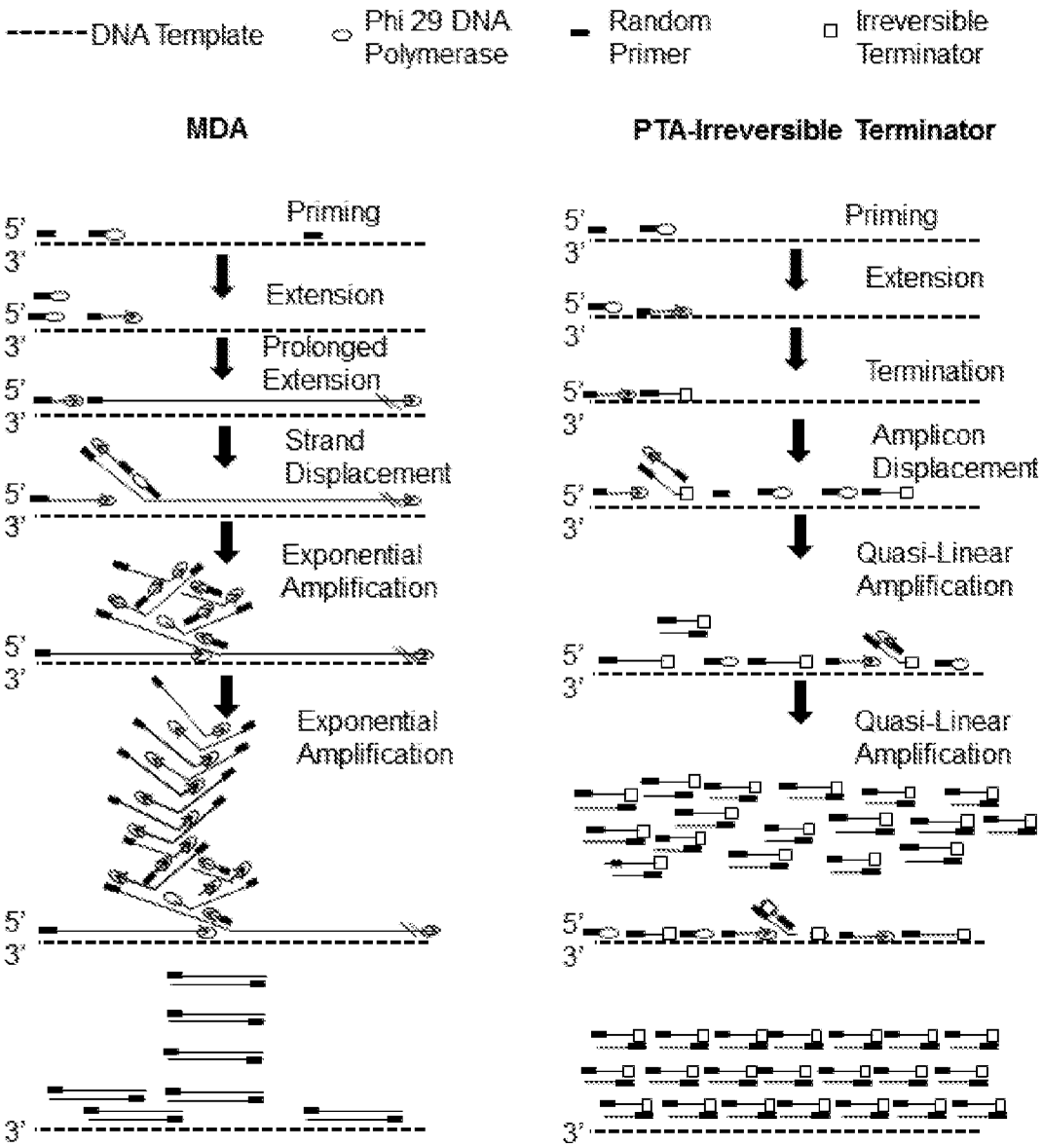

DNA Template
Phi 29 DNA Polymerase
Random Primer
Irreversible Terminator
MDA
PTA-Irreversible Terminator
Priming
Extension
Prolonged Extension
Strand Displacement
Exponential Amplification
Exponential Amplification
Priming
Extension
Termination
Amplicon Displacement
Quasi-Linear Amplification
Quasi-Linear Amplification
5'
3'

FIG. 1A

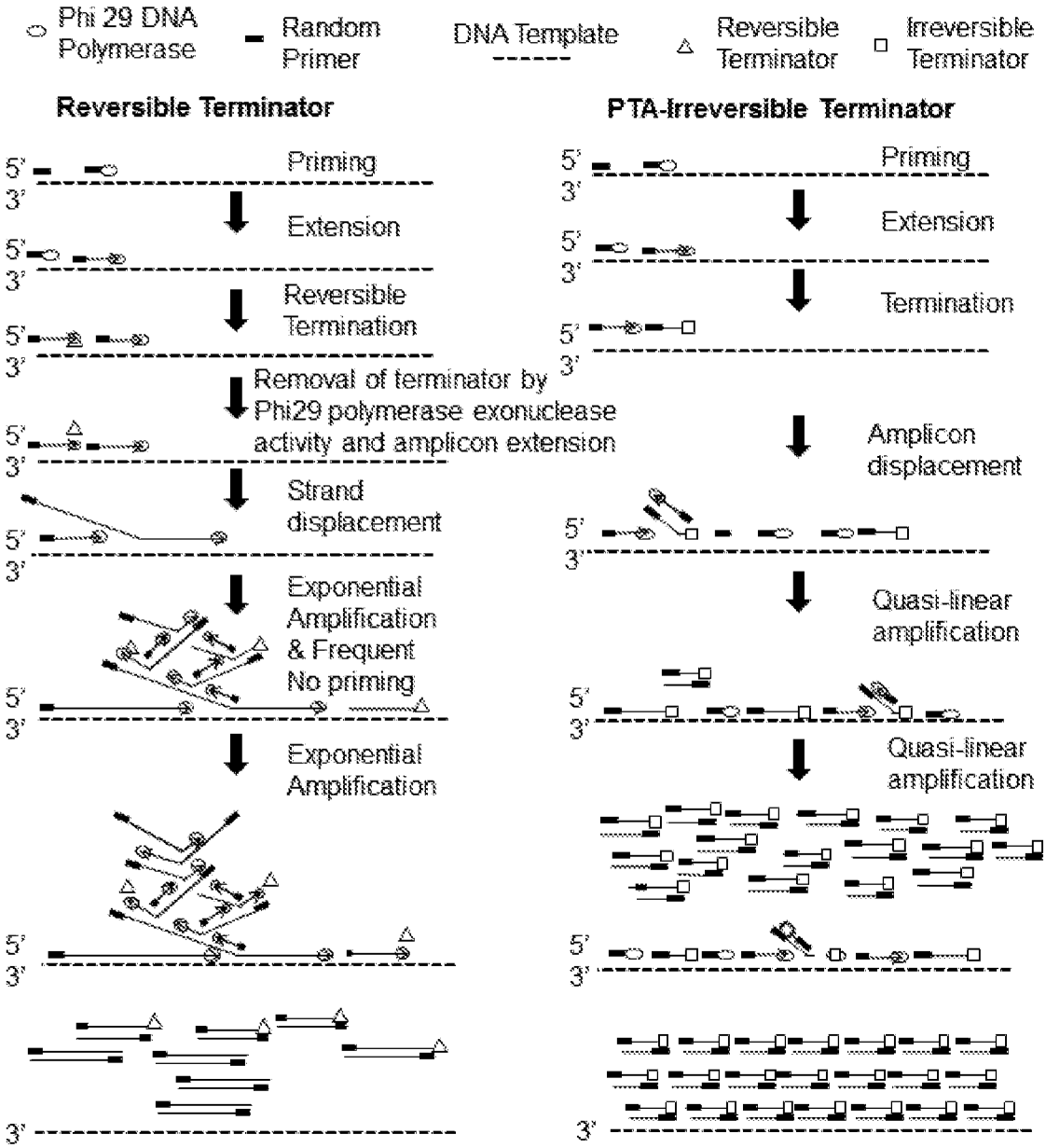

Phi 29 DNA Polymerase
Random Primer
DNA Template
Reversible Terminator
Irreversible Terminator
Reversible Terminator
PTA-Irreversible Terminator
Priming
Extension
Reversible Termination
Removal of terminator by Phi29 polymerase exonuclease activity and amplicon extension
Strand displacement
Exponential Amplification & Frequent No priming
Exponential Amplification
Priming
Extension
Termination
Amplicon displacement
Quasi-linear amplification
Quasi-linear amplification
5'
3'

FIG. 1B

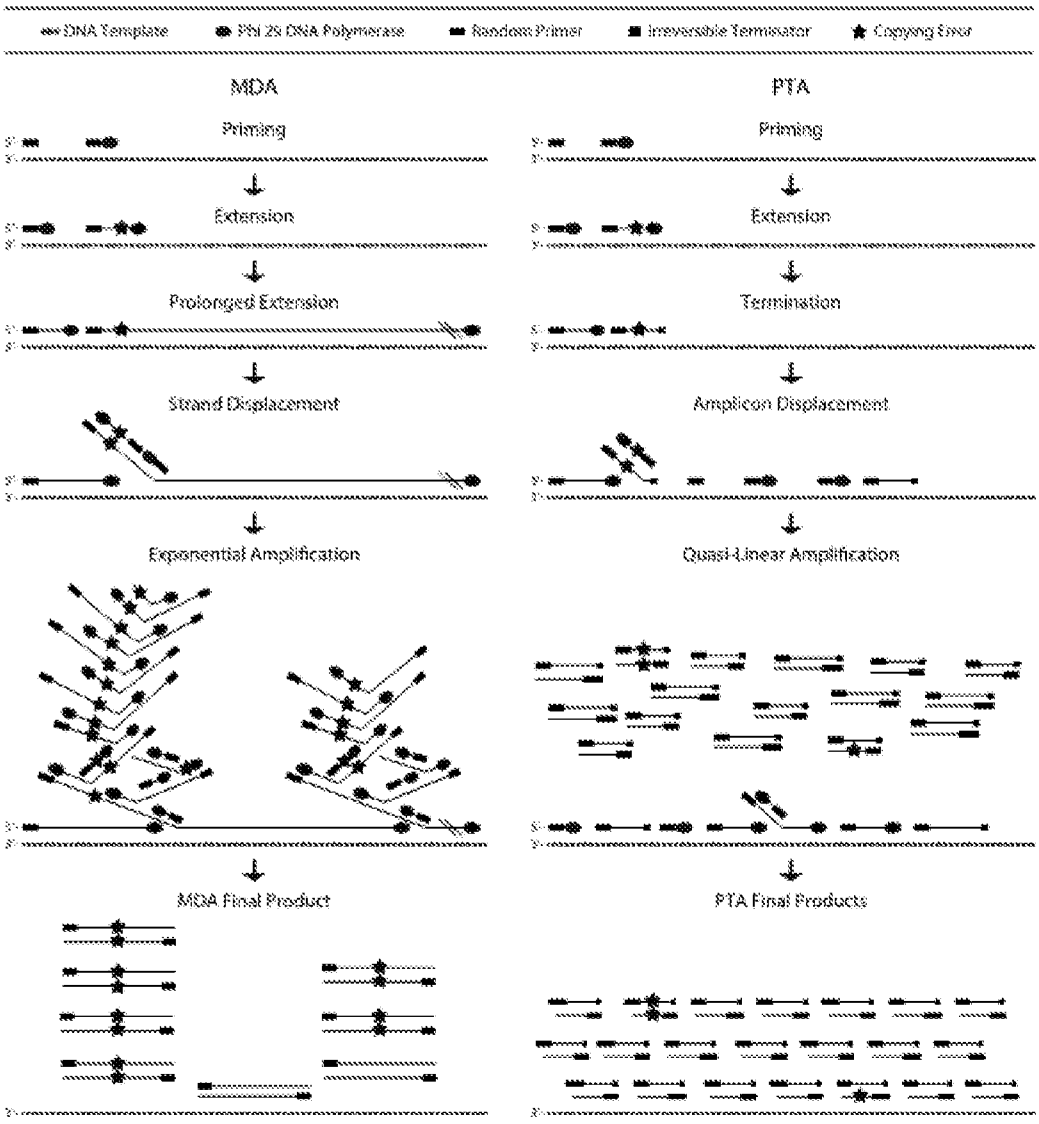

DNA Template
Phi 29 DNA Polymerase
Random Primer
Irreversible Terminator
Copying Error
MDA
Priming
Extension
Prolonged Extension
Strand Displacement
Exponential Amplification
MDA Final Product
PTA
Priming
Extension
Termination
Amplicon Displacement
Quasi-Linear Amplification
PTA Final Products

FIG. 1C

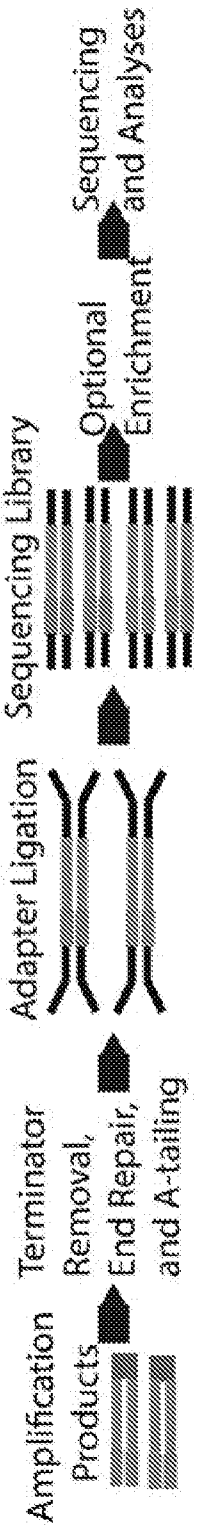

Amplification Products
Terminator Removal, End Repair, and A-tailing
Adapter Ligation
Sequencing Library
Optional Enrichment
Sequencing and Analyses

FIG. 1D

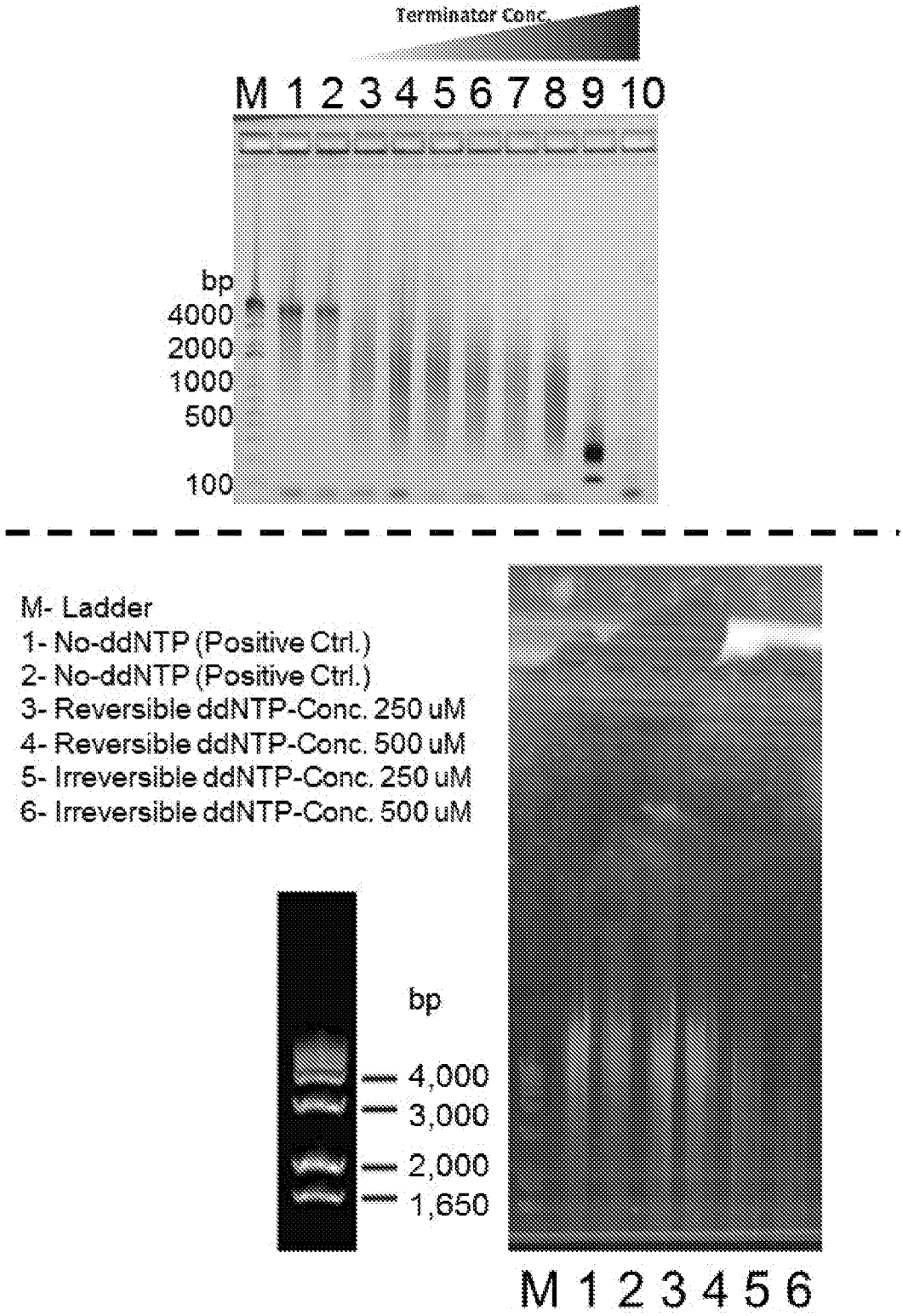

Terminator Conc.
M 1 2 3 4 5 6 7 8 9 10
bp
4000
2000
1000
500
100
M- Ladder
1- No-ddNTP (Positive Ctrl.)
2- No-ddNTP (Positive Ctrl.)
3- Reversible ddNTP-Conc. 250 uM
4- Reversible ddNTP-Conc. 500 uM
5- Irreversible ddNTP-Conc. 250 uM
6- Irreversible ddNTP-Conc. 500 uM
bp
4,000
3,000
2,000
1,650
M 1 2 3 4 5 6

FIG. 2A

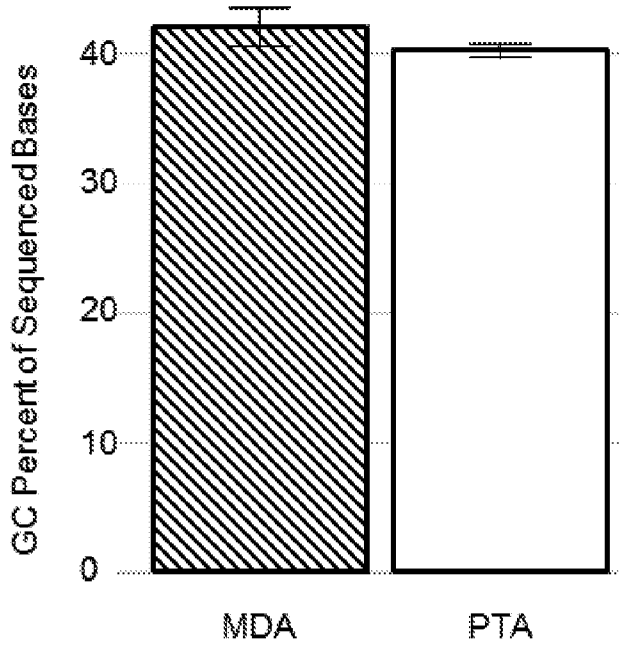

GC Percent of Sequenced Bases
40
30
20
10
0
MDA
PTA

FIG. 2B

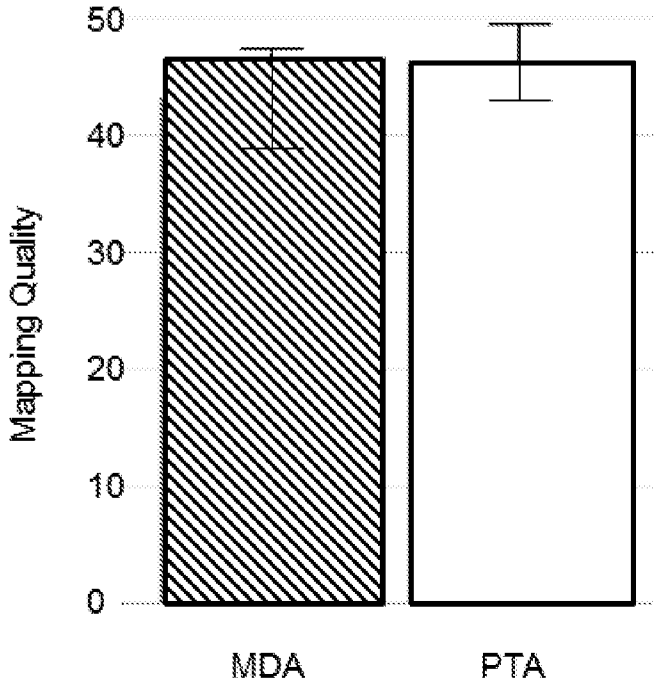

Mapping Quality
50
40
30
20
10
0
MDA
PTA

FIG. 2C

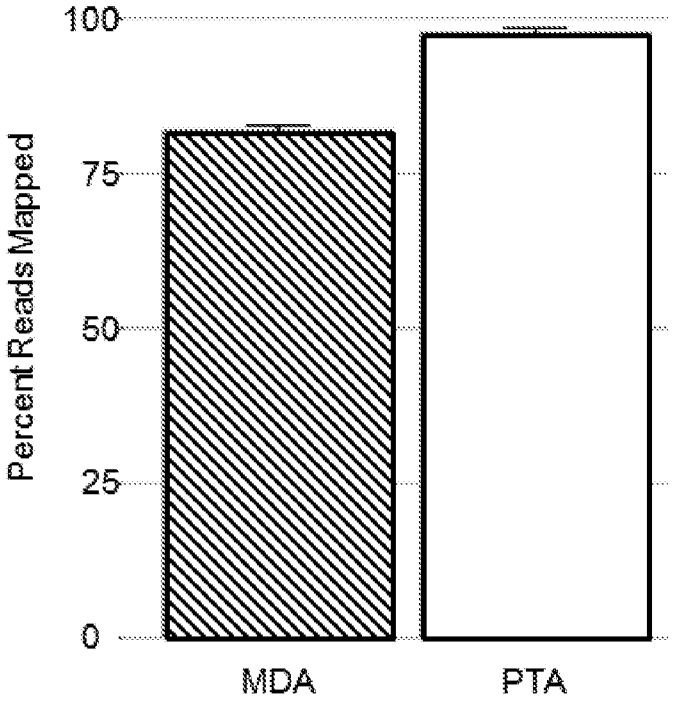

100
75
50
25
0
Percent Reads Mapped
MDA
PTA

FIG. 2D

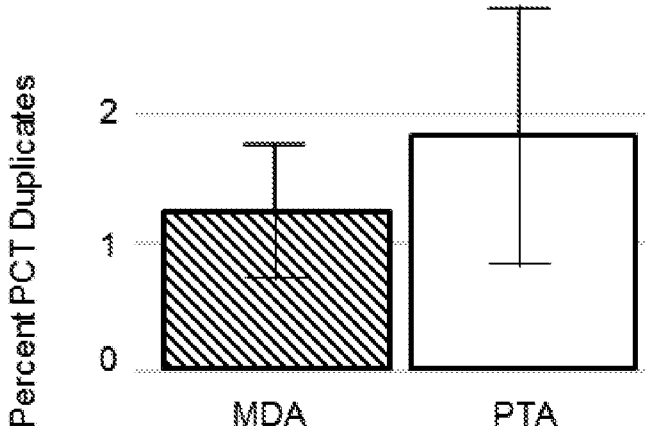

2
1
0
Percent PCT Duplicates
MDA
PTA

FIG. 2E

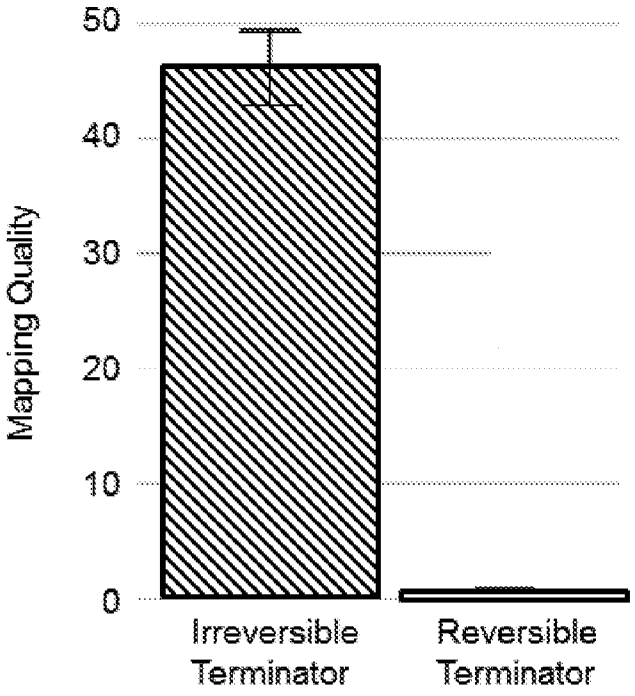

50
40
30
20
10
0
Mapping Quality
Irreversible Terminator
Reversible Terminator

FIG. 3A

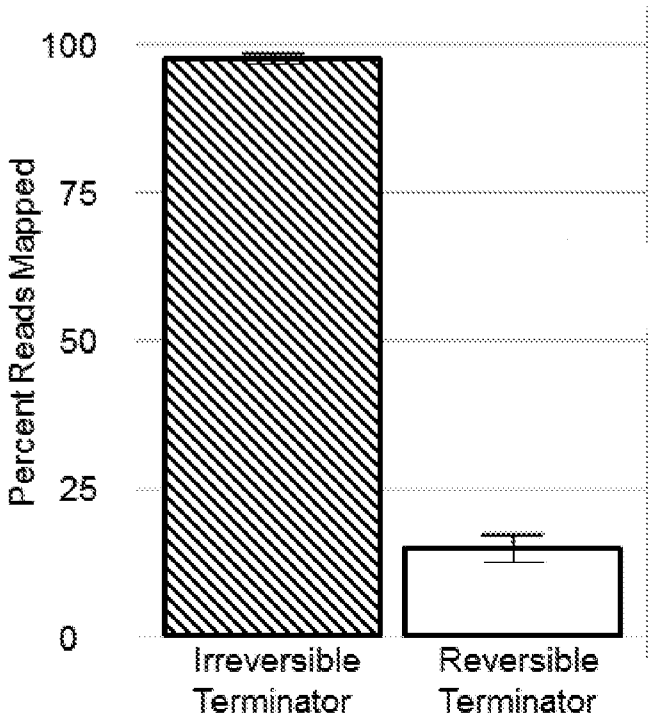

100
75
50
25
0
Percent Reads Mapped
Irreversible Terminator
Reversible Terminator

FIG. 3B

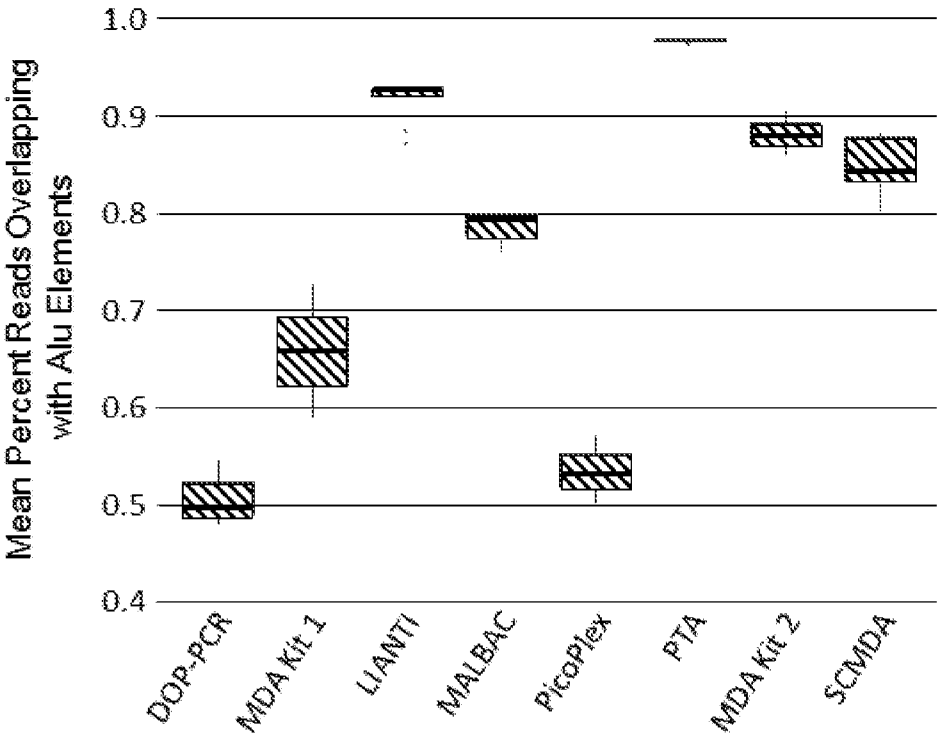

Mean Percent Reads Overlapping with Alu Elements
1.0
0.9
0.8
0.7
0.6
0.5
0.4
DOP-PCR
MDA Kit 1
LIANTI
MALBAC
PicoPlex
PTA
MDA Kit 2
SCMDA

FIG. 3C

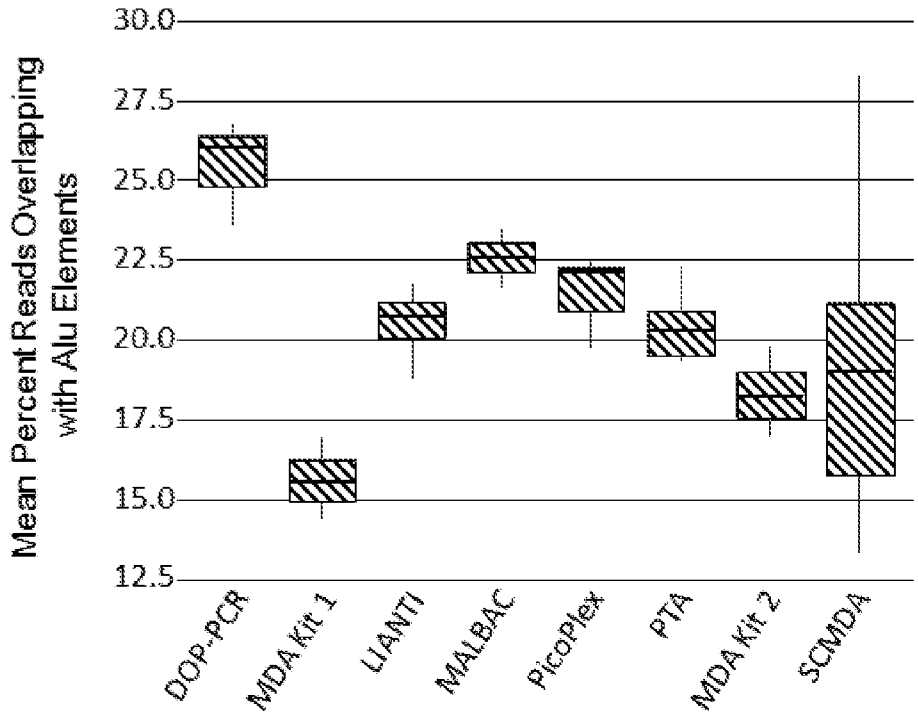

Mean Percent Reads Overlapping with Alu Elements
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
DOP-PCR
MDA Kit 1
LIANTI
MALBAC
PicoPlex
PTA
MDA Kit 2
SCMDA

FIG. 3D

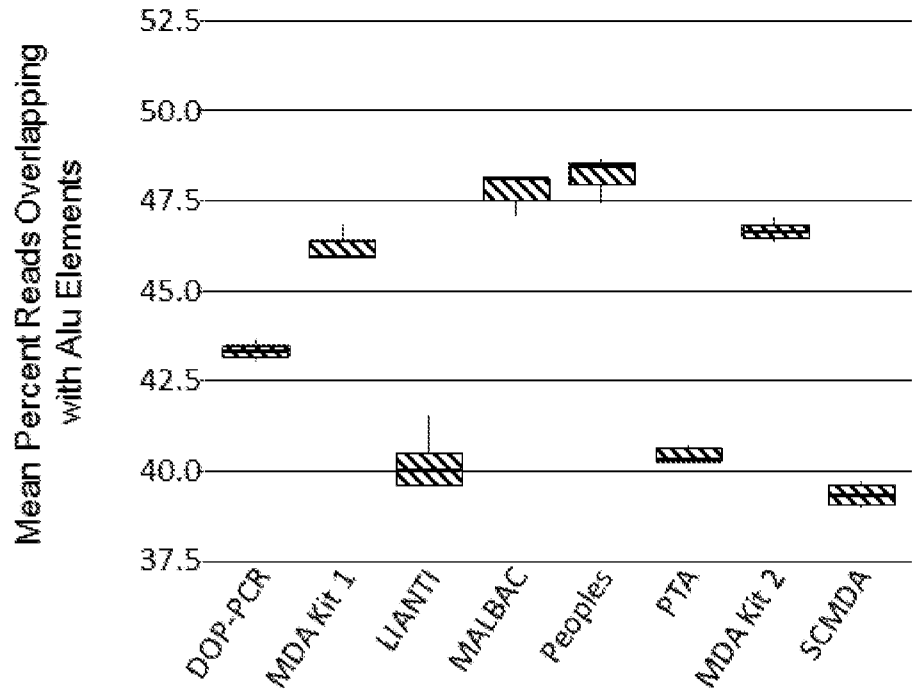

Mean Percent Reads Overlapping with Alu Elements
52.5
50.0
47.5
45.0
42.5
40.0
37.5
DOP-PCR
MDA Kit 1
LIANTI
MALBAC
Peoples
PTA
MDA Kit 2
SCMDA

FIG. 3E

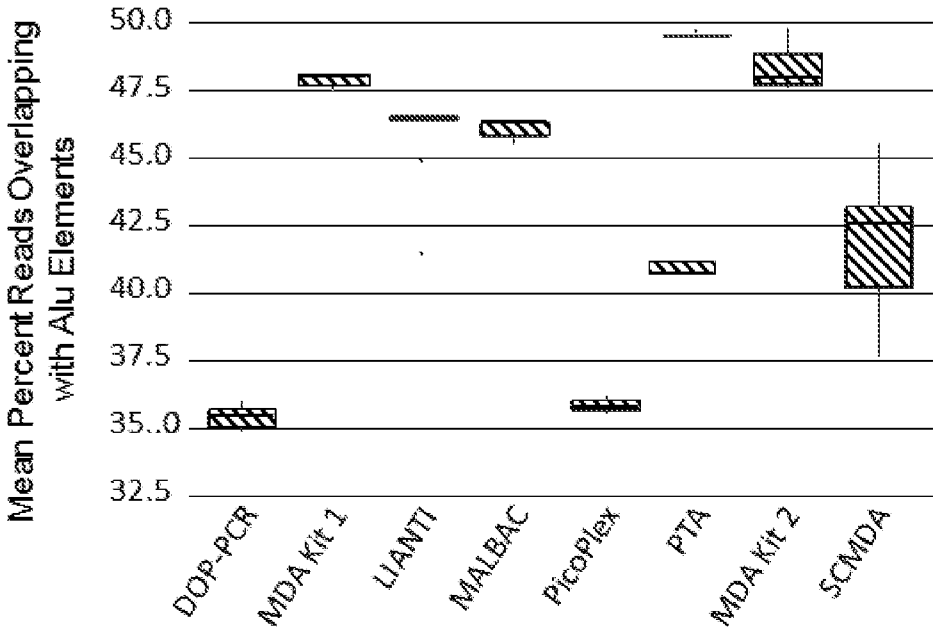

Mean Percent Reads Overlapping with Alu Elements
50.0
47.5
45.0
42.5
40.0
37.5
35..0
32.5
DOP-PCR
MDA Kit 1
LIANTI
MALBAC
PicoPlex
PTA
MDA Kit 2
SCMDA

FIG. 3F

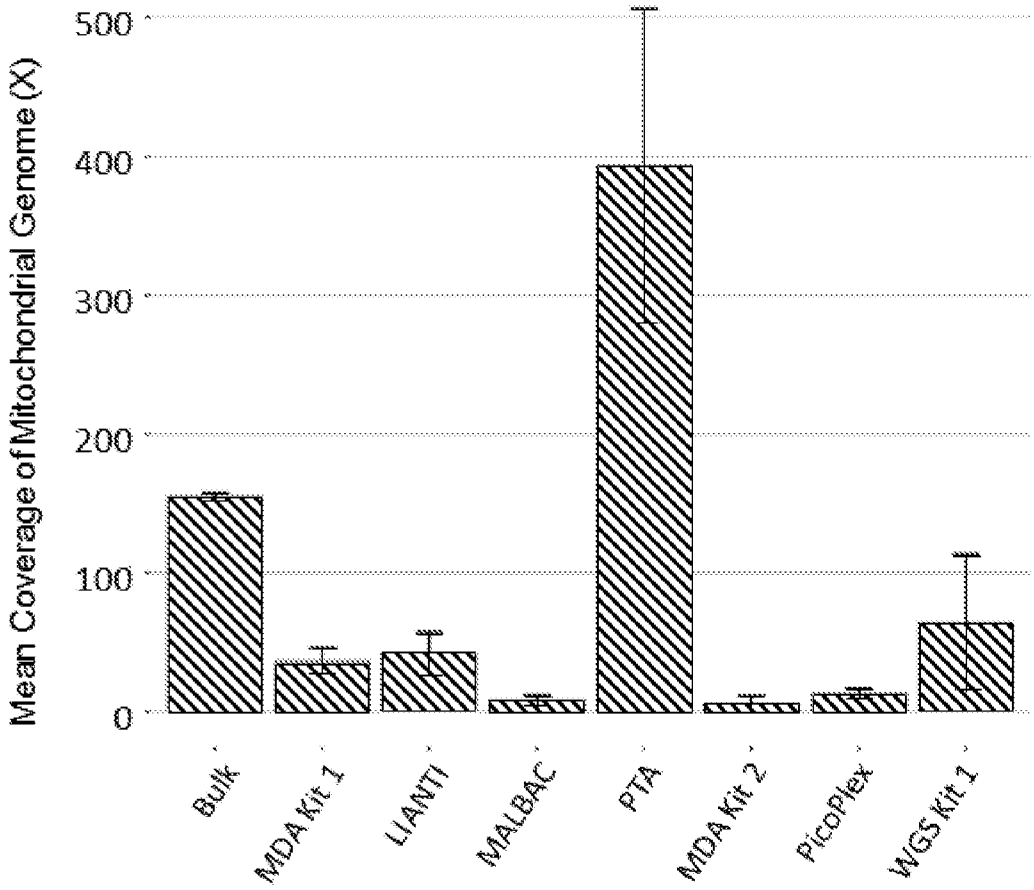

Mean Coverage of Mitochondrial Genome (X)
500
400
300
200
100
0
Bulk
MDA Kit 1
LIANTI
MALBAC
PTA
MDA Kit 2
PicoPlex
WGS Kit 1

FIG. 3G

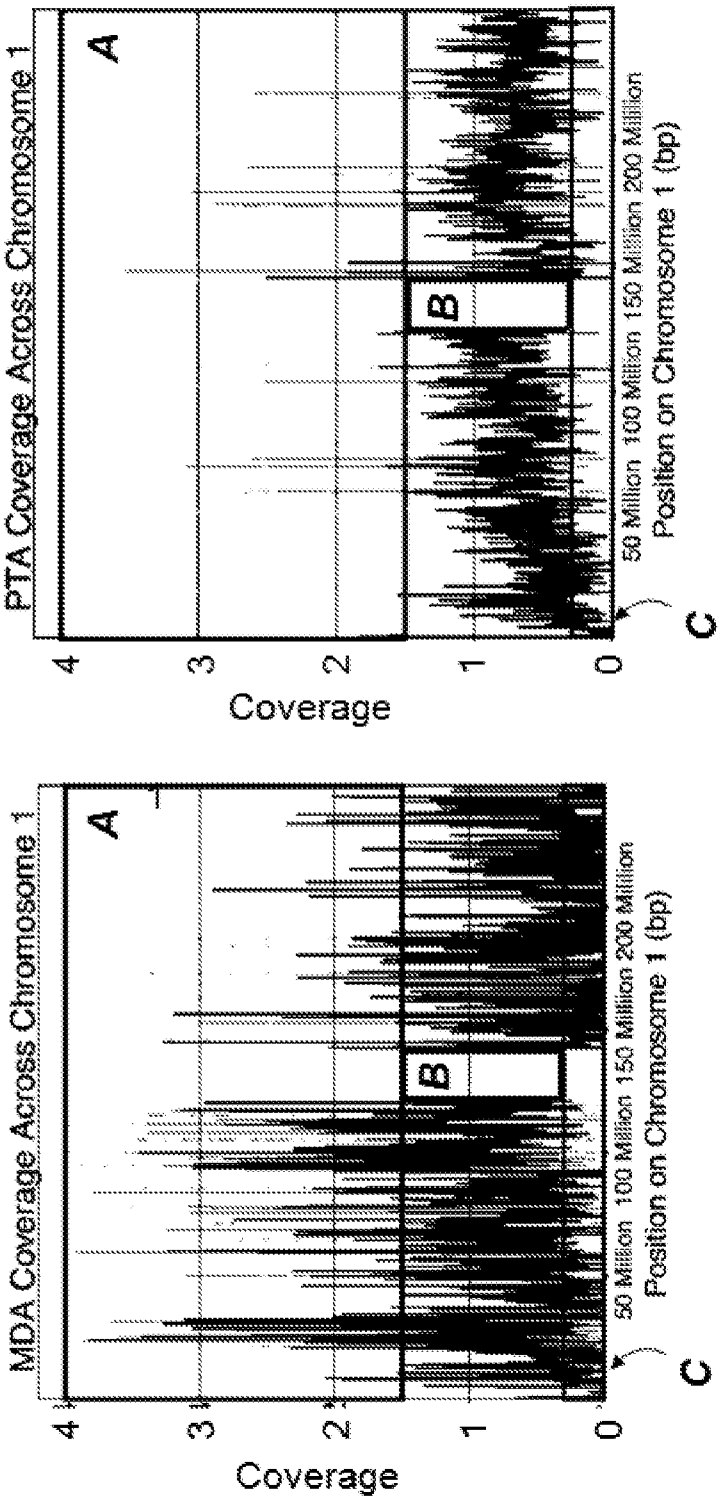

PTA Coverage Across Chromosome 1
A
B
C
Coverage
0
1
2
3
4
50 Million 100 Million 150 Million 200 Million
Position on Chromosome 1 (bp)
MDA Coverage Across Chromosome 1
A
B
C
Coverage
0
1
2
3
4
50 Million 100 Million 150 Million 200 Million
Position on Chromosome 1 (bp)

FIG. 4A

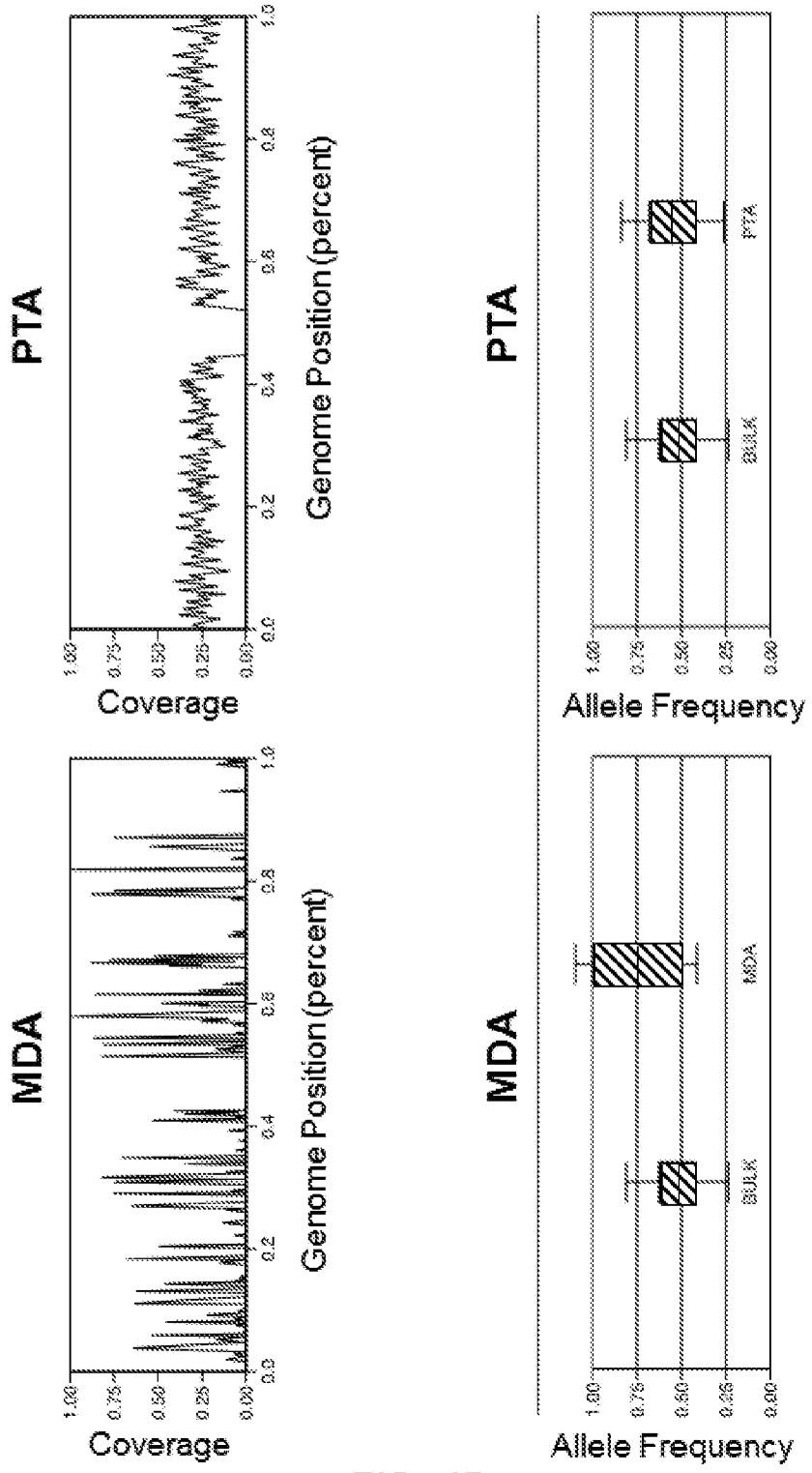

PTA
Genome Position (percent)
Coverage
MDA
Genome Position (percent)
Coverage
PTA
Allele Frequency
PTA
BULK
MDA
Allele Frequency
MDA
BULK

FIG. 4B

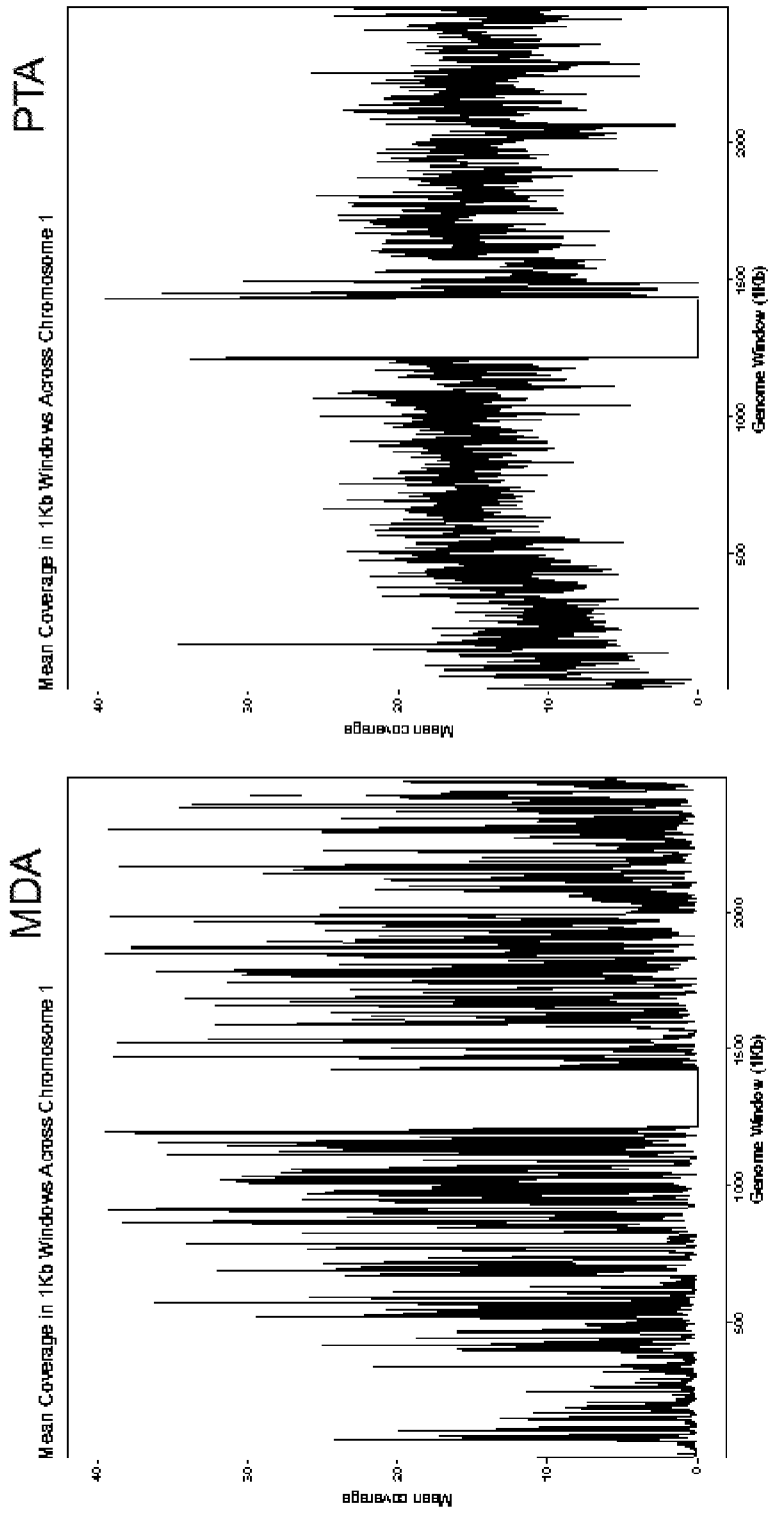

PTA
Mean Coverage in 1Kb Windows Across Chromosome 1
Mean coverage
Genome Window (1Kb)
MDA
Mean Coverage in 1Kb Windows Across Chromosome 1
Mean coverage
Genome Window (1Kb)

FIG. 4C

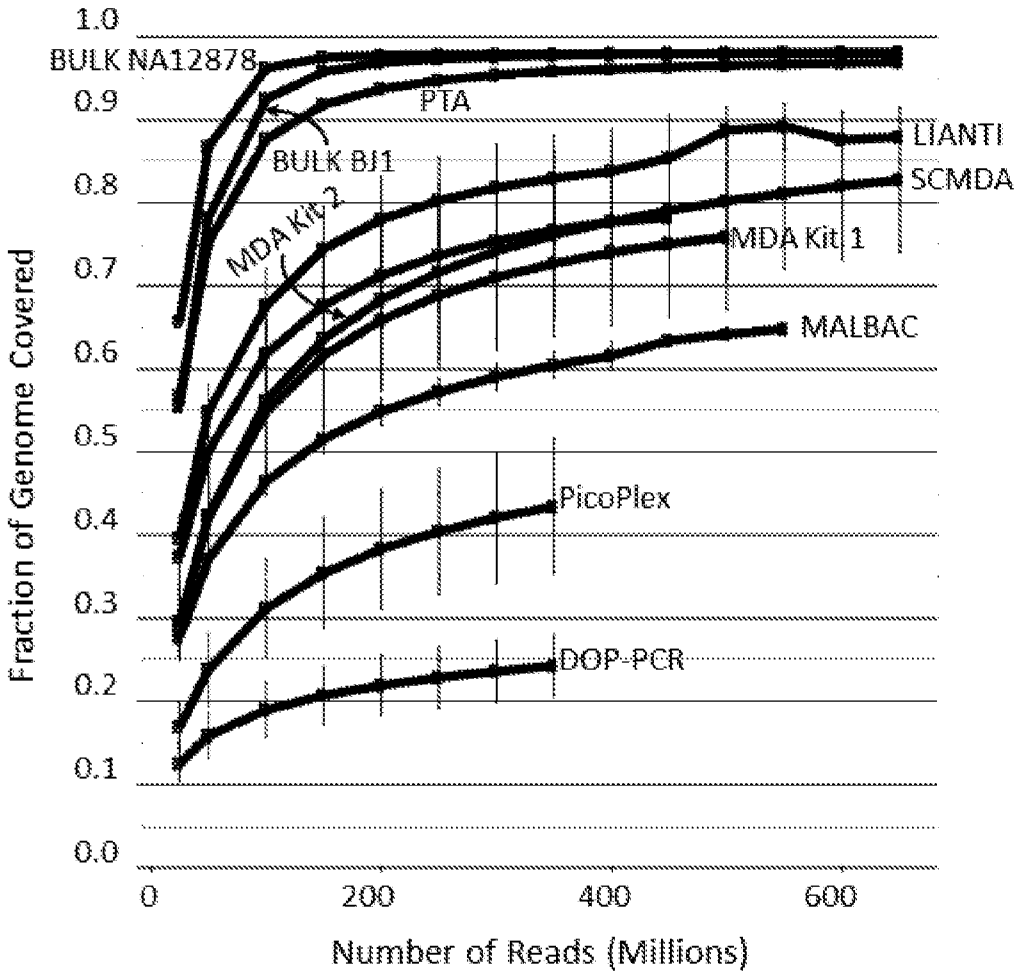

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Fraction of Genome Covered
0
200
400
600
Number of Reads (Millions)
BULK NA12878
PTA
BULK BJ1
MDA Kit 2
LIANTI
SCMDA
MDA Kit 1
MALBAC
PicoPlex
DOP-PCR

FIG. 5A

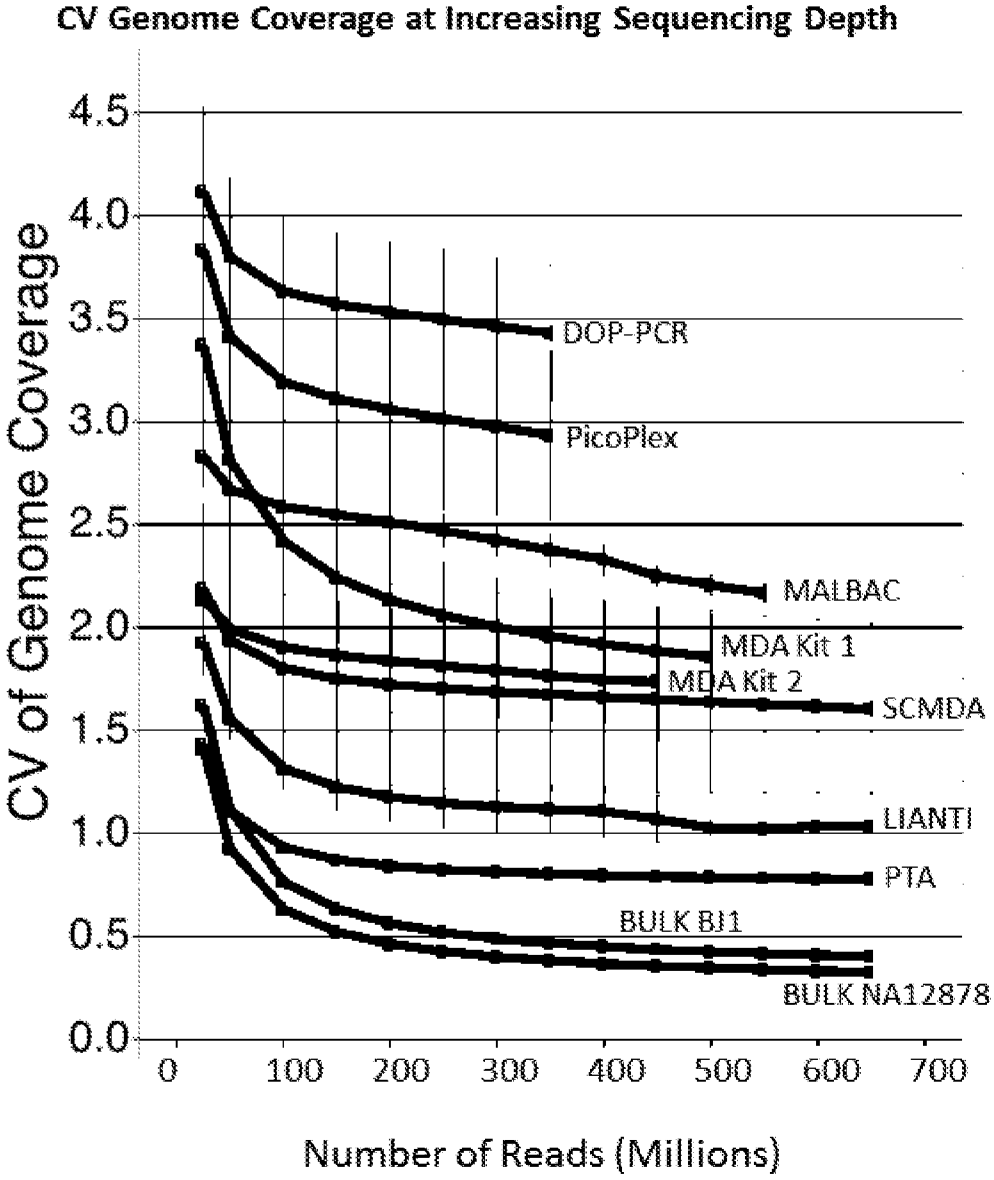

CV Genome Coverage at Increasing Sequencing Depth
CV of Genome Coverage
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
700
Number of Reads (Millions)
DOP-PCR
PicoPlex
MALBAC
MDA Kit 1
MDA Kit 2
SCMDA
LIANTI
PTA
BULK BJ1
BULK NA12878

FIG. 5B

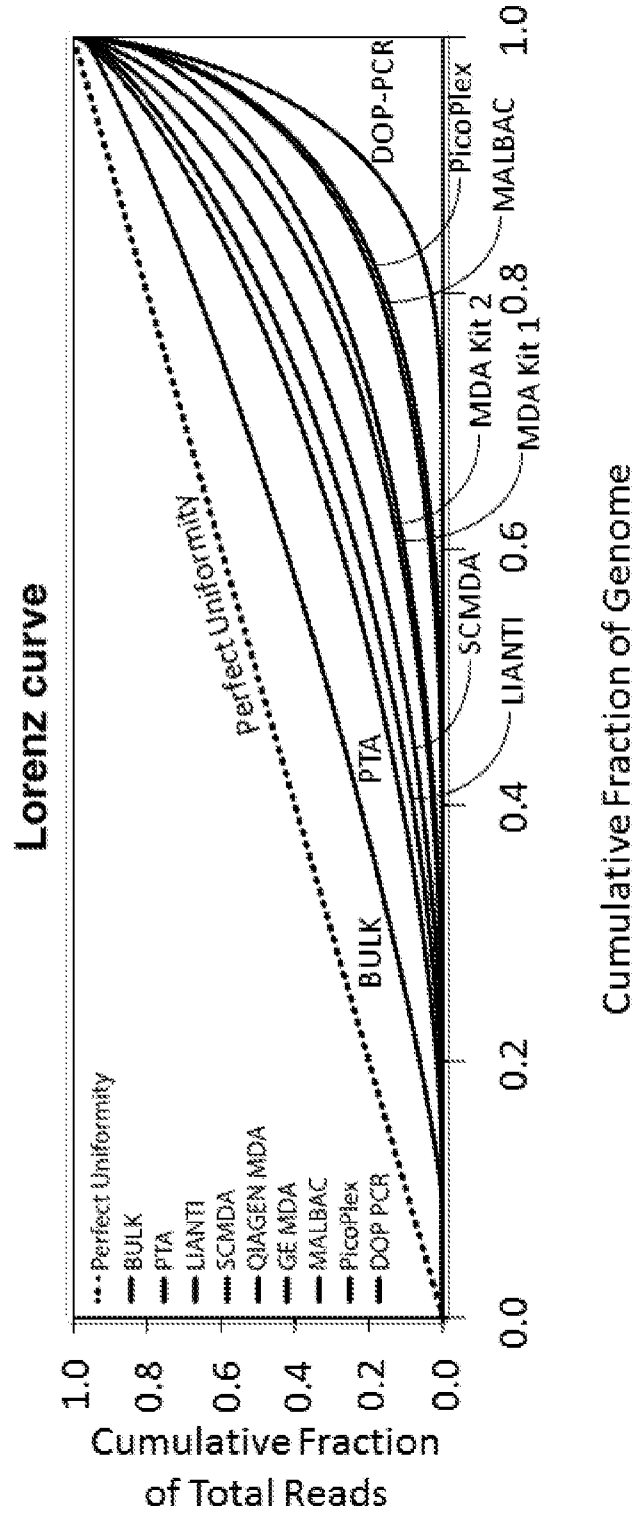
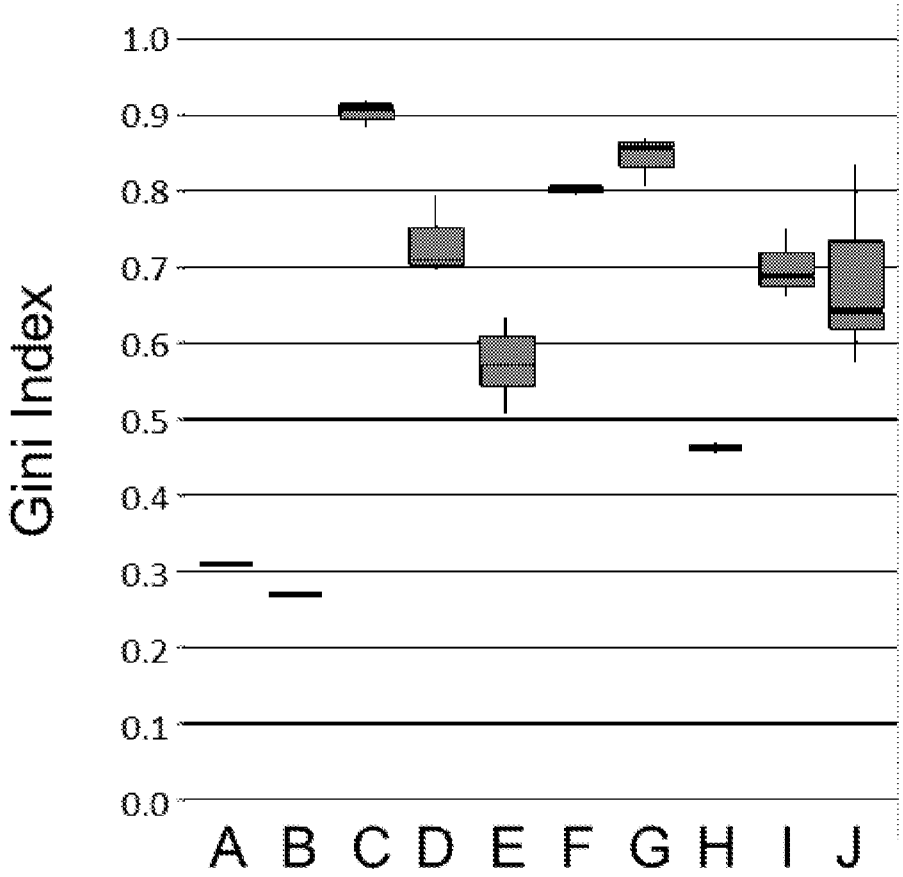

Lorenz curve
Perfect Uniformity
BULK
PTA
LIANTI
SCMDA
QIAGEN MDA
GE MDA
MALBAC
PicoPlex
DOP PCR
DOP-PCR
PicoPlex
MALBAC
MDA Kit 2
MDA Kit 1
SCMDA
LIANTI
PTA
BULK
0.0
0.2
0.4
0.6
0.8
1.0
Cumulative Fraction of Genome
Cumulative Fraction of Total Reads

FIG. 5C



A. BULK BJ1
B. BULK NA12878
C. DOP-PCR
D. MDA Kit 1
E. LIANTI
F. MALBEC
G. PicoPLex
H. PTA
I. MDA Kit 2
J. SCMDA

FIG. 5D

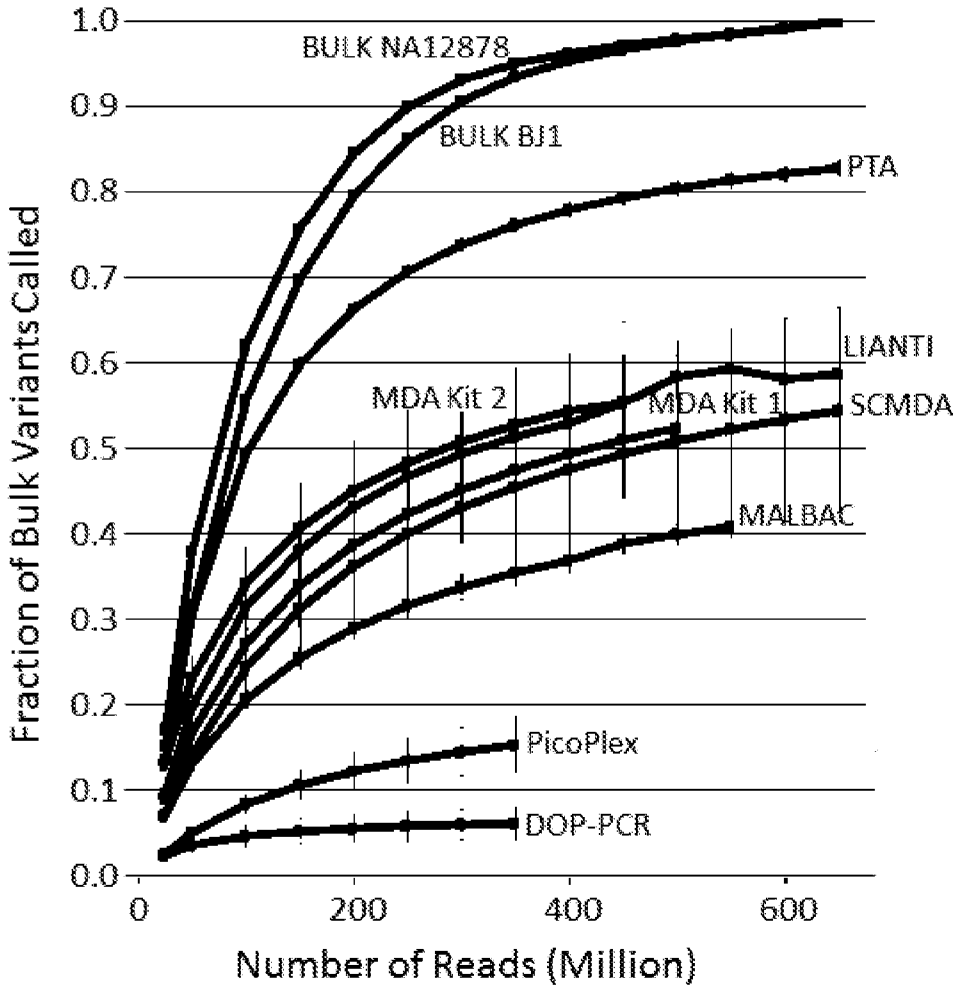

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Fraction of Bulk Variants Called
0
200
400
600
Number of Reads (Million)
BULK NA12878
BULK BJ1
PTA
LIANTI
MDA Kit 2
MDA Kit 1
SCMDA
MALBAC
PicoPlex
DOP-PCR

FIG. 5E

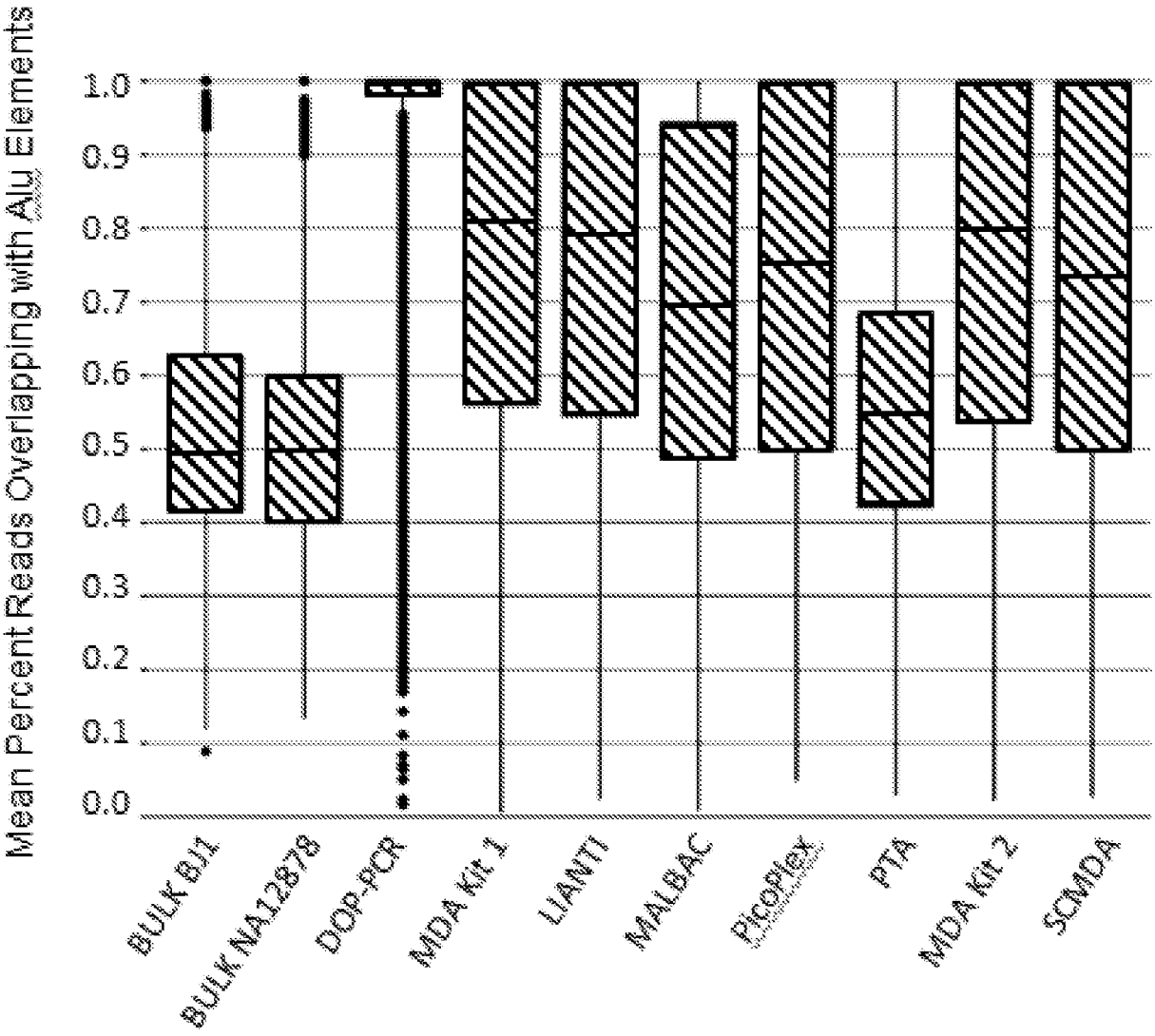

Mean Percent Reads Overlapping with Alu Elements
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
BULK BJ1
BULK NA12878
DOP-PCR
MDA Kit 1
LIANTI
MALBAC
PicoPlex
PTA
MDA Kit 2
SCMDA

FIG. 5F

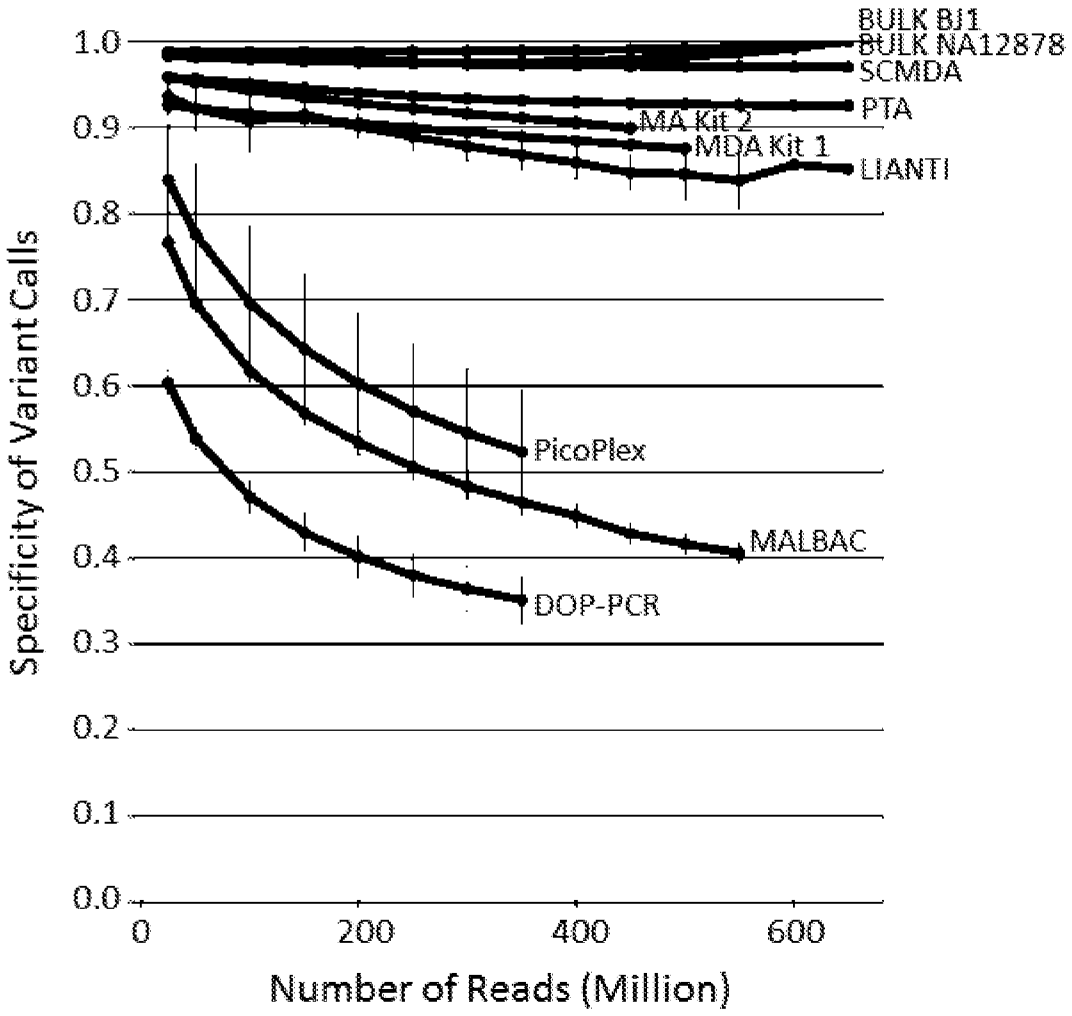

BULK BJ1
BULK NA12878
SCMDA
PTA
MA Kit 2
MDA Kit 1
LIANTI
PicoPlex
MALBAC
DOP-PCR
Specificity of Variant Calls
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
200
400
600
Number of Reads (Million)

FIG. 5G

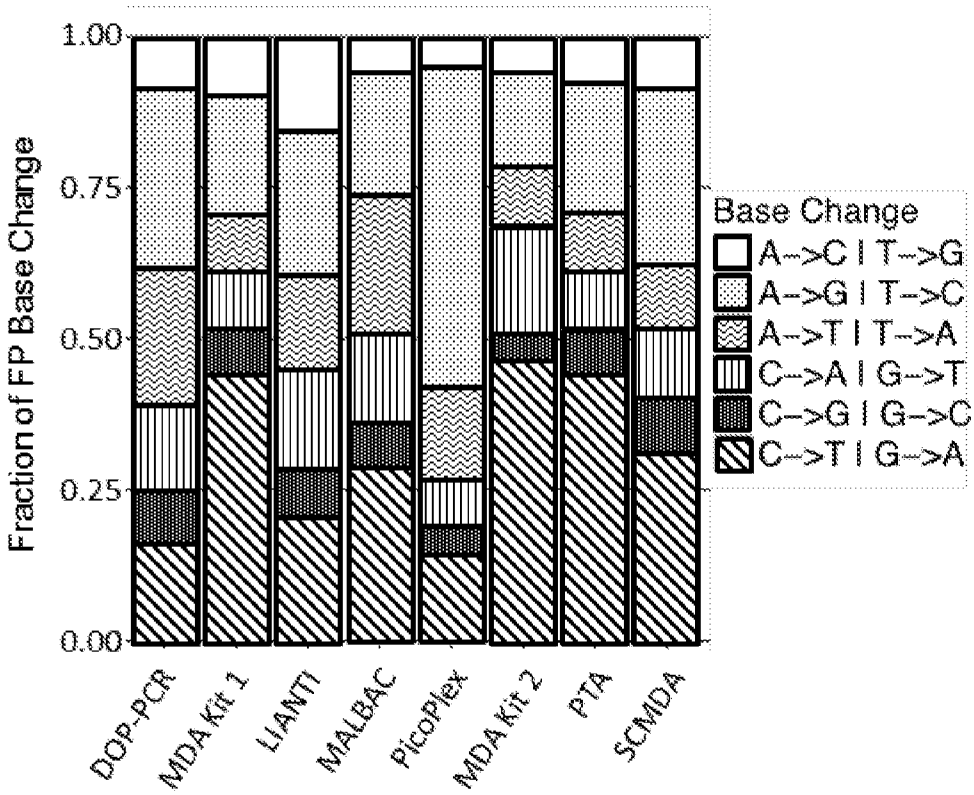

1.00
0.75
0.50
0.25
0.00
Fraction of FP Base Change
DOP-PCR
MDA Kit 1
LIANTI
MALBAC
PicoPlex
MDA Kit 2
PTA
SCMDA
Base Change
A->C | T->G
A->G | T->C
A->T | T->A
C->A | G->T
C->G | G->C
C->T | G->A

FIG. 5H

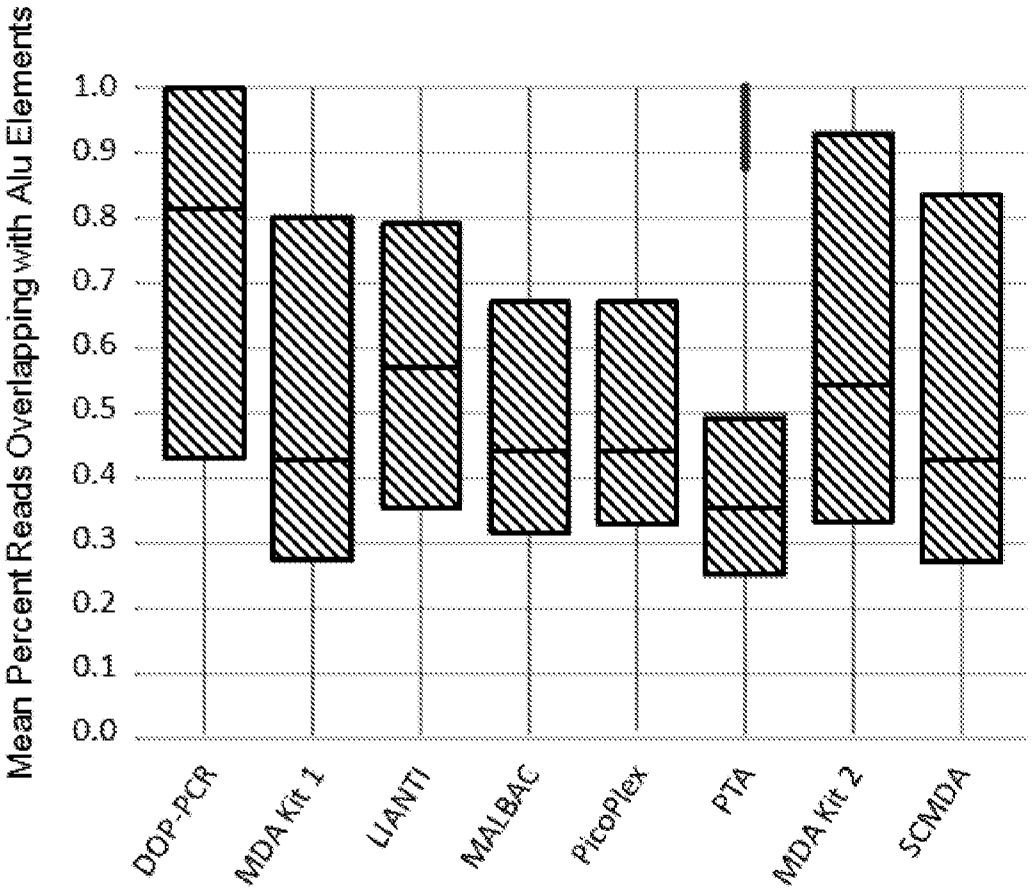

Mean Percent Reads Overlapping with Alu Elements
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
DOP-PCR
MDA Kit 1
LIANTI
MALBAC
PicoPlex
PTA
MDA Kit 2
SCMDA

FIG. 5I

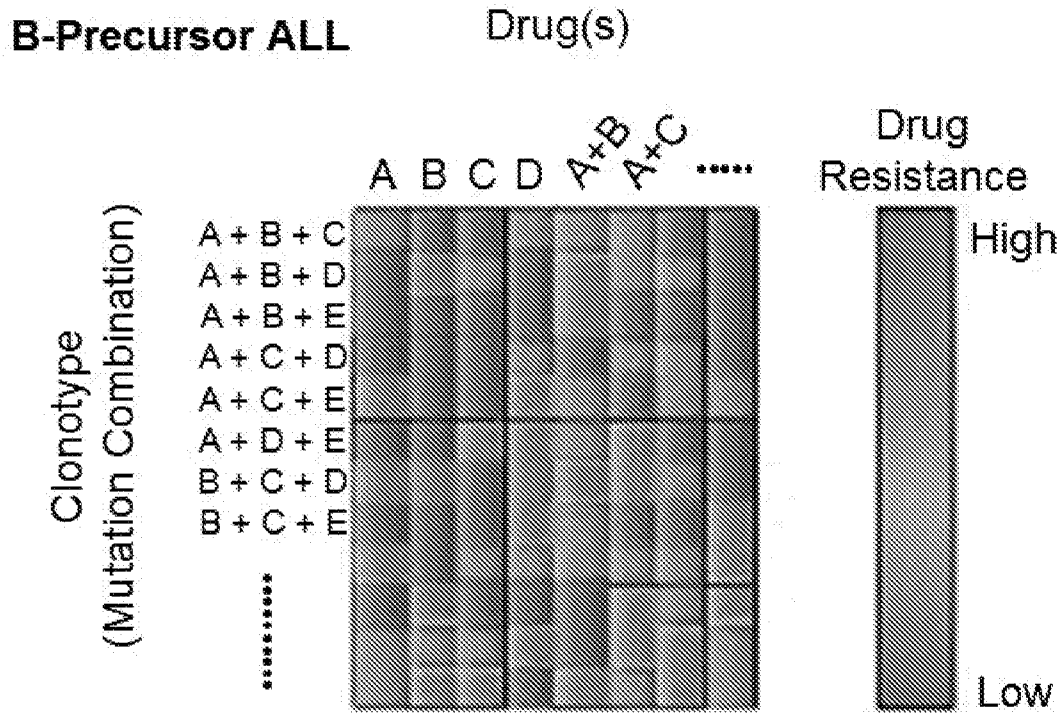

B-Precursor ALL
Drug(s)
A
B
C
D
A+B
A+C
.....
Drug Resistance
High
Low
Clonotype (Mutation Combination)
A + B + C
A + B + D
A + B + E
A + C + D
A + C + E
A + D + E
B + C + D
B + C + E

FIG. 6A

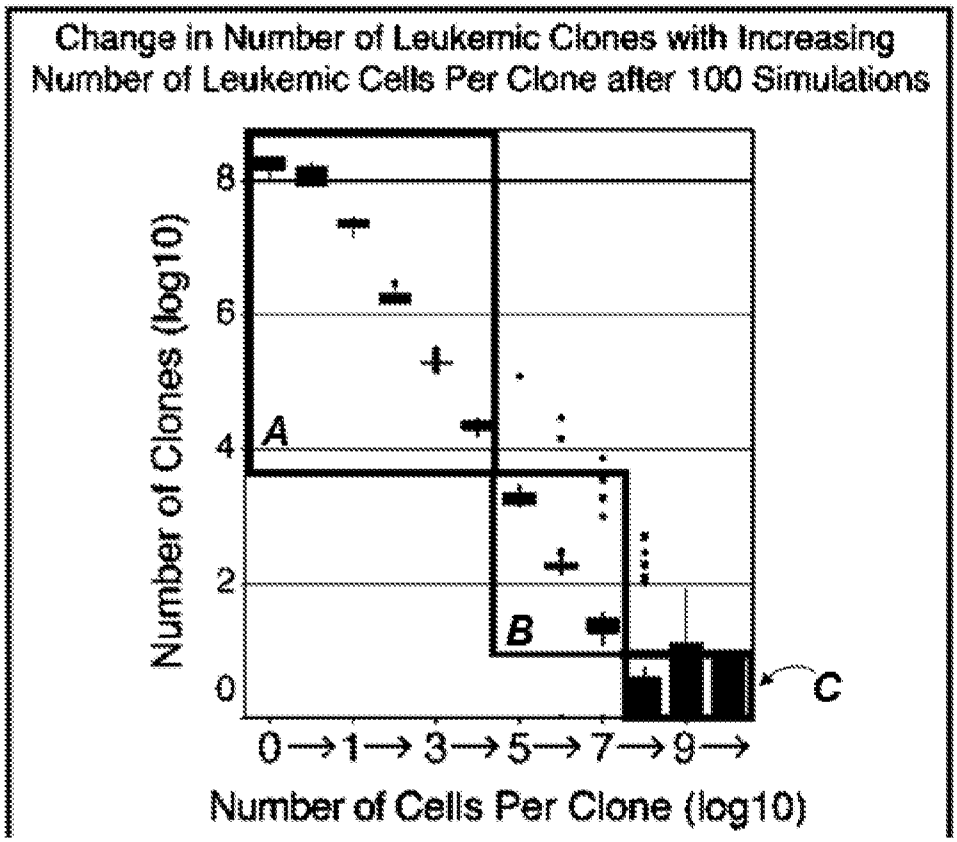

Change in Number of Leukemic Clones with Increasing Number of Leukemic Cells Per Clone after 100 Simulations
Number of Clones (log10)
8
6
4
2
0
A
B
C
0→1→3→5→7→9→
Number of Cells Per Clone (log10)

FIG. 6B

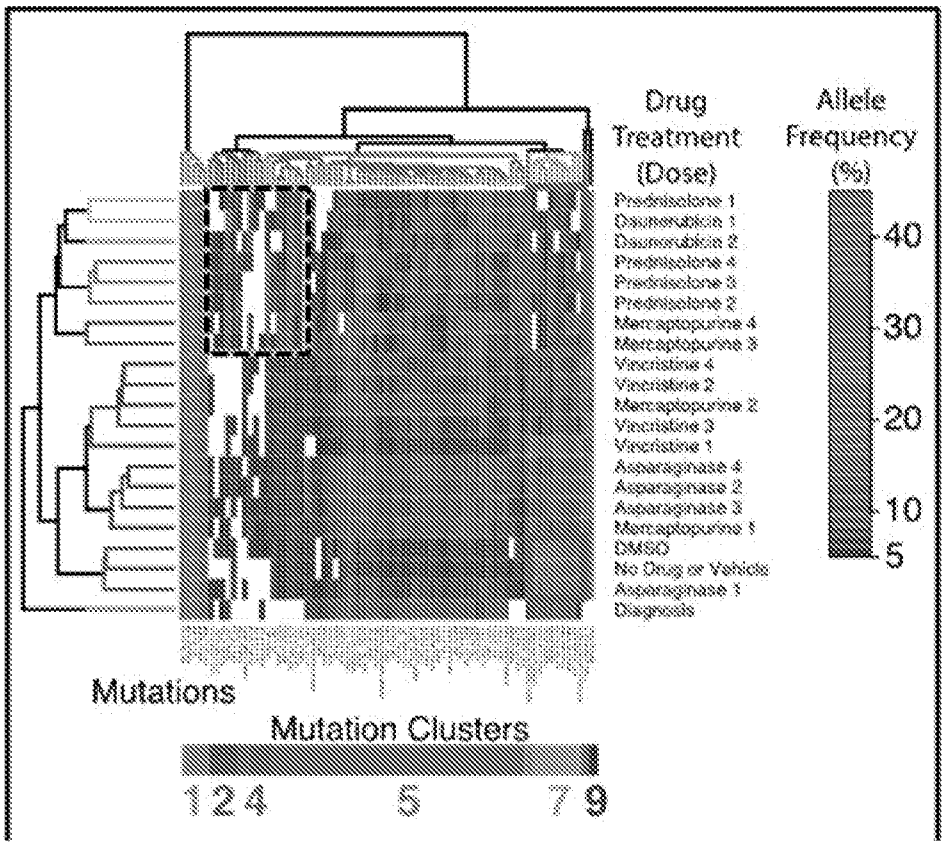

Drug Treatment (Dose)
Allele Frequency (%)
40
30
20
10
5
Mutations
Mutation Clusters
1 2 4
5
7 9

FIG. 7

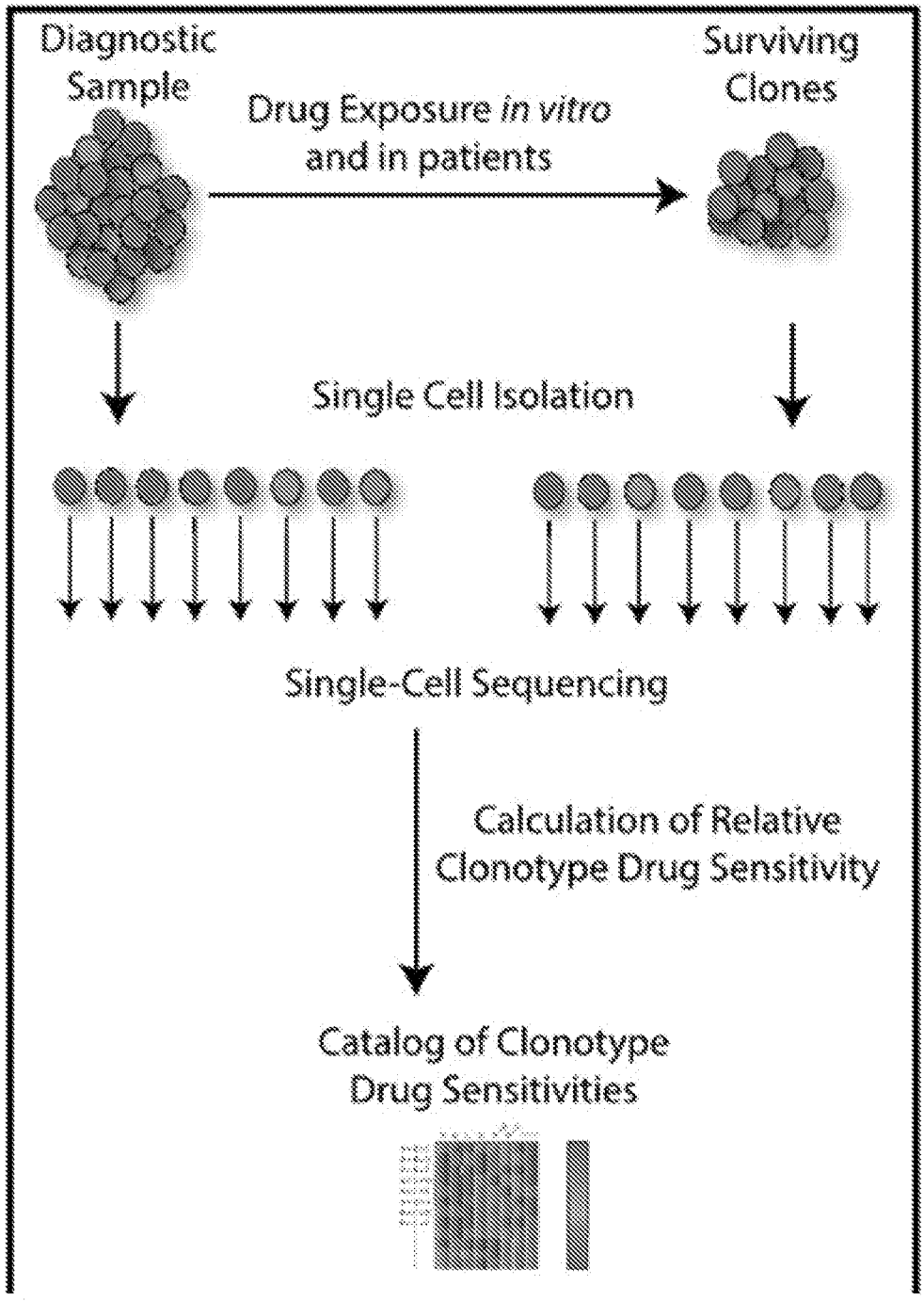

Diagnostic Sample
Drug Exposure *in vitro* and in patients
Surviving Clones
Single Cell Isolation
Single-Cell Sequencing
Calculation of Relative Clonotype Drug Sensitivity
Catalog of Clonotype Drug Sensitivities

FIG. 8

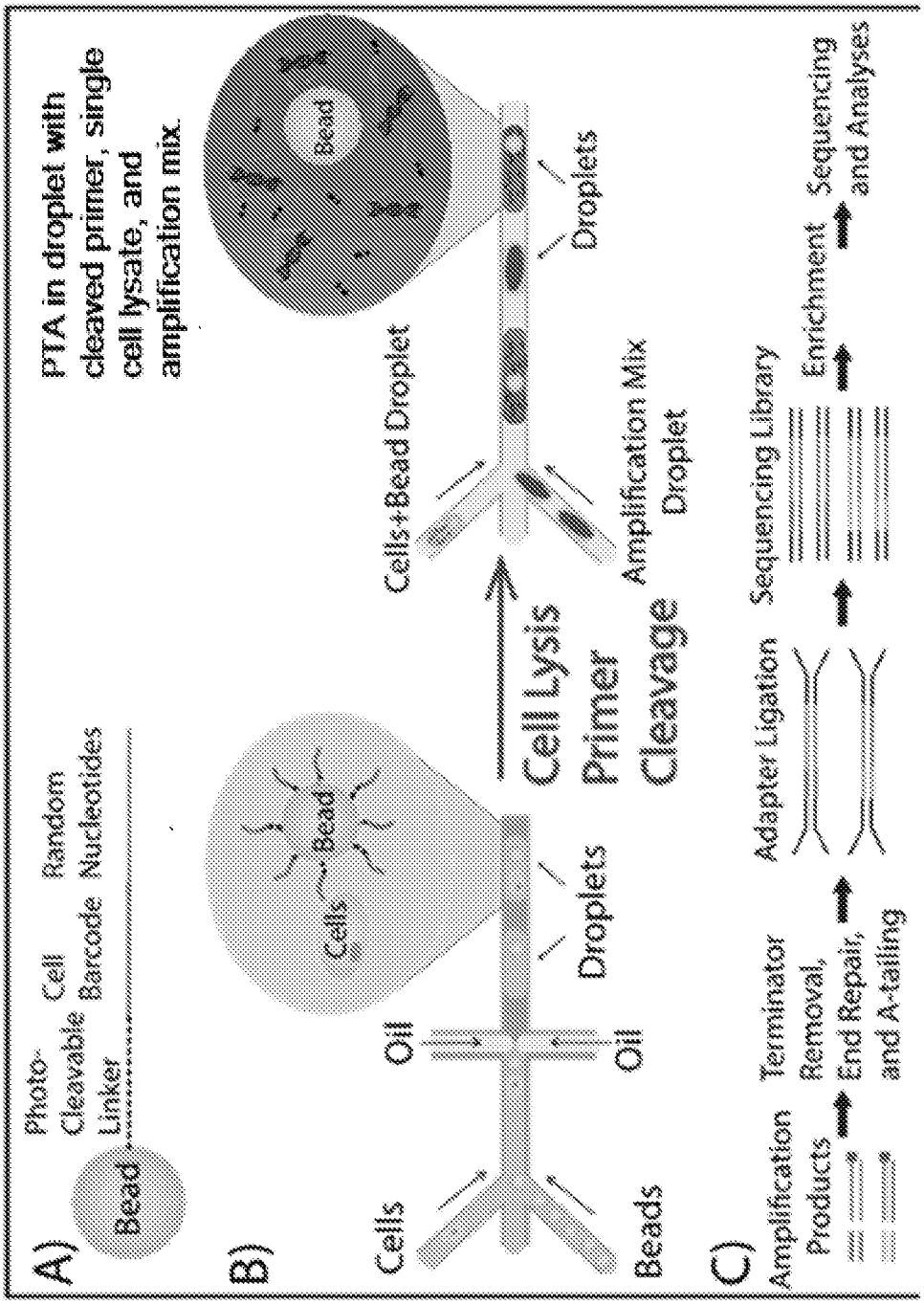

A)
Photo-Cleavable Linker
Cell Barcode
Random Nucleotides
Bead
PTA in droplet with cleaved primer, single cell lysate, and amplification mix
B)
Cells
Oil
Beads
Oil
Droplets
Cells
Bead
Cell Lysis
Primer Cleavage
Cells+Bead Droplet
Amplification Mix Droplet
Droplets
C)
Amplification Products
Terminator Removal, End Repair, and A-tailing
Adapter Ligation
Sequencing Library
Enrichment
Sequencing and Analyses

FIG. 9

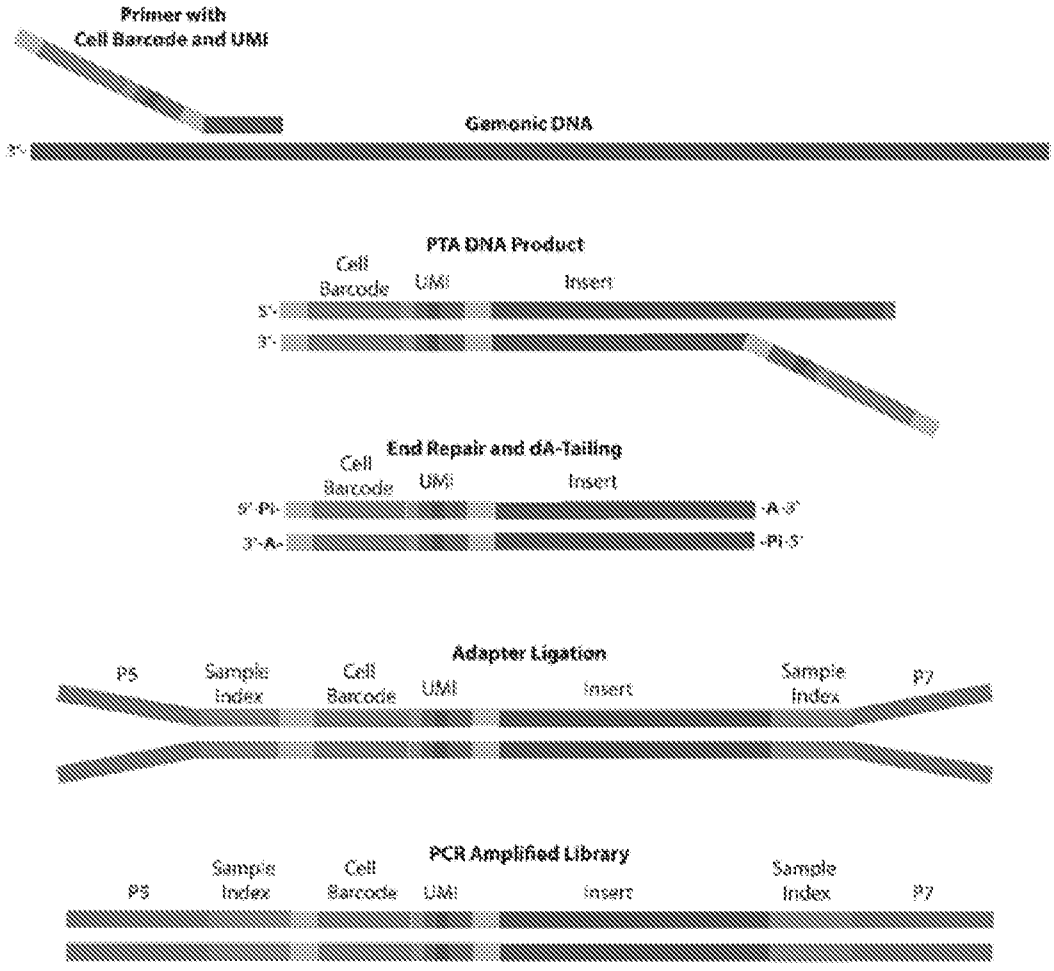

Primer with
Cell Barcode and UMI
Gemonic DNA
PTA DNA Product
Cell Barcode
UMI
Insert
End Repair and dA-Tailing
Adapter Ligation
P5
Sample Index
P7
PCR Amplified Library

FIG. 10A

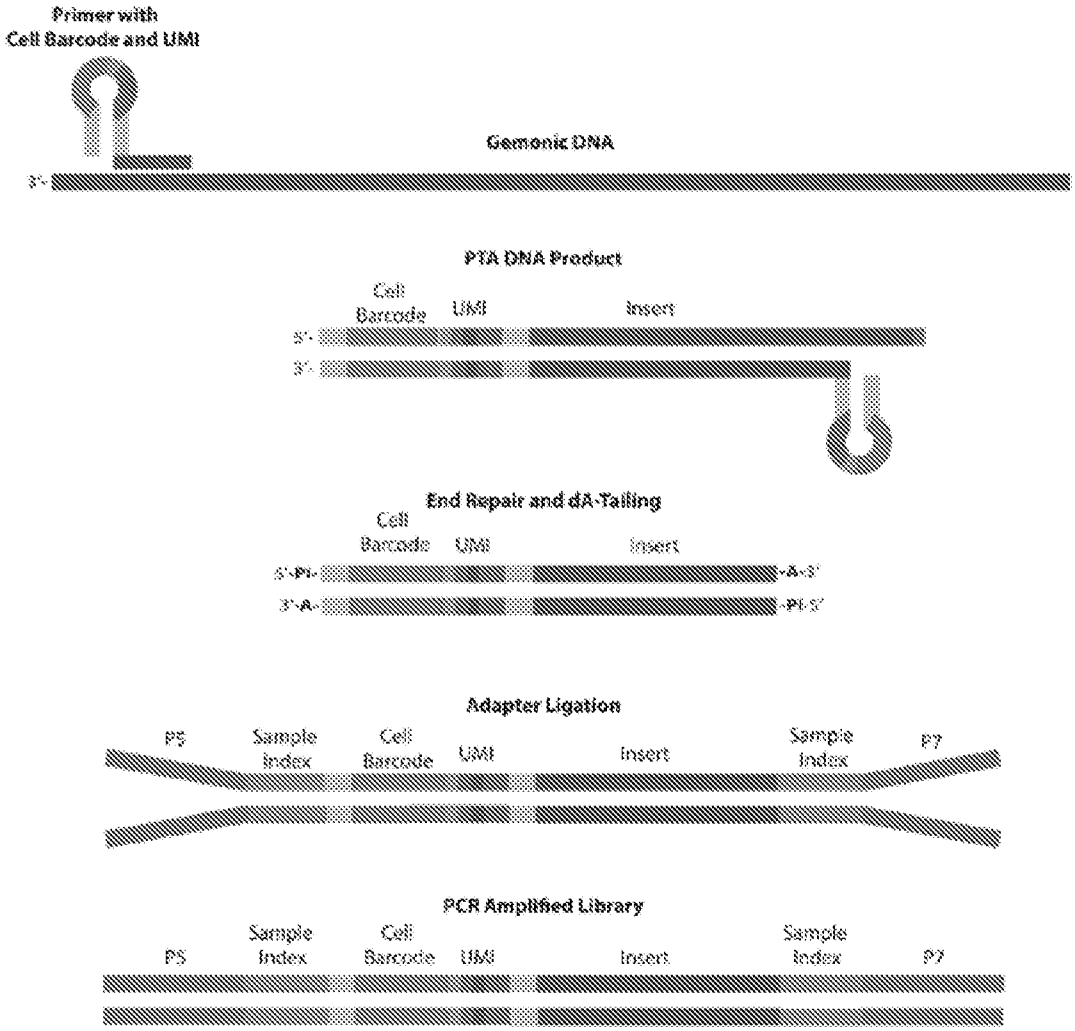

Primer with
Cell Barcode and UMI
Gemonic DNA
PTA DNA Product
Cell
Barcode
UMI
Insert
End Repair and dA-Tailing
Adapter Ligation
P5
Sample
Index
P7
PCR Amplified Library

FIG. 10B

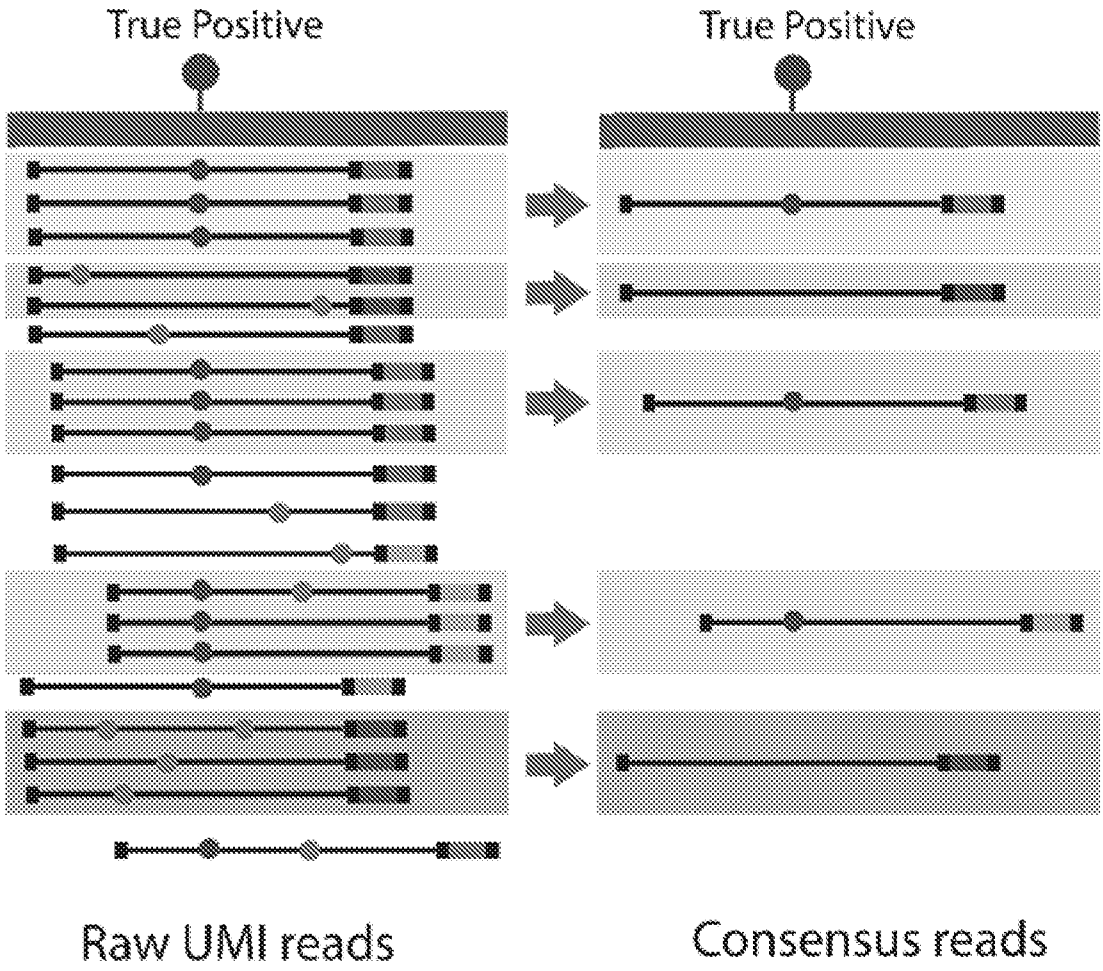


FIG. 11A

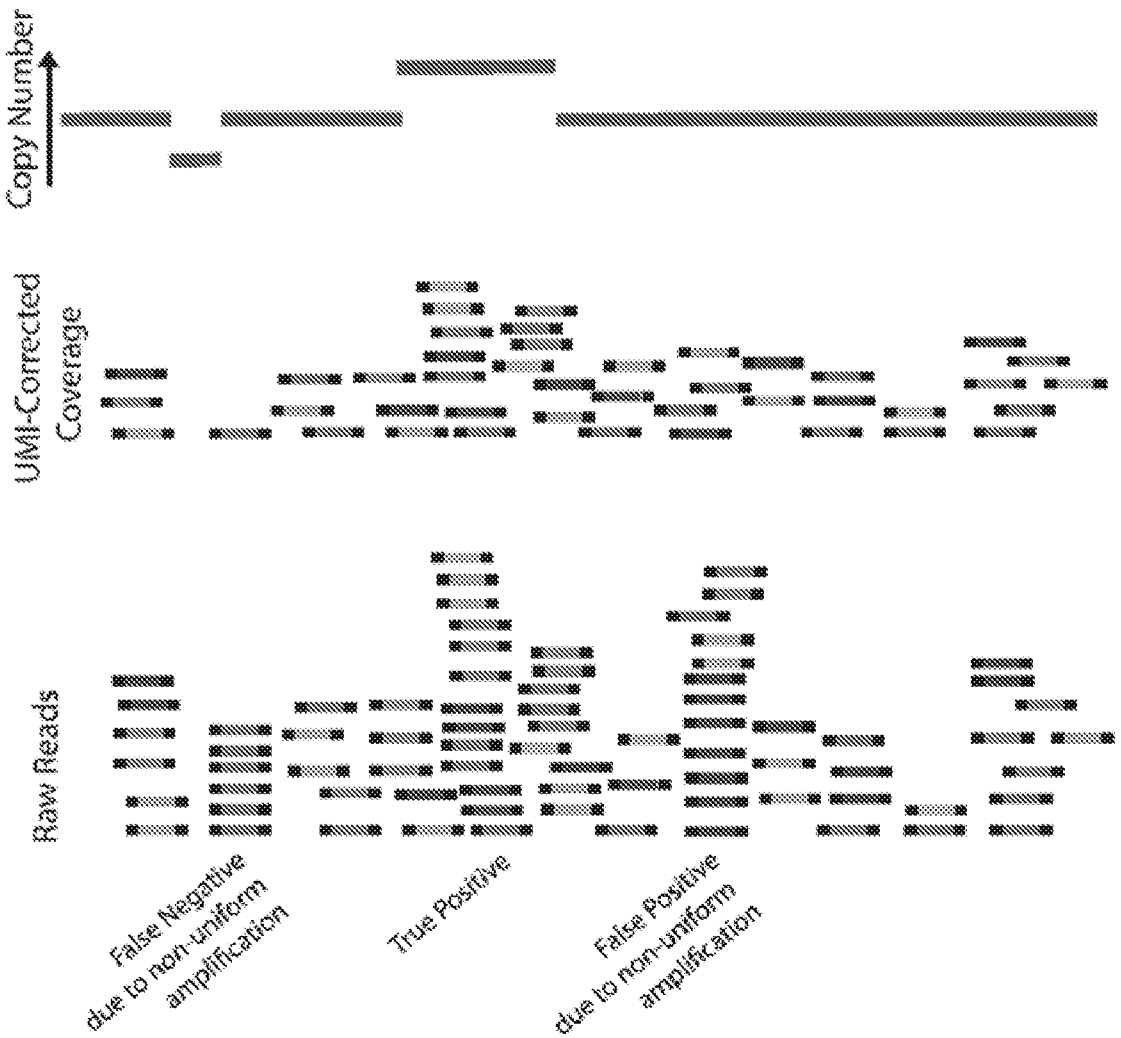

Copy Number
UMI-Corrected Coverage
Raw Reads
False Negative due to non-uniform amplification
True Positive
False Positive due to non-uniform amplification

FIG. 11B

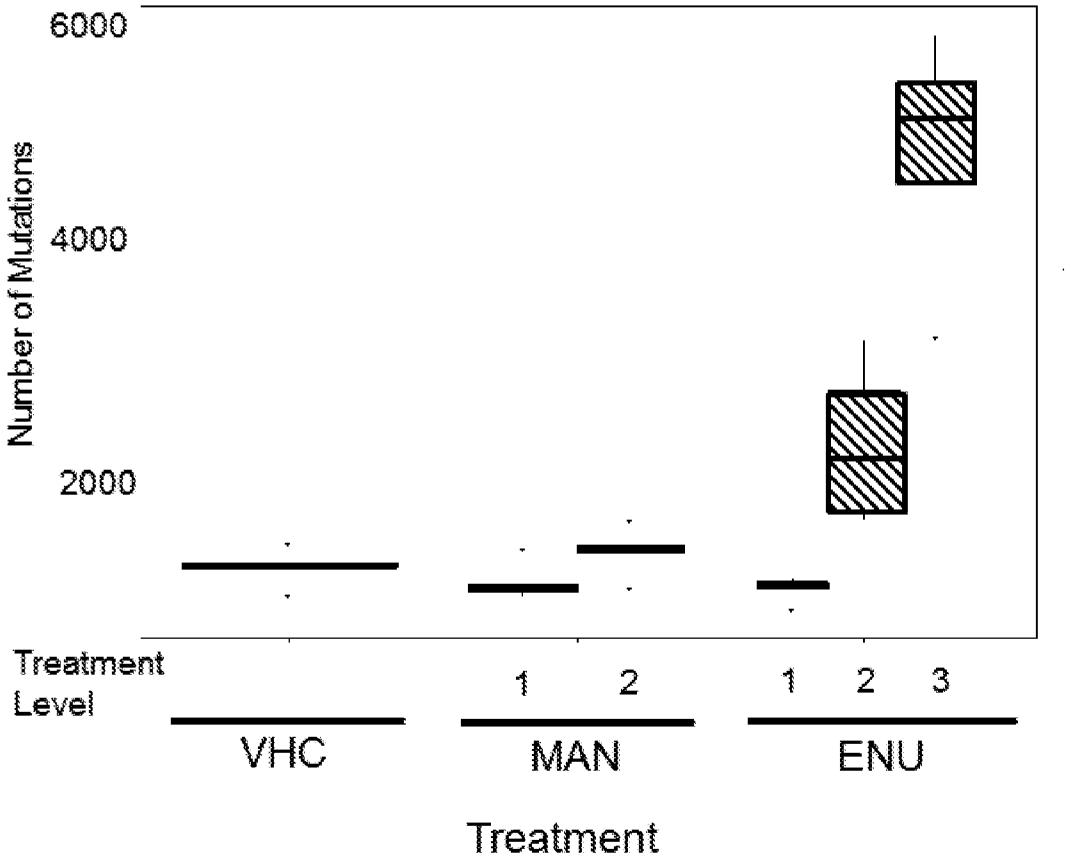

6000
4000
2000
Number of Mutations
Treatment Level
1
2
1
2
3
VHC
MAN
ENU
Treatment

FIG. 12A

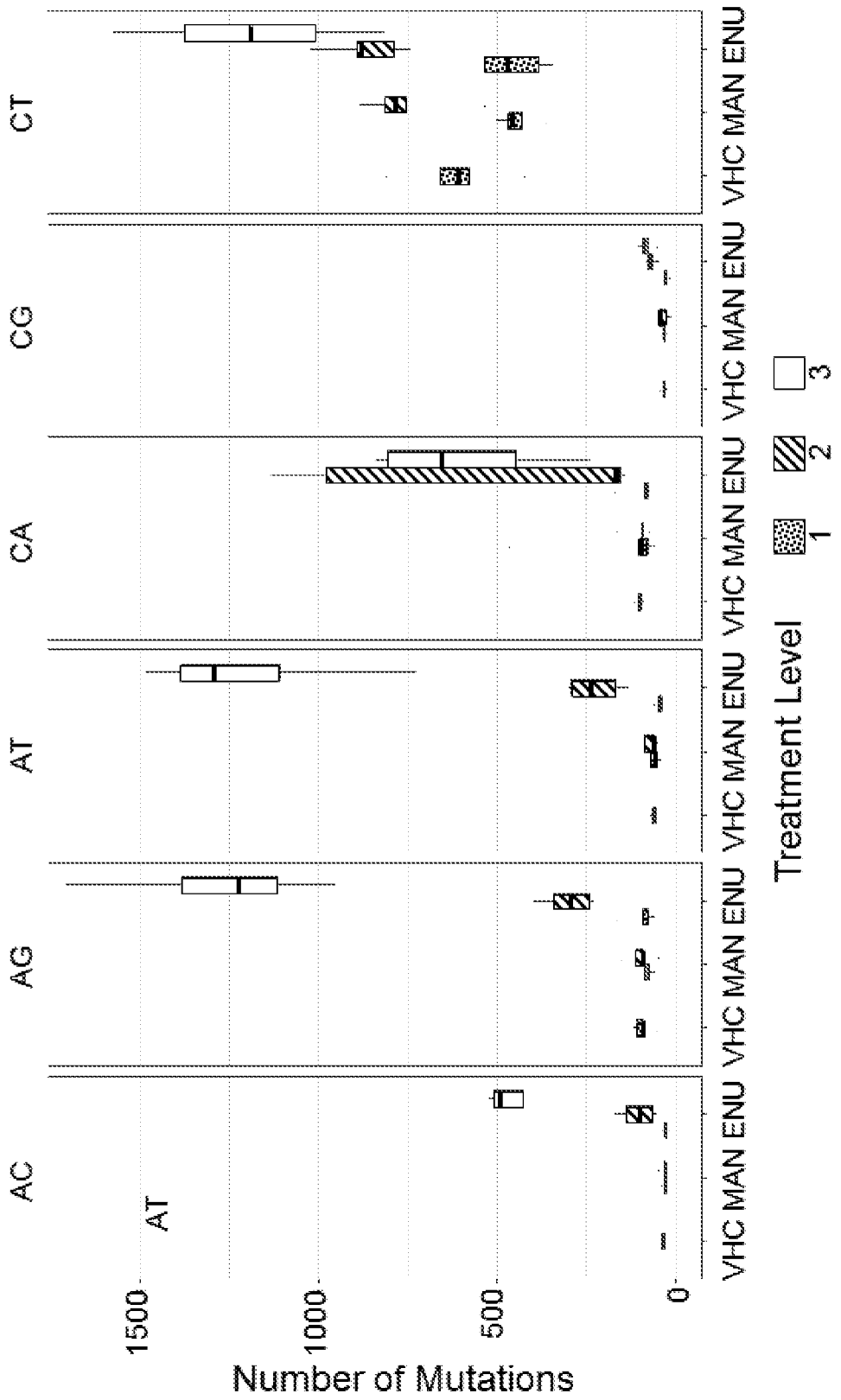

AC
AG
AT
CA
CG
CT
VHC MAN ENU VHC MAN ENU VHC MAN ENU VHC MAN ENU VHC MAN ENU VHC MAN ENU
0
500
1000
1500
AT
Number of Mutations
Treatment Level
1
2
3

FIG. 12B

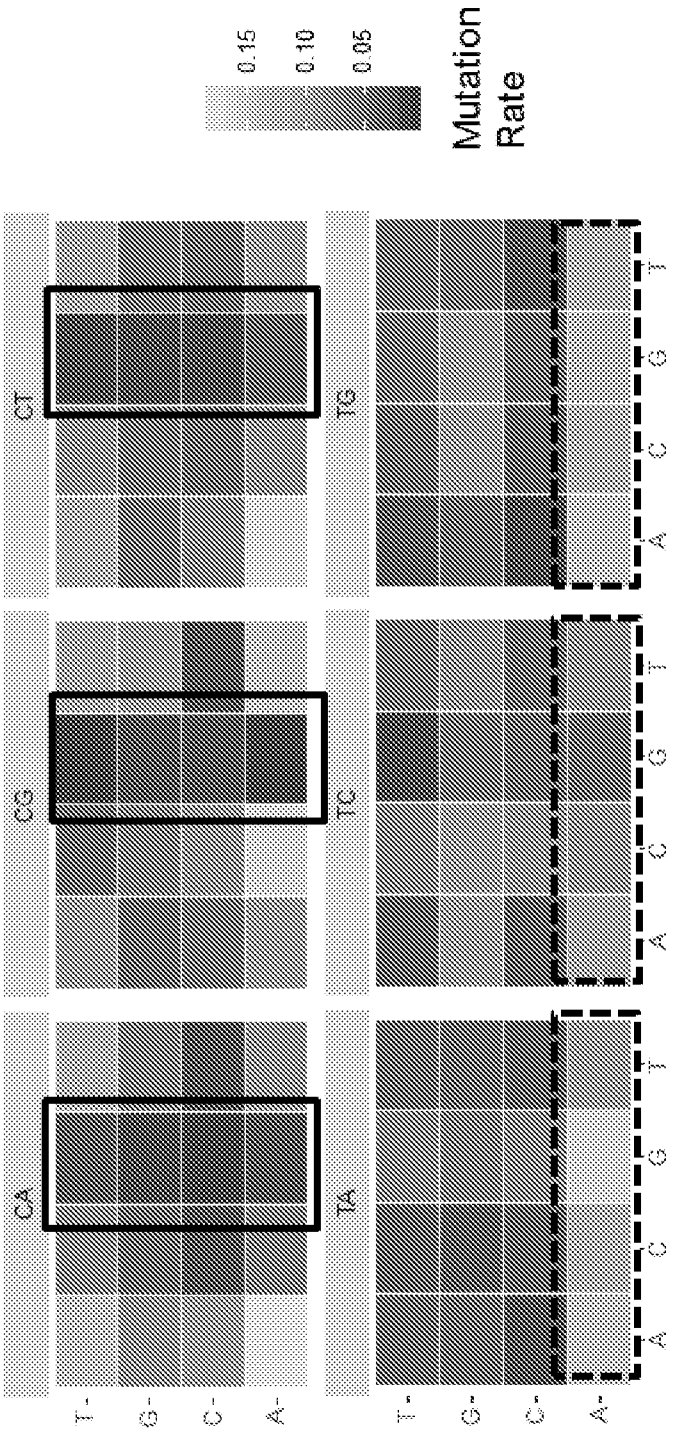


FIG. 12C

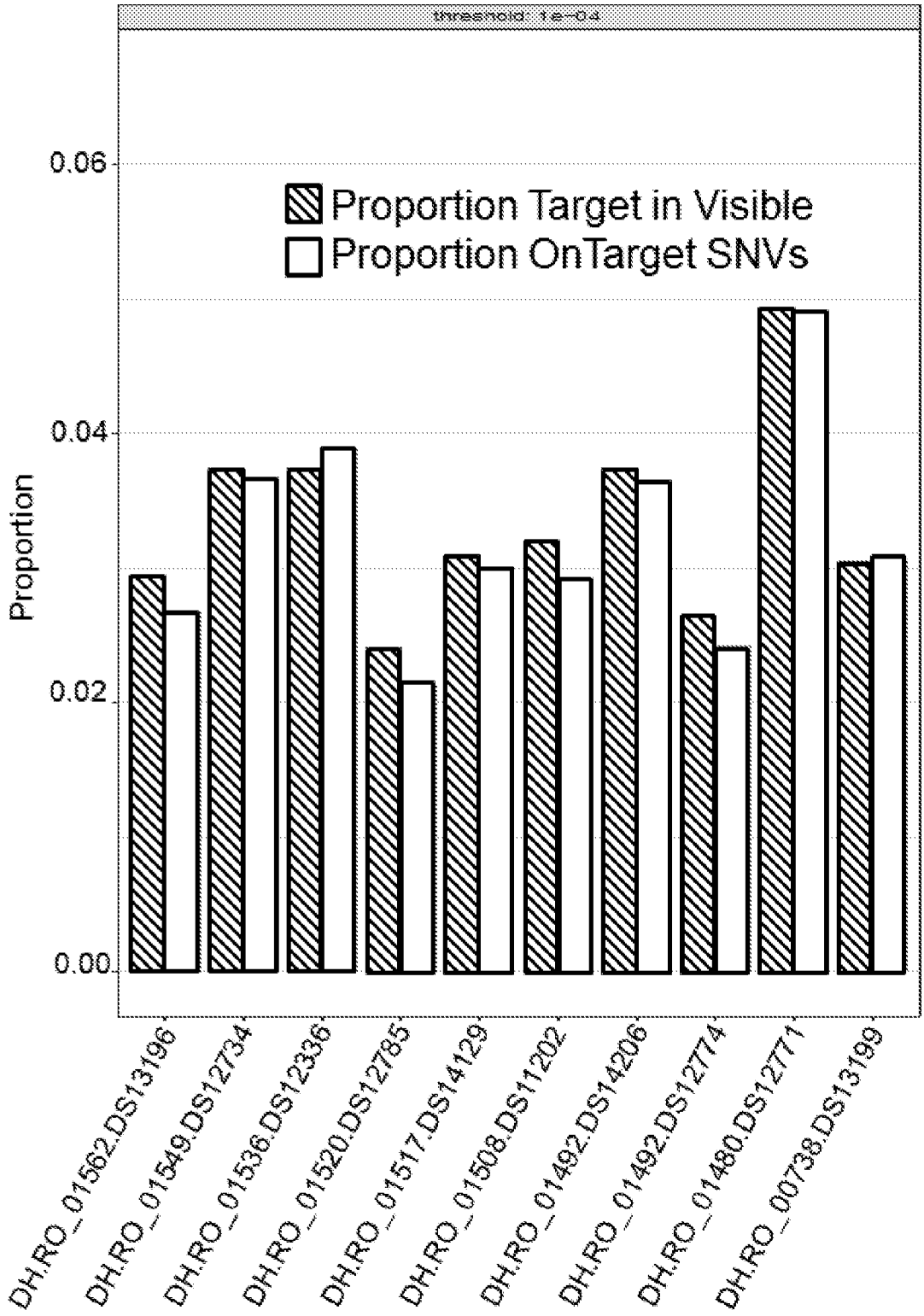

threshold: 1e−04
Proportion Target in Visible
Proportion OnTarget SNVs
Proportion
0.06
0.04
0.02
0.00
DH.RO_01562.DS13196
DH.RO_01549.DS12734
DH.RO_01536.DS12336
DH.RO_01520.DS12785
DH.RO_01517.DS14129
DH.RO_01508.DS11202
DH.RO_01492.DS14206
DH.RO_01492.DS12774
DH.RO_01480.DS12771
DH.RO_00738.DS13199

FIG. 12D

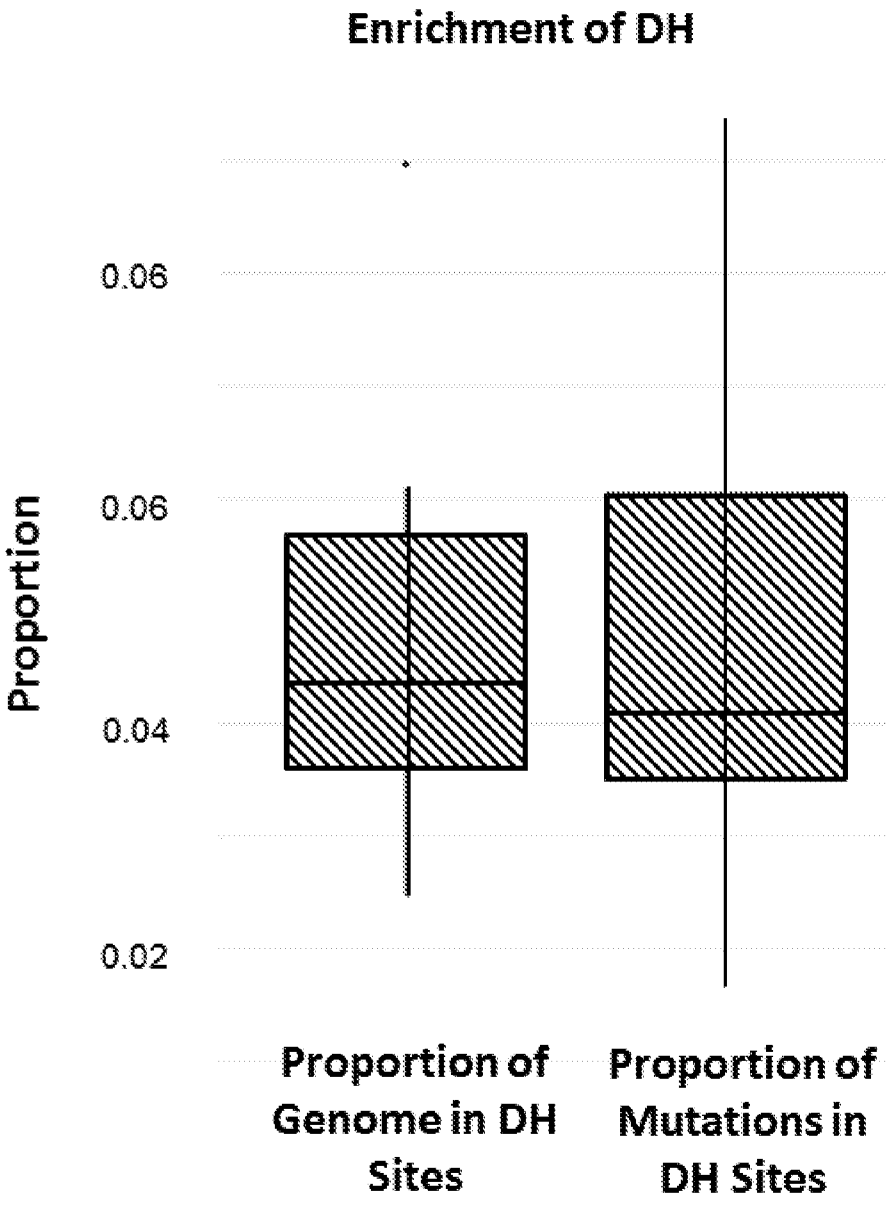

Enrichment of DH
Proportion
0.06
0.06
0.04
0.02
Proportion of Genome in DH Sites
Proportion of Mutations in DH Sites

FIG. 12E

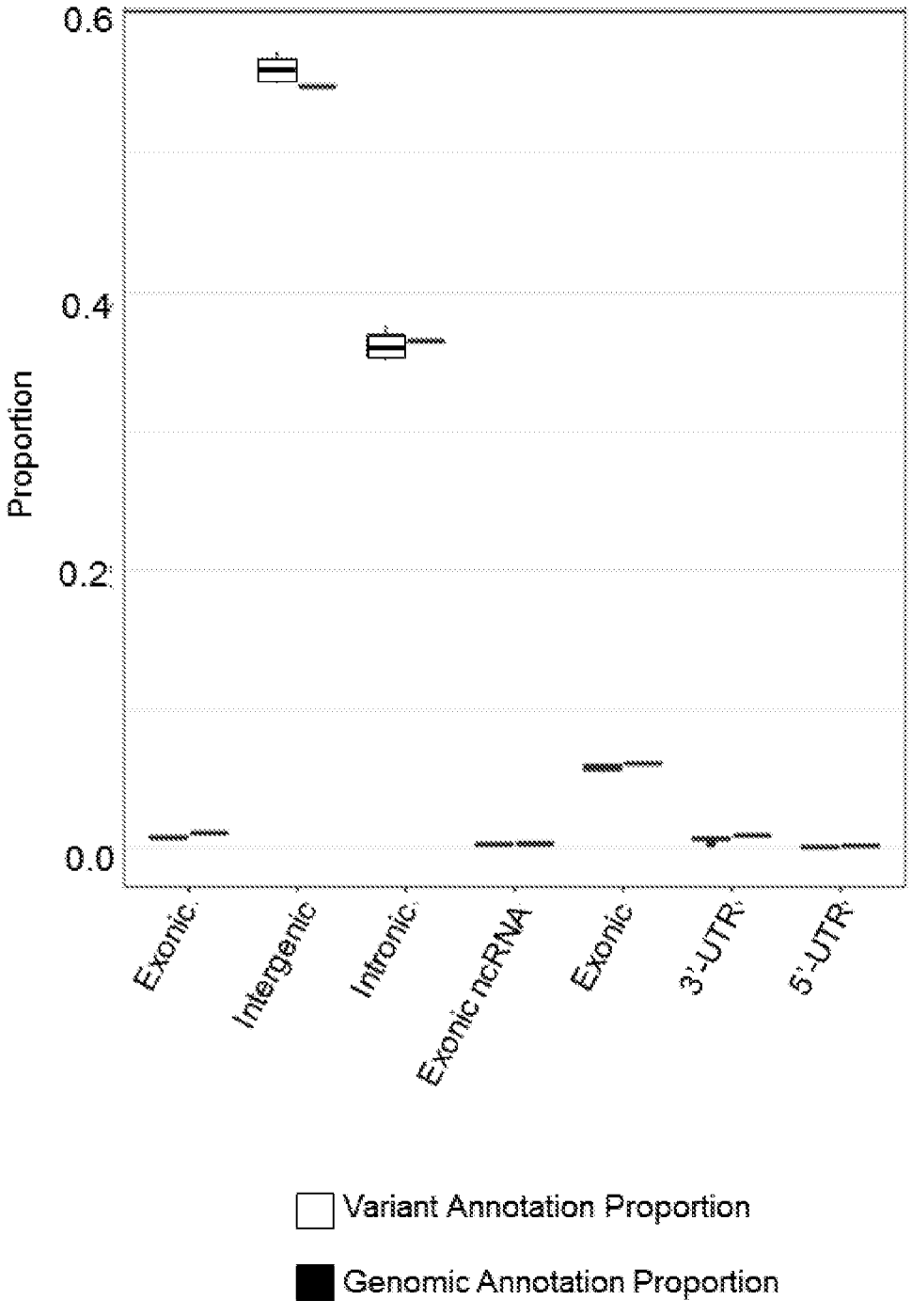

0.6
0.4
0.2
0.0
Proportion
Exonic
Intergenic
Intronic
Exonic ncRNA
Exonic
3'-UTR
5'-UTR
Variant Annotation Proportion
Genomic Annotation Proportion

FIG. 12F