FIG. 1

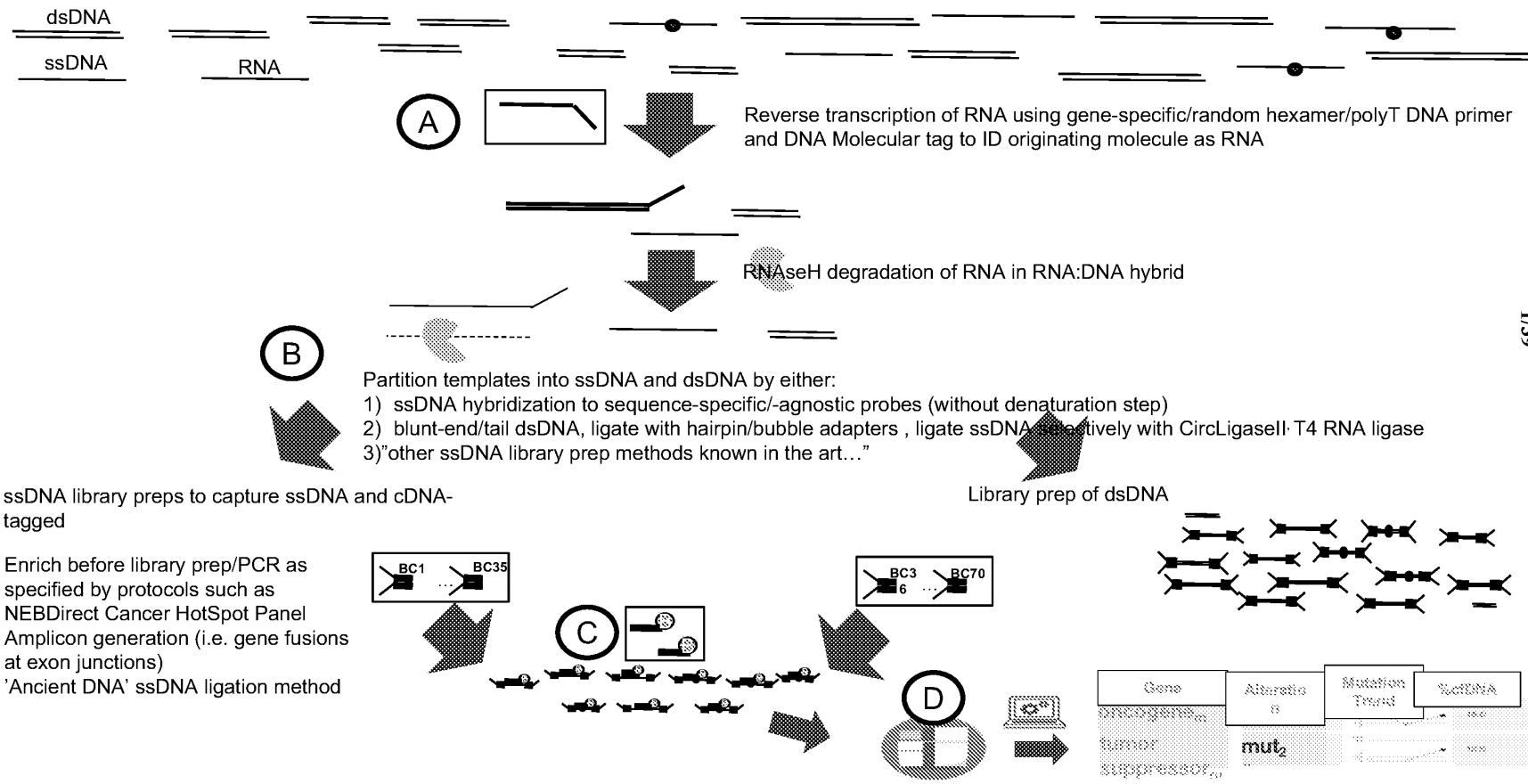
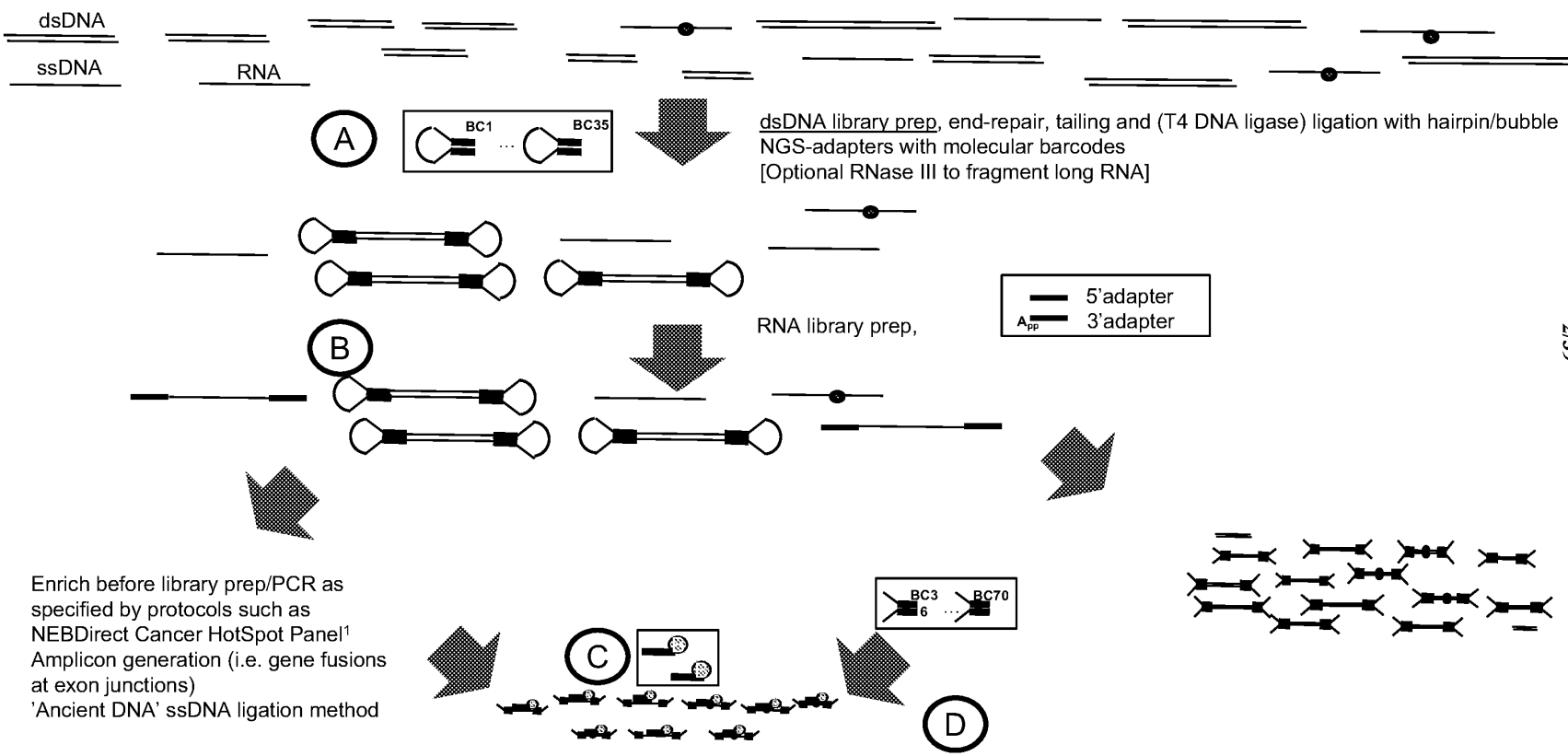

dsDNA
ssDNA
RNA
A
Reverse transcription of RNA using gene-specific/random hexamer/polyT DNA primer and DNA Molecular tag to ID originating molecule as RNA
RNAseH degradation of RNA in RNA:DNA hybrid
B
Partition templates into ssDNA and dsDNA by either:
1) ssDNA hybridization to sequence-specific/-agnostic probes (without denaturation step)
2) blunt-end/tail dsDNA, ligate with hairpin/bubble adapters , ligate ssDNA selectively with CircLigaseII· T4 RNA ligase
3)"other ssDNA library prep methods known in the art…"
ssDNA library preps to capture ssDNA and cDNA-tagged
Library prep of dsDNA
Enrich before library prep/PCR as specified by protocols such as NEBDirect Cancer HotSpot Panel Amplicon generation (i.e. gene fusions at exon junctions)
'Ancient DNA' ssDNA ligation method
BC1
BC35
BC3
6
BC70
C
D
Gene
Alteratio
n
Mutation Trend
%cfDNA
mut2

FIG. 2



Prevention of circularization by T4 RNA ligase 2 truncated K227Q, requires pre-adenylated linker 5' end

FIG. 3

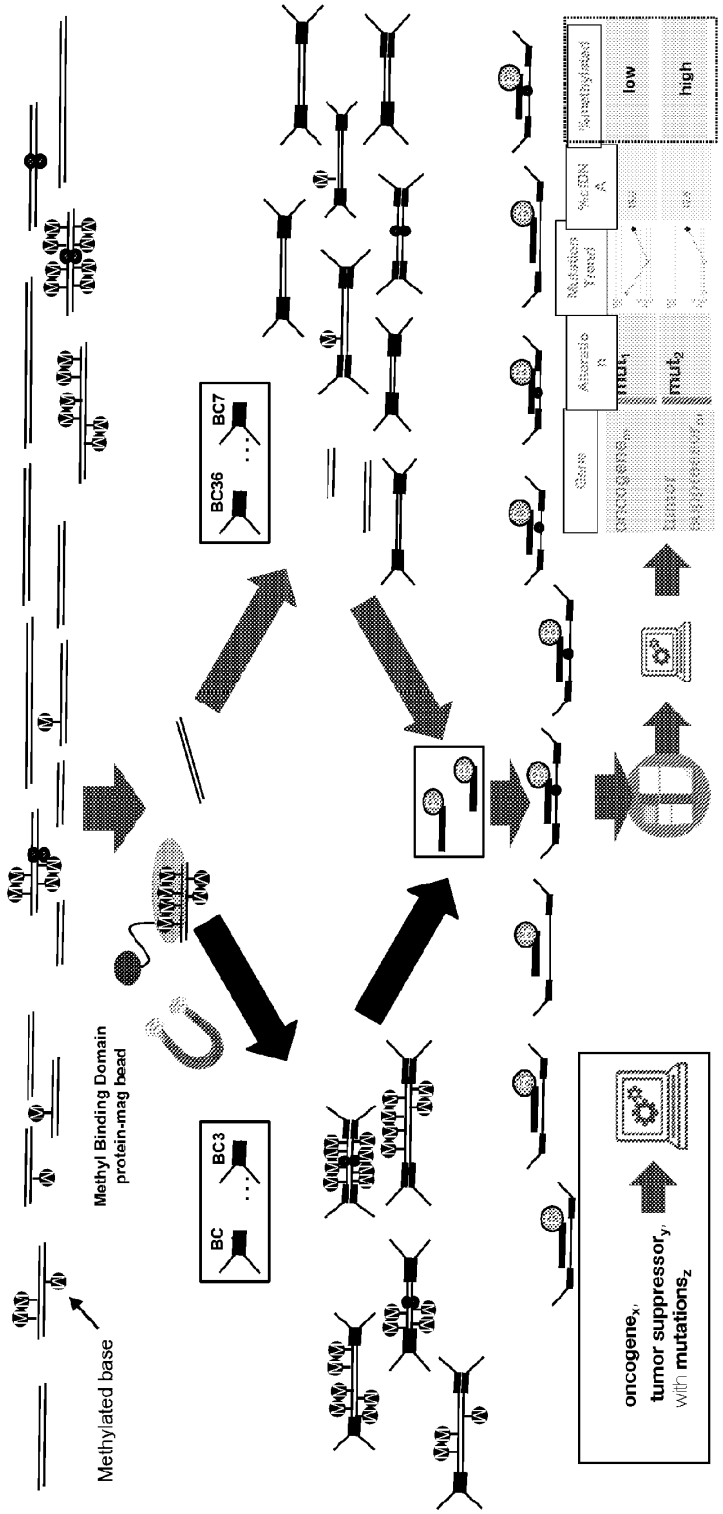

Methylated base
Methyl Binding Domain protein-mag bead
BC
BC3
BC36
BC7
oncogene$_x$,
tumor suppressor$_y$,
with mutations$_z$
low
high

FIG. 4

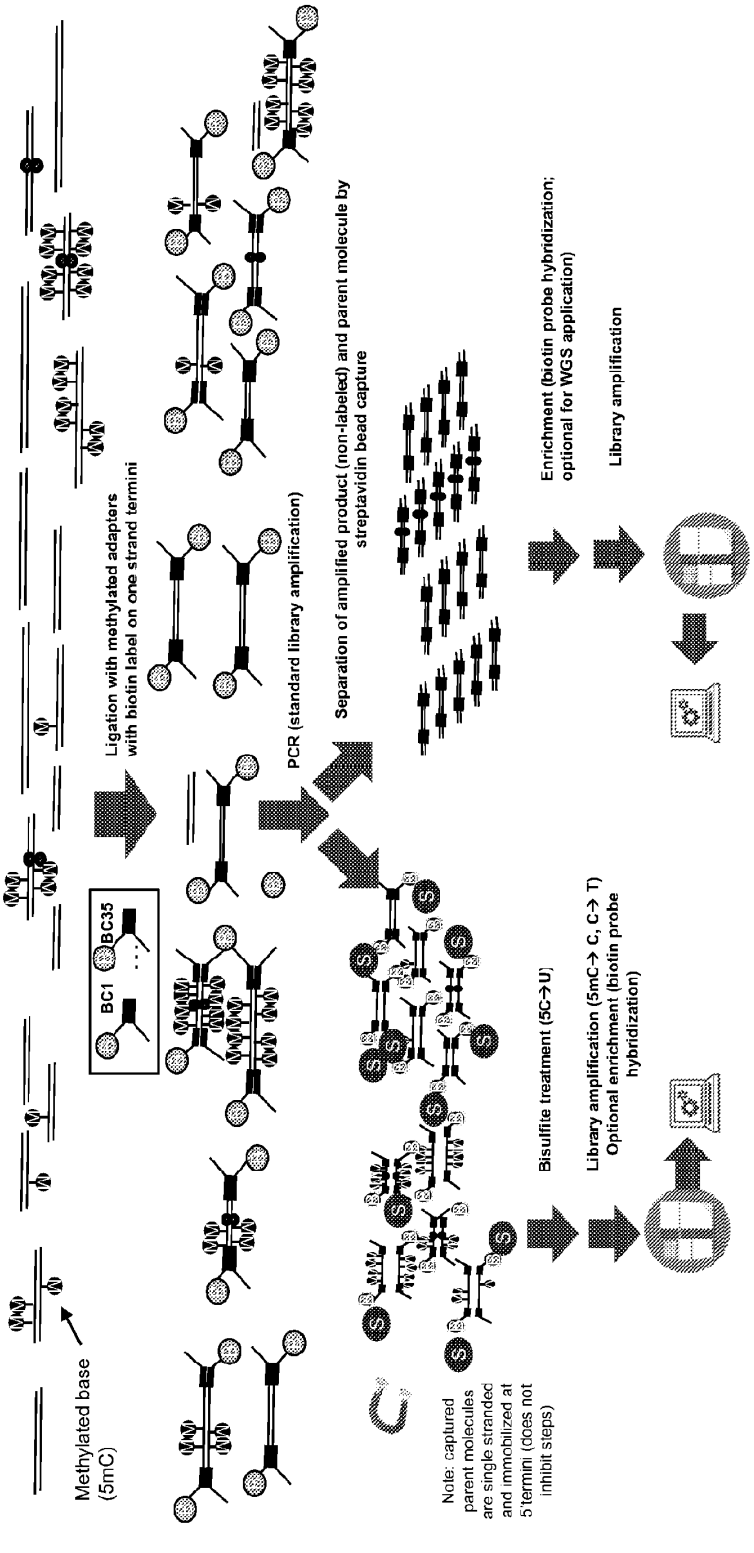

Methylated base (5mC)
BC1
BC35
Ligation with methylated adapters with biotin label on one strand termini
PCR (standard library amplification)
Separation of amplified product (non-labeled) and parent molecule by streptavidin bead capture
Enrichment (biotin probe hybridization; optional for WGS application)
Library amplification
Bisulfite treatment (5C→U)
Library amplification (5mC→ C, C→ T) Optional enrichment (biotin probe hybridization)
Note: captured parent molecules are single stranded and immobilized at 5'termini (does not inhibit steps)

FIG. 5

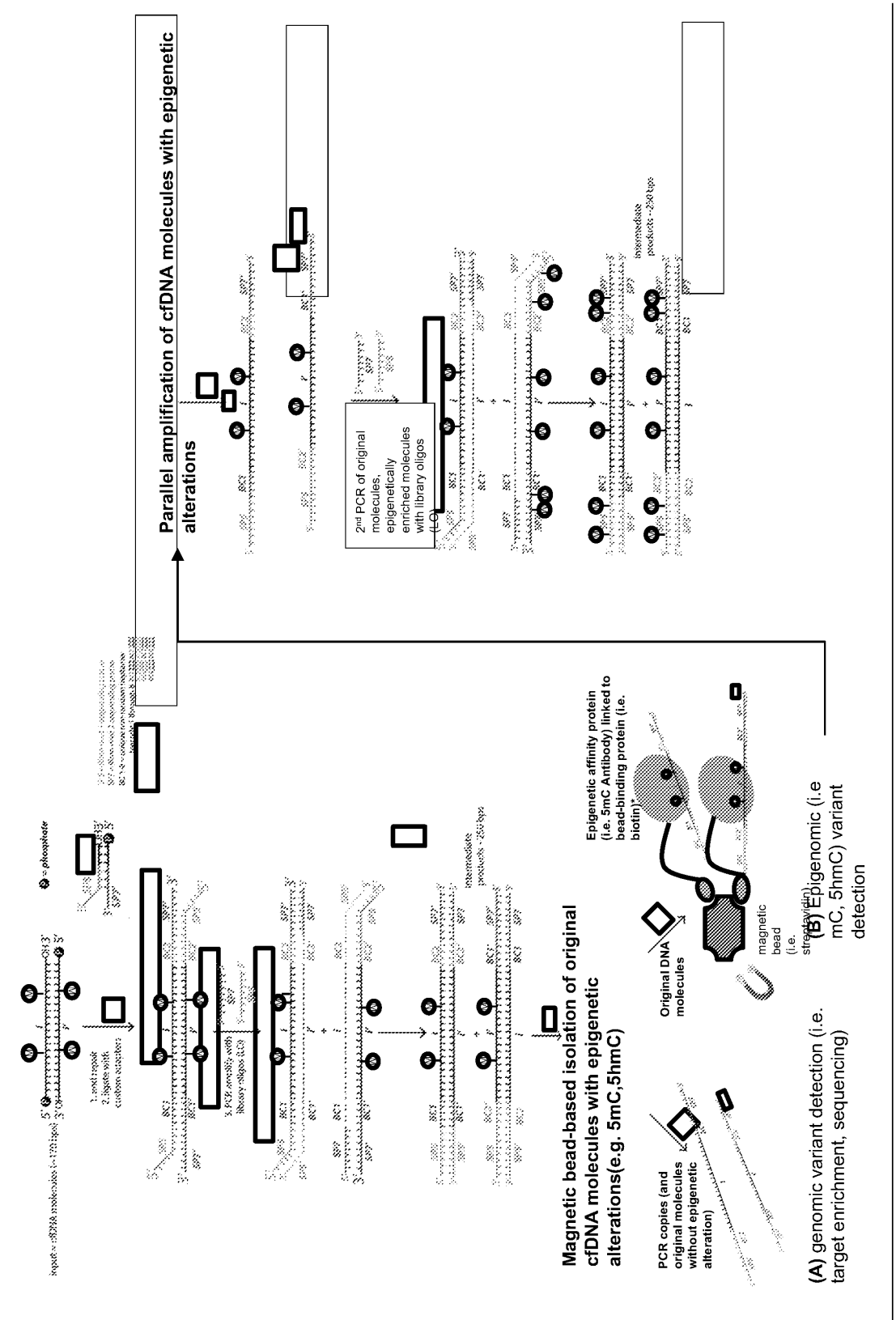
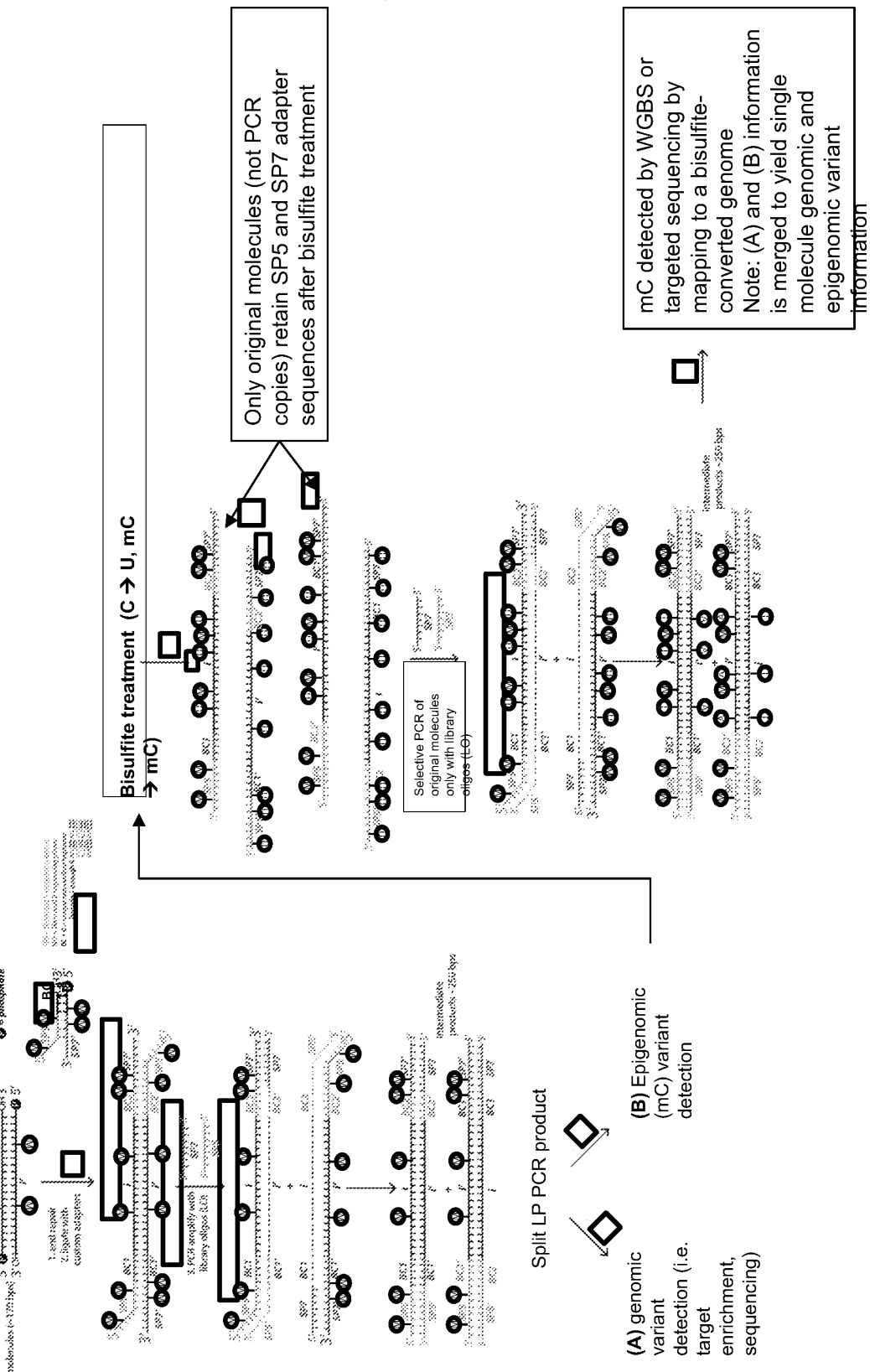

Parallel amplification of cfDNA molecules with epigenetic alterations
2nd PCR of original molecules, epigenetically enriched molecules with library oligos (+O)
Magnetic bead-based isolation of original cfDNA molecules with epigenetic alterations(e.g. 5mC,5hmC)
Epigenetic affinity protein (i.e. 5mC Antibody) linked to bead-binding protein (i.e. biotin)*
Original DNA molecules
magnetic bead (i.e. streptavidin)
PCR copies (and original molecules without epigenetic alteration)
(A) genomic variant detection (i.e. target enrichment, sequencing)
(B) Epigenomic (i.e mC, 5hmC) variant detection

FIG. 6


Bisulfite treatment (C → U, mC → mC)
Only original molecules (not PCR copies) retain SP5 and SP7 adapter sequences after bisulfite treatment
Selective PCR of original molecules only with library oligos (LO)
mC detected by WGBS or targeted sequencing by mapping to a bisulfite-converted genome
Note: (A) and (B) information is merged to yield single molecule genomic and epigenomic variant information
Split LP PCR product
(A) genomic variant detection (i.e. target enrichment, sequencing)
(B) Epigenomic (mC) variant detection

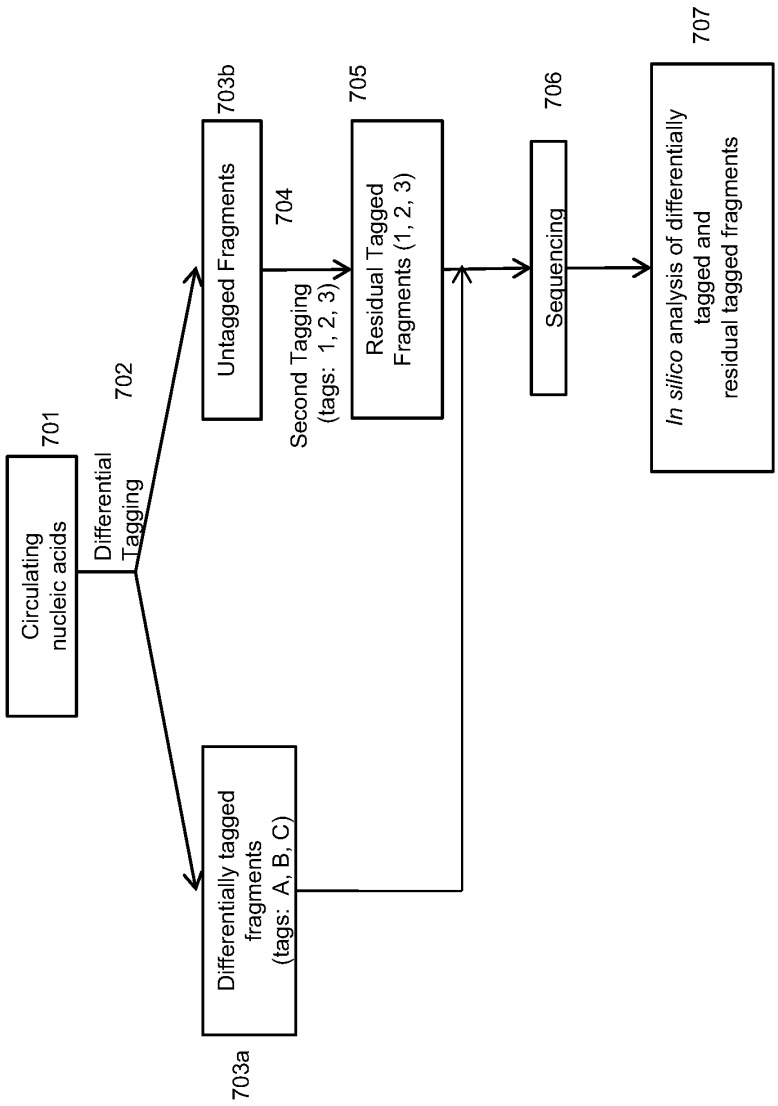

Circulating nucleic acids
701
Differential Tagging
702
Differentially tagged fragments (tags: A, B, C)
703a
Untagged Fragments
703b
704
Second Tagging (tags: 1, 2, 3)
Residual Tagged Fragments (1, 2, 3)
705
Sequencing
706
In silico analysis of differentially tagged and residual tagged fragments
707

FIG. 7

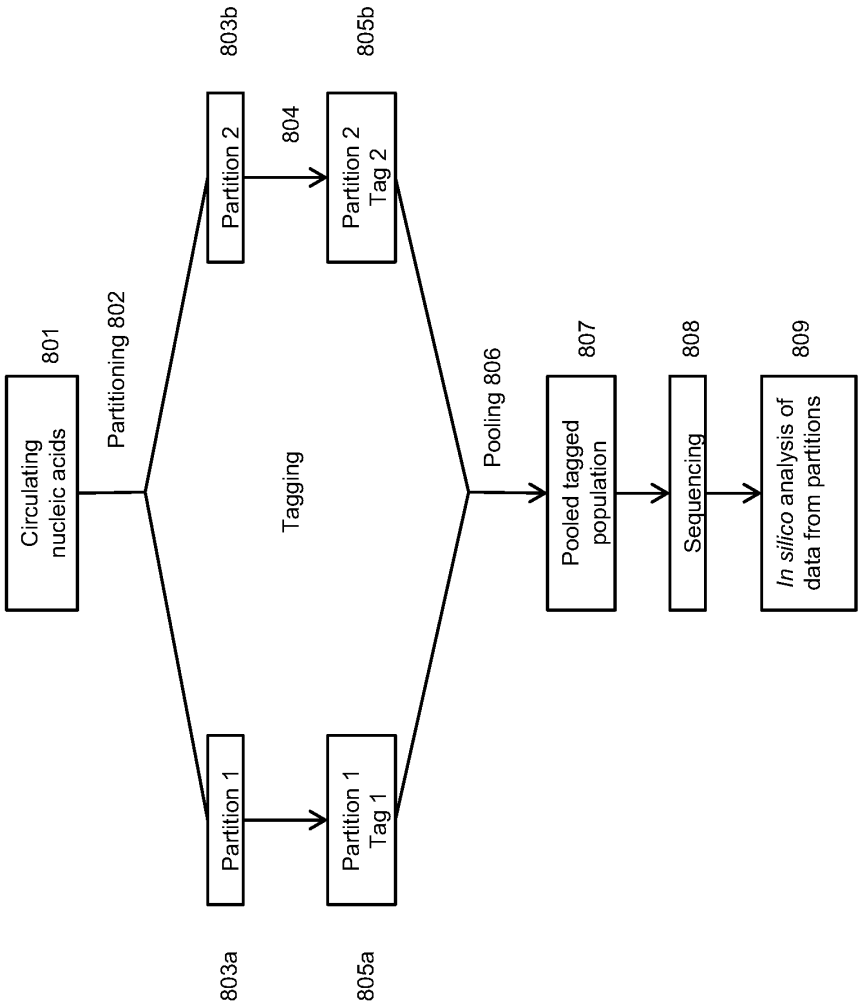

Circulating nucleic acids
801
Partitioning 802
Partition 1
803a
Partition 2
803b
804
Tagging
Partition 1 Tag 1
805a
Partition 2 Tag 2
805b
Pooling 806
Pooled tagged population
807
Sequencing
808
In silico analysis of data from partitions
809

FIG. 8

FIG. 9

1. Use 8 Lung Cancer no MBD samples to identify dyad centers
2. Isolate canonical nucleosomes with well positioned dyad and high occupancy (cov>.5Qu & peak width <.5Qu).
3. Locate dyad centers in combined MBD samples
4. Compare no MBD vs MBD aligned samples to calculate dyad center distances.

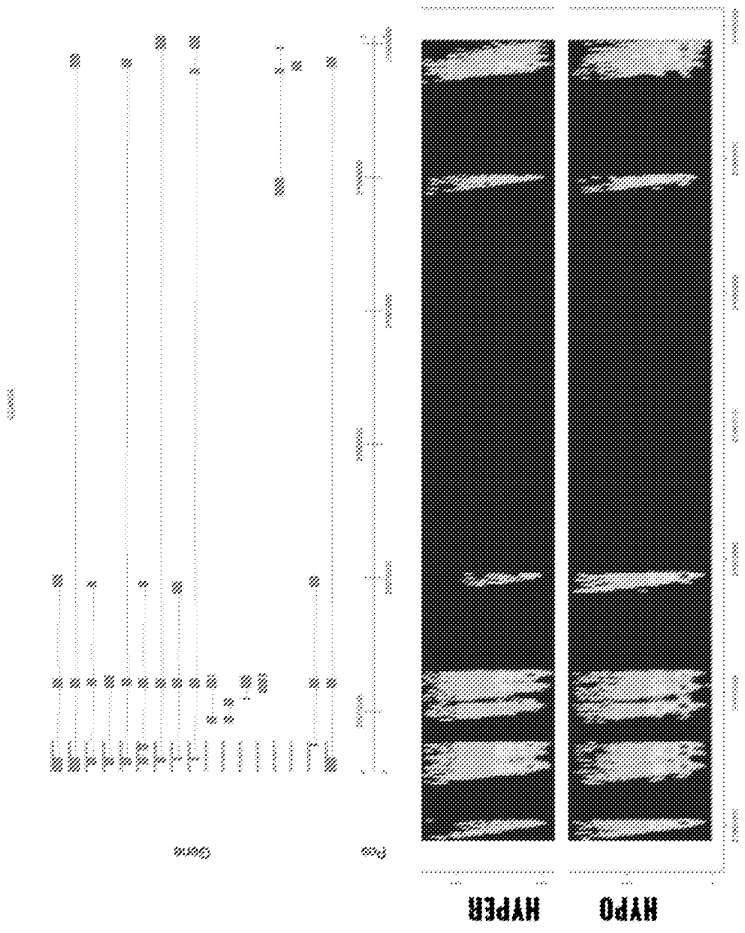


FIG. 10

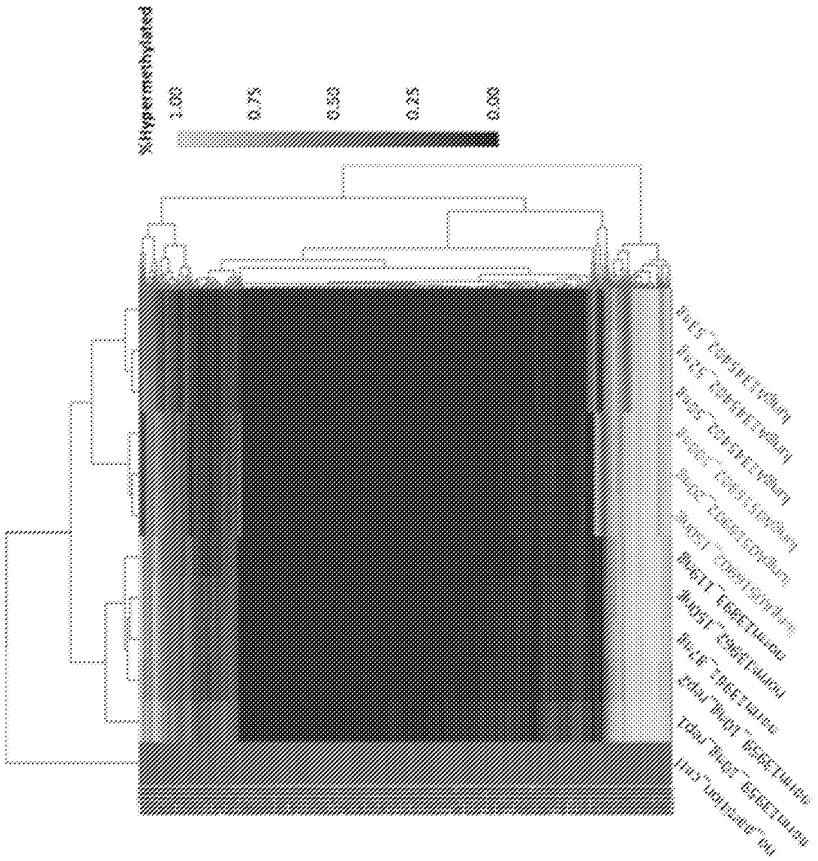


FIG. 11

FIG. 12A-FIG. 12C

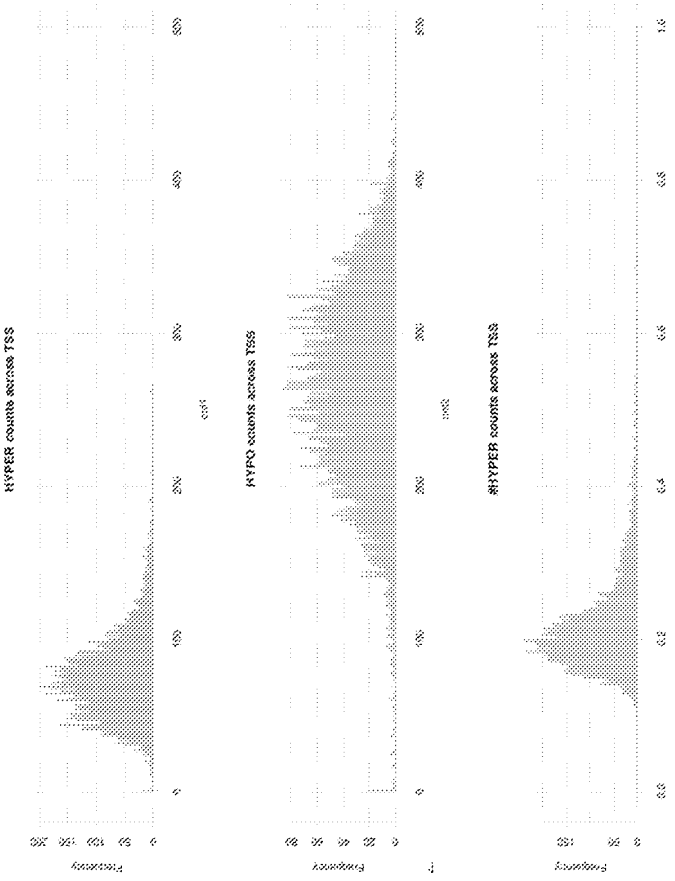


Fig. 12A

Fig. 12B

Fig. 12C

FIG. 13A-FIG.13B

Fig. 13A : MOB3A

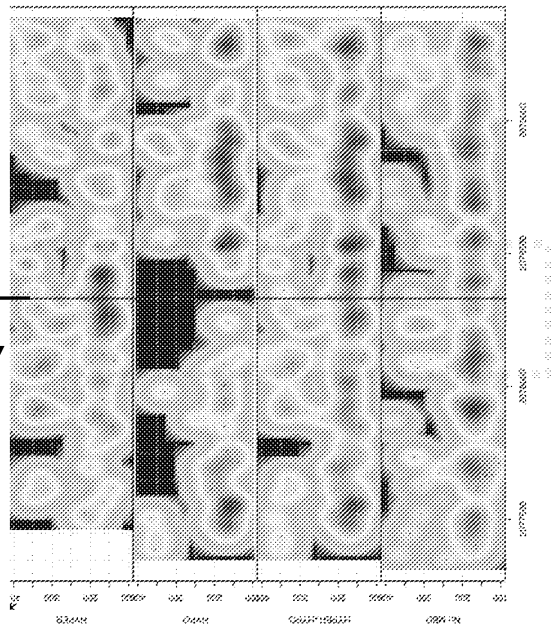


Fig. 13B : WDR88

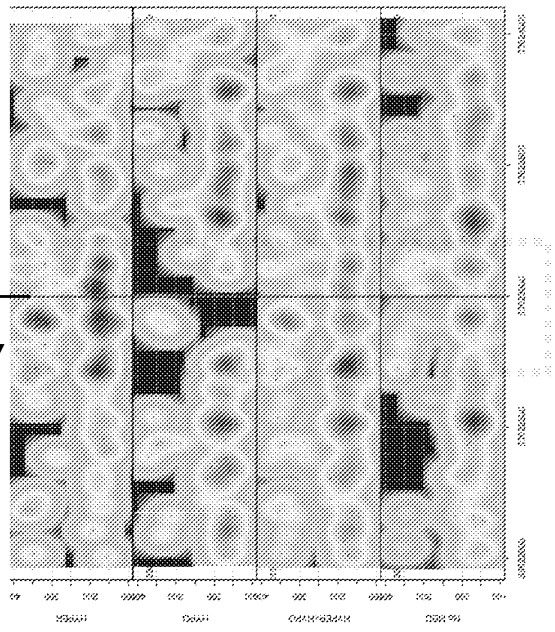


FIG. 14A-FIG.14B

Fig. 14A

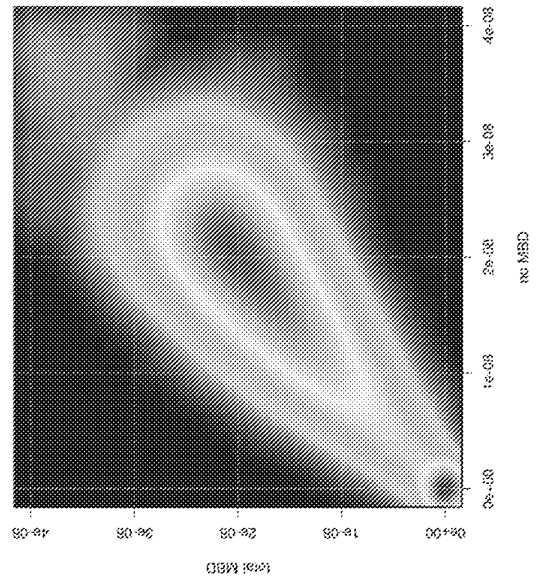
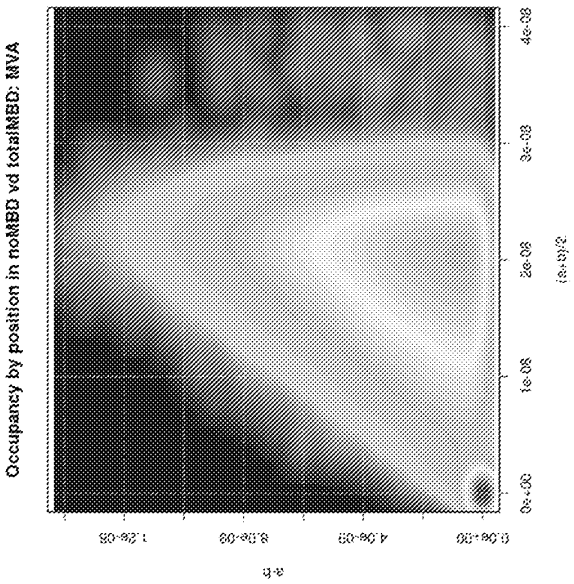


Fig. 14B



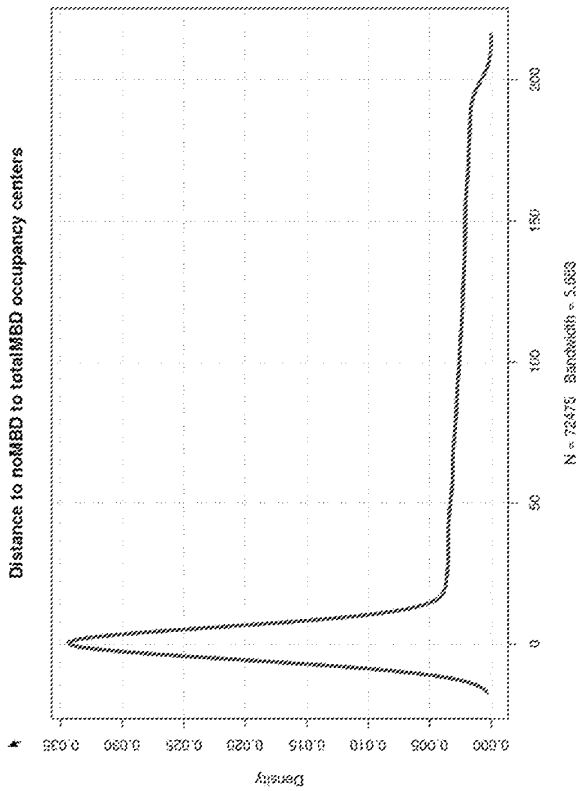
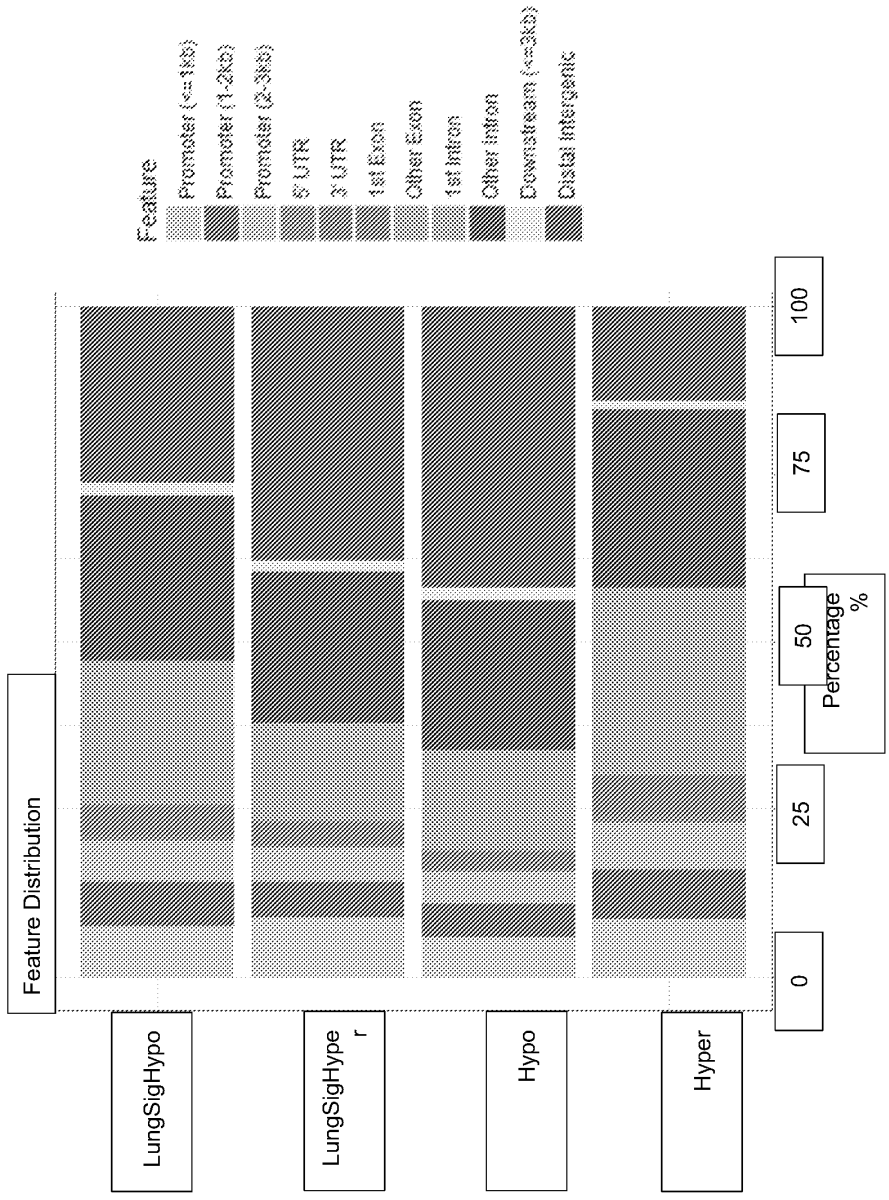

Distance to noMBD to totalMBD occupancy centers
Density
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.035
0
50
100
150
200
N = 72475 Bandwidth = 5.888

FIG. 15

FIG. 16


Feature
Promoter (<=1Kb)
Promoter (1-2Kb)
Promoter (2-3Kb)
5' UTR
3' UTR
1st Exon
Other Exon
1st Intron
Other Intron
Downstream (<=3Kb)
Distal Intergenic
Feature Distribution
LungSigHypo
LungSigHyper
Hypo
Hyper
0
25
50
75
100
Percentage
%

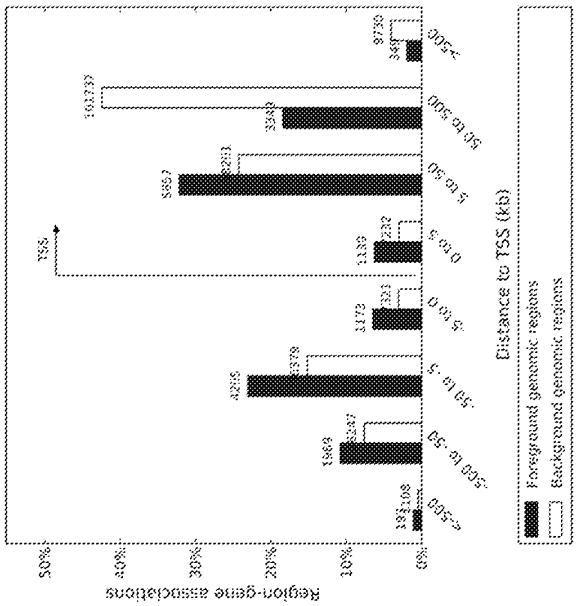
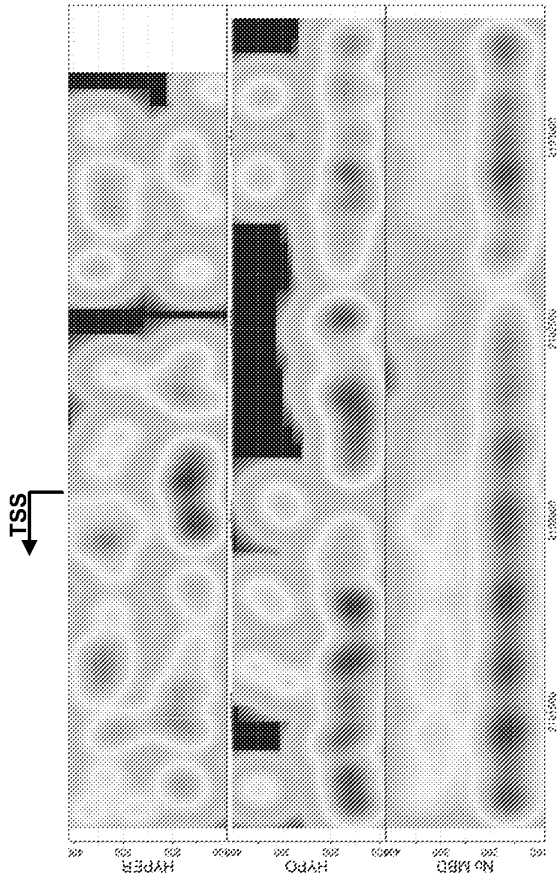

Region-gene associations
50%
40%
30%
20%
10%
0%
TSS
<-500
-500 to -50
-50 to -5
-5 to 0
0 to 5
5 to 50
50 to 500
>500
Distance to TSS (kb)
Foreground genomic regions
Background genomic regions

FIG. 17

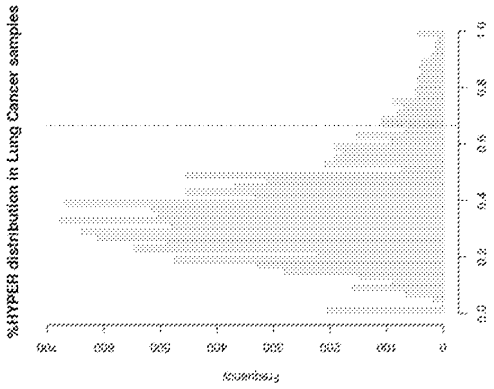
FIG. 18A-FIG. 18B

Fig. 18A



AP3D1

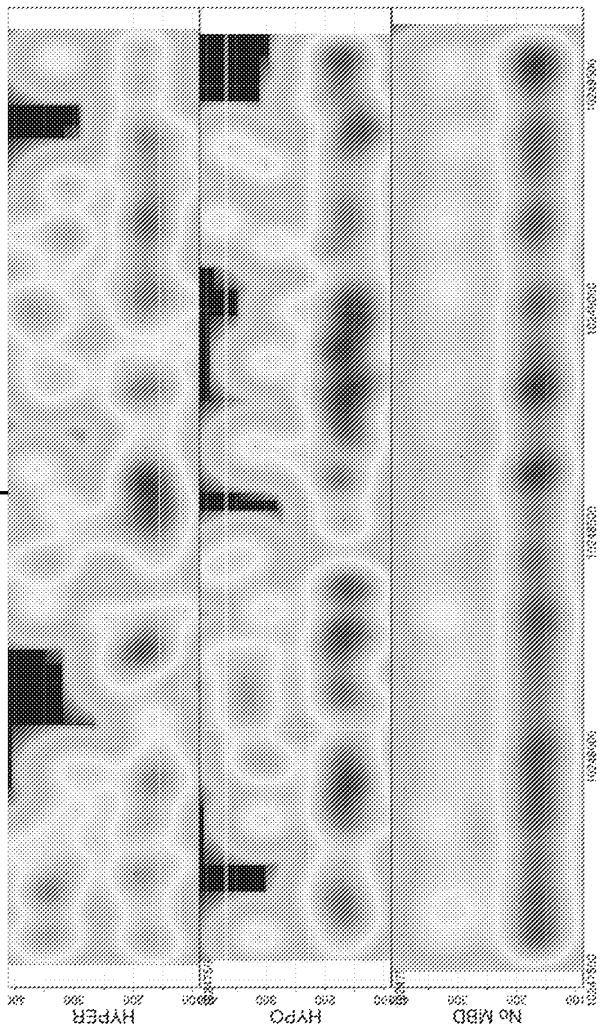
Fig. 18B



AP3D1

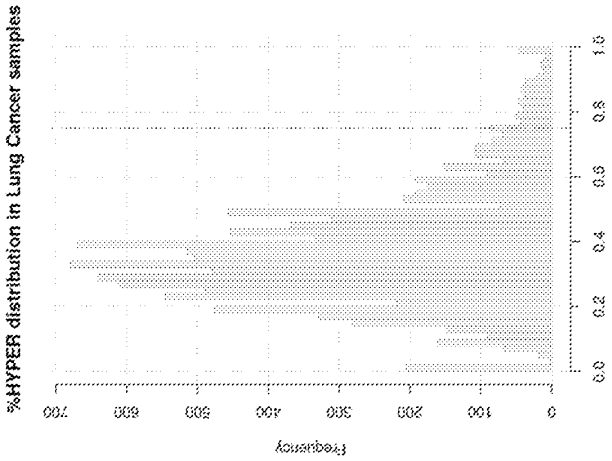
FIG. 19A-FIG.19B

Fig. 19A



DNMT1

Fig. 19B



DNMT1

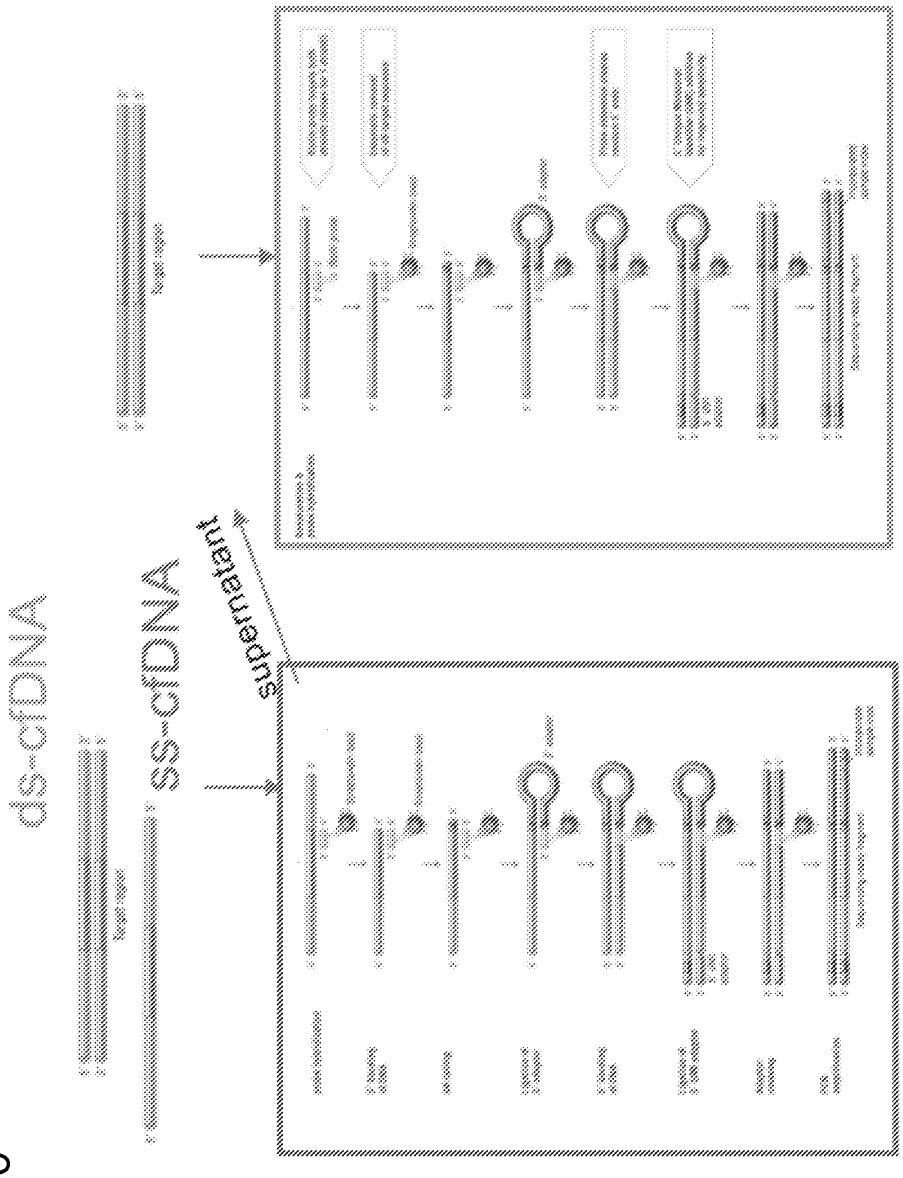


FIG. 20

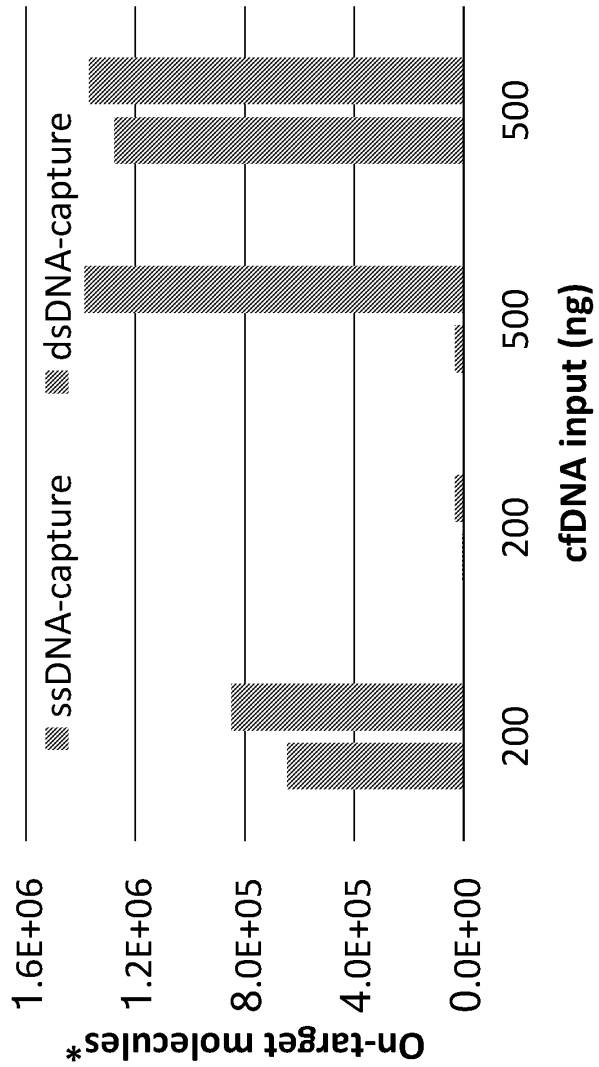

ssDNA-capture
dsDNA-capture
On-target molecules*
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
200
500
200
500
cfDNA input (ng)

FIG. 21

FIG. 22

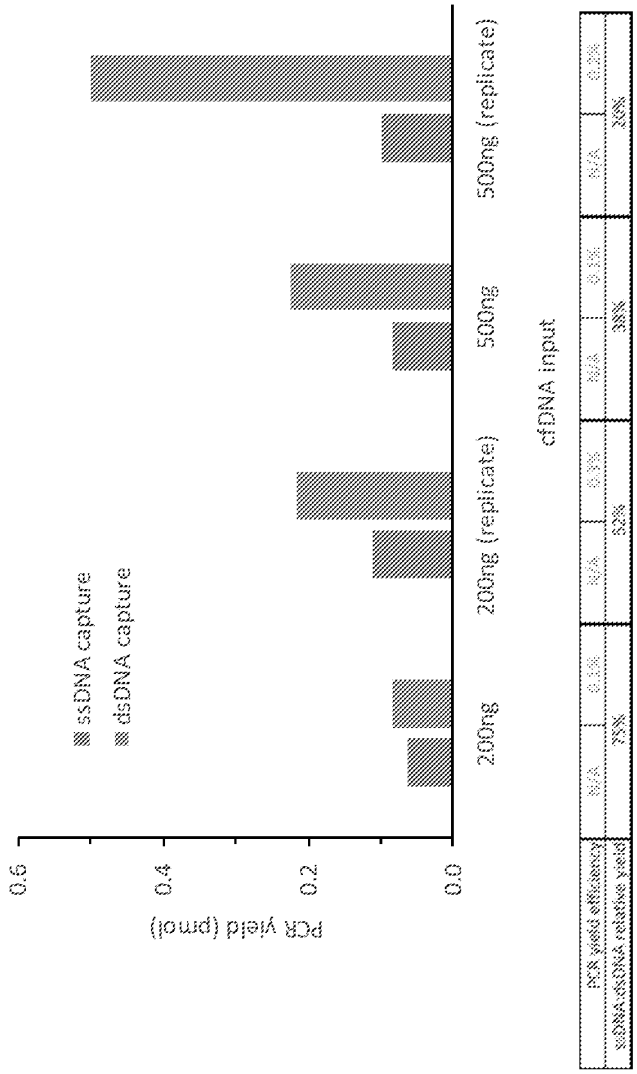
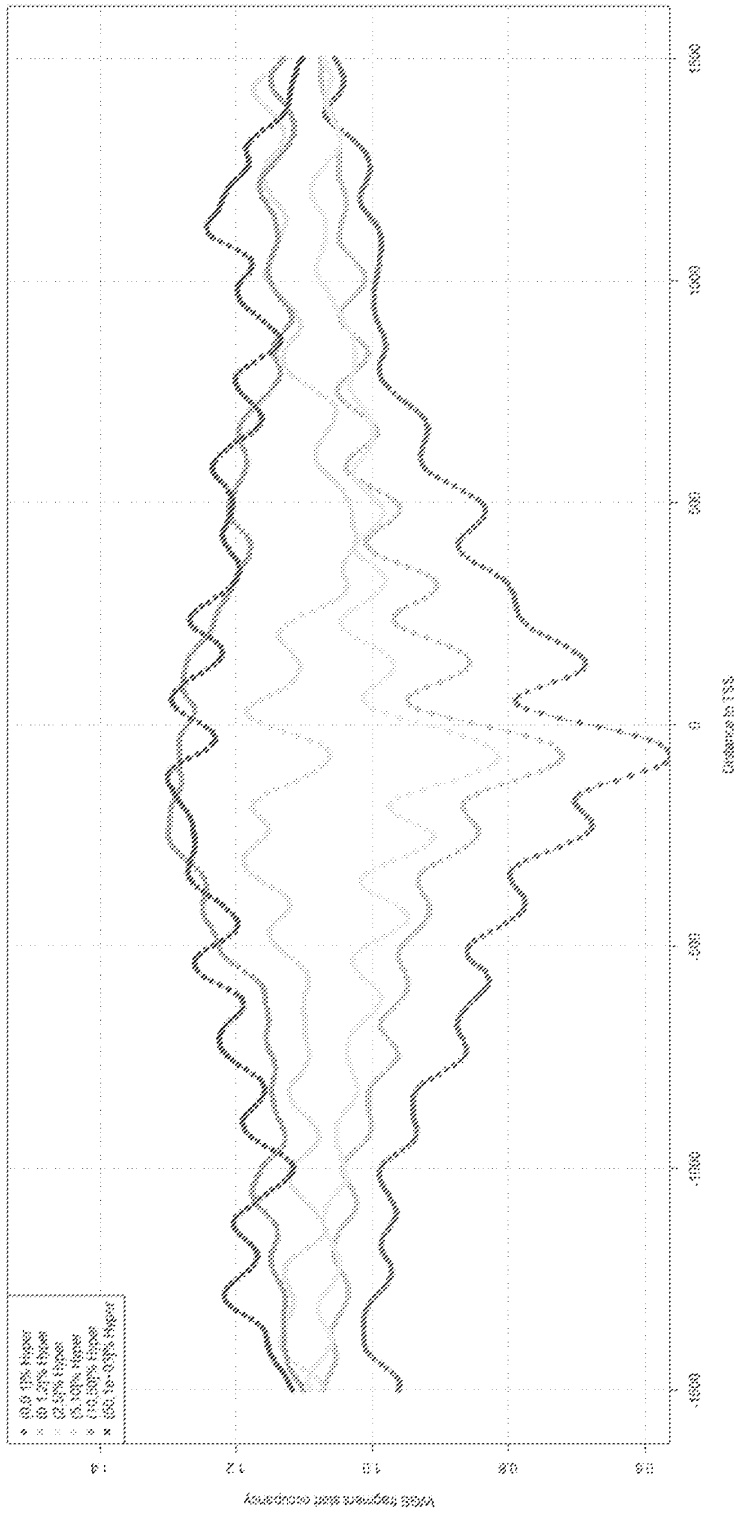

PCR Yield (pmol)
0.0
0.2
0.4
0.6
ssDNA capture
dsDNA capture
200ng
200ng (replicate)
500ng
500ng (replicate)
cfDNA input
PCR yield efficiency
ssDNA:dsDNA relative yield
N/A
75%
0.3%
N/A
52%
0.3%
N/A
38%
0.3%
N/A
20%
0.3%

FIG. 23



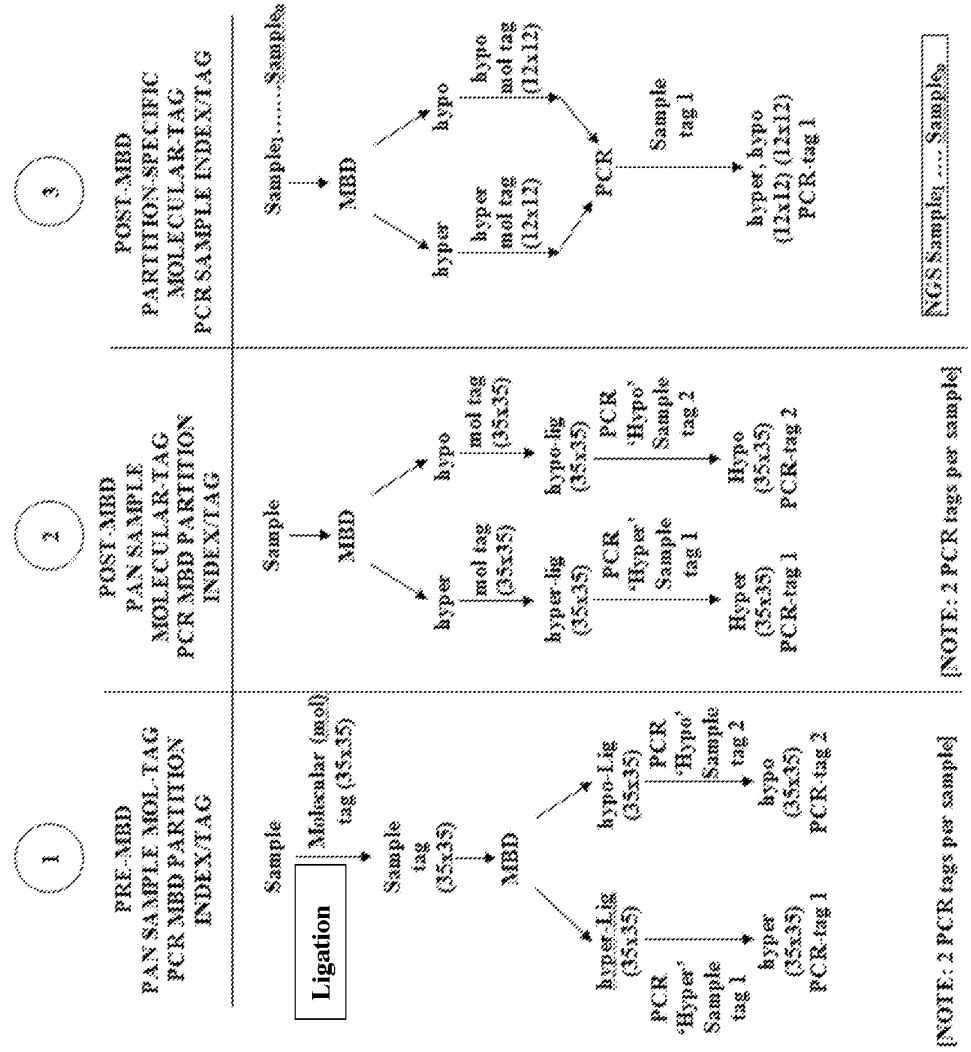

1
PRE-MBD
PAN SAMPLE MOL-TAG
PCR MBD PARTITION
INDEX/TAG
Sample
Molecular (mol) tag (35x35)
Ligation
Sample tag (35x35)
MBD
hyper-Lig (35x35)
hypo-Lig (35x35)
PCR 'Hyper' Sample tag 1
PCR 'Hypo' Sample tag 2
hyper (35x35) PCR-tag 1
hypo (35x35) PCR-tag 2
[NOTE: 2 PCR tags per sample]
2
POST-MBD
PAN SAMPLE
MOLECULAR-TAG
PCR MBD PARTITION
INDEX/TAG
Sample
MBD
hyper
hypo
mol tag (35x35)
hyper-lig (35x35)
hypo-lig (35x35)
PCR 'Hyper' Sample tag 1
PCR 'Hypo' Sample tag 2
Hyper (35x35) PCR-tag 1
Hypo (35x35) PCR-tag 2
[NOTE: 2 PCR tags per sample]
3
POST-MBD
PARTITION-SPECIFIC
MOLECULAR-TAG
PCR SAMPLE INDEX/TAG
Sample1 ..... Sample96
MBD
hyper
hypo
hyper mol tag (12x12)
hypo mol tag (12x12)
PCR
Sample tag 1
hyper, hypo (12x12) (12x12) PCR-tag 1
NGS Sample1 .... Sample96

Fig. 24

Fig. 25A-FIG.25B

FIG. 25A

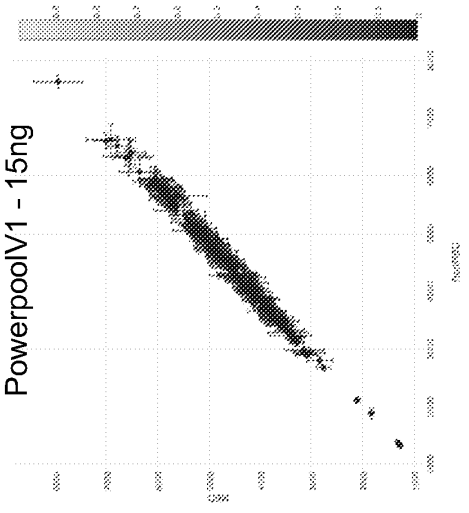
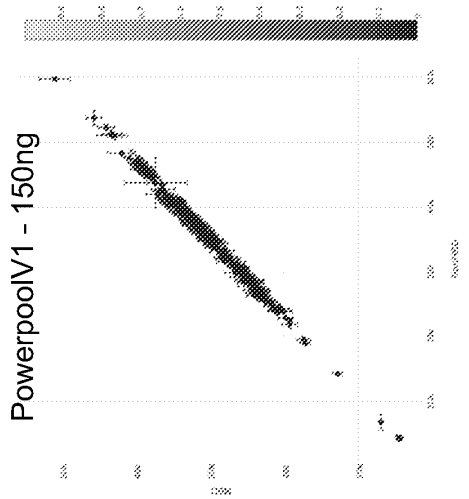


FIG. 25B



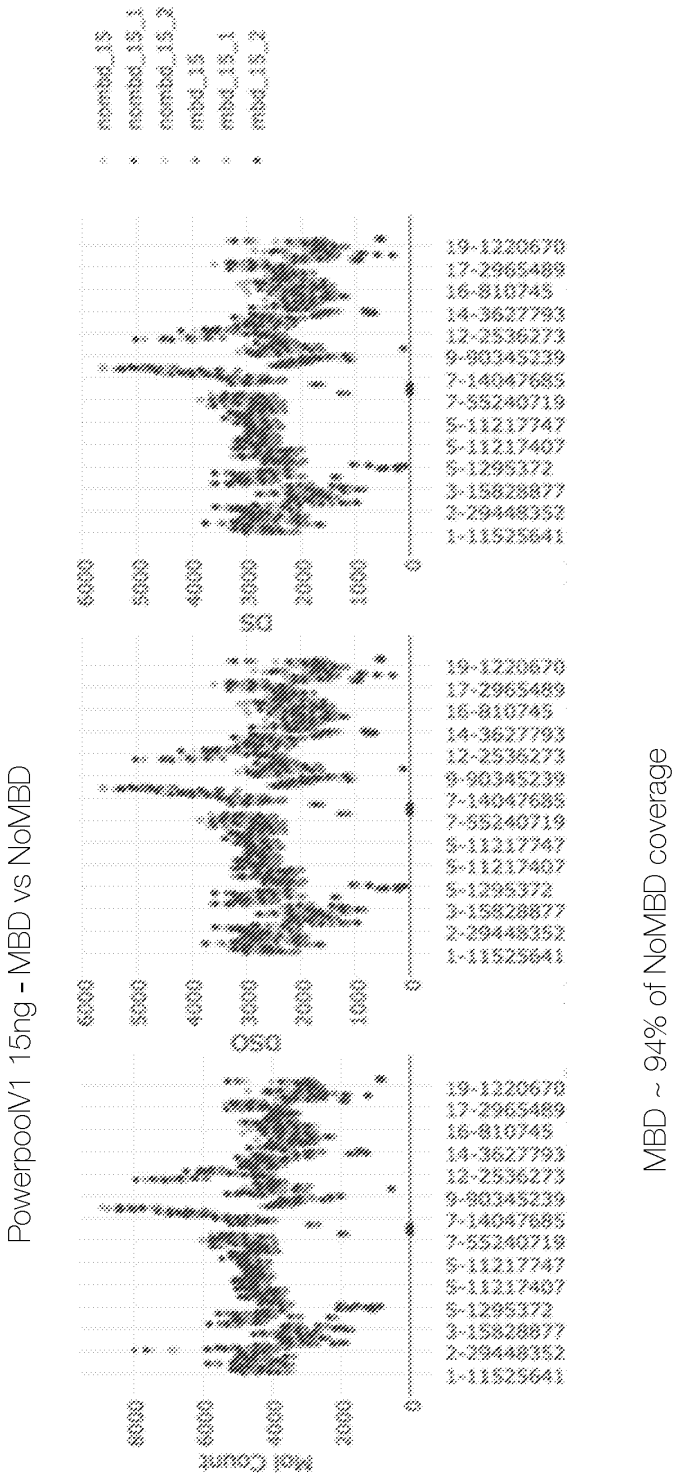

PowerpoolV1 15ng - MBD vs NoMBD
mm9_15
mm9_15_1
mm9_15_2
mm9_15
mm9_15_1
mm9_15_2
Mol Count
8000
6000
4000
2000
0
SO
OSO
19-1220670
17-2965489
16-810745
14-3627793
12-2536273
9-80345239
7-14047685
7-55240719
5-11217747
5-11217407
5-1295372
3-15828877
2-29448352
1-11525641
MBD ~ 94% of NoMBD coverage

Fig. 26A

Fig. 26B

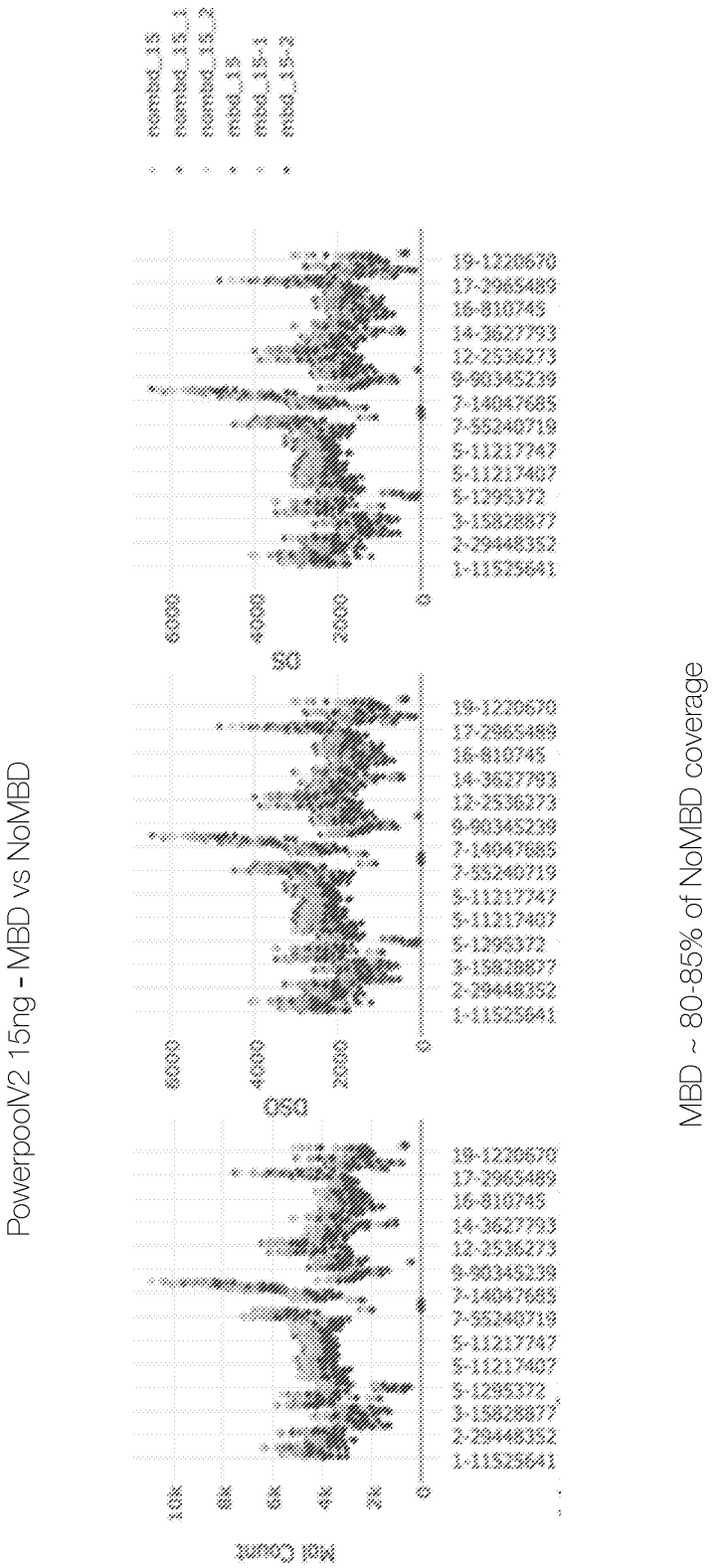

PowerpoolV2 15ng - MBD vs NoMBD
Mdl Count
MBD ~ 80-85% of NoMBD coverage
mmd_15
mmd_15-1
mmd_15-2
mmd_15_1
mmd_15_2
19-1220670
17-2965489
16-810745
14-3627793
12-2536273
9-90345239
7-14047685
7-55240719
5-11217747
5-11217407
5-1295372
3-15828877
2-29448352
1-11525641

Fig. 27A

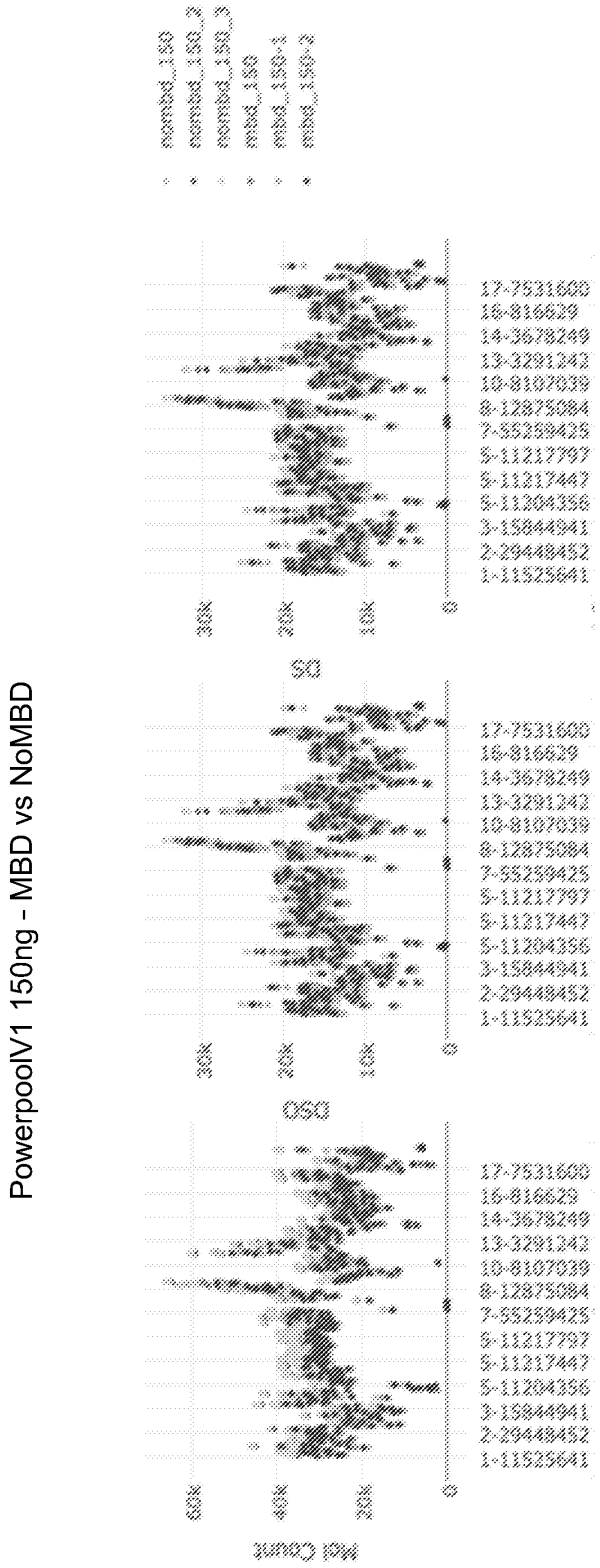

PowerpoolV1 150ng - MBD vs NoMBD
MBD ~ 80-85% of NoMBD coverage
Mol Count
mmd_150
mmd_150_3
mmd_150_2
mm_150
md_150_1
md_150_2
17-7531600
16-816629
14-3678249
13-3291242
10-8107039
8-12875084
7-55259425
5-11217797
5-11217447
5-11204356
3-15844941
2-29448452
1-11525641
50
250

Fig. 27B

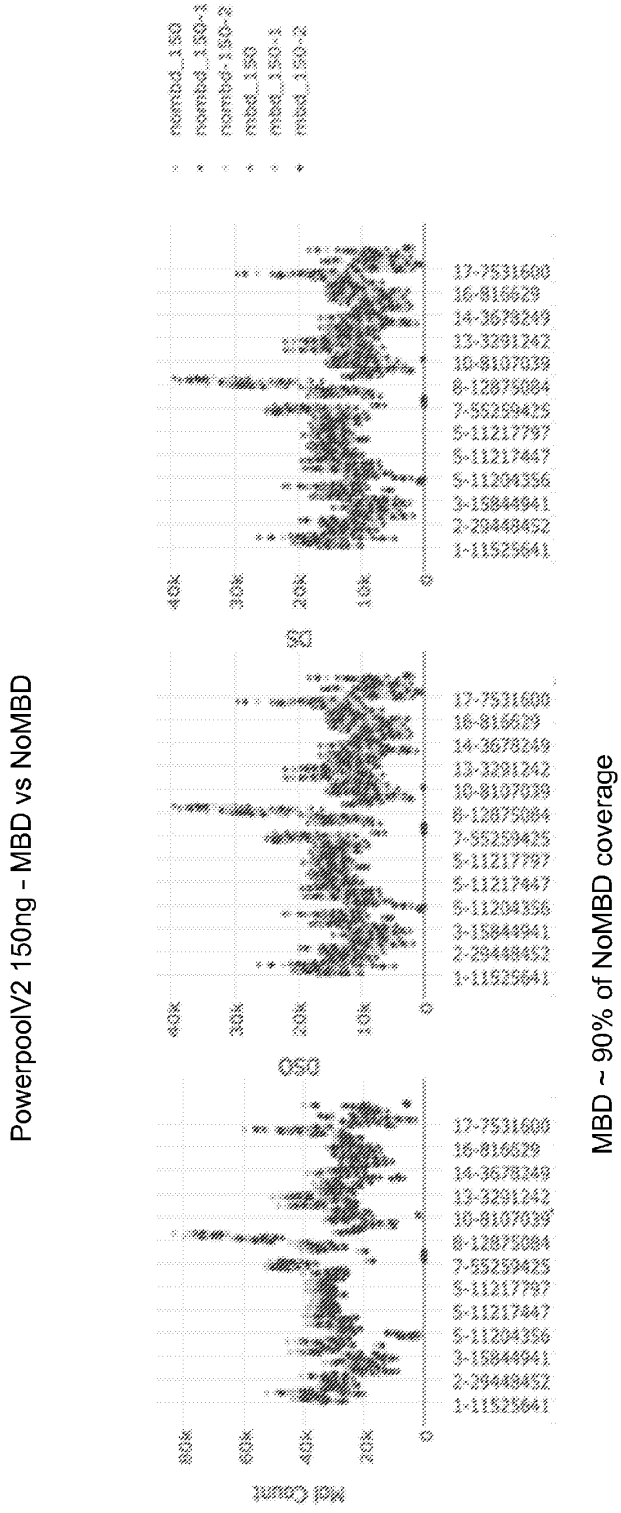

PowerpoolV2 150ng - MBD vs NoMBD
nomd_150
nomd_150-1
nomd_150-2
md_150
md_150-1
md_150-2
Mcf Count
80K
60K
40K
20K
0
17-7531600
16-816629
14-3678249
13-3291242
10-8107039
8-12875084
7-55259425
5-11217787
5-11217447
5-11204356
3-15844941
2-29448452
1-11525641
MBD ~ 90% of NoMBD coverage

Fig. 28A-FIG.28B

FIG. 28A

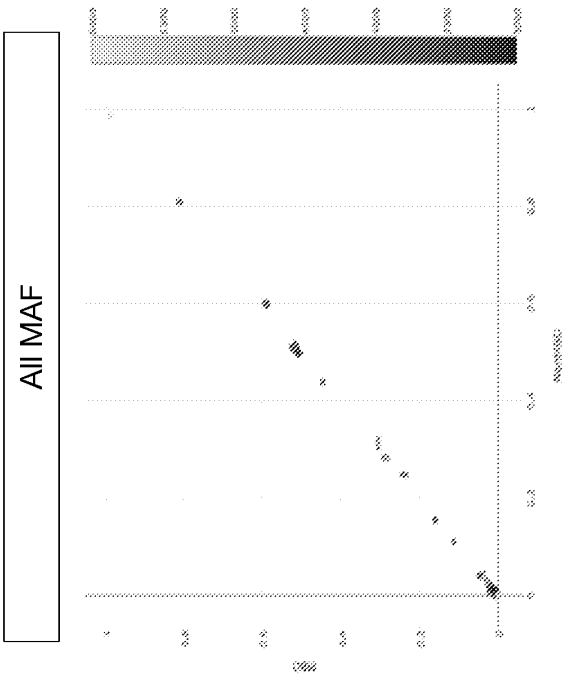
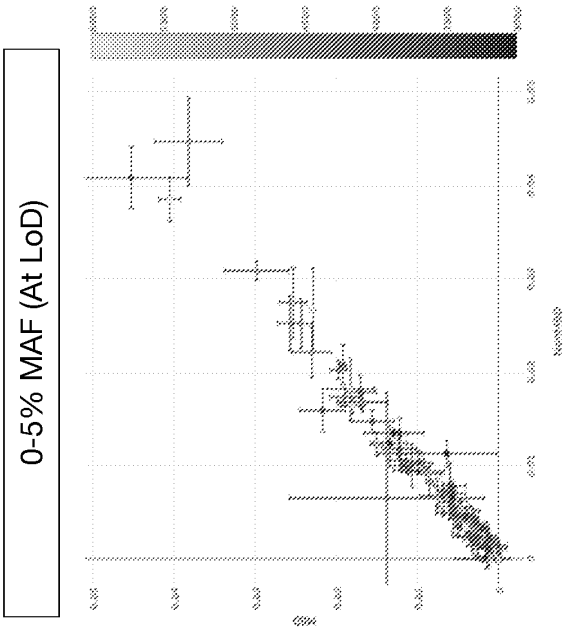


FIG. 28B



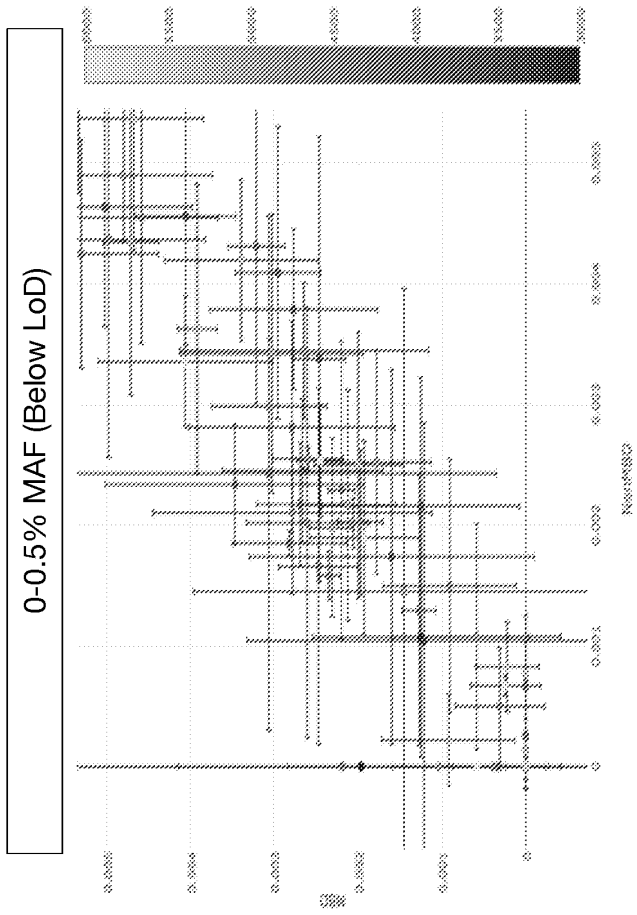


Fig. 28C

Fig. 29A-FIG.29B

FIG. 29A

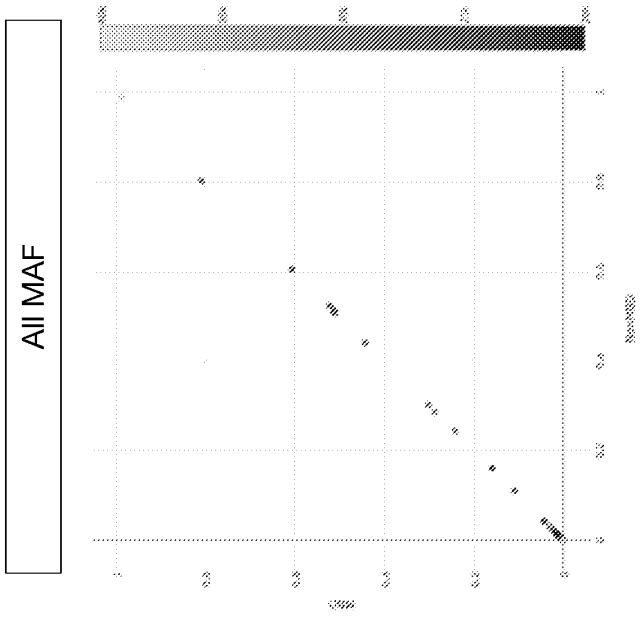
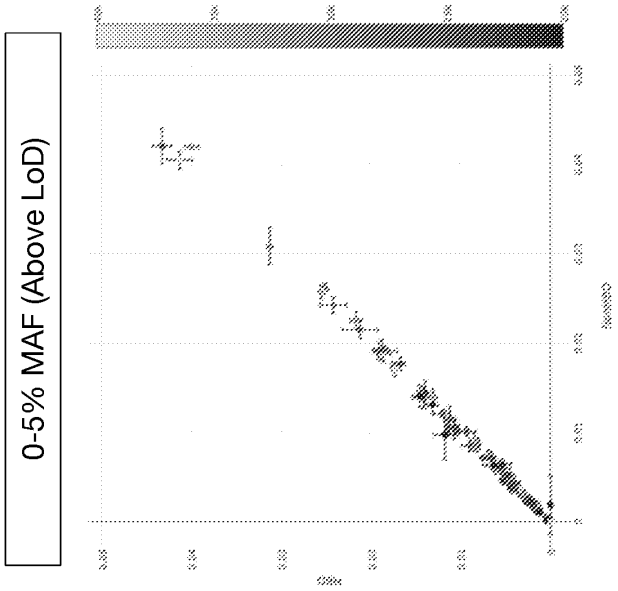


FIG. 29B



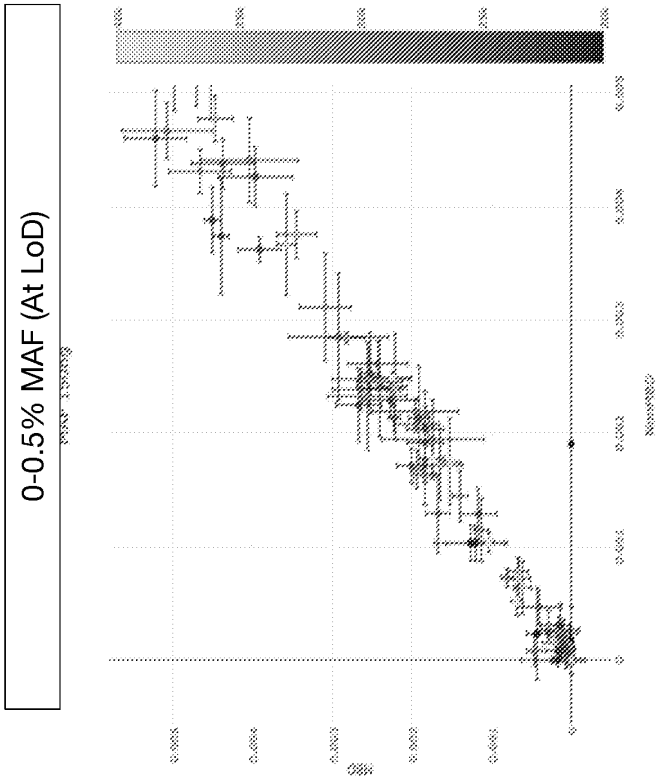


Fig. 29C

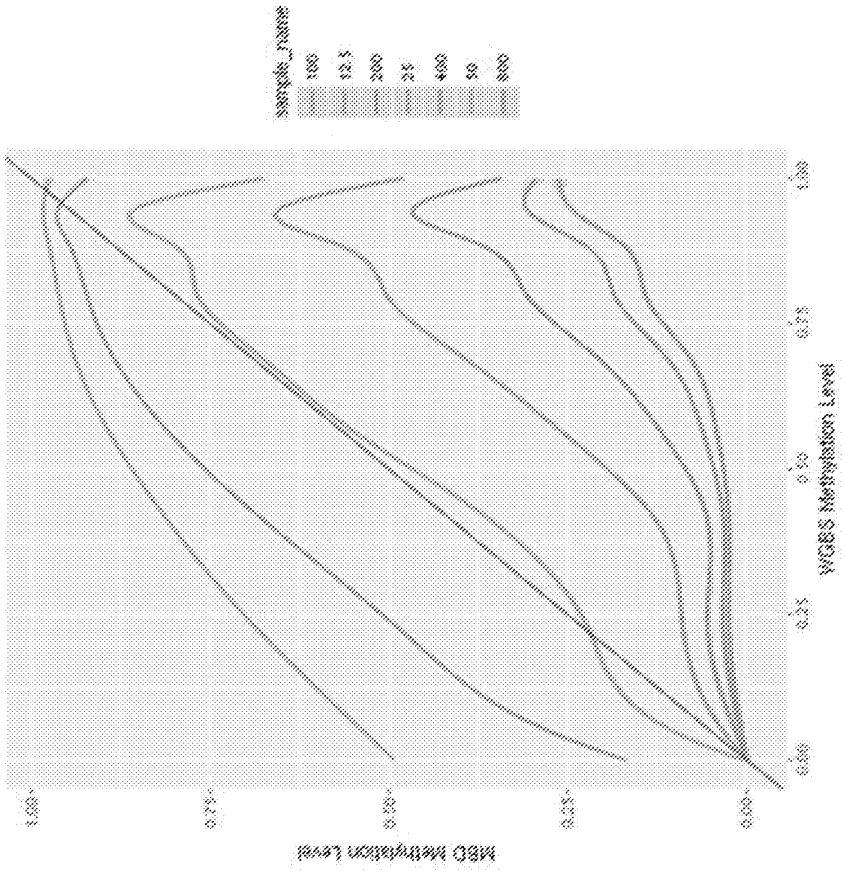


Fig. 30

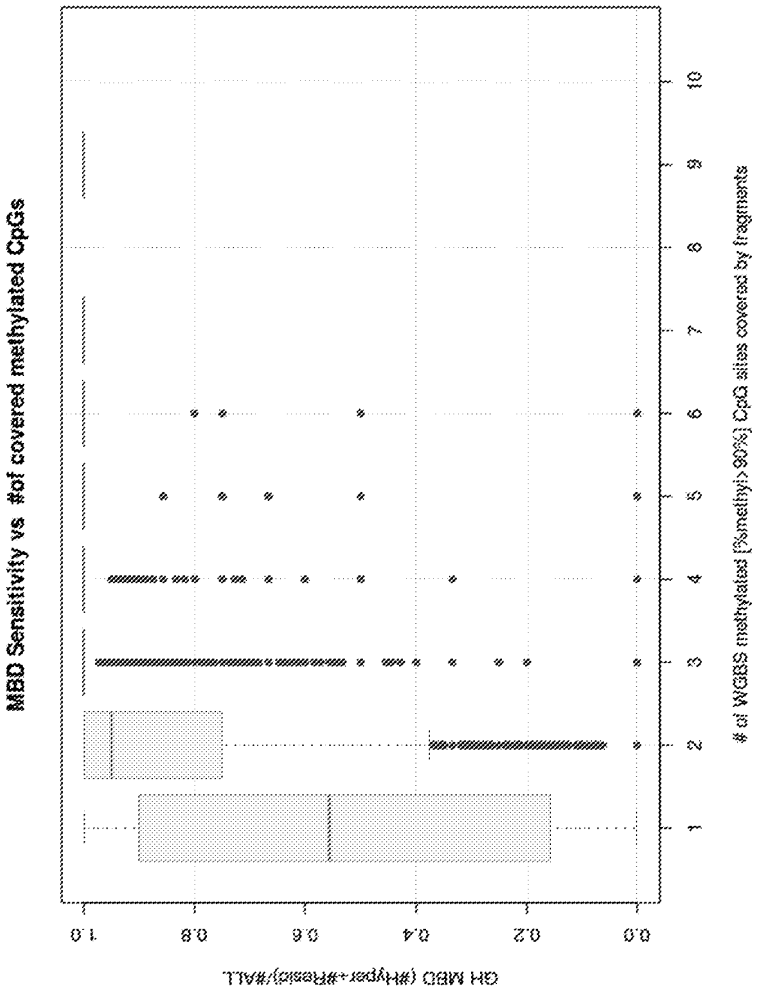

MBD Sensitivity vs #of covered methylated CpGs
CH MBD (#Hyper+#Hemi)/#ALL
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
7
8
9
10
# of WGBS methylated [%methyl>80%] CpG sites covered by fragments

Fig. 31A

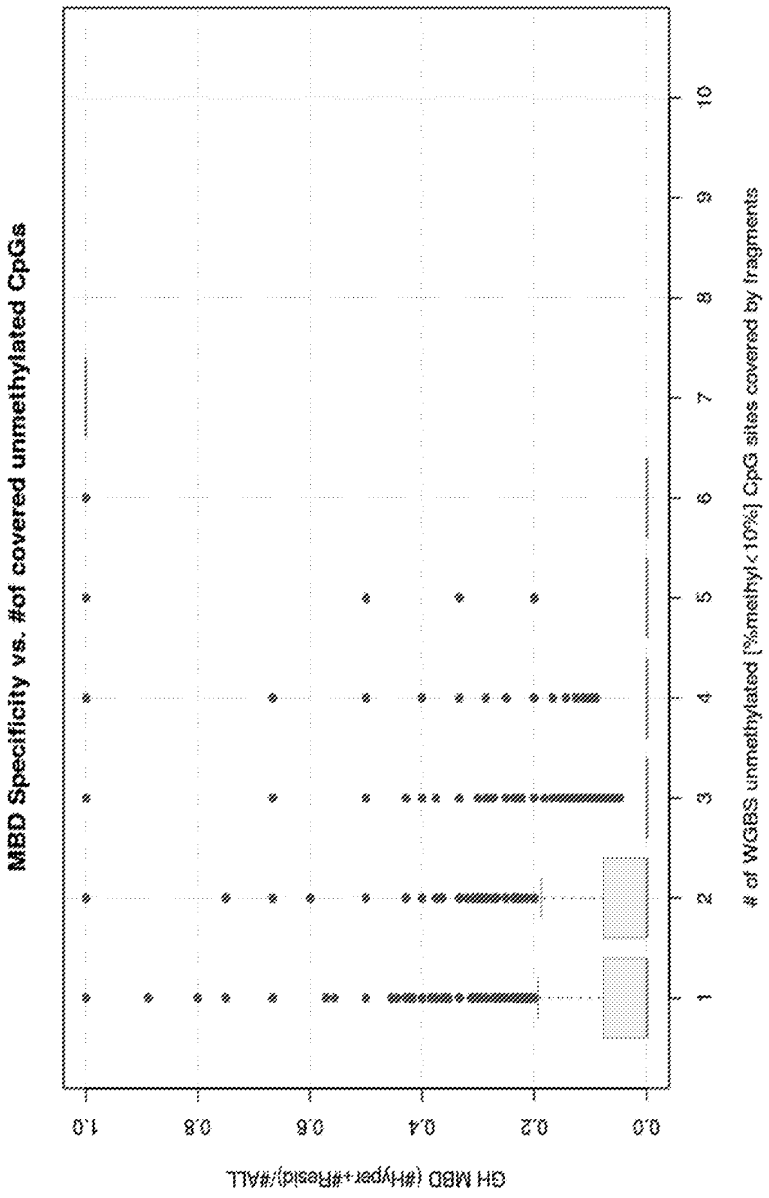

MBD Specificity vs. #of covered unmethylated CpGs
GH MBD (#Hyper+#Resid)/#ALL
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
7
8
9
10
# of WGBS unmethylated [%methyl<10%] CpG sites covered by fragments

Fig. 31B

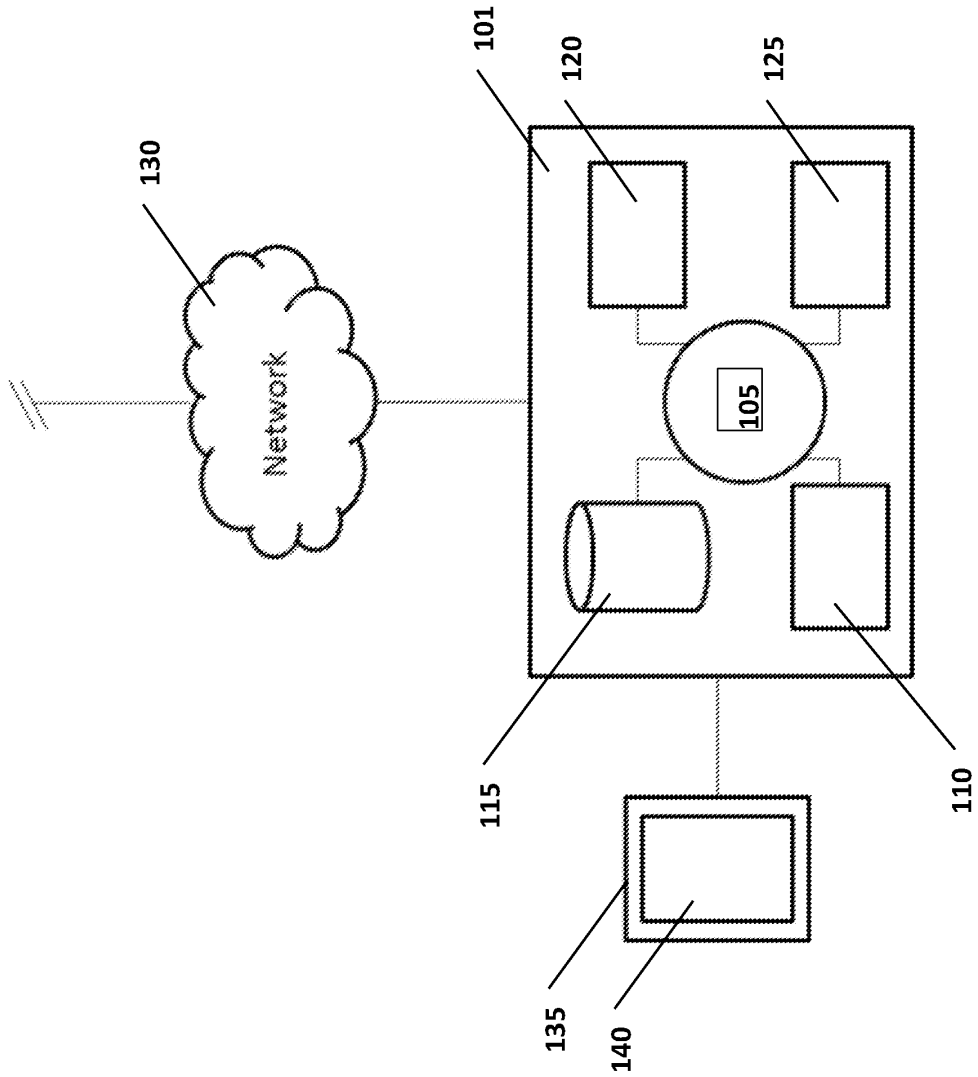


Fig. 32

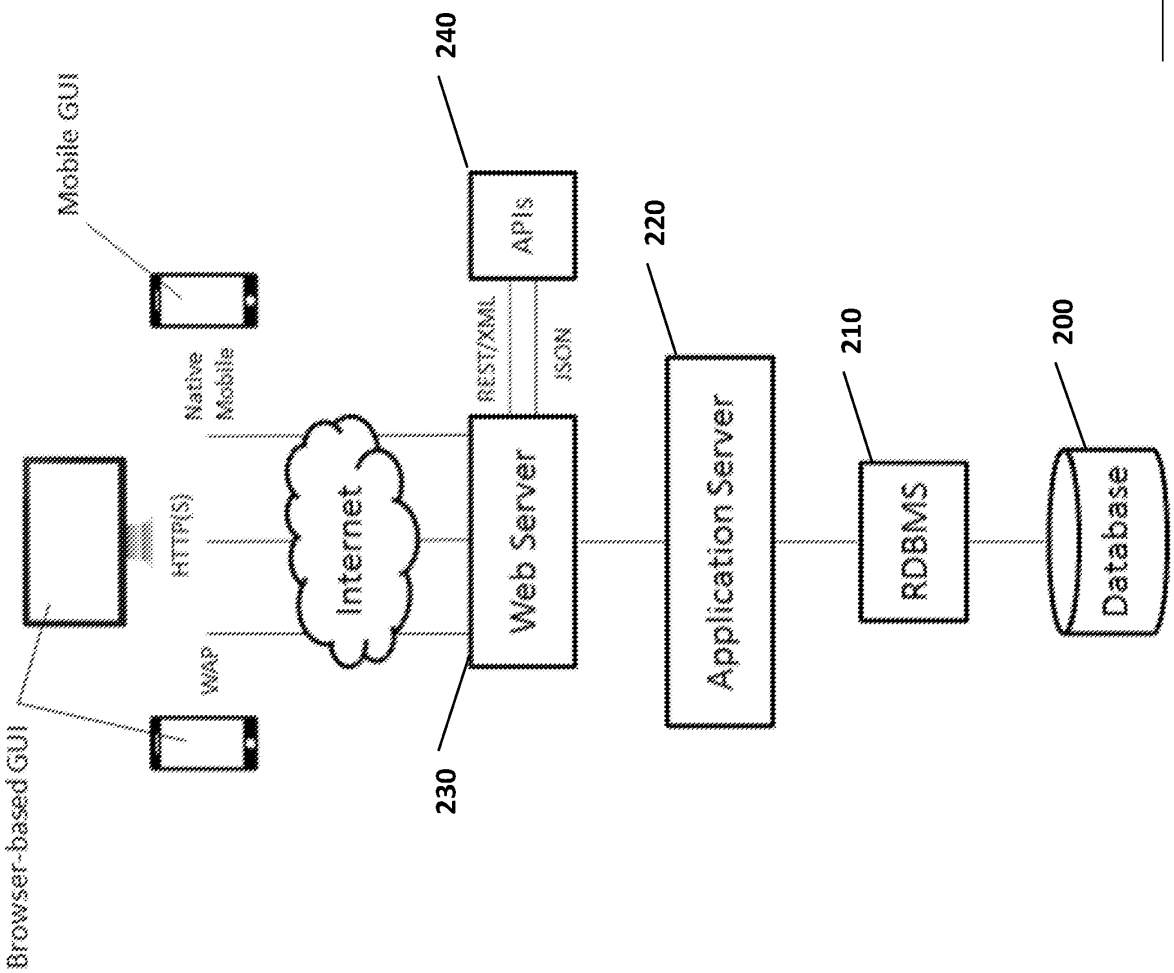

Mobile GUI
Native Mobile
HTTP(S)
WAP
Browser-based GUI
Internet
Web Server
APIs
REST/XML
JSON
Application Server
RDBMS
Database
240
220
210
200
230

Fig. 33

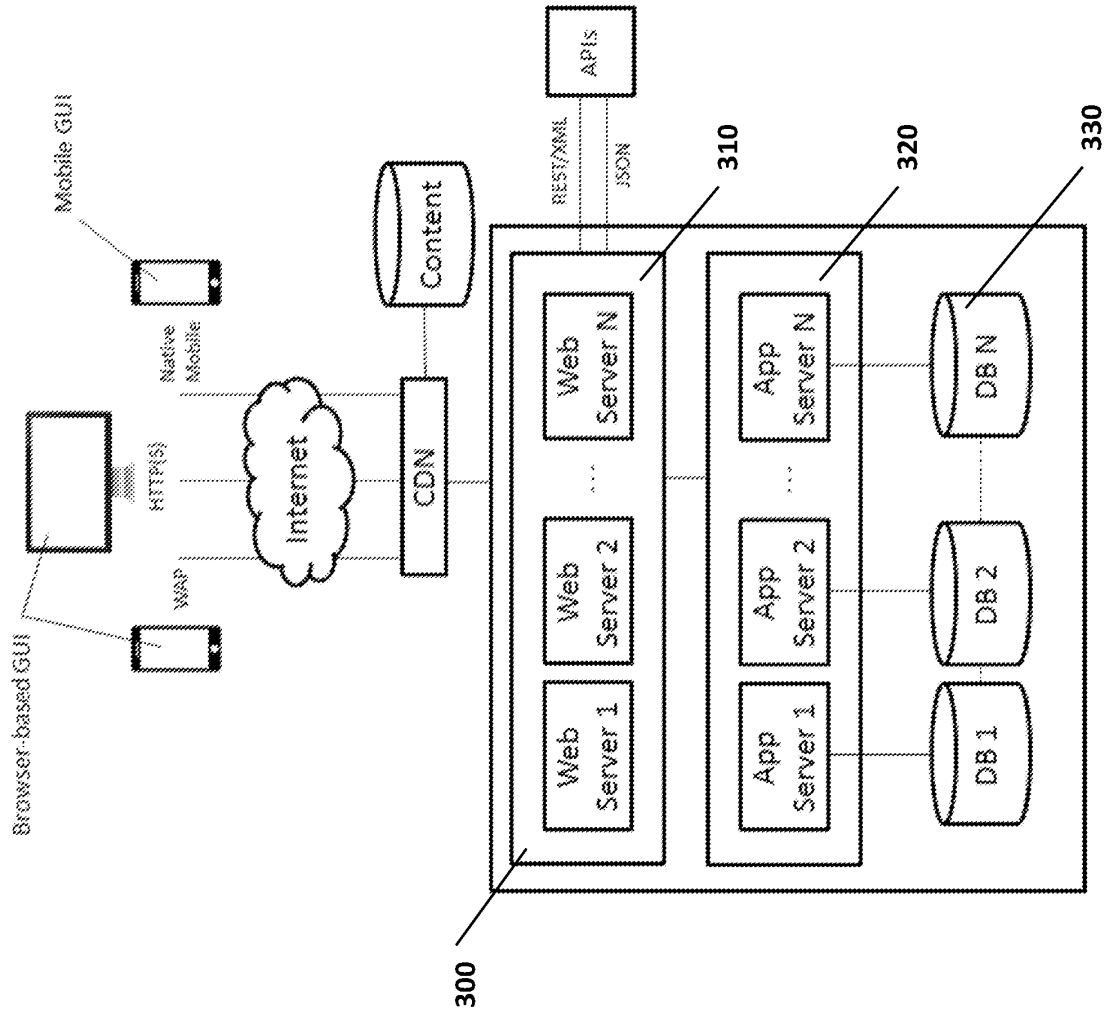

Mobile GUI
Native Mobile
HTTP(S)
WAP
Browser-based GUI
Internet
CDN
Content
APIs
REST/XML
JSON
Web Server 1
Web Server 2
Web Server N
App Server 1
App Server 2
App Server N
DB 1
DB 2
DB N
300
310
320
330

Fig. 34