FIG. 1A

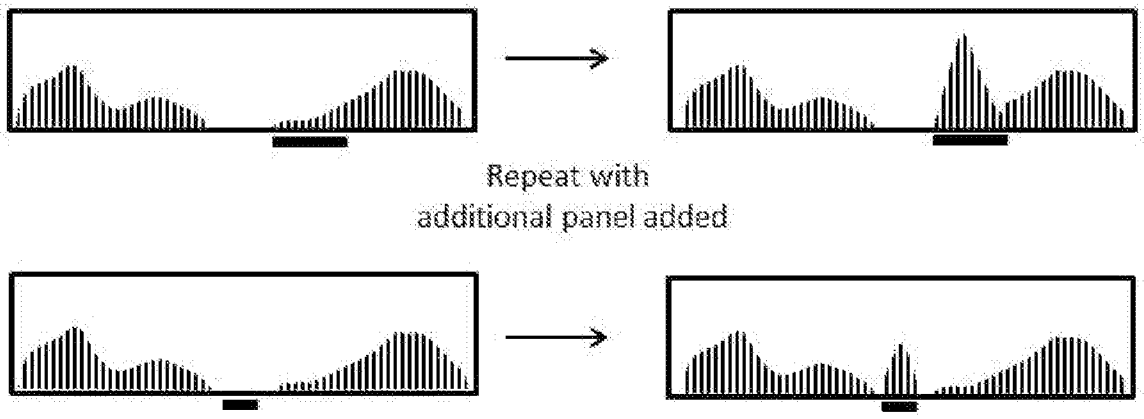


FIG. 1B

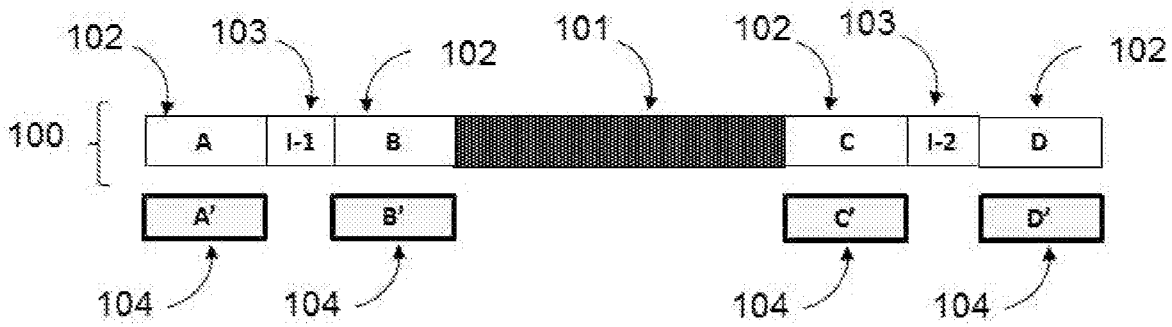
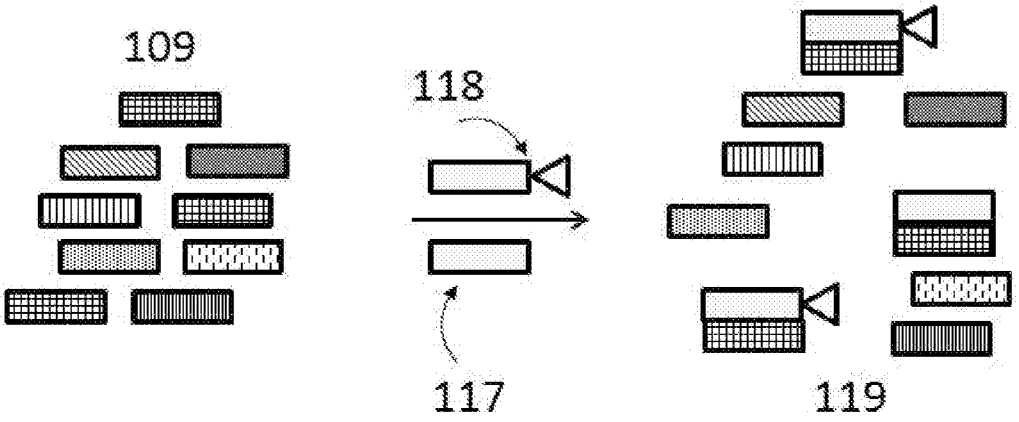


FIG. 1C



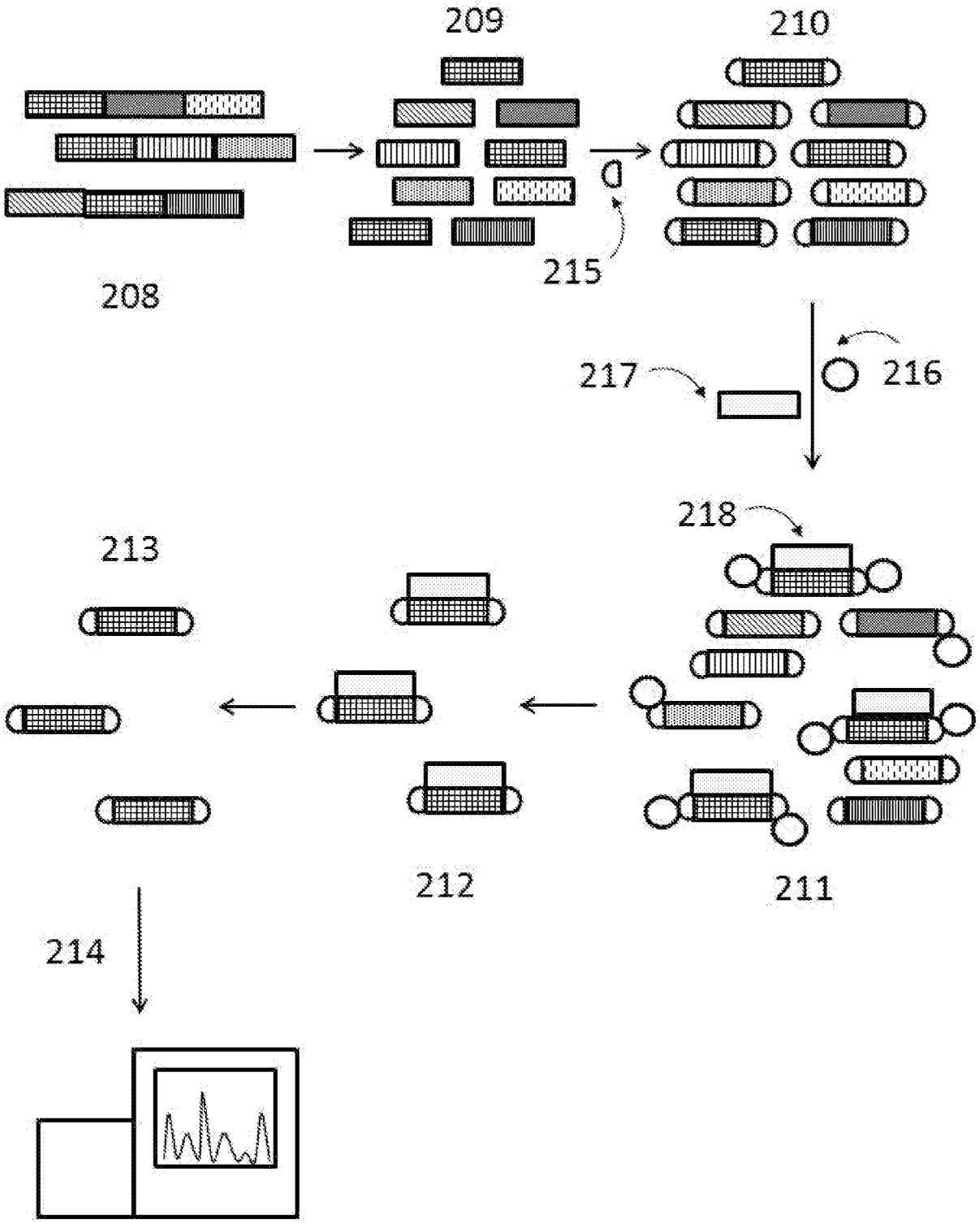


FIG. 2

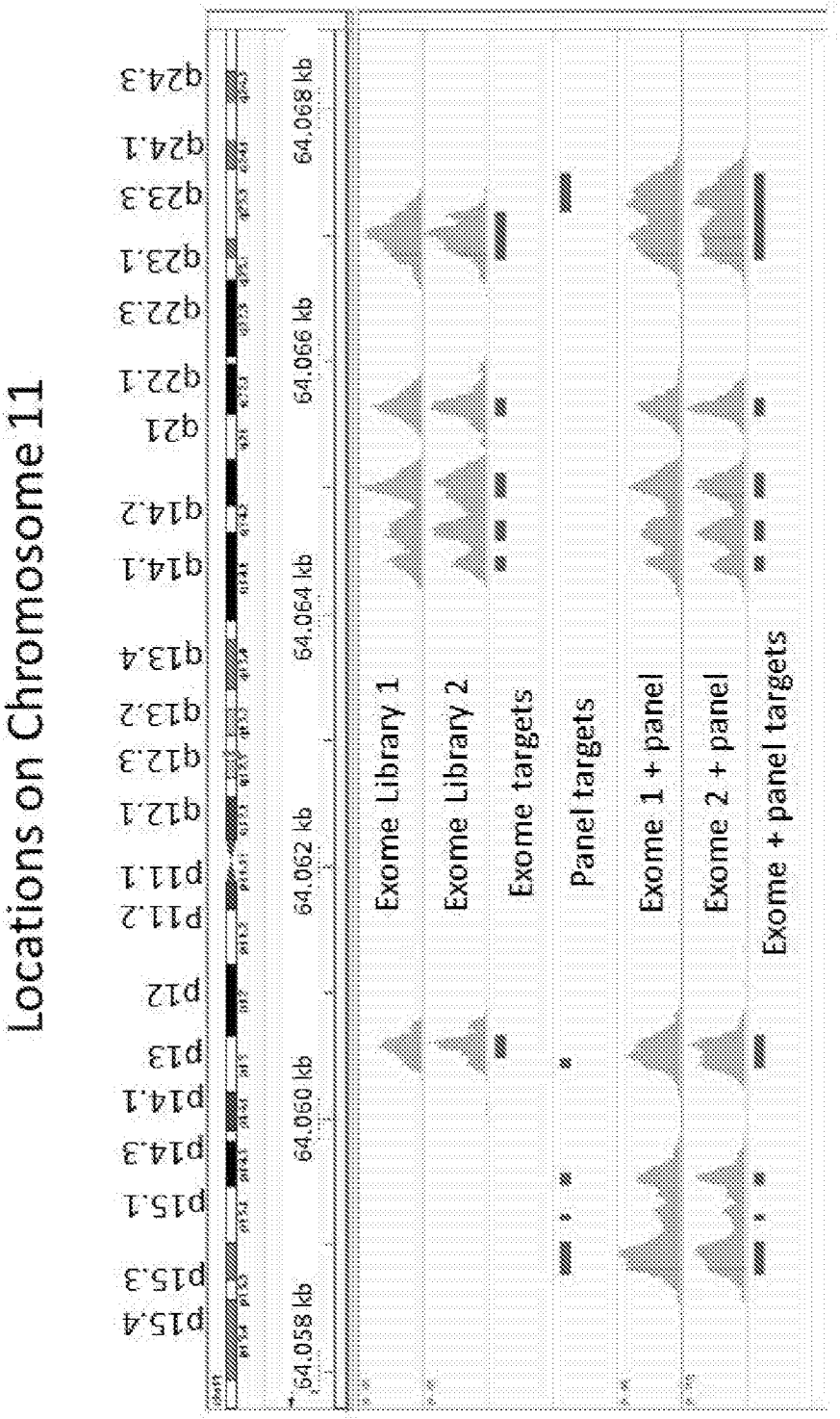

Locations on Chromosome 11
p15.4
p15.3
p15.1
p14.3
p14.1
p13
p12
p11.2
p11.1
q12.1
q12.3
q13.2
q13.4
q14.1
q14.2
q21
q22.1
q22.3
q23.1
q23.3
q24.1
q24.3
64.058 kb
64.060 kb
64.062 kb
64.064 kb
64.066 kb
64.068 kb
Exome Library 1
Exome Library 2
Exome targets
Panel targets
Exome 1 + panel
Exome 2 + panel
Exome + panel targets

FIG. 3

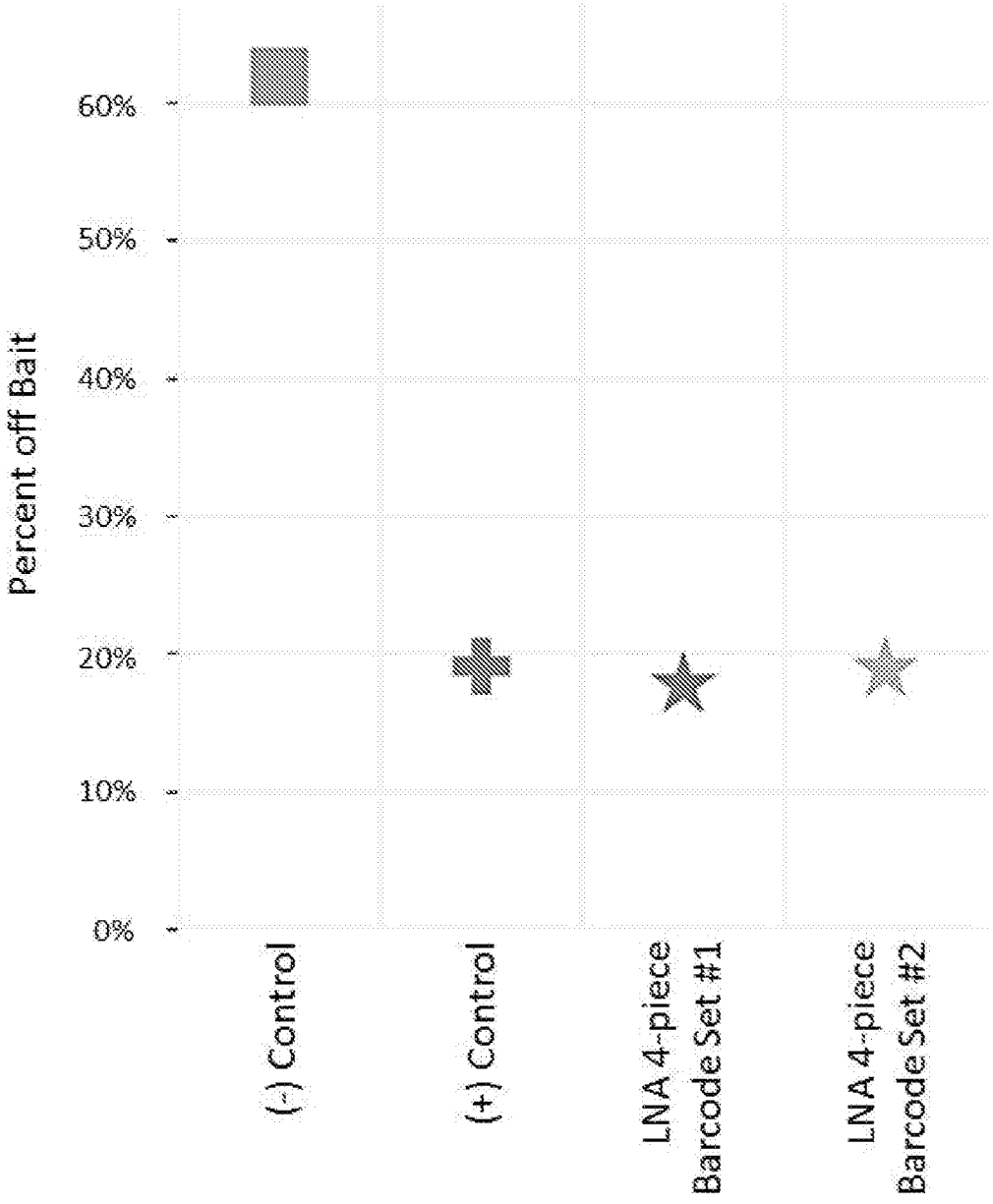

Percent off Bait
60%
50%
40%
30%
20%
10%
0%
(-) Control
(+) Control
LNA 4-piece Barcode Set #1
LNA 4-piece Barcode Set #2

FIG. 4A

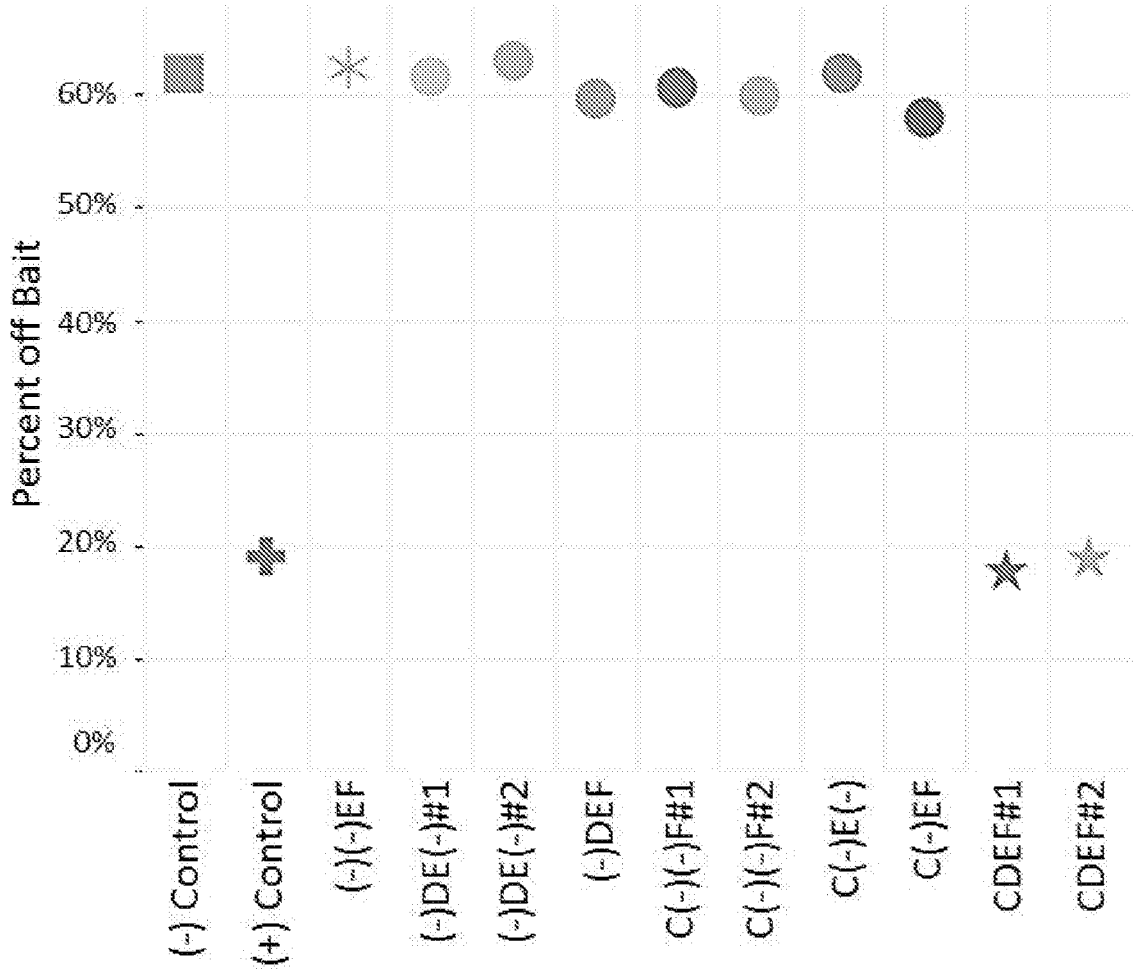

Percent off Bait
60%
50%
40%
30%
20%
10%
0%
(-) Control
(+) Control
(-)(-)EF
(-)DE(-)#1
(-)DE(-)#2
(-)DEF
C(-)(-)F#1
C(-)(-)F#2
C(-)E(-)
C(-)EF
CDEF#1
CDEF#2

FIG. 4B

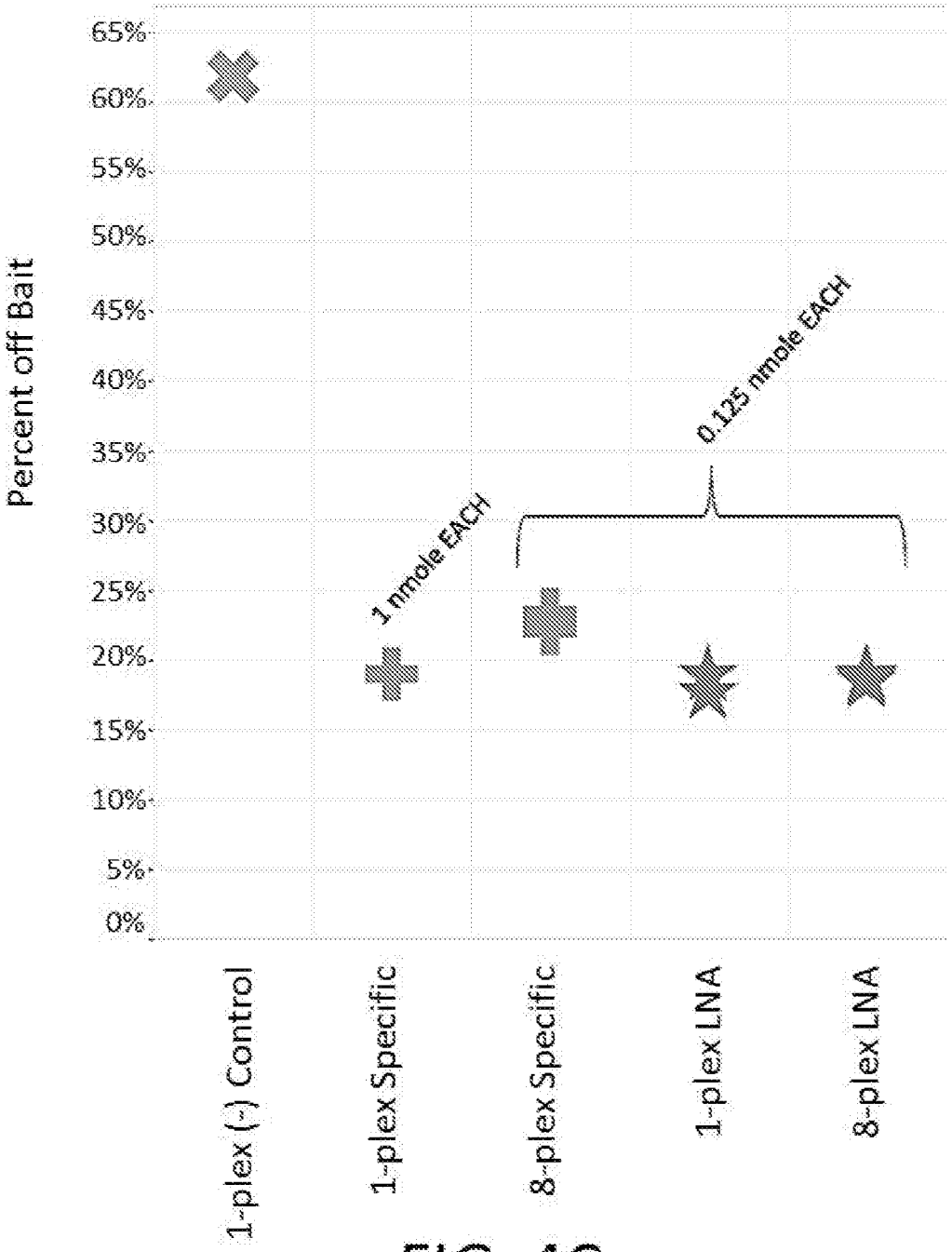

Percent off Bait
65%
60%
55%
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
1 nmole EACH
0.125 nmole EACH
1-plex (-) Control
1-plex Specific
8-plex Specific
1-plex LNA
8-plex LNA

FIG. 4C

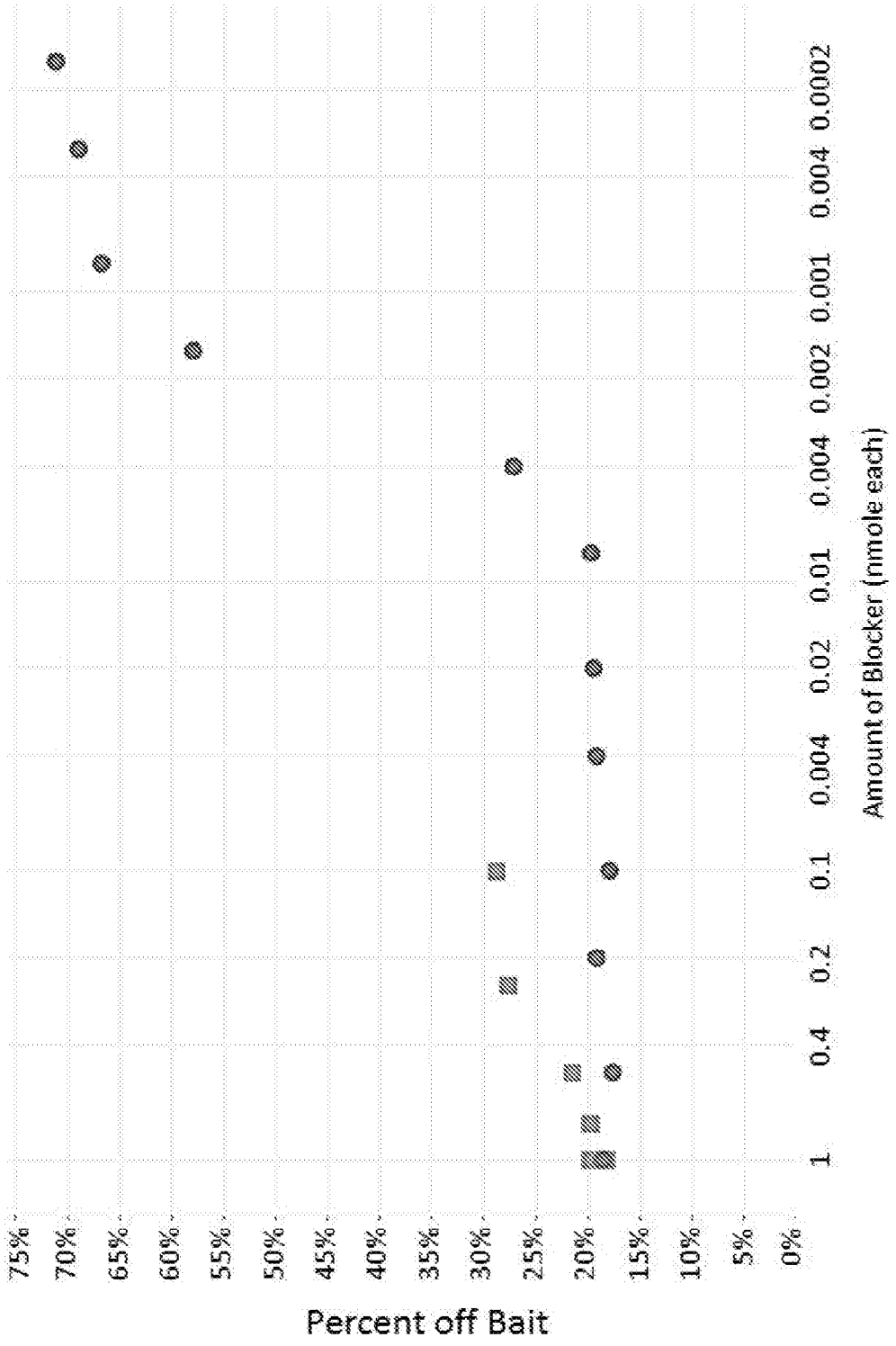

75%
70%
65%
60%
55%
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Percent off Bait
1
0.4
0.2
0.1
0.004
0.02
0.01
0.004
0.002
0.001
0.004
0.0002
Amount of Blocker (nmole each)

FIG. 4D

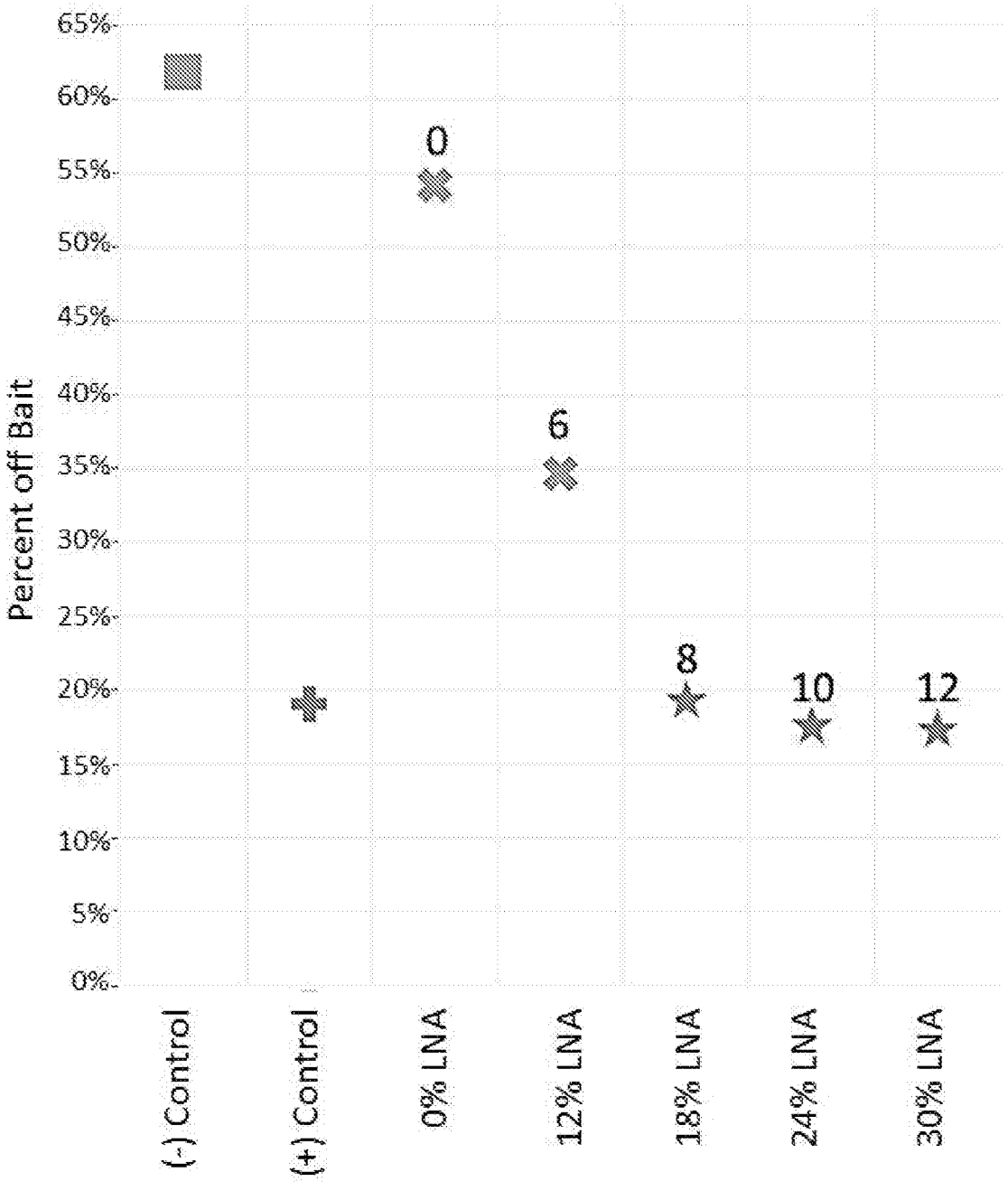

Percent off Bait
65%
60%
55%
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
0
6
8
10
12
(-) Control
(+) Control
0% LNA
12% LNA
18% LNA
24% LNA
30% LNA

FIG. 4E

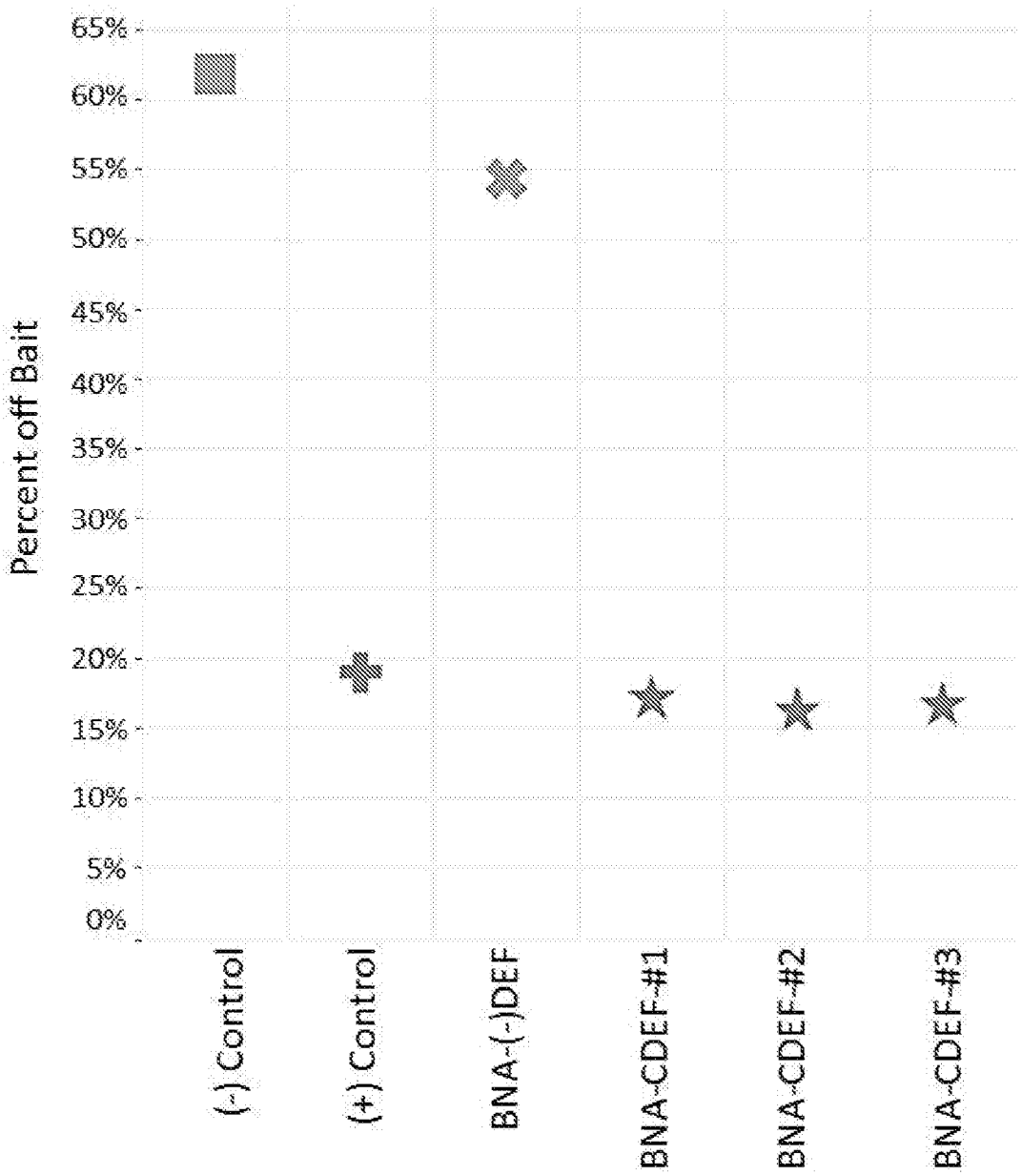

Percent off Bait
65%
60%
55%
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
(-) Control
(+) Control
BNA-(-)DEF
BNA-CDEF-#1
BNA-CDEF-#2
BNA-CDEF-#3

FIG. 4F

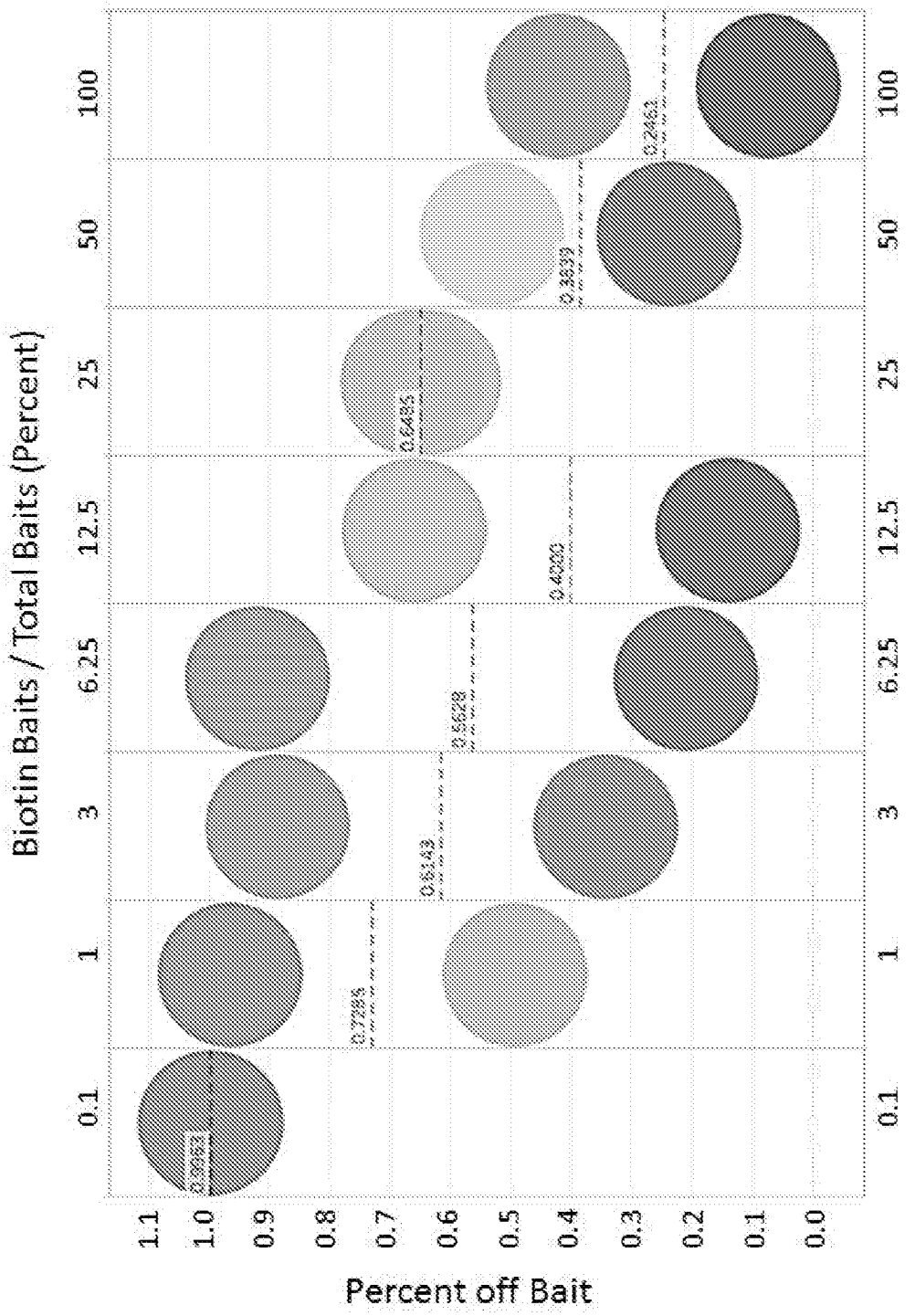

Biotin Baits / Total Baits (Percent)
0.1
1
3
6.25
12.5
25
50
100
Percent off Bait
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.7285
0.6143
0.5628
0.6485
0.4500
0.3839
0.2461

FIG. 5A

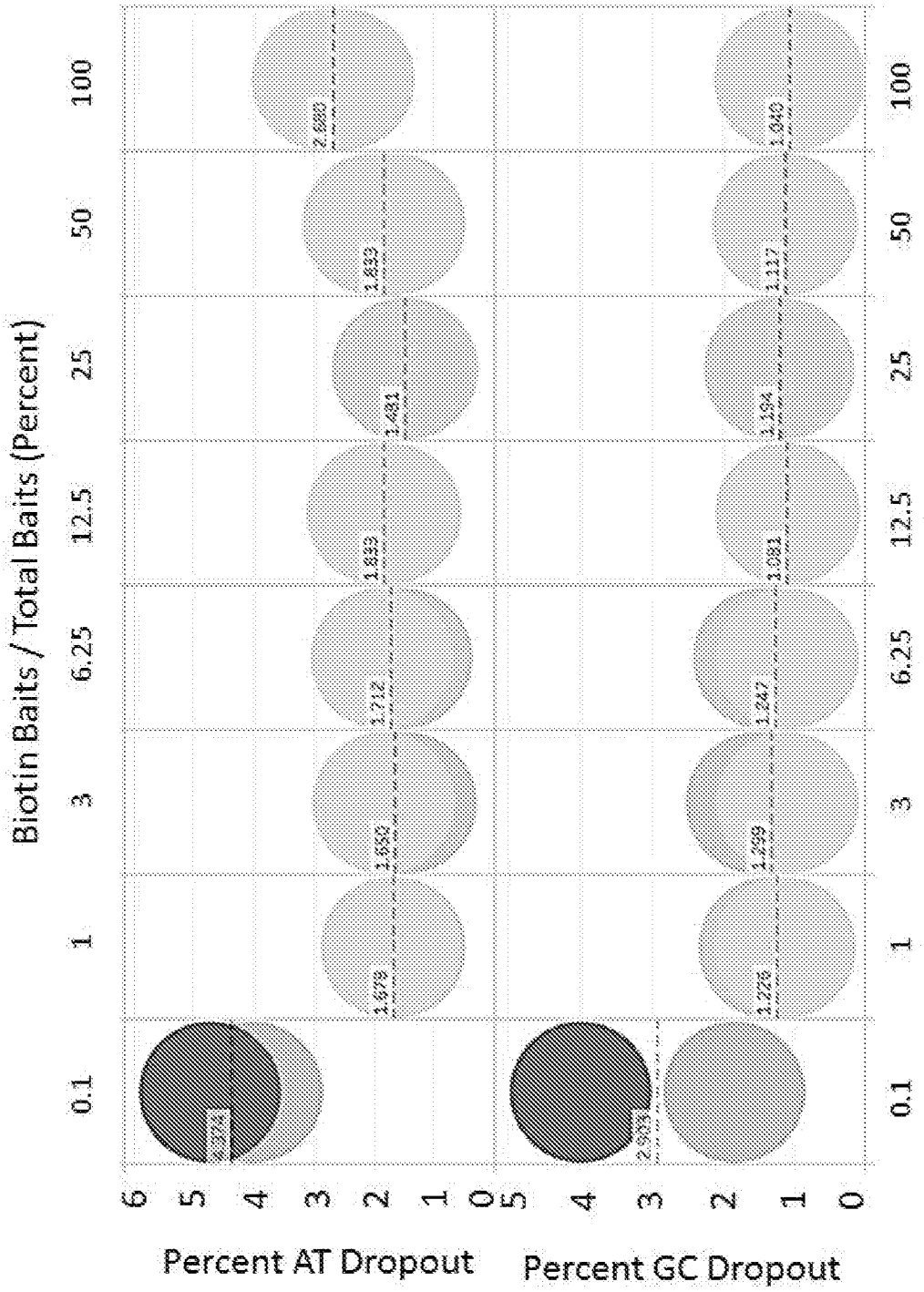

Biotin Baits / Total Baits (Percent)
0.1
1
3
6.25
12.5
25
50
100
Percent AT Dropout
Percent GC Dropout
4.374
1.679
1.650
1.712
1.833
1.481
1.833
2.680
2.903
1.226
1.299
1.247
1.081
1.194
1.117
1.040

FIG. 5B

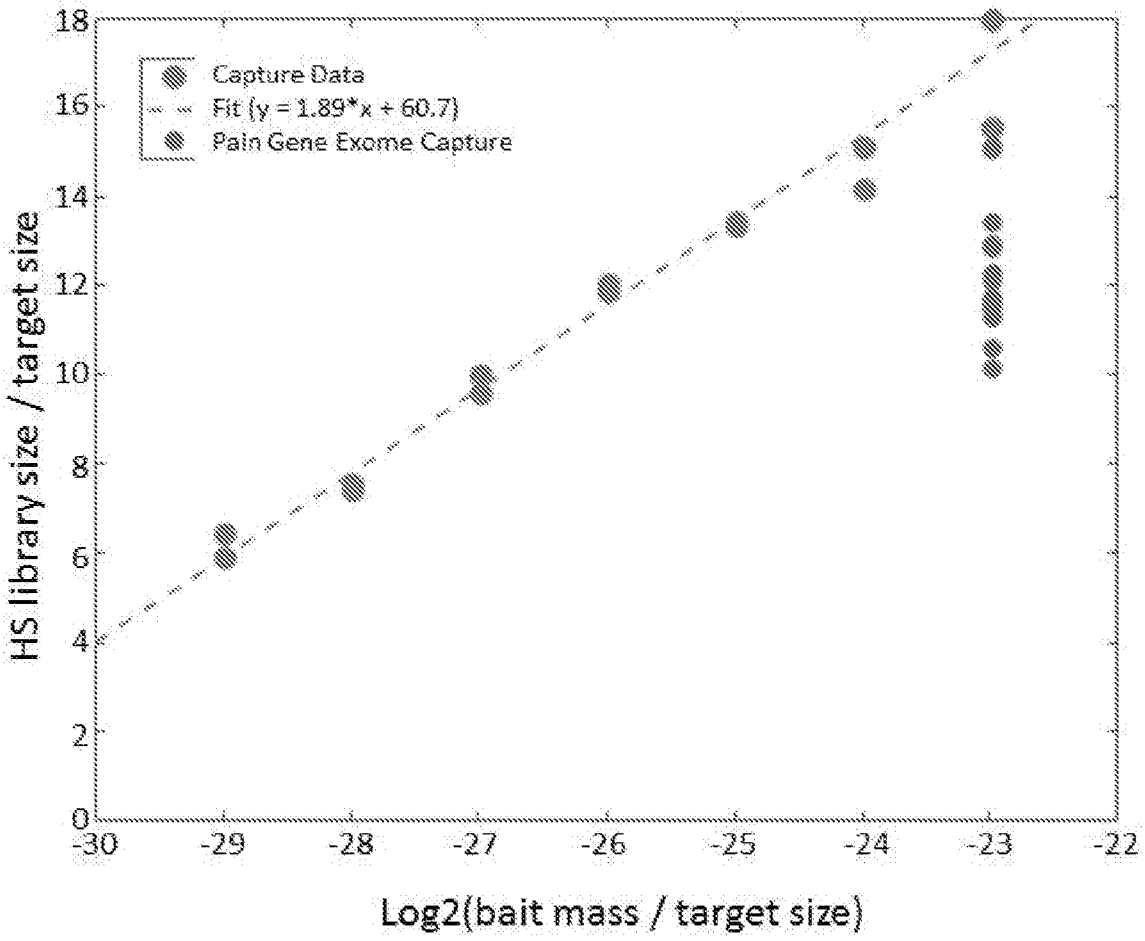

Capture Data
Fit (y = 1.89*x + 60.7)
Pain Gene Exome Capture
HS library size / target size
Log2(bait mass / target size)
18
16
14
12
10
8
6
4
2
0
-30
-29
-28
-27
-26
-25
-24
-23
-22

FIG. 6A

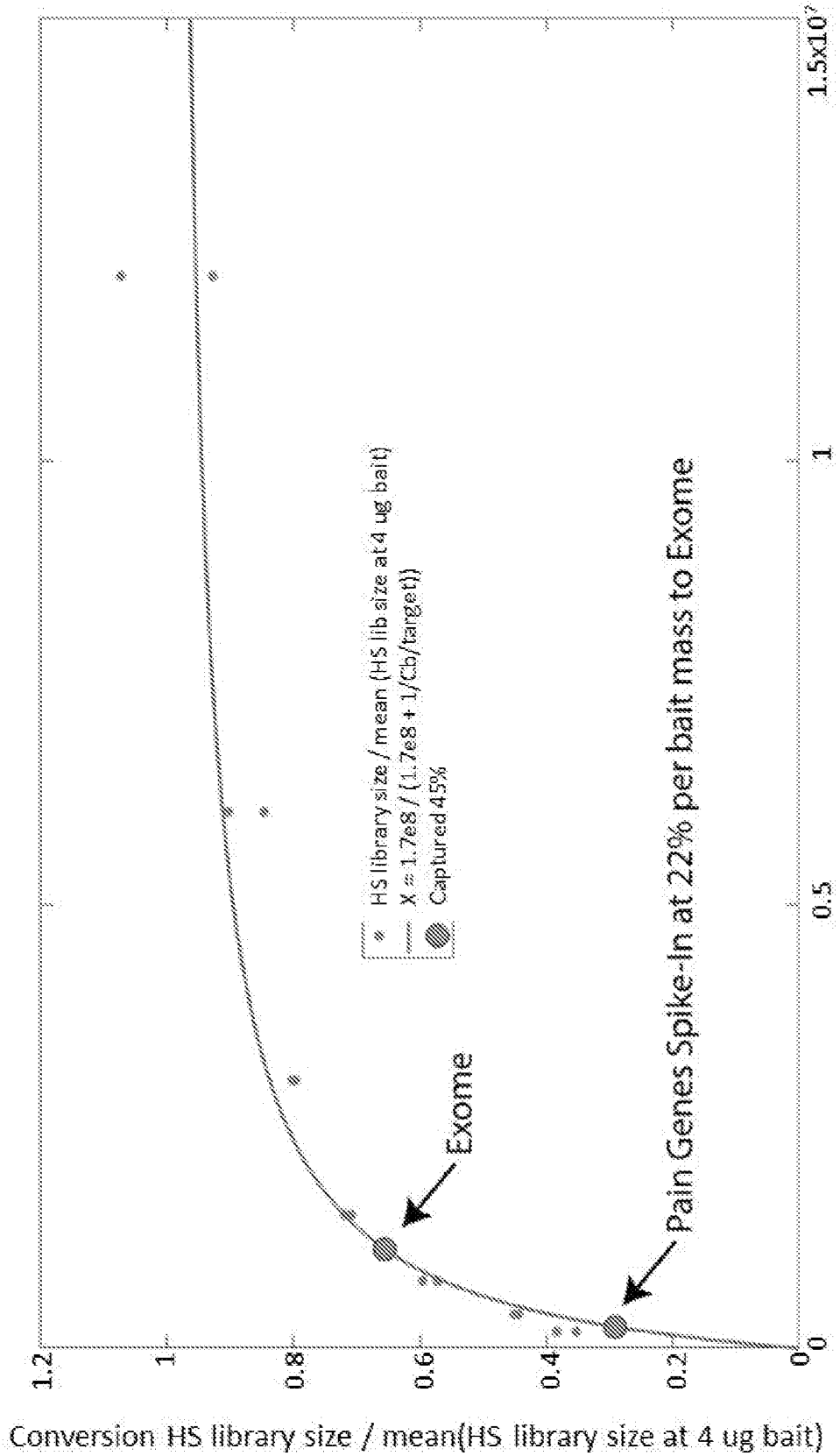

HS library size / mean (HS lib size at 4 ug bait)
X = 1.7e8 / (1.7e8 + 1/Cb/target))
Captured 45%
Exome
Pain Genes Spike-In at 22% per bait mass to Exome
Conversion HS library size / mean(HS library size at 4 ug bait)
Bait mass (ug) / Target size (nt)
0
0.2
0.4
0.6
0.8
1
1.2
0.5
1
1.5x10^7

FIG. 6B

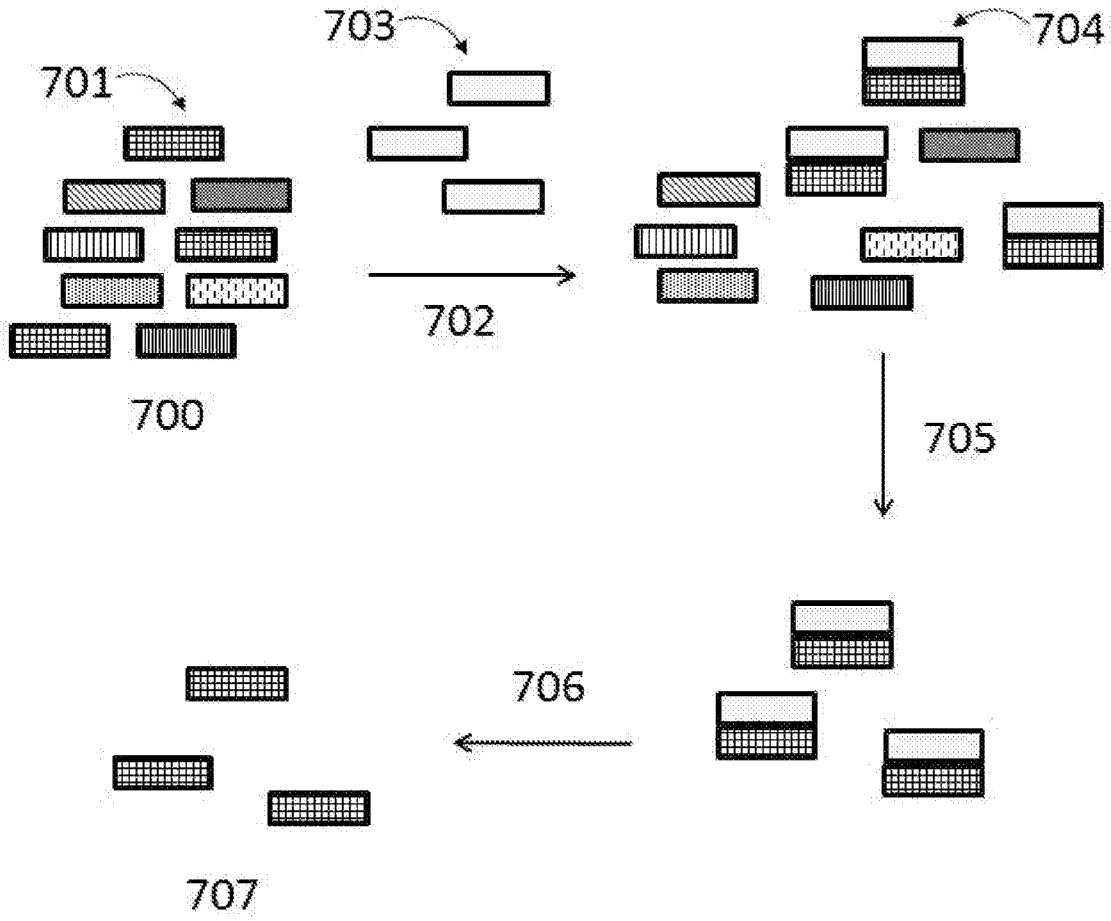


FIG. 7

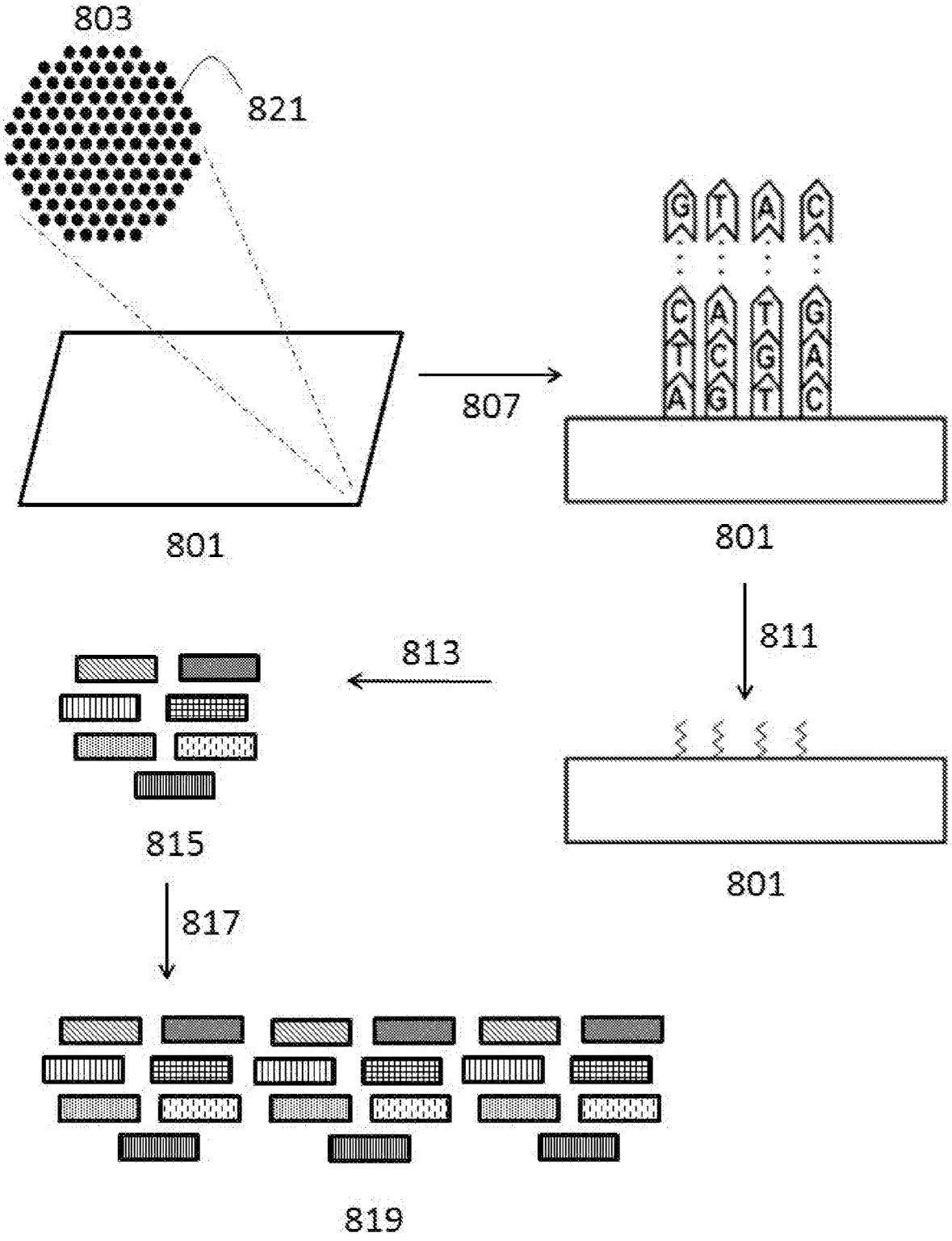


FIG. 8

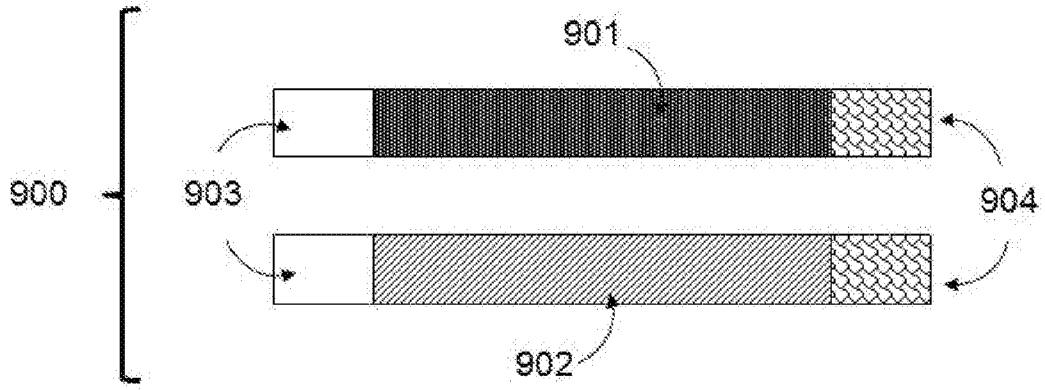


FIG. 9A

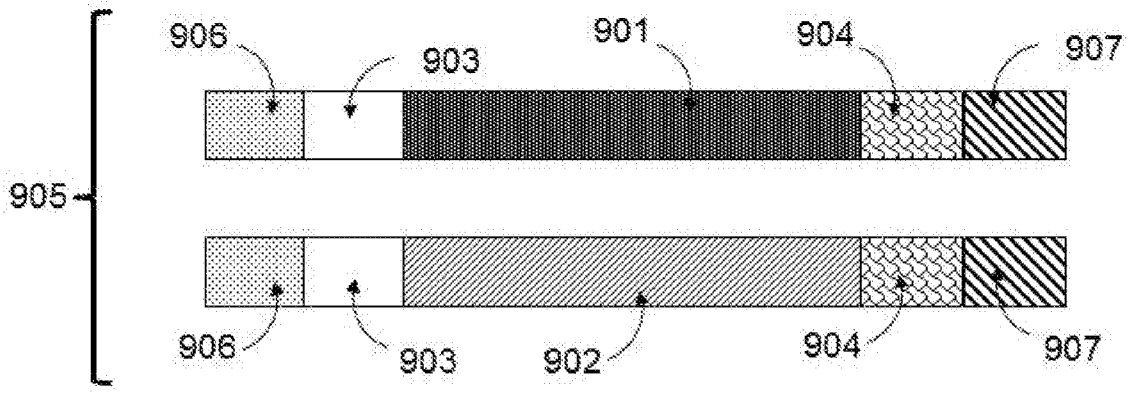


FIG. 9B

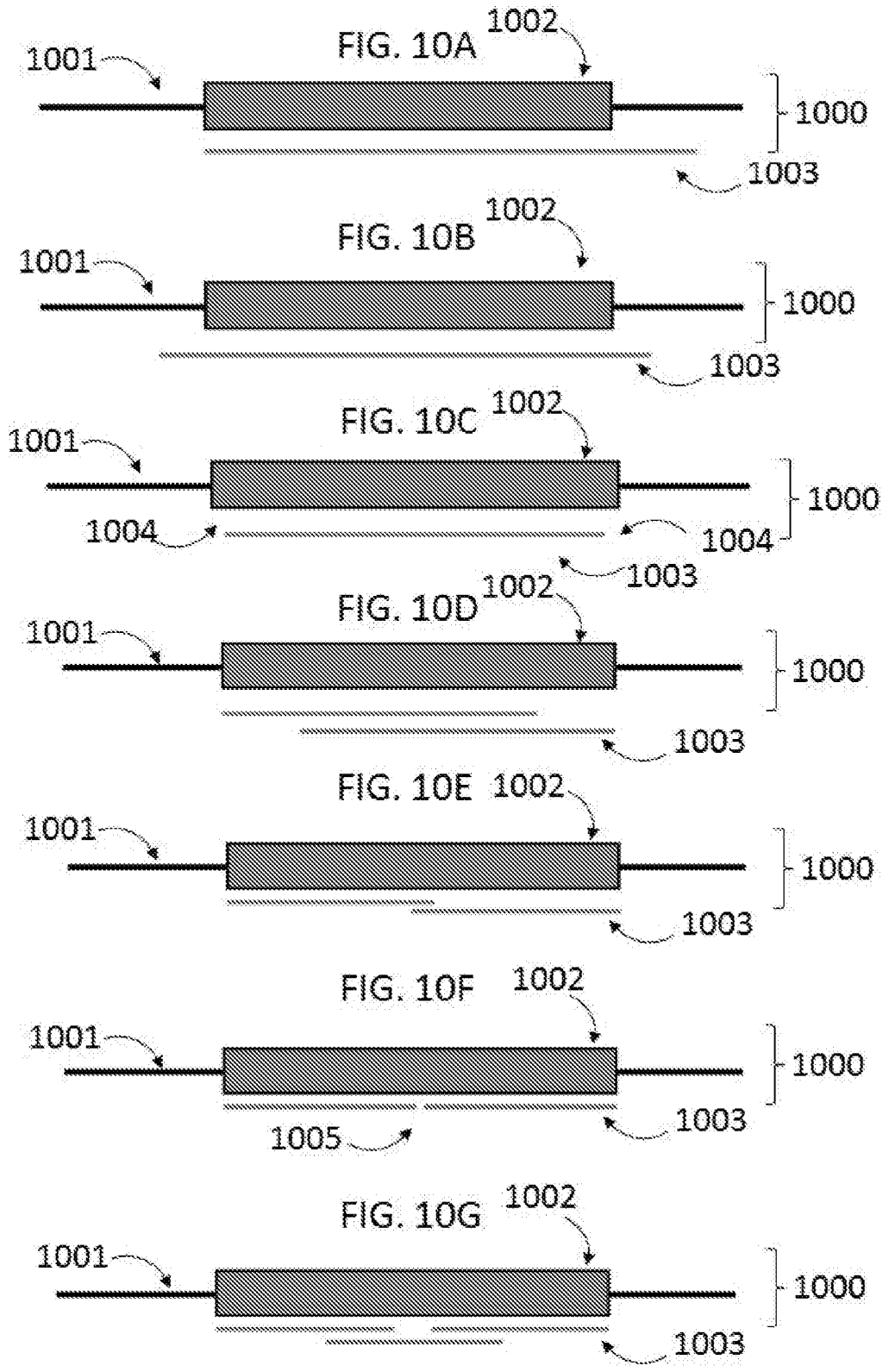


FIG. 10A
1001
1002
1000
1003
FIG. 10B
1001
1002
1000
1003
FIG. 10C
1001
1002
1000
1004
1004
1003
FIG. 10D
1001
1002
1000
1003
FIG. 10E
1001
1002
1000
1003
FIG. 10F
1001
1002
1000
1005
1003
FIG. 10G
1001
1002
1000
1003

FIGS. 10A-10G

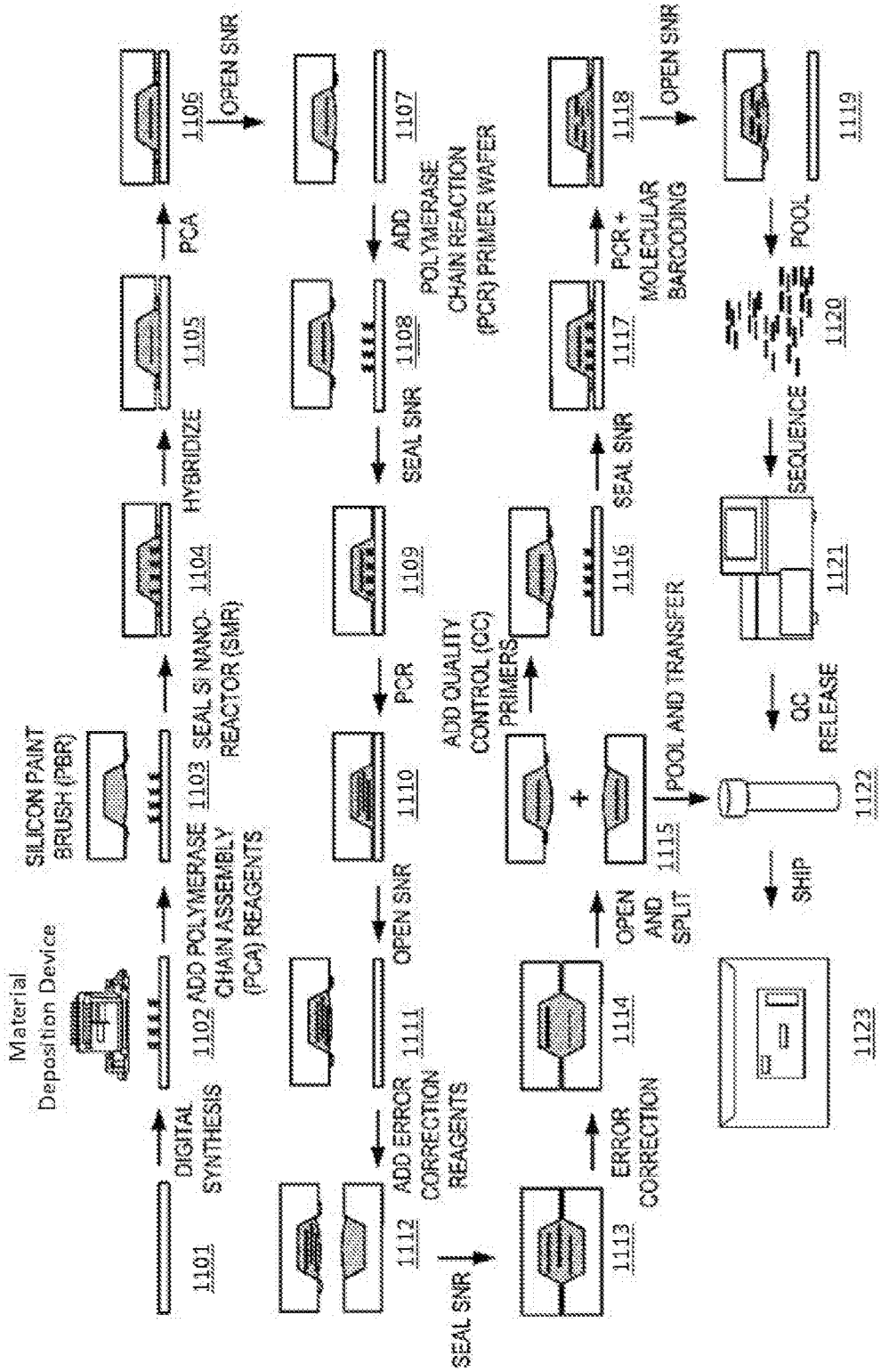

Material Deposition Device
SILICON PAINT BRUSH (PBR)
1101
DIGITAL SYNTHESIS
1102 ADD POLYMERASE CHAIN ASSEMBLY (PCA) REAGENTS
1103 SEAL Si NANO-REACTOR (SMR)
1104 HYBRIDIZE
1105 PCA
1106 OPEN SMR
1107 ADD POLYMERASE CHAIN REACTION (PCR) PRIMER WAFER
1108 SEAL SMR
1109 PCR
1110 OPEN SMR
1111 ADD ERROR CORRECTION REAGENTS
1112 SEAL SMR
1113 ERROR CORRECTION
1114 OPEN AND SPLIT
1115 POOL AND TRANSFER
1116 SEAL SMR
1117 PCR + MOLECULAR BARCODING
1118 OPEN SMR
1119 POOL
1120 SEQUENCE
1121 QC RELEASE
1122 SHIP
1123
ADD QUALITY CONTROL (QC) PRIMERS

FIG. 11

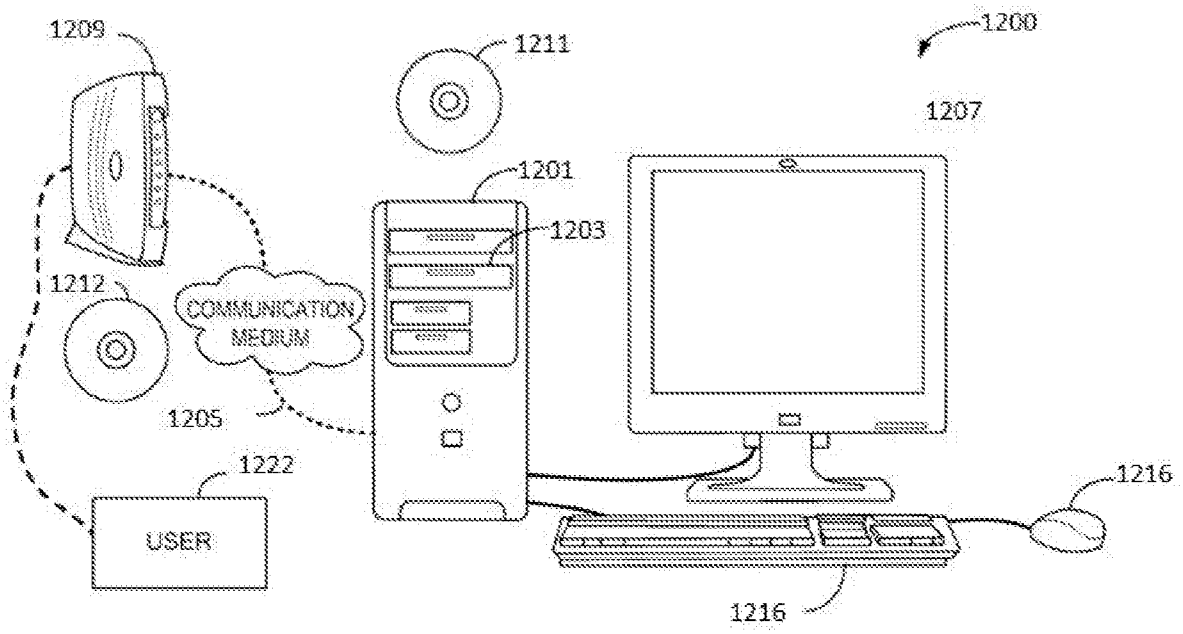

1209
1211
1200
1207
1201
1203
1212
COMMUNICATION MEDIUM
1205
1222
USER
1216
1216

FIG. 12

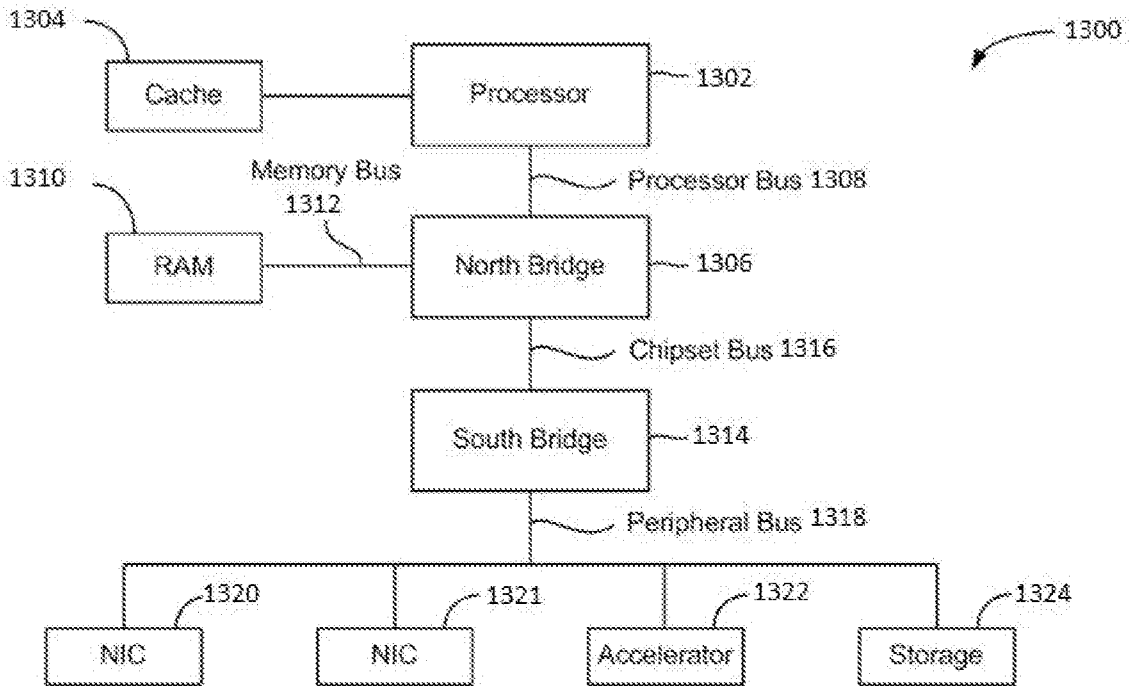

1304
Cache
Processor
1302
1300
1310
Memory Bus
1312
Processor Bus 1308
RAM
North Bridge
1306
Chipset Bus 1316
South Bridge
1314
Peripheral Bus 1318
1320
1321
1322
1324
NIC
NIC
Accelerator
Storage

FIG. 13

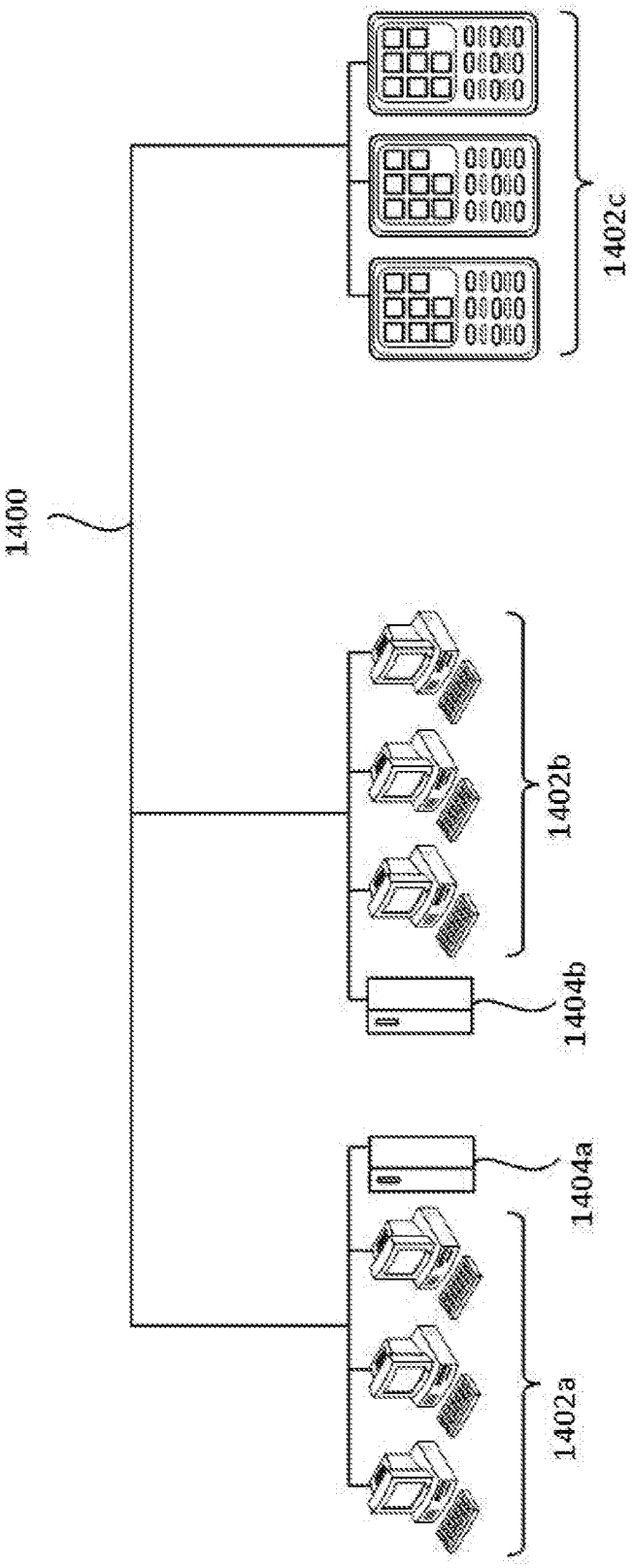


FIG. 14

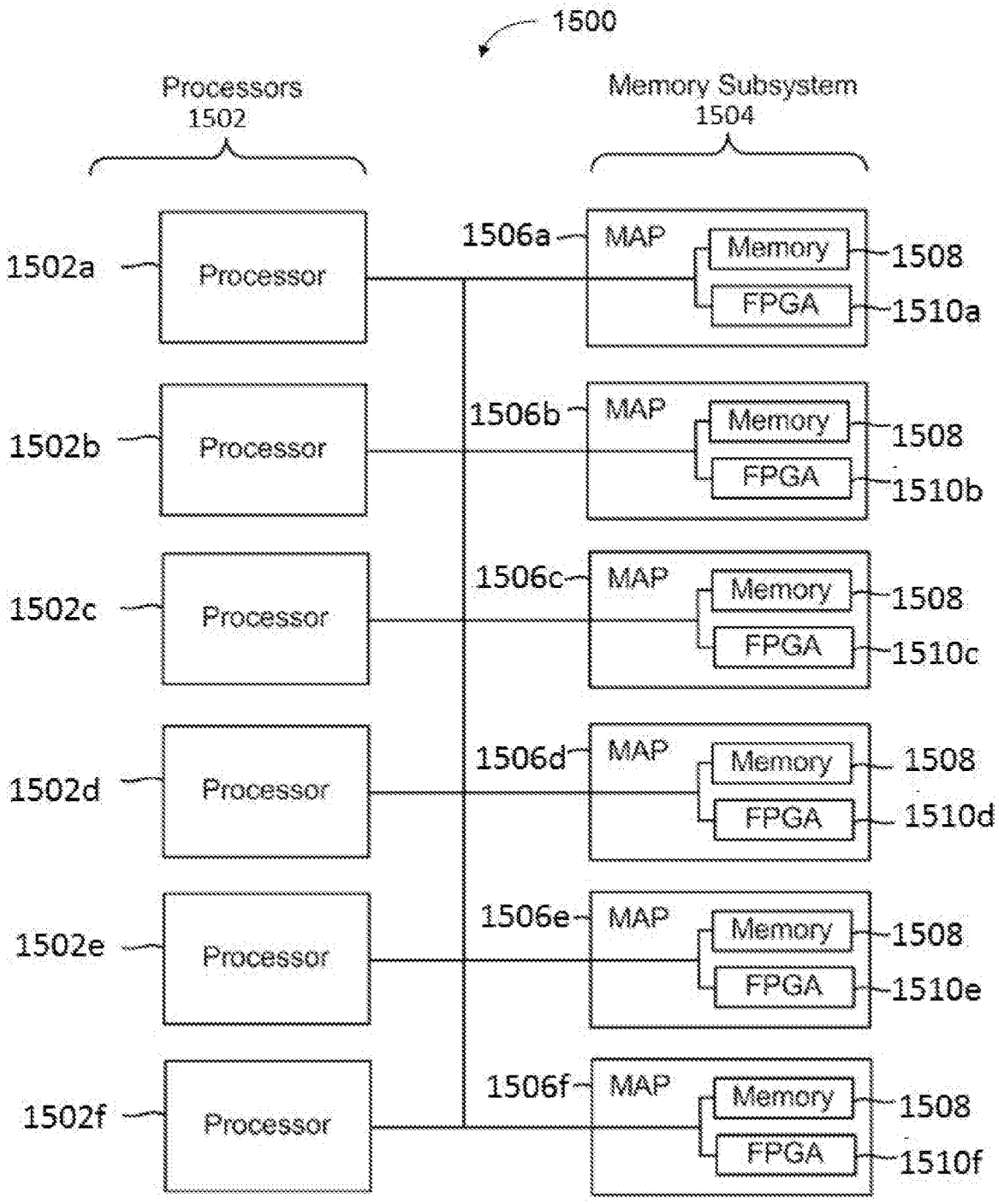

1500
Processors
1502
Memory Subsystem
1504
1502a
Processor
1506a
MAP
Memory
1508
FPGA
1510a
1502b
Processor
1506b
MAP
Memory
1508
FPGA
1510b
1502c
Processor
1506c
MAP
Memory
1508
FPGA
1510c
1502d
Processor
1506d
MAP
Memory
1508
FPGA
1510d
1502e
Processor
1506e
MAP
Memory
1508
FPGA
1510e
1502f
Processor
1506f
MAP
Memory
1508
FPGA
1510f

FIG. 15

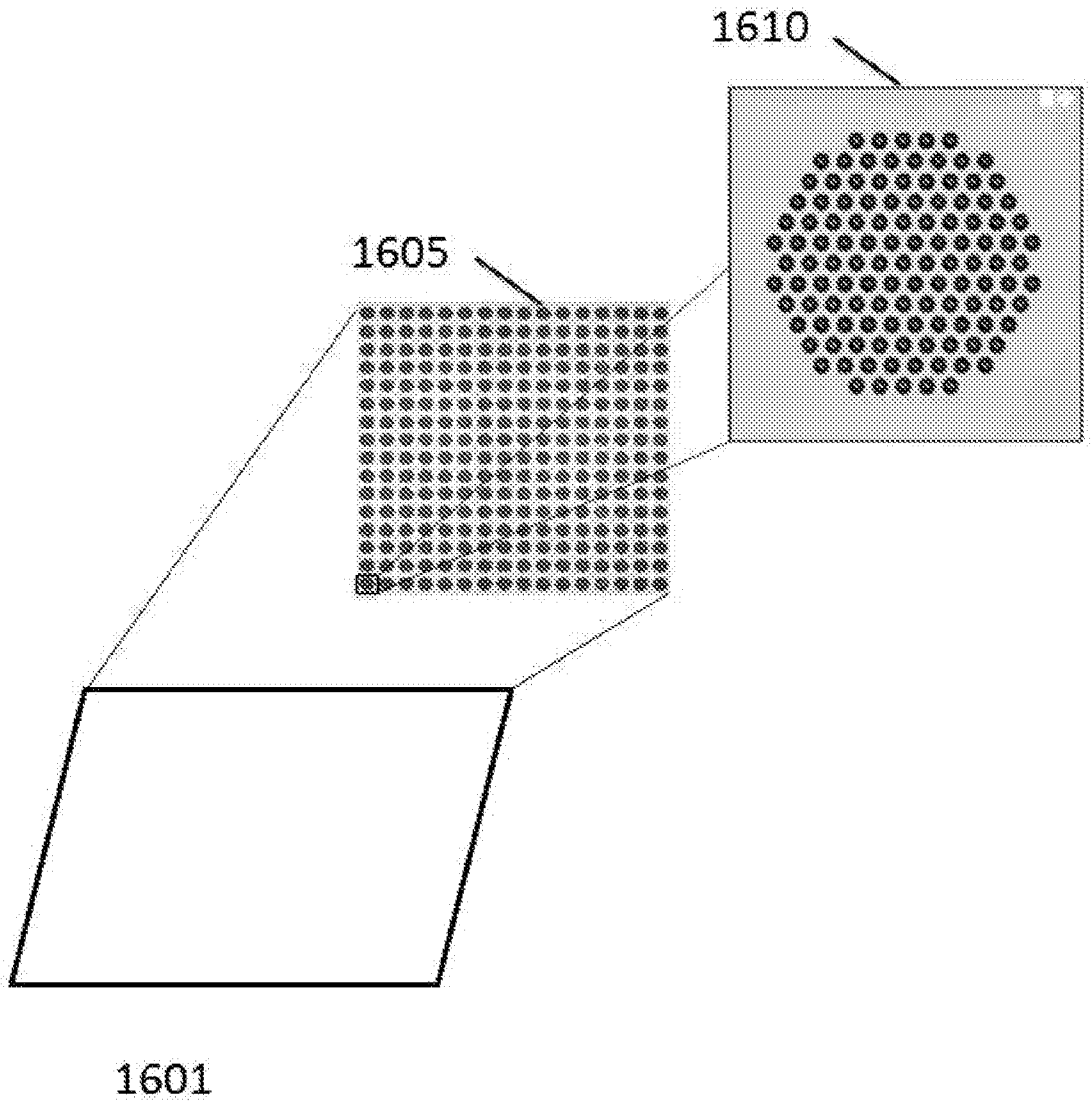


FIG. 16

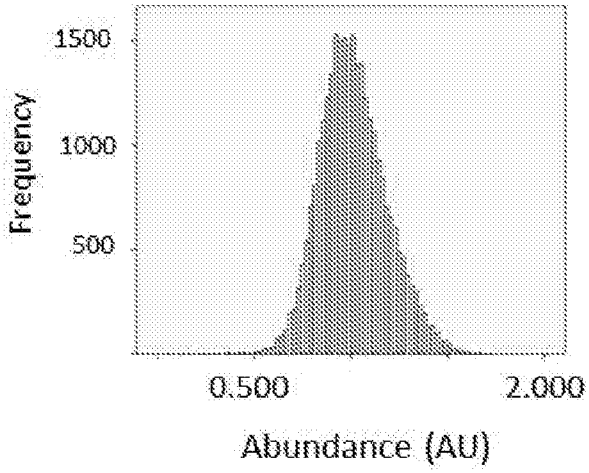


FIG. 17A

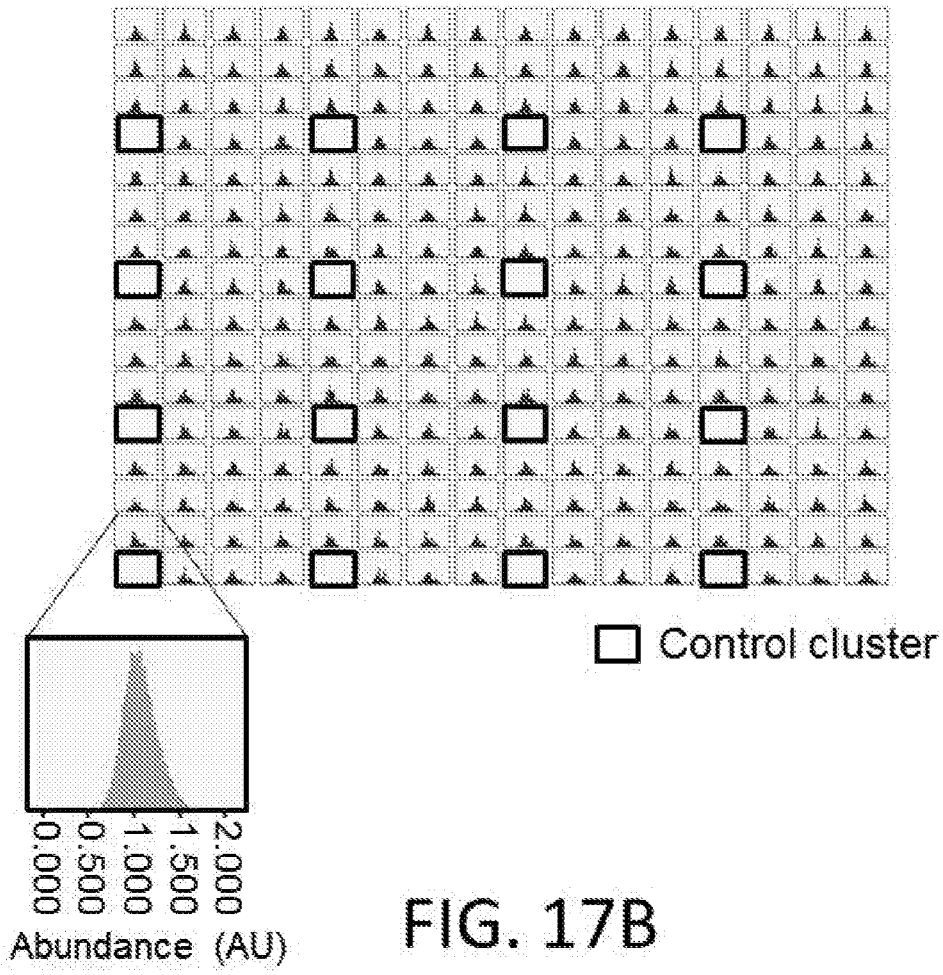


FIG. 17B

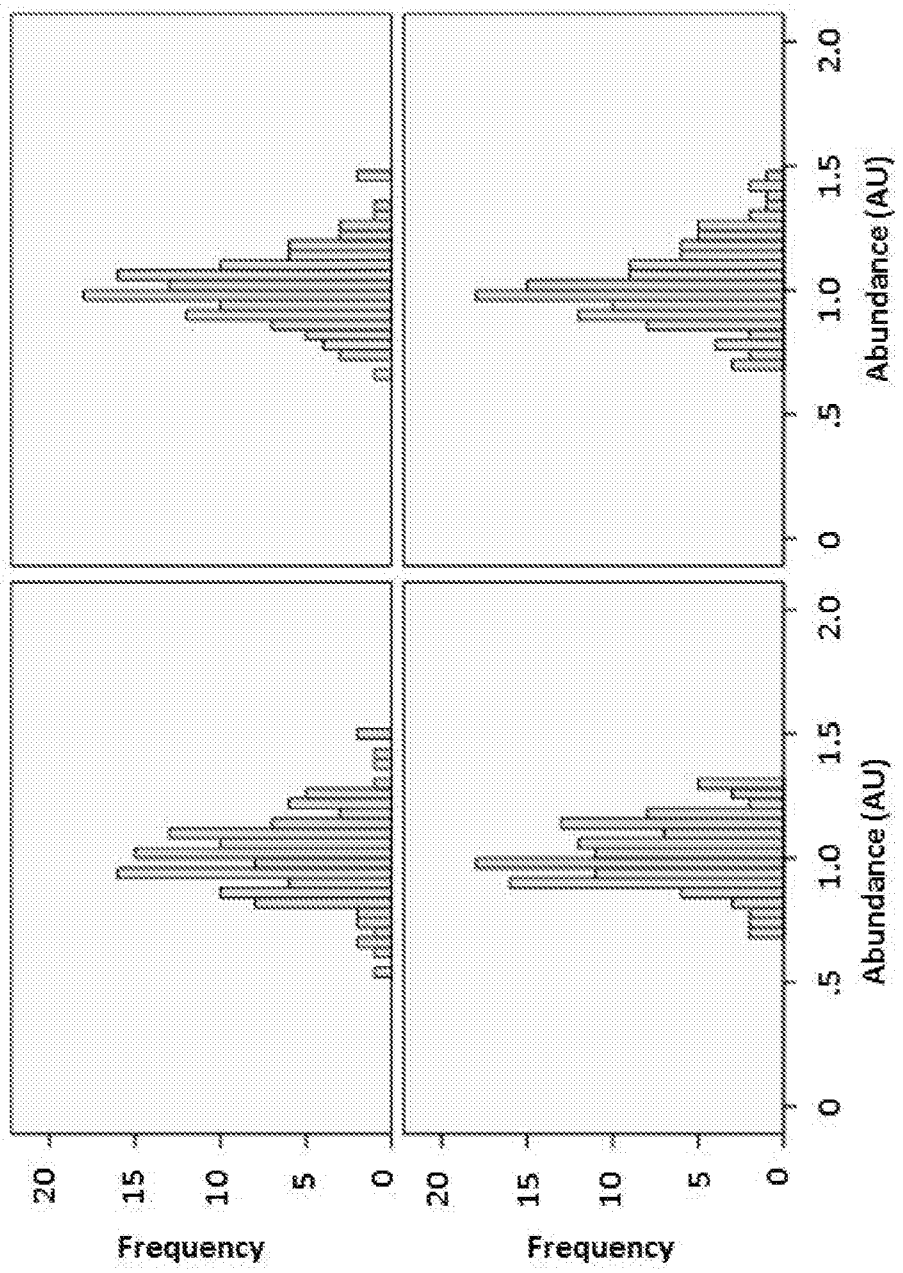


FIG. 18

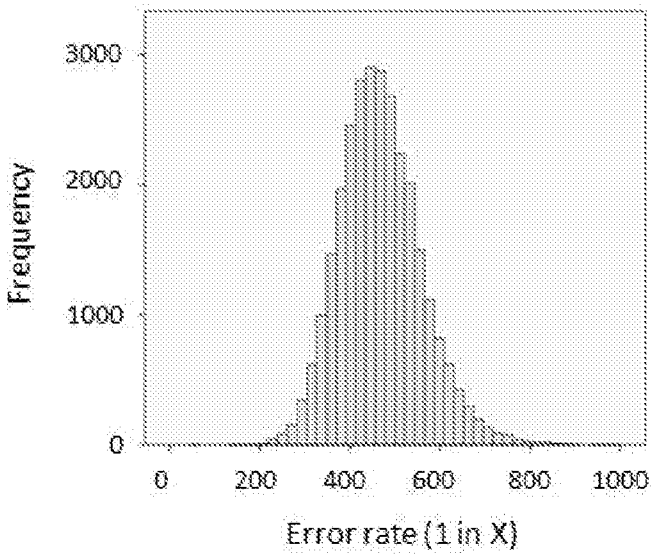


FIG. 19A

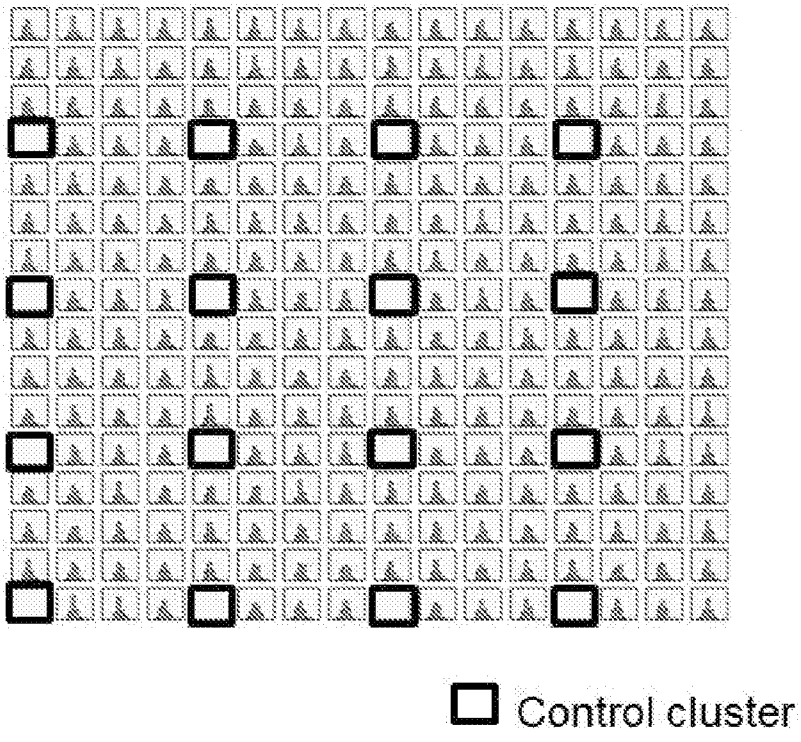


FIG. 19B

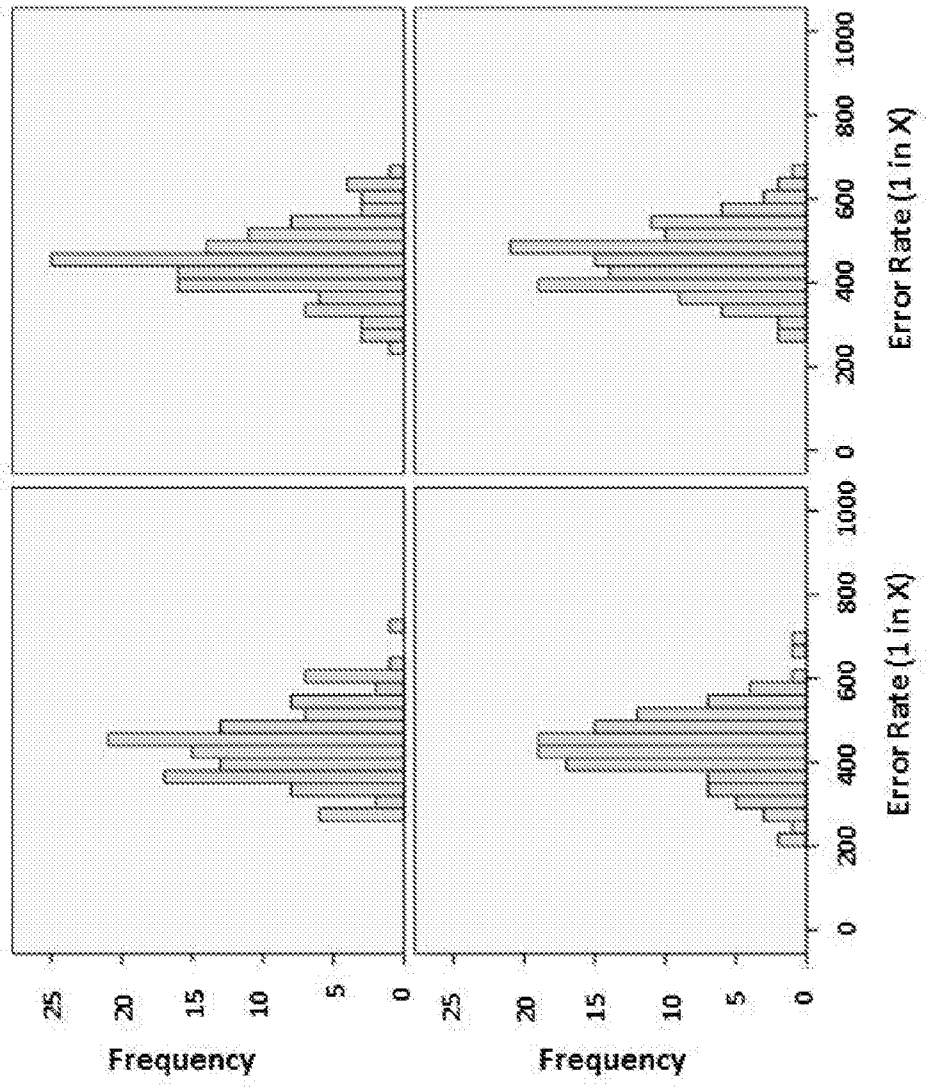

Frequency
Frequency
Error Rate (1 in X)
Error Rate (1 in X)
0
5
10
15
20
25
0
200
400
600
800
1000

FIG. 20

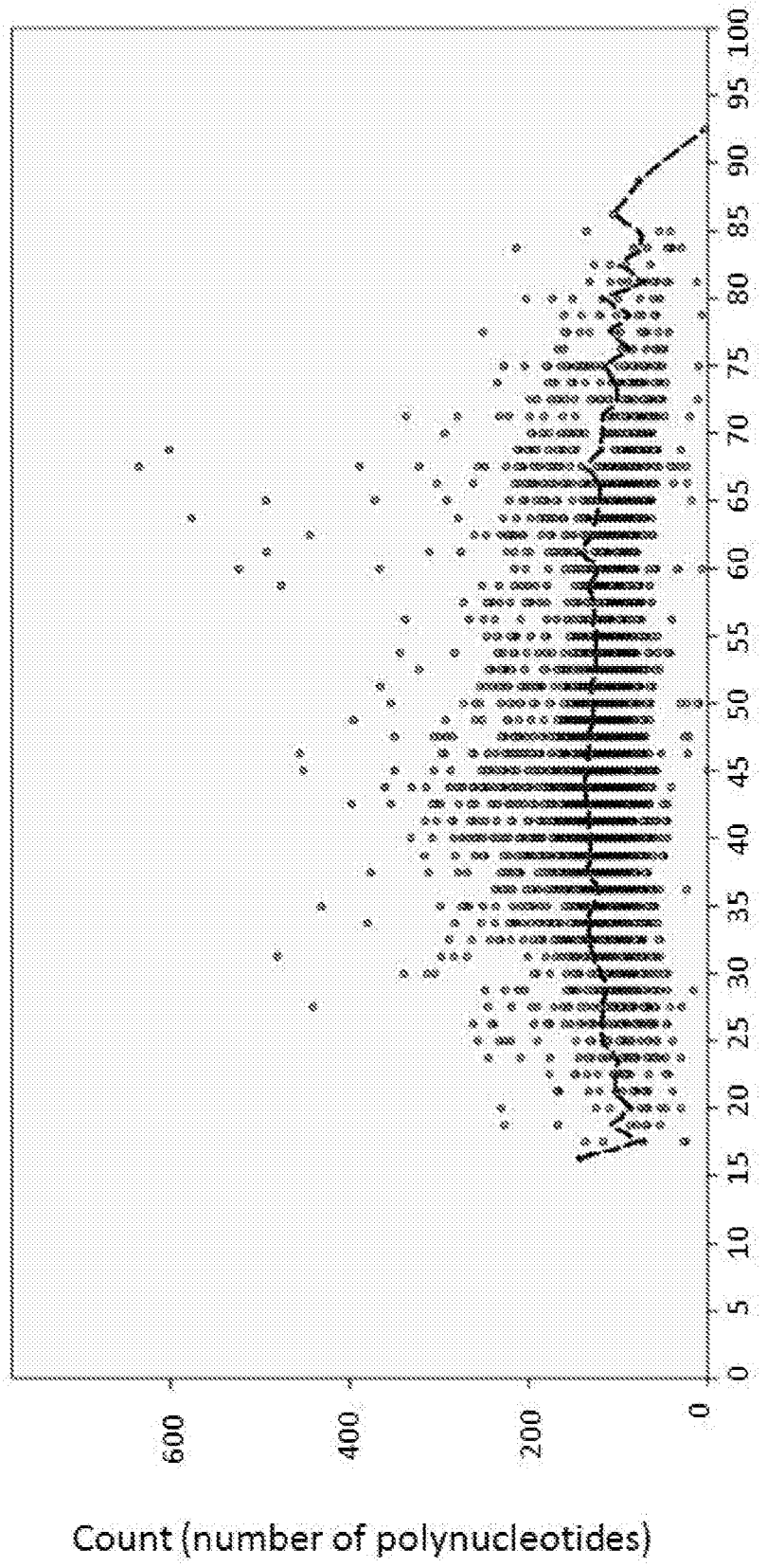

0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
GC%
0
200
400
600
Count (number of polynucleotides)

FIG. 21

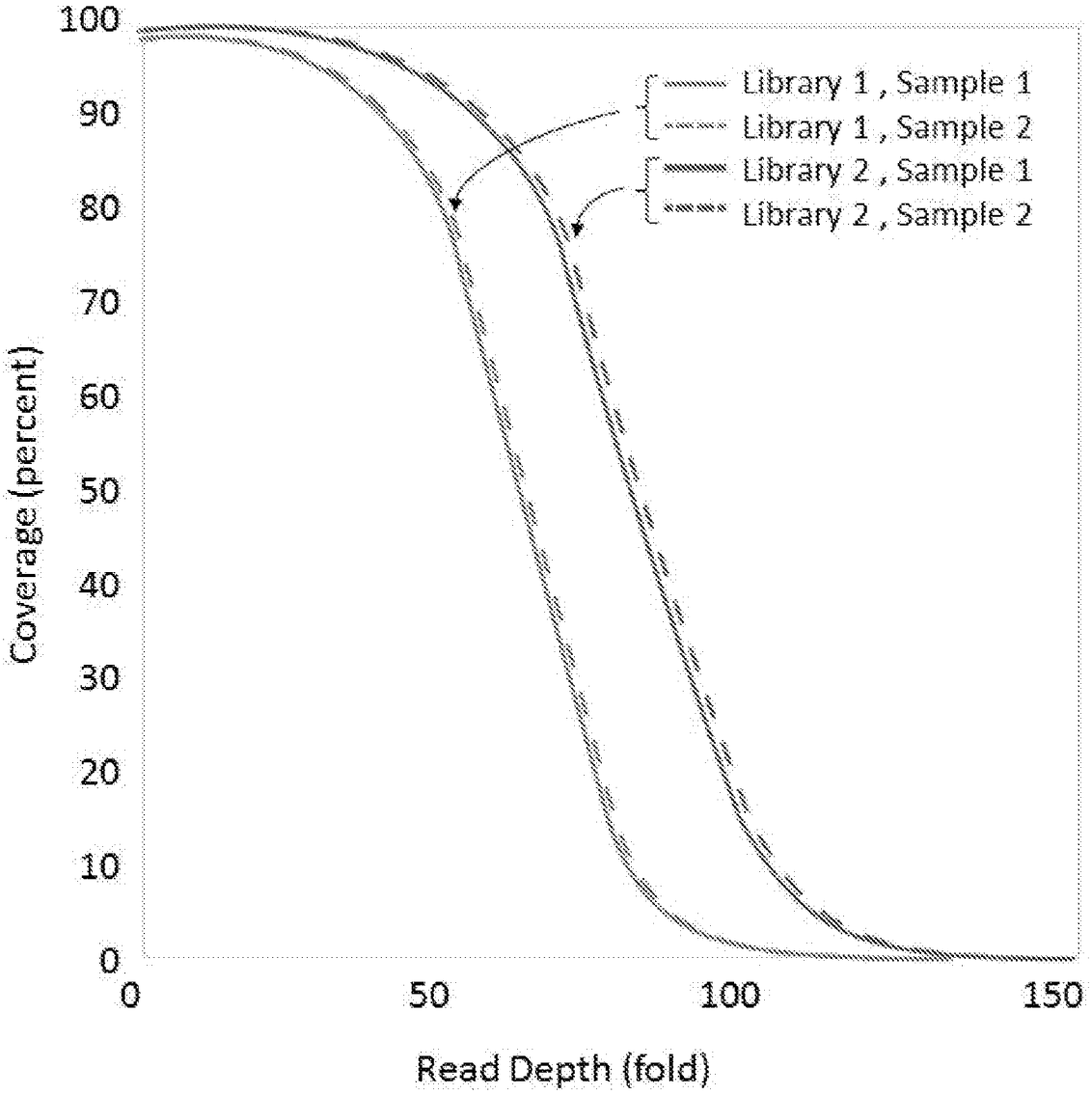

100
90
80
70
60
50
40
30
20
10
0
Coverage (percent)
0
50
100
150
Read Depth (fold)
Library 1 , Sample 1
Library 1 , Sample 2
Library 2 , Sample 1
Library 2 , Sample 2

FIG. 22


UB
INSERT
UB
INSERT
UB
INSERT
UB
INSERT
Single Index
8 bps
Combinatorial
Dual Index
8 bps
Unique Dual Index
10 bps
UMI + UMI
10 bps

FIG. 23A

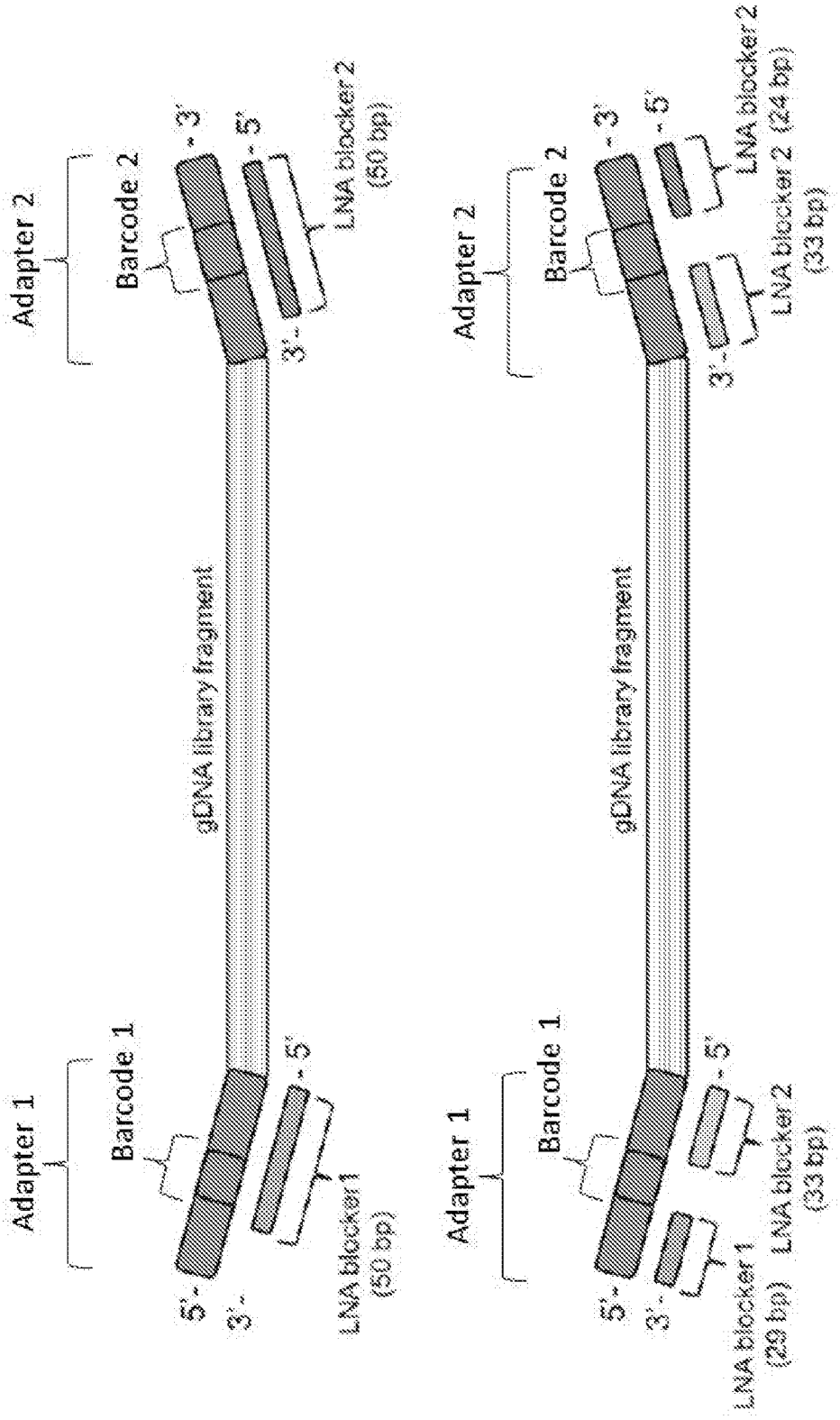

Adapter 1
Barcode 1
5'-
3'-
gDNA library fragment
- 5'
LNA blocker 1
(50 bp)
Adapter 2
Barcode 2
- 3'
- 5'
3'-
LNA blocker 2
(50 bp)
Adapter 1
Barcode 1
5'-
3'-
LNA blocker 1
(29 bp)
gDNA library fragment
- 5'
LNA blocker 2
(33 bp)
Adapter 2
Barcode 2
- 3'
- 5'
3'-
LNA blocker 2 (24 bp)
(33 bp)

FIG. 23B

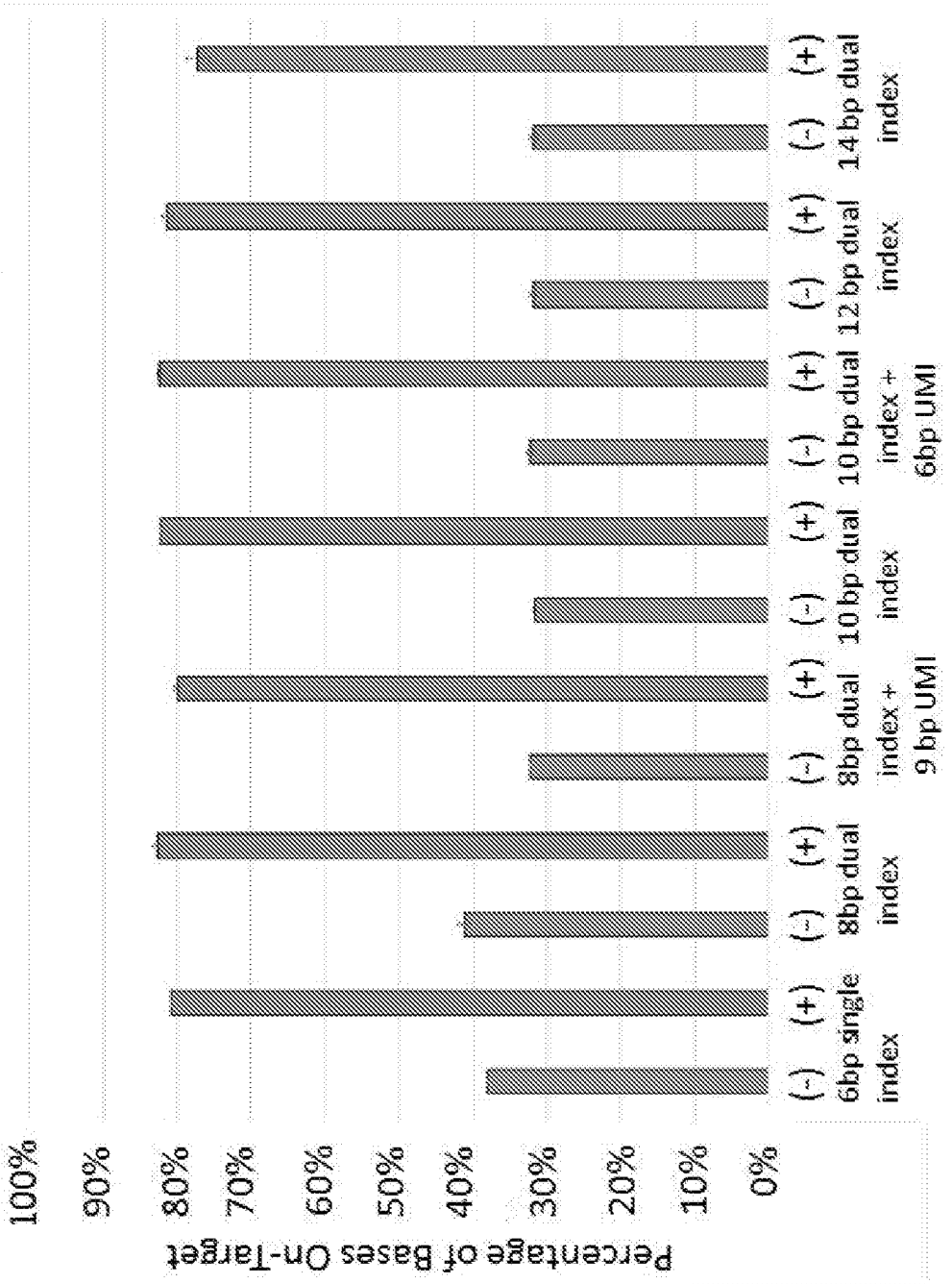

Percentage of Bases On-Target
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
(-) (+)
6bp single index
(-) (+)
8bp dual index
(-) (+)
8bp dual index + 9 bp UMI
(-) (+)
10 bp dual index
(-) (+)
10 bp dual index + 6bp UMI
(-) (+)
12 bp dual index
(-) (+)
14 bp dual index

FIG. 24

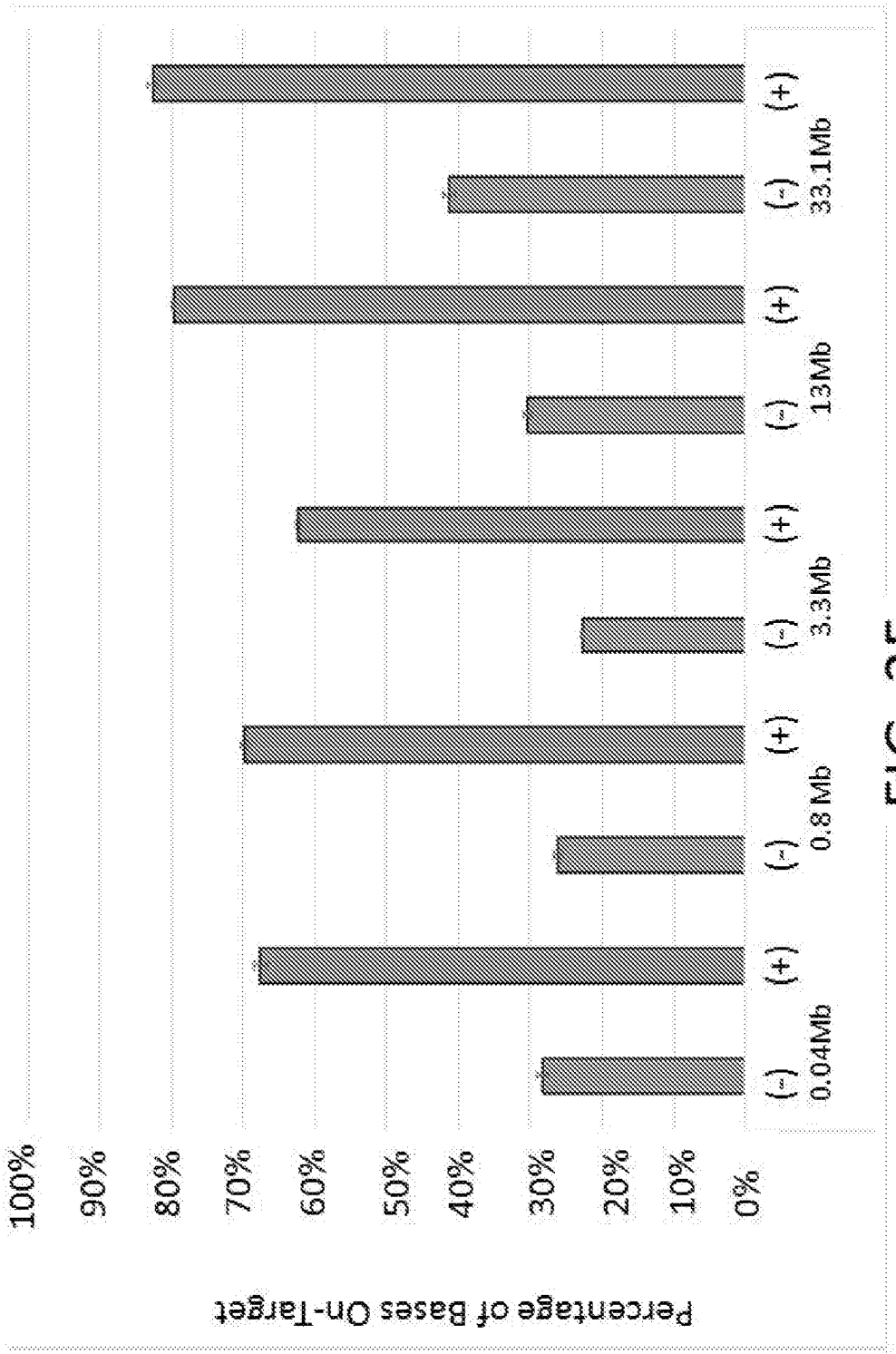

Percentage of Bases On-Target
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
(-)
(+)
0.04Mb
0.8 Mb
3.3Mb
13Mb
33.1Mb

FIG. 25

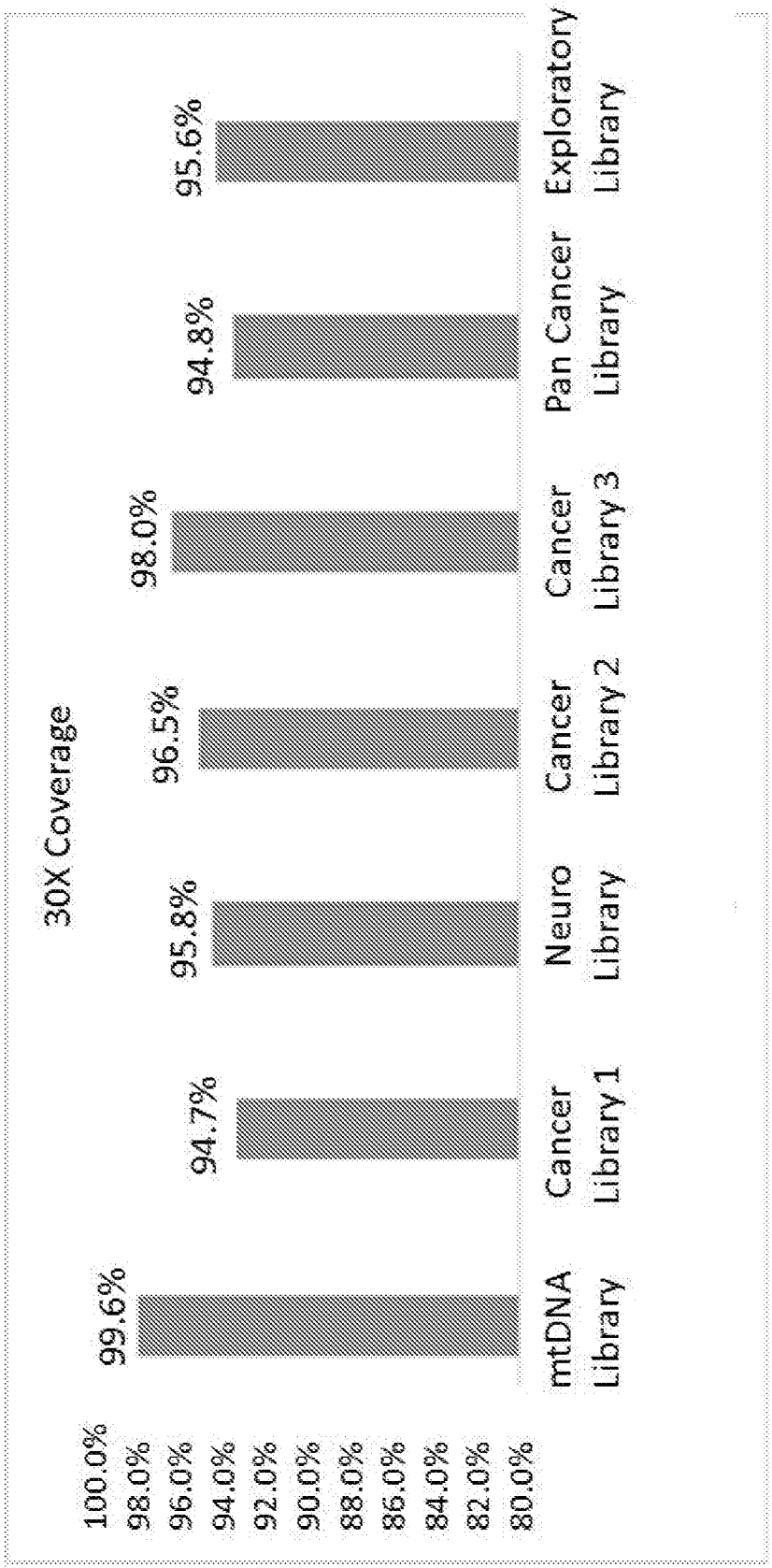

30X Coverage
100.0%
98.0%
96.0%
94.0%
92.0%
90.0%
88.0%
86.0%
84.0%
82.0%
80.0%
99.6%
94.7%
95.8%
96.5%
98.0%
94.8%
95.6%
mtDNA Library
Cancer Library 1
Neuro Library
Cancer Library 2
Cancer Library 3
Pan Cancer Library
Exploratory Library

FIG. 26A

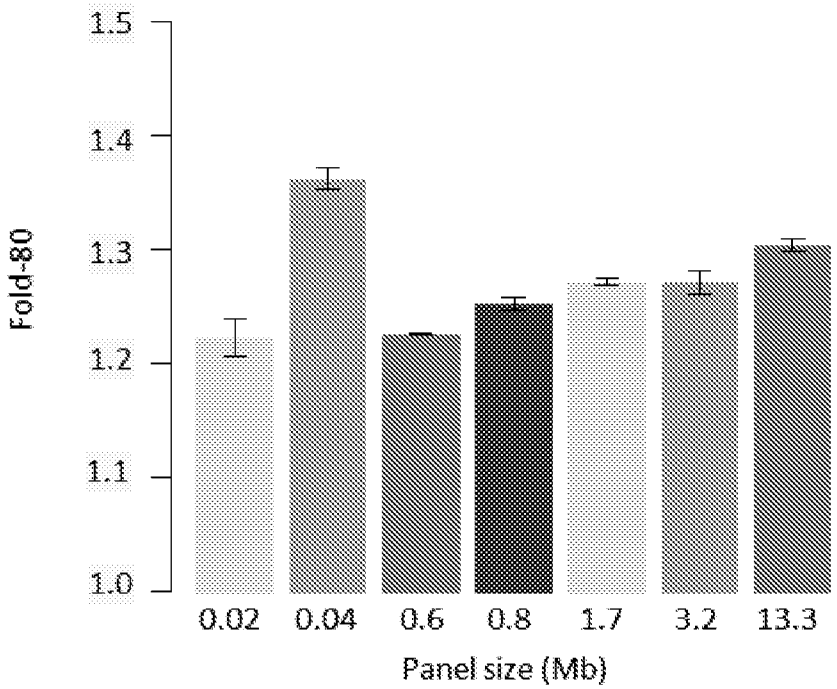


FIG. 26B

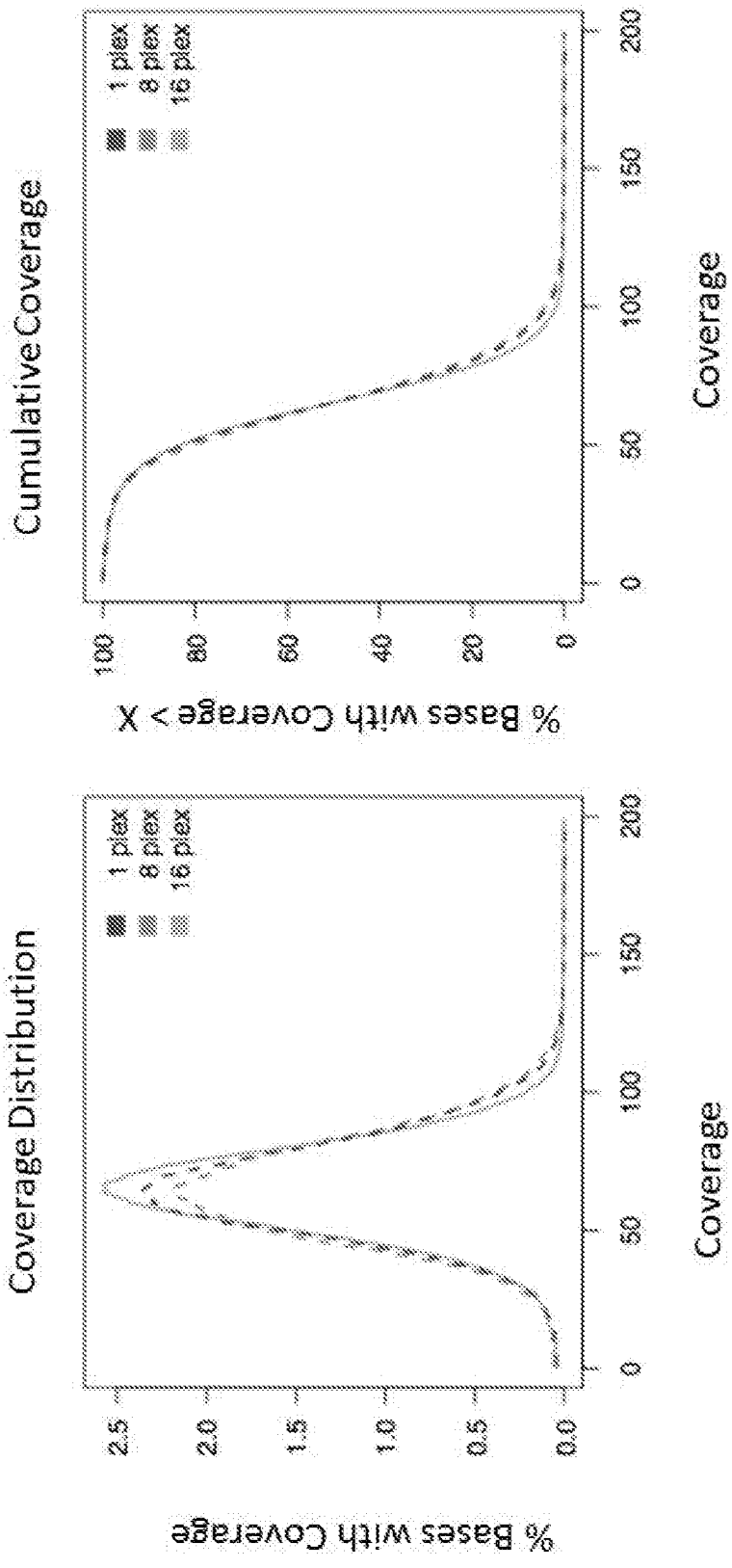

Coverage Distribution
% Bases with Coverage
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Coverage
1 plex
8 plex
16 plex
Cumulative Coverage
% Bases with Coverage > X
100
80
60
40
20
0
0
50
100
150
200
Coverage
1 plex
8 plex
16 plex

FIG. 27A

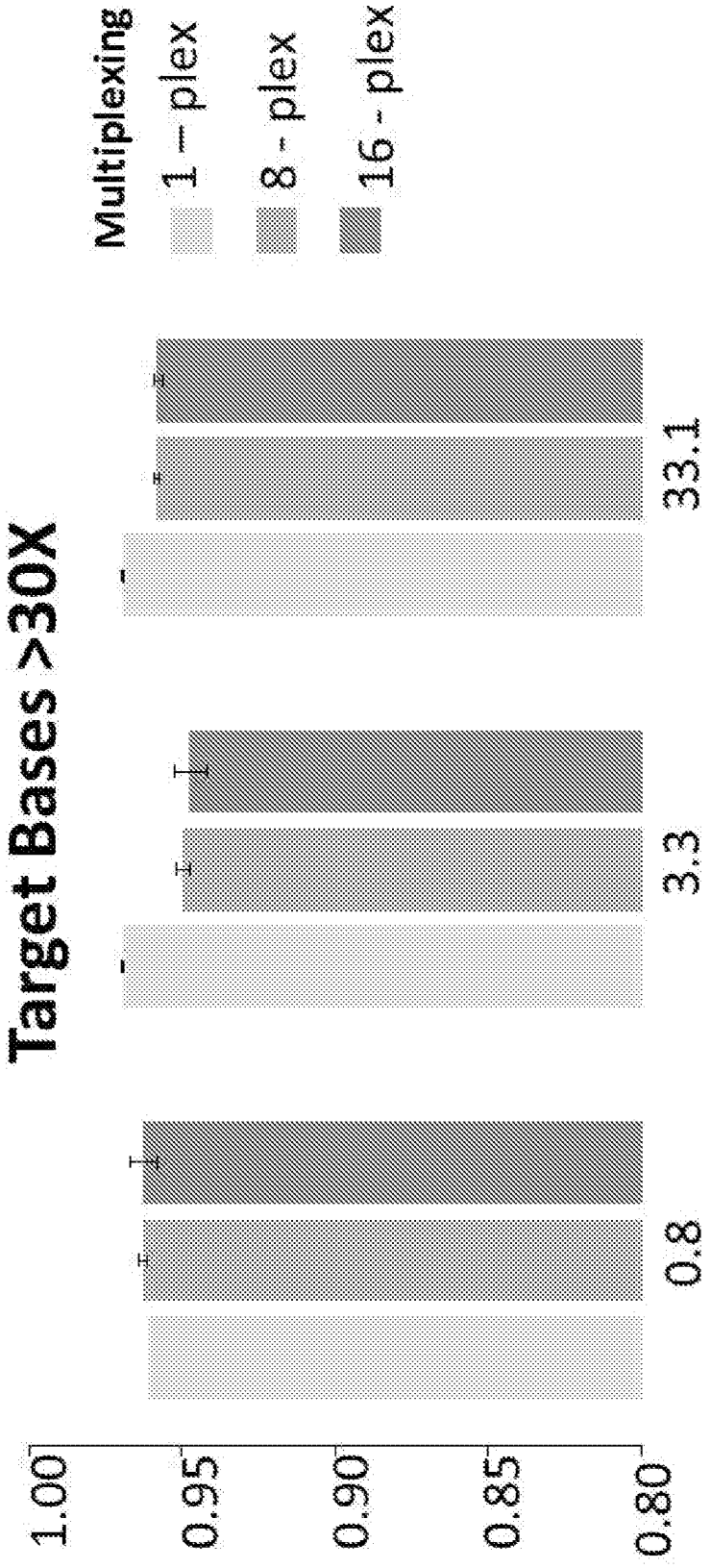

Multiplexing
1 – plex
8 - plex
16 - plex
Target Bases >30X
1.00
0.95
0.90
0.85
0.80
0.8
3.3
33.1
Panel Size (Mb)

FIG. 27B

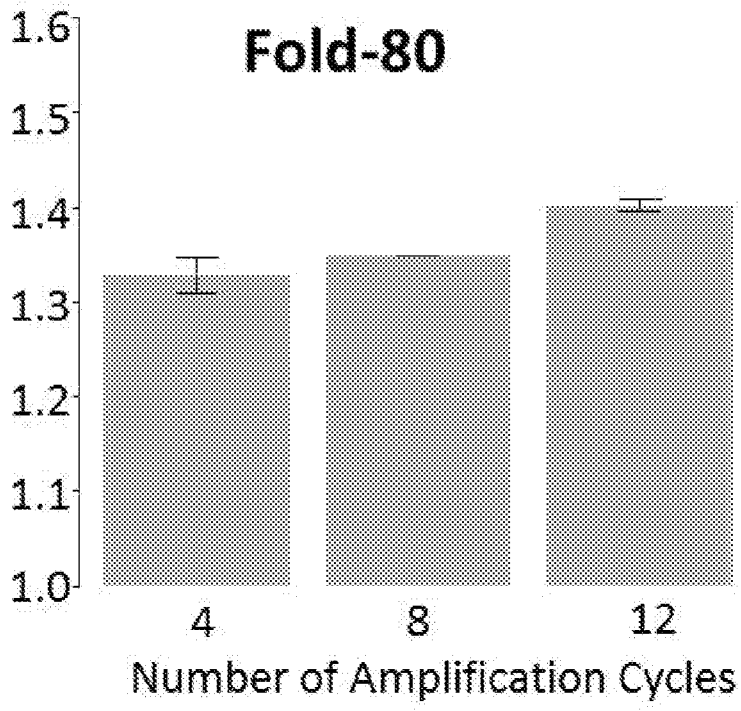


FIG. 27C

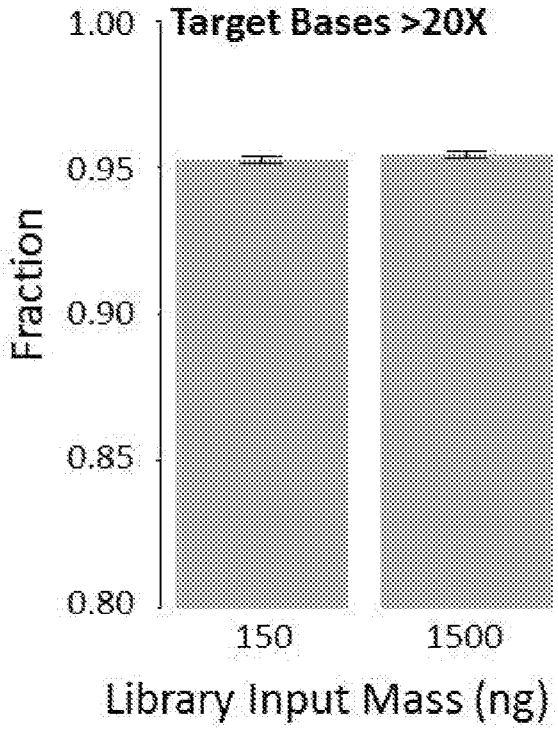


FIG. 27D

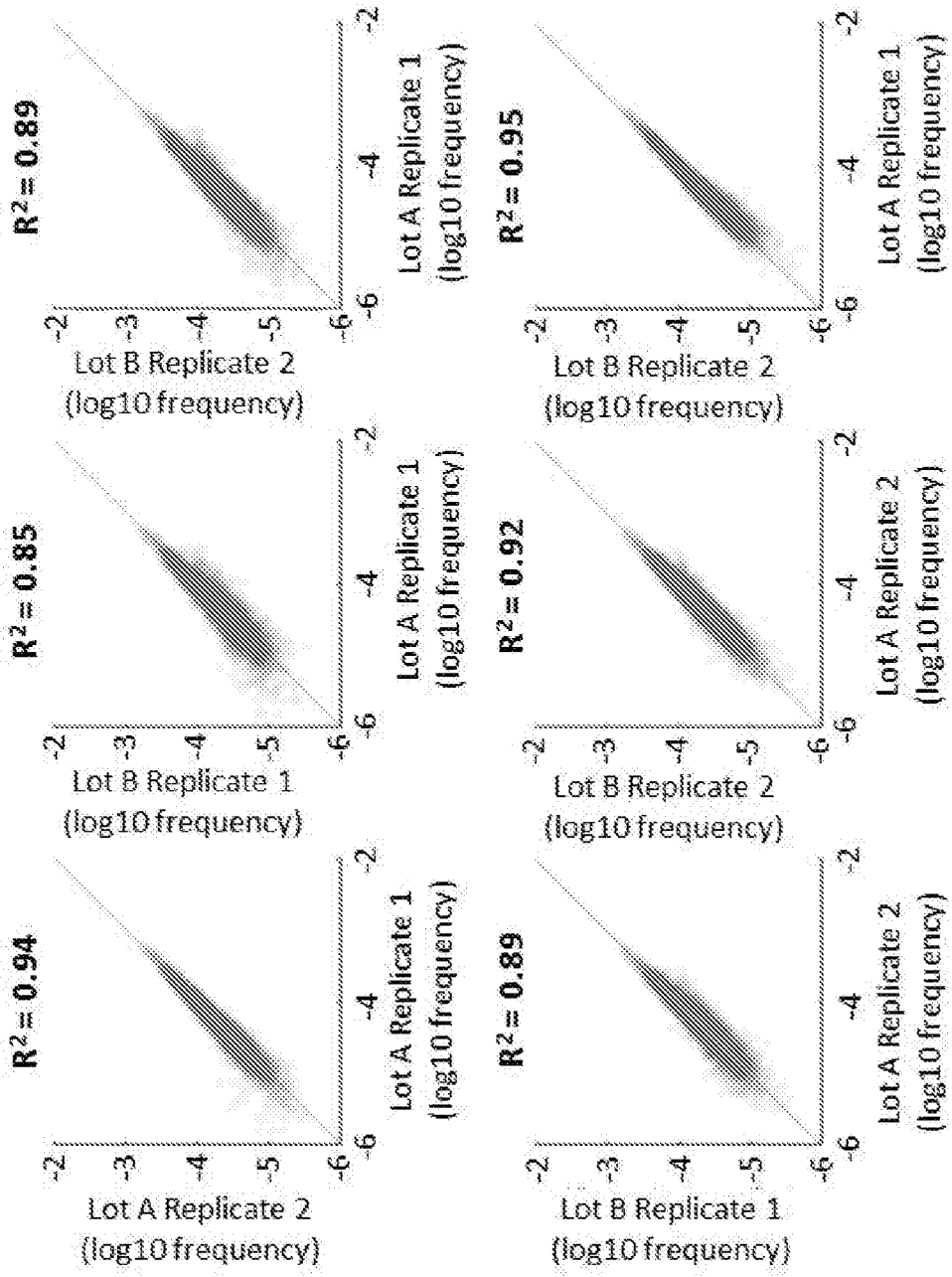

R² = 0.89
Lot B Replicate 2 (log10 frequency)
Lot A Replicate 1 (log10 frequency)
R² = 0.95
Lot B Replicate 2 (log10 frequency)
Lot A Replicate 1 (log10 frequency)
R² = 0.85
Lot B Replicate 1 (log10 frequency)
Lot A Replicate 1 (log10 frequency)
R² = 0.92
Lot B Replicate 2 (log10 frequency)
Lot A Replicate 2 (log10 frequency)
R² = 0.94
Lot A Replicate 2 (log10 frequency)
Lot A Replicate 1 (log10 frequency)
R² = 0.89
Lot B Replicate 1 (log10 frequency)
Lot A Replicate 2 (log10 frequency)

FIG. 28A

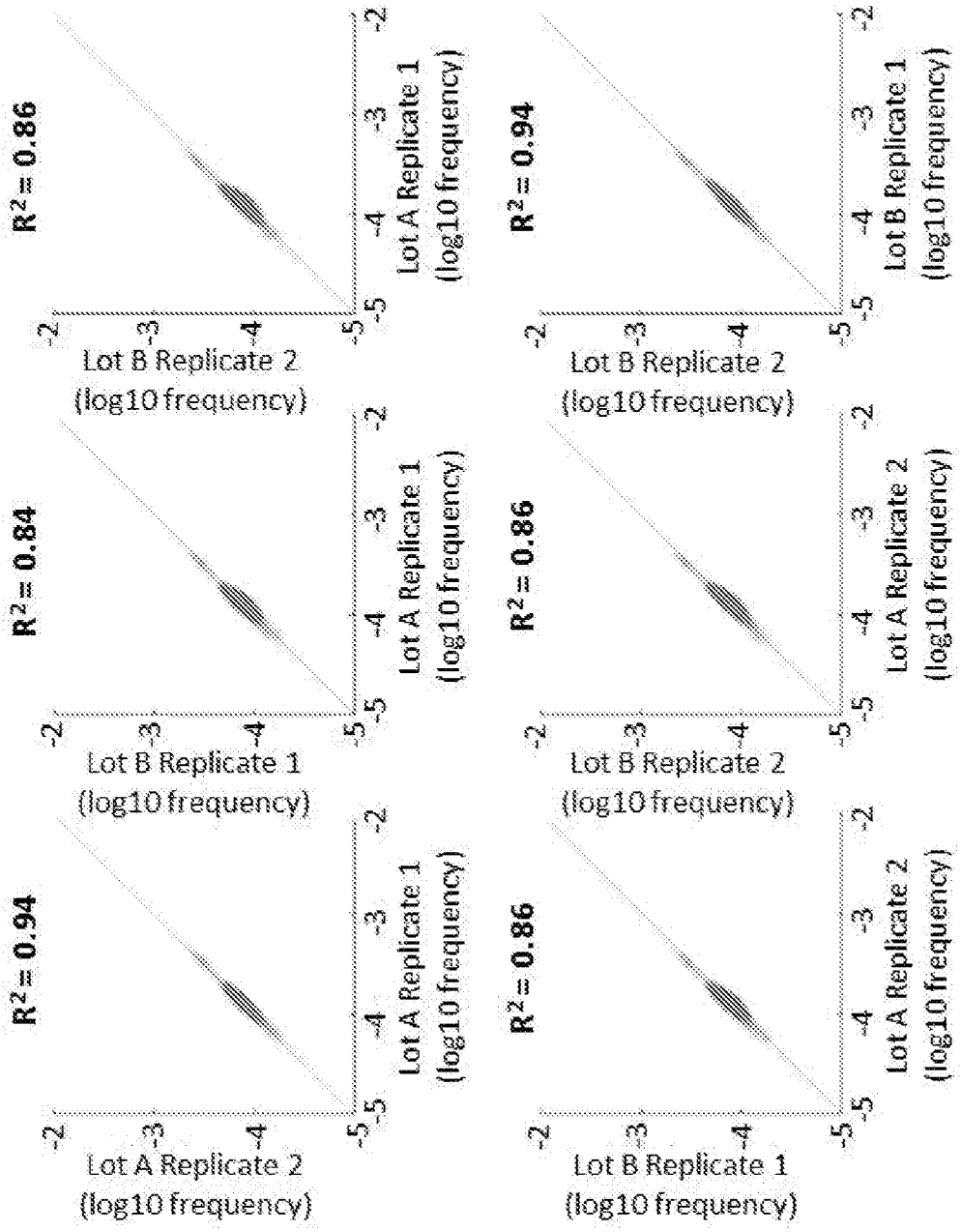

R² = 0.86
Lot B Replicate 2 (log10 frequency)
Lot A Replicate 1 (log10 frequency)
R² = 0.94
Lot B Replicate 2 (log10 frequency)
Lot B Replicate 1 (log10 frequency)
R² = 0.84
Lot B Replicate 1 (log10 frequency)
Lot A Replicate 1 (log10 frequency)
R² = 0.86
Lot B Replicate 2 (log10 frequency)
Lot A Replicate 2 (log10 frequency)
R² = 0.94
Lot A Replicate 2 (log10 frequency)
Lot A Replicate 1 (log10 frequency)
R² = 0.86
Lot B Replicate 1 (log10 frequency)
Lot A Replicate 2 (log10 frequency)

FIG. 28B

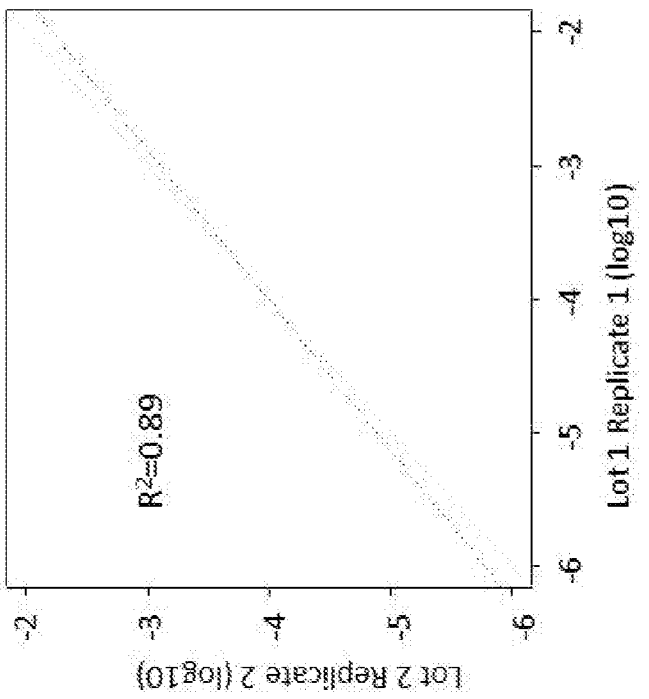


FIG. 28D

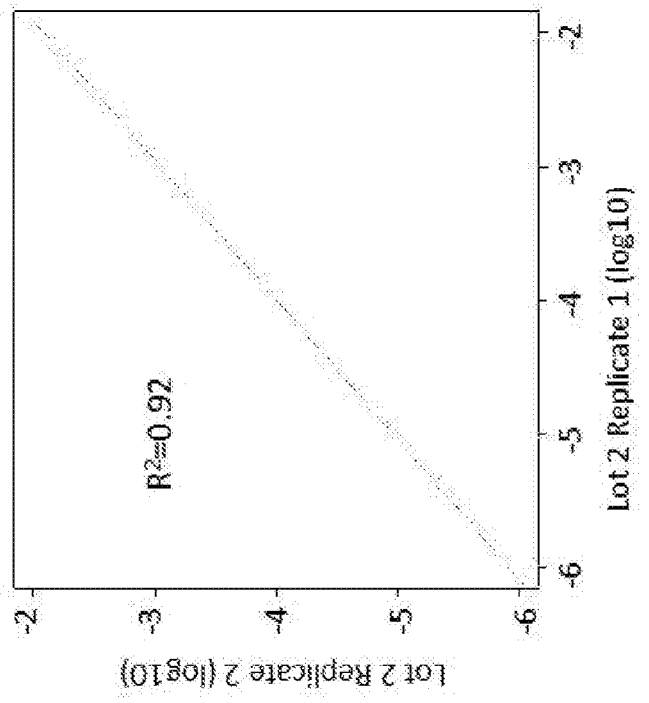


FIG. 28C

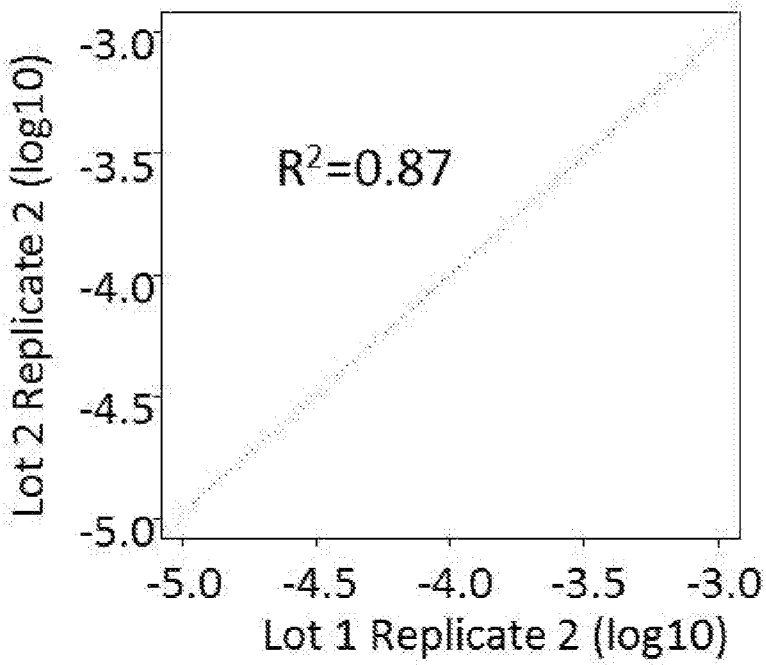


FIG. 28E

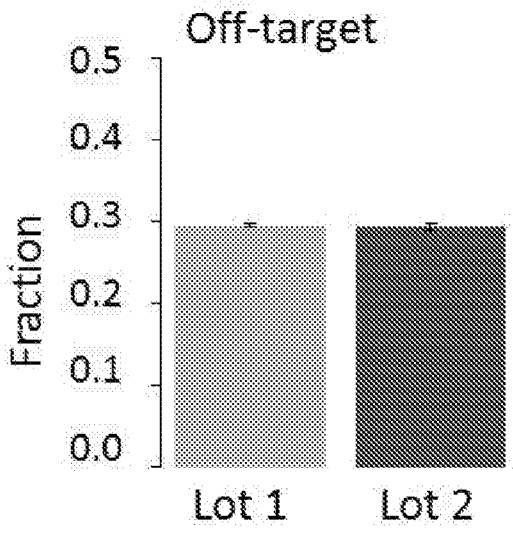


FIG. 28F

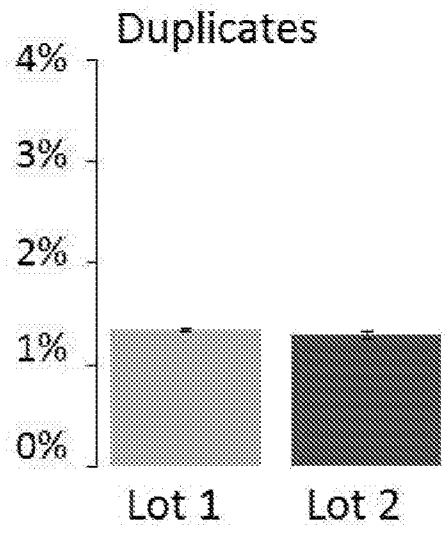


FIG. 28G

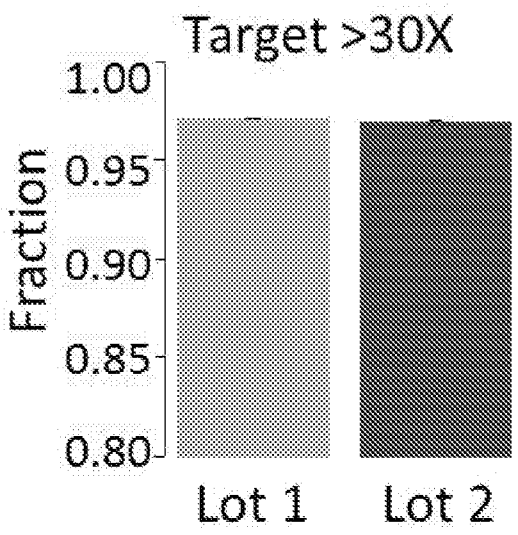


FIG. 28H

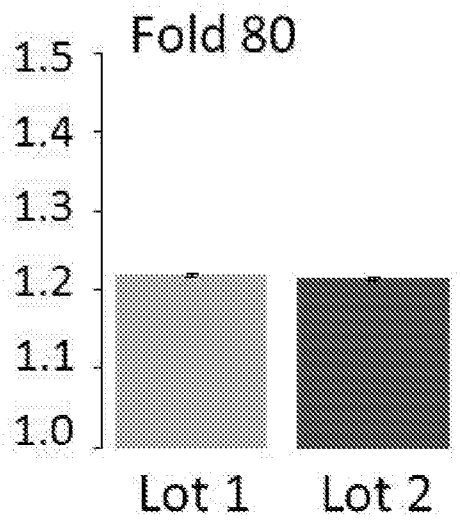


FIG. 28I

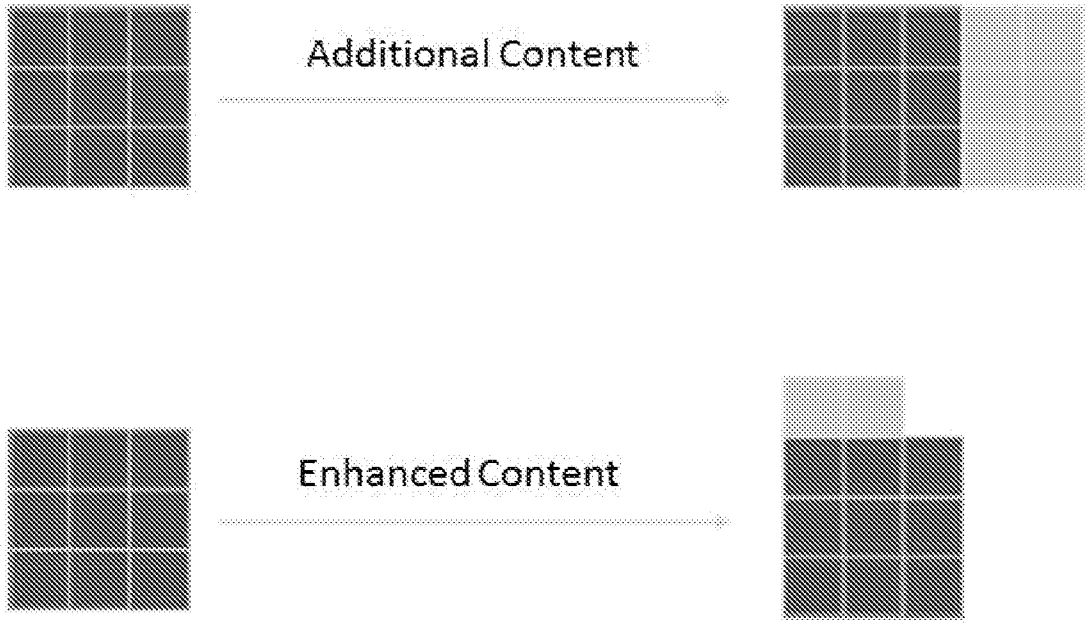


FIG. 29A

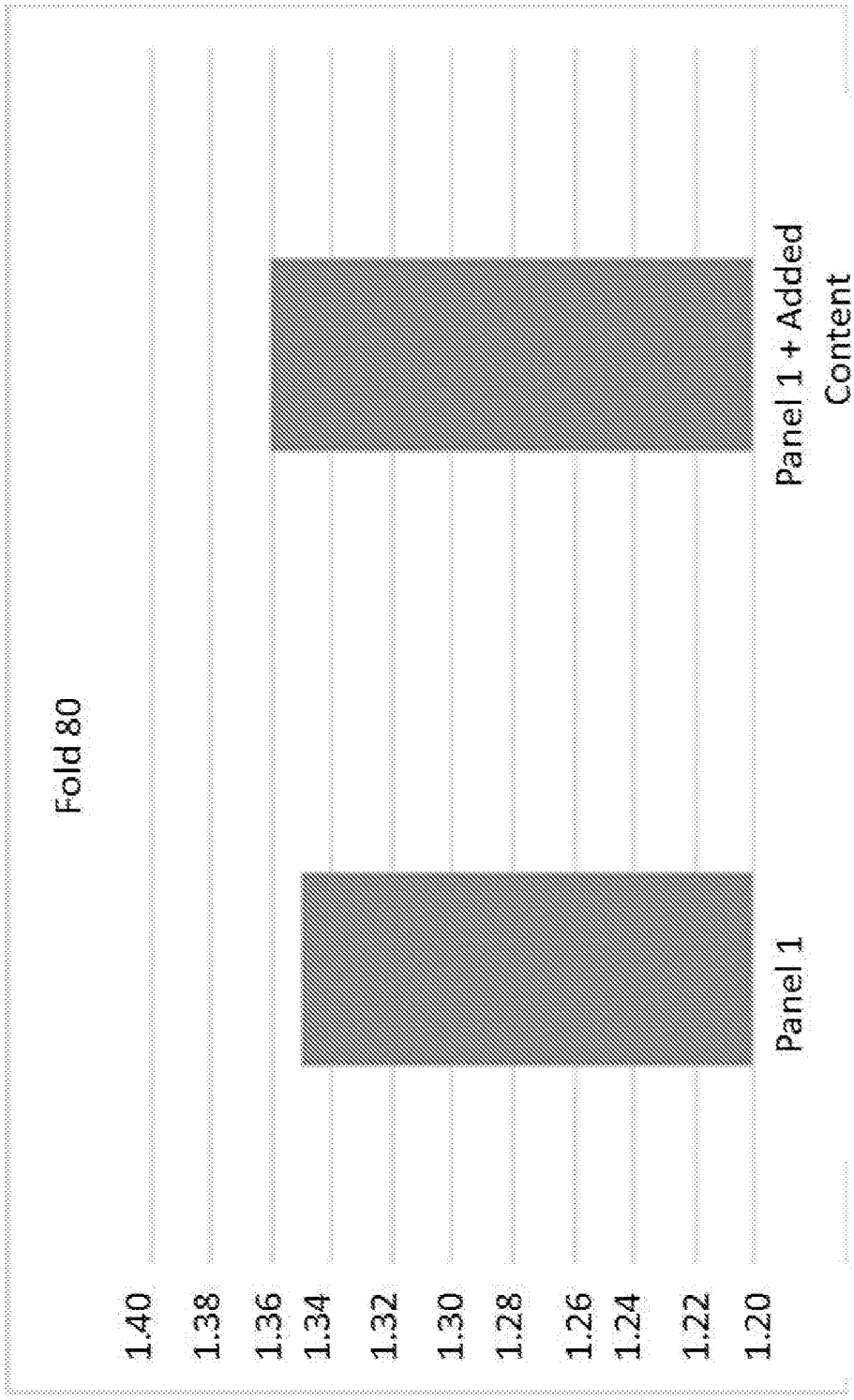

Fold 80
1.40
1.38
1.36
1.34
1.32
1.30
1.28
1.26
1.24
1.22
1.20
Panel 1
Panel 1 + Added Content

FIG. 29B

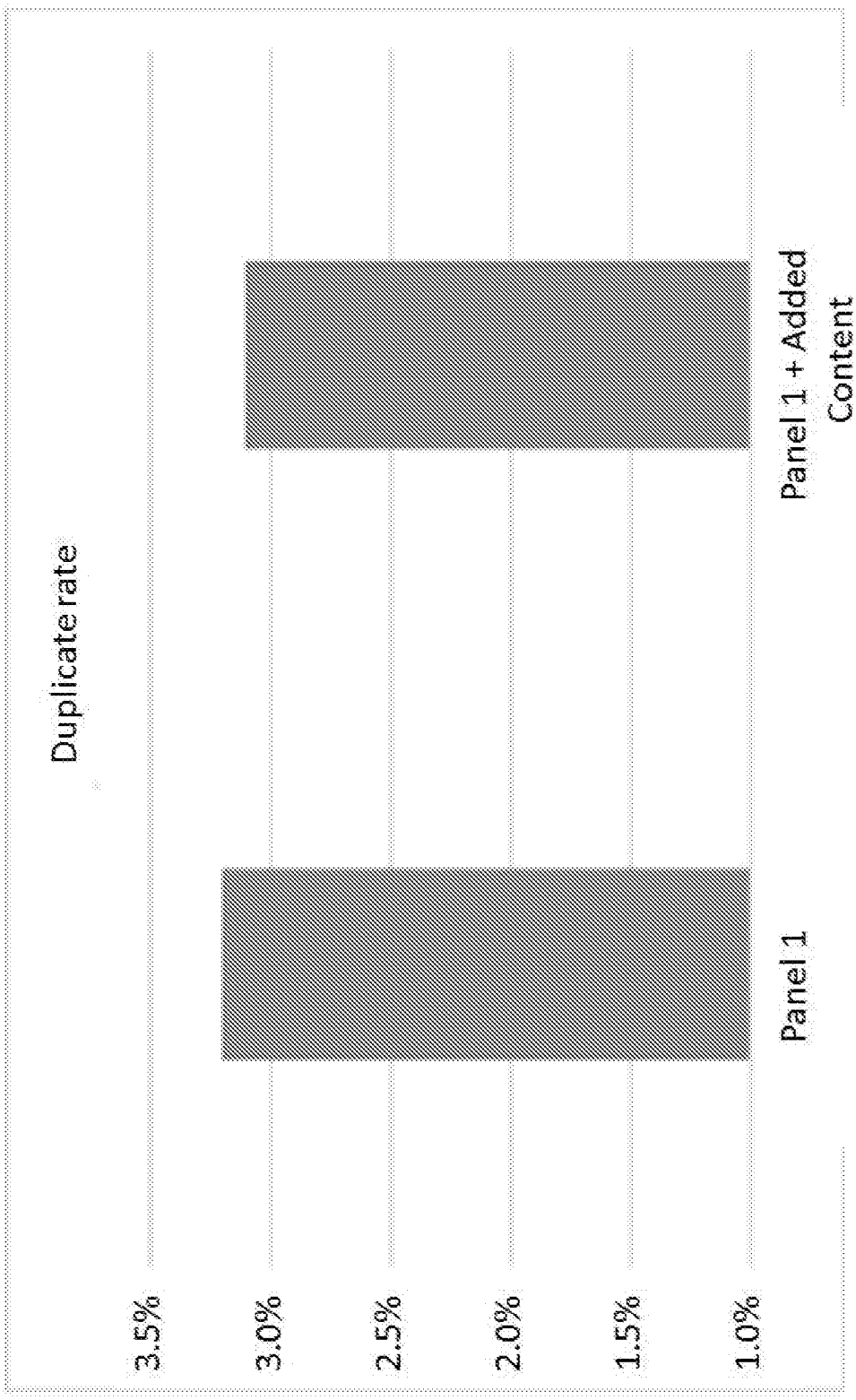


FIG. 29C

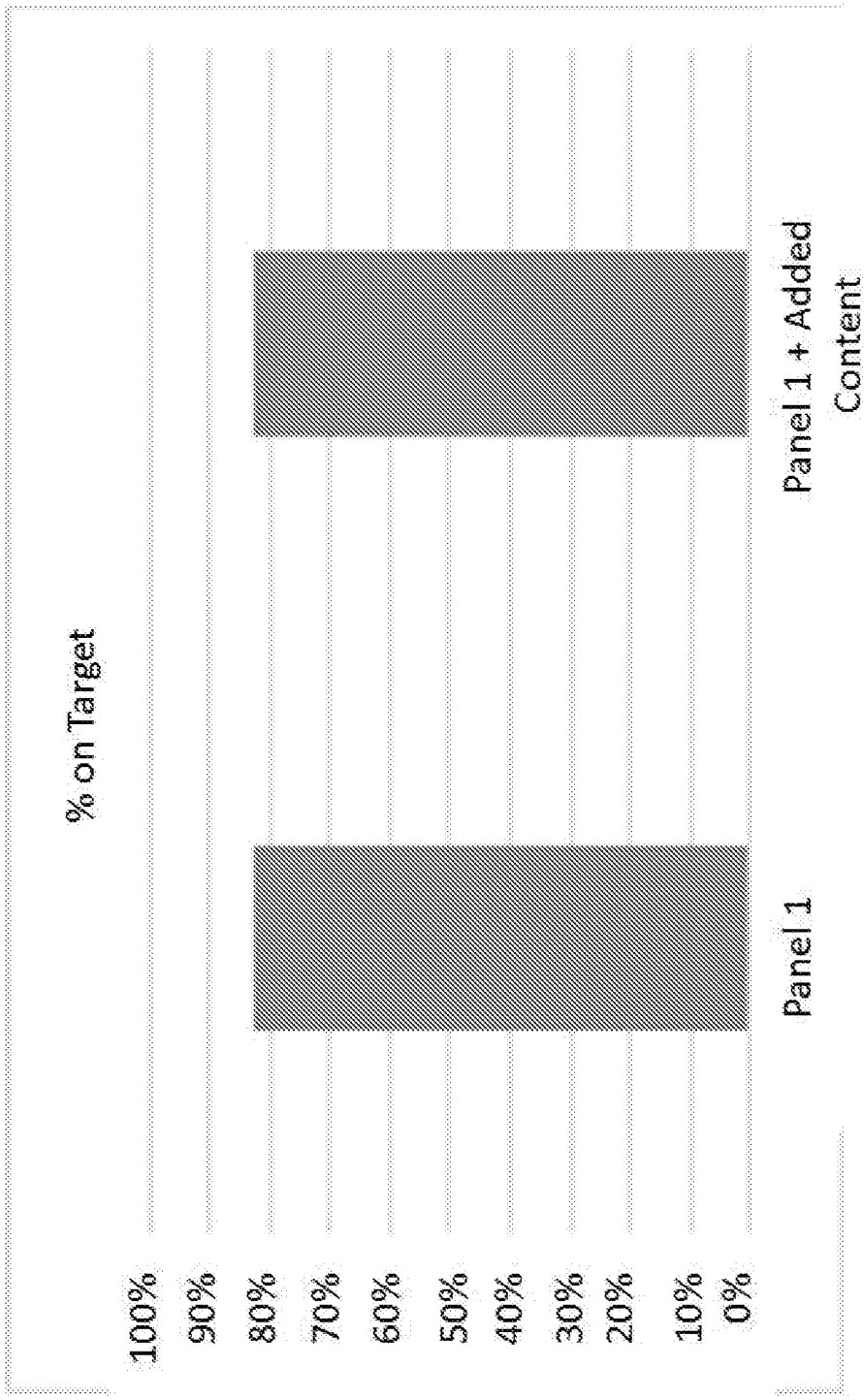

% on Target
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Panel 1
Panel 1 + Added Content

FIG. 29D

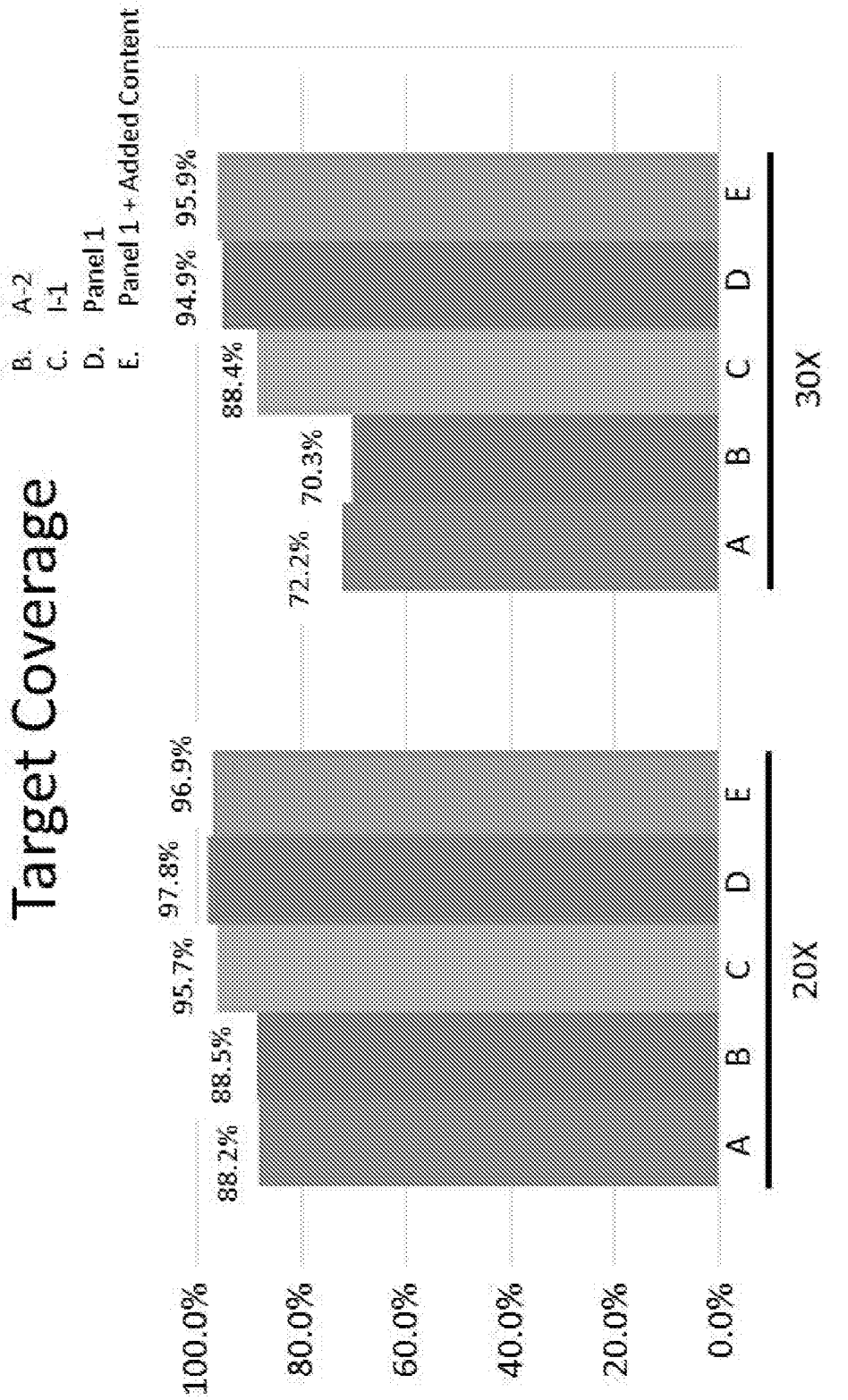

Target Coverage
A. A-1
B. A-2
C. I-1
D. Panel 1
E. Panel 1 + Added Content
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
88.2%
88.5%
95.7%
97.8%
96.9%
72.2%
70.3%
88.4%
94.9%
95.9%
A B C D E
20X
A B C D E
30X

FIG. 29E

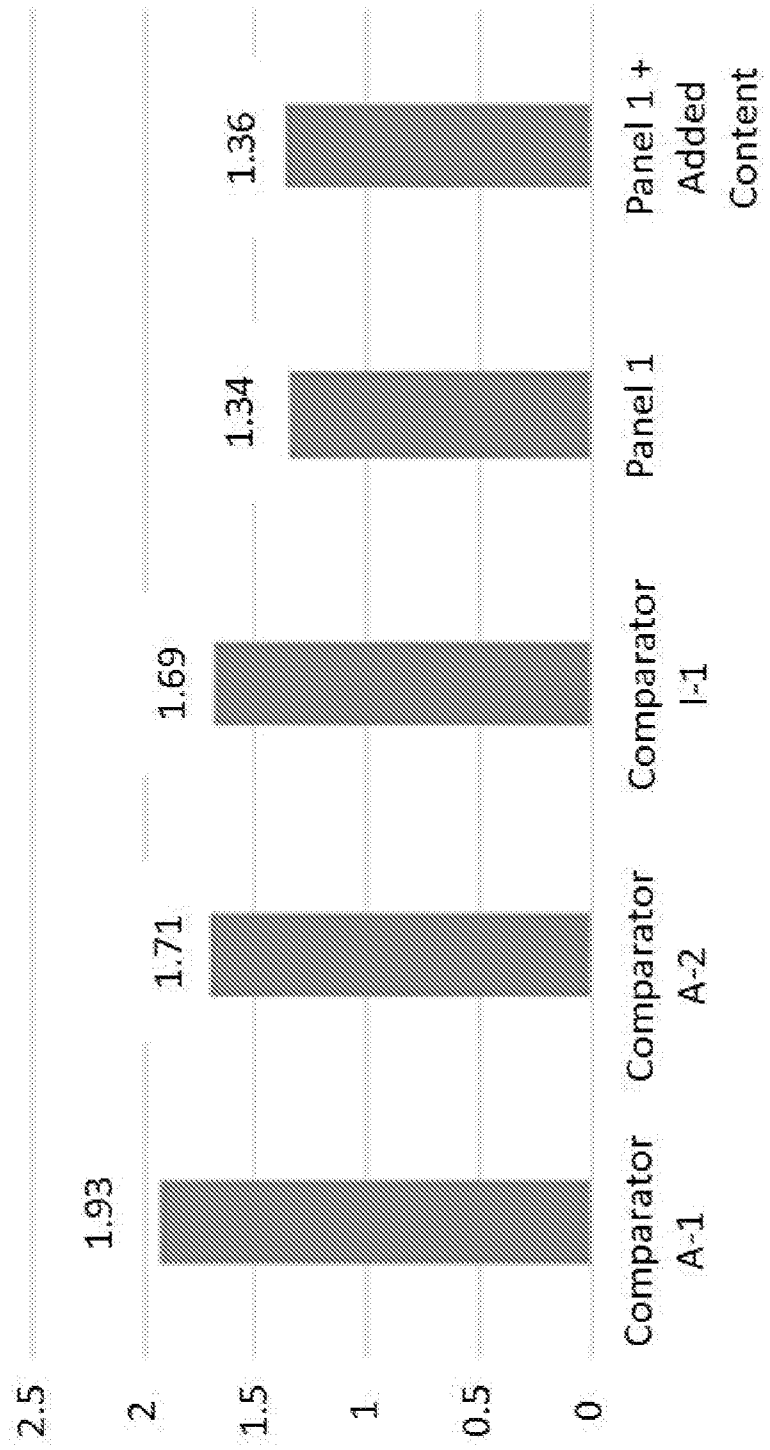

Fold 80
2.5
2
1.5
1
0.5
0
1.93
1.71
1.69
1.34
1.36
Comparator A-1
Comparator A-2
Comparator I-1
Panel 1
Panel 1 + Added Content

FIG. 29F

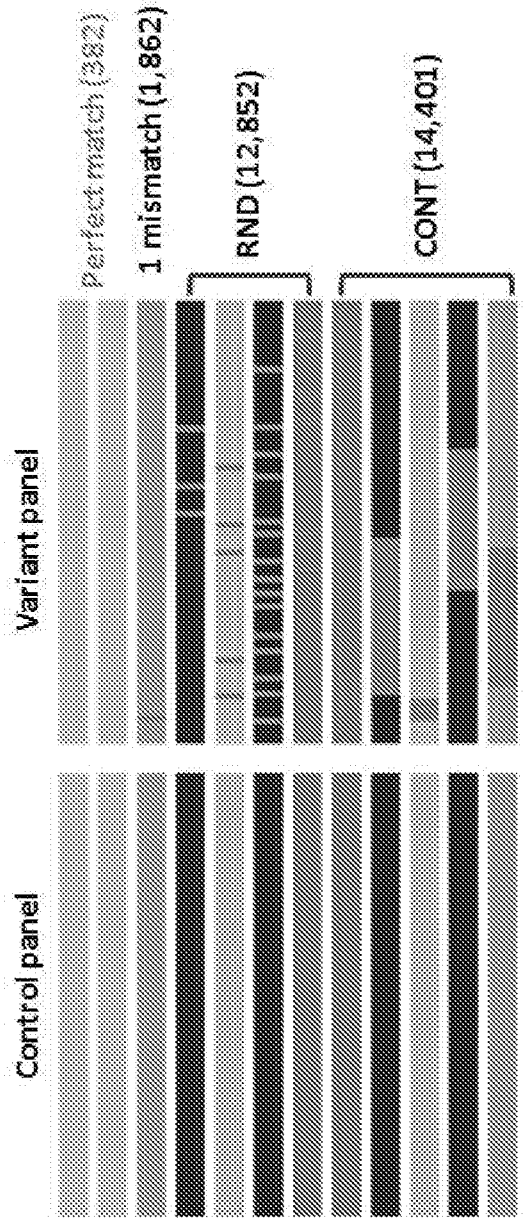

Perfect match (382)
1 mismatch (1,862)
RND (12,852)
CONT (14,401)
Variant panel
Control panel

FIG. 30A

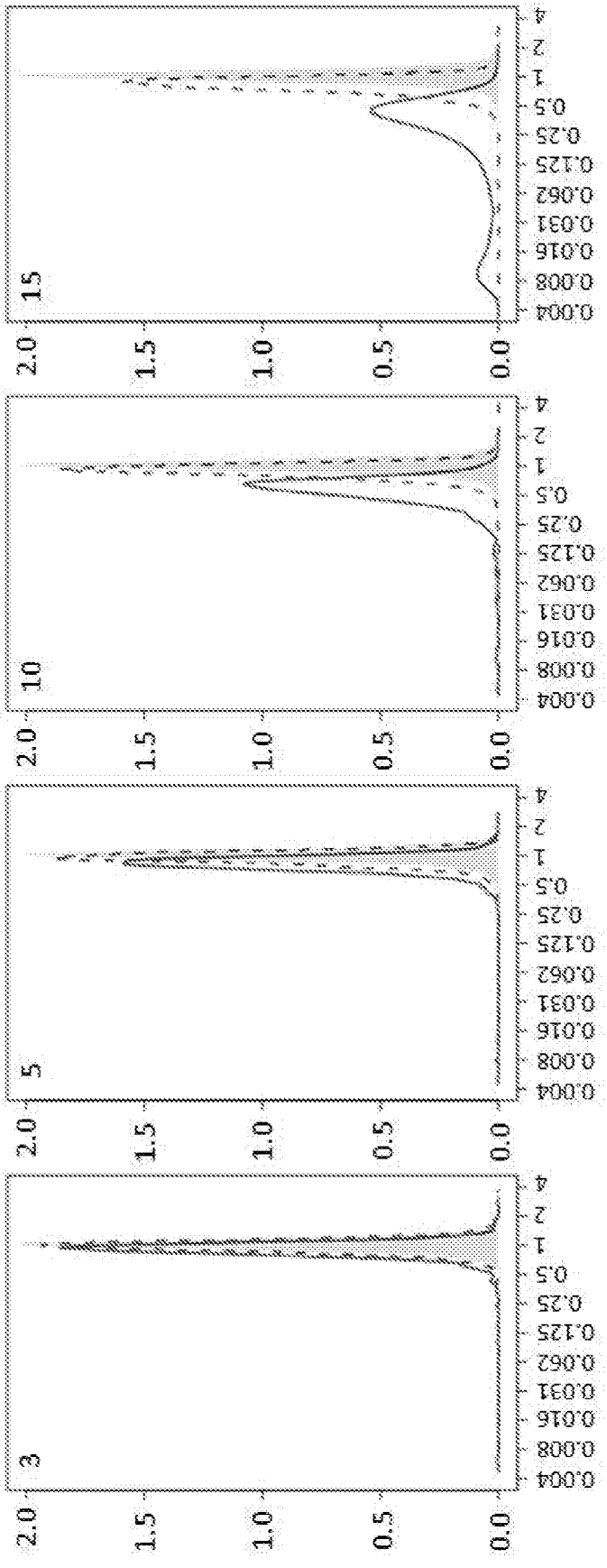


FIG. 30B

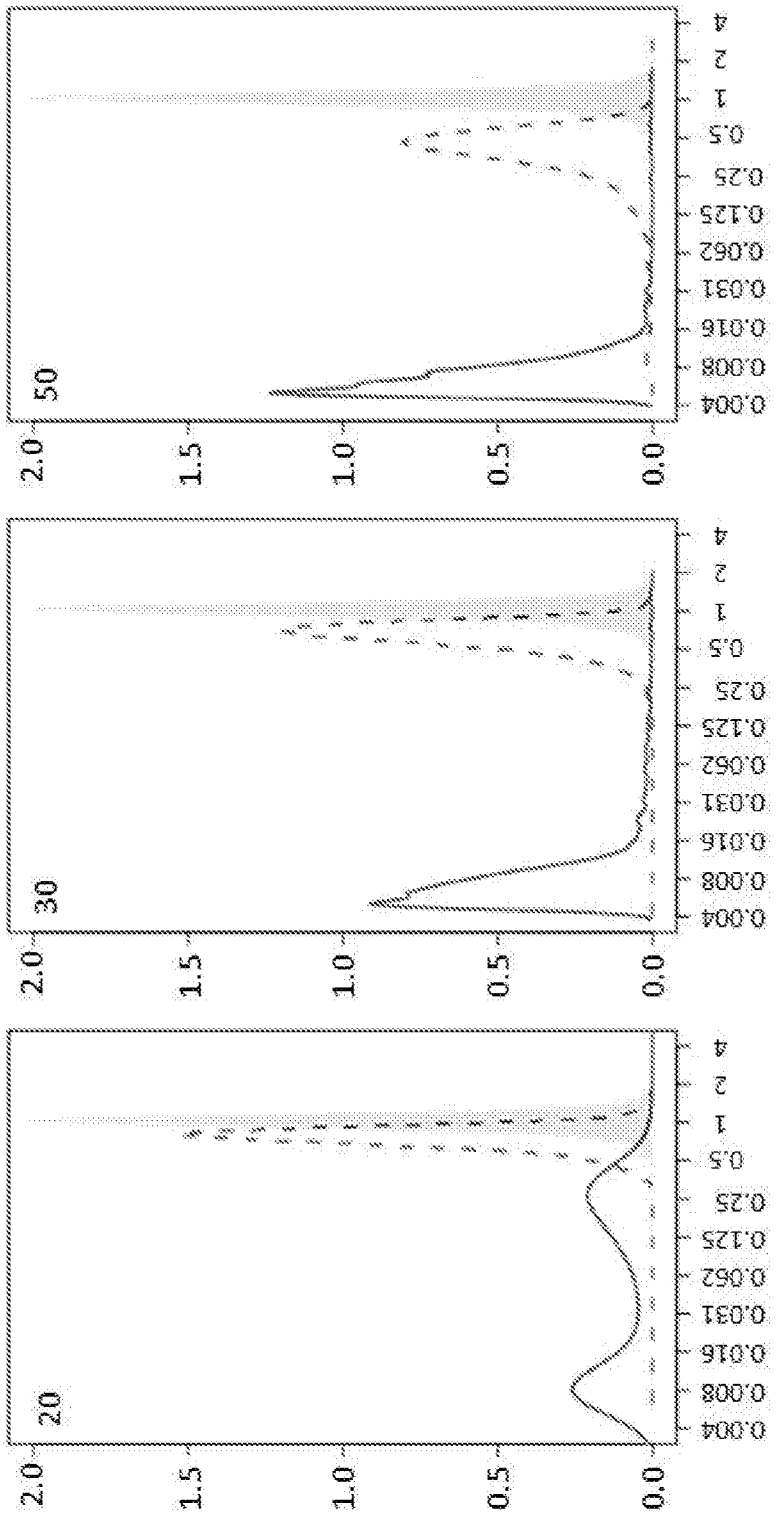


FIG. 30C

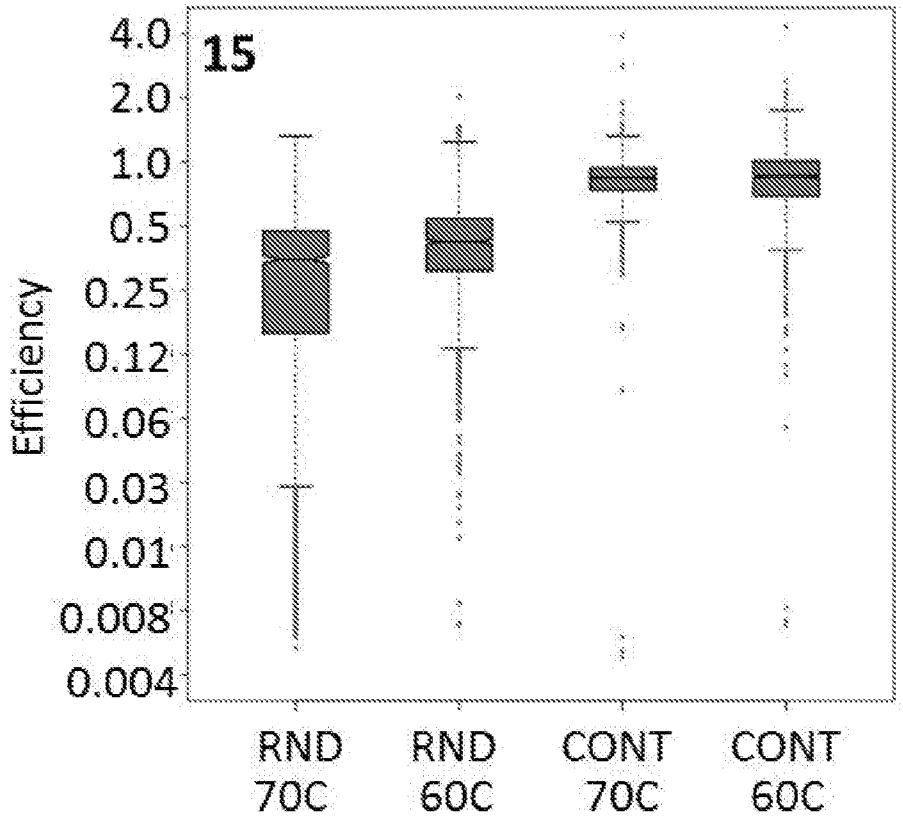

15
Efficiency
4.0
2.0
1.0
0.5
0.25
0.12
0.06
0.03
0.01
0.008
0.004
RND 70C
RND 60C
CONT 70C
CONT 60C

FIG. 30D

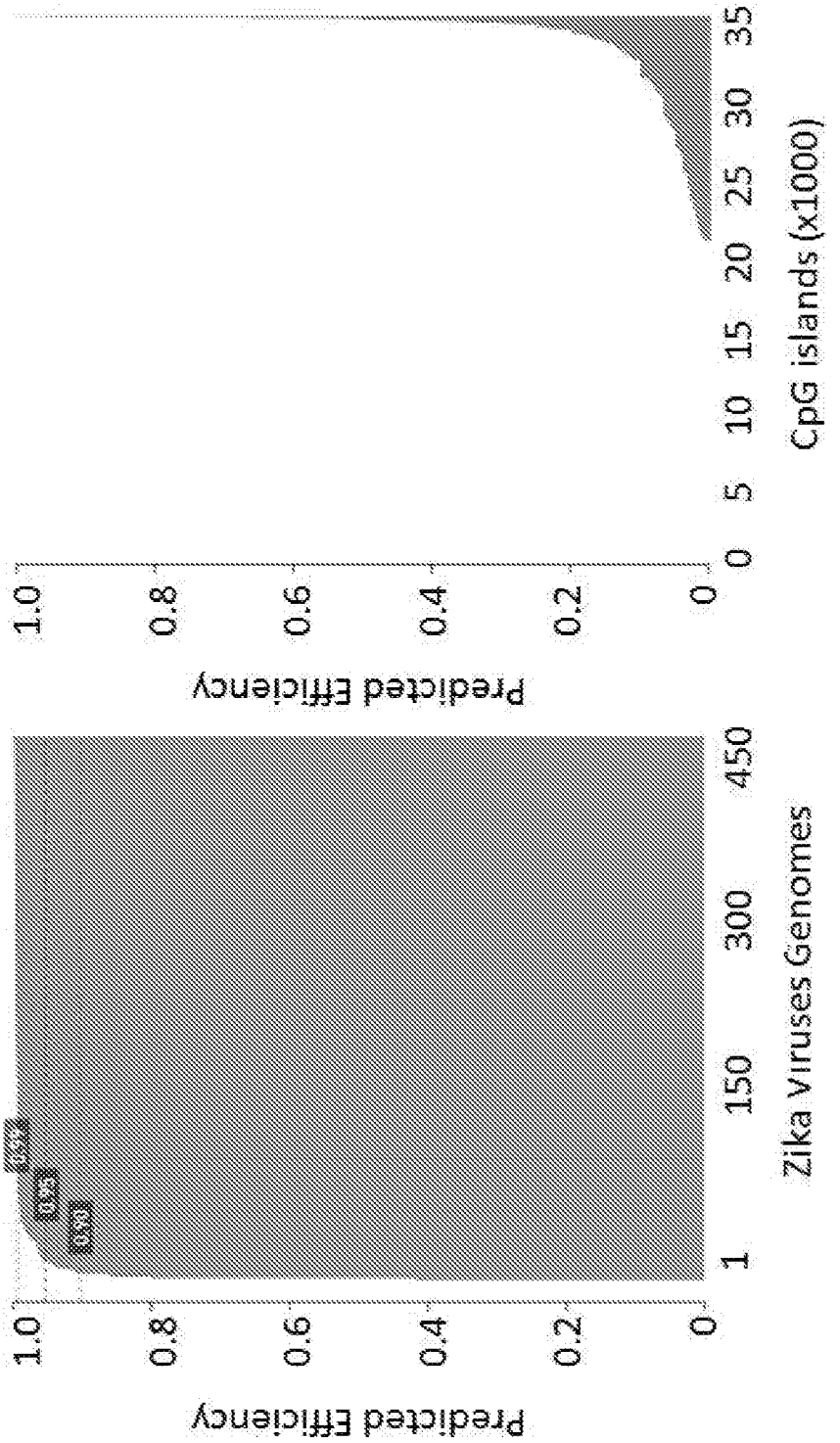


FIG. 30E

FIG. 30F

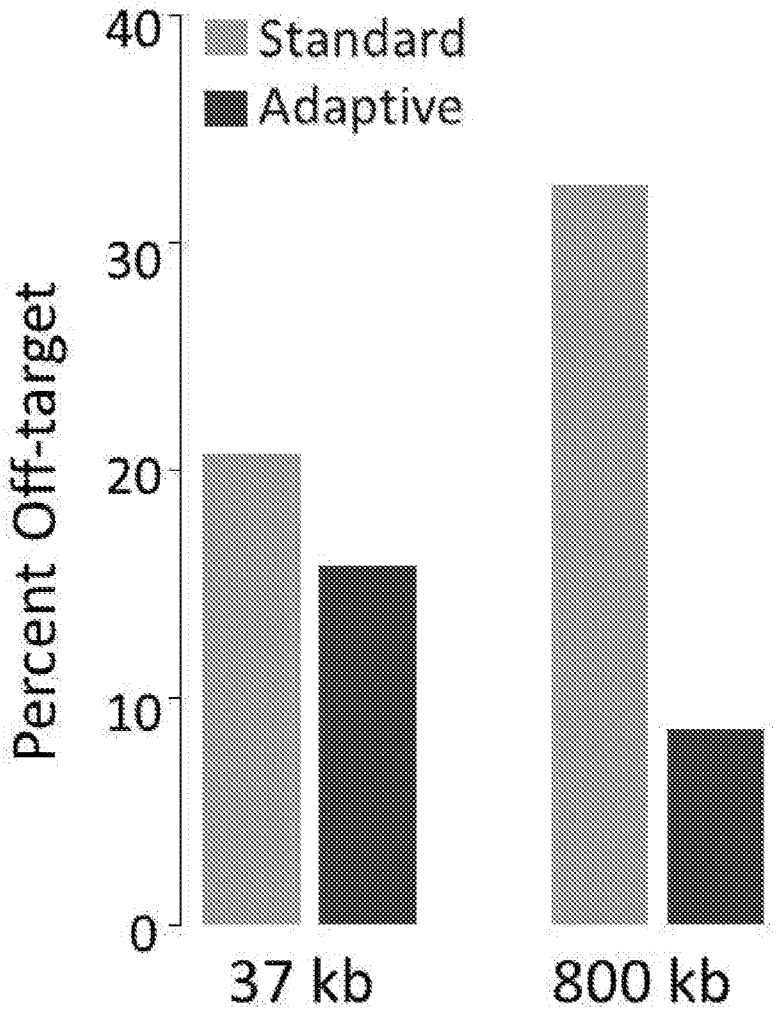

40
30
20
10
0
Percent Off-target
Standard
Adaptive
37 kb
800 kb

FIG. 31A

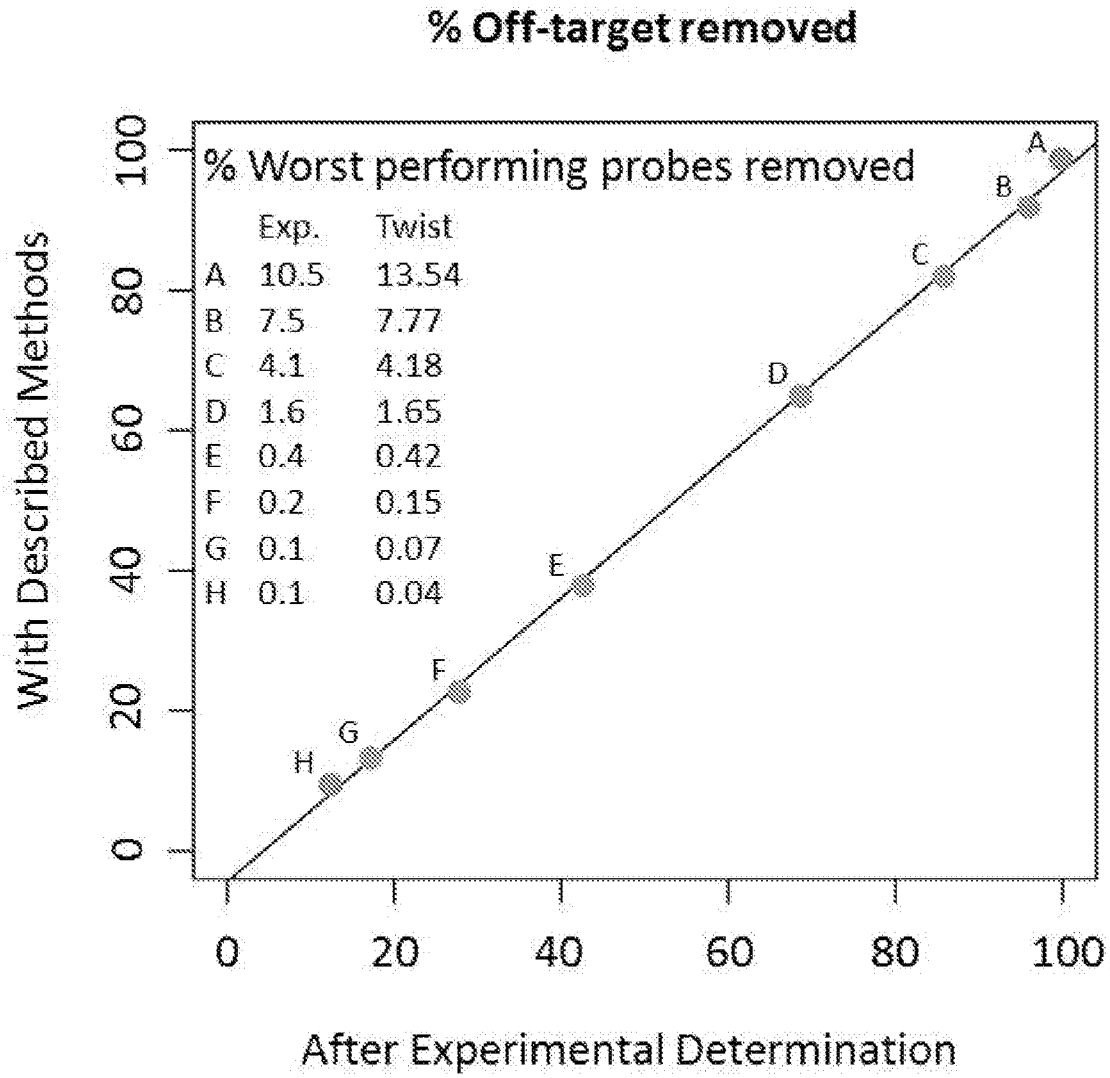

% Off-target removed
% Worst performing probes removed
Exp. Twist
A 10.5 13.54
B 7.5 7.77
C 4.1 4.18
D 1.6 1.65
E 0.4 0.42
F 0.2 0.15
G 0.1 0.07
H 0.1 0.04
With Described Methods
After Experimental Determination
0
20
40
60
80
100

FIG. 31B

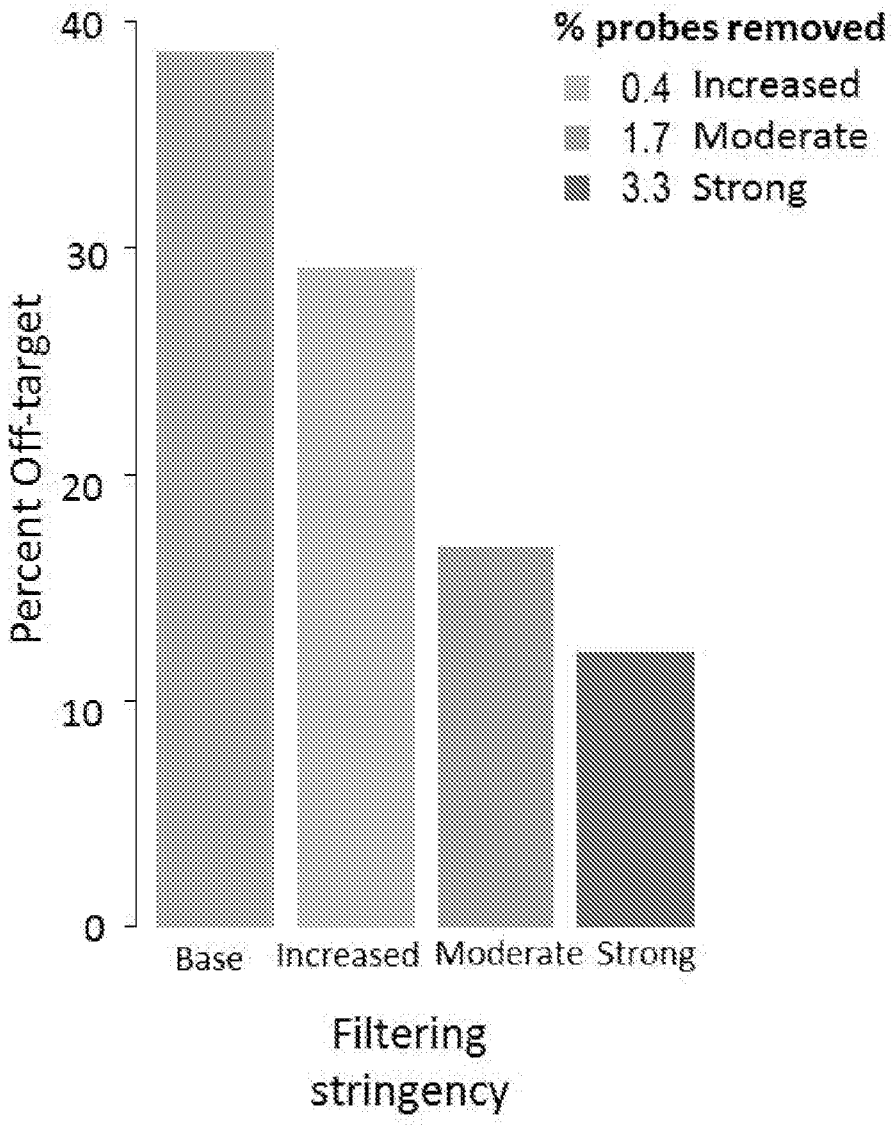

% probes removed
0.4 Increased
1.7 Moderate
3.3 Strong
Percent Off-target
40
30
20
10
0
Base
Increased
Moderate
Strong
Filtering stringency

FIG. 31C

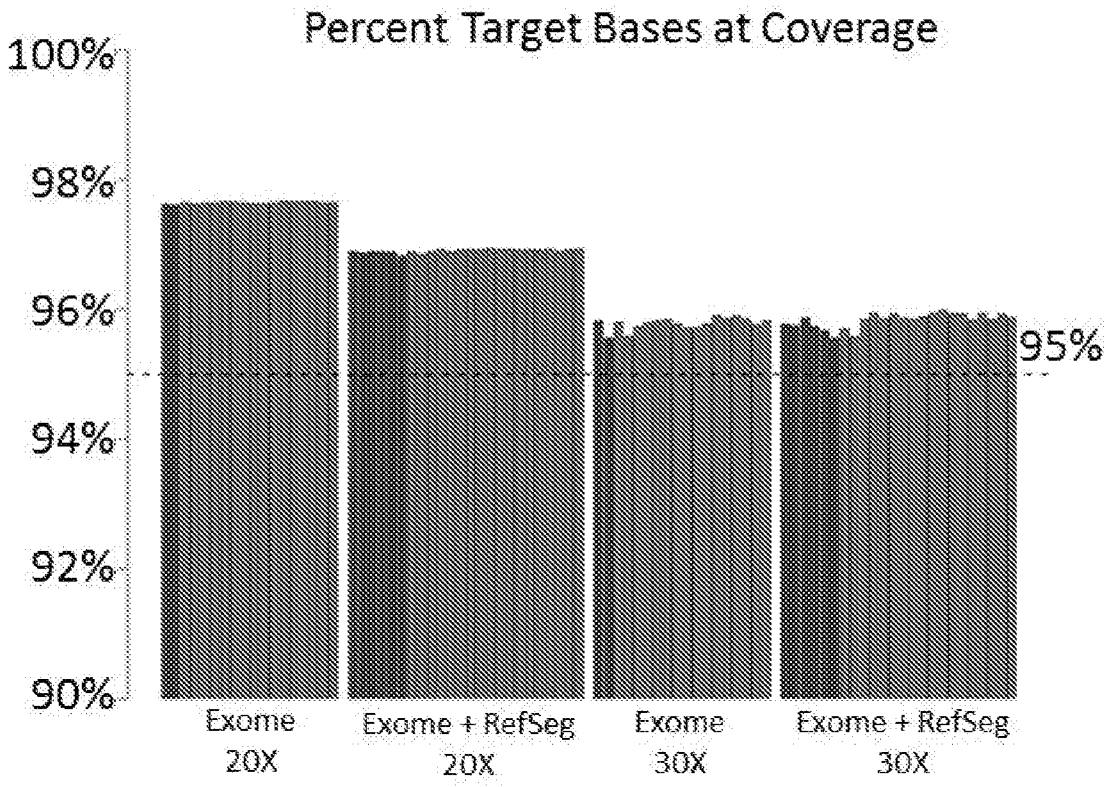


FIG. 32A

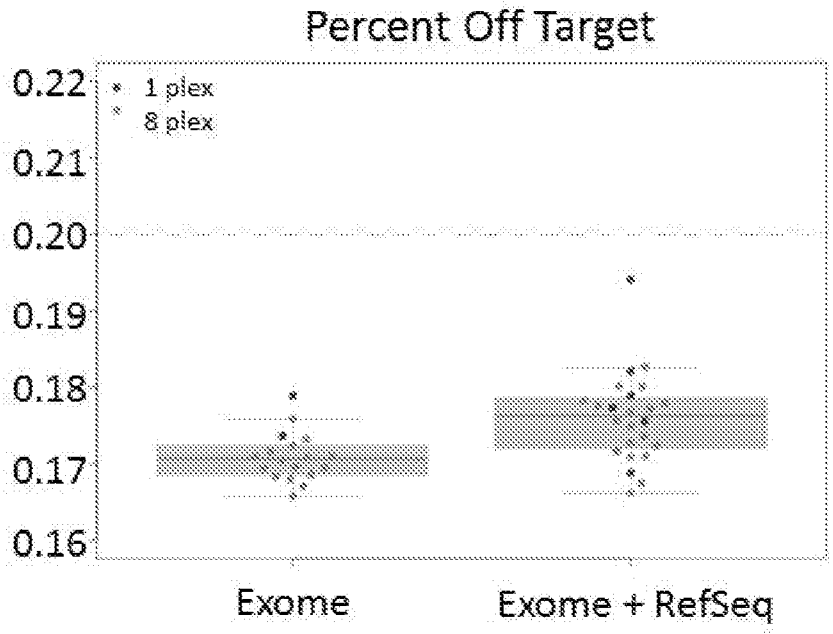


FIG. 32B

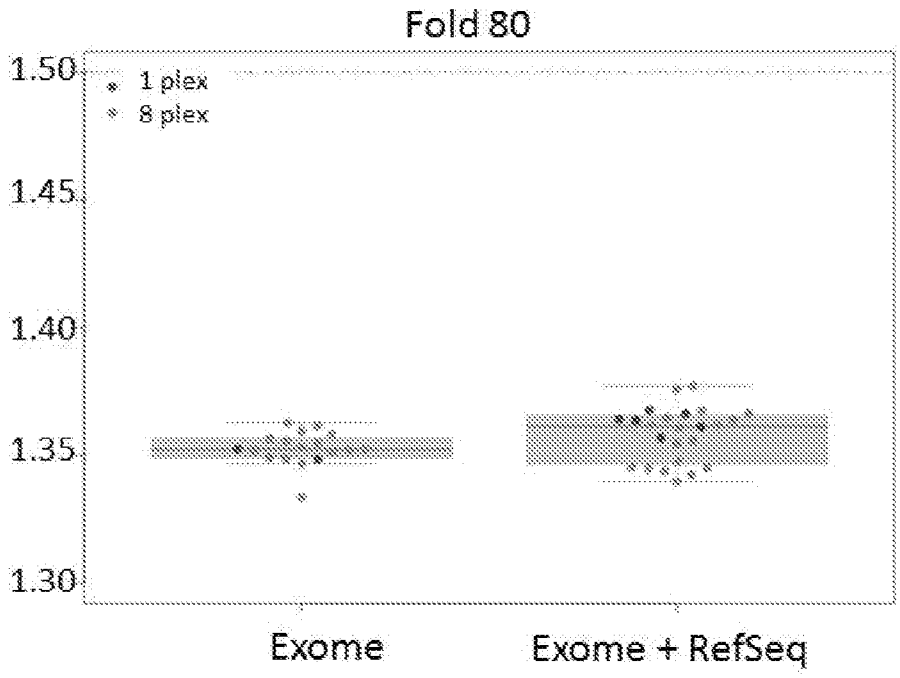


FIG. 32C

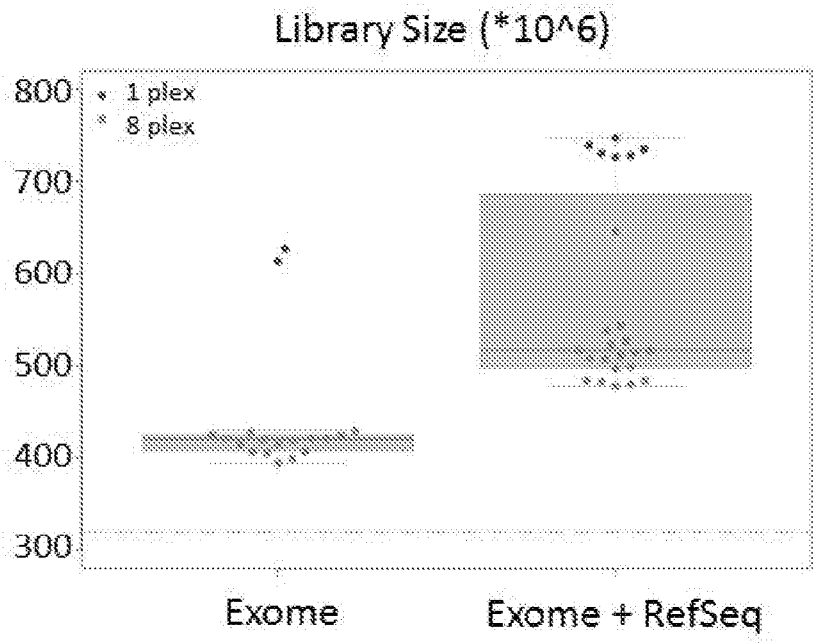


FIG. 32D

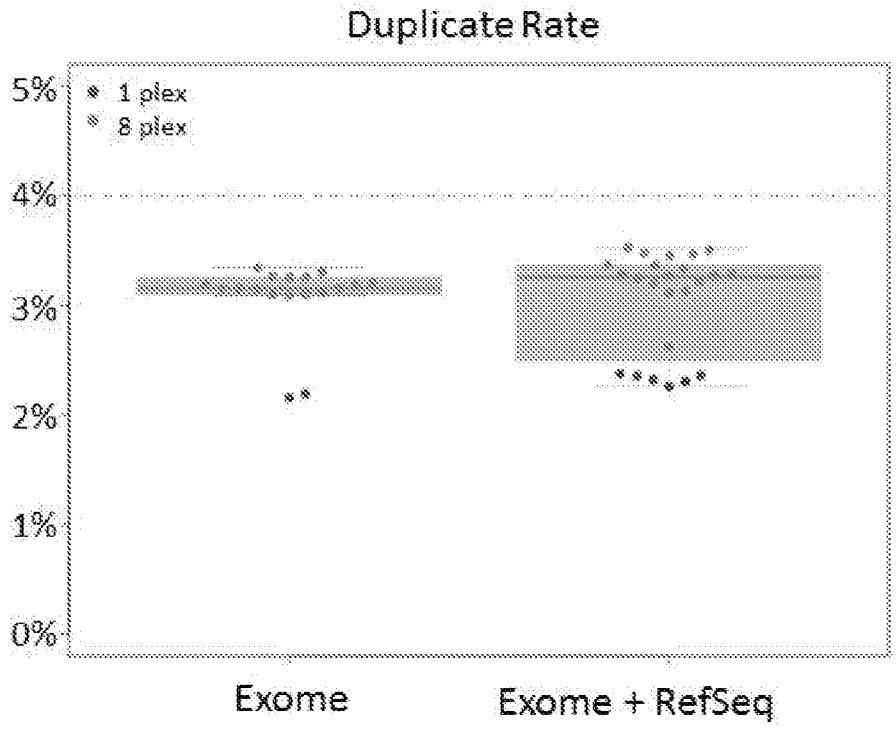


FIG. 32E

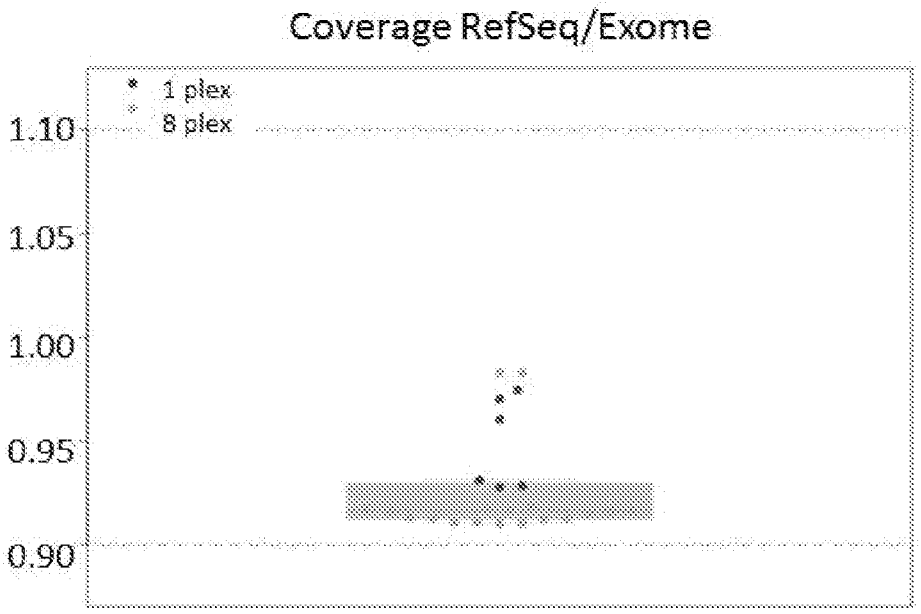


FIG. 32F

FIG. 33A

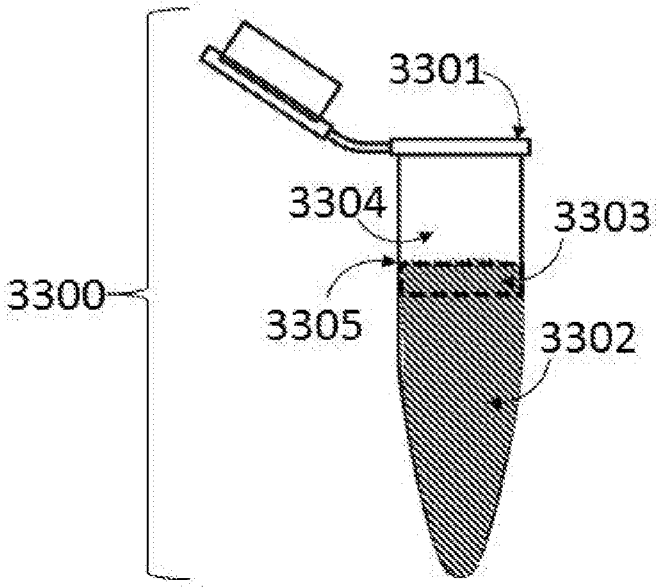
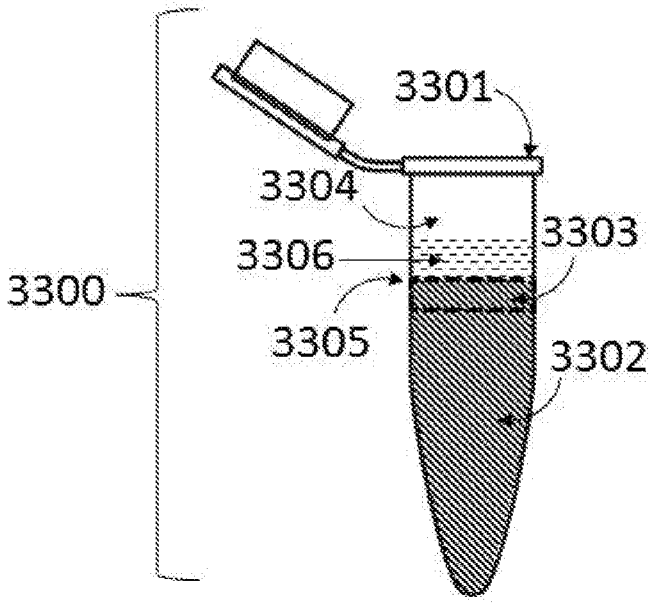


FIG. 33B



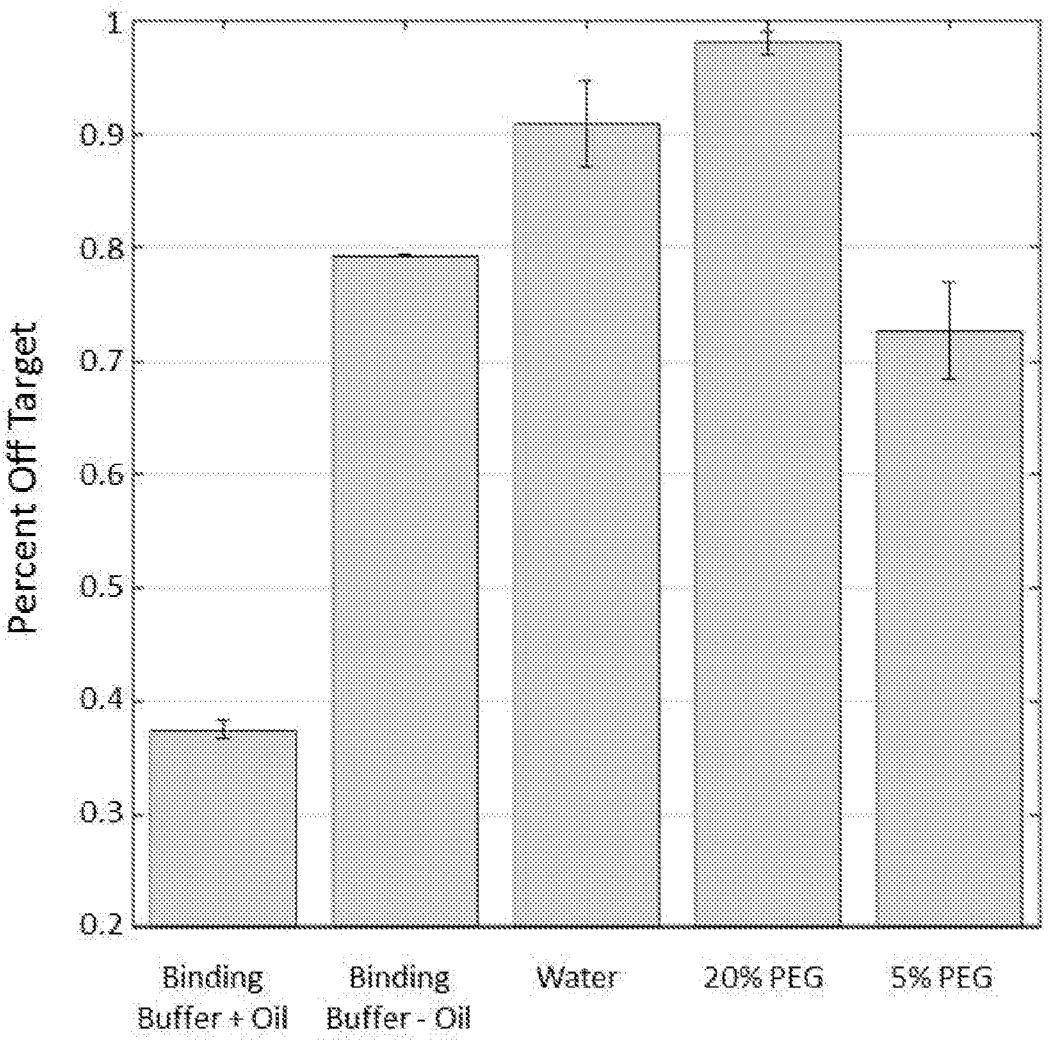

Percent Off Target
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Binding Buffer + Oil
Binding Buffer - Oil
Water
20% PEG
5% PEG

FIG. 33C

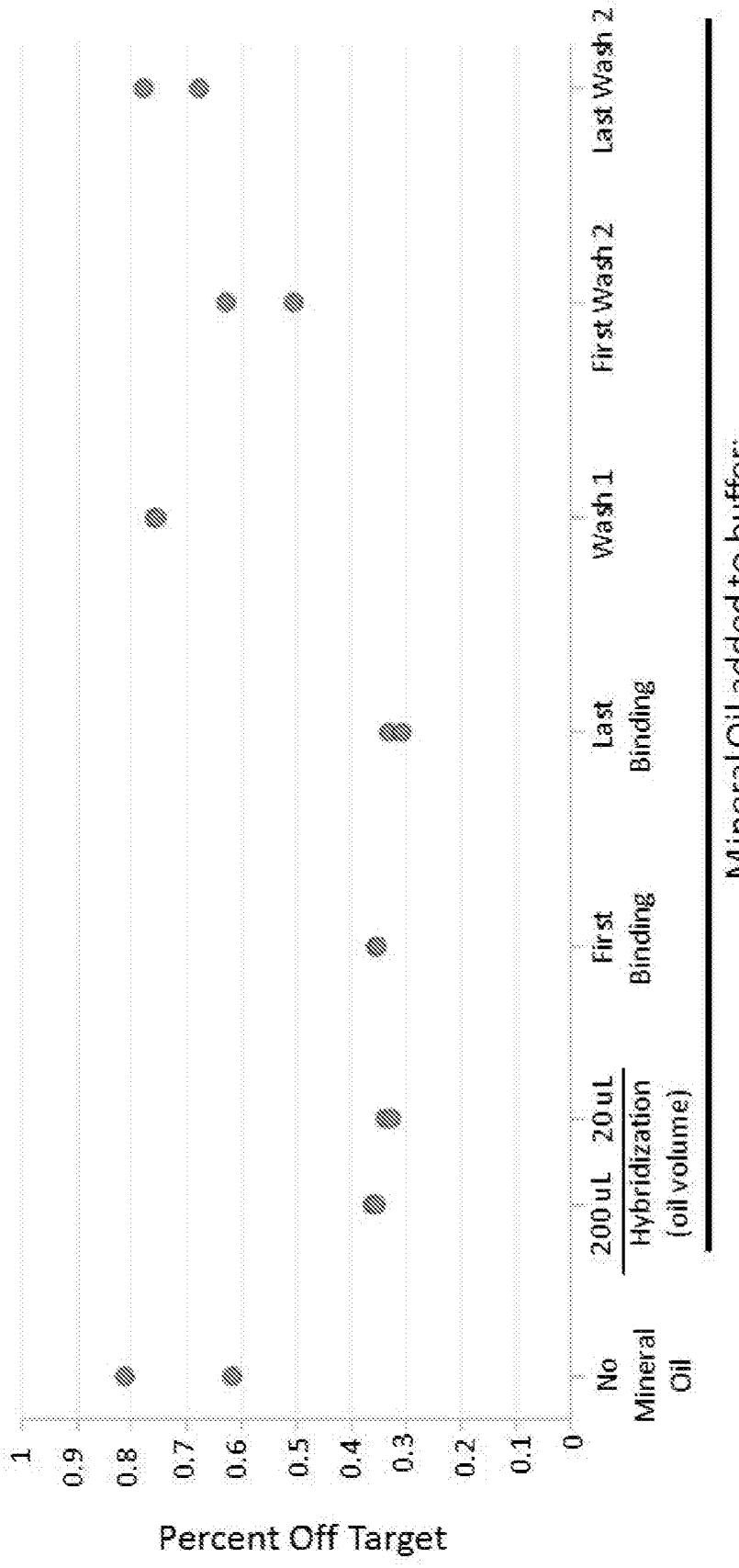

Percent Off Target
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
No Mineral Oil
200 uL Hybridization (oil volume)
20 uL Hybridization (oil volume)
First Binding
Last Binding
Wash 1
First Wash 2
Last Wash 2
Mineral Oil added to buffer:

FIG. 34A

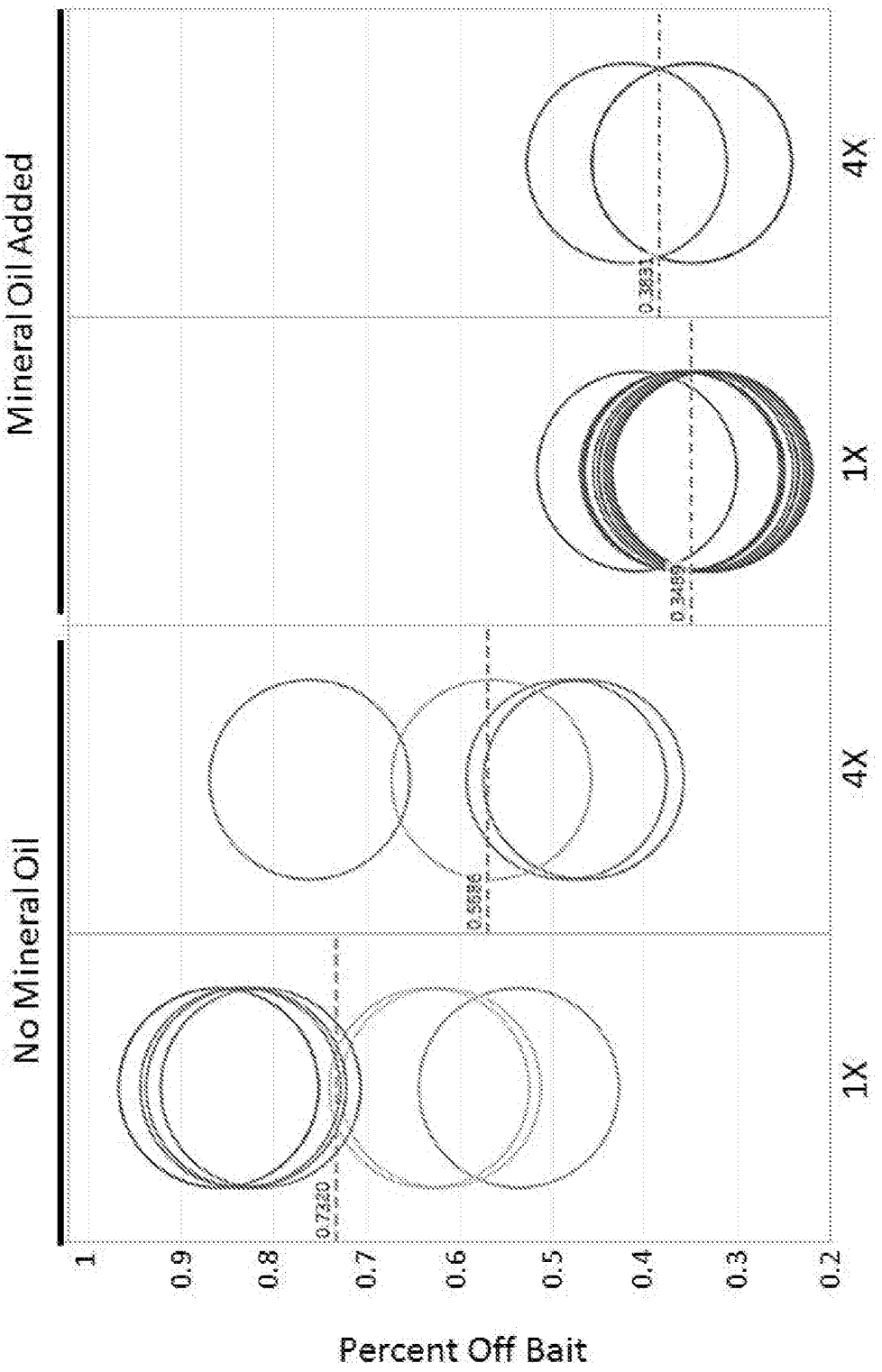

No Mineral Oil
Mineral Oil Added
1X
4X
1X
4X
0.7320
0.5525
0.3469
0.3831
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Percent Off Bait
Number of Washes with Wash Buffer 1

FIG. 34B

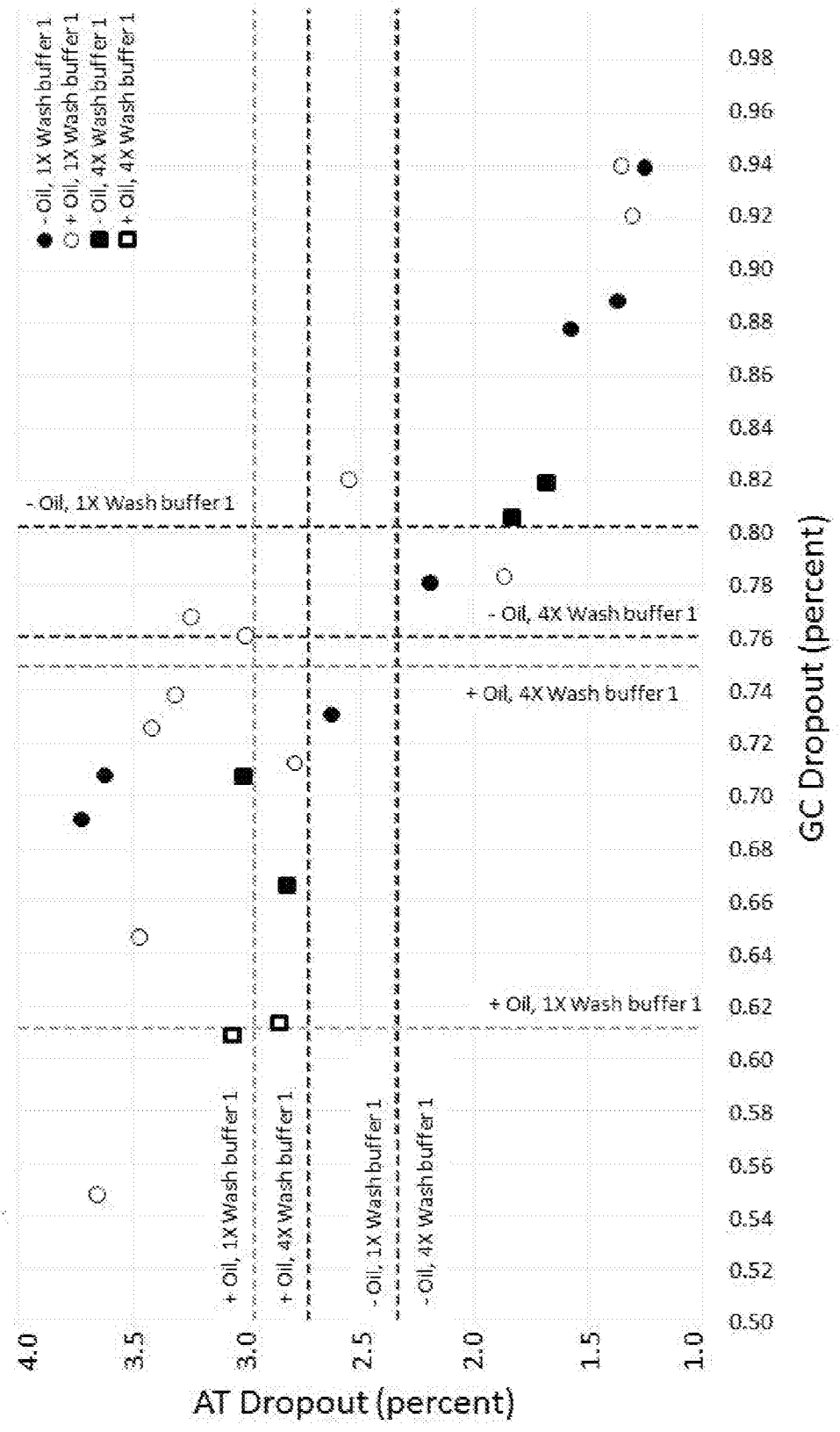

- Oil, 1X Wash buffer 1
+ Oil, 1X Wash buffer 1
- Oil, 4X Wash buffer 1
+ Oil, 4X Wash buffer 1
- Oil, 1X Wash buffer 1
- Oil, 4X Wash buffer 1
+ Oil, 4X Wash buffer 1
+ Oil, 1X Wash buffer 1
+ Oil, 1X Wash buffer 1
+ Oil, 4X Wash buffer 1
- Oil, 1X Wash buffer 1
- Oil, 4X Wash buffer 1
4.0
3.5
3.0
2.5
2.0
1.5
1.0
AT Dropout (percent)
0.98
0.96
0.94
0.92
0.90
0.88
0.86
0.84
0.82
0.80
0.78
0.76
0.74
0.72
0.70
0.68
0.66
0.64
0.62
0.60
0.58
0.56
0.54
0.52
0.50
GC Dropout (percent)

FIG. 34C

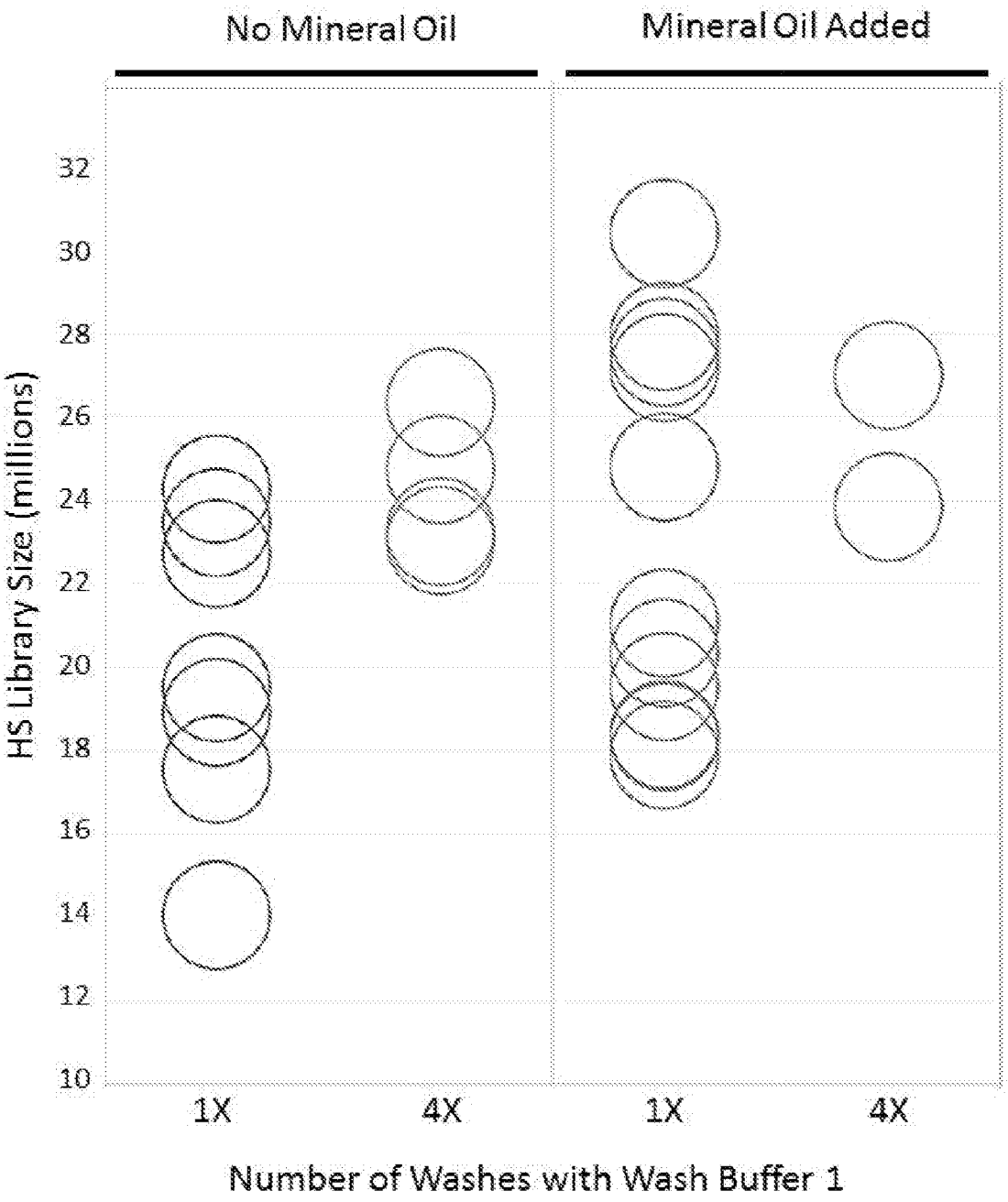

No Mineral Oil
Mineral Oil Added
HS Library Size (millions)
32
30
28
26
24
22
20
18
16
14
12
10
1X
4X
1X
4X
Number of Washes with Wash Buffer 1

FIG. 34D

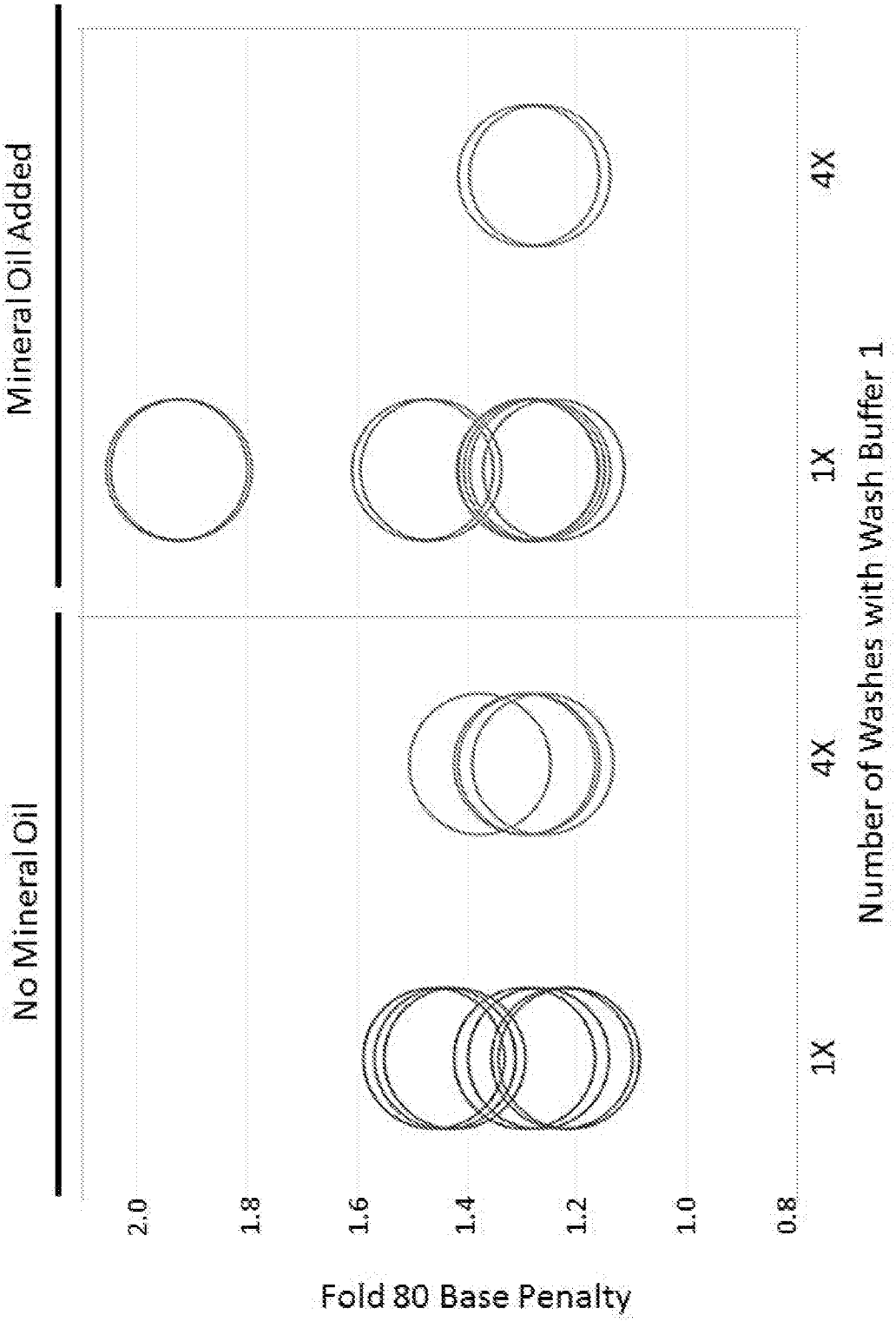

No Mineral Oil
Mineral Oil Added
2.0
1.8
1.6
1.4
1.2
1.0
0.8
Fold 80 Base Penalty
1X
4X
1X
4X
Number of Washes with Wash Buffer 1

FIG. 34E

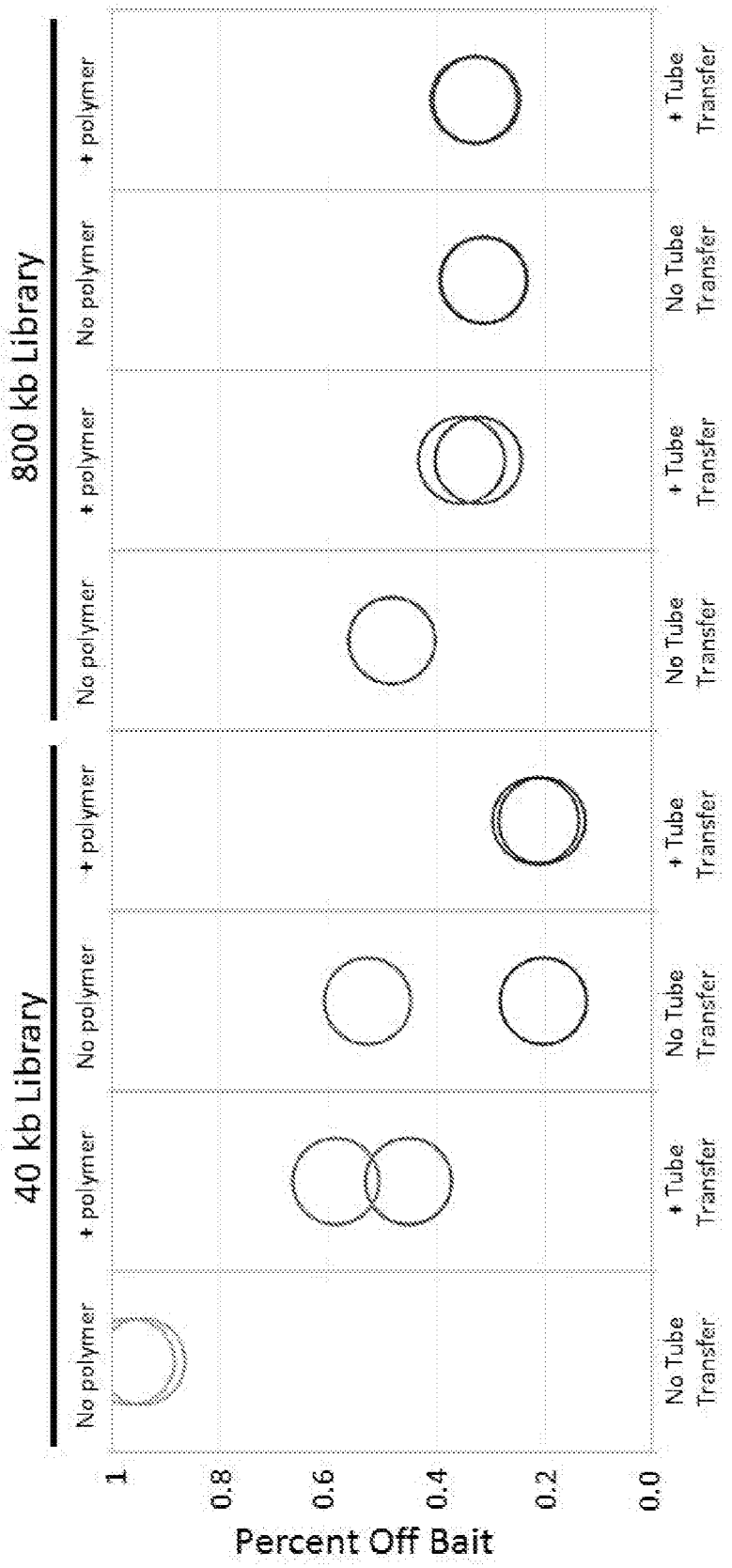

40 kb Library
800 kb Library
No polymer
+ polymer
No polymer
+ polymer
No polymer
+ polymer
No polymer
+ polymer
No Tube Transfer
+ Tube Transfer
No Tube Transfer
+ Tube Transfer
No Tube Transfer
+ Tube Transfer
No Tube Transfer
+ Tube Transfer
1
0.8
0.6
0.4
0.2
0.0
Percent Off Bait

FIG. 35A

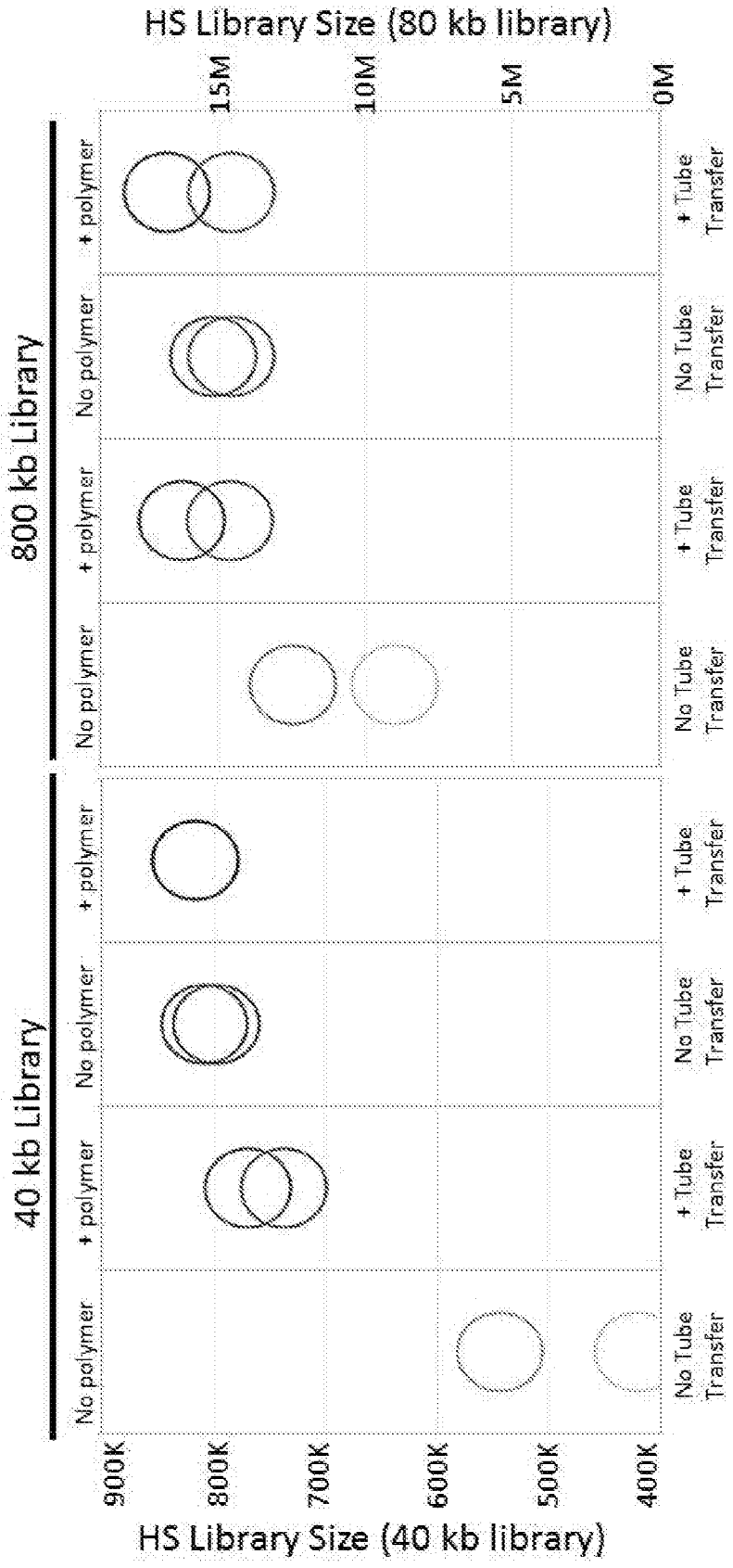

HS Library Size (80 kb library)
15M
10M
5M
0M
800 kb Library
40 kb Library
No polymer
+ polymer
No polymer
+ polymer
No polymer
+ polymer
No polymer
+ polymer
No Tube Transfer
+ Tube Transfer
No Tube Transfer
+ Tube Transfer
No Tube Transfer
+ Tube Transfer
No Tube Transfer
+ Tube Transfer
900K
800K
700K
600K
500K
400K
HS Library Size (40 kb library)

FIG. 35B

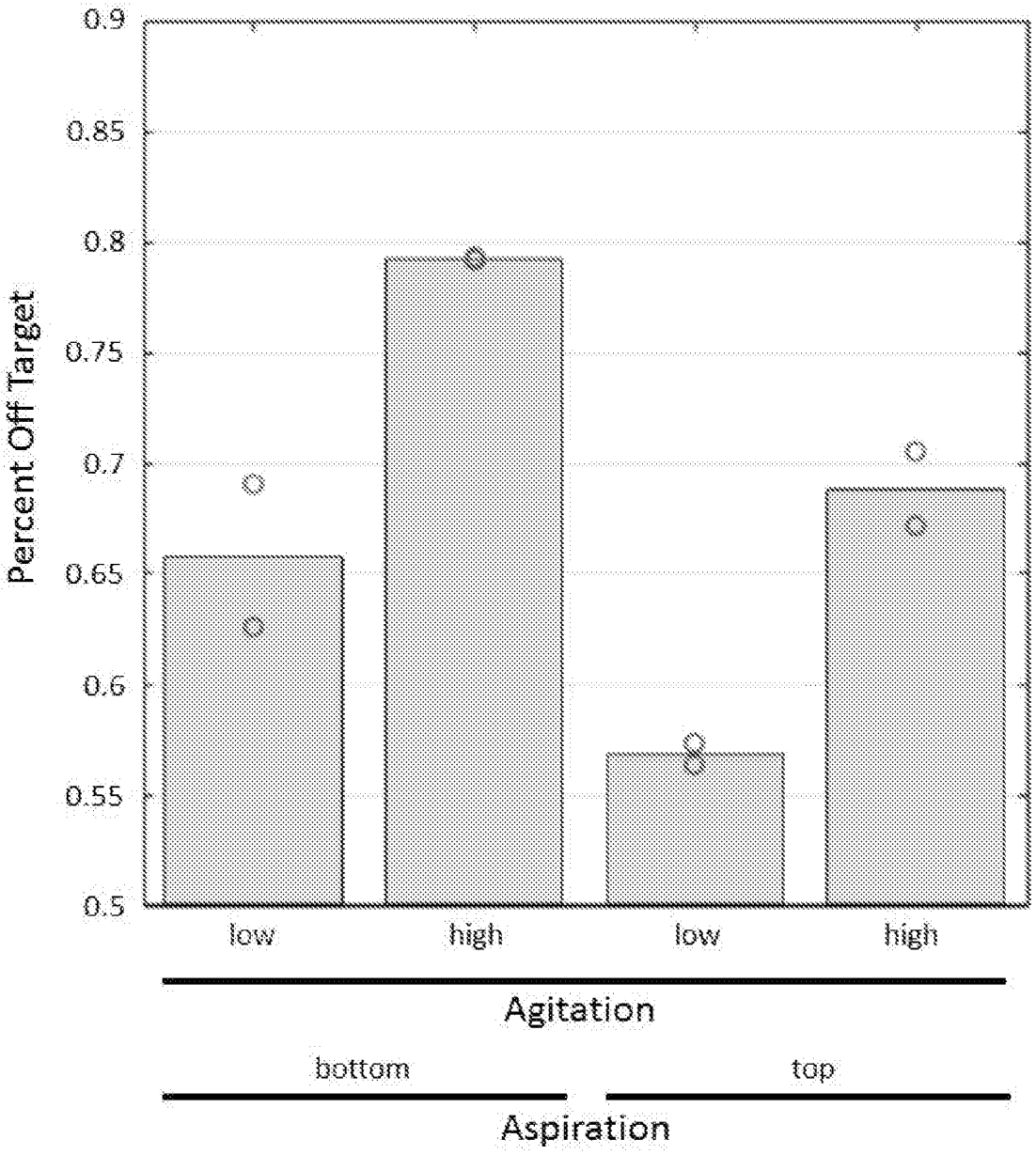

0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
Percent Off Target
low
high
low
high
Agitation
bottom
top
Aspiration

FIG. 36

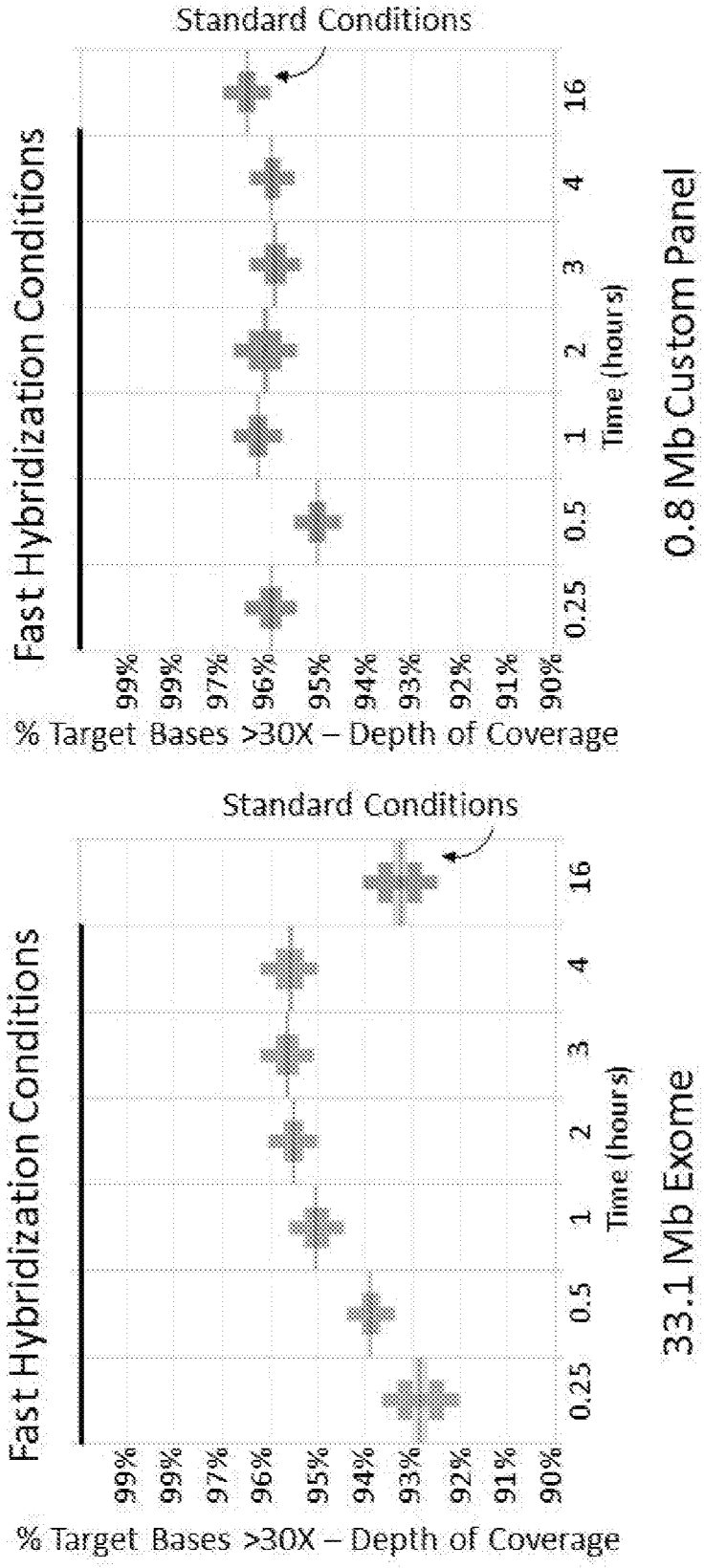

Standard Conditions
Fast Hybridization Conditions
99%
99%
97%
96%
95%
94%
93%
92%
91%
90%
% Target Bases >30X – Depth of Coverage
0.25
0.5
1
2
3
4
16
Time (hours)
0.8 Mb Custom Panel
Standard Conditions
Fast Hybridization Conditions
99%
99%
97%
96%
95%
94%
93%
92%
91%
90%
% Target Bases >30X – Depth of Coverage
0.25
0.5
1
2
3
4
16
Time (hours)
33.1 Mb Exome

FIG. 37A

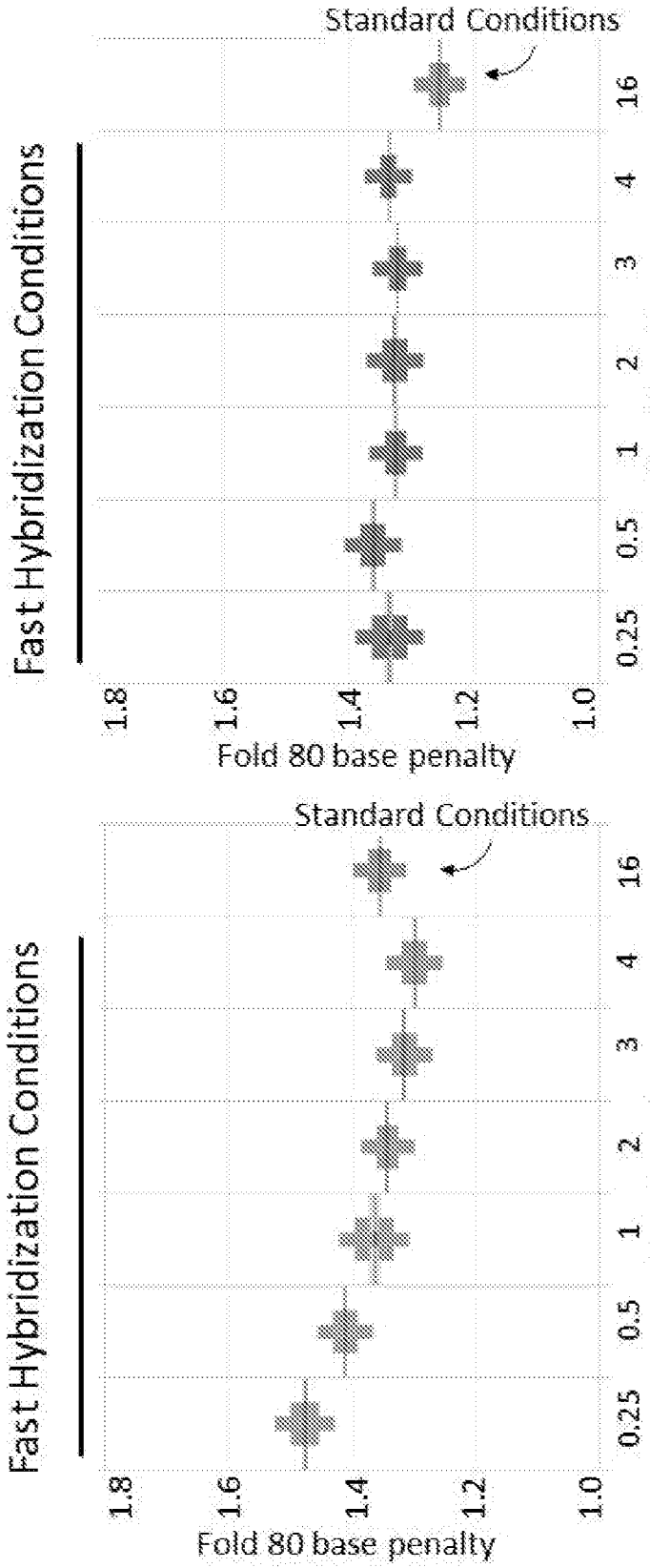

Standard Conditions
Fast Hybridization Conditions
1.8
1.6
1.4
1.2
1.0
Fold 80 base penalty
0.25
0.5
1
2
3
4
16
Time (hours)
0.8 Mb Custom Panel
Standard Conditions
Fast Hybridization Conditions
1.8
1.6
1.4
1.2
1.0
Fold 80 base penalty
0.25
0.5
1
2
3
4
16
Time (hours)
33.1 Mb Exome

FIG. 37B

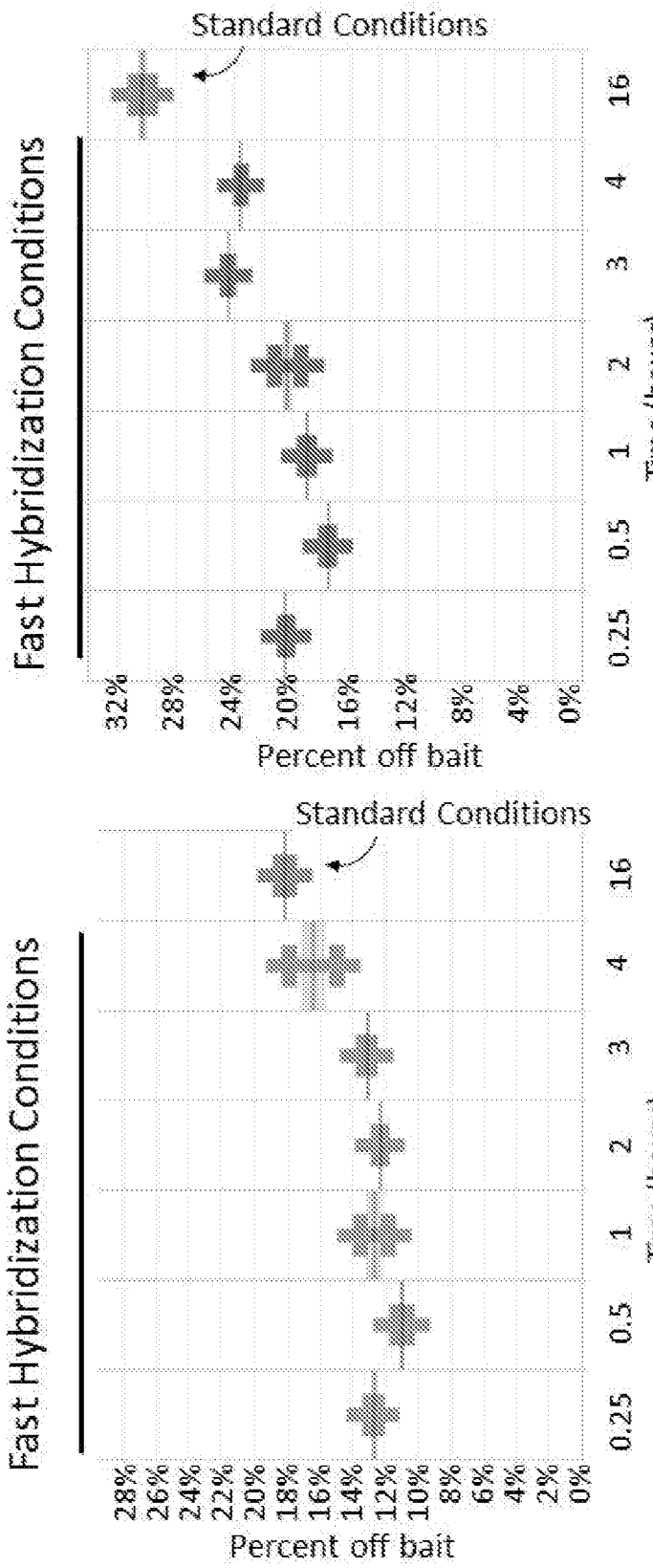

Standard Conditions
Fast Hybridization Conditions
32%
28%
24%
20%
16%
12%
8%
4%
0%
Percent off bait
0.25
0.5
1
2
3
4
16
Time (hours)
0.8 Mb Custom Panel
Standard Conditions
Fast Hybridization Conditions
28%
26%
24%
22%
20%
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
Percent off bait
0.25
0.5
1
2
3
4
16
Time (hours)
33.1 Mb Exome

FIG. 37C

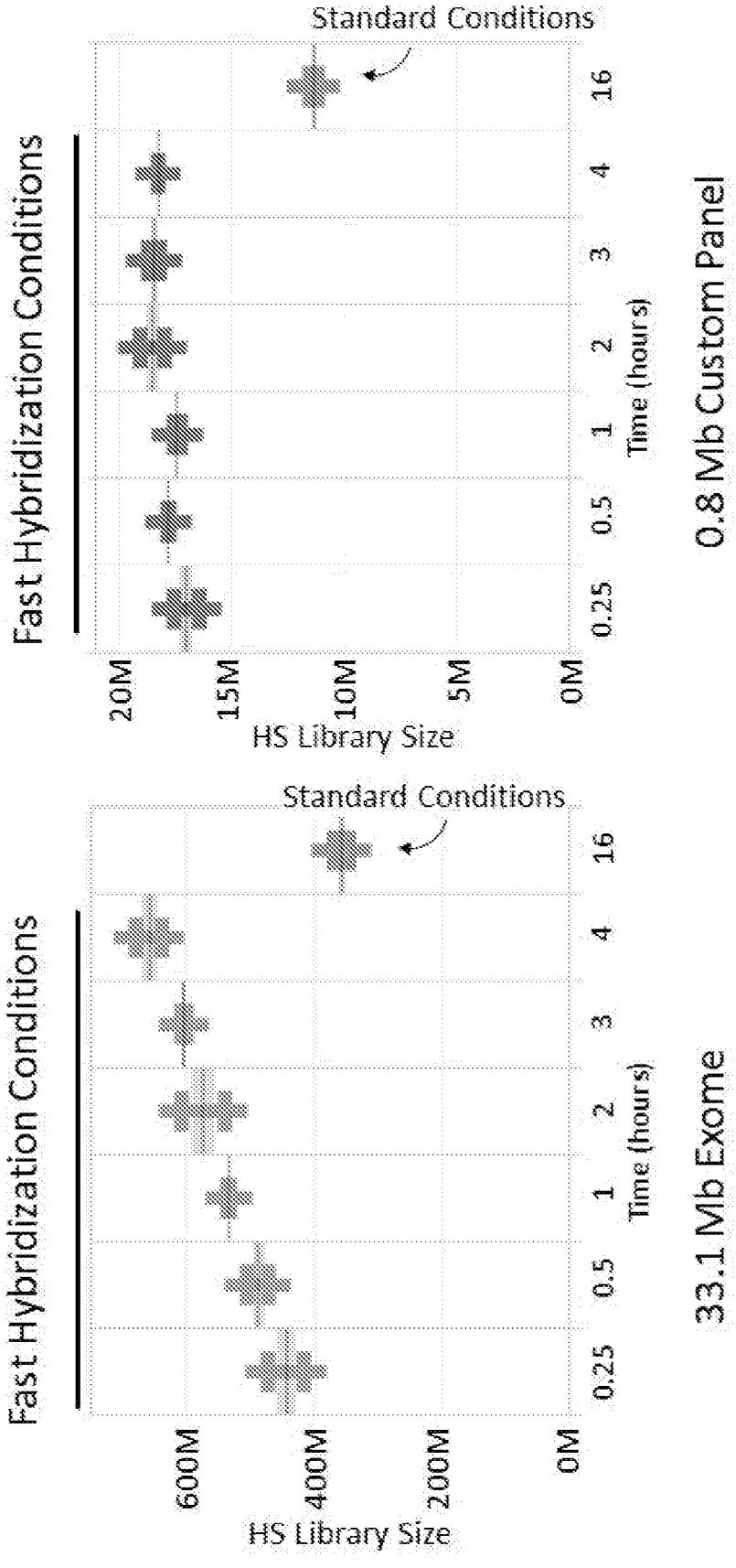

Standard Conditions
Fast Hybridization Conditions
20M
15M
10M
5M
0M
HS Library Size
0.25
0.5
1
2
3
4
16
Time (hours)
0.8 Mb Custom Panel
Standard Conditions
Fast Hybridization Conditions
600M
400M
200M
0M
HS Library Size
0.25
0.5
1
2
3
4
16
Time (hours)
33.1 Mb Exome

FIG. 37D

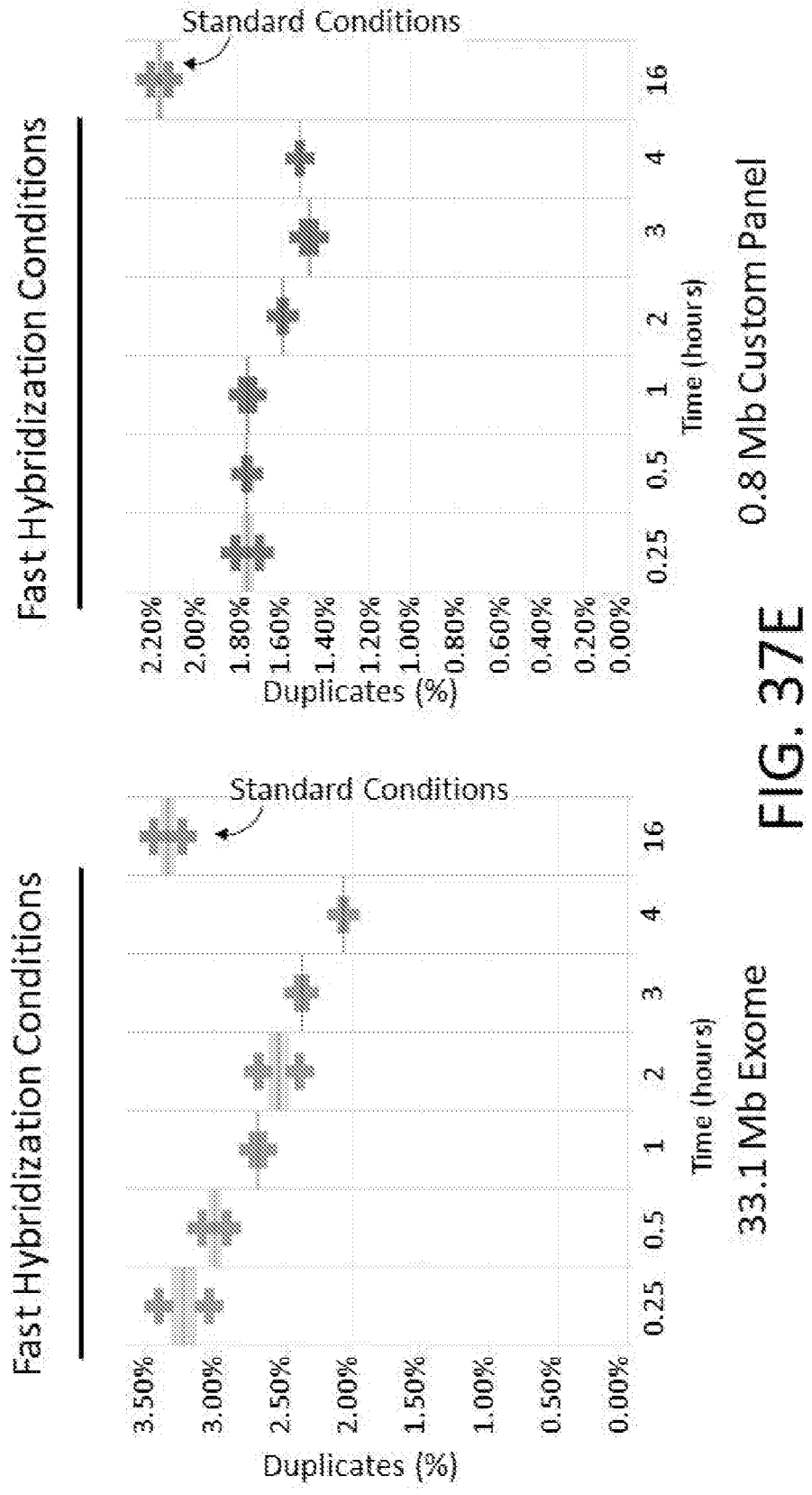

Fast Hybridization Conditions
Standard Conditions
Duplicates (%)
3.50%
3.00%
2.50%
2.00%
1.50%
1.00%
0.50%
0.00%
0.25
0.5
1
2
3
4
16
Time (hours)
33.1 Mb Exome
Fast Hybridization Conditions
Standard Conditions
Duplicates (%)
2.20%
2.00%
1.80%
1.60%
1.40%
1.20%
1.00%
0.80%
0.60%
0.40%
0.20%
0.00%
Time (hours)
0.8 Mb Custom Panel

FIG. 37E

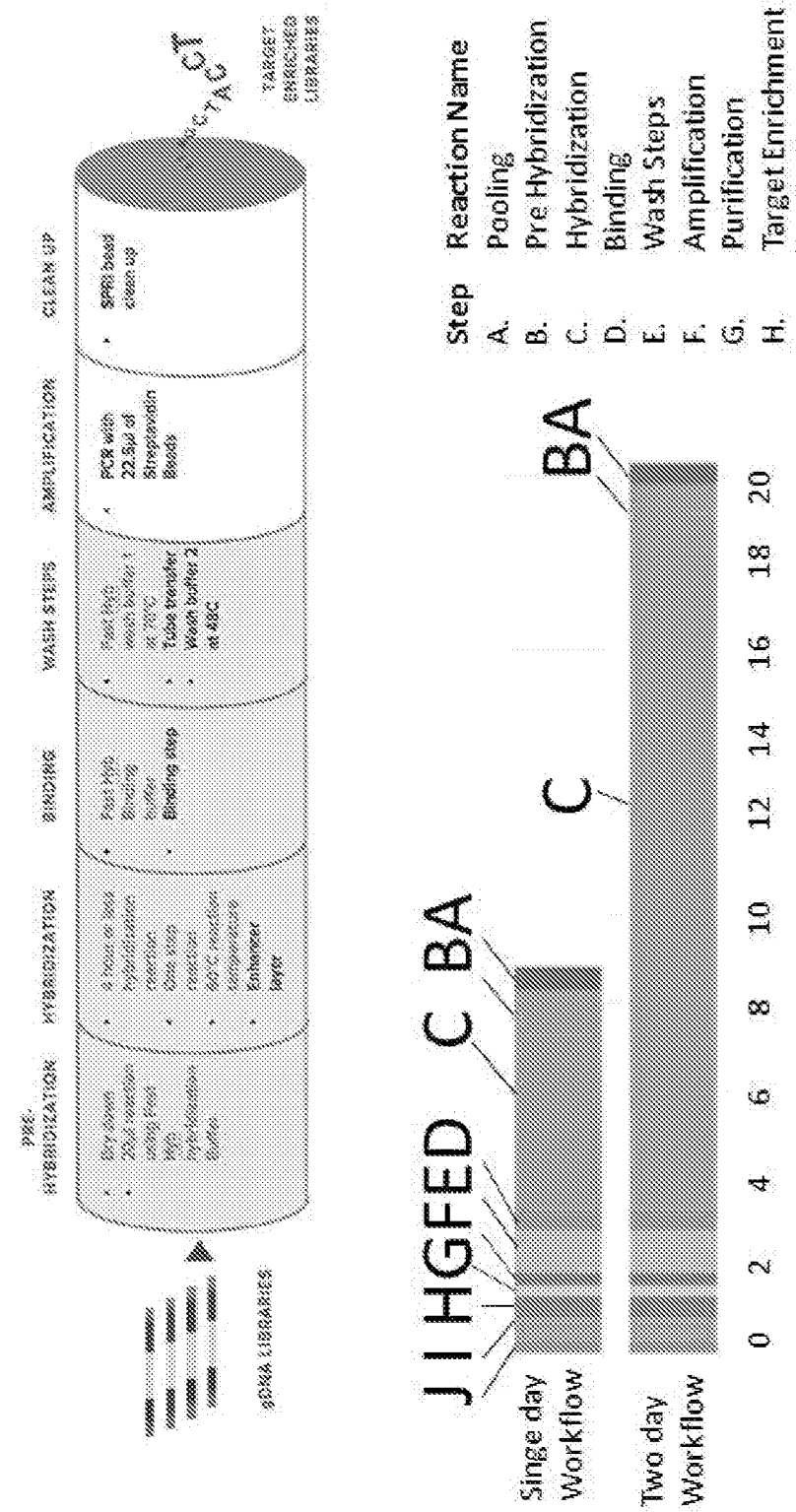

PRE-HYBRIDIZATION
HYBRIDIZATION
BINDING
WASH STEPS
AMPLIFICATION
CLEAN UP
PCR with 22.5µl of Streptavidin Beads
SPRI bead clean up
gDNA LIBRARIES
TARGET ENRICHED LIBRARIES
Step
Reaction Name
A. Pooling
B. Pre Hybridization
C. Hybridization
D. Binding
E. Wash Steps
F. Amplification
G. Purification
H. Target Enrichment QC
I. Pooling 2
J. NGS Prep
J I H G F E D C B A
C
B A
Singe day Workflow
Two day Workflow
0 2 4 6 8 10 12 14 16 18 20
Time (Hours)

FIG. 38

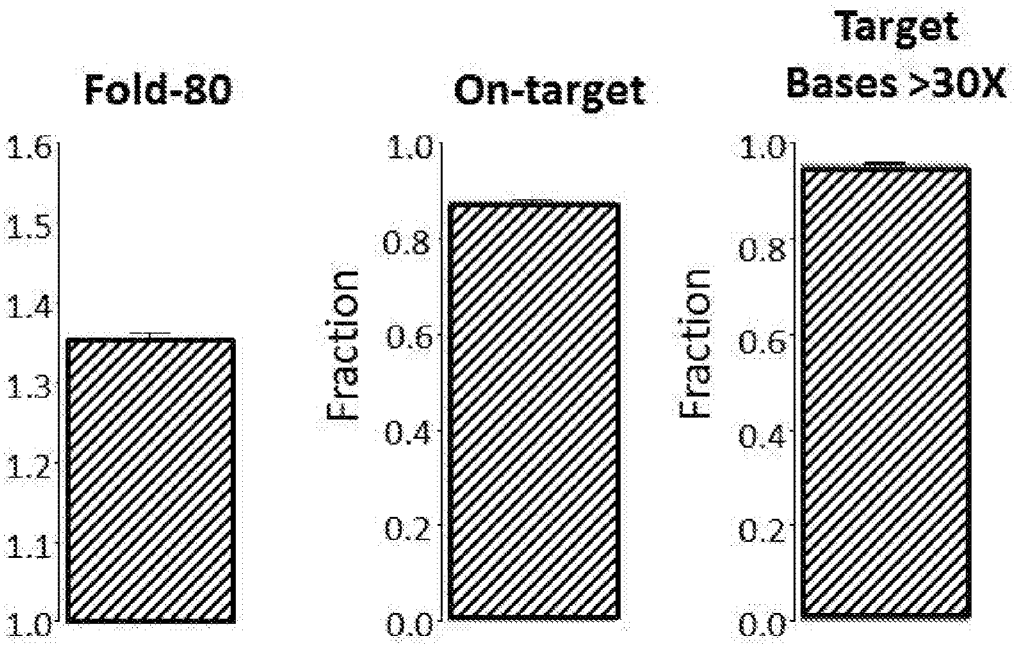


FIG. 39A

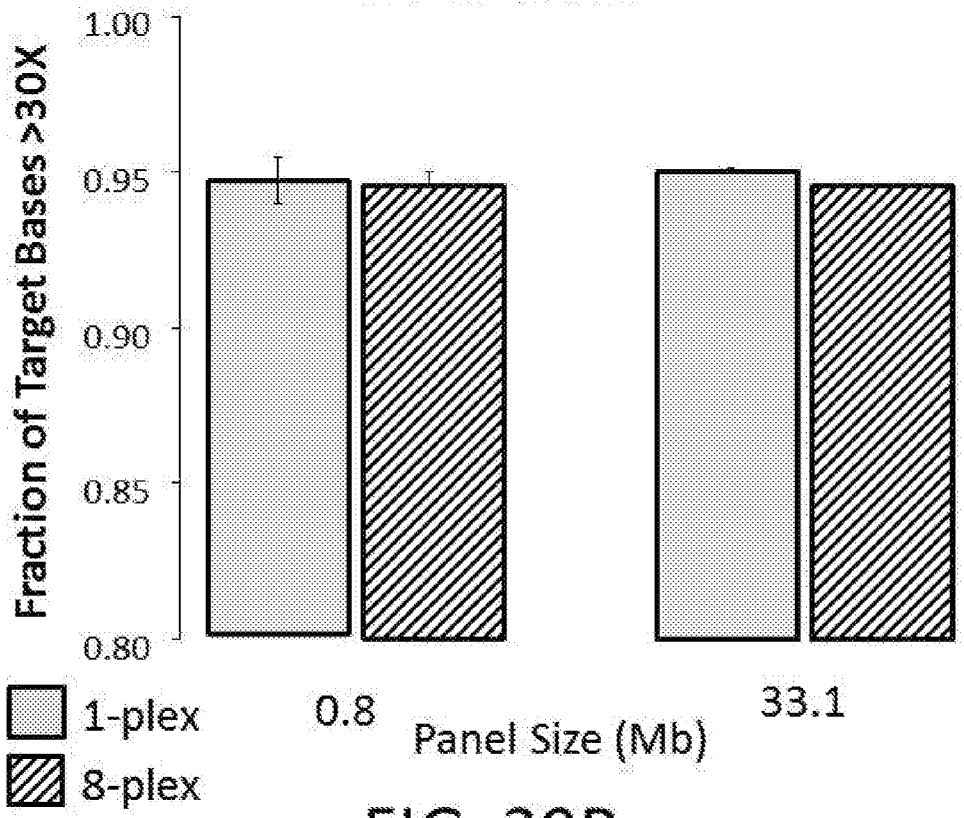


FIG. 39B

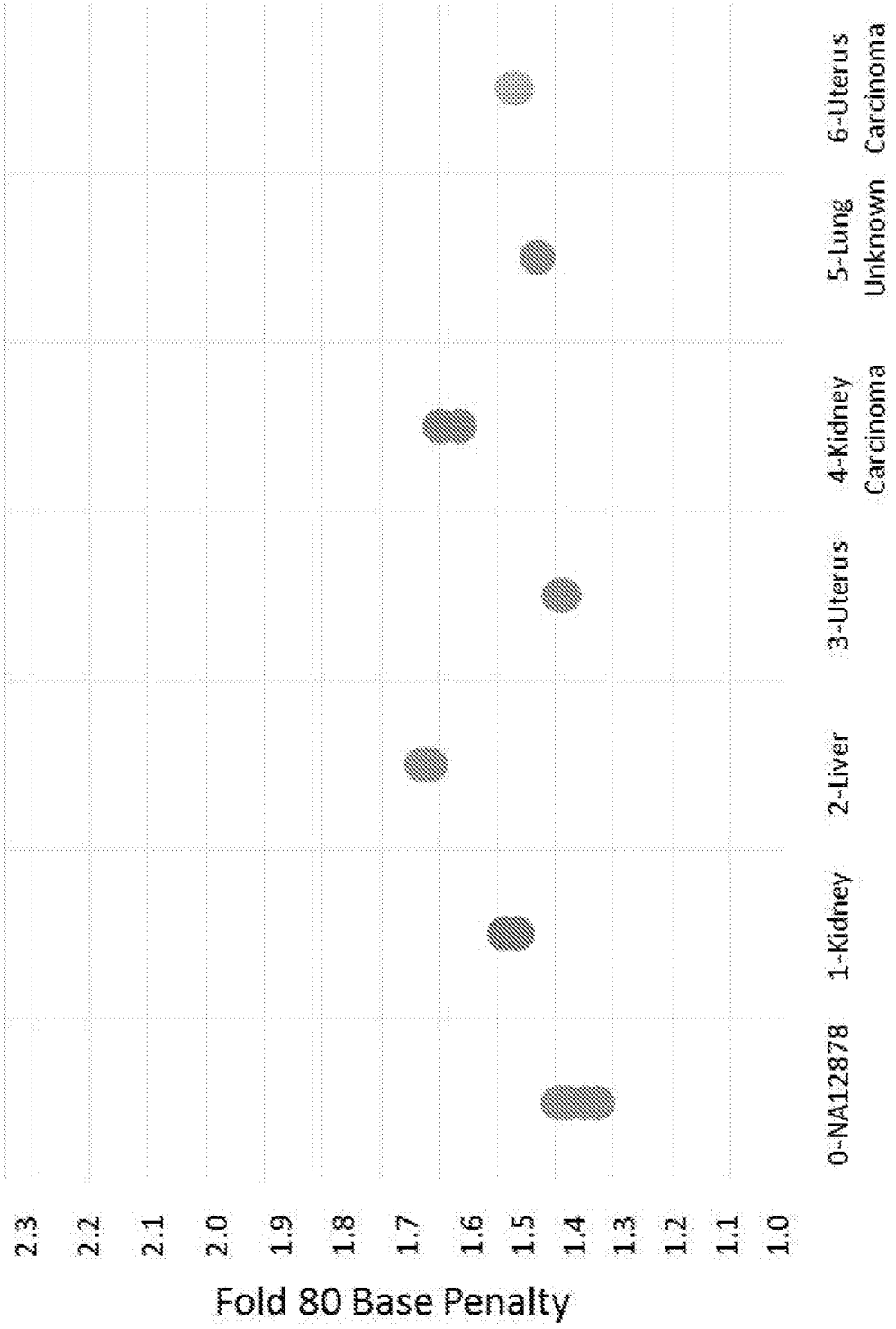

0-NA12878
1-Kidney
2-Liver
3-Uterus
4-Kidney Carcinoma
5-Lung Unknown
6-Uterus Carcinoma
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
Fold 80 Base Penalty

FIG. 39C

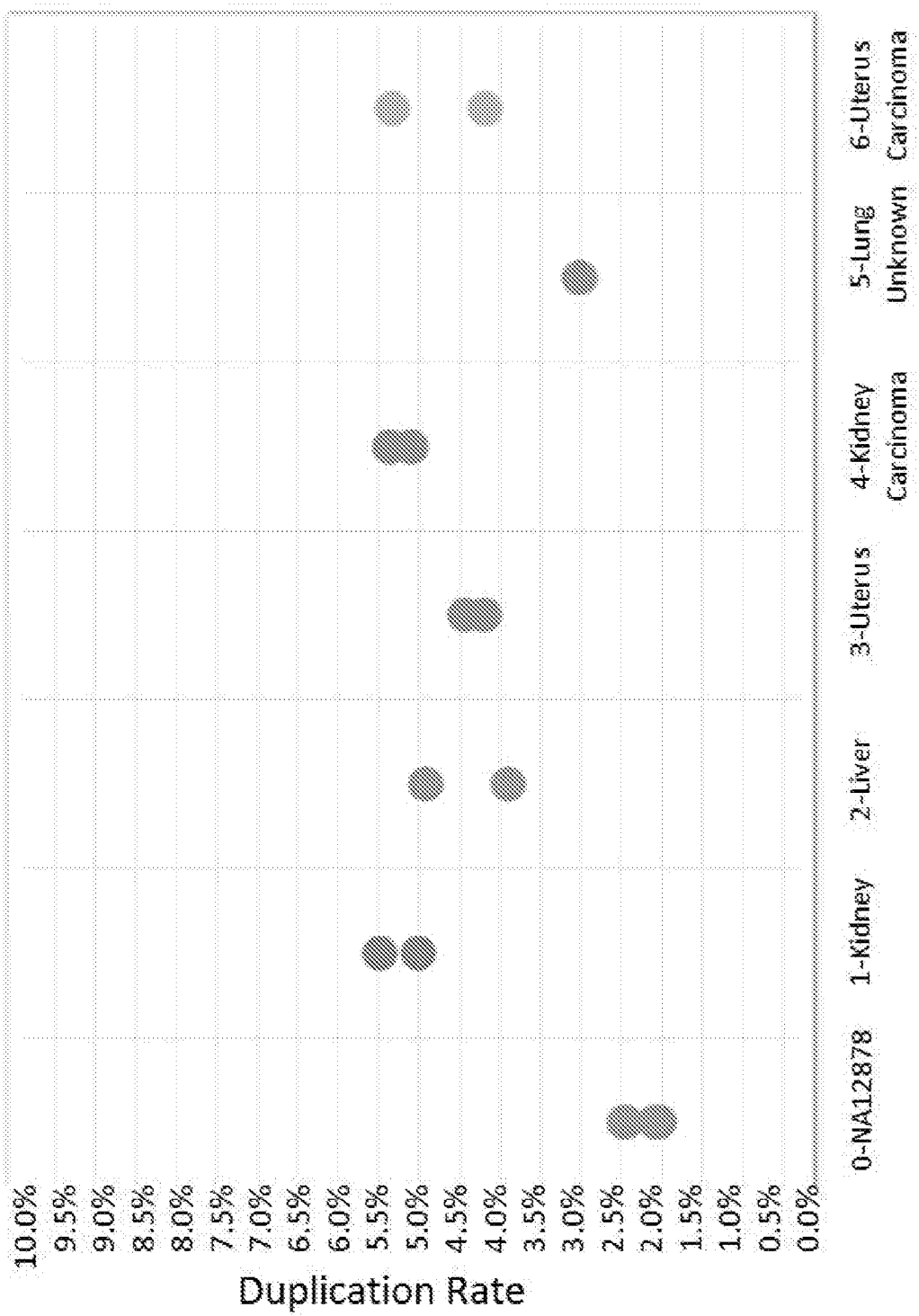

10.0%
9.5%
9.0%
8.5%
8.0%
7.5%
7.0%
6.5%
6.0%
5.5%
5.0%
4.5%
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
Duplication Rate
0-NA12878
1-Kidney
2-Liver
3-Uterus
4-Kidney Carcinoma
5-Lung Unknown
6-Uterus Carcinoma

FIG. 39D

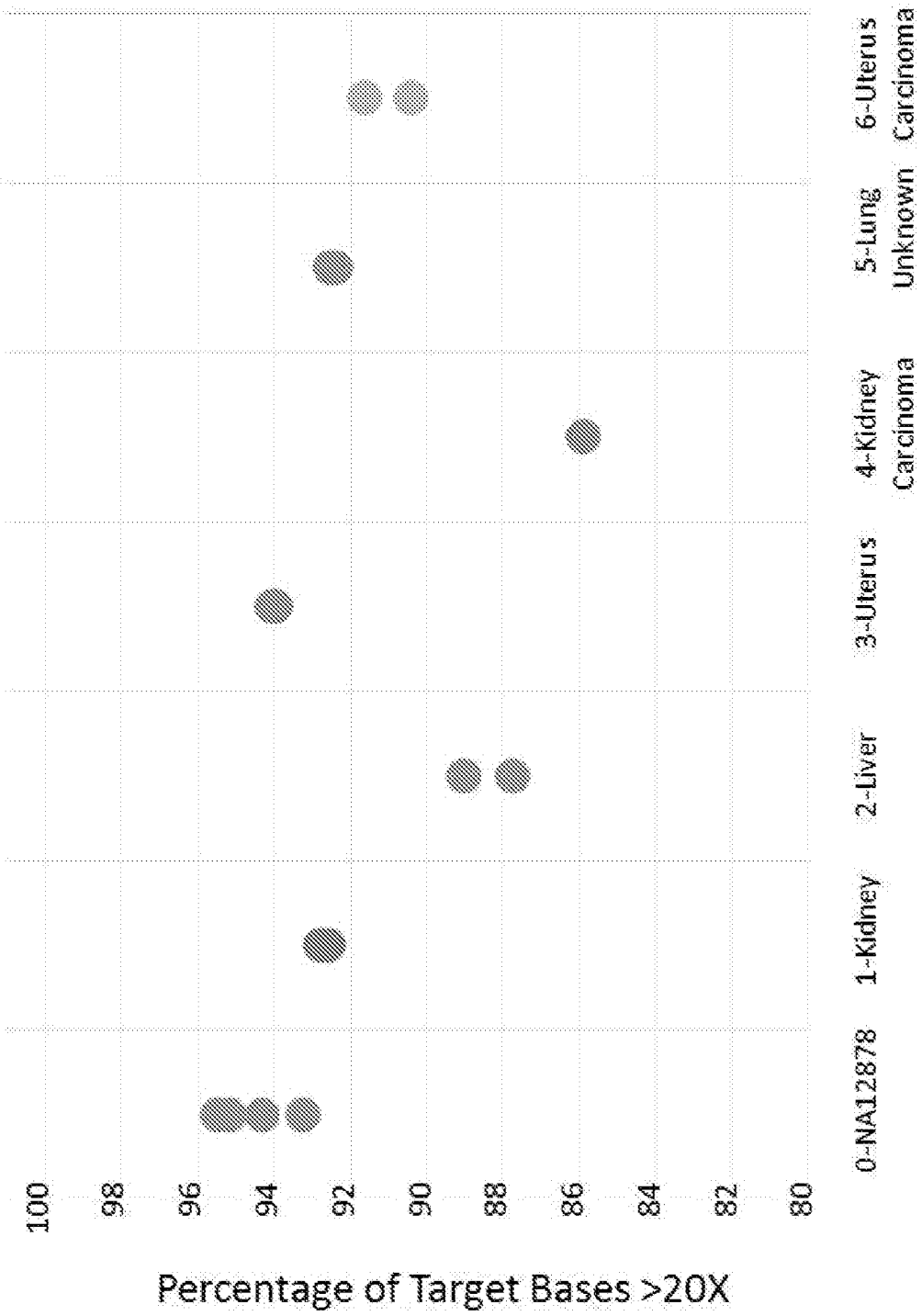

0-NA12878
1-Kidney
2-Liver
3-Uterus
4-Kidney Carcinoma
5-Lung Unknown
6-Uterus Carcinoma
100
98
96
94
92
90
88
86
84
82
80
Percentage of Target Bases >20X

FIG. 39E

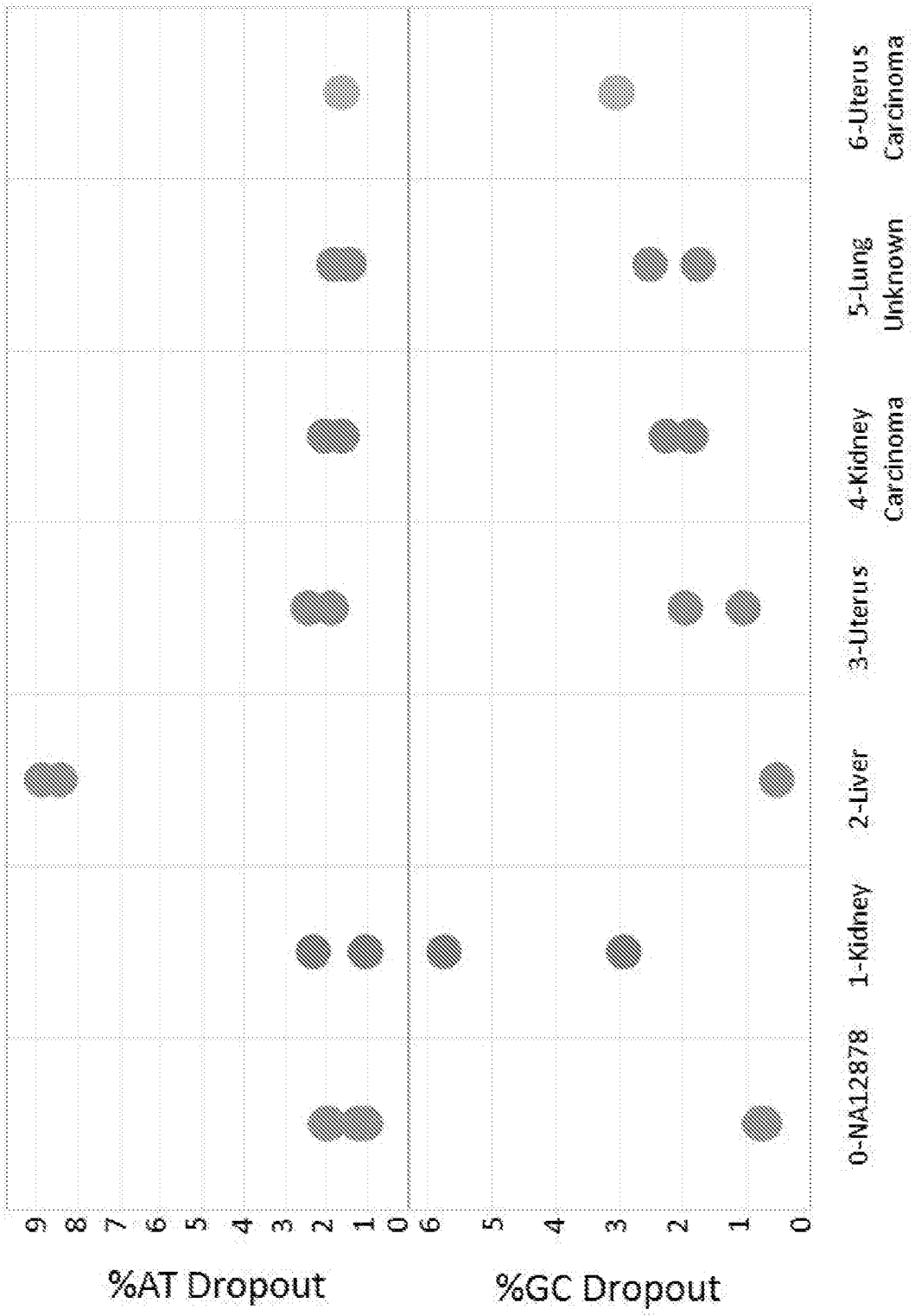

0-NA12878
1-Kidney
2-Liver
3-Uterus
4-Kidney Carcinoma
5-Lung Unknown
6-Uterus Carcinoma
9 8 7 6 5 4 3 2 1 0
%AT Dropout
6 5 4 3 2 1 0
%GC Dropout

FIG. 39F

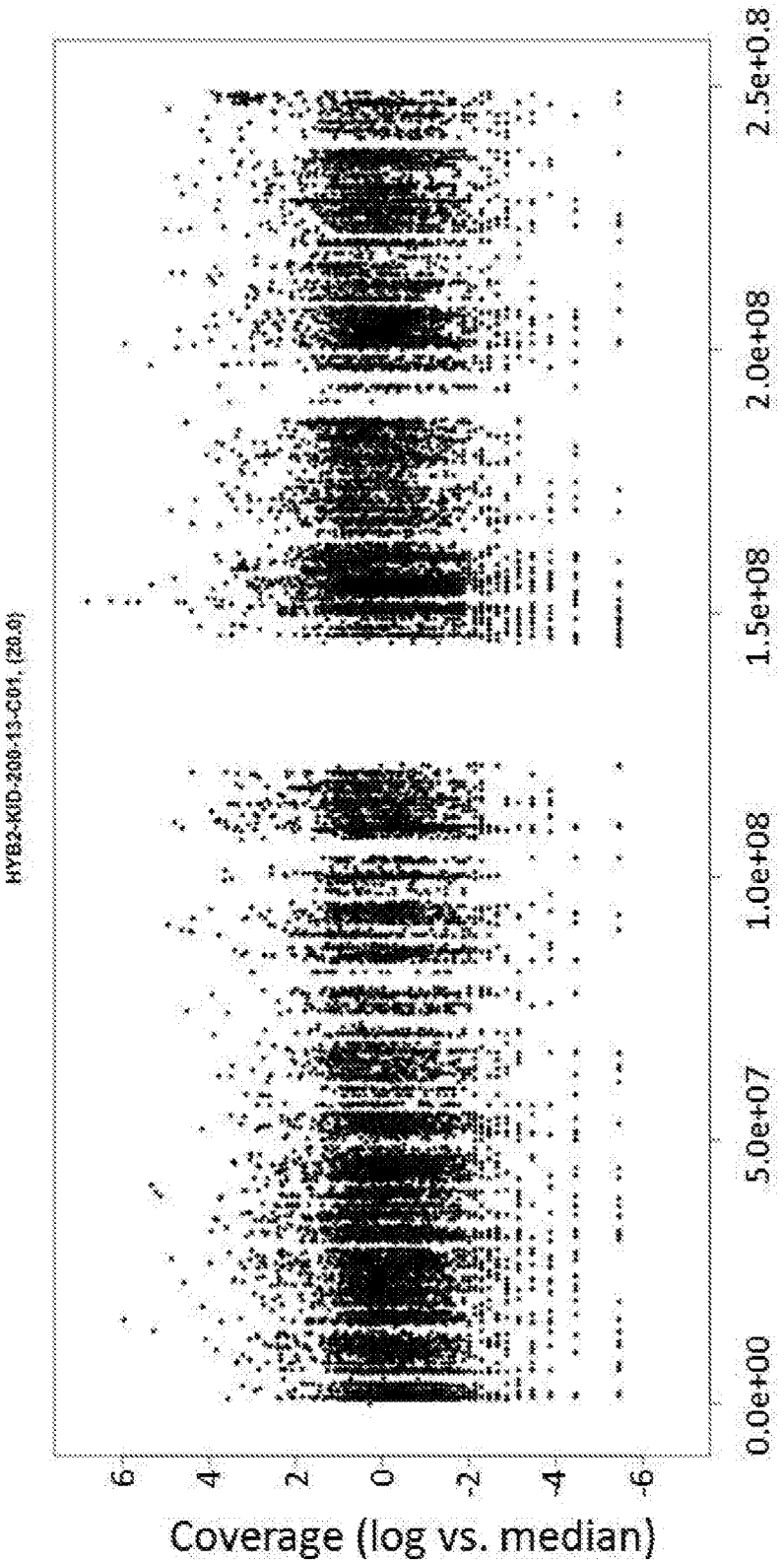

HYB2-KID-208-13-C01, (20.0)
6
4
2
0
-2
-4
-6
Coverage (log vs. median)
0.0e+00
5.0e+07
1.0e+08
1.5e+08
2.0e+08
2.5e+0.8
Chr 1 position

FIG. 39G

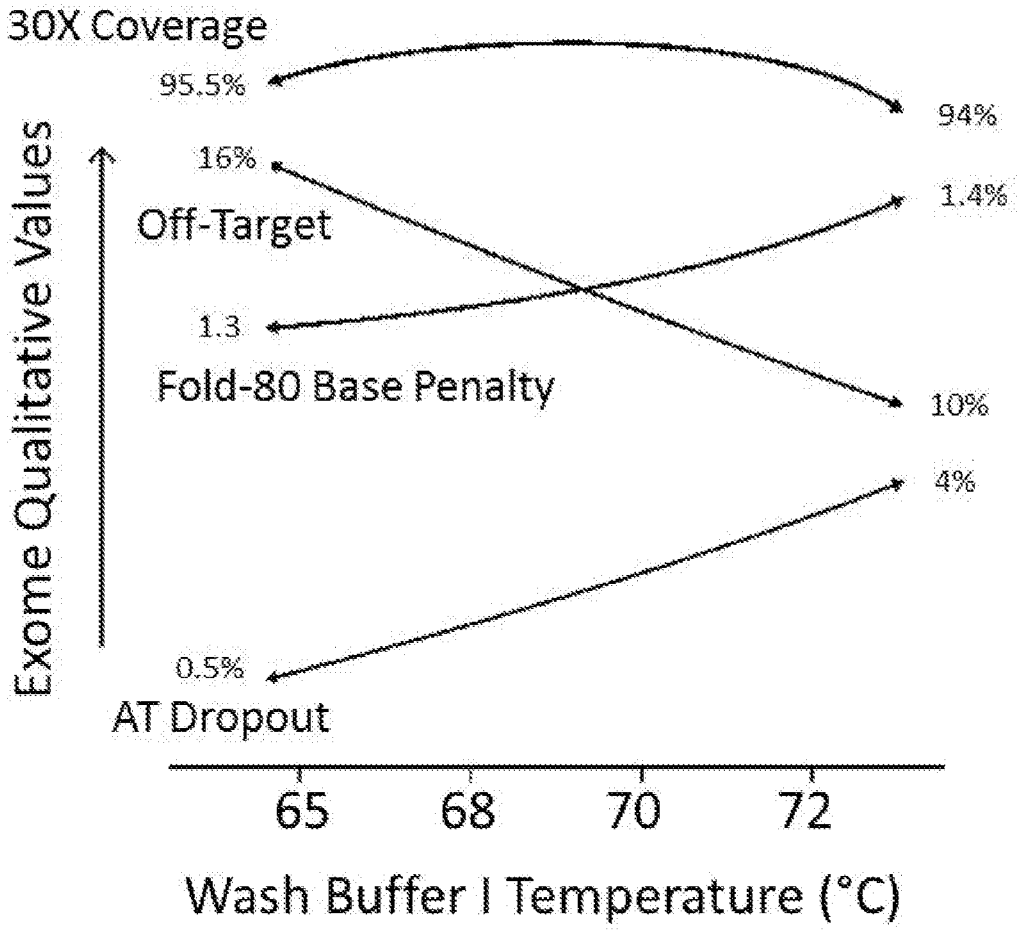

30X Coverage
95.5%
94%
16%
1.4%
Off-Target
1.3
Fold-80 Base Penalty
10%
4%
0.5%
AT Dropout
Exome Qualitative Values
65
68
70
72
Wash Buffer I Temperature (°C)

FIG. 40

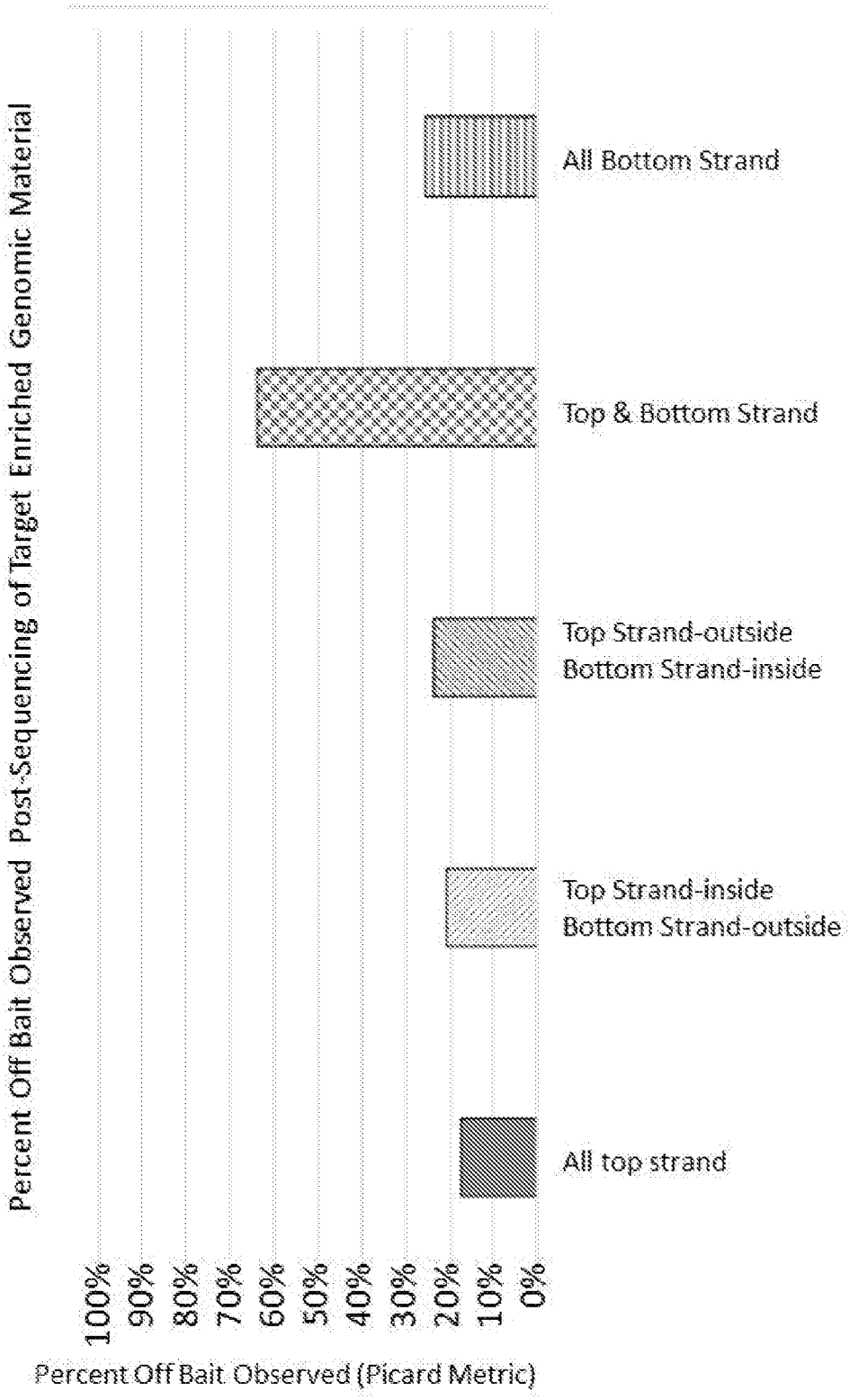

Percent Off Bait Observed Post-Sequencing of Target Enriched Genomic Material
All Bottom Strand
Top & Bottom Strand
Top Strand-outside
Bottom Strand-inside
Top Strand-inside
Bottom Strand-outside
All top strand
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Off Bait Observed (Picard Metric)

FIG. 41

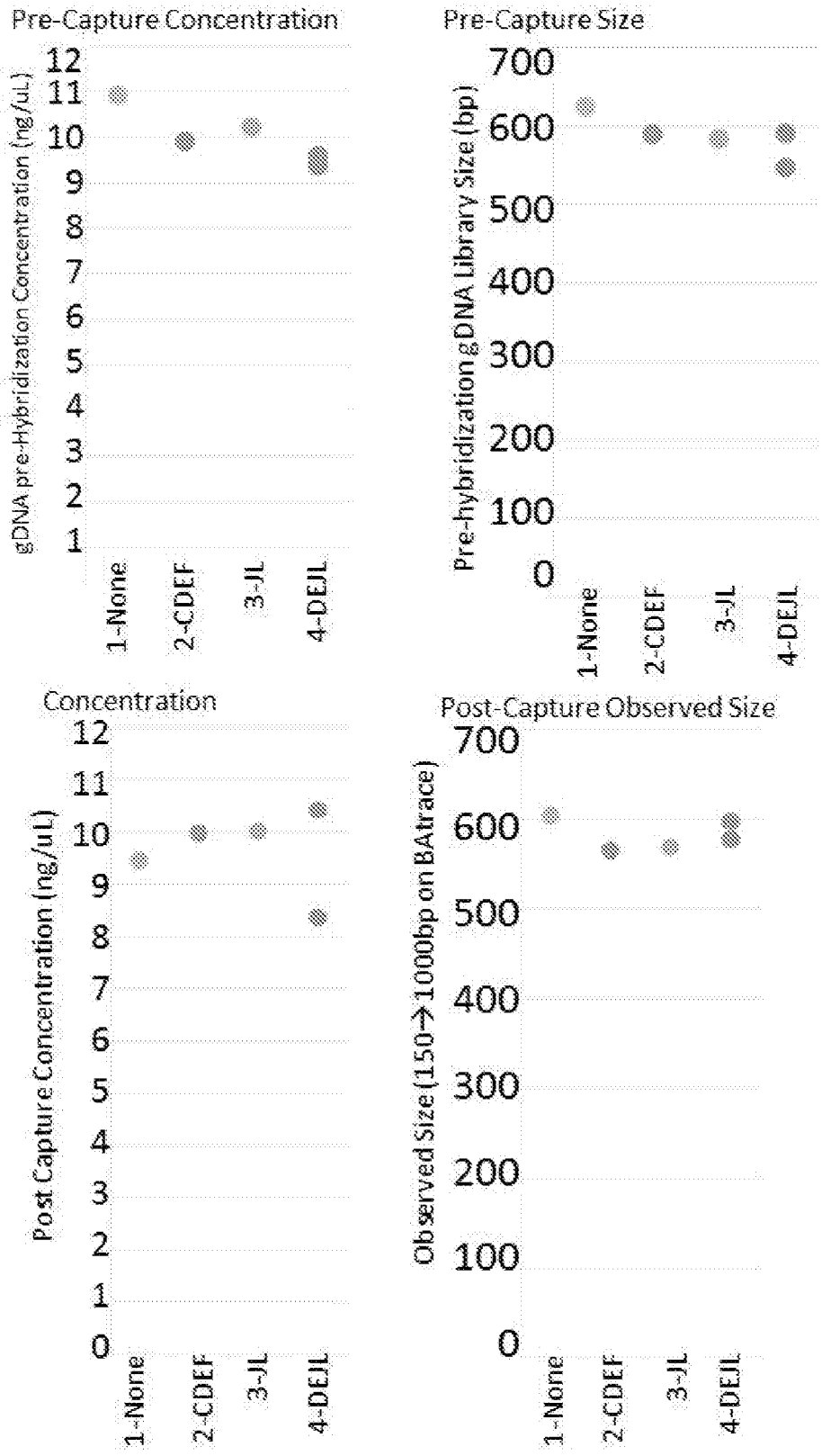

Pre-Capture Concentration
gDNA pre-Hybridization Concentration (ng/uL)
12
11
10
9
8
7
6
5
4
3
2
1
1-None
2-CDEF
3-JL
4-DEJL
Pre-Capture Size
Pre-hybridization gDNA Library Size (bp)
700
600
500
400
300
200
100
0
1-None
2-CDEF
3-JL
4-DEJL
Concentration
Post Capture Concentration (ng/uL)
12
11
10
9
8
7
6
5
4
3
2
1
0
1-None
2-CDEF
3-JL
4-DEJL
Post-Capture Observed Size
Observed Size (150→1000bp on BAtrace)
700
600
500
400
300
200
100
0
1-None
2-CDEF
3-JL
4-DEJL

FIG. 42A

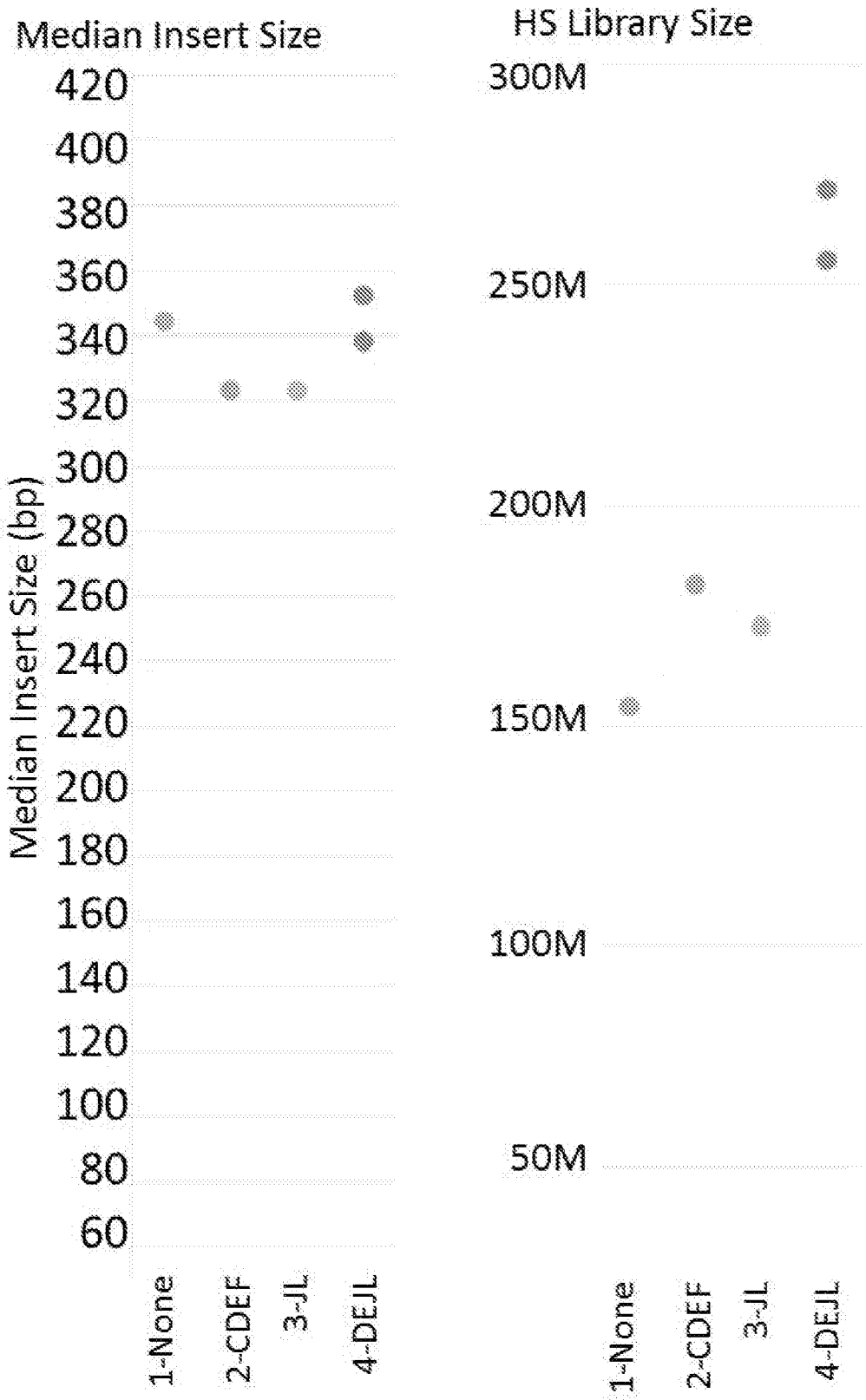

Median Insert Size
Median Insert Size (bp)
420
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
1-None
2-CDEF
3-JL
4-DEJL
HS Library Size
300M
250M
200M
150M
100M
50M
1-None
2-CDEF
3-JL
4-DEJL

FIG. 42B

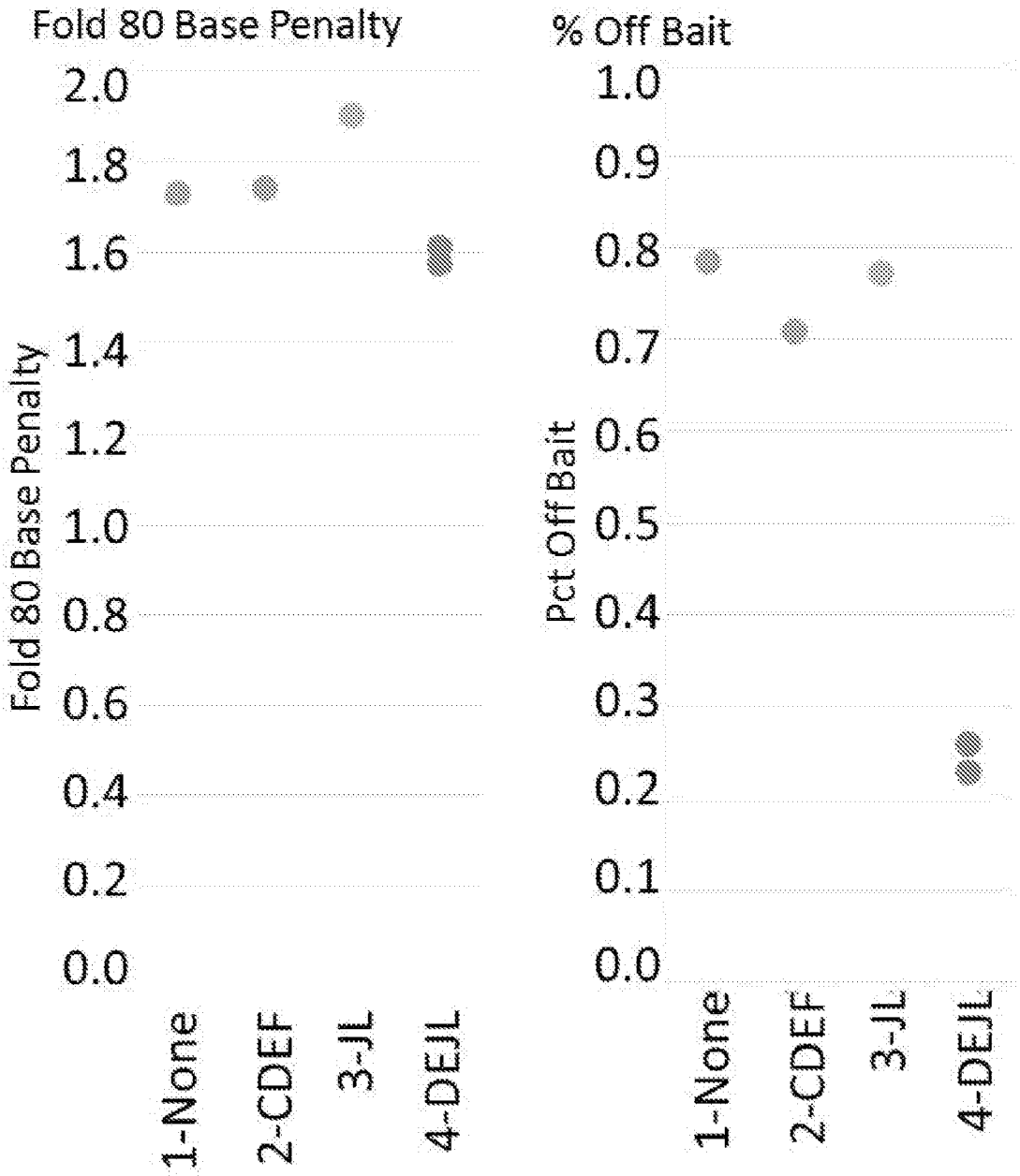

Fold 80 Base Penalty
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Fold 80 Base Penalty
1-None
2-CDEF
3-JL
4-DEJL
% Off Bait
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Pct Off Bait
1-None
2-CDEF
3-JL
4-DEJL

FIG. 42C

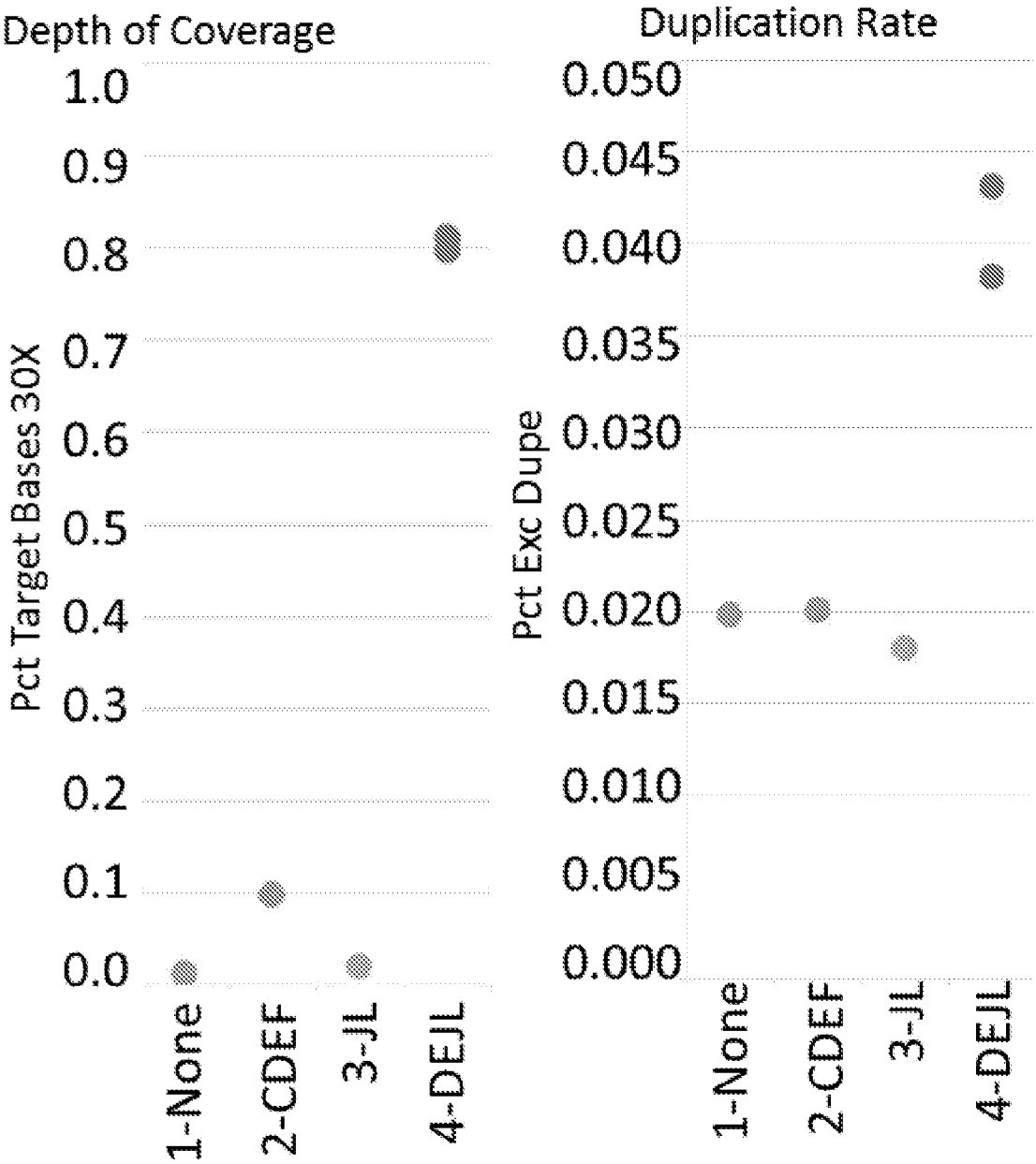

Depth of Coverage
Pct Target Bases 30X
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1-None
2-CDEF
3-JL
4-DEJL
Duplication Rate
Pct Exc Dupe
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
1-None
2-CDEF
3-JL
4-DEJL

FIG. 42D

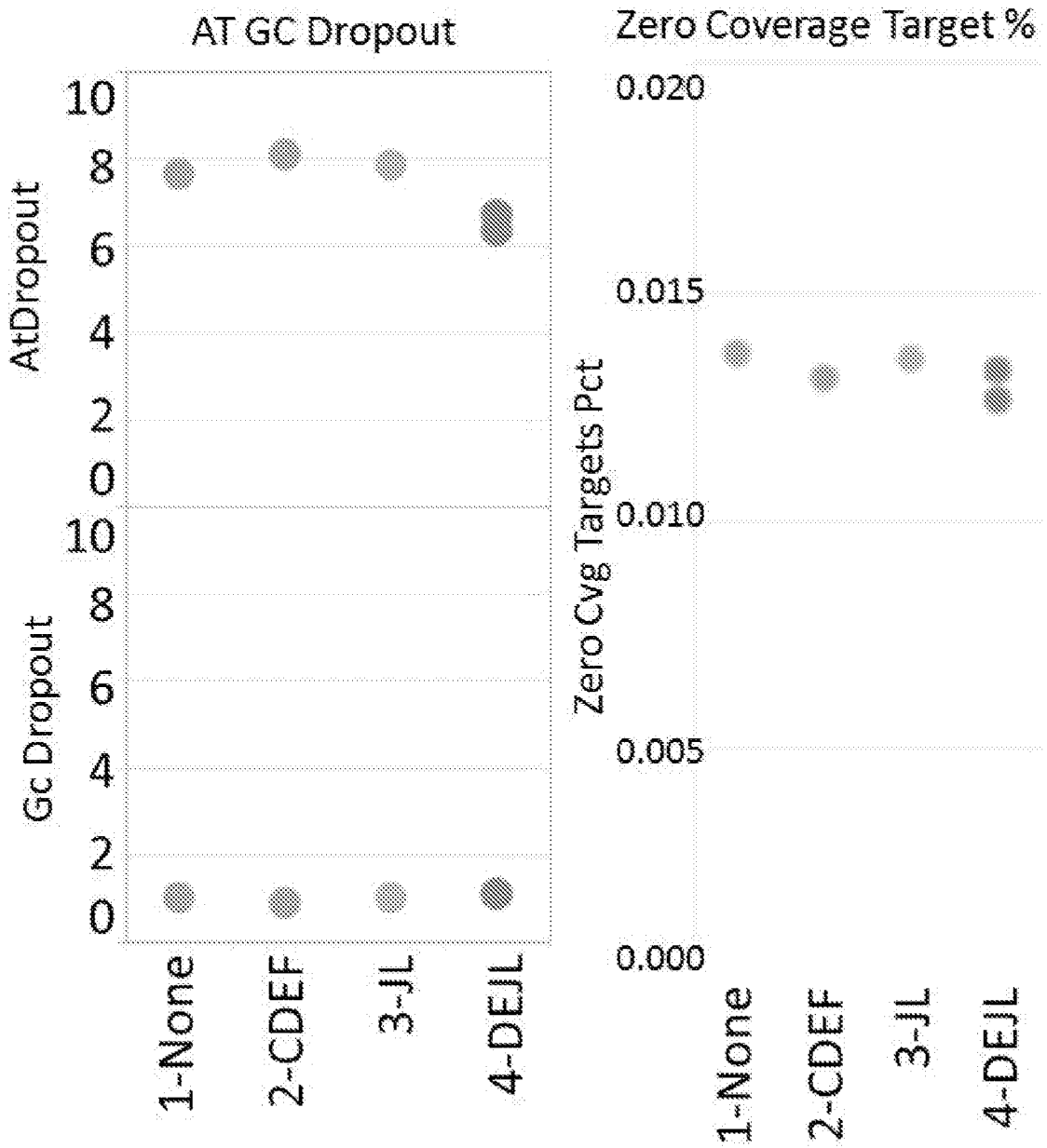

AT GC Dropout
AtDropout
Gc Dropout
10
8
6
4
2
0
1-None
2-CDEF
3-JL
4-DEJL
Zero Coverage Target %
Zero Cvg Targets Pct
0.020
0.015
0.010
0.005
0.000

FIG. 42E

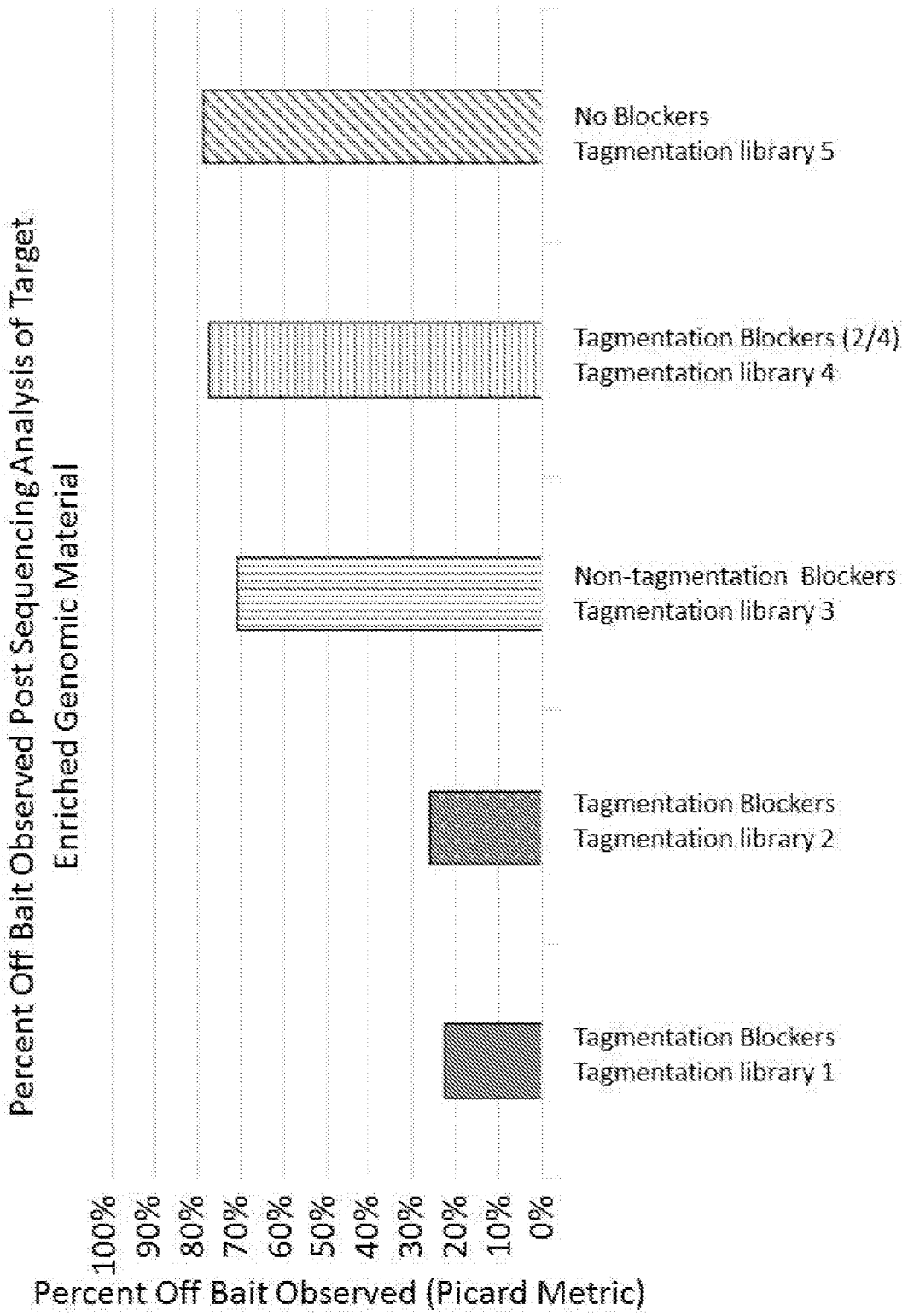

No Blockers
Tagmentation library 5
Tagmentation Blockers (2/4)
Tagmentation library 4
Non-tagmentation Blockers
Tagmentation library 3
Tagmentation Blockers
Tagmentation library 2
Tagmentation Blockers
Tagmentation library 1
Percent Off Bait Observed Post Sequencing Analysis of Target Enriched Genomic Material
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Off Bait Observed (Picard Metric)

FIG. 43

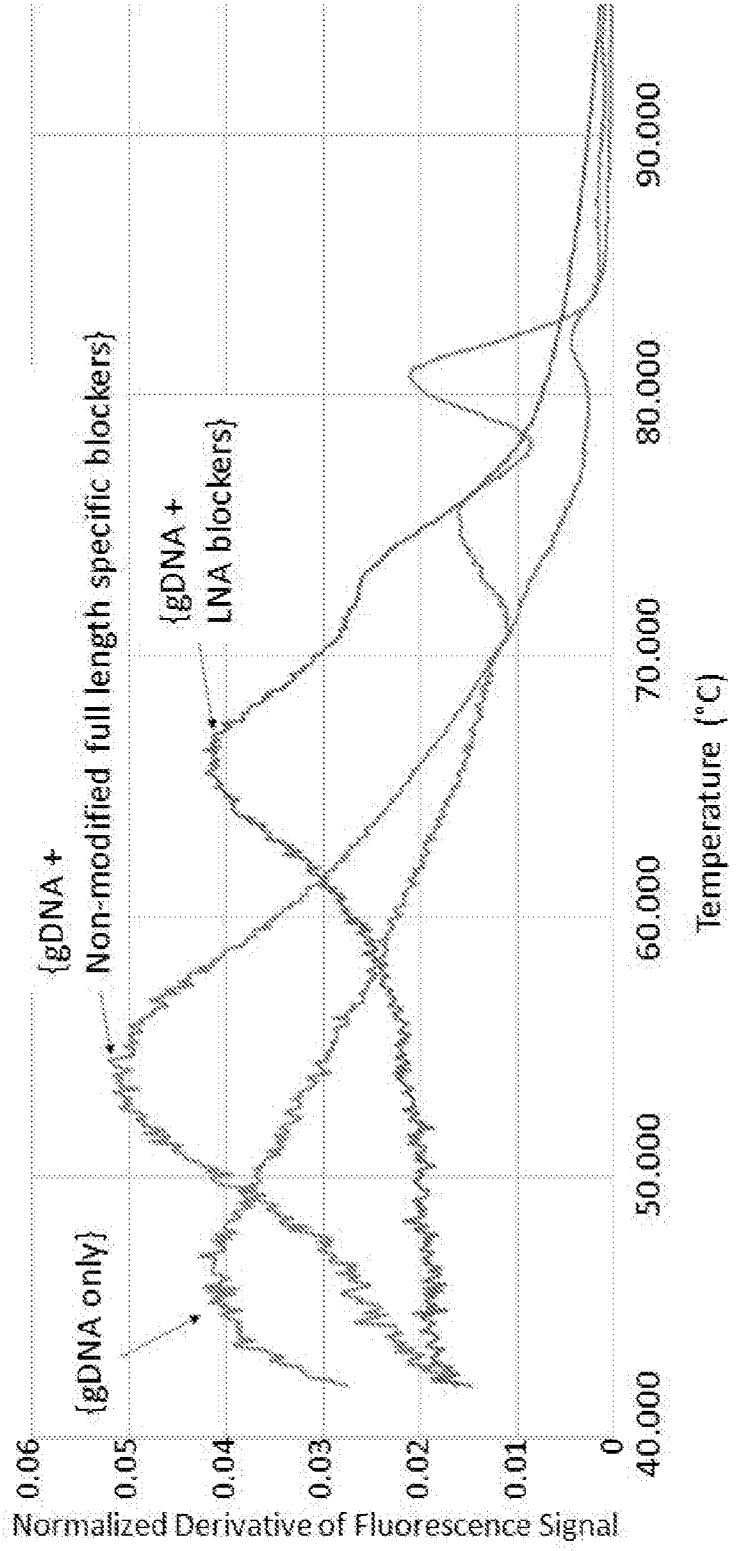

Melt Curves in the Presence or Absence of Blockers
{gDNA +
Non-modified full length specific blockers}
{gDNA +
LNA blockers}
{gDNA only}
0.06
0.05
0.04
0.03
0.02
0.01
0
Normalized Derivative of Fluorescence Signal
40.000
50.000
60.000
70.000
80.000
90.000
Temperature (°C)

FIG. 44