72.0%	73.2%
11	58.5%	69.6%	70.2%	70.4%	70.2%	71.6%
12	62.0%	71.0%	71.6%	71.7%	71.5%	72.7%
13	60.2%	72.8%	73.2%	73.4%	73.2%	74.3%
14	59.2%	70.2%	70.7%	70.9%	70.8%	72.0%
15	61.0%	70.5%	70.6%	71.0%	70.9%	72.0%
16	60.7%	71.3%	72.4%	71.8%	71.6%	73.3%
17	60.2%	68.6%	69.3%	69.4%	69.3%	70.8%
18	60.0%	72.7%	72.9%	73.1%	73.0%	74.2%
19	61.0%	66.9%	68.1%	68.1%	67.6%	69.3%
20	57.8%	69.6%	70.3%	70.3%	70.3%	71.6%
21	57.7%	67.0%	64.0%	64.5%	65.7%	67.4%
22	63.8%	71.4%	72.5%	72.0%	71.9%	73.3%
All autosomes	59.7%	70.8%	71.2%	71.3%	71.2%	72.5%

FIG. 22

2200

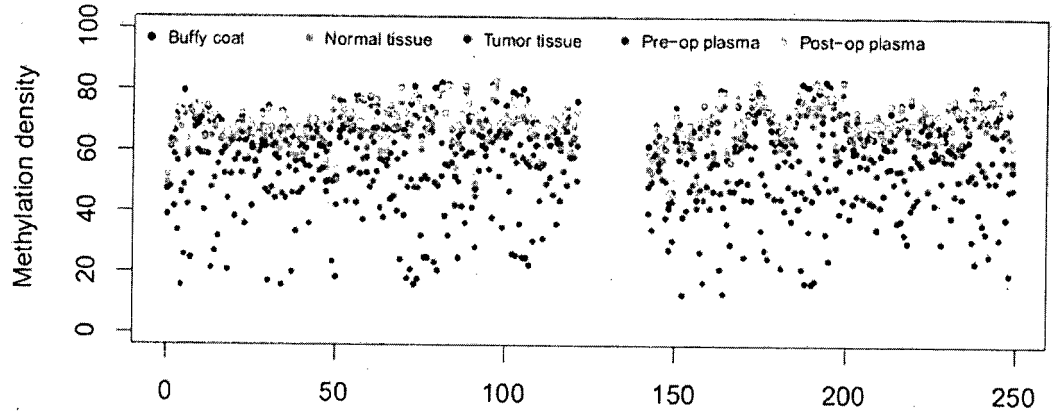


FIG. 23A

2300

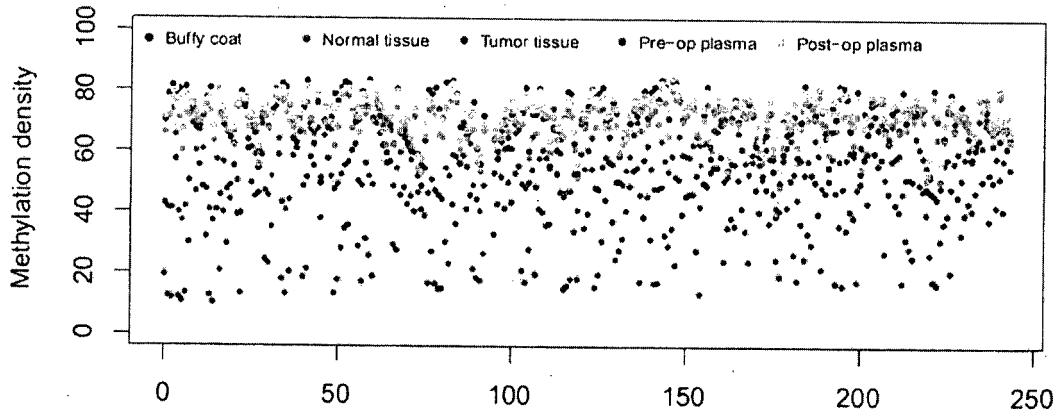


FIG. 23B

2350

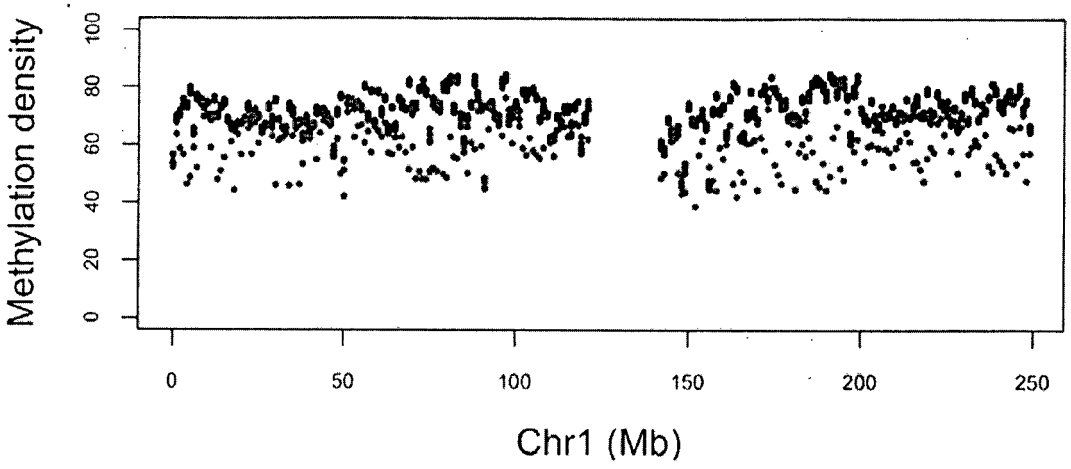


FIG. 24A

2400

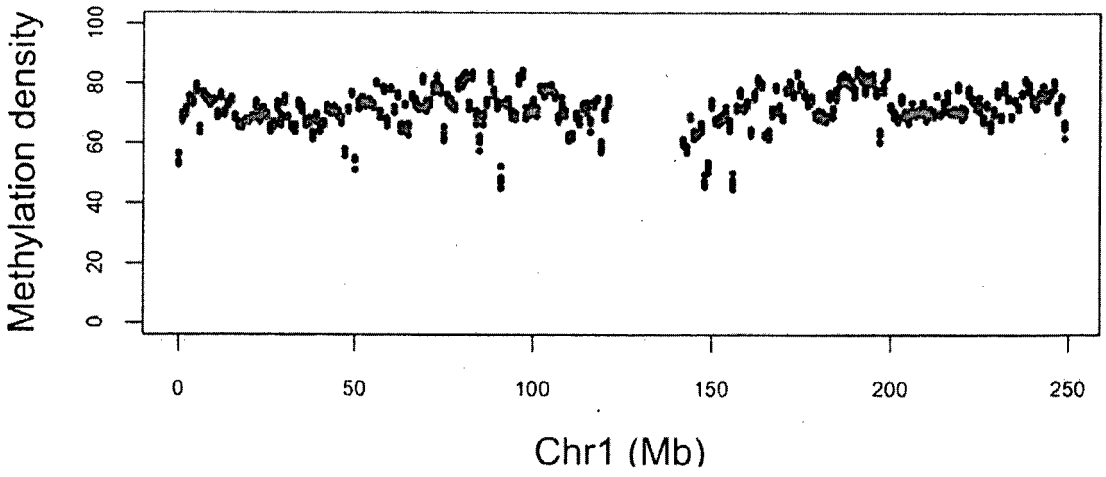


FIG. 24B

2450

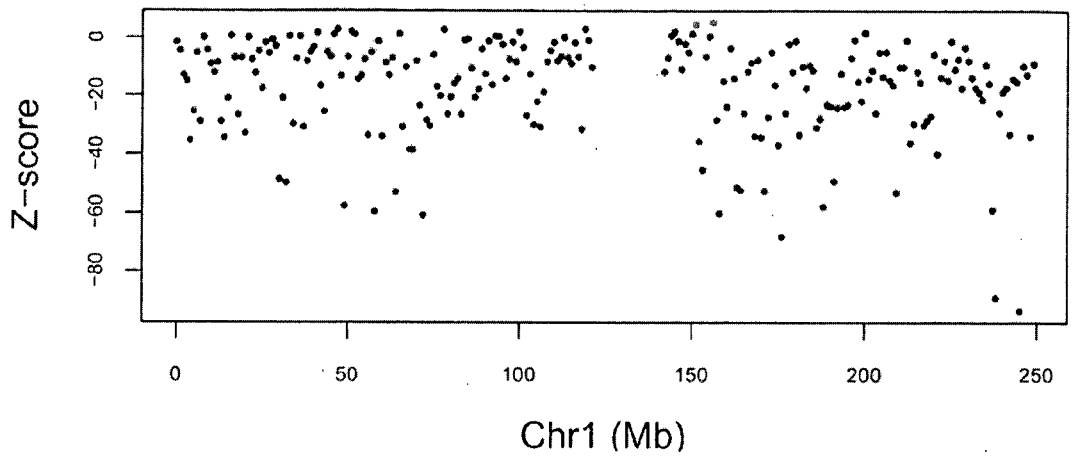


FIG. 25A

2500

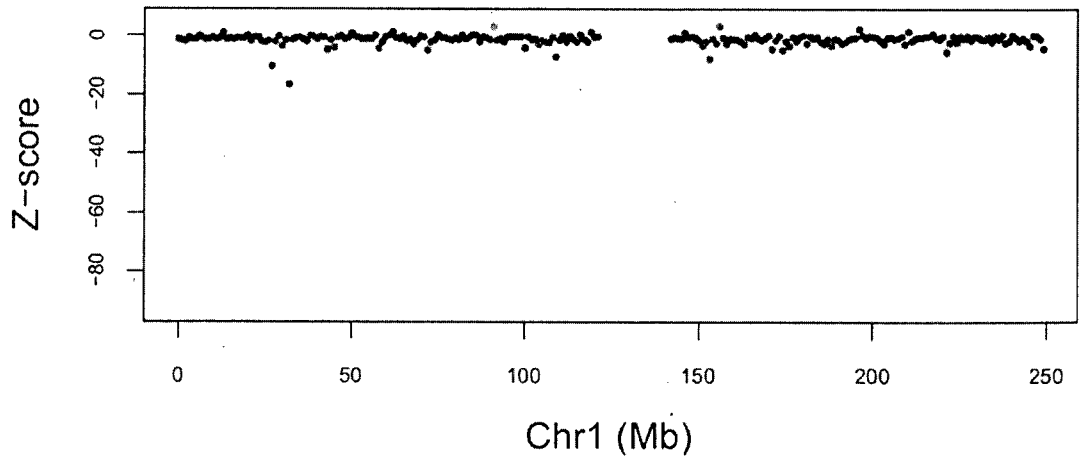


FIG. 25B

2550

z-scores	Pre-operative plasma (No. of bins)	Post-operative plasma (No. of bins)
<-3	186 (80.9%)	19 (8.3%)
-3 to 3	42 (18.3%)	209 (90.3%)
>3	2 (0.9%)	2 (0.9%)
Total	230 (100%)	230 (100%)

FIG. 26A

2600

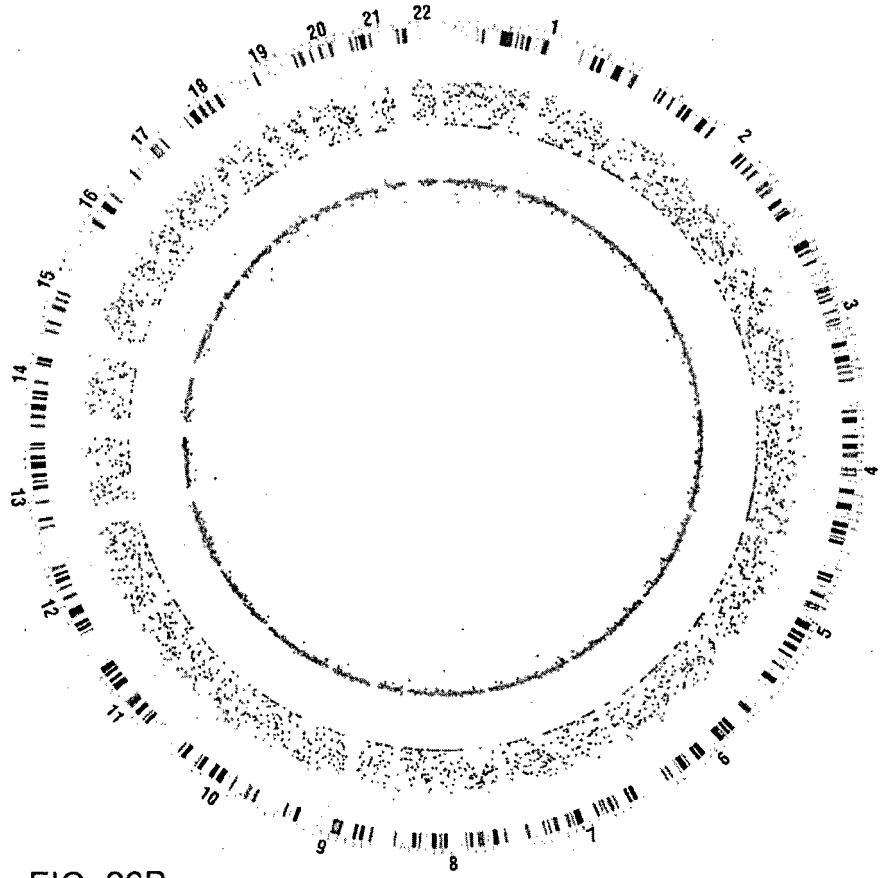


FIG. 26B

2620

z-score	Pre-operative plasma (No. of bins)	Post-operative plasma (No. of bins)	C06	Control 1 (No. of bins)	Control 2 (No. of bins)	Control 3 (No. of bins)	Control 4 (No. of bins)
<-3	2330 (85.2%)	171 (6.3%)	29 (1.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
-3 to 3	362 (13.3%)	2556 (93.5%)	2688 (98.3%)	2734 (100%)	2734 (100%)	2734 (100%)	2734 (100%)
>3	42 (1.5%)	7 (0.2%)	17 (0.62%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	2734 (100%)	2734 (100%)	2734 (100%)	2734 (100%)	2734 (100%)	2734 (100%)	2734 (100%)

FIG. 26C 2640

	Proportion of methylated CHH	Proportion of methylated CHG	Proportion of methylated cytosines	Proportion of methylated CpG (methylation density)
Buffy coat of HCC patient	0.13	0.14	5.74	67.08
Non-tumor liver tissue	0.16	0.15	5.45	66.85
tumor tissue	0.09	0.09	3.29	42.88
Pre-op plasma	0.09	0.10	3.99	59.67
Post-op plasma	1.25	0.96	6.52	70.75
Control plasma 1	0.12	0.12	6.38	71.17
Control plasma 2	0.10	0.10	5.62	71.33
Control plasma 3	0.09	0.09	5.57	71.20
Control plasma 4	0.10	0.10	5.93	72.48

FIG. 26D 2660

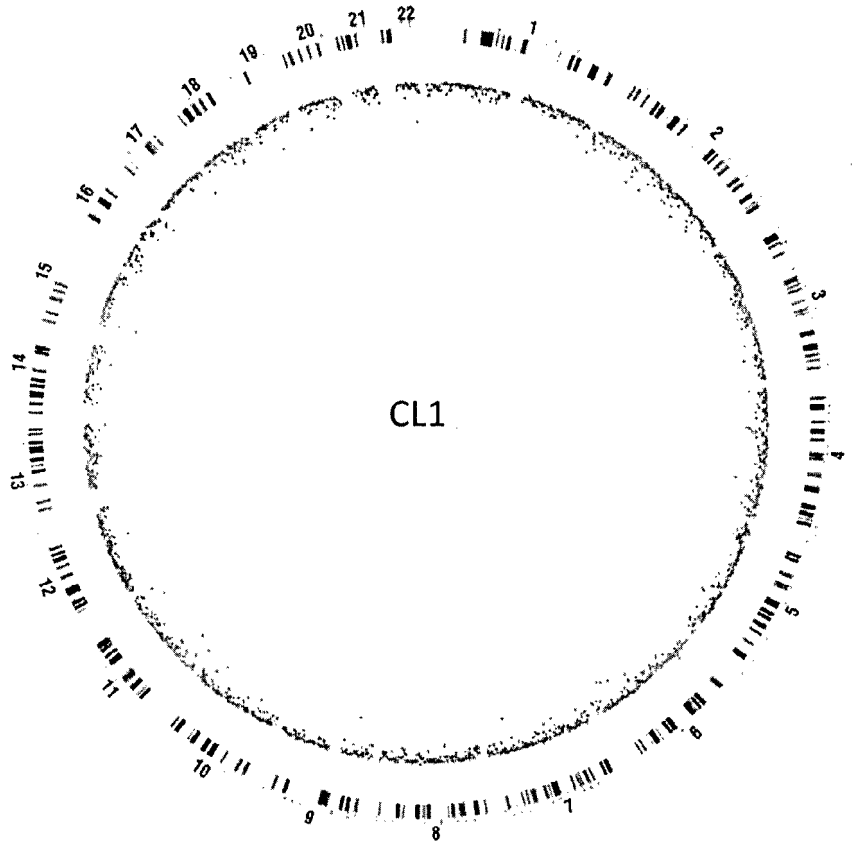


FIG. 27A

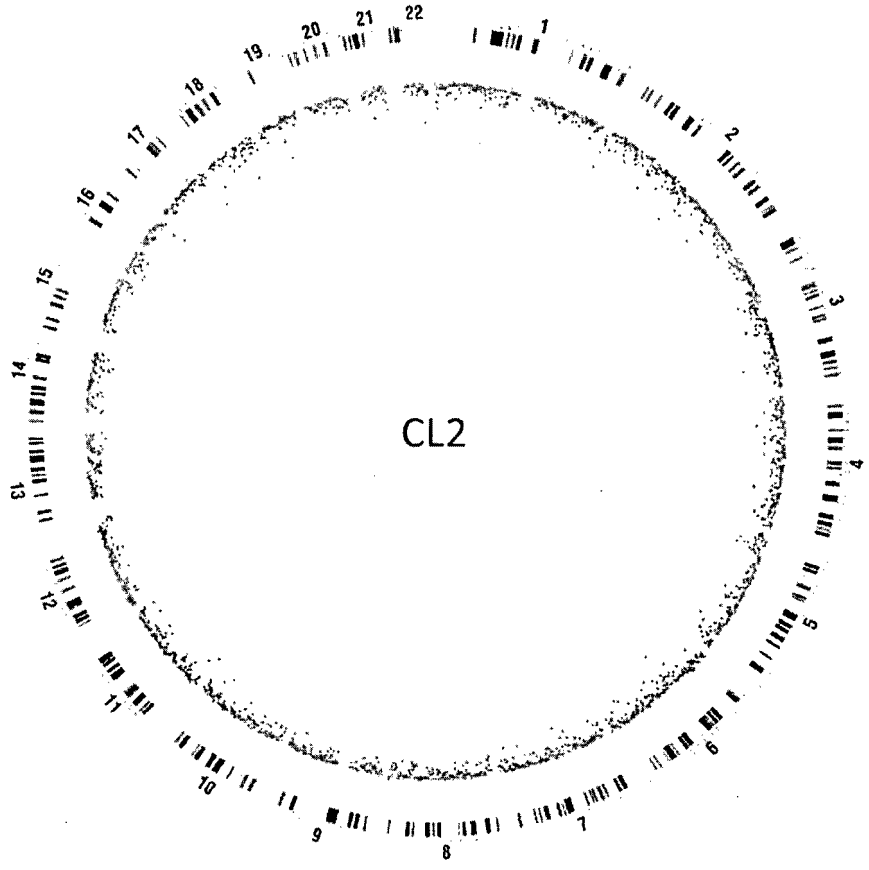


FIG. 27B

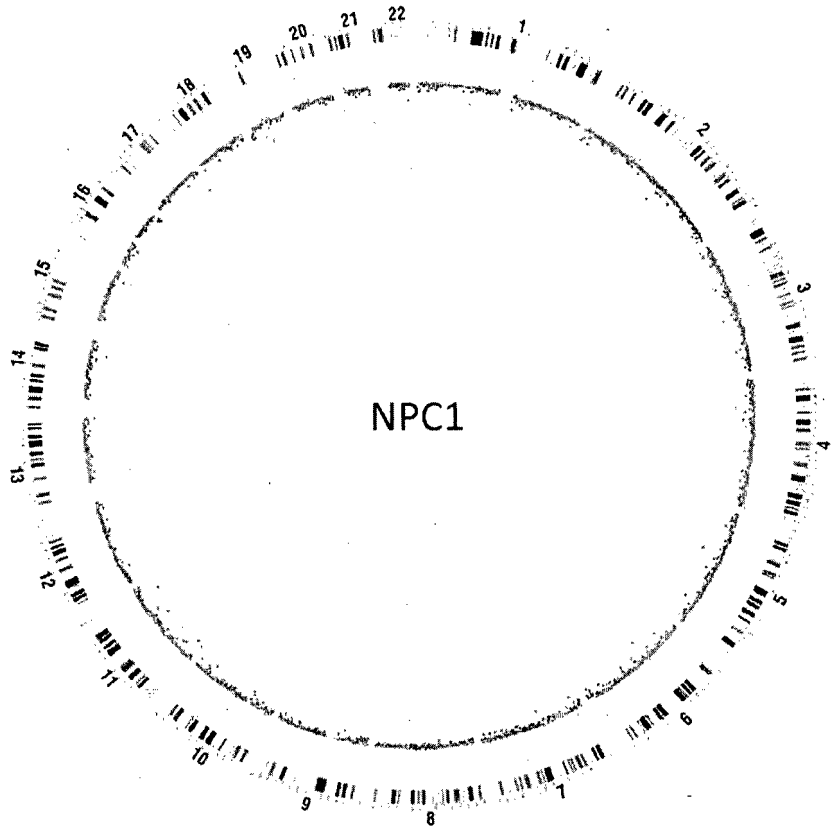


FIG. 27C

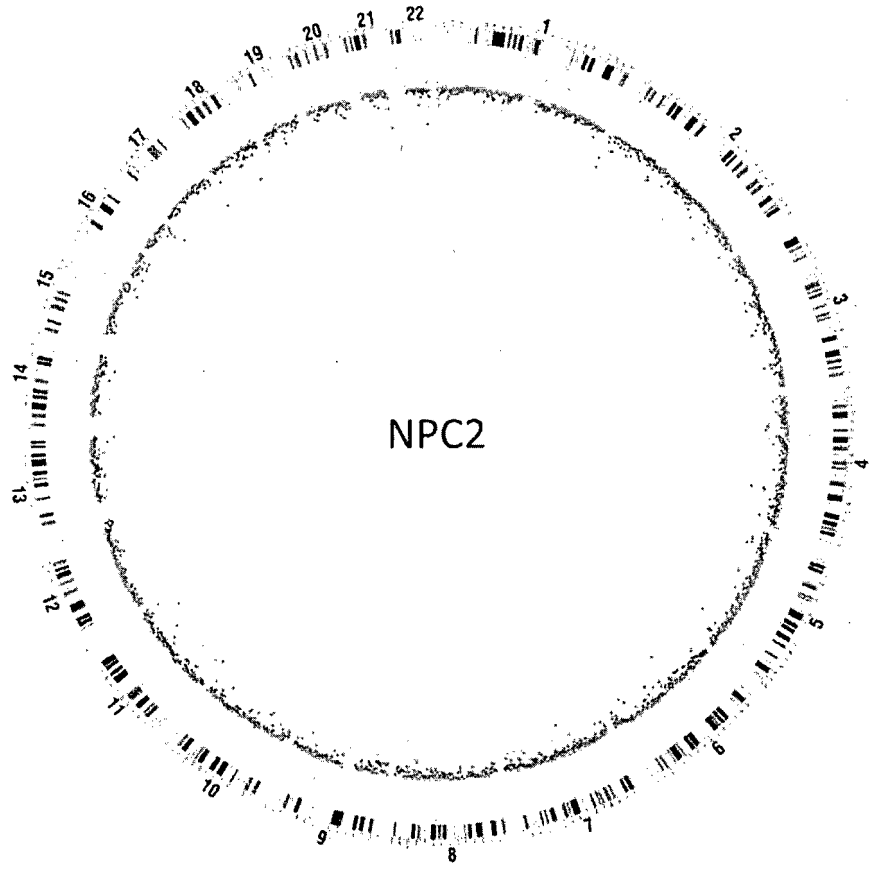


FIG. 27D

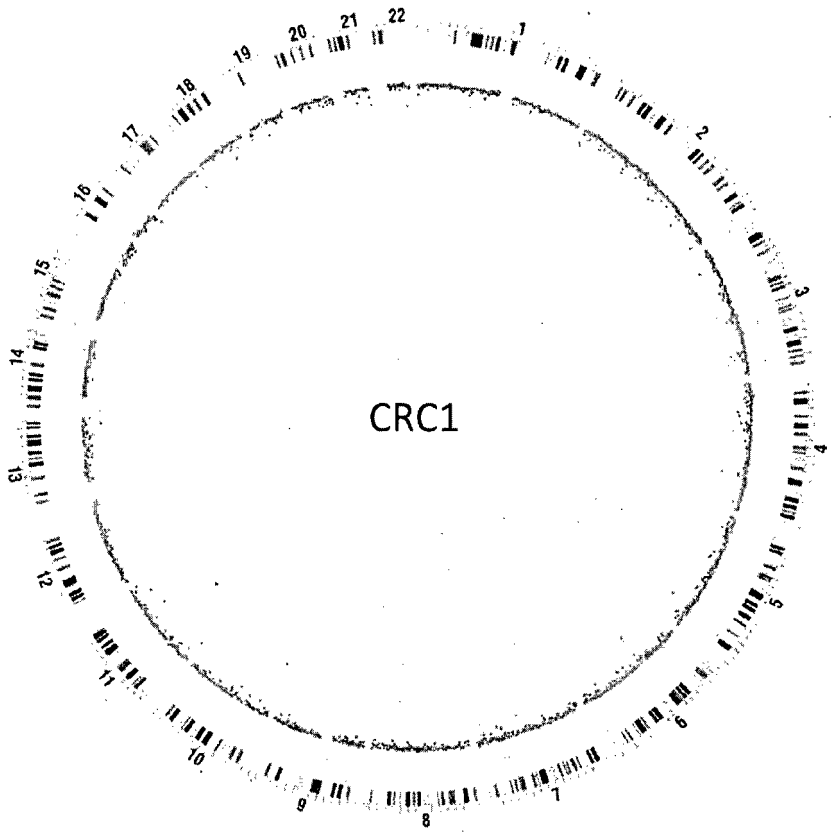


FIG. 27E

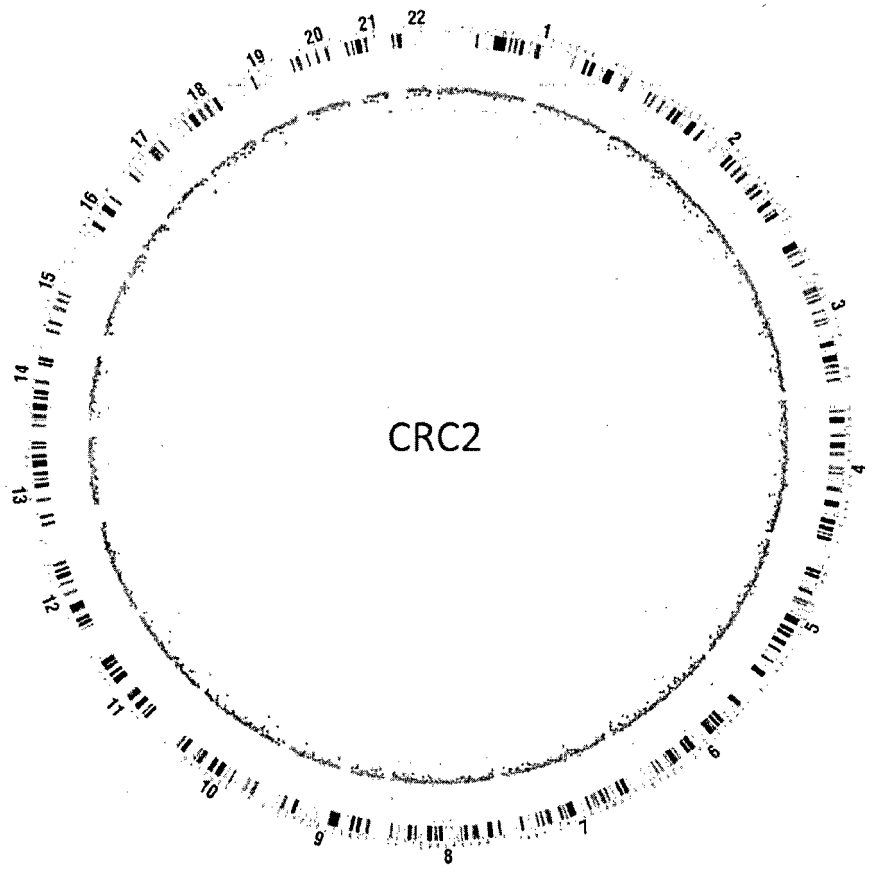


FIG. 27F

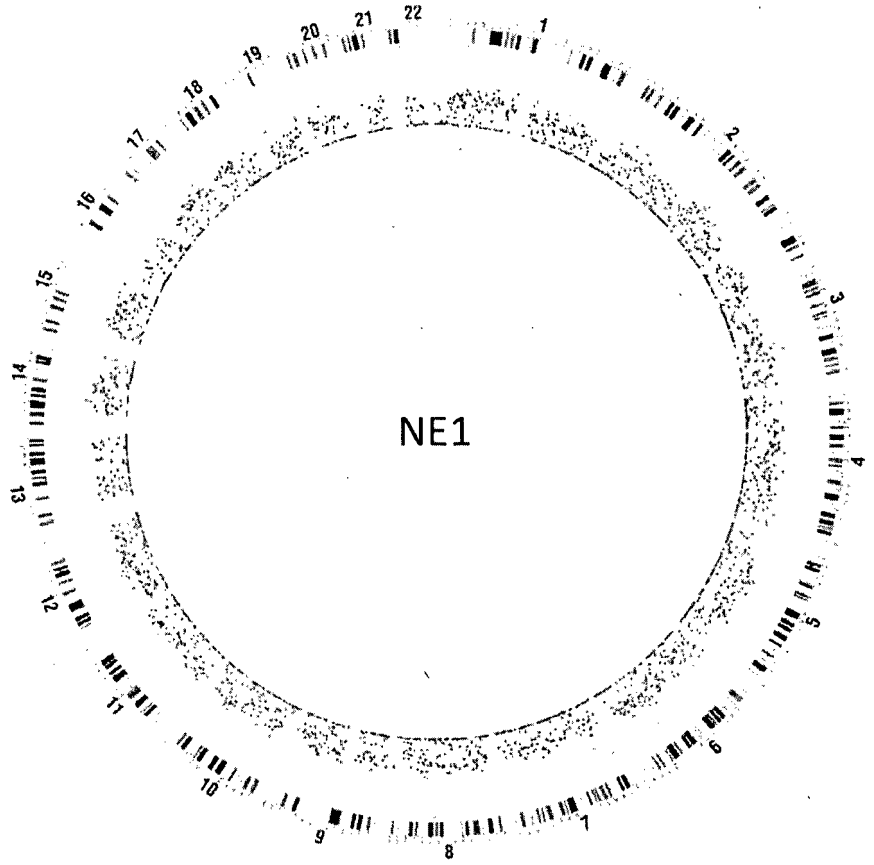


FIG. 27G

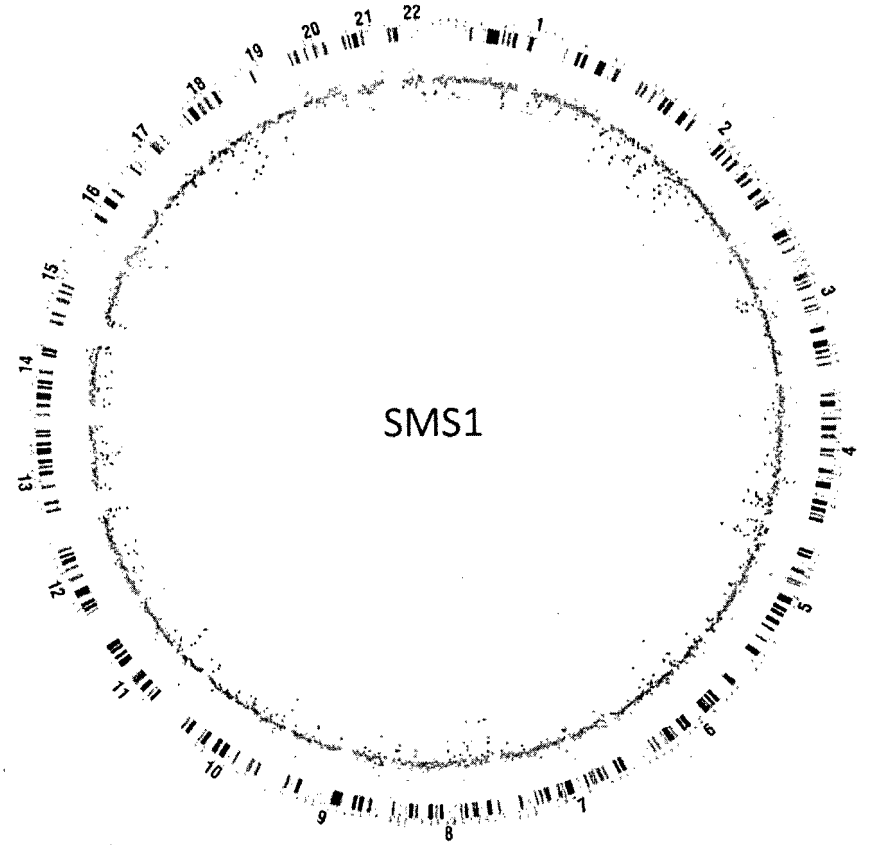


FIG. 27H

	raw read count	Mapped read count	Nonduplicate fragments	Proportion of reads usable %	Depth per strand	CpG coverage %	Depth on CpG sites with reads covered oer strand
CL1	120807588	91750018	76890830	64%	0.69	33.32%	2.87
CL2	101344850	76473792	66246435	65%	0.59	30.26%	2.79
NPC1	101883643	75281330	57236188	56%	0.51	27.87%	2.72
NPC2	102745073	74211781	56841611	55%	0.51	28.74%	2.70
CRC1	87732645	66456695	56112543	64%	0.50	27.38%	2.63
CRC2	109955730	82594007	67253101	61%	0.60	29.91%	2.90
NE1	105514000	79923333	64534726	61%	0.58	27.86%	2.58
SMS1	94727279	71698718	64426529	68%	0.58	29.23%	2.79

FIG. 27I

2780

z-score	CL1	CL2	NPC1	NPC2	CRC1	CRC2	NE1	SMS1
< -3	34.9%	33.9%	17.6%	54.3%	13.5%	9.8%	98.1%	11.2%
-3 to 3	65.0%	65.9%	82.4%	45.6%	86.5%	90.2%	1.7%	70.8%
>3	0.07%	0.15%	0.04%	0%	0.07%	0%	0.15%	18.0%

FIG. 27J

2790

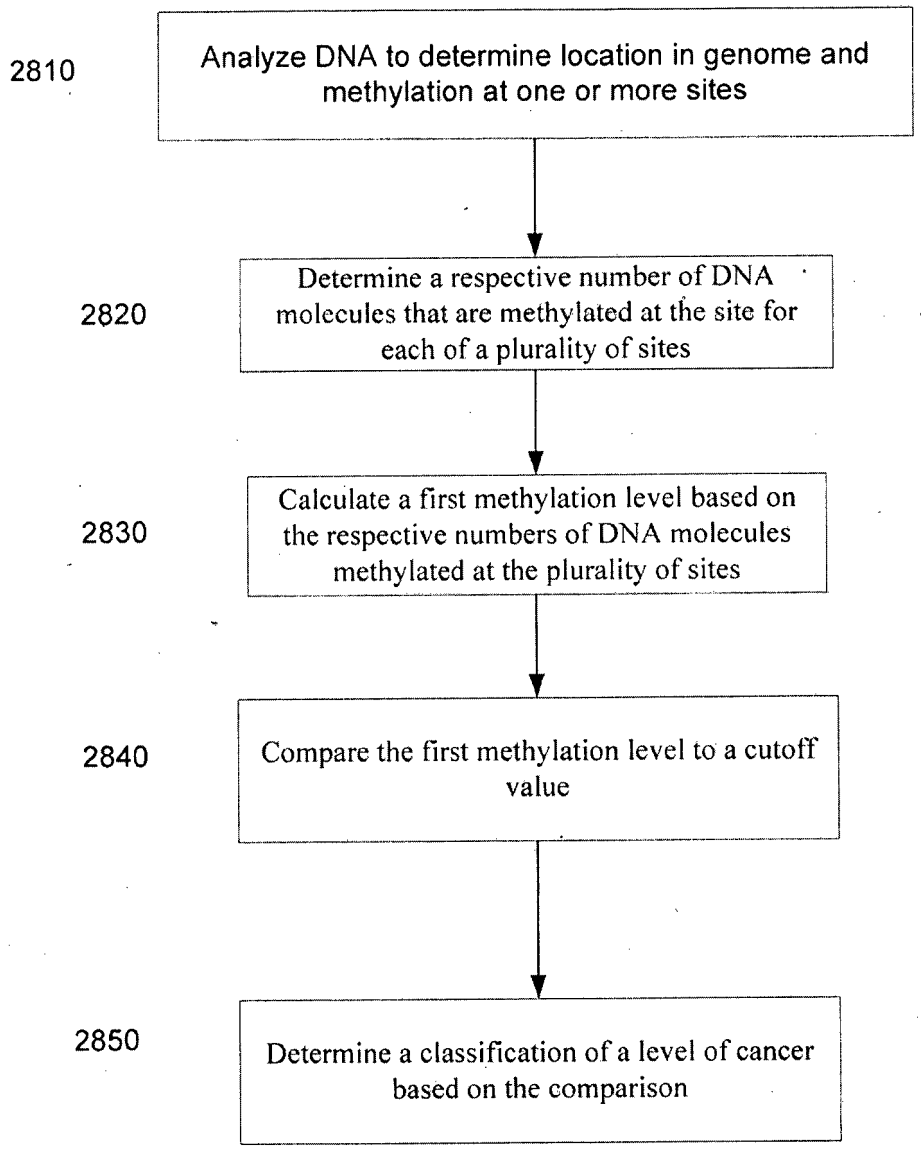

2810
Analyze DNA to determine location in genome and methylation at one or more sites
2820
Determine a respective number of DNA molecules that are methylated at the site for each of a plurality of sites
2830
Calculate a first methylation level based on the respective numbers of DNA molecules methylated at the plurality of sites
2840
Compare the first methylation level to a cutoff value
2850
Determine a classification of a level of cancer based on the comparison

FIG. 28

2800

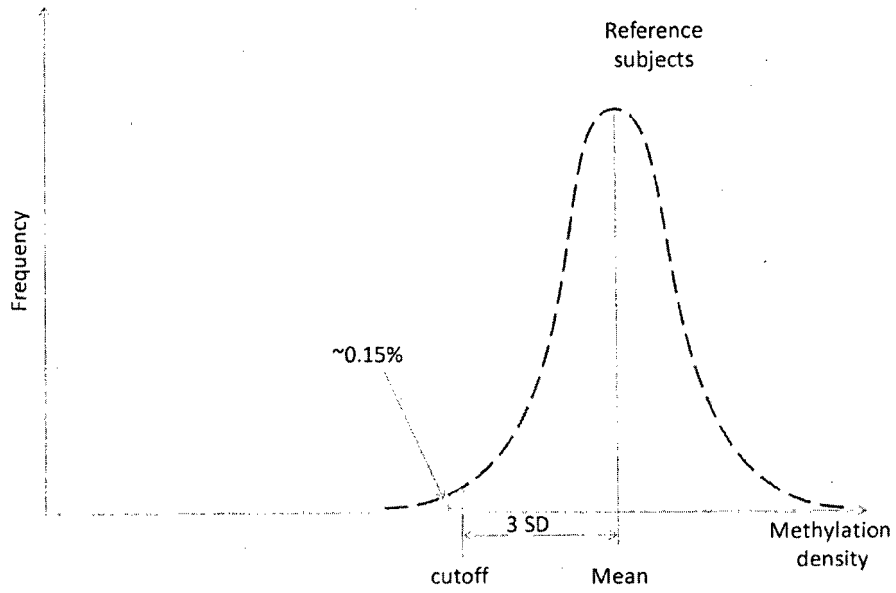


FIG. 29A 2900

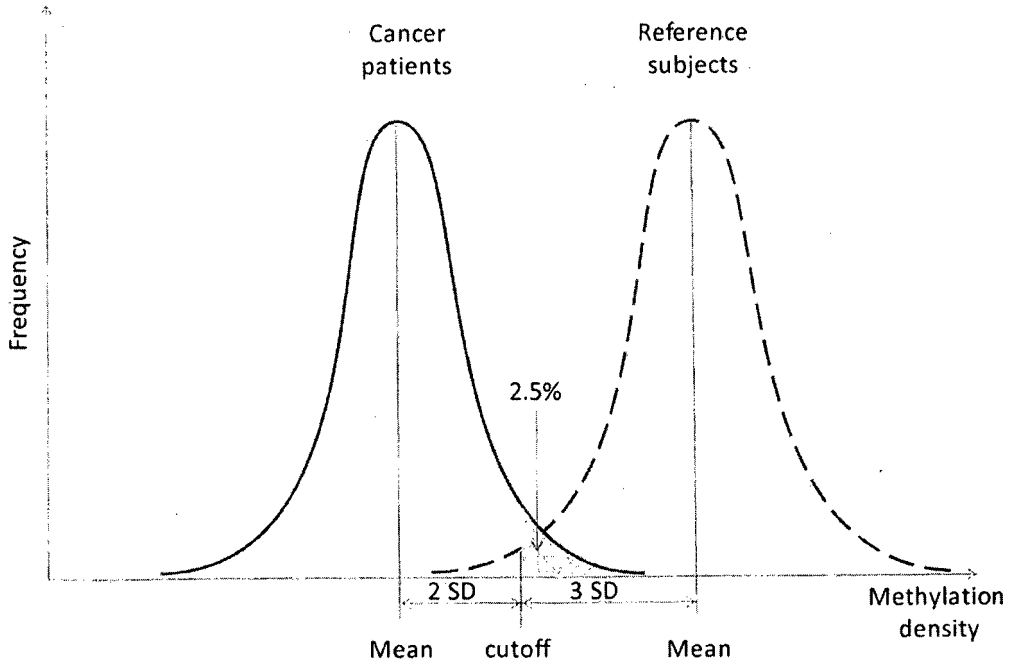


FIG. 29B 2950

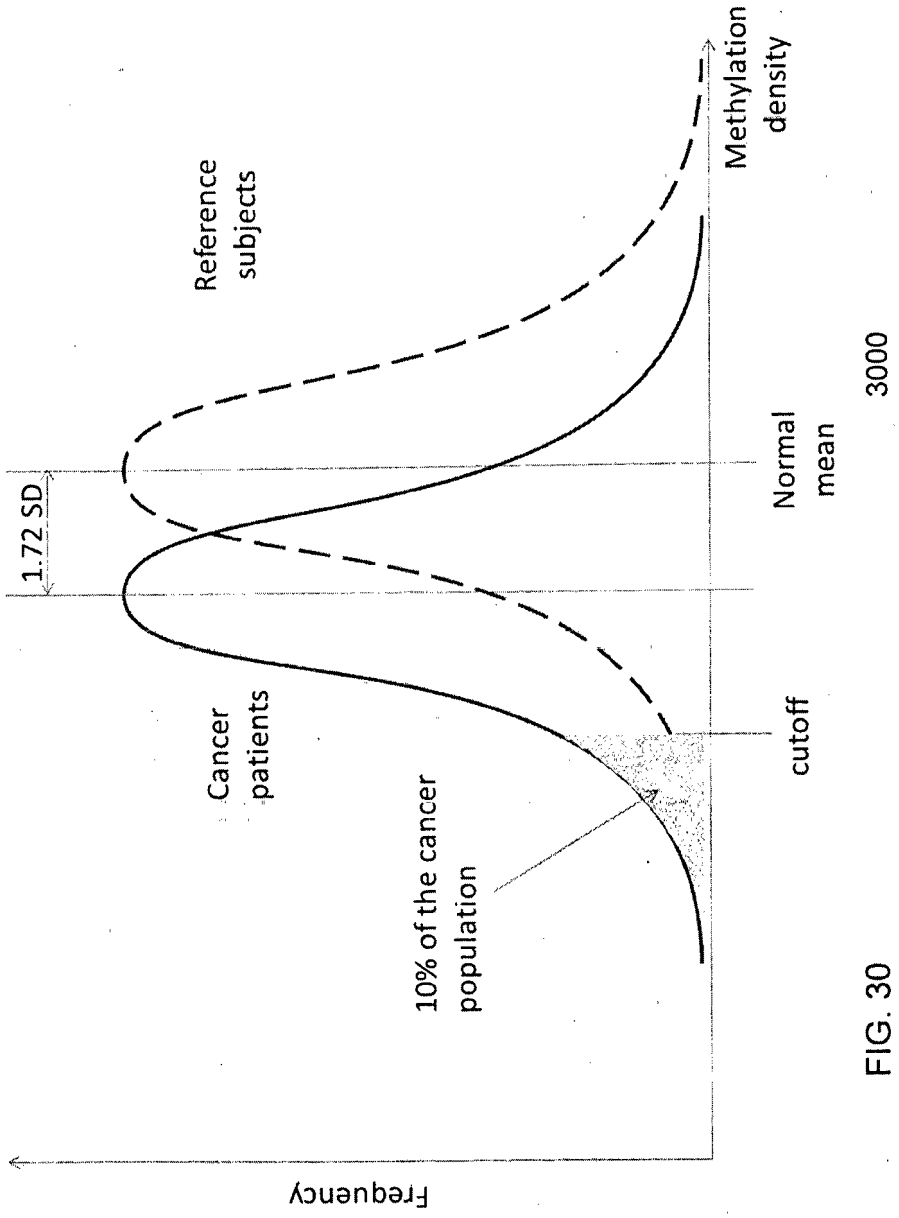

Methylation density
Reference subjects
1.72 SD
Normal mean
3000
Cancer patients
cutoff
10% of the cancer population
Frequency

FIG. 30

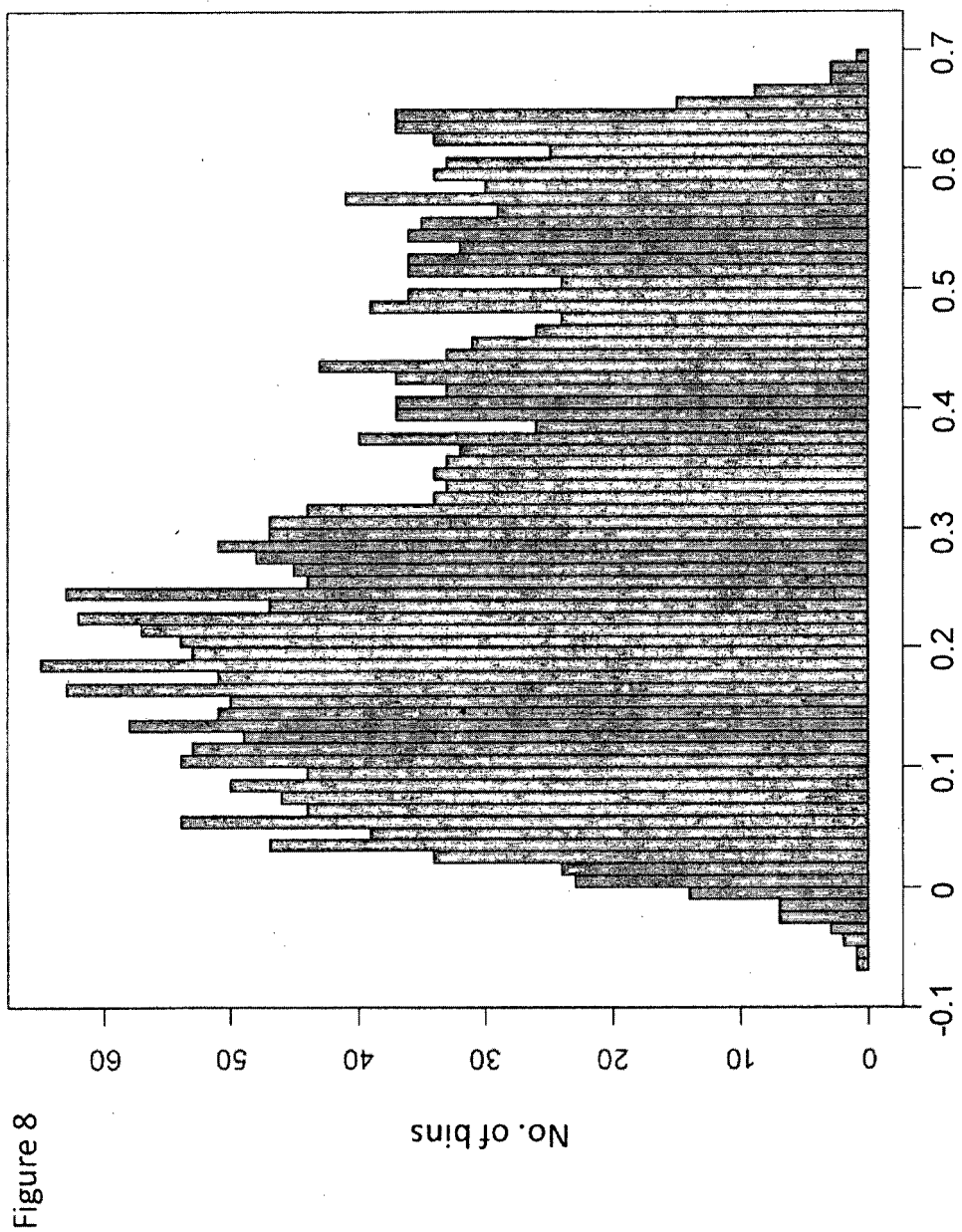


Figure 8

FIG. 31

depth diluting factor at 5%	Sequencing depth (times haploid genome)	No. of hypomethylated bins (z-scores <-3) in Pre-operative plasma	% of hypomethylated bins (z-scores <-3) in Pre-operative plasma
0.8	1.76	1314	48.06
0.6	1.32	1323	48.39
0.4	0.88	1220	44.62
0.2	0.44	1094	40.01
0.1	0.22	900	32.92
0.05	0.11	645	23.59
0.01	0.022	207	7.57

depth diluting factor at 2%	Sequencing depth (times haploid genome)	No. of hypomethylated bins (z-scores <-3) in Pre-operative plasma	% of hypomethylated bins (z-scores <-3) in Pre-operative plasma
0.8	1.76	491	17.96
0.6	1.32	493	18.03
0.4	0.88	460	16.83
0.2	0.44	439	16.06
0.1	0.22	356	13.02
0.05	0.11	297	10.86
0.01	0.022	231	8.45

FIG. 32A 3200

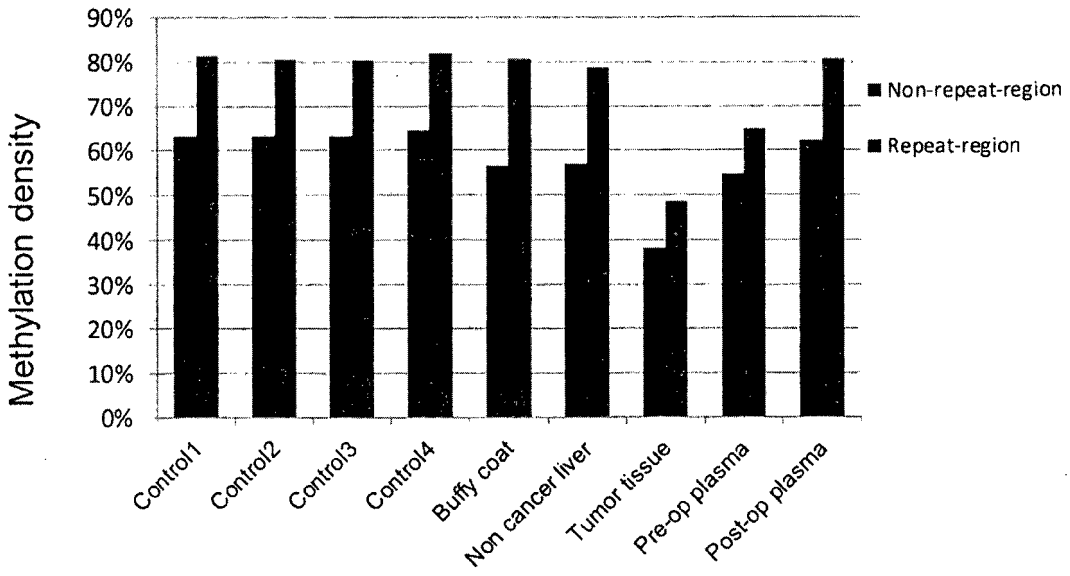


FIG. 32B 3250

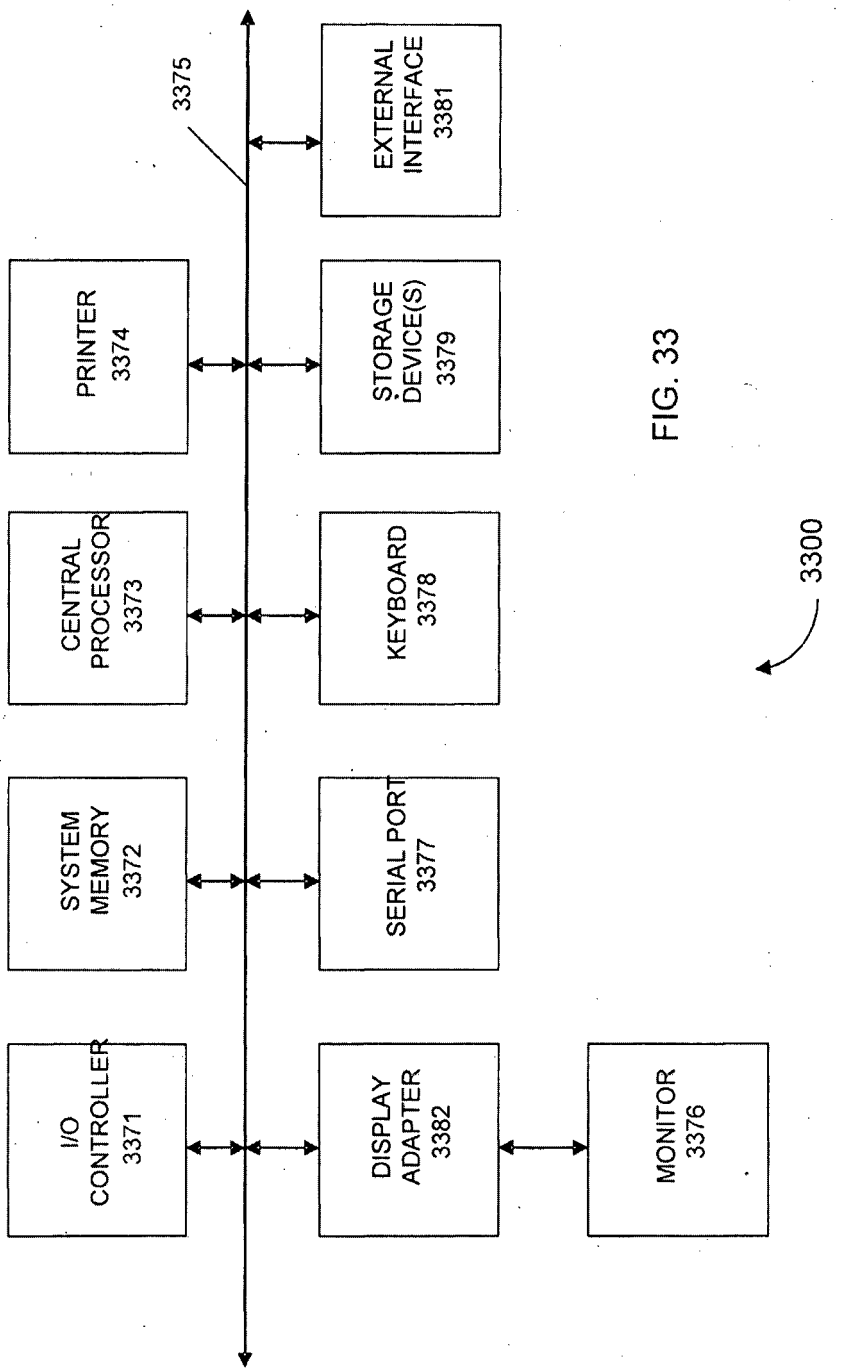

3375
EXTERNAL INTERFACE 3381
PRINTER 3374
STORAGE DEVICE(S) 3379
CENTRAL PROCESSOR 3373
KEYBOARD 3378
SYSTEM MEMORY 3372
SERIAL PORT 3377
I/O CONTROLLER 3371
DISPLAY ADAPTER 3382
MONITOR 3376
3300

FIG. 33

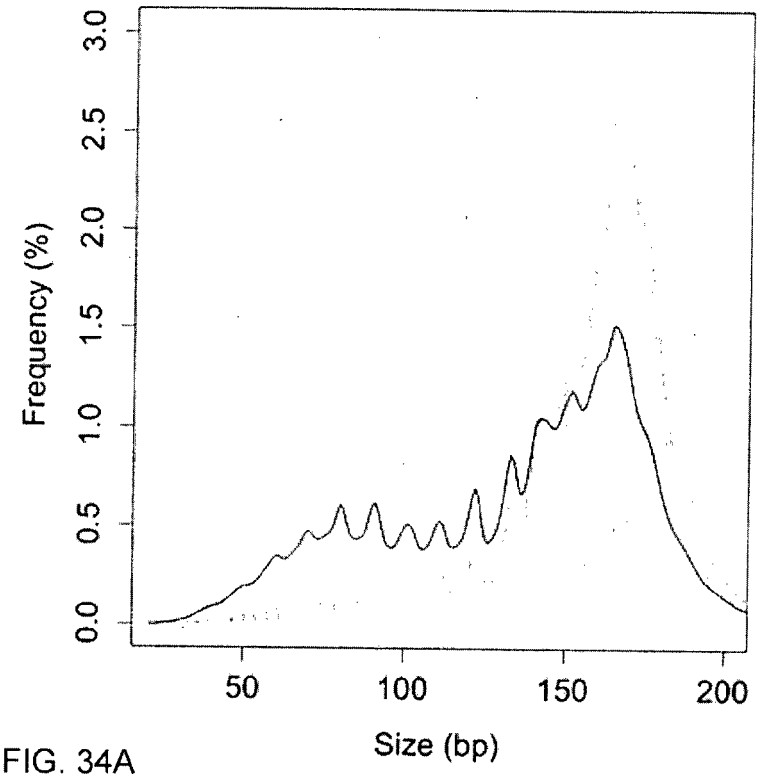


FIG. 34A

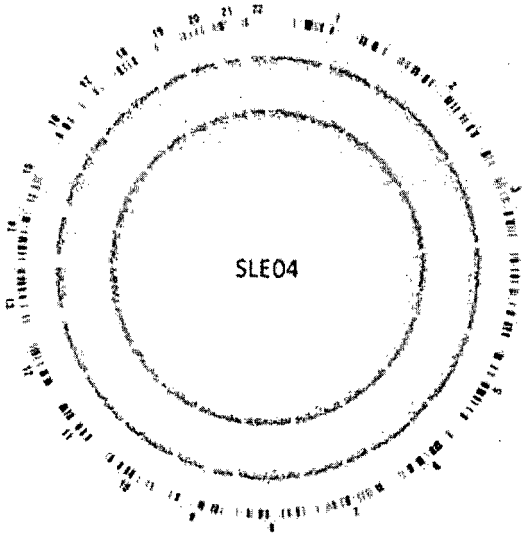


FIG. 34B

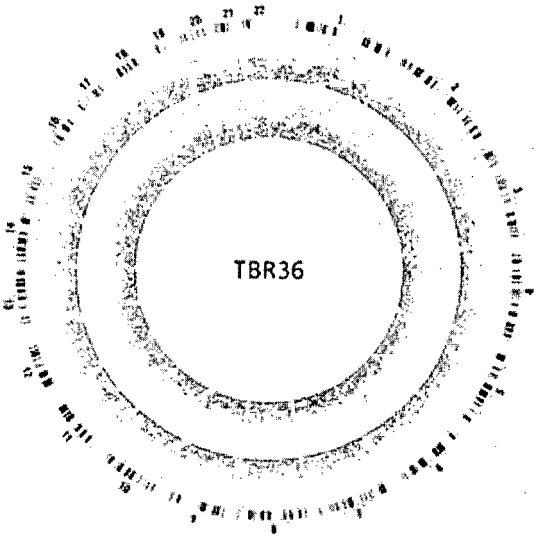


FIG. 34C

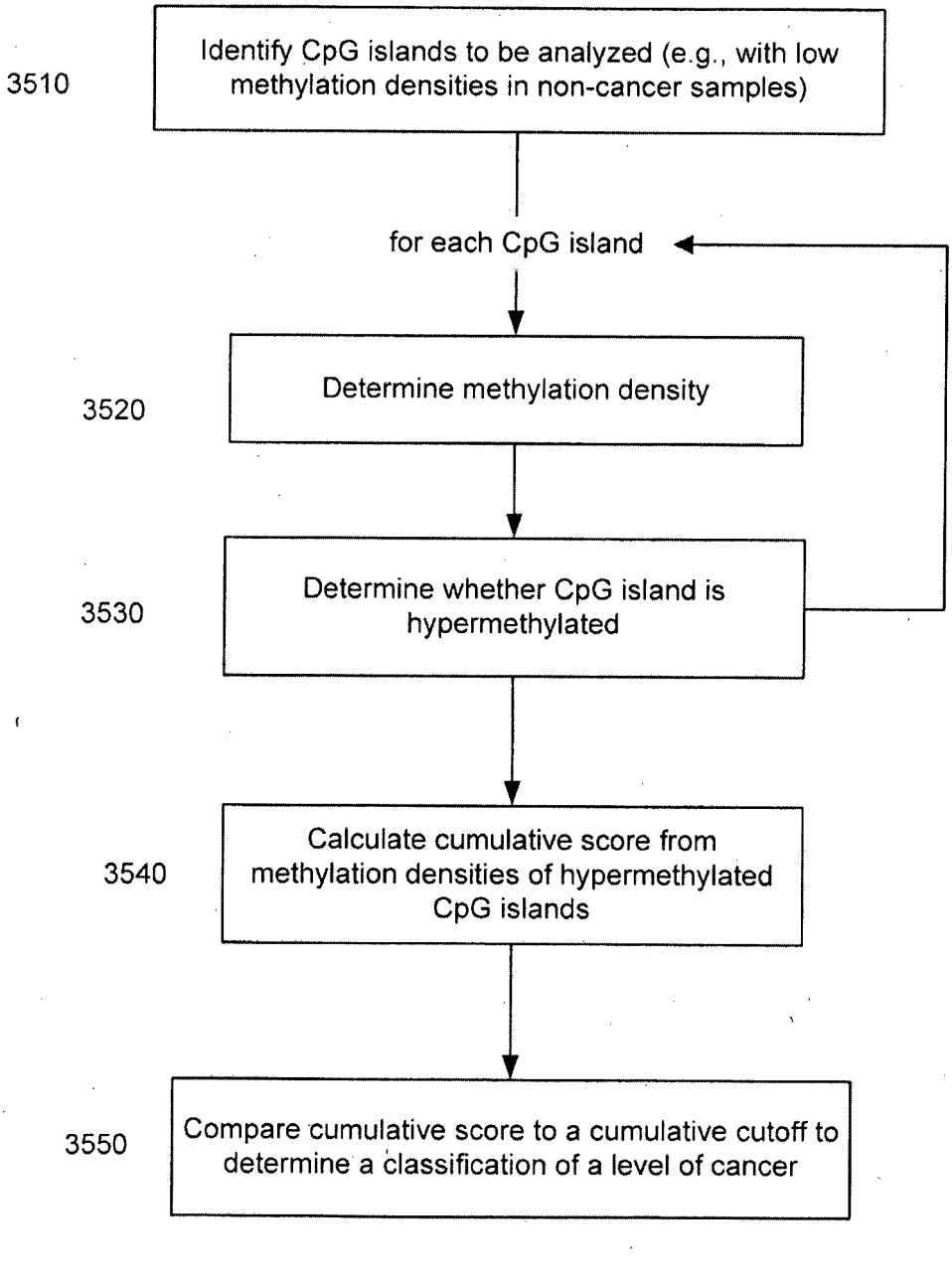

3510
Identify CpG islands to be analyzed (e.g., with low methylation densities in non-cancer samples)
for each CpG island
3520
Determine methylation density
3530
Determine whether CpG island is hypermethylated
3540
Calculate cumulative score from methylation densities of hypermethylated CpG islands
3550
Compare cumulative score to a cumulative cutoff to determine a classification of a level of cancer
3500

FIG. 35

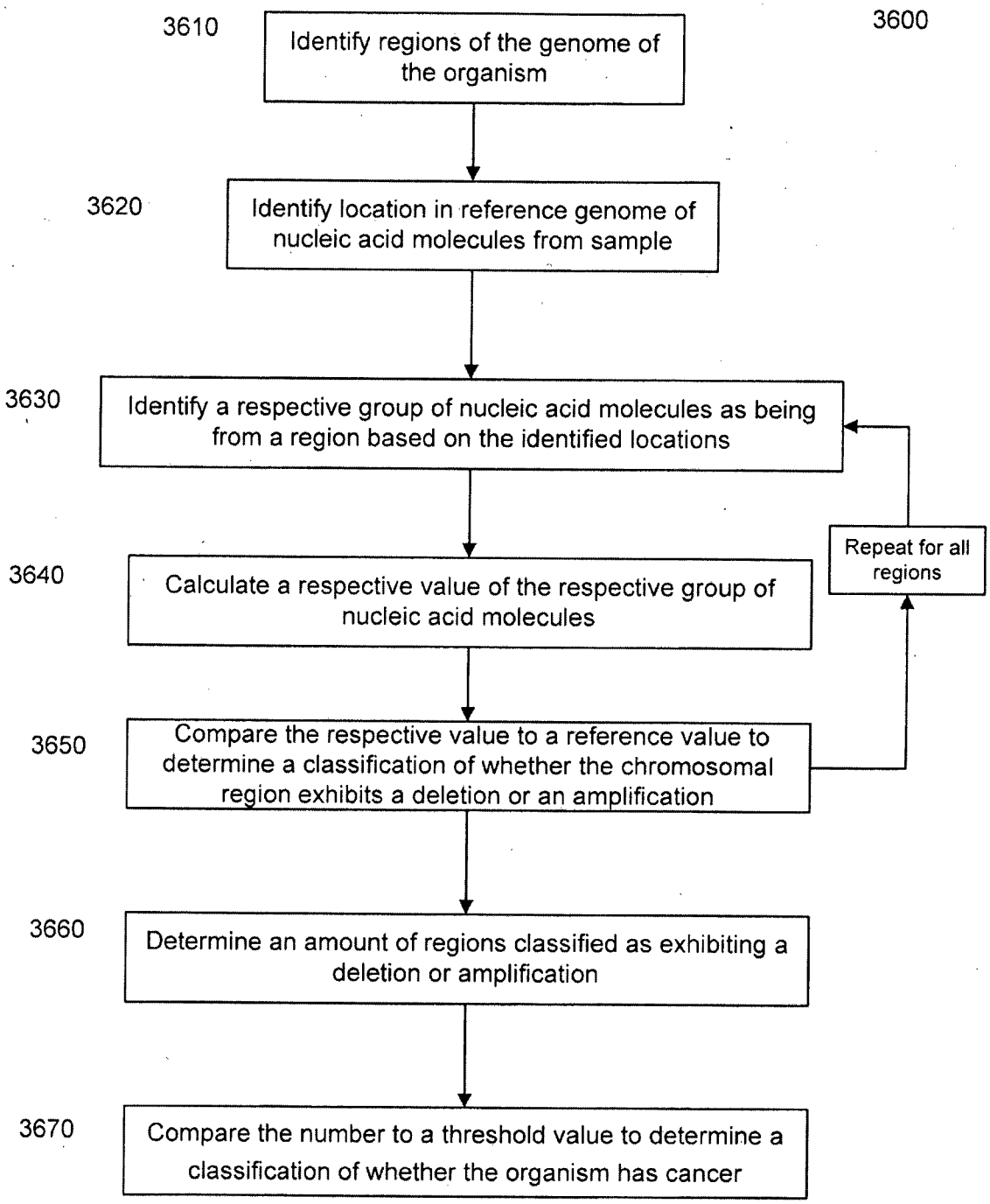

3600
3610
Identify regions of the genome of the organism
3620
Identify location in reference genome of nucleic acid molecules from sample
3630
Identify a respective group of nucleic acid molecules as being from a region based on the identified locations
Repeat for all regions
3640
Calculate a respective value of the respective group of nucleic acid molecules
3650
Compare the respective value to a reference value to determine a classification of whether the chromosomal region exhibits a deletion or an amplification
3660
Determine an amount of regions classified as exhibiting a deletion or amplification
3670
Compare the number to a threshold value to determine a classification of whether the organism has cancer

FIG. 36

Sample: TBR36
Tumor DNA fraction: 40%

From inside to outside:
1) Tumor CNA
2) Plasma CNA (non-BS)
3) Plasma CNA (BS)

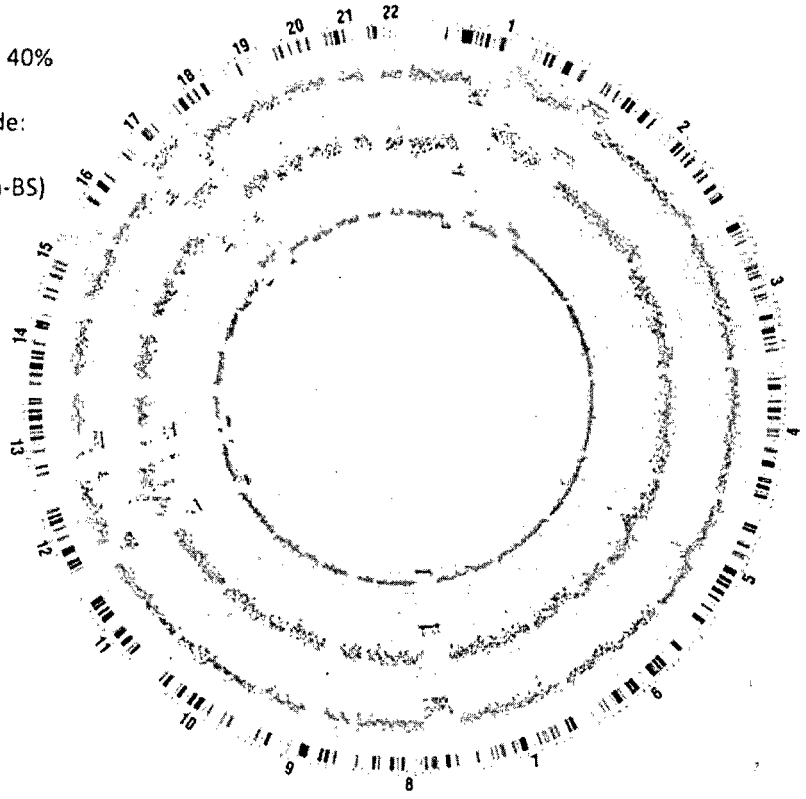


FIG. 37A

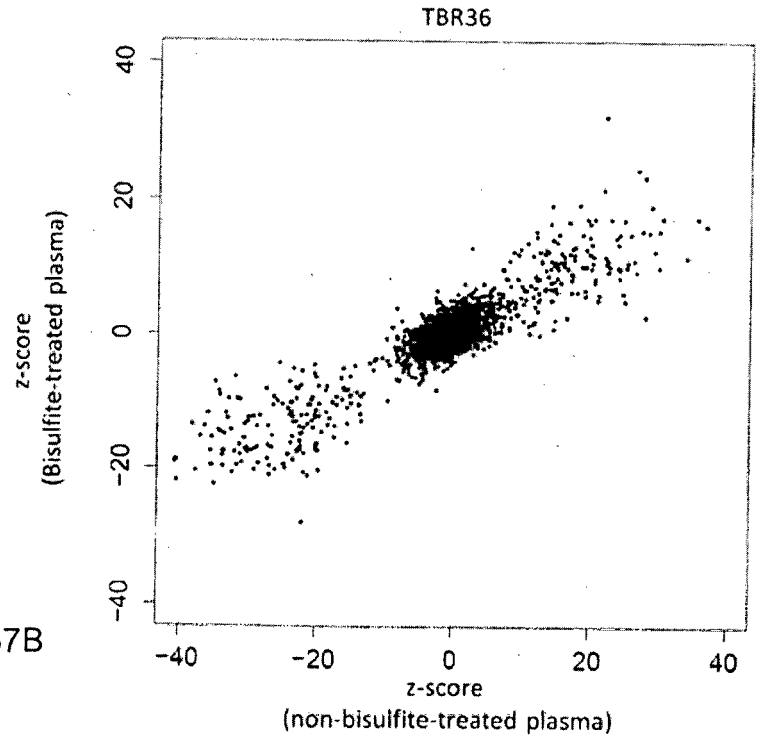


FIG. 37B

Sample: TBR34
Tumor DNA fraction: 30%

From inside to outside:
1) Tumor CNA
2) Plasma CNA (non-BS)
3) Plasma CNA (BS)

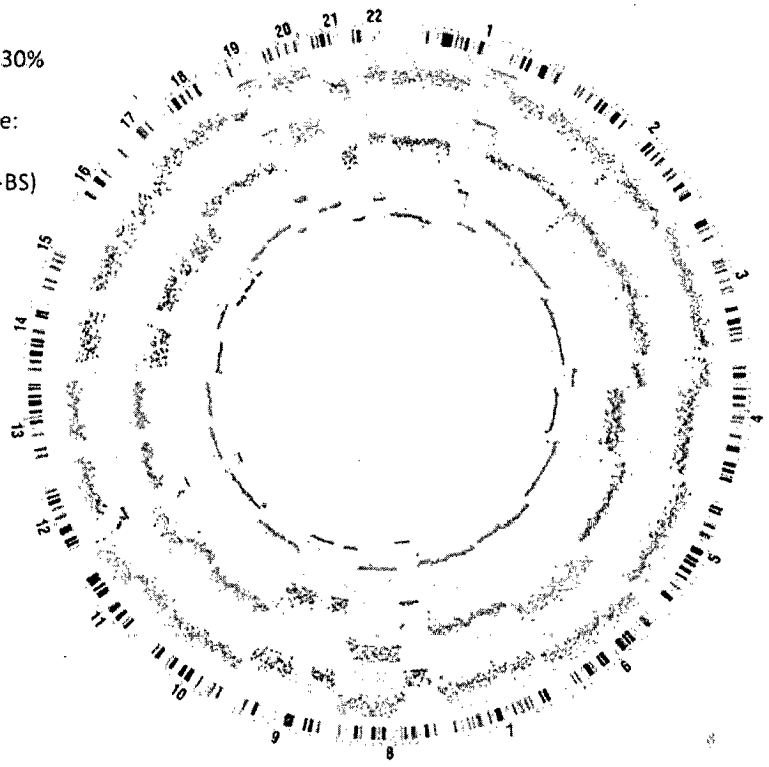


FIG. 38A

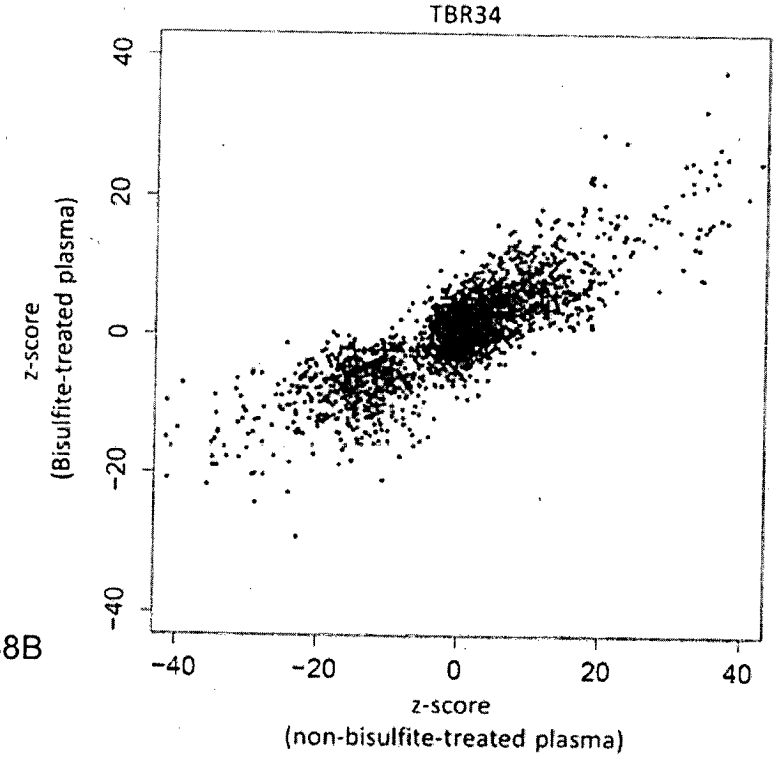


FIG. 38B

Sample: TBR240
Tumor size: 2.8cm x 2.3cm

From inside to outside:
1) Plasma CNA z-score
2) Plasma methylation z-score

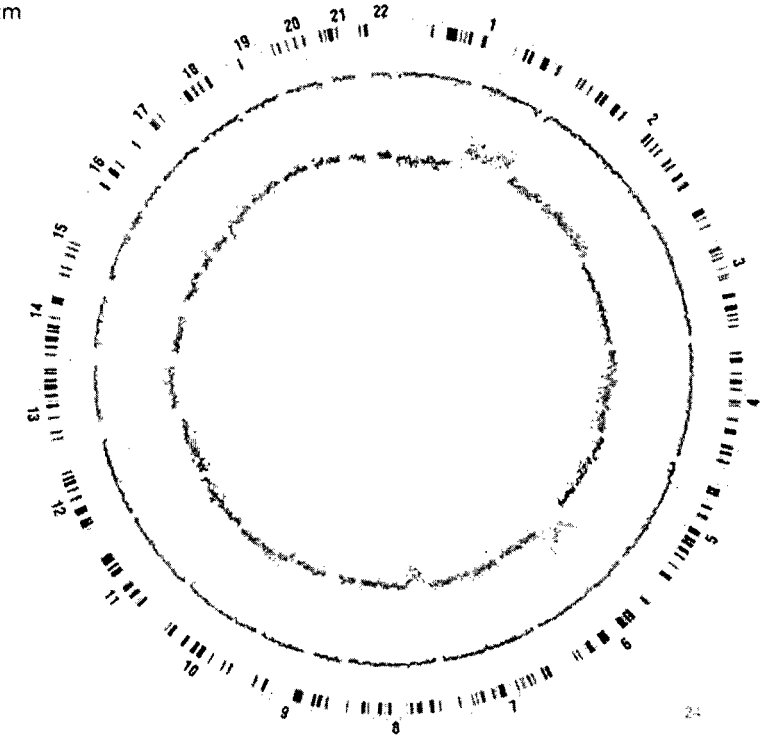


FIG. 39A

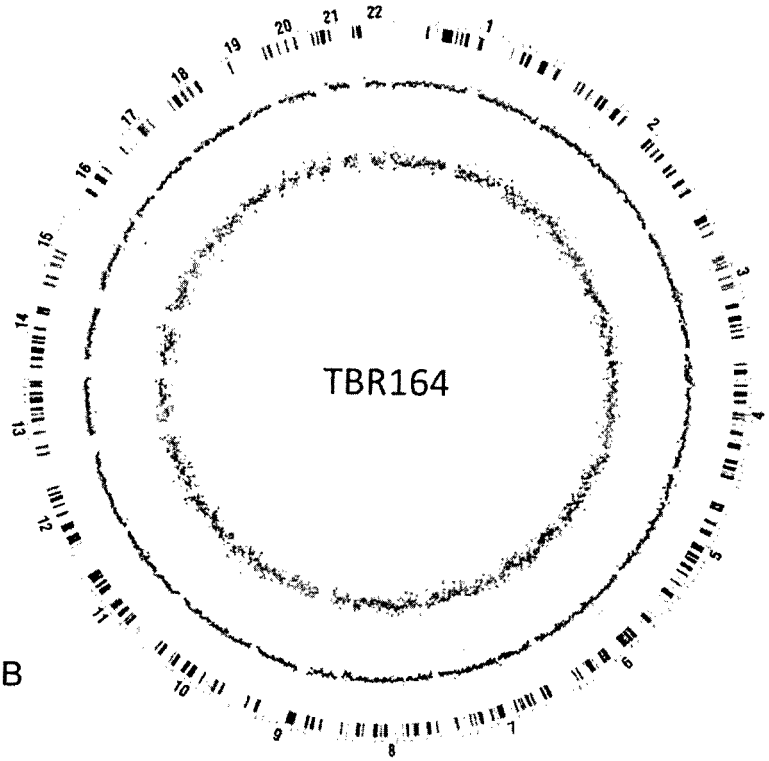


FIG. 39B

Inner ring:
Pre-treatment plasma

Outer ring:
Post-treatment plasma

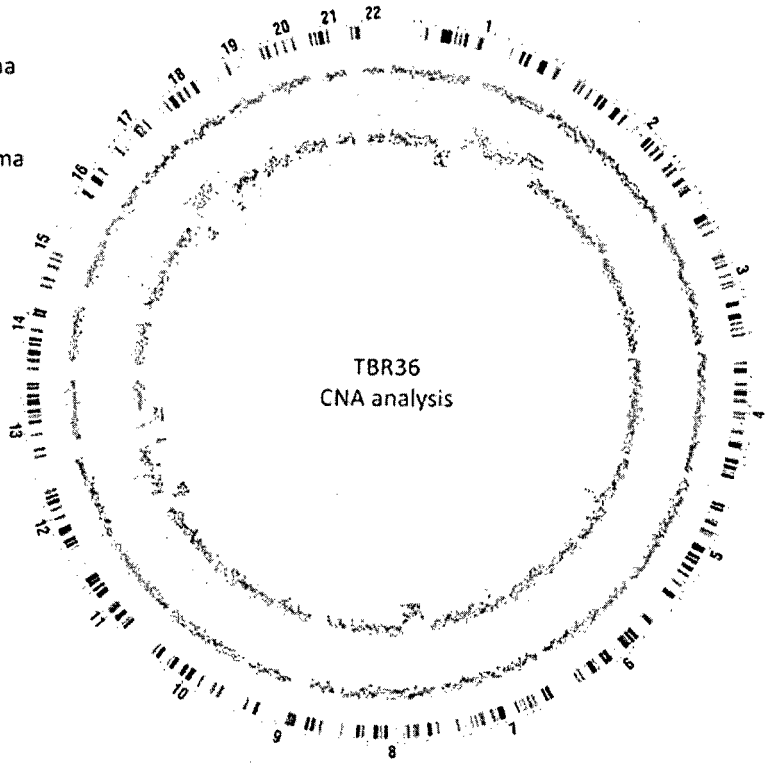


FIG. 40A

Inner ring:
Pre-treatment plasma

Outer ring:
Post-treatment plasma

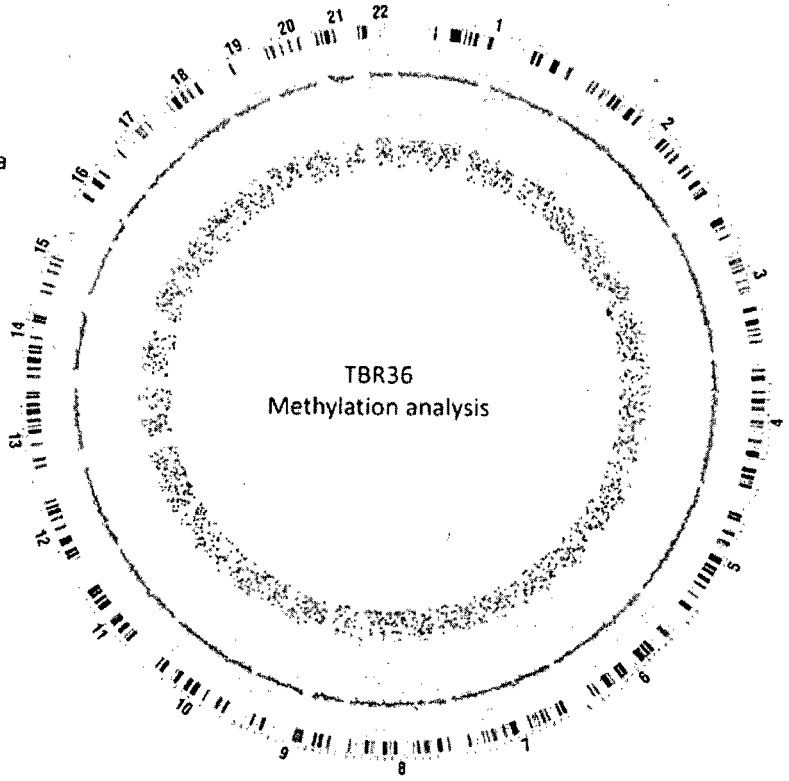


FIG. 40B

Sample: TBR34
Tumor DNA fraction: 30%
From outside to inside:
1) Post-op CNV
2) Pre-op CNV

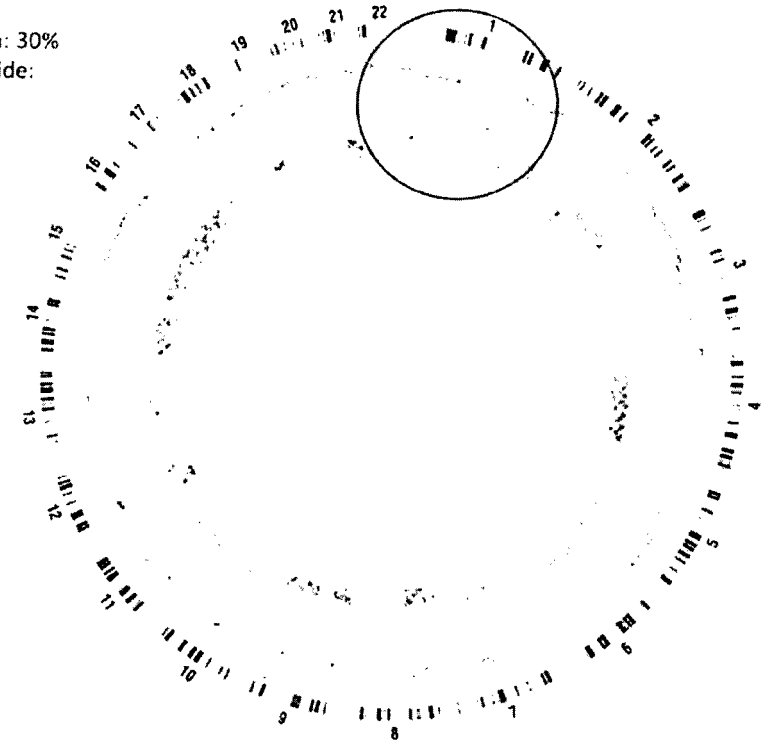


FIG. 41A

Inner ring:
Pre-treatment plasma

Outer ring:
Post-treatment plasma

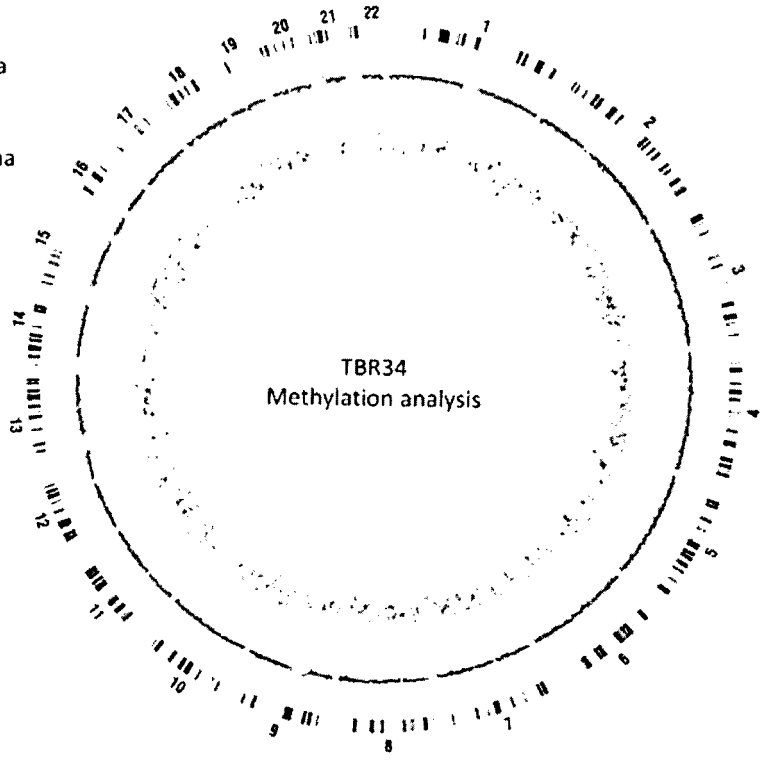


FIG. 41B

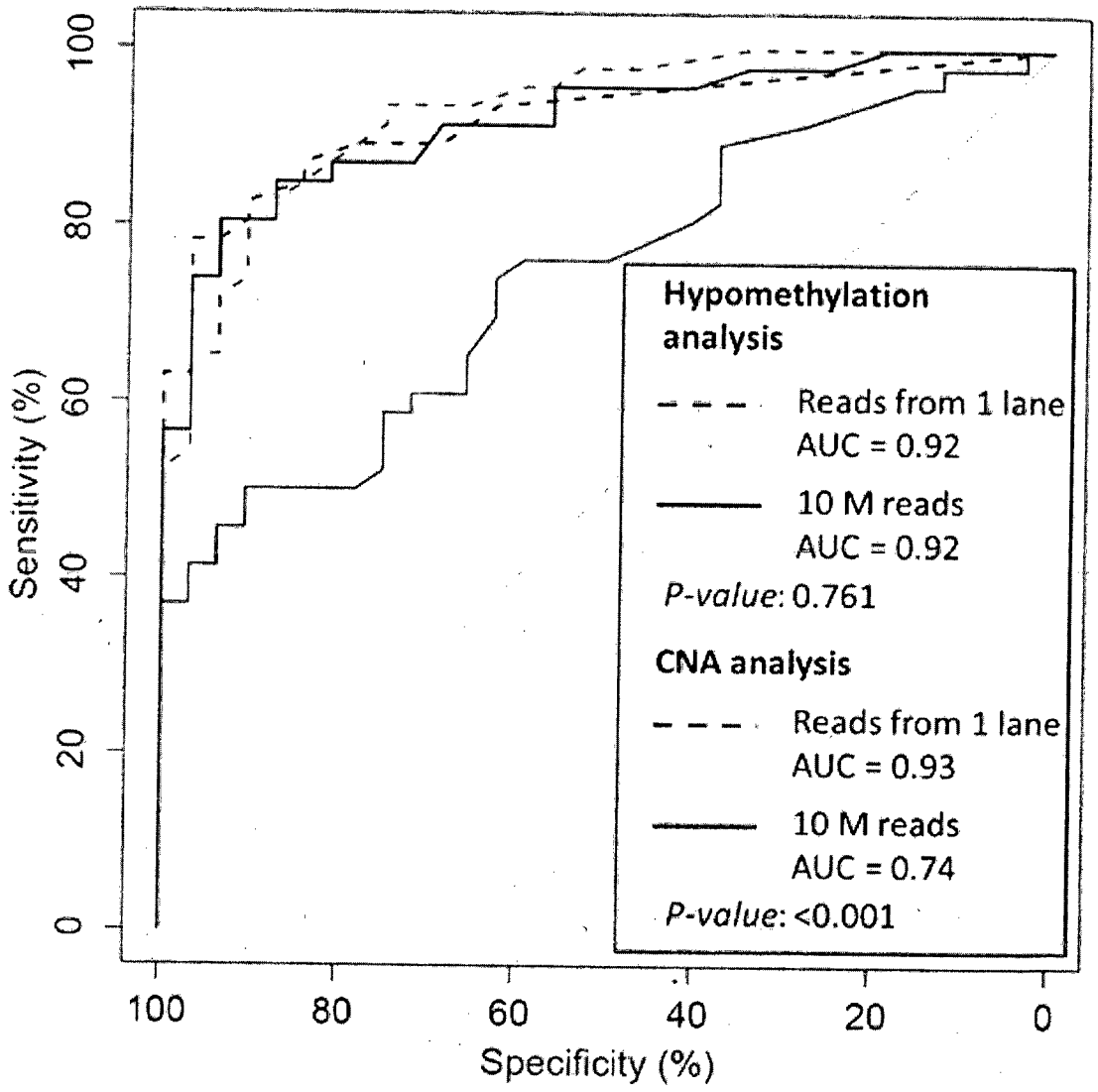

Sensitivity (%)
100
80
60
40
20
0
100
80
60
40
20
0
Specificity (%)
Hypomethylation analysis
Reads from 1 lane
AUC = 0.92
10 M reads
AUC = 0.92
P-value: 0.761
CNA analysis
Reads from 1 lane
AUC = 0.93
10 M reads
AUC = 0.74
P-value: <0.001

FIG. 42

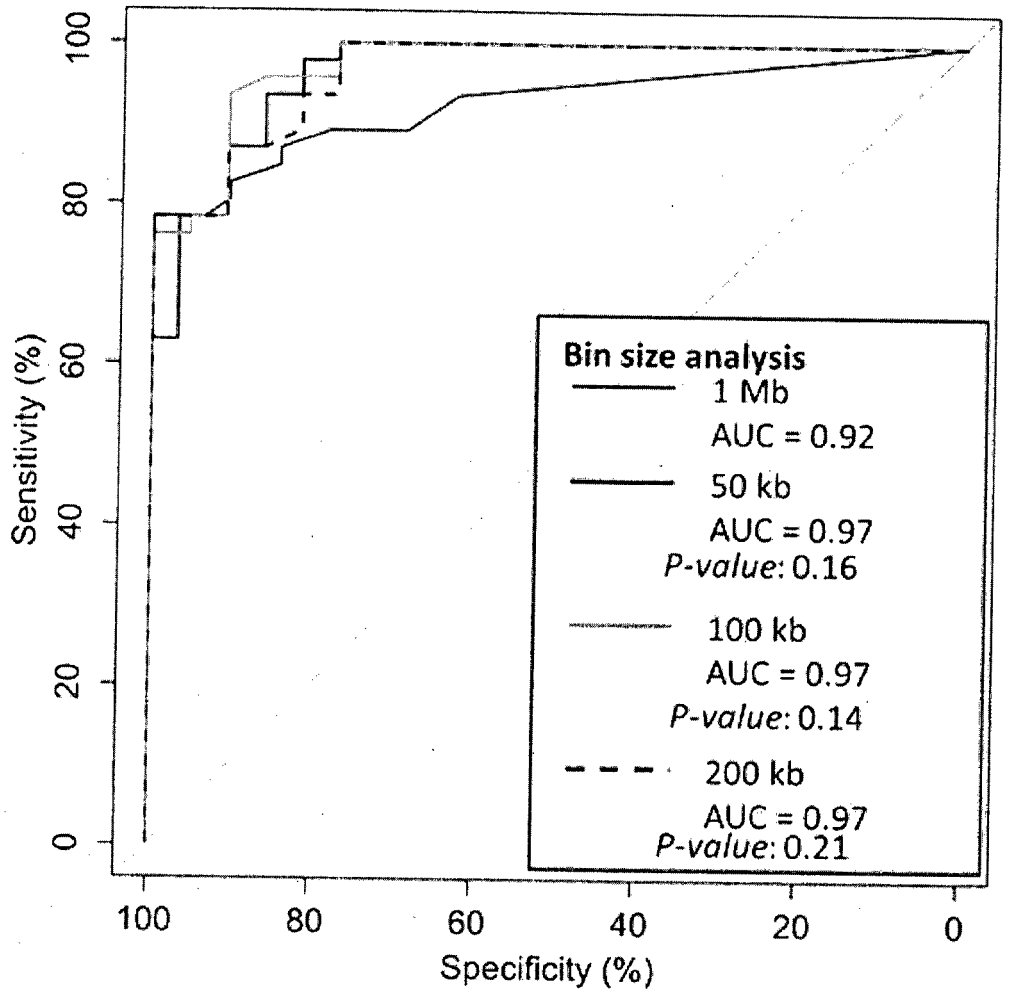

Sensitivity (%)
100
80
60
40
20
0
100
80
60
40
20
0
Specificity (%)
Bin size analysis
1 Mb
AUC = 0.92
50 kb
AUC = 0.97
P-value: 0.16
100 kb
AUC = 0.97
P-value: 0.14
200 kb
AUC = 0.97
P-value: 0.21

FIG. 43

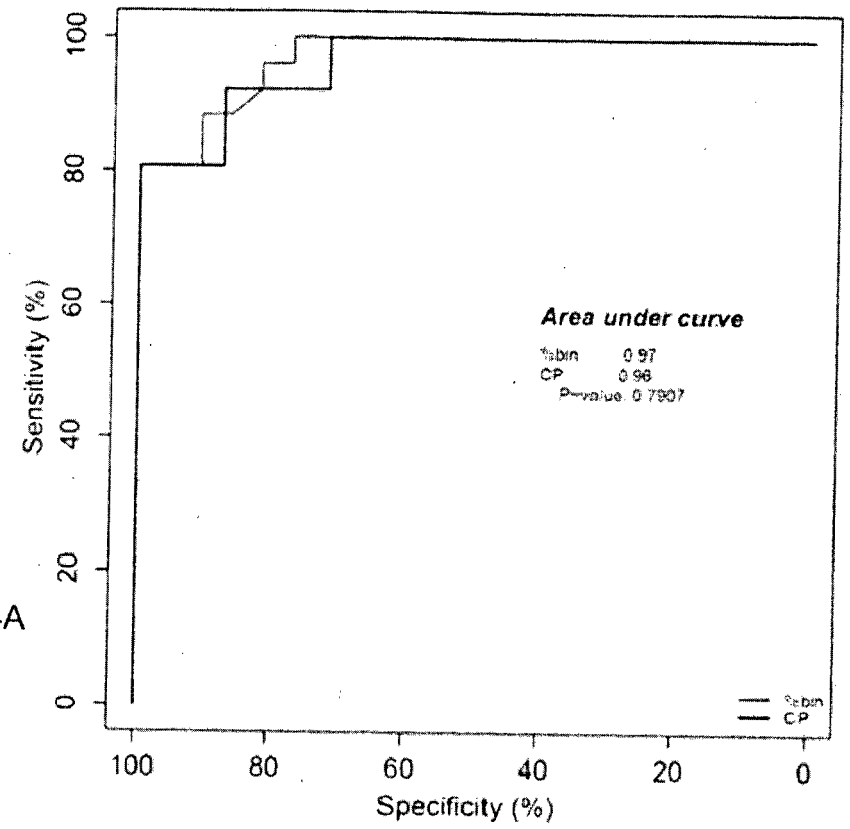


FIG. 44A

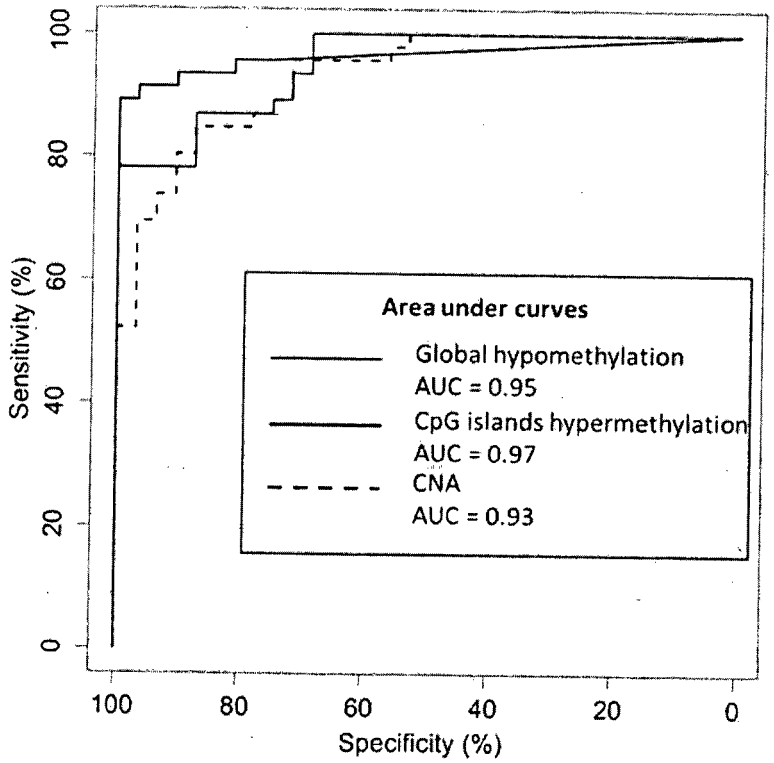


FIG. 44B

Case no	BCLC stage	Tumor(s) largest dimension (cm)	CP score			Presence of any type of changes
			General hypomethylation	Hypermethylation of CpG islands	CNA	
TBR36	A1	16	**88585**	**80.9**	**3777**	Positive
TBR34	A1	13	**37573**	**221.7**	**8650**	Positive
HOT198	A1	12	**93132**	**52.5**	**6803**	Positive
HOT205	A1	6	**1146**	1.7	175	Positive
HOT192	A1	5.5	**25914**	**119.5**	**1668**	Positive
HOT227	A1	5.5	**36333**	**155.4**	**5008**	Positive
HOT238	A1	5.5	**12187**	**105.3**	**1978**	Positive
HOT197	B	5.3	**1714**	**9.5**	28	Positive
HOT156	A1	5	**16529**	**115.9**	**1947**	Positive
HOT236	A1	3.7	**7993**	**5.9**	85	Positive
HOT170	A1	3.5	**22271**	**81.9**	**1542**	Positive
HOT229	A1	3.1	383	**5.4**	71	Positive
HOT233	A1	3	189	**7.5**	**322**	Positive
HOT240	A1	2.8	**1277**	**14.0**	**972**	Positive
HOT222	A1	2.6	**18832**	**10.5**	172	Positive
HOT162	A1	2.5	**26112**	**41.0**	**3048**	Positive
HOT172	A1	2.5	**12909**	**98.1**	**1305**	Positive
HOT164	A1	2.3	**12604**	**4.8**	210	Positive
HOT215	A1	2.3	**25824**	**54.8**	**714**	Positive
HOT207	A1	2.1	**3377**	**12.0**	101	Positive
HOT224	A1	2	367	0.0	13	Negative
HOT159	A1	1.5	**1367**	1.4	26	Positive
HOT204	A1	1.5	102	**4.5**	198	Positive
HOT151	A1	1.5	153	2.8	9	Negative
HOT208	A1	1.2	**5867**	**4.7**	**214**	Positive
HOT167	A1	1	**21544**	**4.5**	54	Positive

FIG. 45

Cancer type	Case no.	Staging / tumor max dimension	CP score			Presence of either type of changes
			General hypomethylation	Hypermethylation of Cp G islands	CNA	
Breast cancer	TBR150	Metastatic	**14581**	**146.7**	**5123**	Positive
	TBR126	T2N1M0 4.5 cm	**10415**	**34.9**	**1951**	Positive
	TBR127	T2N1M0 2.5 cm	**2598**	**4.3**	54	Positive
	TBR128	T2N0M0 2 cm	157	**7.7**	**2250**	Positive
	TBR111	T1N0M0 0.9 cm	298	0.0	128	Negative
Smooth muscle sarcoma	TBR051	Metastatic	**4787**	**79.8**	**7986**	Positive
Neuroendocrine tumor	TBR052	Metastatic	**112518**	**119.8**	**14681**	Positive
Lung cancer	TBR164	Metastatic	**51818**	**47.3**	**4714**	Positive
	TBR012	Metastatic	**4351**	**7.7**	**2213**	Positive
	TBR014	Metastatic	**2356**	**12.2**	**825**	Positive
	TBR177	T2N1M0 3.9 cm	**1072**	**10.6**	25	Positive
Nasopharyngeal cancer	TBR031	Metastatic	**1028**	**4.4**	**1860**	Positive
	TBR125	Metastatic	**20949**	**162.5**	**6708**	Positive
	TBR124	T4N1	270	**4.9**	37	Positive
	TBR123	T1N2M0	**6871**	**11.8**	80	Positive
	TBR062	T3N1M0	111	**30.9**	41	Positive
	TBR108	T3N1M0	93	**7.7**	26	Positive
	TBR107	T3N0M0	**2439**	**41.2**	205	Positive
	TBR106	T1N1M0	**2281**	**5.9**	97	Positive
	TBR099	T1N0M0	**6187**	**10.2**	26	Positive

FIG. 46

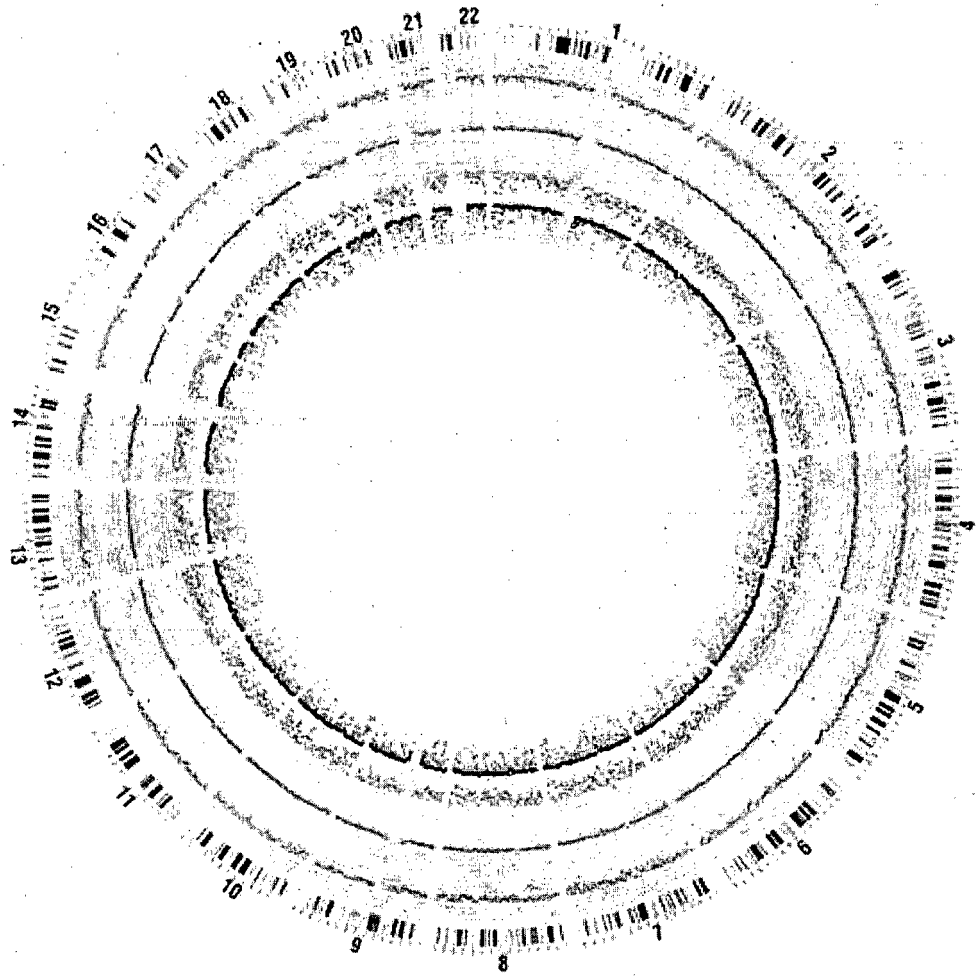


FIG. 47

Sample: TBR36
Tumor DNA fraction: 40%

From inside to outside:
1) CNA z-score
2) Methylation z-score

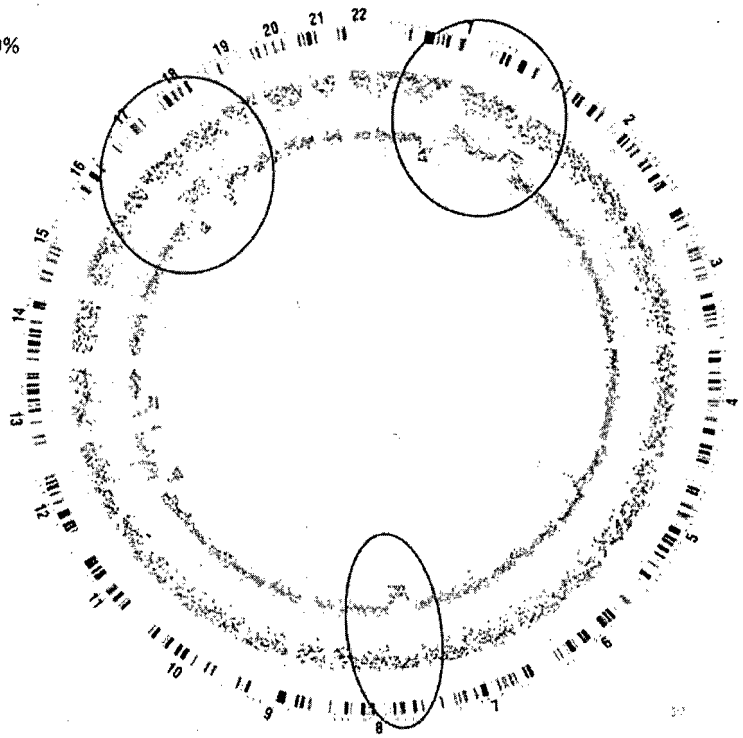


FIG. 48A

Sample: TBR36
Tumor DNA fraction: 40%

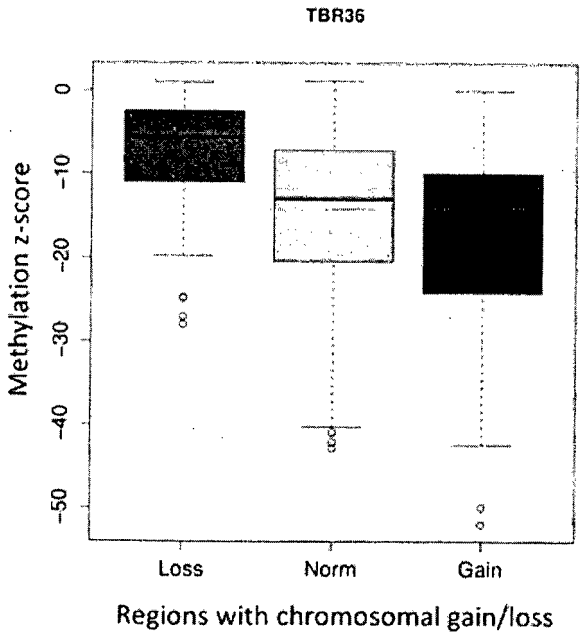


FIG. 48B

Sample: TBR34
Tumor DNA fraction: 30%

From inside to outside:
1) CNA z-score
2) Methylation z-score

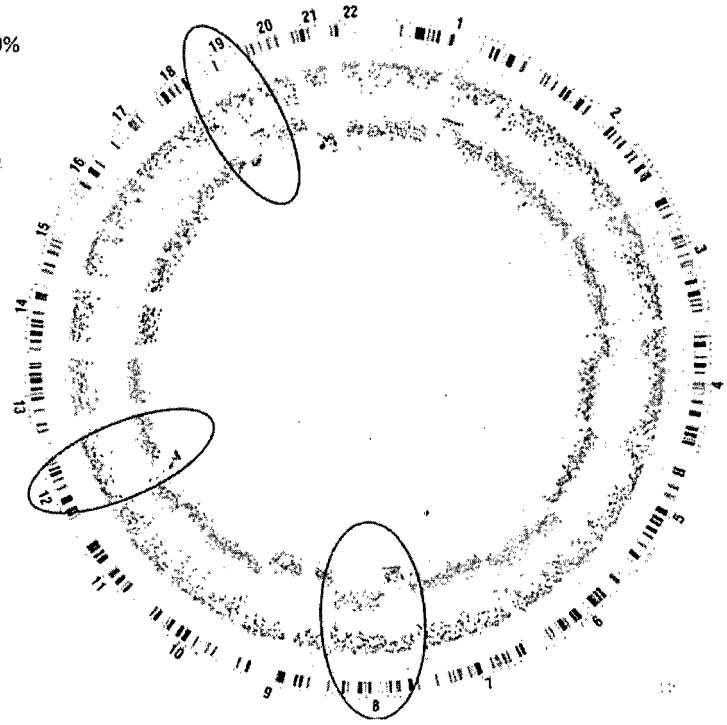


FIG. 49A

Sample: TBR34
Tumor DNA fraction: 30%

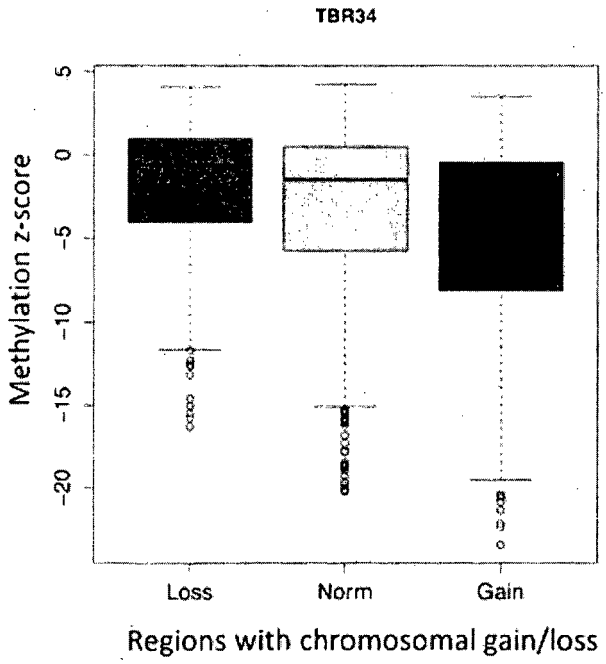


FIG. 49B

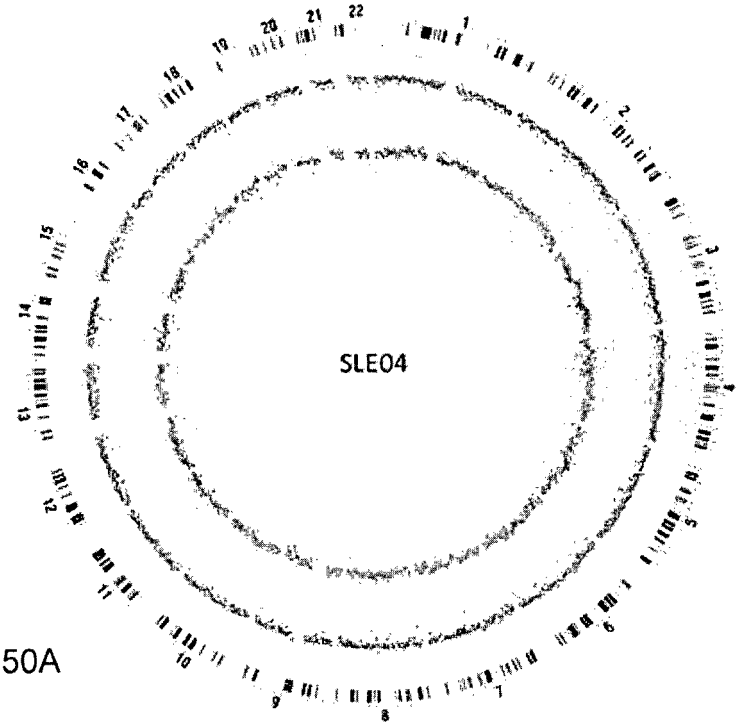


FIG. 50A

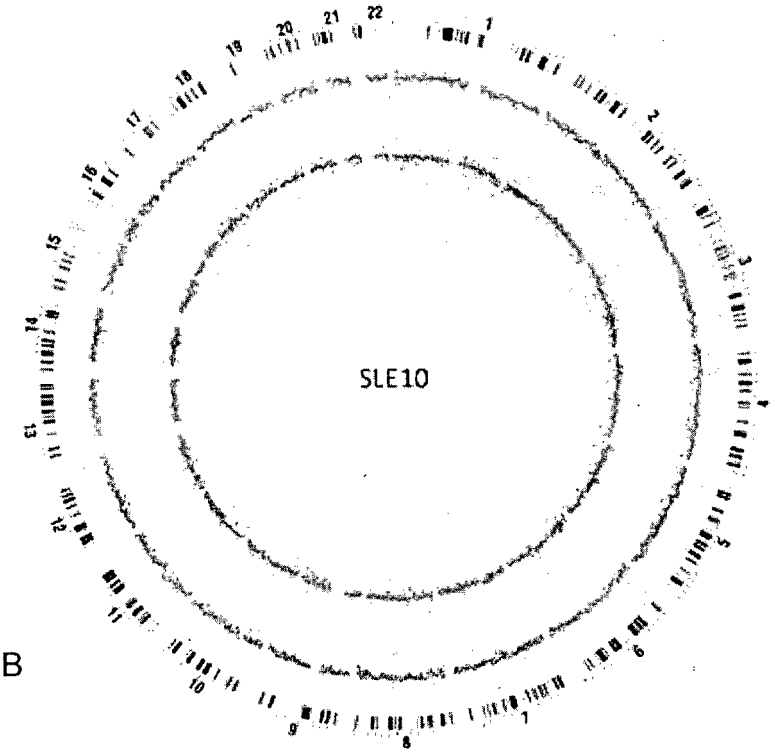


FIG. 50B

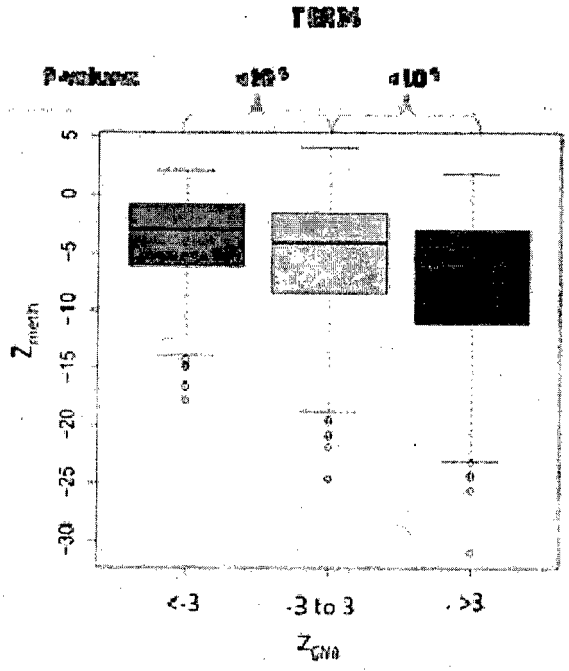


FIG. 51A

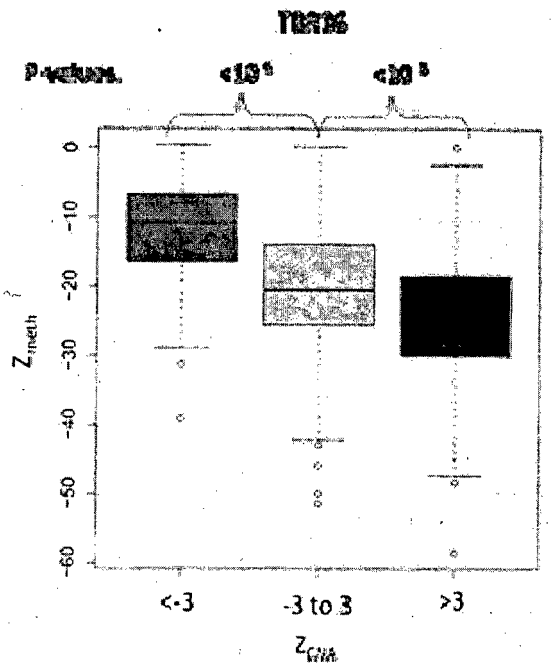


FIG. 51B

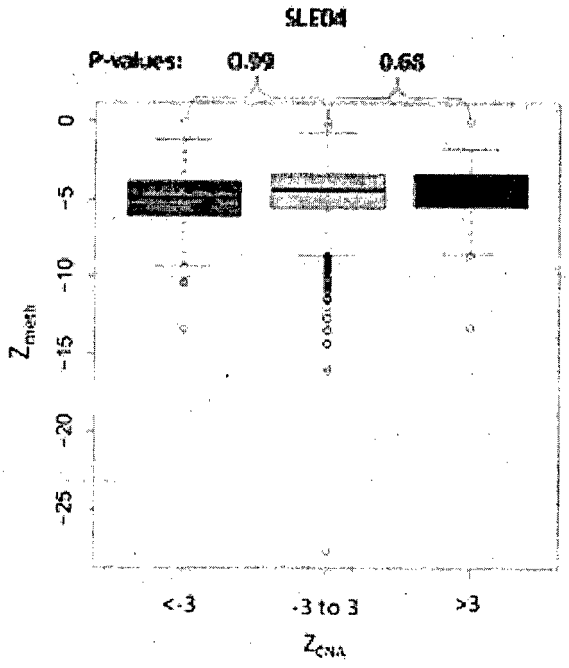


FIG. 51C

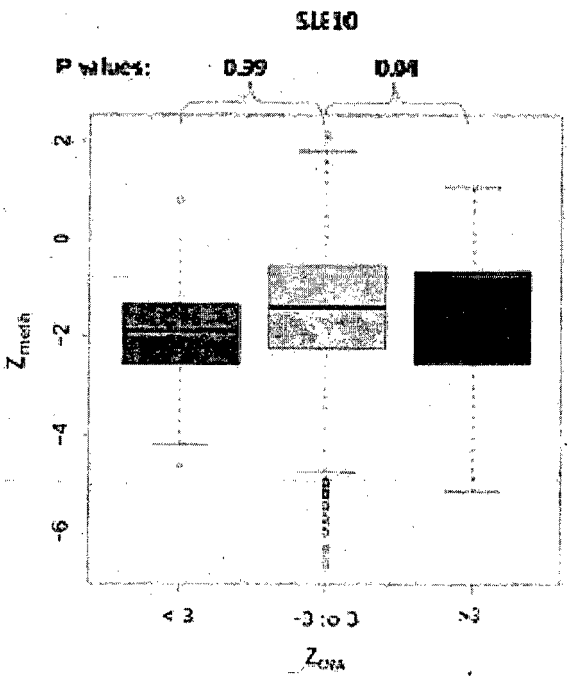


FIG. 51D

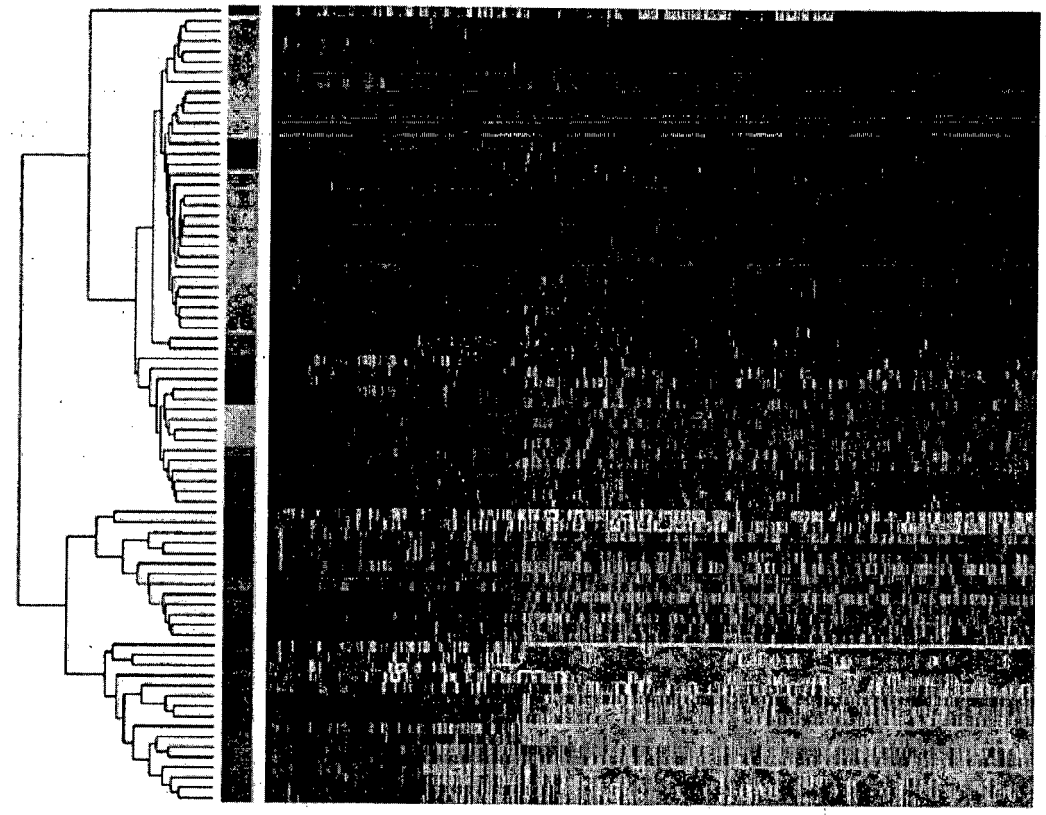


FIG. 52B

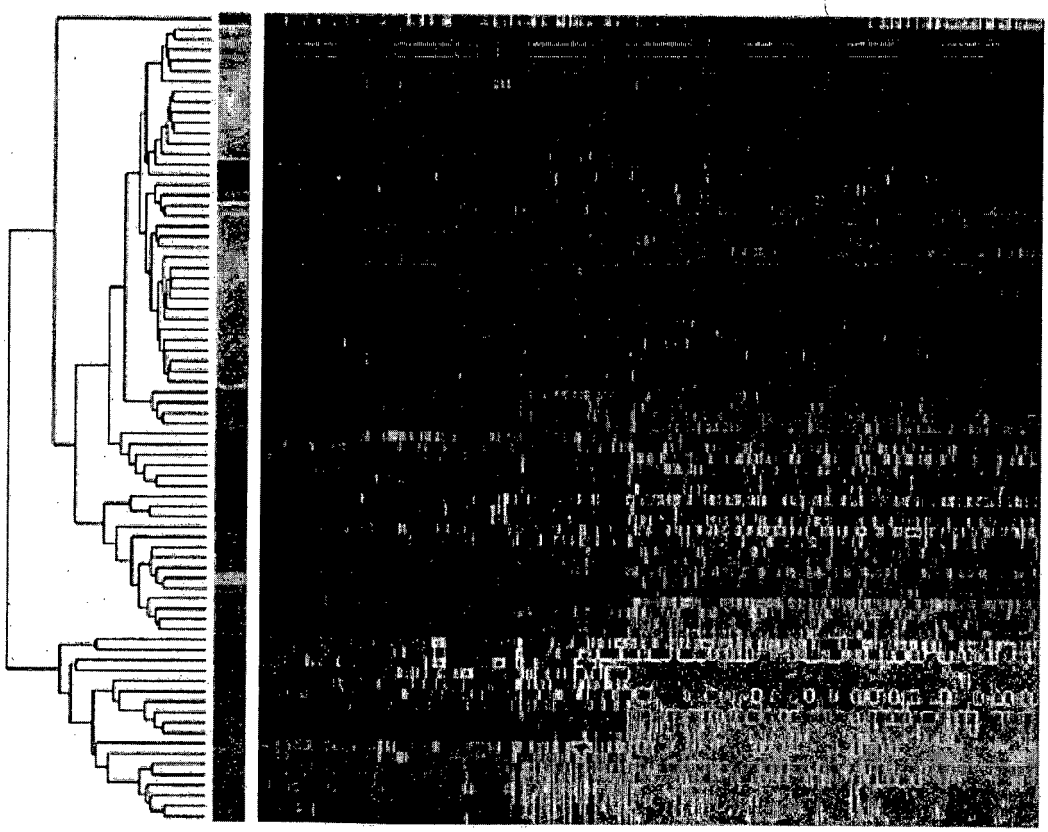


FIG. 52A

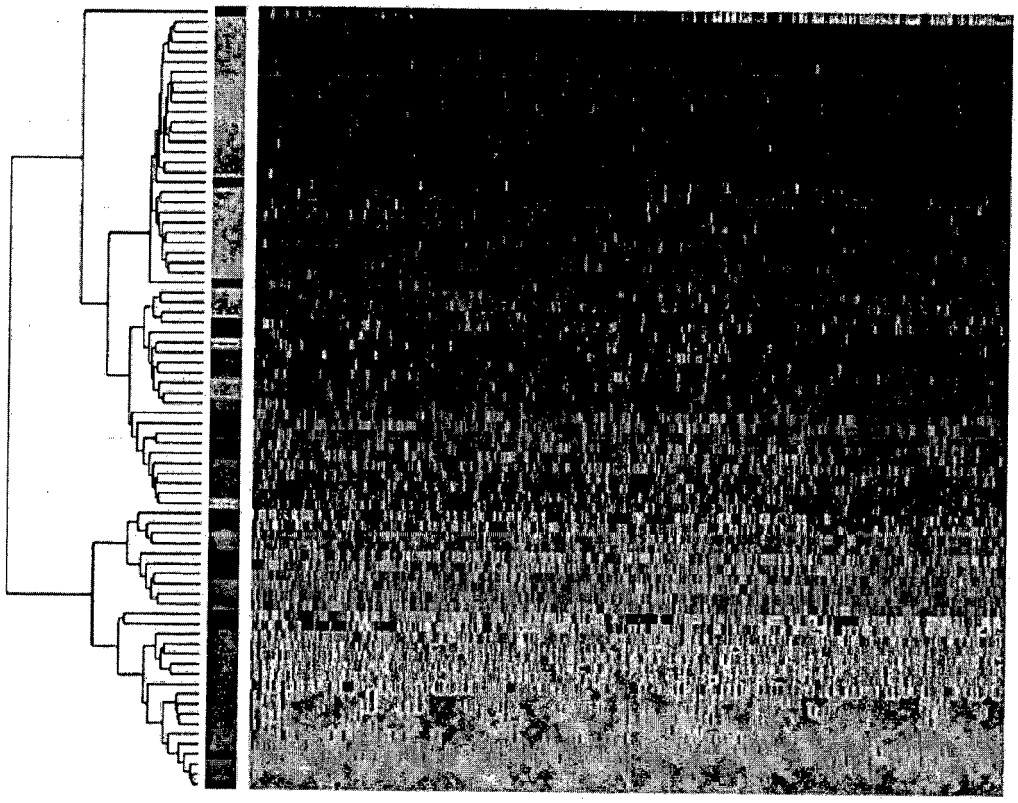


FIG. 53B

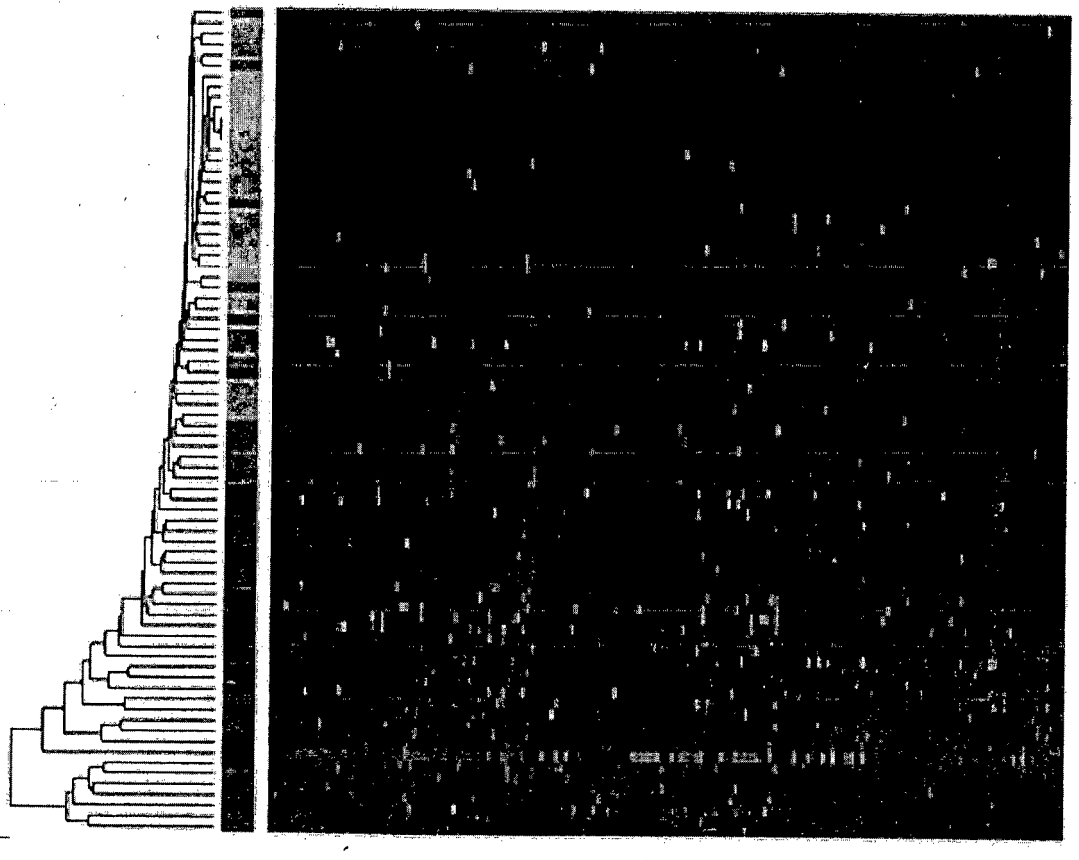


FIG. 53A

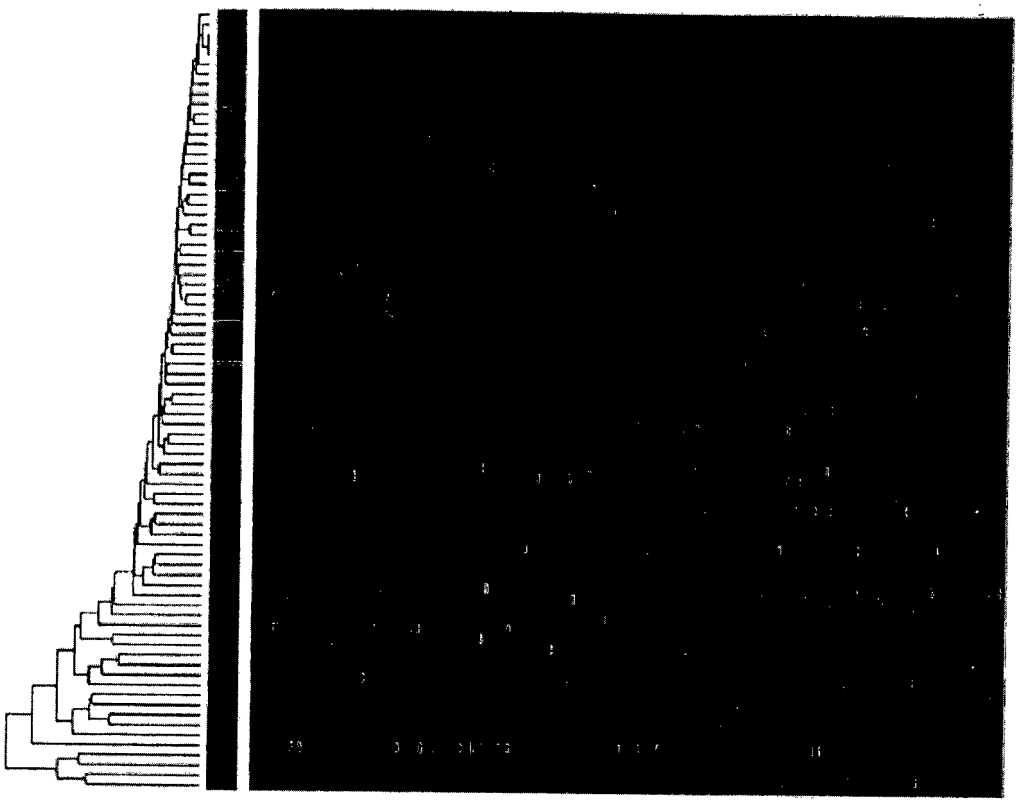


FIG. 54B

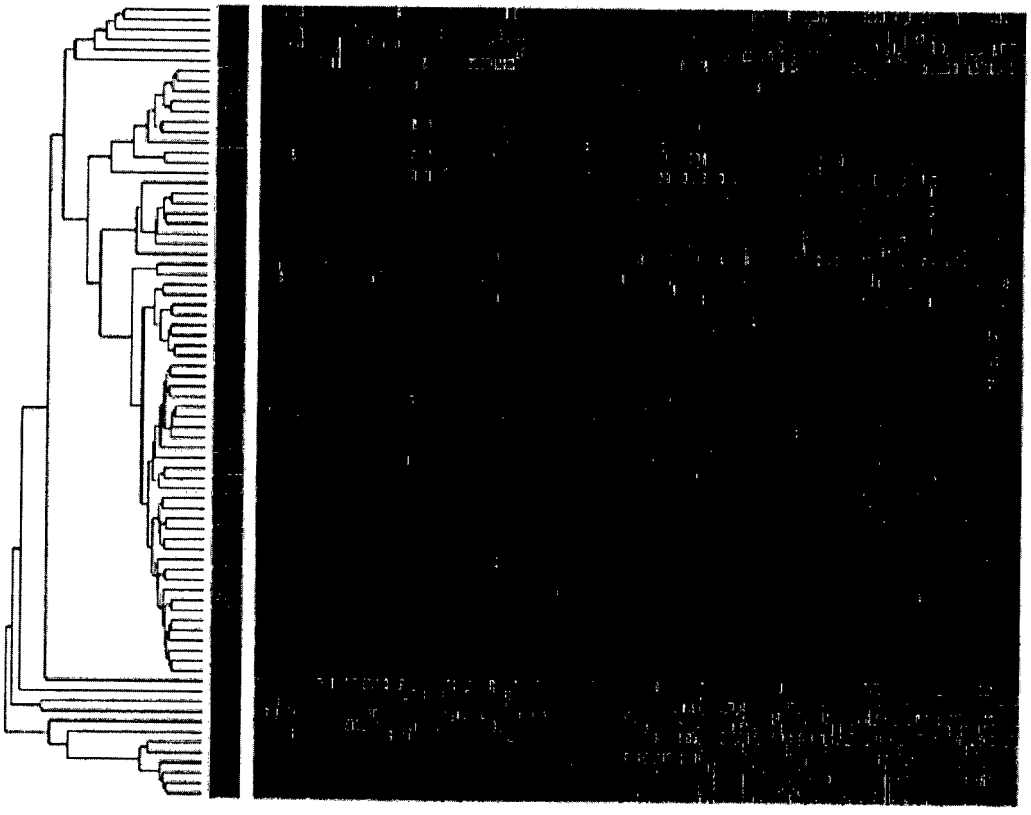


FIG. 54A

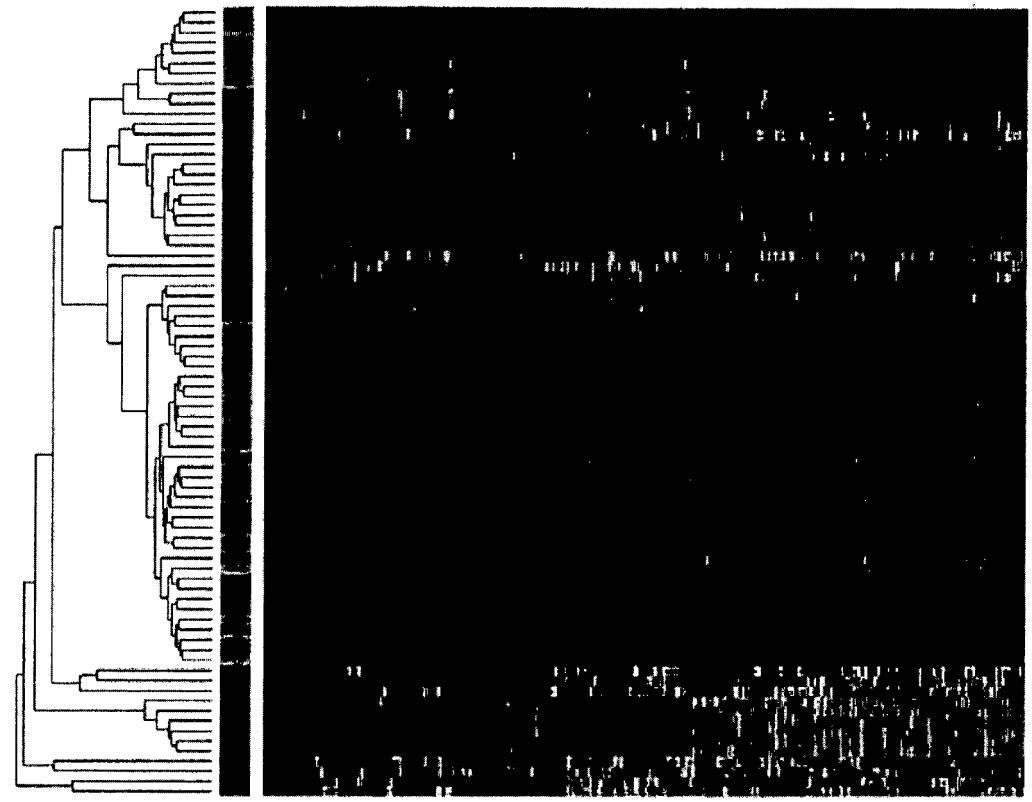


FIG. 55B

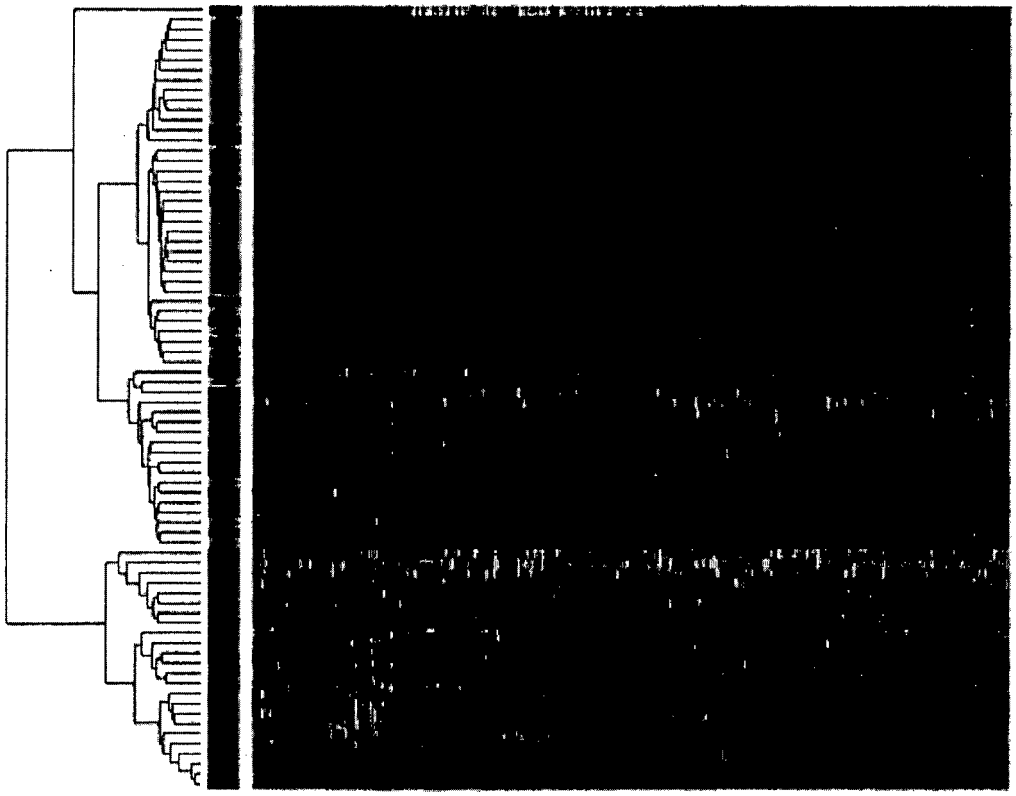
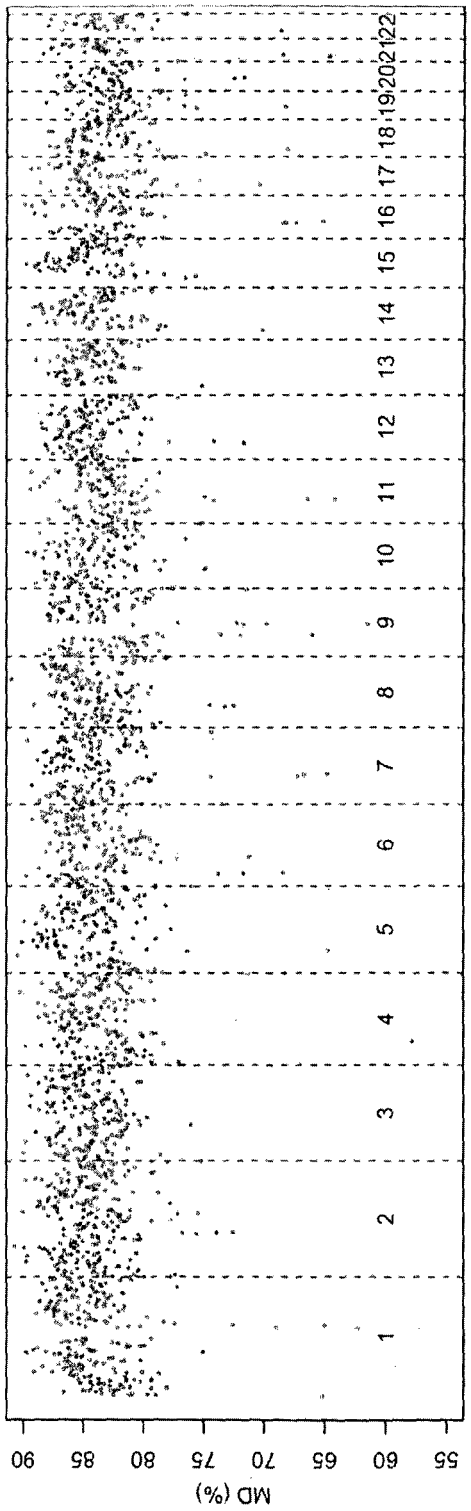


FIG. 55A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21
22
90
85
80
75
70
65
60
55
MD (%)

FIG. 56