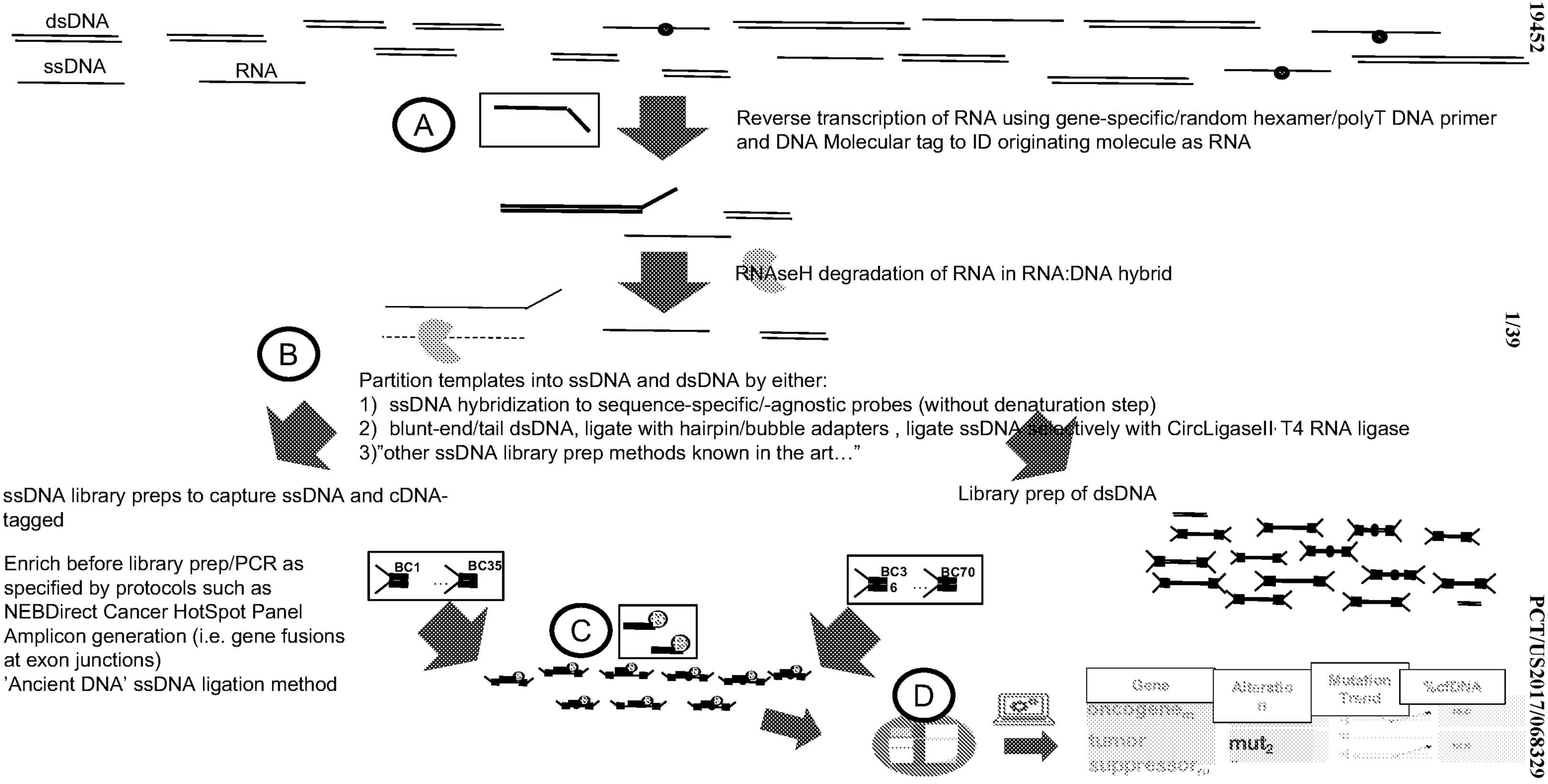
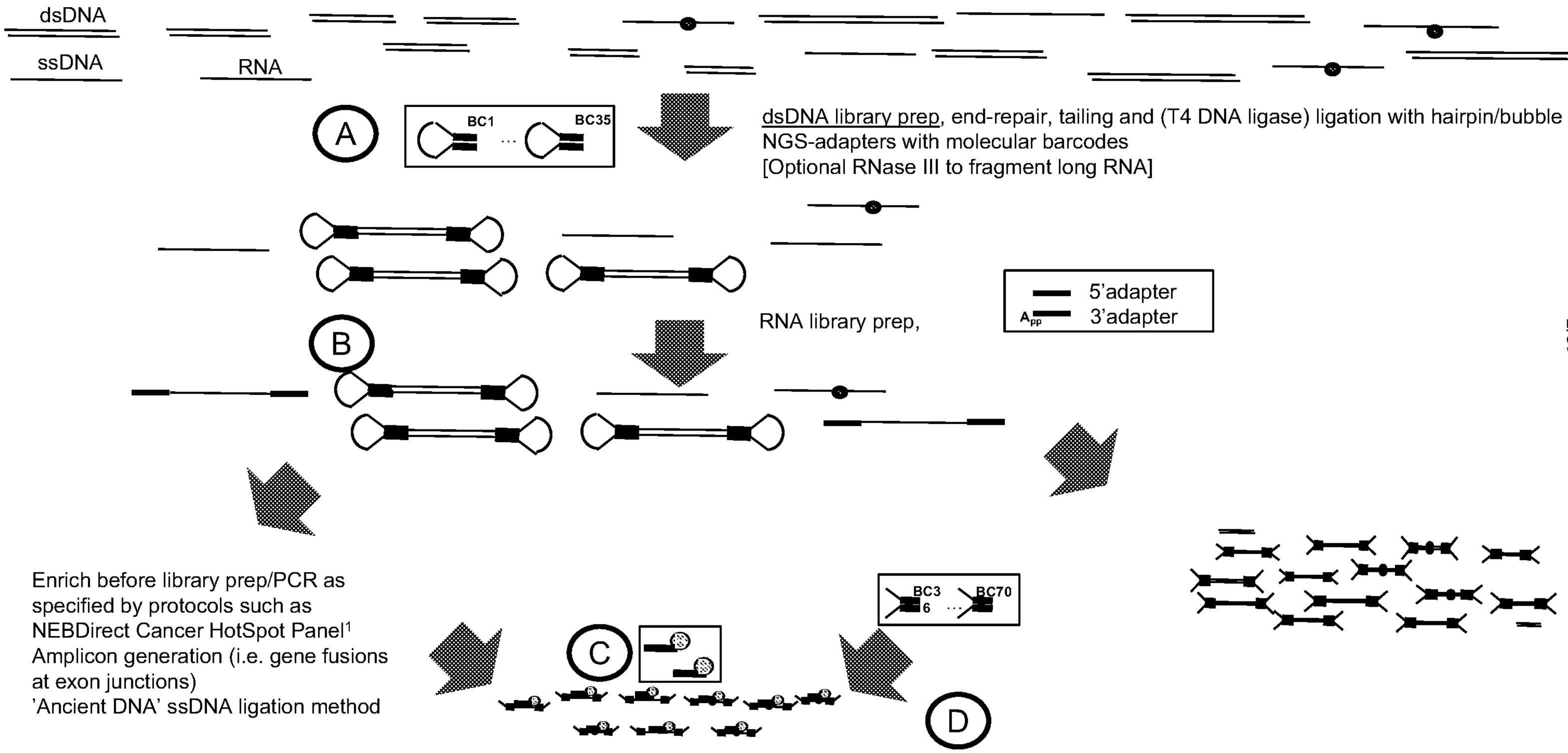
FIG. 1



PCT/US2017/068329

FIG. 2



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

FIG. 3

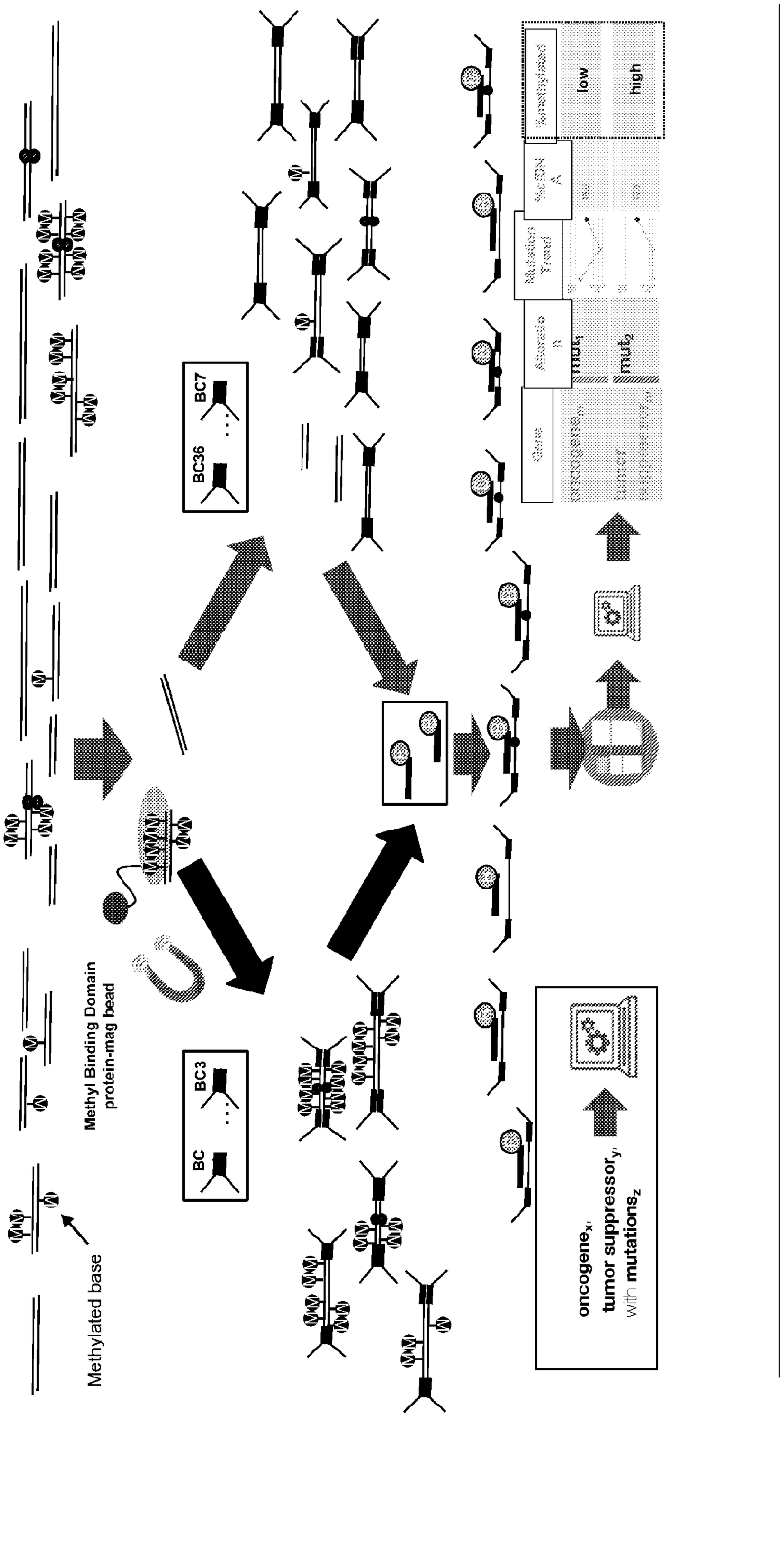

Methylated base
Methyl Binding Domain protein-mag bead
BC
BC3
BC7
BC36
low
high
oncogene$_x$,
tumor suppressor$_y$,
with mutations$_z$

FIG. 4

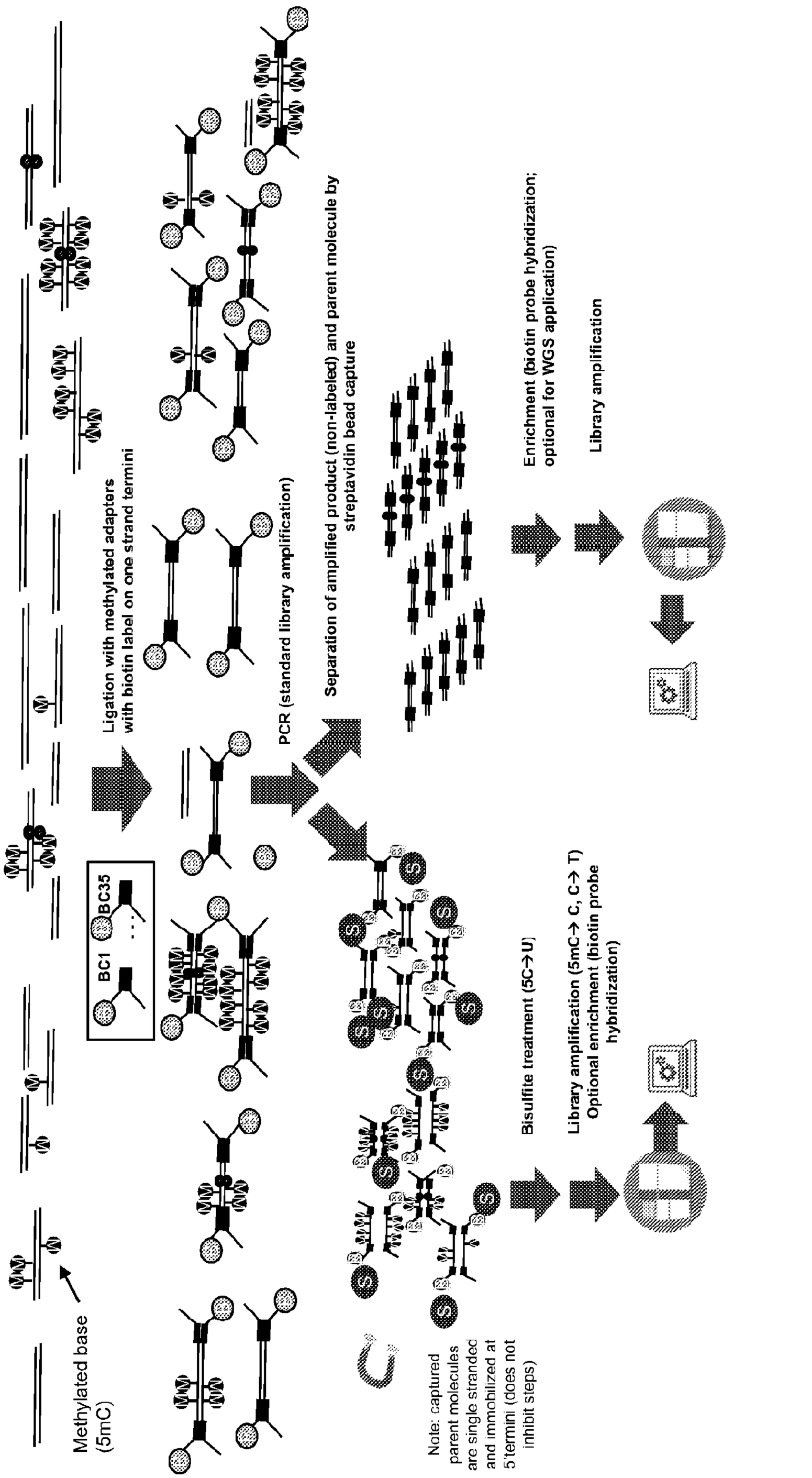

Methylated base (5mC)
Ligation with methylated adapters with biotin label on one strand termini
BC1
BC35
PCR (standard library amplification)
Separation of amplified product (non-labeled) and parent molecule by streptavidin bead capture
Bisulfite treatment (5C→U)
Library amplification (5mC→ C, C→ T)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

FIG. 5

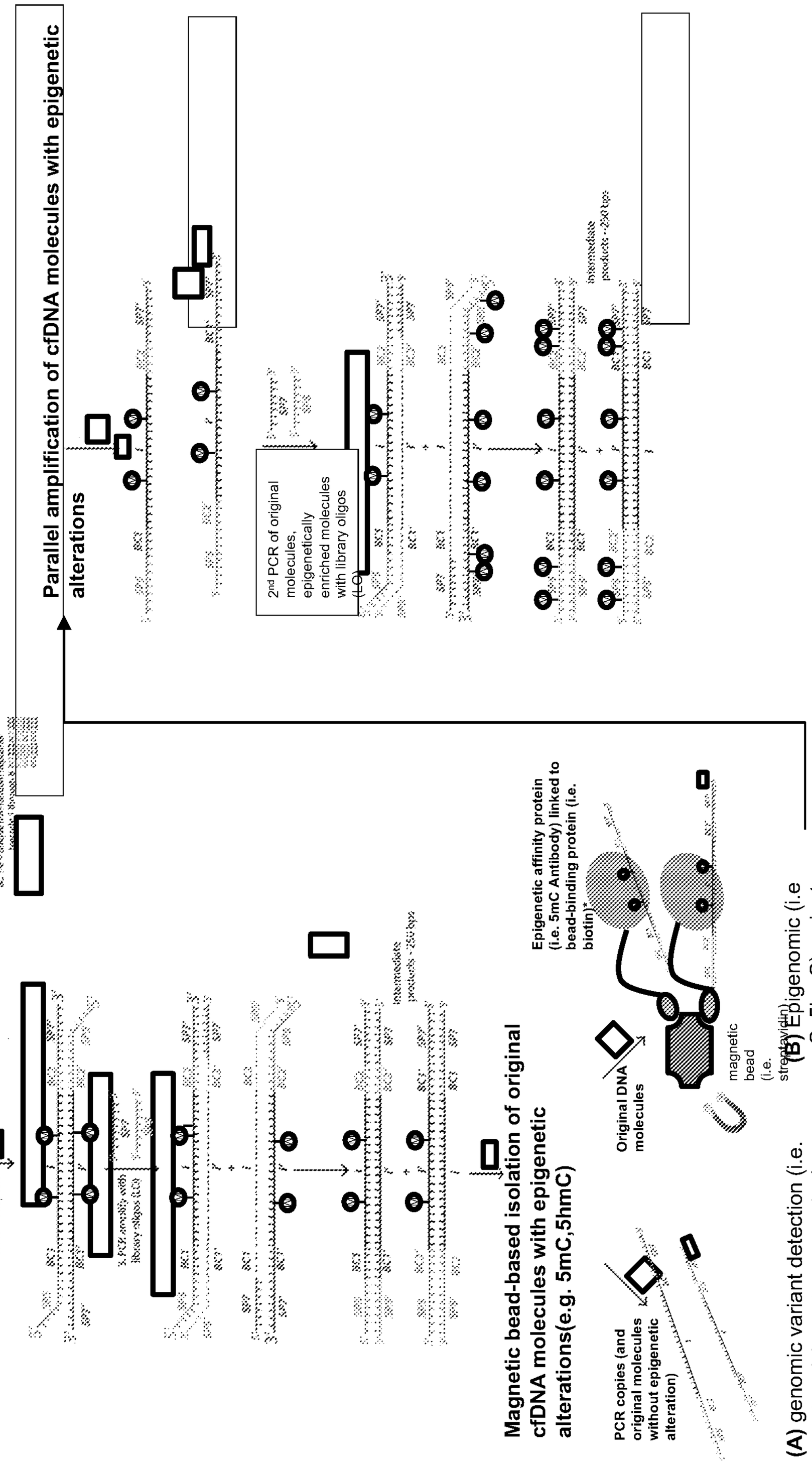
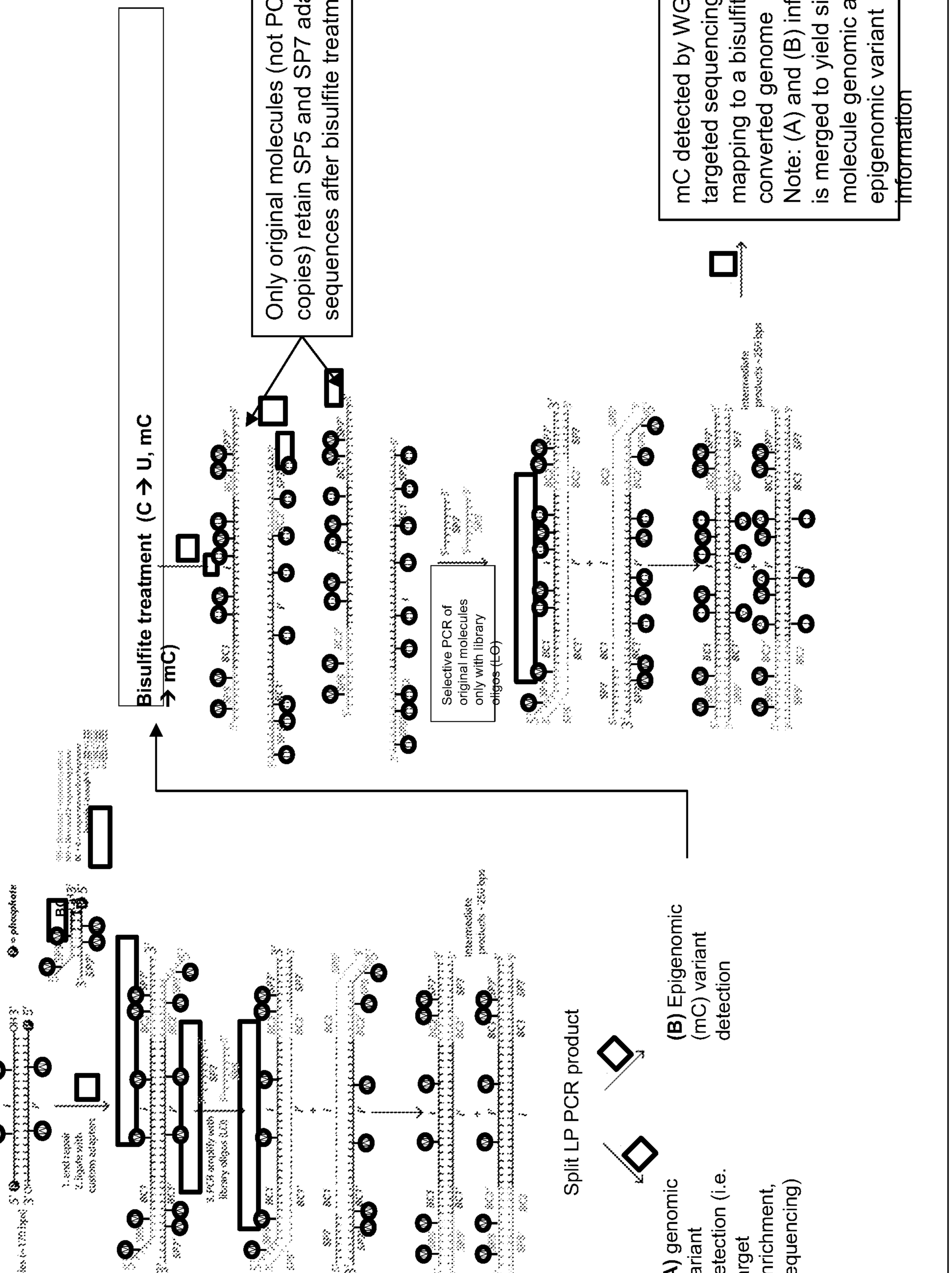

(A) genomic variant detection (i.e. target enrichment, sequencing)
PCR copies (and original molecules without epigenetic alteration)
Magnetic bead-based isolation of original cfDNA molecules with epigenetic alterations(e.g. 5mC,5hmC)
Original DNA molecules
Epigenetic affinity protein (i.e. 5mC Antibody) linked to bead-binding protein (i.e. biotin)*
magnetic bead (i.e. streptavidin)
(B) Epigenomic (i.e mC, 5hmC) variant detection
Parallel amplification of cfDNA molecules with epigenetic alterations
2nd PCR of original molecules, epigenetically enriched molecules with library oligos (LO)
intermediate products ~250 bps
Input = cfDNA molecules with epigenetic alterations
= phosphate

FIG. 6


Only original molecules (not PCR copies) retain SP5 and SP7 adapter sequences after bisulfite treatment
Bisulfite treatment (C → U, mC → mC)
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

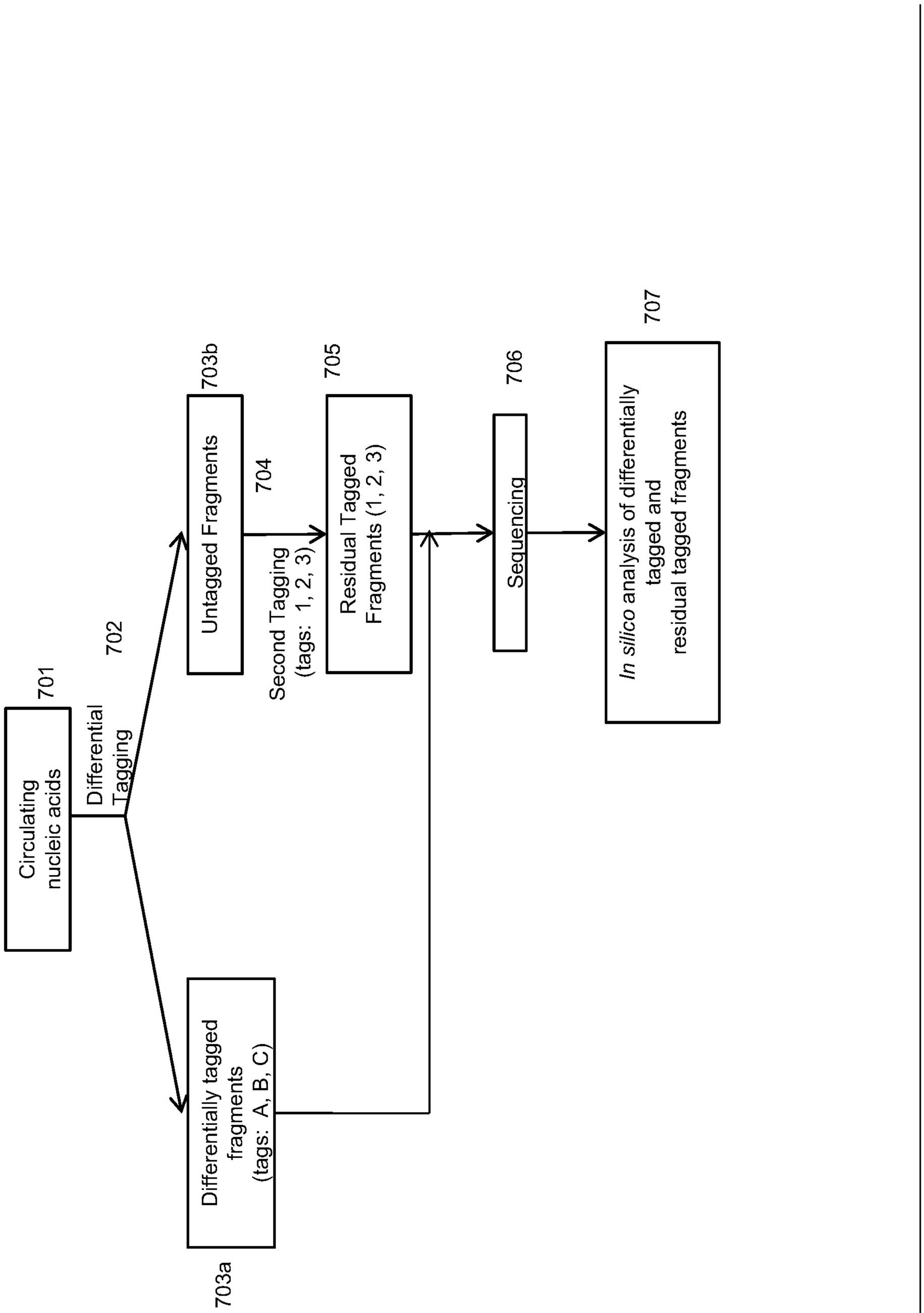

701
Circulating nucleic acids
702
Differential Tagging
703a
Differentially tagged fragments (tags: A, B, C)
703b
Untagged Fragments
704
Second Tagging (tags: 1, 2, 3)
705
Residual Tagged Fragments (1, 2, 3)
706
Sequencing
707
In silico analysis of differentially tagged and residual tagged fragments

FIG. 7

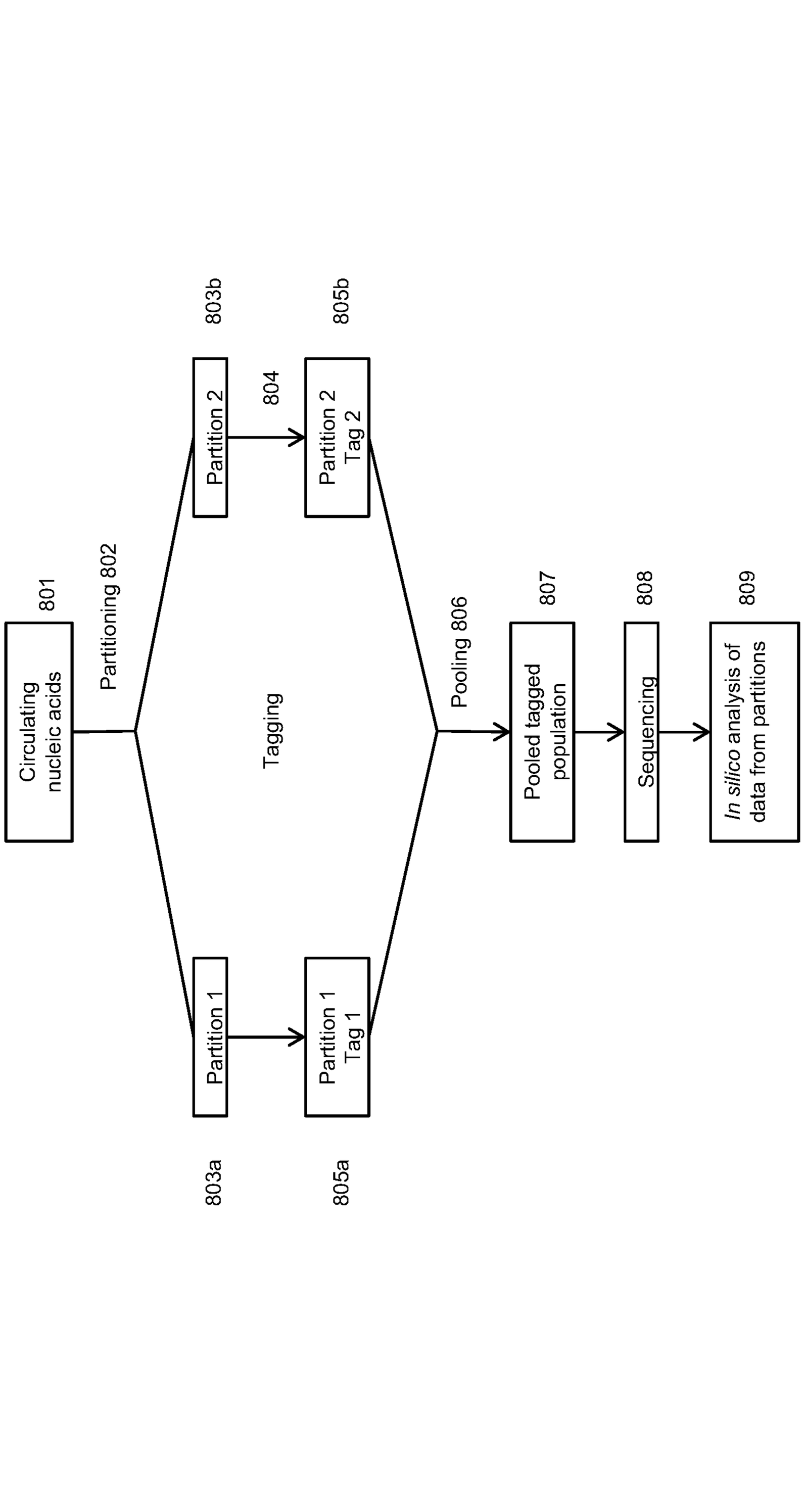

801
Circulating nucleic acids
Partitioning 802
Partition 2
803b
804
Partition 2 Tag 2
805b
Tagging
Partition 1
803a
Partition 1 Tag 1
805a
Pooling 806
807
Pooled tagged population
808
Sequencing
809
In silico analysis of data from partitions

FIG. 8

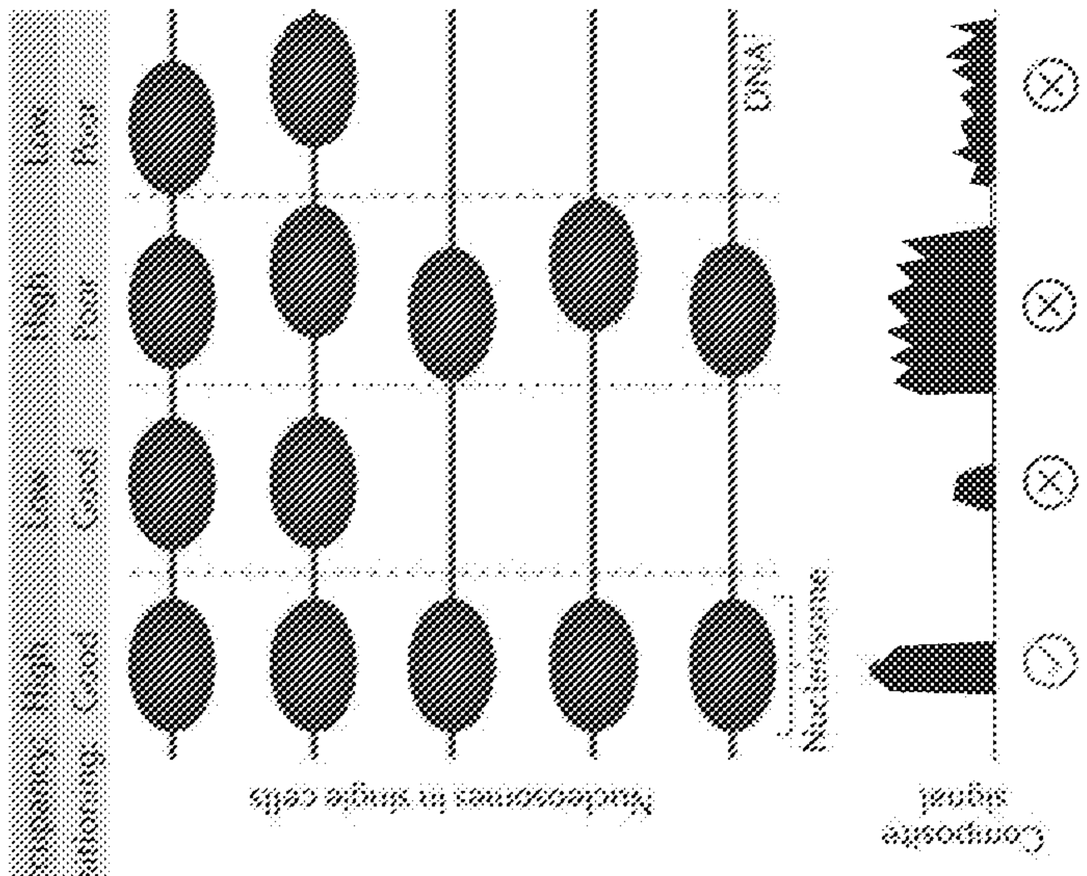


FIG. 9

1. Use 8 Lung Cancer no MBD samples to identify dyad centers in combined MBD samples
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.

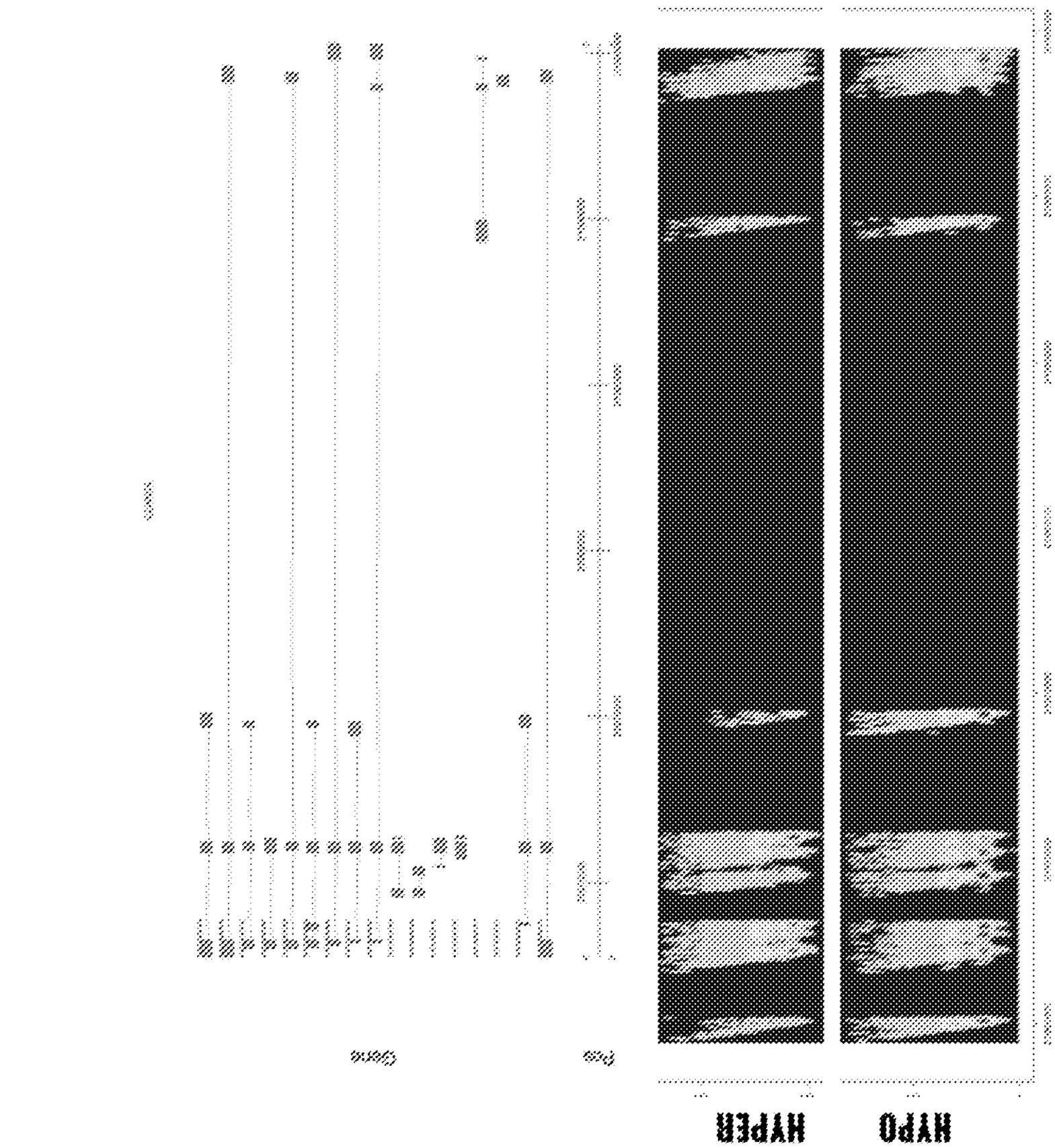
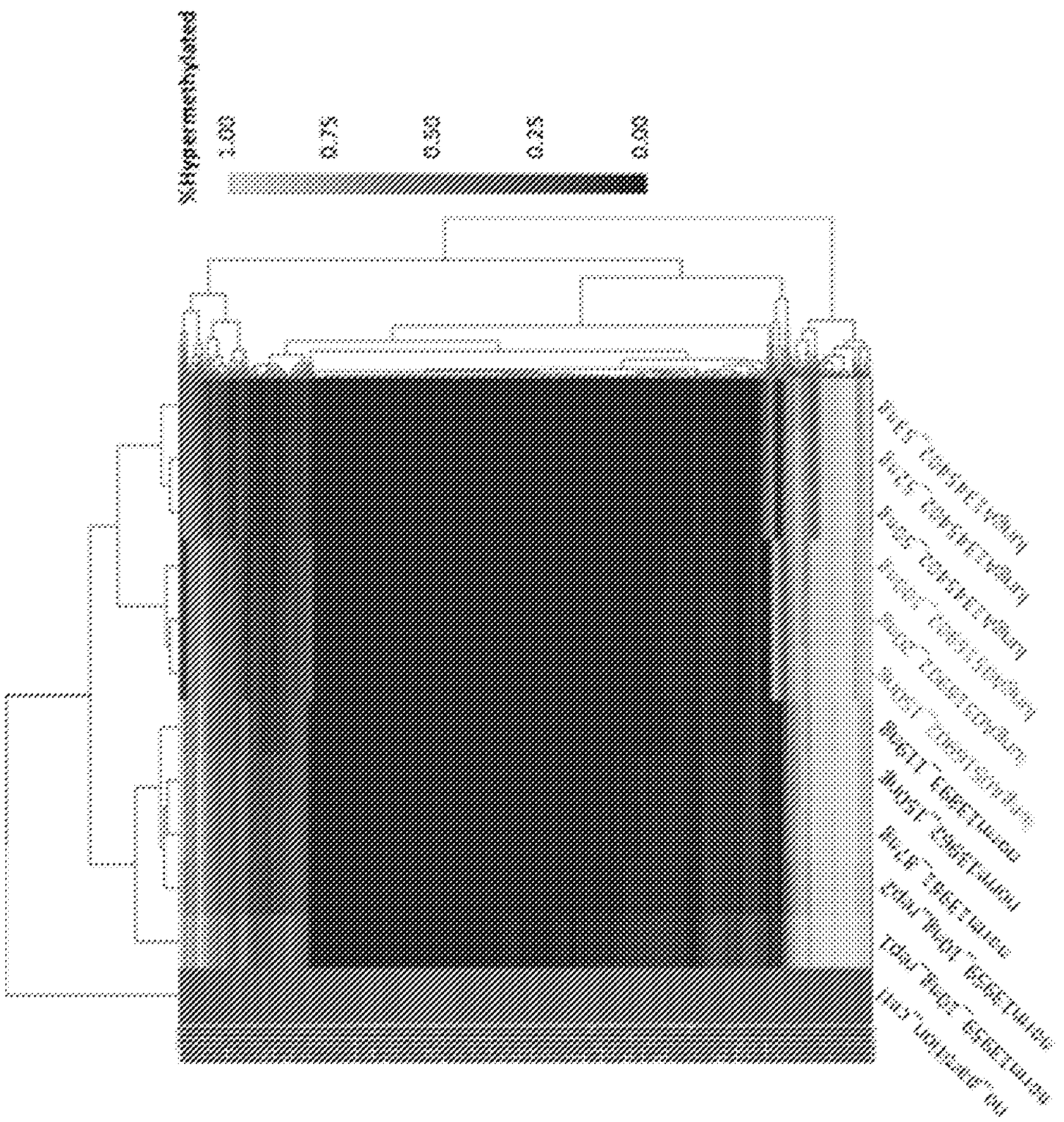


FIG. 10


Wavelength (nm)
700
650
600
550
500
Body Temperature
Body Temperature
Body Temperature

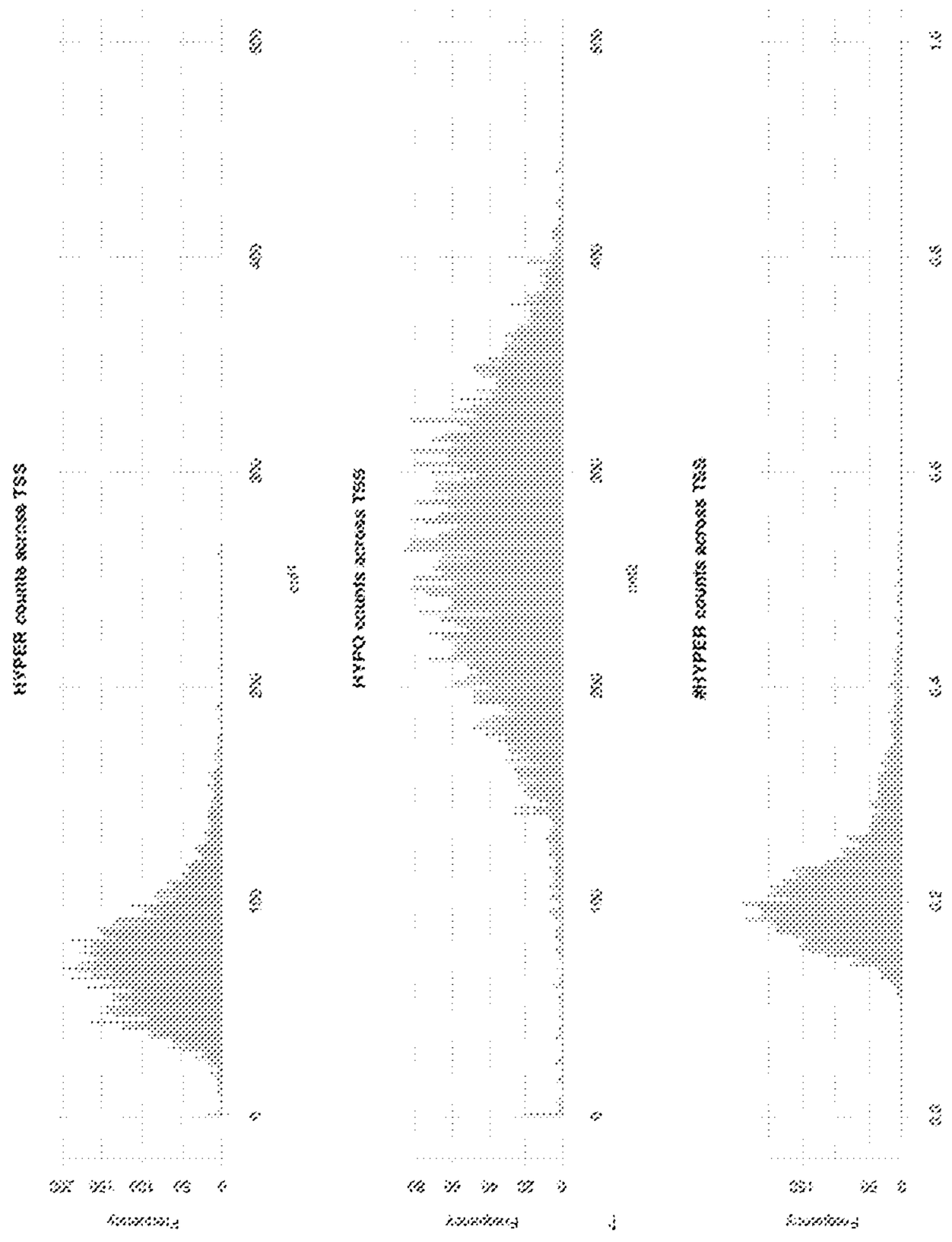


FIG. 12A-FIG. 12C

Fig. 12A

Fig. 12B

Fig. 12C

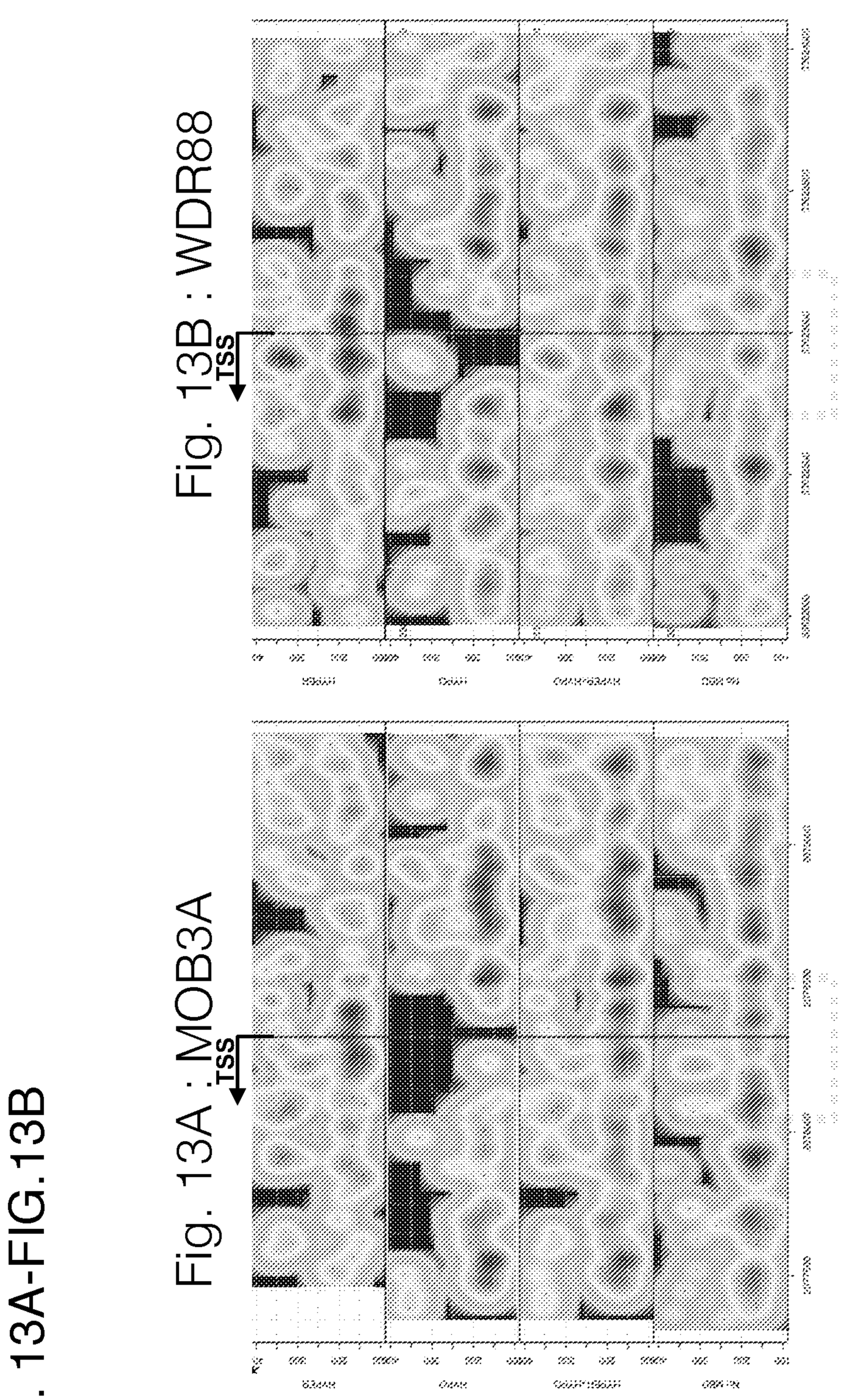

Fig. 13B : WDR88
TSS
Fig. 13A : MOB3A
TSS
FIG. 13A-FIG.13B

FIG. 14A-FIG.14B

Fig. 14A

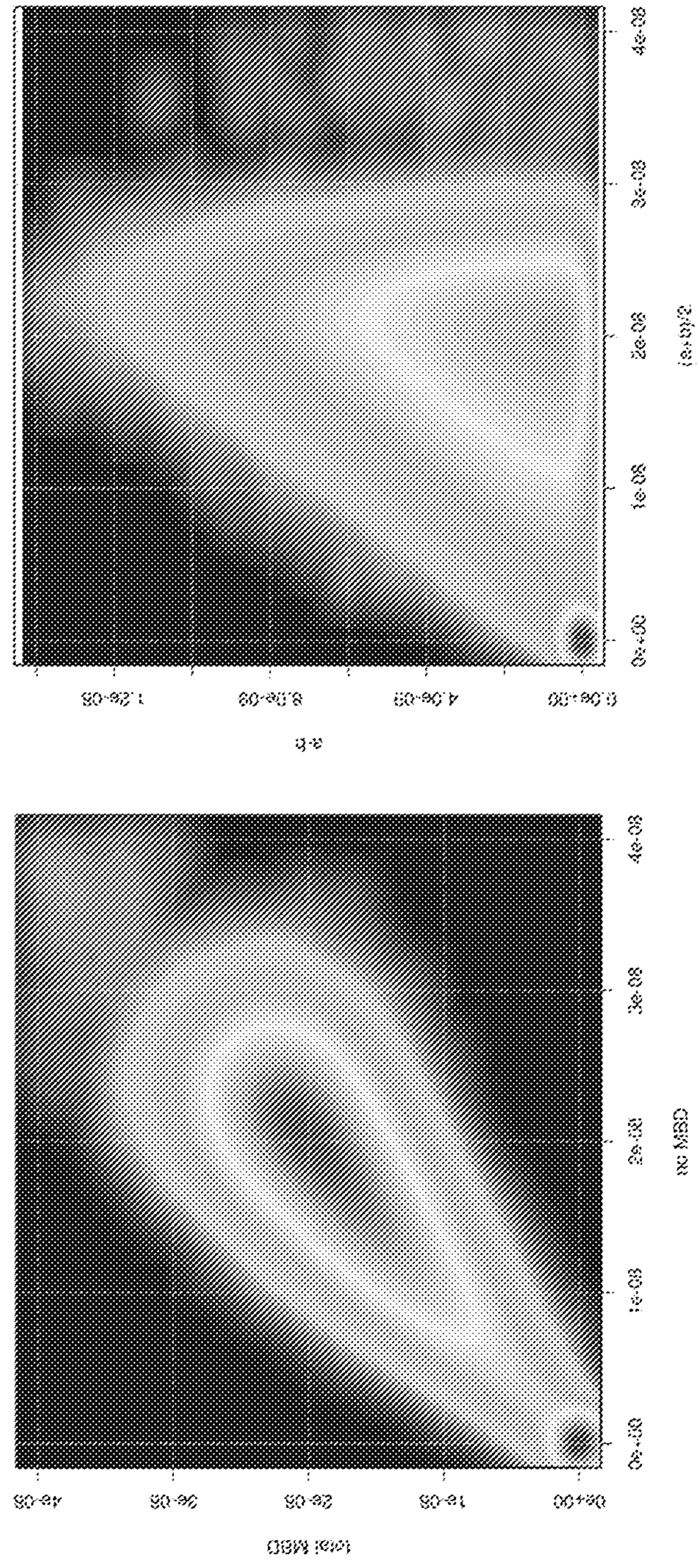


Fig. 14B

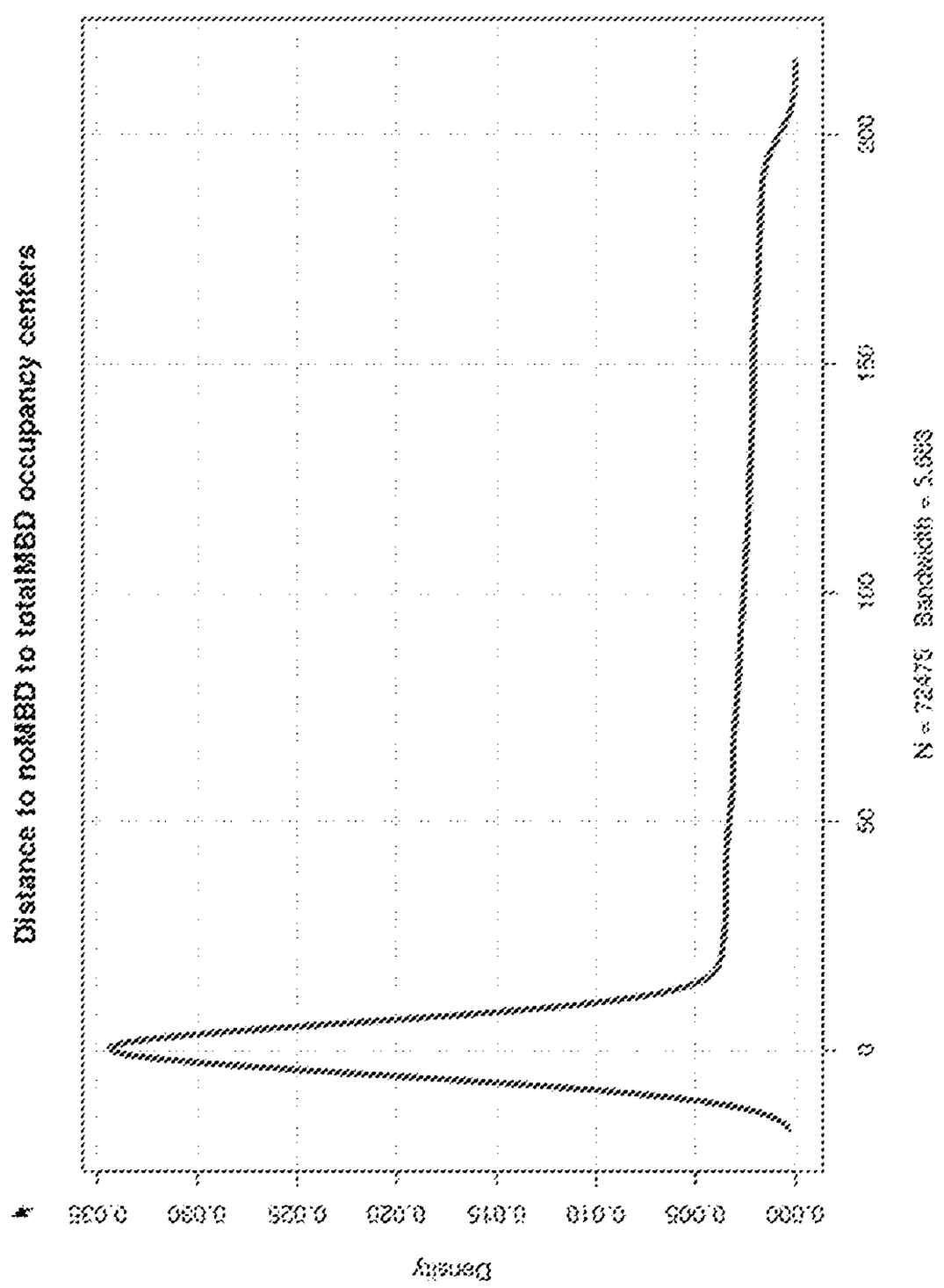

Distance to noMBD to totalMBD occupancy centers
Density
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.035
0
50
100
150
200
N = 728736 Bandwidth = 5.883

FIG. 15

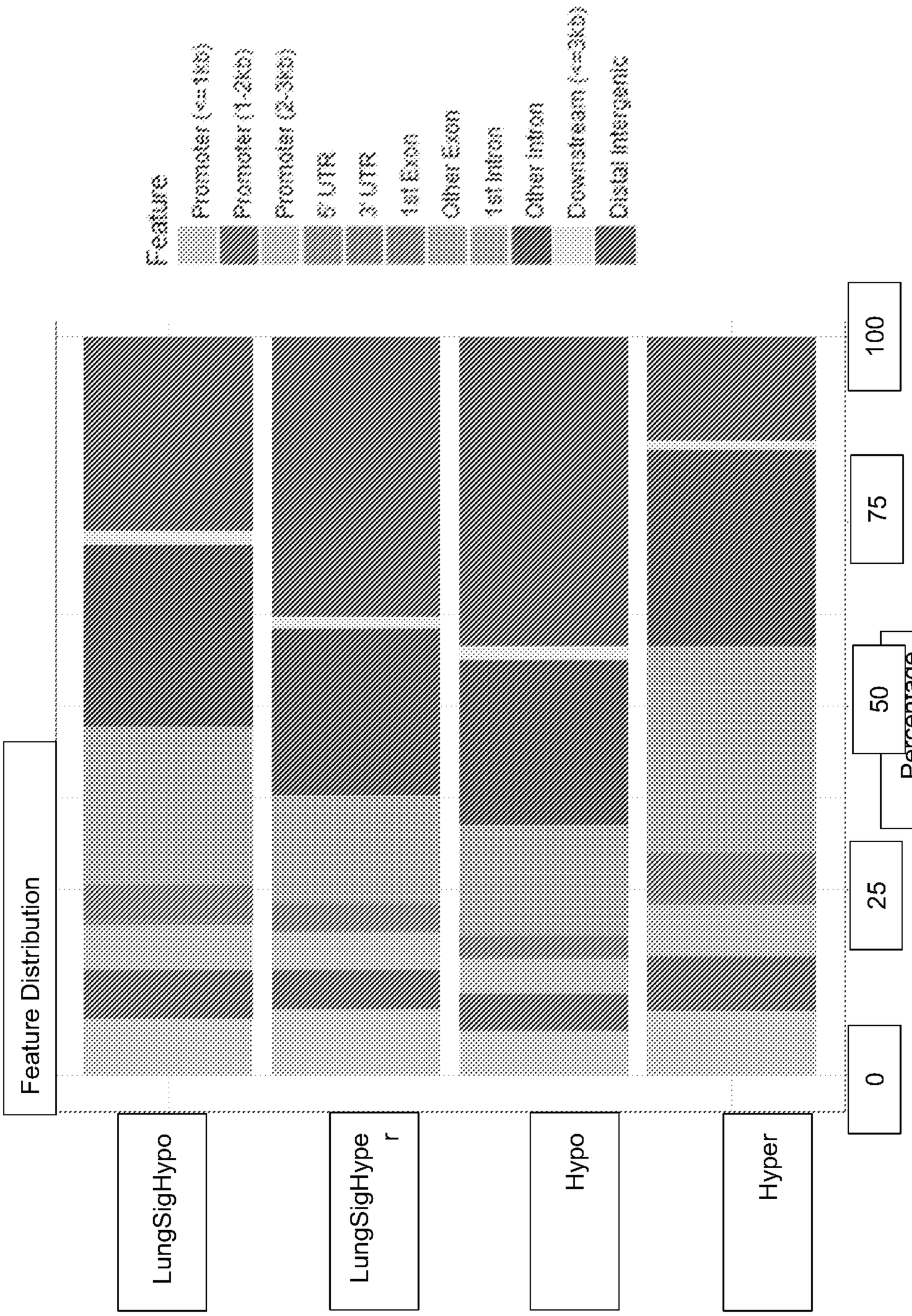

Feature
Promoter (<-1kb)
Promoter (1-2kb)
Promoter (2-3kb)
5' UTR
1st Exon
Other Exon
1st Intron
Other Intron
Downstream (<=1kb)
Distal Intergenic
100
75
50
25
0
Percentage %
Feature Distribution
LungSigHypo
LungSigHyper
Hypo
Hyper

FIG. 16

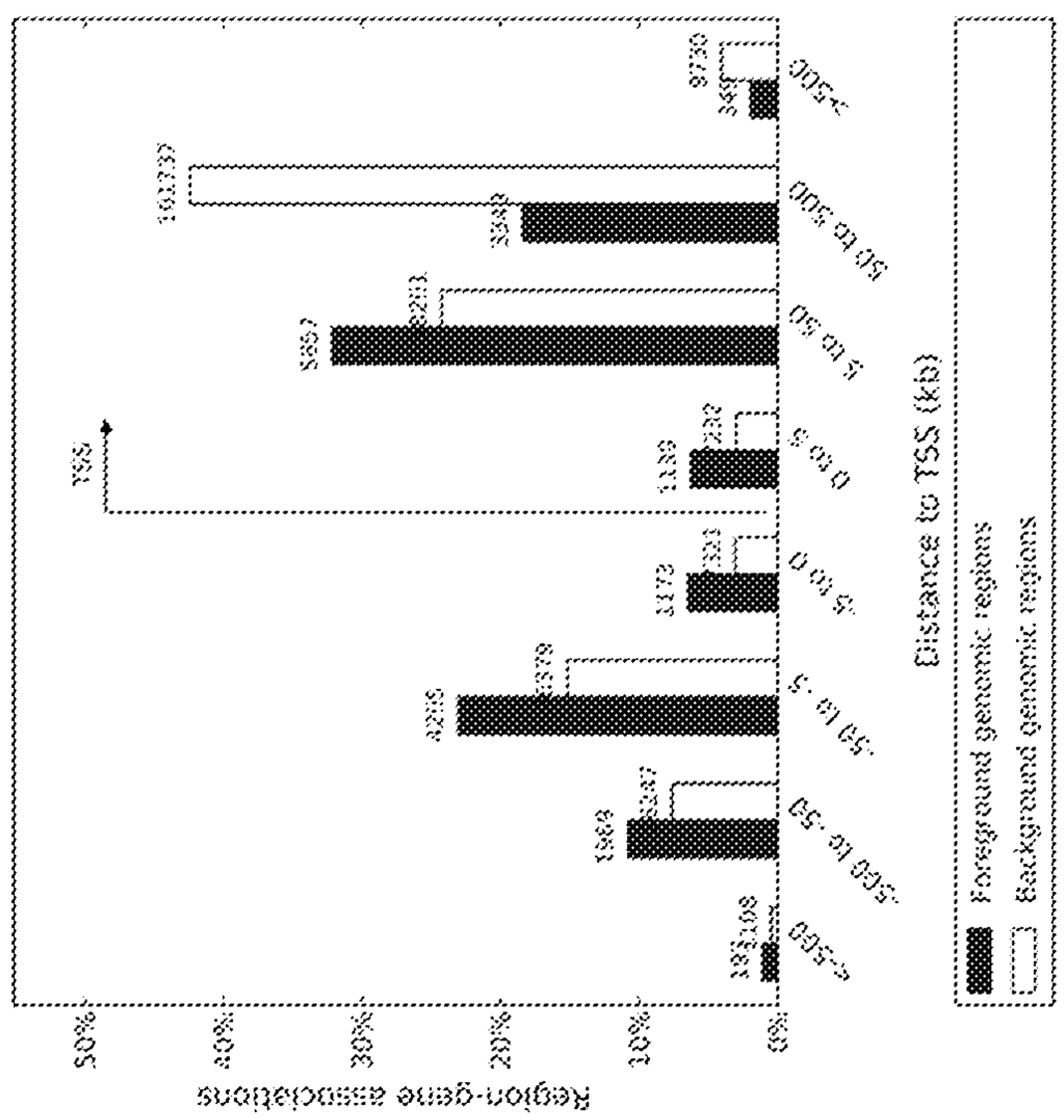

Region-gene associations
50%
40%
30%
20%
10%
0%
Distance to TSS (kb)
Background genomic regions
Foreground genomic regions

FIG. 17

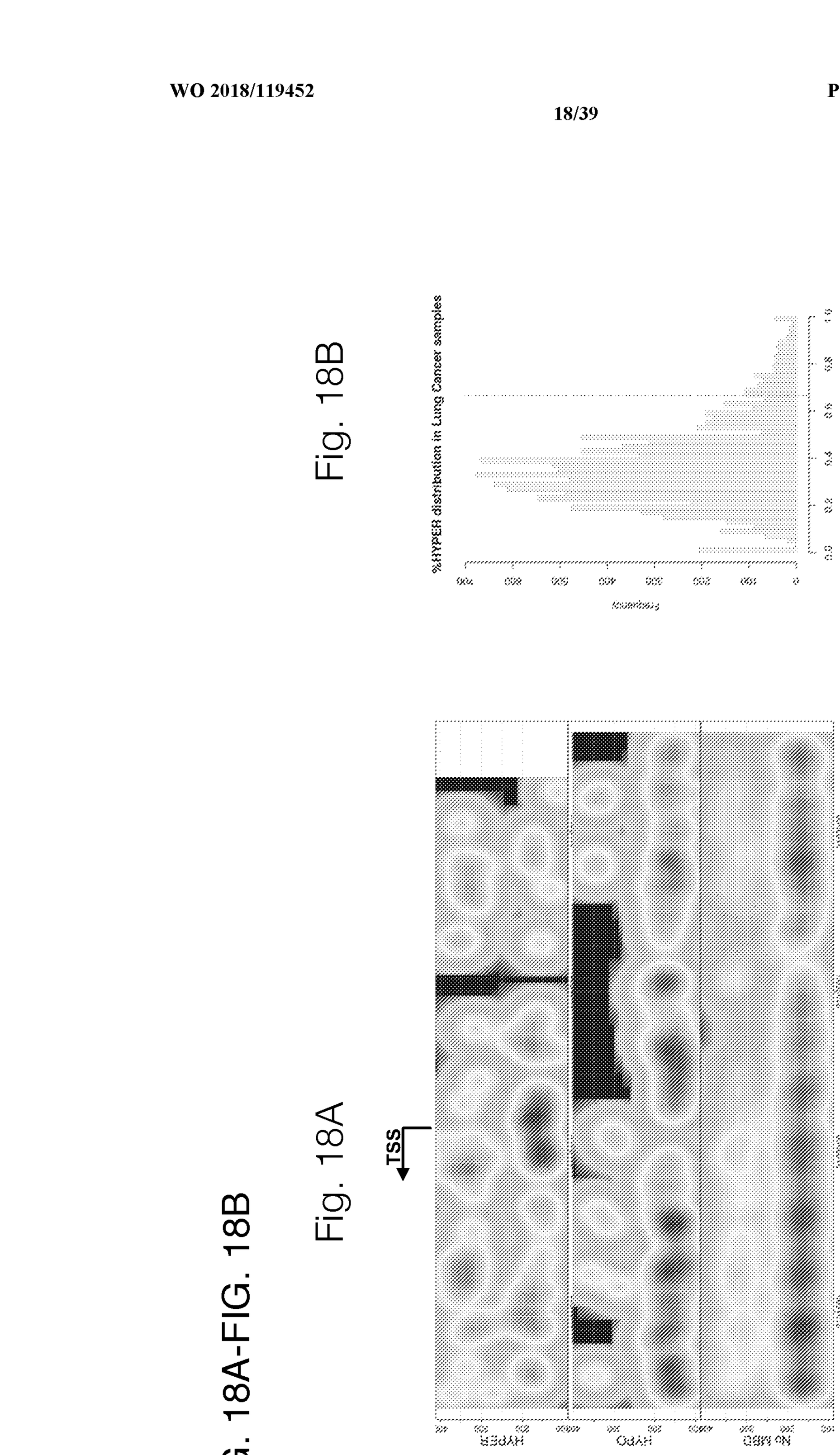


Fig. 18A

Fig. 18B

FIG. 18A-FIG. 18B

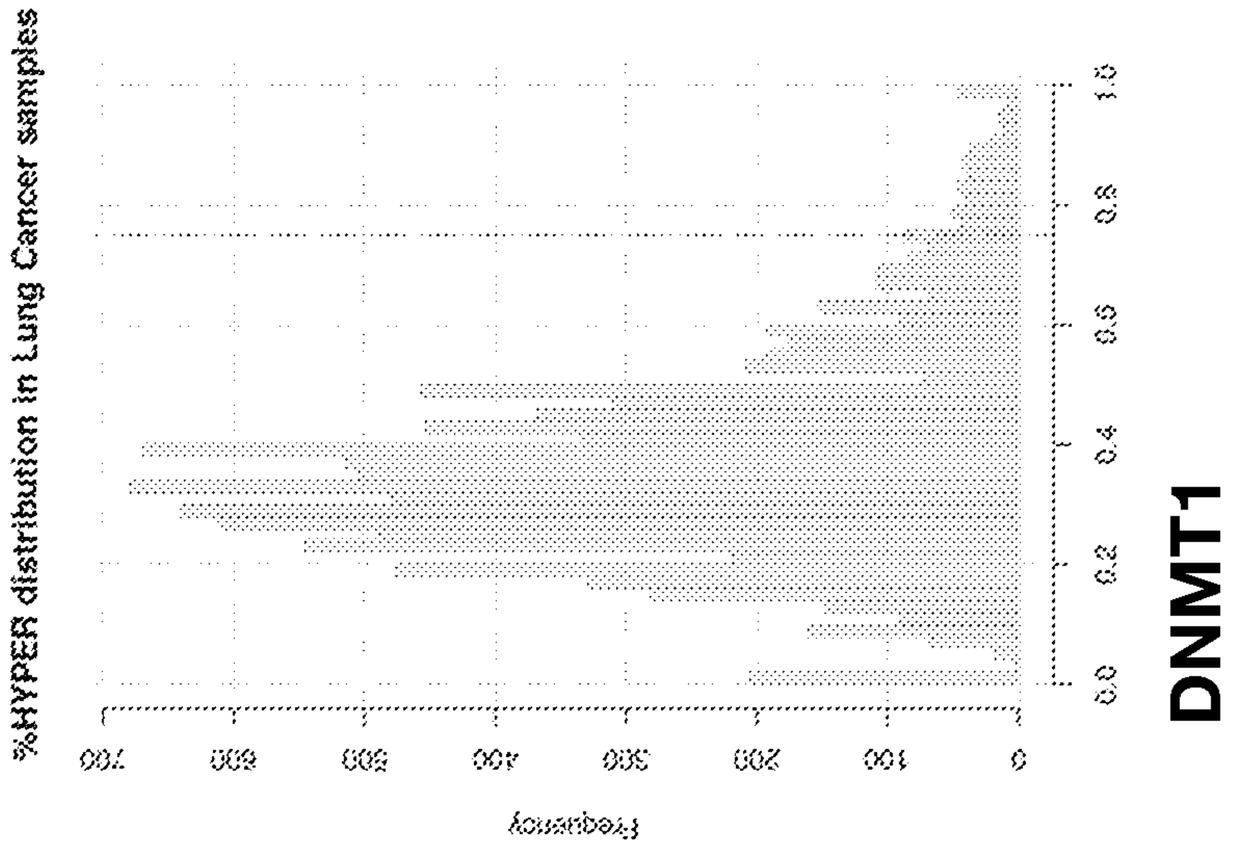


Fig. 19B

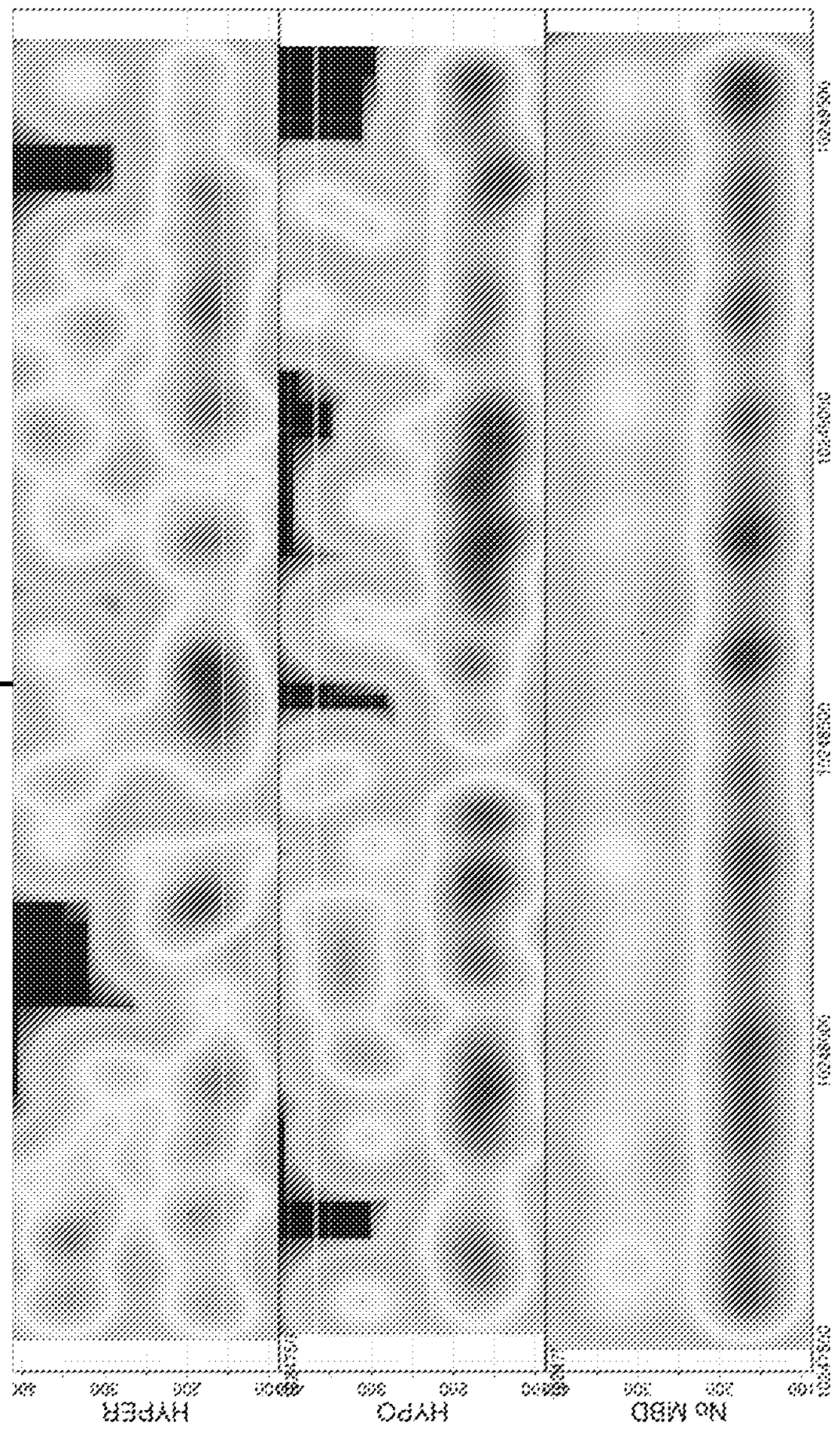


FIG. 19A-FIG.19B
Fig. 19A

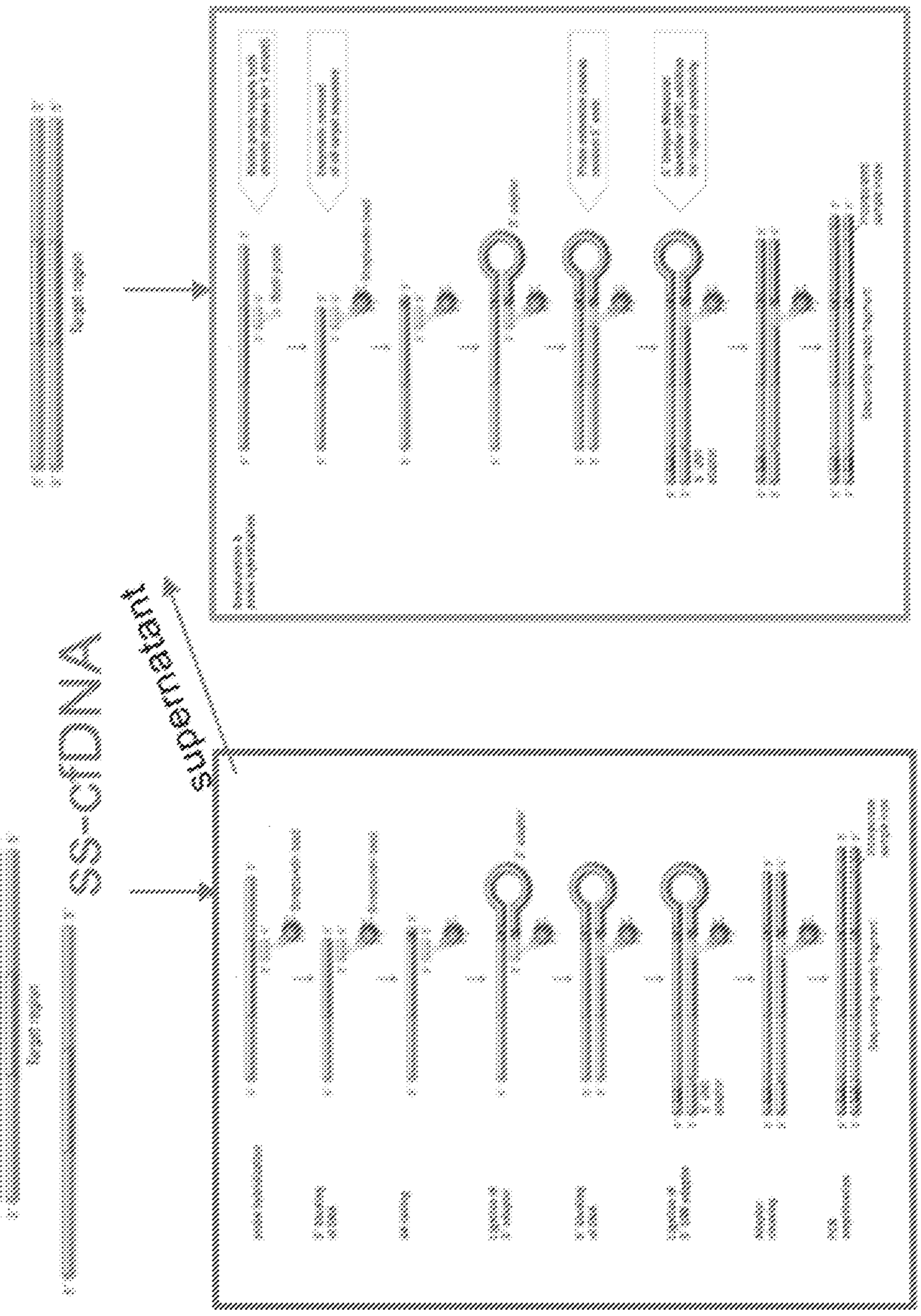


FIG. 20

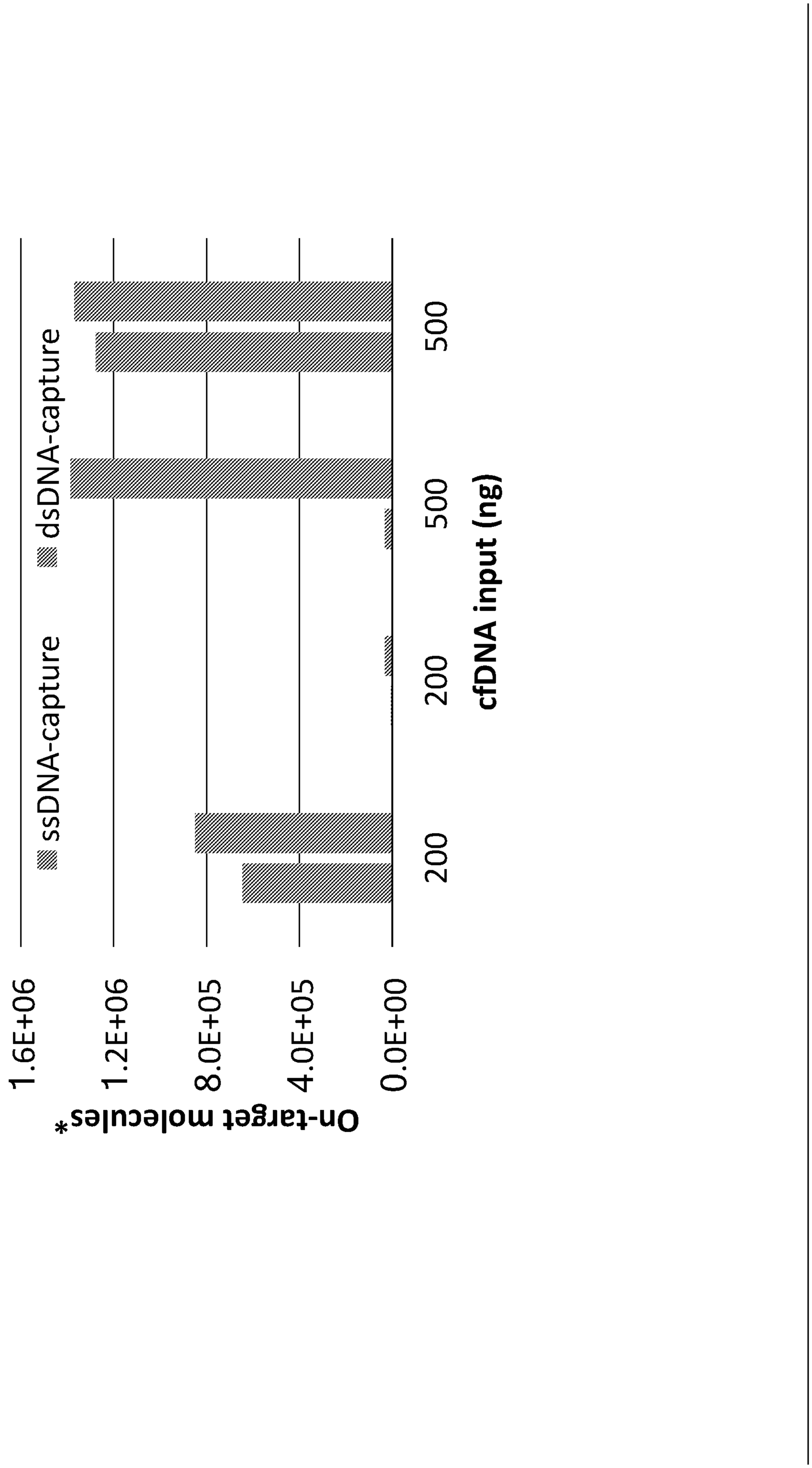

dsDNA-capture
ssDNA-capture
1.6E+06
1.2E+06
8.0E+05
4.0E+05
0.0E+00
On-target molecules*
200
500
200
500
cfDNA input (ng)

FIG. 21

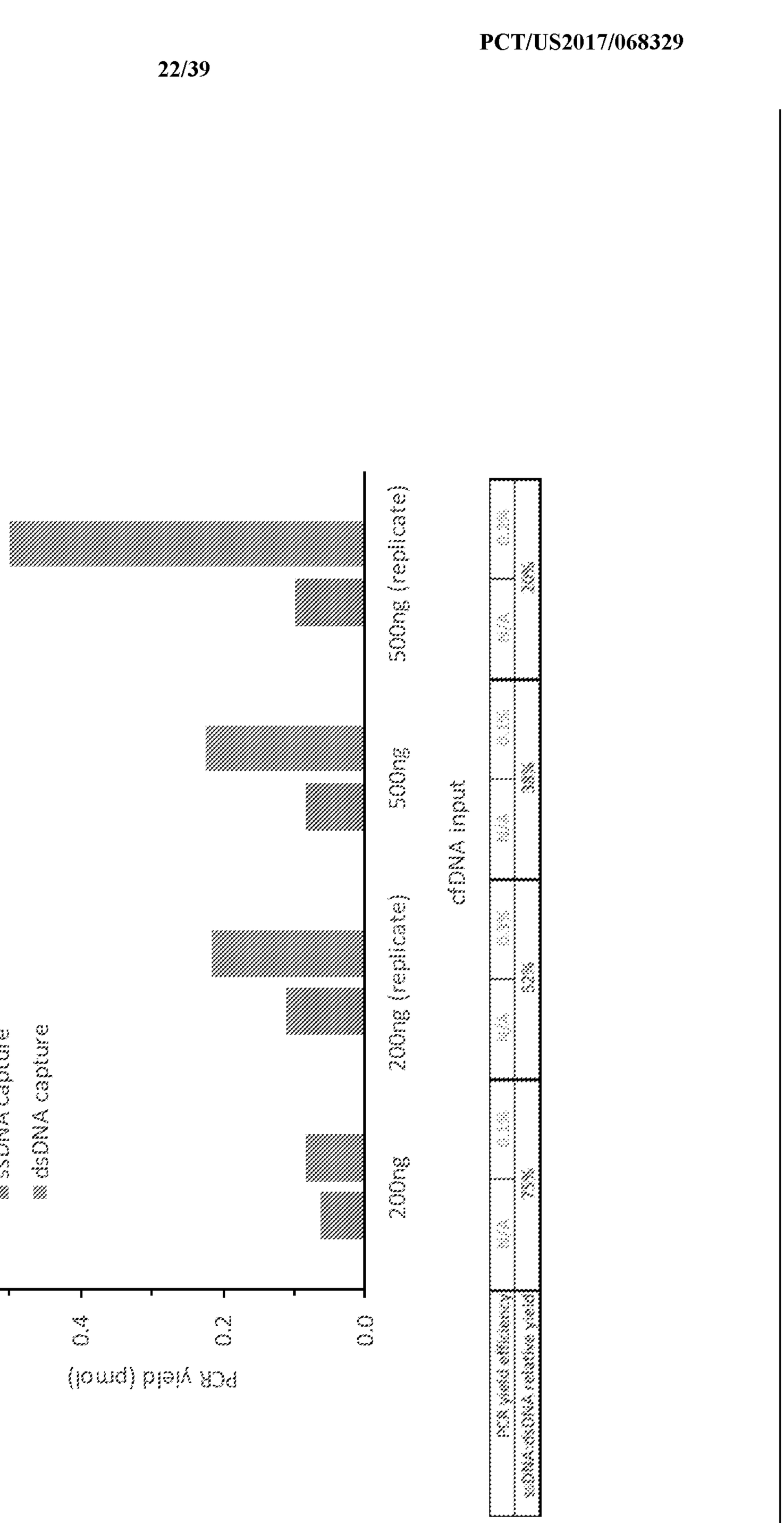
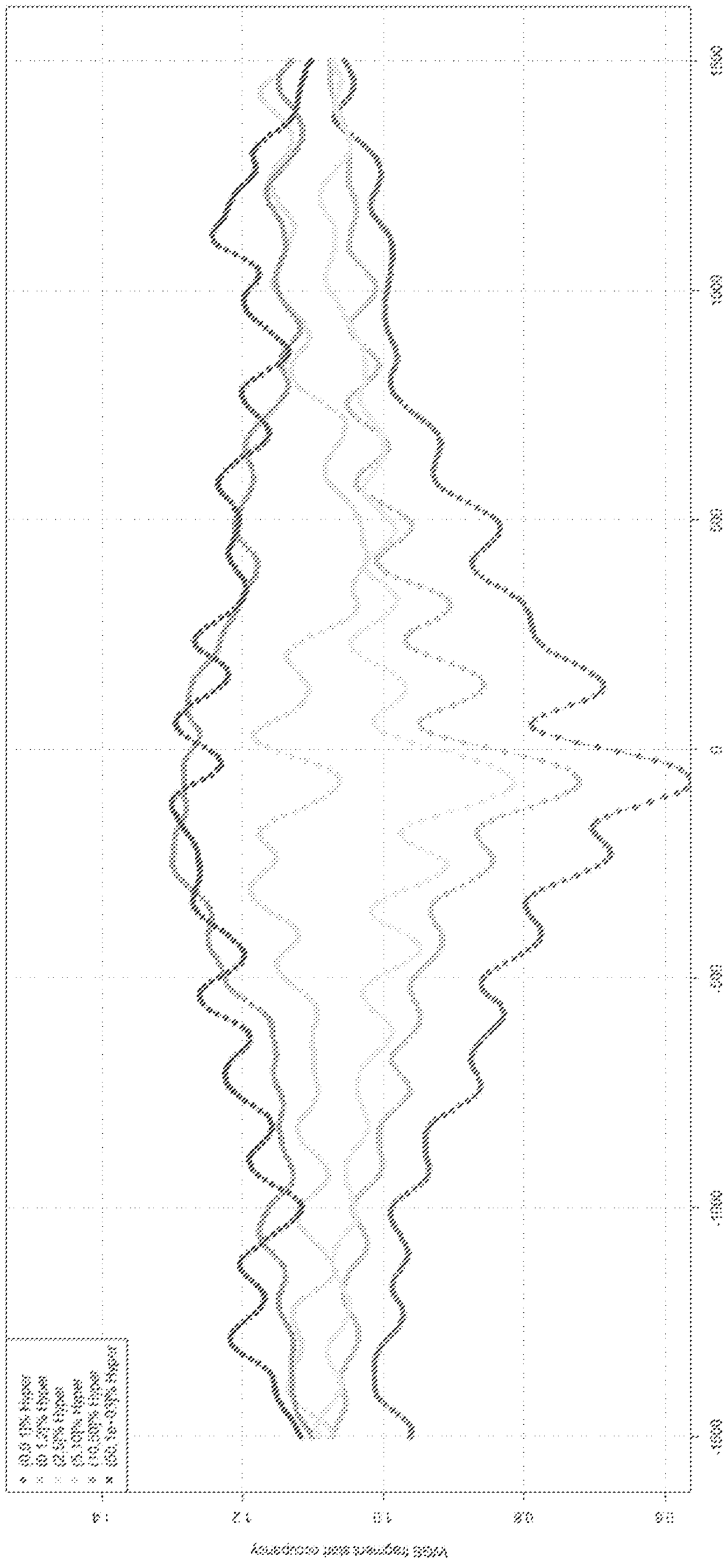

PCR Yield (pmol)
0.0
0.2
0.4
0.6
ssDNA capture
dsDNA capture
200ng
200ng (replicate)
500ng
500ng (replicate)
cfDNA input
PCR yield comparison
ssDNA capture
dsDNA capture

FIG. 22


Wide sagittal slot aperture
Wide sagittal slot aperture
Wide sagittal slot aperture

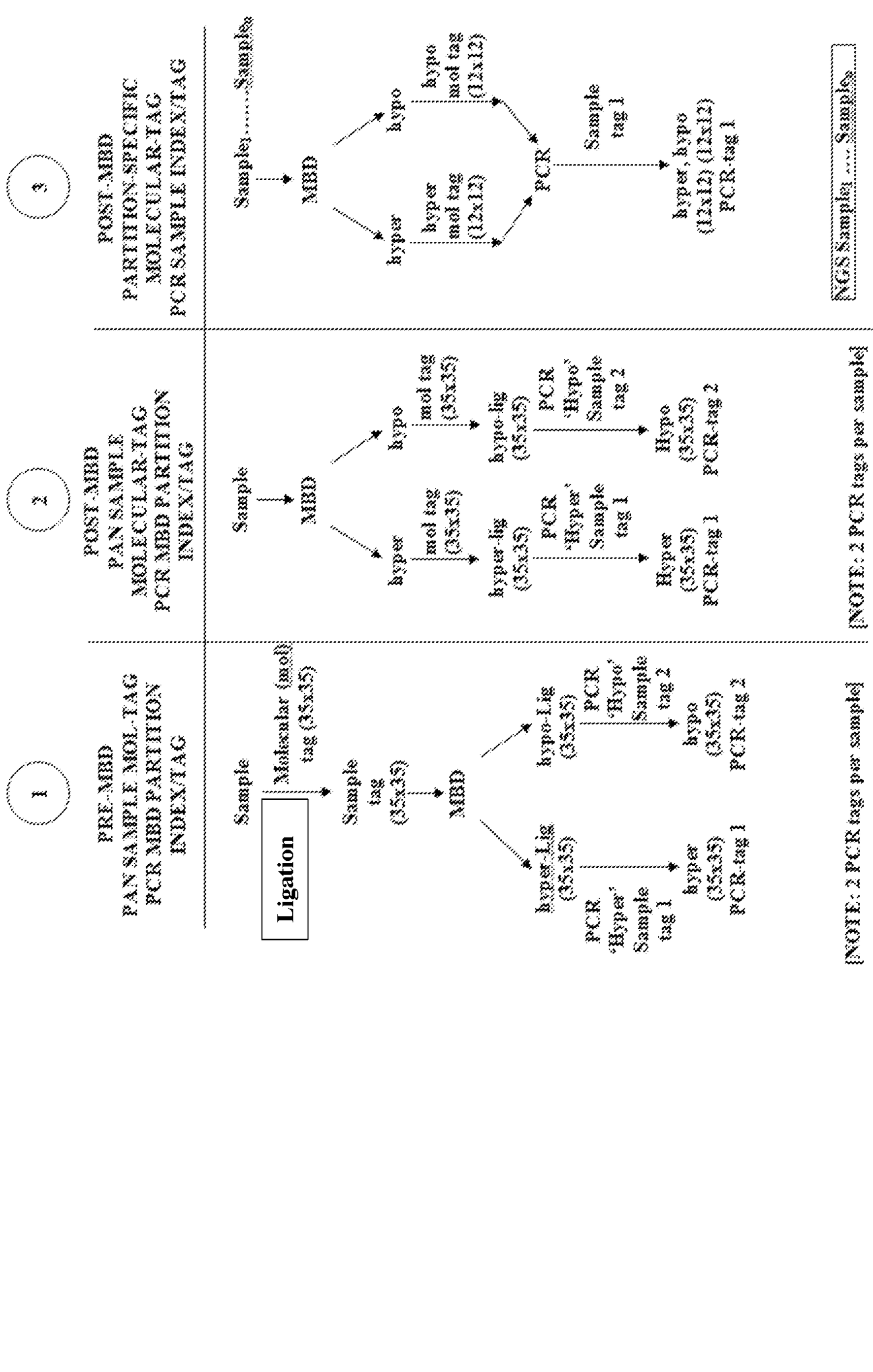

1
PRE-MBD
PAN-SAMPLE MOL-TAG
PCR-MBD PARTITION
INDEX-TAG
Sample
Molecular (well) tag (5x15)
Ligation
Sample tag (5x15)
MBD
mut-Lig (5x15)
PCR "Hyper" Sample tag 1
Hyper (5x15)
PCR-tag 1
wild-Lig (5x15)
PCR "Hyper" Sample tag 2
wild (5x15)
PCR-tag 2
2
POST-MBD
PAN-SAMPLE
MOLECULAR-TAG
PCR-MBD PARTITION
INDEX-TAG
Sample
MBD
wild
mut (5x15)
wild-Lig (5x15)
PCR "Hyper" Sample tag 2
wild (5x15)
PCR-tag 2
mut
mut-tag (5x15)
mut-Lig (5x15)
PCR "Hyper" Sample tag 1
Hyper (5x15)
PCR-tag 1
NOTE: 2 PCR tags per sample
3
PRE-MBD
PAN-SAMPLE
MOLECULAR-TAG
PCR-MBD PARTITION
INDEX-TAG
Sample
MBD
wild
wild-tag (5x15)
wild-tag (5x15)
PCR
mut
mut-tag (5x15)
mut-tag (5x15)
PCR
Sample tag 1
Hyper (5x15)
PCR-tag 1
4
POST-MBD
PARTITION-SPECIFIC
MOLECULAR-TAG
PCR SAMPLE INDEX-TAG
Sample
MBD
wild
wild-tag (5x15)
wild-tag (5x15)
PCR
mut
mut-tag (5x15)
mut-tag (5x15)
PCR
Sample tag 1
Hyper (5x15)
PCR-tag 1
Non-sample
NOTE: 2 PCR tags per sample

Fig. 24

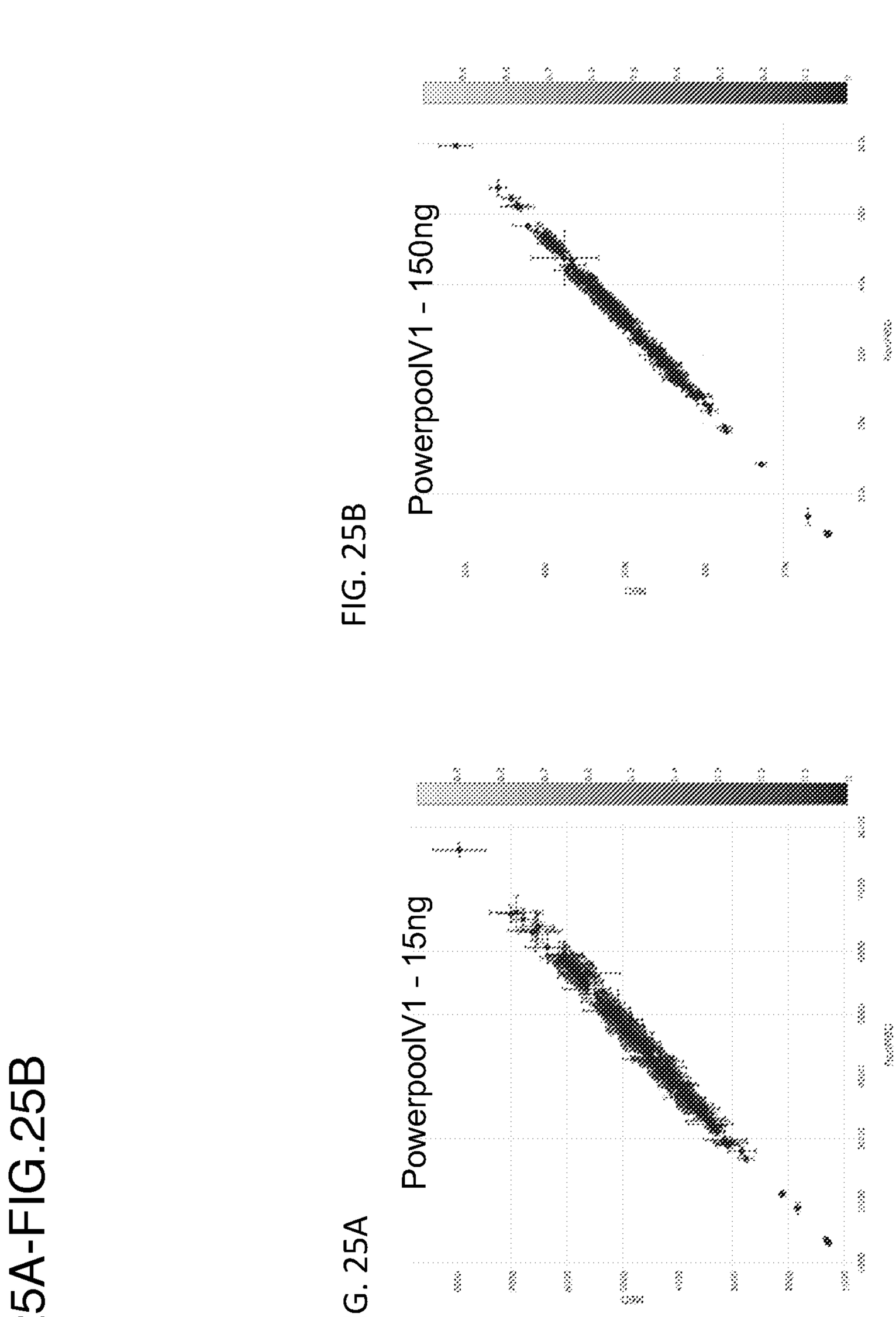


FIG. 25A

FIG. 25B

Fig. 25A-FIG. 25B

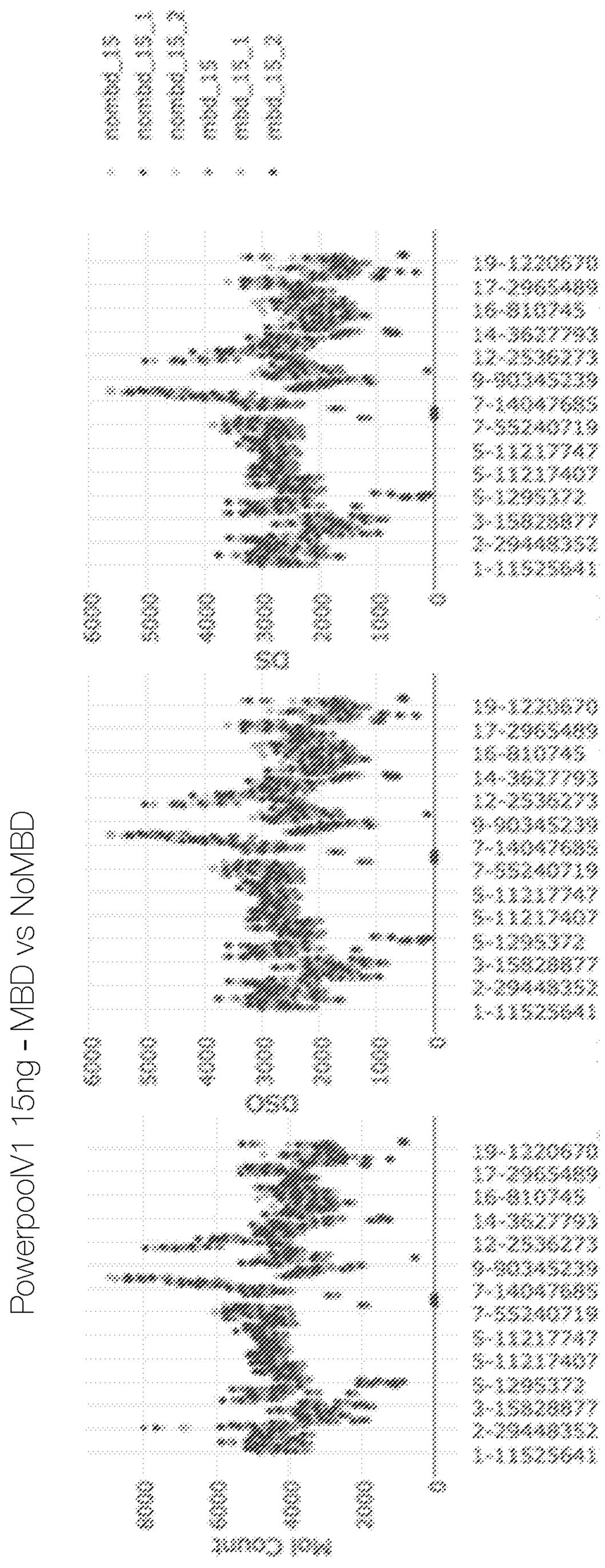

PowerpoolV1 15ng - MBD vs NoMBD
MBD ~ 94% of NoMBD coverage
19~1220670
17~2965489
16~810345
14~3627793
12~2536273
9~9034533
7~14047685
7~5524071
6~11217747
6~11217467
6~1295372
3~15828877
2~29448352
1~11525641
50
Mod Count

Fig. 26A

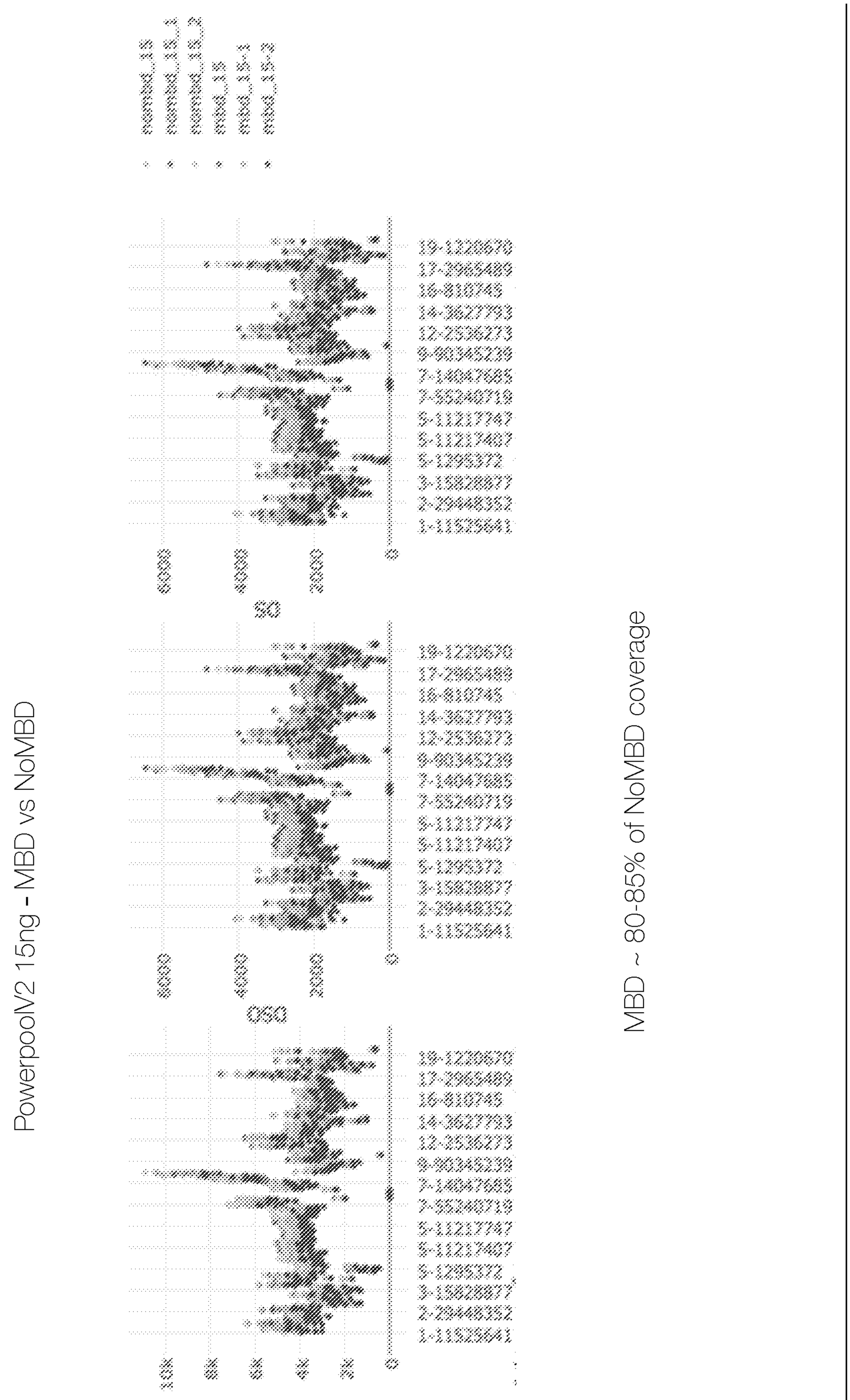

PowerpoolV2 15ng - MBD vs NoMBD
MBD ~ 80-85% of NoMBD coverage
19-1220670
17-2965489
16-810745
14-3627793
12-2536273
9-60345239
7-14047685
7-55240719
5-11217747
5-11217407
5-1295372
3-15828877
2-29448352
1-11525641
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
Mb Cover

Fig. 26B

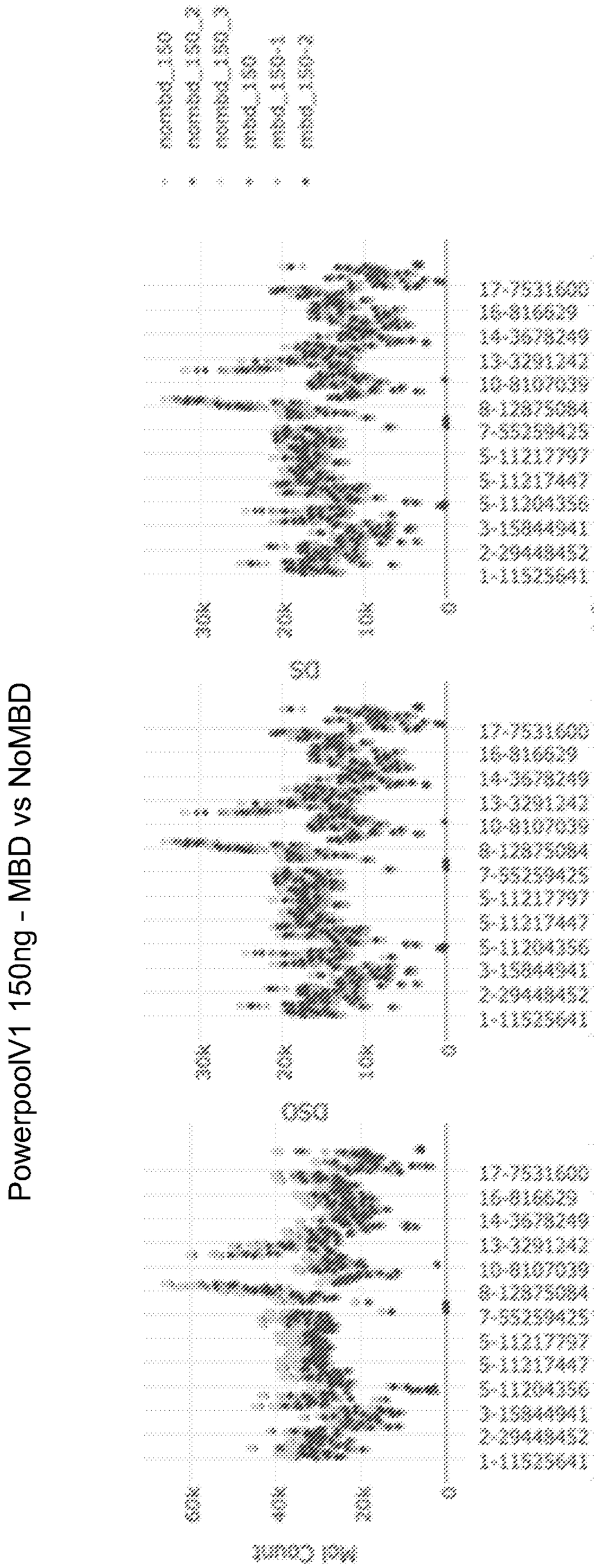
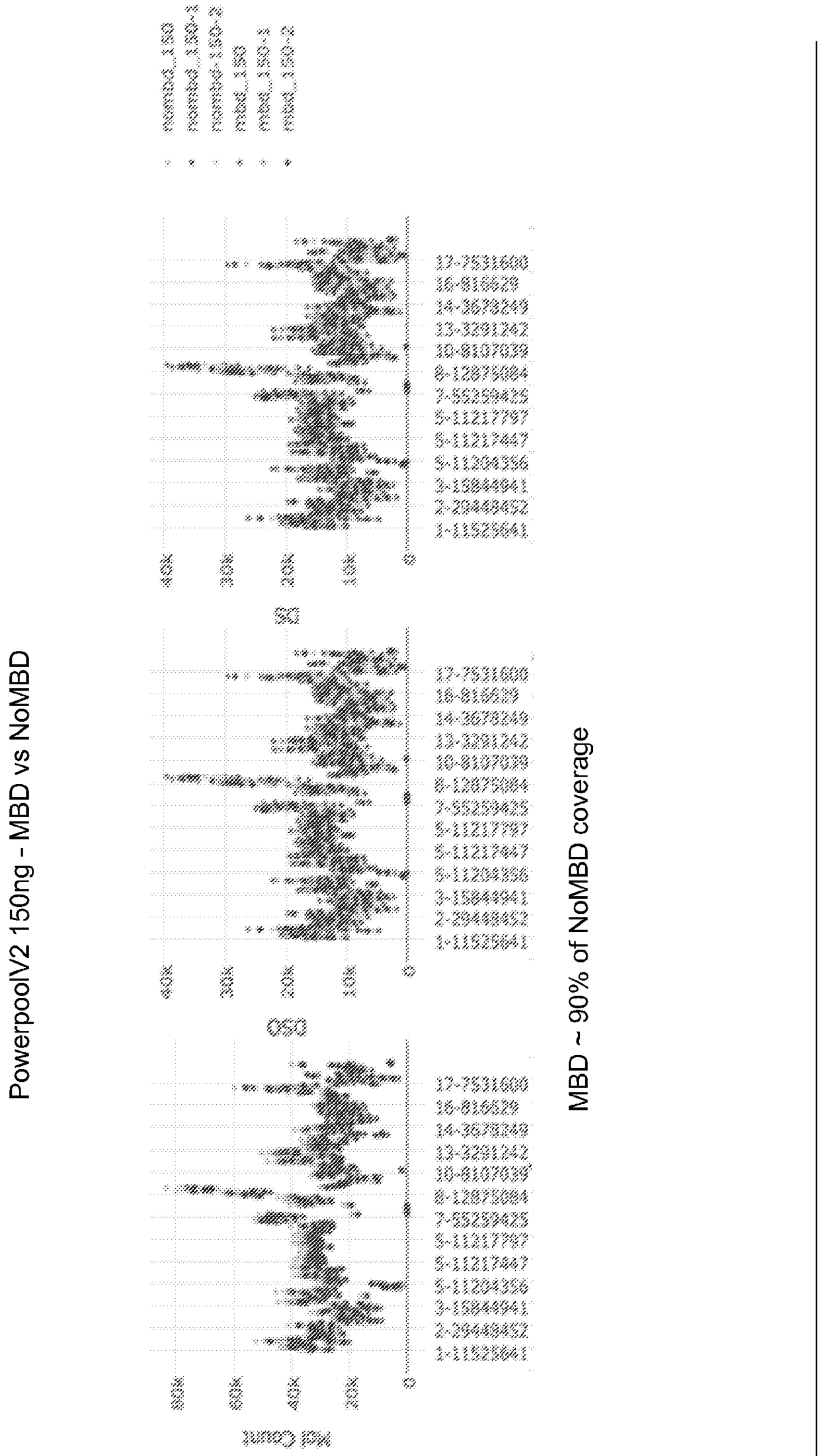

PowerpoolV1 150ng - MBD vs NoMBD
MBD ~ 80-85% of NoMBD coverage
Mid Count
17-7531600
16-816629
14-3678249
13-3291343
10-8107039
8-12875084
7-55259425
5-11217797
5-11317447
5-11204356
3-15844941
2-29448452
1-11525641

Fig. 27A


PowerpoolV2 150ng - MBD vs NoMBD
MBD ~ 90% of NoMBD coverage
Fig. 27B
Avg Count
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
620
640
660
680
700
720
740
760
780
800
820
840
860
880
900
920
940
960
980
1000
1020
1040
1060
1080
1100
1120
1140
1160
1180
1200
1220
1240
1260
1280
1300
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1380
1400
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1440
1460
1480
1500
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1600
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1900
1920
1940
1960
1980
2000
2020
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7000
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7180
7200
7220
7240
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8000
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8080
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14-36778249
13-32912242
10-81070339
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7-55259425
5-11217797
5-11217647
5-11204356
3-15044941
2-29448452
1-11525641

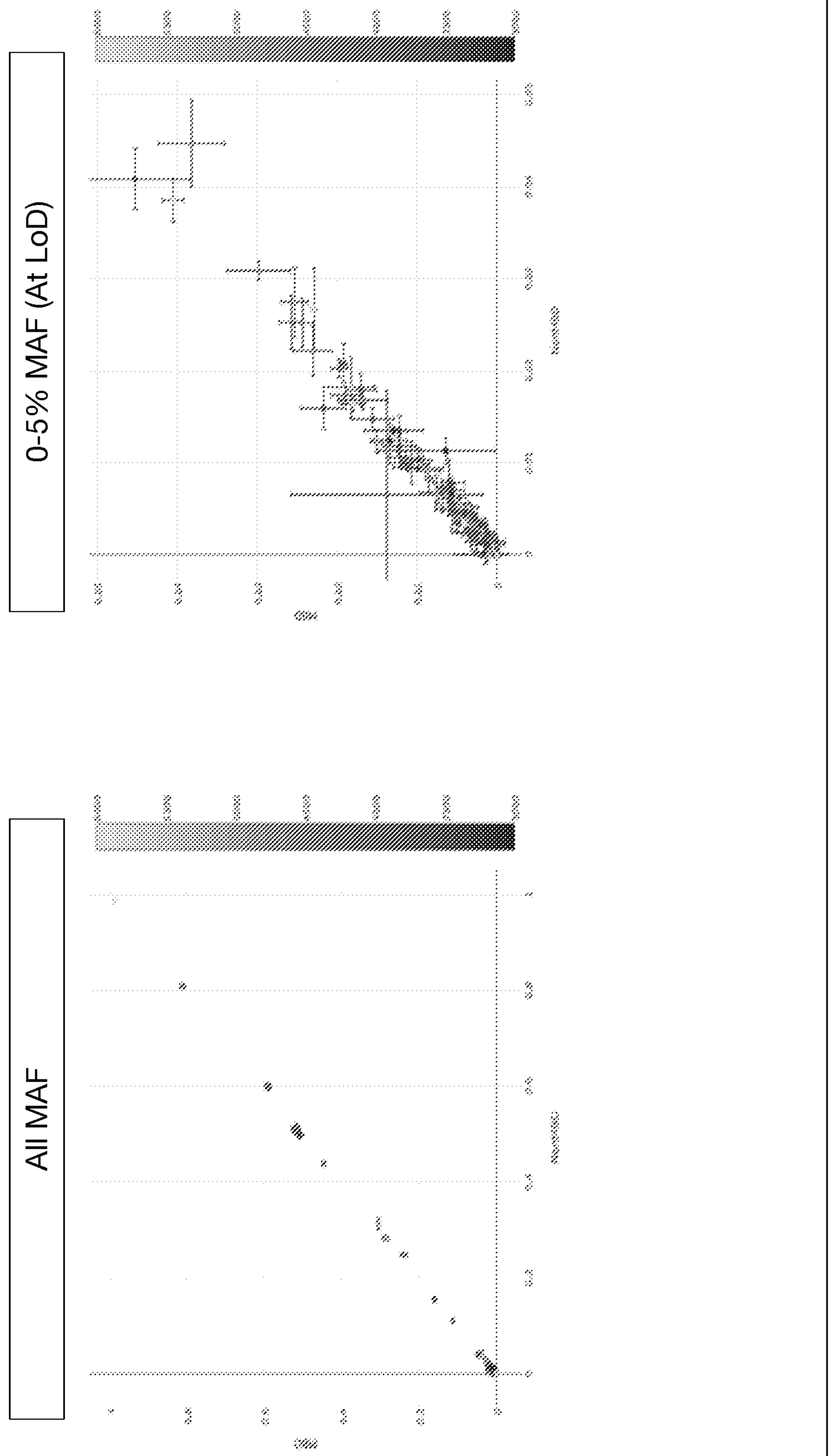


FIG. 28A FIG. 28B

Fig. 28A-FIG.28B

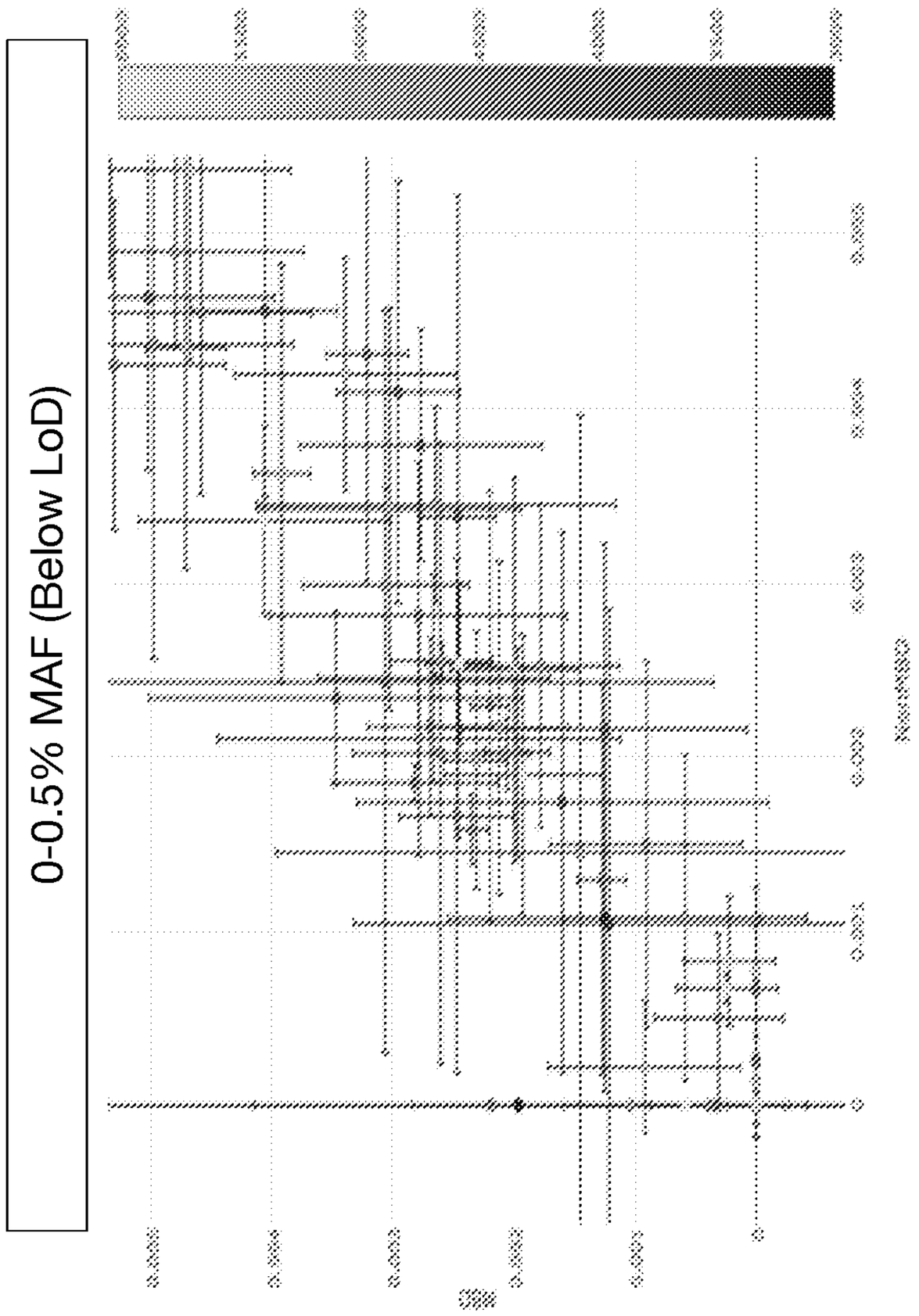


Fig. 28C

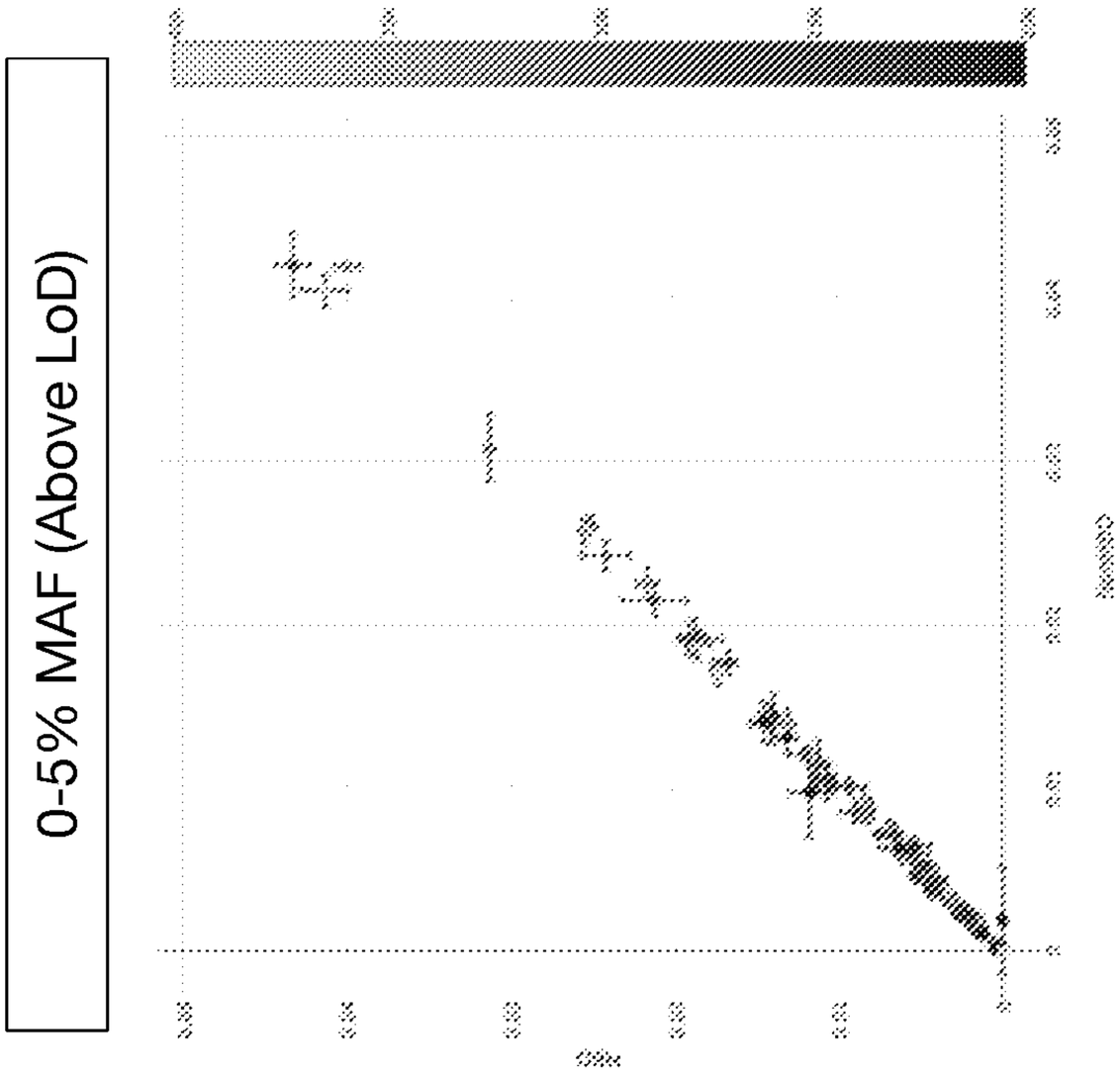


FIG. 29A

FIG. 29B

Fig. 29A-FIG.29B

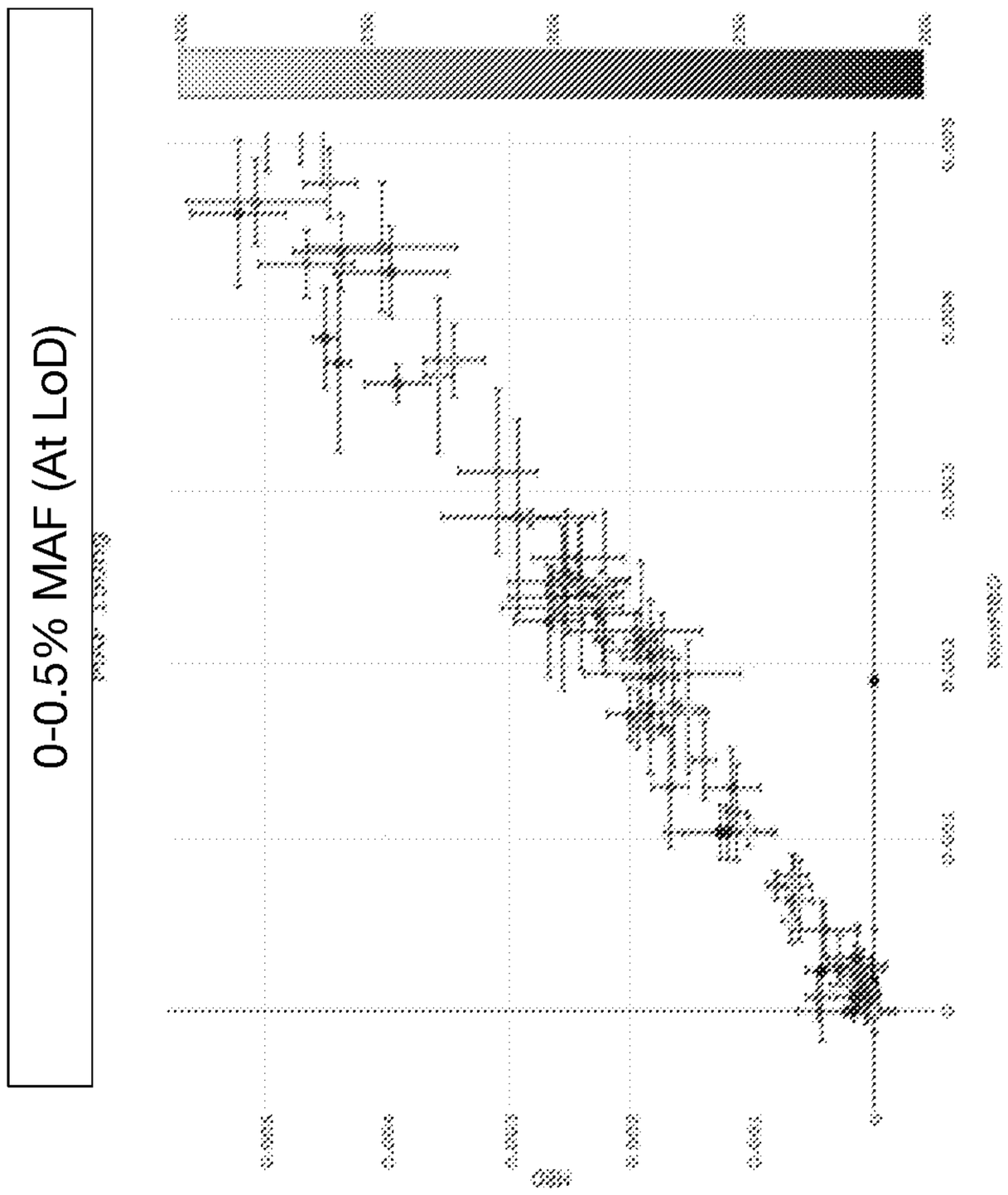


Fig. 29C

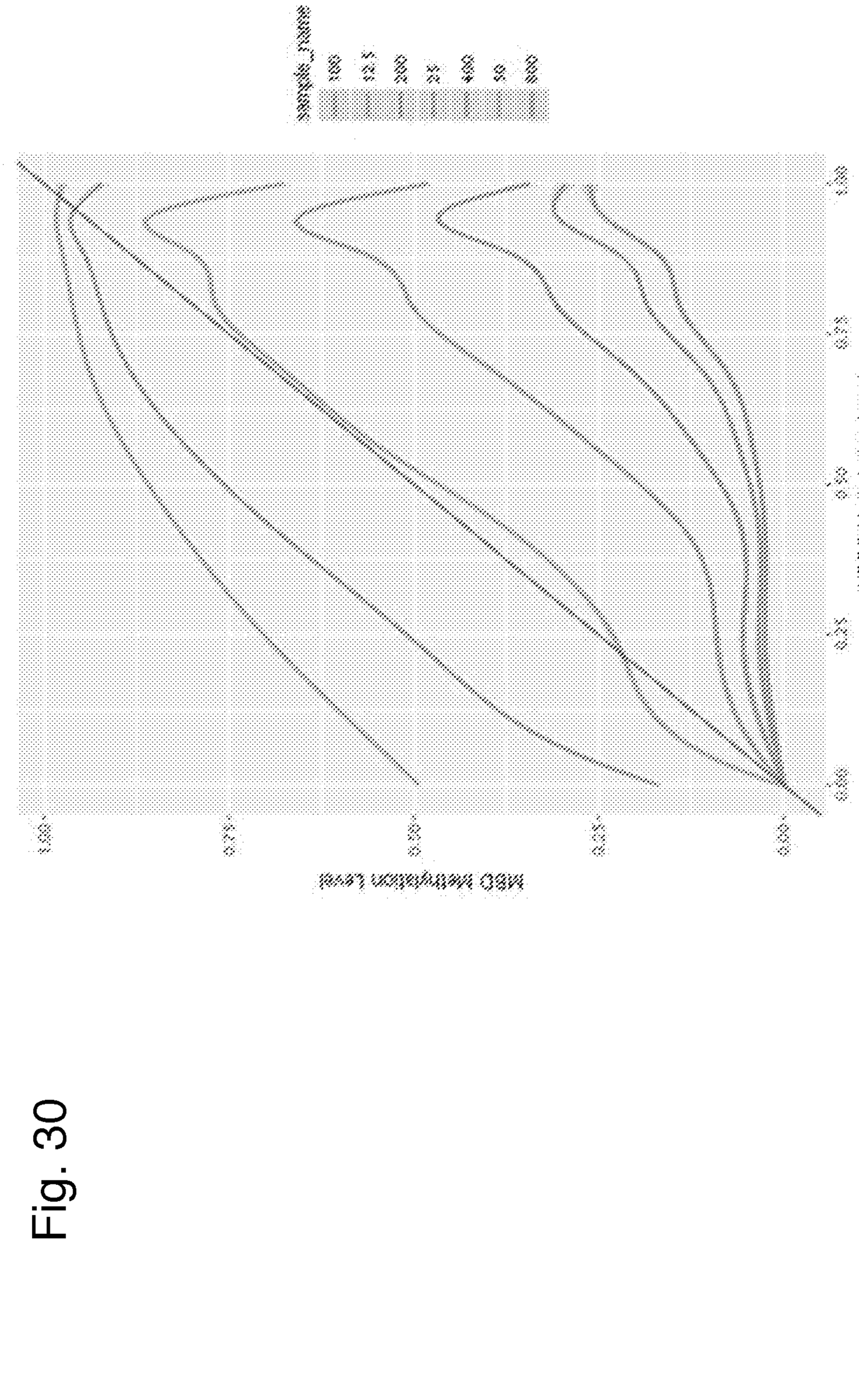


Fig. 30

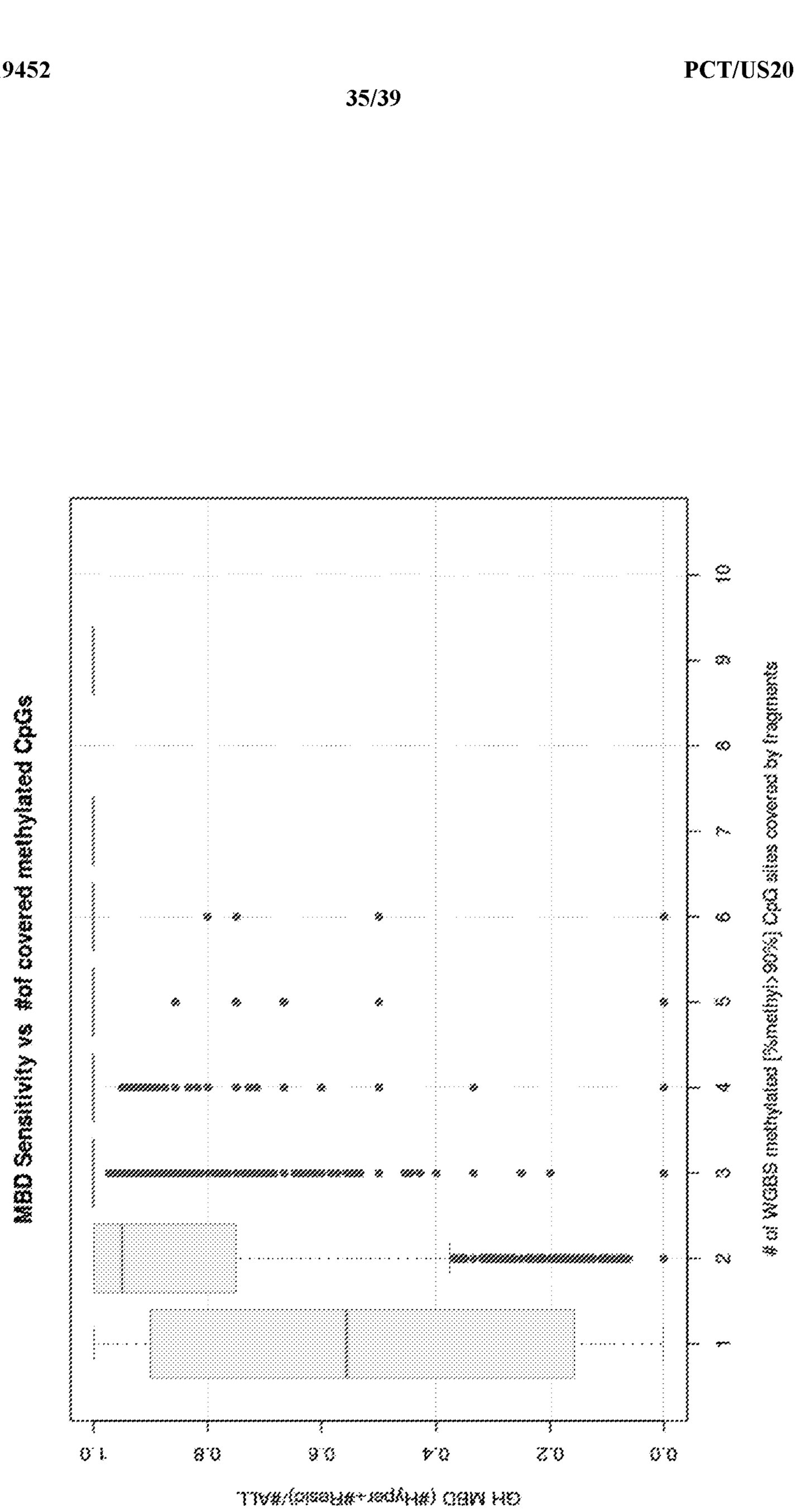

MBD Sensitivity vs # of covered methylated CpGs
# of WGBS methylated (5mC/all) CpG sites covered by fragments
Cell MBD (5mC+5hmC/all)
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
7
8
9
10

Fig. 31A

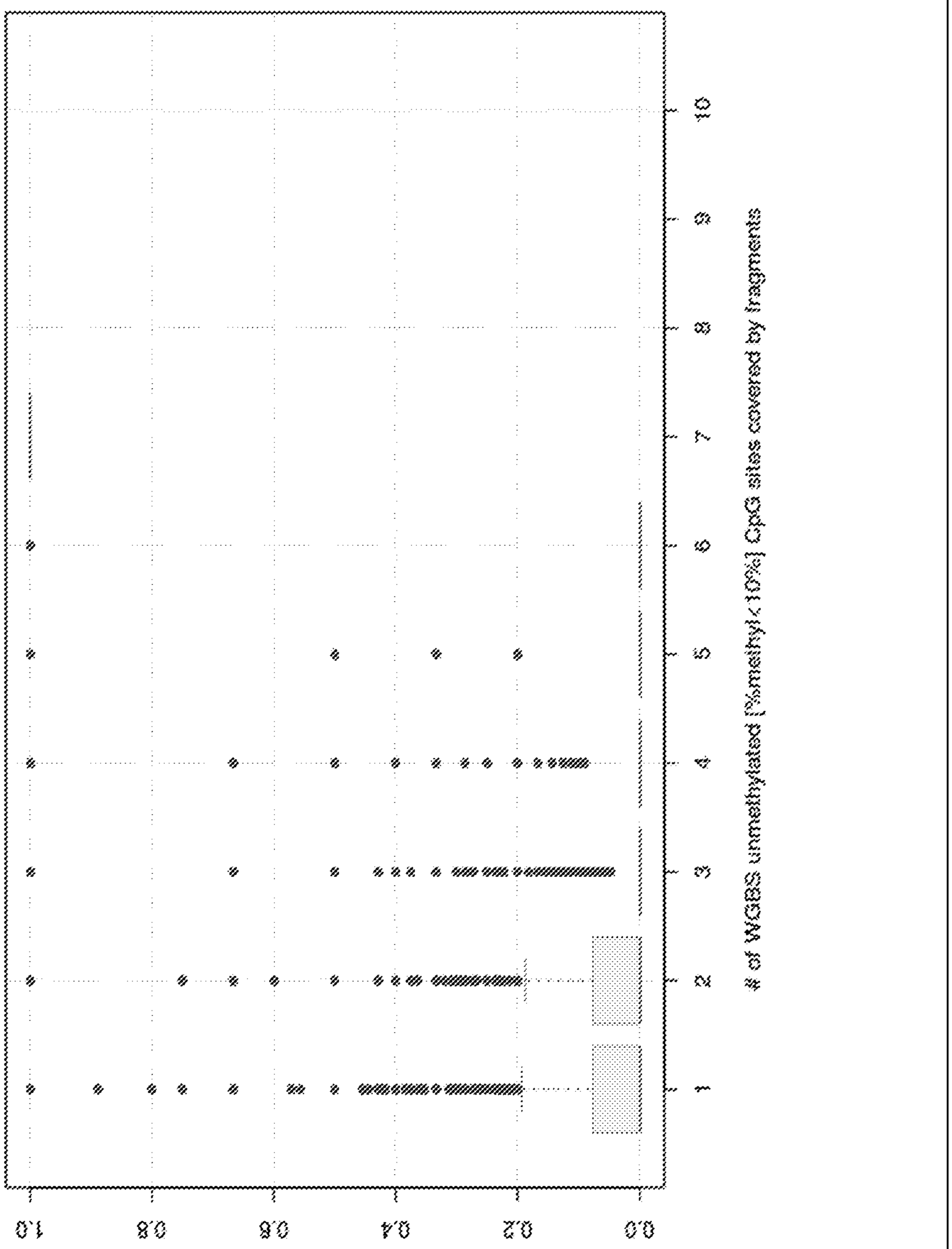

MBD Specificity vs. #of covered unmethylated CpGs
GH MBD (#Hyper+#Resid)/#ALL
0.0
0.2
0.4
0.6
0.8
1.0
# of WGBS unmethylated [%methyl<10%] CpG sites covered by fragments
1
2
3
4
5
6
7
8
9
10

Fig. 31B

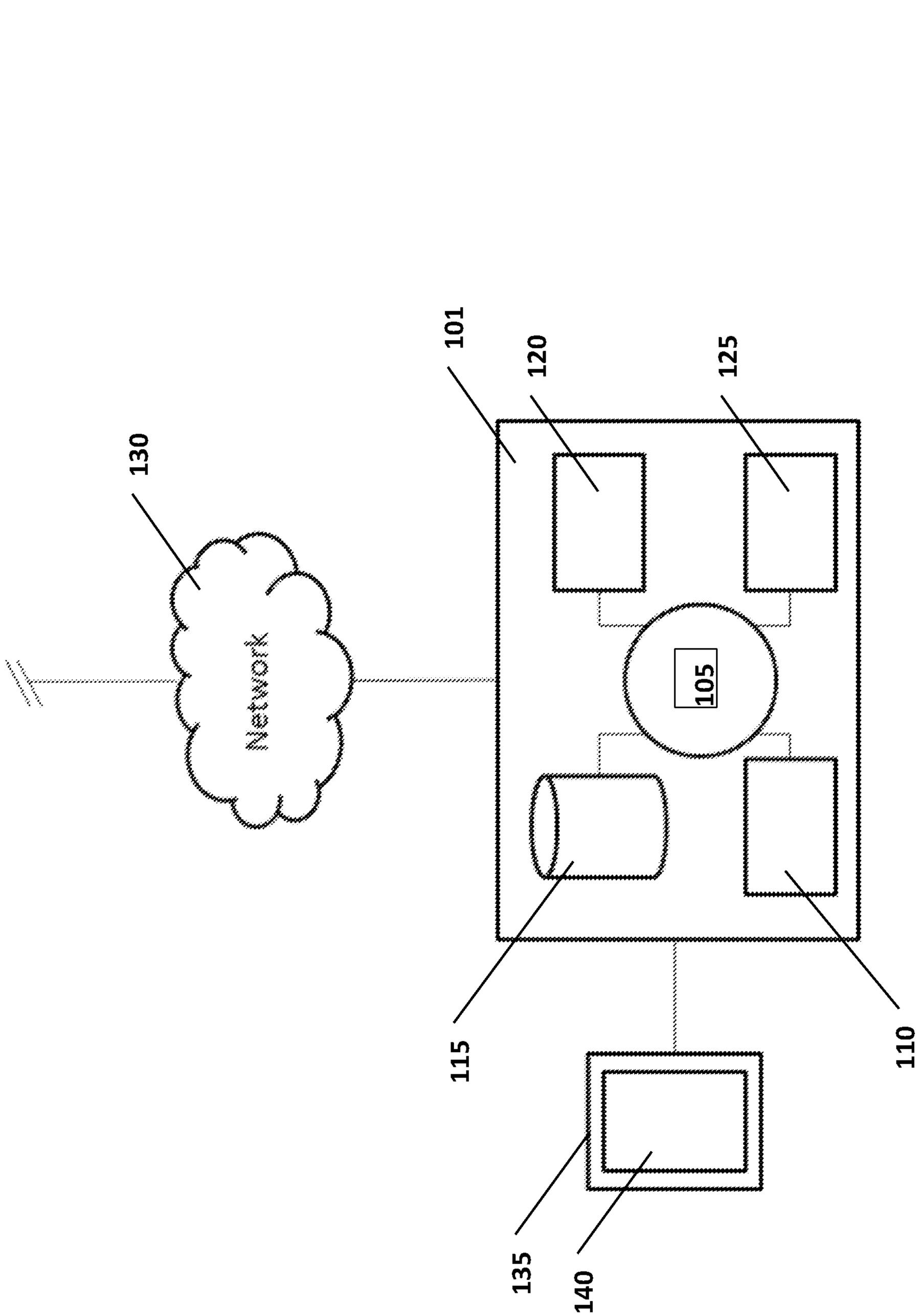


Fig. 32

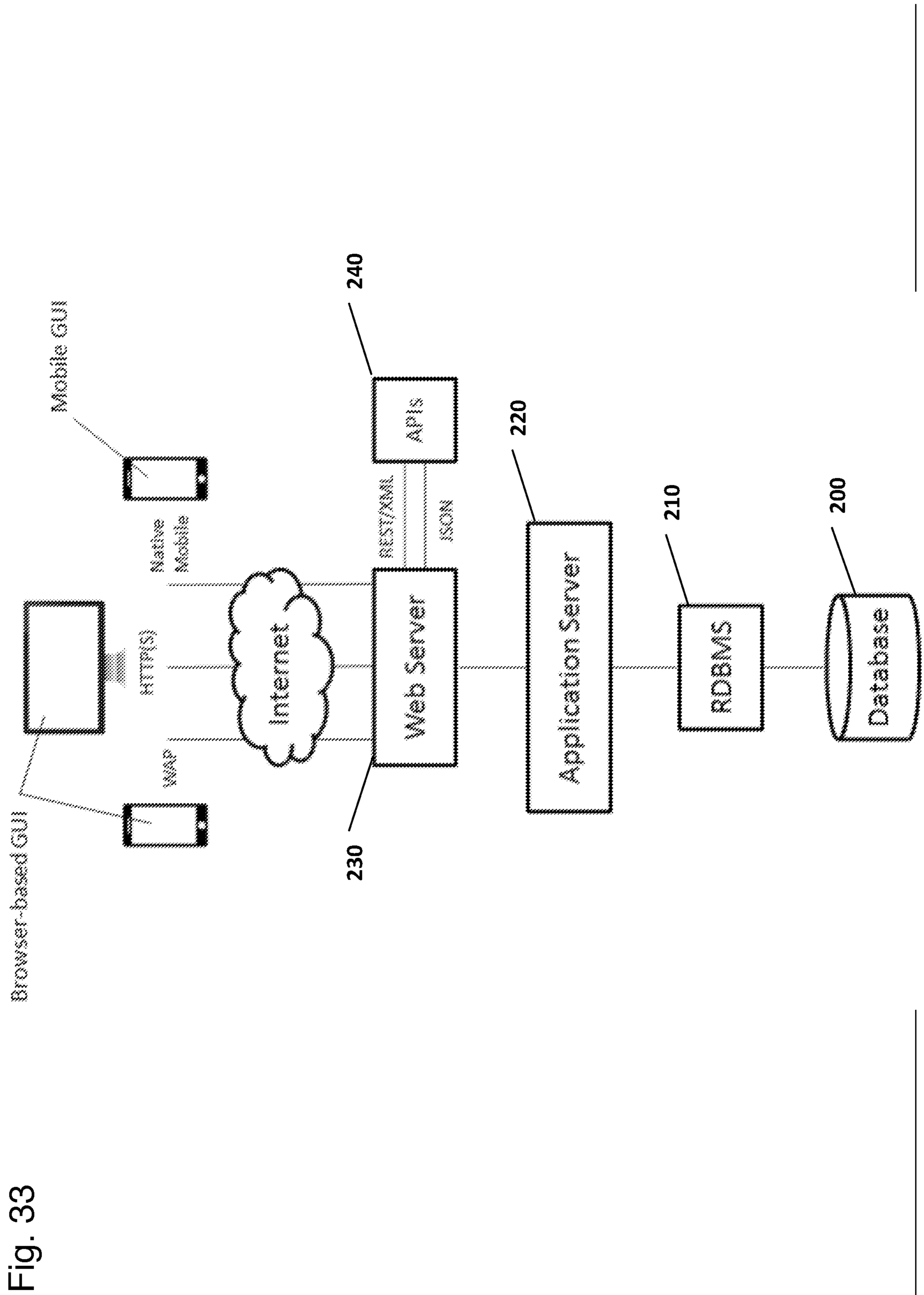

240
Mobile app
Mobile app
Internet
SMS
SMS
Browser-based app
Internet
Web Server
230
Application Server
220
eGenS
210
Database
200

Fig. 33

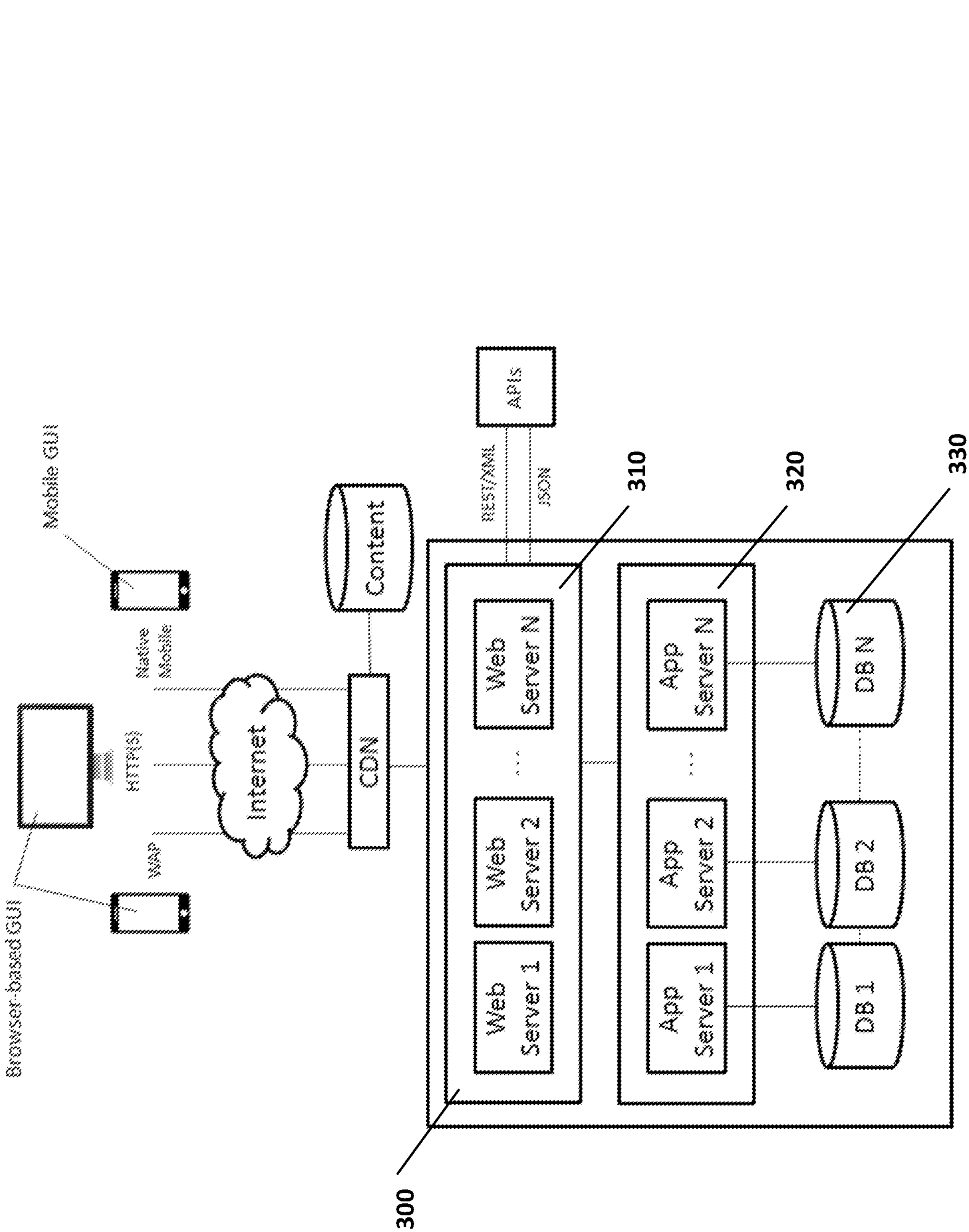

Mobile GUI
Native
Mobile
Content
REST/XML
JSON
APIs
310
320
330
Web Server N
App Server N
DB N
Web Server 2
App Server 2
DB 2
Web Server 1
App Server 1
DB 1
Internet
Native
Mobile
Browser-based GUI
Web
300

Fig. 34