

Cutting positions relative to CpG sites

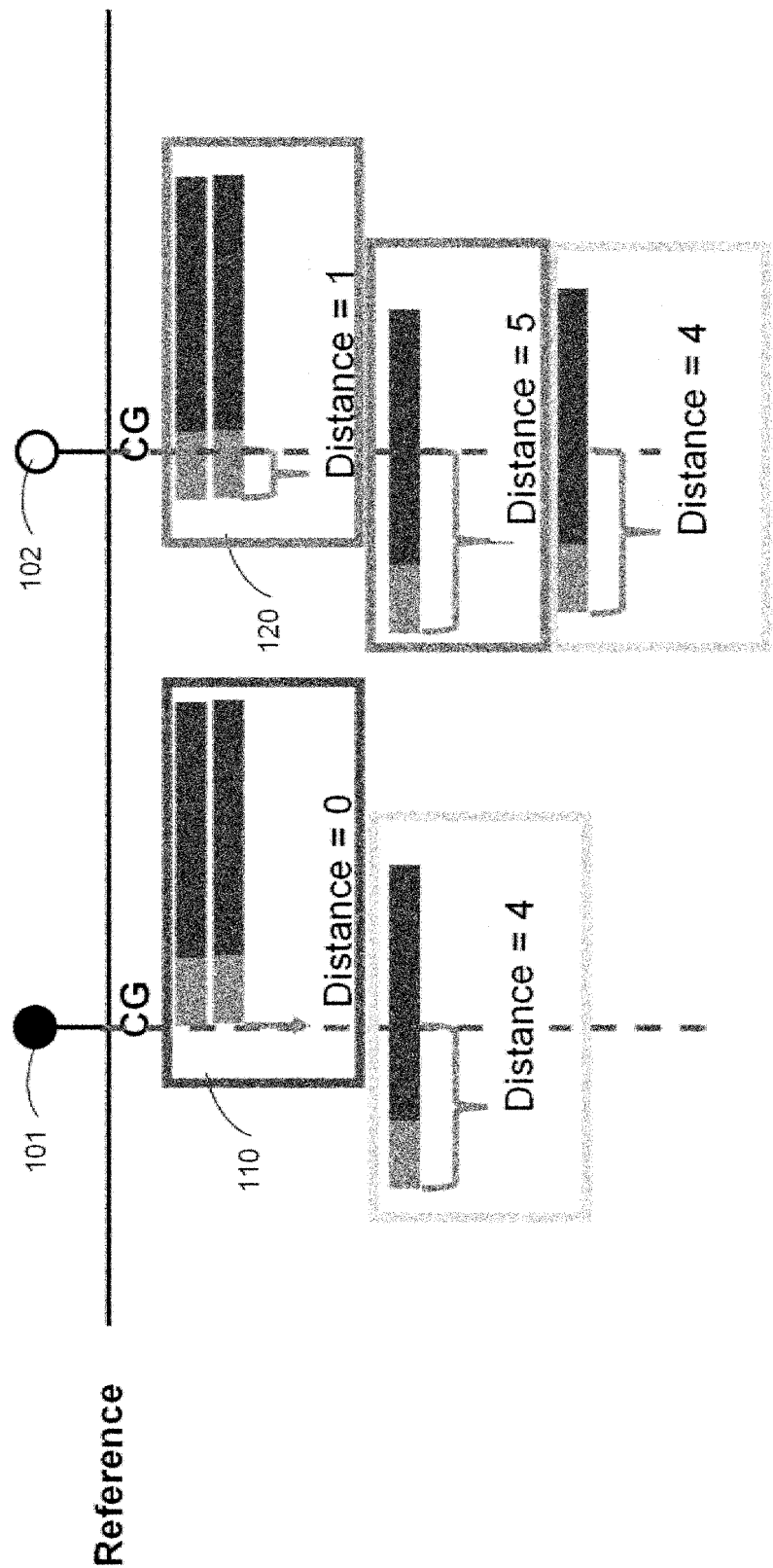


FIG. 1

FIG. 2A cfDNA fragment 5' end

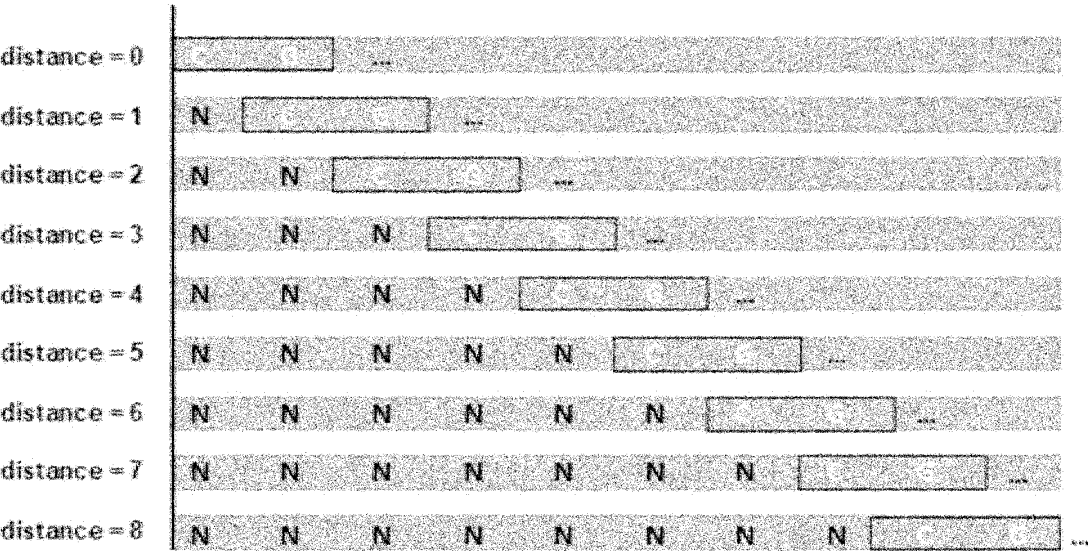
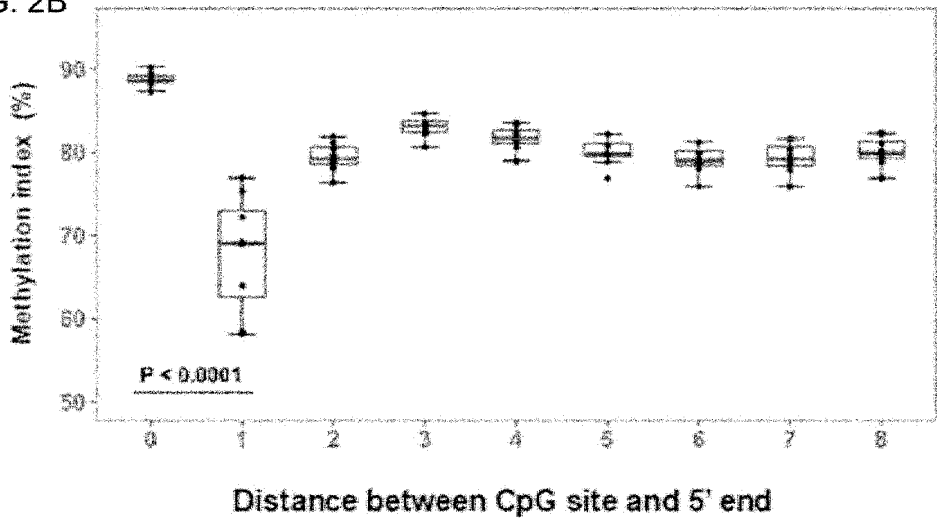
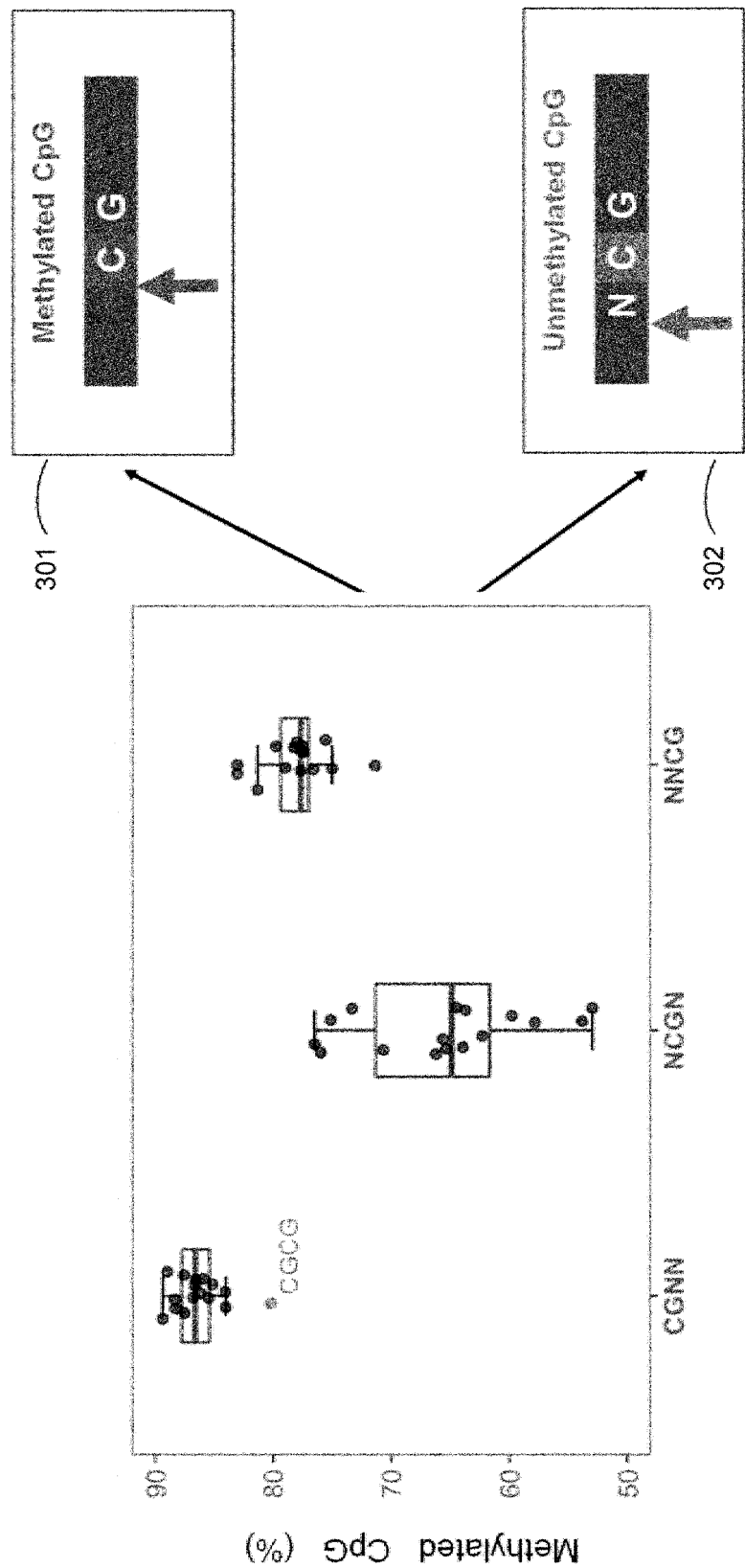


FIG. 2B



Methylation levels are varied according to different CpG-containing 4-mer motifs



(Healthy Control, N = 8)

FIG. 3

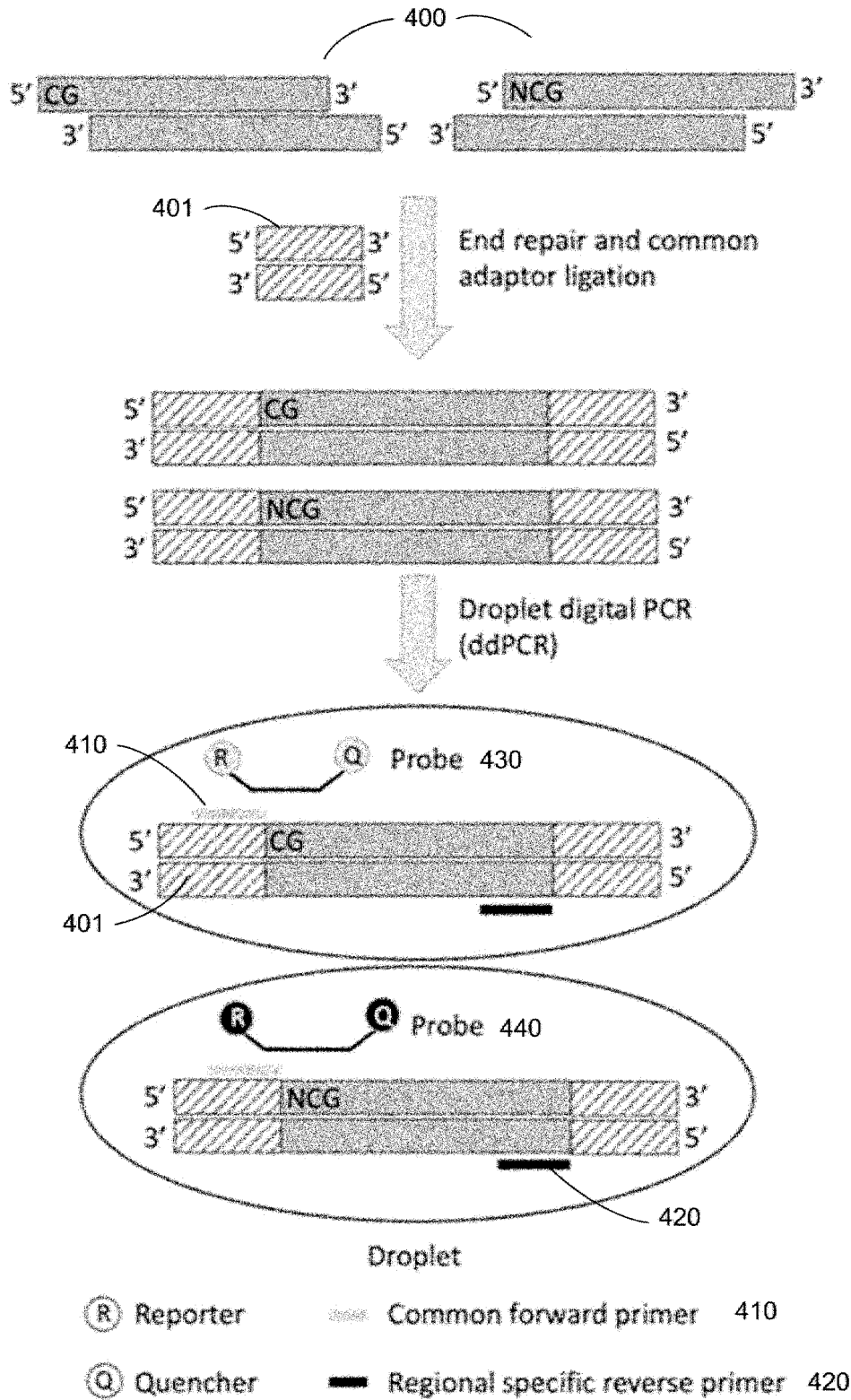


FIG. 4

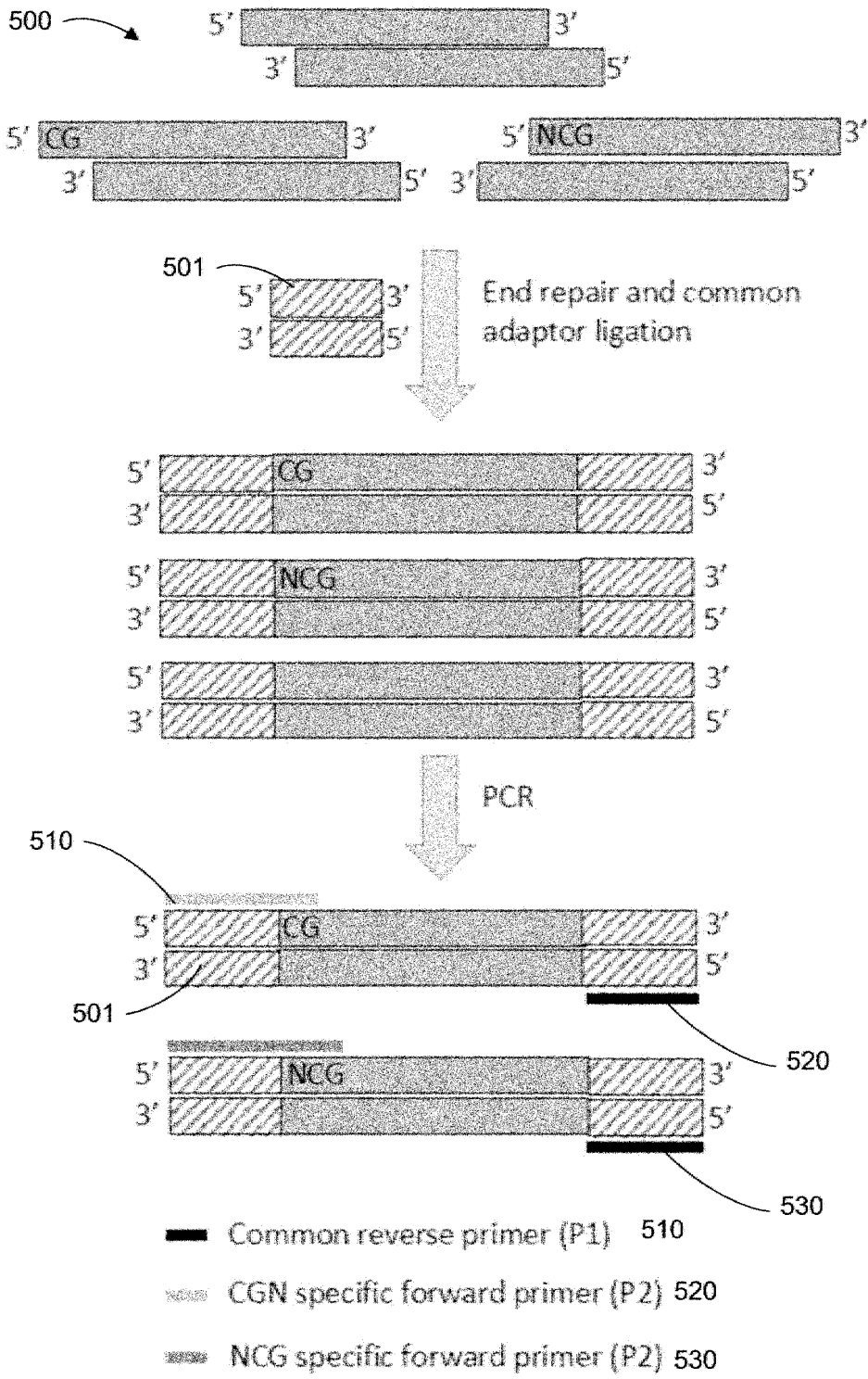
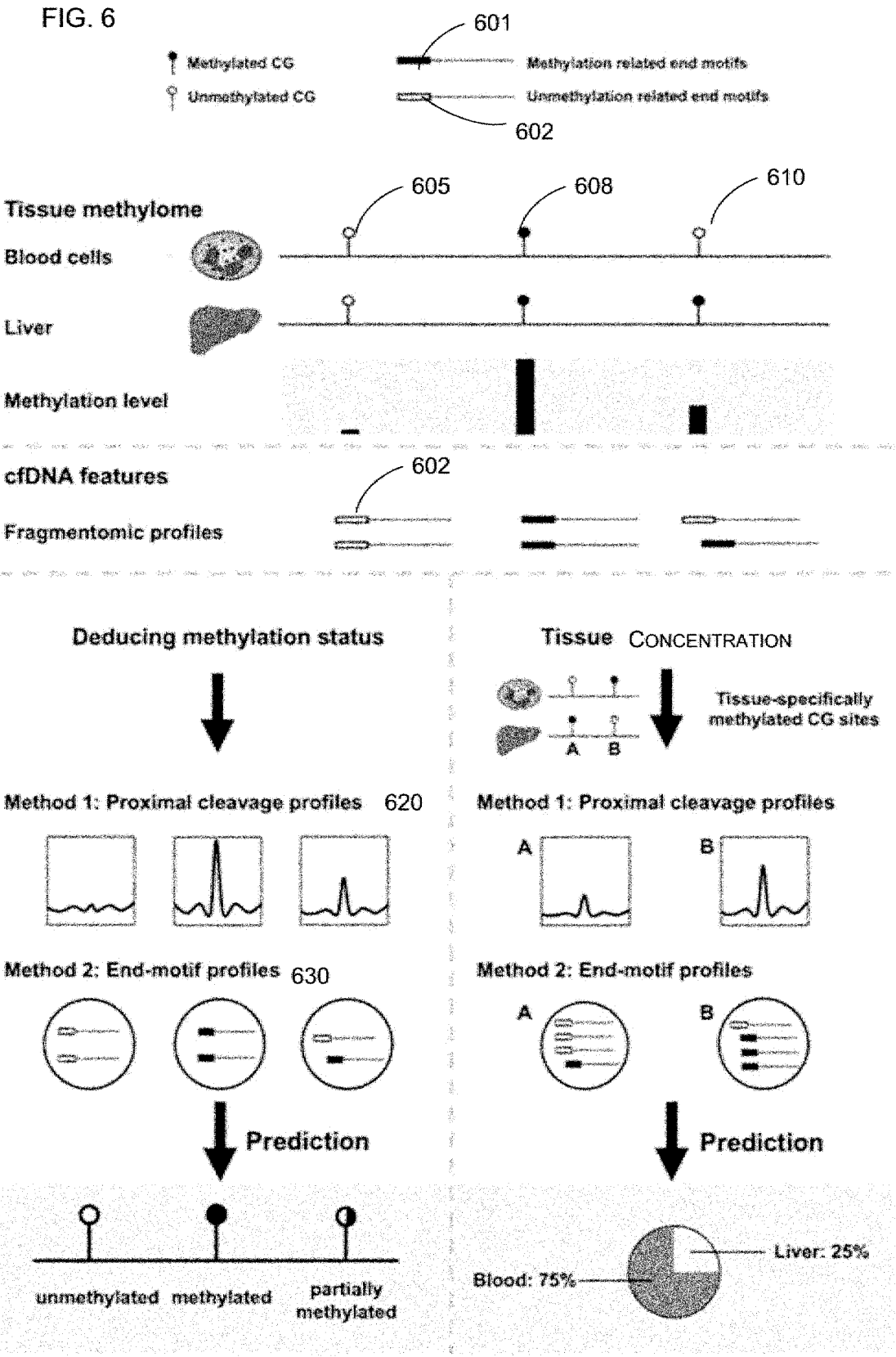


FIG. 5

FIG. 6



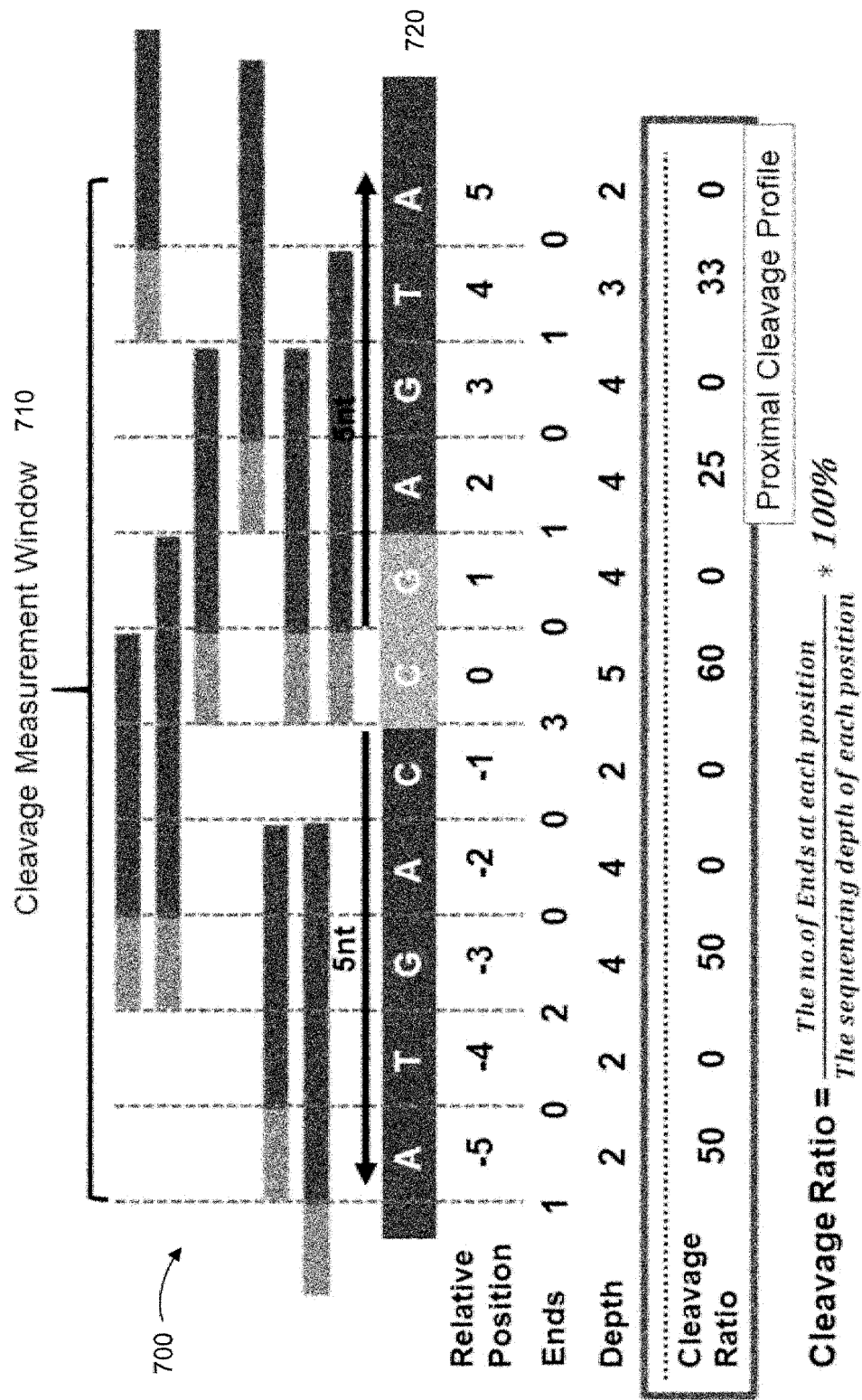
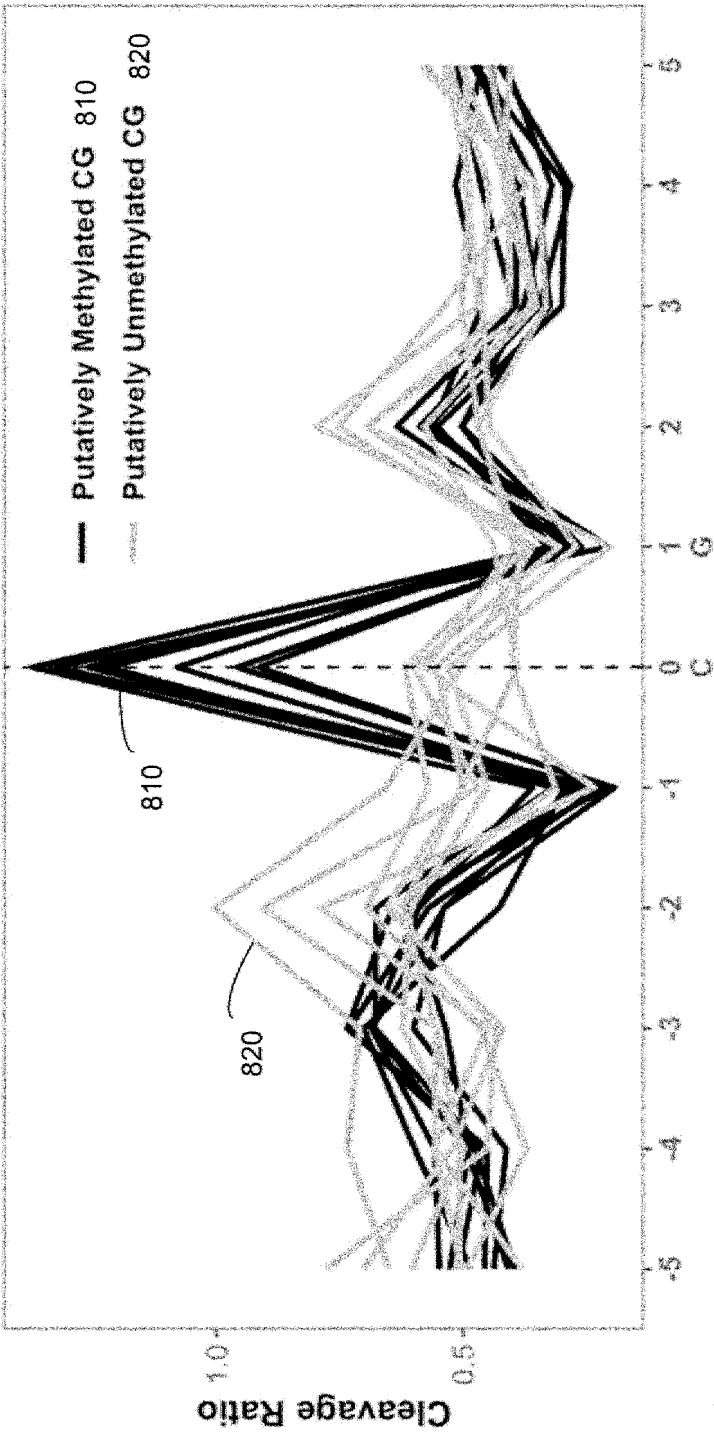


FIG. 7



Based on leukocyte array data

Hyper = 1000 putative methylated CG sites

Hypo = 1000 putative unmethylated CG sites

FIG. 8

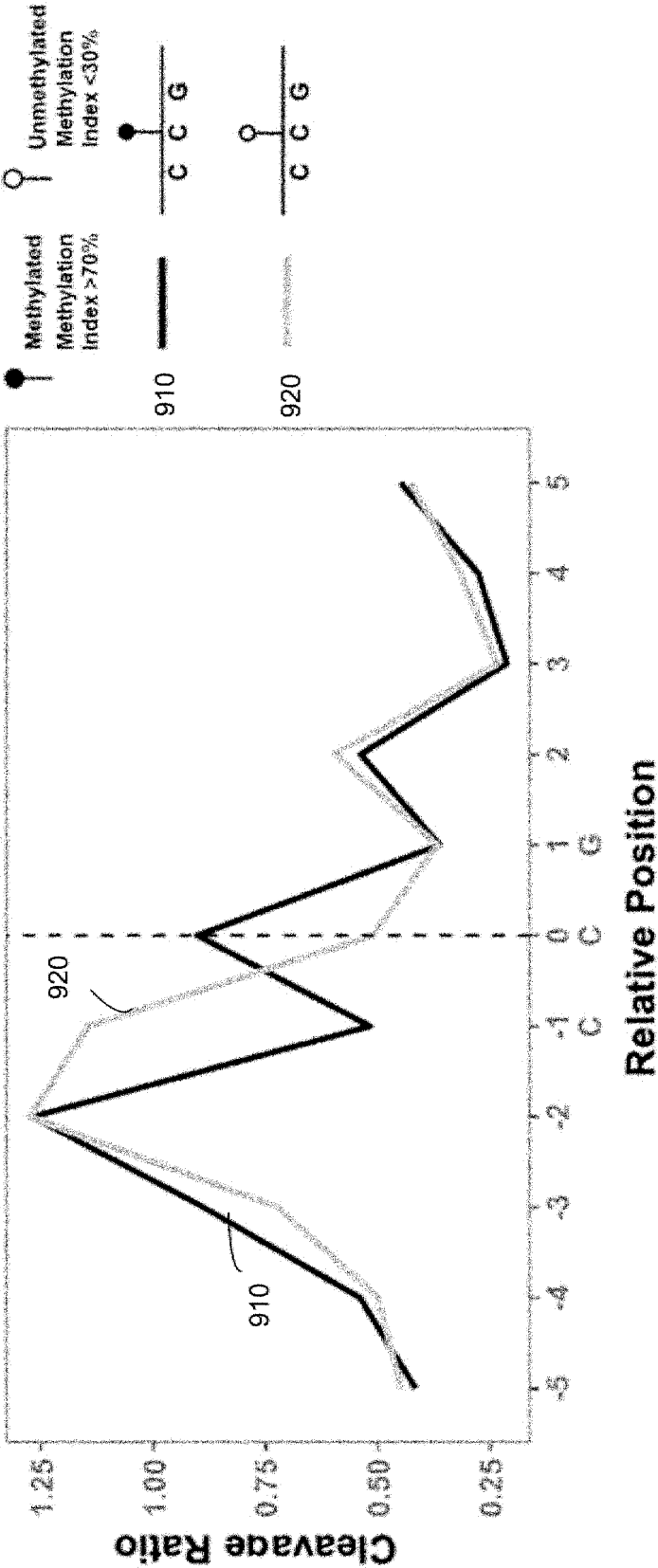


FIG. 9

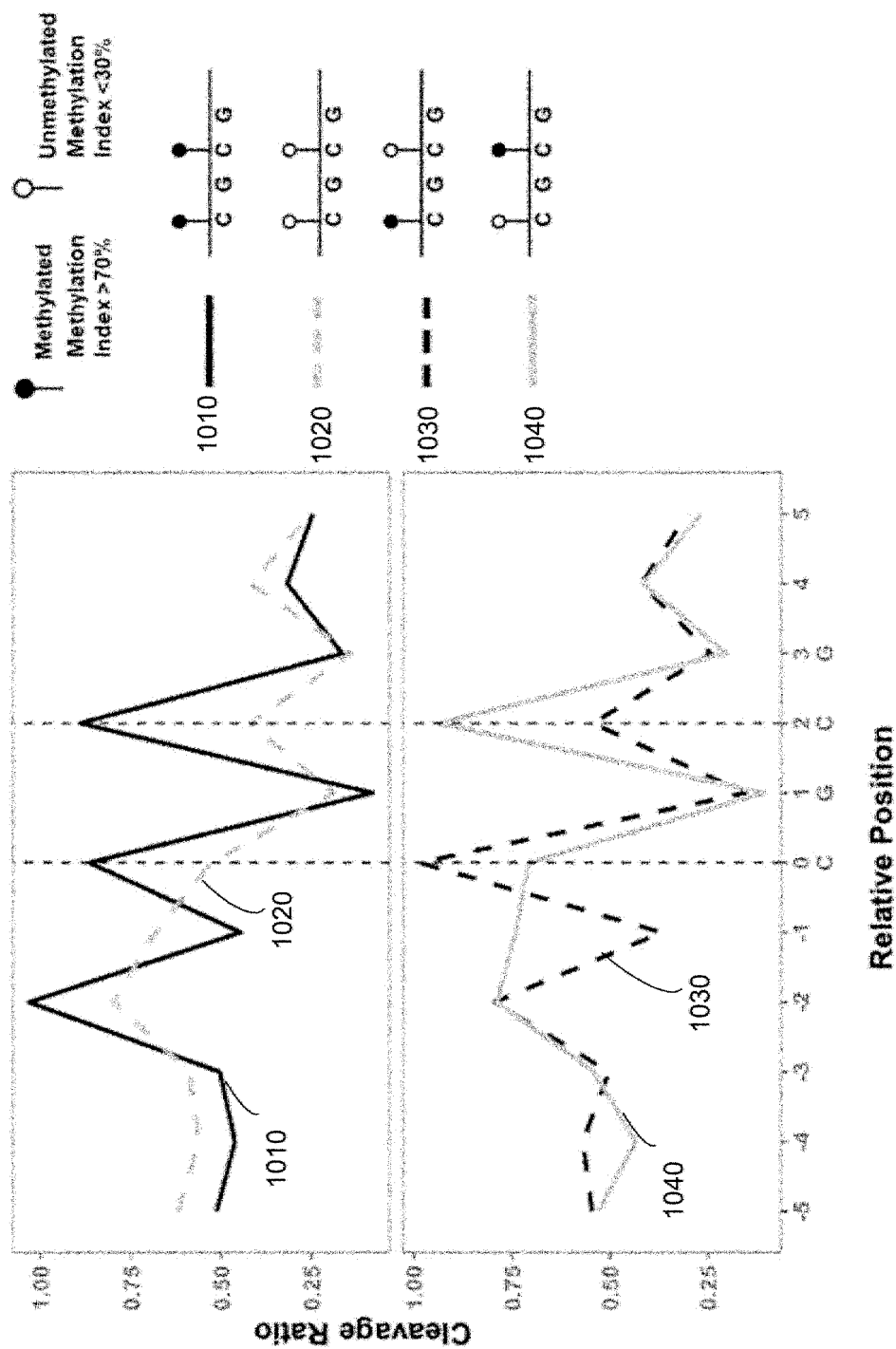


FIG. 10

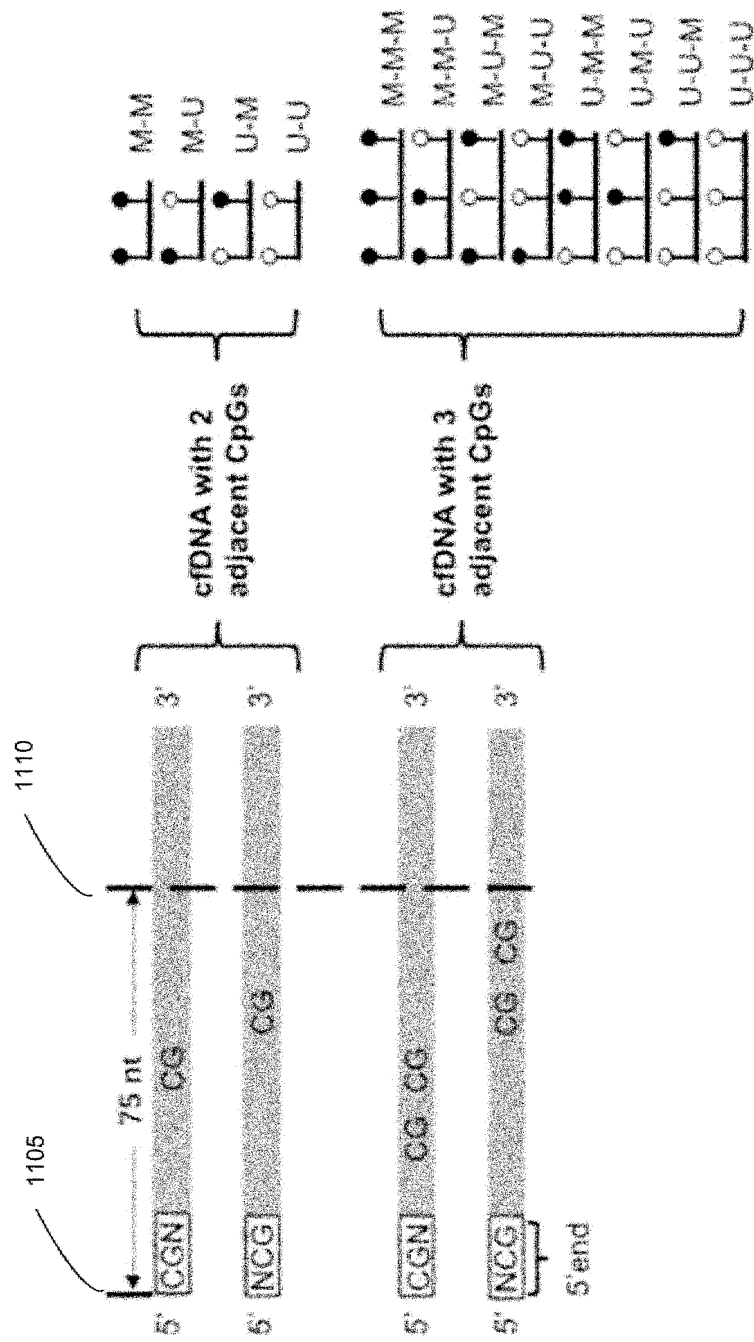


FIG. 11

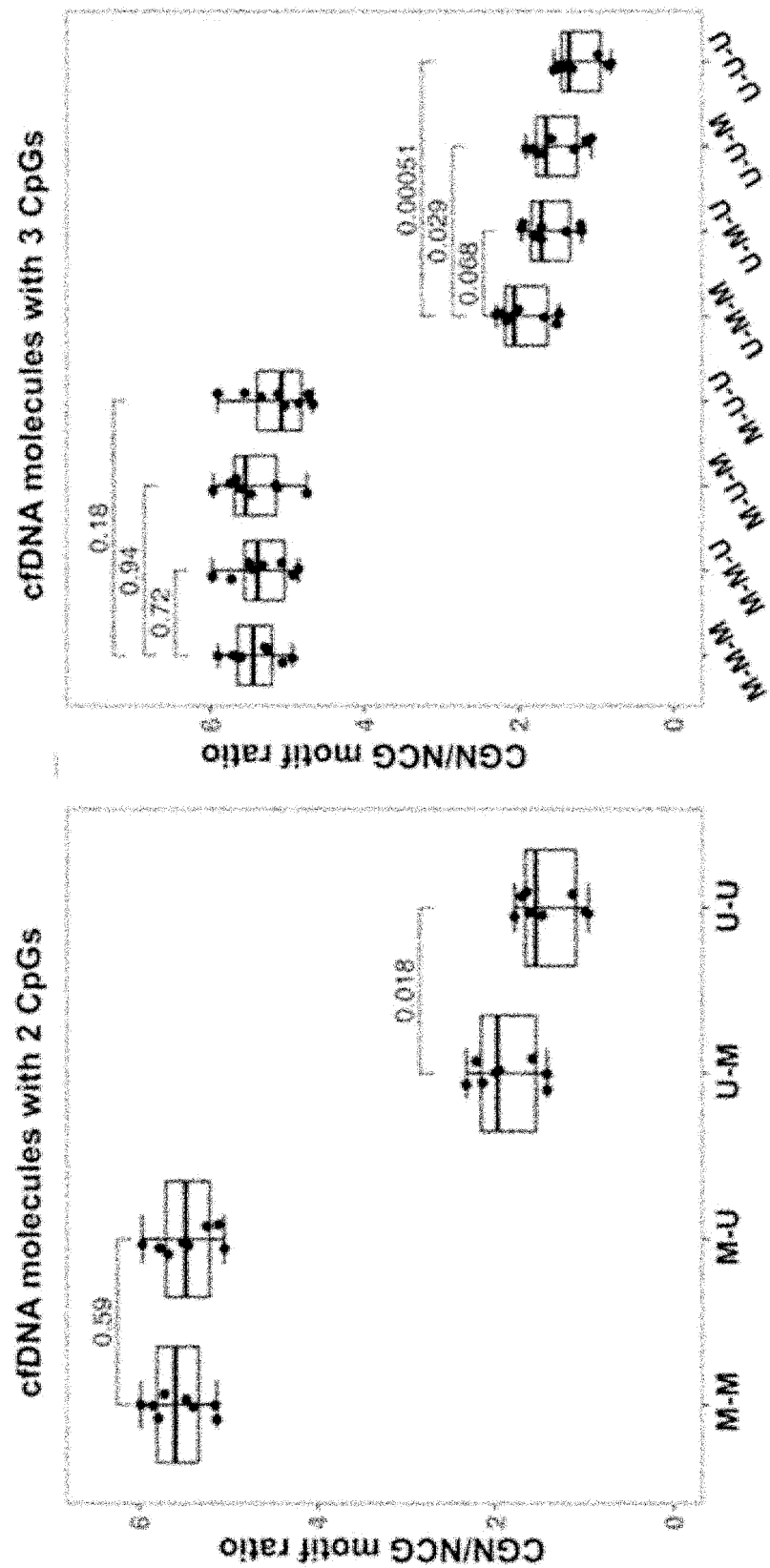
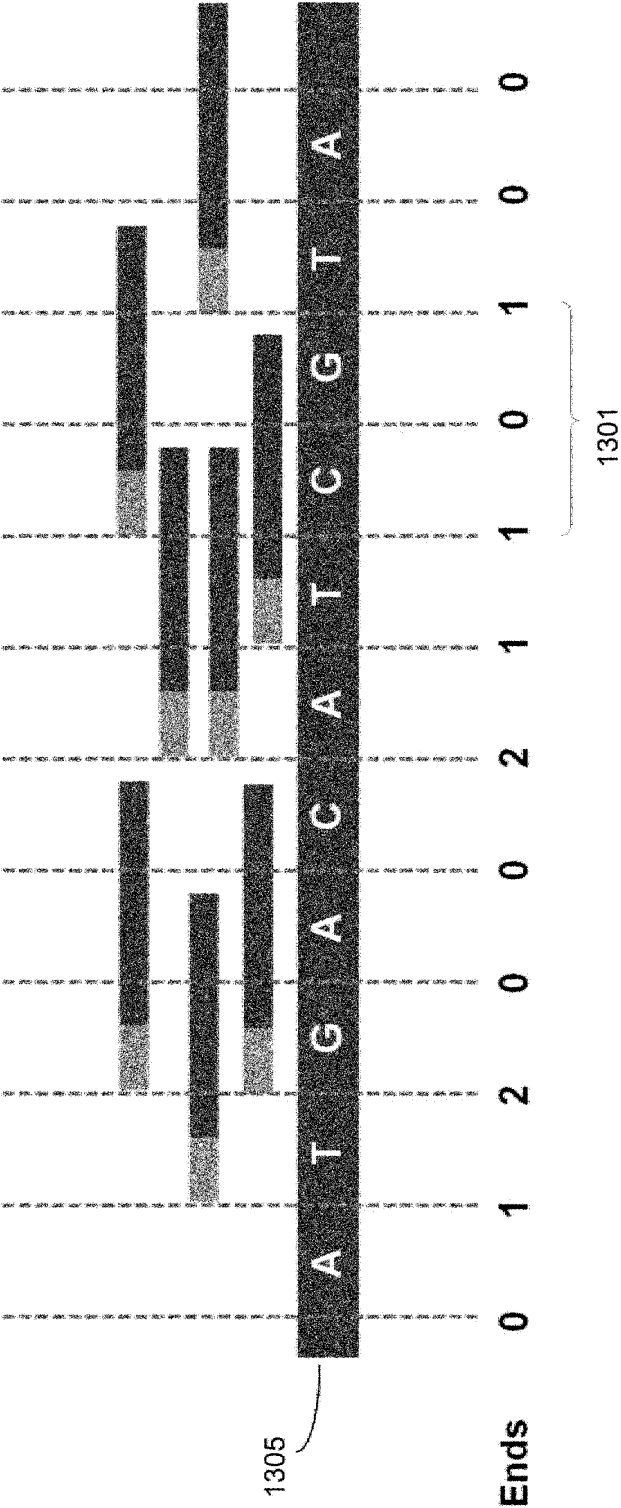


FIG. 12B

Definition of normalized end density



Cleavage Density = Ends at Each Position / Total Ends in This Region

FIG. 13

DNases cutting is highly preferred to methylated CG

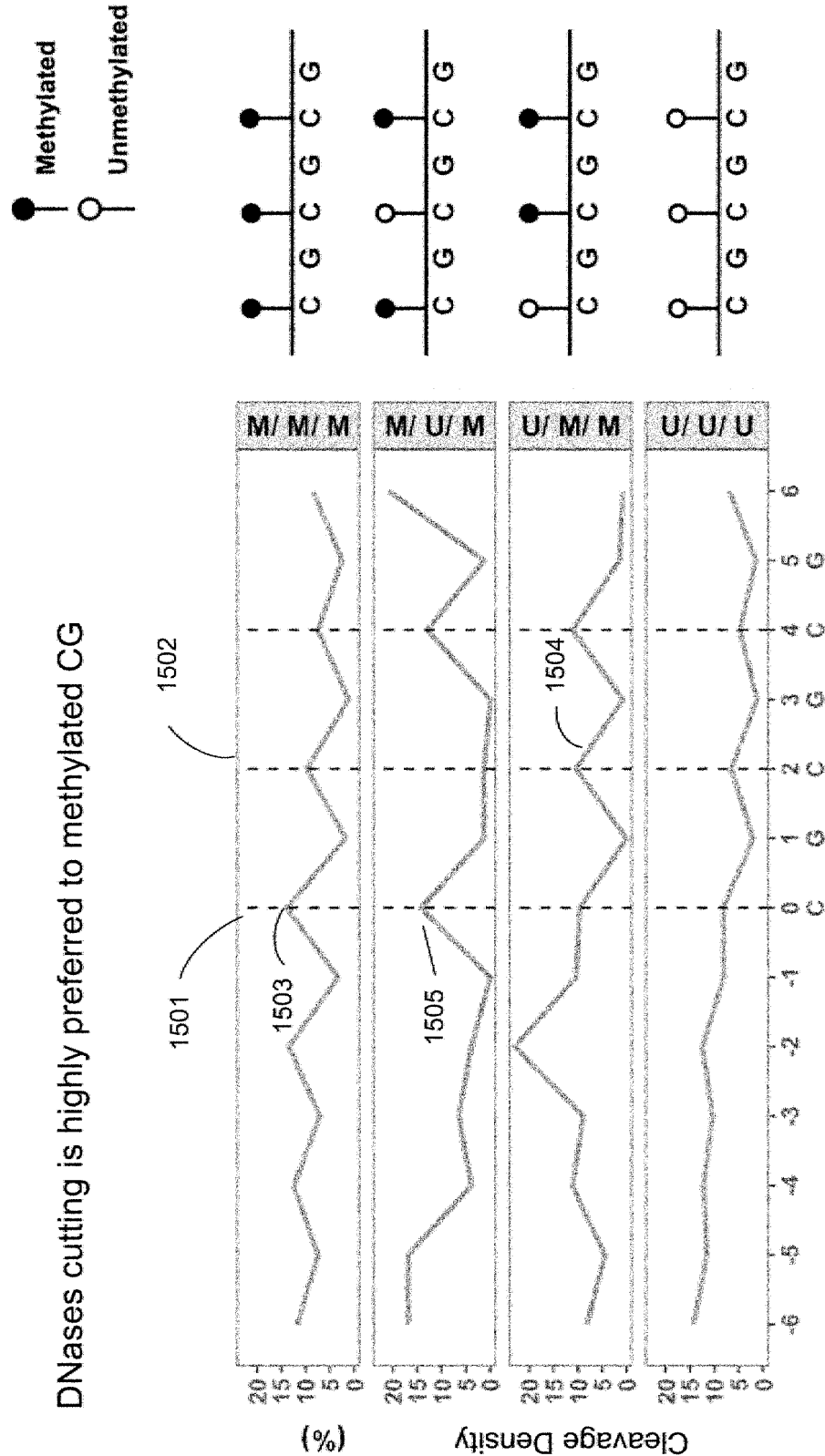


FIG. 15

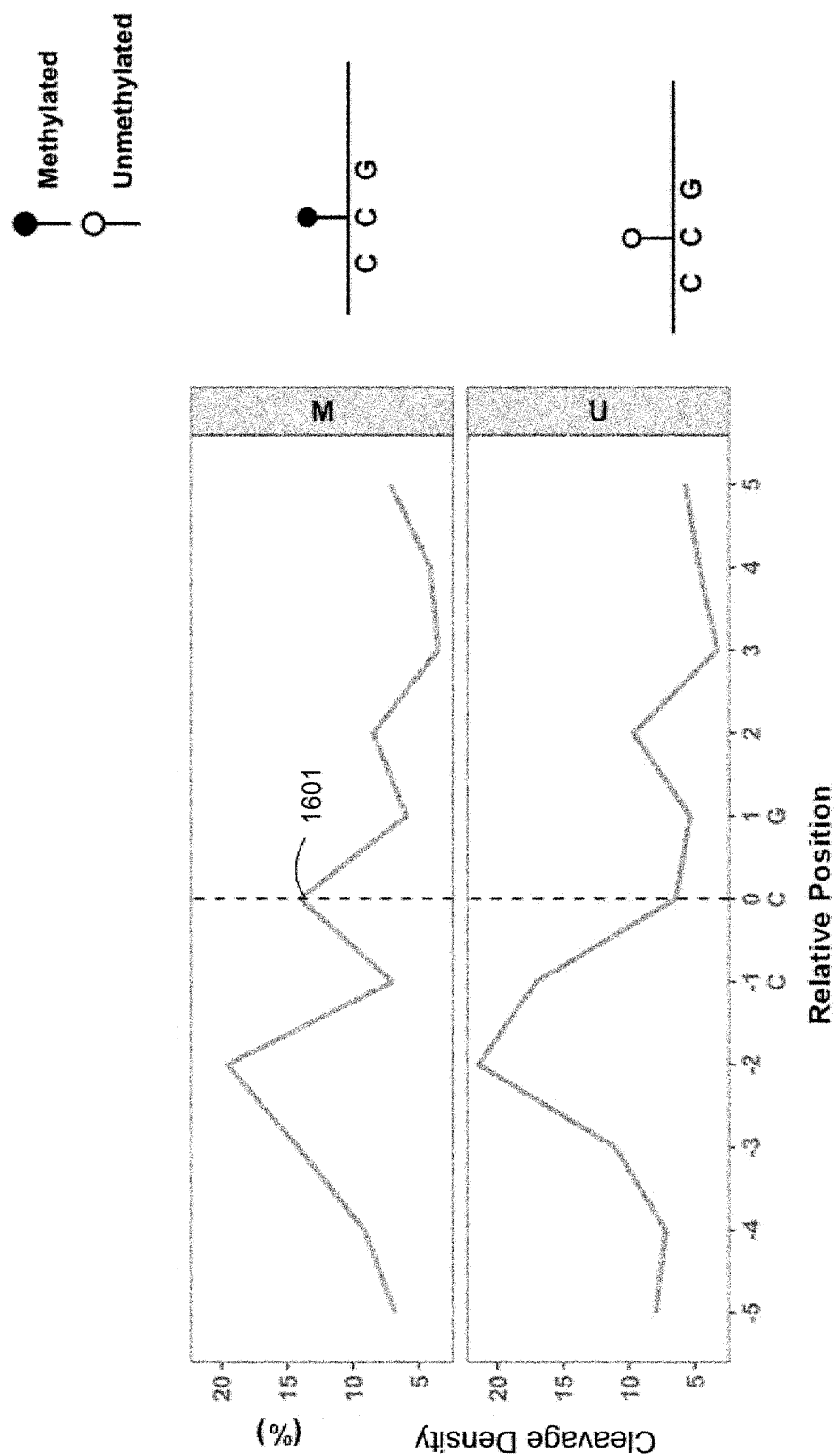
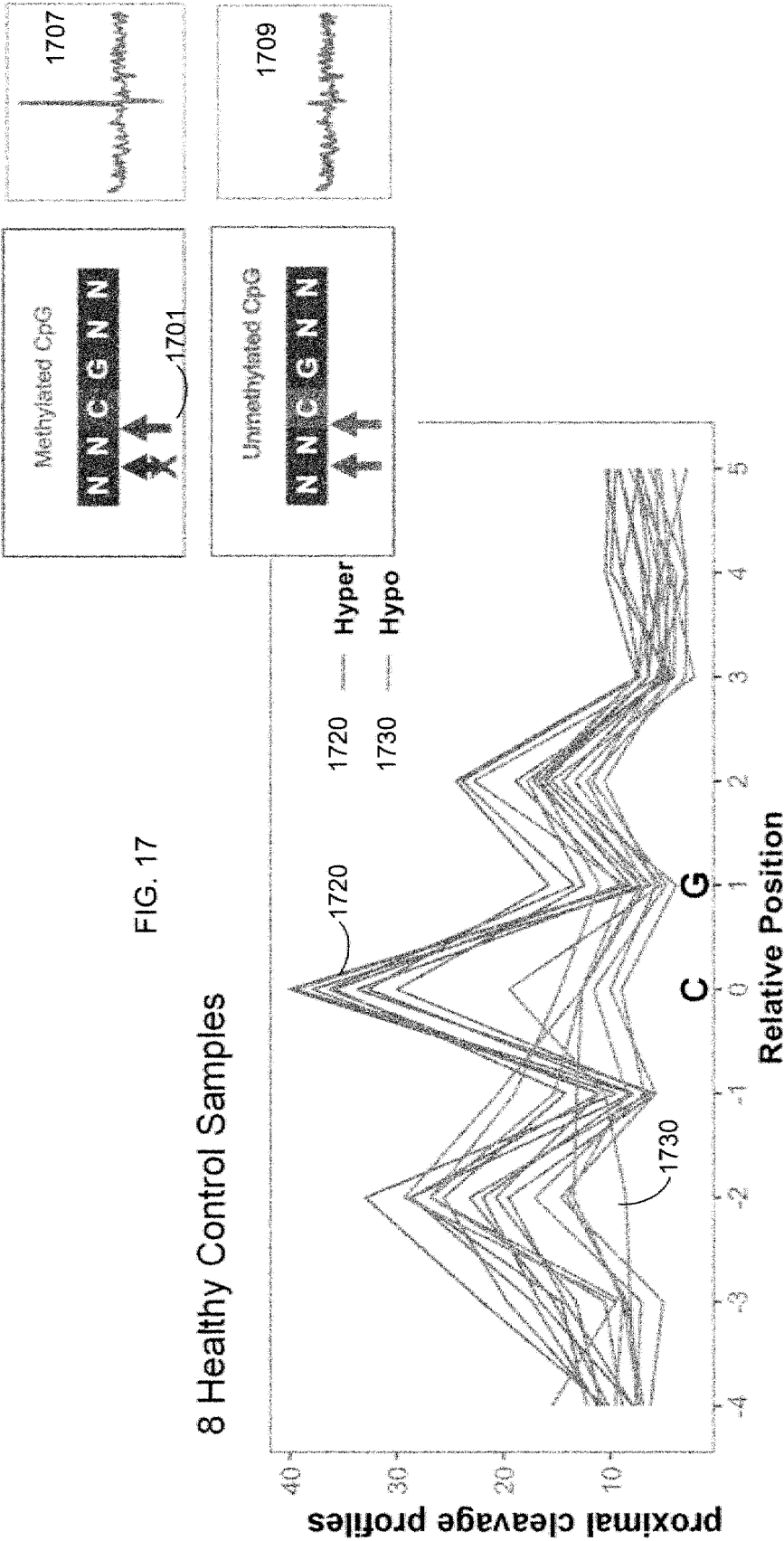


FIG. 16



Based on leukocyte array data

Hyper = 1000 putative methylated CG sites

Hypo = 1000 putative unmethylated CG sites

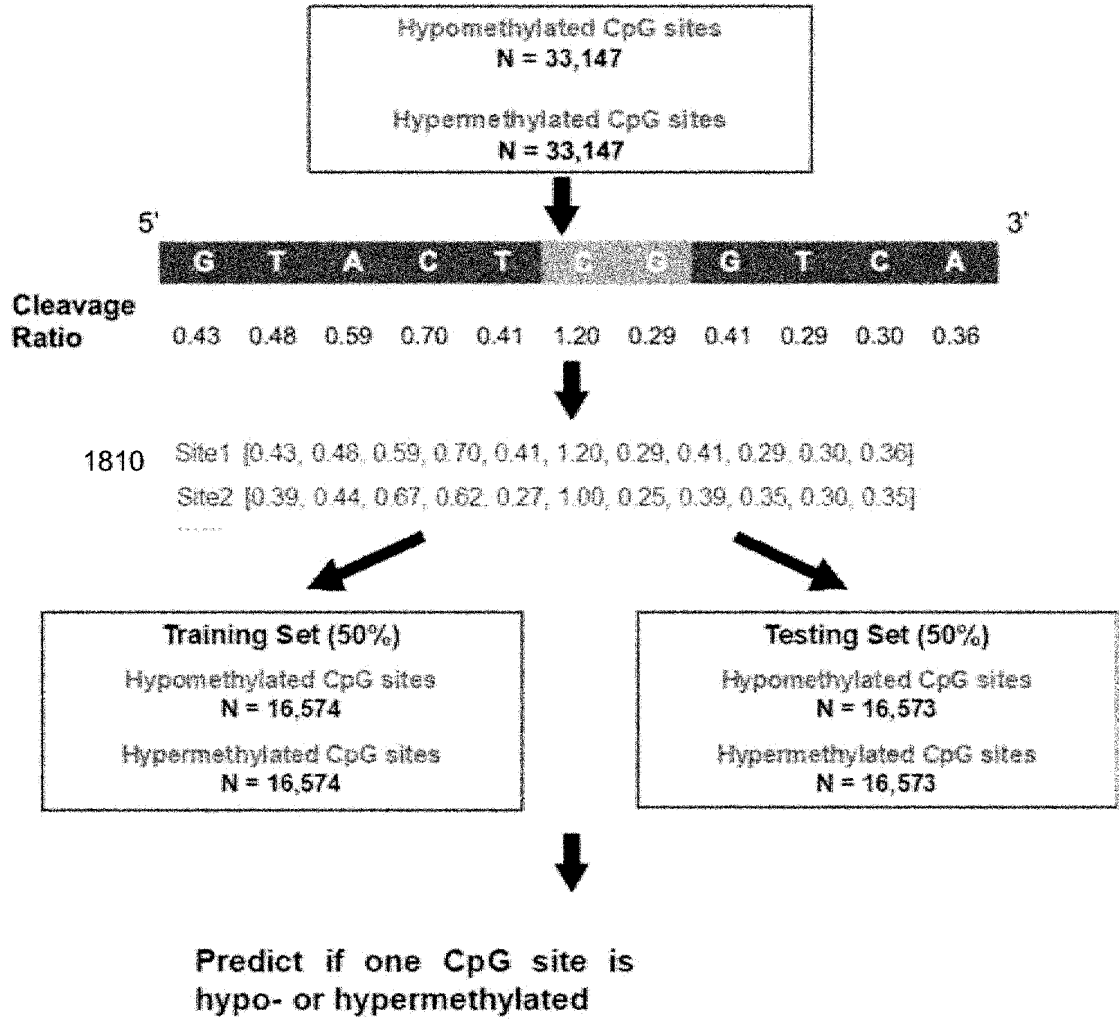


FIG. 18

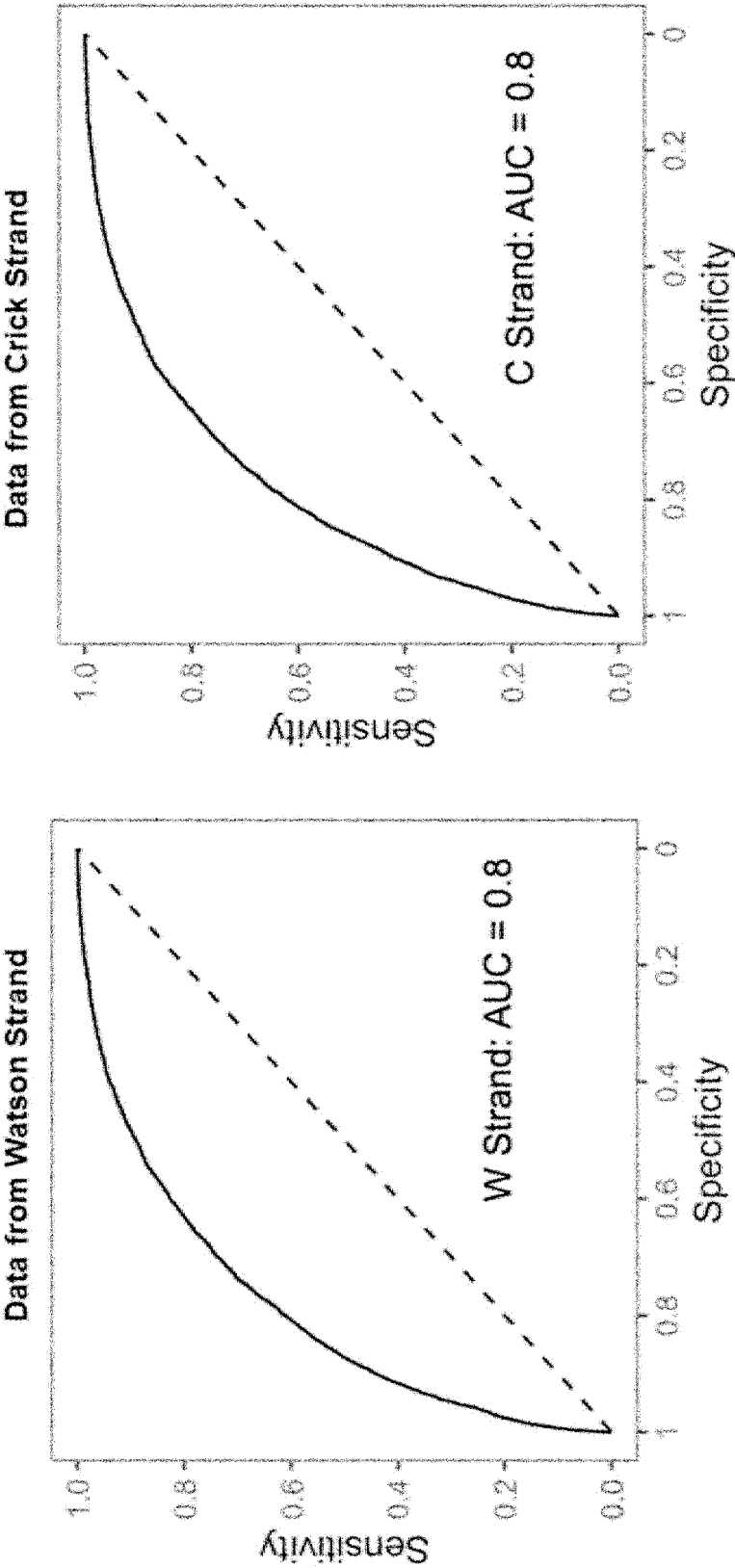


FIG. 19

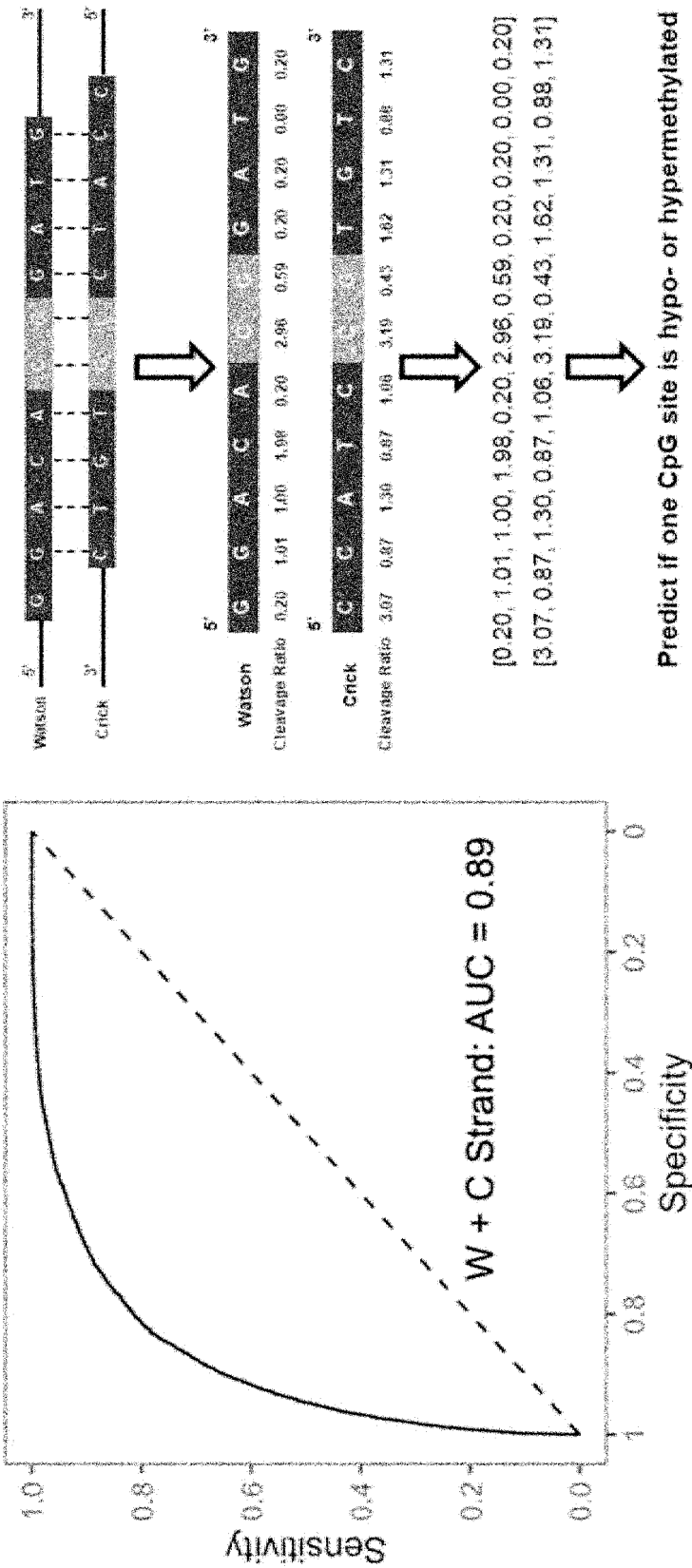


FIG. 20

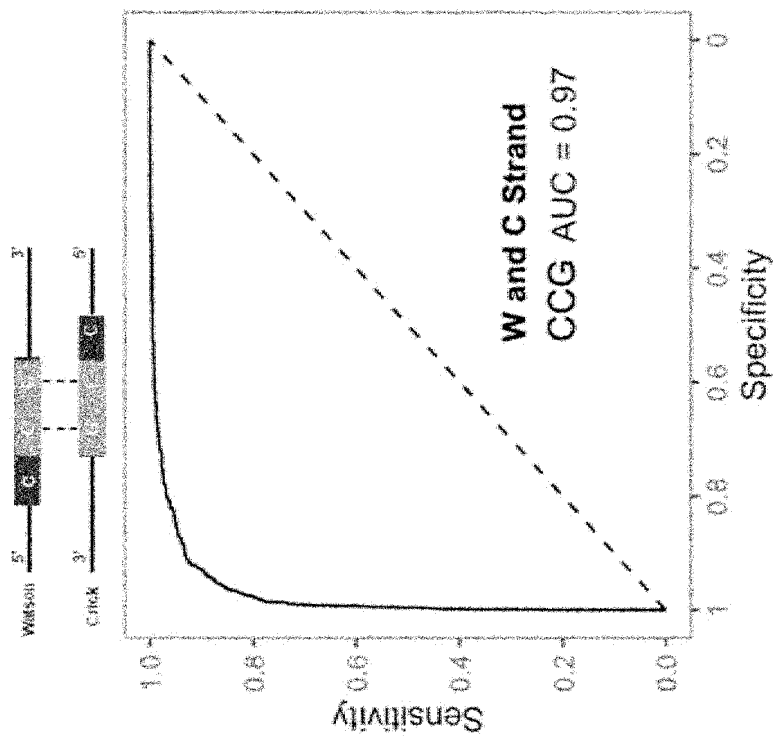


FIG. 21A

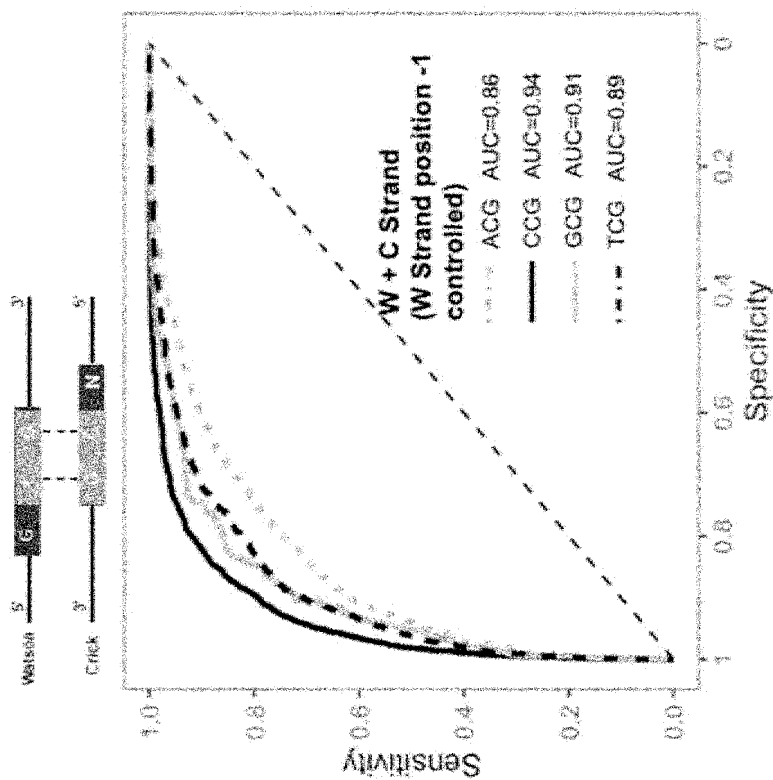
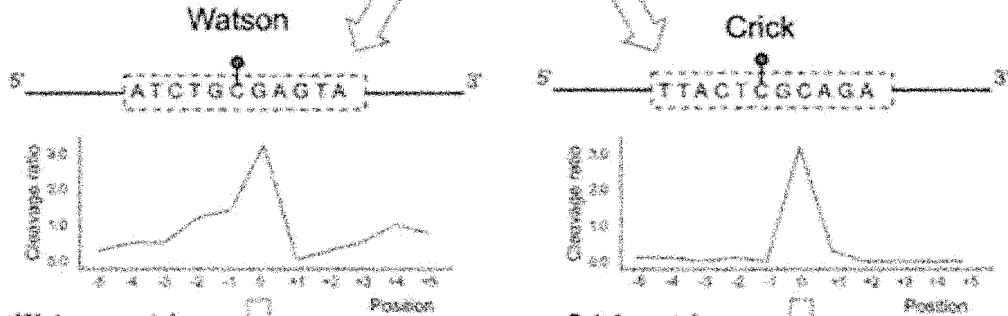


FIG. 21B

Cleavage measurement window



Cleavage ratio calculation



Watson matrix

	A	T	C	T	G	C	G	A	G	T	A
A	0.25	0	0	0	0	0	0.25	0	0	0.70	
G	0	0	0	0	1.40	0	0.05	0	0.50	0	0
C	0	0	0.45	0	0	3.10	0	0	0	0	0
T	0	0.45	0	1.10	0	0	0	0	0	0.95	0

Crick matrix

	T	T	A	C	T	C	G	C	A	G	A
A	0	0	0.05	0	0	0	0	0.05	0	0.05	
G	0	0	0	0	0	0	0.45	0	0	0.05	0
C	0	0	0	0.15	0	5.00	0	0.05	0	0	0
T	0.15	0.15	0	0	0.50	0	0	0	0	0	0

Combined matrix

		A	T	C	T	G	C	G	A	G	T	A
		-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5
Watson strand data	A	0.25	0	0	0	0	0	0	0.25	0	0	0.70
	G	0	0	0	0	1.40	0	0.05	0	0.50	0	0
	C	0	0	0.45	0	0	3.10	0	0	0	0	0
	T	0	0.45	0	1.10	0	0	0	0	0	0.95	0
Crick strand data	A	0	0	0.05	0	0	0	0	0	0.05	0	0.05
	G	0	0	0	0	0	0	0.45	0	0	0.05	0
	C	0	0	0	0.15	0	5.00	0	0.05	0	0	0
	T	0.15	0.15	0	0	0.50	0	0	0	0	0	0
		-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5
		T	T	A	C	T	C	G	C	A	G	A

Model structure

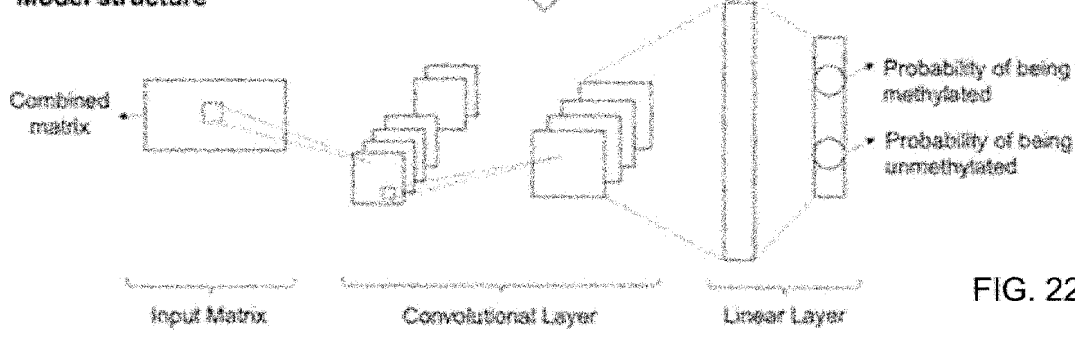
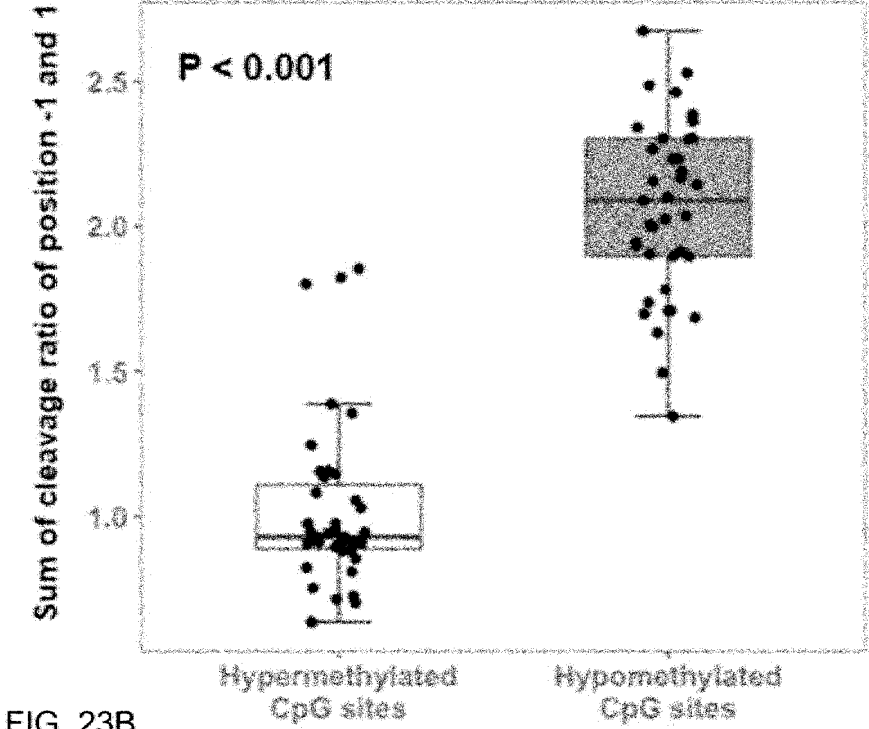
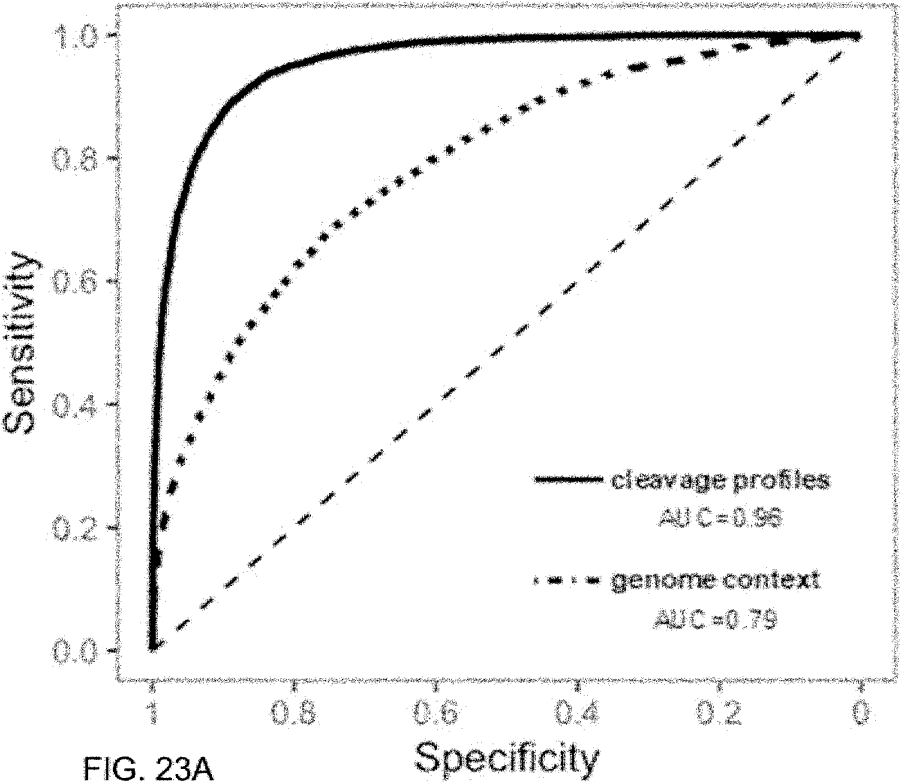


FIG. 22



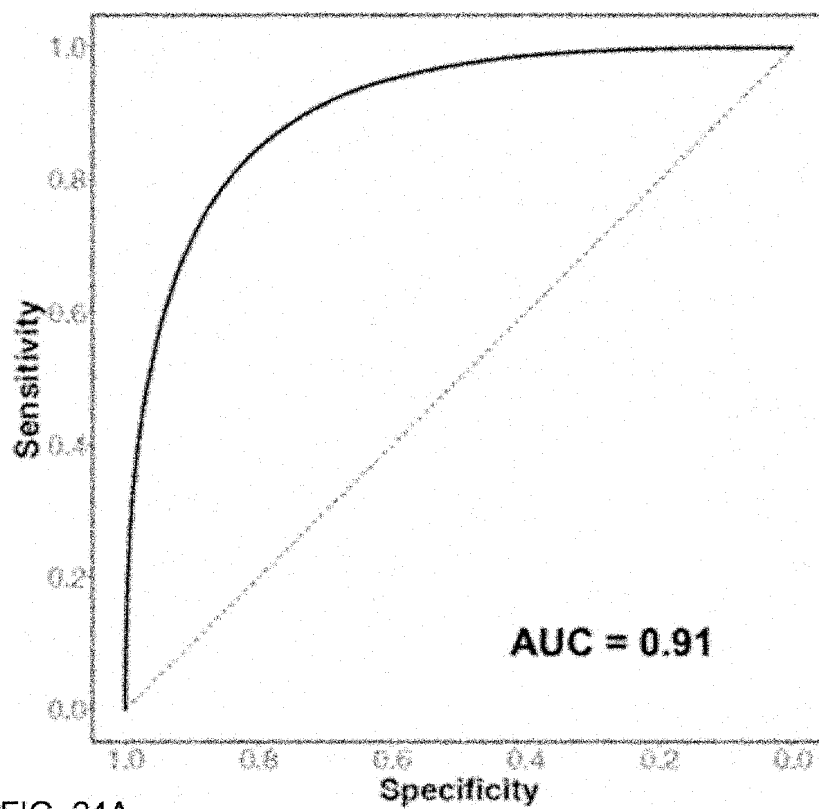


FIG. 24A

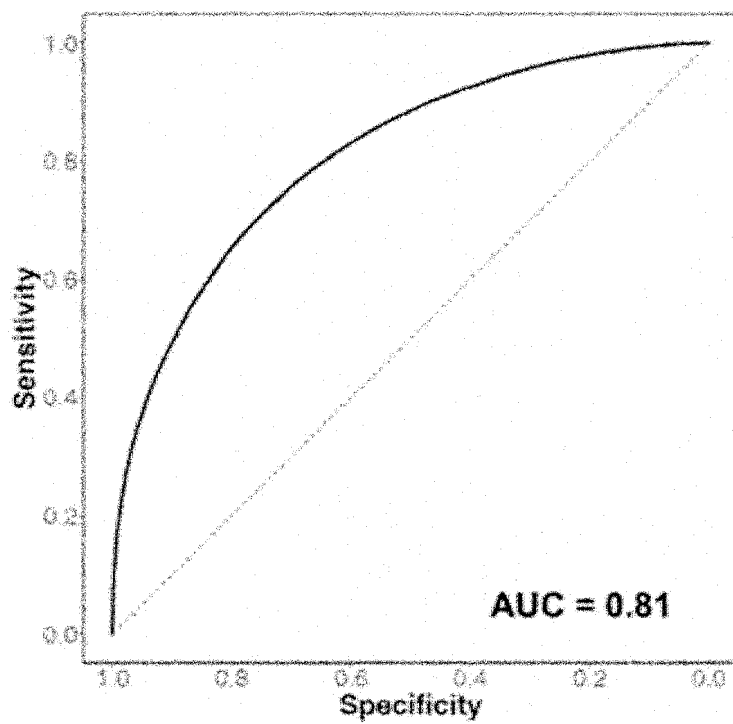


FIG. 24B

FIG. 25A

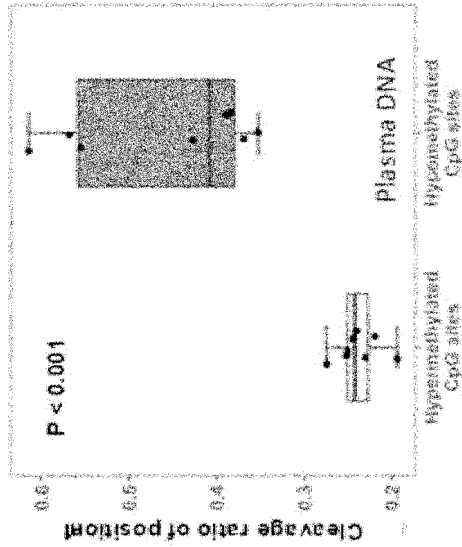


FIG. 25B

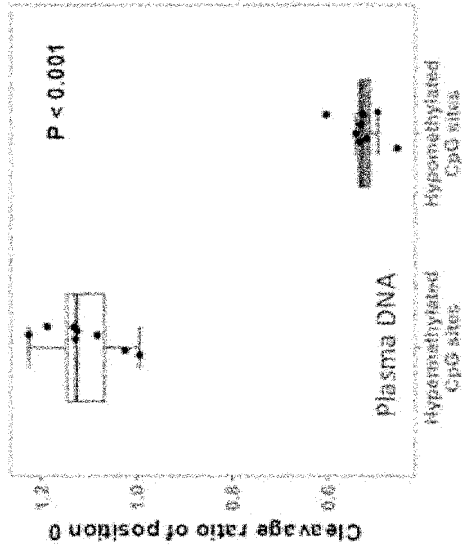


FIG. 25C

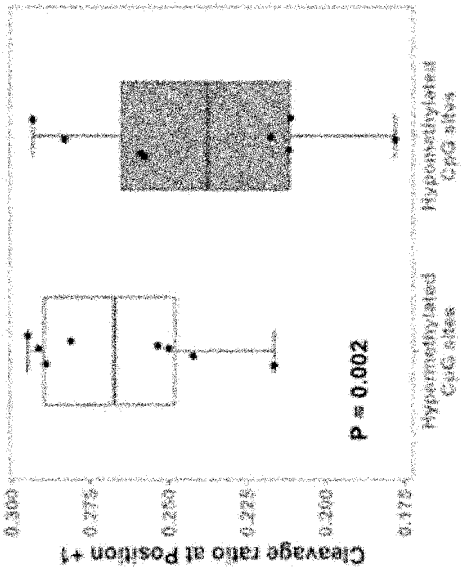


FIG. 25D

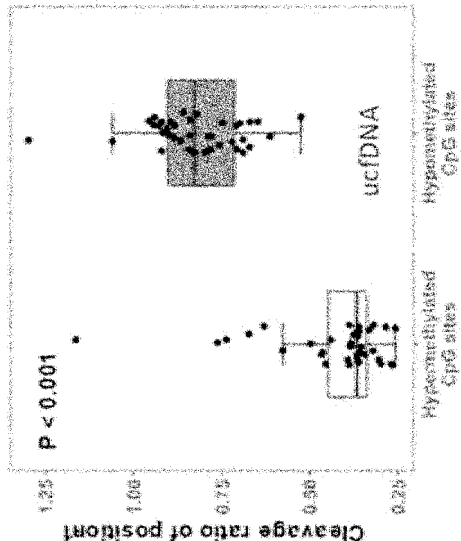


FIG. 25E

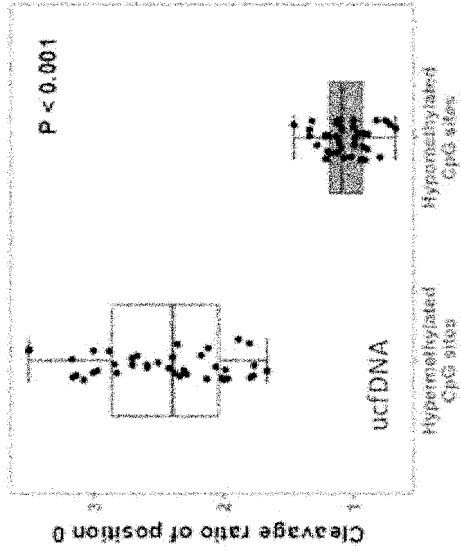
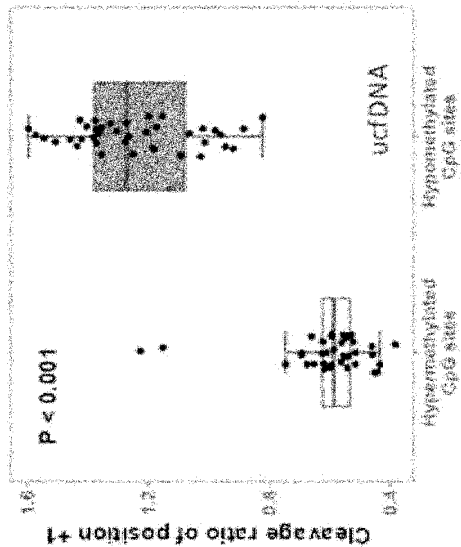


FIG. 25F



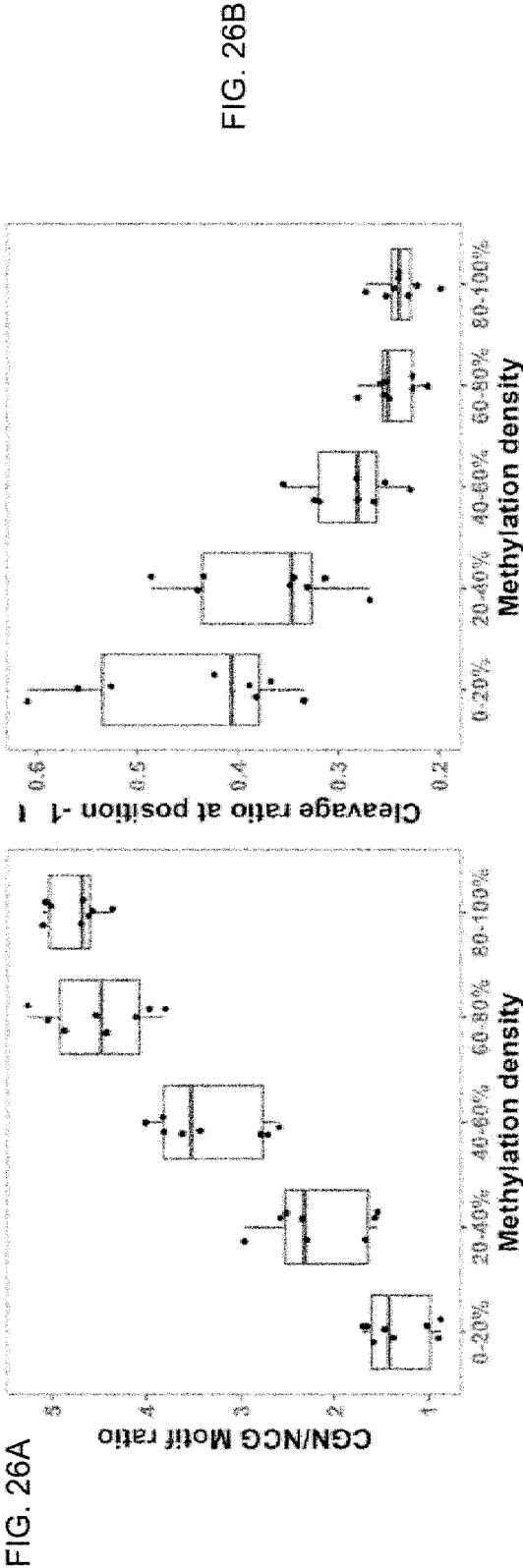
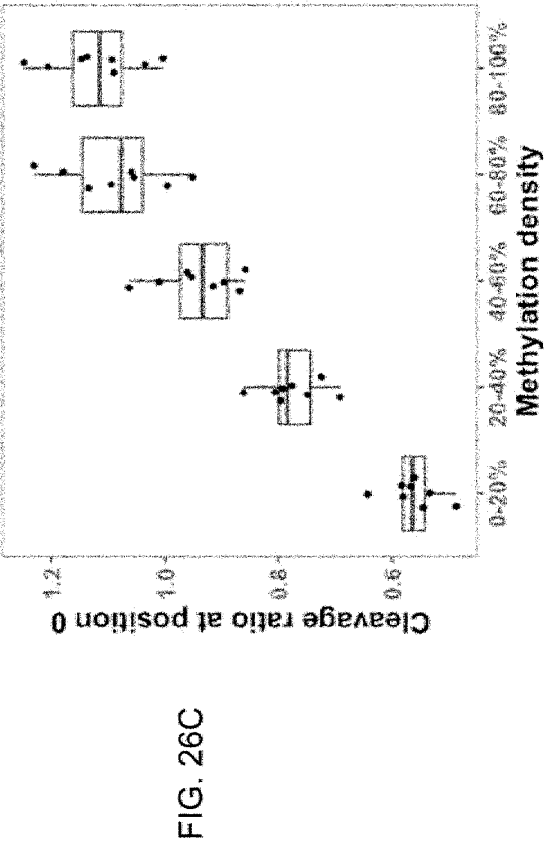


FIG. 26B



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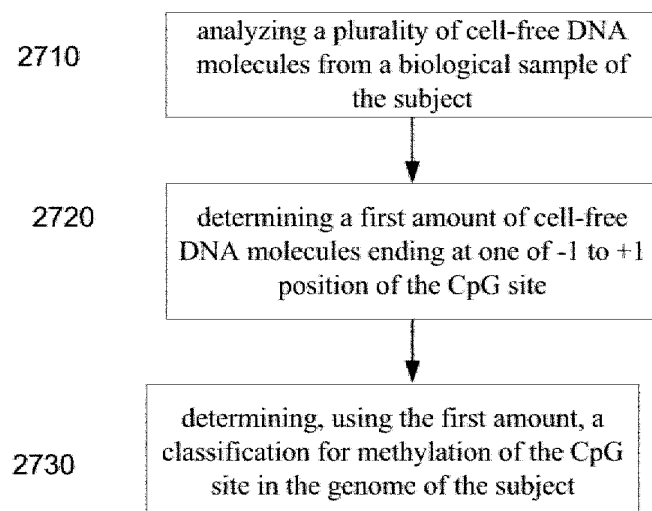


FIG. 27

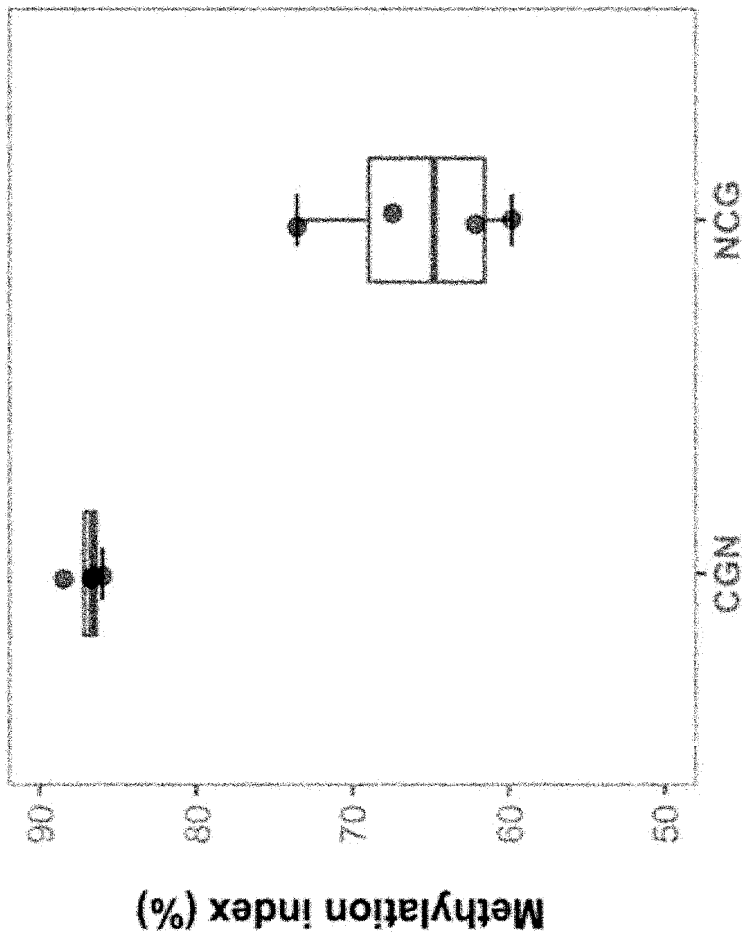
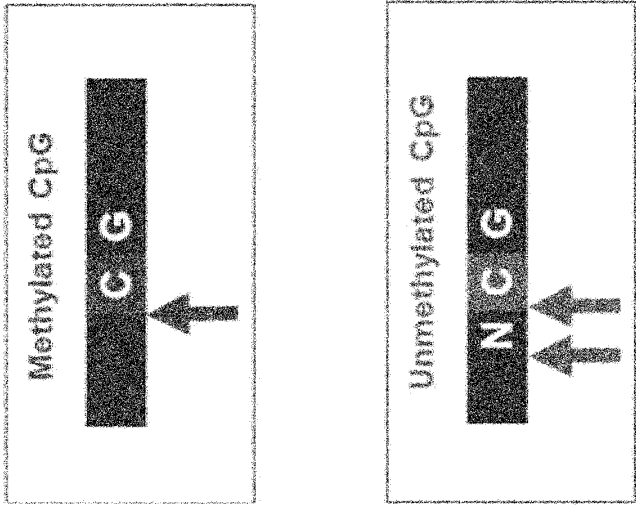


FIG. 28



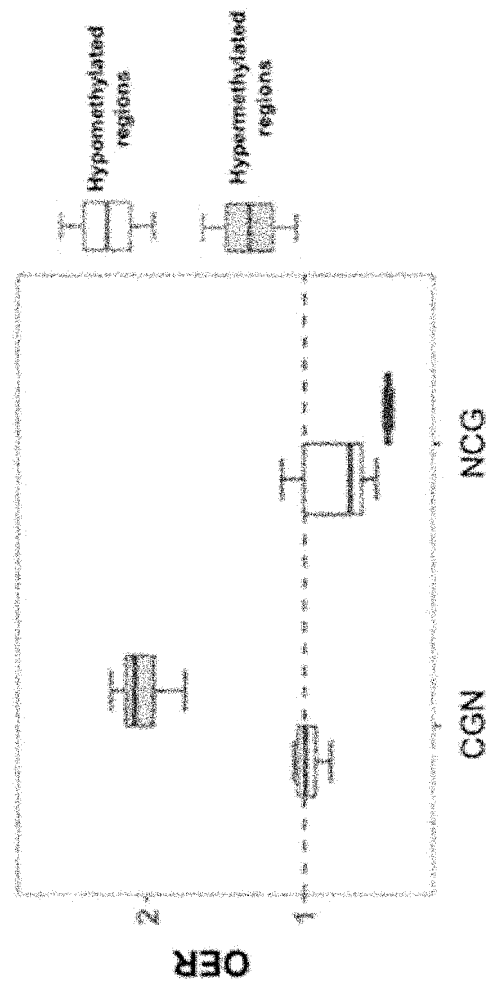


FIG. 29

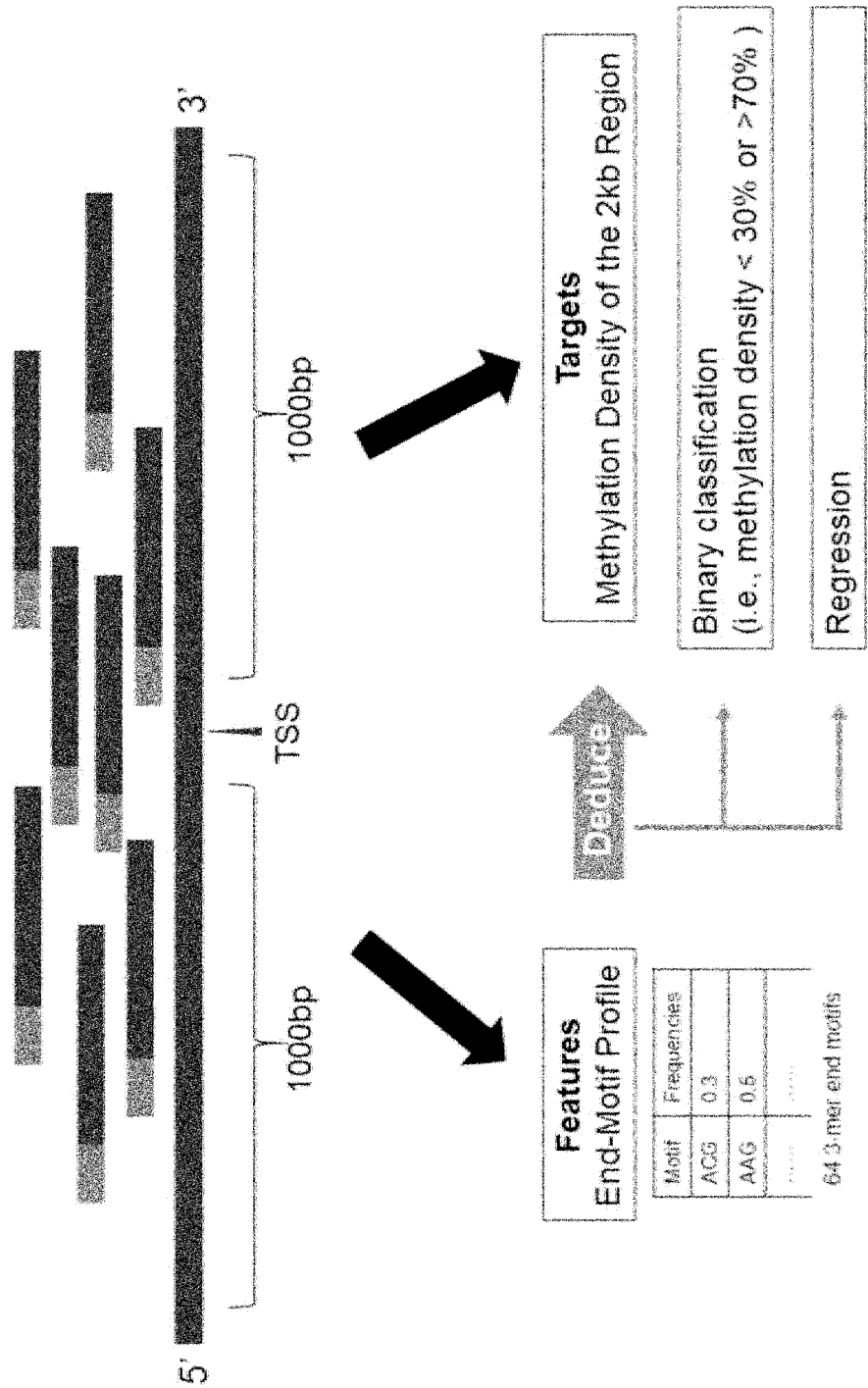


FIG. 30

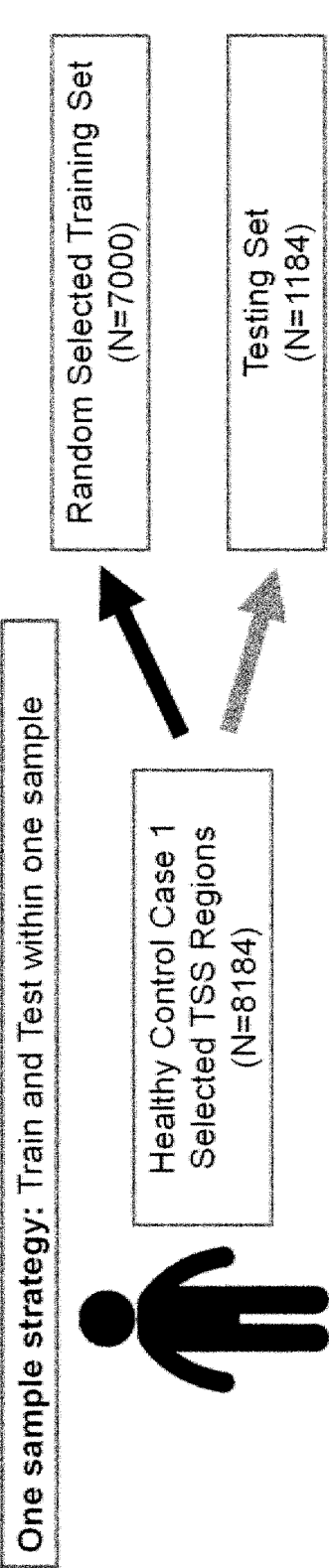


FIG. 31A

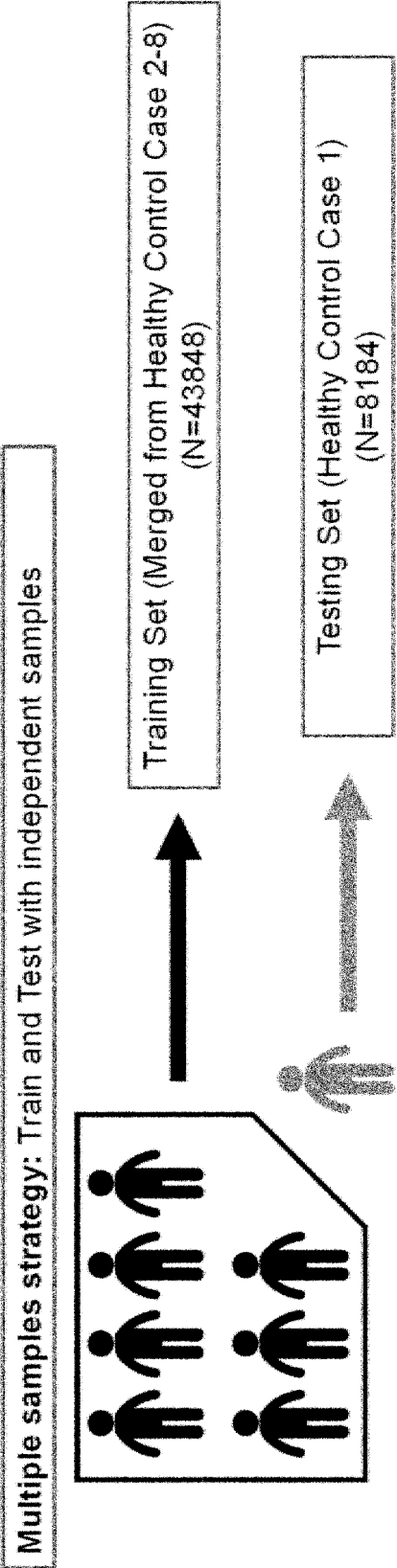
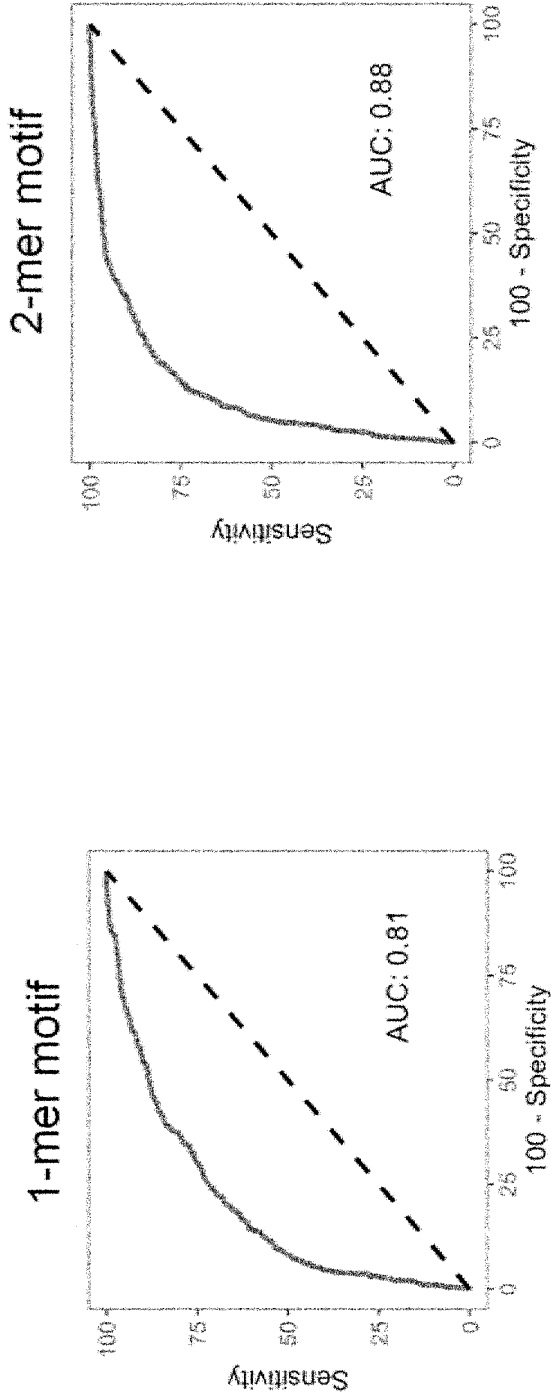


FIG. 31B

Binary Classification by end-motifs (One sample strategy)

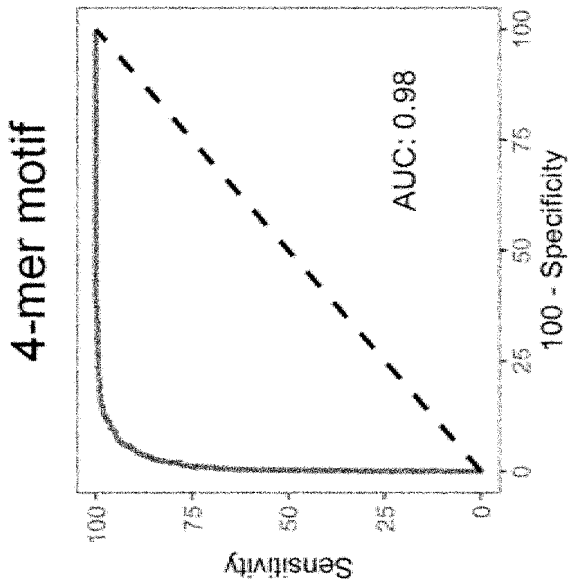


	Hypo	Hyper
Predicted Hypo	417	197
Predicted Hyper	145	425
Accuracy	74.2%	68.3%

FIG. 32A

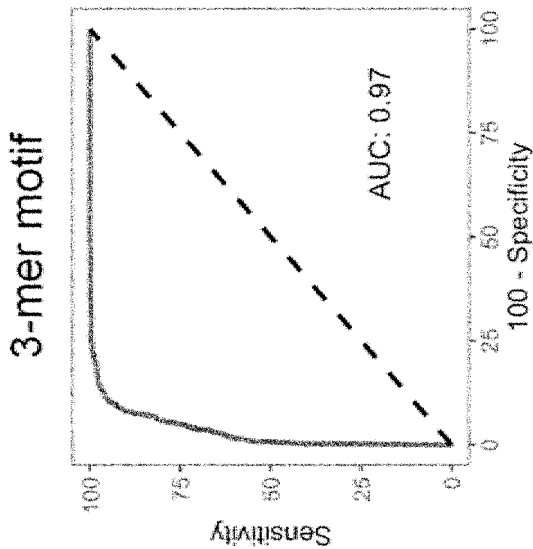
	Hypo	Hyper
Predicted Hypo	430	108
Predicted Hyper	120	526
Accuracy	78.2%	83.0%

FIG. 32B



	Hypo	Hyper
Predicted Hypo	552	25
Predicted Hyper	66	630
Accuracy	89.3%	96.2%

FIG. 33B



	Hypo	Hyper
Predicted Hypo	544	24
Predicted Hyper	74	631
Accuracy	88.0%	96.3%

FIG. 33A

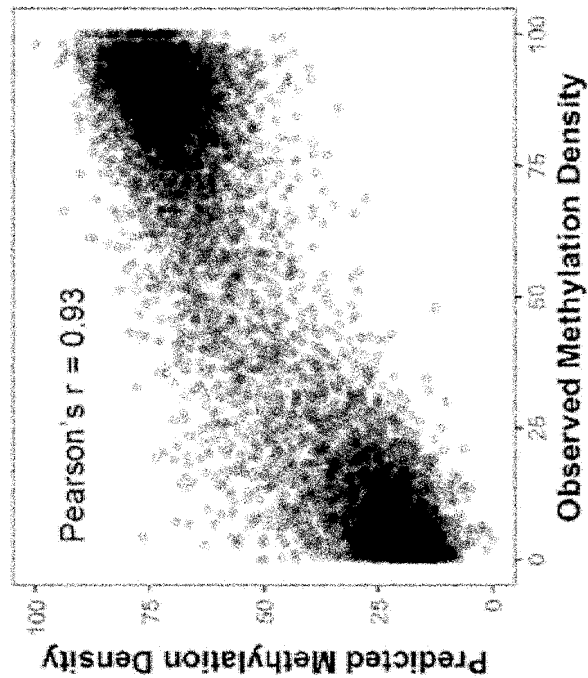


FIG. 34B

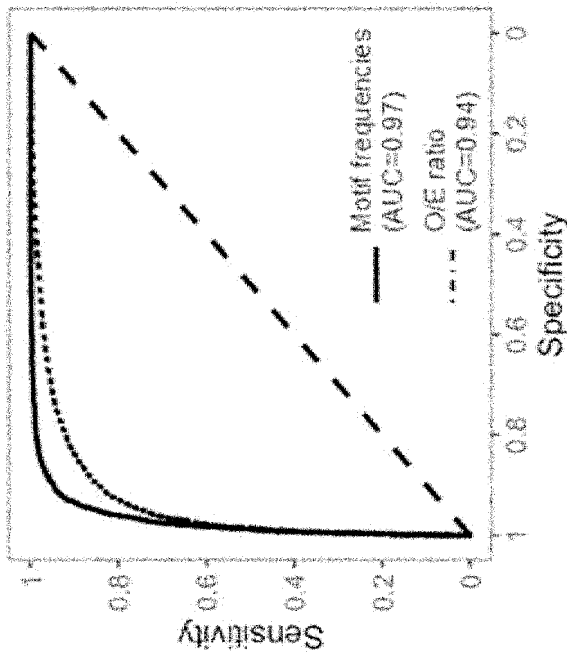


FIG. 34A

Normalization of observed motif frequency by genome context

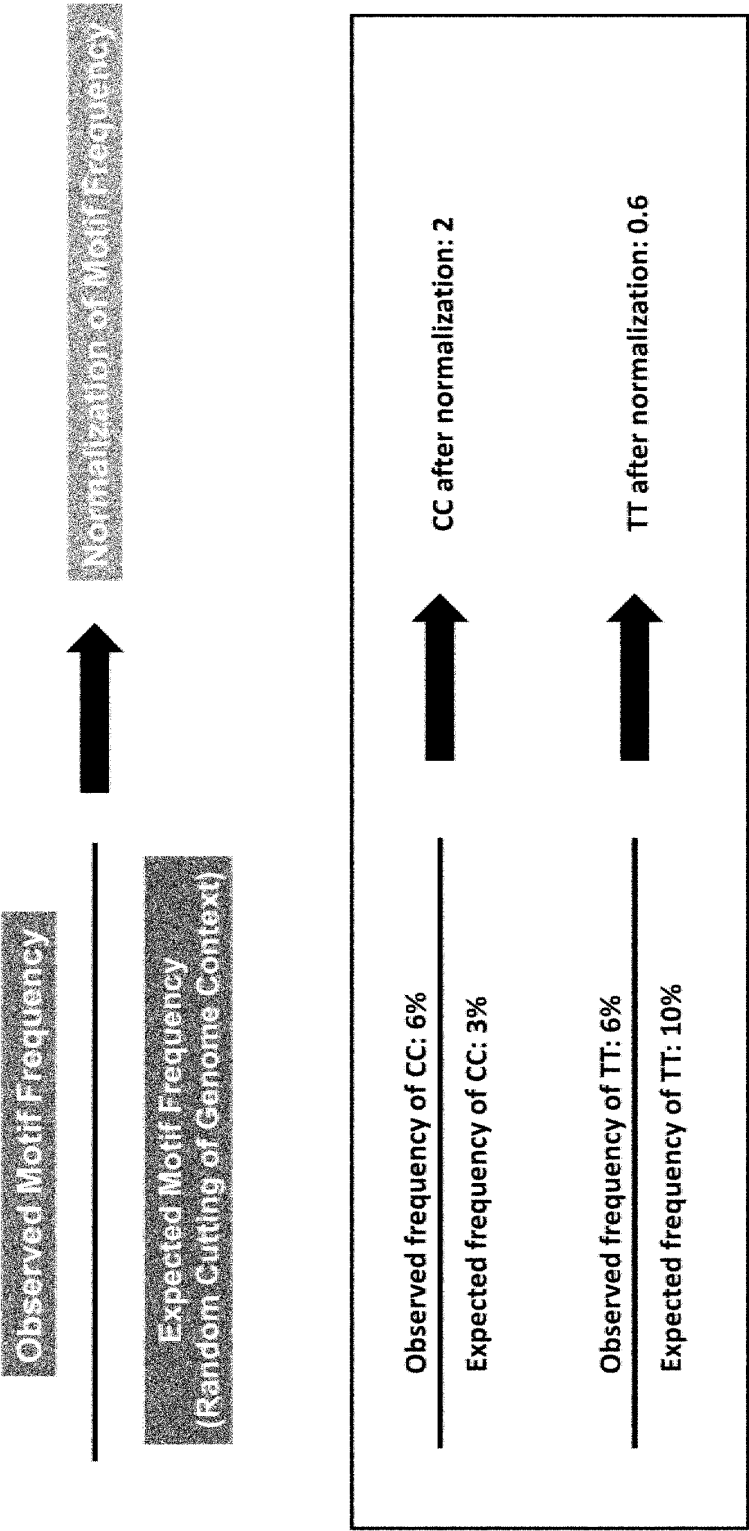


FIG. 35

Binary Classification by context normalized end-motifs

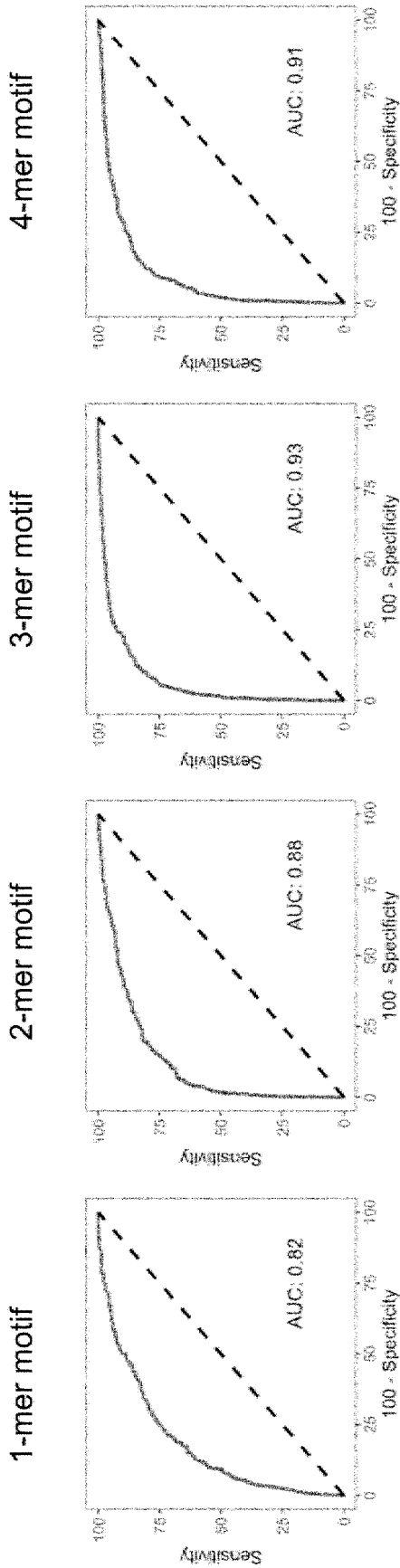


FIG. 36

Normalization of observed motif frequency by per molecule context

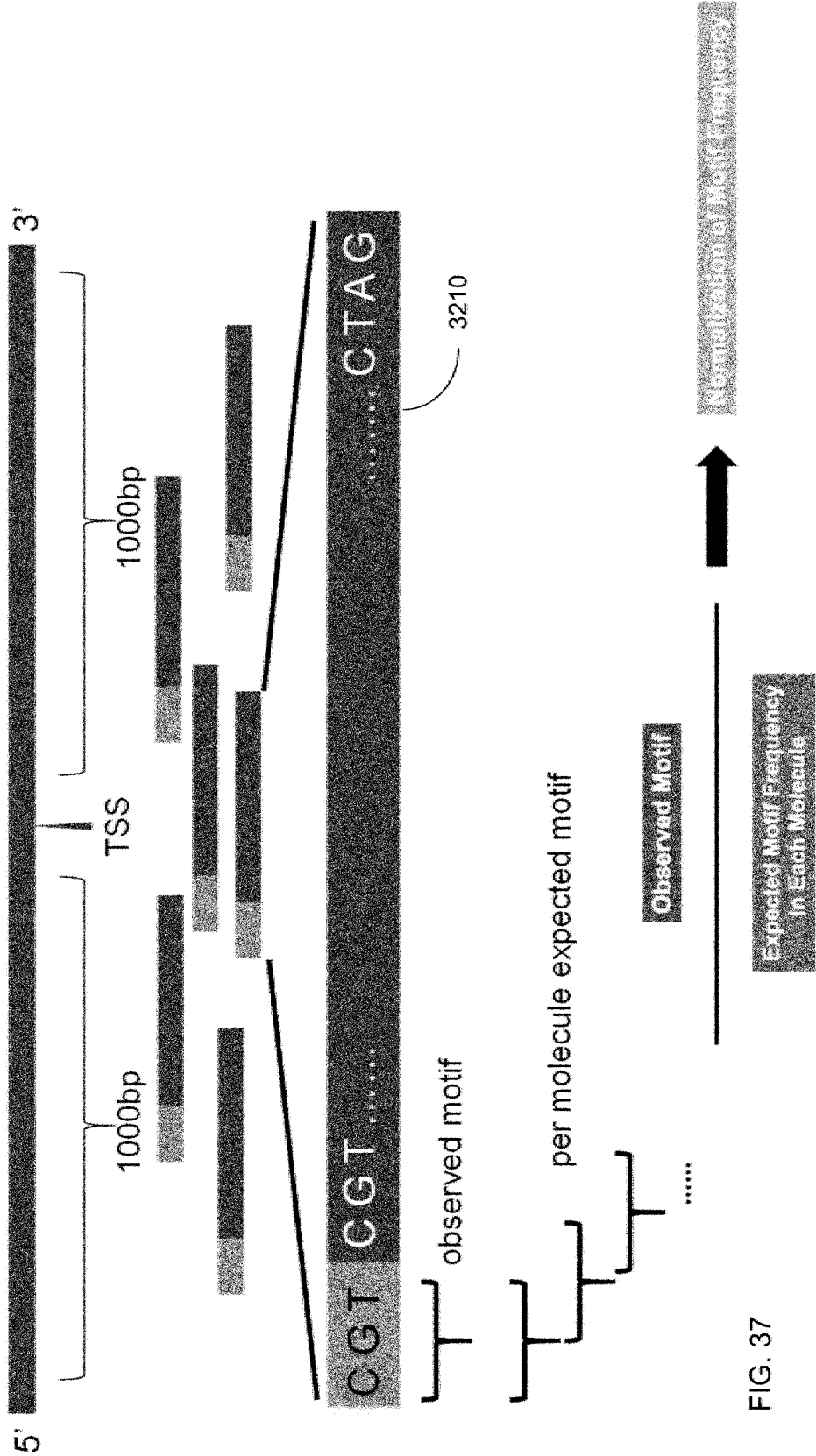


FIG. 37

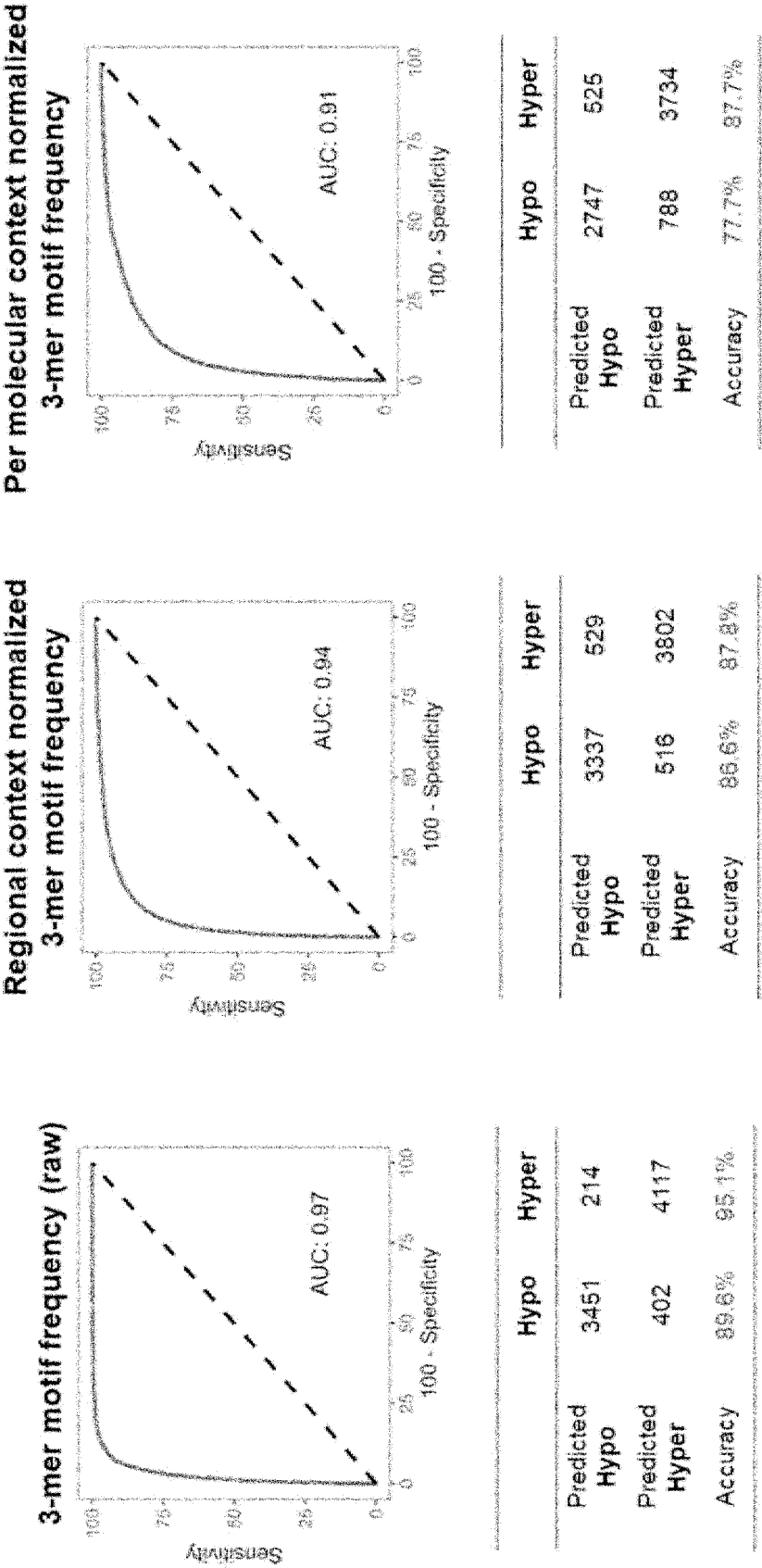
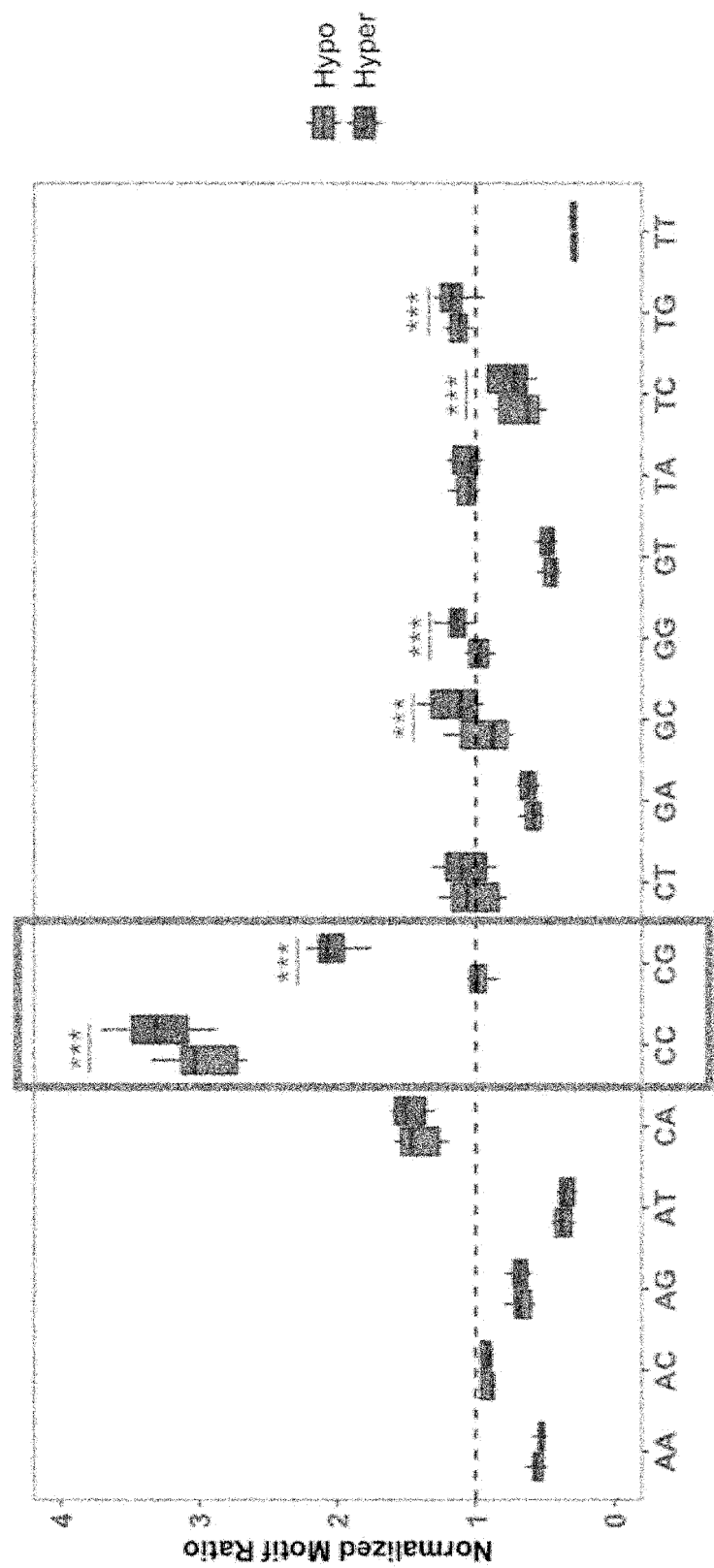


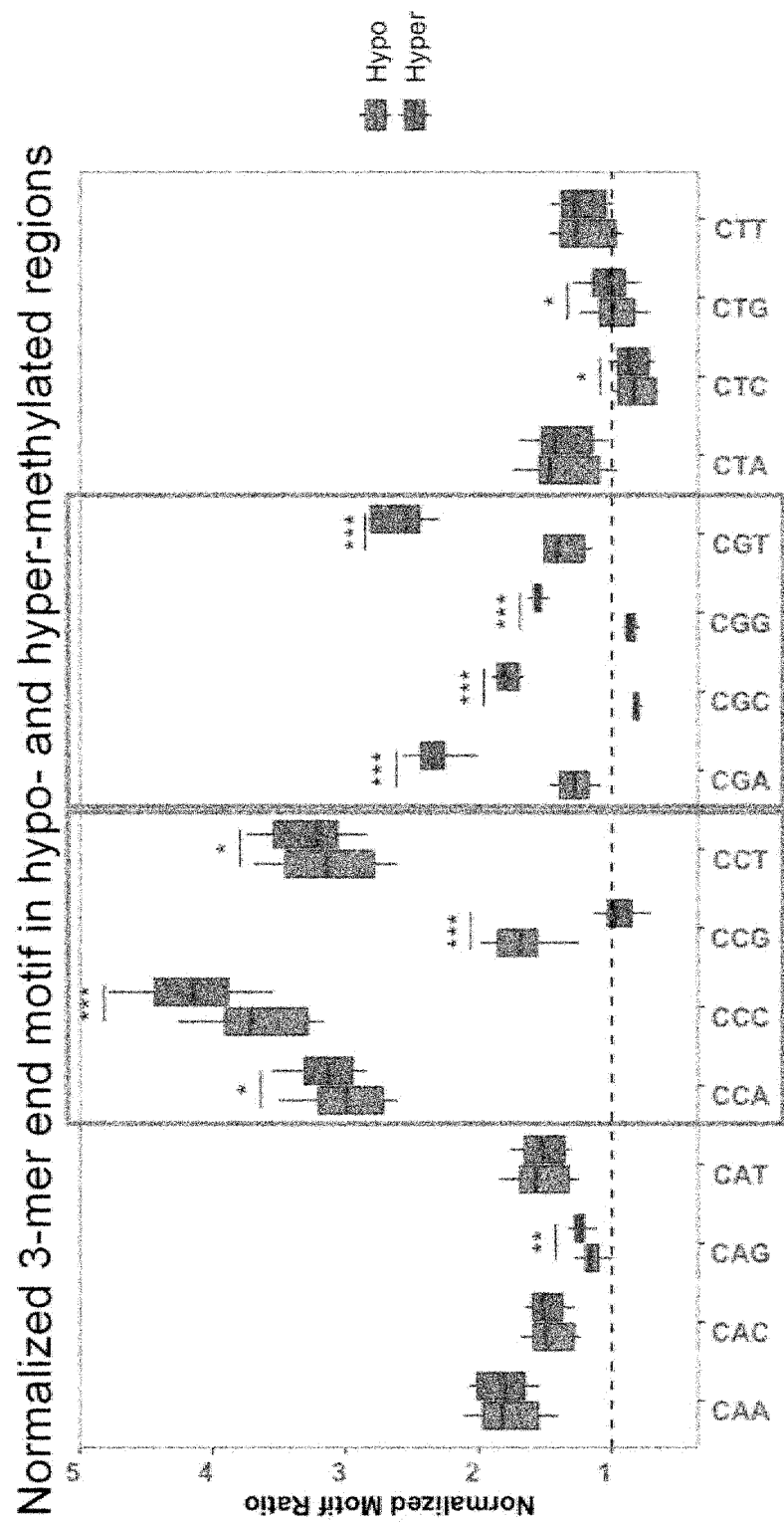
FIG. 38

Normalized 2-mer end motif in hypo- and hyper-methylated regions



*** Paired t-test after Bonferroni Adjustment $P < 0.0001$

FIG. 39



Paired t-test after Bonferroni Adjustment: *** Adjusted $P < 0.0001$; ** Adjusted $P < 0.001$; * Adjusted $P < 0.05$

FIG. 40

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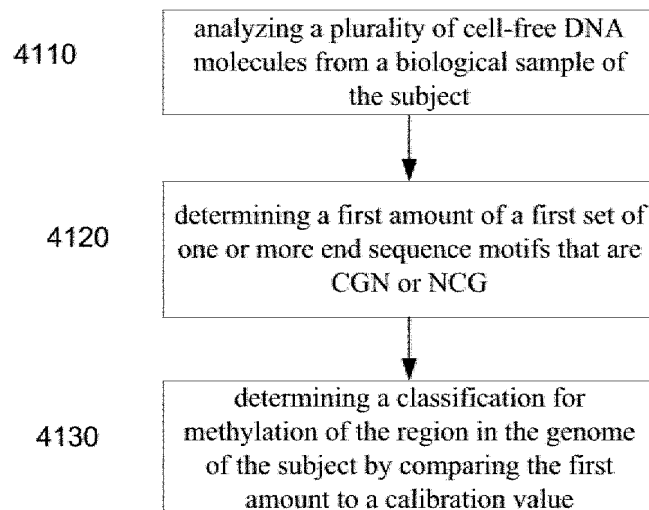


FIG. 41

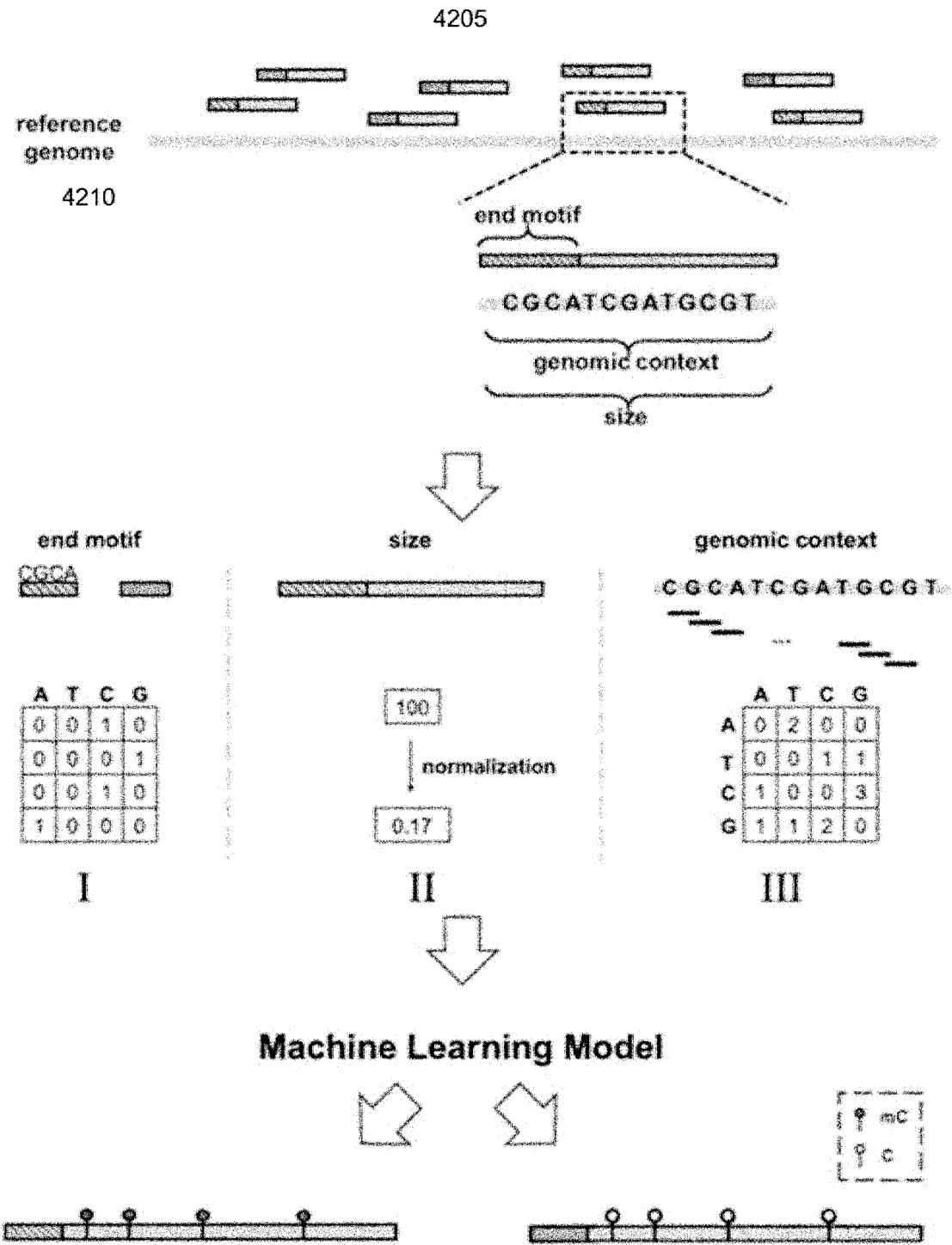


FIG. 42

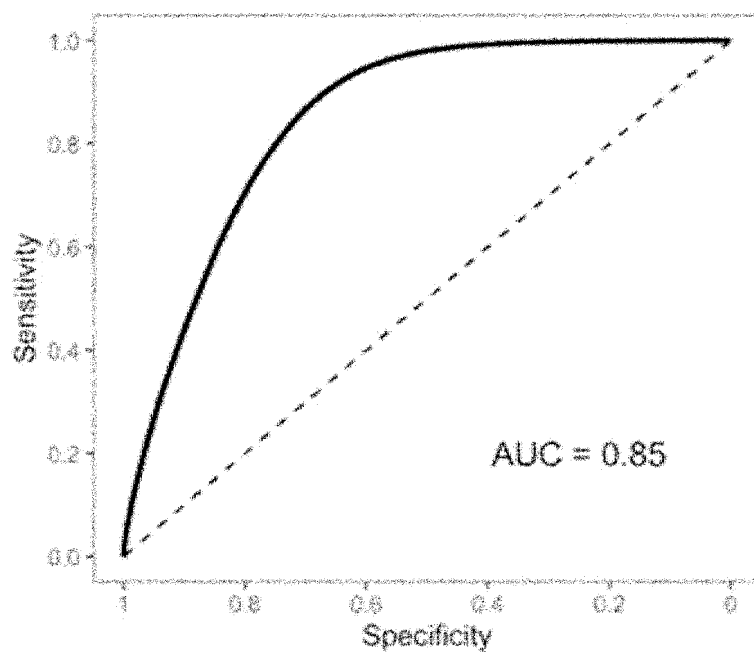


FIG. 43A

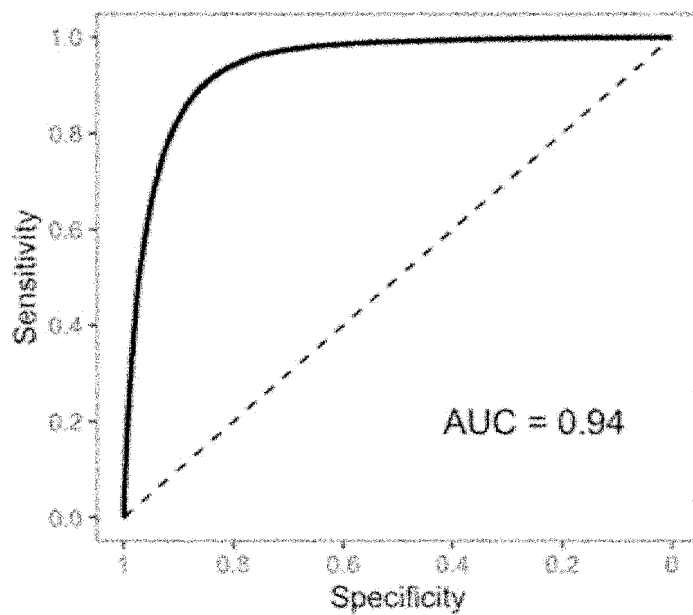


FIG. 43B

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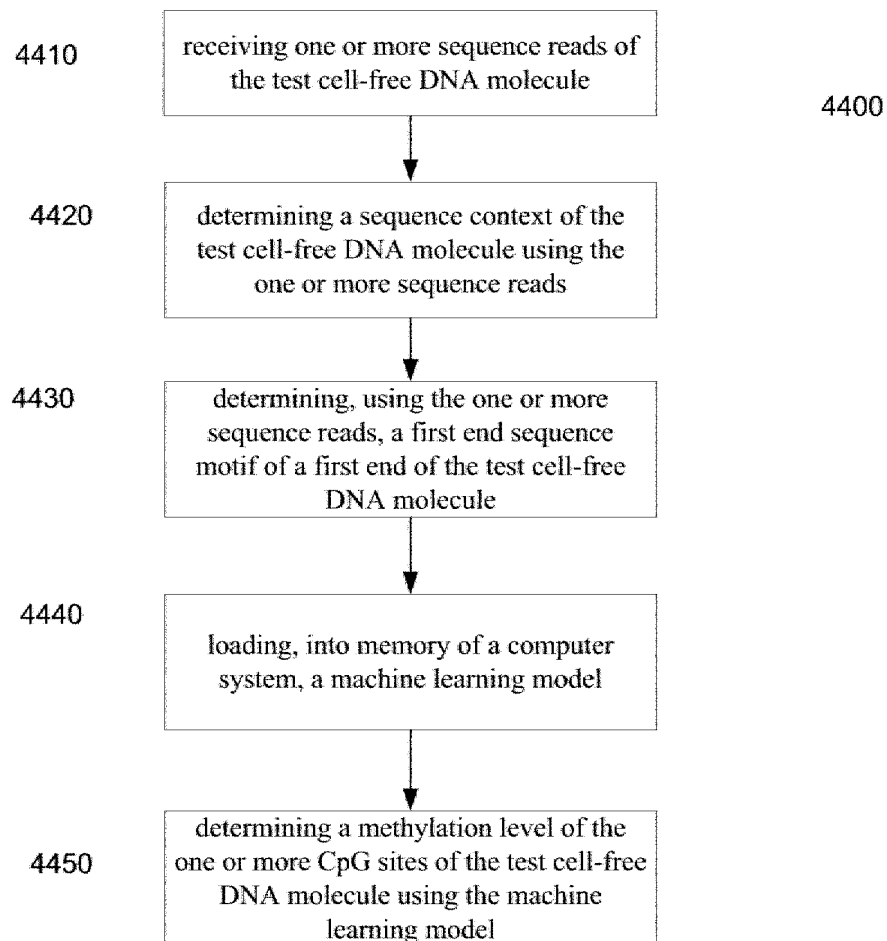


FIG. 44

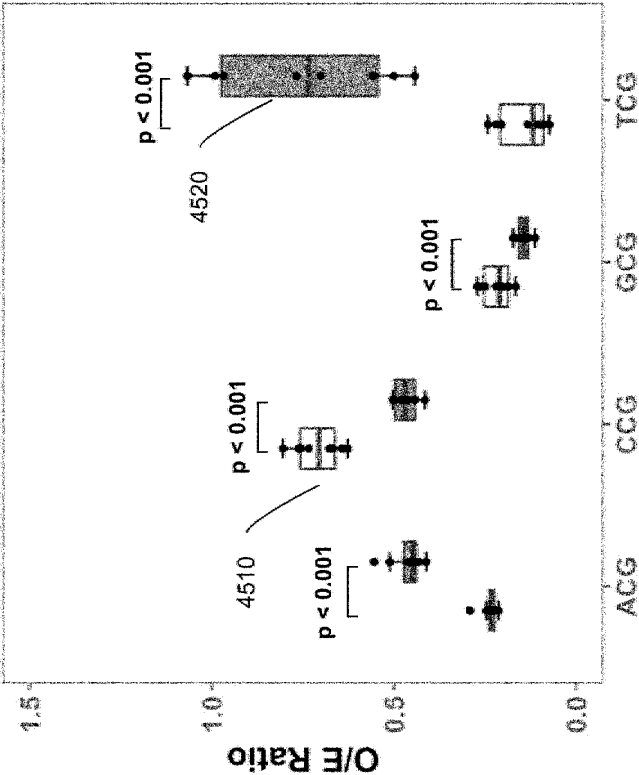


FIG. 45B

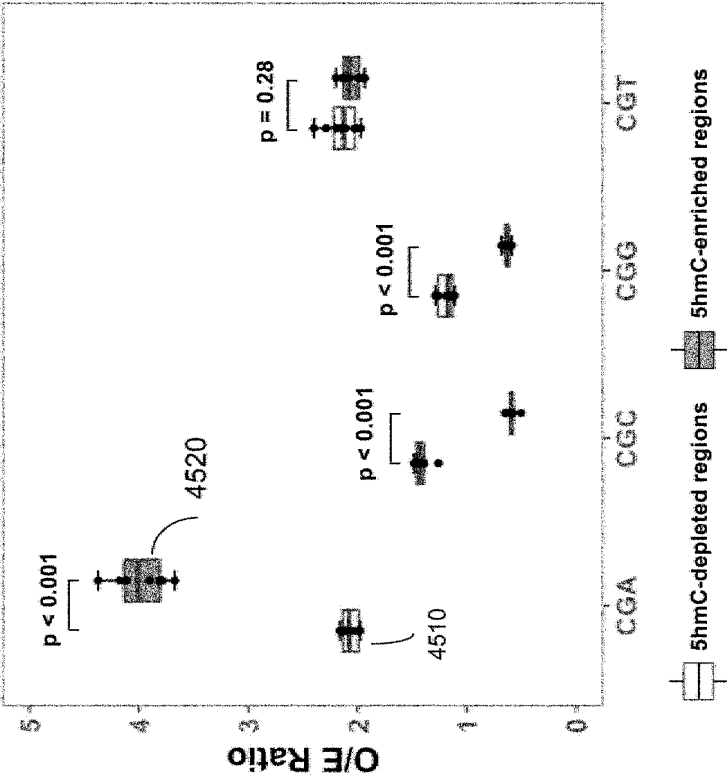


FIG. 45A

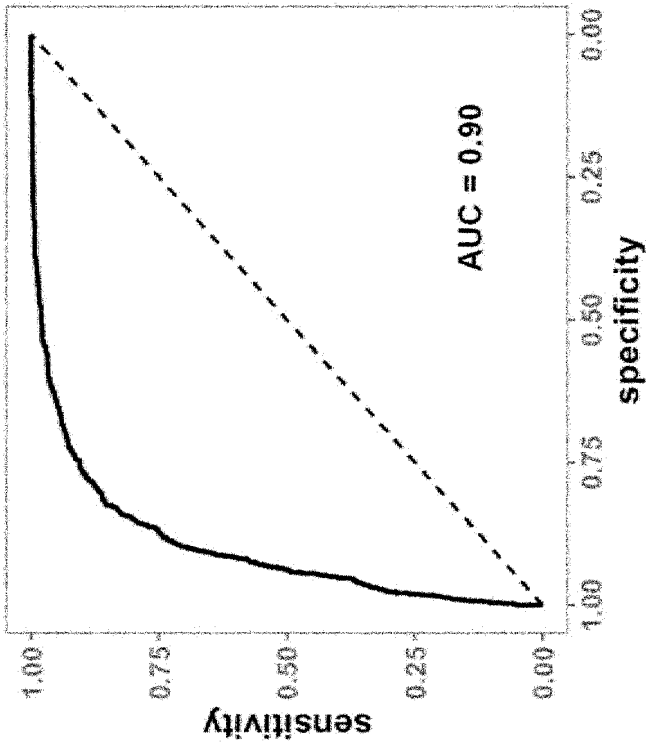


FIG. 46B

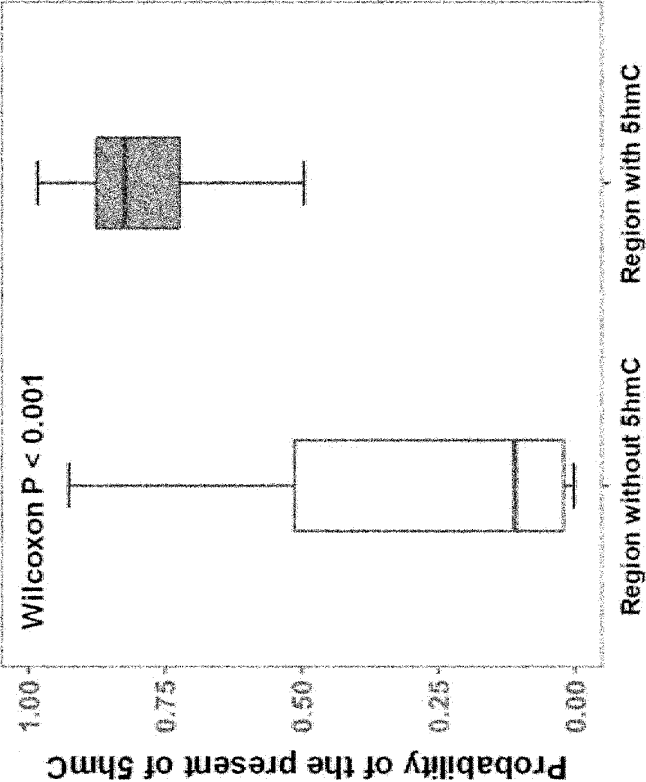


FIG. 46A

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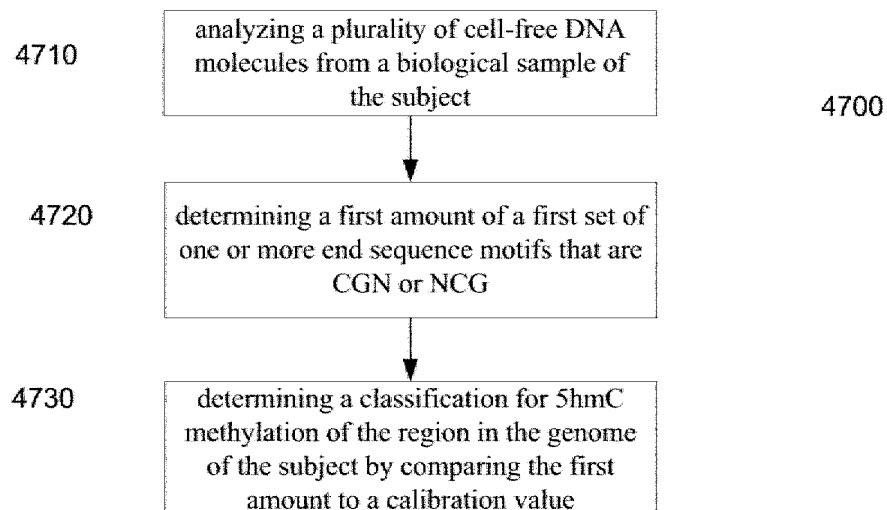


FIG. 47

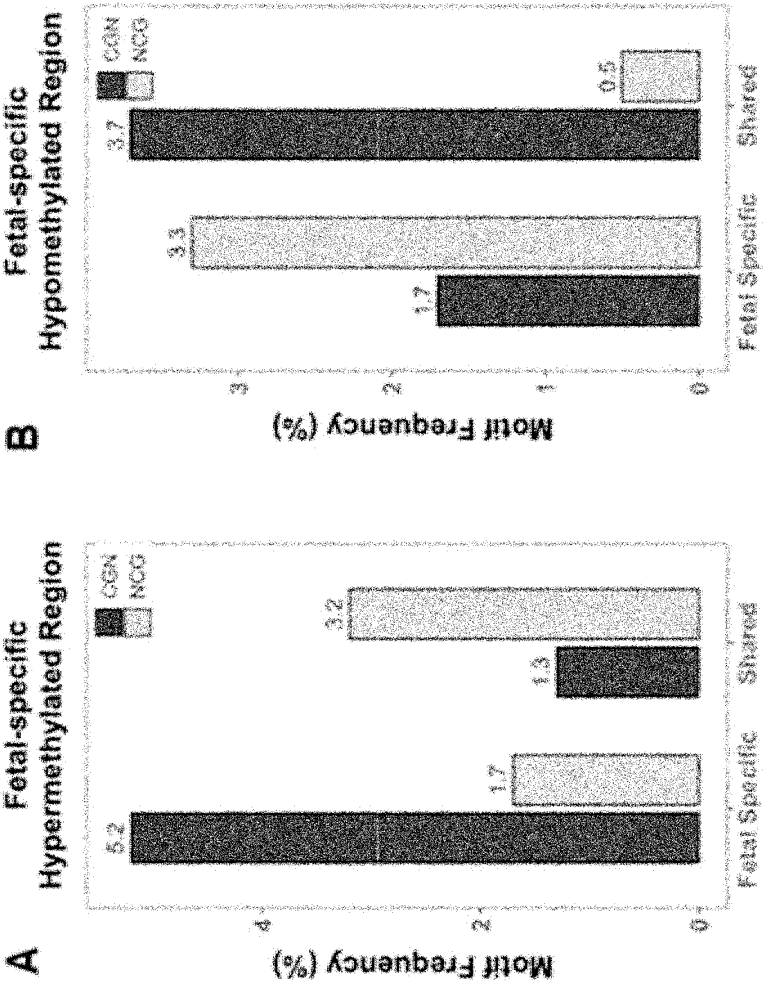


FIG. 48B

FIG. 48A

FIG. 49A Placenta-specific hypermethylated CpGs

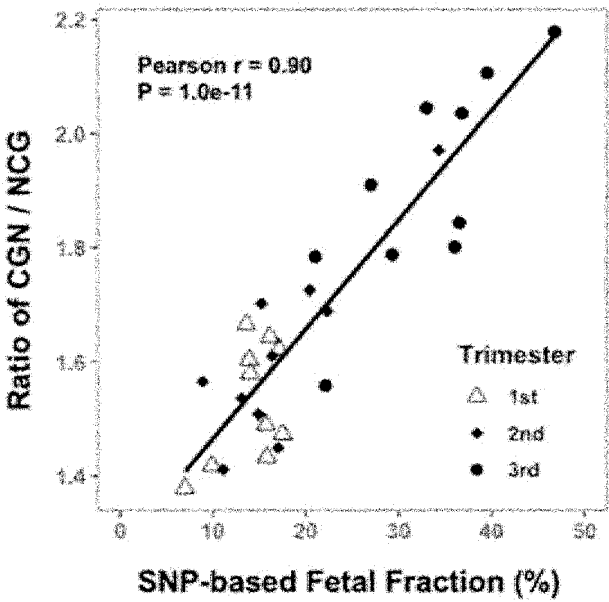
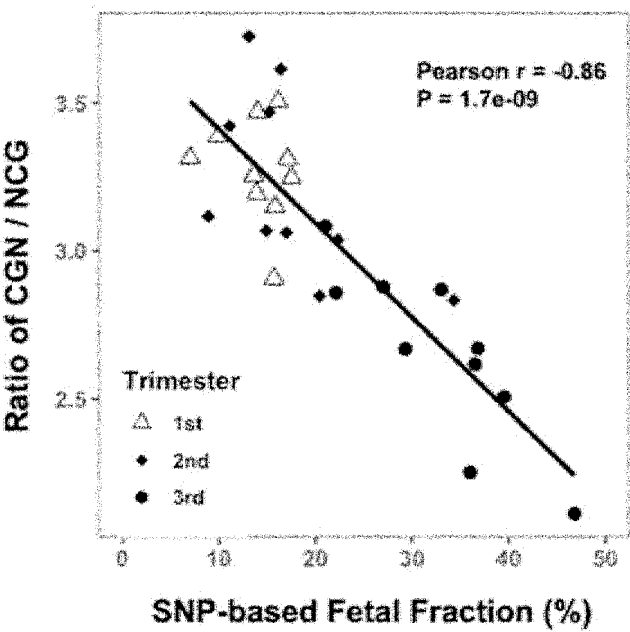


FIG. 49B Placenta-specific hypomethylated CpGs



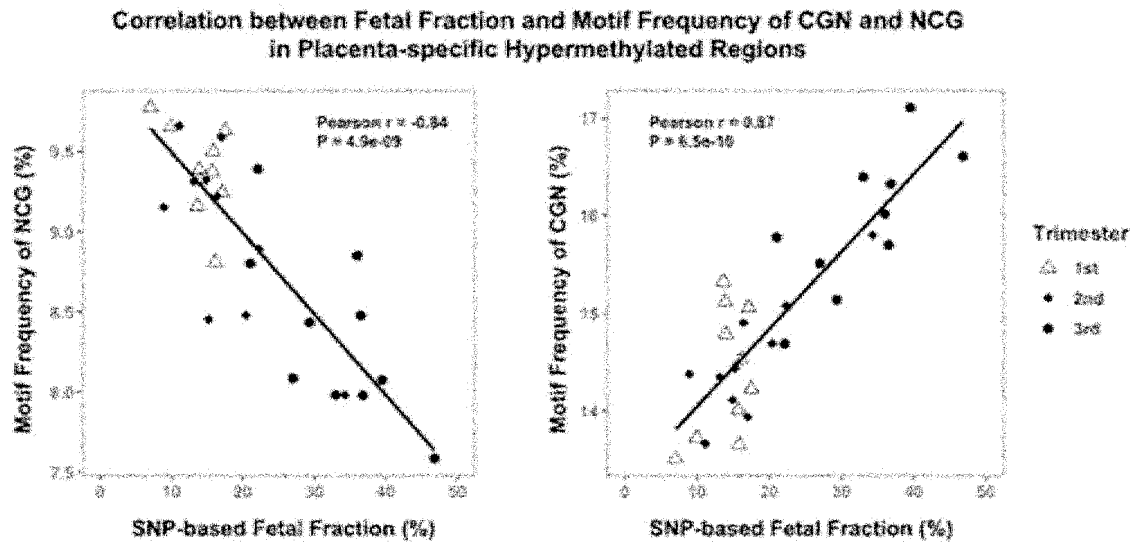


FIG. 50A

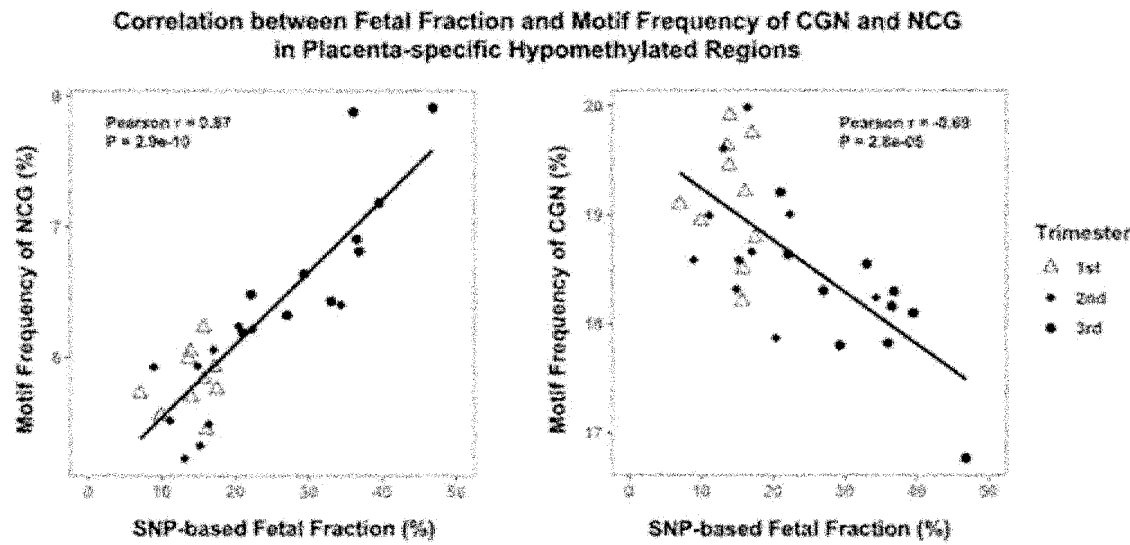


FIG. 50B

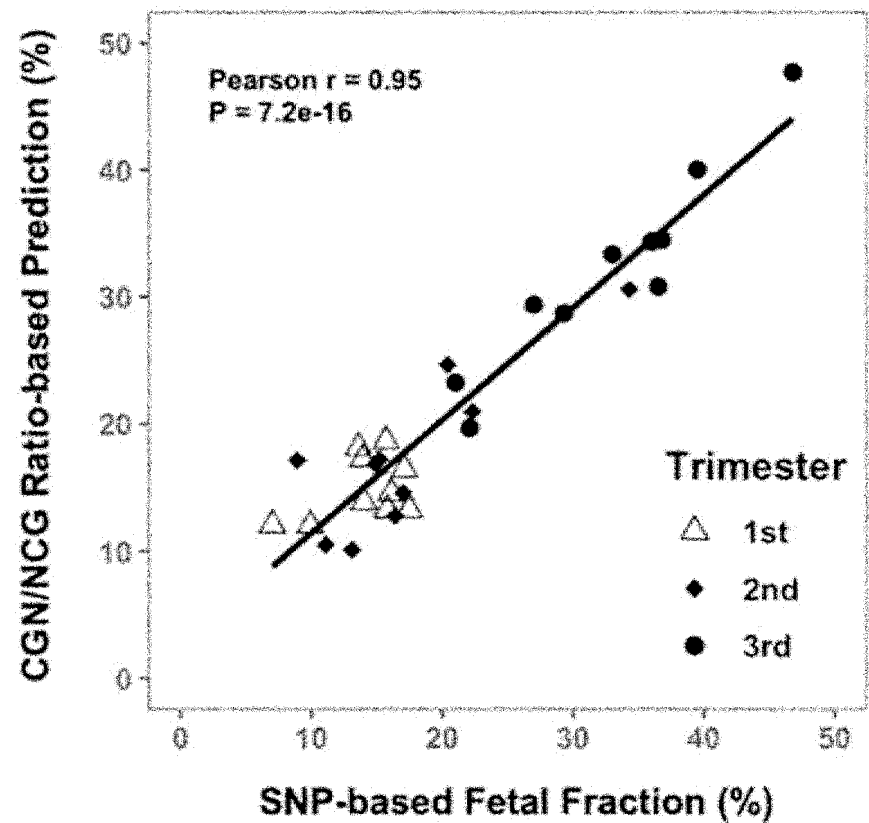


FIG. 51

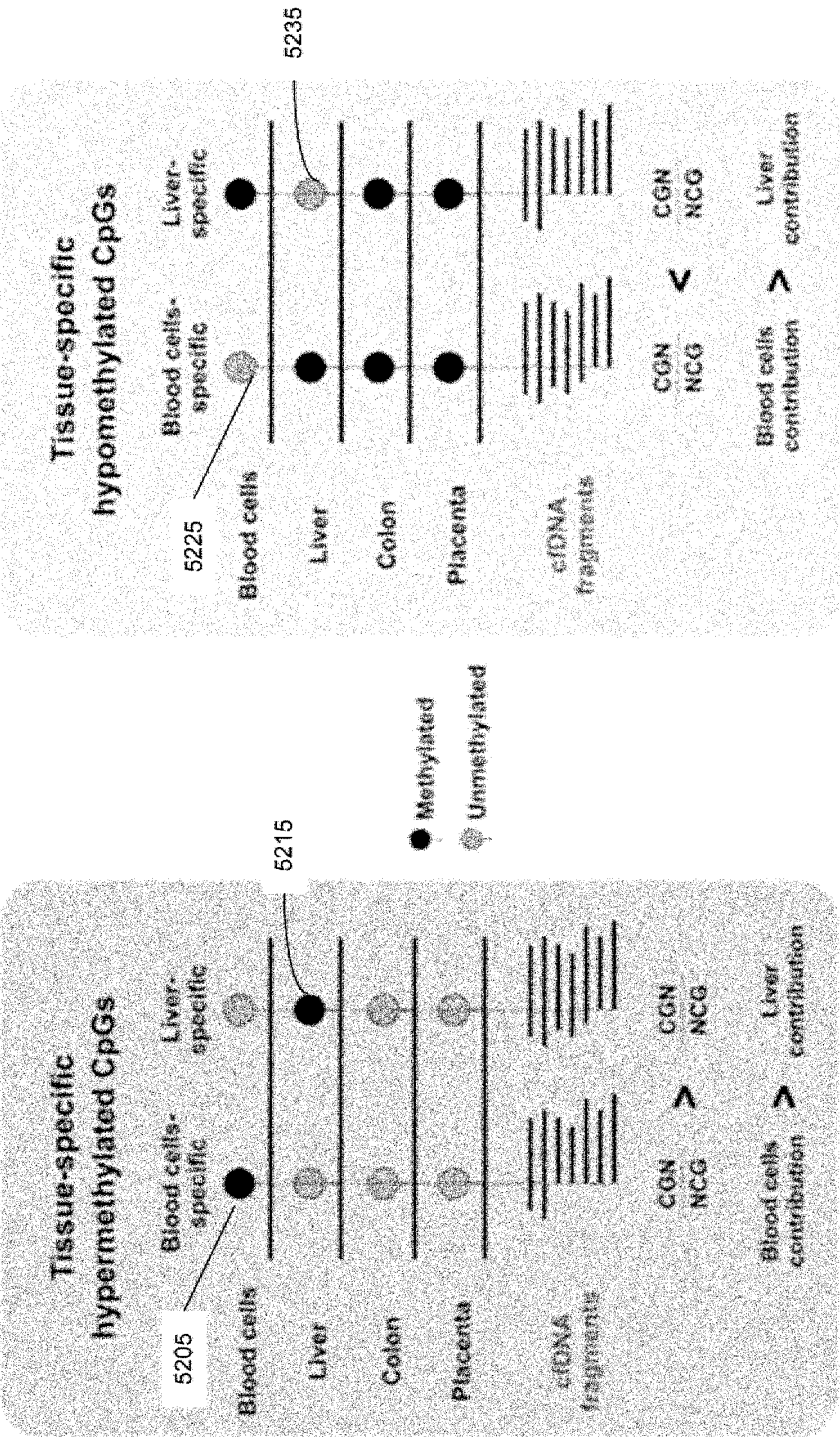


FIG. 52

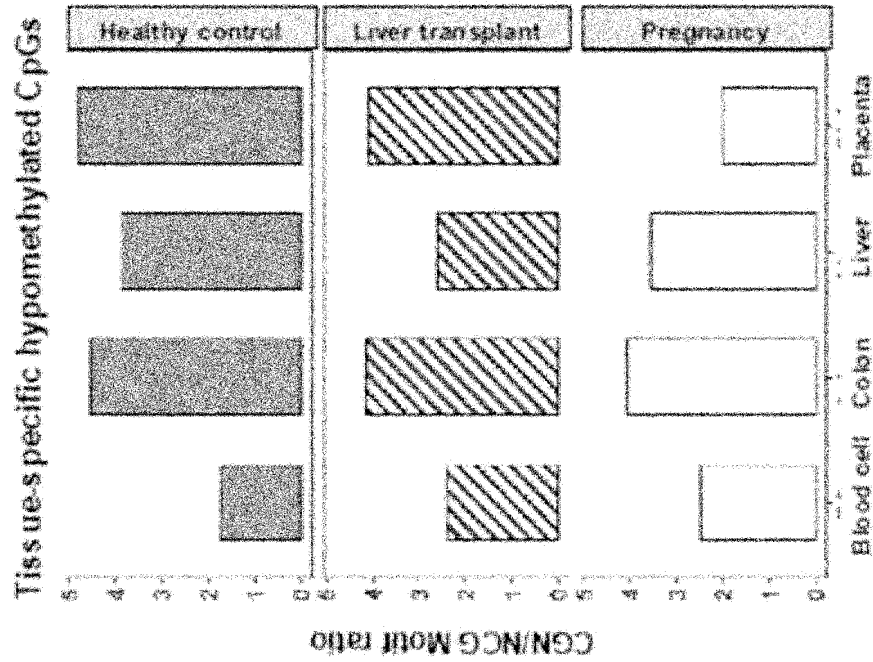


FIG. 53B

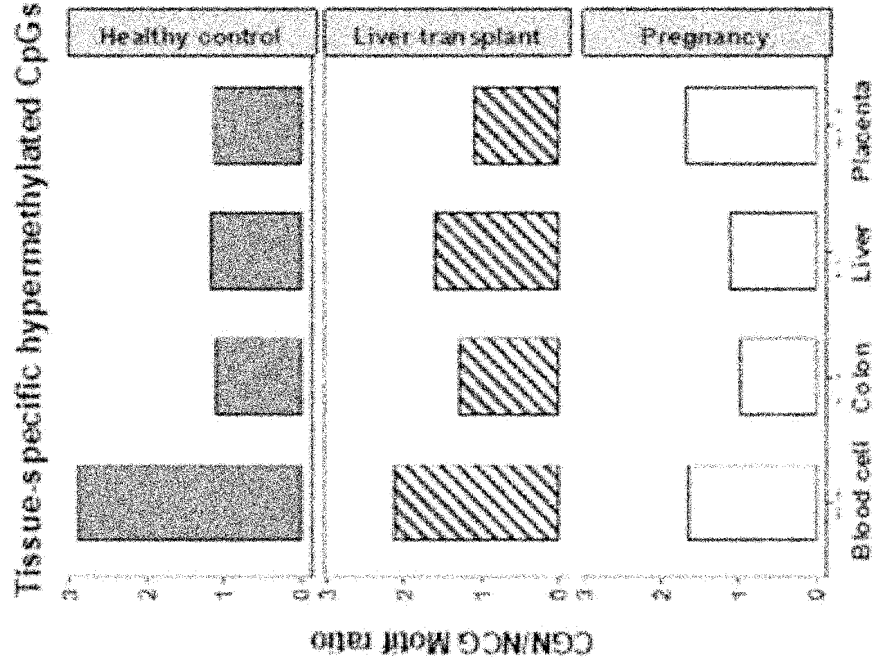


FIG. 53A

FIG. 54A Placenta Specific Hypermethylated CpG Sites

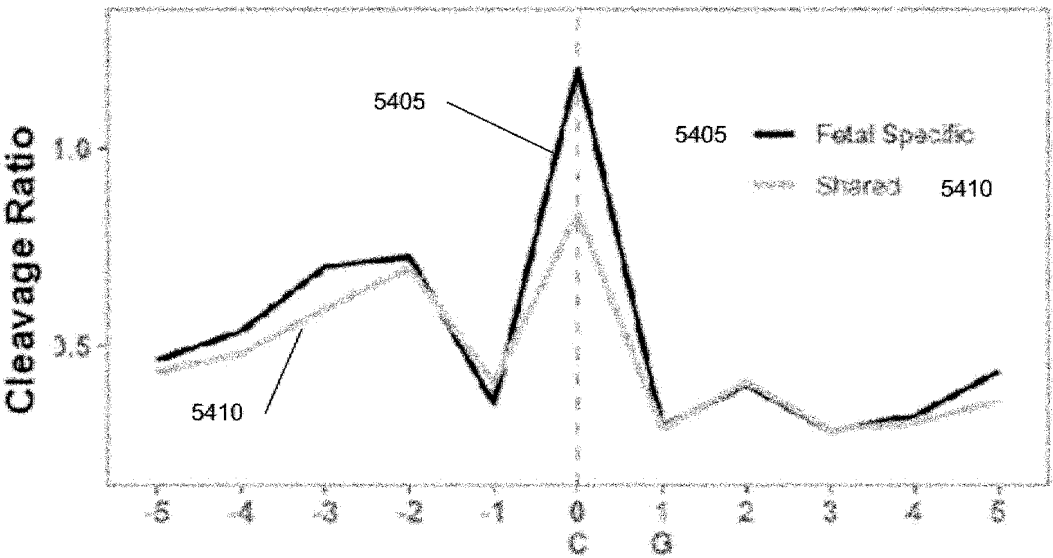
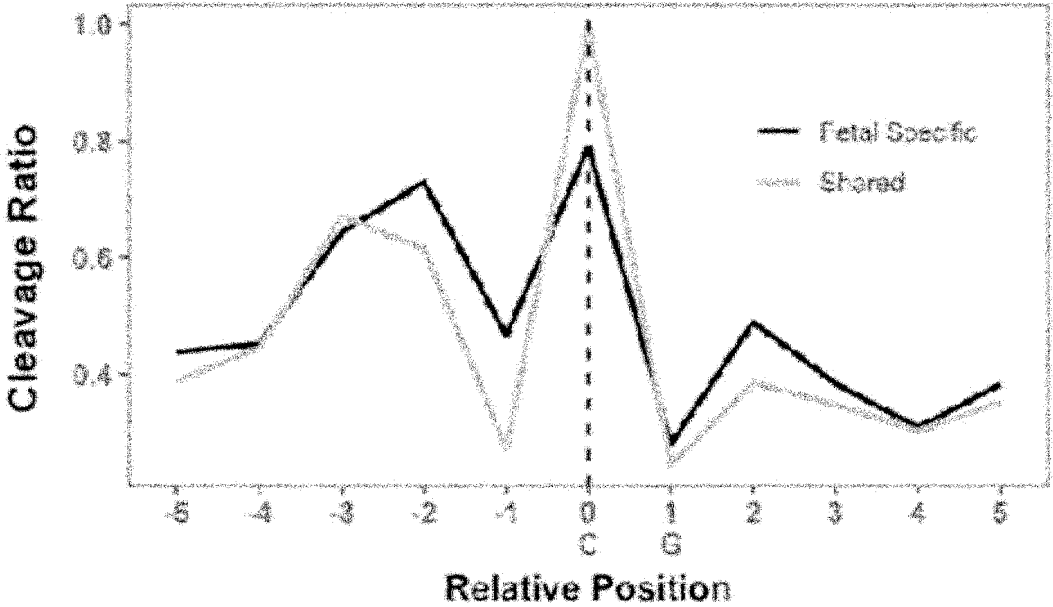


FIG. 54B Placenta Specific Hypomethylated CpG Sites



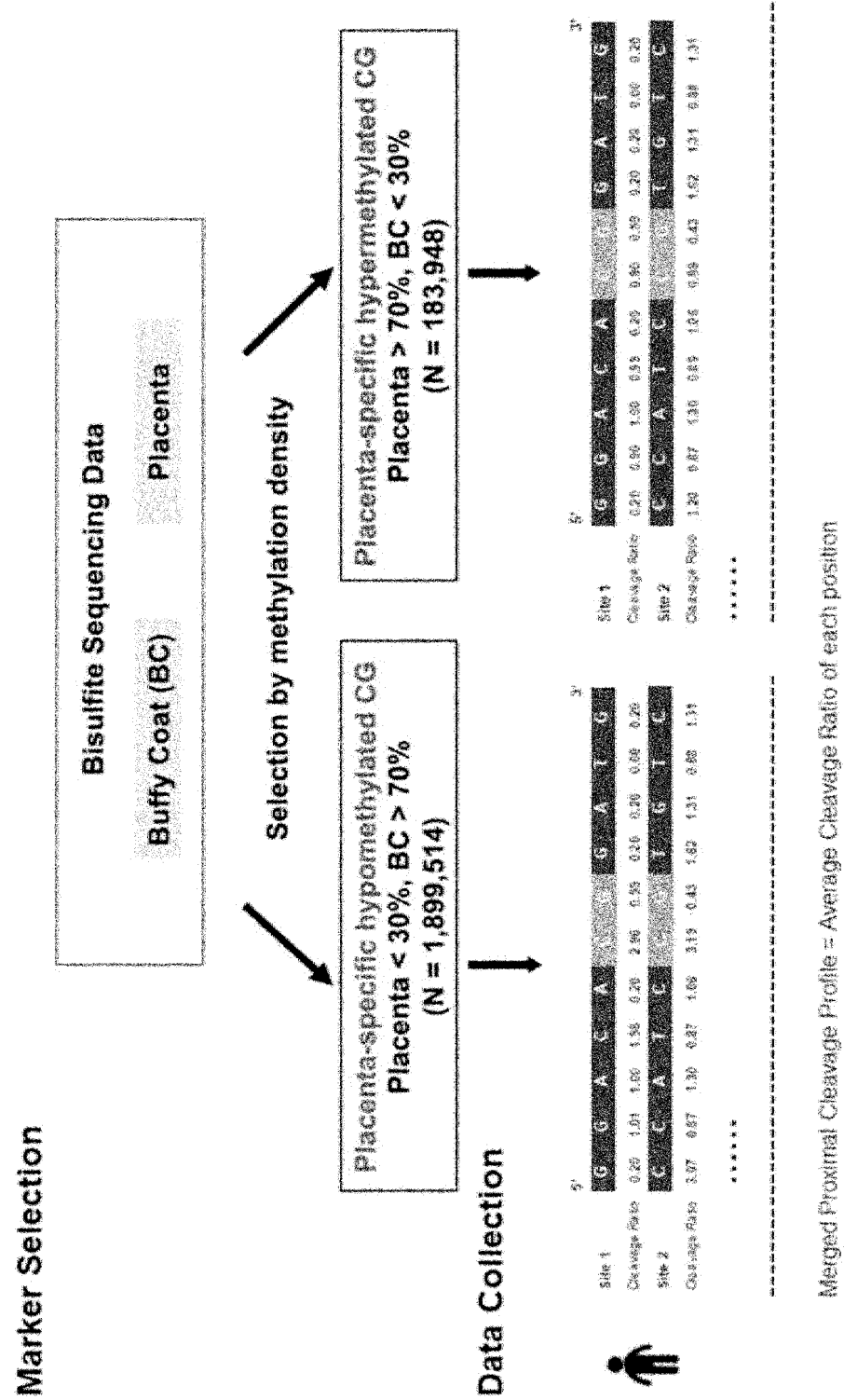


FIG. 55



FIG. 56

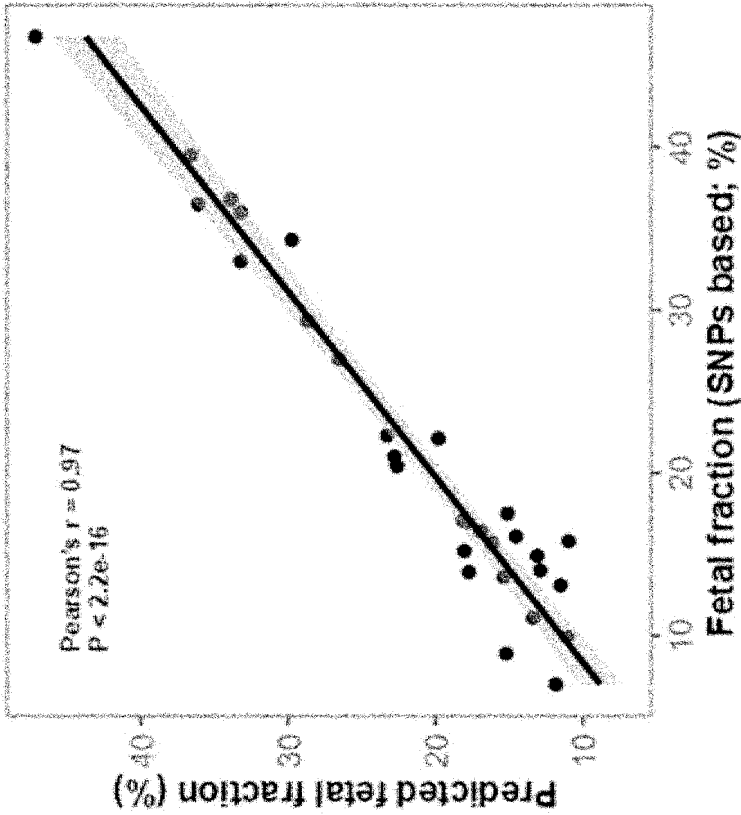


FIG. 57

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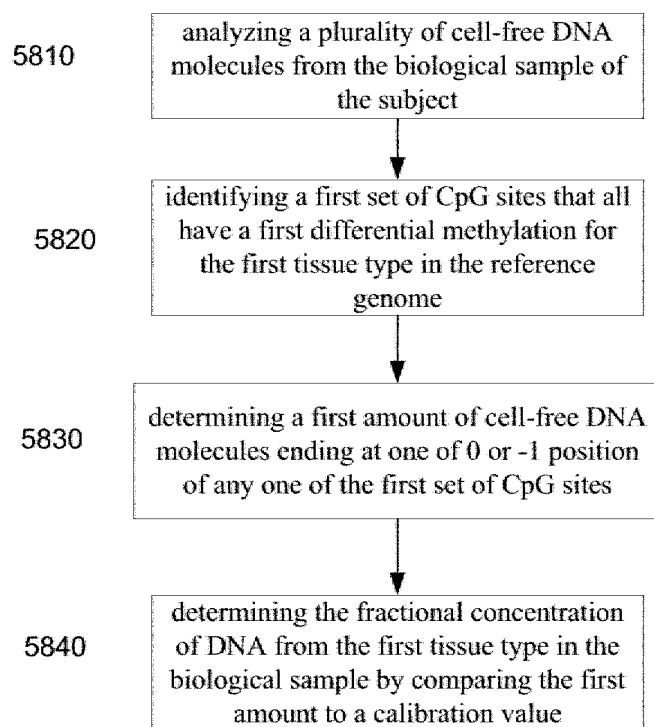


FIG. 58

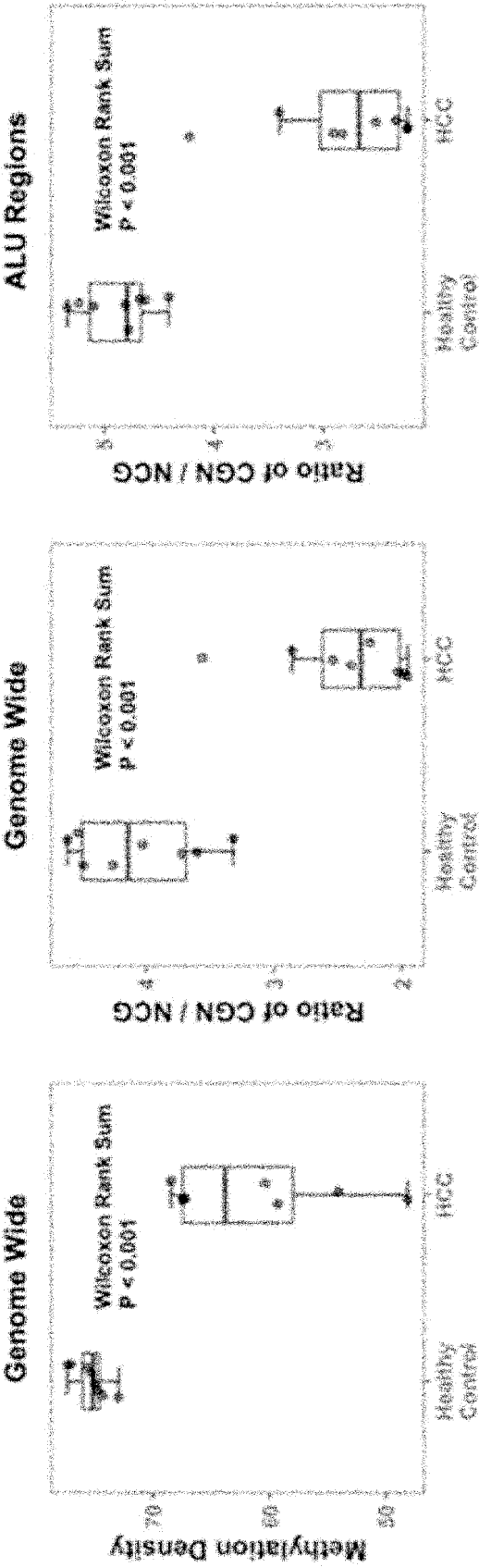


FIG. 59A

FIG. 59B

FIG. 59C

FIG. 60C

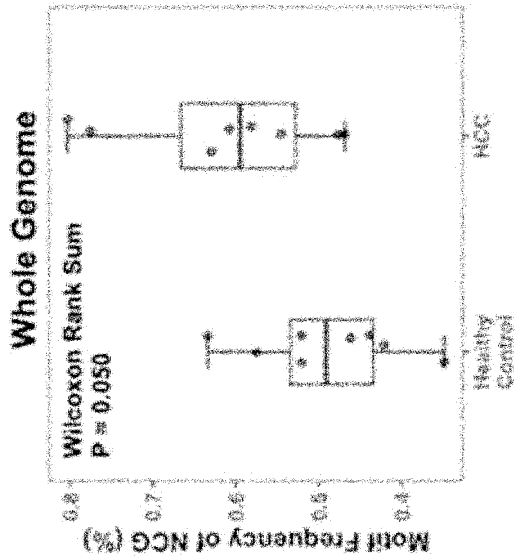


FIG. 60D

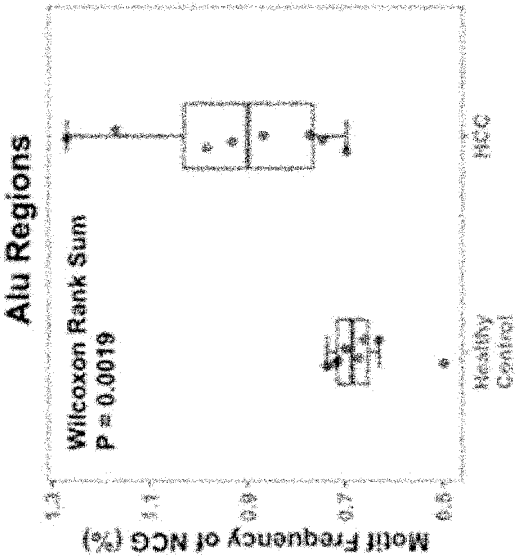


FIG. 60A

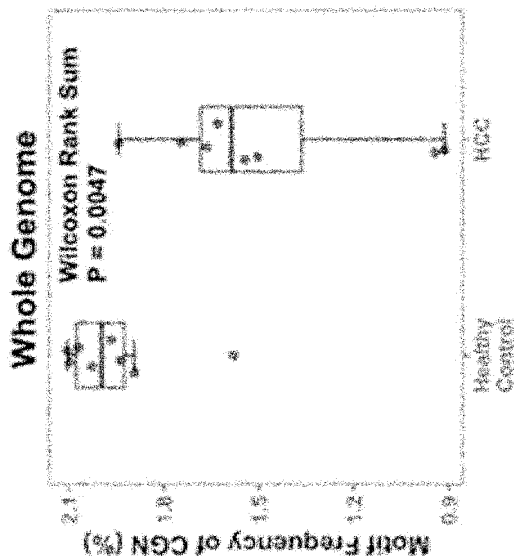
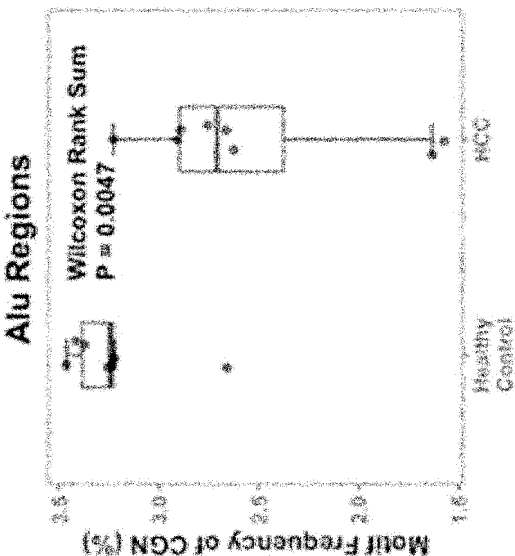


FIG. 60B



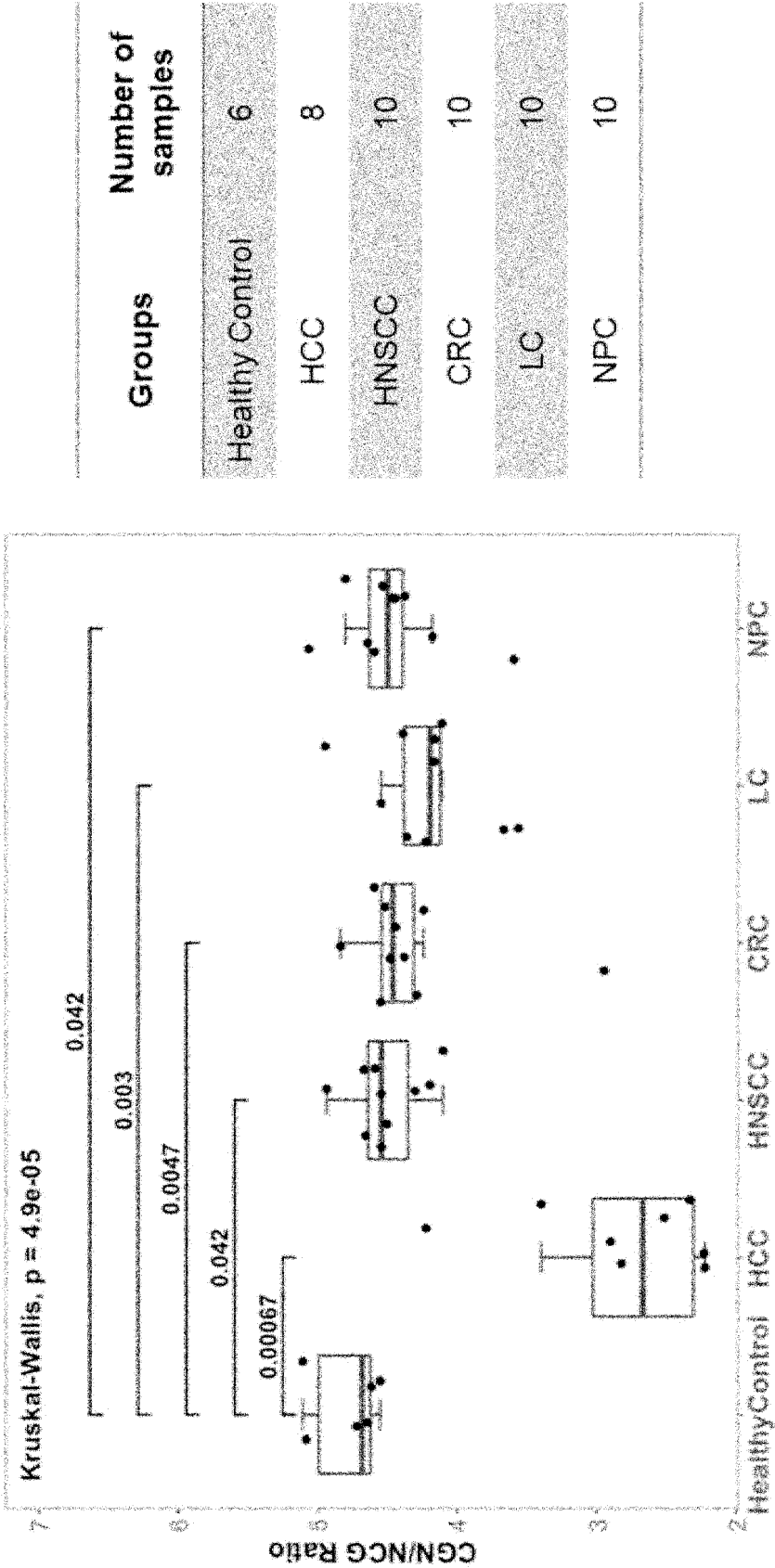


FIG. 61

ddPCR probe design

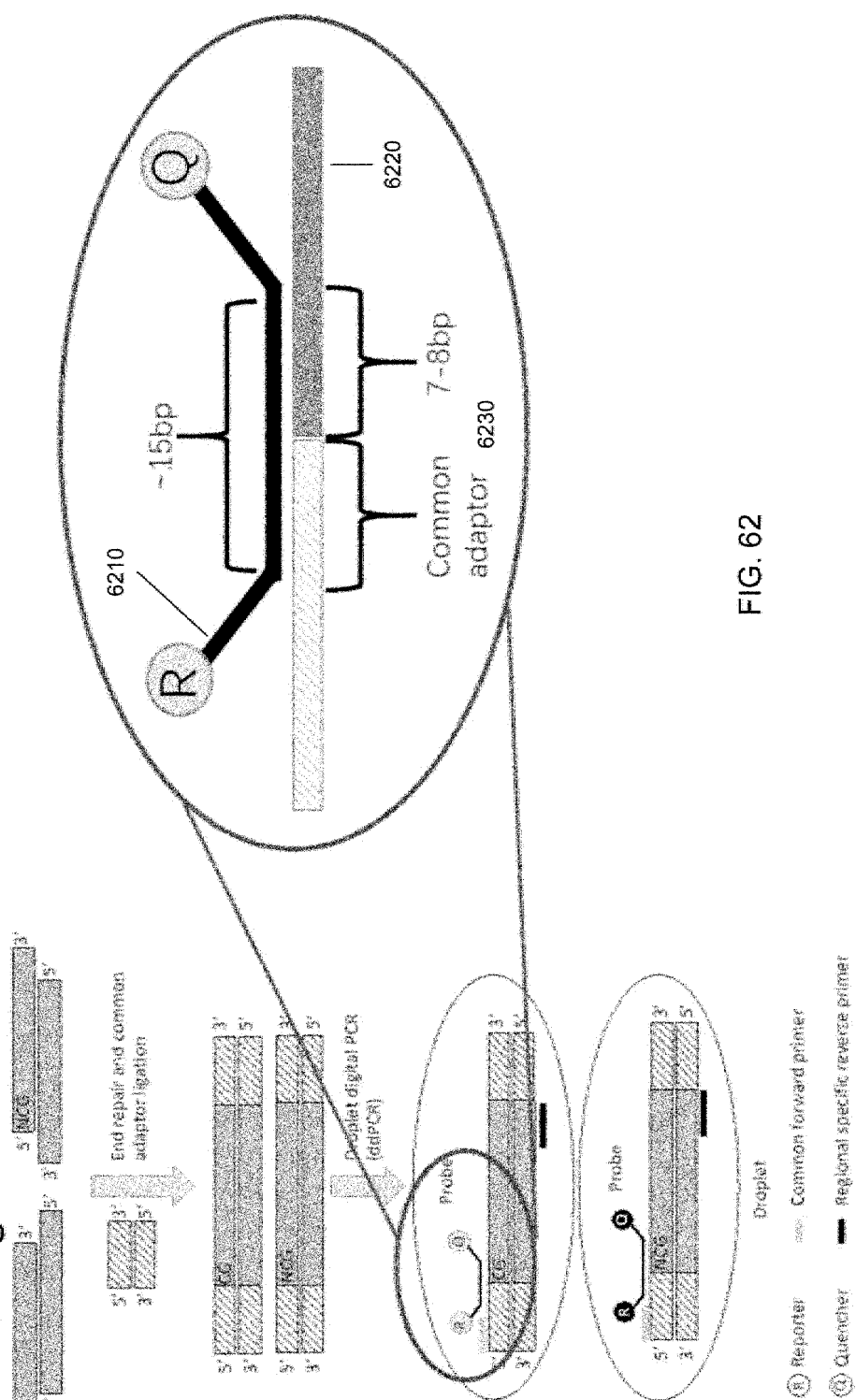
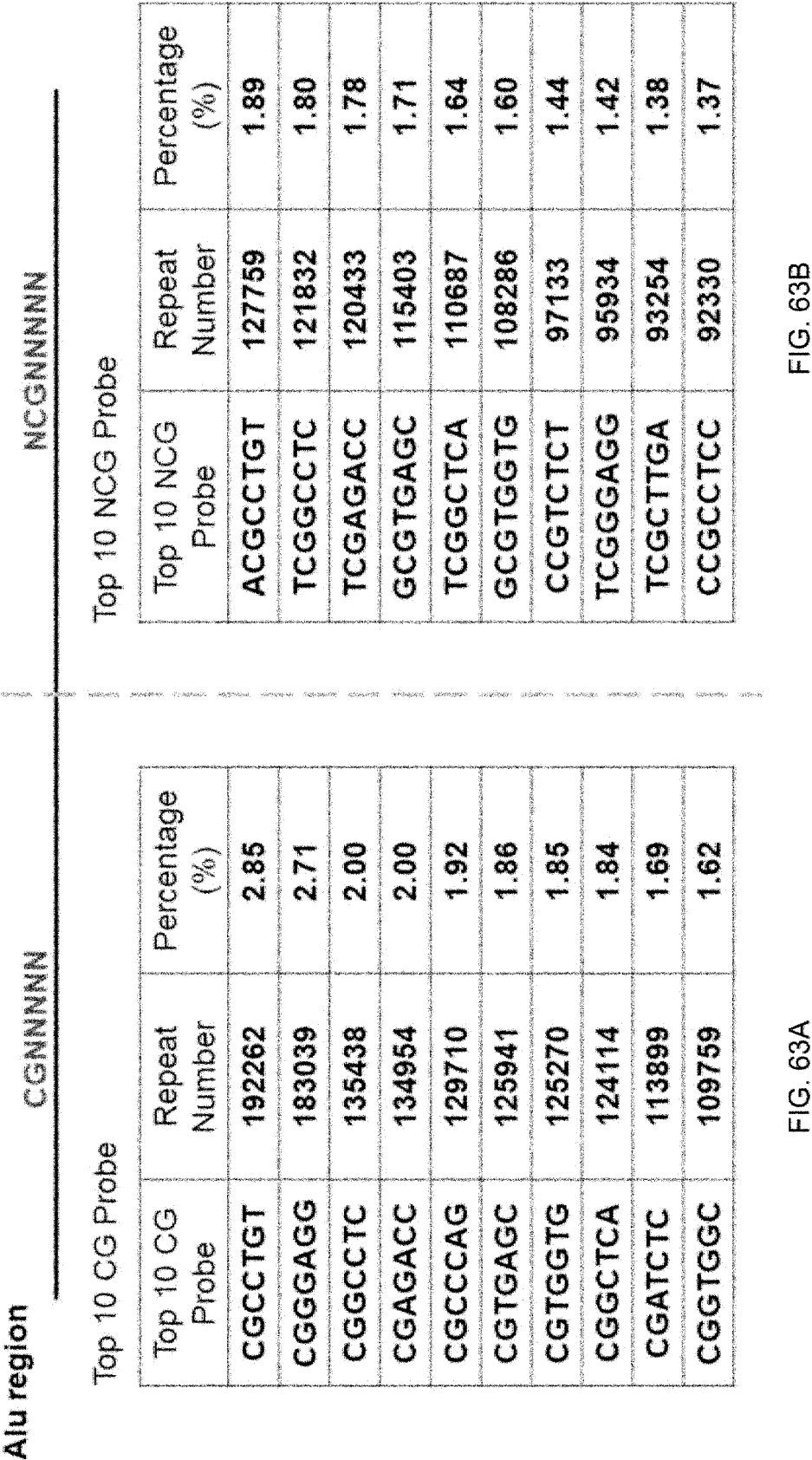


FIG. 62



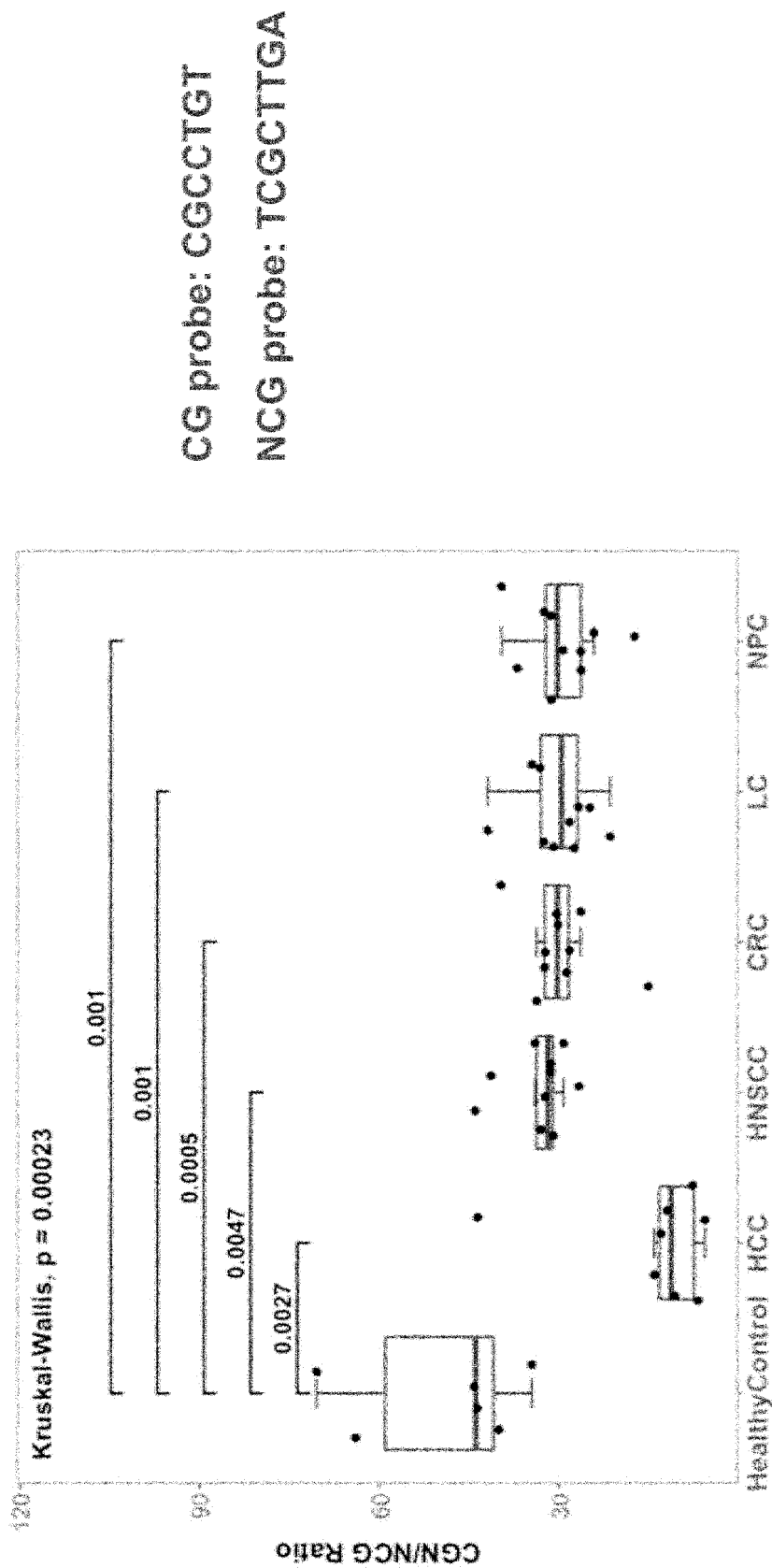


FIG. 64

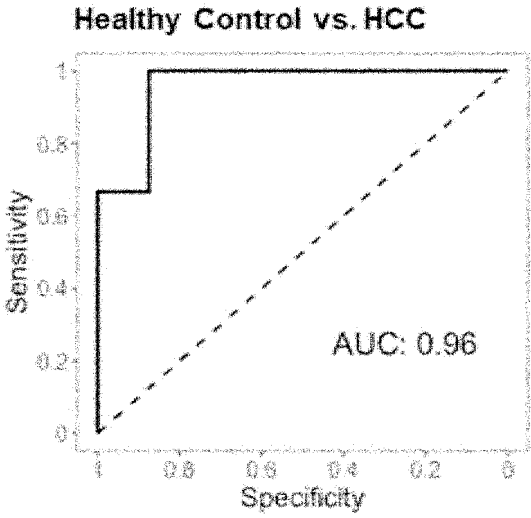


FIG. 65A

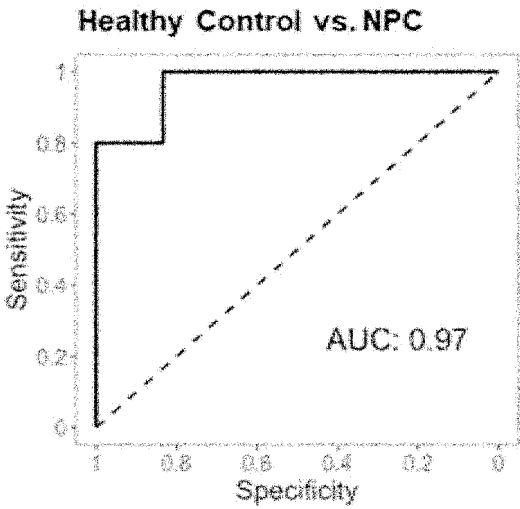


FIG. 65B

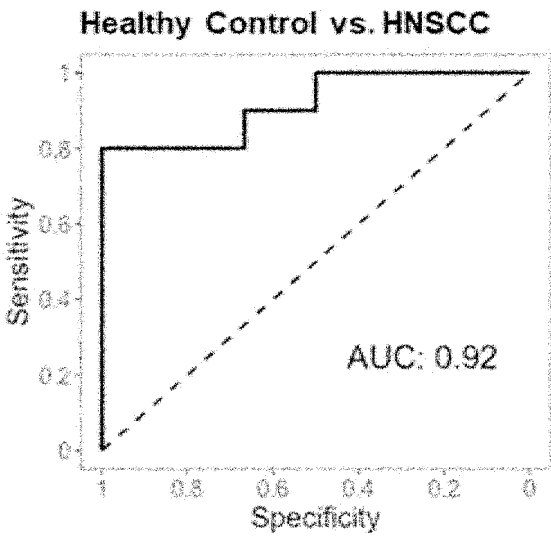


FIG. 65C

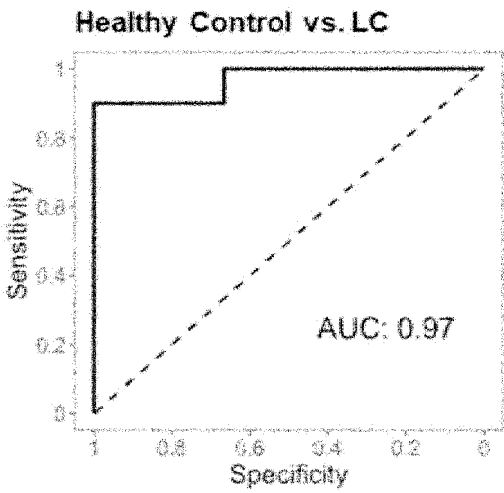


FIG. 65D

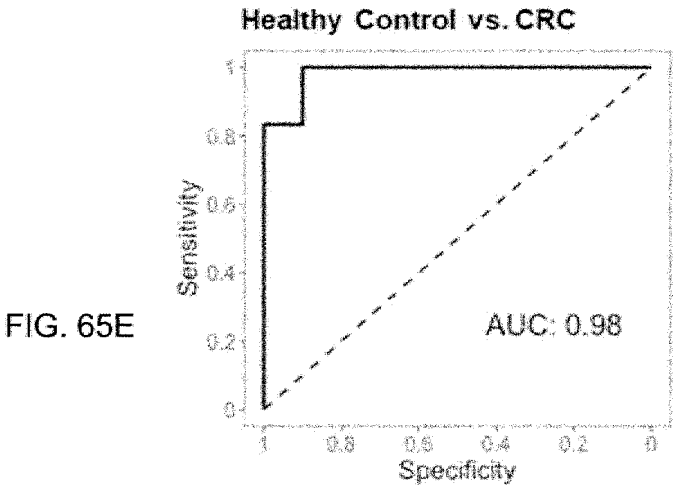


FIG. 65E

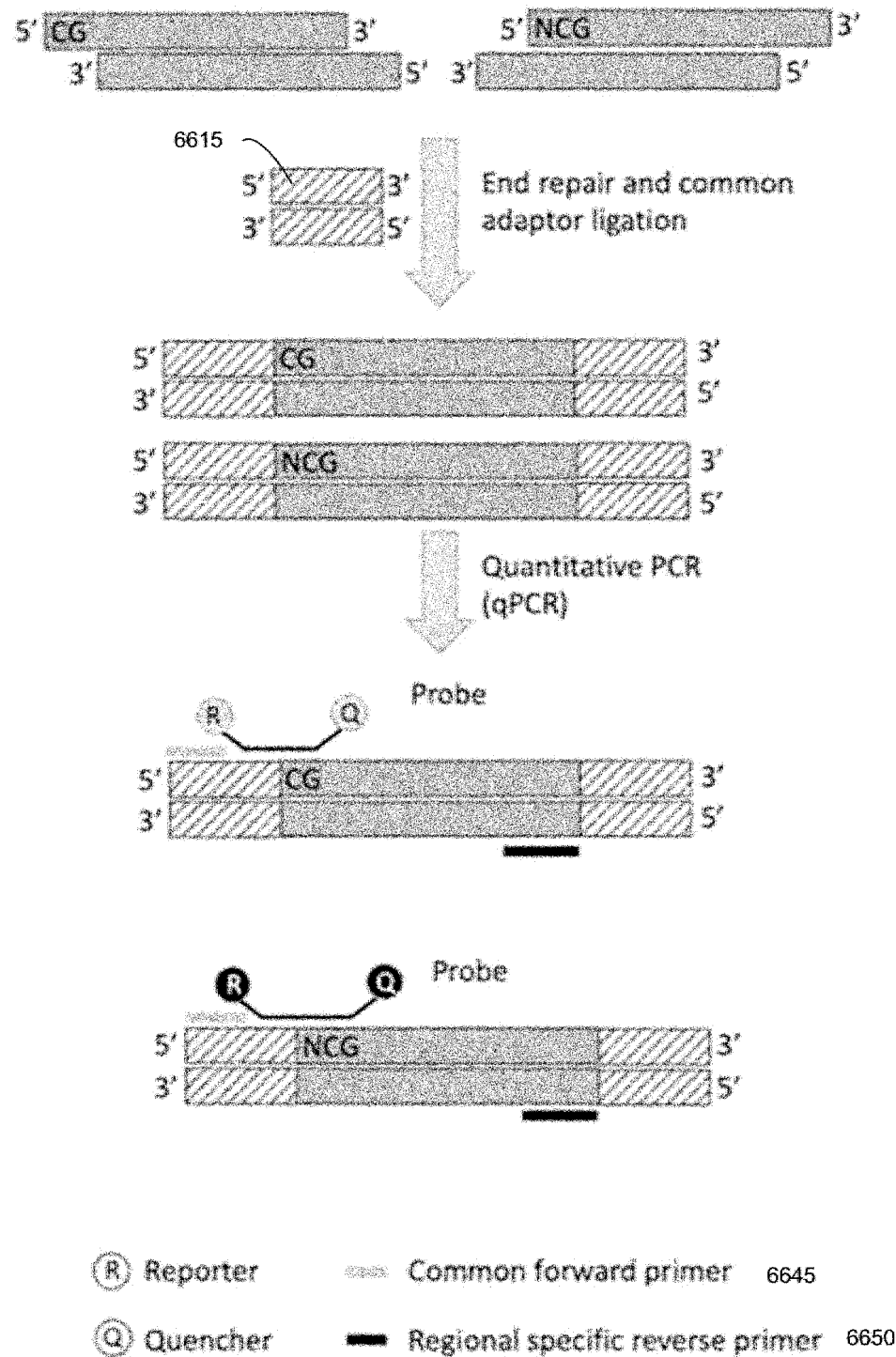


FIG. 66

FIG. 67A

Alu consensus sequence:

GGAGGCCGAGGTGGGTGGATCAGCTGAGGTCAGAGTTCAGAGCCAGCCTGGCCAACATGGTGAAC
CCCCGTCTCTACTAAATAACAAAATTAGCCGGCGGTGGTGCCGGGCTGTAAATCCCAGCTACTCG
GGAGGCTGAGGCAGGAGAAATCGCTTGAACCCGGGAGGTGGAGTTGCAGTGAGCCGAGATCGCGG
GGAGCTCCAGCCCTG

FIG. 67B

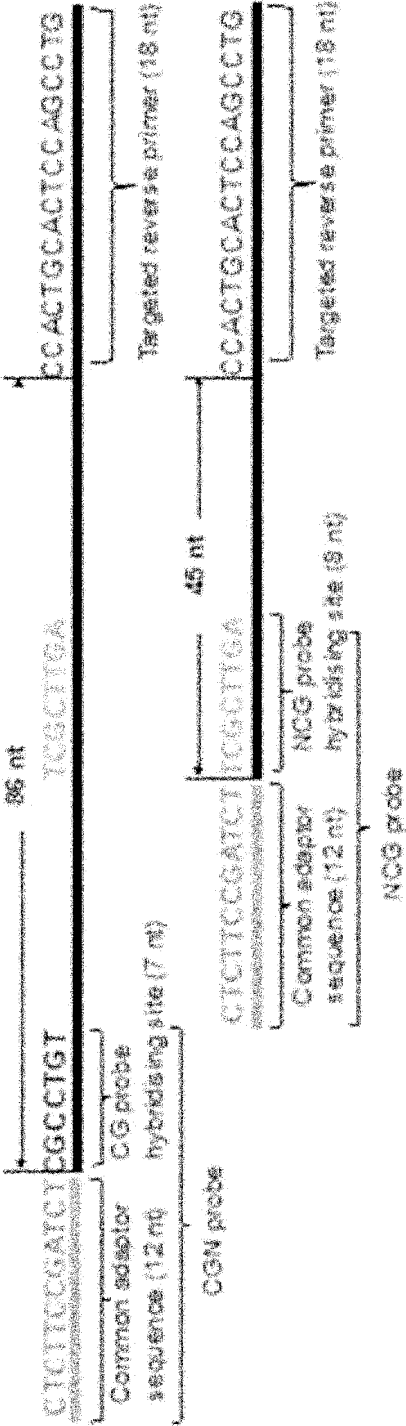


FIG. 67C

Region	no. of CGN probe hybridising sites	no. of NCG probe hybridising sites
Alu region ^a	23676	22413
Outside of Alu region ^b	71	98

^a200 bp before reverse primer

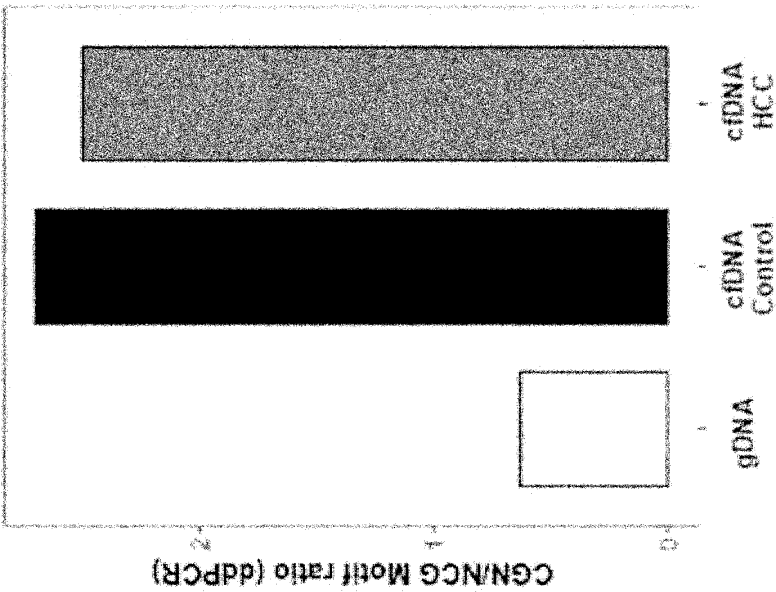


FIG. 68B

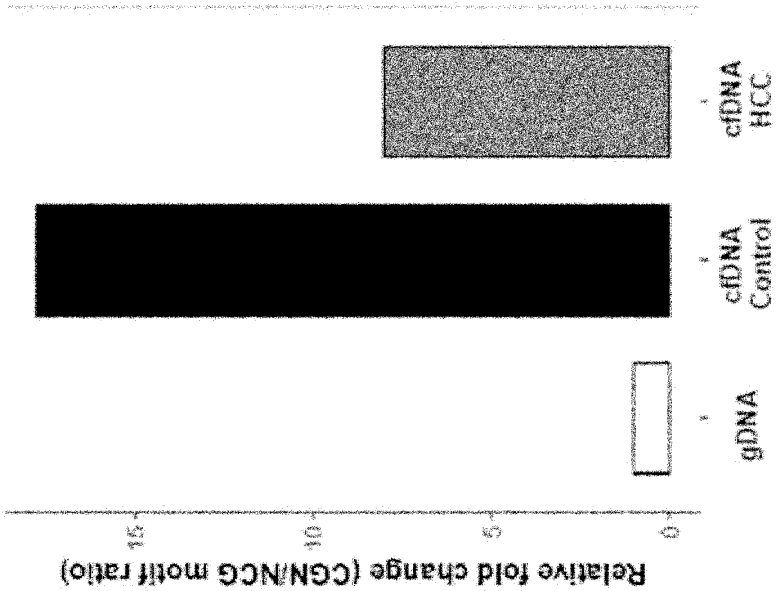


FIG. 68A

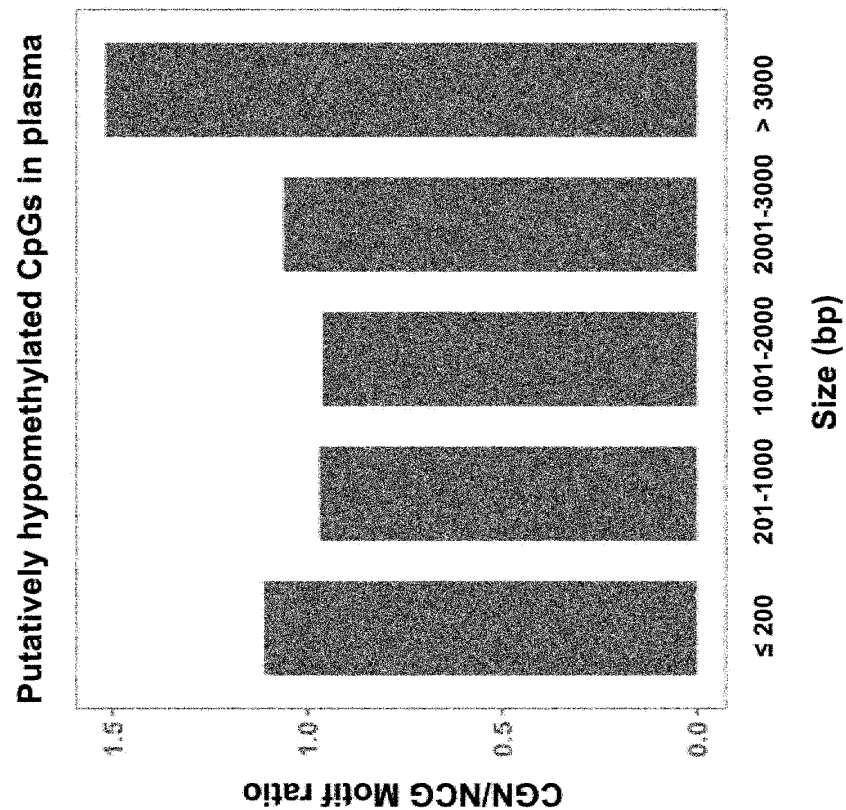


FIG. 69B

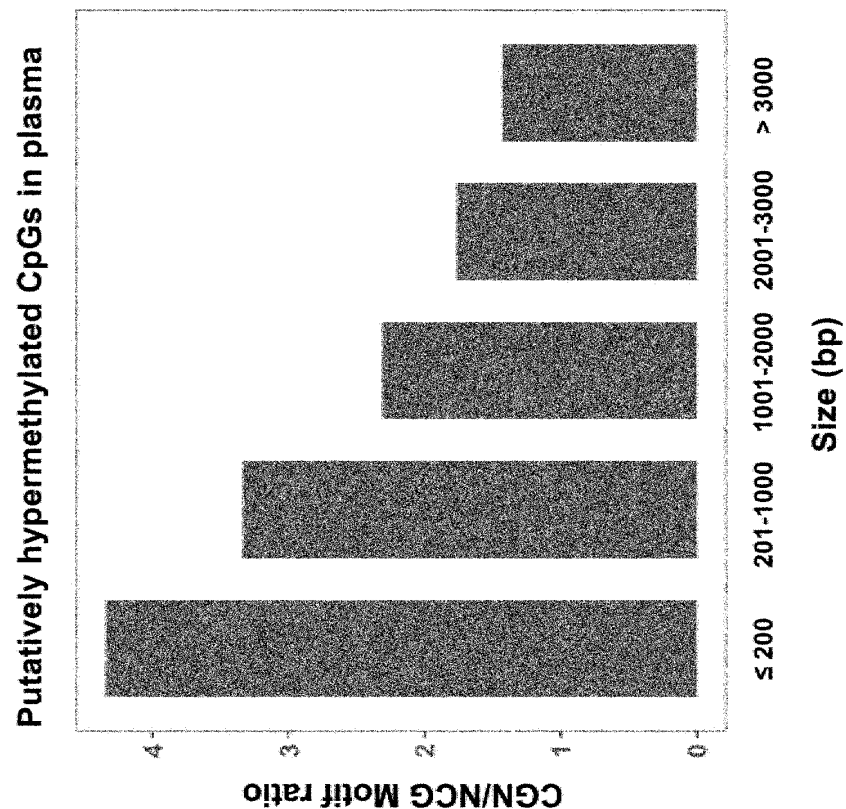
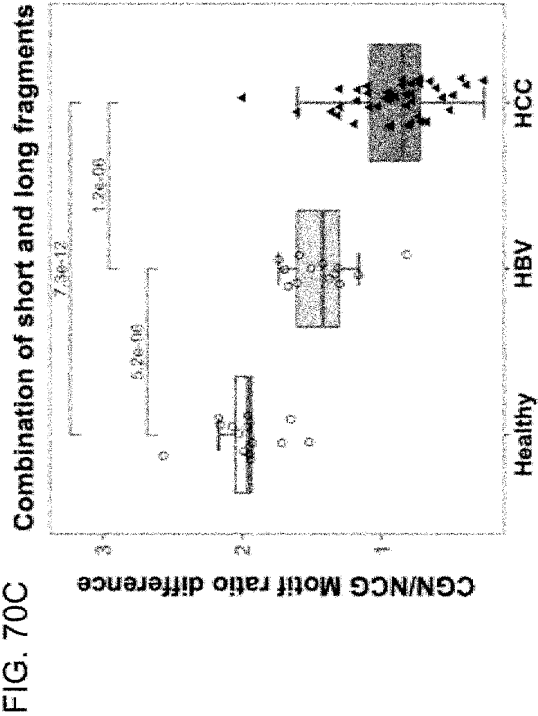
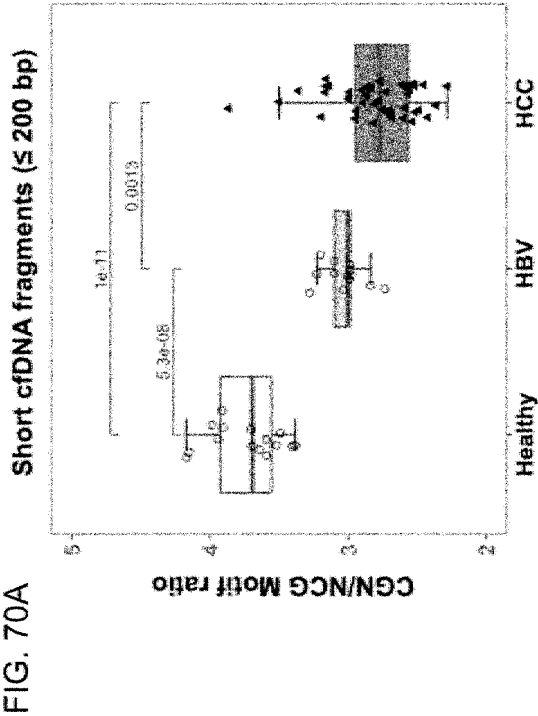
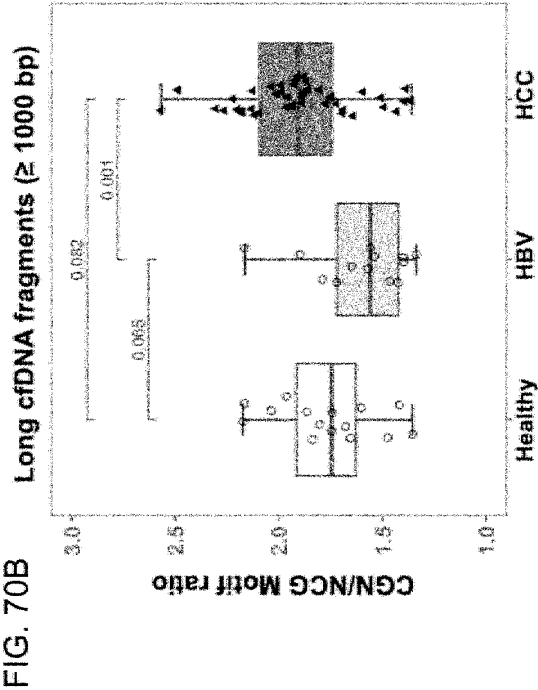


FIG. 69A



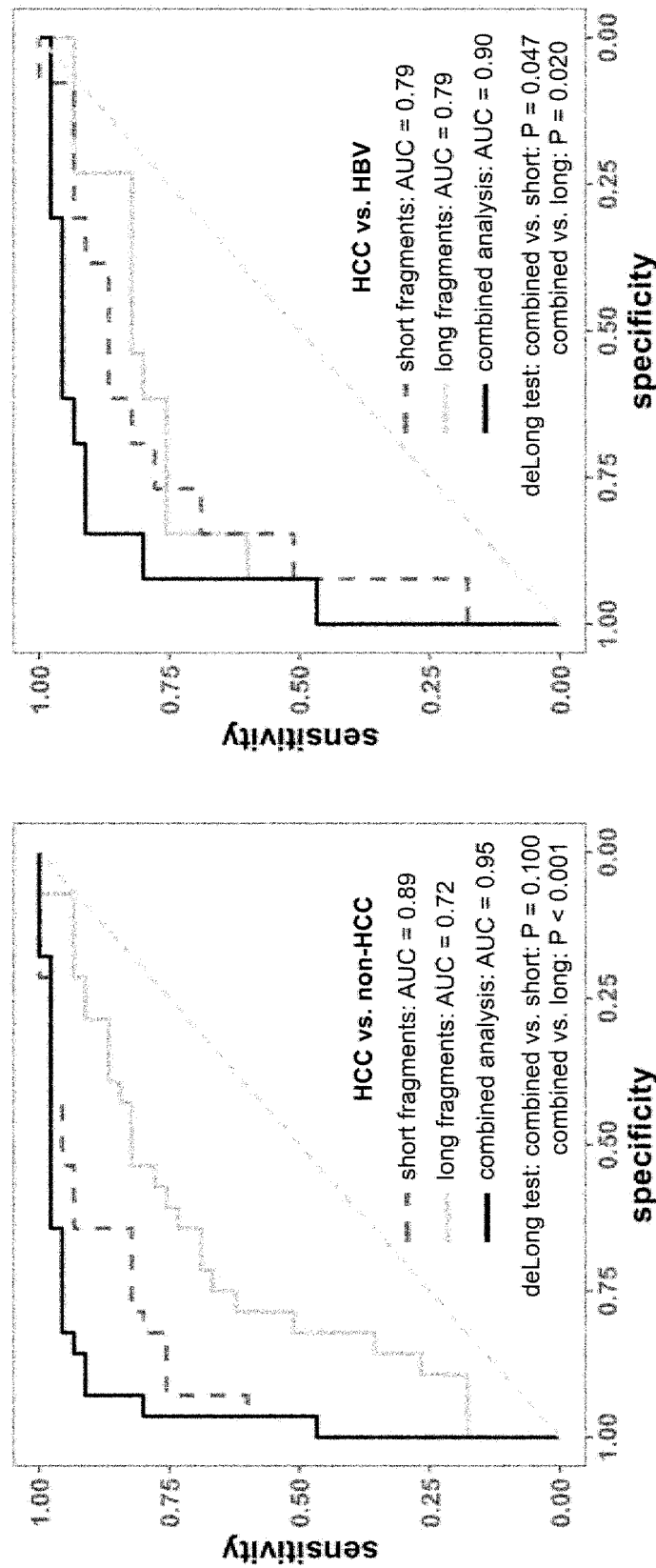


FIG. 71B

FIG. 71A

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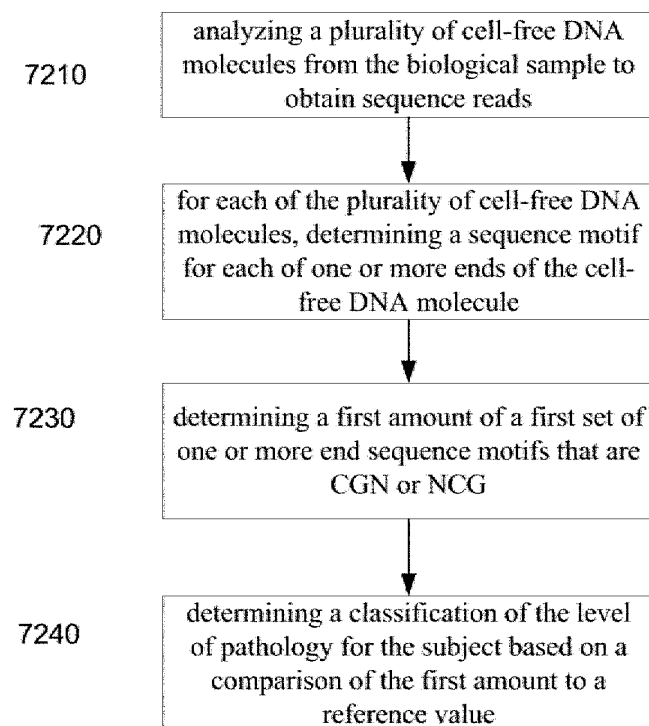


FIG. 72

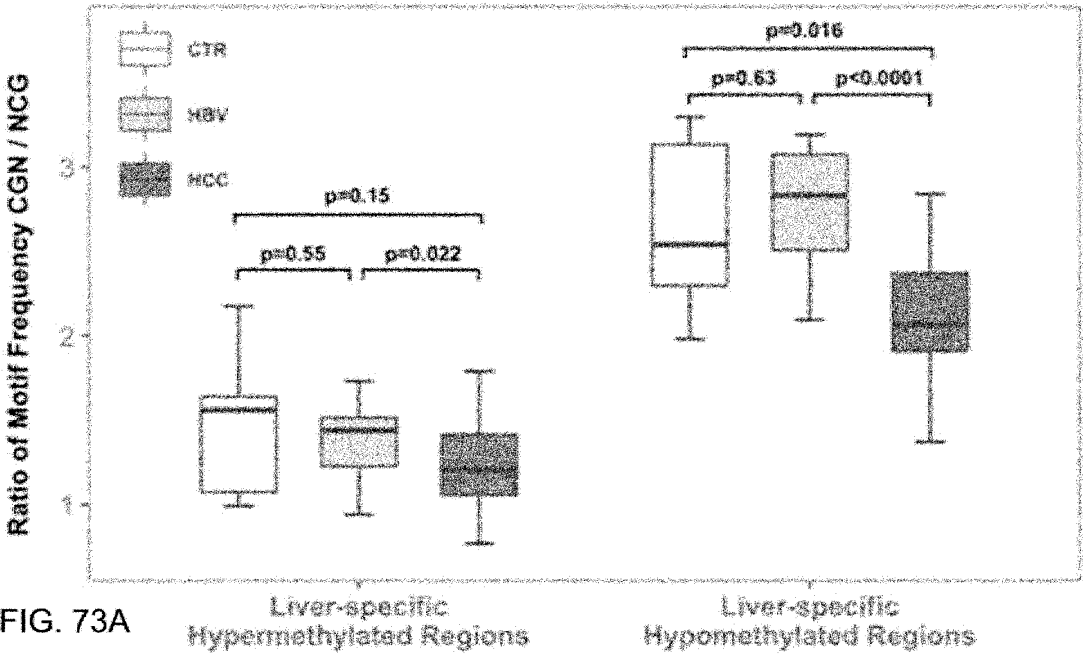


FIG. 73A

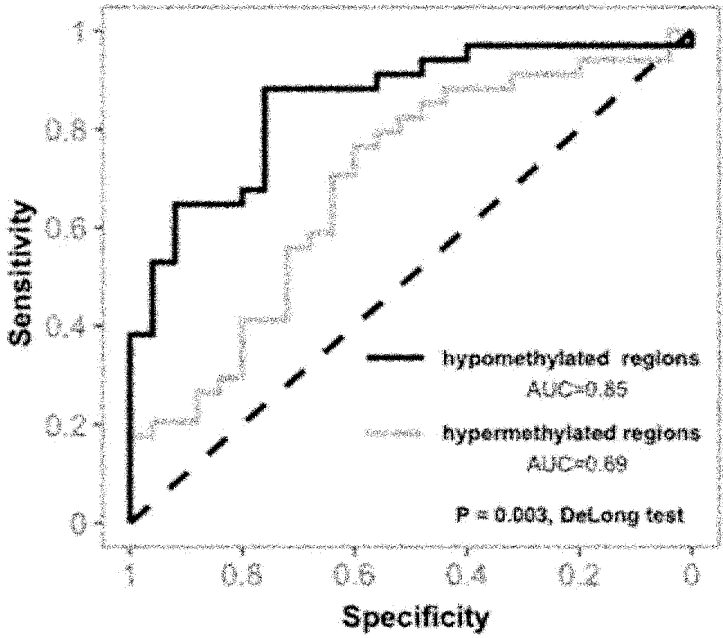


FIG. 73B

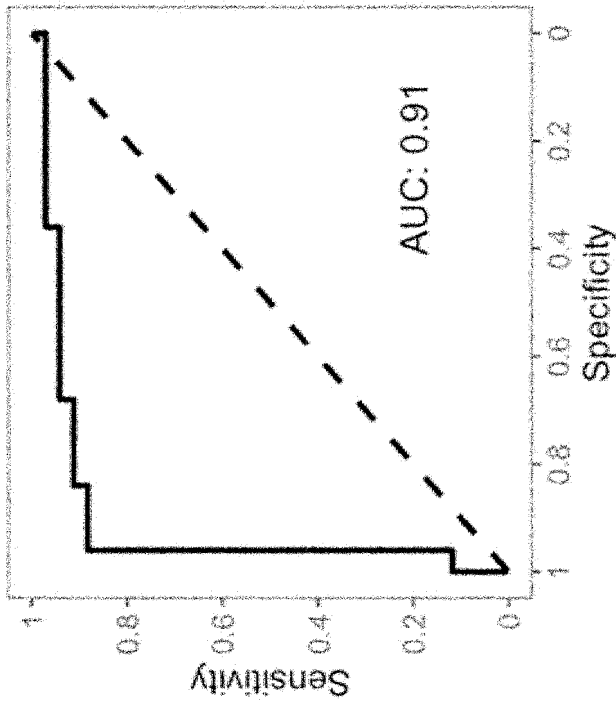


FIG. 74B

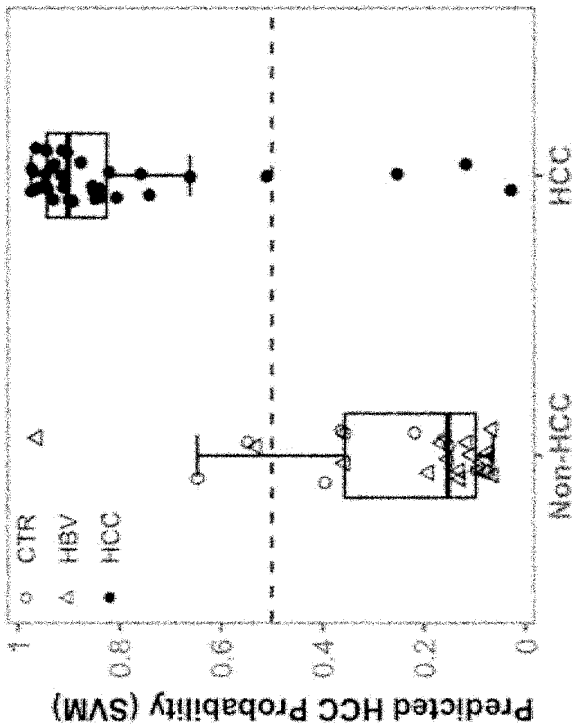


FIG. 74A

FIG. 75A HCC-specific hypermethylated CpGs

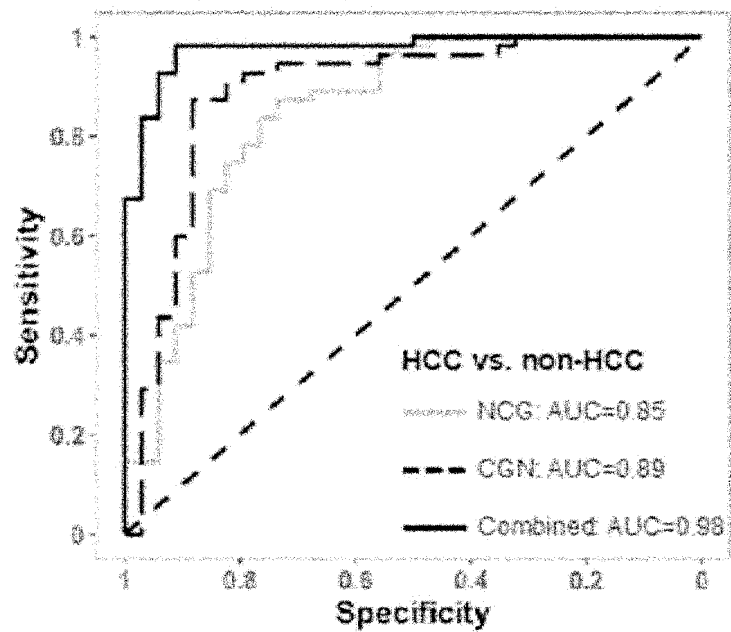
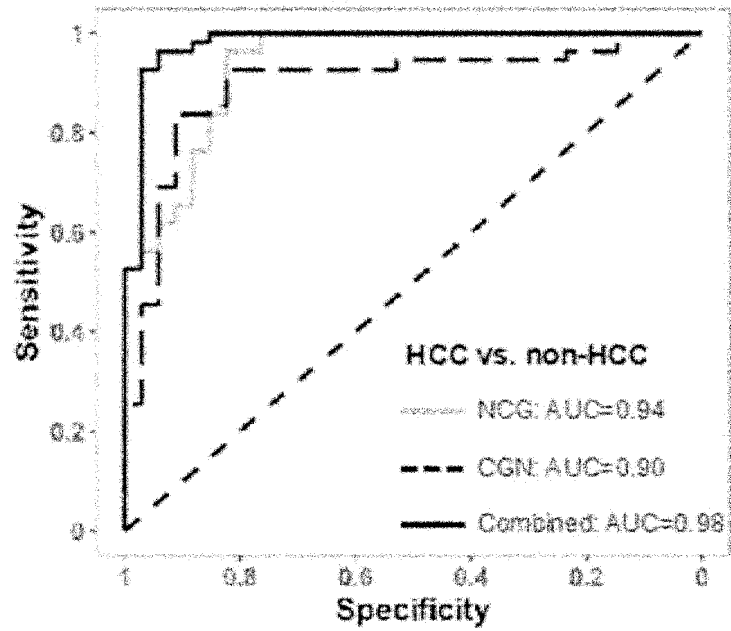


FIG. 75B HCC-specific hypomethylated CpGs



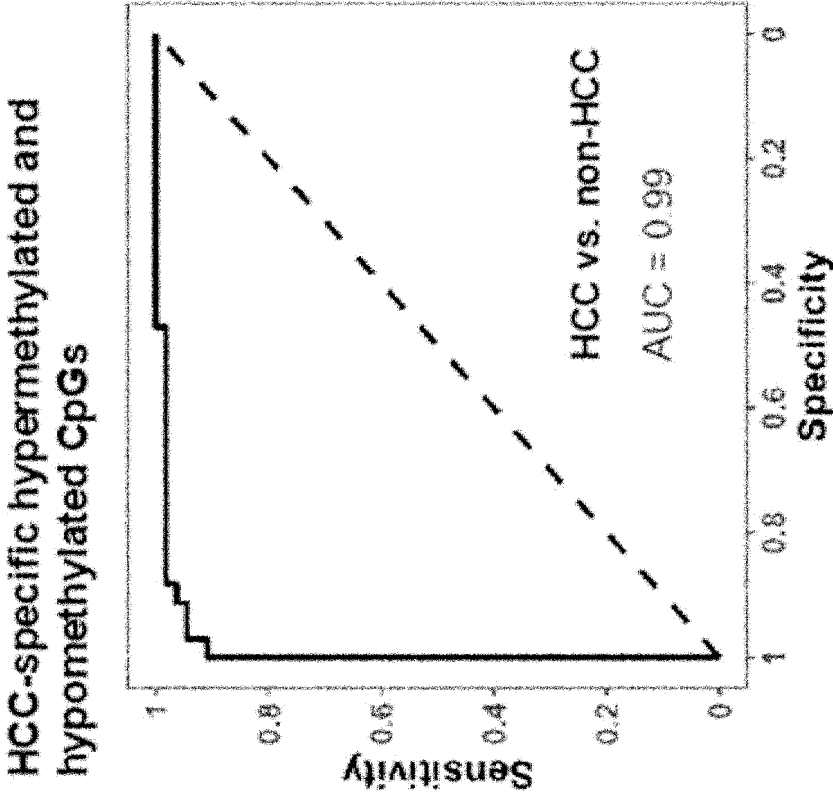


FIG. 76

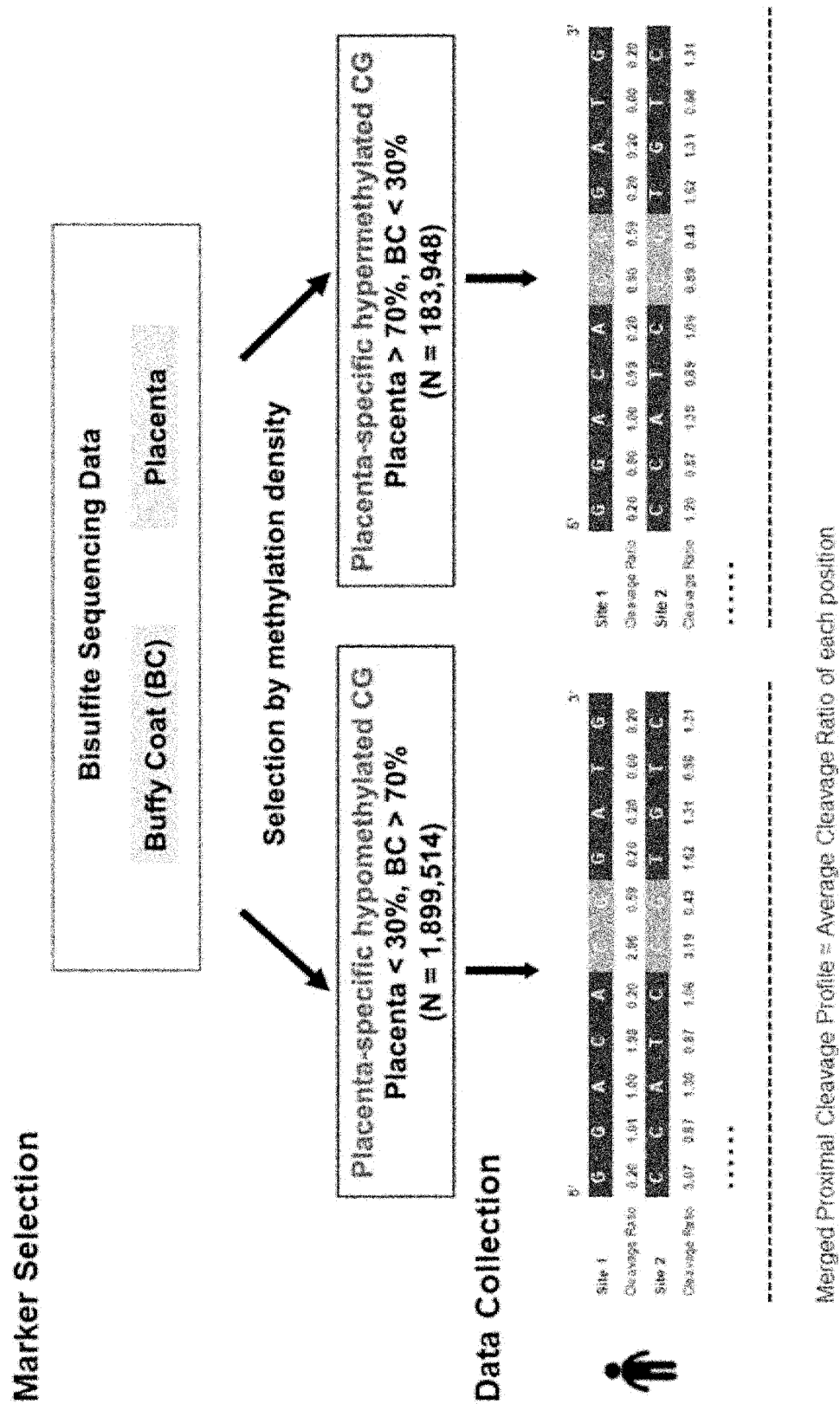


FIG. 77

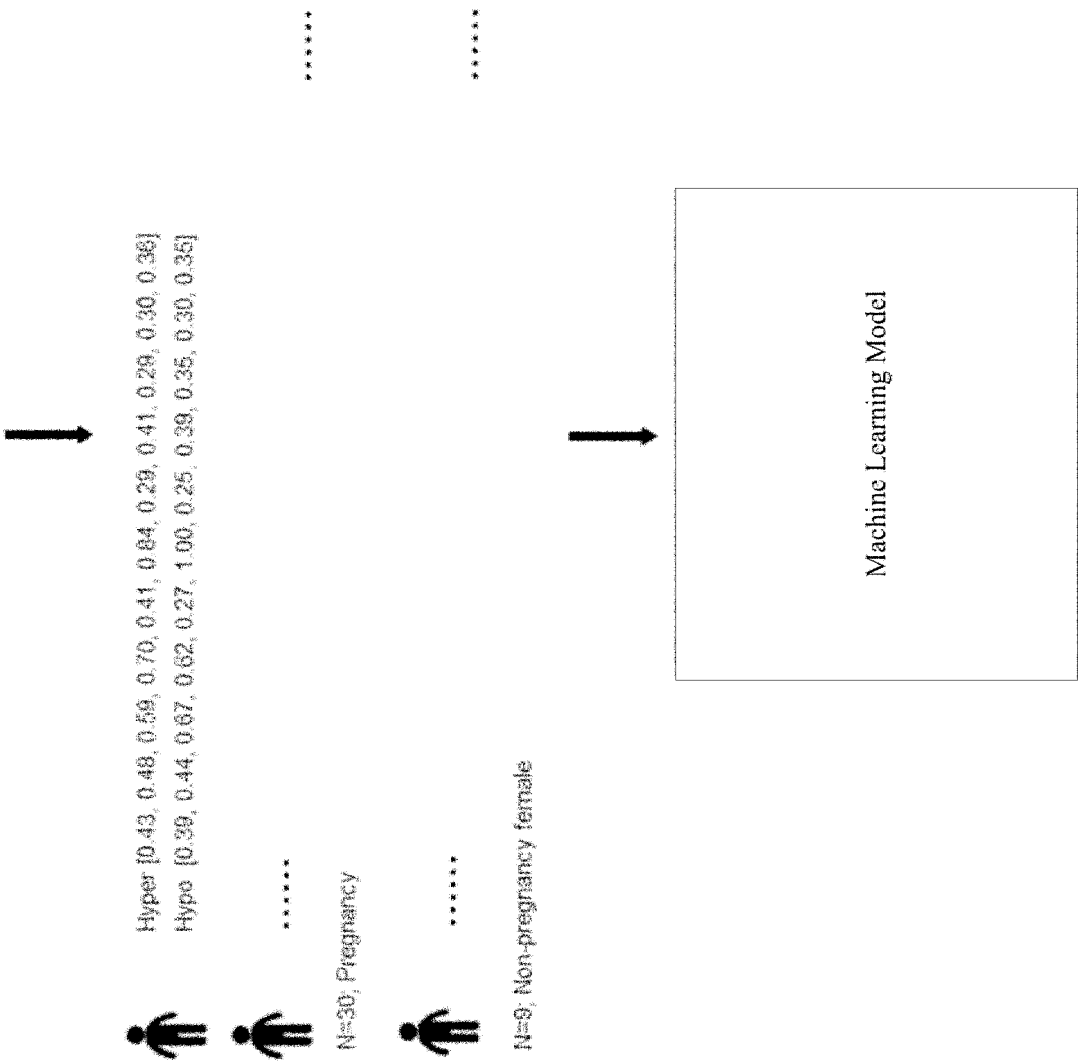


FIG. 78

Placenta specific hypermethylated windows

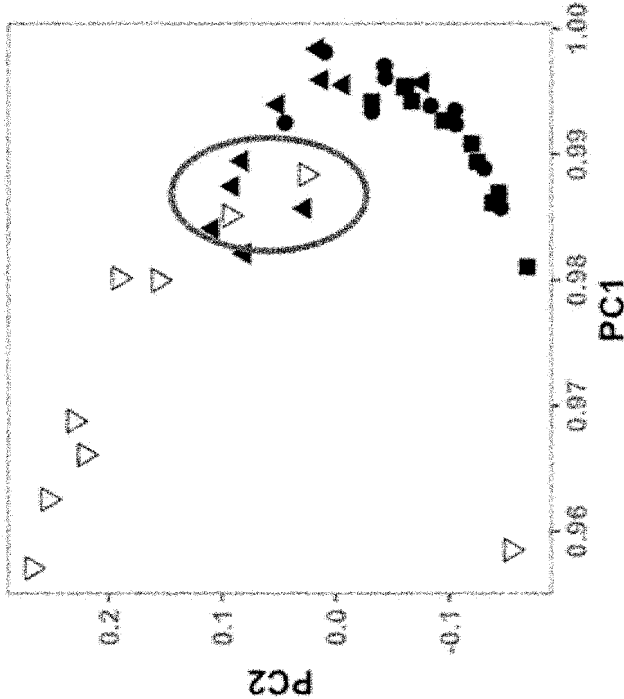


FIG. 79A

Placenta specific hypomethylated windows

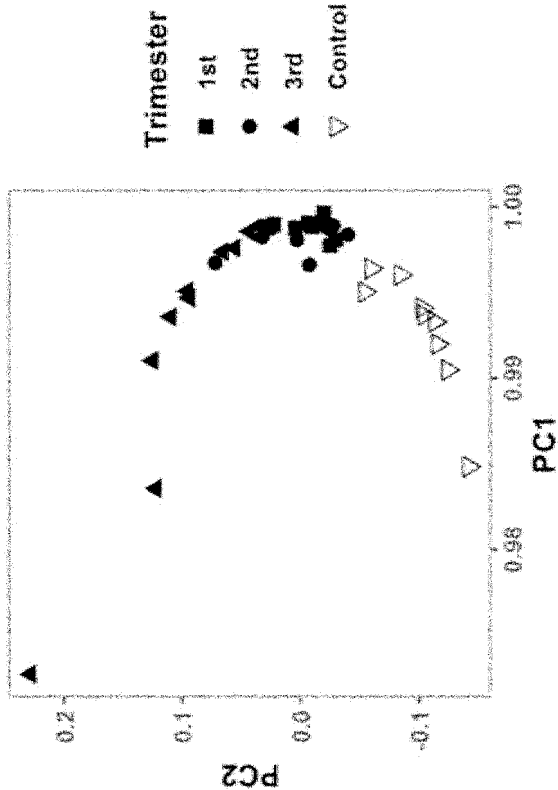


FIG. 79B

Combine placenta specific hyper- and hypo- methylated windows

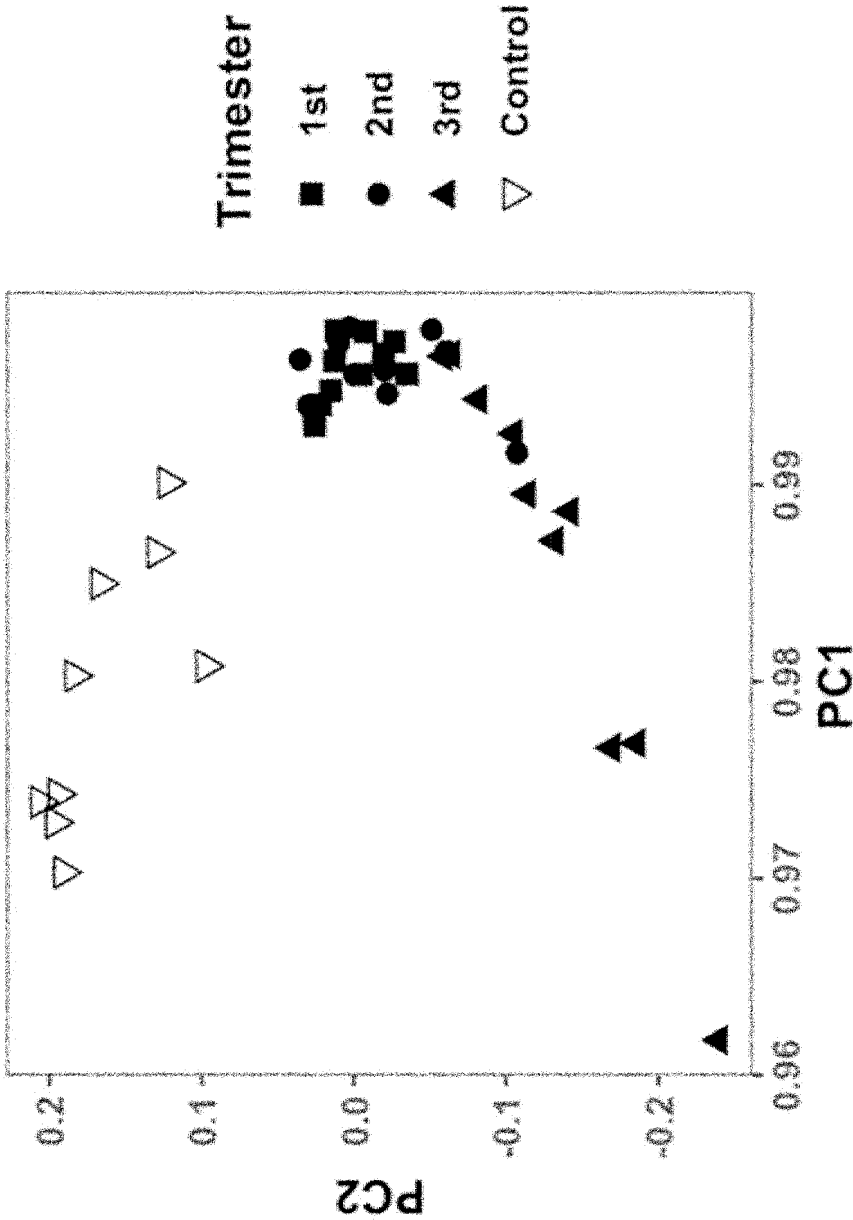


FIG. 80

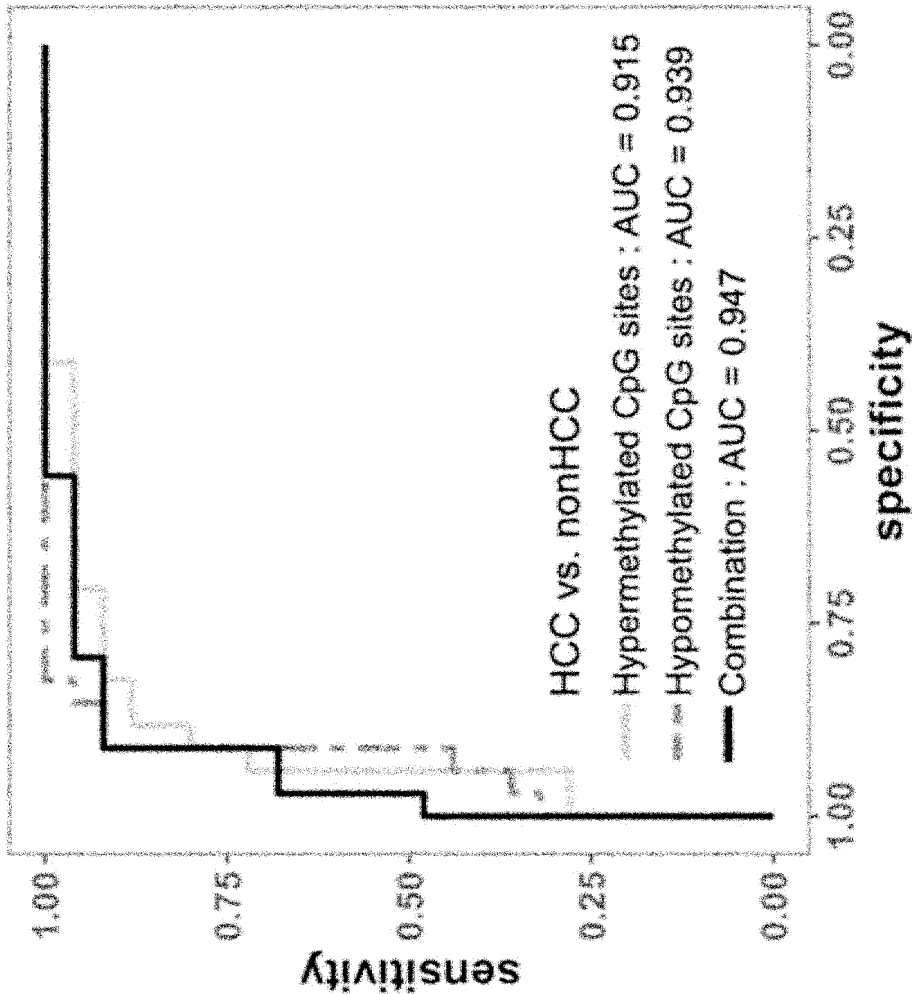


FIG. 81

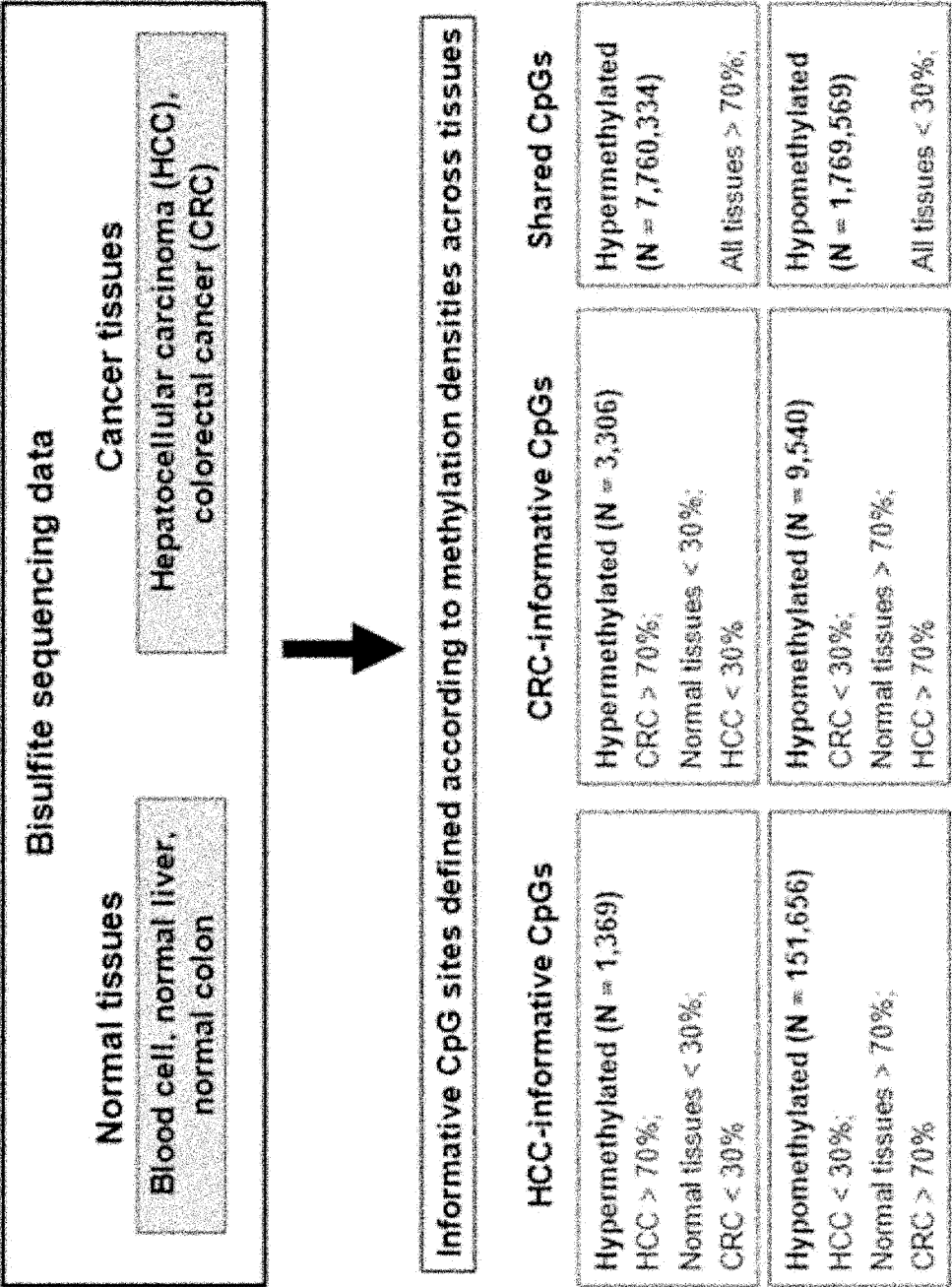


FIG. 82

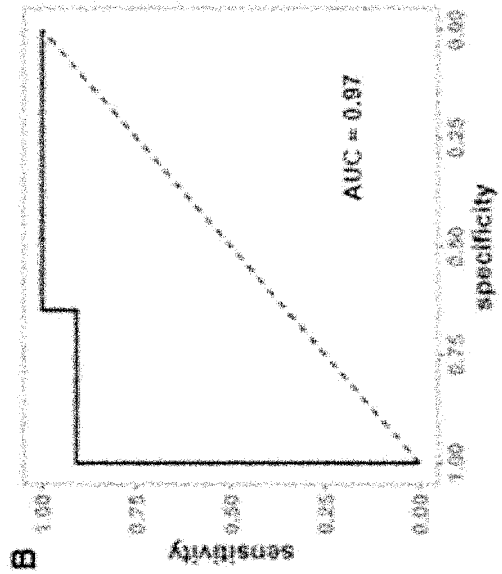
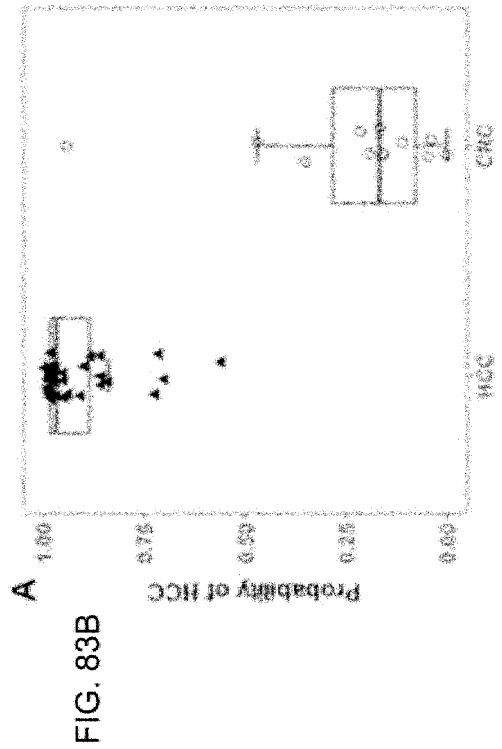
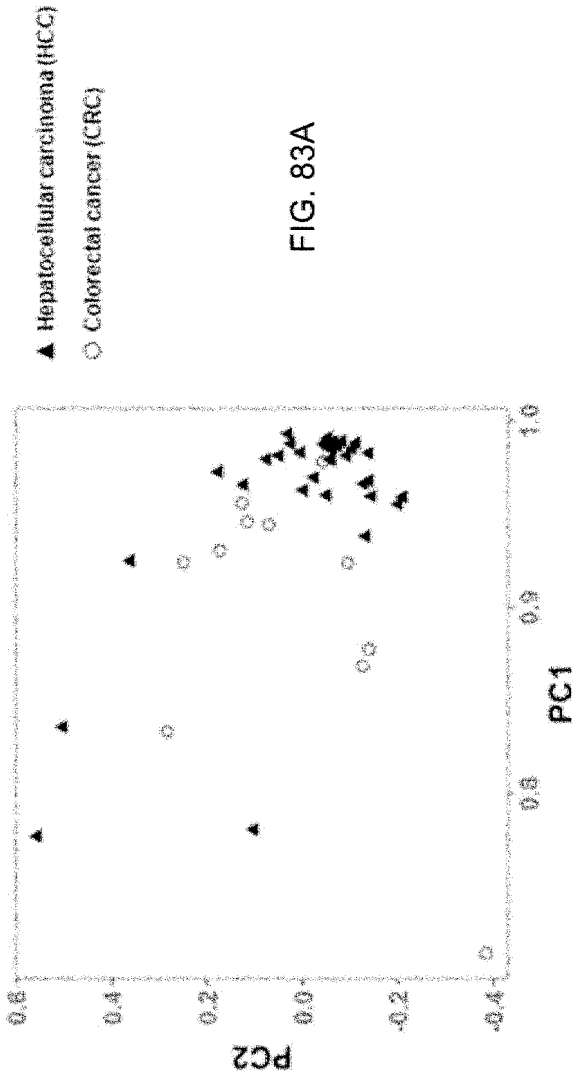


FIG. 83C

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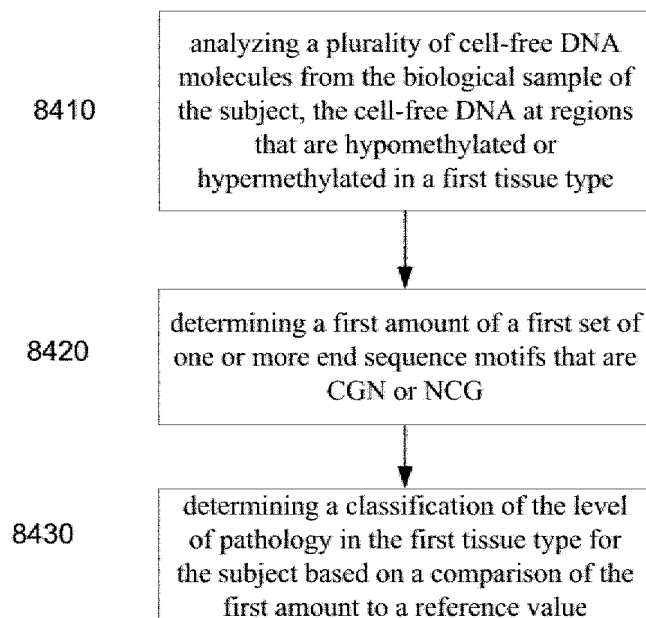


FIG. 84

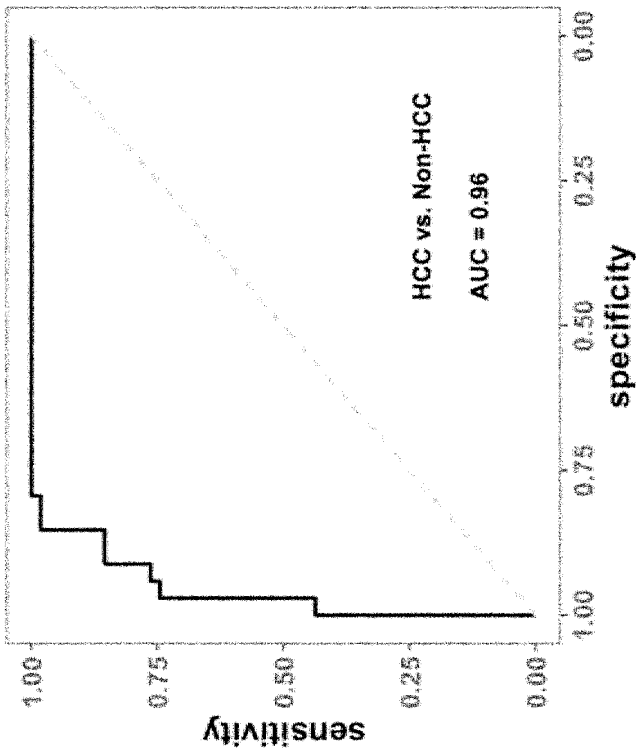


FIG. 85B

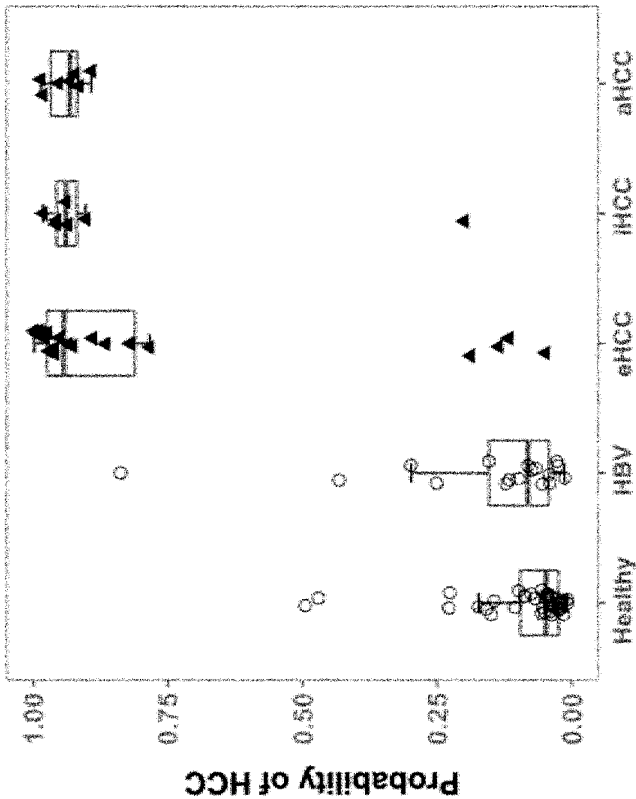


FIG. 85A

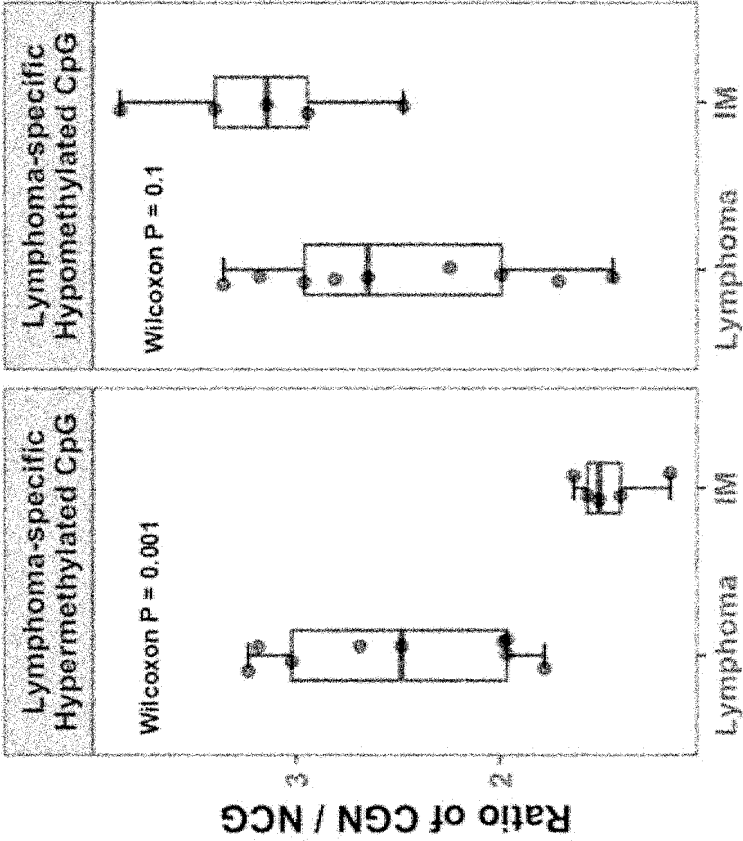


FIG. 86A

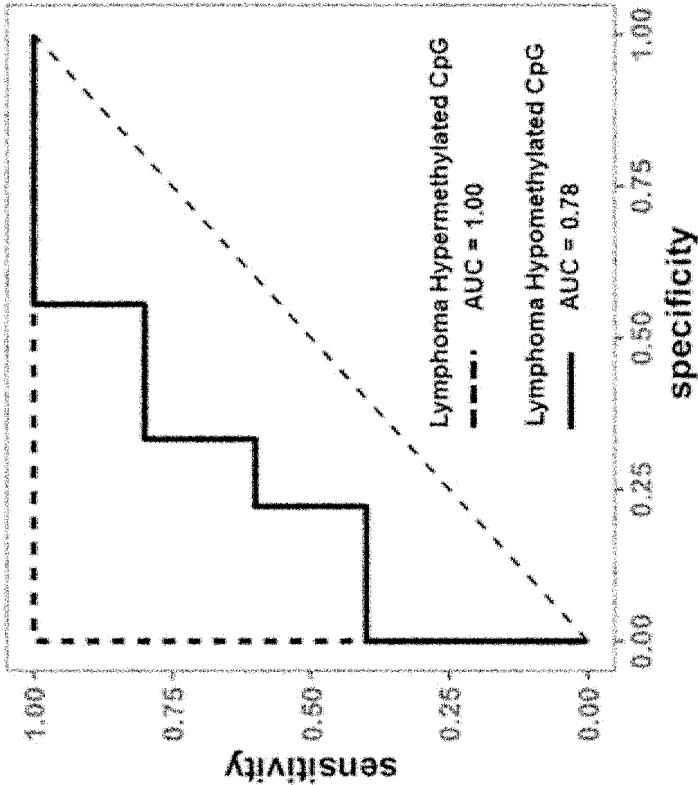


FIG. 86B

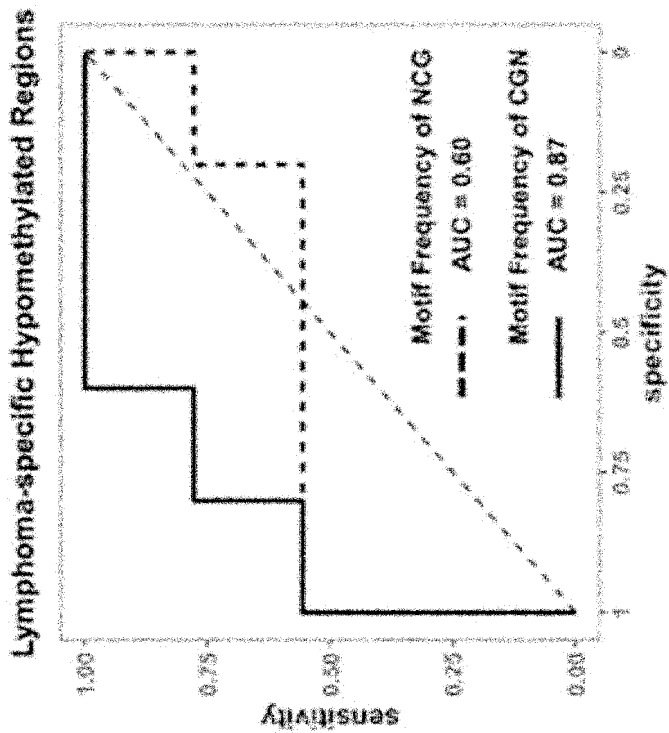


FIG. 87B

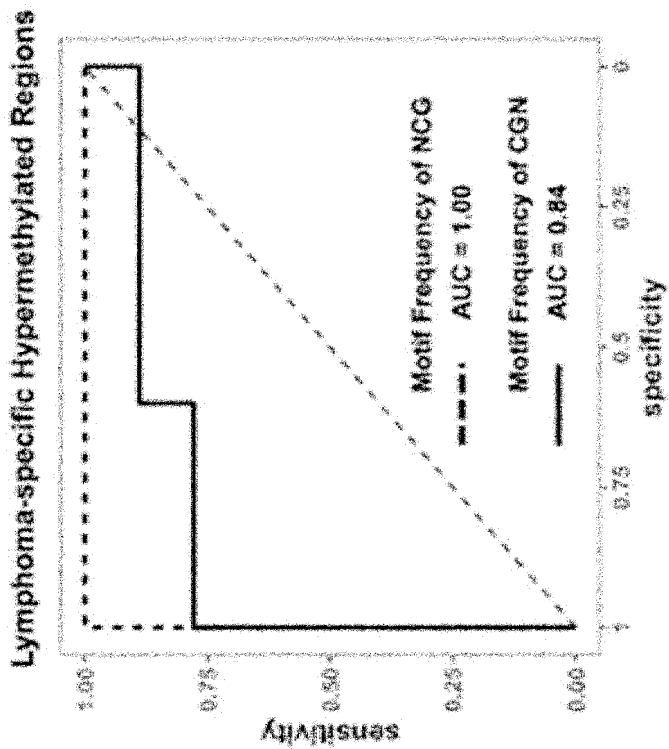


FIG. 87A

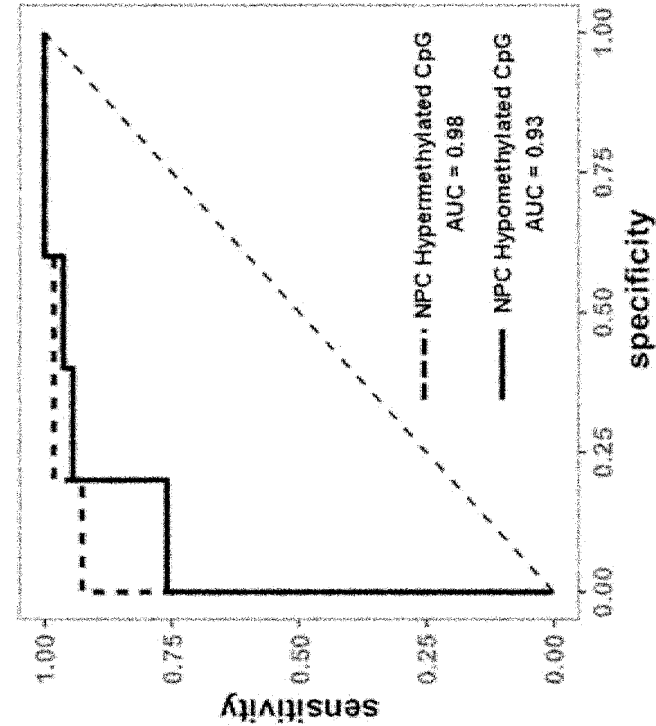


FIG. 88B

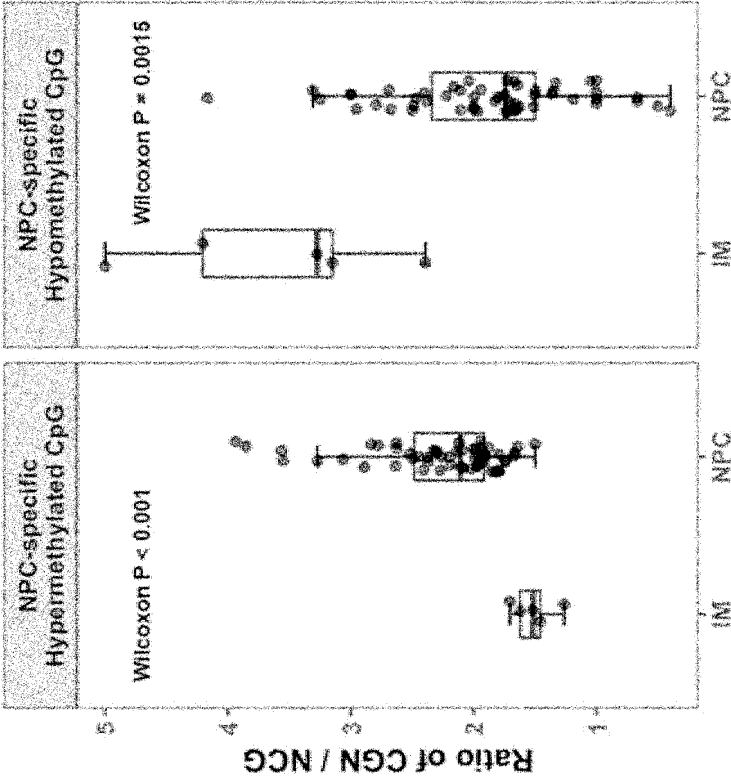


FIG. 88A

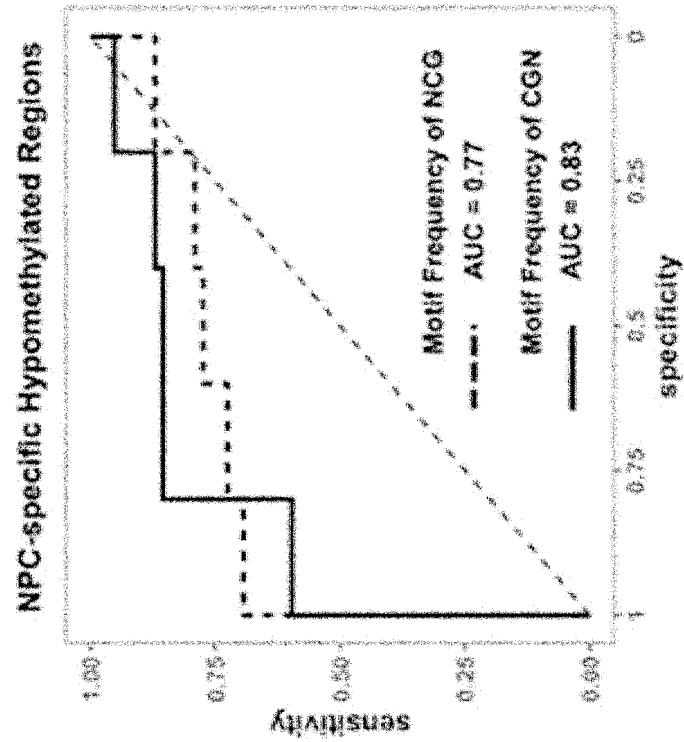


FIG. 89B

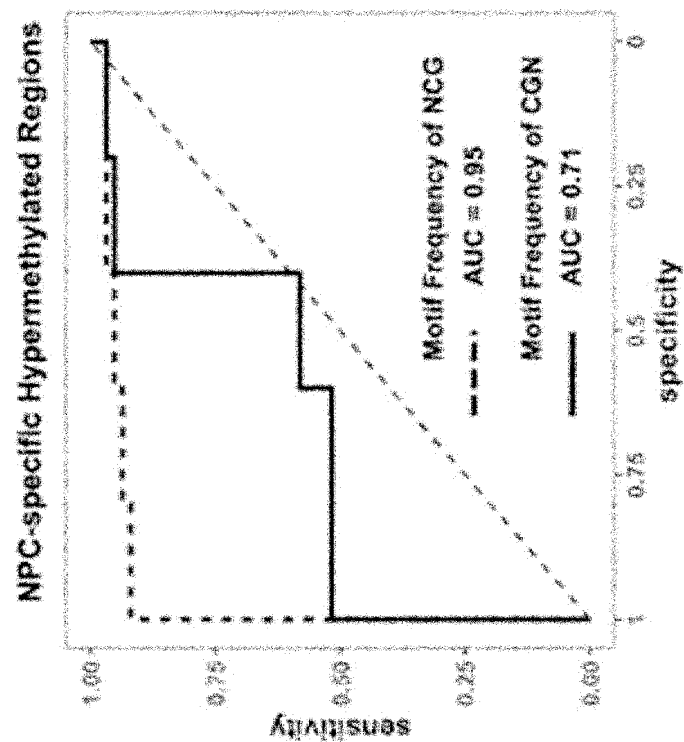


FIG. 89A

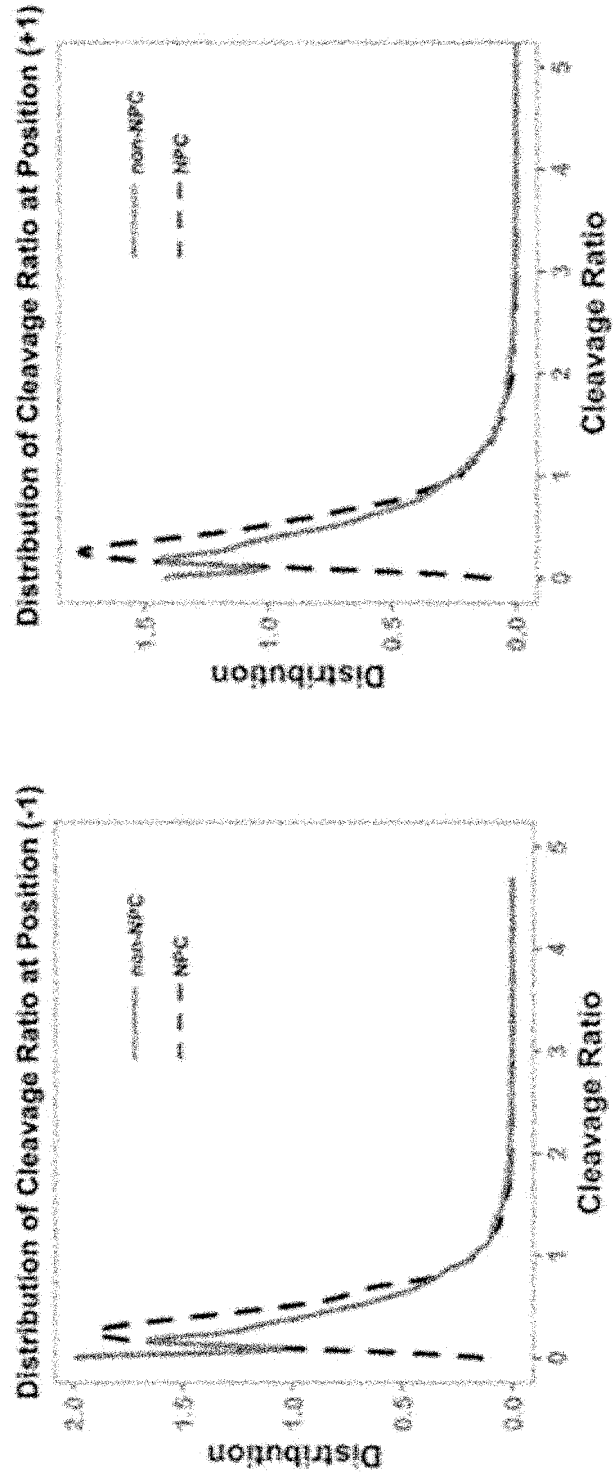
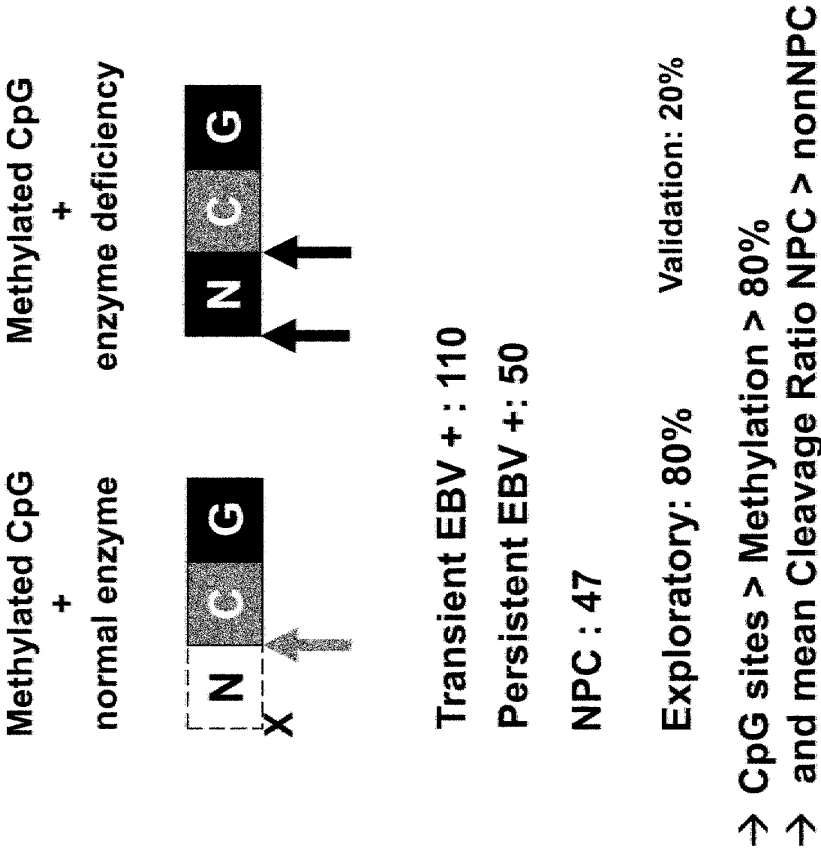


FIG. 90A

FIG. 90B

Hypothesis



CpG site with methylation density > 80%

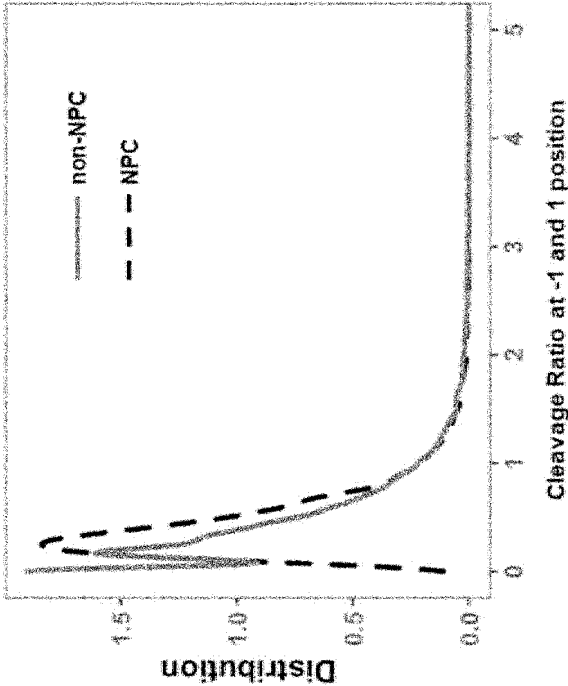


FIG. 91A

FIG. 91B

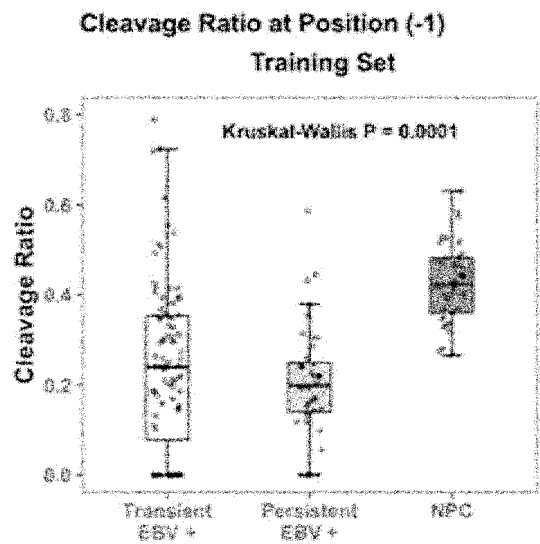


FIG. 92A

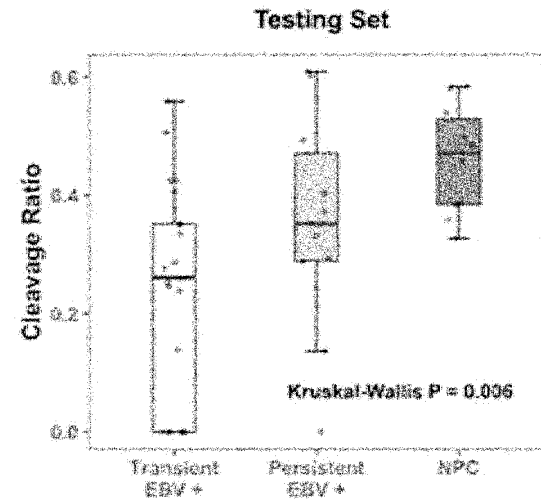


FIG. 92B

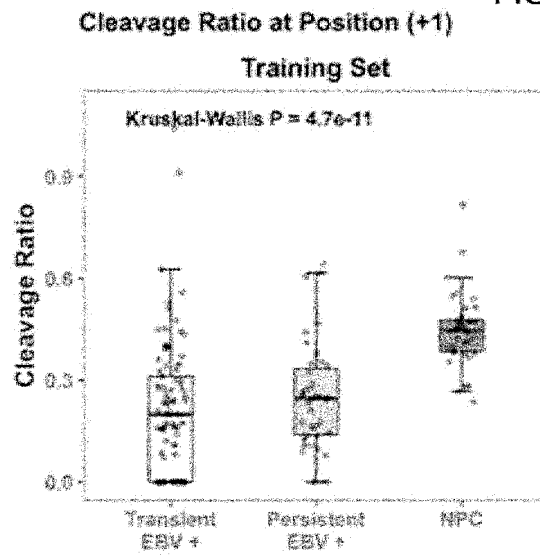


FIG. 92C

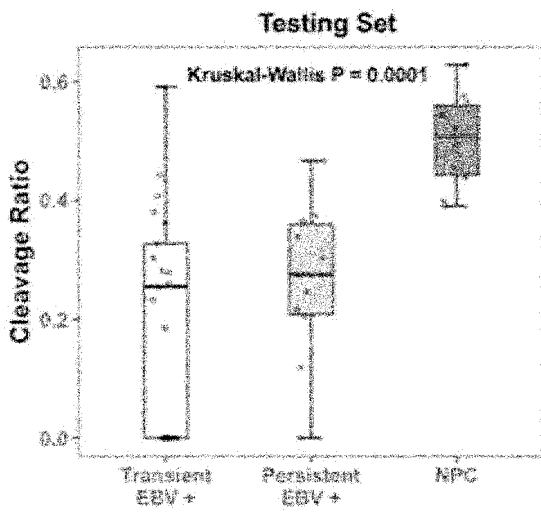


FIG. 92D

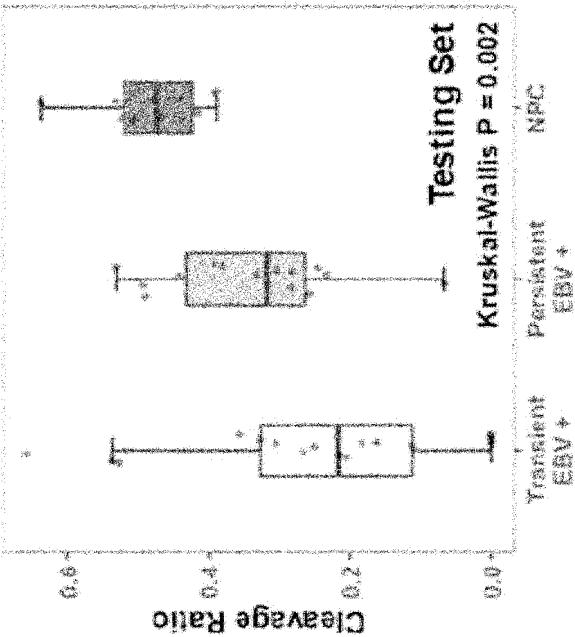


FIG. 93A

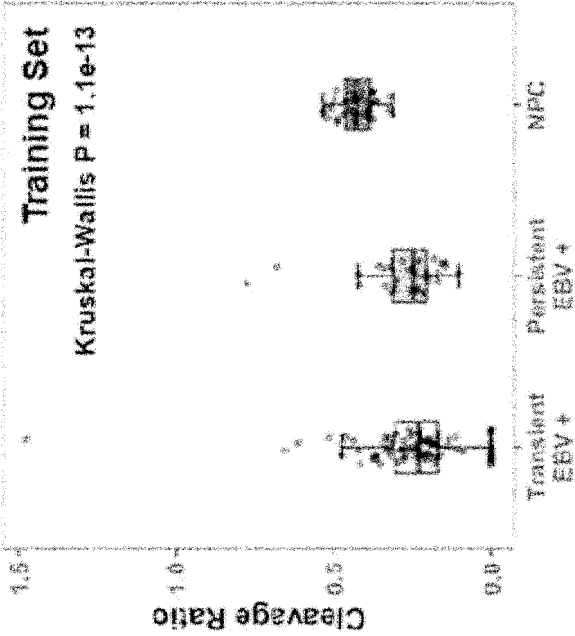


FIG. 93B

FIG. 94A

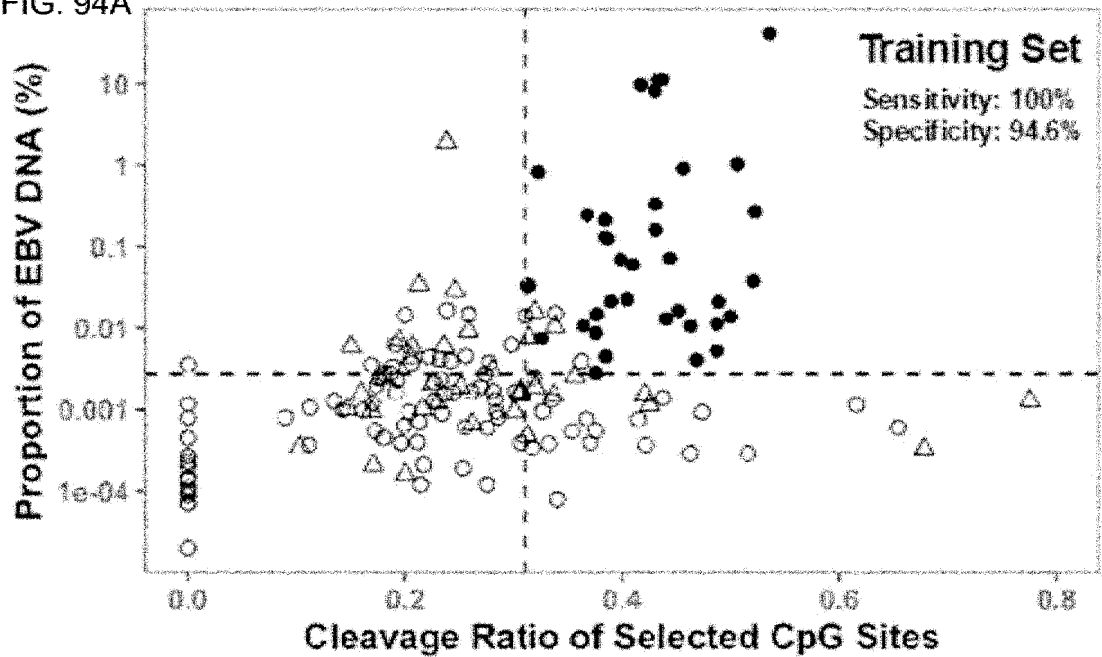
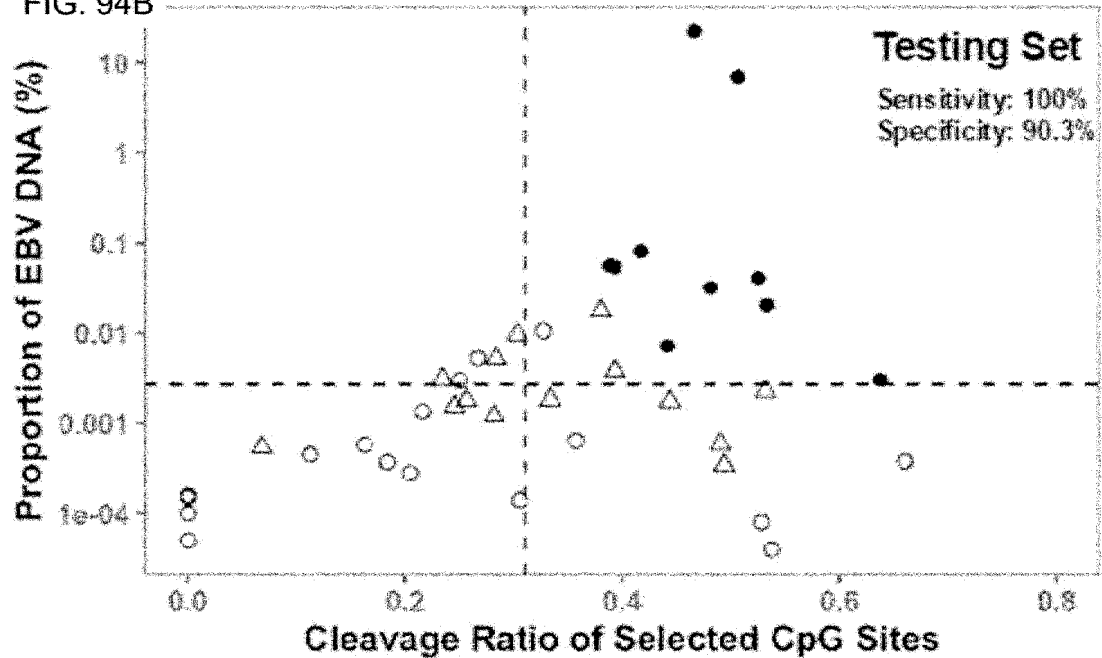


FIG. 94B



Method	Sensitivity (Training Set)	Specificity (Training Set)	Sensitivity (Testing Set)	Specificity (Testing Set)
Count	100% (37/37)	73.6% (95/129)	100% (10/10)	74.2% (23/31)
Size	100% (37/37)	34.9% (45/129)	100% (10/10)	38.7% (12/31)
Cleavage Ratio	100% (37/37)	73.6% (95/129)	100% (10/10)	61.3% (19/31)
Count + Size	100% (37/37)	80.6% (104/129)	100% (10/10)	87.1% (27/31)
Count + Cleavage Ratio	100% (37/37)	94.6% (122/129)	100% (10/10)	90.3% (28/31)
Count + Size + Cleavage Ratio	100% (37/37)	96.9% (125/129)	100% (10/10)	96.8% (30/31)

FIG. 95

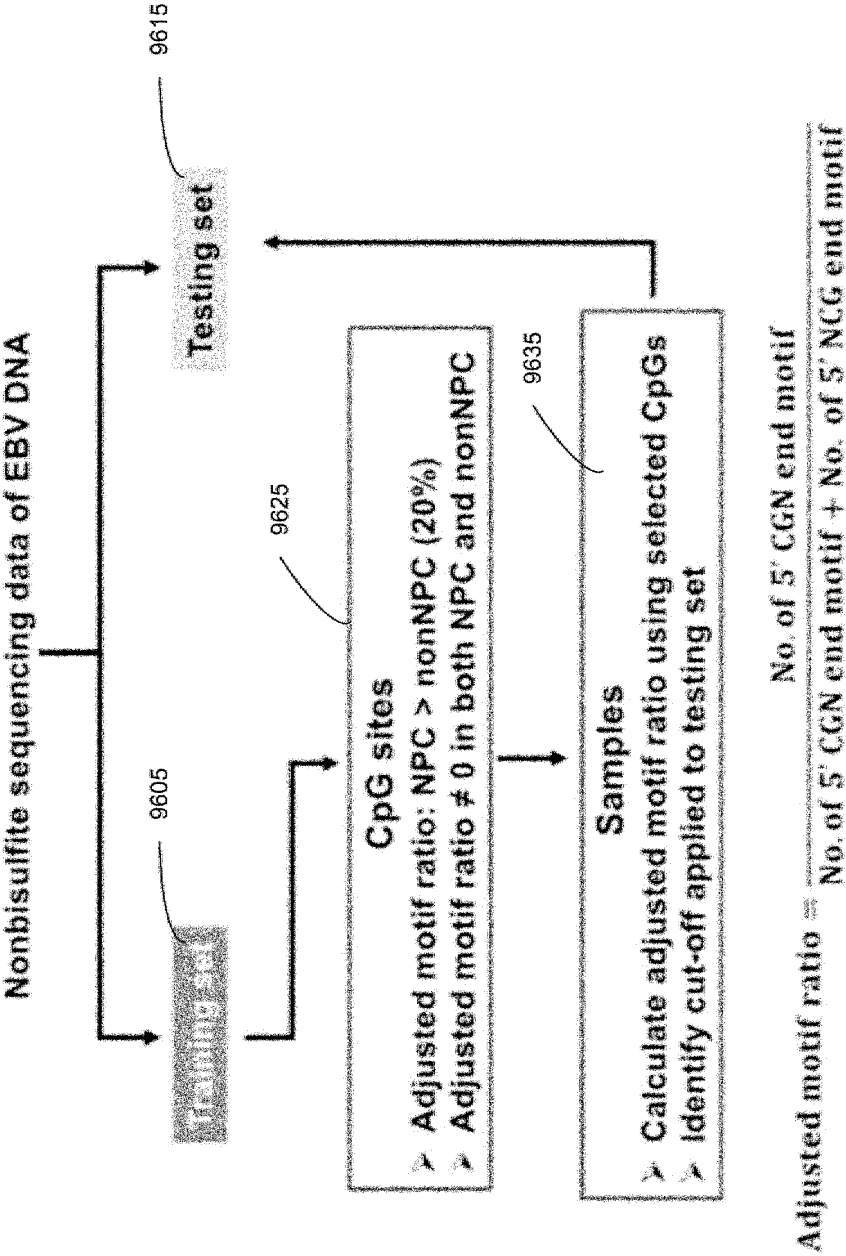


FIG. 96

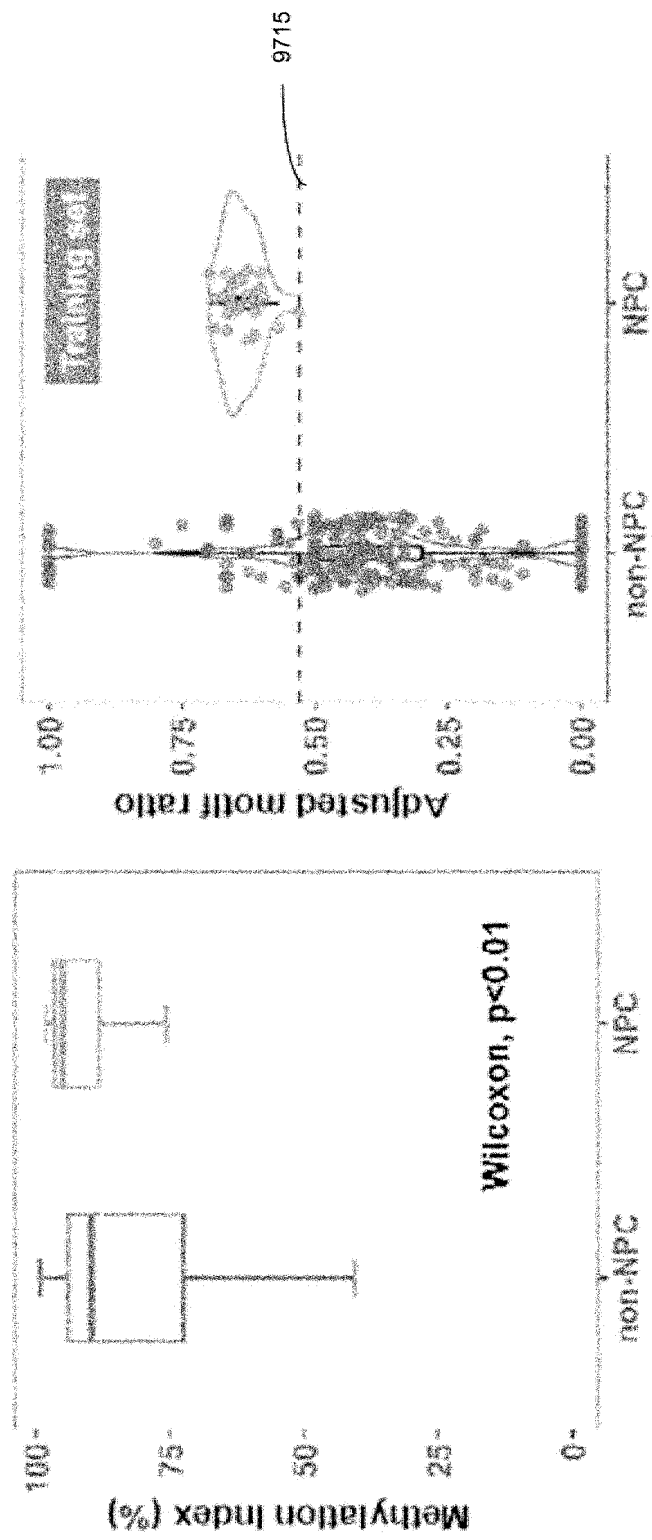


FIG. 97B

FIG. 97A

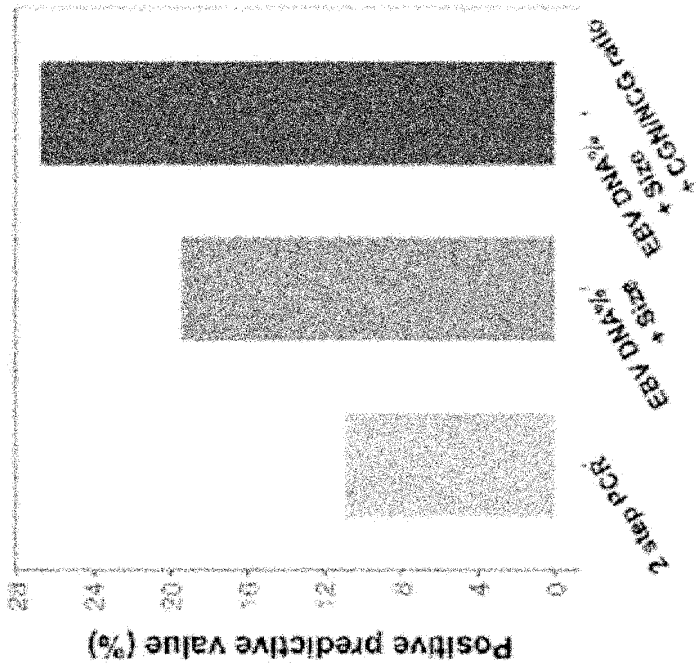


FIG. 98B

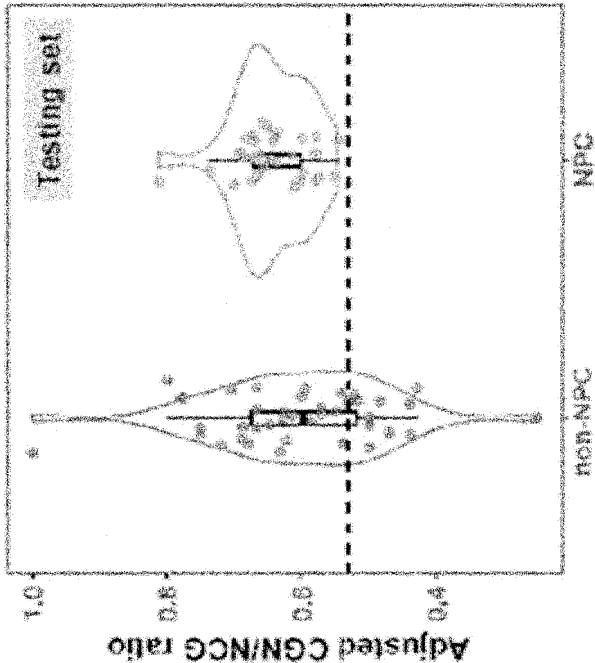


FIG. 98A

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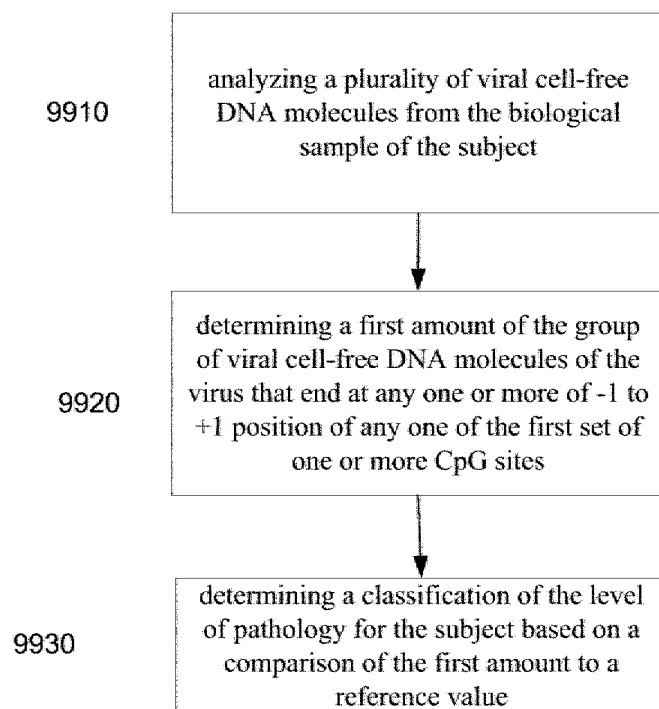


FIG. 99

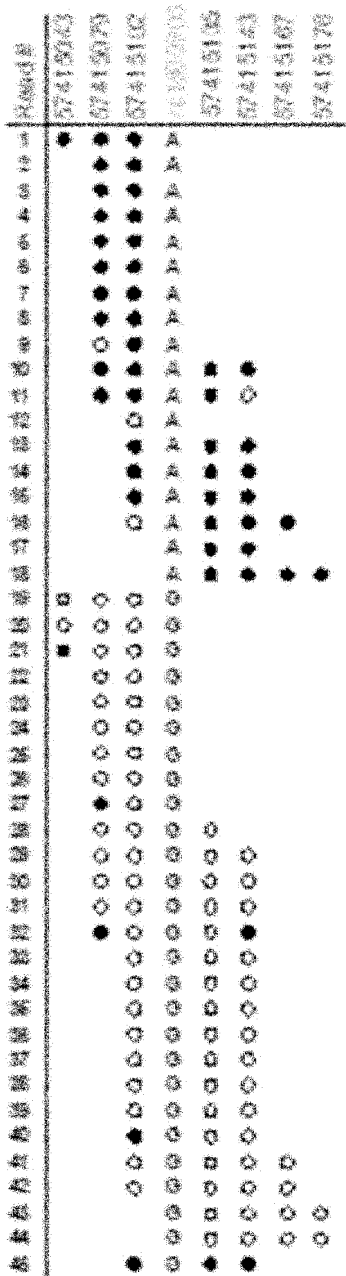
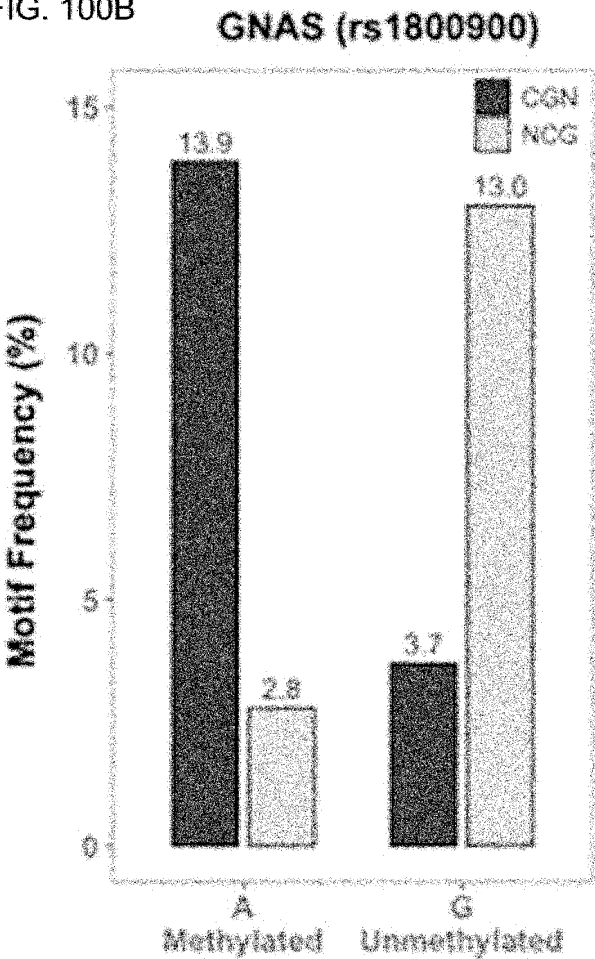


FIG. 100A

FIG. 100B



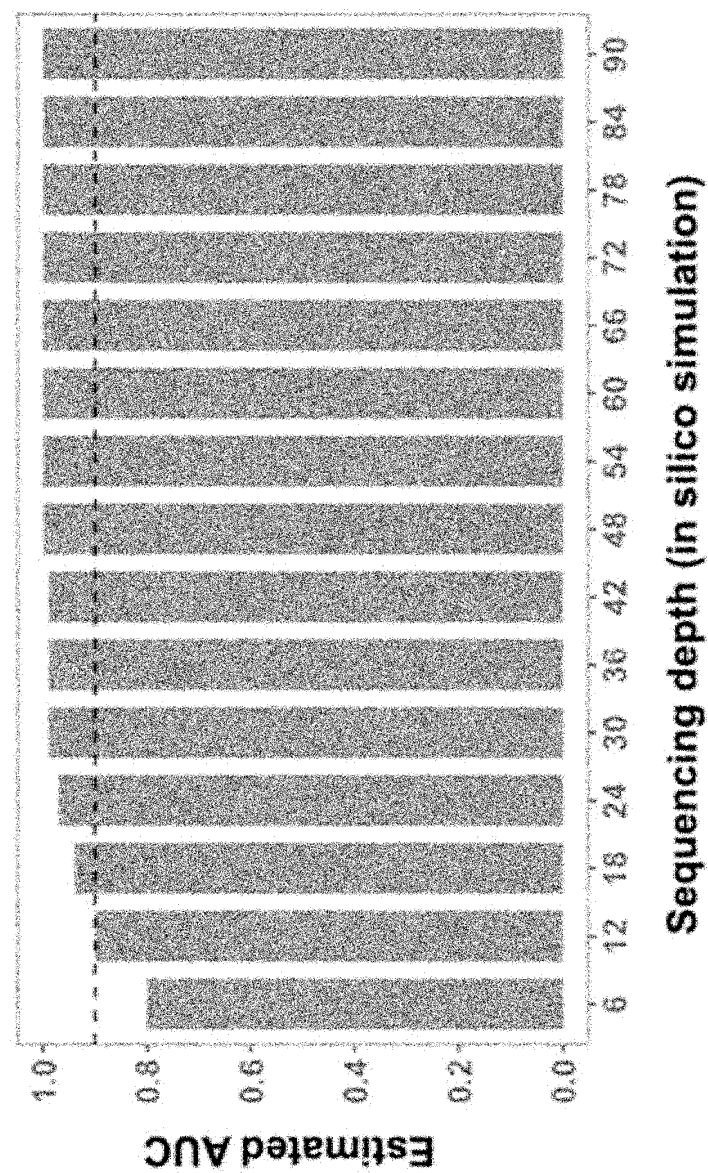


FIG. 101

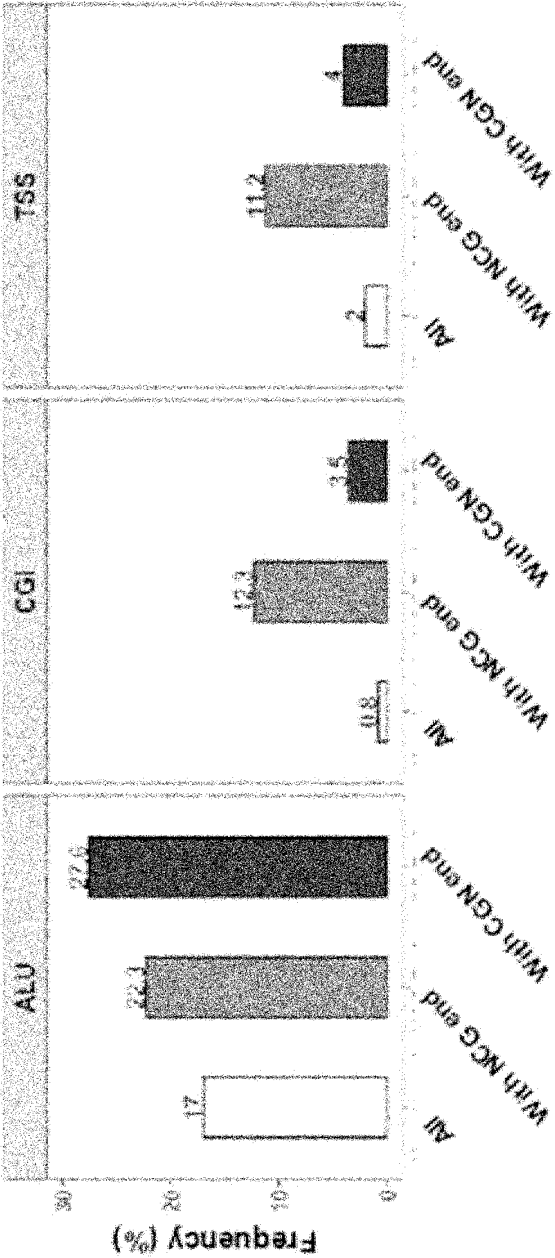


FIG. 102

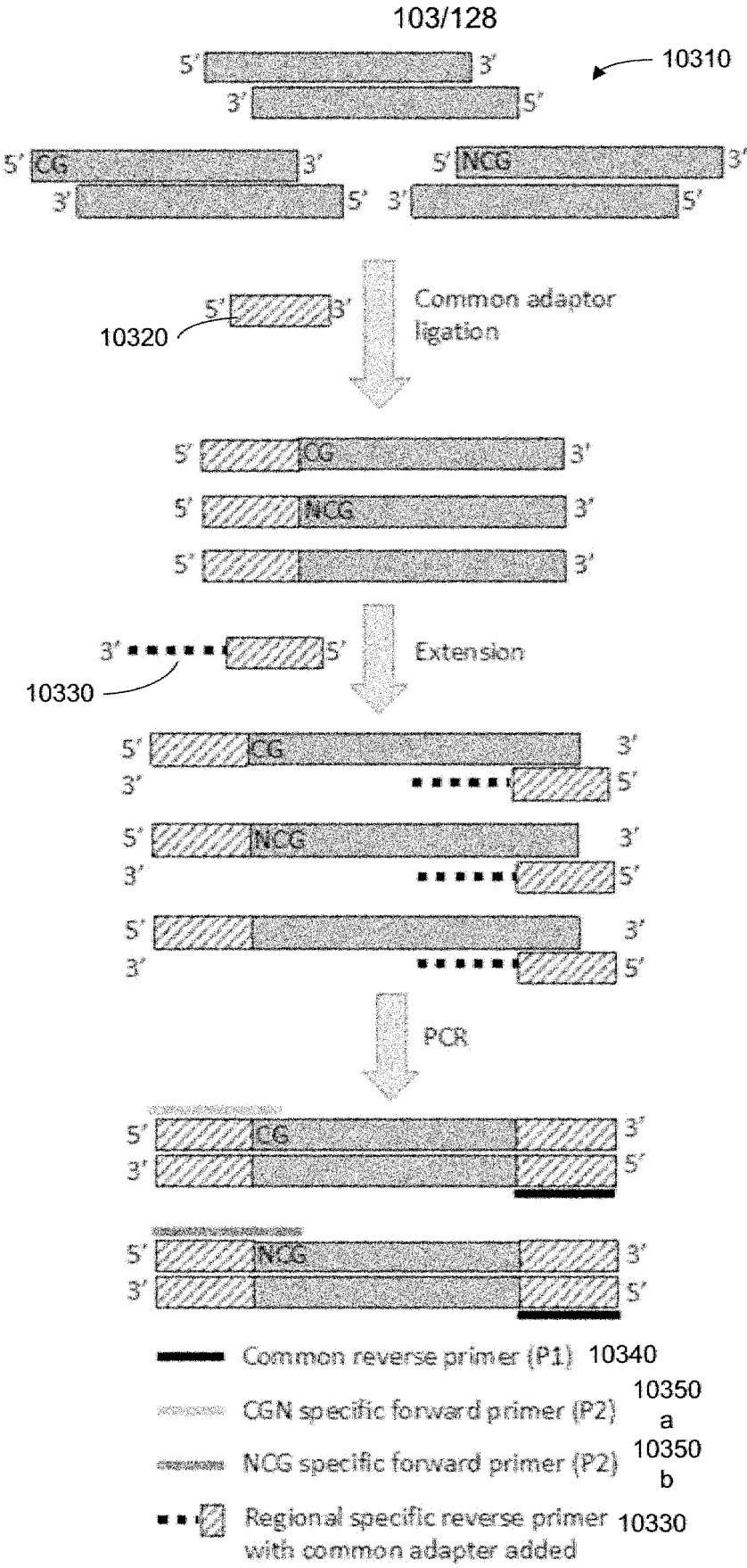


FIG. 103

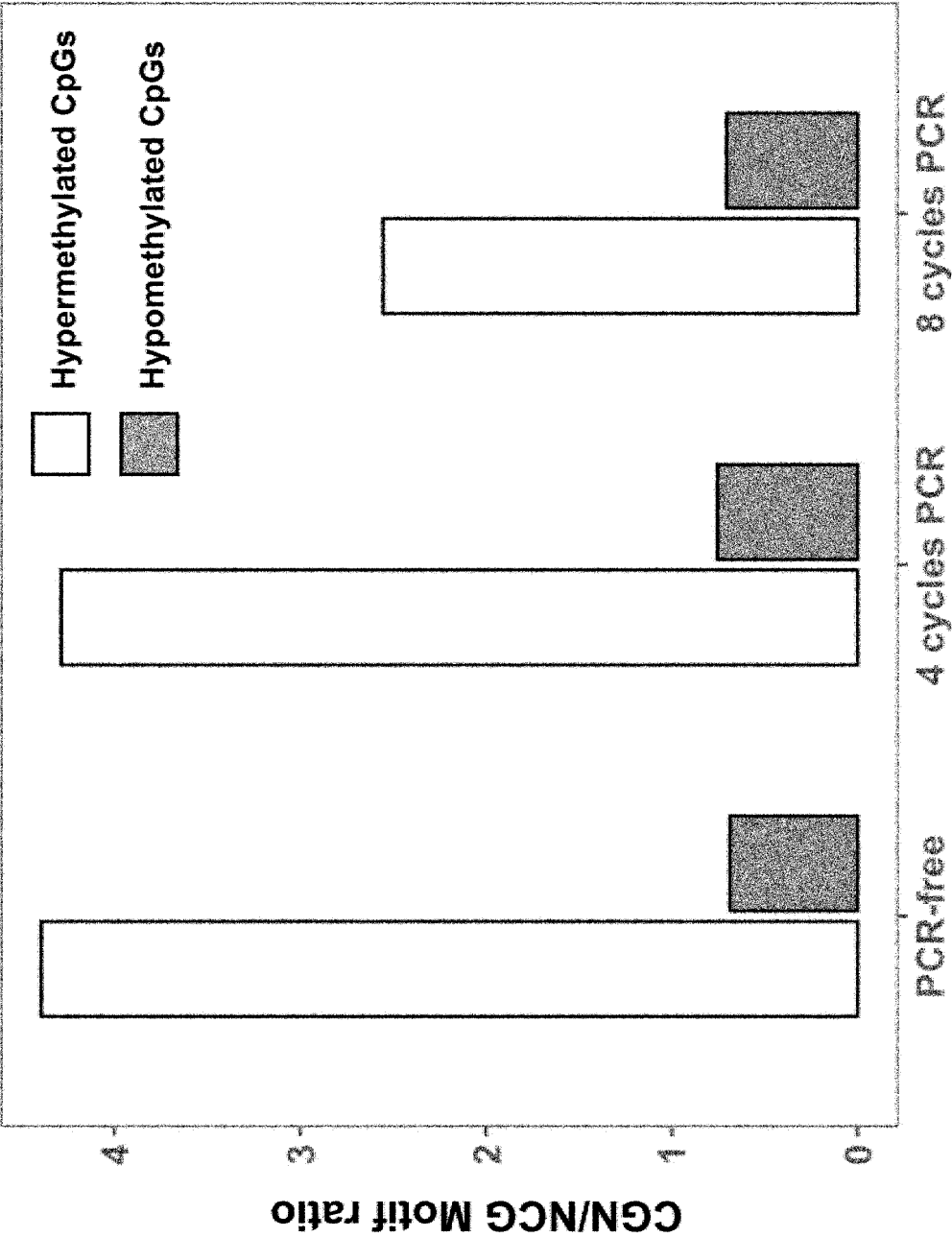


FIG. 104

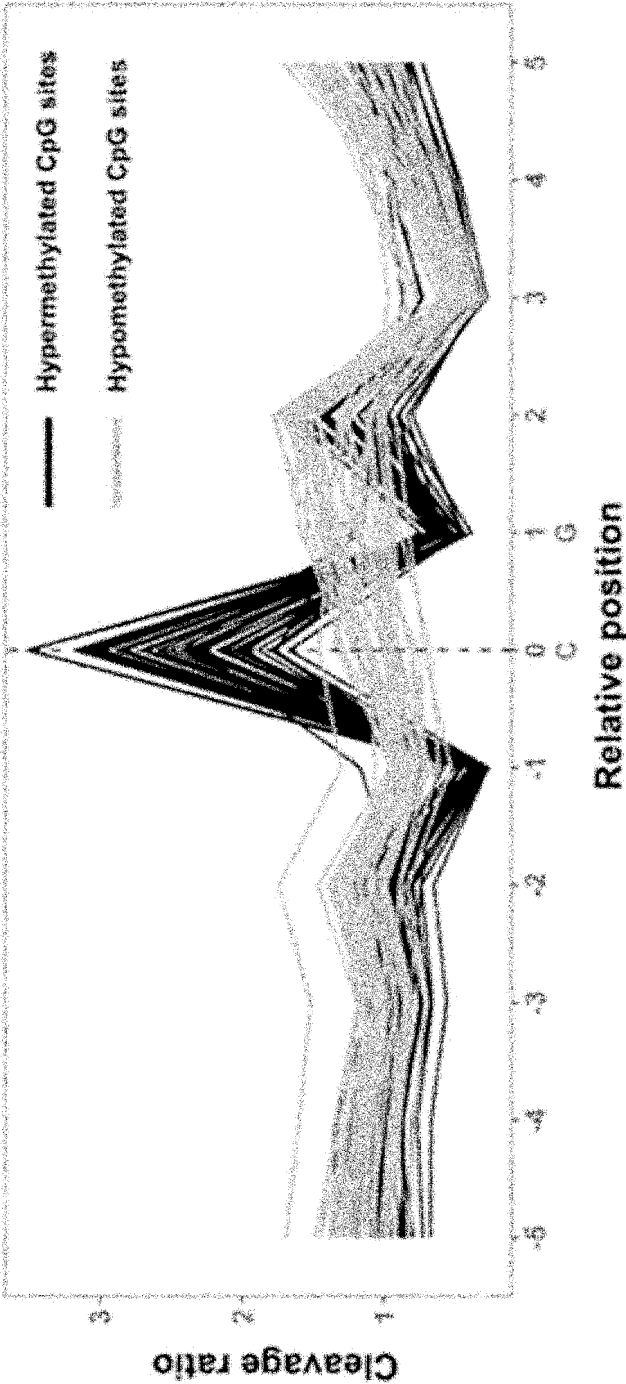


FIG. 105

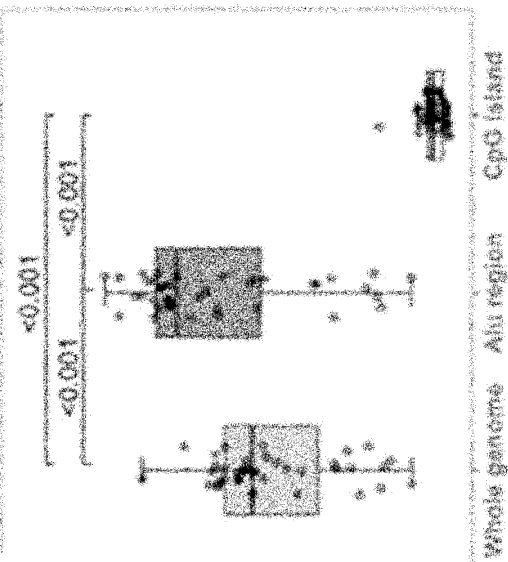


FIG. 106B

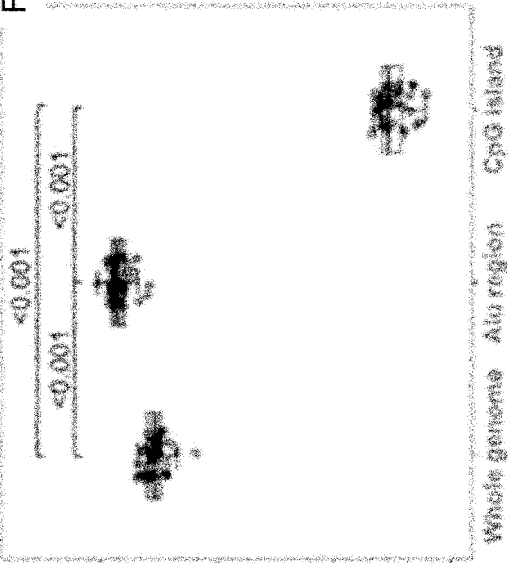


FIG. 106A

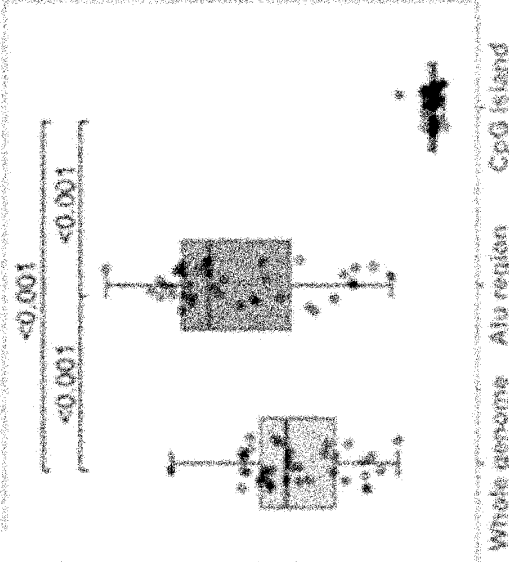


FIG. 106D

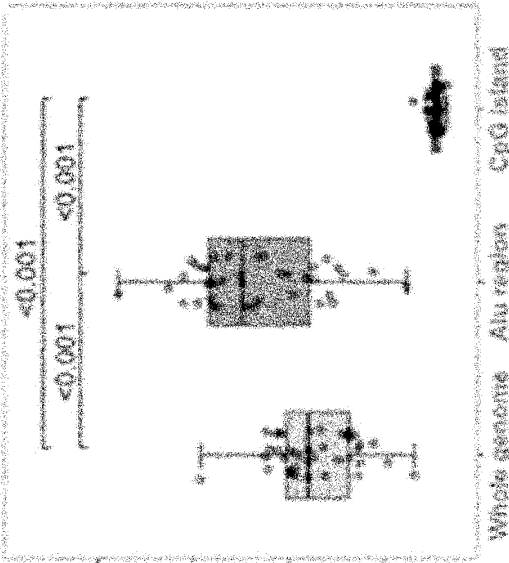


FIG. 106C

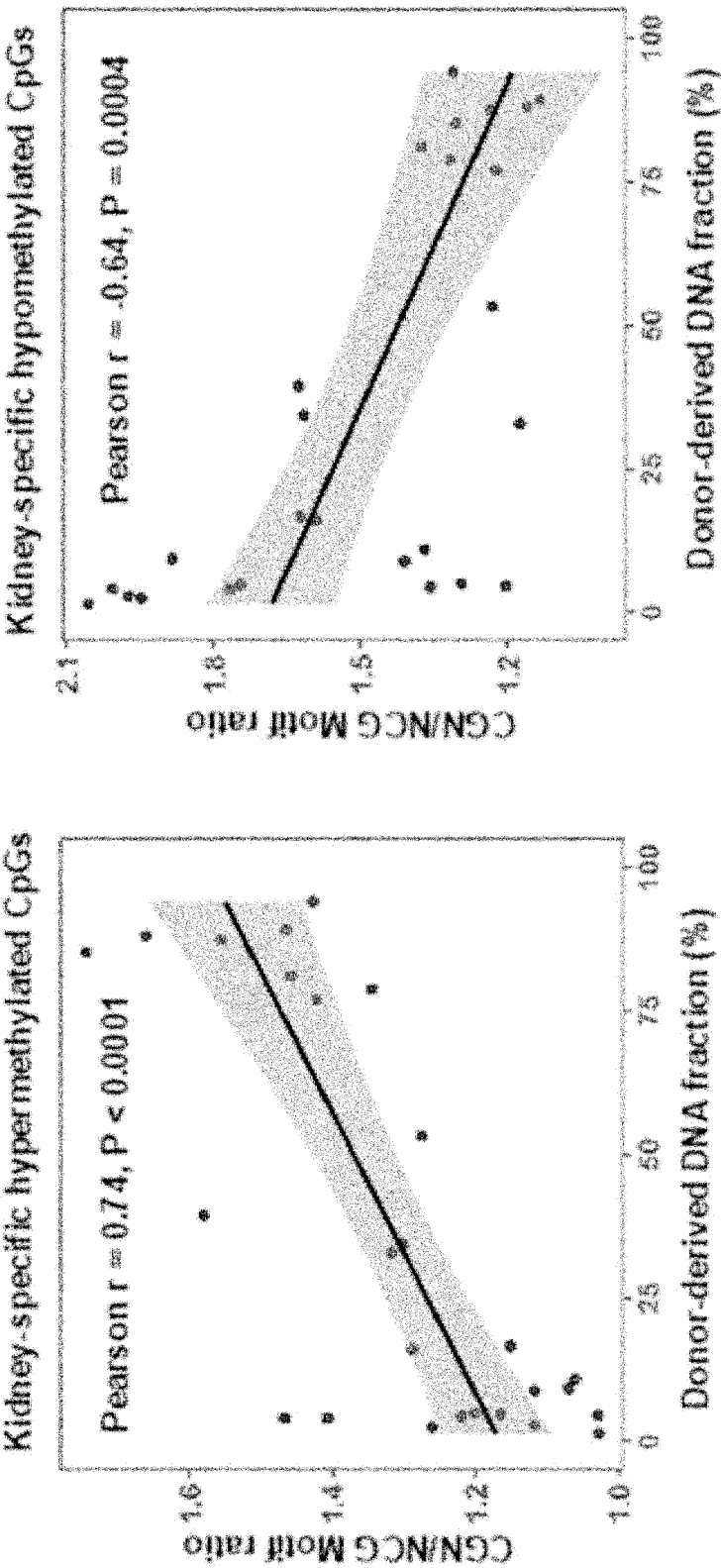
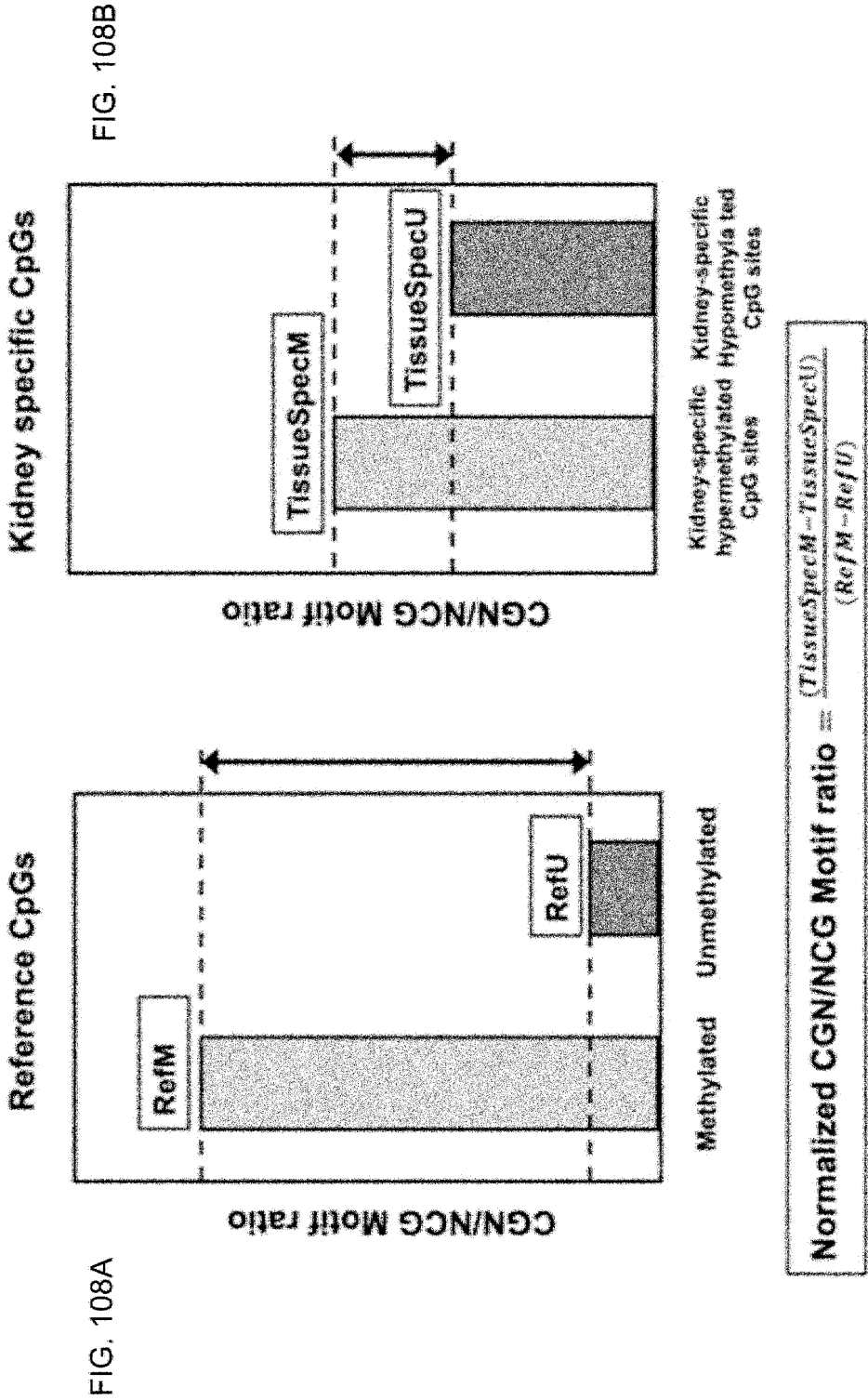
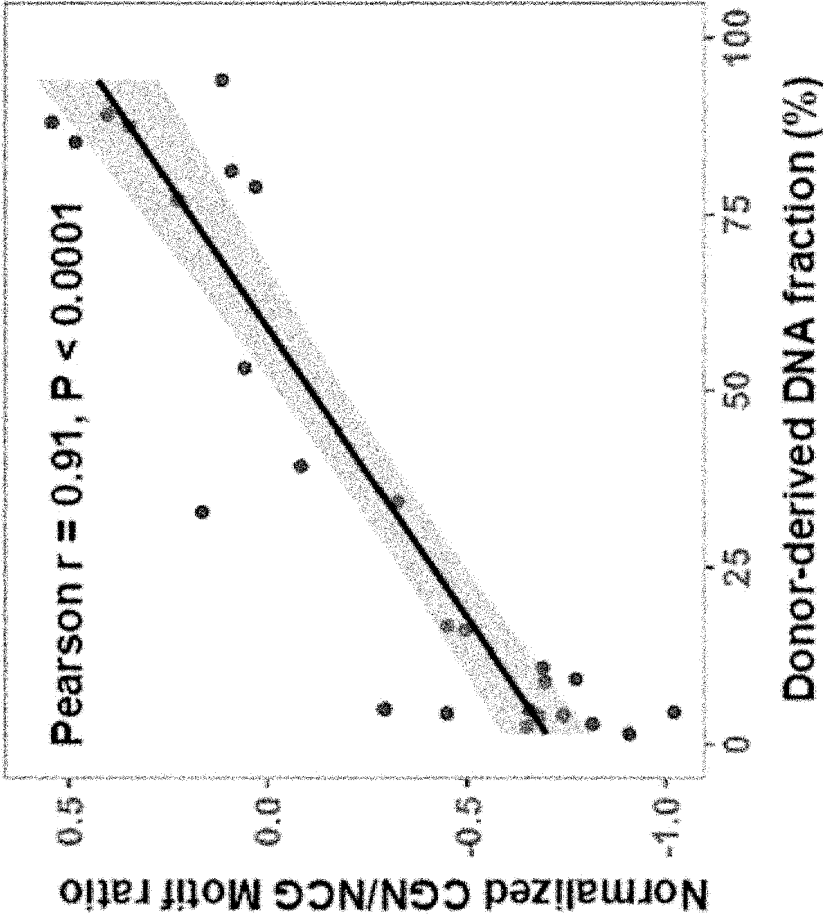


FIG. 107A

FIG. 107B





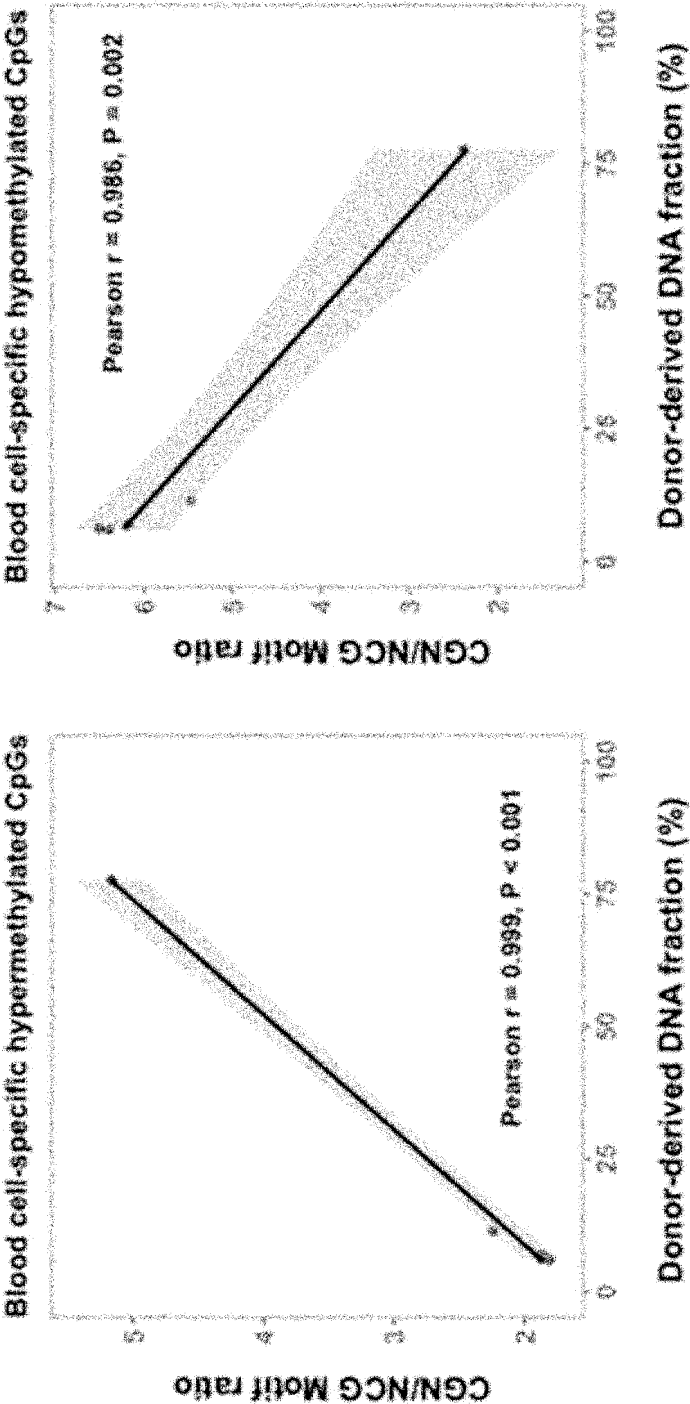


FIG. 110B

FIG. 110A

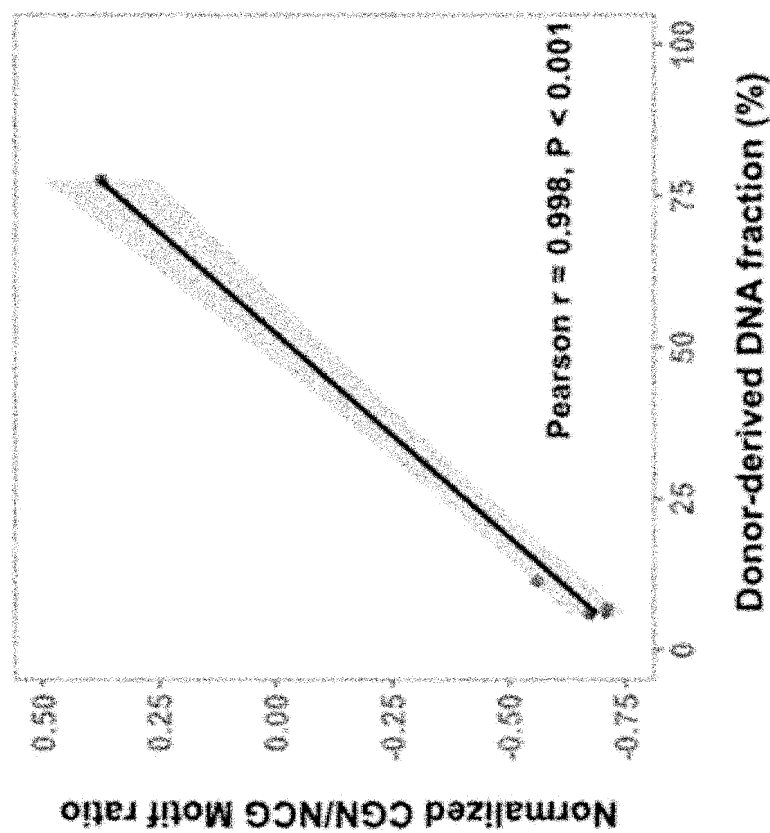


FIG. 111

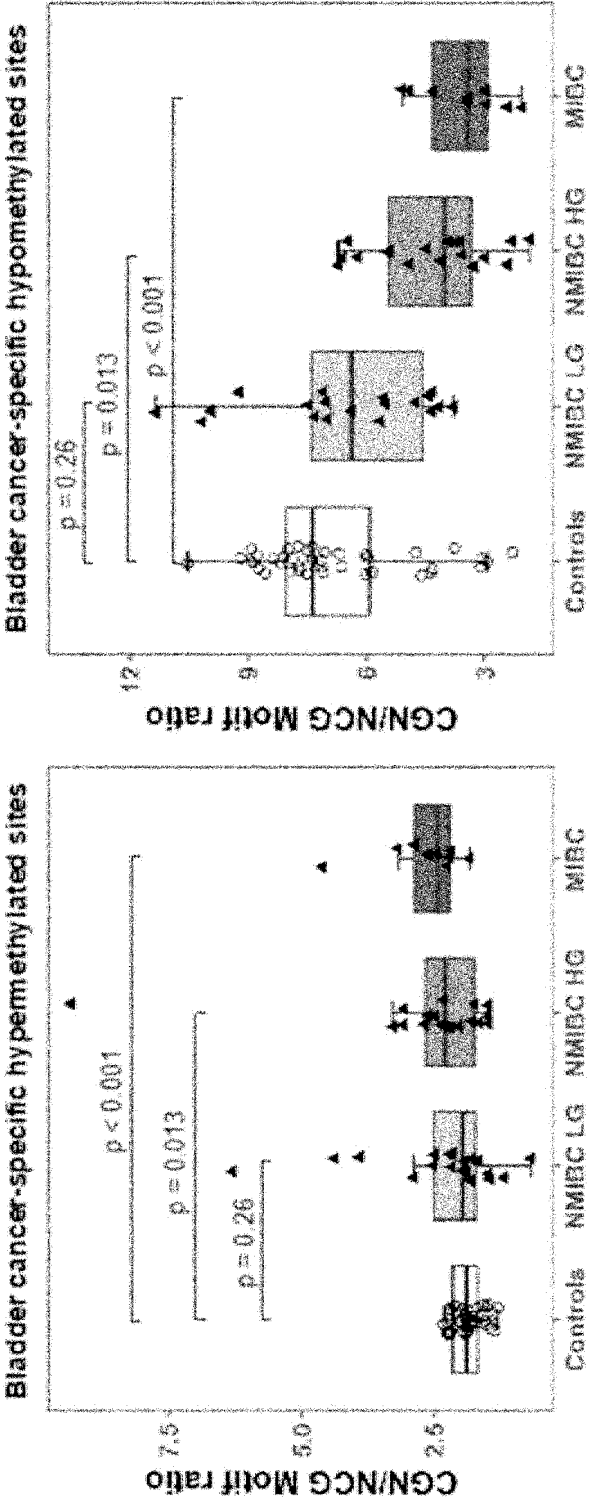


FIG. 112B

FIG. 112A

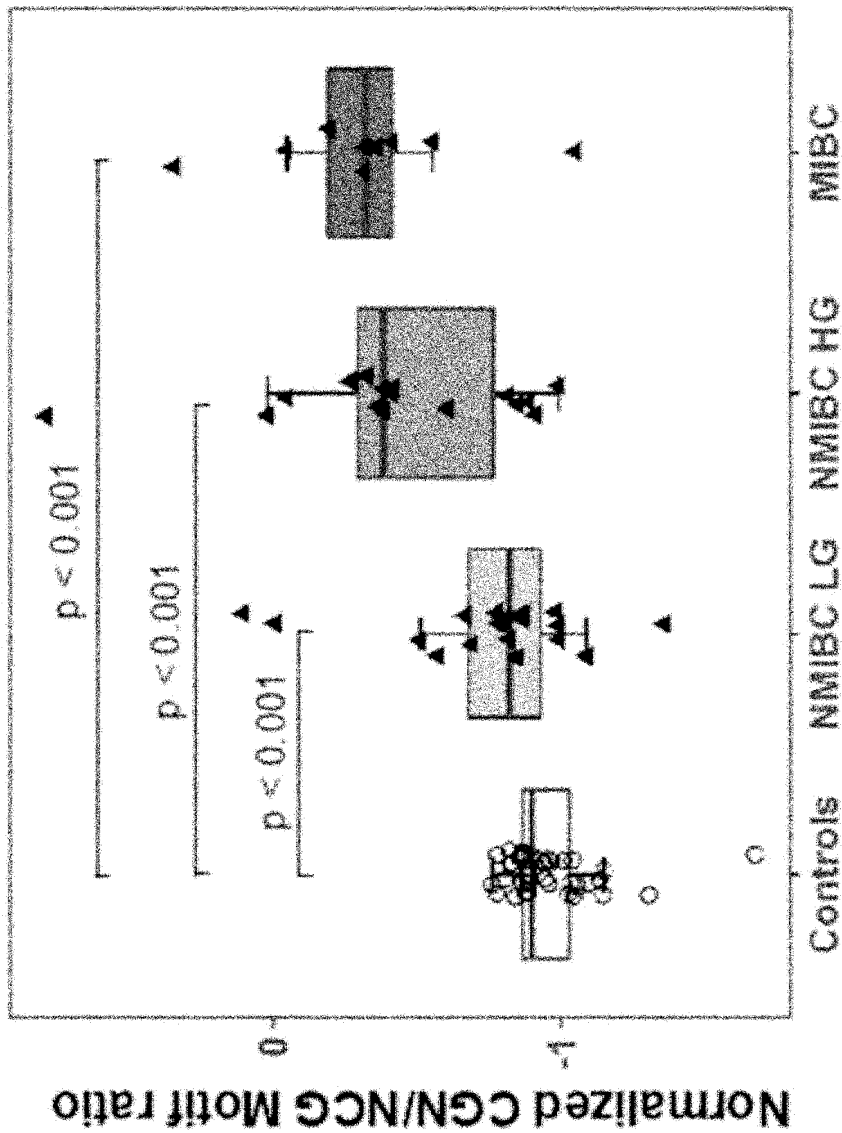


FIG. 113

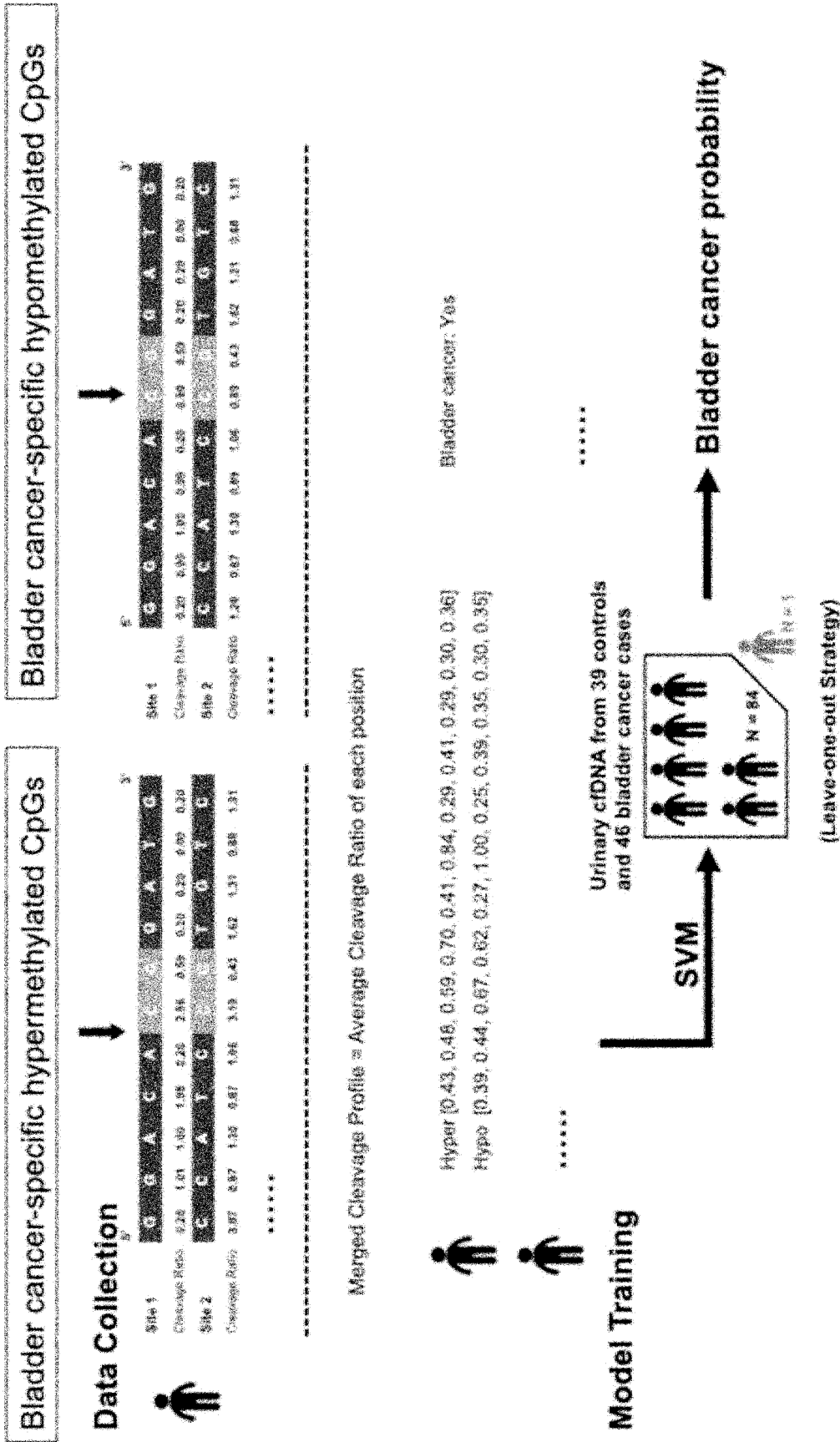


FIG. 114

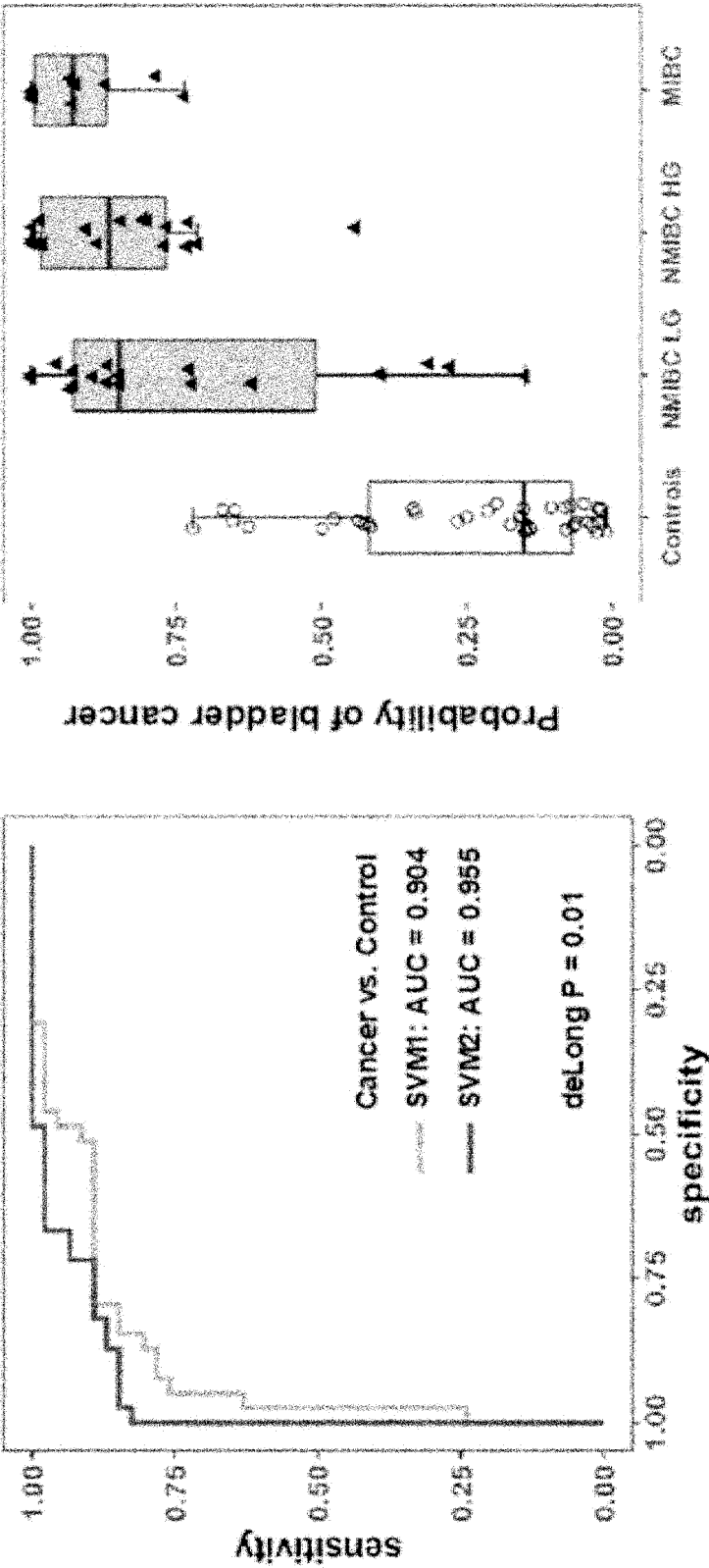


FIG. 115B

FIG. 115A

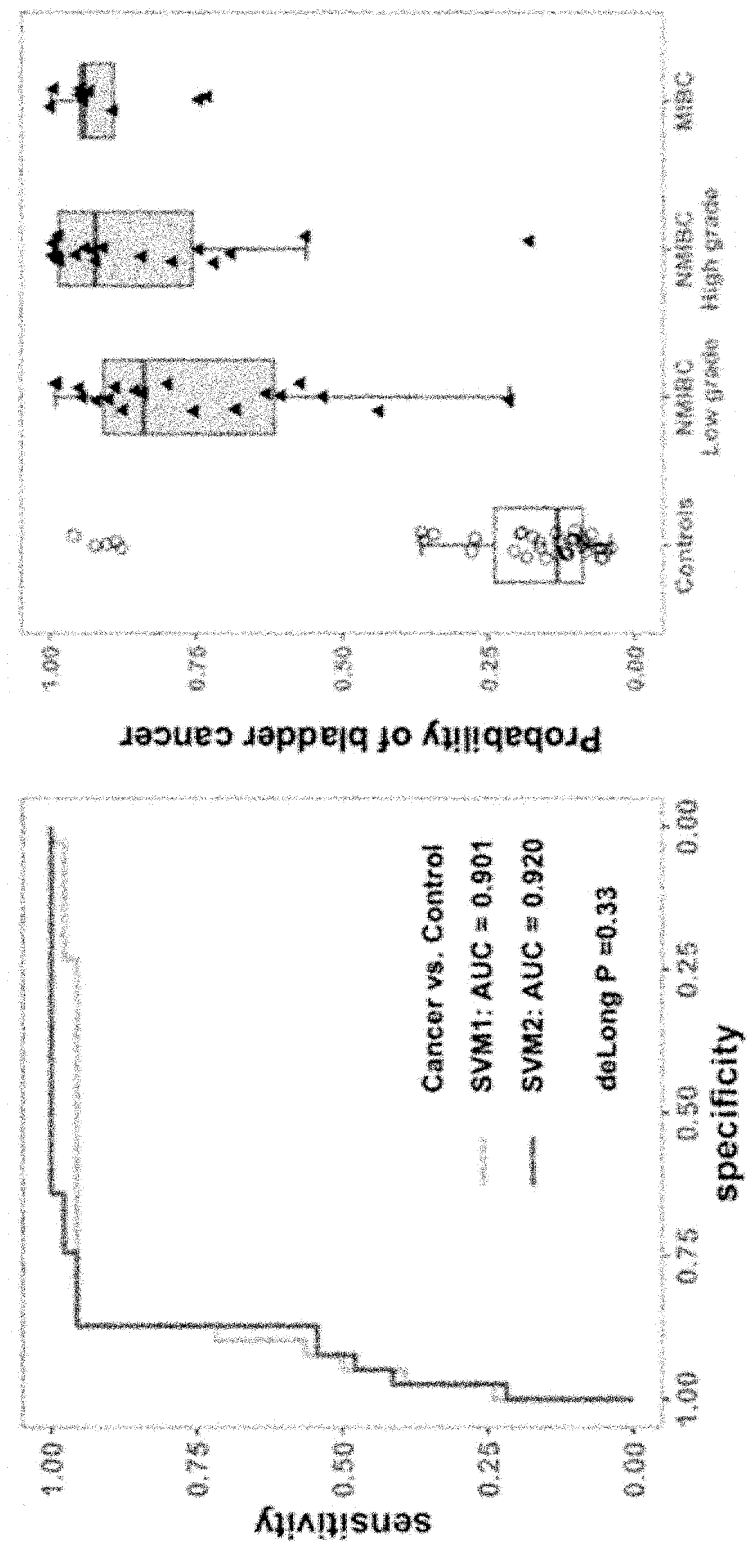


FIG. 116B

FIG. 116A

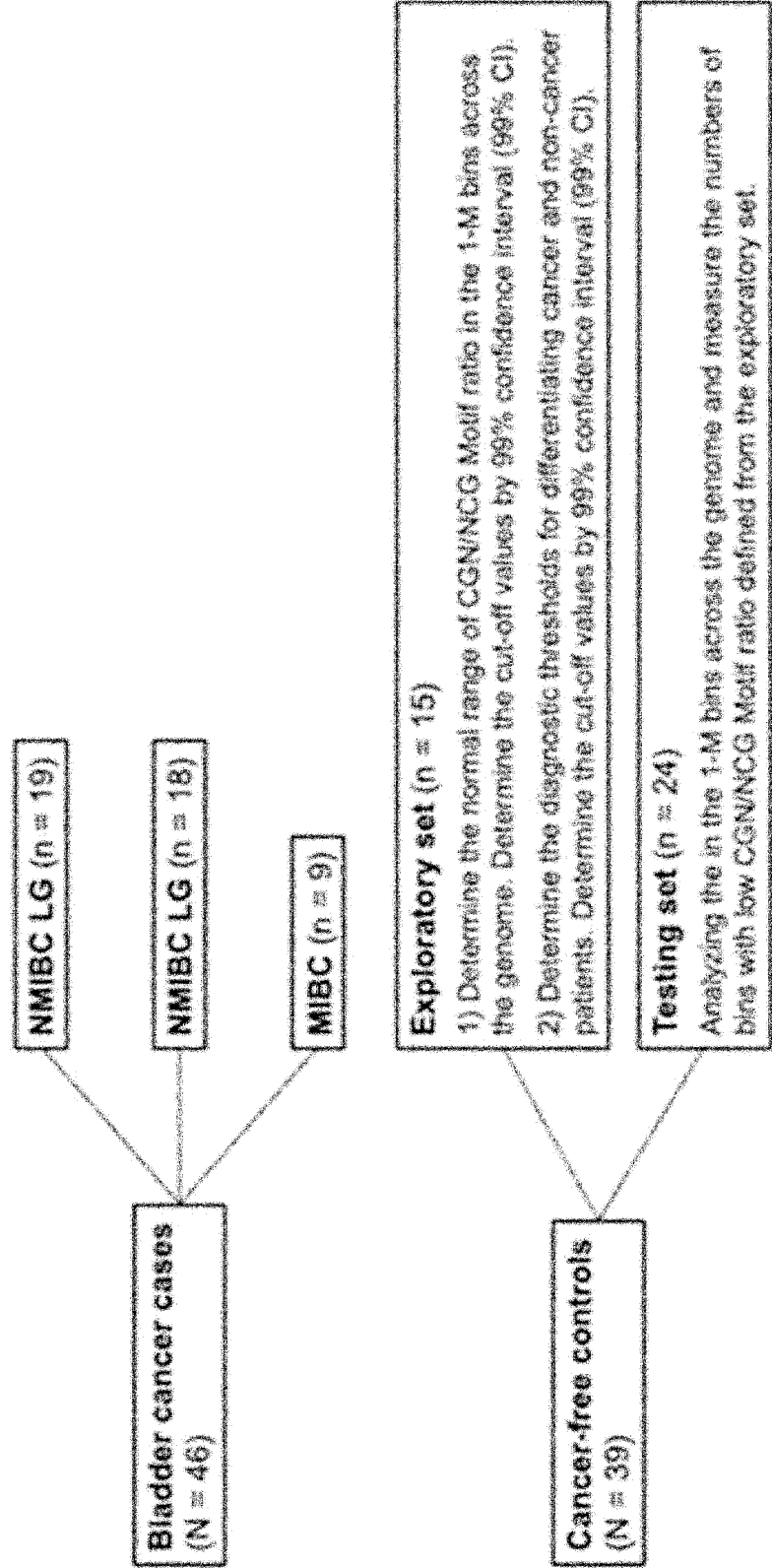


FIG. 117

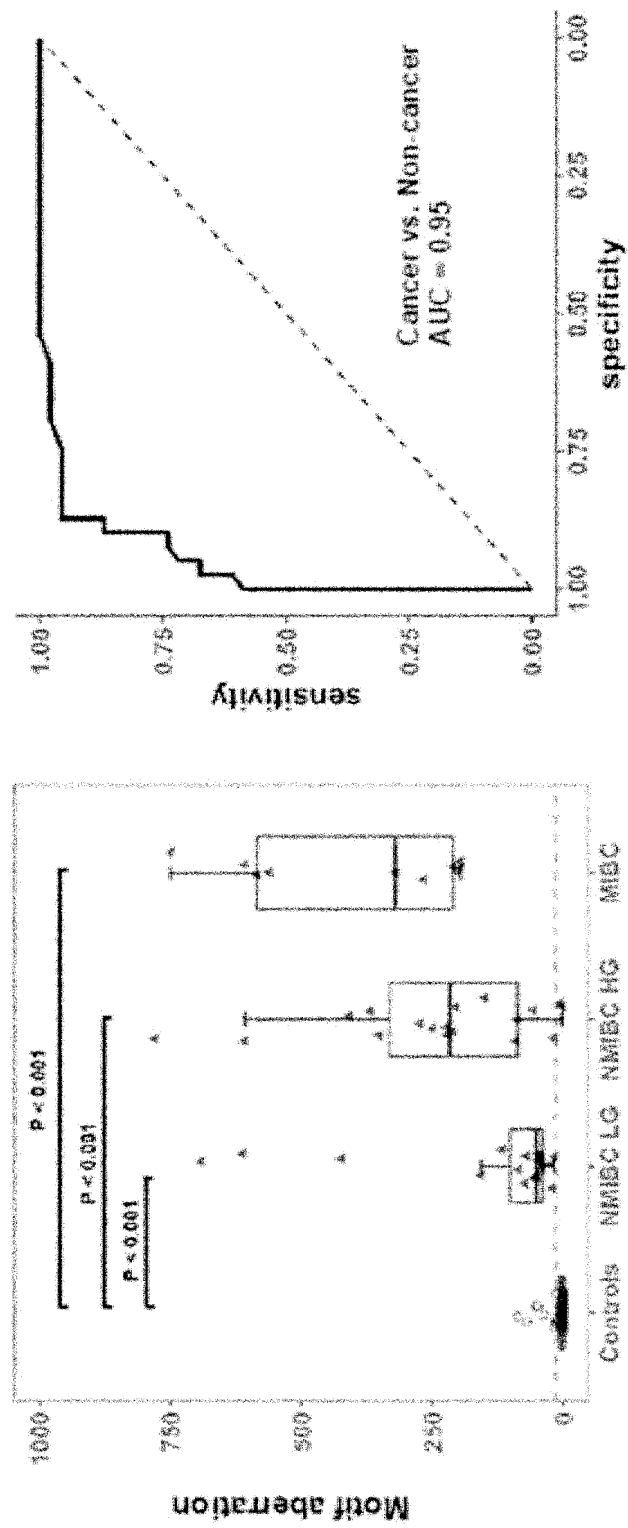


FIG. 118B

FIG. 118A

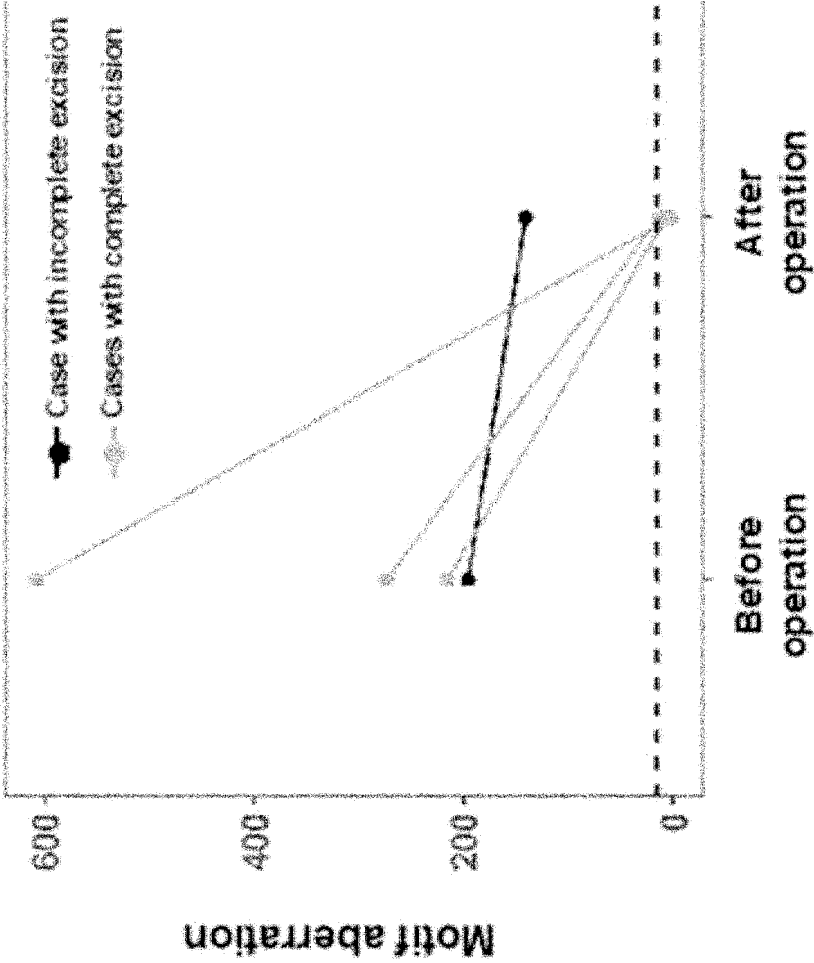


FIG. 119

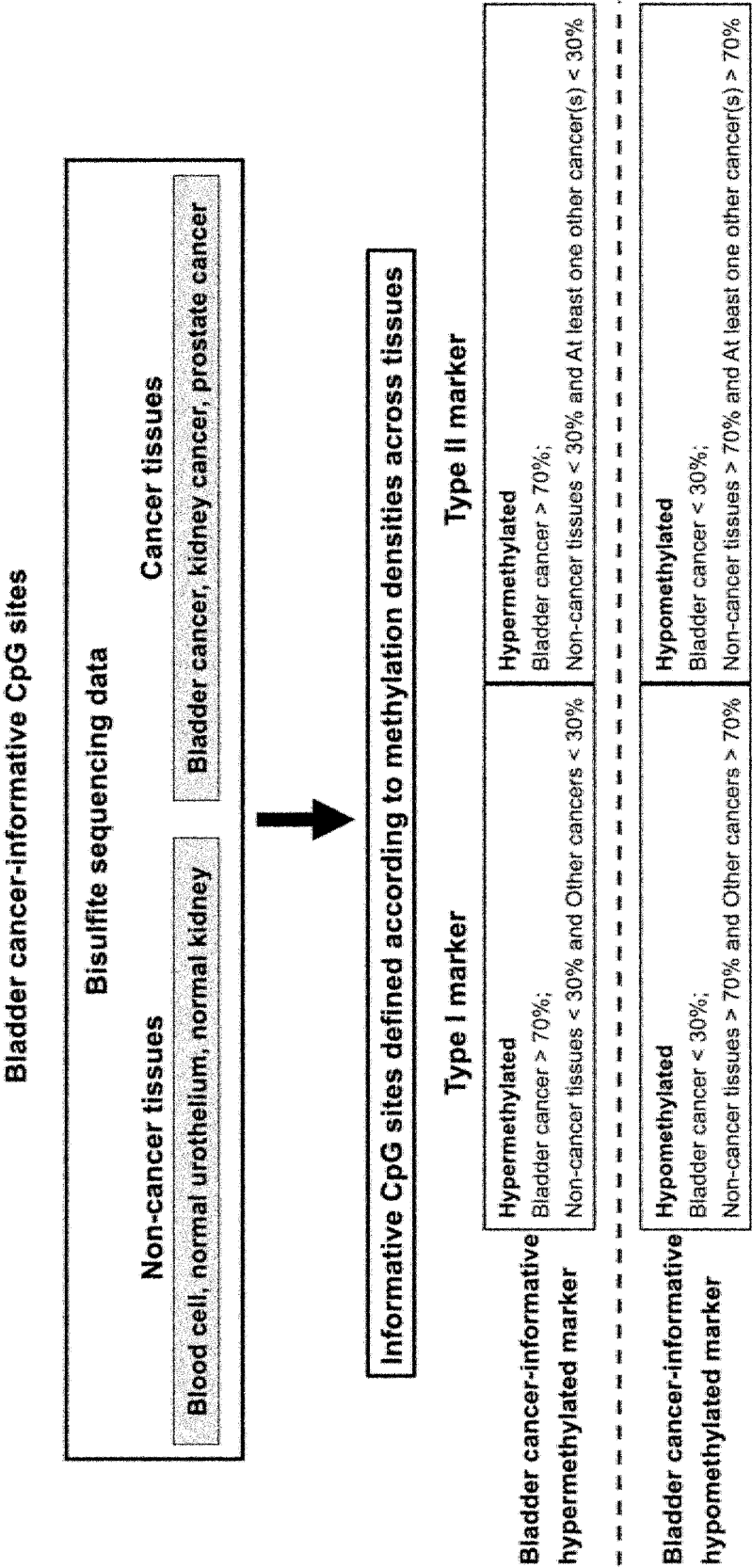


FIG. 120

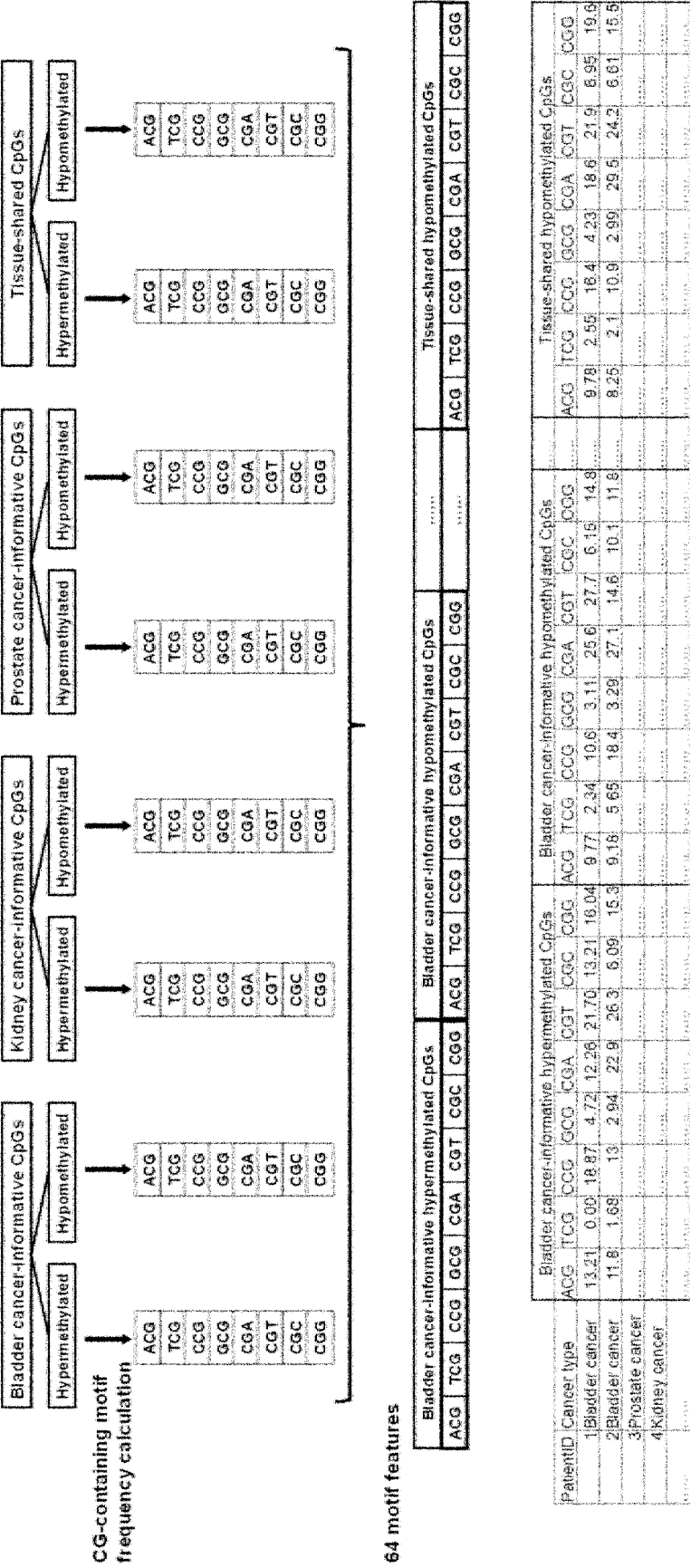


FIG. 121

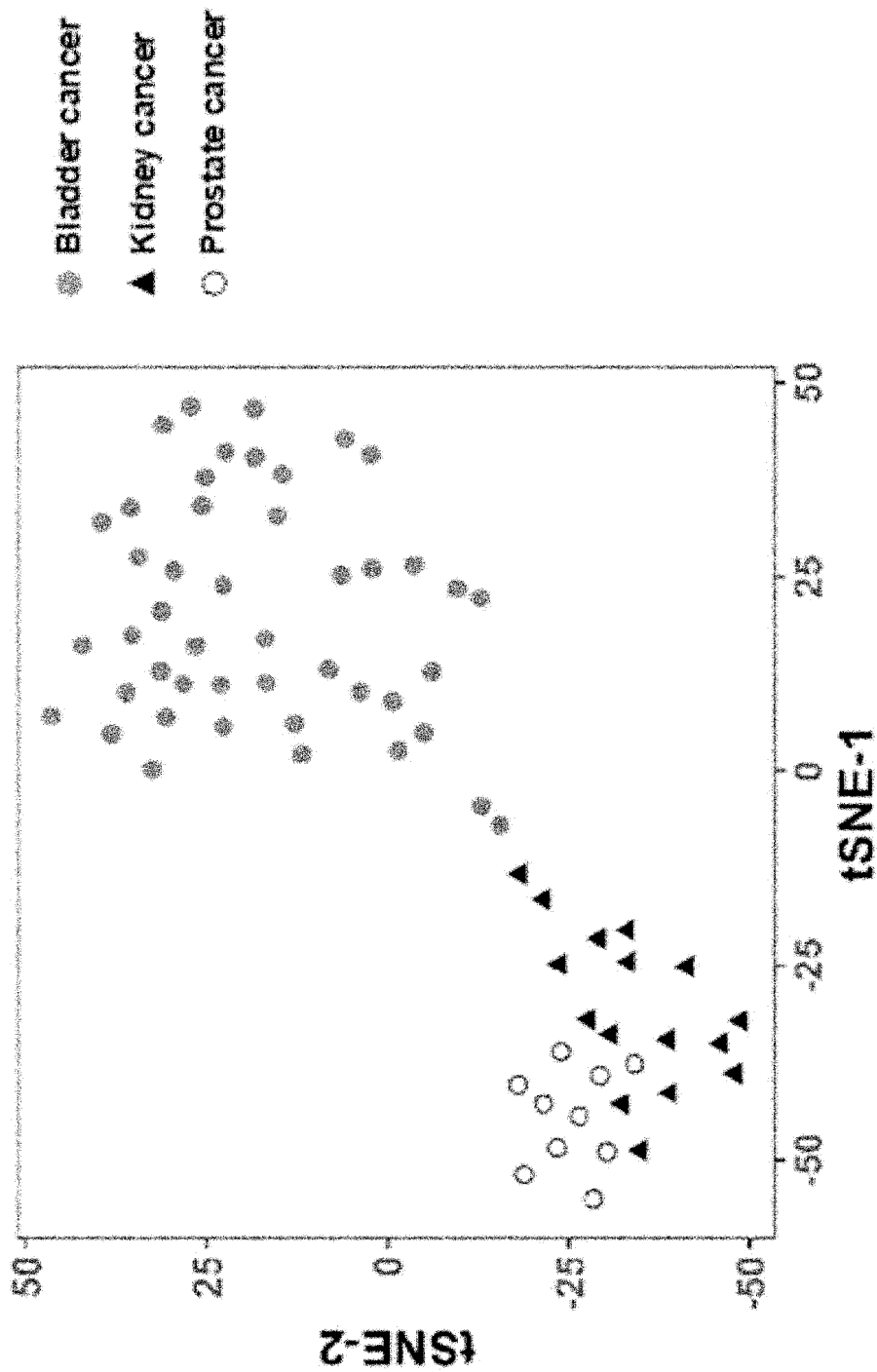


FIG. 122

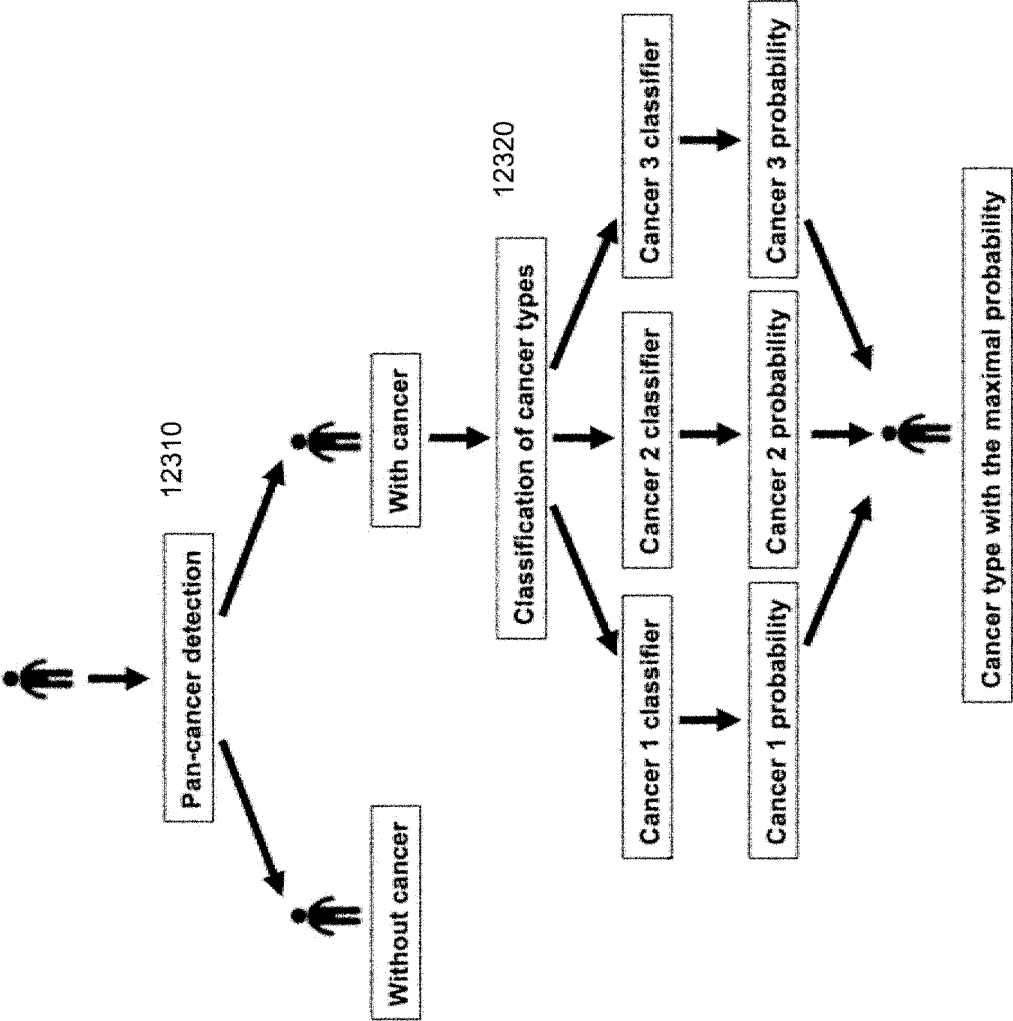


FIG. 123

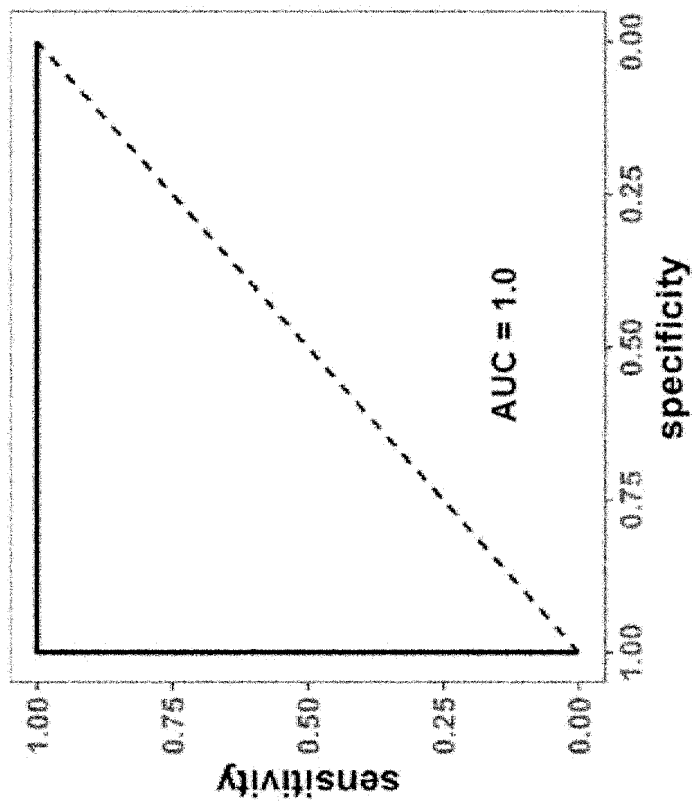


FIG. 124B

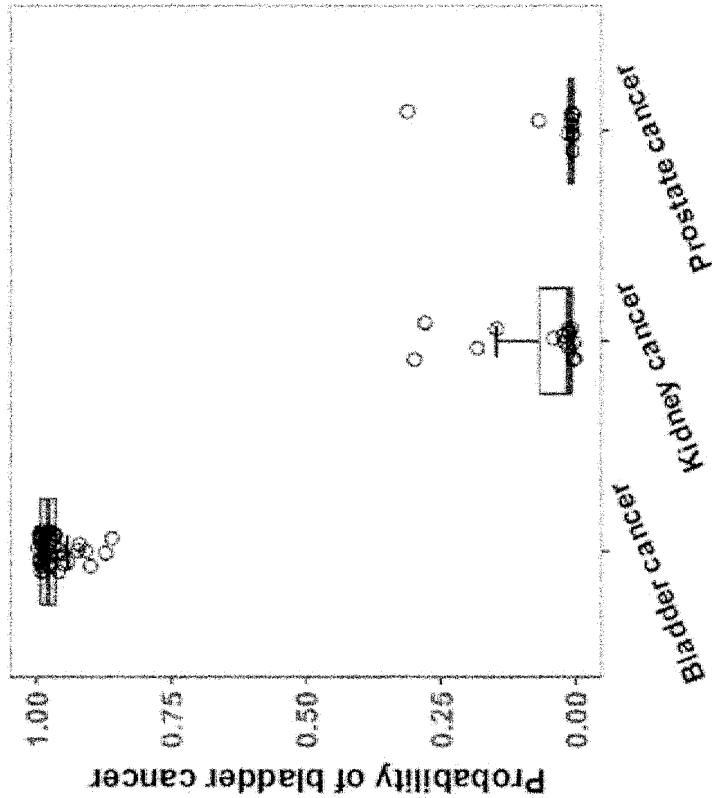


FIG. 124A

FIG. 125A

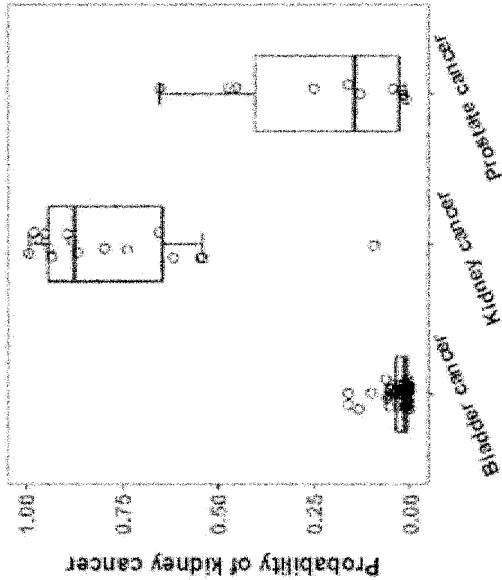


FIG. 125B

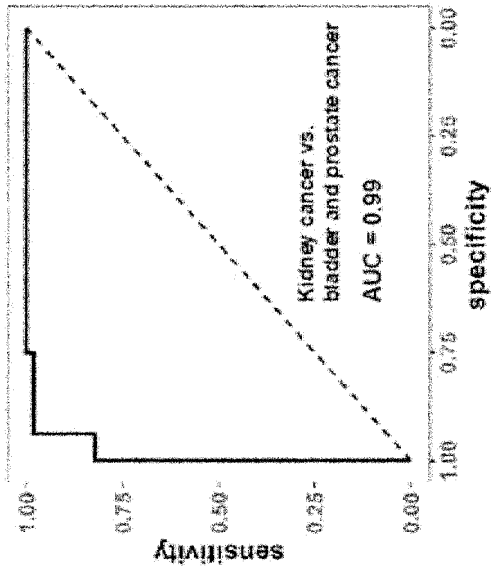


FIG. 125C

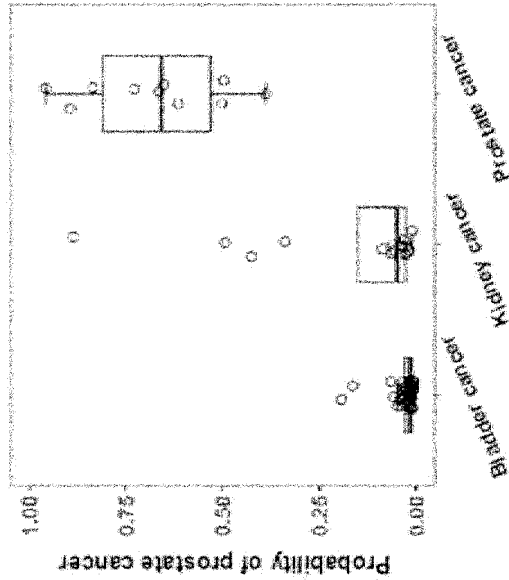
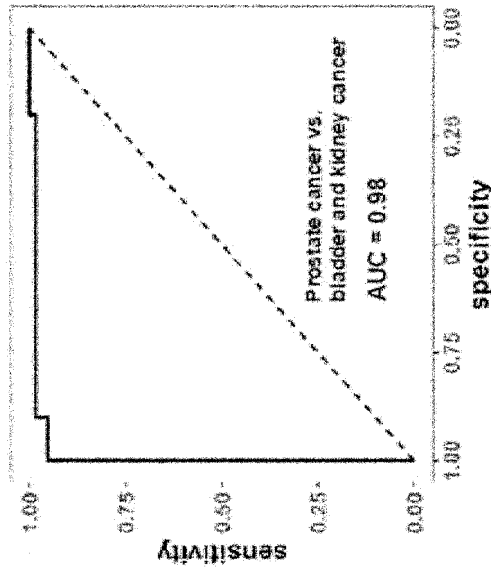


FIG. 125D



	ACG	TCG	CCG	GCG	CGA	CGT	CGC	CGG
Bladder cancer-informative Hypermethylation	13.21	0.00	18.87	4.72	12.26	21.70	13.21	18.04
Bladder cancer-informative Hypomethylation	9.77	2.34	10.58	3.11	25.57	27.74	6.15	14.75
Kidney cancer-informative Hypermethylation	8.18	5.65	18.35	3.29	27.06	14.59	10.12	11.76
Kidney cancer-informative Hypomethylation	11.76	1.69	13.03	2.94	22.90	26.28	6.09	15.34
Prostate cancer-informative Hypermethylation	8.40	2.78	17.78	4.69	19.96	19.74	10.41	19.25
Prostate cancer-informative Hypomethylation	8.25	2.10	10.90	2.99	29.45	24.19	6.61	15.52
Tissue-shared Hypermethylation	8.31	1.21	10.07	4.56	20.10	27.24	11.14	17.38
Tissue-shared Hypomethylation	9.78	2.55	18.42	4.23	18.63	21.89	6.95	19.55

12620

FIG. 126A

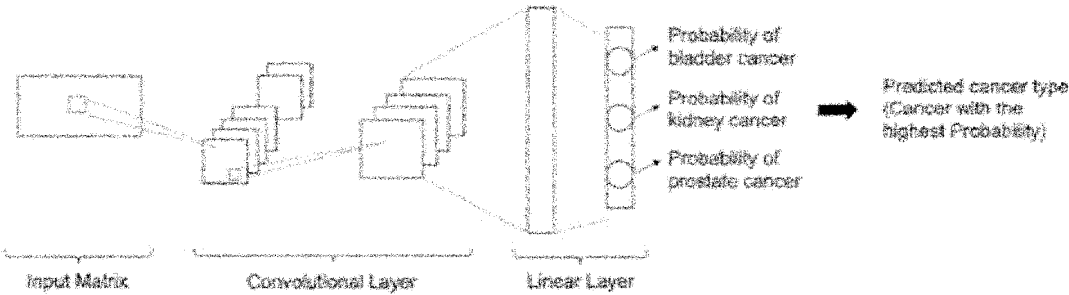


FIG. 126B

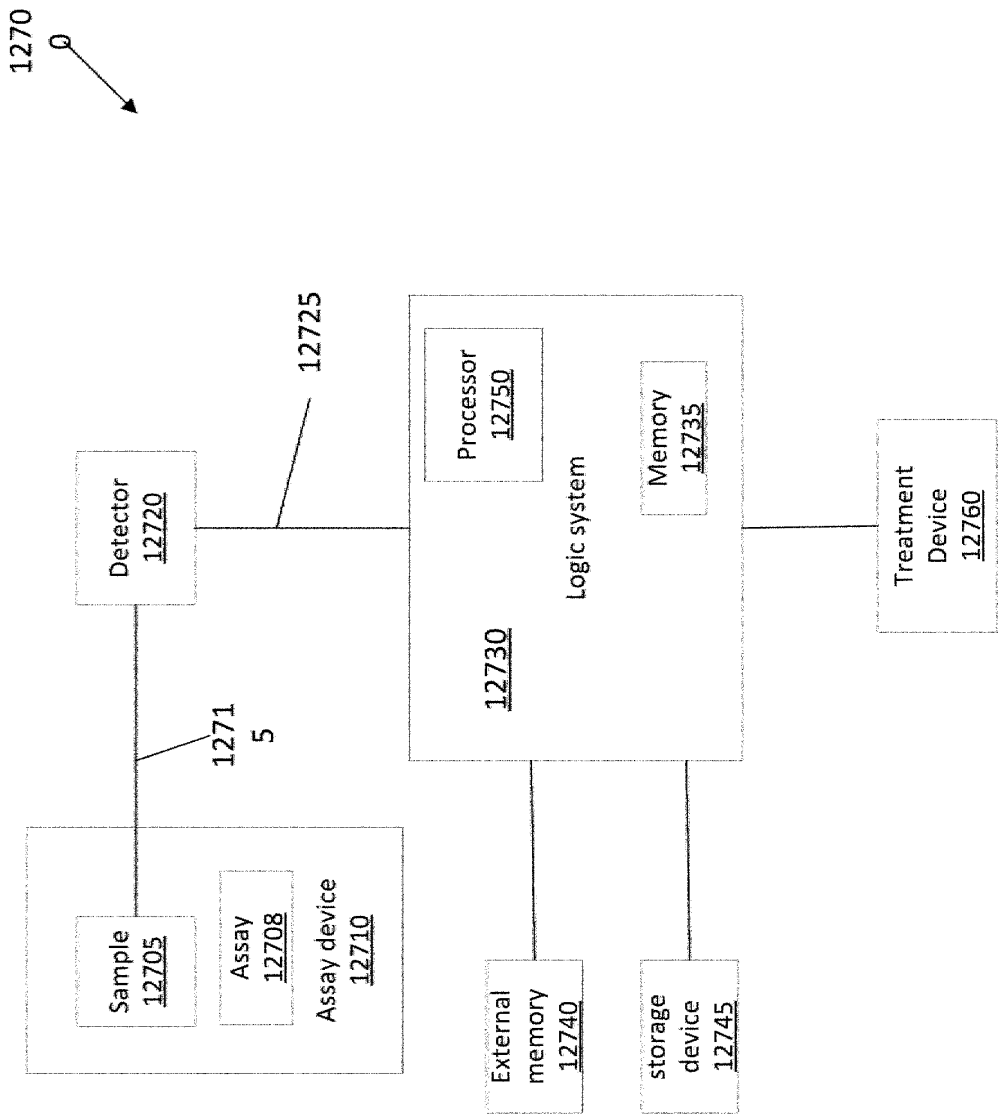


FIG. 127

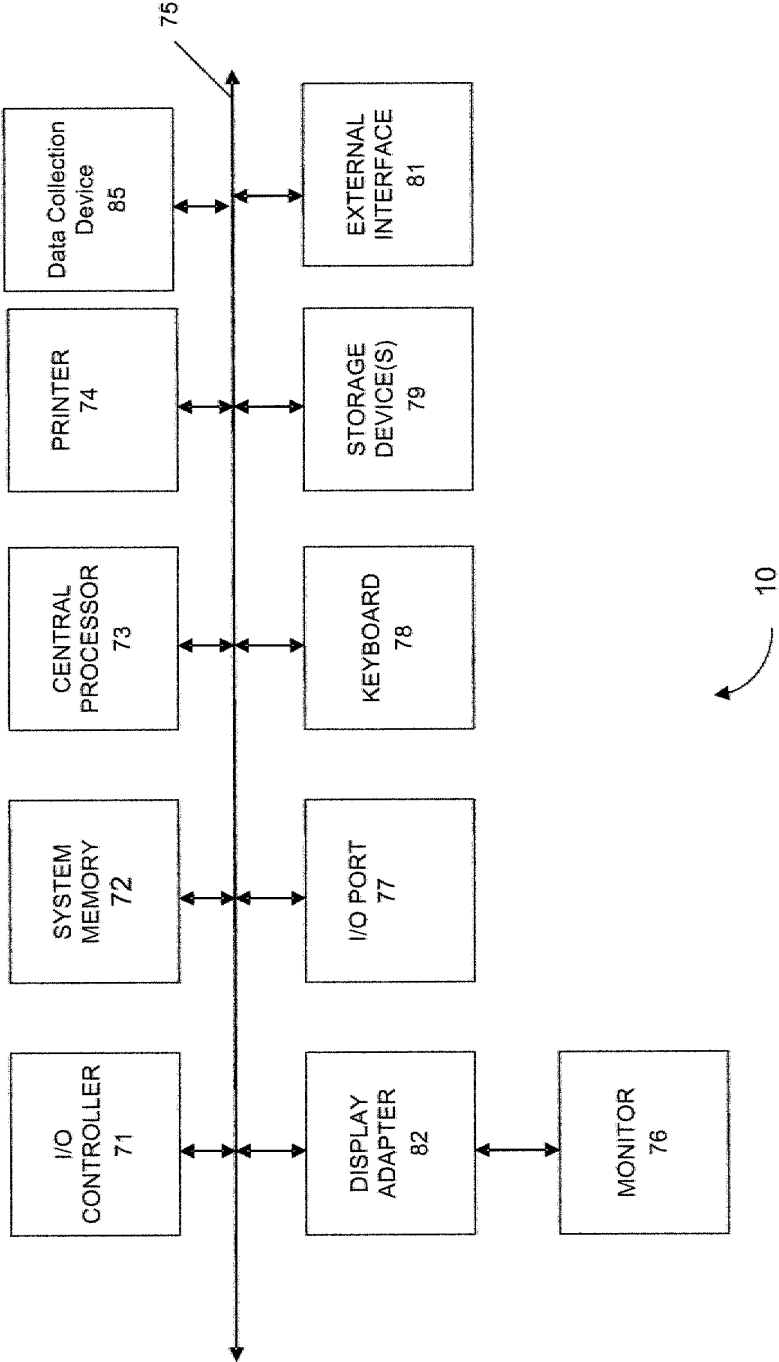


FIG. 128