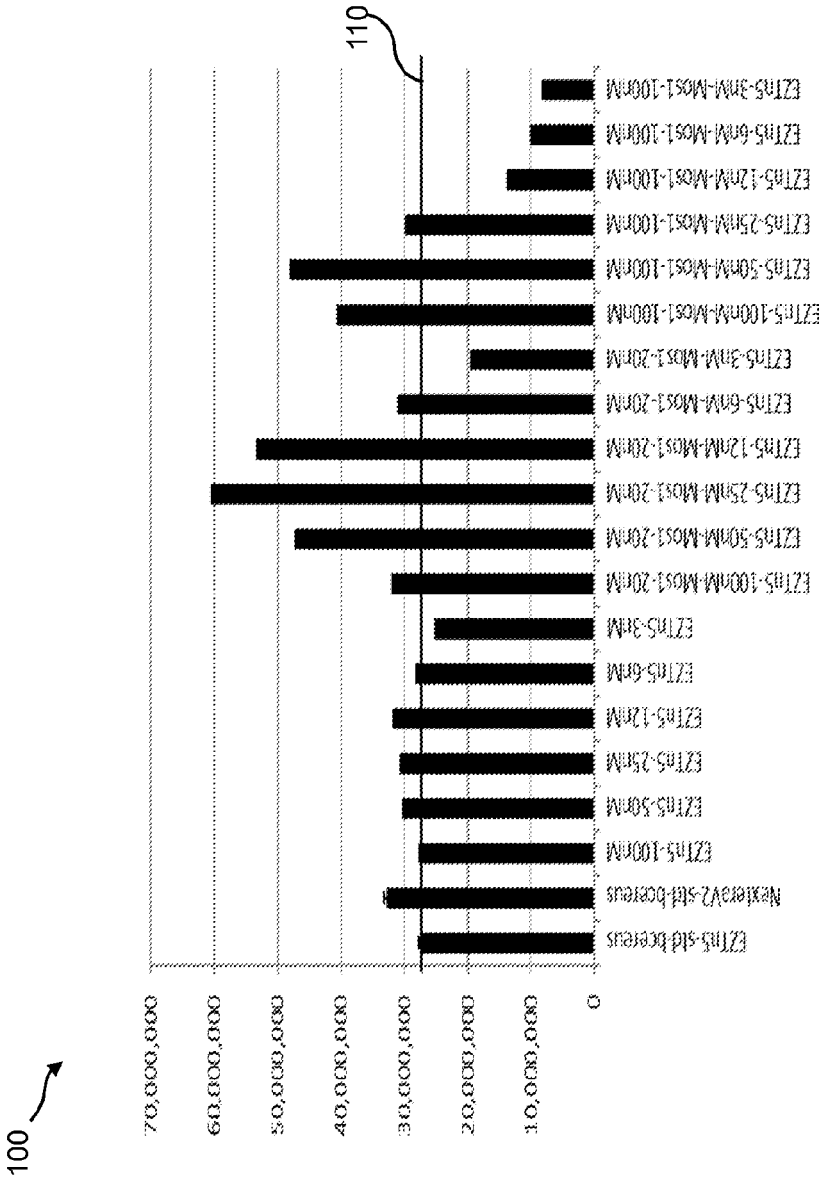

100
110
70,000,000
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
0
EZTn5-sld bcerevs
NextEraV2-sld-bcerevs
EZTn5-100NM
EZTn5-50NM
EZTn5-25NM
EZTn5-12NM
EZTn5-6NM
EZTn5-3NM
EZTn5-100nM-Mos1-20NM
EZTn5-50NM-Mos1-20NM
EZTn5-25NM-Mos1-20NM
EZTn5-12NM-Mos1-20NM
EZTn5-6NM-Mos1-20NM
EZTn5-3NM-Mos1-20NM
EZTn5-100nM-Mos1-100NM
EZTn5-50NM-Mos1-100NM
EZTn5-25NM-Mos1-100NM
EZTn5-12NM-Mos1-100NM
EZTn5-6NM-Mos1-100NM
EZTn5-3NM-Mos1-100NM

Figure 1

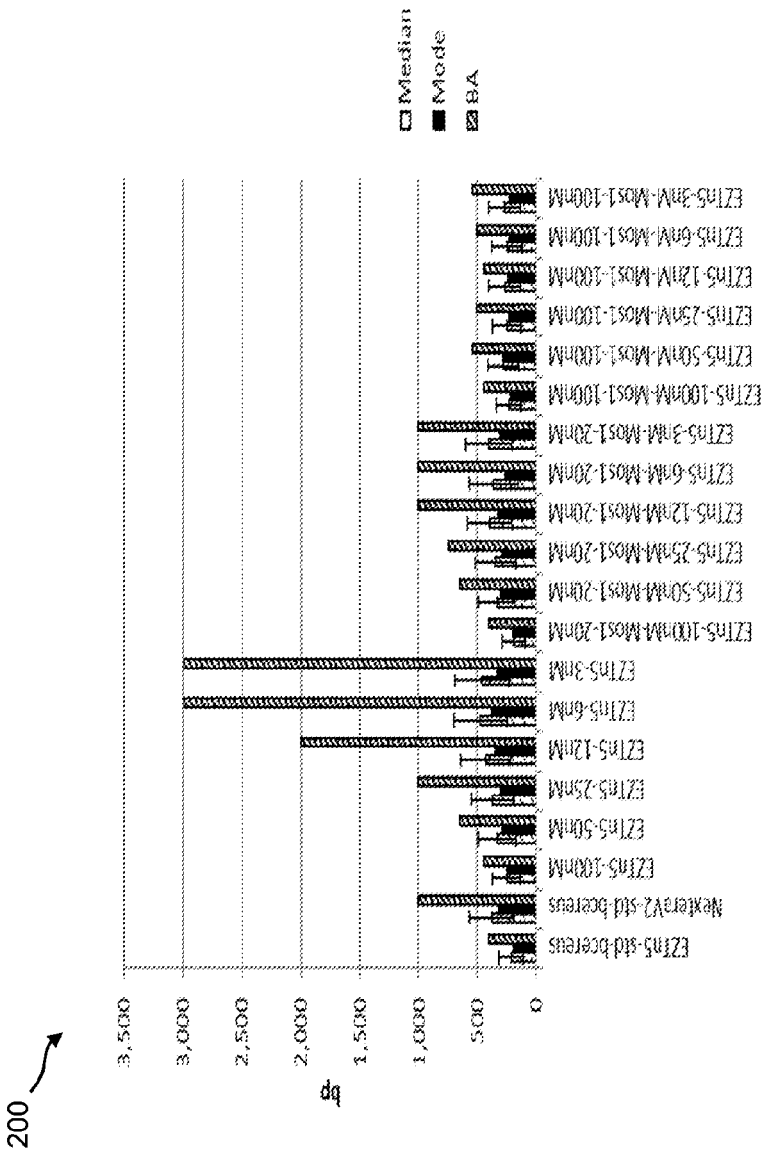

200
Median
Mode
BA
bp
3,500
3,000
2,500
2,000
1,500
1,000
500
0
EZTn5-std bcereus
NextEraV2-std bcereus
EZTn5-10OnM
EZTn5-50nM
EZTn5-25nM
EZTn5-12nM
EZTn5-6nM
EZTn5-3nM
EZTn5-100nM-Mos1-20nM
EZTn5-50nM-Mos1-20nM
EZTn5-25nM-Mos1-20nM
EZTn5-12nM-Mos1-20nM
EZTn5-6nM-Mos1-20nM
EZTn5-3nM-Mos1-20nM
EZTn5-100nM-Mos1-100nM
EZTn5-50nM-Mos1-100nM
EZTn5-25nM-Mos1-100nM
EZTn5-12nM-Mos1-100nM
EZTn5-6nM-Mos1-100nM
EZTn5-3nM-Mos1-100nM

Figure 2

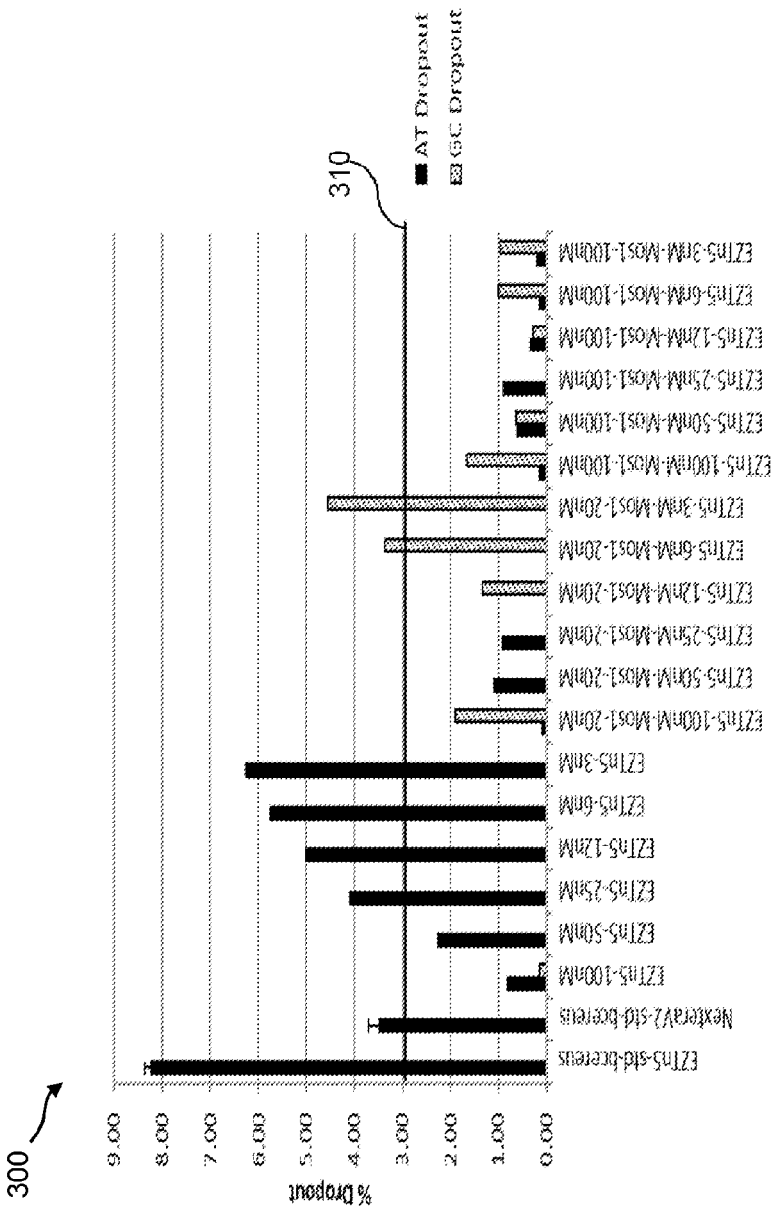

300
310
AT Dropout
GC Dropout
% Dropout
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
EZTn5-std-bcereus
NexteraV2-std-bcereus
EZTn5-100nM
EZTn5-50nM
EZTn5-25nM
EZTn5-12nM
EZTn5-6nM
EZTn5-3nM
EZTn5-100nM-Mos1-20nM
EZTn5-50nM-Mos1-20nM
EZTn5-25nM-Mos1-20nM
EZTn5-12nM-Mos1-20nM
EZTn5-6nM-Mos1-20nM
EZTn5-3nM-Mos1-20nM
EZTn5-100nM-Mos1-100nM
EZTn5-50nM-Mos1-100nM
EZTn5-25nM-Mos1-100nM
EZTn5-12nM-Mos1-100nM
EZTn5-6nM-Mos1-100nM
EZTn5-3nM-Mos1-100nM

Figure 3

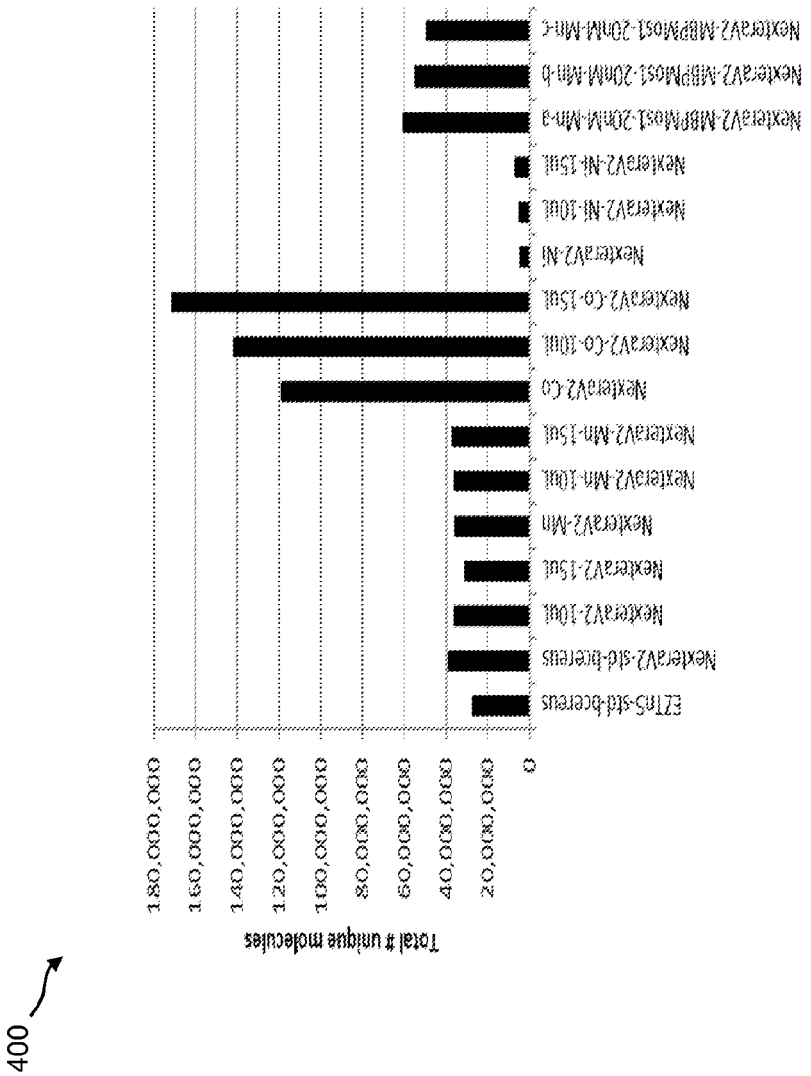

400
Total # unique molecules
180,000,000
160,000,000
140,000,000
120,000,000
100,000,000
80,000,000
60,000,000
40,000,000
20,000,000
0
EZTn5-std-bcereus
NexteraV2-std-bcereus
NexteraV2-10ul
NexteraV2-15ul
NexteraV2-Mn
NexteraV2-Mn-10ul
NexteraV2-Mn-15ul
NexteraV2-Co
NexteraV2-Co-10ul
NexteraV2-Co-15ul
NexteraV2-Ni
NexteraV2-Ni-10ul
NexteraV2-Ni-15ul
NexteraV2-MBPMo51-20nM-Mn-a
NexteraV2-MBPMo51-20nM-Mn-b
NexteraV2-MBPMo51-20nM-Mn-c

Figure 4

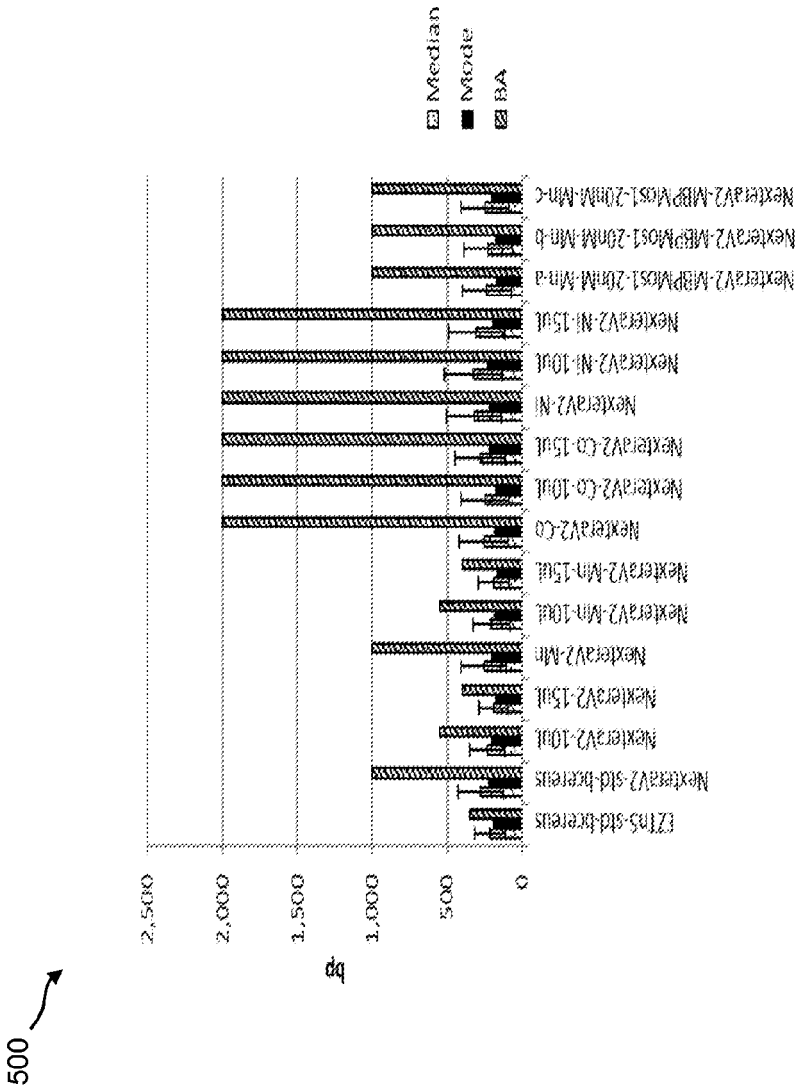

500
bp
2,500
2,000
1,500
1,000
500
0
Median
Mode
BA
EZTrS-std-bcrelous
NextGenV2-std-bcrelous
NextGenV2-10uL
NextGenV2-15uL
NextGenV2-Mn
NextGenV2-Mn-10uL
NextGenV2-Mn-15uL
NextGenV2-Co
NextGenV2-Co-10uL
NextGenV2-Co-15uL
NextGenV2-Ni
NextGenV2-Ni-10uL
NextGenV2-Ni-15uL
NextGenV2-MBPMos1-20mM-Mn-a
NextGenV2-MBPMos1-20mM-Mn-b
NextGenV2-MBPMos1-20mM-Mn-c

Figure 5

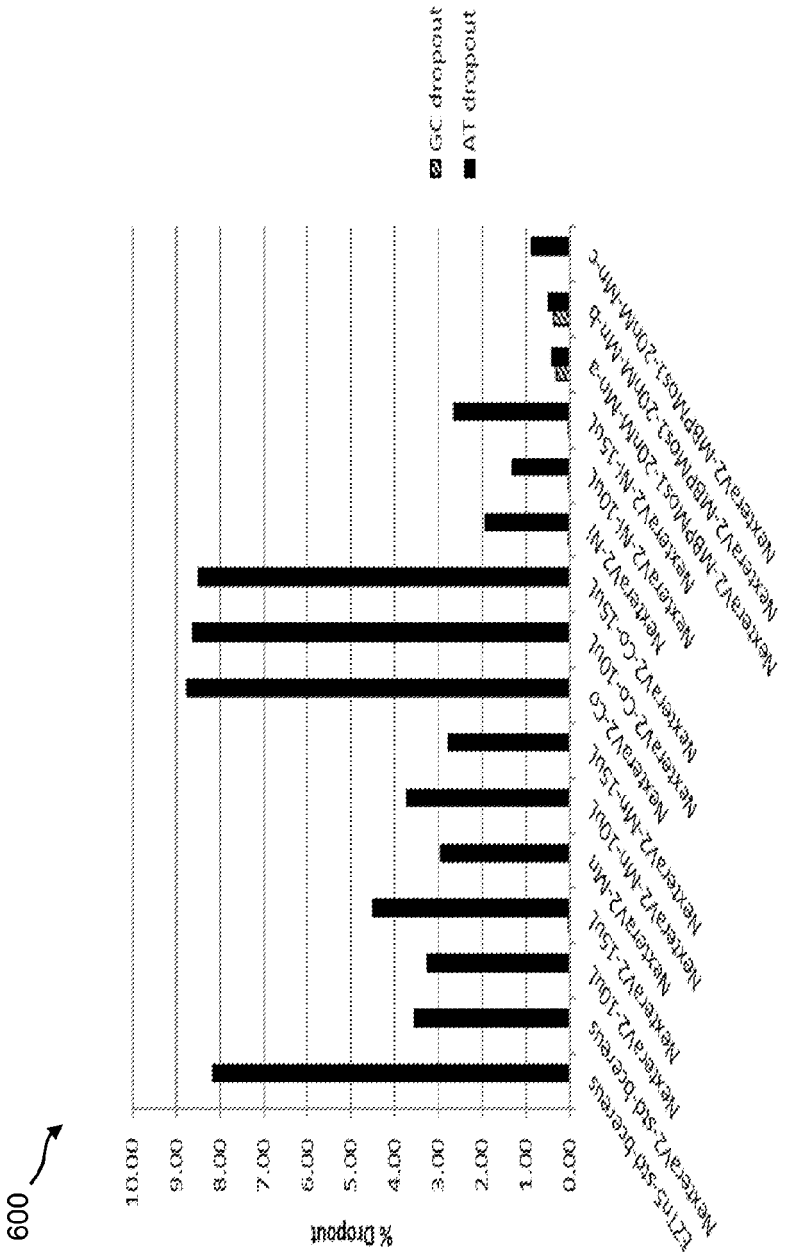

600
GC dropout
AT dropout
% Dropout
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
ECNTS-Sub-Bacteroides
NexteraV2-Sub-Bacteroides
NexteraV2-10uL
NexteraV2-15uL
NexteraV2-Mn
NexteraV2-Mn-10uL
NexteraV2-Mn-15uL
NexteraV2-Co
NexteraV2-Co-10uL
NexteraV2-Co-15uL
NexteraV2-Ni
NexteraV2-Ni-10uL
NexteraV2-Ni-15uL
NexteraV2-MBPMo51-20mM-Mn-a
NexteraV2-MBPMo51-20mM-Mn-b
NexteraV2-MBPMo51-20mM-Mn-c

Figure 6

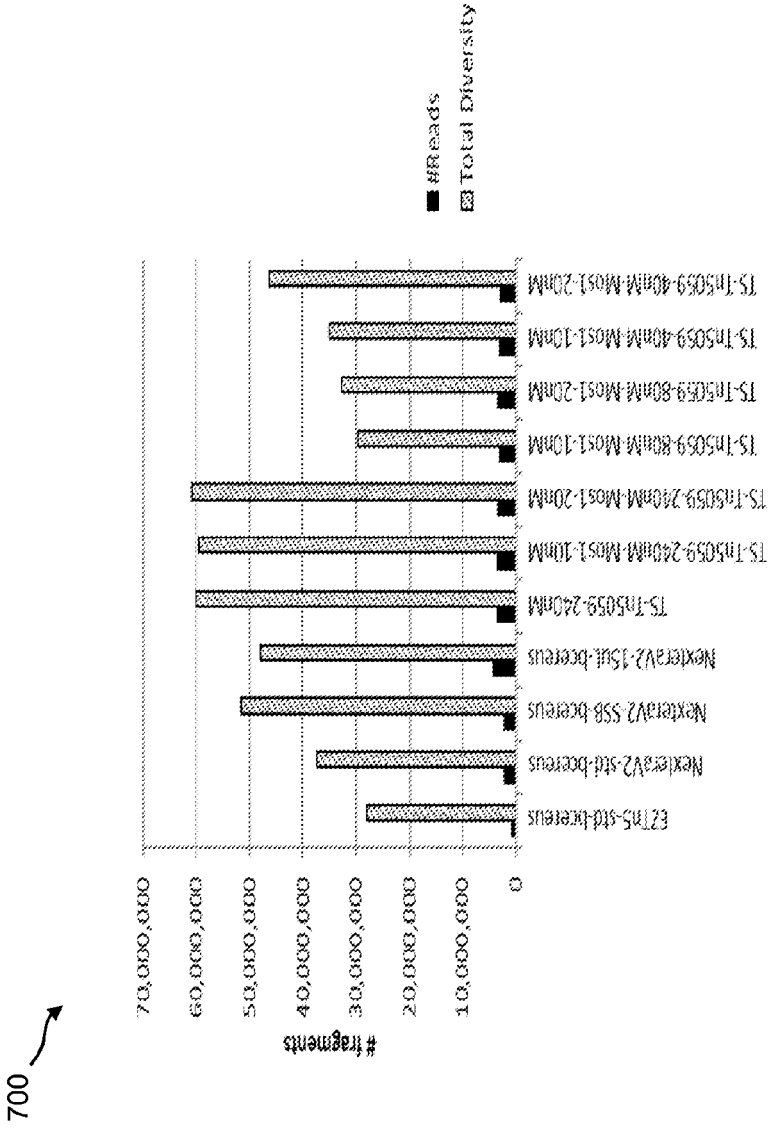

700
fragments
70,000,000
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
0
#Reads
Total Diversity
E2TN5-std-bcereus
NextEraV2-std-bcereus
NexteraV2 SSB bcereus
NexteraV2-15ul-bcereus
TS-TN5059-240nM
TS-TN5059-240nM-Mos1-10nM
TS-TN5059-240nM-Mos1-20nM
TS-TN5059-80nM-Mos1-10nM
TS-TN5059-80nM-Mos1-20nM
TS-TN5059-40nM-Mos1-10nM
TS-TN5059-40nM-Mos1-20nM

Figure 7

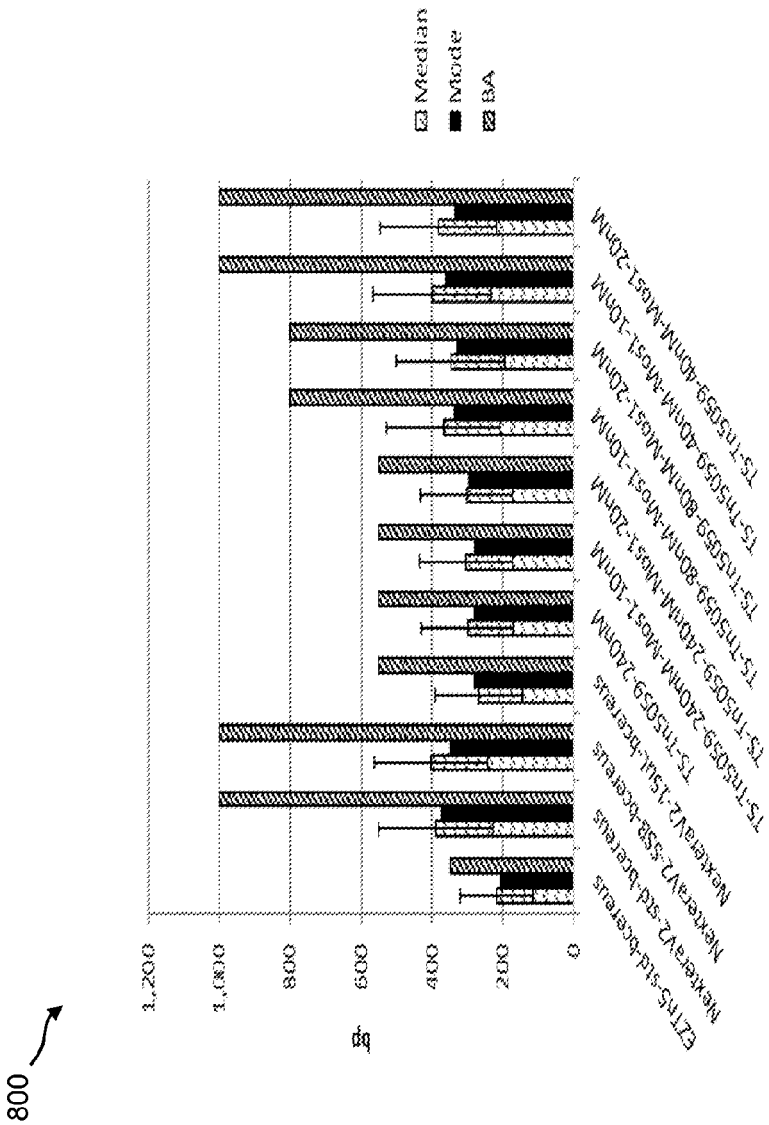

800
Median
Mode
BA
bp
0
200
400
600
800
1,000
1,200
E.Chi5-sla-bcereus
Mekera-V2-sla-bcereus
Mekera-V2-SSB-bcereus
Mekera-V2-15sl-bcereus
15-Tn5C59-240hM-bcereus
15-Tn5C59-240hM-Mas1-10hM
15-Tn5C59-240hM-Mas1-20hM
15-Tn5C59-80hM-Mas1-10hM
15-Tn5C59-80hM-Mas1-20hM
15-Tn5C59-40hM-Mas1-10hM
15-Tn5C59-40hM-Mas1-20hM

Figure 8

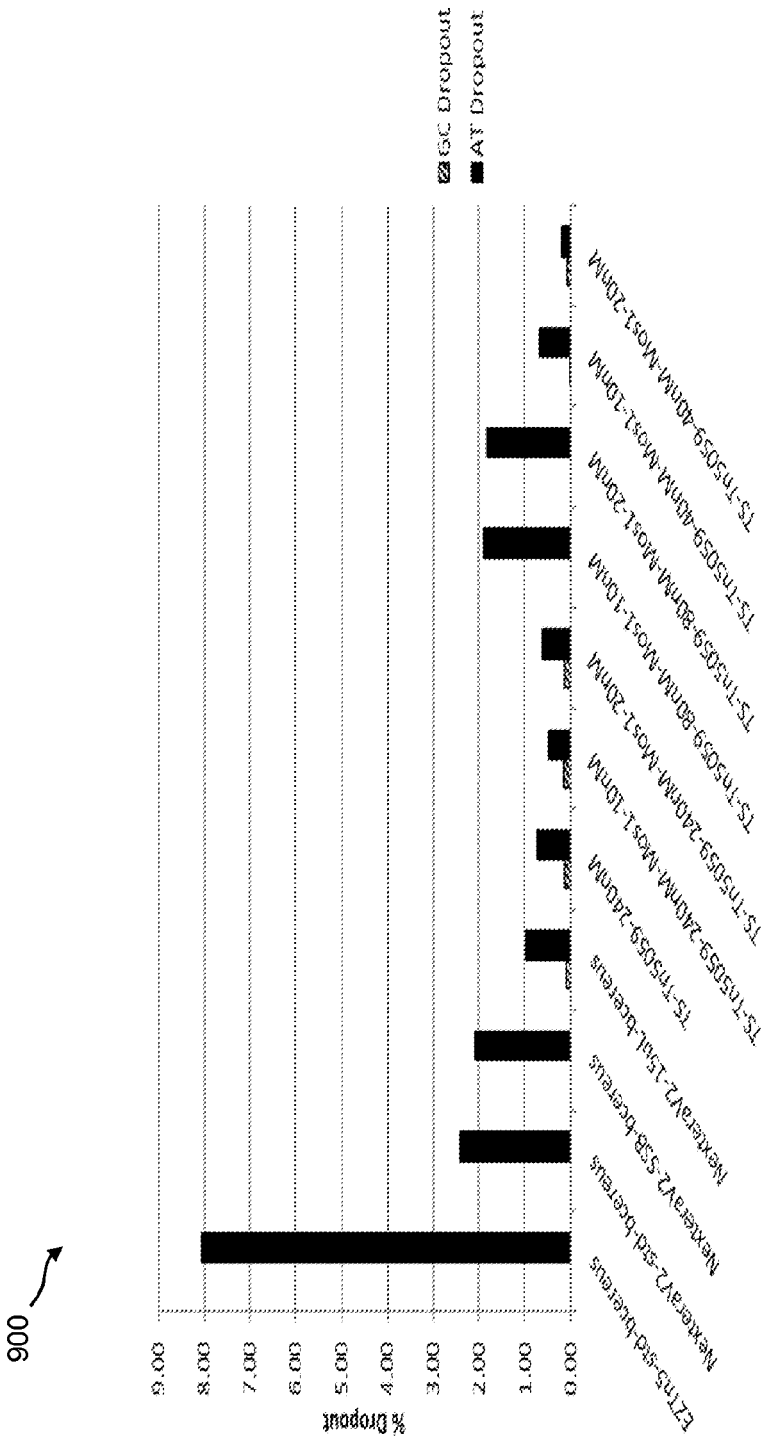

900
% Dropout
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
EZTN3-std-bucterus
Nextera V2-std-bucterus
Nextera V2-SSB-bucterus
Nextera V2-1.5xL-bucterus
TS-TN5059-240nM
TS-TN5059-240nM-MO51-10nM
TS-TN5059-240nM-MO51-20nM
TS-TN5059-80nM-MO51-10nM
TS-TN5059-80nM-MO51-20nM
TS-TN5059-40nM-MO51-10nM
TS-TN5059-40nM-MO51-20nM
5C Dropout
AT Dropout

Figure 9

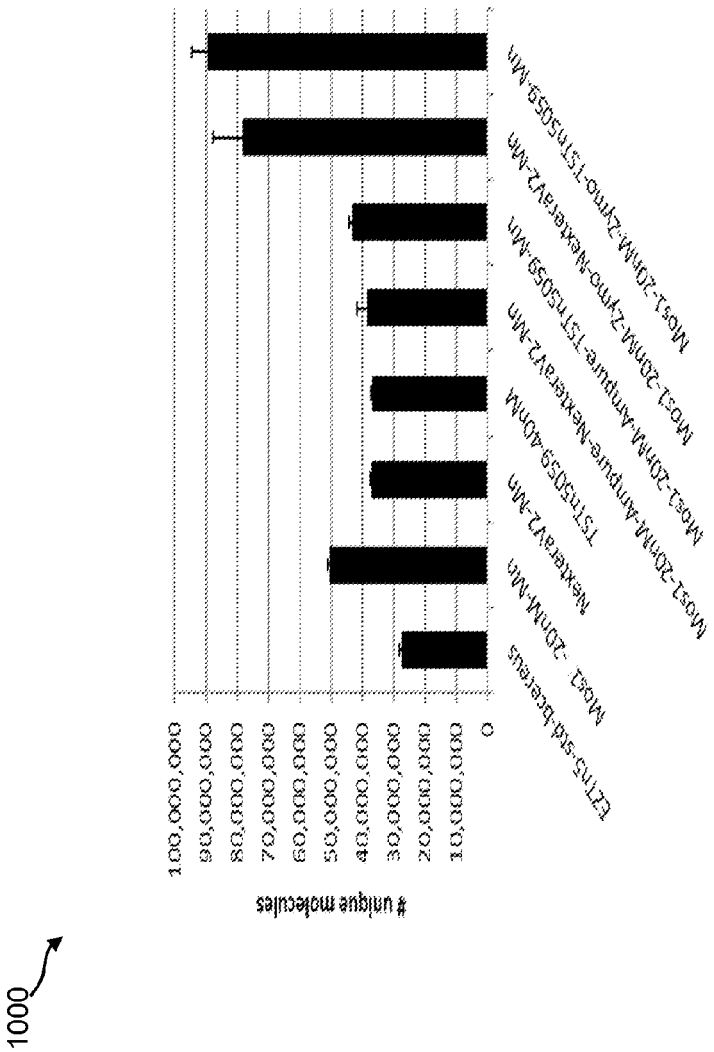

1000
unique molecules
100,000,000
90,000,000
80,000,000
70,000,000
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
0
EZTn5-std-bcerus
Mos1 -20nM-Mn
Nextera-V2-Mn
T5Tn5059-40nM
Mos1-20nM-Ampure-Nextera-V2-Mn
Mos1-20nM-Ampure-T5Tn5059-Mn
Mos1-20nM-Zymo-Nextera-V2-Mn
Mos1-20nM-Zymo-T5Tn5059-Mn

Figure 10

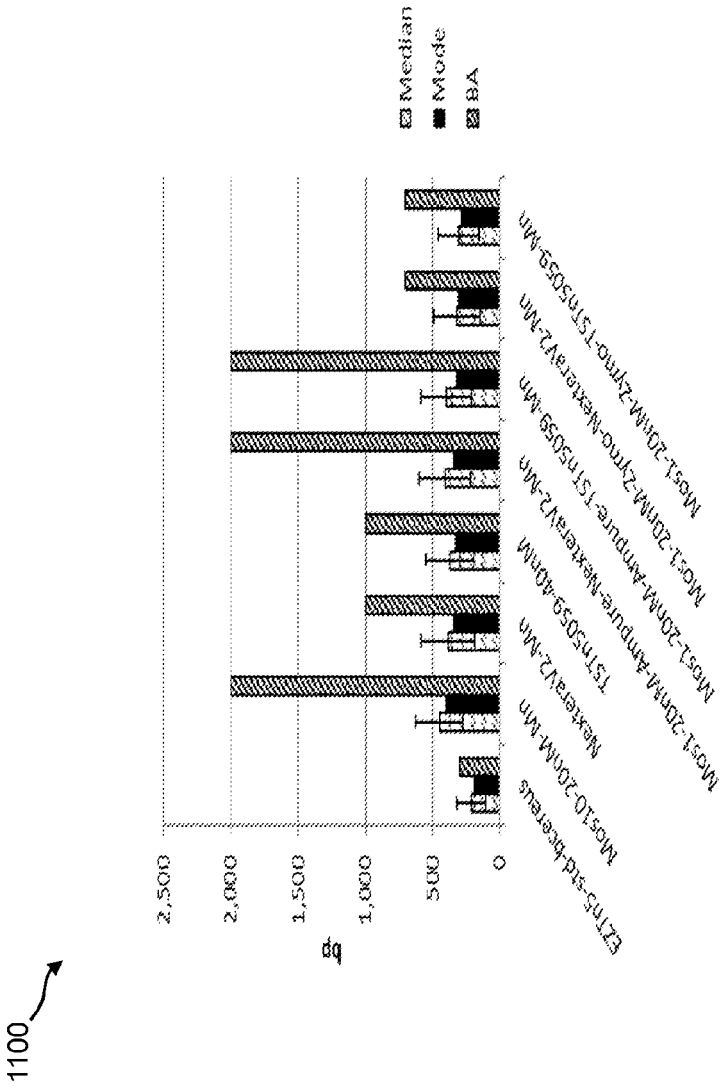

1100
Median
Mode
BA
bp
2,500
2,000
1,500
1,000
500
0
EZTn5-std-bfereus
MoS10-20nM-Nm
Mexterra V2-Nm
TST n5059-40nM
MoS1-20nM-Ampure-Mexterra V2-Nm
MoS1-20nM-Ampure-TST n5059-Nm
MoS1-20nM-Zymo-Mexterra V2-Nm
MoS1-20nM-Zymo-TST n5059-Nm

Figure 11

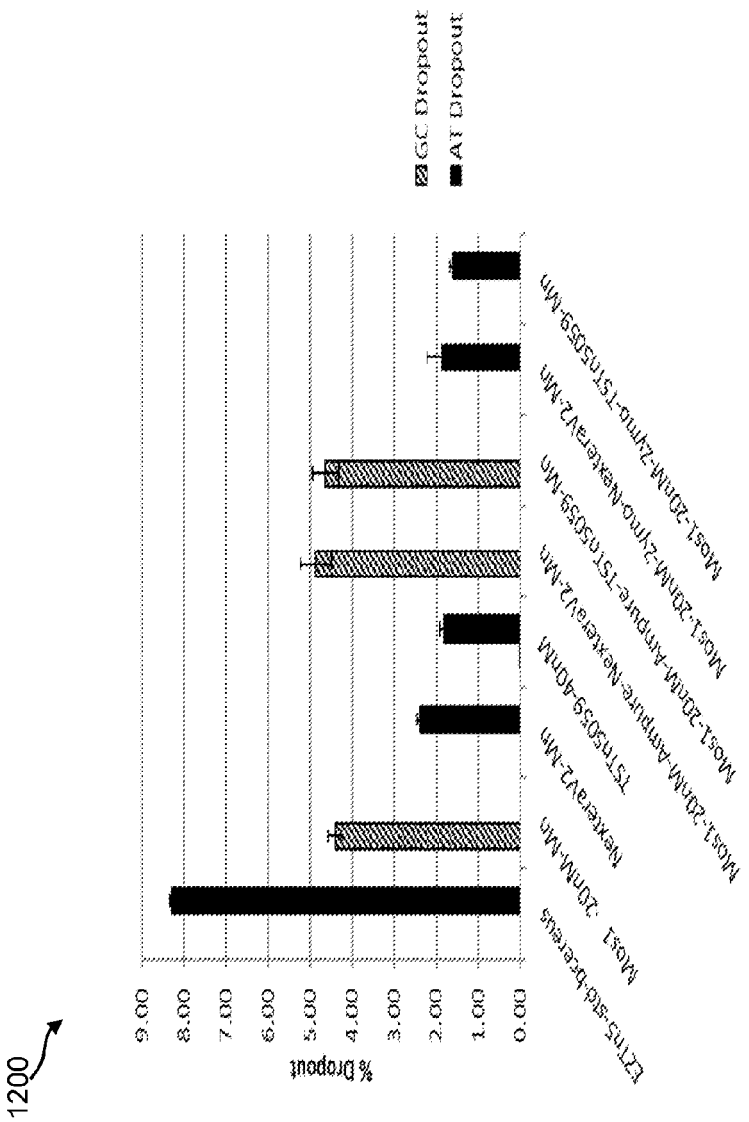

1200
GC Dropout
AT Dropout
% Dropout
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
EZTN5-sig-bzeres
Mos1_20nM-Mn
Nextera V2-1Mn
TSTN5059-40nM
Mos1_20nM-Rampure-Nextera V2-1Mn
Mos1_20nM-Rampure-TSTN5059-Mn
Mos1_20nM-2xMo-Nextera V2-1Mn
Mos1_20nM-2xMo-TSTN5059-Mn

Figure 12

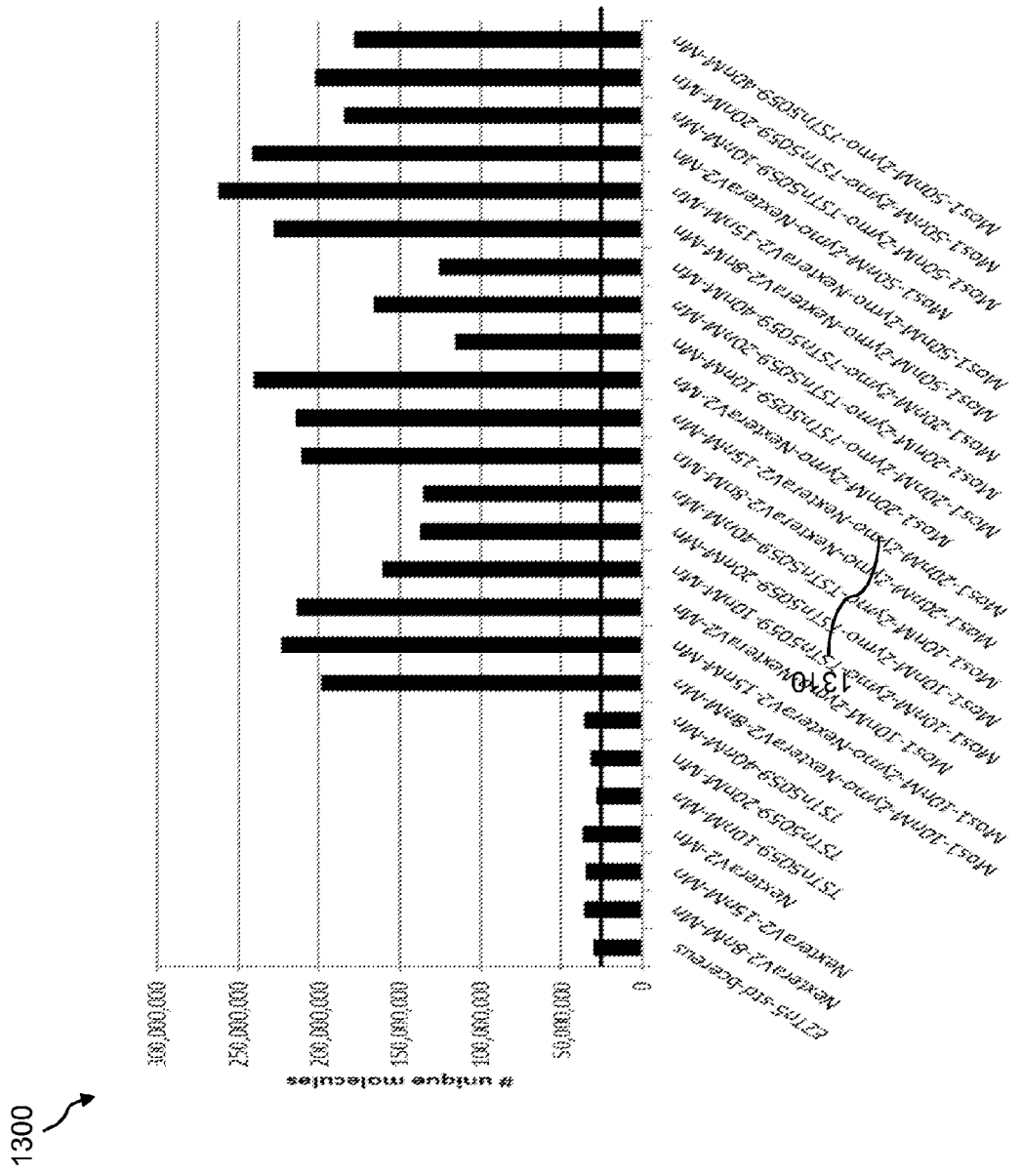

1300
unique molecules
0
50,000,000
100,000,000
150,000,000
200,000,000
250,000,000
300,000,000
1310
c2ms-std-bcerens
Nextera-50nM-Am
Nextera-25nM-Am
Nextera-10nM-Am
15TM5059-10nM-Am
Nextera-50nM-Am
15TM5059-40nM-Am
Nextera-25nM-Am
15TM5059-20nM-Am
Mos1-10nM-Zymo-Nextera-50nM-Am
Mos1-10nM-Zymo-Nextera-25nM-Am
Mos1-10nM-Zymo-15TM5059-10nM-Am
Mos1-10nM-Zymo-15TM5059-40nM-Am
Mos1-10nM-Zymo-15TM5059-20nM-Am
Mos1-20nM-Zymo-Nextera-50nM-Am
Mos1-20nM-Zymo-Nextera-25nM-Am
Mos1-20nM-Zymo-15TM5059-10nM-Am
Mos1-20nM-Zymo-15TM5059-40nM-Am
Mos1-20nM-Zymo-15TM5059-20nM-Am
Mos1-50nM-Zymo-Nextera-50nM-Am
Mos1-50nM-Zymo-Nextera-25nM-Am
Mos1-50nM-Zymo-15TM5059-10nM-Am
Mos1-50nM-Zymo-15TM5059-40nM-Am
Mos1-50nM-Zymo-15TM5059-20nM-Am

Figure 13

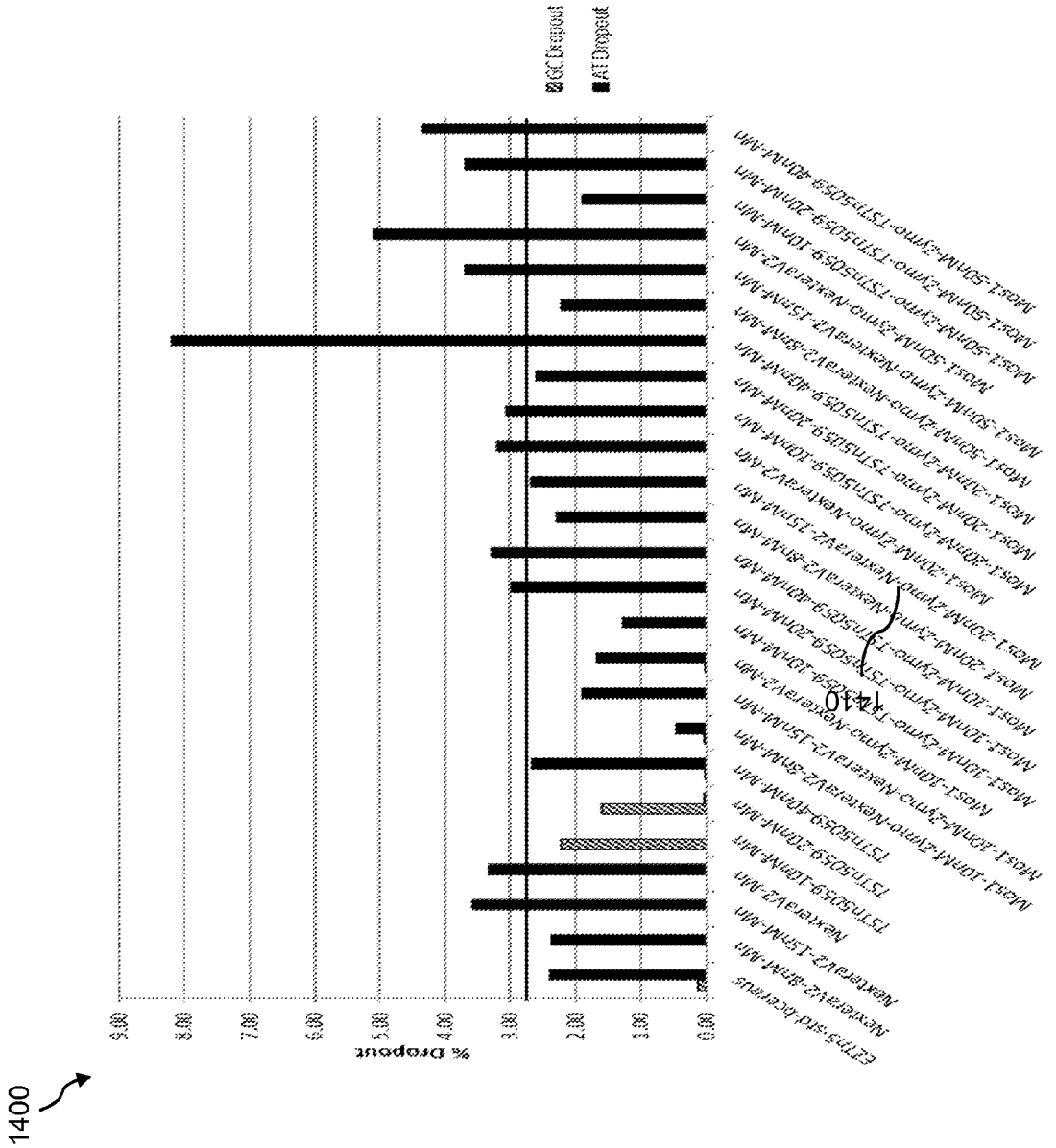

1400
1410
% Dropout
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
GC Dropout
AT Dropout

Figure 14

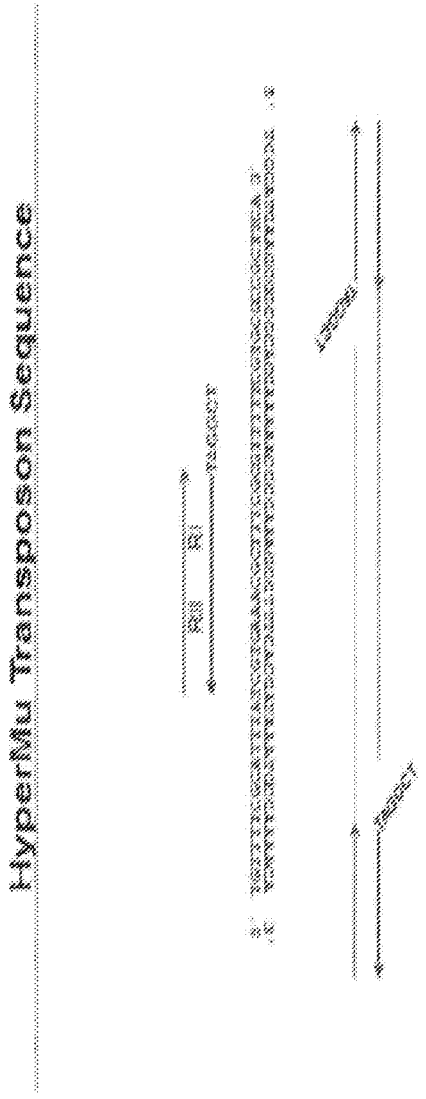


Figure 15

Oligo Sequences Used for MuTsm

MM1141:
TGTTTTCGCATTTATCGTGAAACGCTTTCGCGTTTTTCGTGCGCCGCTTCA

MM1138:
TCGGATGAAGCGGCGCACGAAAAACGCGAAAGCGTTTCACGATAAATGCGAAAACA

```
MM1141  1   TGTTTTCGCATTTATCGTGAAACGCTTTCGCGTTTTTCGTGCGCCGCTTCA  51
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
MM1138  56  TGTTTTCGCATTTATCGTGAAACGCTTTCGCGTTTTTCGTGCGCCGCTTCA  6
```

Sequencing Primer (MuPCRts):
CGTTTTTCGTGCGCCGCTTCA

P7MUTS:
CAAGCAGAAGACGGCATACGAGATCGTTTTTCGTGCGCCGCTTCA

P5MUTS:
AATGATACGGCGACCACCGAGATCTACACCGTTTTTCGTGCGCCGCTTCA

Figure 16

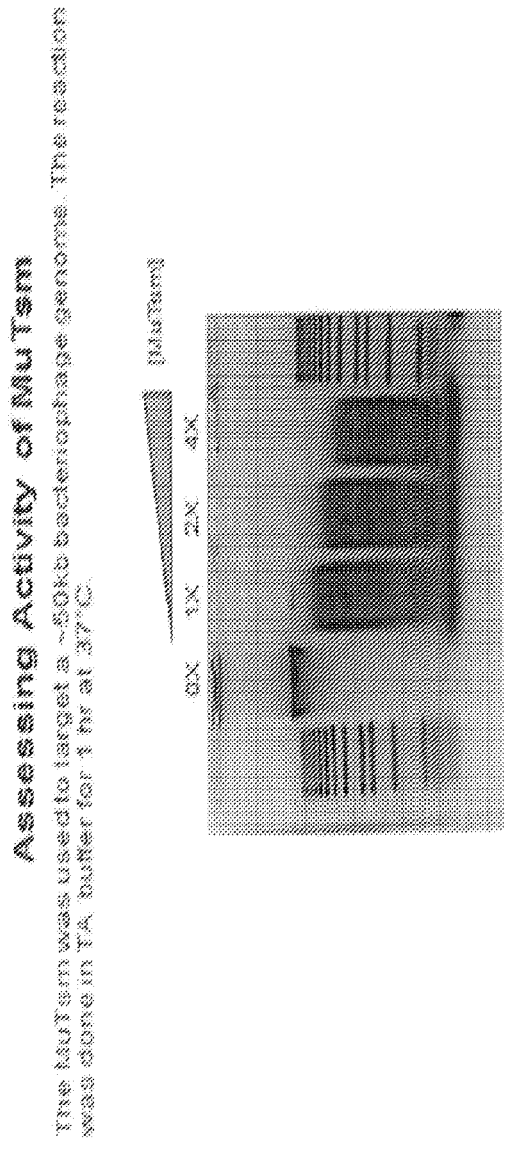

Assessing Activity of MuTem
The MuTem was used to target a ~50kb bacteriophage genome. The reaction was done in TA buffer for 1 hr at 37°C.
[MuTem]
0X
1X
2X
4X

Figure 17

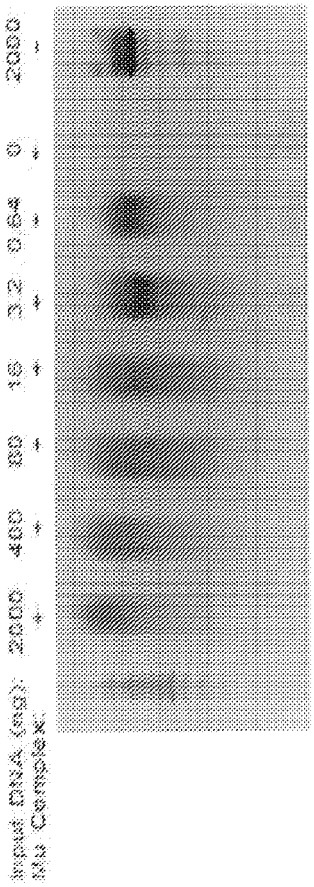


Figure 18

Some Statistics of the Sequencing Run

total # of reads	30,071,951
# of reads passed eland alignment	16,775,955
# of unique reads	4,629,392
# of unique reads <2000bp	4,599,574
mean coverage	69.39X

Figure 19

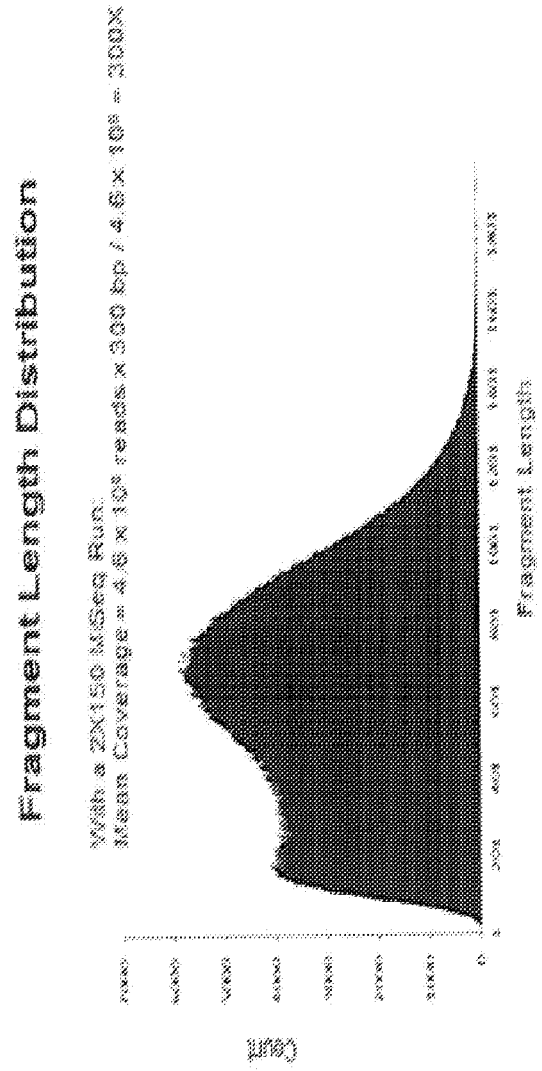


Figure 20

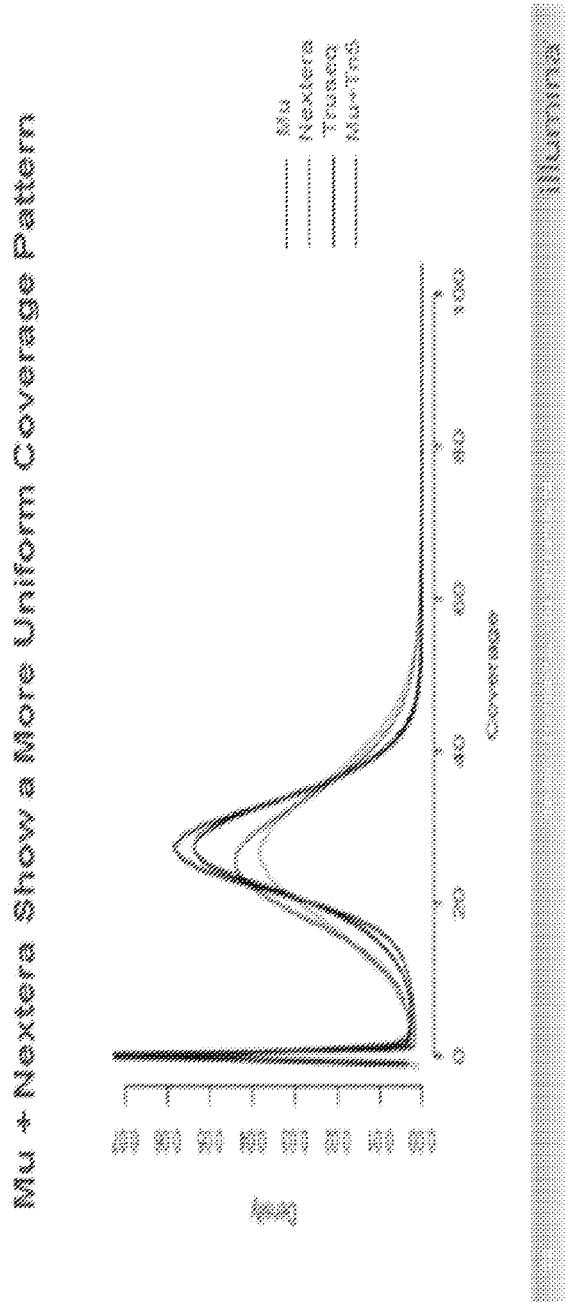

Mu + Nextera Show a More Uniform Coverage Pattern
Mu
Nextera
Truseq
Illumina
Coverage
0
200
400
600
800
1000
Illumina

Figure 21

Figure 22A

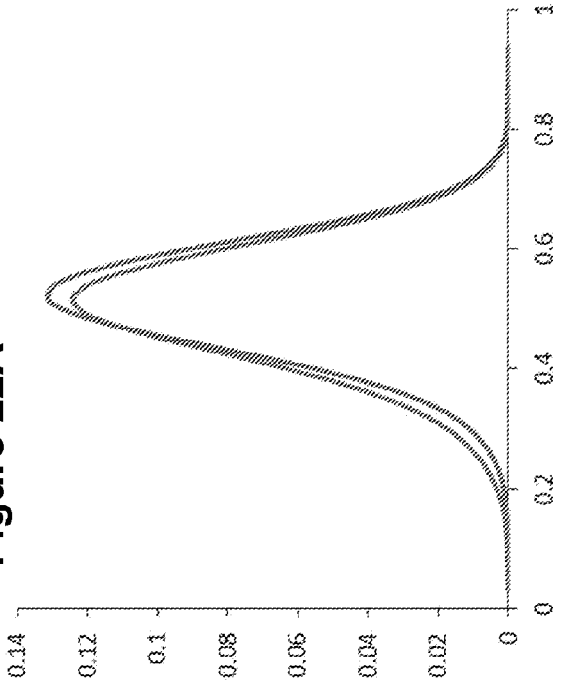


Figure 22B

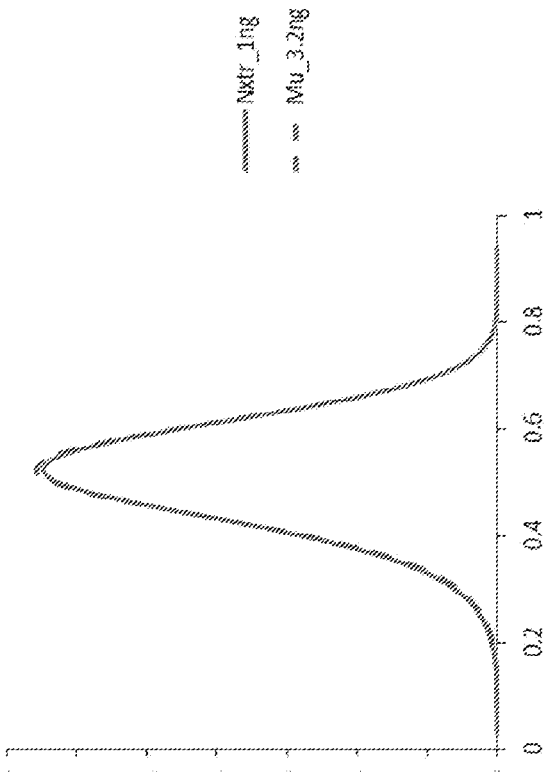


Figure 22C

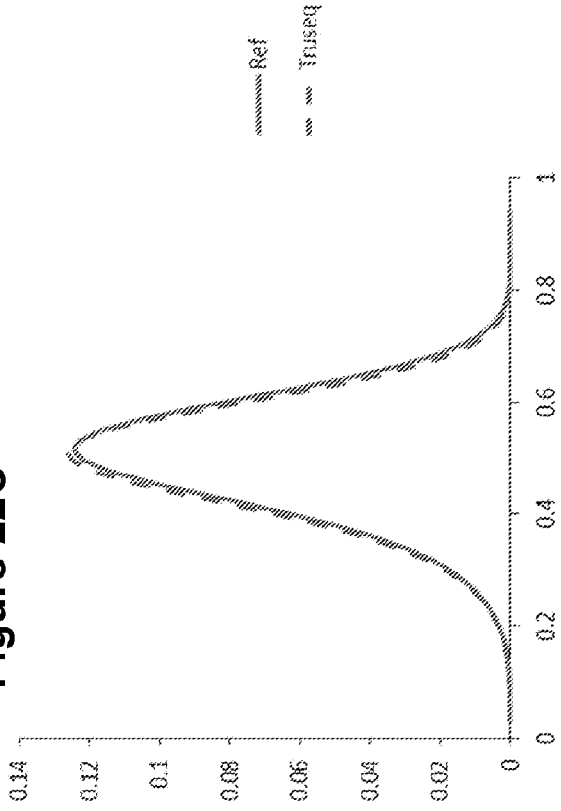
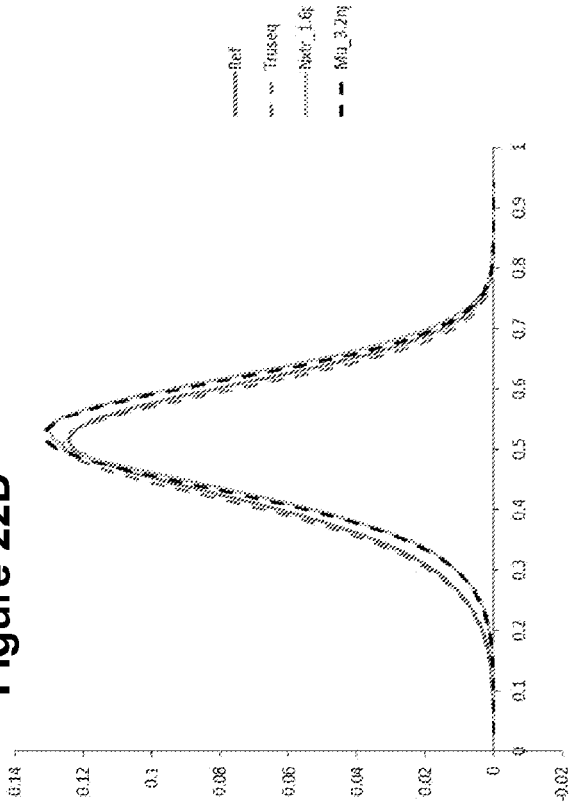


Figure 22D



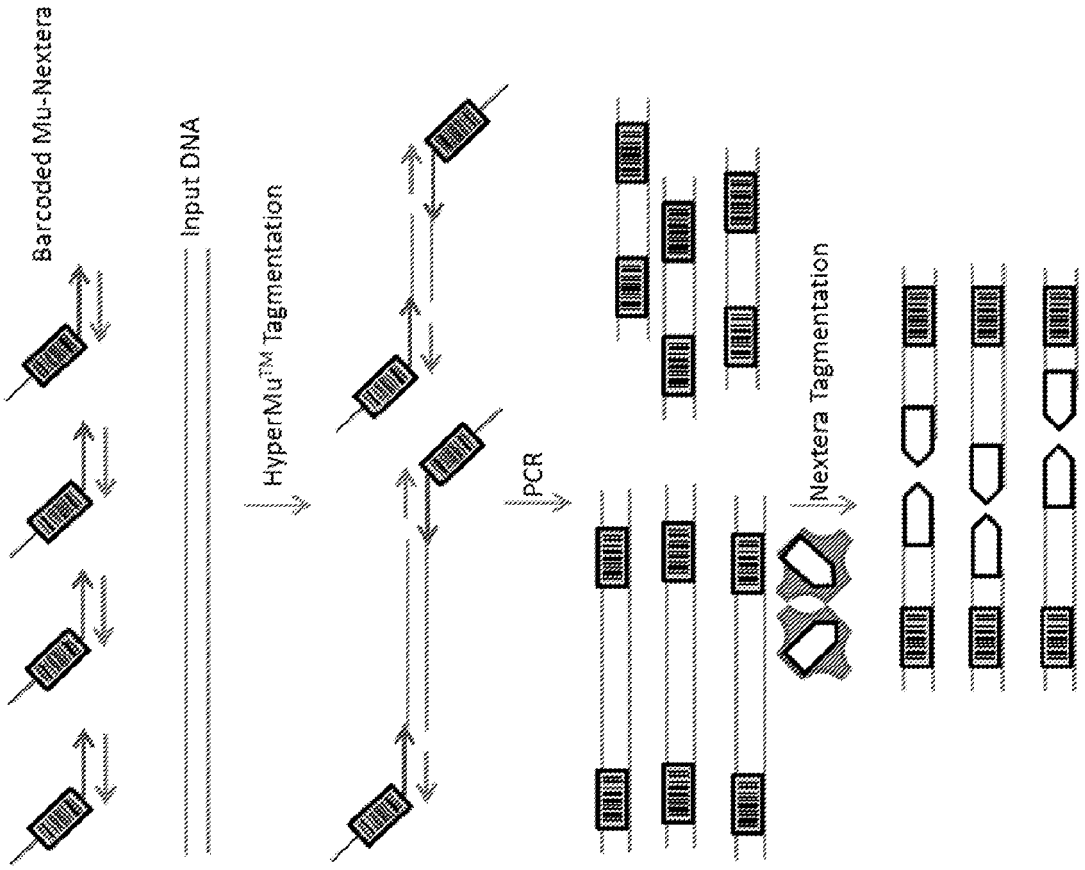

Barcoded Mu-Nextera
Input DNA
HyperMu™ Tagmentation
PCR
Nextera Tagmentation

Figure 23

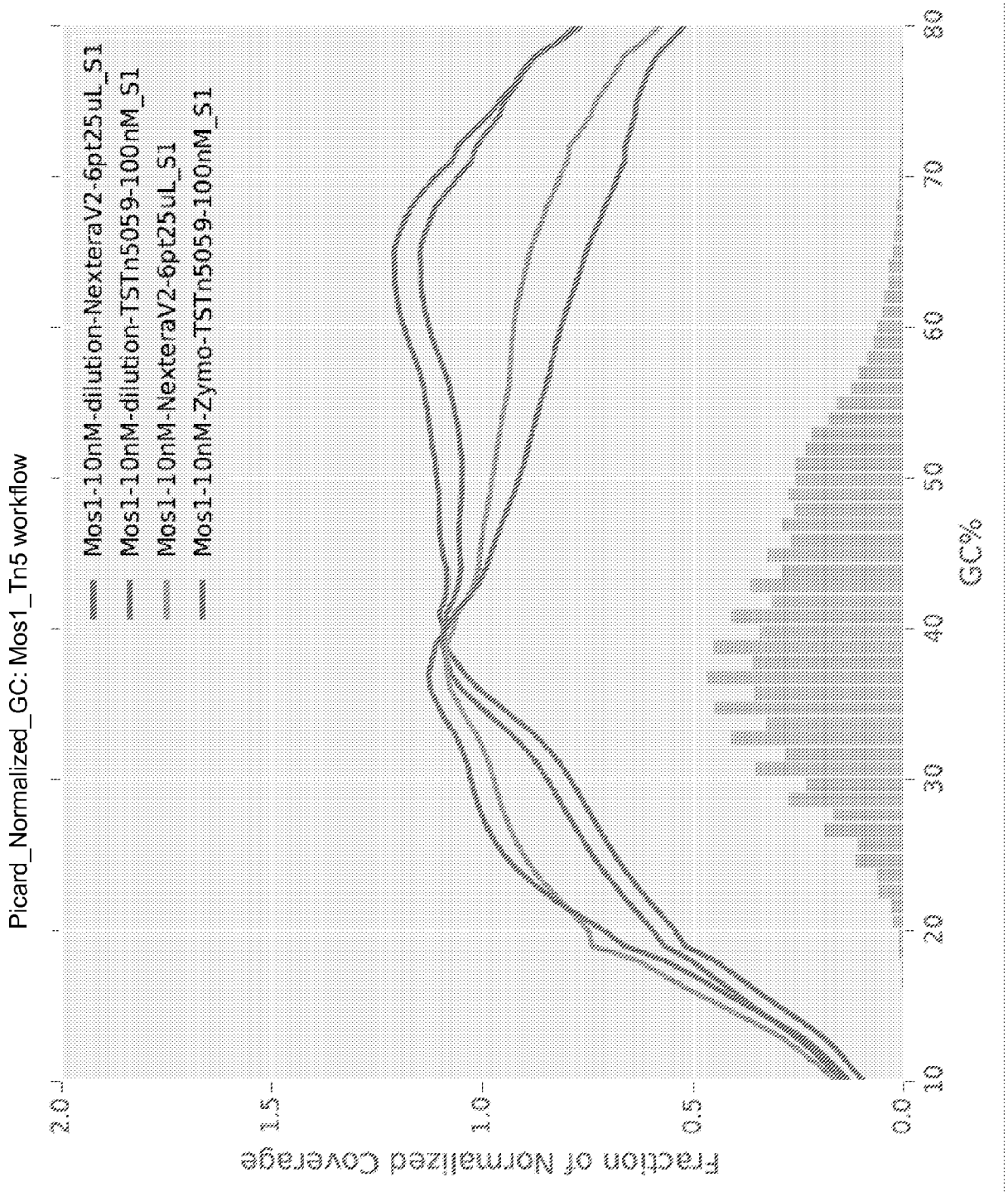

Picard_Normalized_GC: Mos1_Tn5 workflow
Mos1-10nM-dilution-NexteraV2-6pt25uL_S1
Mos1-10nM-dilution-TSTn5059-100nM_S1
Mos1-10nM-NexteraV2-6pt25uL_S1
Mos1-10nM-Zymo-TSTn5059-100nM_S1
Fraction of Normalized Coverage
2.0
1.5
1.0
0.5
0.0
10
20
30
40
50
60
70
80
GC%

Figure 24

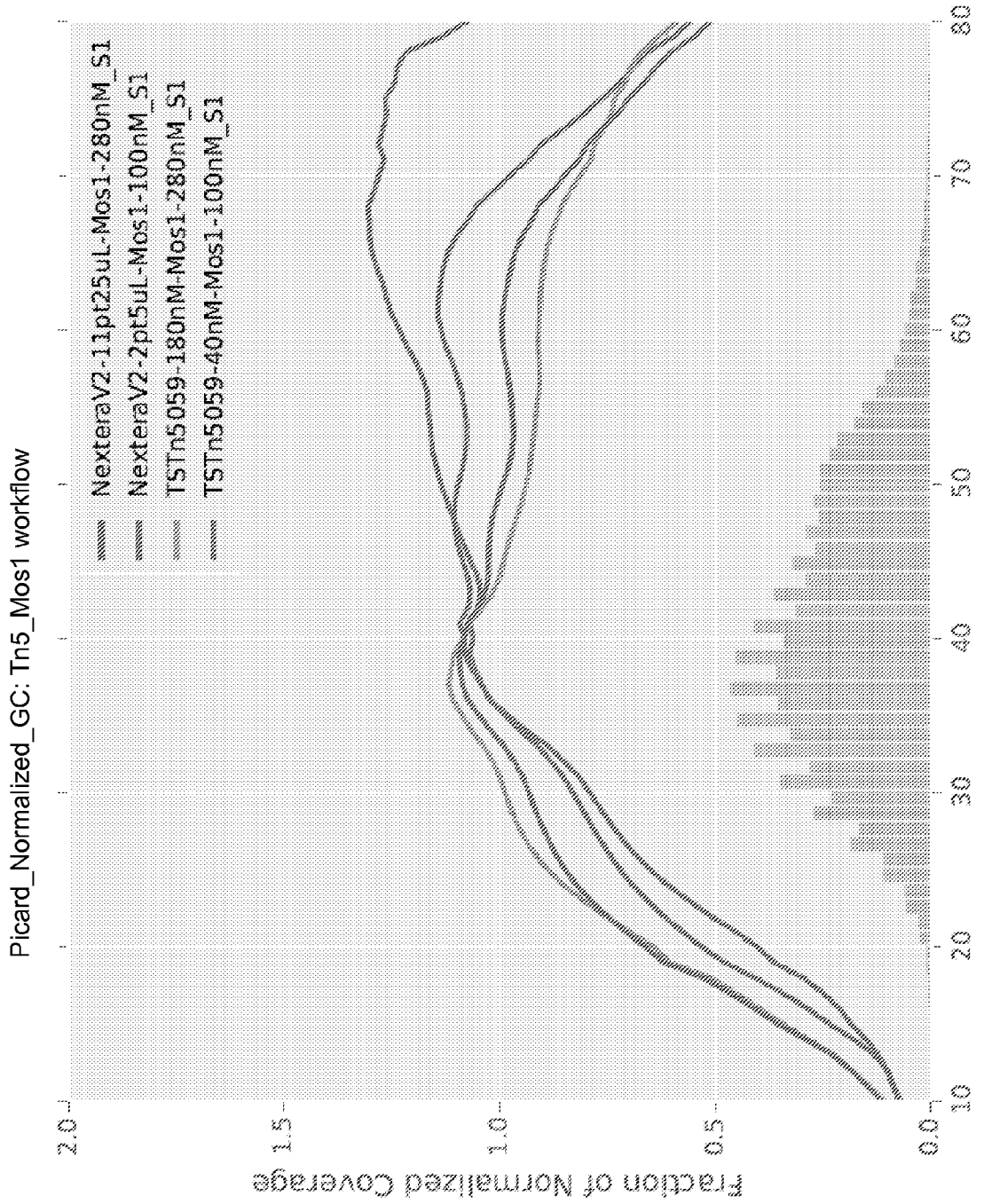

Picard_Normalized_GC: Tn5_Mos1 workflow
NexteraV2-11pt25uL-Mos1-280nM_S1
NexteraV2-2pt5uL-Mos1-100nM_S1
TSTn5059-180nM-Mos1-280nM_S1
TSTn5059-40nM-Mos1-100nM_S1
Fraction of Normalized Coverage
2.0
1.5
1.0
0.5
0.0
10
20
30
40
50
60
70
80
GC%

Figure 25

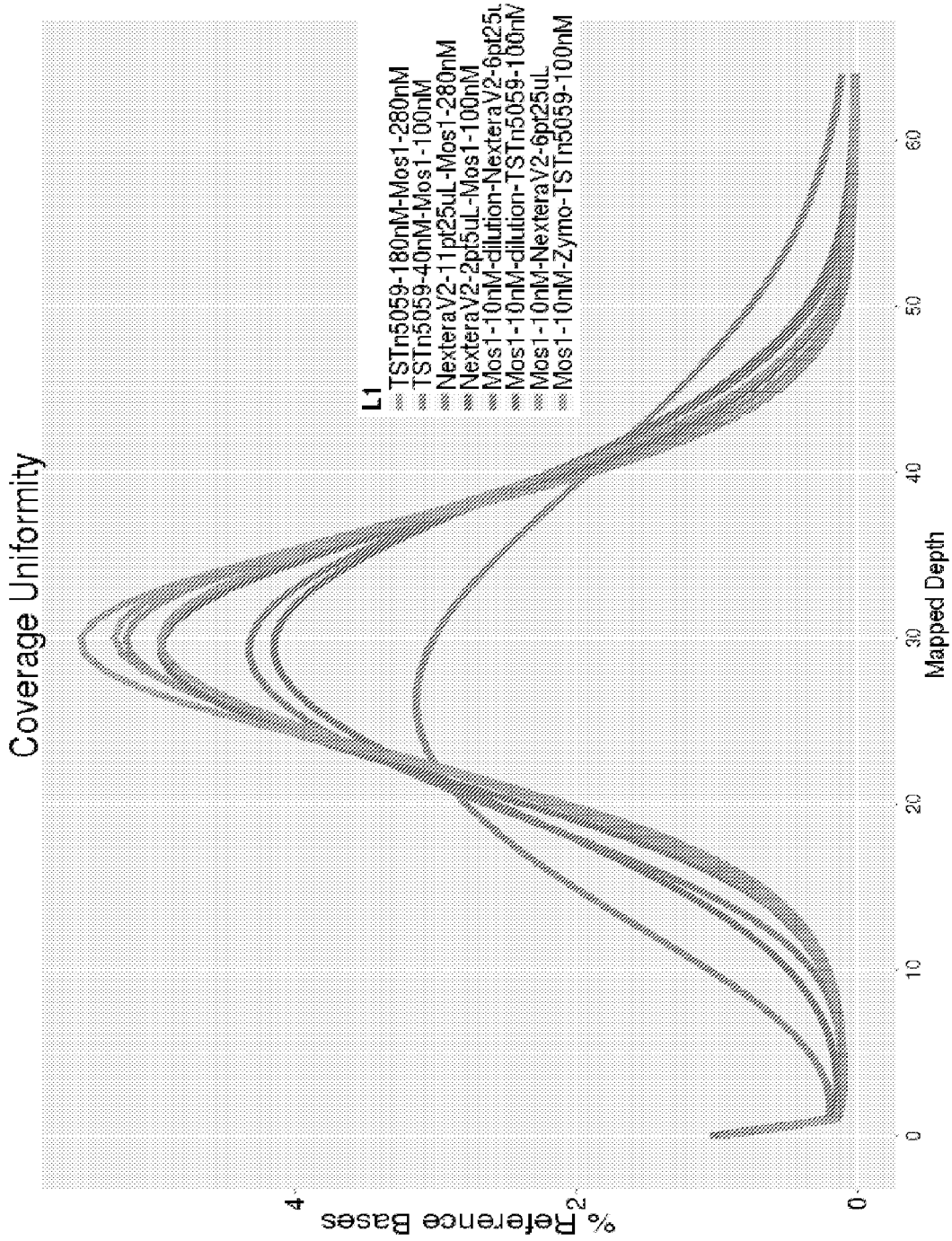

Coverage Uniformity
% Reference Bases
Mapped Depth
0
2
4
0
10
20
30
40
50
60
L1
TSTn5059-180nM-Mos1-280nM
TSTn5059-40nM-Mos1-100nM
NexteraV2-11pt25uL-Mos1-280nM
NexteraV2-2pt5uL-Mos1-100nM
Mos1-10nM-dilution-NexteraV2-6pt25uL
Mos1-10nM-dilution-TSTn5059-100nM
Mos1-10nM-NexteraV2-6pt25uL
Mos1-10nM-Zymo-TSTn5059-100nM

Figure 26

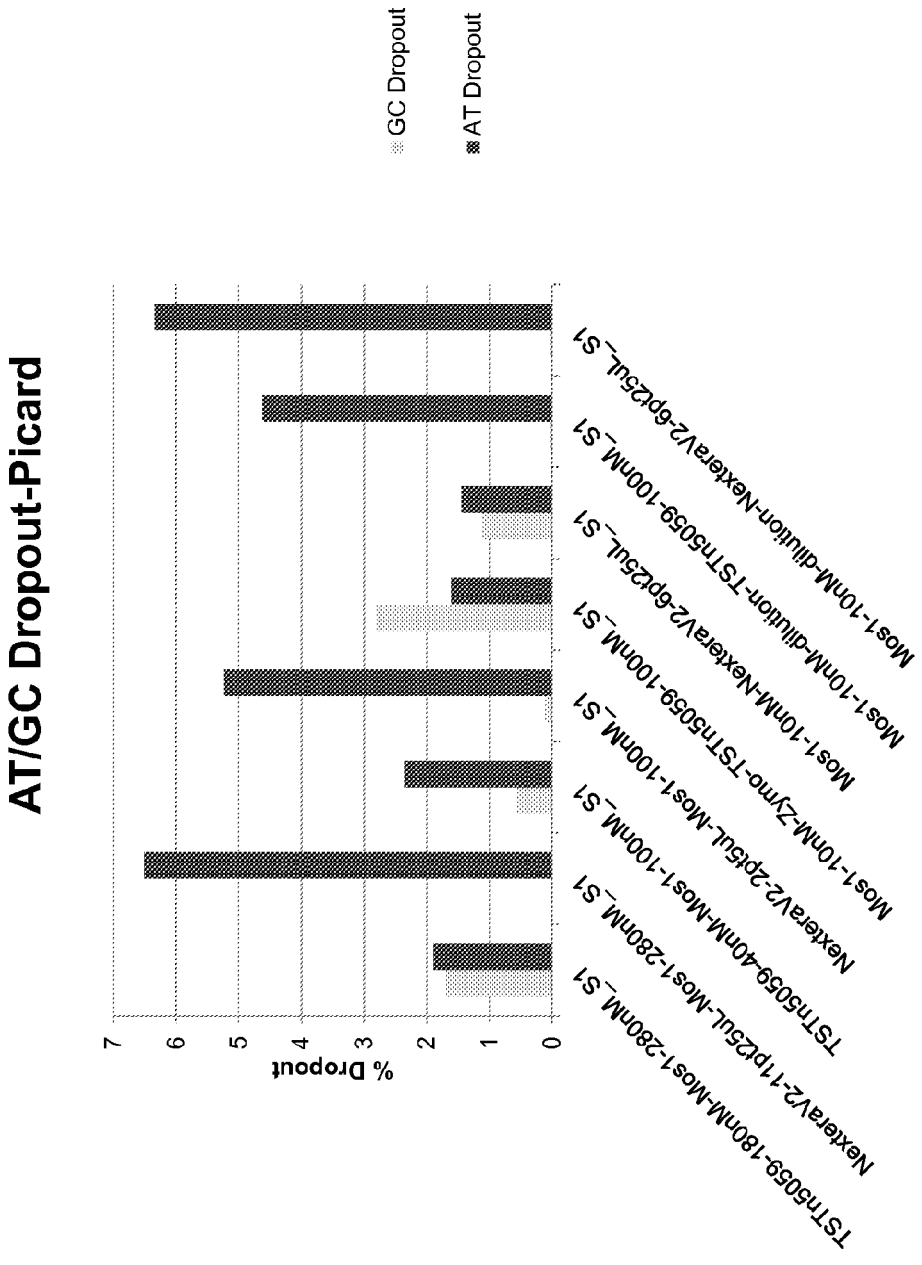

AT/GC Dropout-Picard
GC Dropout
AT Dropout
% Dropout
0
1
2
3
4
5
6
7
TSTn5059-180nM-Mos1-280nM_S1
NextseraV2-11pt25uL-Mos1-280nM_S1
TSTn5059-40nM-Mos1-100nM_S1
NextseraV2-2pt5uL-Mos1-100nM_S1
Mos1-10nM-Zymo-TSTn5059-100nM_S1
Mos1-10nM-NextseraV2-6pt25uL_S1
Mos1-10nM-dilution-TSTn5059-100nM_S1
Mos1-10nM-dilution-NextseraV2-6pt25uL_S1

Figure 27

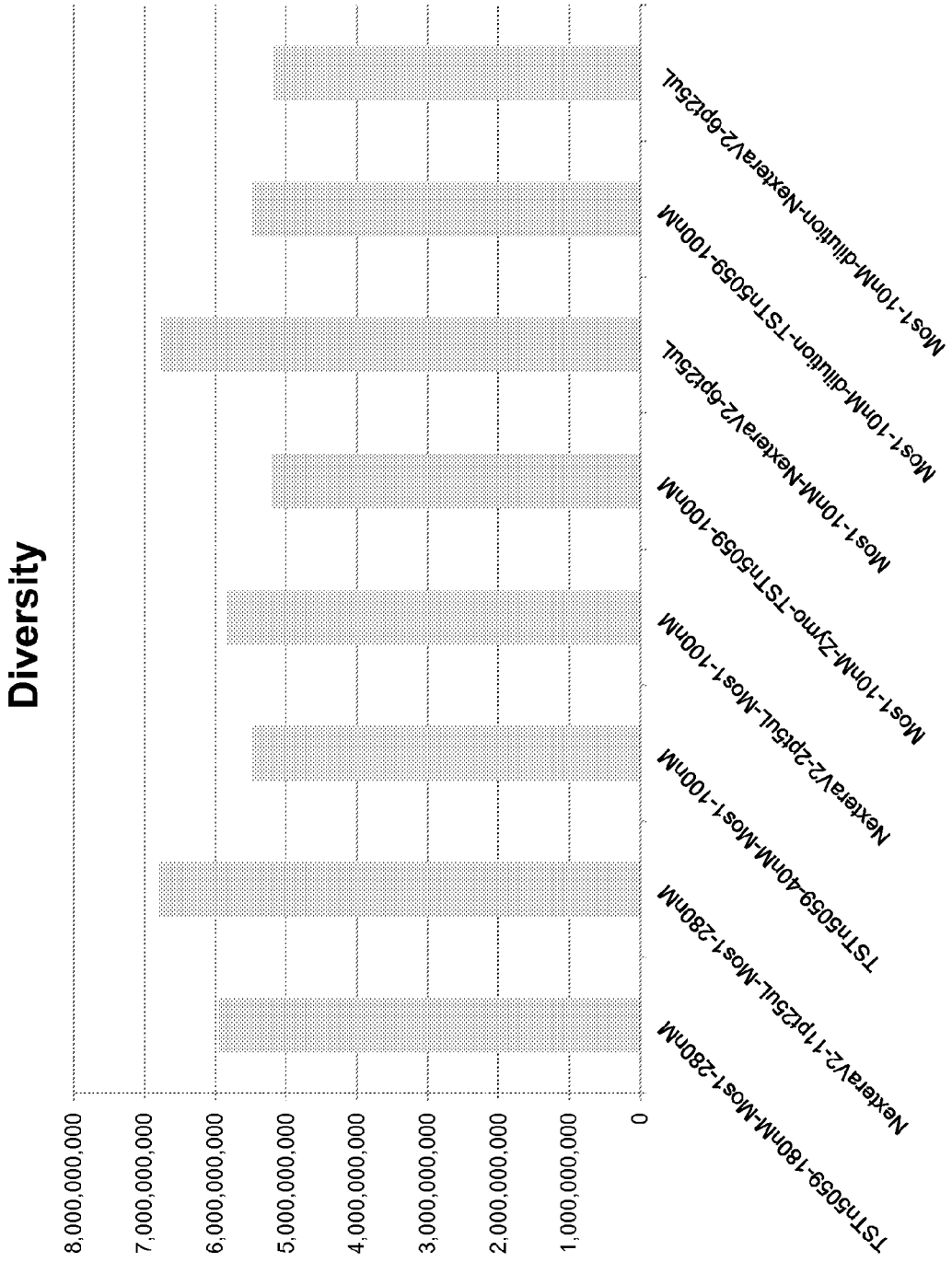

Diversity
8,000,000,000
7,000,000,000
6,000,000,000
5,000,000,000
4,000,000,000
3,000,000,000
2,000,000,000
1,000,000,000
0
TSTn5059-180nM-Mos1-280nM
NextEraV2-1-1pt25uL-Mos1-280nM
TSTn5059-40nM-Mos1-100nM
NextEraV2-2pt5uL-Mos1-100nM
Mos1-10nM-Zymo-TSTn5059-100nM
Mos1-10nM-NextEraV2-6pt25uL
Mos1-10nM-dilution-TSTn5059-100nM
Mos1-10nM-dilution-NextEraV2-6pt25uL

Figure 28