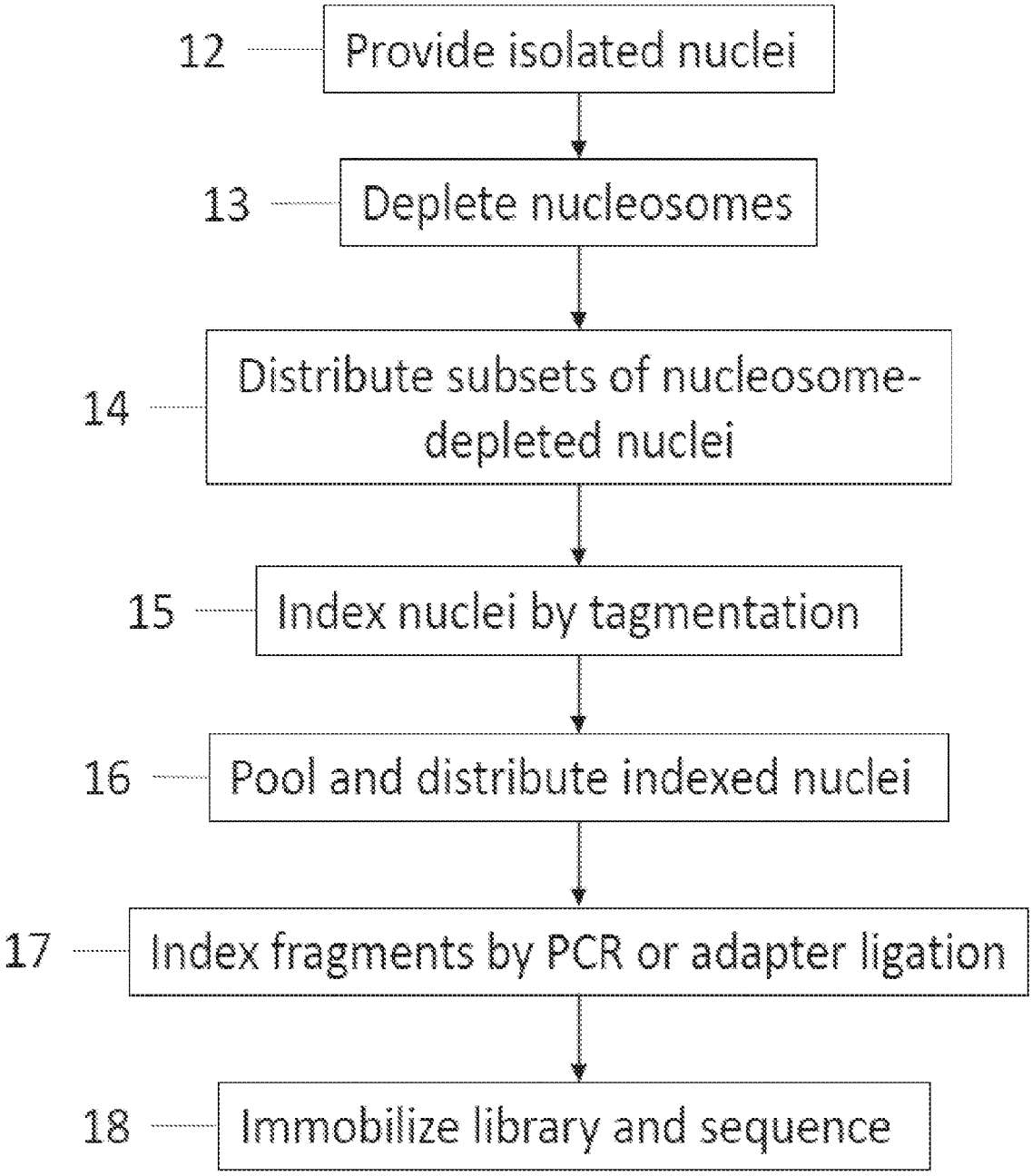

12
Provide isolated nuclei
13
Deplete nucleosomes
14
Distribute subsets of nucleosome-depleted nuclei
15
Index nuclei by tagmentation
16
Pool and distribute indexed nuclei
17
Index fragments by PCR or adapter ligation
18
Immobilize library and sequence

FIG. 1

FIG. 2

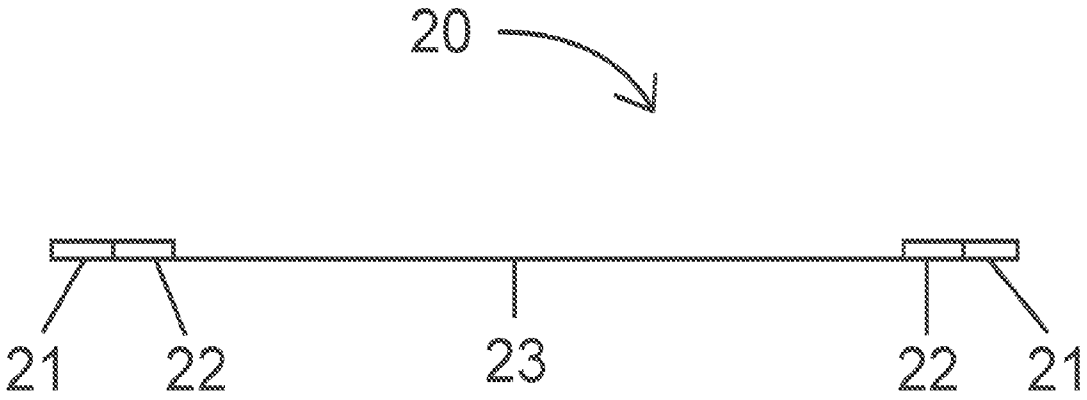


FIG. 3

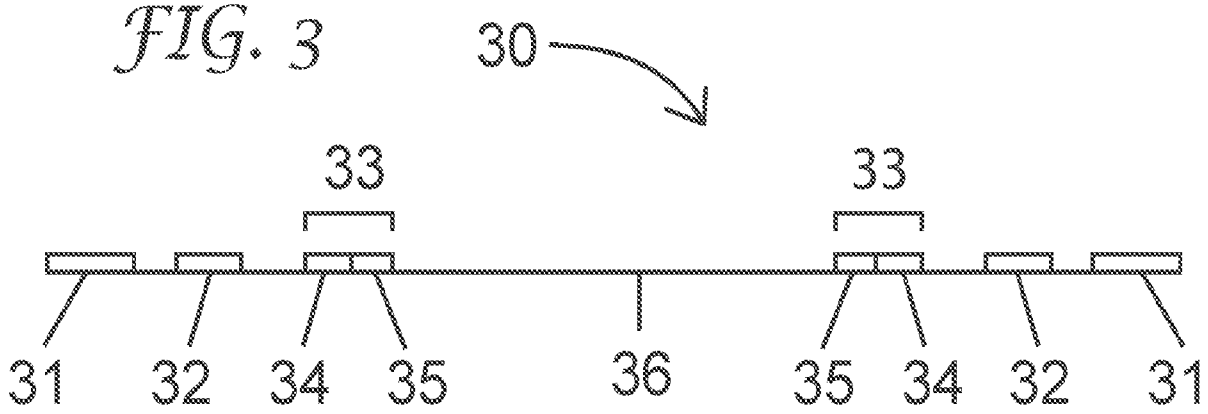


FIG. 4A

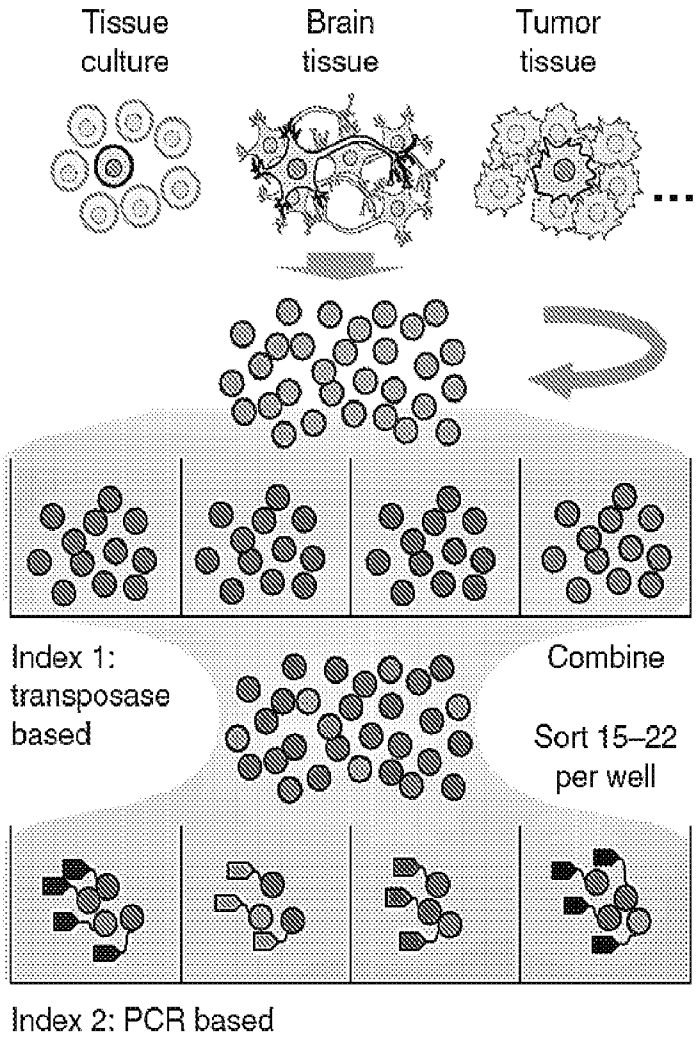
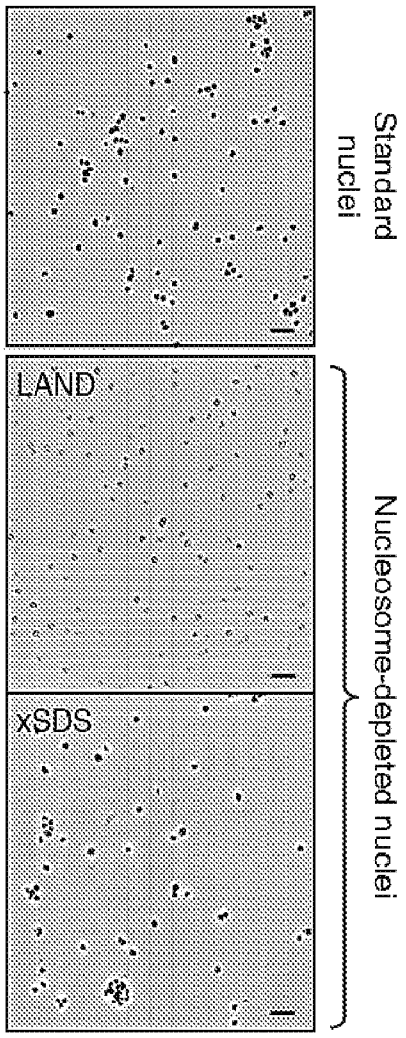

Tissue culture
Brain tissue
Tumor tissue
Index 1: transposase based
Combine
Sort 15–22 per well
Index 2: PCR based

FIG. 4B


Standard nuclei
LAND
xSDS
Nucleosome-depleted nuclei

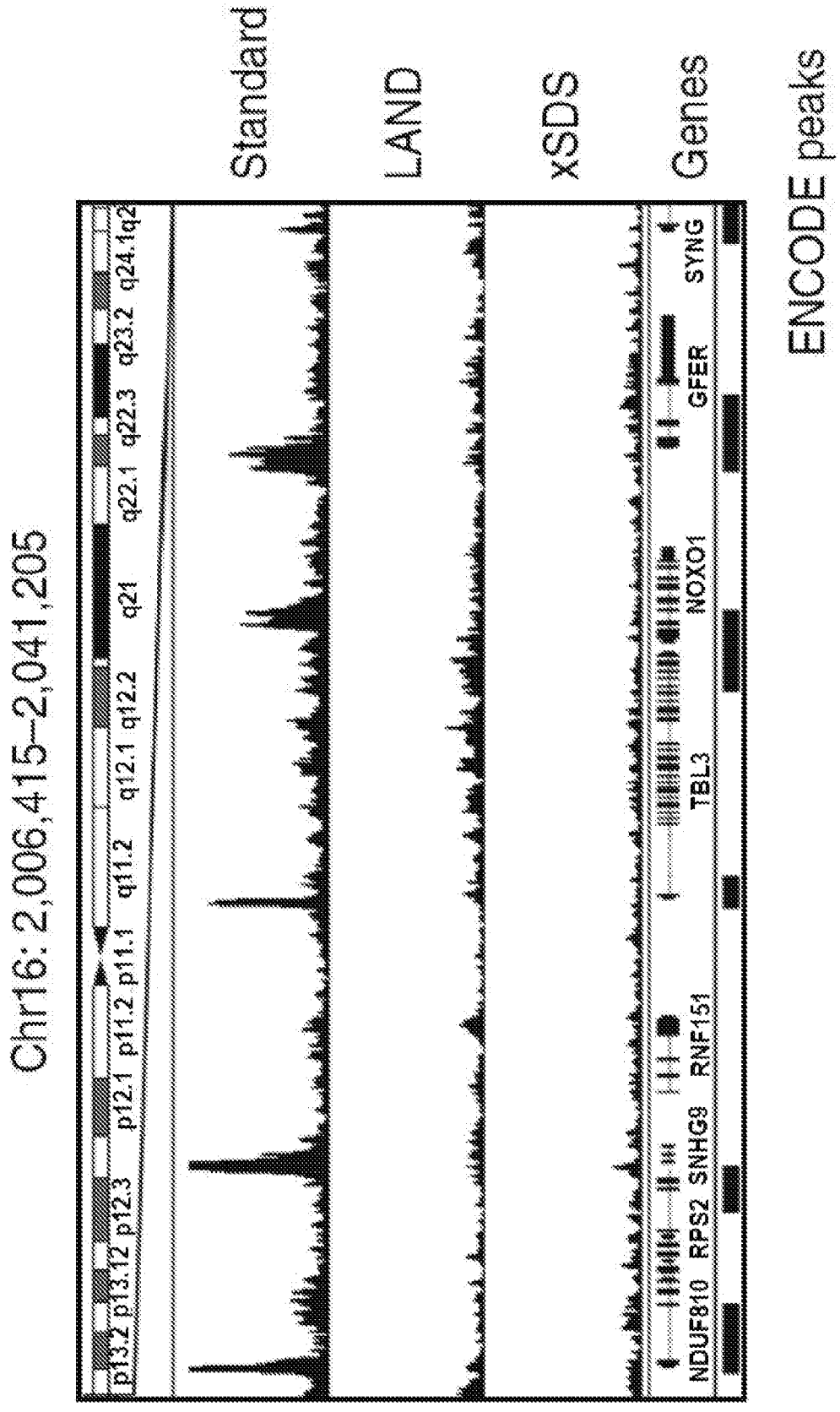

Chr16: 2,006,415–2,041,205
p13.2 p13.12 p12.3 p12.1 p11.2 p11.1 q11.2 q12.1 q12.2 q21 q22.1 q22.3 q23.2 q24.1q2
Standard
LAND
xSDS
Genes
ENCODE peaks
NDUFB10 RPS2 SNHG8 RNF151 TBL3 NOXO1 GFER SYNG

FIG. 4C

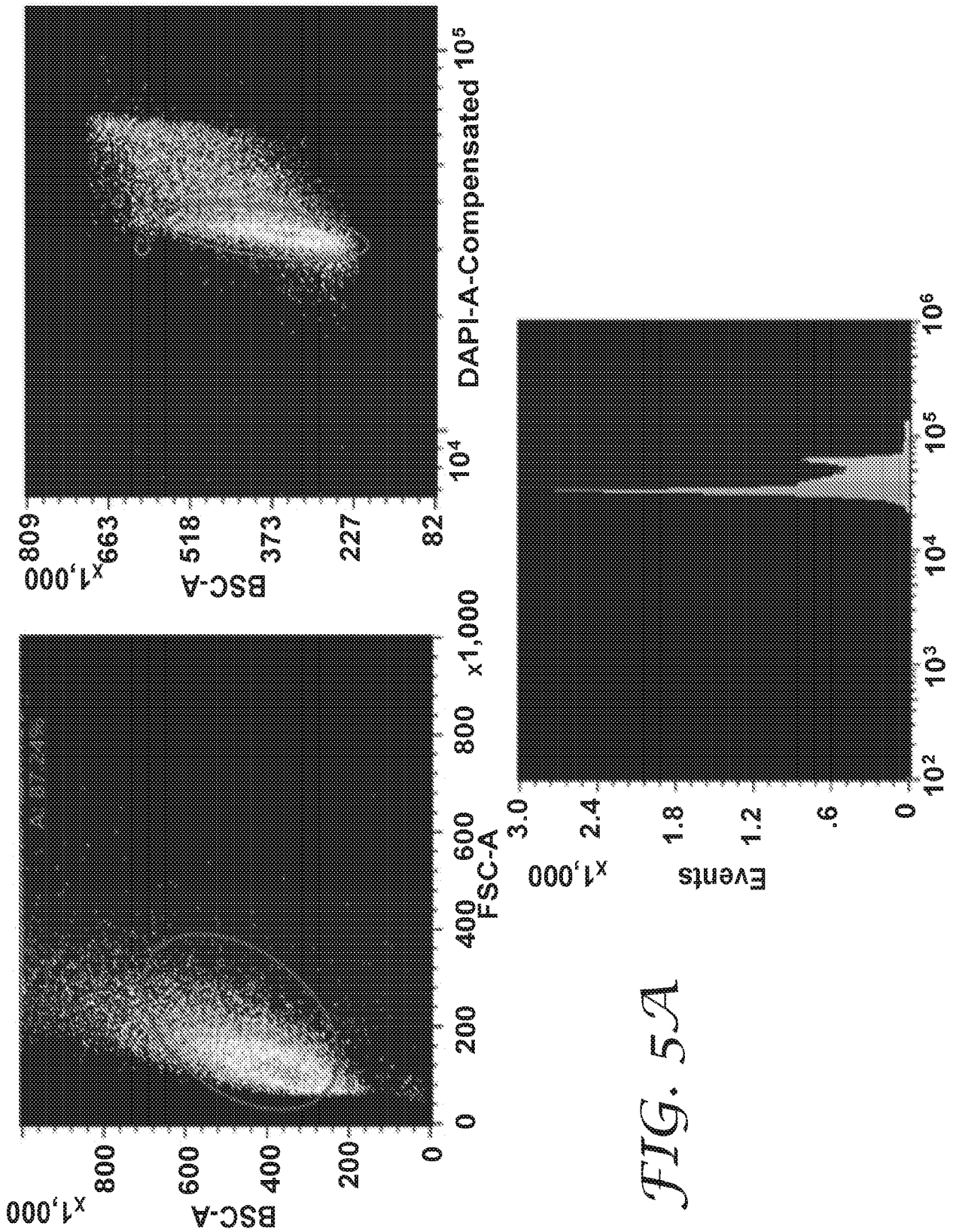

BSC-A x1,000
800
600
400
200
0
0
200
400
600
800
x1,000
FSC-A
BSC-A x1,000
809
663
518
373
227
82
10^4
10^5
DAPI-A-Compensated
Events x1,000
3.0
2.4
1.8
1.2
.6
0
10^2
10^3
10^4
10^5
10^6

FIG. 5A

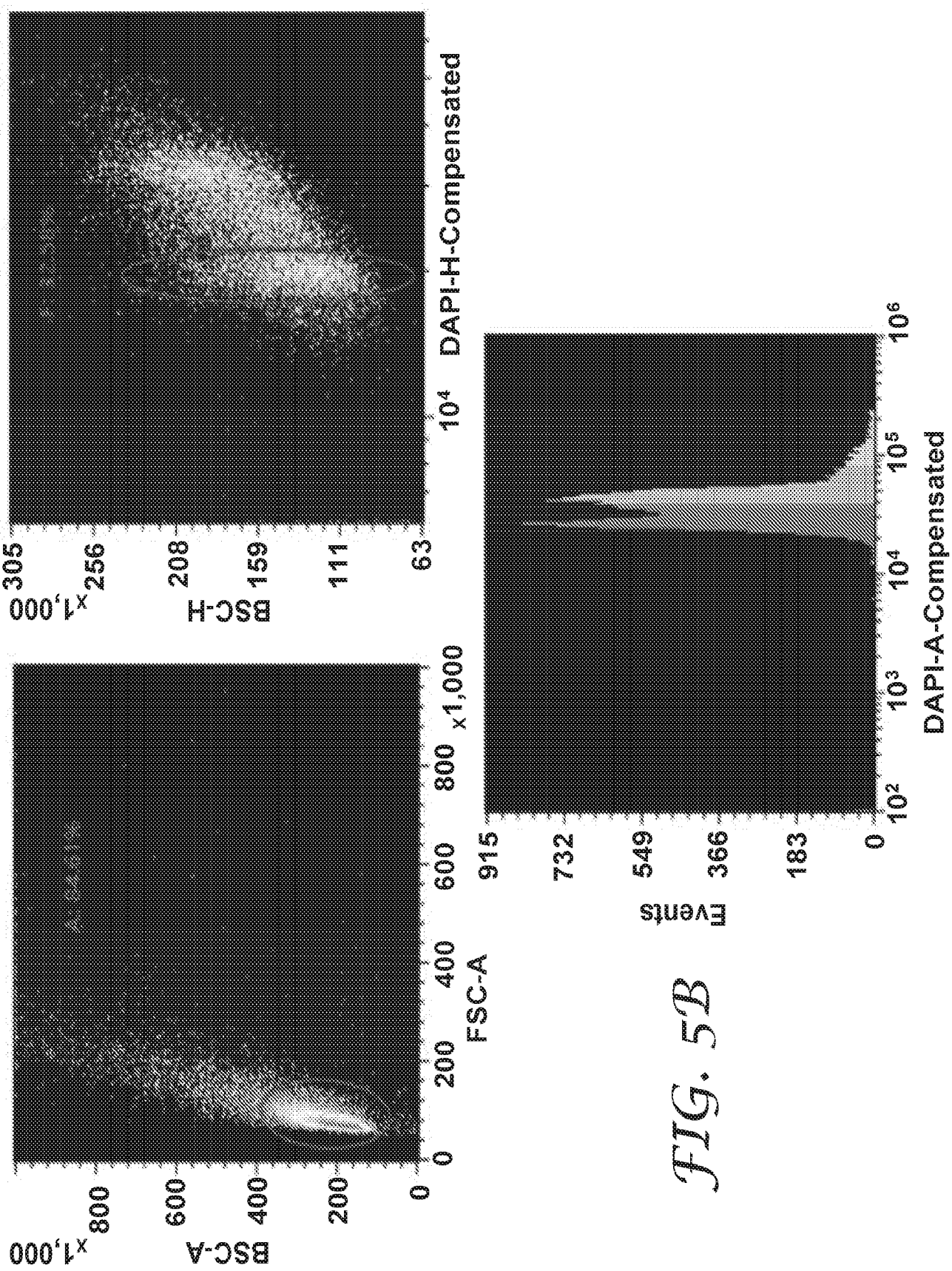

BSC-A
x1,000
800
600
400
200
0
0
200
400
600
800
x1,000
FSC-A
BSC-H
x1,000
305
256
208
159
111
63
10⁴
DAPI-H-Compensated
Events
915
732
549
366
183
0
10²
10³
10⁴
10⁵
10⁶
DAPI-A-Compensated

FIG. 5B

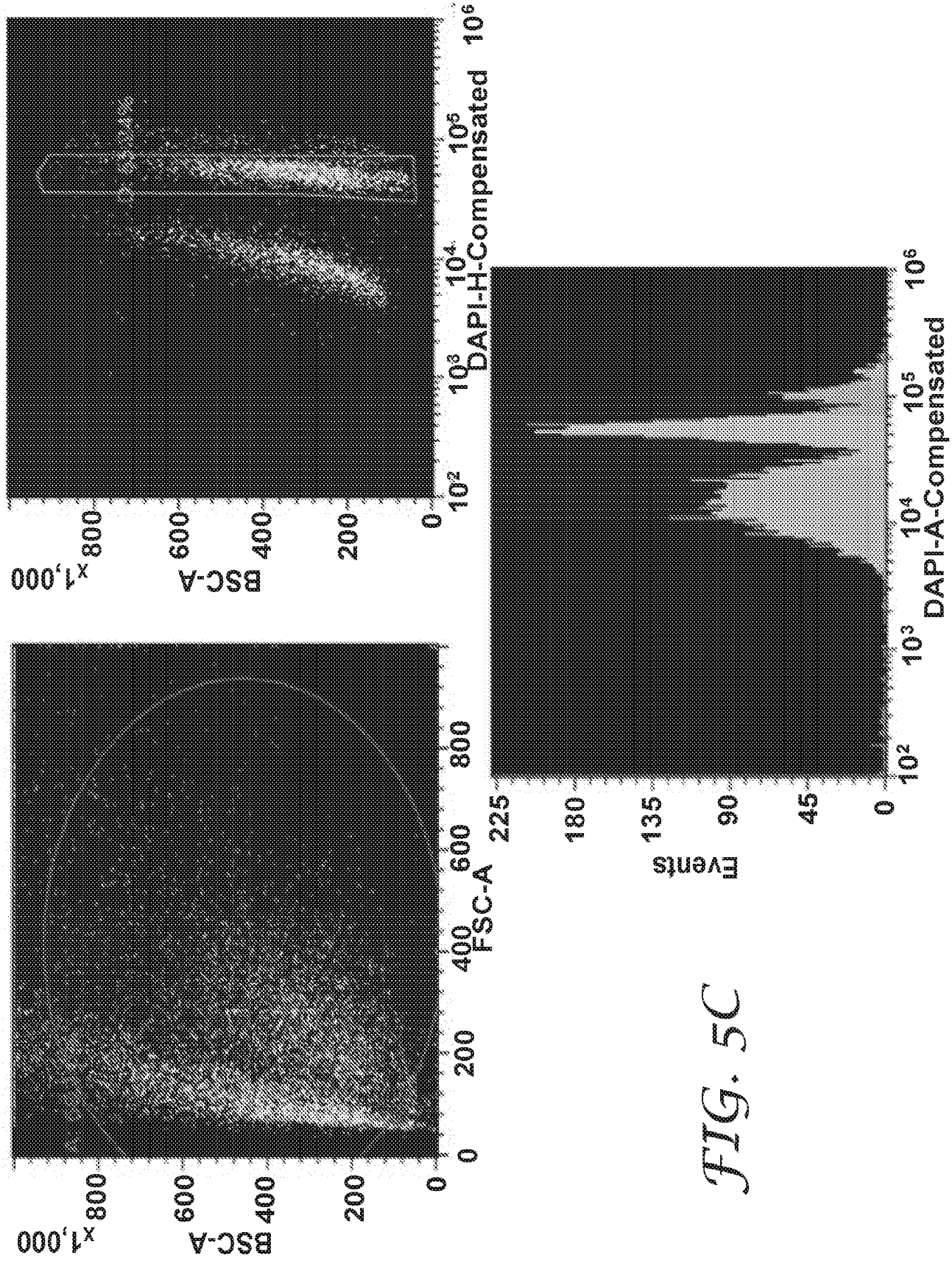

BSC-A
x1,000
800
600
400
200
0
FSC-A
0
200
400
600
800
DAPI-H-Compensated
10^2
10^3
10^4
10^5
10^6
Events
225
180
135
90
45
0
DAPI-A-Compensated
10^2
10^3
10^4
10^5
10^6

FIG. 5C

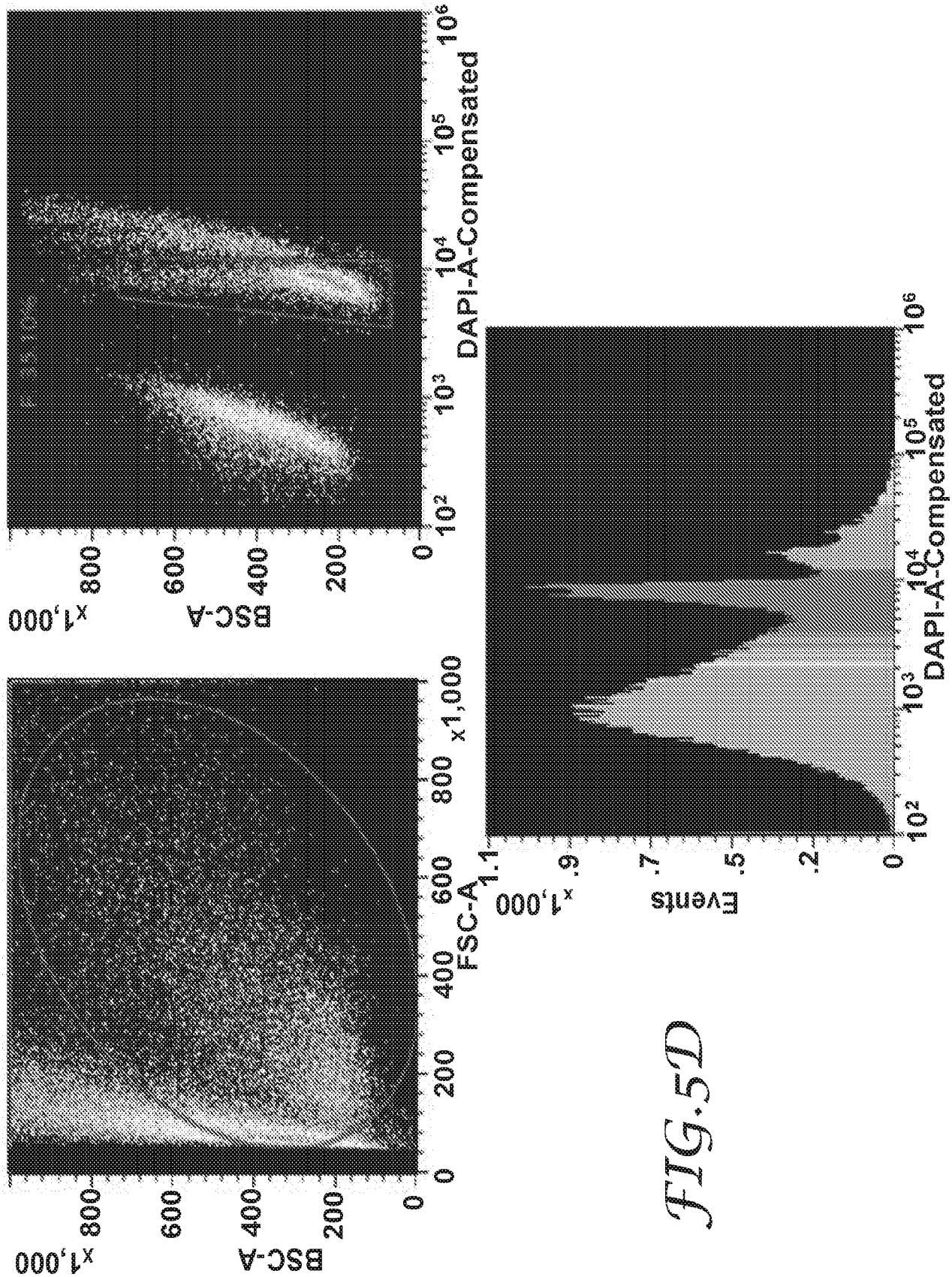

BSC-A
x1,000
800
600
400
200
0
0
200
400
600
800
x1,000
FSC-A
BSC-A
x1,000
800
600
400
200
0
0
10²
10³
10⁴
10⁵
10⁶
DAPI-A-Compensated
Events
x1,000
1.1
.9
.7
.5
.2
0
10²
10³
10⁴
10⁵
10⁶
DAPI-A-Compensated

FIG. 5D

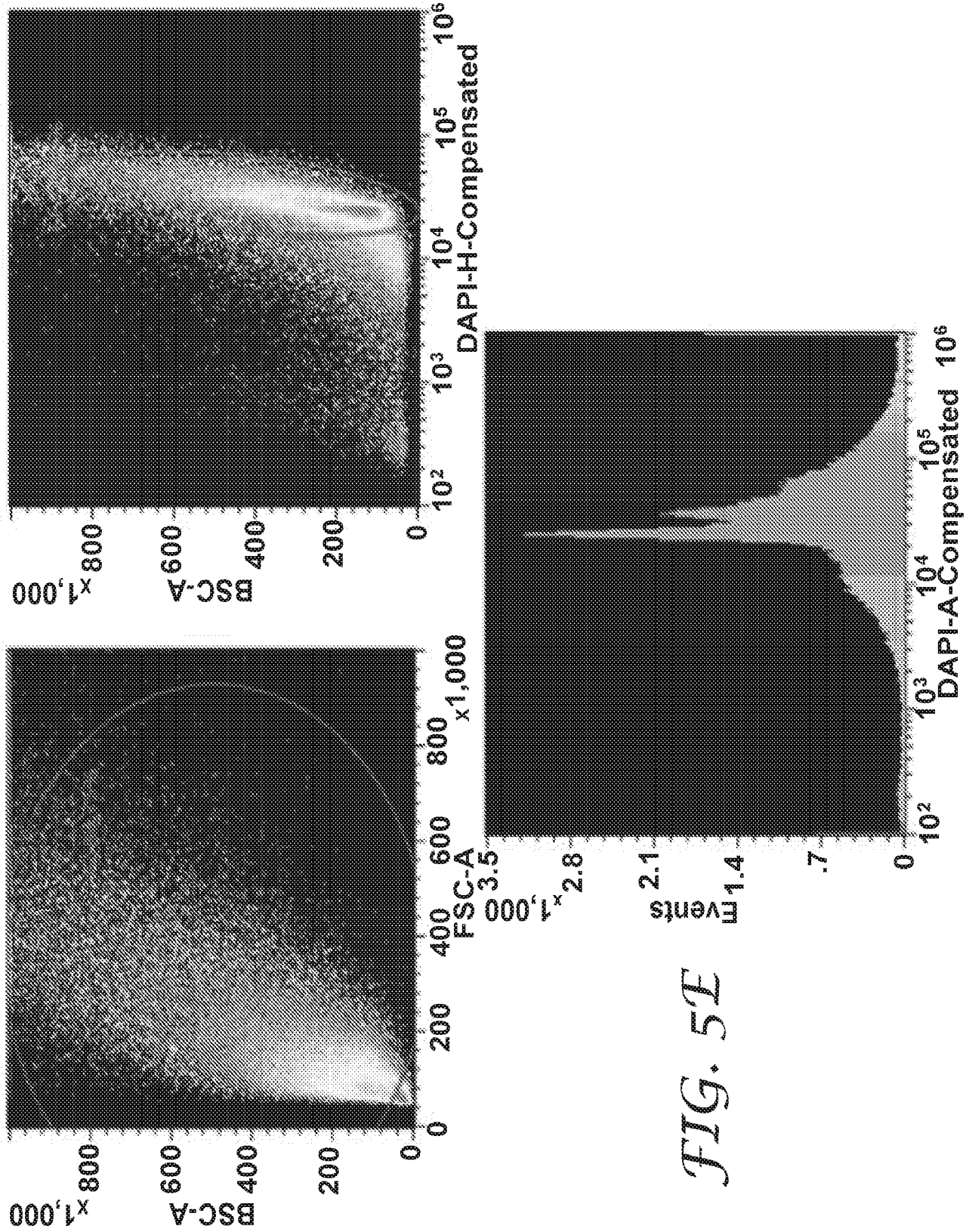

BSC-A
x1,000
800
600
400
200
0
0
200
400
600
800
FSC-A
x1,000
DAPI-H-Compensated
10^2
10^3
10^4
10^5
10^6
Events
3.5
2.8
2.1
1.4
.7
0
DAPI-A-Compensated

FIG. 5E

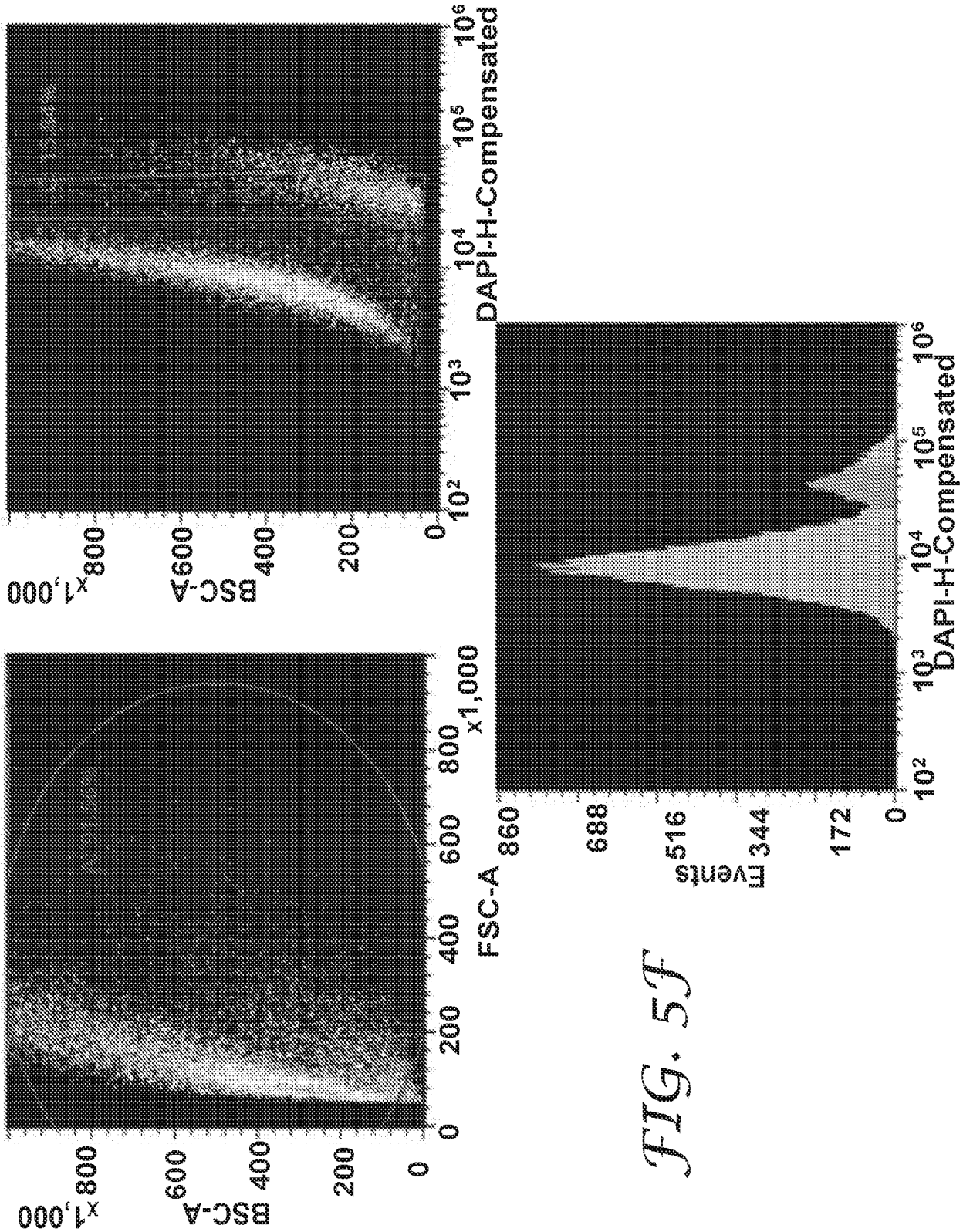

BSC-A
x1,000
800
600
400
200
0
FSC-A
x1,000
0
200
400
600
800
DAPI-H-Compensated
10^2
10^3
10^4
10^5
10^6
Events
860
688
516
344
172
0

FIG. 5F

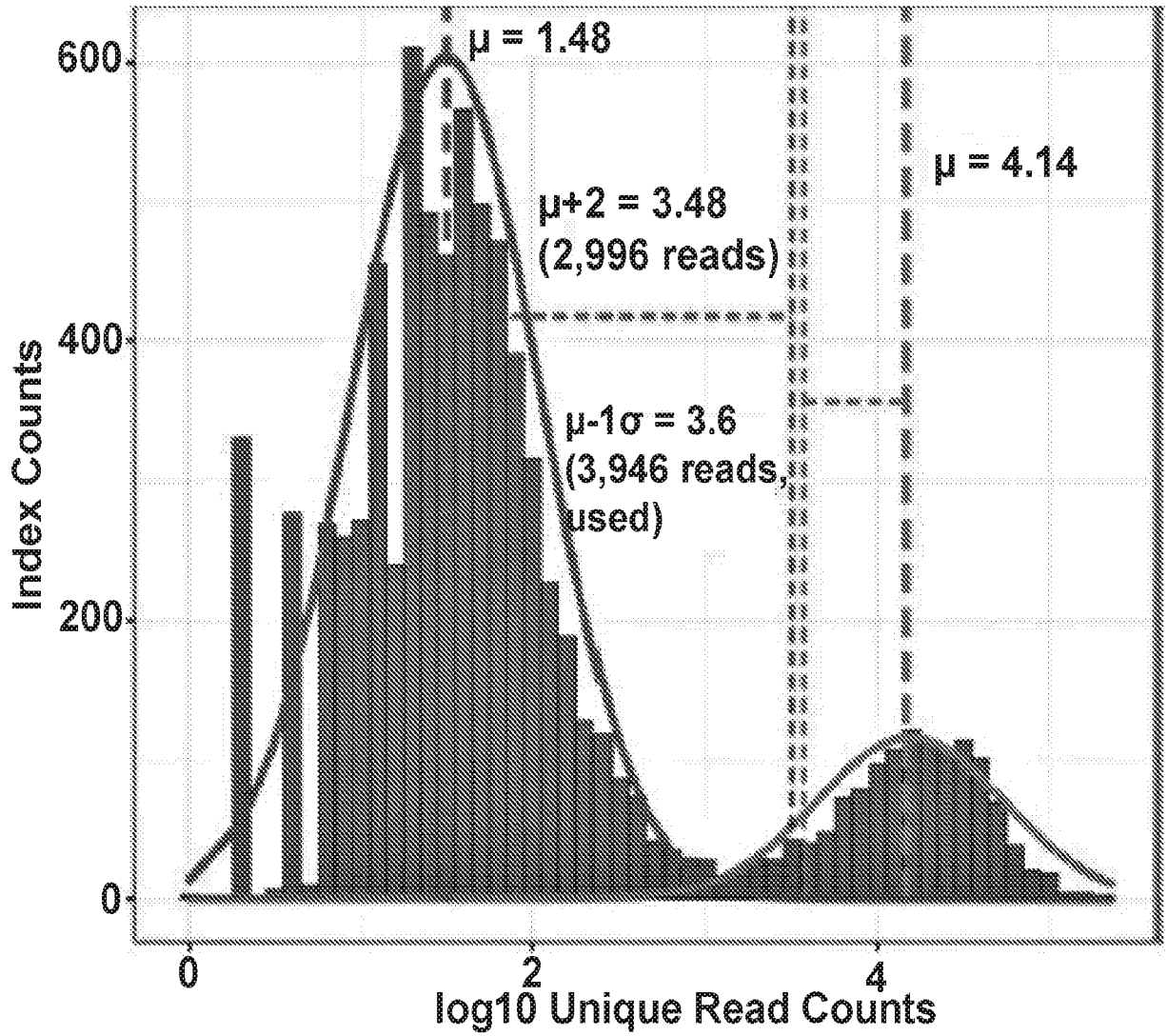

μ = 1.48
μ = 4.14
μ+2 = 3.48
(2,996 reads)
μ-1σ = 3.6
(3,946 reads,
used)
600
400
200
0
Index Counts
0
2
4
log10 Unique Read Counts

FIG. 6

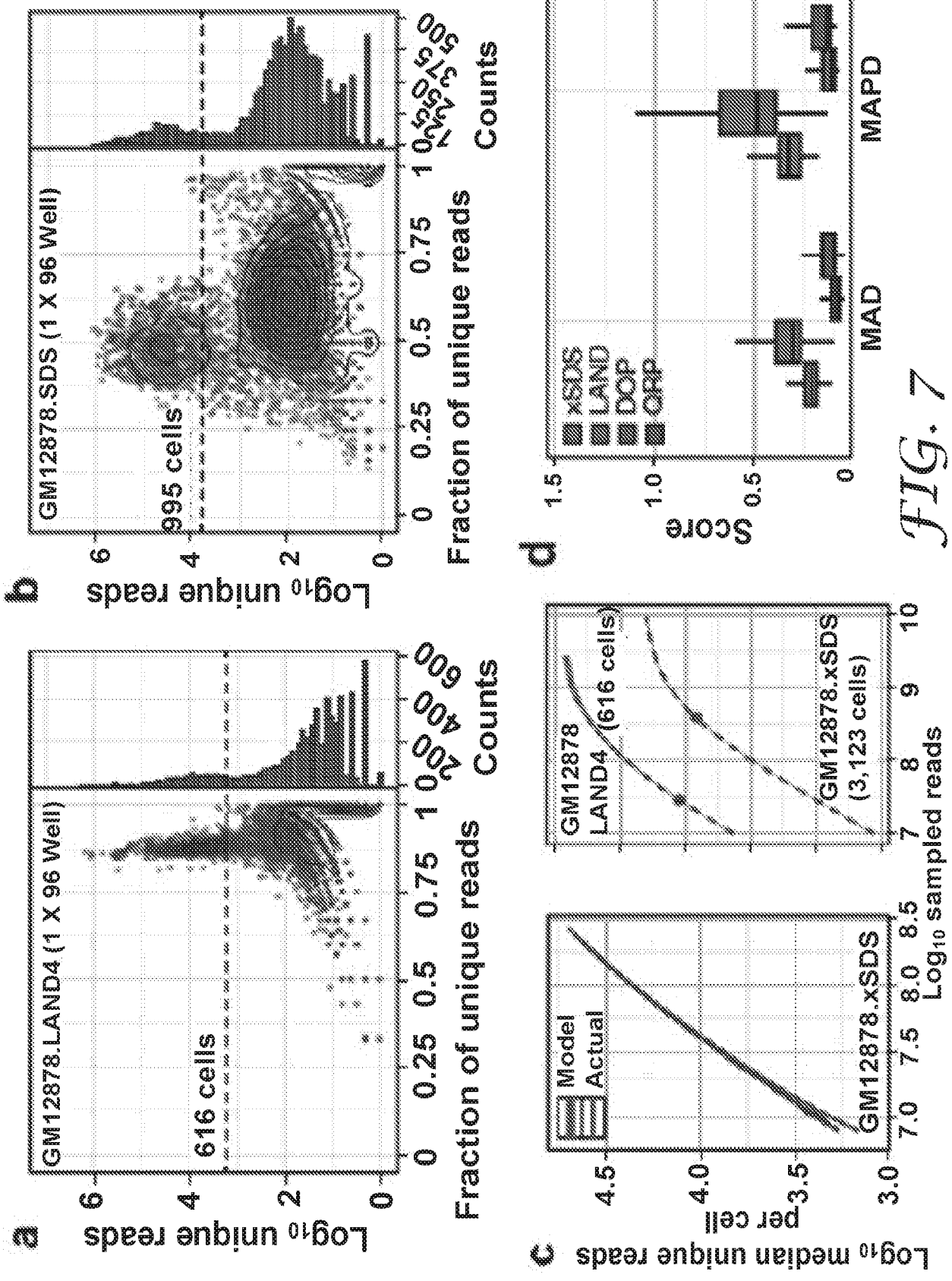

a
GM12878.LAND4 (1 X 96 Well)
616 cells
Log10 unique reads
Fraction of unique reads
Counts
b
GM12878.SDS (1 X 96 Well)
995 cells
Log10 unique reads
Fraction of unique reads
Counts
c
Model
Actual
GM12878.xSDS
Log10 median unique reads
per cell
GM12878 LAND4 (616 cells)
GM12878.xSDS (3,123 cells)
Log10 sampled reads
d
xSDS
LAND
DOP
ORP
Score
MAD
MAPD

FIG. 7

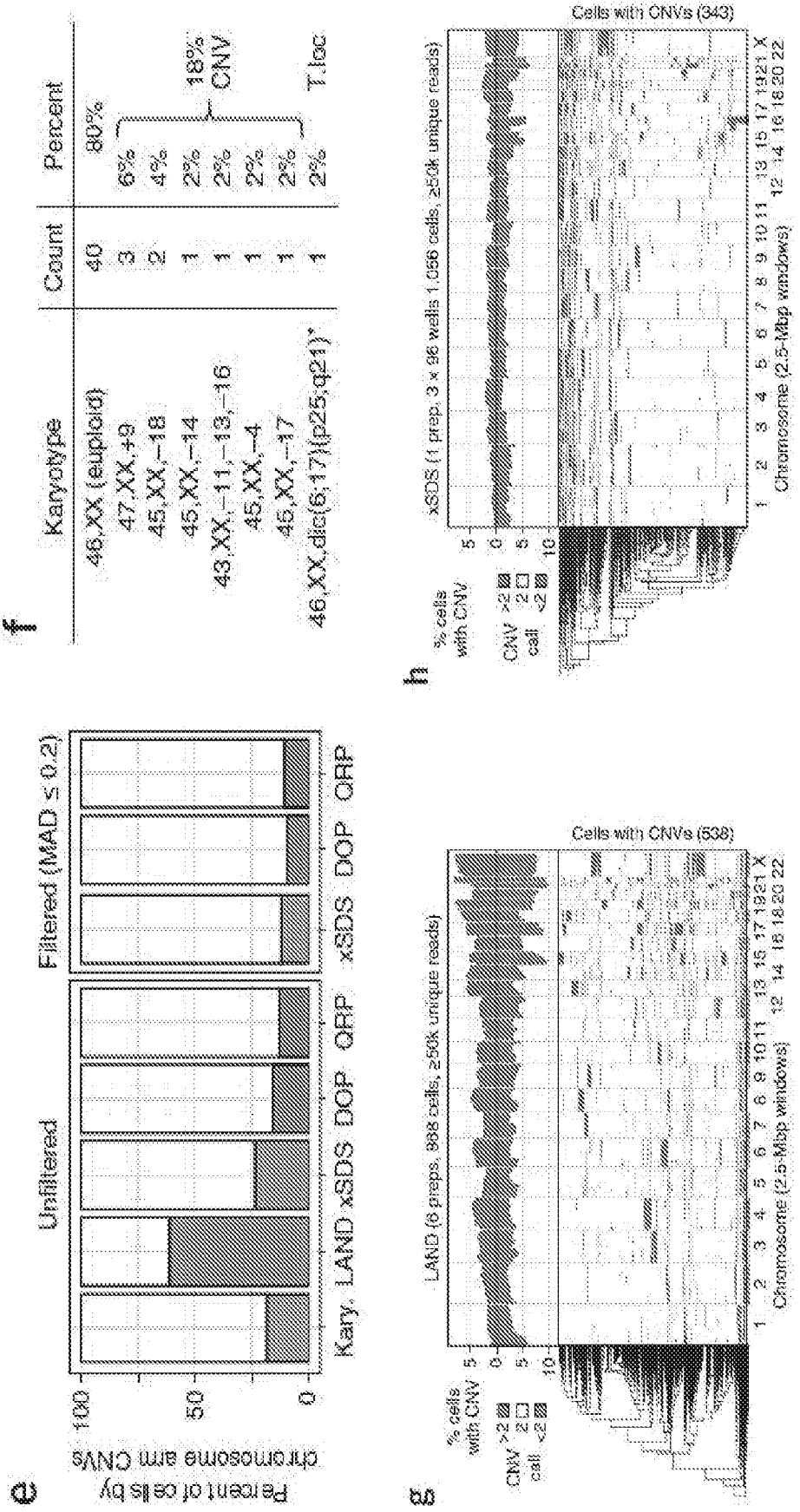

f
Karyotype
Count
Percent
46,XX (euploid)
40
80%
47,XX,+9
3
6%
45,XX,−18
2
4%
45,XX,−14
1
2%
18% CNV
43,XX,−11,−13,−16
1
2%
45,XX,−4
1
2%
45,XX,−17
1
2%
T-loc
46,XX,dic(6;17)(p25;q21)*
1
2%
e
Unfiltered
Filtered (MAD ≤ 0.2)
Percent of cells by chromosome arm CNVs
100
50
0
Kary. LAND xSDS DOP ORP xSDS DOP ORP
g
LAND (6 preps, 868 cells, ≥50k unique reads)
Cells with CNVs (538)
% cells with CNV
CNV call
>2
2
<2
Chromosome (2.5-Mbp windows)
h
xSDS (1 prep, 3 × 96 wells 1,056 cells, ≥50k unique reads)
Cells with CNVs (343)

FIG. 7

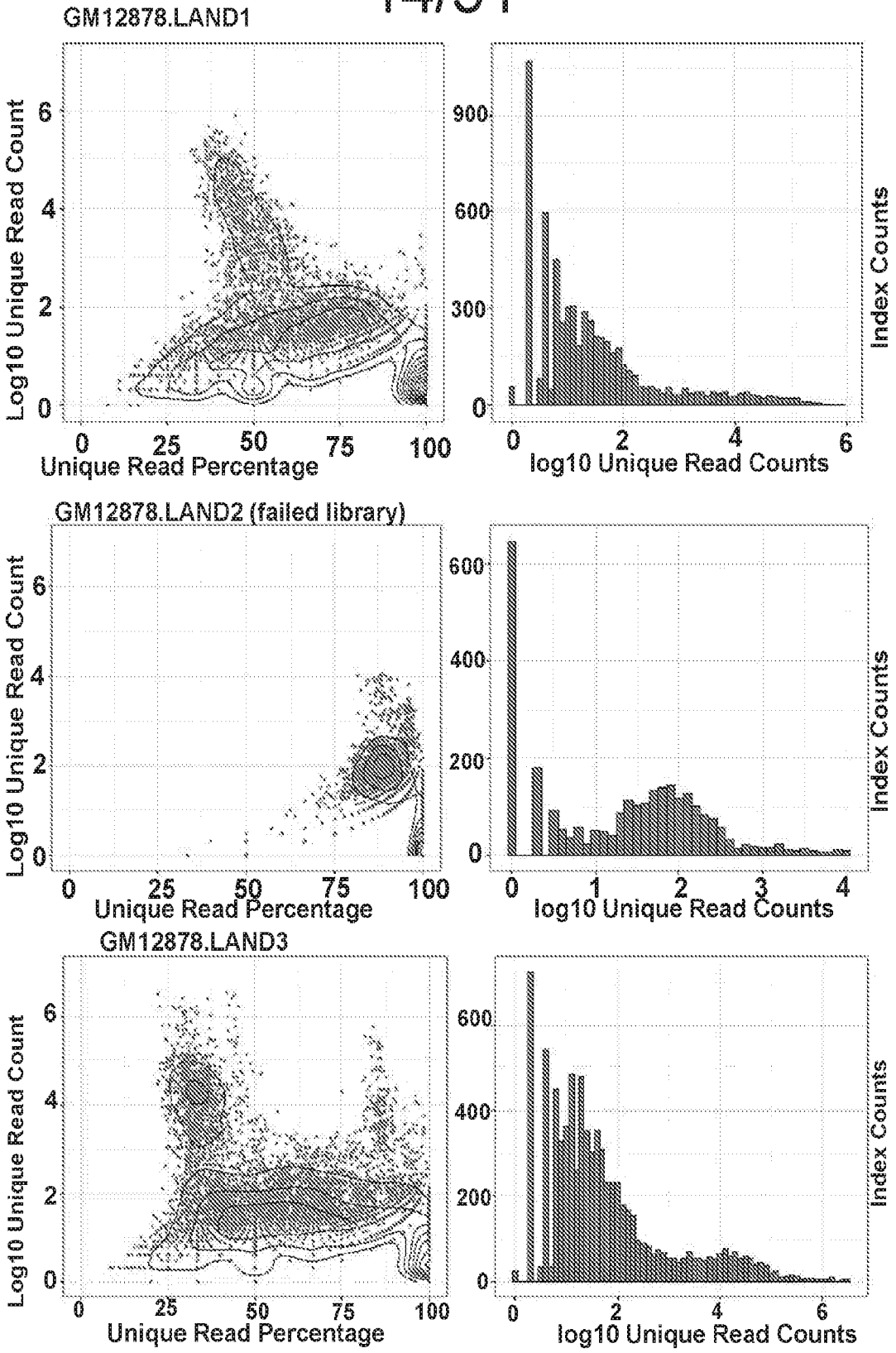

GM12878.LAND1
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
GM12878.LAND2 (failed library)
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
GM12878.LAND3
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts

FIG. 8-1

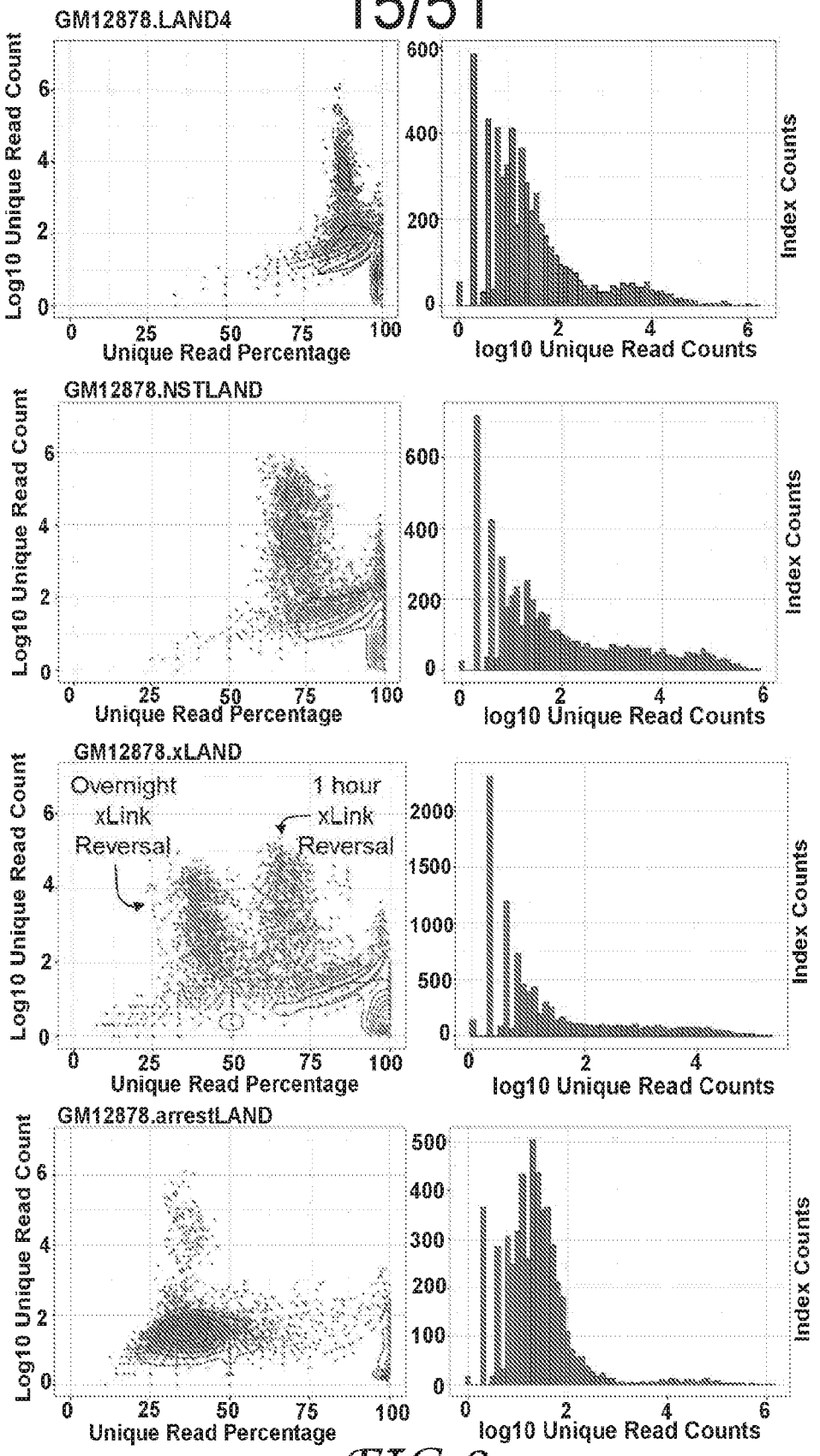

GM12878.LAND4
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
GM12878.NSTLAND
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
GM12878.xLAND
Overnight xLink Reversal
1 hour xLink Reversal
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
GM12878.arrestLAND
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts

FIG.8-2

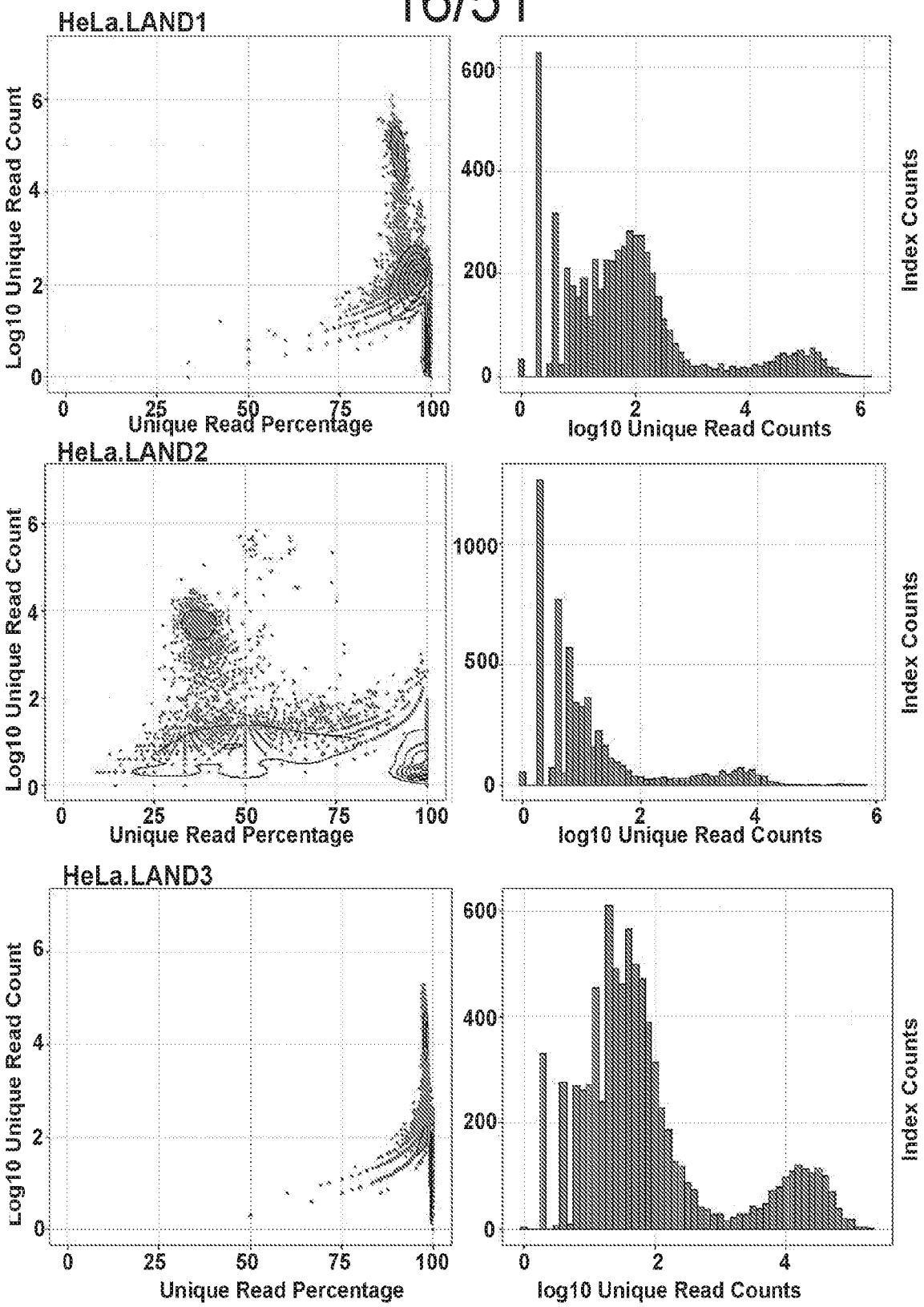

HeLa.LAND1
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
HeLa.LAND2
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
HeLa.LAND3
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts

FIG. 8-3

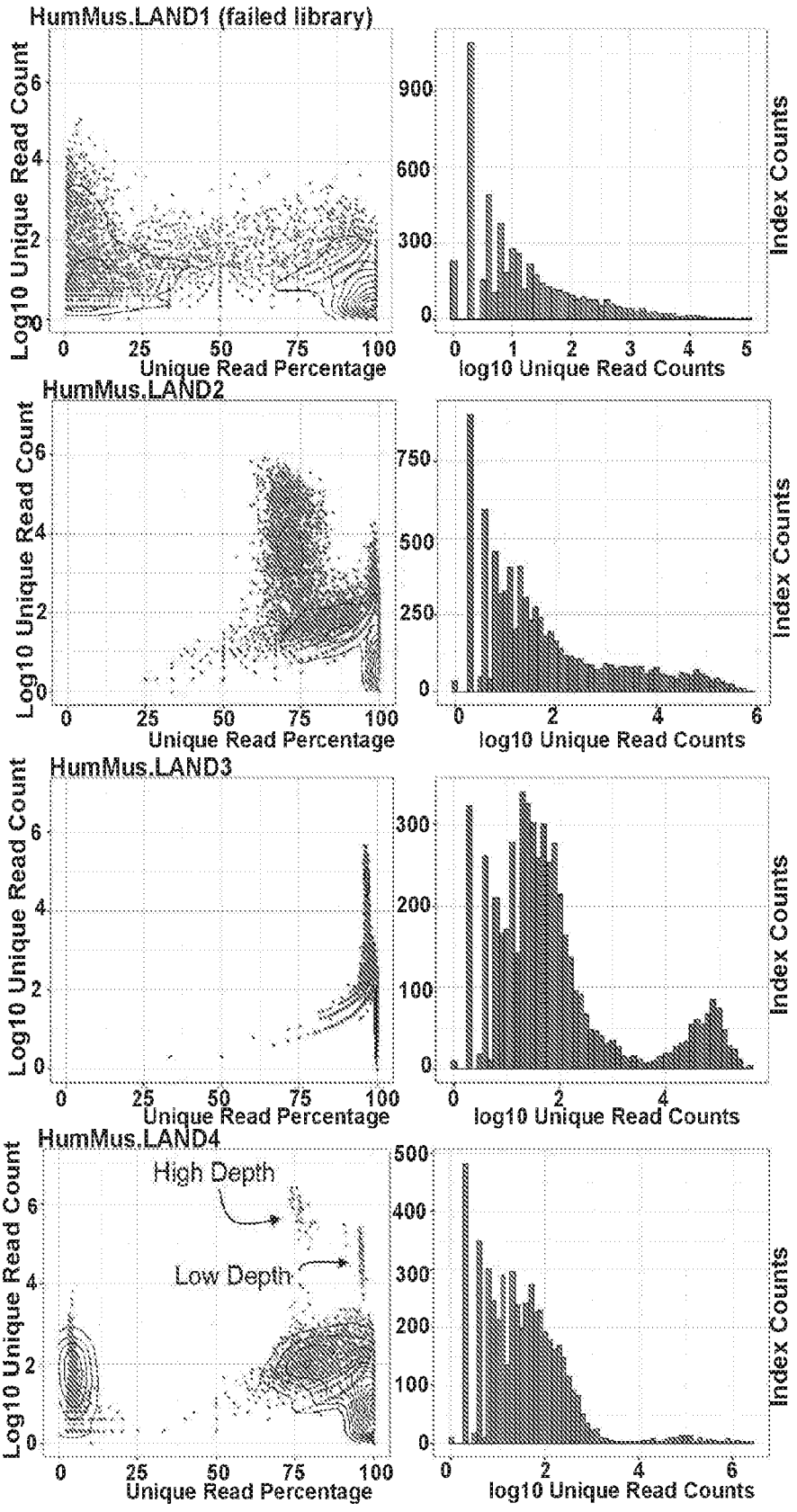

HumMus.LAND1 (failed library)
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
HumMus.LAND2
HumMus.LAND3
HumMus.LAND4
High Depth
Low Depth

FIG. 8-4

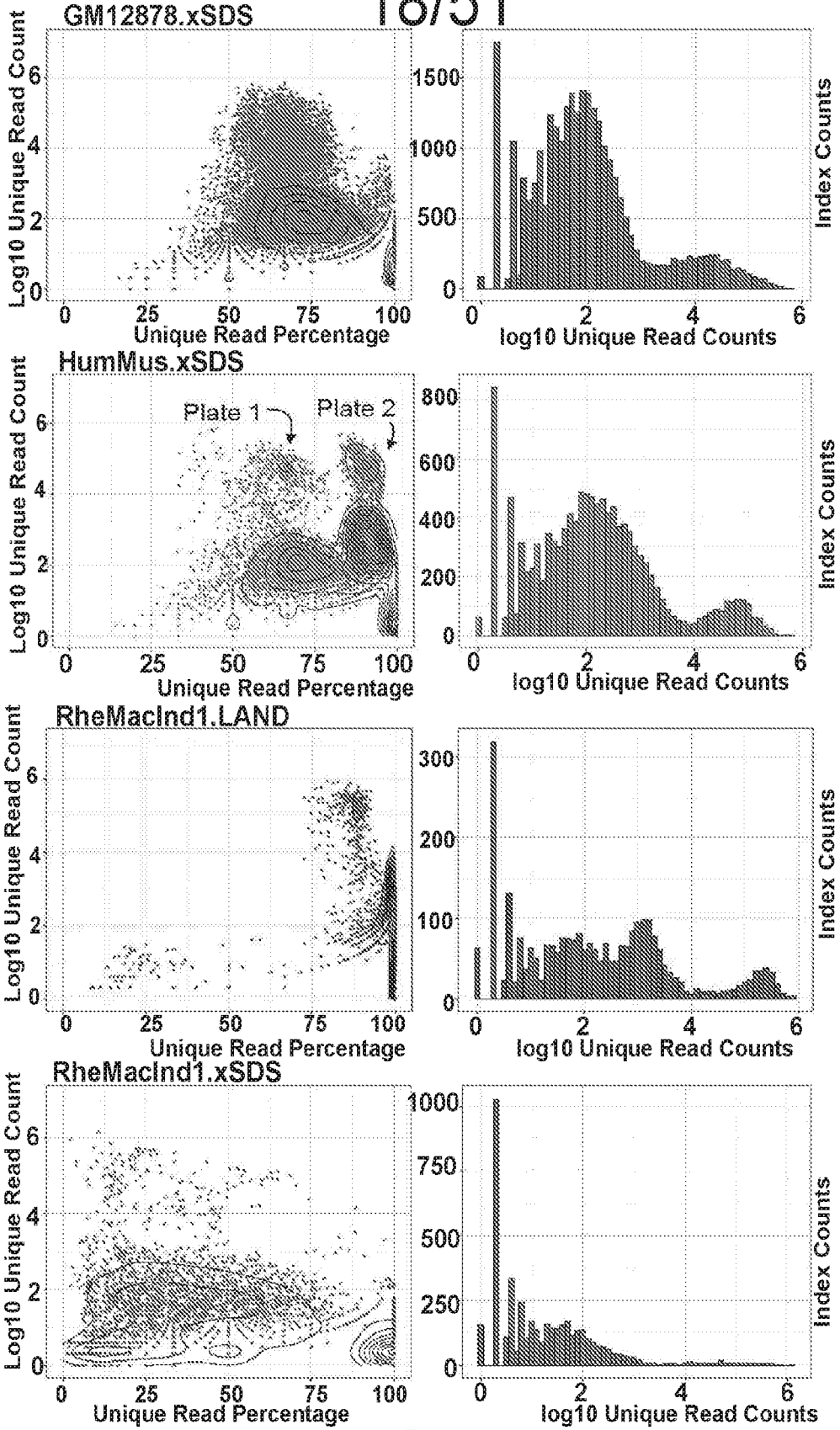

GM12878.xSDS
HumMus.xSDS
Plate 1
Plate 2
RheMacInd1.LAND
RheMacInd1.xSDS
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts

FIG. 8-5

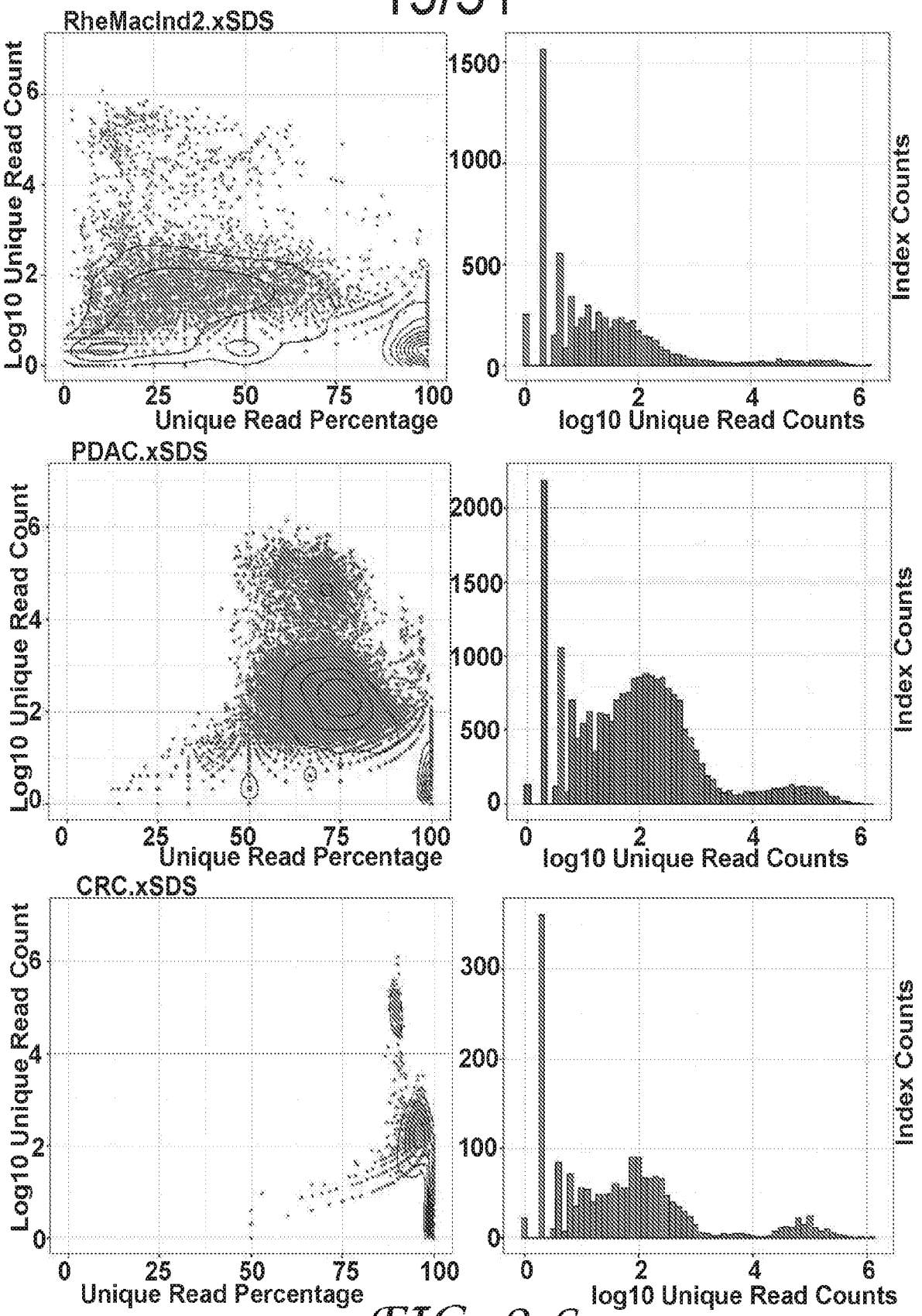

RheMacInd2.xSDS
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
PDAC.xSDS
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts
CRC.xSDS
Log10 Unique Read Count
Unique Read Percentage
log10 Unique Read Counts
Index Counts

FIG. 8-6

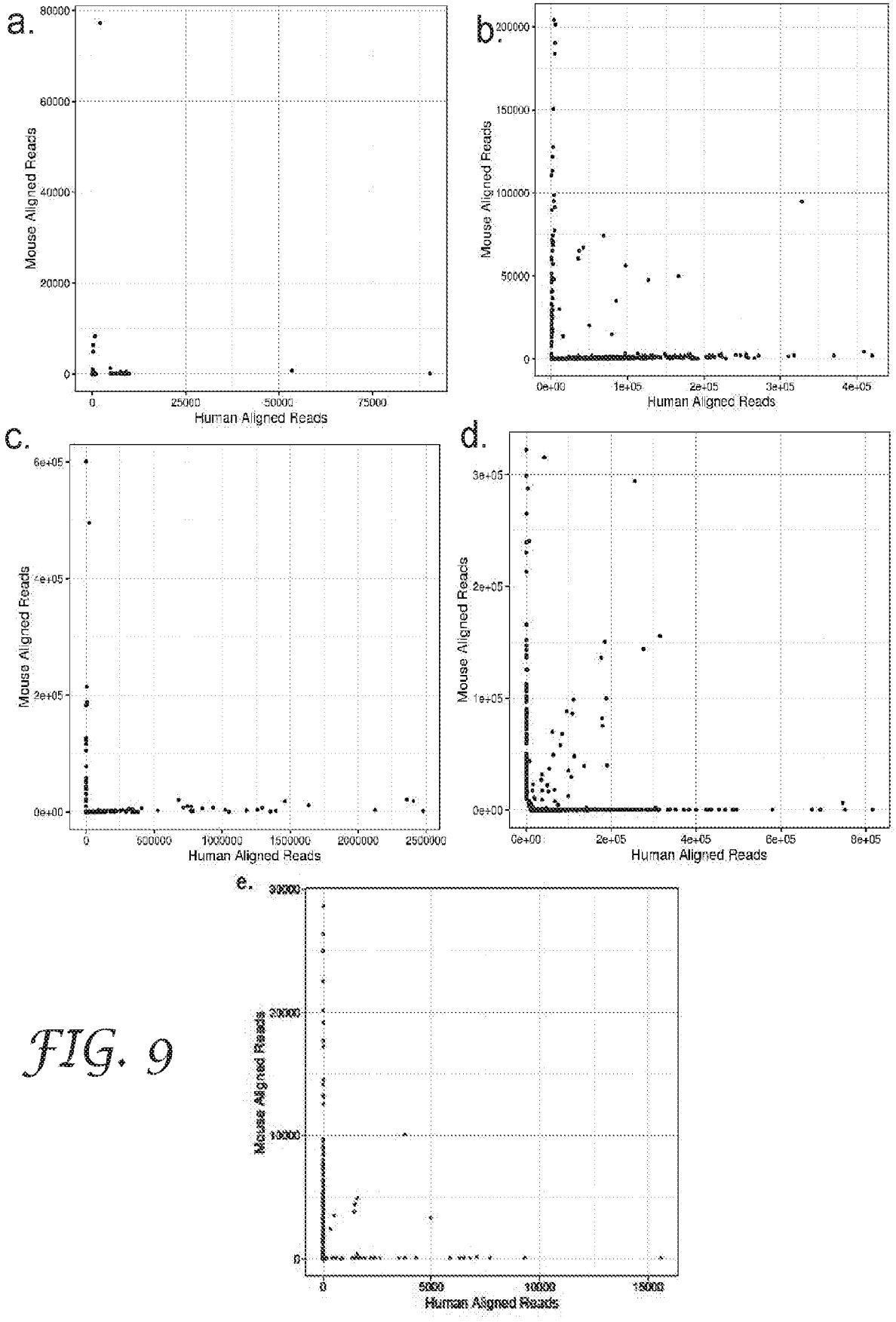

a.
Mouse Aligned Reads
Human Aligned Reads
b.
Mouse Aligned Reads
Human Aligned Reads
c.
Mouse Aligned Reads
Human Aligned Reads
d.
Mouse Aligned Reads
Human Aligned Reads
e.
Mouse Aligned Reads
Human Aligned Reads

FIG. 9

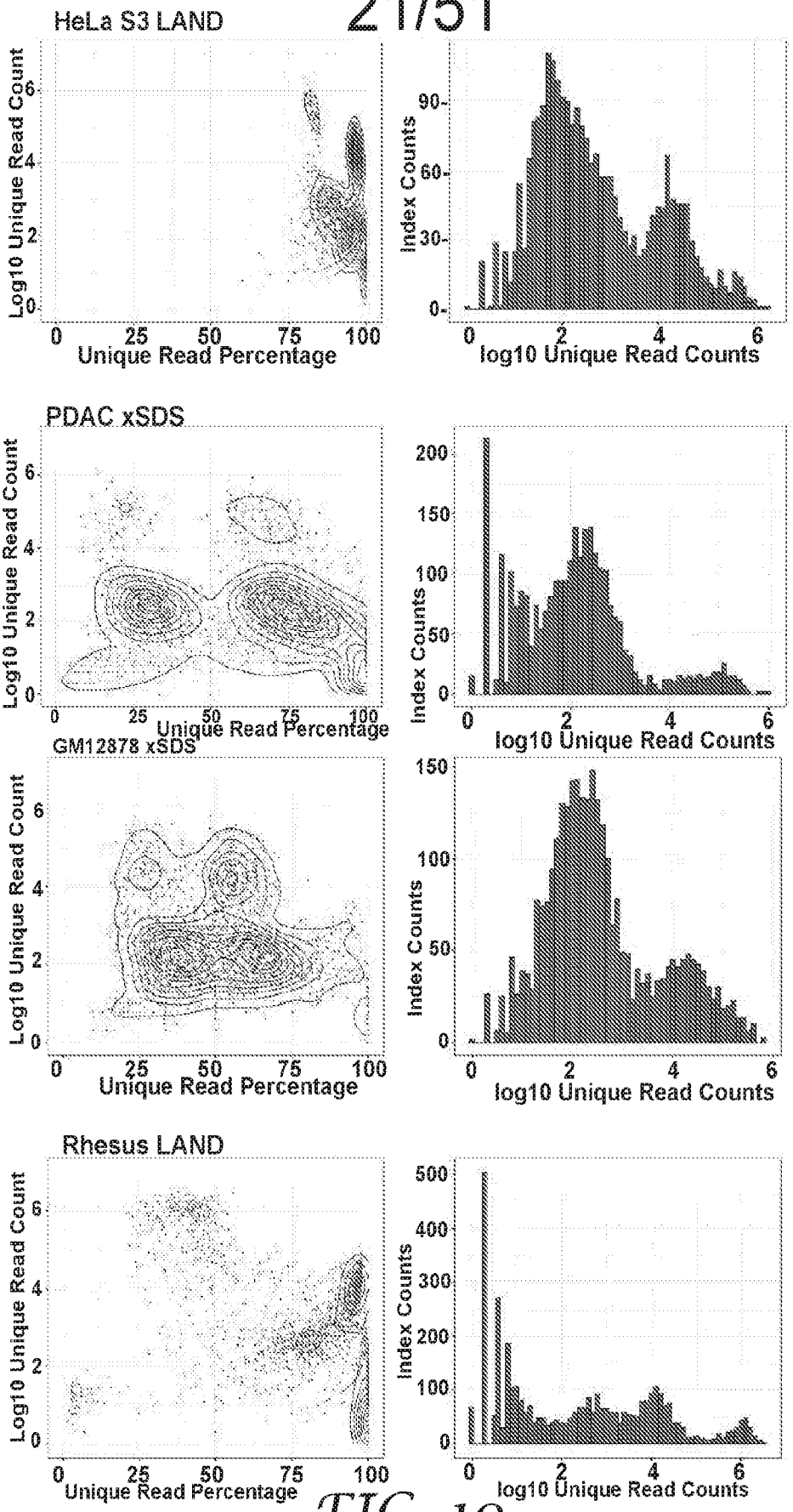

HeLa S3 LAND
Log10 Unique Read Count
Unique Read Percentage
Index Counts
log10 Unique Read Counts
PDAC xSDS
GM12878 xSDS
Rhesus LAND

FIG. 10

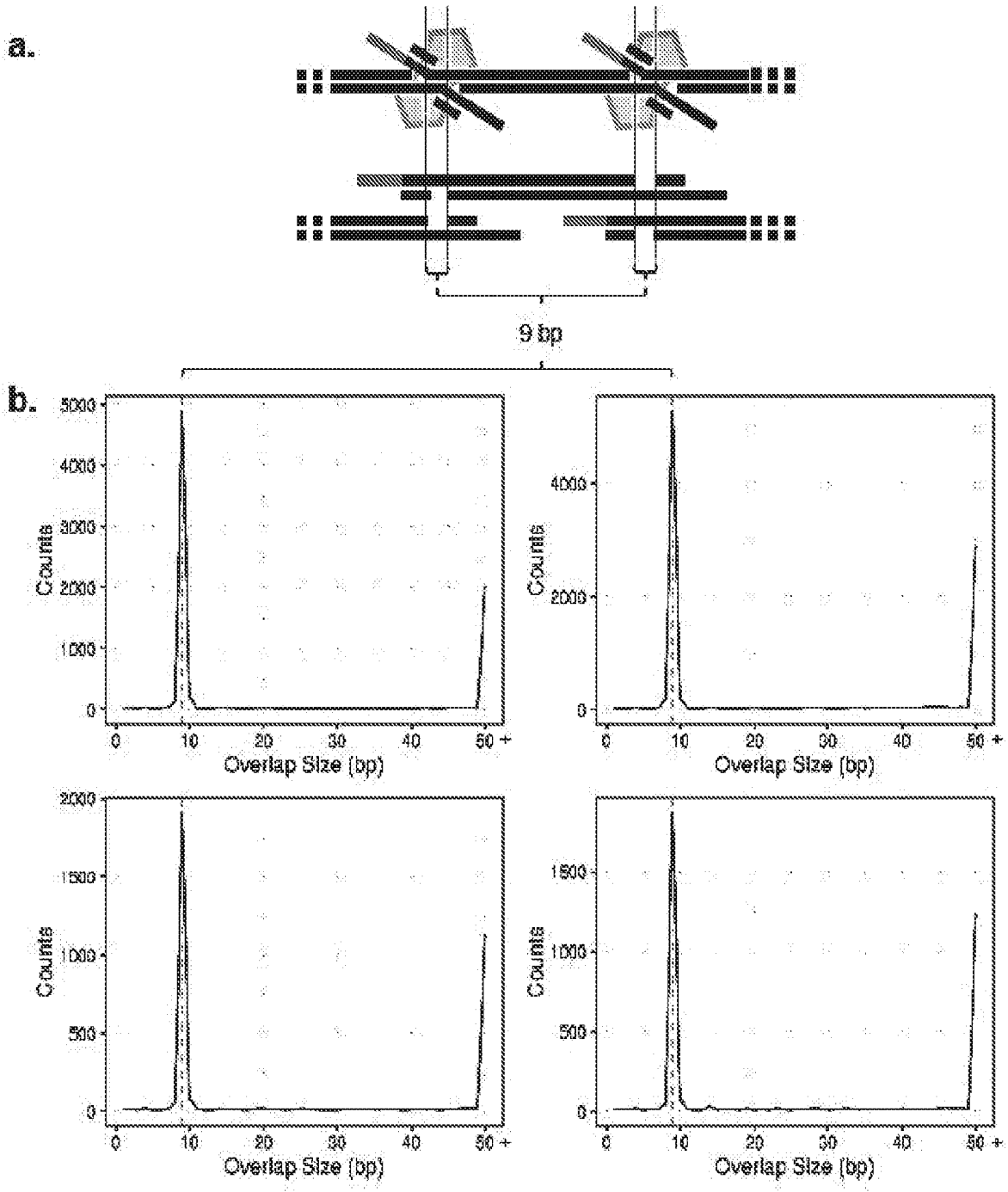

a.
9 bp
b.
Counts
Overlap Size (bp)
0
10
20
30
40
50 +
1000
2000
3000
4000
5000
500
1500

FIG. 11

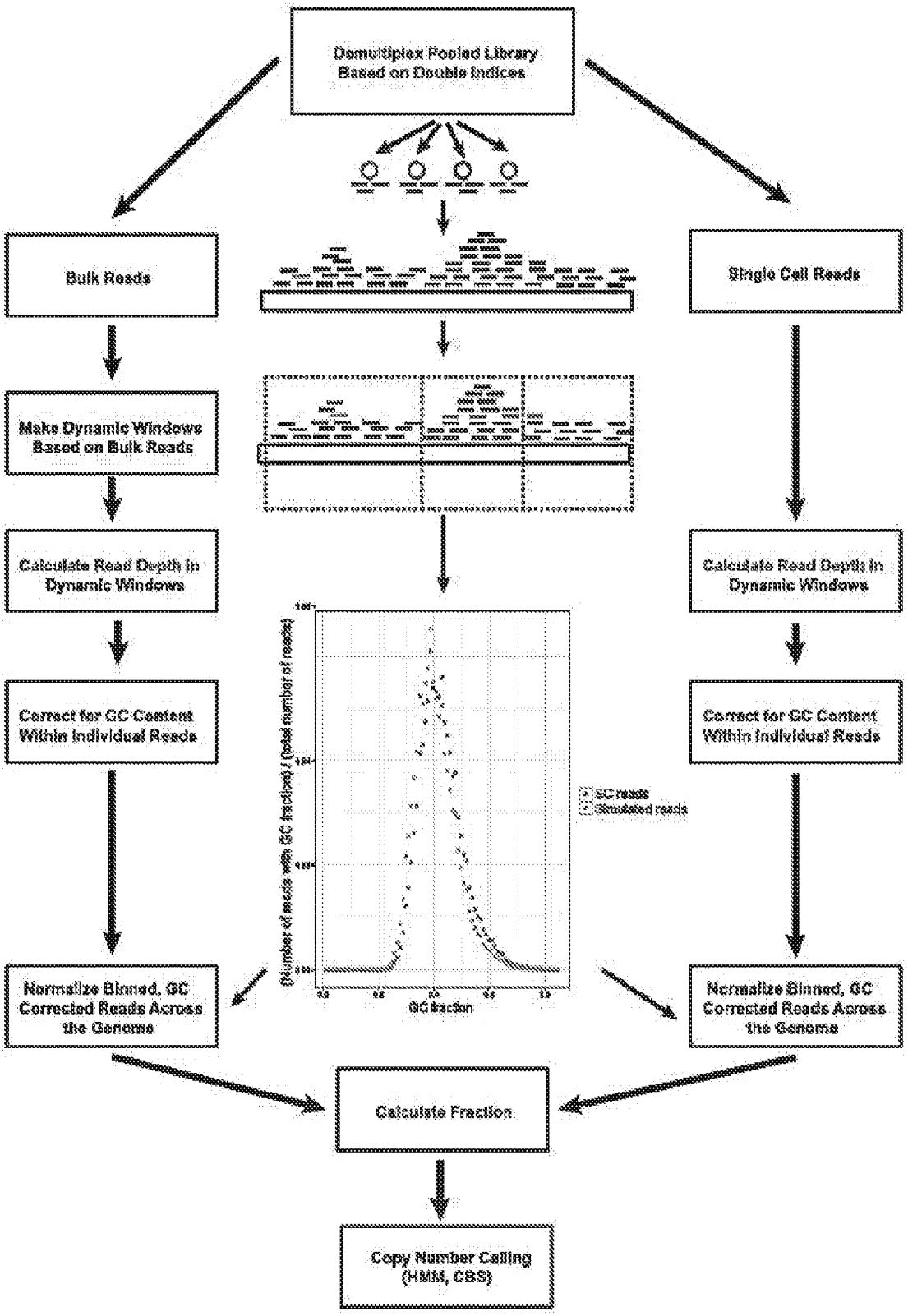

Demultiplex Pooled Library Based on Double Indices
Bulk Reads
Single Cell Reads
Make Dynamic Windows Based on Bulk Reads
Calculate Read Depth in Dynamic Windows
Calculate Read Depth in Dynamic Windows
Correct for GC Content Within Individual Reads
Correct for GC Content Within Individual Reads
SC reads
Simulated reads
GC fraction
Normalize Binned, GC Corrected Reads Across the Genome
Normalize Binned, GC Corrected Reads Across the Genome
Calculate Fraction
Copy Number Calling (HMM, CBS)

FIG. 12

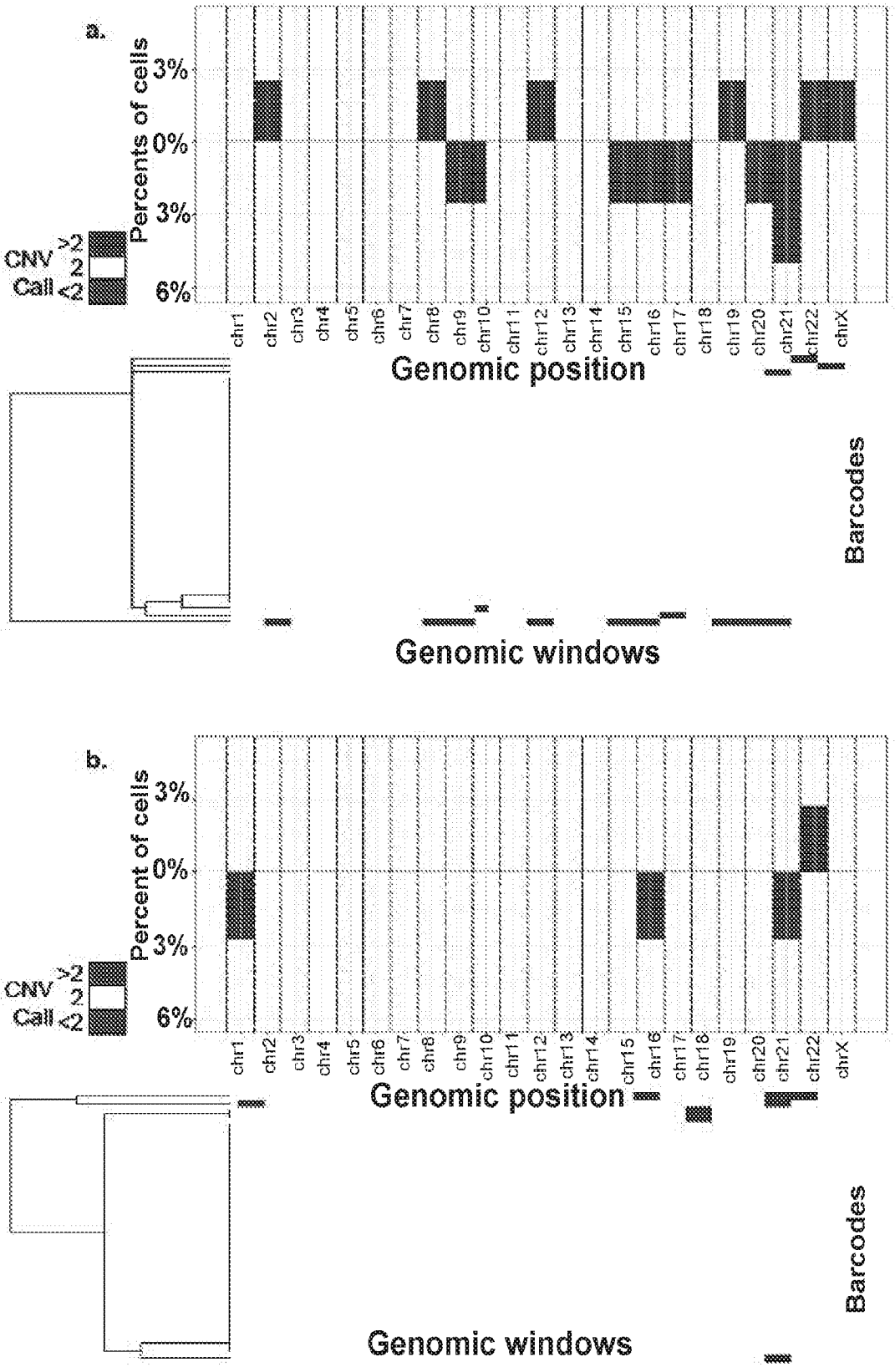

a.
Percents of cells
3%
0%
3%
6%
CNV Call
> 2
2
< 2
chr1 chr2 chr3 chr4 chr5 chr6 chr7 chr8 chr9 chr10 chr11 chr12 chr13 chr14 chr15 chr16 chr17 chr18 chr19 chr20 chr21 chr22 chrX
Genomic position
Barcodes
Genomic windows
b.
Percent of cells
3%
0%
3%
6%
CNV Call
> 2
2
< 2
chr1 chr2 chr3 chr4 chr5 chr6 chr7 chr8 chr9 chr10 chr11 chr12 chr13 chr14 chr15 chr16 chr17 chr18 chr19 chr20 chr21 chr22 chrX
Genomic position
Barcodes
Genomic windows

FIG. 13

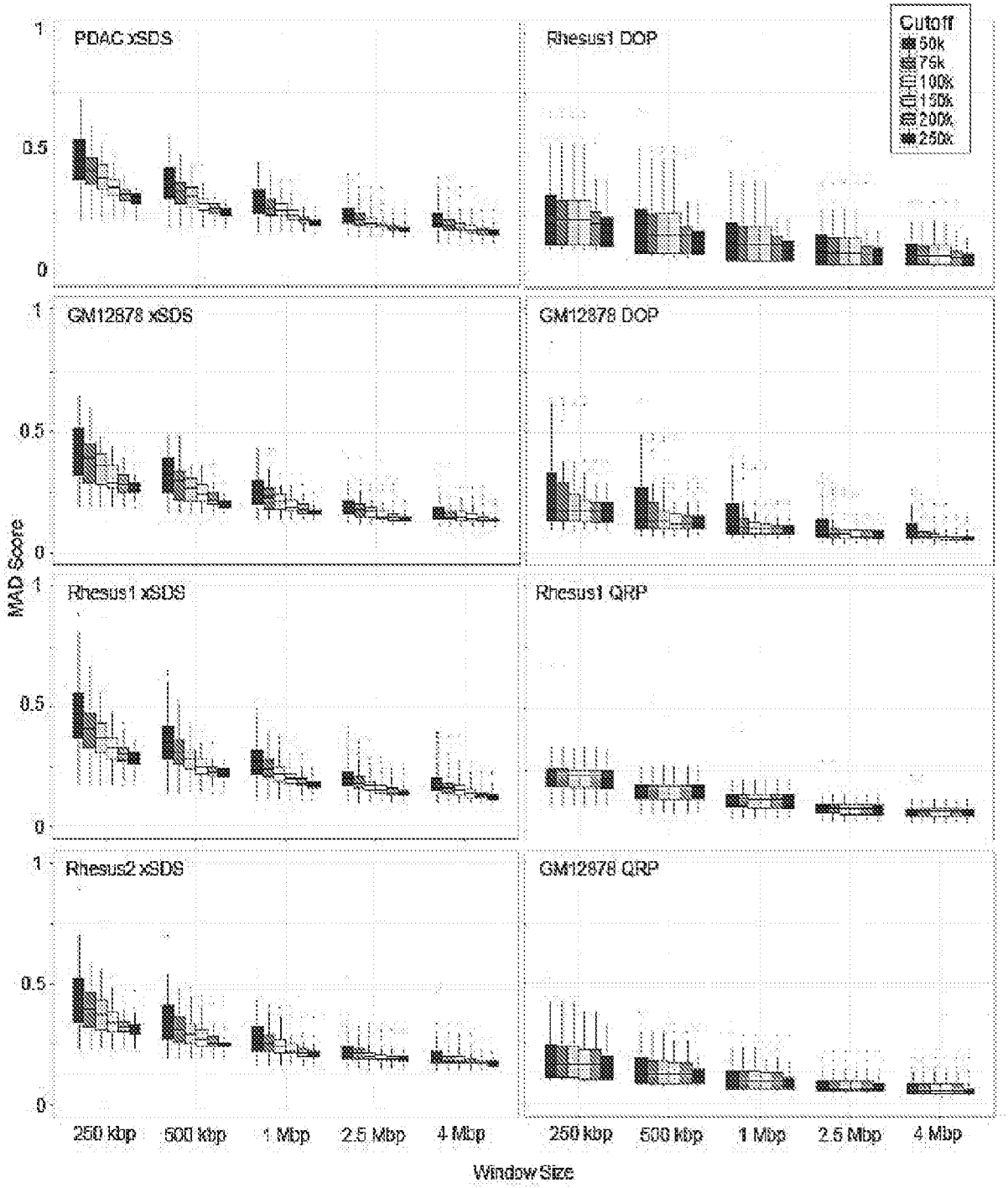

PDAC xSDS
Rhesus1 DOP
GM12878 xSDS
GM12878 DOP
Rhesus1 xSDS
Rhesus1 QRP
Rhesus2 xSDS
GM12878 QRP
Cutoff
50k
75k
100k
150k
200k
250k
MAD Score
1
0.5
0
250 kbp
500 kbp
1 Mbp
2.5 Mbp
4 Mbp
Window Size

FIG. 14-1

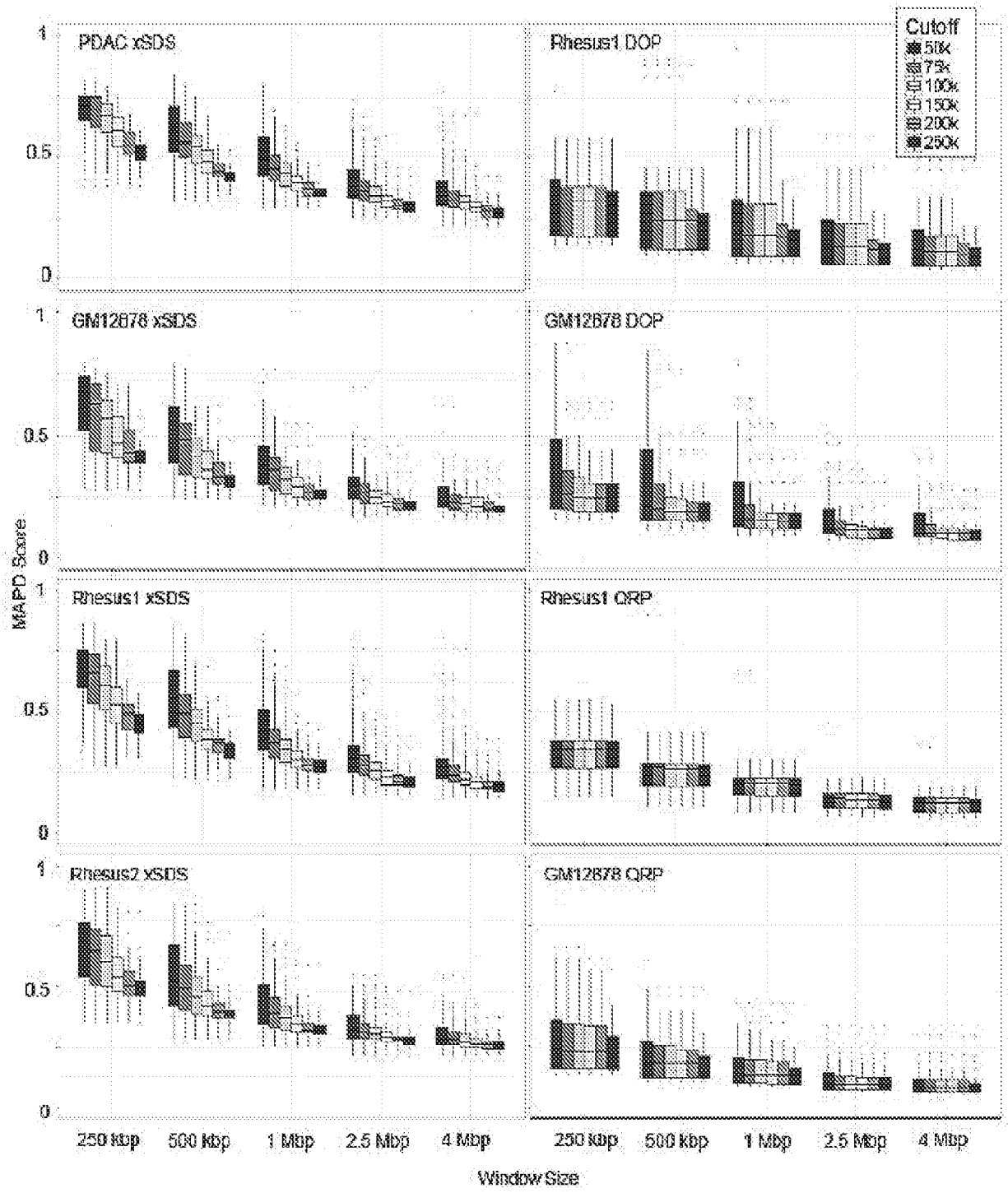

PDAC xSDS
Rhesus1 DOP
GM12878 xSDS
GM12878 DOP
Rhesus1 xSDS
Rhesus1 QRP
Rhesus2 xSDS
GM12878 QRP
Cutoff
50k
75k
100k
150k
200k
250k
MAPD Score
1
0.5
0
250 kbp
500 kbp
1 Mbp
2.5 Mbp
4 Mbp
Window Size

FIG. 14-2

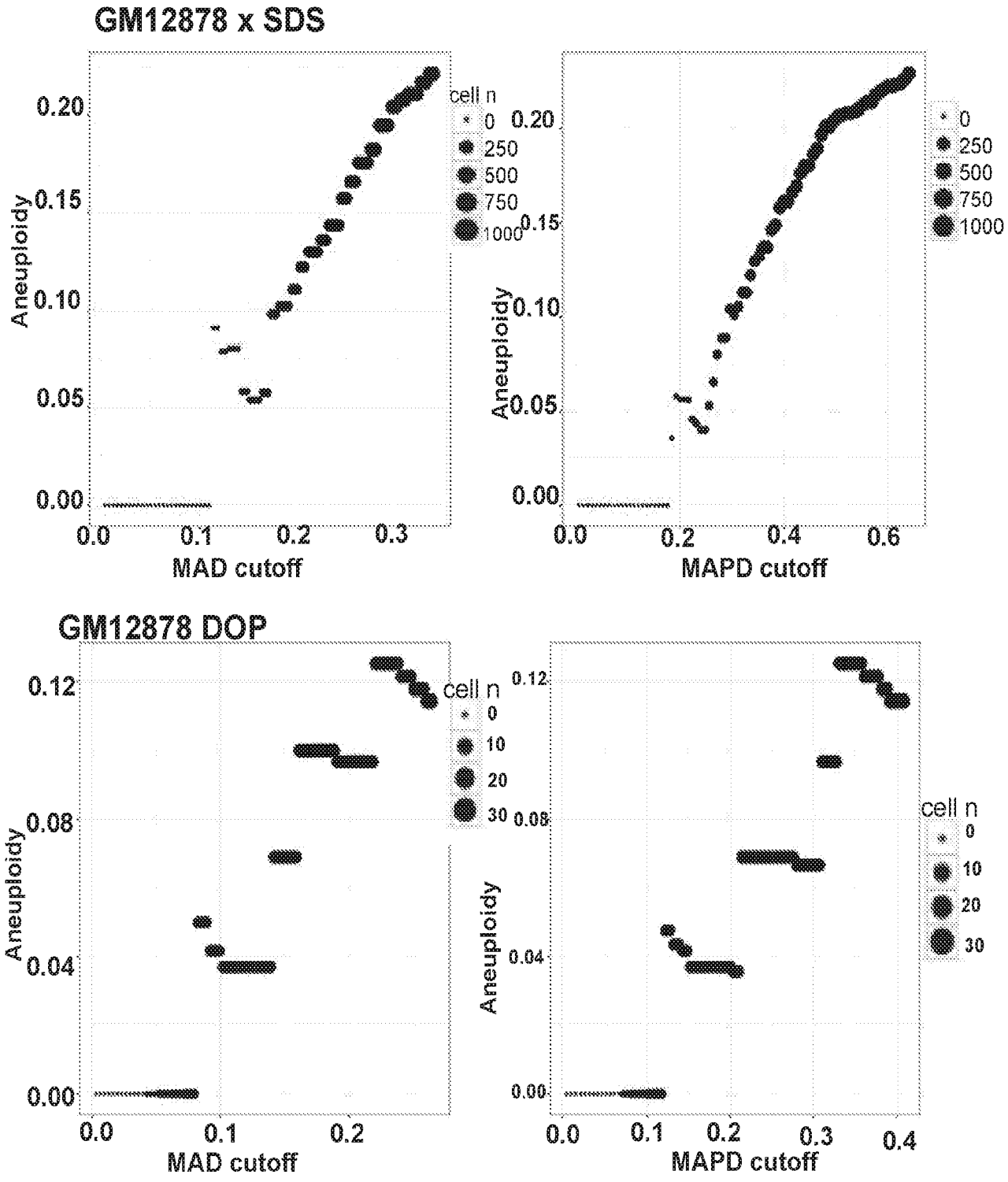

GM12878 x SDS
Aneuploidy
MAD cutoff
MAPD cutoff
cell n
0
250
500
750
1000
GM12878 DOP
10
20
30

FIG. 15-1

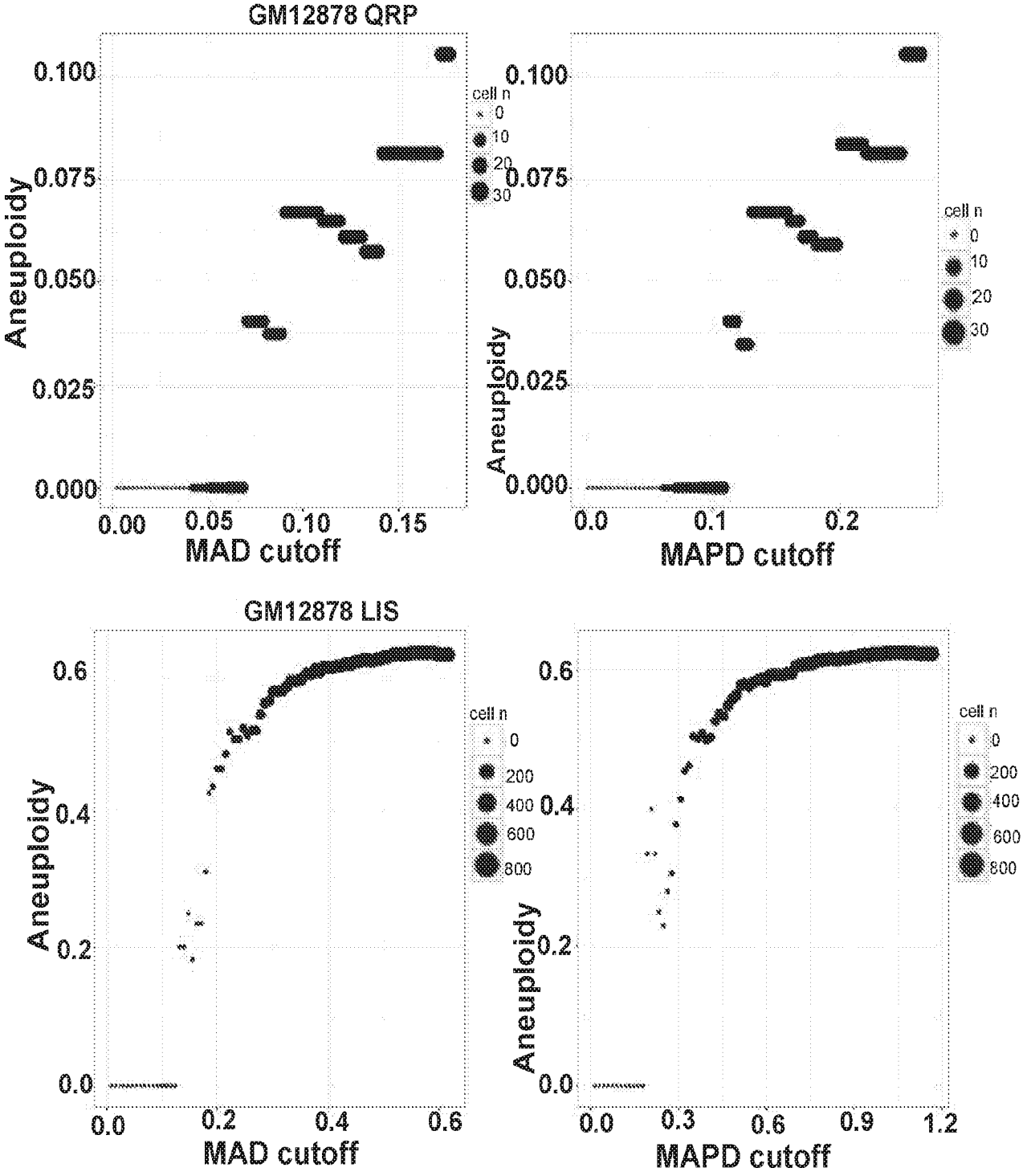

GM12878 QRP
Aneuploidy
0.100
0.075
0.050
0.025
0.000
0.00
0.05
0.10
0.15
MAD cutoff
cell n
0
10
20
30
0.0
0.1
0.2
MAPD cutoff
GM12878 LIS
0.6
0.4
0.2
0.0
0.3
0.6
0.9
1.2
200
400
600
800

FIG. 15-2

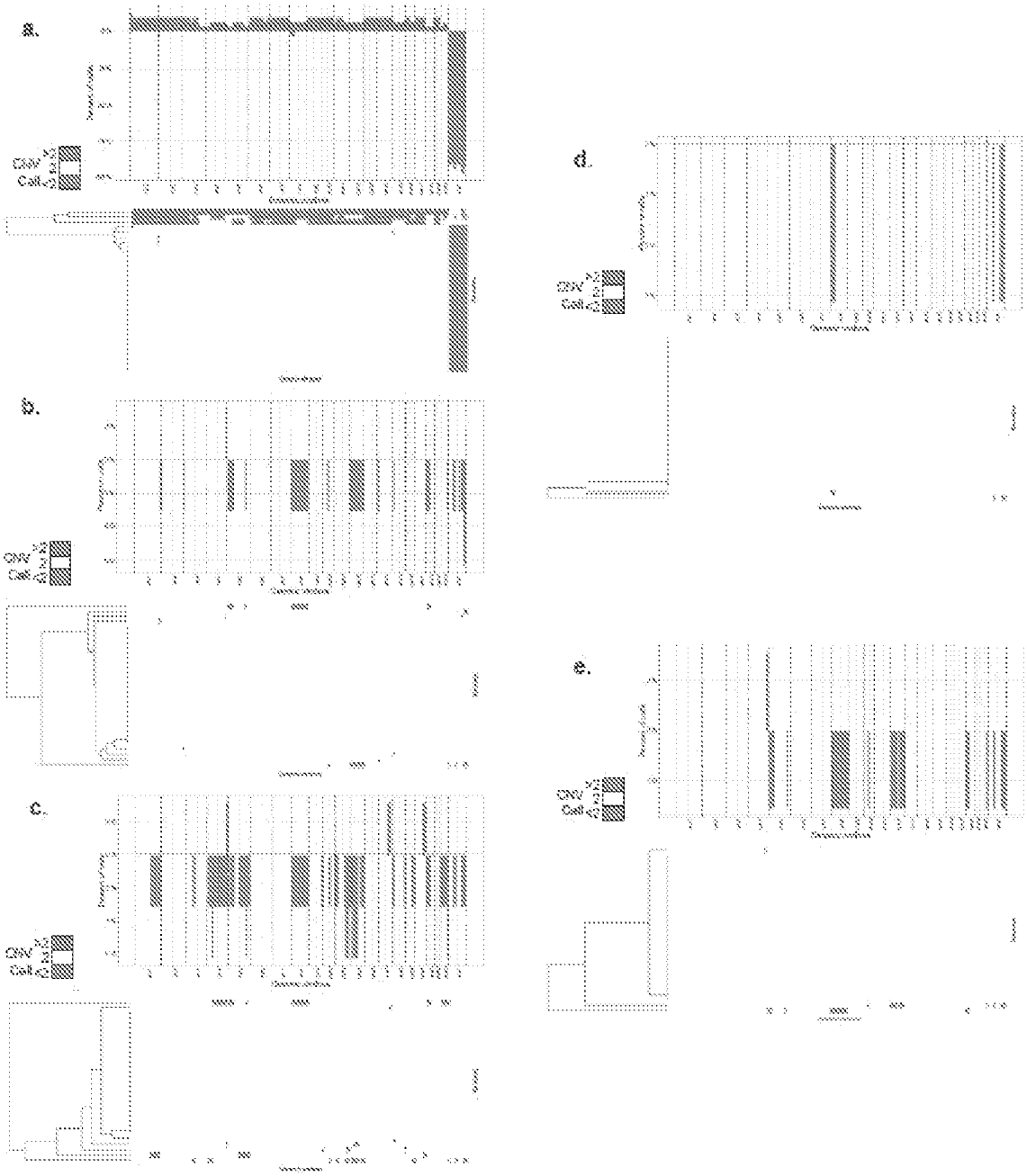


FIG. 16

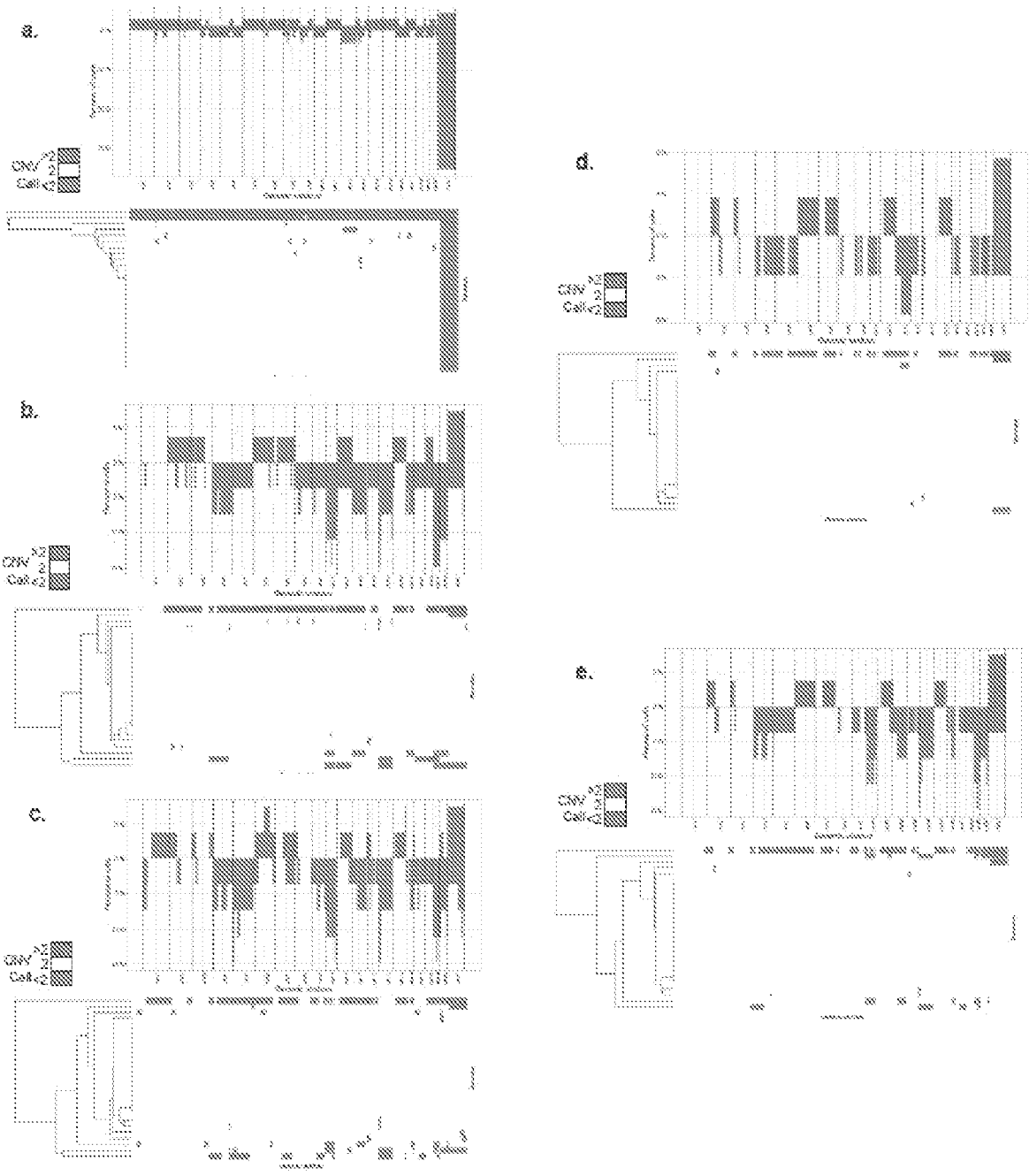


FIG. 17

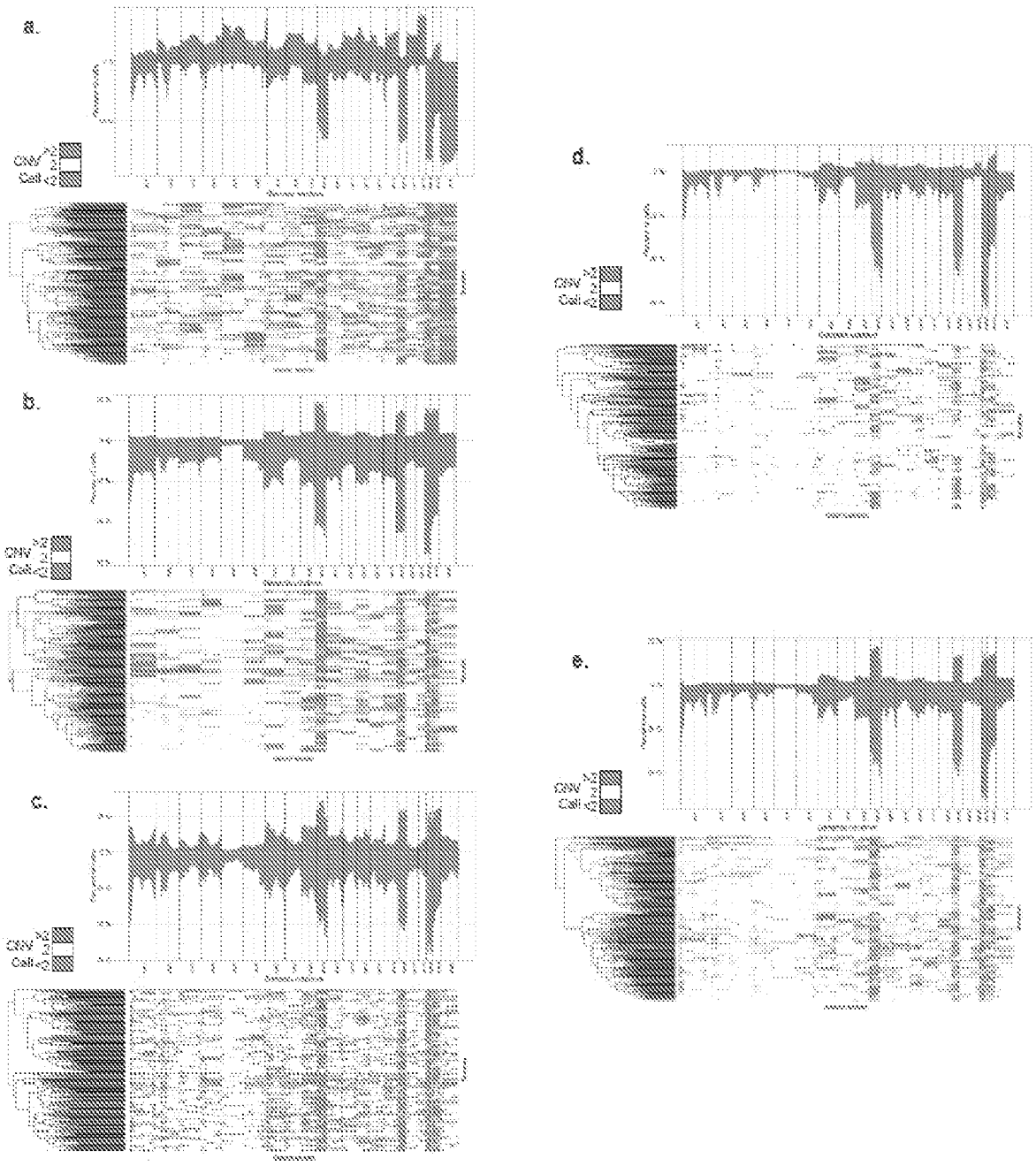


FIG. 18

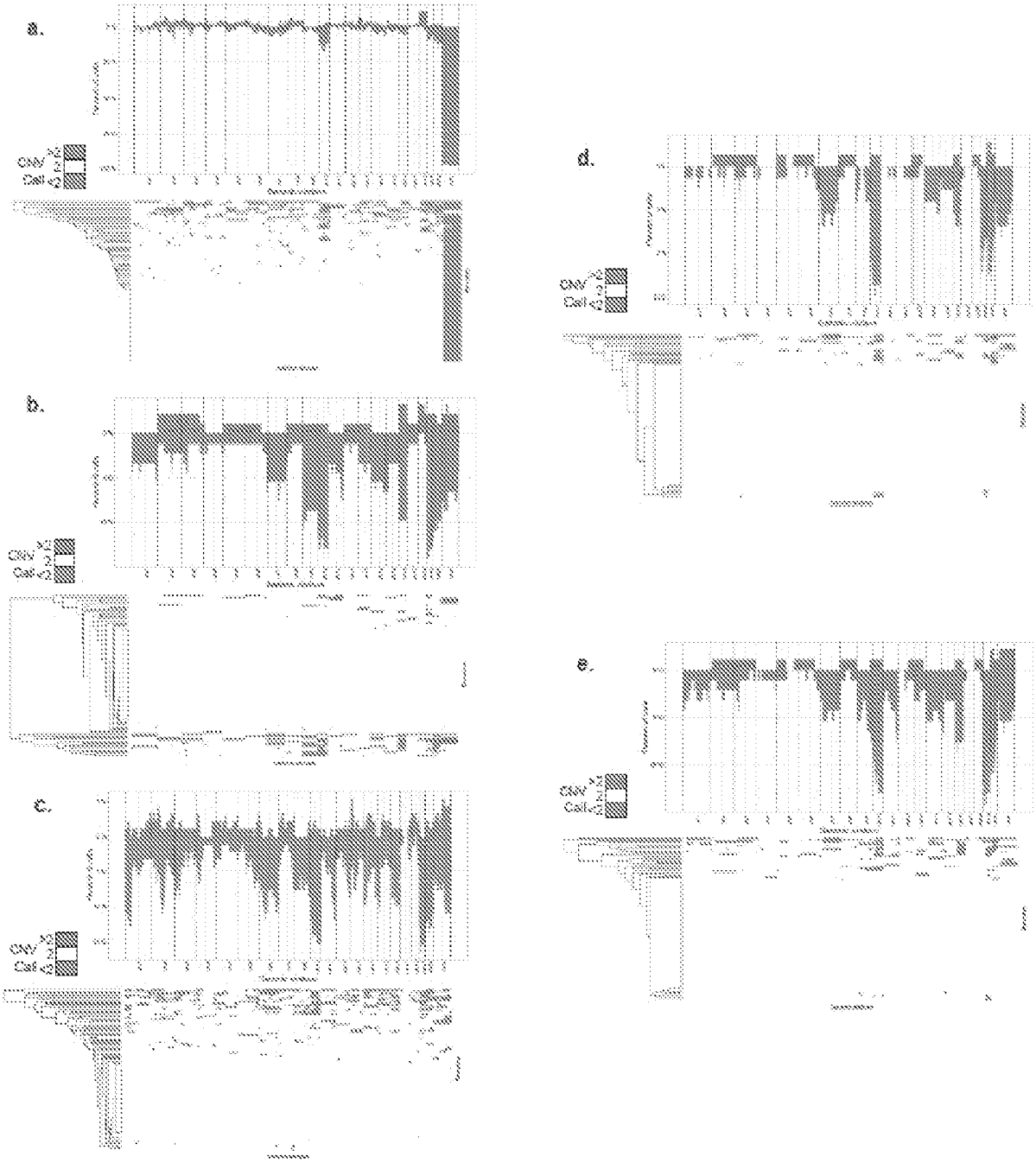


FIG. 19

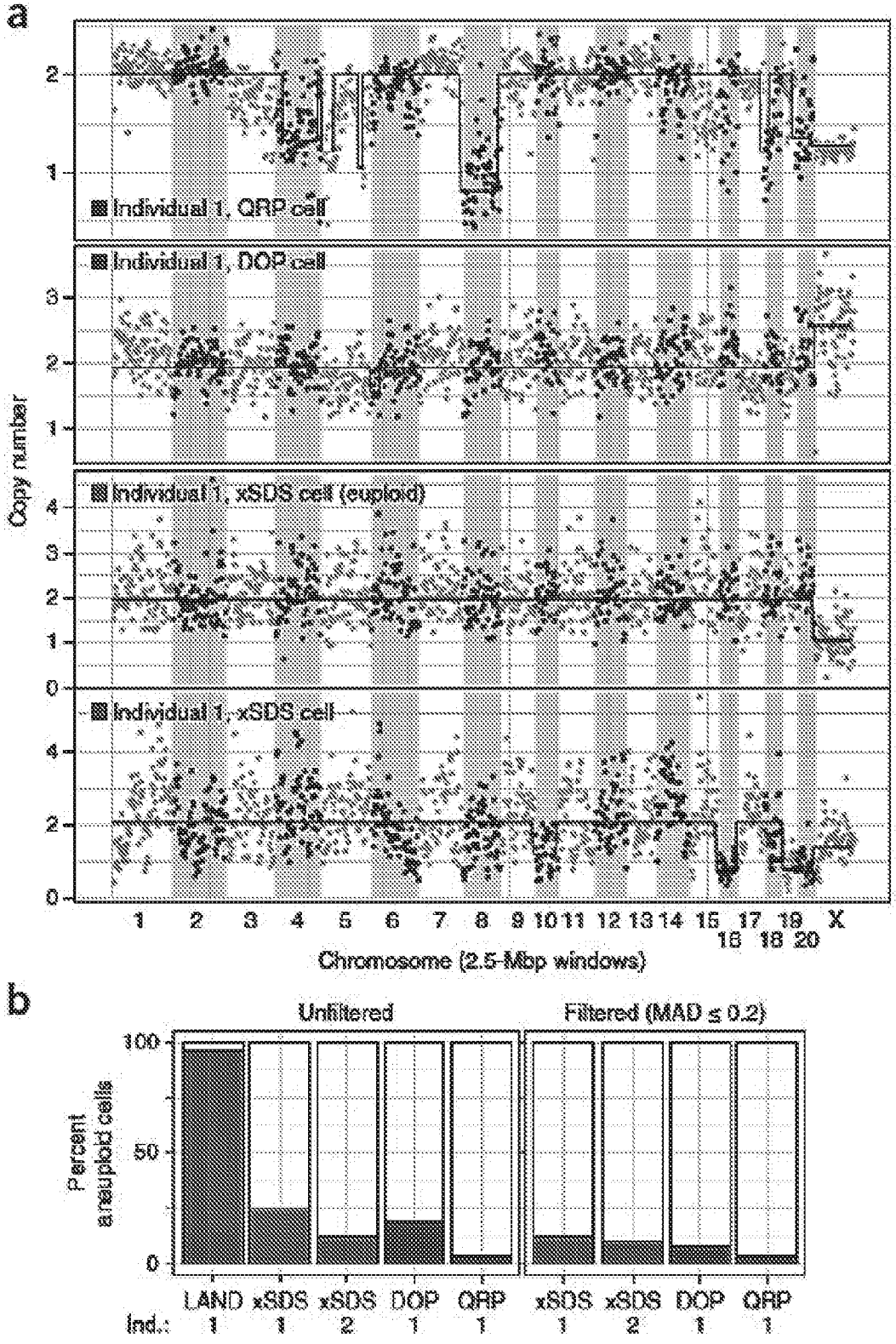

a
Individual 1, QRP cell
Individual 1, DOP cell
Individual 1, xSDS cell (euploid)
Individual 1, xSDS cell
Copy number
Chromosome (2.5-Mbp windows)
b
Unfiltered
Filtered (MAD ≤ 0.2)
Percent aneuploid cells
LAND xSDS xSDS DOP QRP xSDS xSDS DOP QRP
Ind.: 1 1 2 1 1 1 2 1 1

FIG. 20

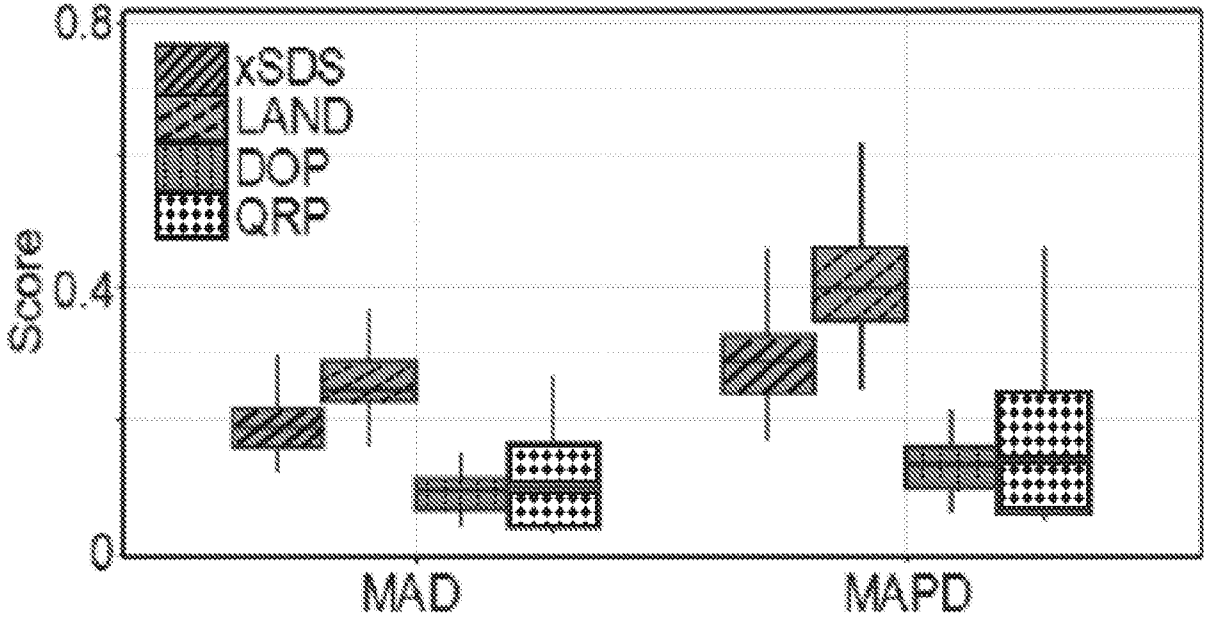


FIG. 21

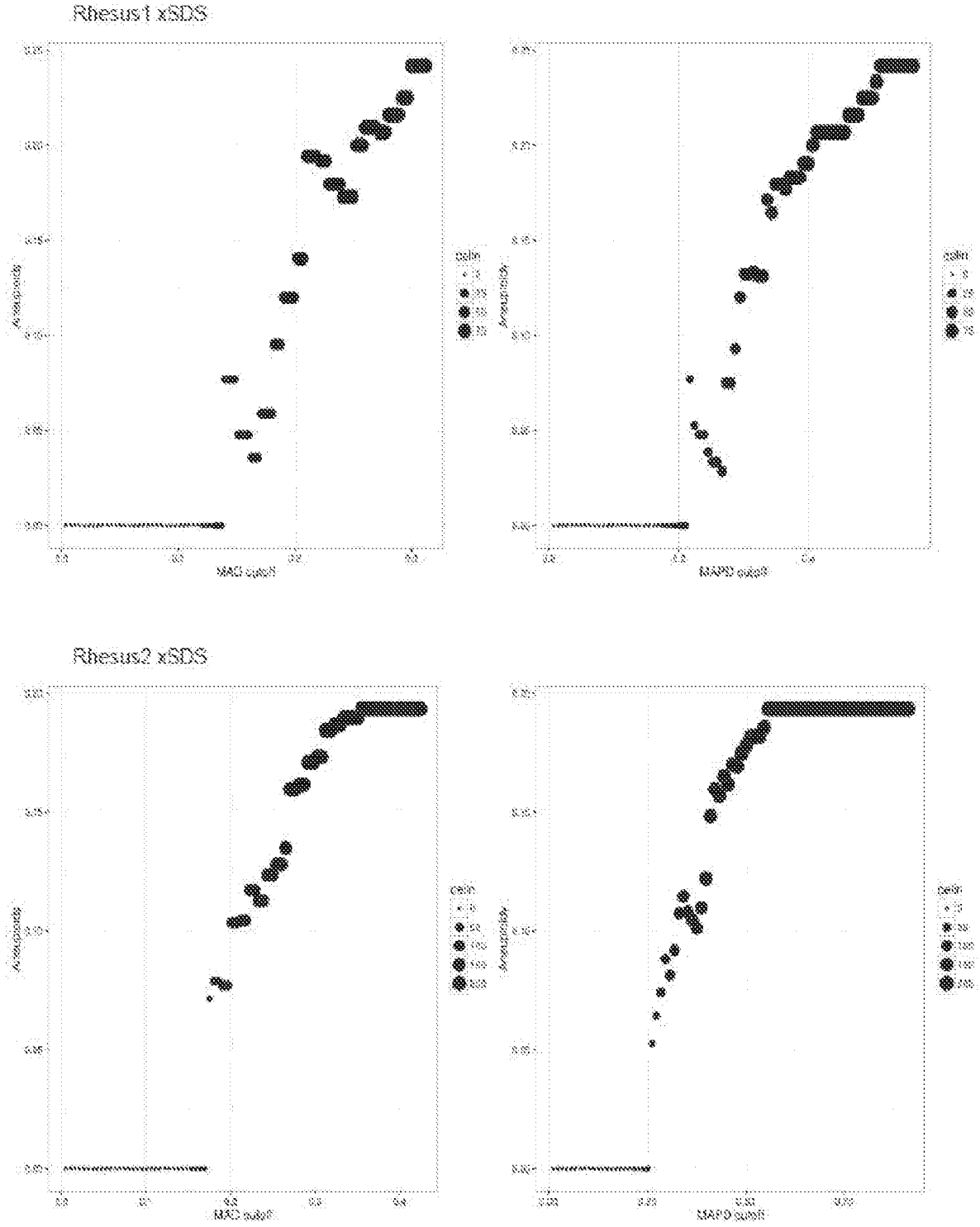


FIG. 22-1

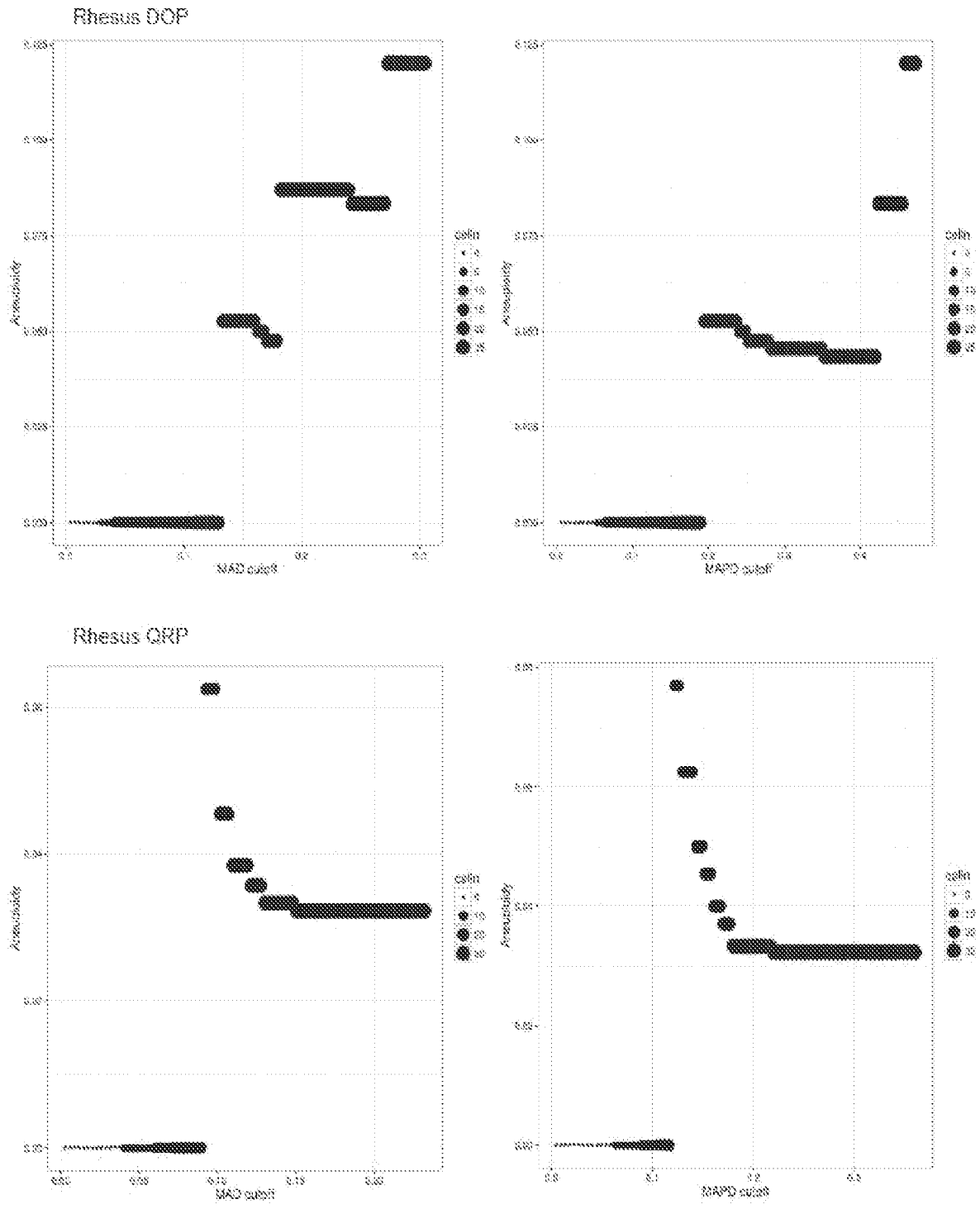


FIG. 22-2

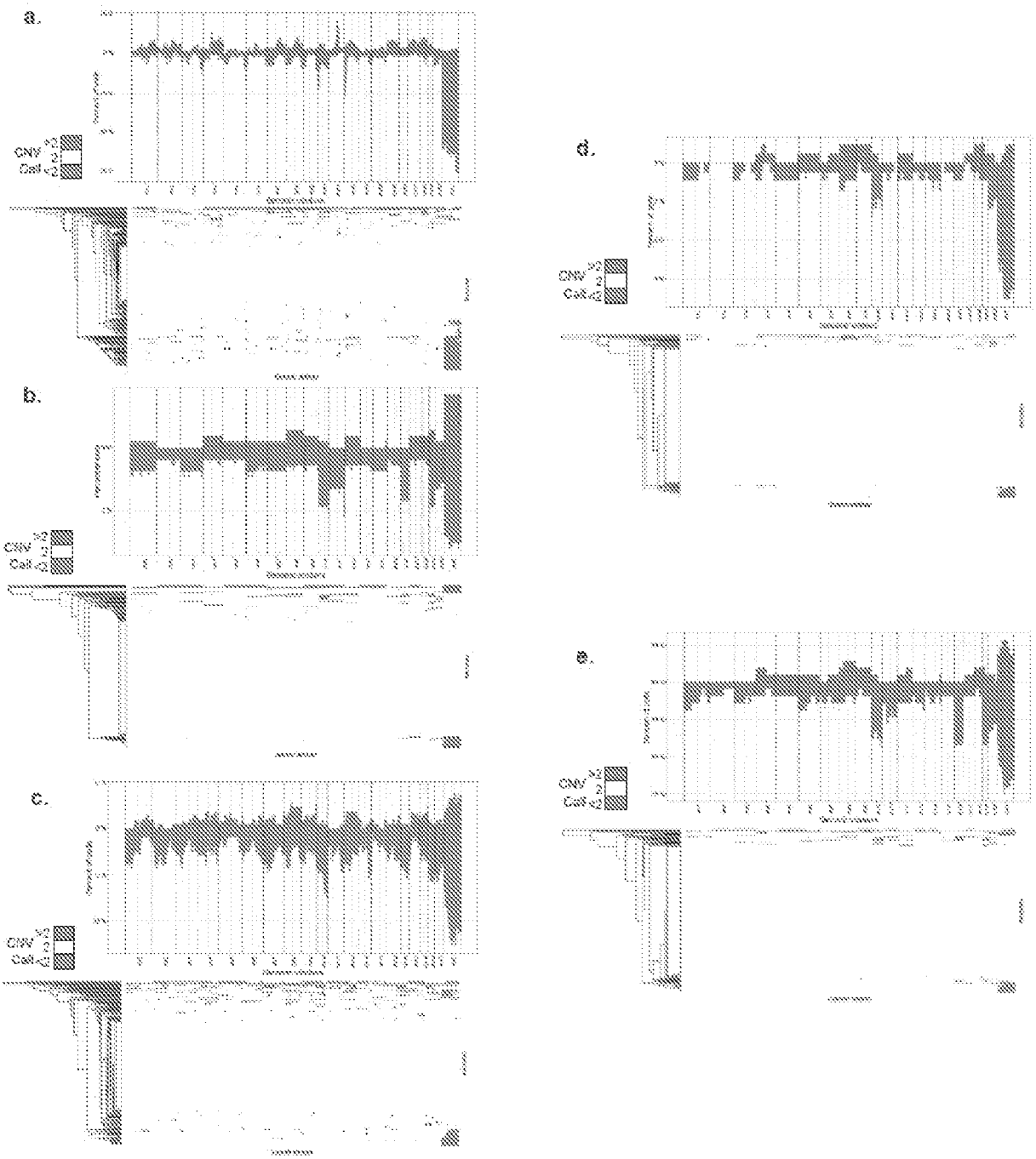


FIG. 23

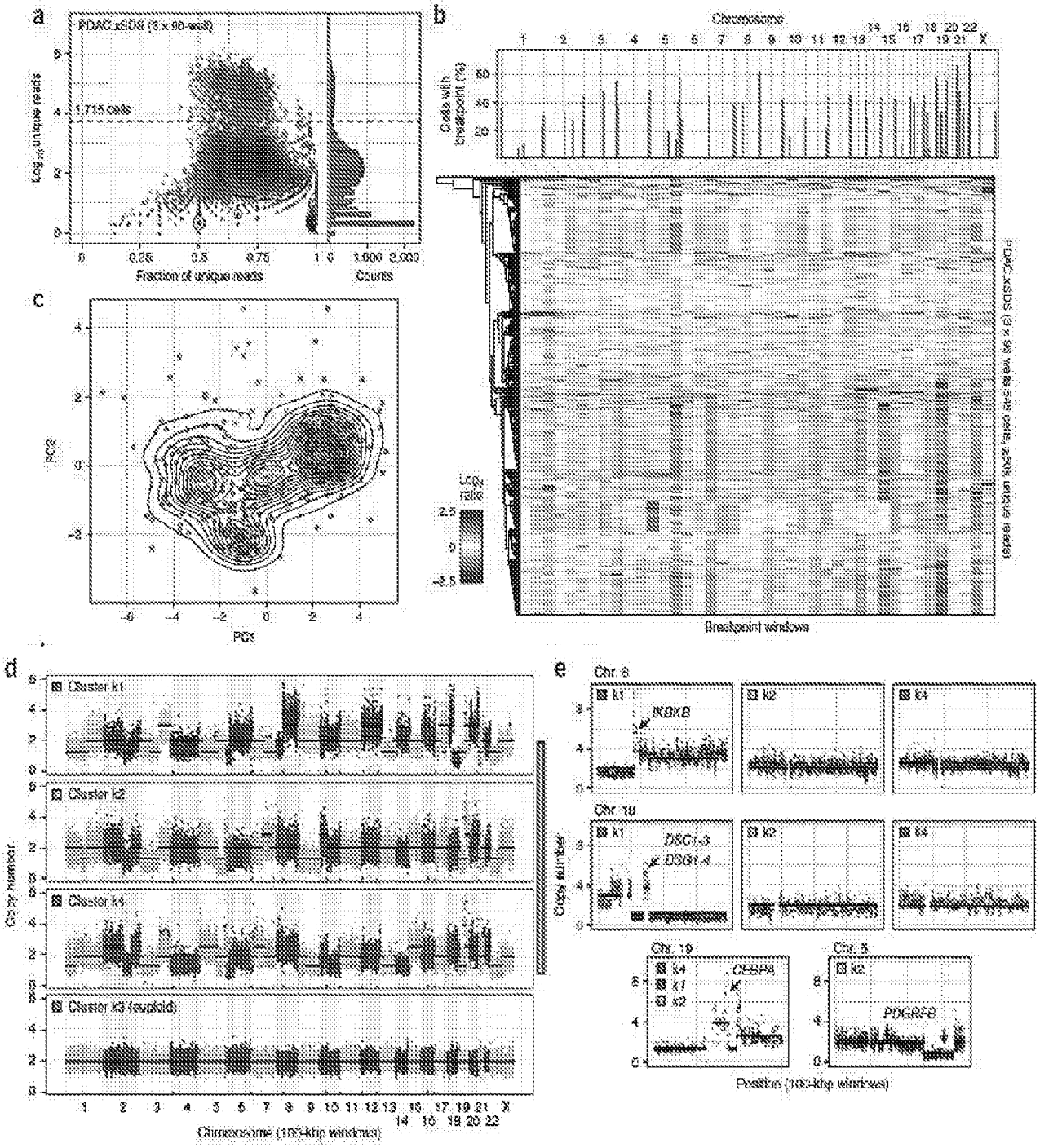

a
PDAC_xSDS (3 x 96-well)
1,715 cells
Log10 unique reads
Fraction of unique reads
Counts
b
Chromosome
Cells with breakpoint (%)
Log2 ratio
Breakpoint windows
c
PC2
PC1
d
Cluster k1
Cluster k2
Cluster k4
Cluster k3 (euploid)
Copy number
Chromosome (100-kbp windows)
e
Chr. 8
IKBKB
Chr. 18
DSC1-3
DSG1-4
Chr. 19
CEBPA
Chr. 5
PDGFRB
Copy number
Position (100-kbp windows)

FIG. 24

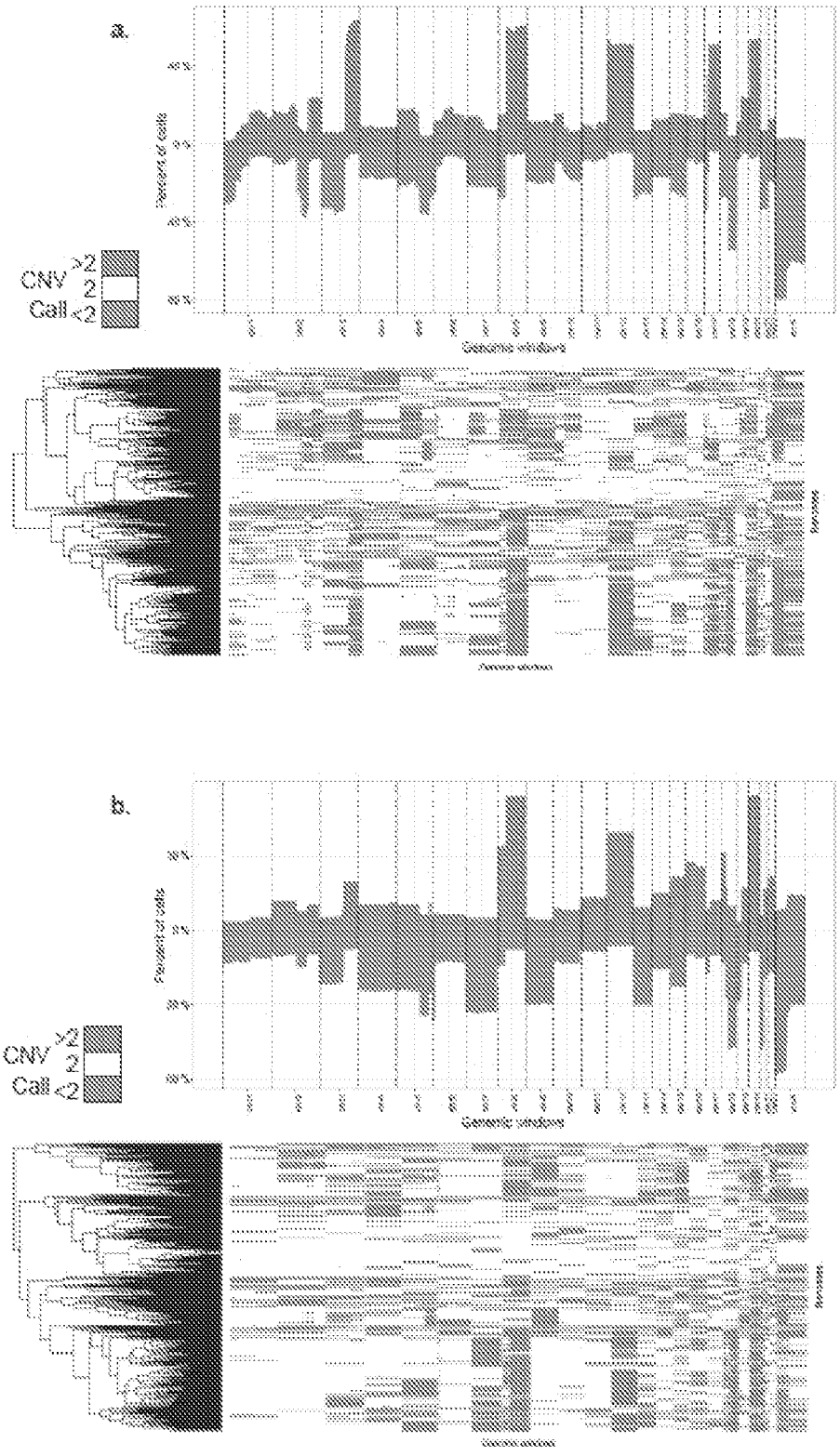


FIG. 25-1

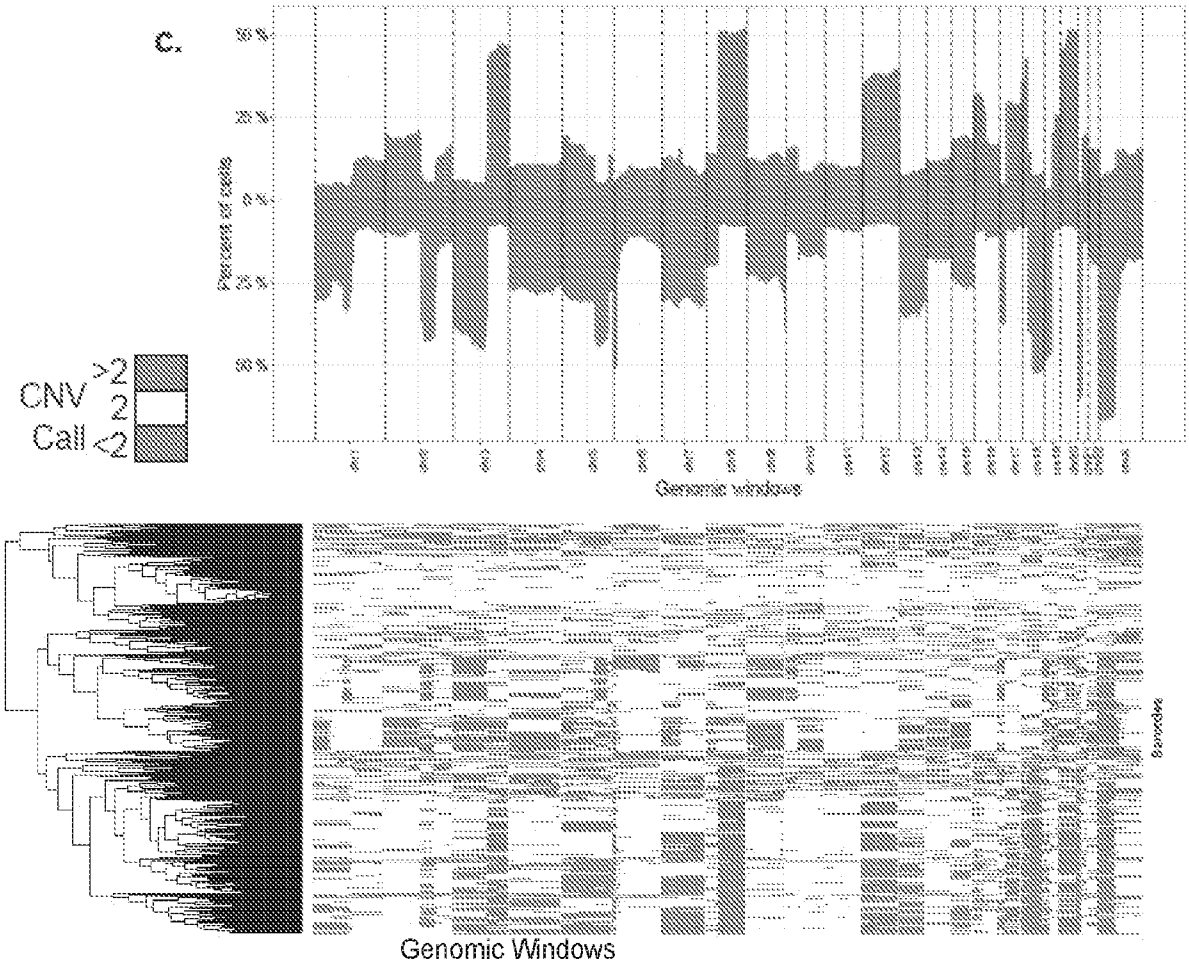


FIG. 25-2

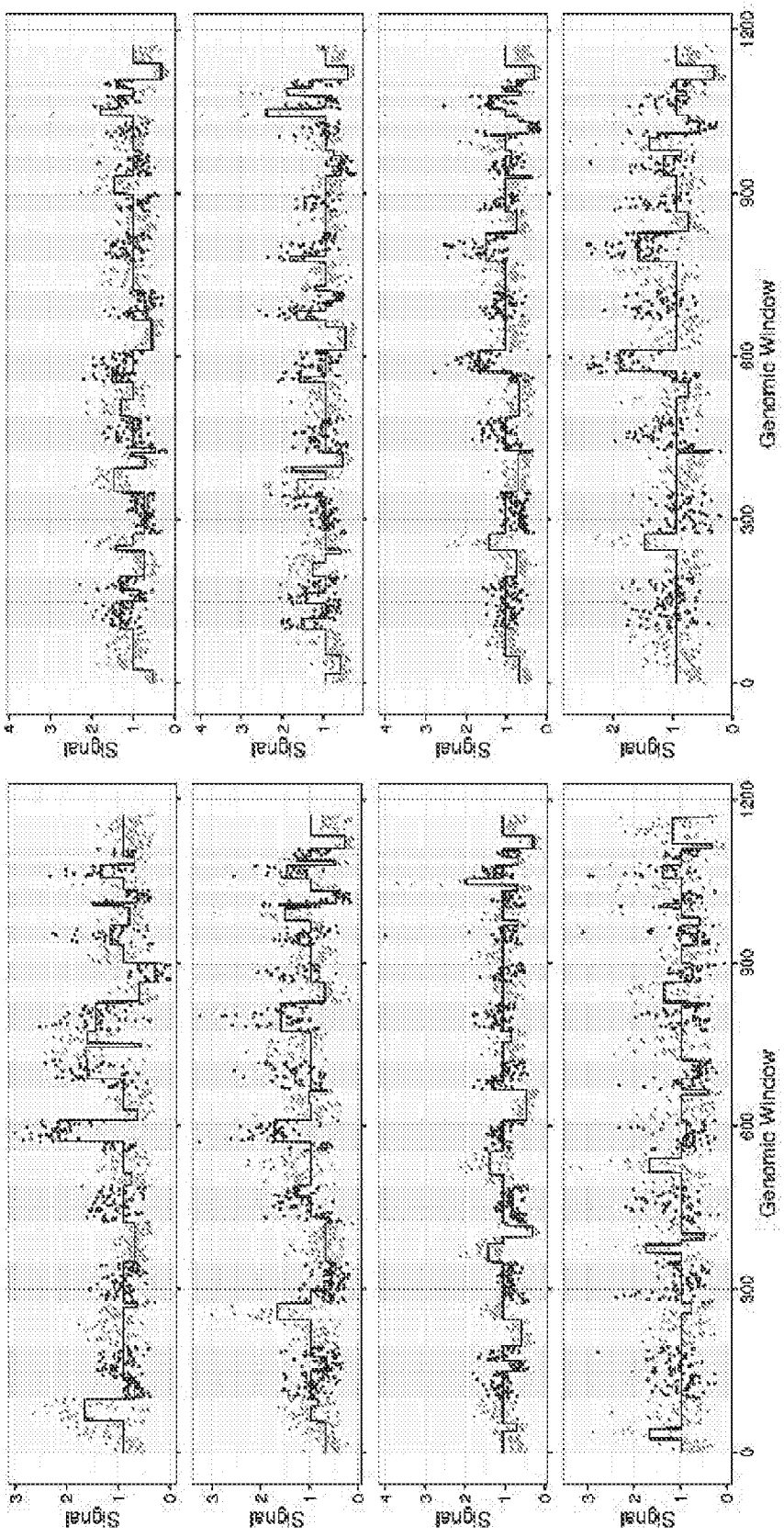


FIG. 26

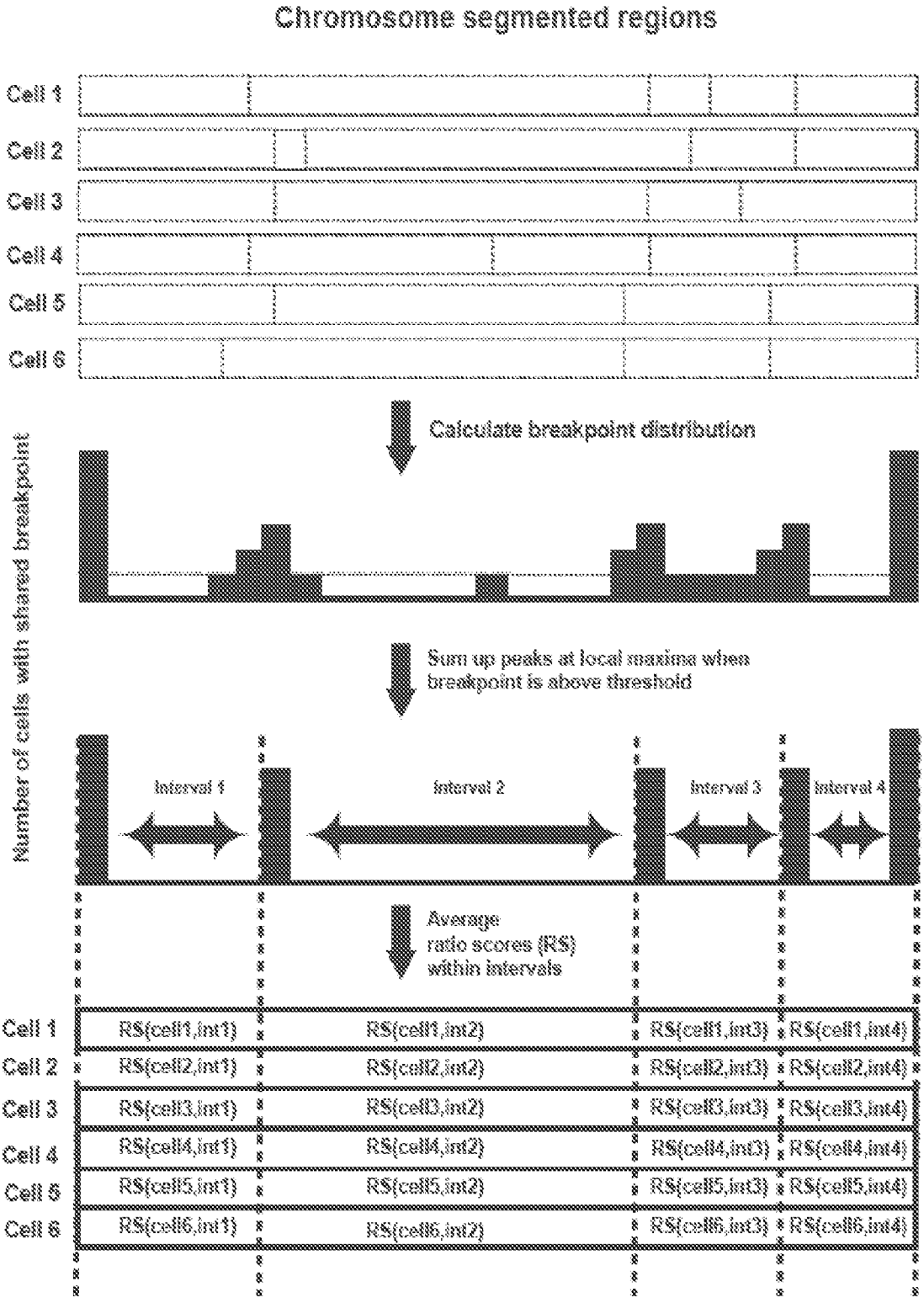

Chromosome segmented regions
Cell 1
Cell 2
Cell 3
Cell 4
Cell 5
Cell 6
Calculate breakpoint distribution
Number of cells with shared breakpoint
Sum up peaks at local maxima when breakpoint is above threshold
Interval 1
Interval 2
Interval 3
Interval 4
Average ratio scores (RS) within intervals
RS(cell1,int1)
RS(cell1,int2)
RS(cell1,int3)
RS(cell1,int4)
RS(cell2,int1)
RS(cell2,int2)
RS(cell2,int3)
RS(cell2,int4)
RS(cell3,int1)
RS(cell3,int2)
RS(cell3,int3)
RS(cell3,int4)
RS(cell4,int1)
RS(cell4,int2)
RS(cell4,int3)
RS(cell4,int4)
RS(cell5,int1)
RS(cell5,int2)
RS(cell5,int3)
RS(cell5,int4)
RS(cell6,int1)
RS(cell6,int2)
RS(cell6,int3)
RS(cell6,int4)

FIG. 27

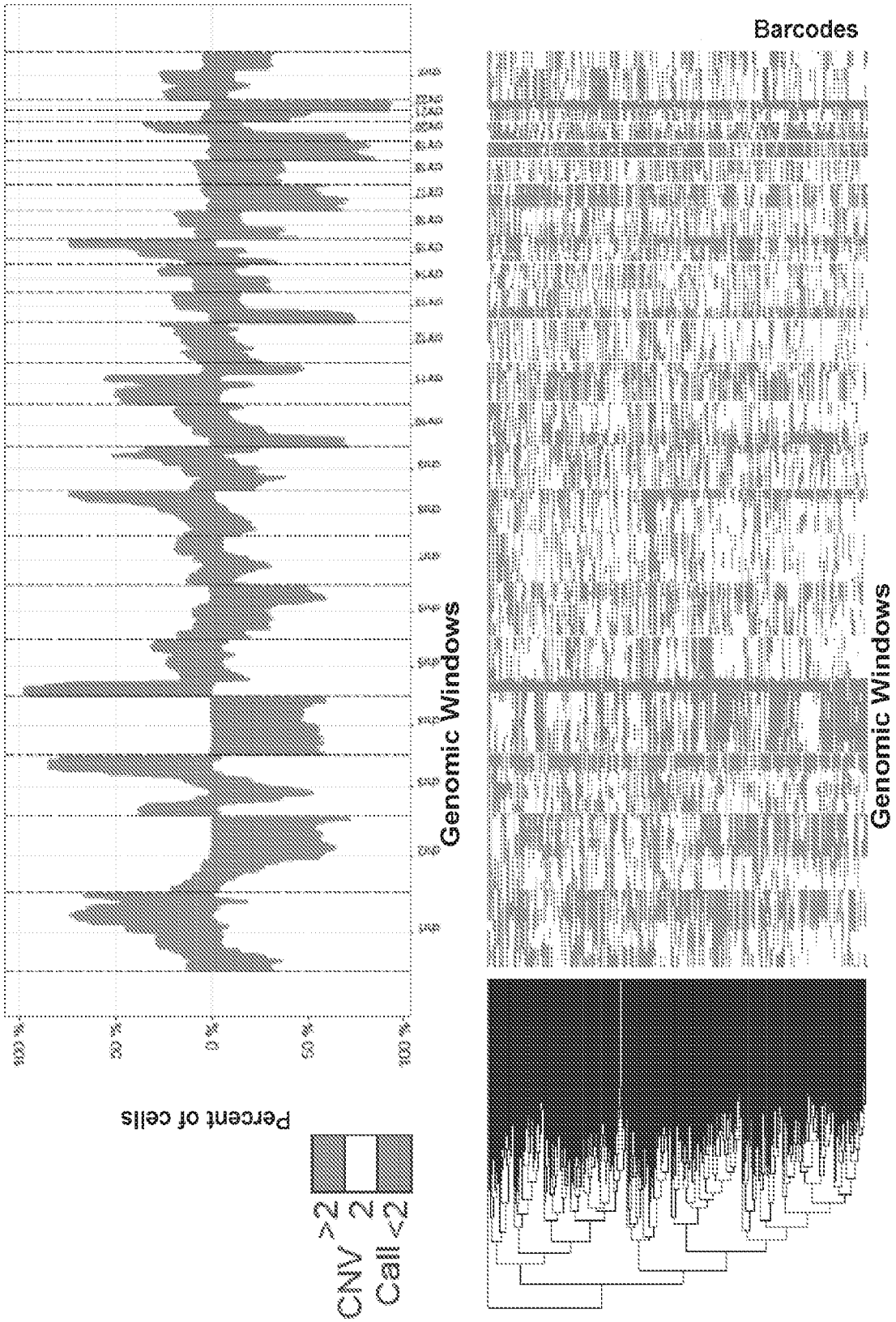

Barcodes
Genomic Windows
Genomic Windows
Percent of cells
100 %
50 %
0 %
50 %
100 %
CNV
Call
>2
2
<2

FIG. 28

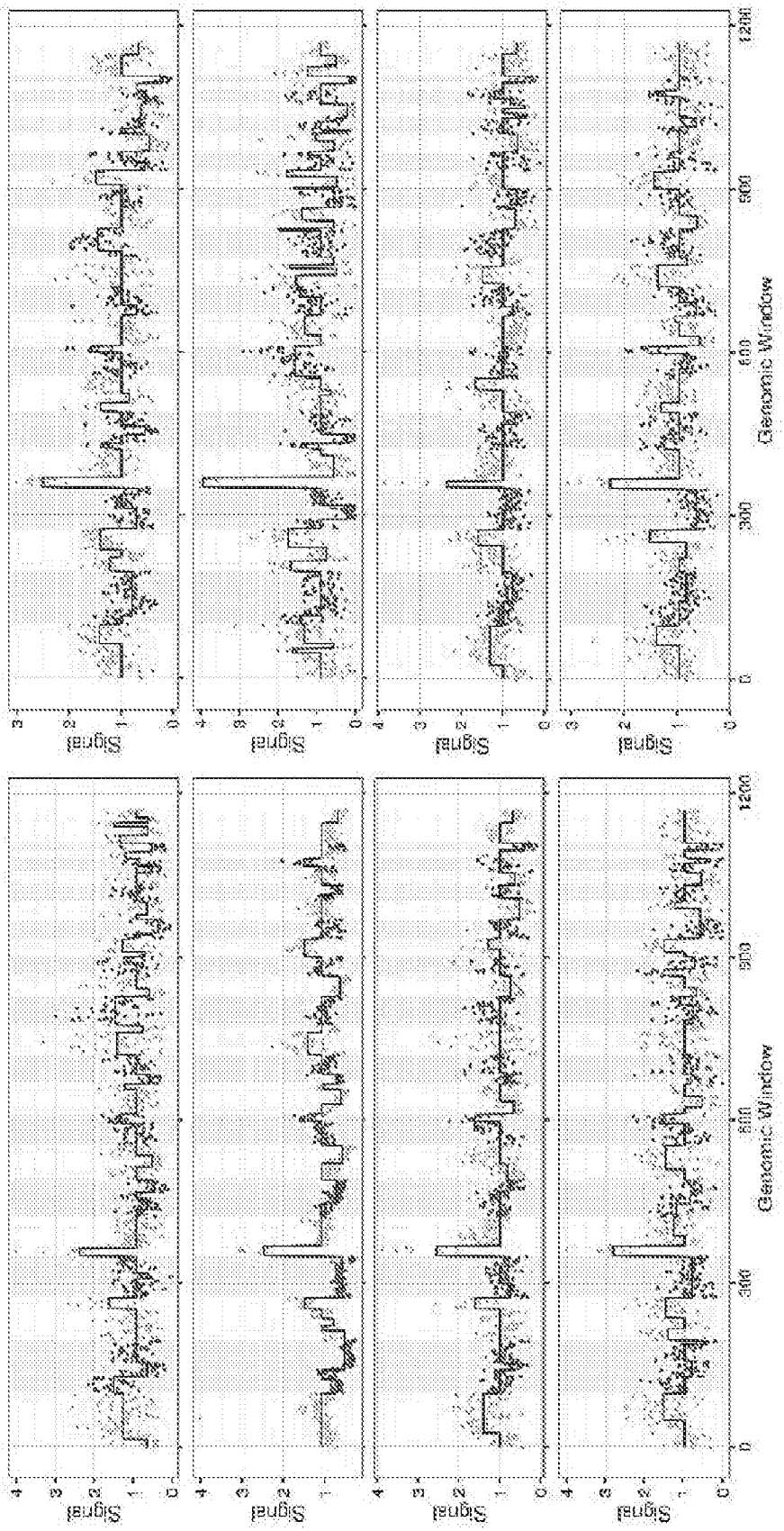

Signal
Signal
Signal
Signal
Signal
Signal
Signal
Signal
0
300
600
900
1200
Genomic Window
Genomic Window

FIG. 29

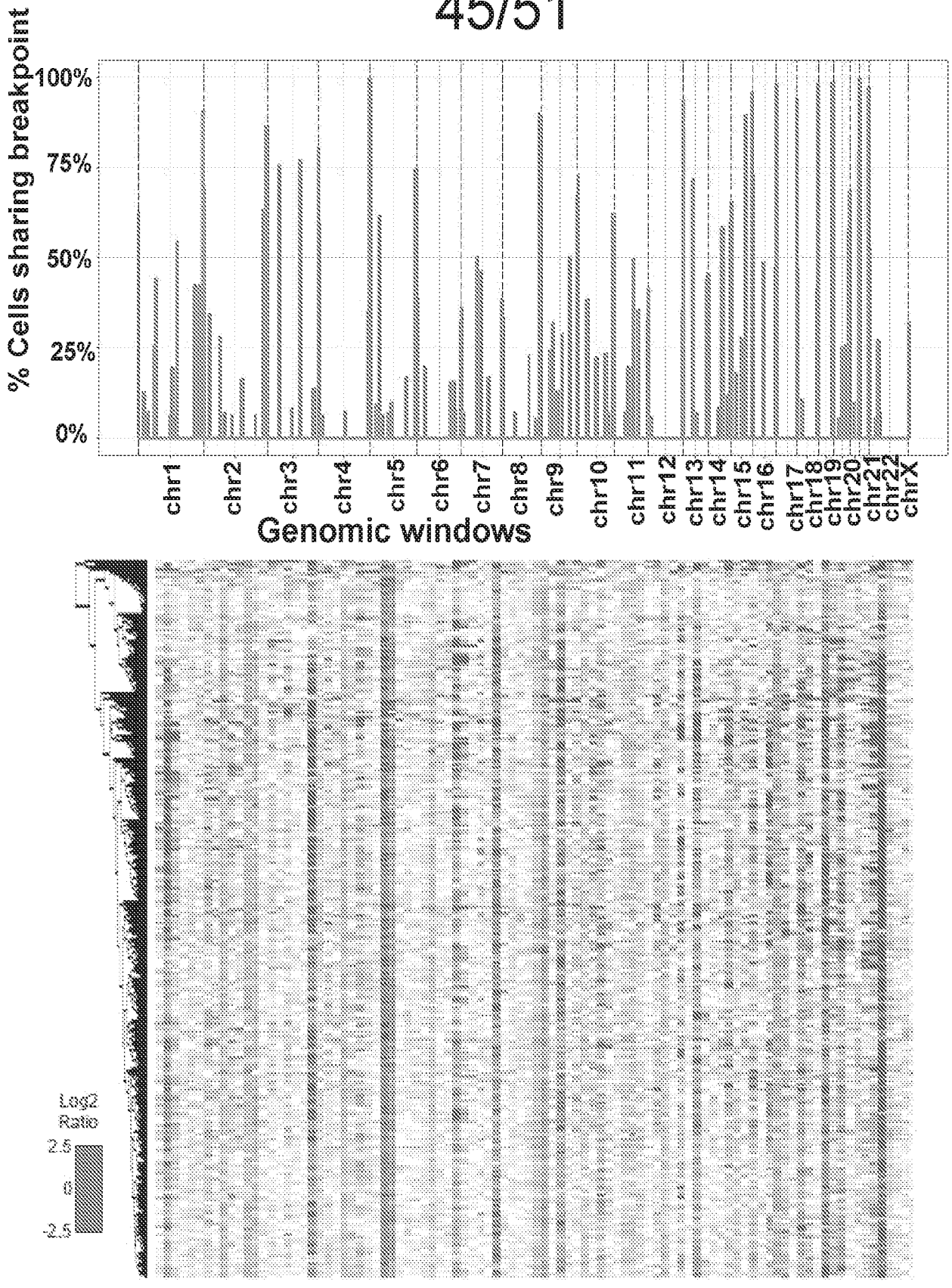

% Cells sharing breakpoint
100%
75%
50%
25%
0%
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
chrX
Genomic windows
Log2 Ratio
2.5
0
-2.5

FIG. 30

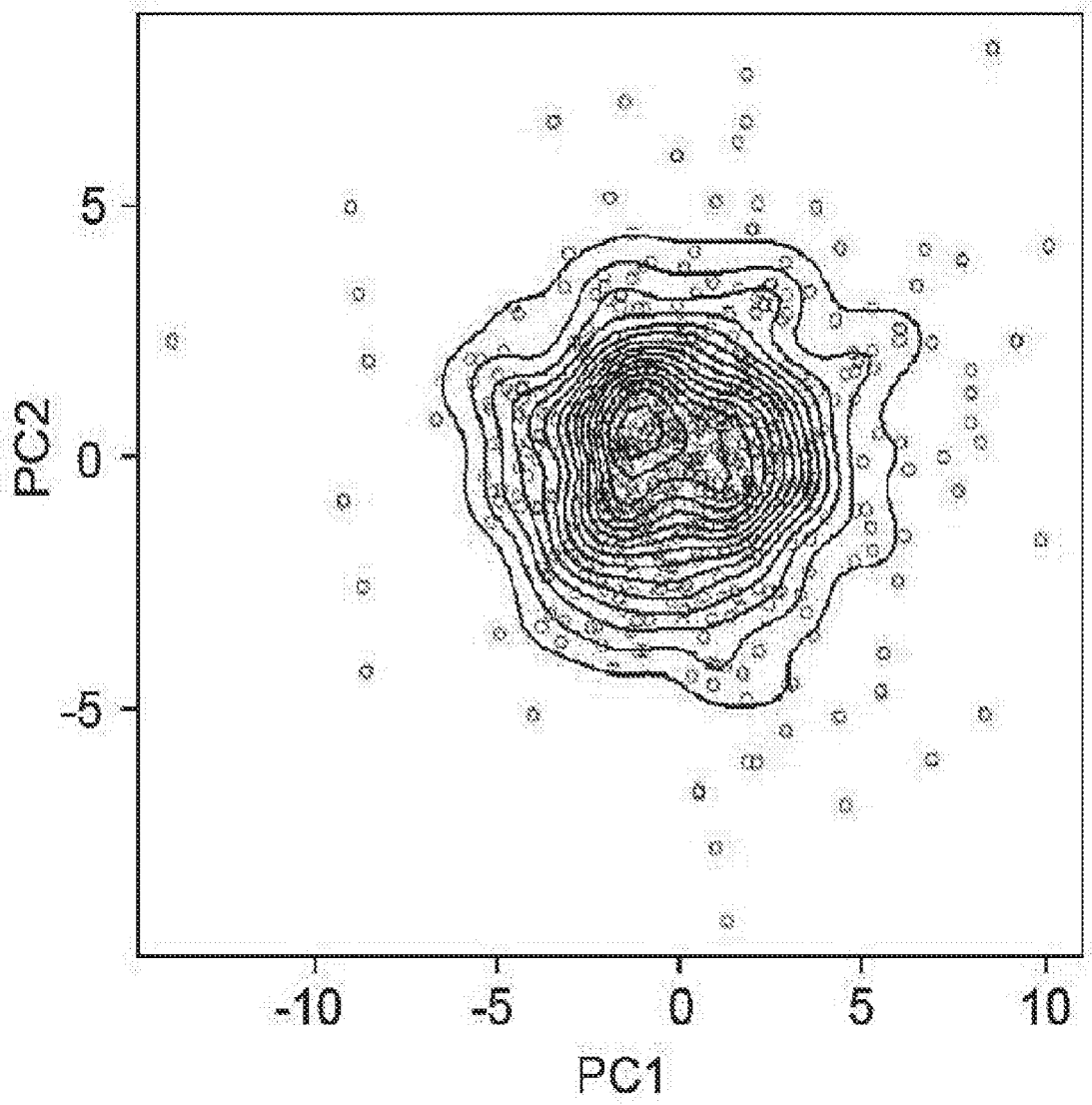


FIG. 31

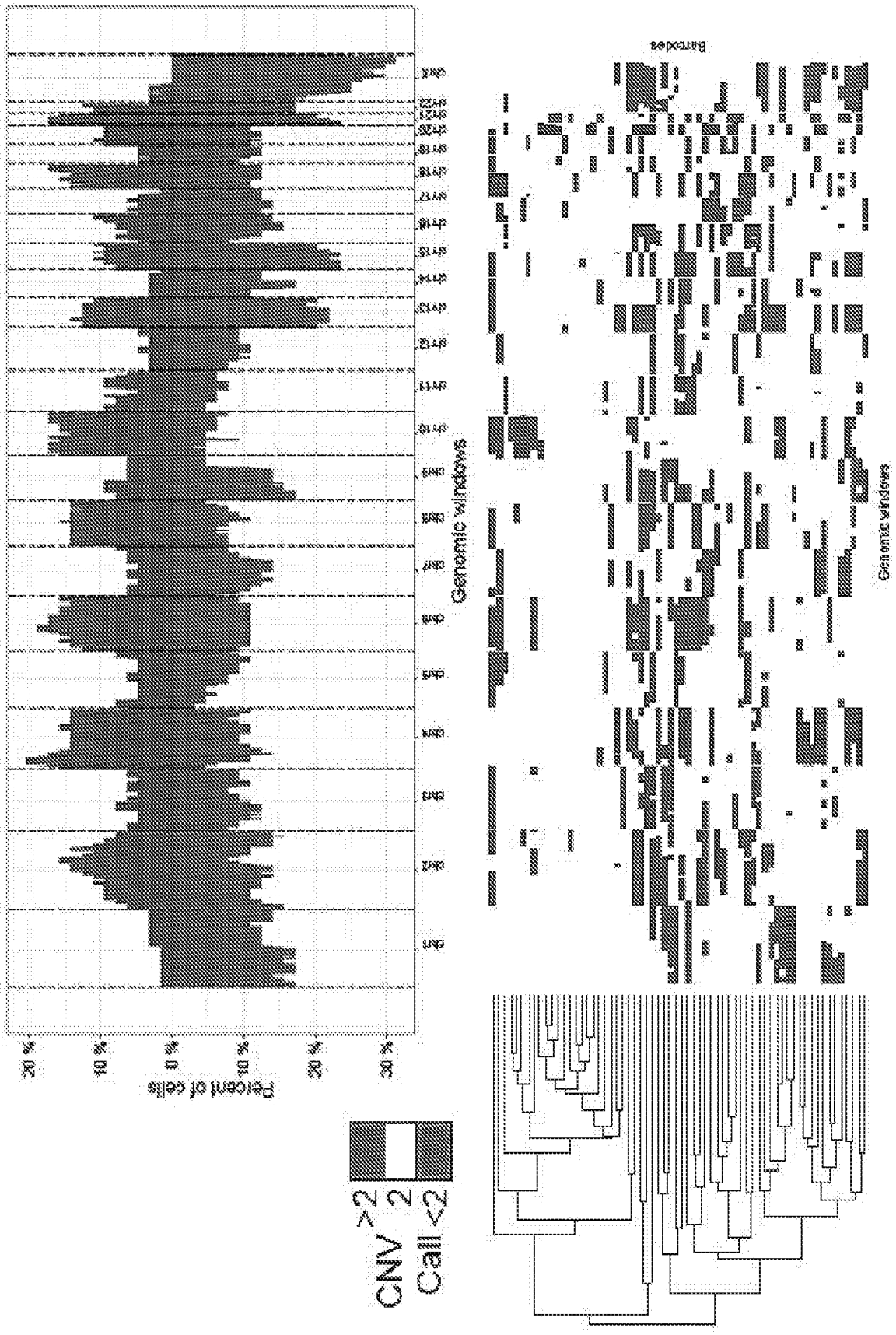

Genomic windows
Percent of cells
20 %
10 %
0 %
10 %
20 %
30 %
CNV
Call
>2
2
<2
Genomic windows
Barcodes

FIG. 32

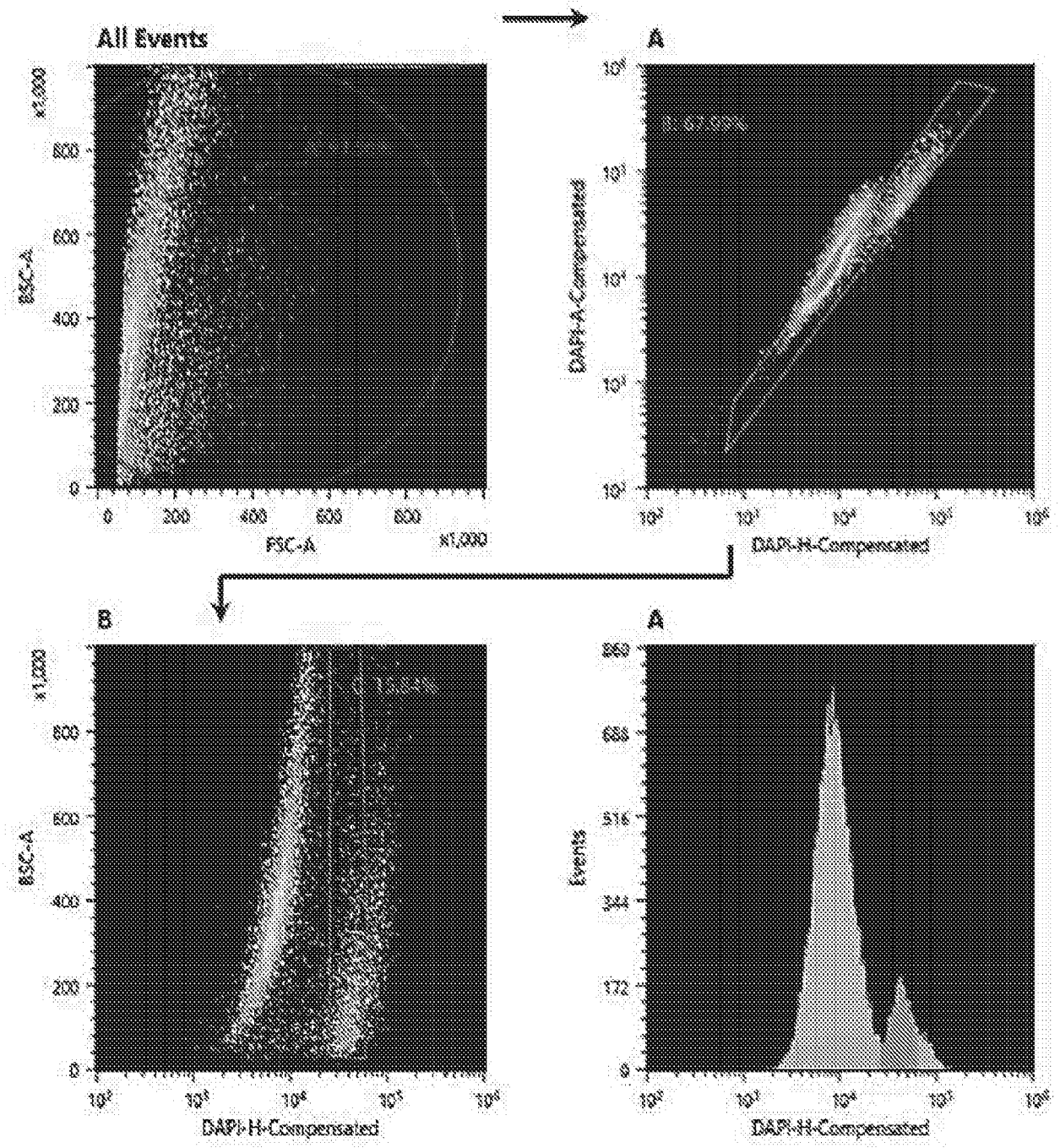


FIG. 33

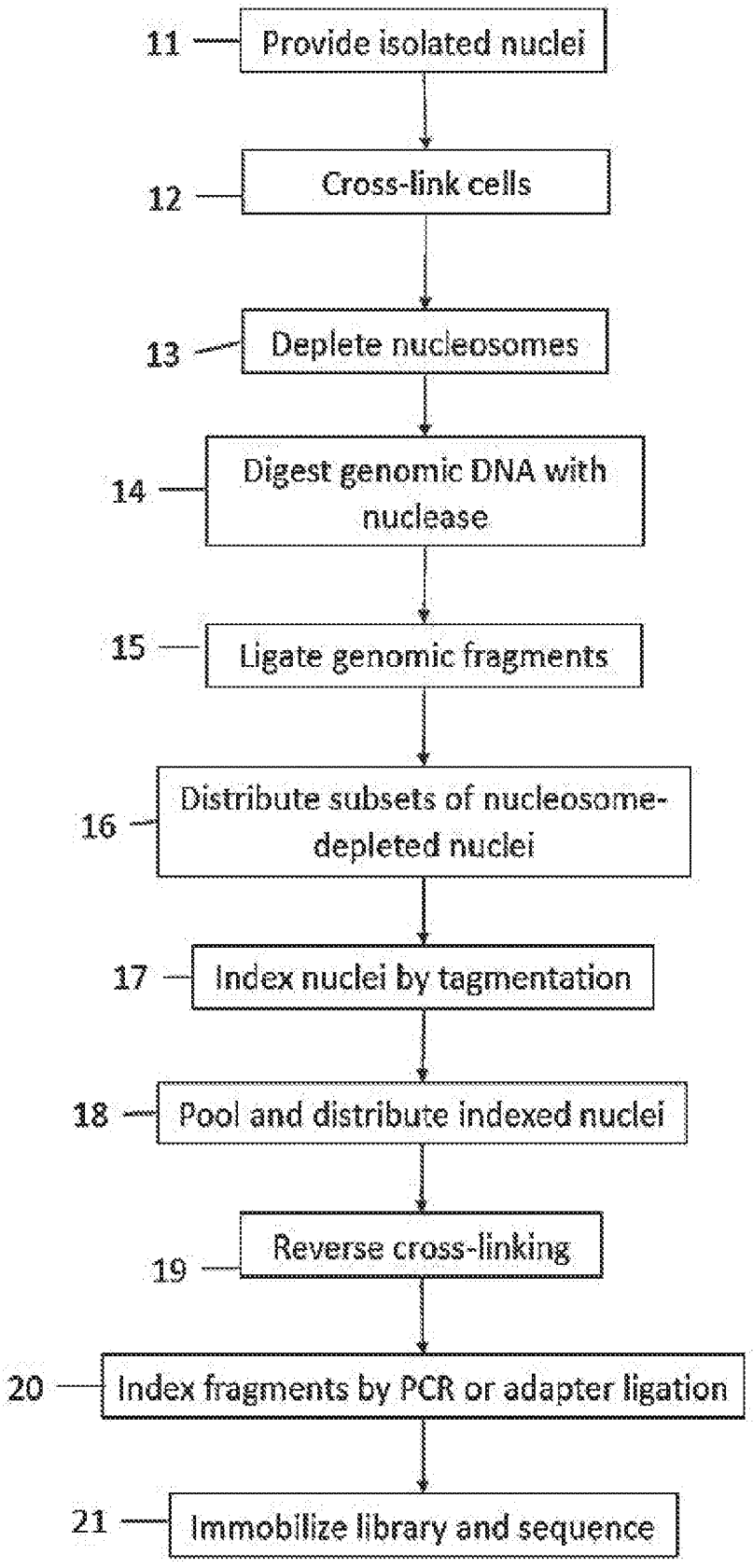

11
Provide isolated nuclei
12
Cross-link cells
13
Deplete nucleosomes
14
Digest genomic DNA with nuclease
15
Ligate genomic fragments
16
Distribute subsets of nucleosome-depleted nuclei
17
Index nuclei by tagmentation
18
Pool and distribute indexed nuclei
19
Reverse cross-linking
20
Index fragments by PCR or adapter ligation
21
Immobilize library and sequence

FIG. 34

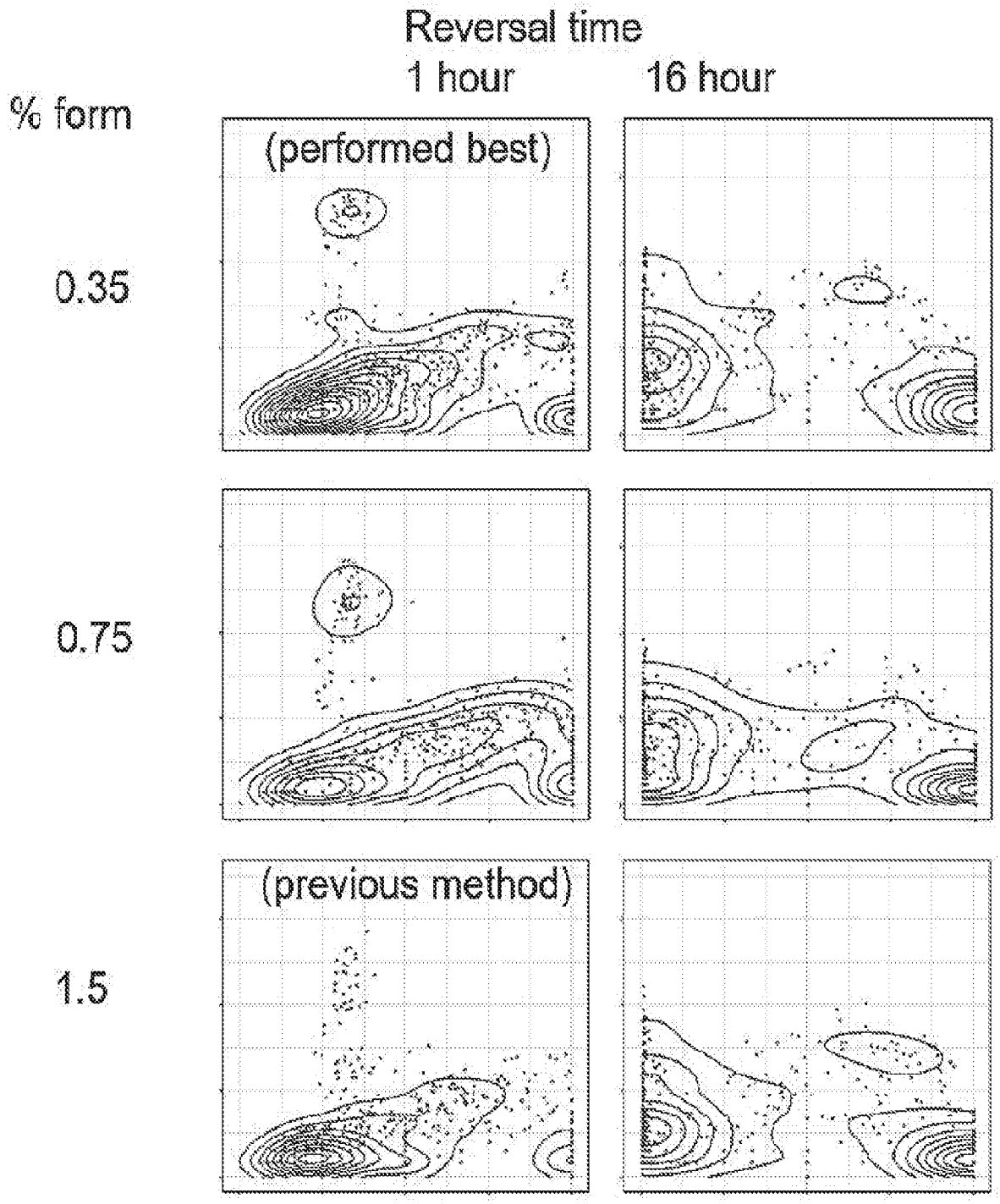

Reversal time
1 hour
16 hour
% form
(performed best)
0.35
0.75
(previous method)
1.5

FIG. 35

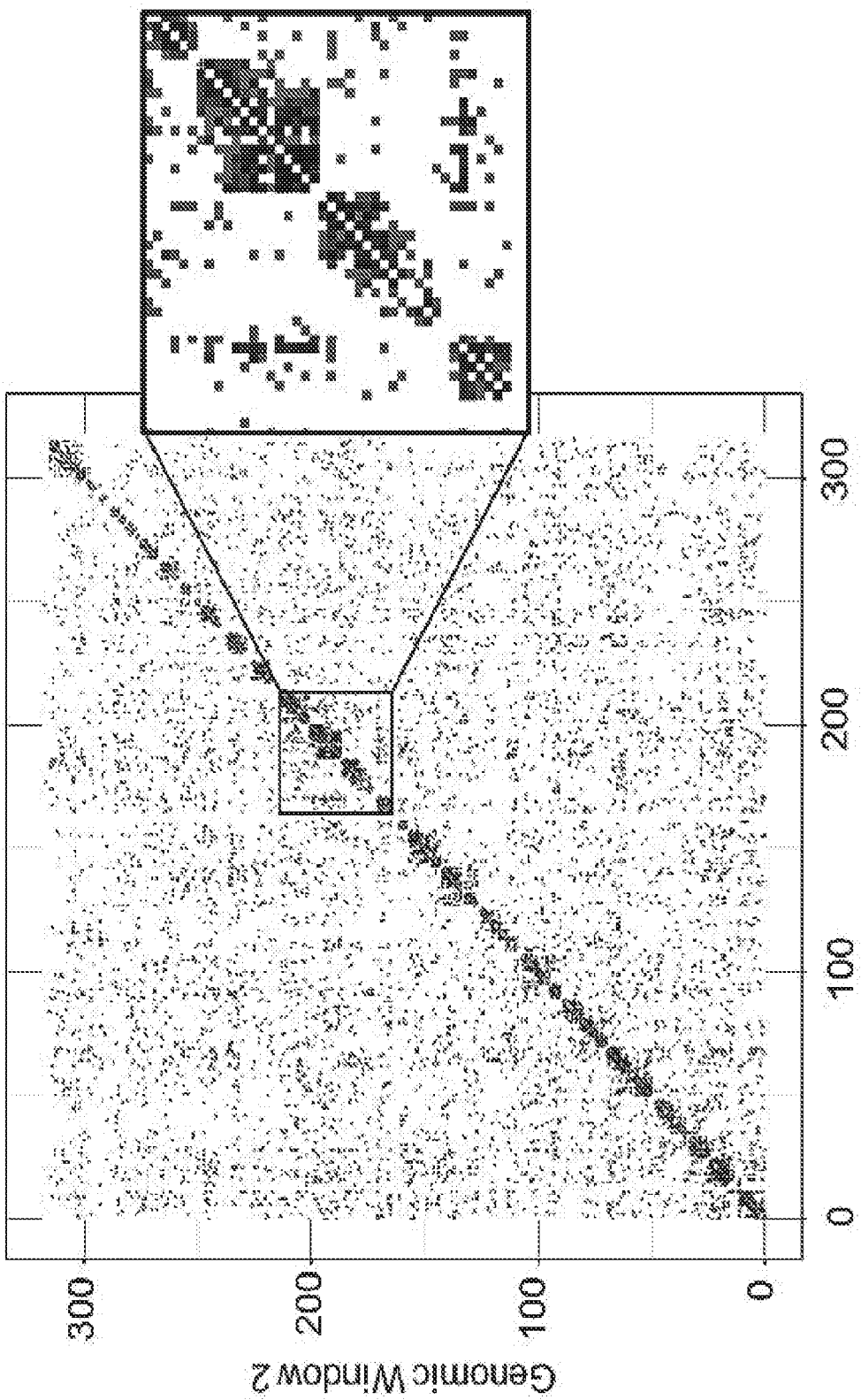


FIG. 36