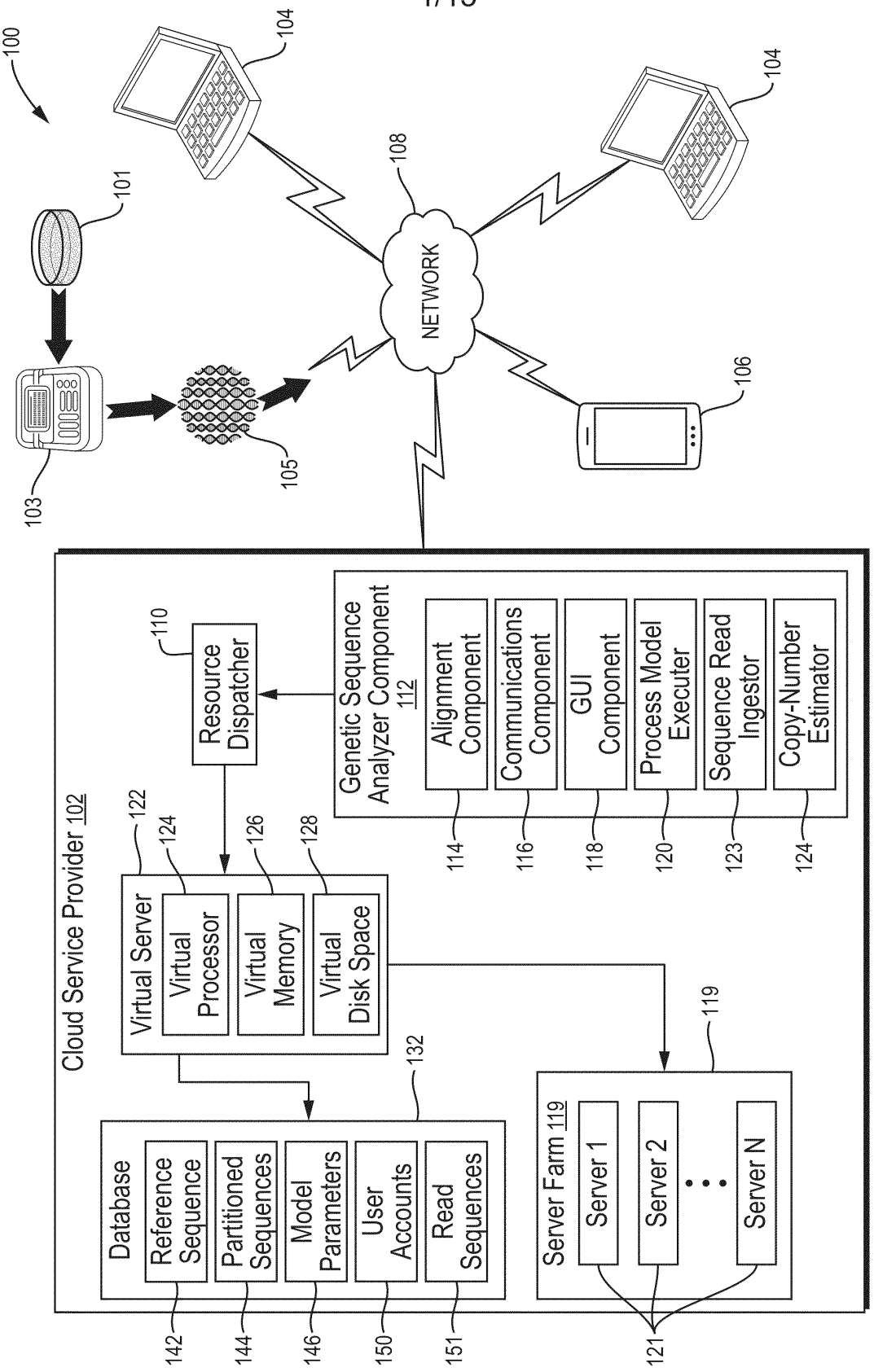
100
104
104
101
108
NETWORK
106
103
105
Cloud Service Provider 102
Resource Dispatcher
110
Genetic Sequence Analyzer Component 112
Alignment Component
Communications Component
GUI Component
Process Model Executer
Sequence Read Ingestor
Copy-Number Estimator
114
116
118
120
123
124
Virtual Server
122
Virtual Processor
124
Virtual Memory
126
Virtual Disk Space
128
Database
132
Reference Sequence
Partitioned Sequences
Model Parameters
User Accounts
Read Sequences
142
144
146
150
151
Server Farm 119
119
Server 1
Server 2
Server N
121

FIG. 1

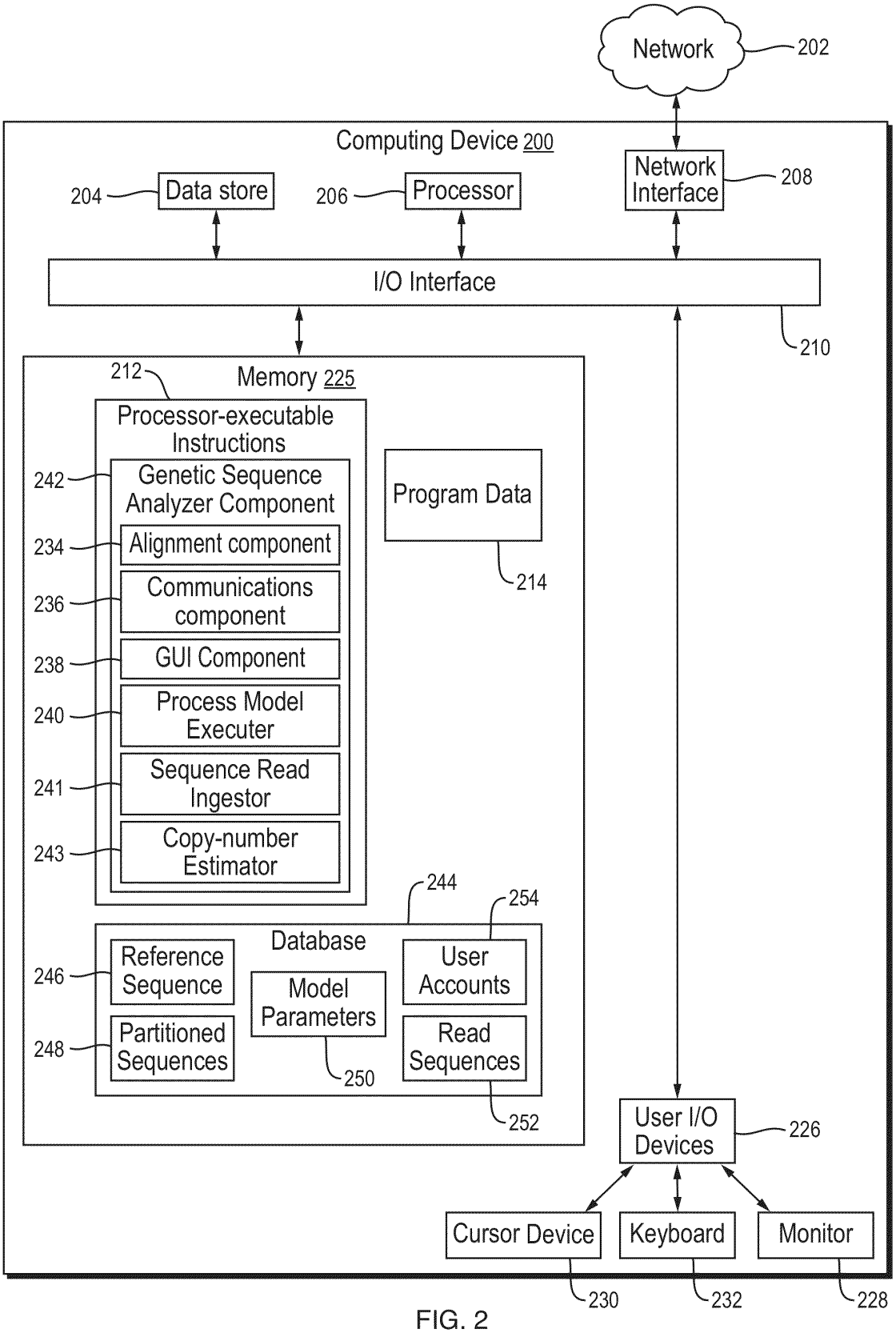
Network
202
Computing Device 200
204
Data store
206
Processor
Network Interface
208
I/O Interface
210
212
Memory 225
Processor-executable Instructions
242
Genetic Sequence Analyzer Component
234
Alignment component
236
Communications component
238
GUI Component
240
Process Model Executer
241
Sequence Read Ingestor
243
Copy-number Estimator
Program Data
214
244
254
Database
246
Reference Sequence
248
Partitioned Sequences
Model Parameters
250
User Accounts
Read Sequences
252
User I/O Devices
226
Cursor Device
Keyboard
Monitor
230
232
228

FIG. 2

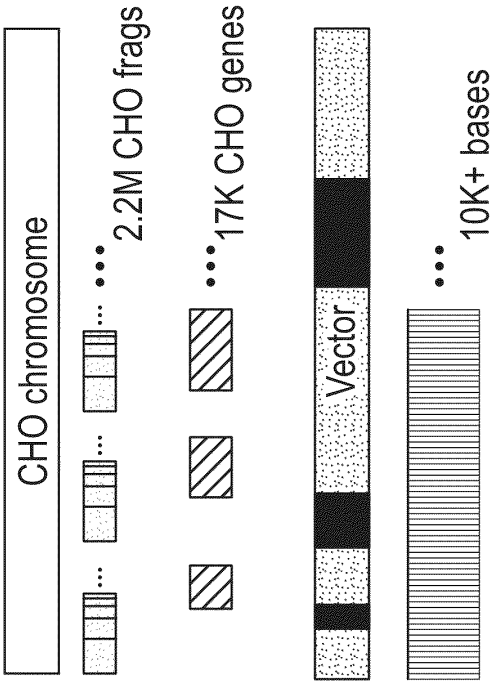
CHO chromosome
2.2M CHO frags
17K CHO genes
Vector
10K+ bases

FIG. 4

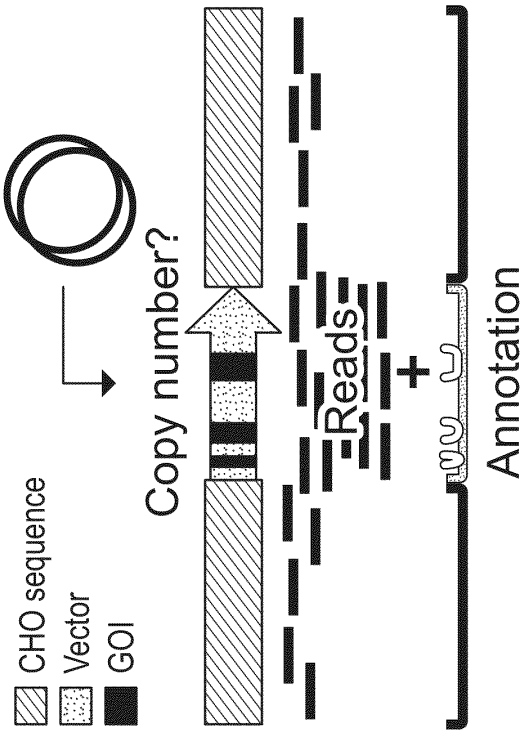
CHO sequence
Vector
GOI
Copy number?
Reads
Annotation

FIG. 3

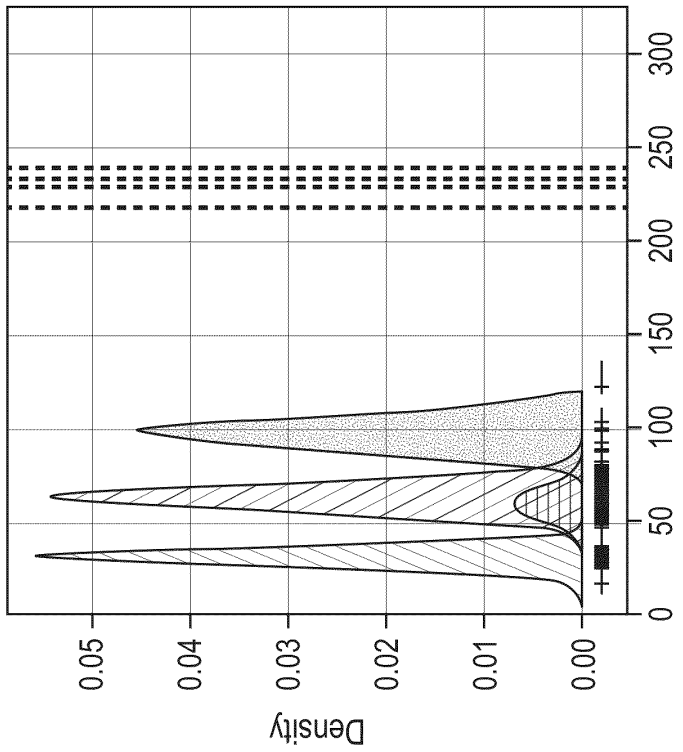
Density
0.05
0.04
0.03
0.02
0.01
0.00
0
50
100
150
200
250
300
Coverage

FIG. 6

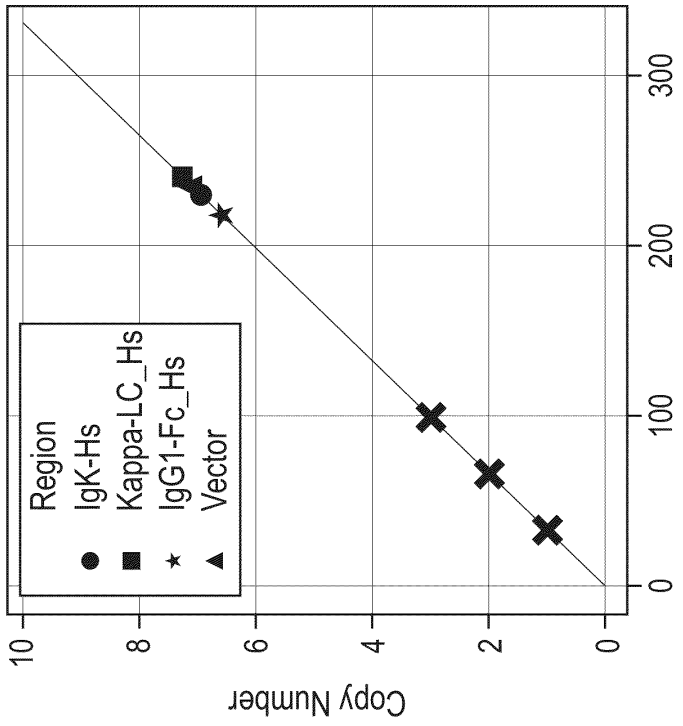
Copy Number
10
8
6
4
2
0
0
100
200
300
Coverage
Region
IgK-Hs
Kappa-LC_Hs
IgG1-Fc_Hs
Vector

FIG. 5

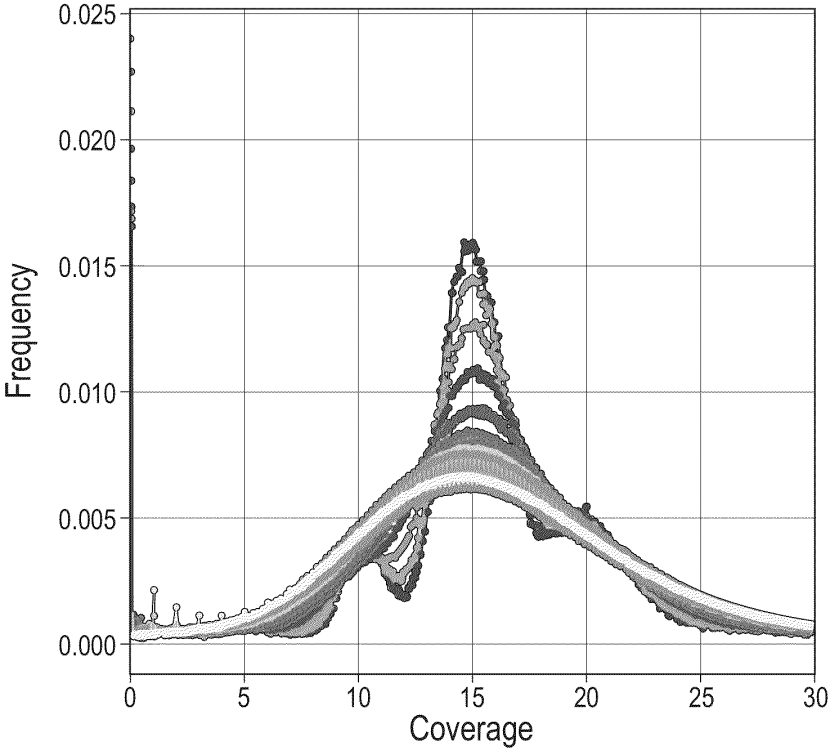
0.025
0.020
0.015
0.010
0.005
0.000
Frequency
0
5
10
15
20
25
30
Coverage

FIG. 7

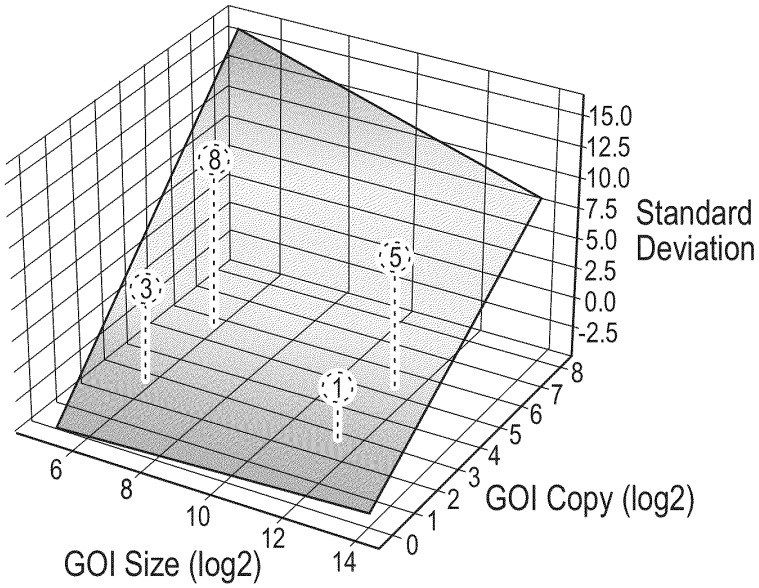
15.0
12.5
10.0
7.5
5.0
2.5
0.0
-2.5
Standard Deviation
8
5
3
1
6
8
10
12
14
GOI Size (log2)
0
1
2
3
4
5
6
7
8
GOI Copy (log2)

FIG. 8

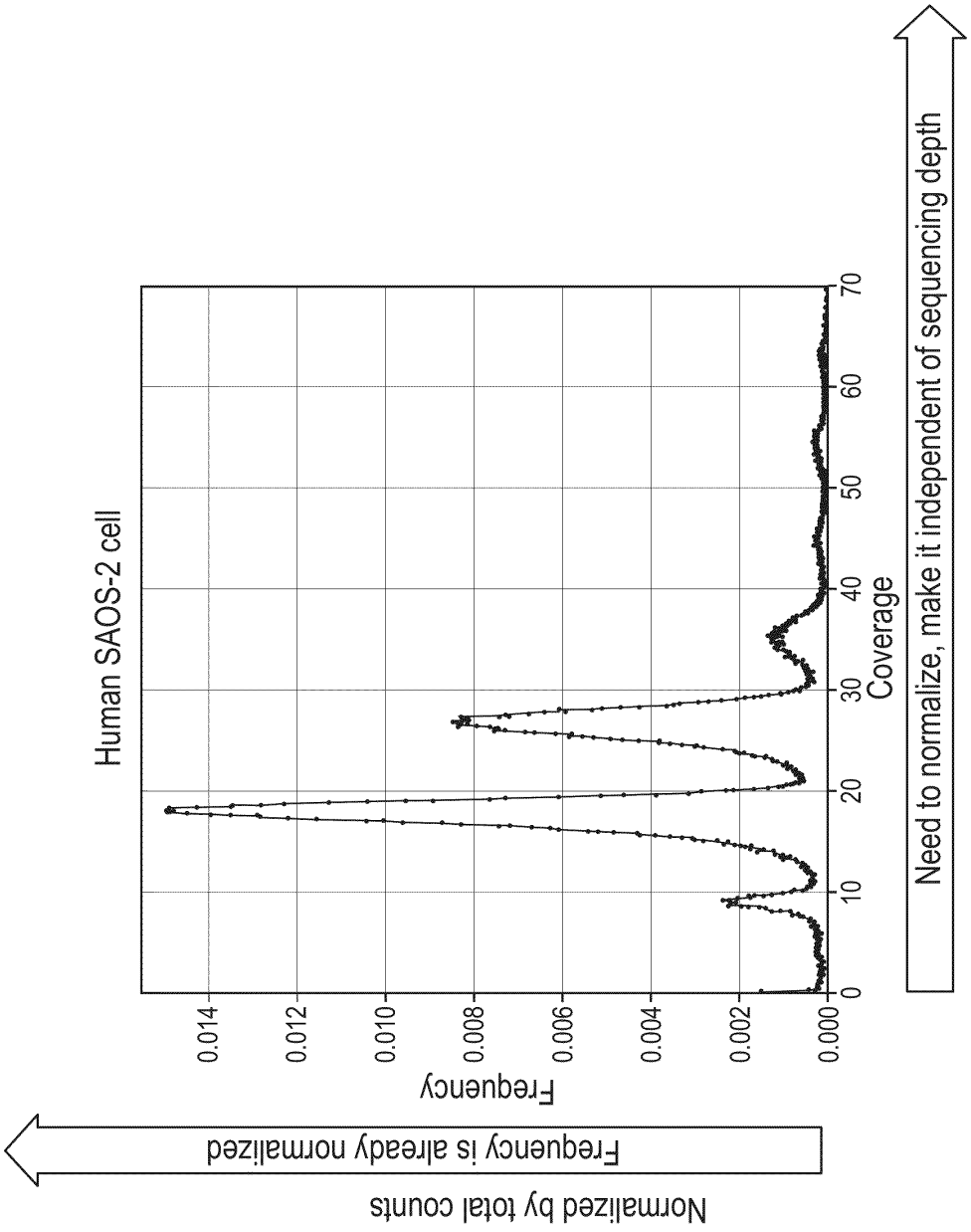
Human SAOS-2 cell
Frequency
0.000
0.002
0.004
0.006
0.008
0.010
0.012
0.014
Coverage
0
10
20
30
40
50
60
70
Normalized by total counts
Frequency is already normalized
Need to normalize, make it independent of sequencing depth

FIG. 9

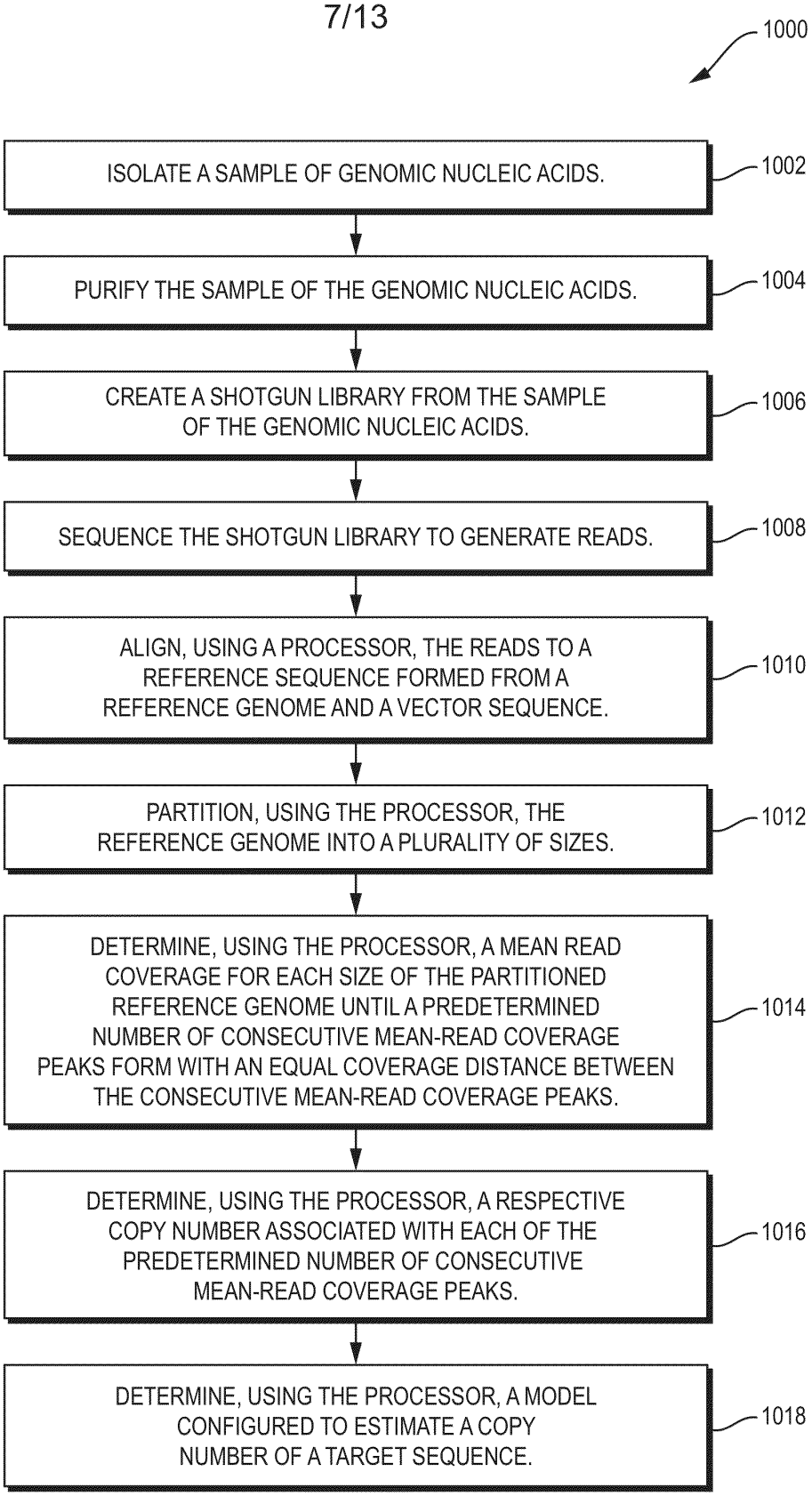
1000
ISOLATE A SAMPLE OF GENOMIC NUCLEIC ACIDS.
1002
PURIFY THE SAMPLE OF THE GENOMIC NUCLEIC ACIDS.
1004
CREATE A SHOTGUN LIBRARY FROM THE SAMPLE OF THE GENOMIC NUCLEIC ACIDS.
1006
SEQUENCE THE SHOTGUN LIBRARY TO GENERATE READS.
1008
ALIGN, USING A PROCESSOR, THE READS TO A REFERENCE SEQUENCE FORMED FROM A REFERENCE GENOME AND A VECTOR SEQUENCE.
1010
PARTITION, USING THE PROCESSOR, THE REFERENCE GENOME INTO A PLURALITY OF SIZES.
1012
DETERMINE, USING THE PROCESSOR, A MEAN READ COVERAGE FOR EACH SIZE OF THE PARTITIONED REFERENCE GENOME UNTIL A PREDETERMINED NUMBER OF CONSECUTIVE MEAN-READ COVERAGE PEAKS FORM WITH AN EQUAL COVERAGE DISTANCE BETWEEN THE CONSECUTIVE MEAN-READ COVERAGE PEAKS.
1014
DETERMINE, USING THE PROCESSOR, A RESPECTIVE COPY NUMBER ASSOCIATED WITH EACH OF THE PREDETERMINED NUMBER OF CONSECUTIVE MEAN-READ COVERAGE PEAKS.
1016
DETERMINE, USING THE PROCESSOR, A MODEL CONFIGURED TO ESTIMATE A COPY NUMBER OF A TARGET SEQUENCE.
1018

FIG. 10

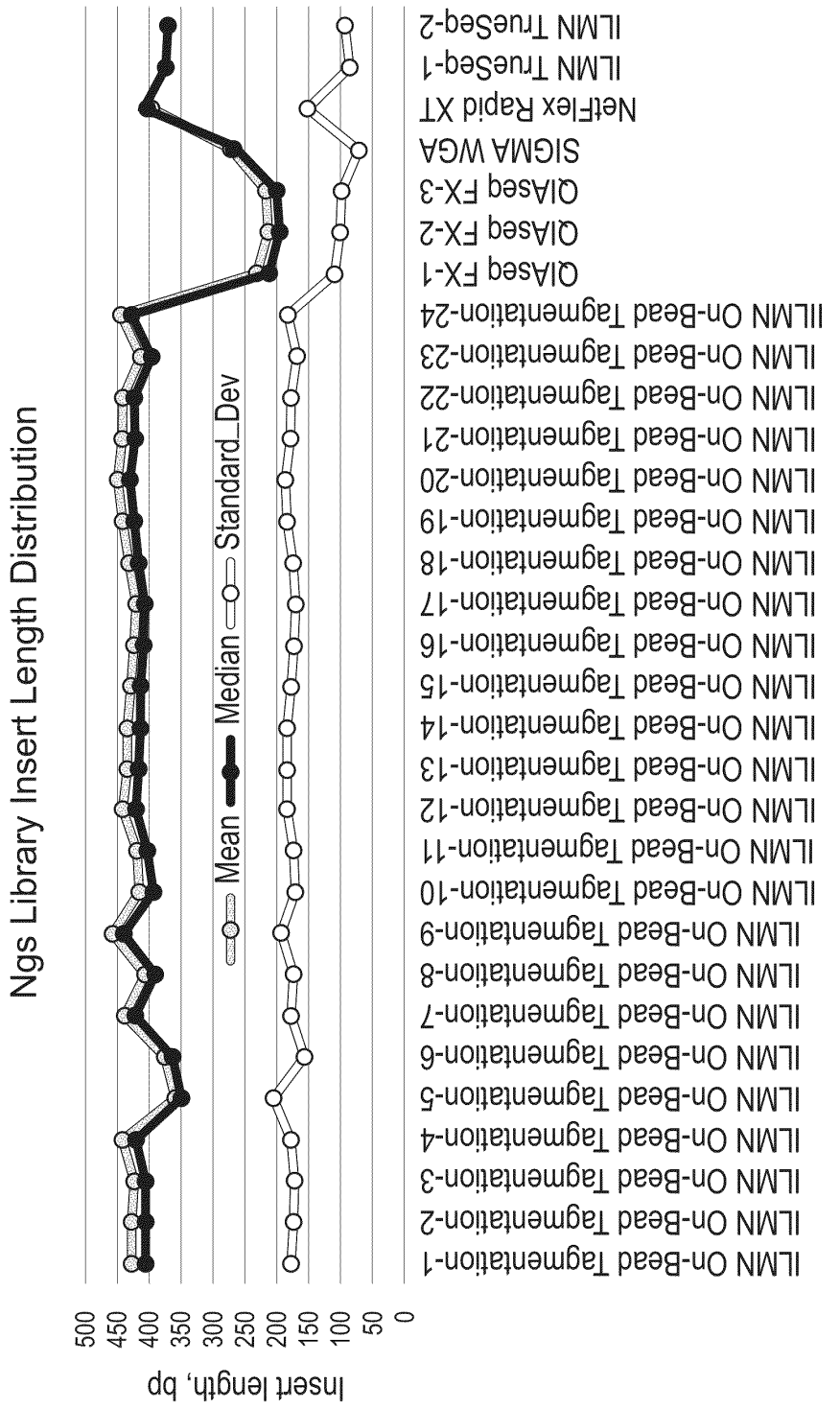
Ngs Library Insert Length Distribution
Mean
Median
Standard_Dev
Insert length, bp
0
50
100
150
200
250
300
350
400
450
500
ILMN On-Bead Tagmentation-1
ILMN On-Bead Tagmentation-2
ILMN On-Bead Tagmentation-3
ILMN On-Bead Tagmentation-4
ILMN On-Bead Tagmentation-5
ILMN On-Bead Tagmentation-6
ILMN On-Bead Tagmentation-7
ILMN On-Bead Tagmentation-8
ILMN On-Bead Tagmentation-9
ILMN On-Bead Tagmentation-10
ILMN On-Bead Tagmentation-11
ILMN On-Bead Tagmentation-12
ILMN On-Bead Tagmentation-13
ILMN On-Bead Tagmentation-14
ILMN On-Bead Tagmentation-15
ILMN On-Bead Tagmentation-16
ILMN On-Bead Tagmentation-17
ILMN On-Bead Tagmentation-18
ILMN On-Bead Tagmentation-19
ILMN On-Bead Tagmentation-20
ILMN On-Bead Tagmentation-21
ILMN On-Bead Tagmentation-22
ILMN On-Bead Tagmentation-23
ILMN On-Bead Tagmentation-24
QIAseq FX-1
QIAseq FX-2
QIAseq FX-3
SIGMA WGA
NetFlex Rapid XT
ILMN TrueSeq-1
ILMN TrueSeq-2

FIG. 11

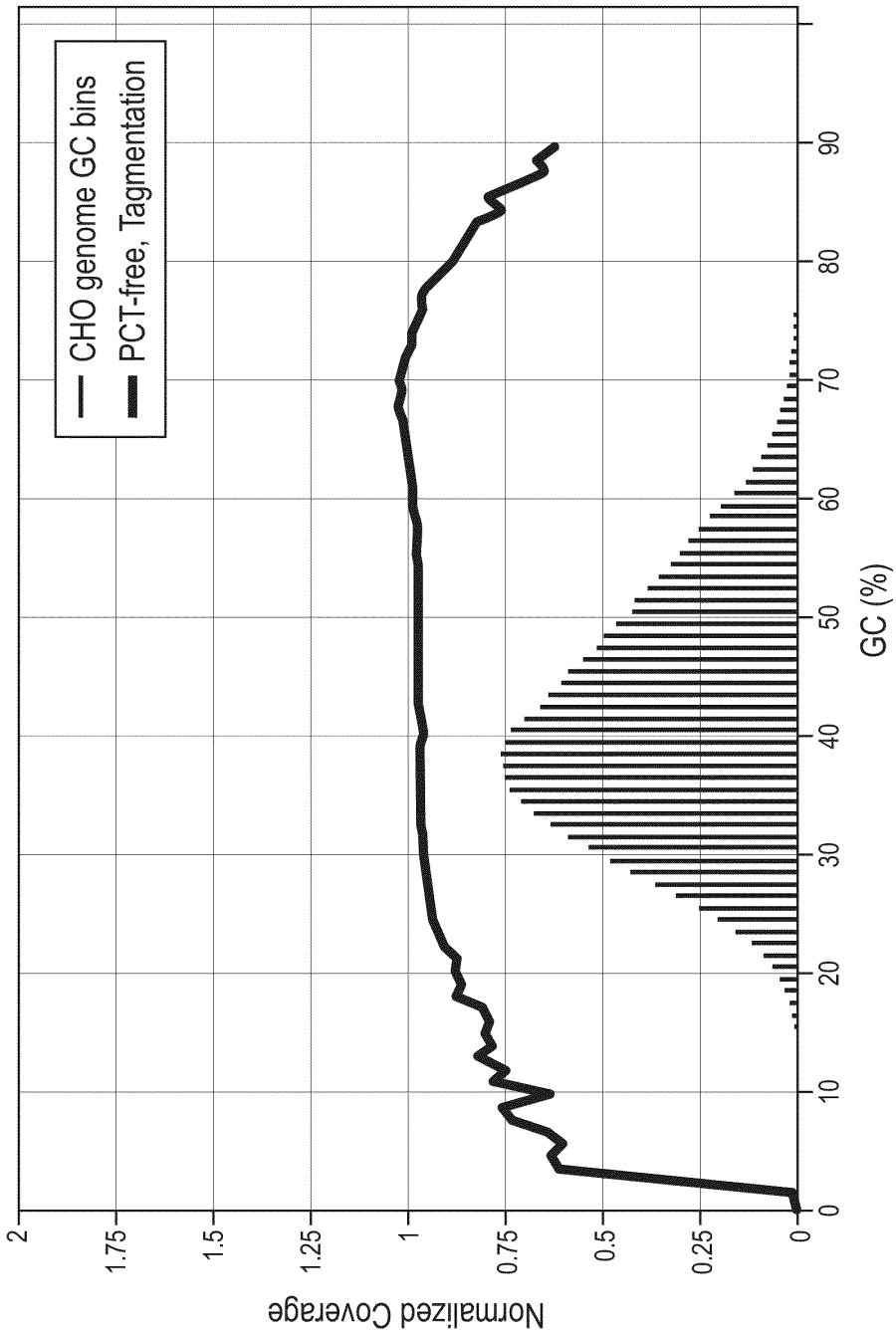
CHO genome GC bins
PCT-free, Tagmentation
Normalized Coverage
0
0.25
0.5
0.75
1
1.25
1.5
1.75
2
GC (%)
0
10
20
30
40
50
60
70
80
90

FIG. 12

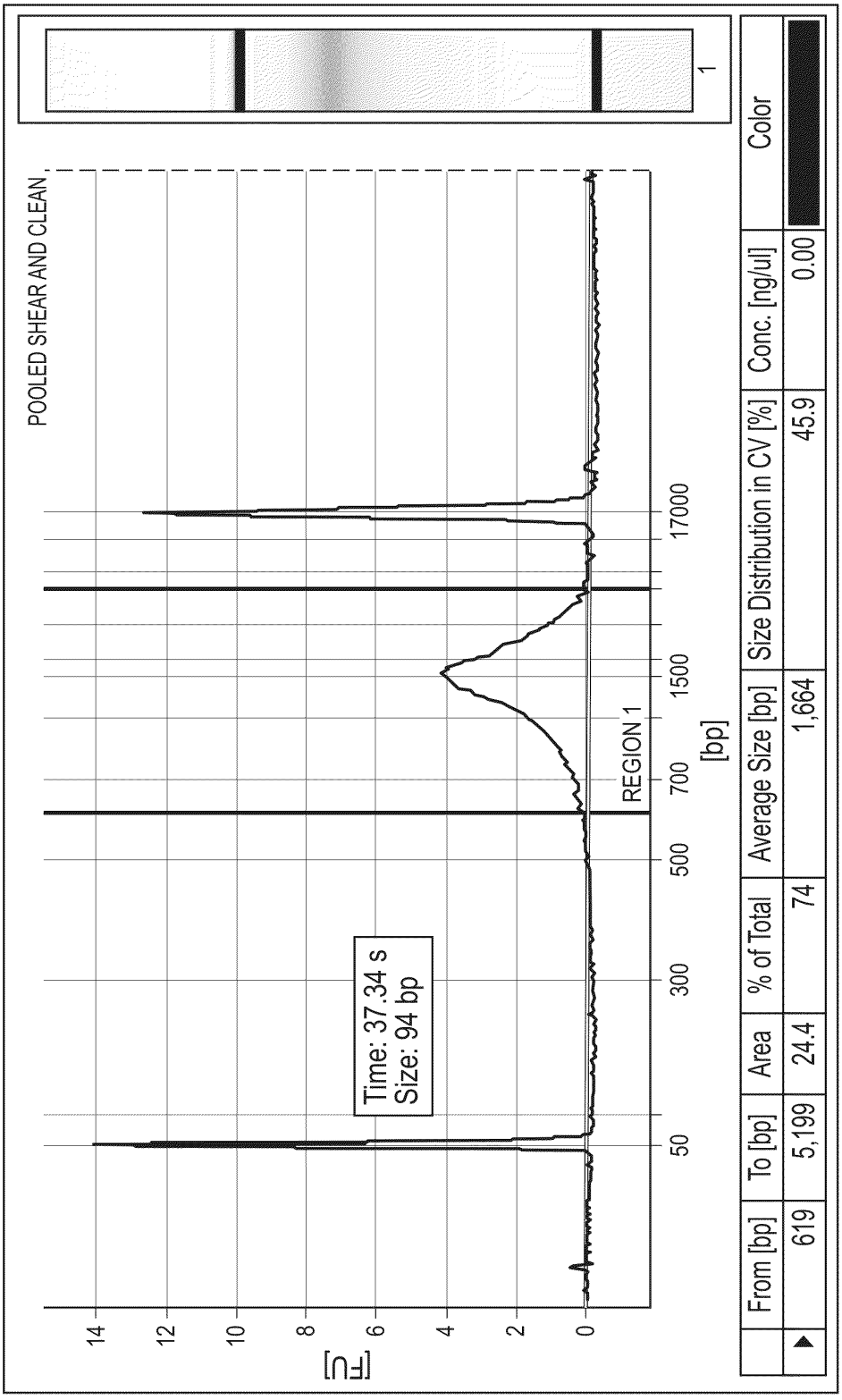
POOLED SHEAR AND CLEAN
[FU]
0
2
4
6
8
10
12
14
50
300
500
700
1500
17000
[bp]
REGION 1
Time: 37.34 s
Size: 94 bp
1
From [bp] | To [bp] | Area | % of Total | Average Size [bp] | Size Distribution in CV [%] | Conc. [ng/ul] | Color
619 | 5,199 | 24.4 | 74 | 1,664 | 45.9 | 0.00

FIG. 13

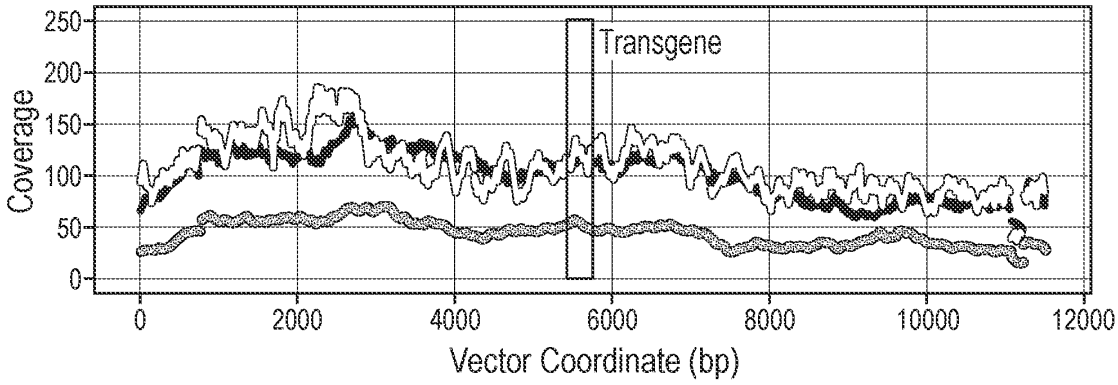


FIG. 14A

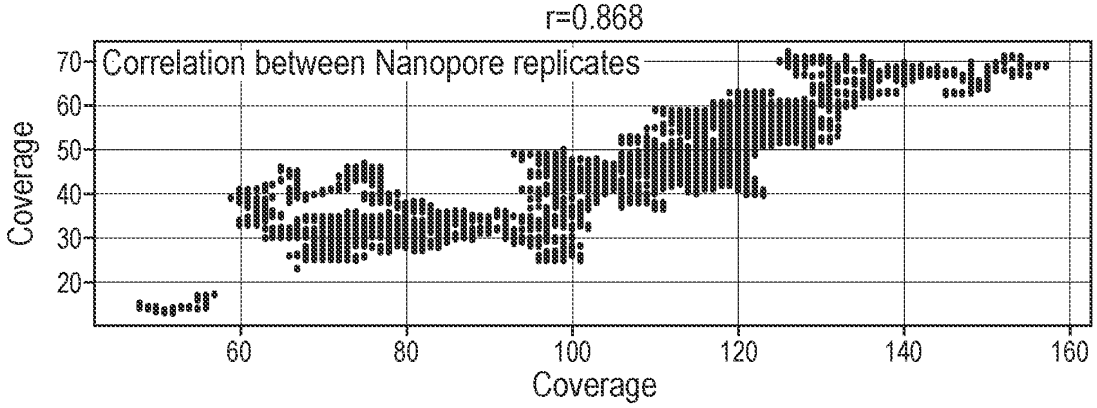


FIG. 14B

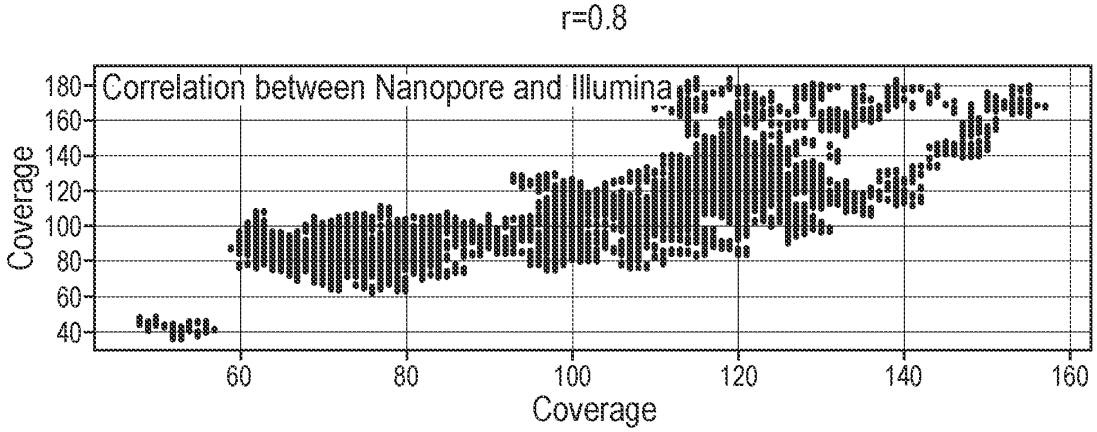


FIG. 14C

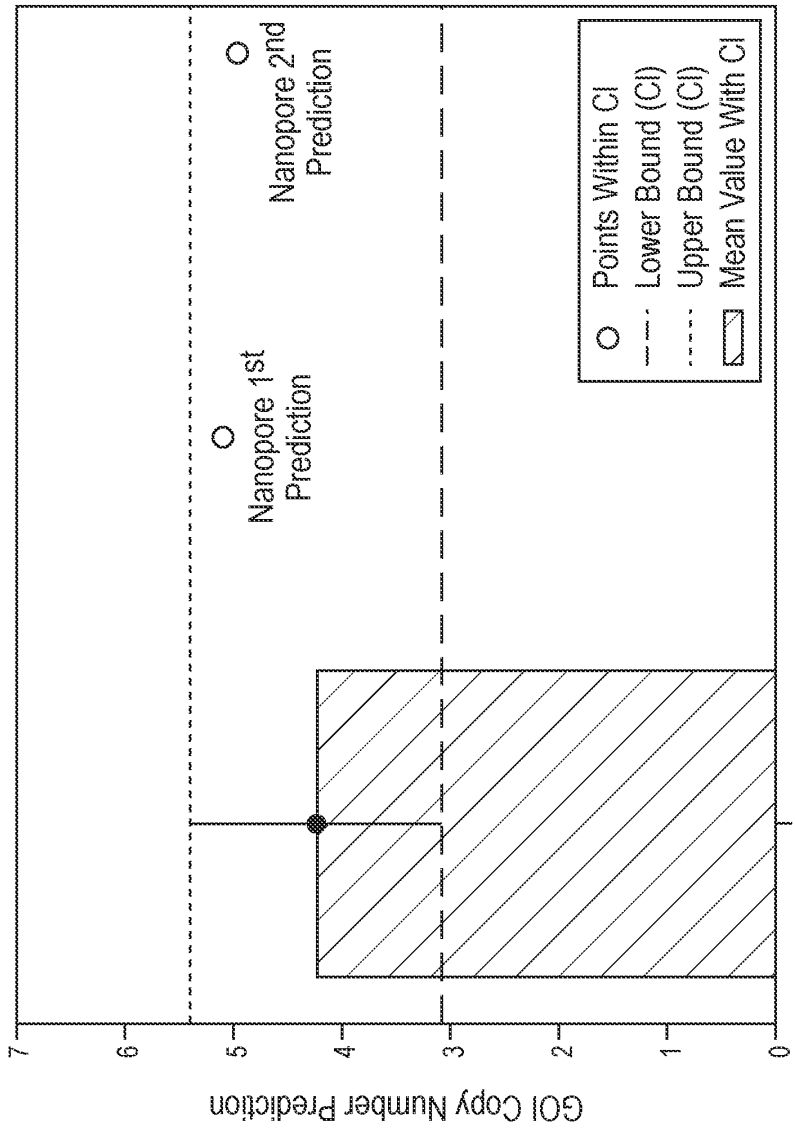
GOI Copy Number Prediction
0
1
2
3
4
5
6
7
Current Aptegra by Illumina
Nanopore 1st Prediction
Nanopore 2nd Prediction
Points Within CI
Lower Bound (CI)
Upper Bound (CI)
Mean Value With CI

FIG. 15

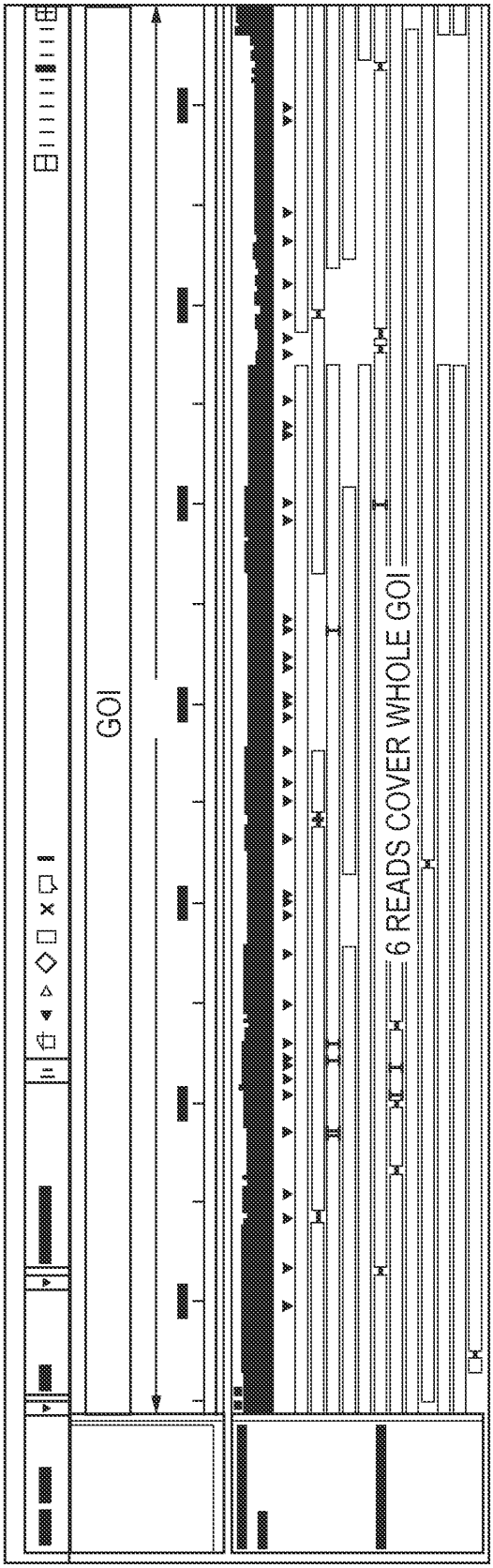


FIG. 16