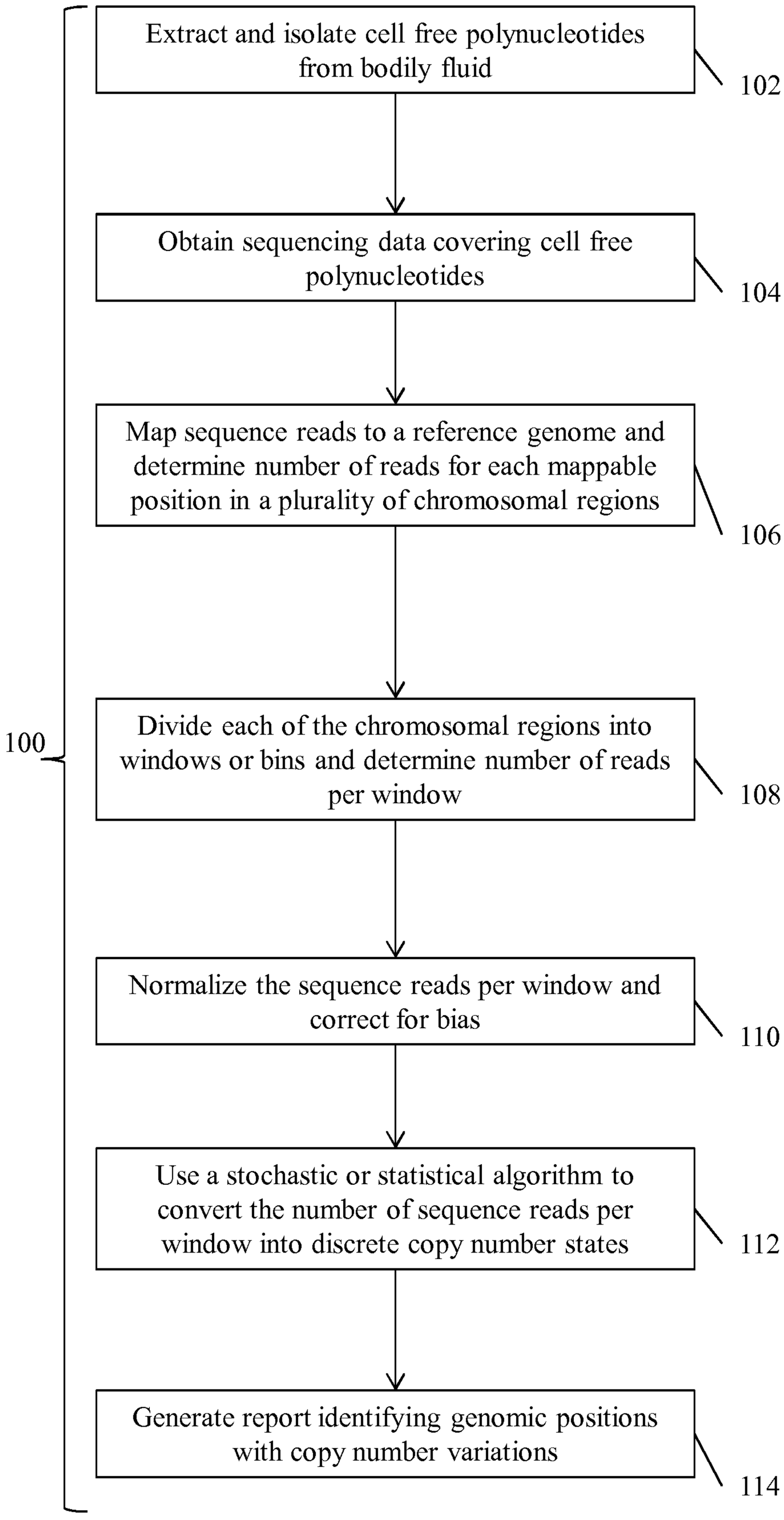

Extract and isolate cell free polynucleotides from bodily fluid
102
Obtain sequencing data covering cell free polynucleotides
104
Map sequence reads to a reference genome and determine number of reads for each mappable position in a plurality of chromosomal regions
106
100
Divide each of the chromosomal regions into windows or bins and determine number of reads per window
108
Normalize the sequence reads per window and correct for bias
110
Use a stochastic or statistical algorithm to convert the number of sequence reads per window into discrete copy number states
112
Generate report identifying genomic positions with copy number variations
114

Fig. 1

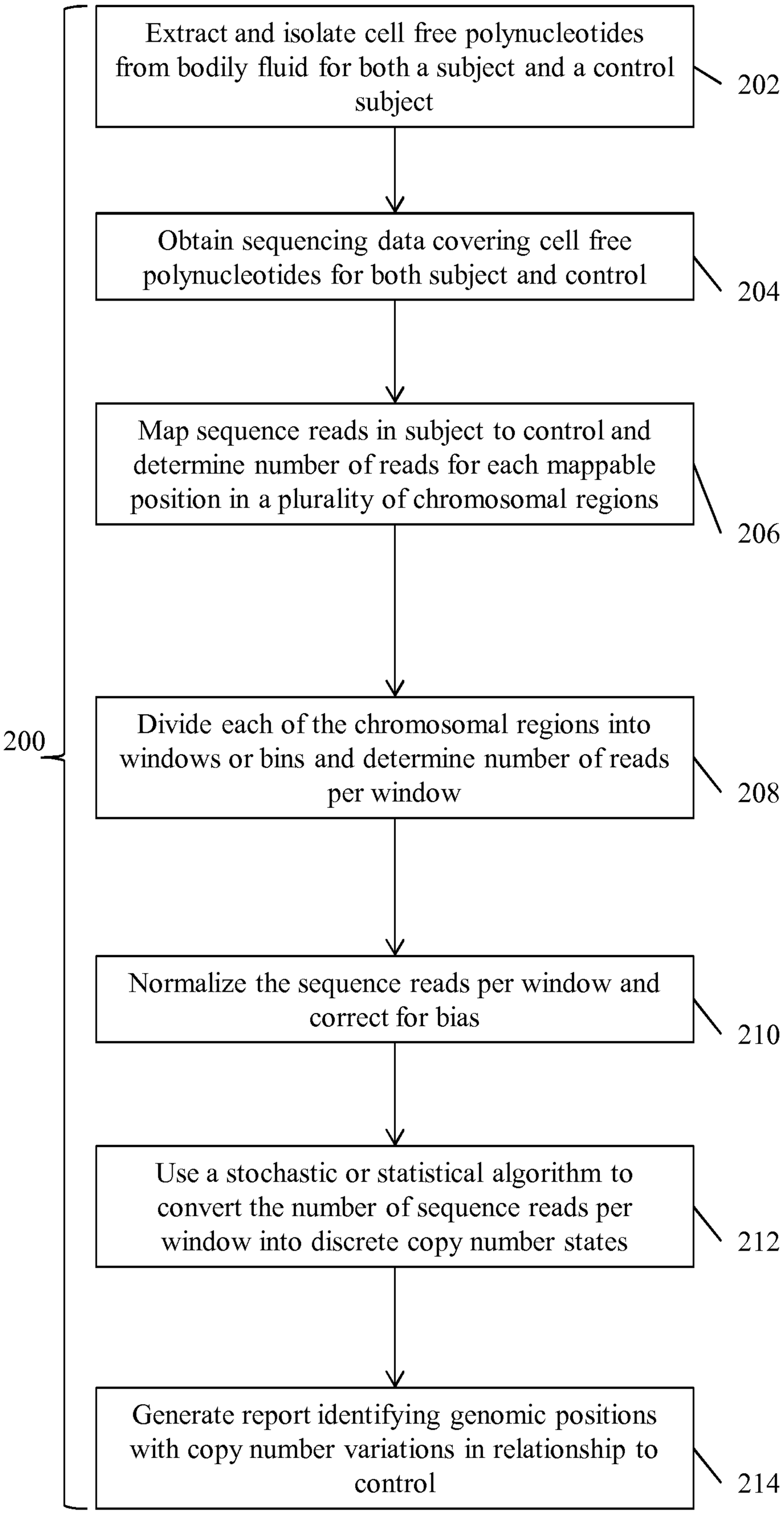

Extract and isolate cell free polynucleotides from bodily fluid for both a subject and a control subject
202
Obtain sequencing data covering cell free polynucleotides for both subject and control
204
Map sequence reads in subject to control and determine number of reads for each mappable position in a plurality of chromosomal regions
206
200
Divide each of the chromosomal regions into windows or bins and determine number of reads per window
208
Normalize the sequence reads per window and correct for bias
210
Use a stochastic or statistical algorithm to convert the number of sequence reads per window into discrete copy number states
212
Generate report identifying genomic positions with copy number variations in relationship to control
214

Fig. 2

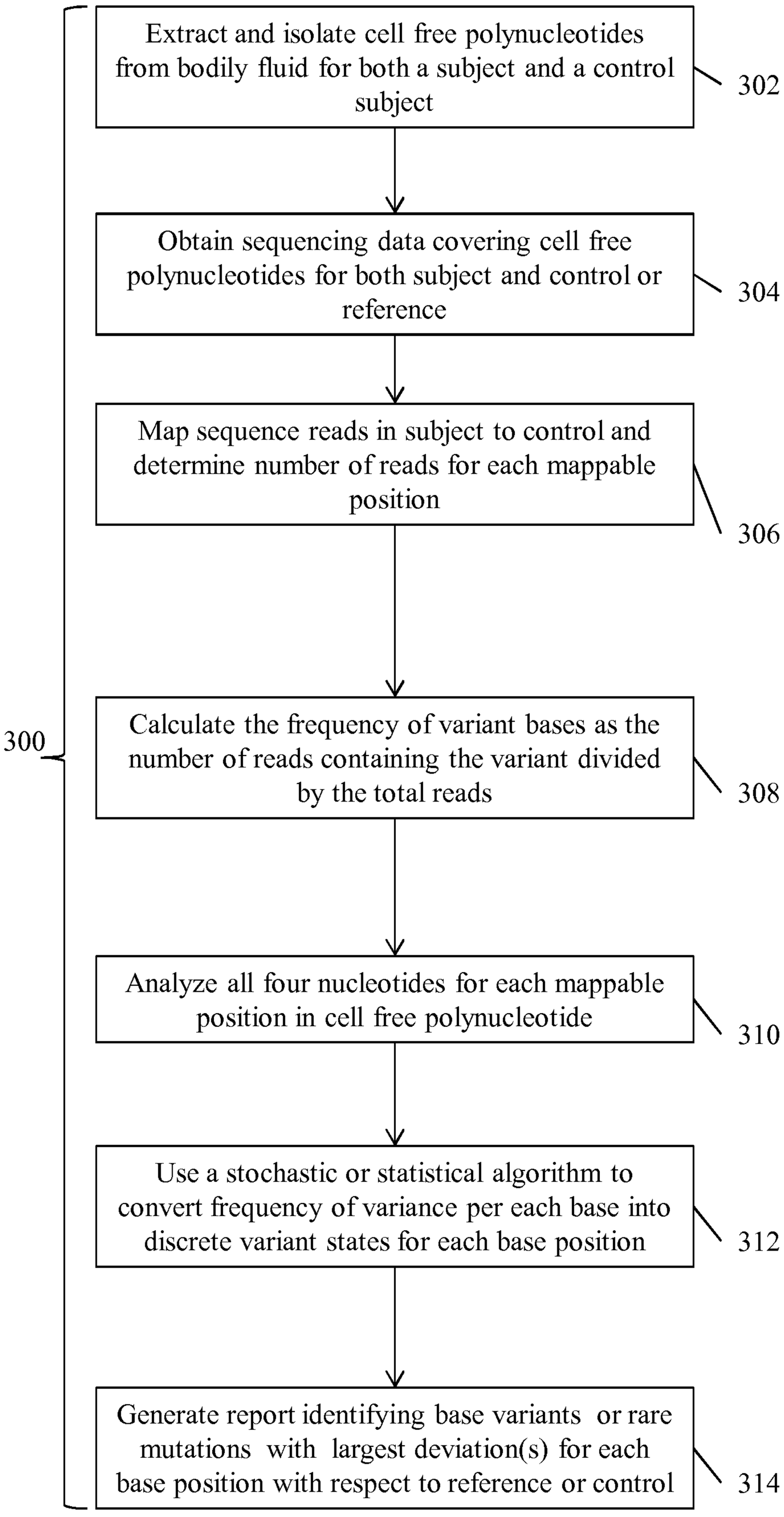
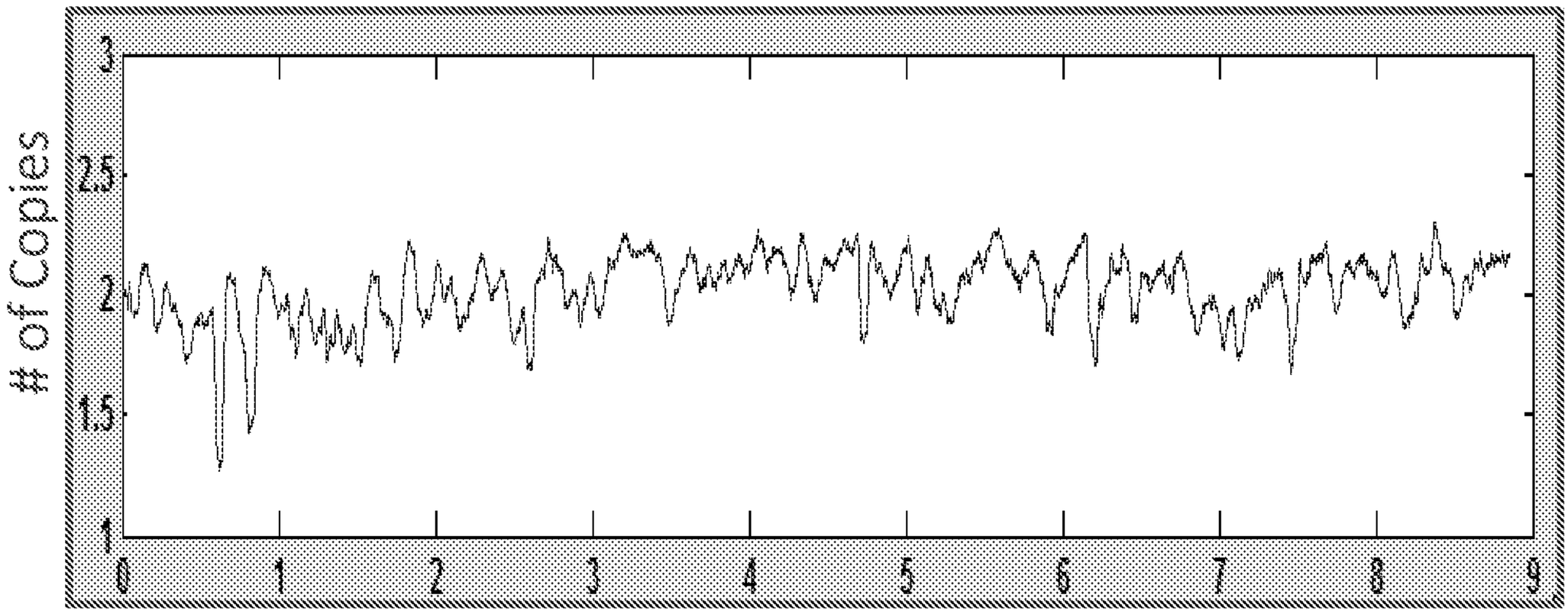
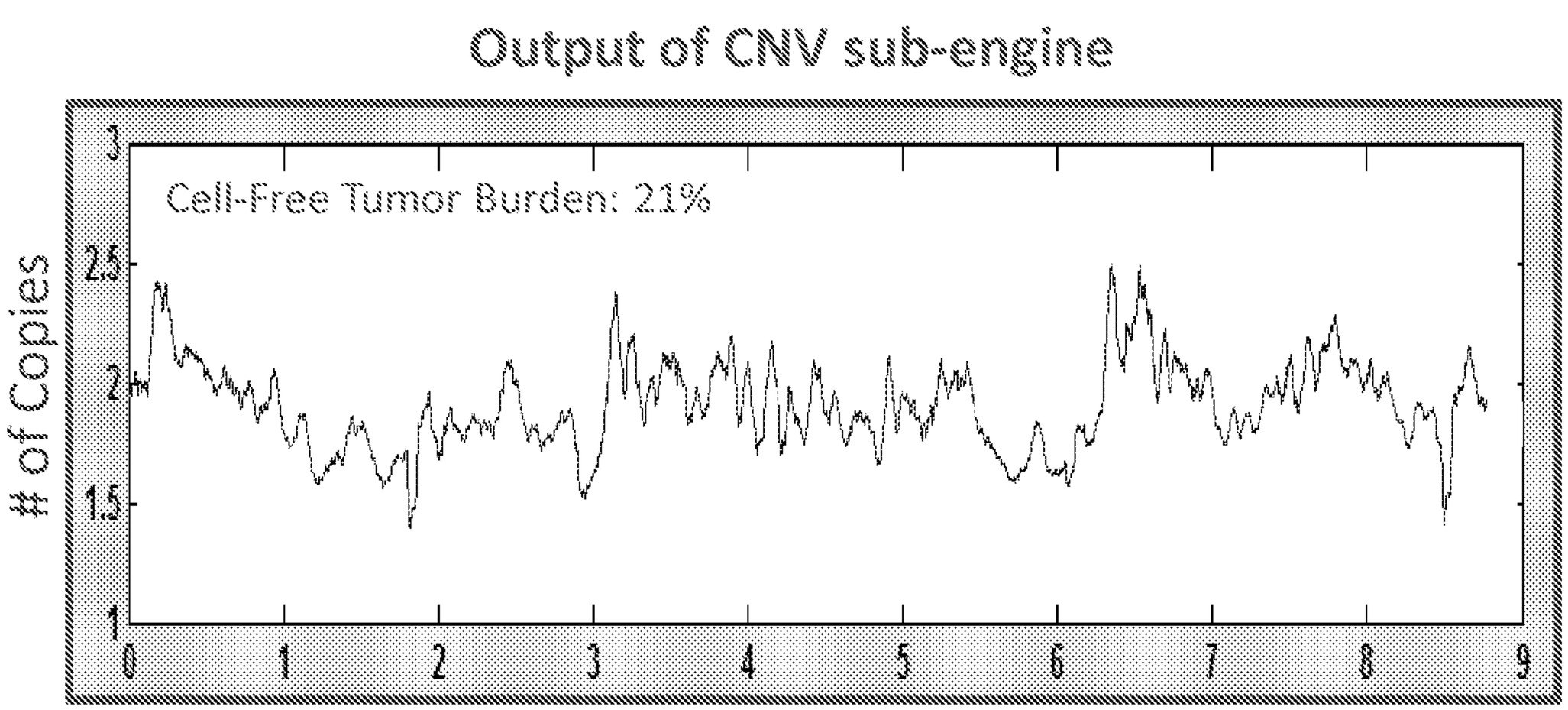

Extract and isolate cell free polynucleotides from bodily fluid for both a subject and a control subject
302
Obtain sequencing data covering cell free polynucleotides for both subject and control or reference
304
Map sequence reads in subject to control and determine number of reads for each mappable position
306
300
Calculate the frequency of variant bases as the number of reads containing the variant divided by the total reads
308
Analyze all four nucleotides for each mappable position in cell free polynucleotide
310
Use a stochastic or statistical algorithm to convert frequency of variance per each base into discrete variant states for each base position
312
Generate report identifying base variants or rare mutations with largest deviation(s) for each base position with respect to reference or control
314

Fig. 3

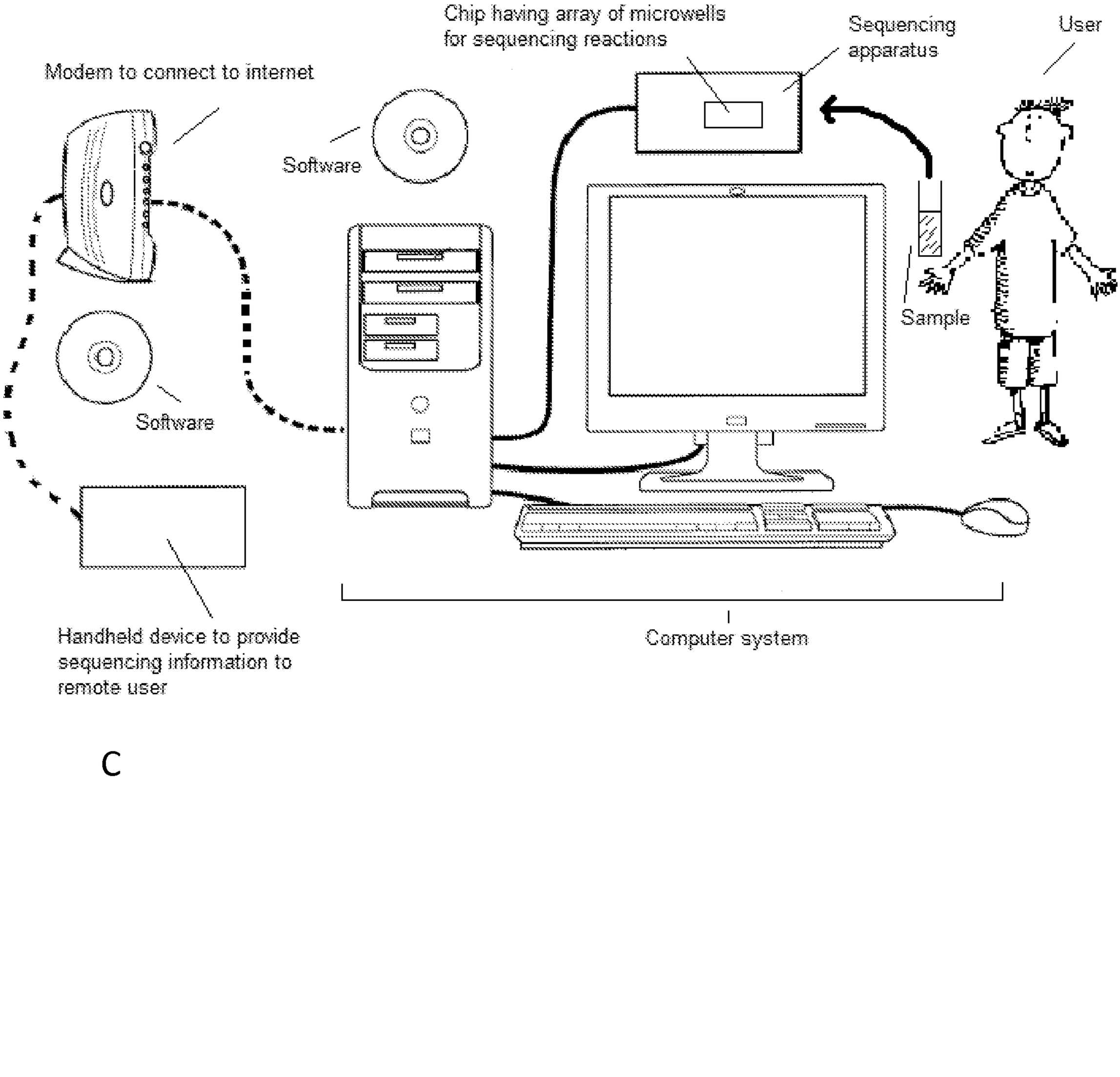

Output of CNV sub-engine
# of Copies
3
2.5
2
1.5
1
0
1
2
3
4
5
6
7
8
9
Normal Sample 1

A


Output of CNV sub-engine
Cell-Free Tumor Burden: 21%
# of Copies
3
2.5
2
1.5
1
0
1
2
3
4
5
6
7
8
9
Prostate Cancer Patient 1

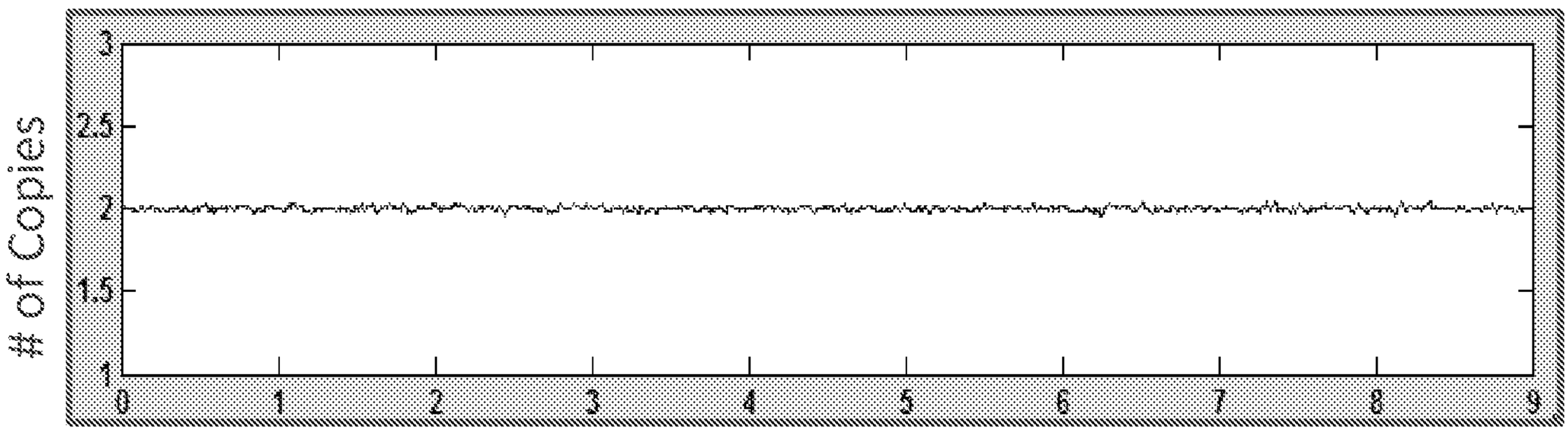
B

Fig. 4


Chip having array of microwells for sequencing reactions
Sequencing apparatus
User
Modem to connect to internet
Software
Sample
Software
Handheld device to provide sequencing information to remote user
Computer system

C

Fig. 4


Output of CNV sub-engine
# of Copies
3
2.5
2
1.5
1
0
1
2
3
4
5
6
7
8
9
Prostate Cancer Patient 2
A

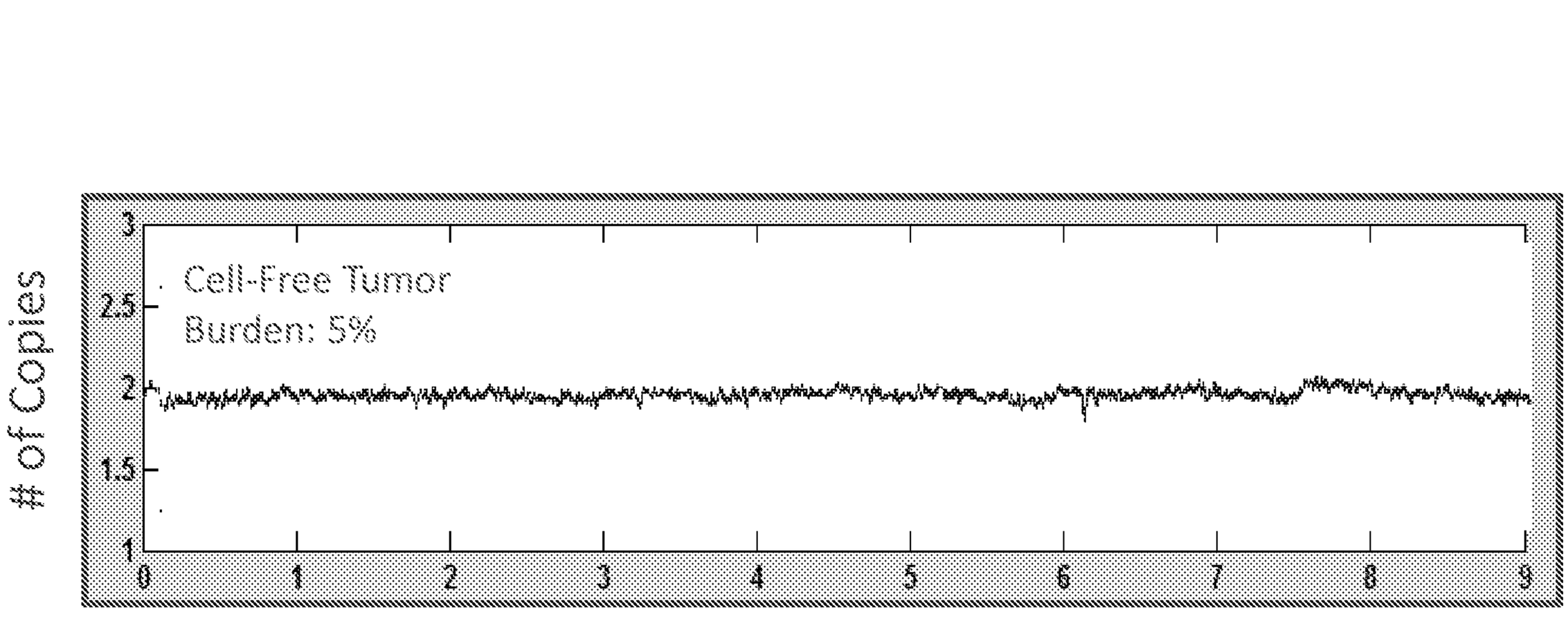
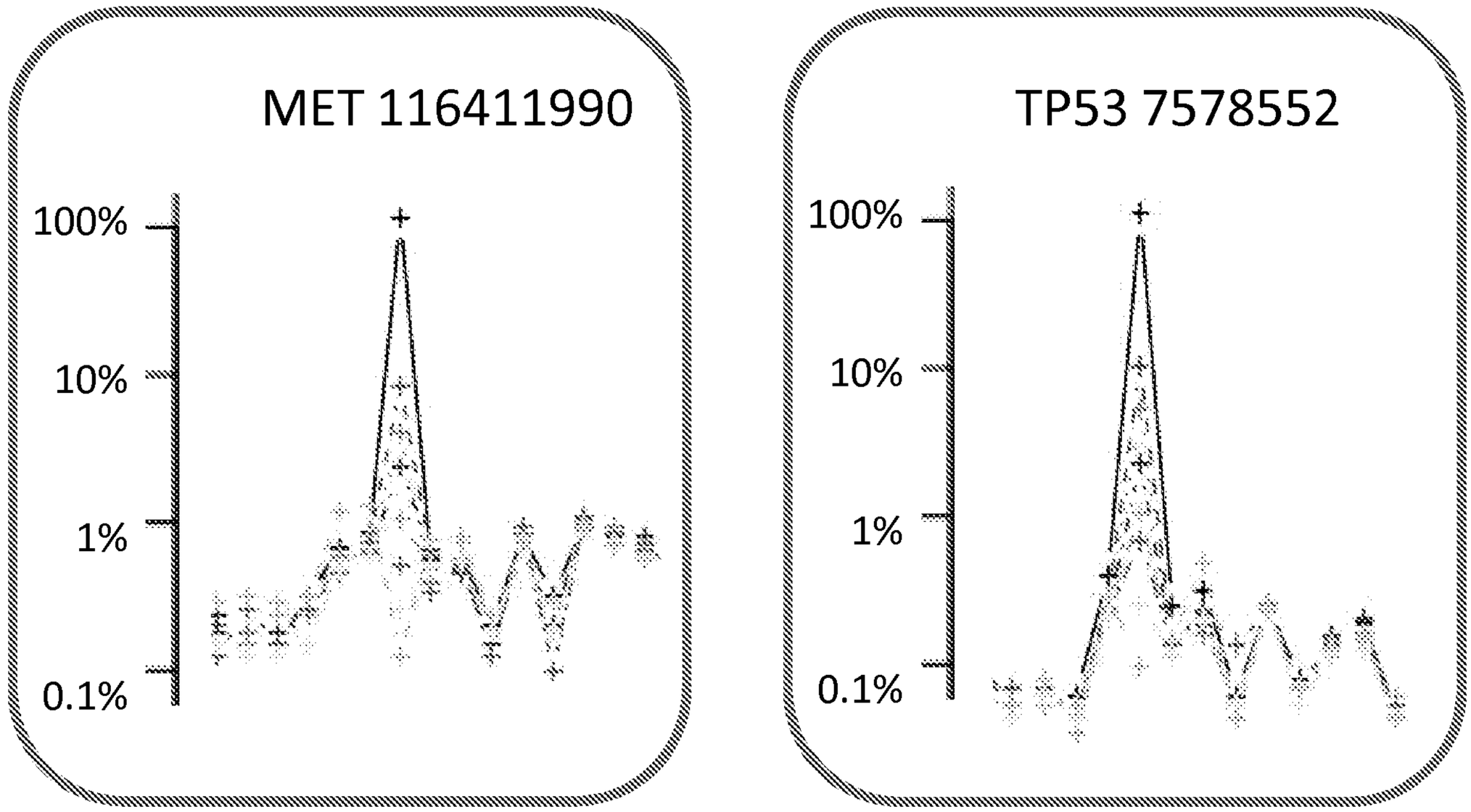
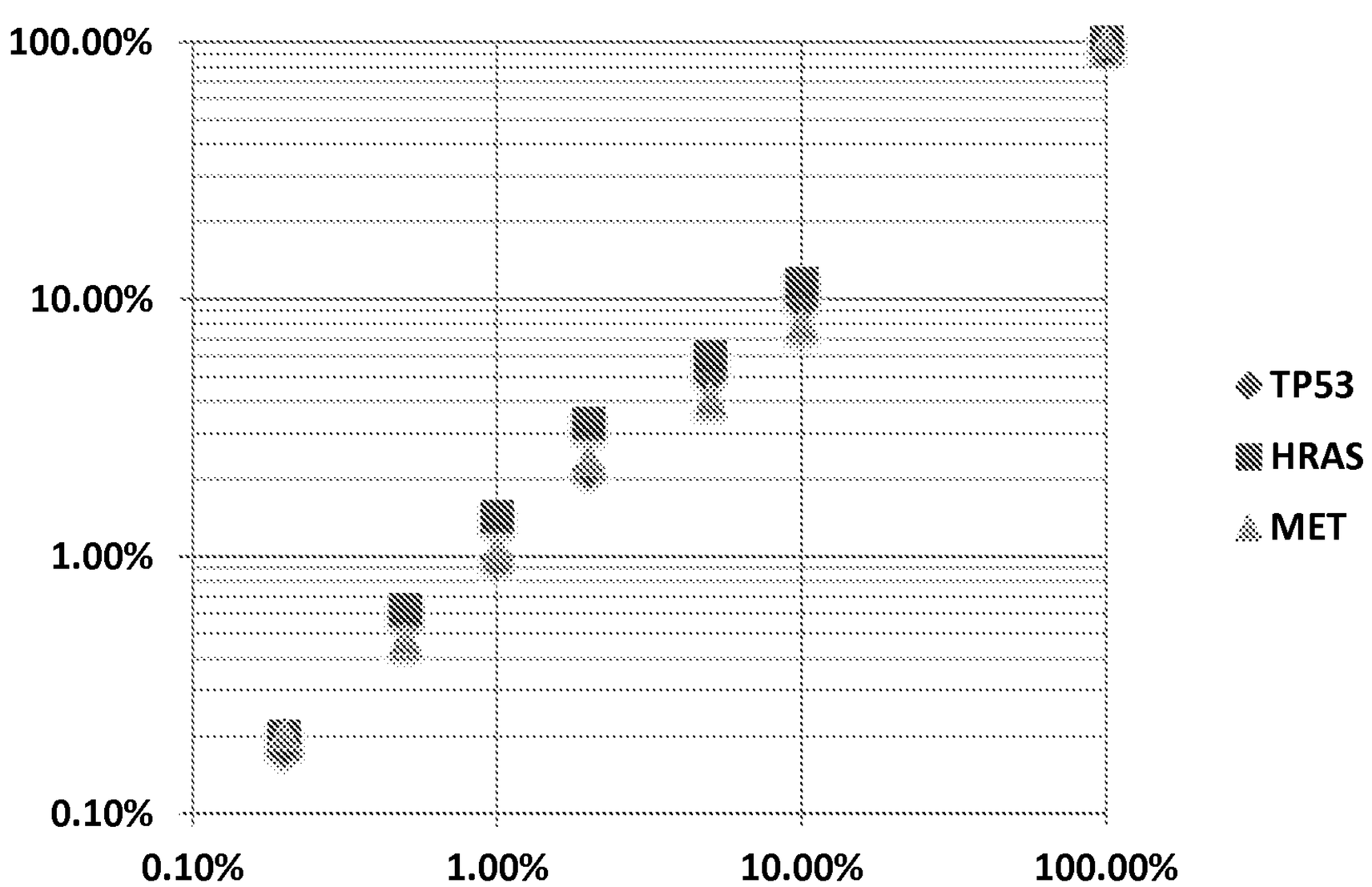

Cell-Free Tumor
Burden: 5%
# of Copies
3
2.5
2
1.5
1
0
1
2
3
4
5
6
7
8
9
Prostate Cancer Patient 3
B

Fig. 5

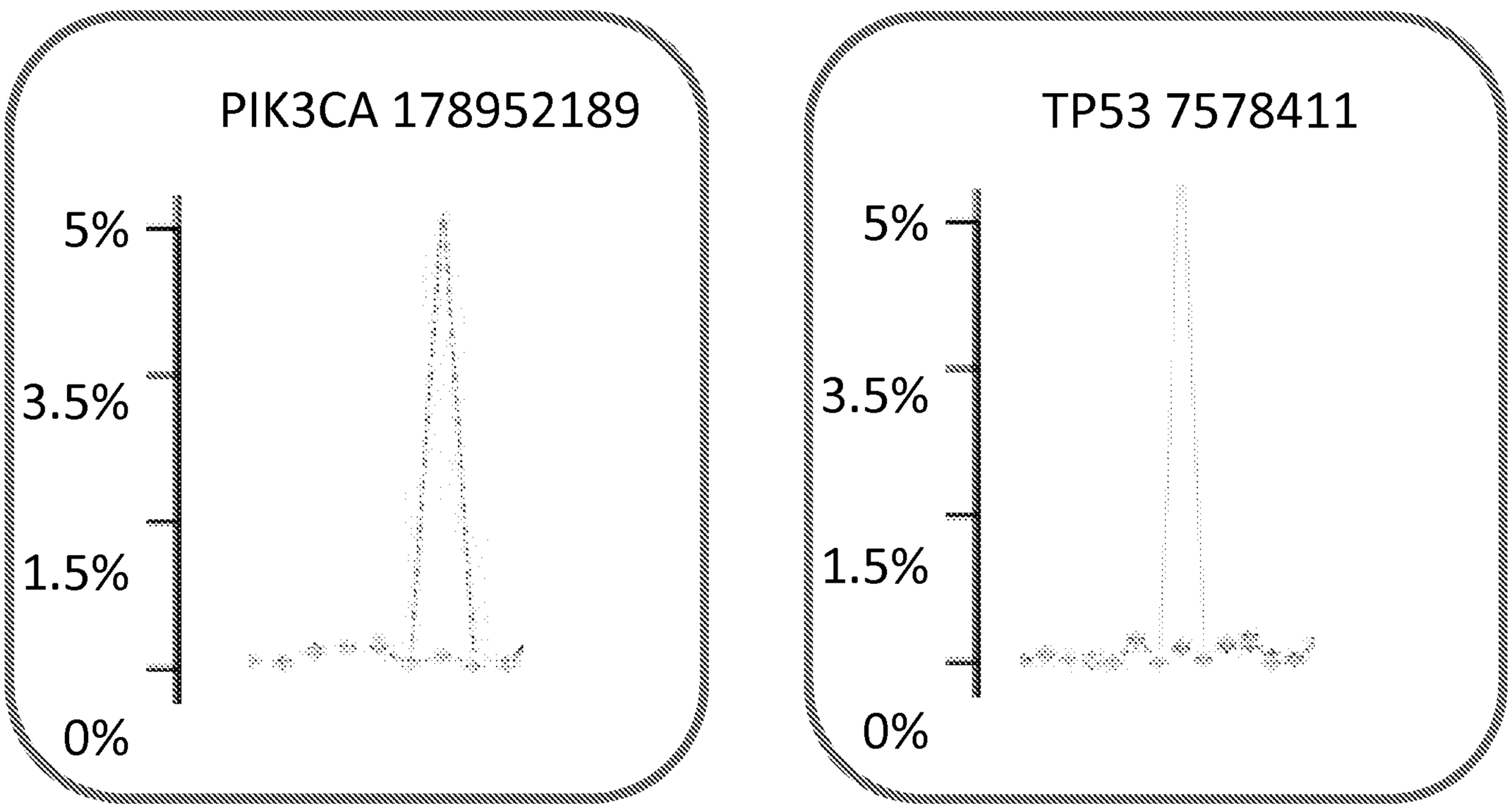

MET 116411990
100%
10%
1%
0.1%
TP53 7578552
100%
10%
1%
0.1%

A

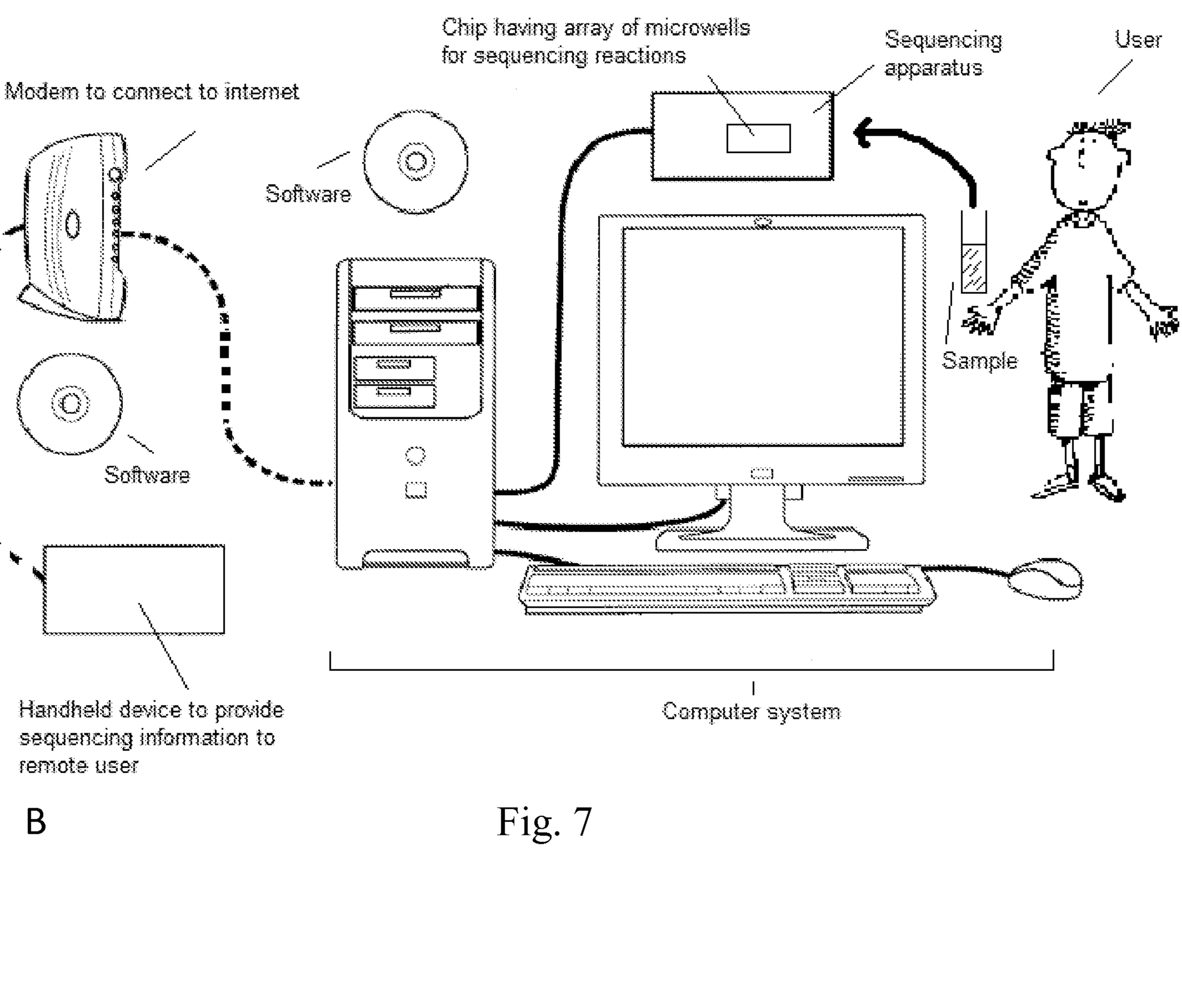

100.00%
10.00%
1.00%
0.10%
0.10%
1.00%
10.00%
100.00%
TP53
HRAS
MET

B

Fig. 6


PIK3CA 178952189
5%
3.5%
1.5%
0%
TP53 7578411
5%
3.5%
1.5%
0%

A


Chip having array of microwells for sequencing reactions
Sequencing apparatus
User
Modem to connect to internet
Software
Sample
Software
Handheld device to provide sequencing information to remote user
Computer system

B

Fig. 7

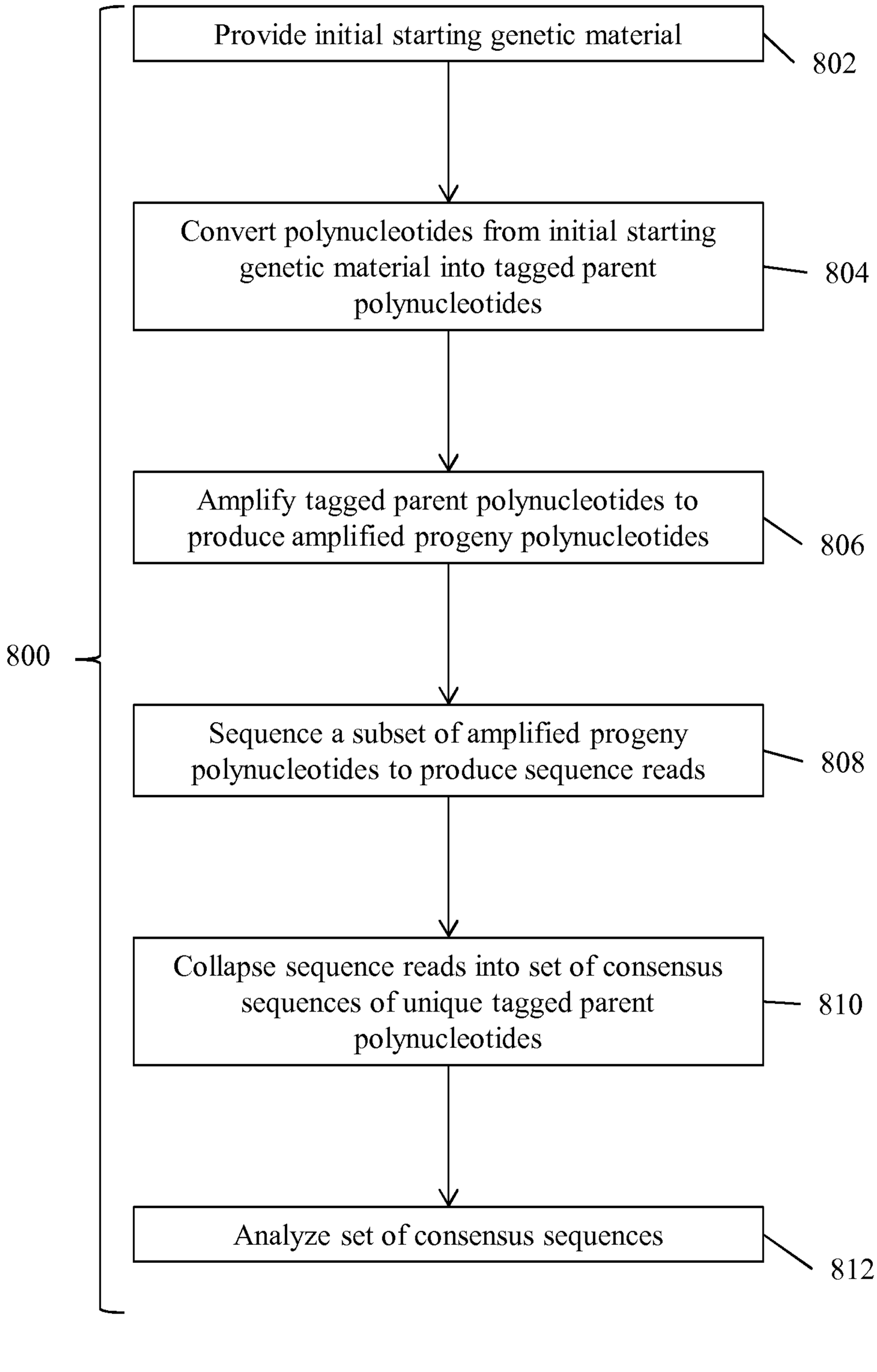

Provide initial starting genetic material
802
Convert polynucleotides from initial starting genetic material into tagged parent polynucleotides
804
Amplify tagged parent polynucleotides to produce amplified progeny polynucleotides
806
800
Sequence a subset of amplified progeny polynucleotides to produce sequence reads
808
Collapse sequence reads into set of consensus sequences of unique tagged parent polynucleotides
810
Analyze set of consensus sequences
812

Fig. 8

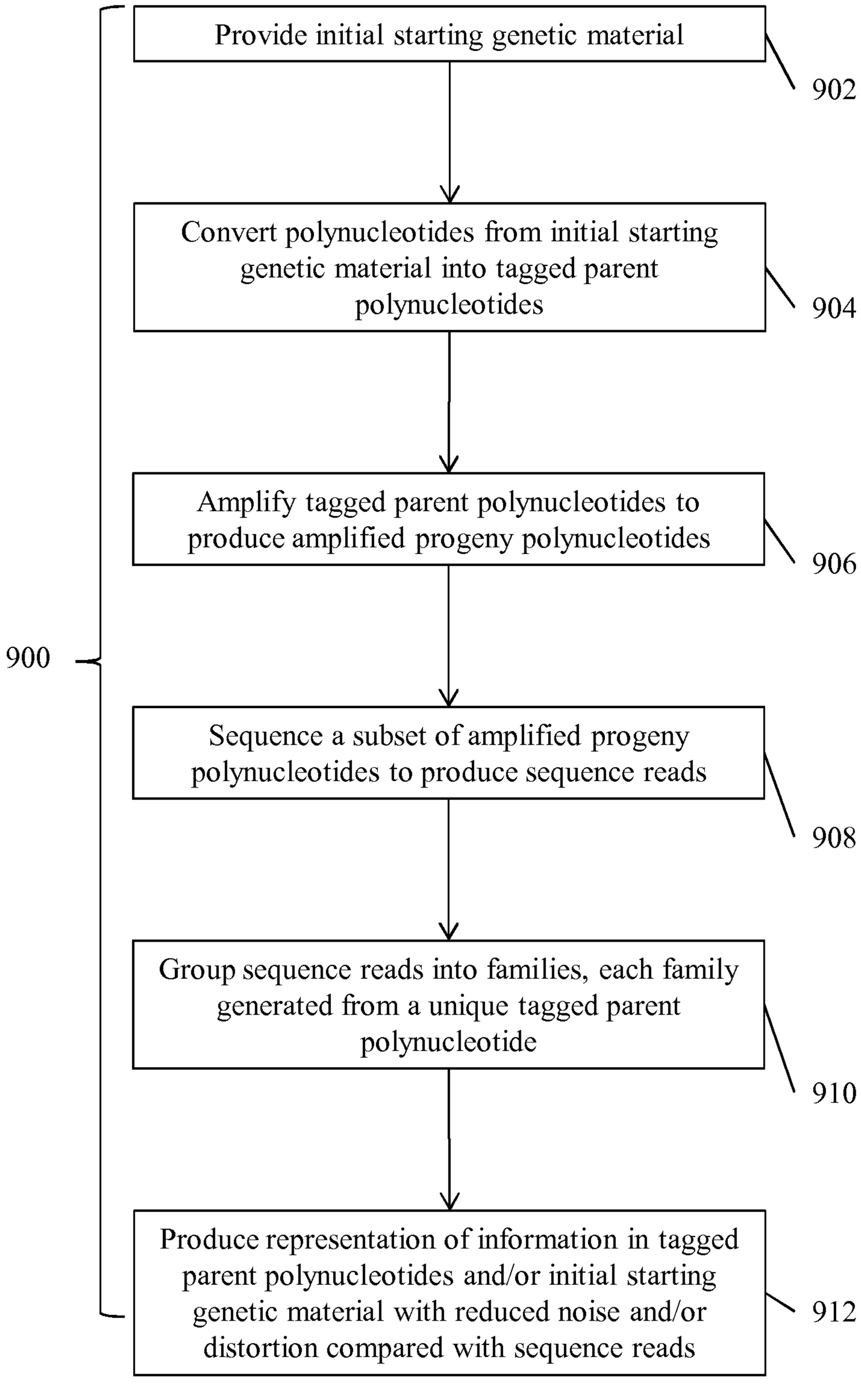

Provide initial starting genetic material
902
Convert polynucleotides from initial starting genetic material into tagged parent polynucleotides
904
Amplify tagged parent polynucleotides to produce amplified progeny polynucleotides
906
900
Sequence a subset of amplified progeny polynucleotides to produce sequence reads
908
Group sequence reads into families, each family generated from a unique tagged parent polynucleotide
910
Produce representation of information in tagged parent polynucleotides and/or initial starting genetic material with reduced noise and/or distortion compared with sequence reads
912

Fig. 9

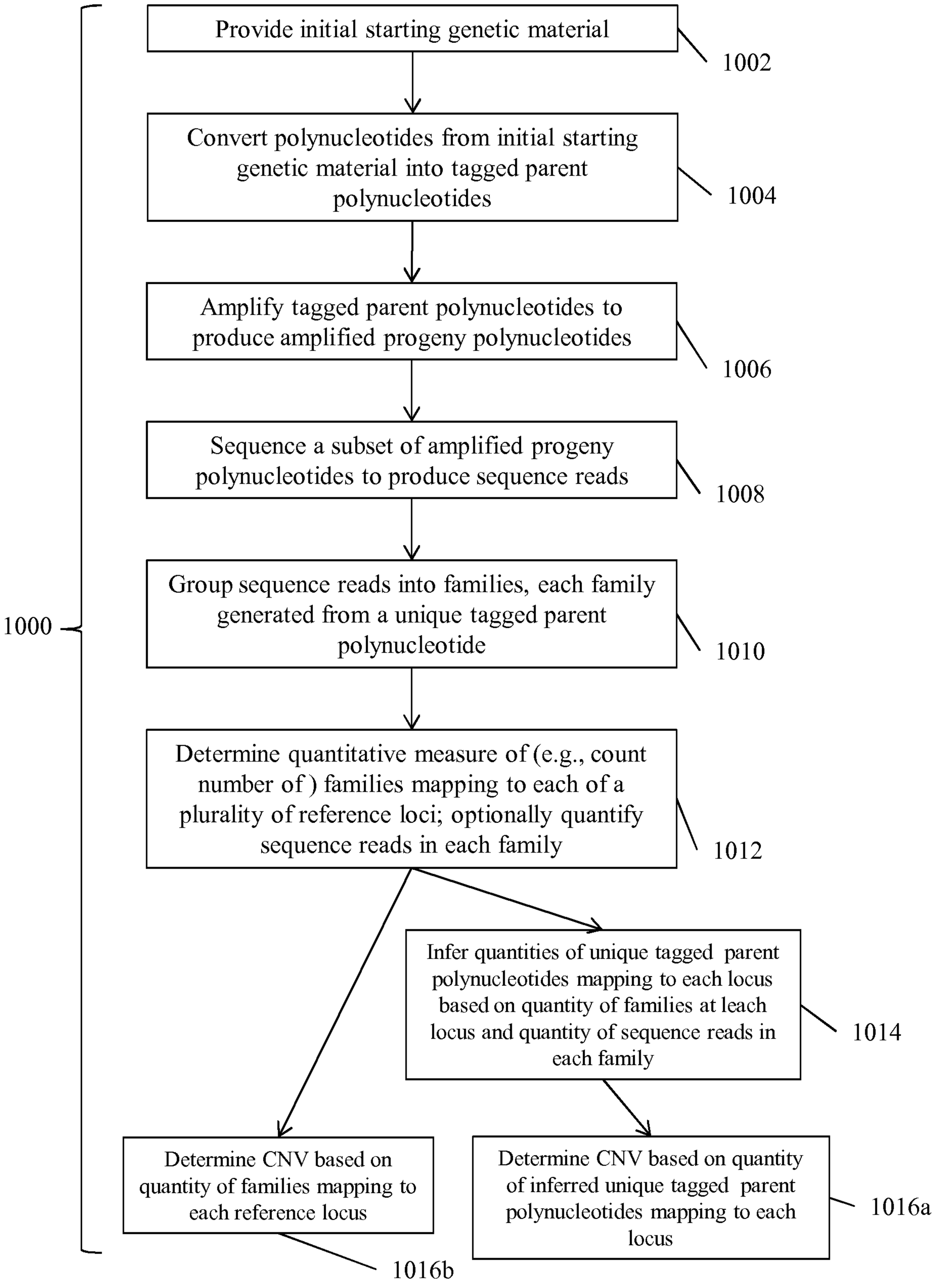

Provide initial starting genetic material
1002
Convert polynucleotides from initial starting genetic material into tagged parent polynucleotides
1004
Amplify tagged parent polynucleotides to produce amplified progeny polynucleotides
1006
Sequence a subset of amplified progeny polynucleotides to produce sequence reads
1008
Group sequence reads into families, each family generated from a unique tagged parent polynucleotide
1010
Determine quantitative measure of (e.g., count number of) families mapping to each of a plurality of reference loci; optionally quantify sequence reads in each family
1012
Infer quantities of unique tagged parent polynucleotides mapping to each locus based on quantity of families at leach locus and quantity of sequence reads in each family
1014
Determine CNV based on quantity of families mapping to each reference locus
1016b
Determine CNV based on quantity of inferred unique tagged parent polynucleotides mapping to each locus
1016a
1000

Fig. 10

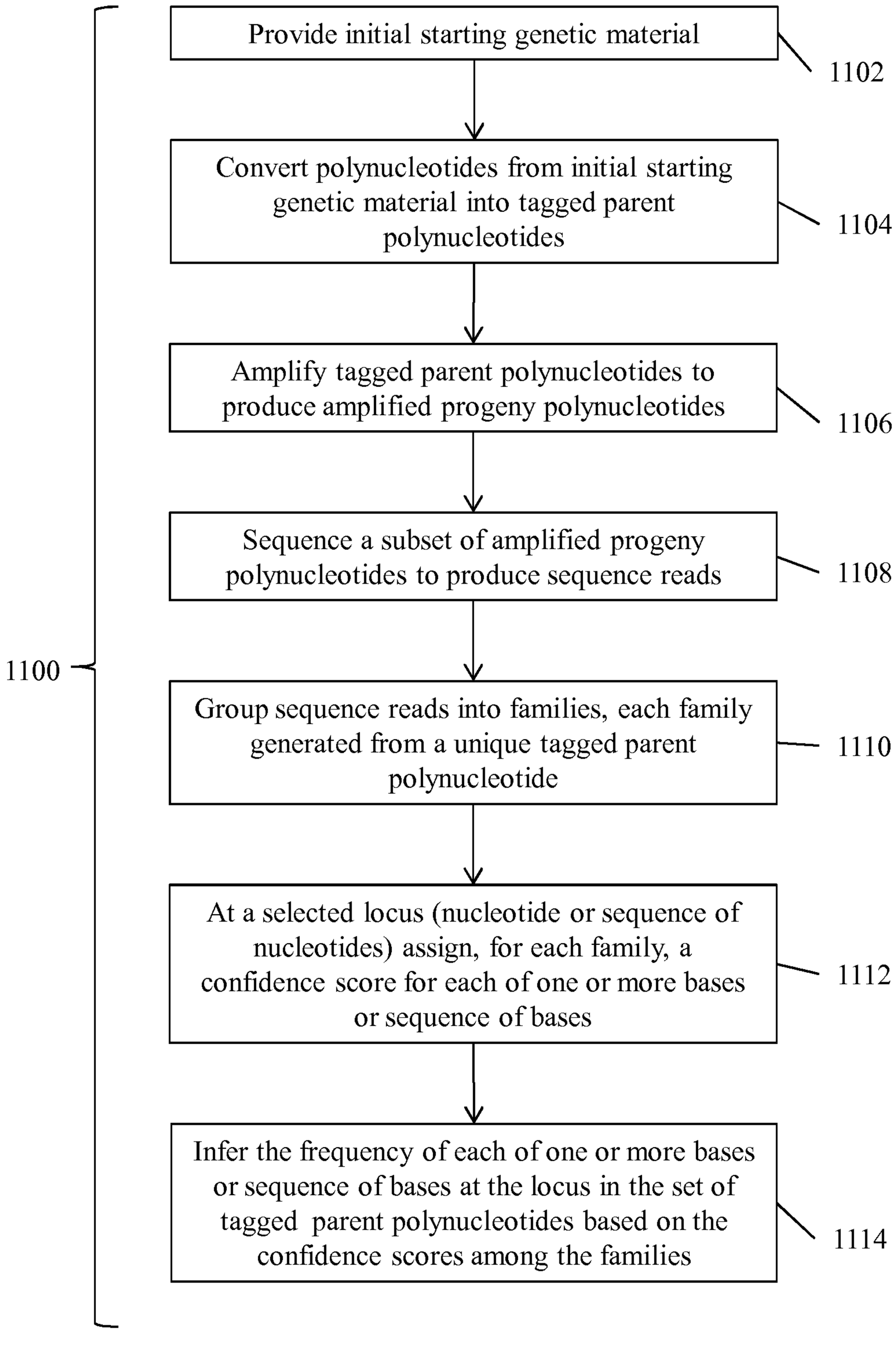

Provide initial starting genetic material
1102
Convert polynucleotides from initial starting genetic material into tagged parent polynucleotides
1104
Amplify tagged parent polynucleotides to produce amplified progeny polynucleotides
1106
Sequence a subset of amplified progeny polynucleotides to produce sequence reads
1108
1100
Group sequence reads into families, each family generated from a unique tagged parent polynucleotide
1110
At a selected locus (nucleotide or sequence of nucleotides) assign, for each family, a confidence score for each of one or more bases or sequence of bases
1112
Infer the frequency of each of one or more bases or sequence of bases at the locus in the set of tagged parent polynucleotides based on the confidence scores among the families
1114

Fig. 11

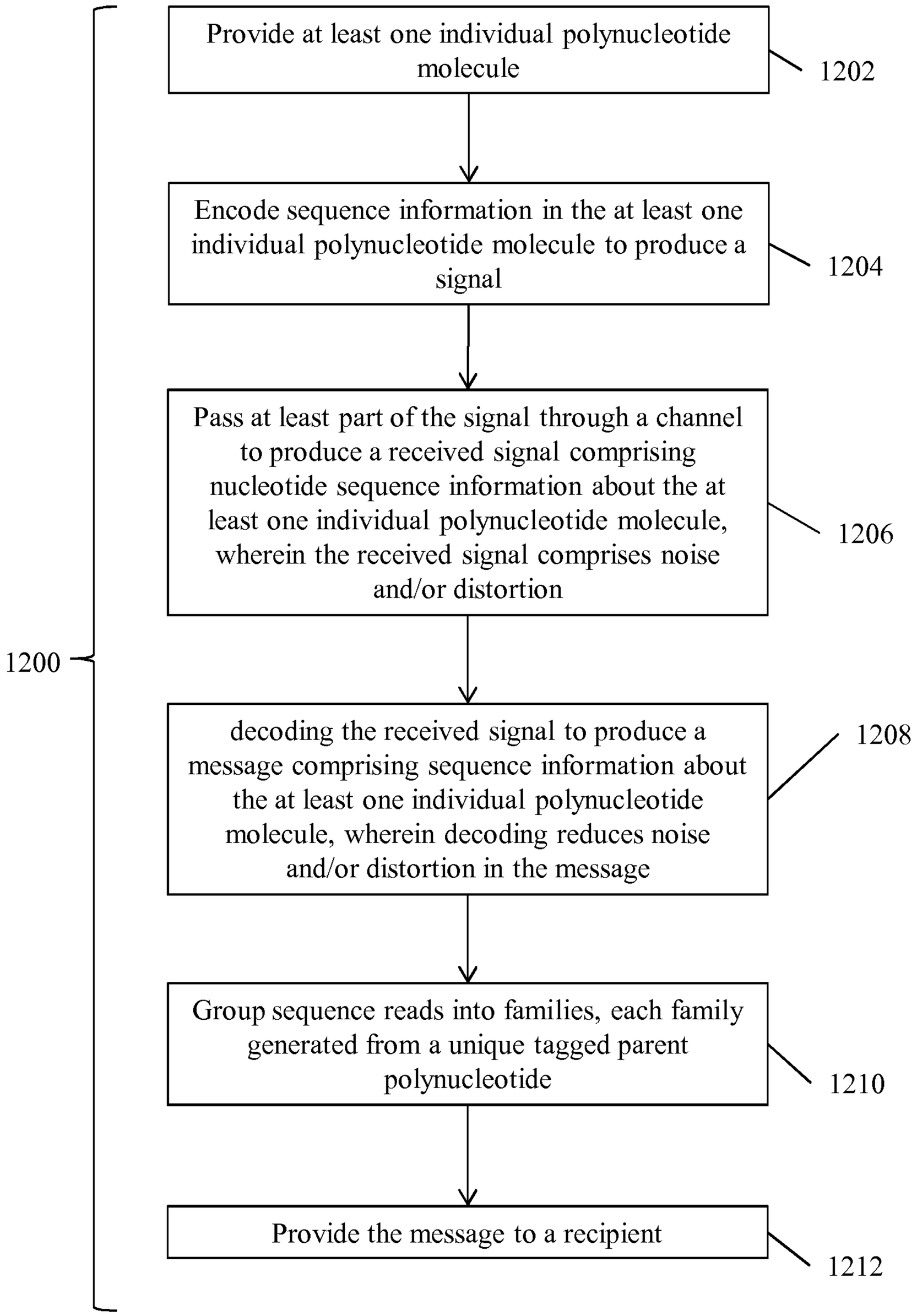

Provide at least one individual polynucleotide molecule
1202
Encode sequence information in the at least one individual polynucleotide molecule to produce a signal
1204
Pass at least part of the signal through a channel to produce a received signal comprising nucleotide sequence information about the at least one individual polynucleotide molecule, wherein the received signal comprises noise and/or distortion
1206
1200
decoding the received signal to produce a message comprising sequence information about the at least one individual polynucleotide molecule, wherein decoding reduces noise and/or distortion in the message
1208
Group sequence reads into families, each family generated from a unique tagged parent polynucleotide
1210
Provide the message to a recipient
1212

Fig. 12

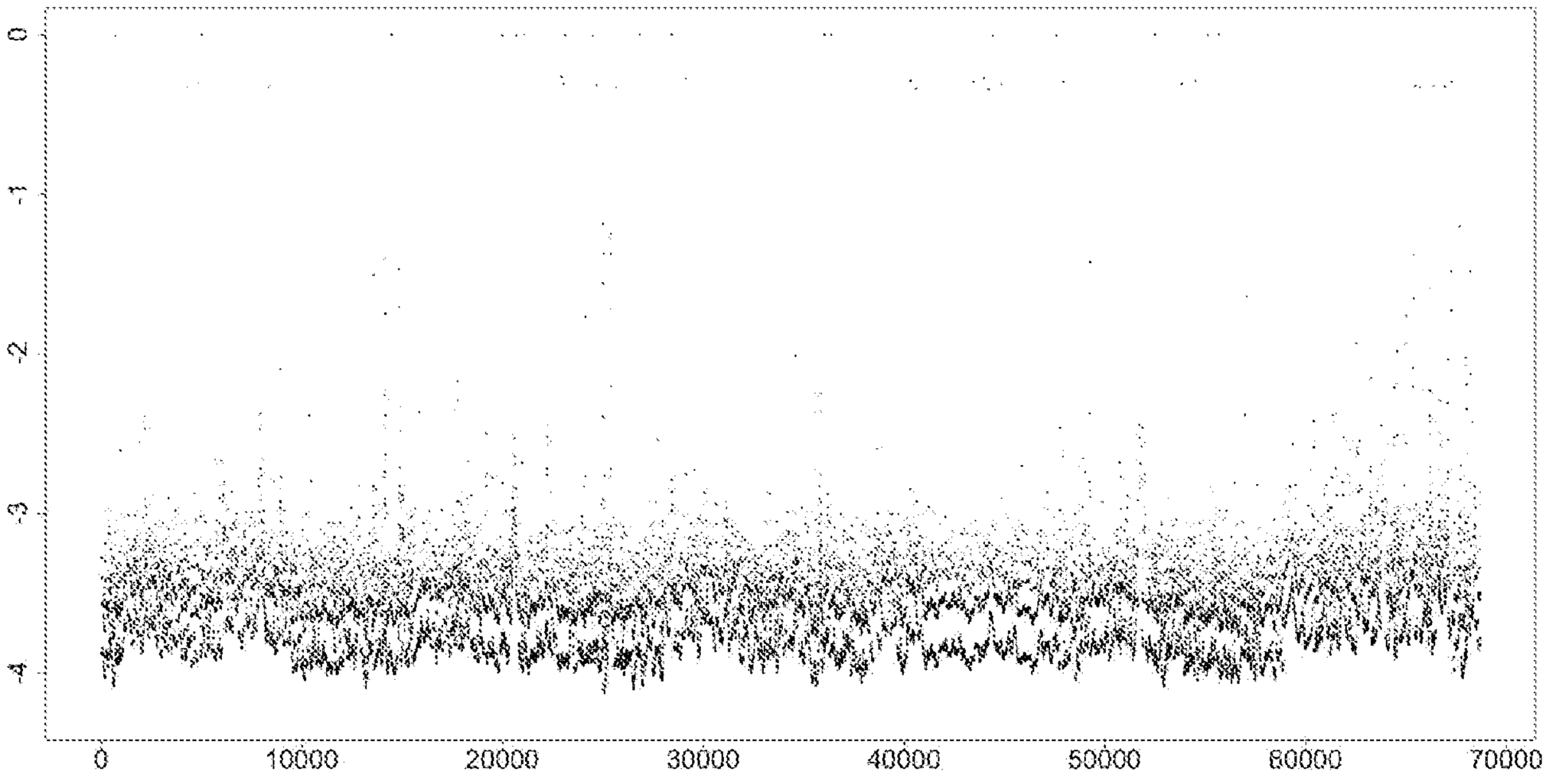


Fig. 13A

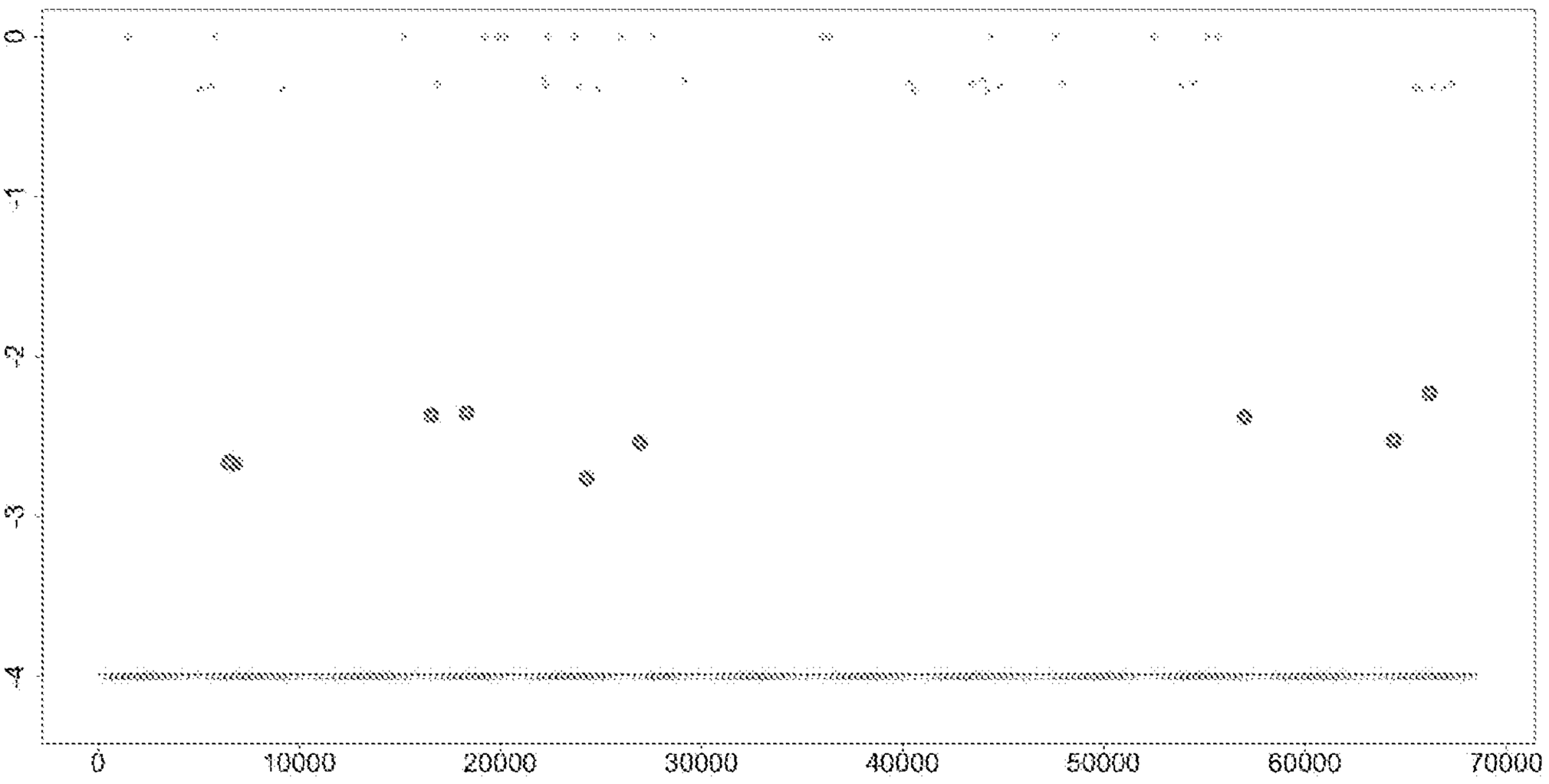


Fig. 13B

Fig. 13

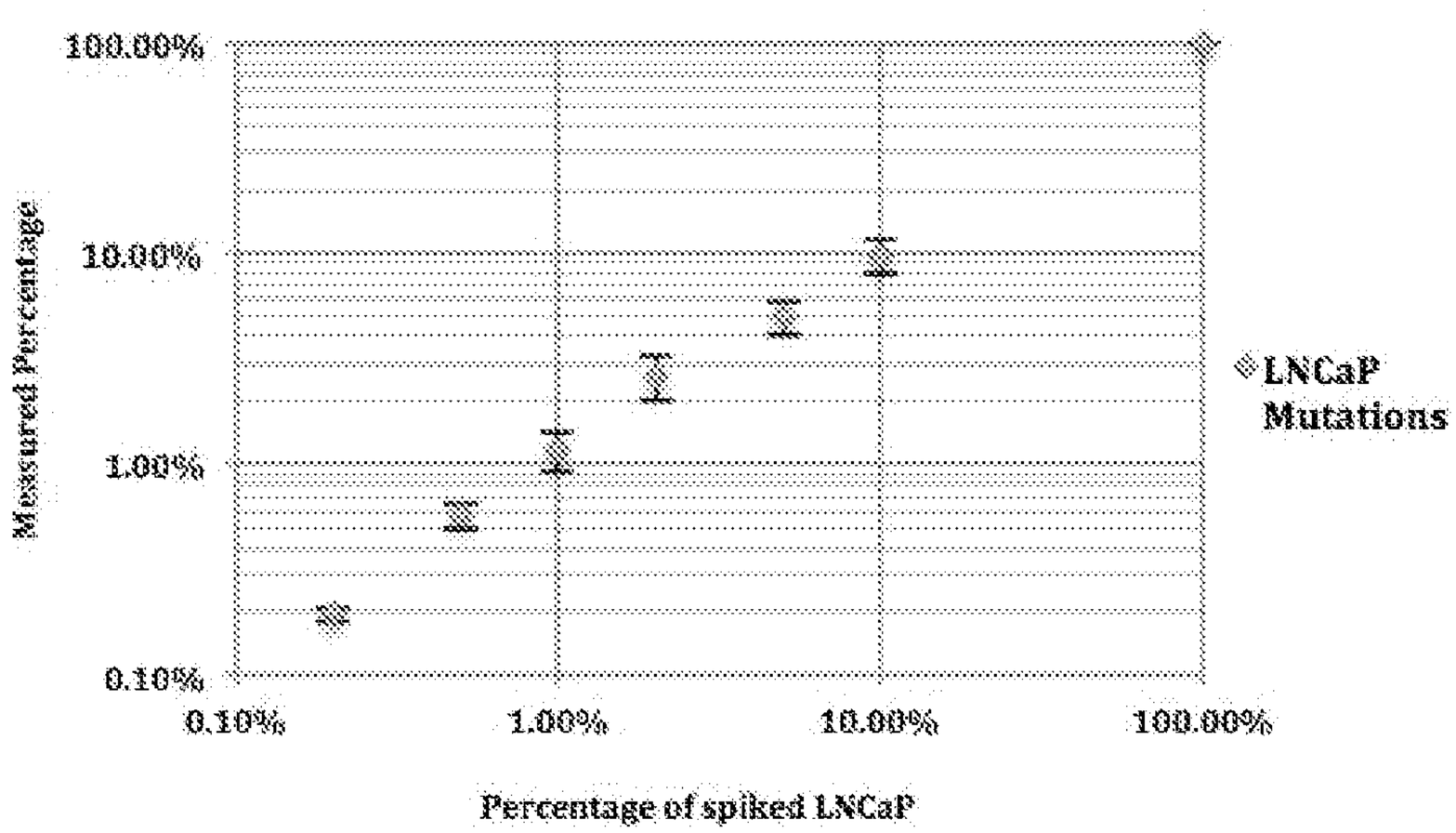

100.00%
10.00%
1.00%
0.10%
Measured Percentage
0.10%
1.00%
10.00%
100.00%
Percentage of spiked LNCaP
LNCaP Mutations

Fig. 14

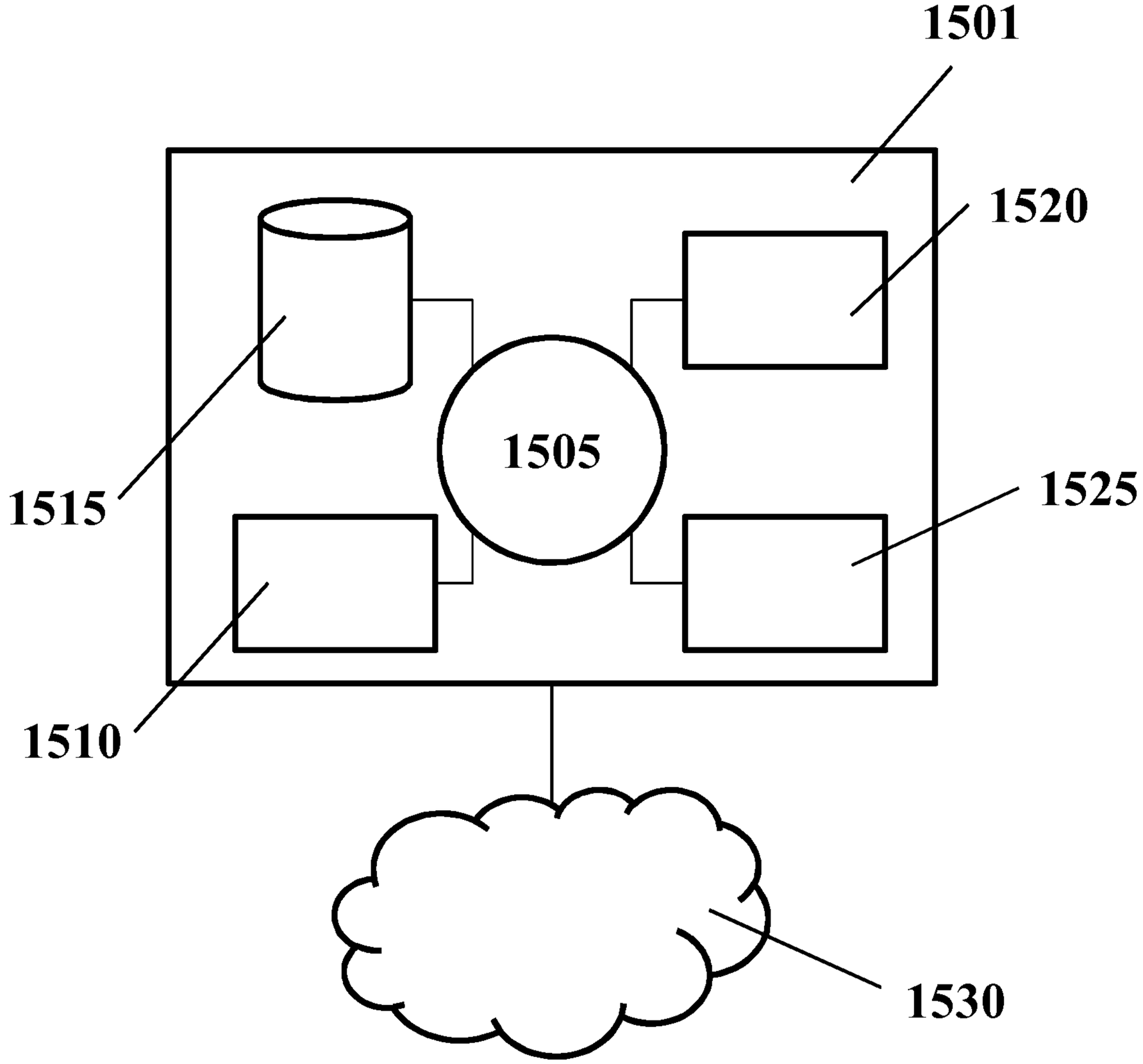


Fig. 15