

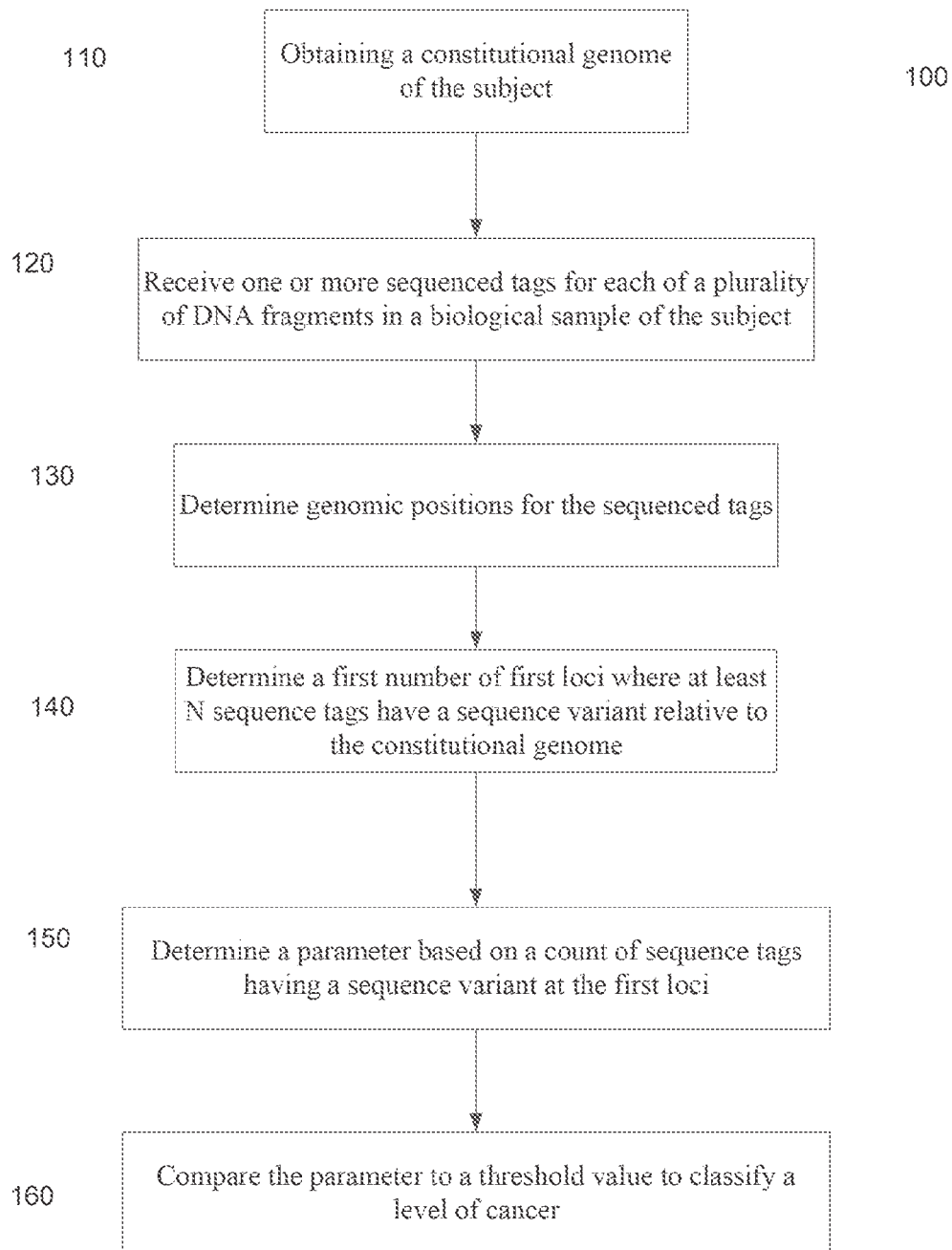


FIG. 1

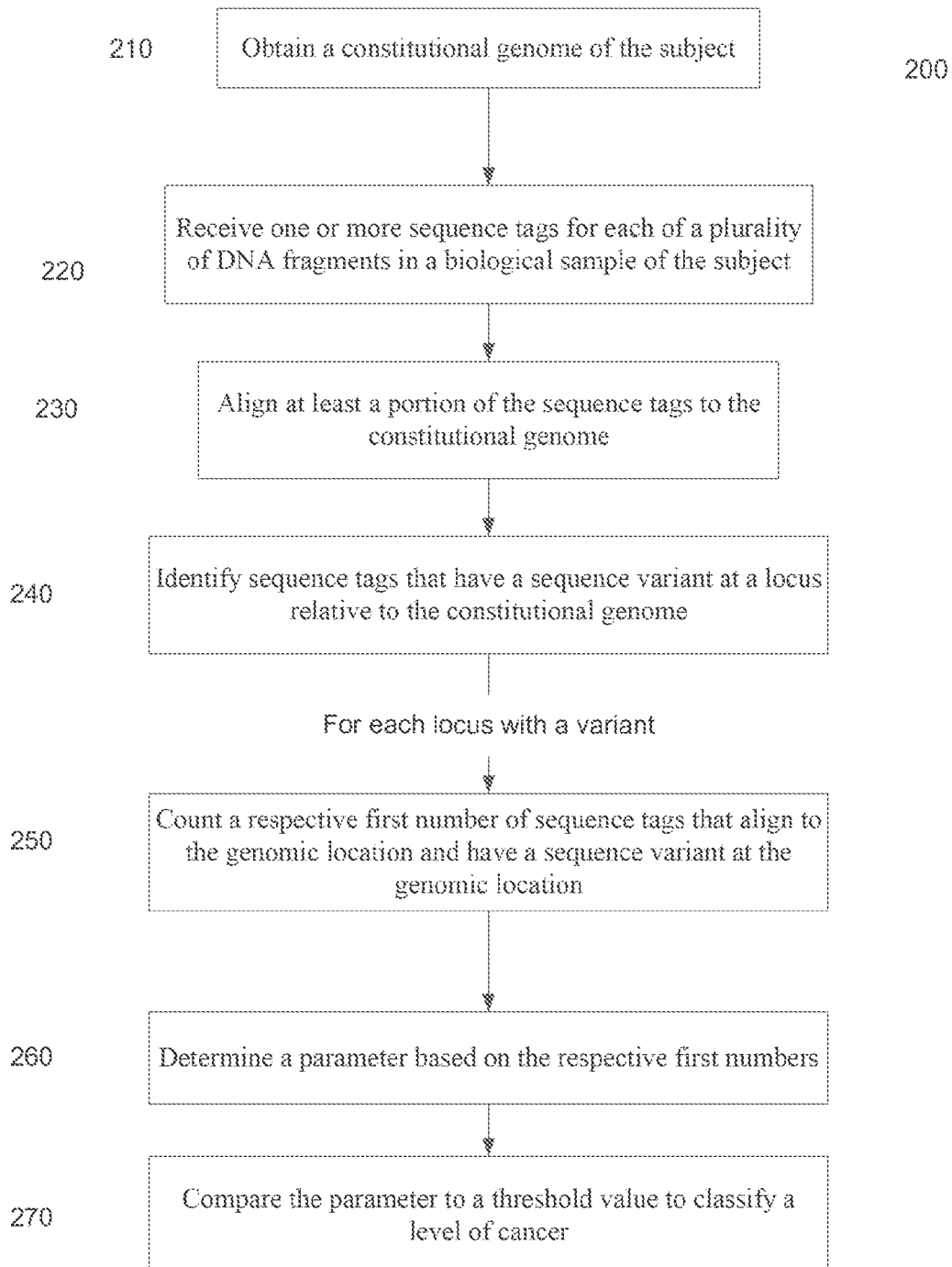


FIG. 2

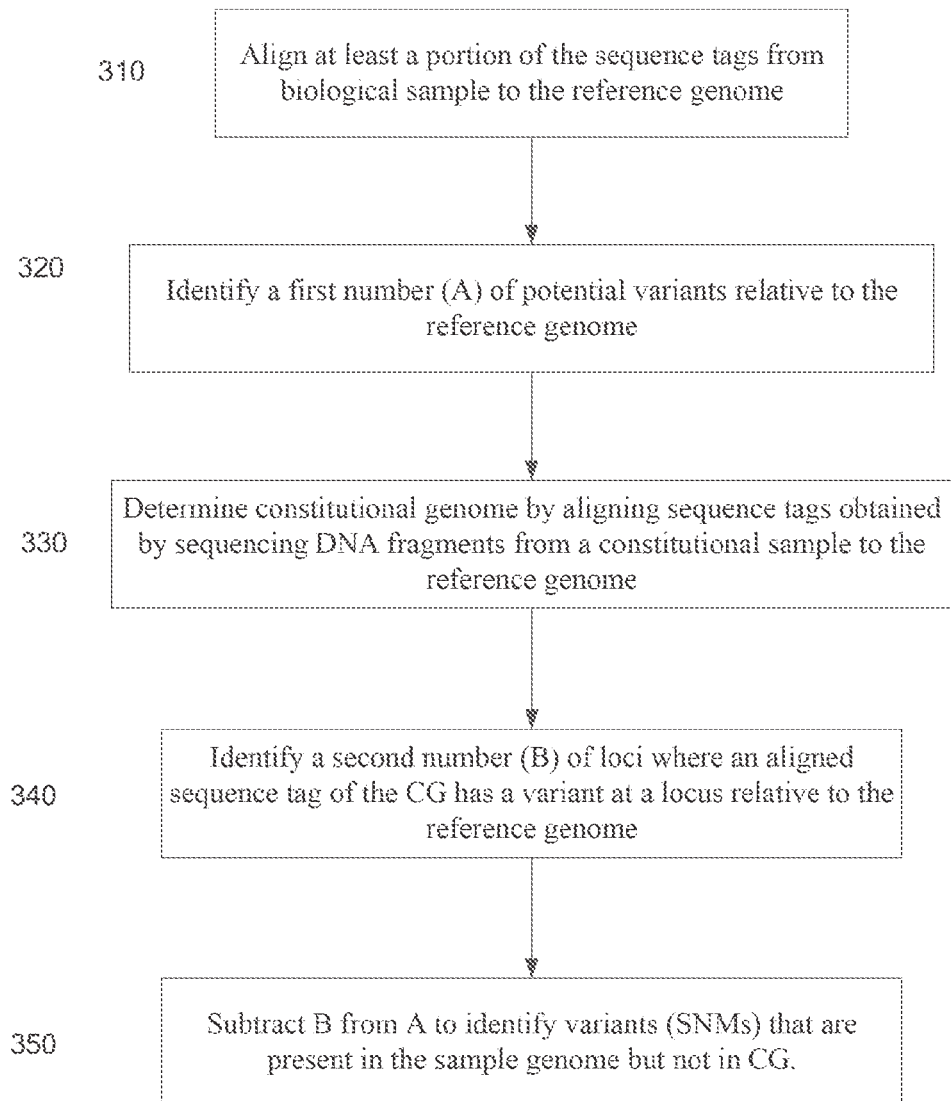


FIG. 3

No. of times seen	1x		2x		3x		4x		5x		6x	
	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified
1	2,998,500	146	1,499	4	0	0	0	0	0	0	0	0
10	29,330,499	1,130	45,000	271	40	43	0	3	0	1	0	0
20	59,403,980	1,896	190,000	793	360	241	1	57	0	11	0	2
40	117,631,683	2,594	780,000	1,752	3,293	970	10	429	0	158	0	50
60	174,706,399	2,851	1,770,000	2,403	11,407	1,730	54	1,038	0	554	0	252
80	230,650,961	2,945	3,160,000	2,725	27,387	2,286	176	1,700	1	1,113	0	645
100	285,487,746	2,980	4,950,000	2,879	53,900	2,626	436	2,205	3	1,679	0	1,152
120	339,236,690	2,993	7,140,000	2,948	93,613	2,814	913	2,546	7	2,145	0	1,663
140	391,925,294	2,997	9,730,000	2,978	149,193	2,911	1,703	2,755	15	2,481	0	2,098
160	443,568,633	2,999	12,730,000	2,991	223,307	2,959	2,922	2,873	30	2,701	0	2,426
180	494,189,366	3,000	16,110,000	2,996	318,620	2,981	4,700	2,936	55	2,835	1	2,653
200	543,807,741	3,000	19,900,000	2,999	437,800	2,992	7,187	2,969	94	2,912	1	2,799

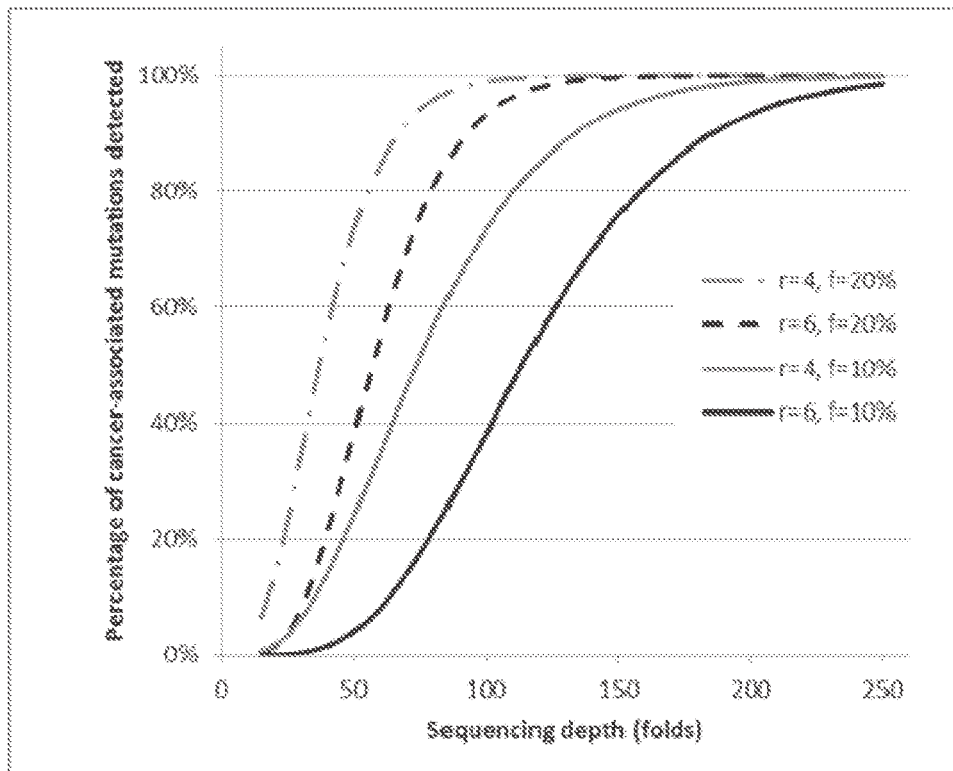
400

FIG. 4

no. of times seen	1x		2x		3x		4x		5x		6x	
	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified	Expect no. false positives loci for sequencing errors	Expect no. of mutations identified
Sequencing depth												
1	2,908,500	74	1,499	1	0	0	0	0	0	0	0	0
10	29,850,499	664	45,000	79	40	6	0	0	0	0	0	0
20	59,403,980	1,180	190,000	271	380	43	1	5	0	1	0	0
40	117,631,683	1,896	780,000	793	3,293	241	10	57	0	11	0	2
60	174,706,399	2,331	1,770,000	1,327	11,407	573	54	197	0	56	0	13
80	230,650,961	2,594	3,160,000	1,782	27,387	970	176	429	1	158	0	50
100	285,487,746	2,754	4,950,000	2,138	53,900	1,369	436	727	3	326	0	126
120	339,238,690	2,851	7,140,000	2,403	93,613	1,730	913	1,058	7	554	0	252
140	391,925,594	2,909	9,730,000	2,592	149,193	2,037	1,703	1,390	15	824	0	427
160	443,568,633	2,945	12,720,000	2,725	223,307	2,286	2,922	1,700	30	1,113	0	645
180	494,189,366	2,967	16,110,000	2,817	318,620	2,479	4,780	1,973	55	1,404	1	891
200	543,807,741	2,980	19,900,000	2,879	437,800	2,626	7,187	2,205	94	1,679	1	1,152

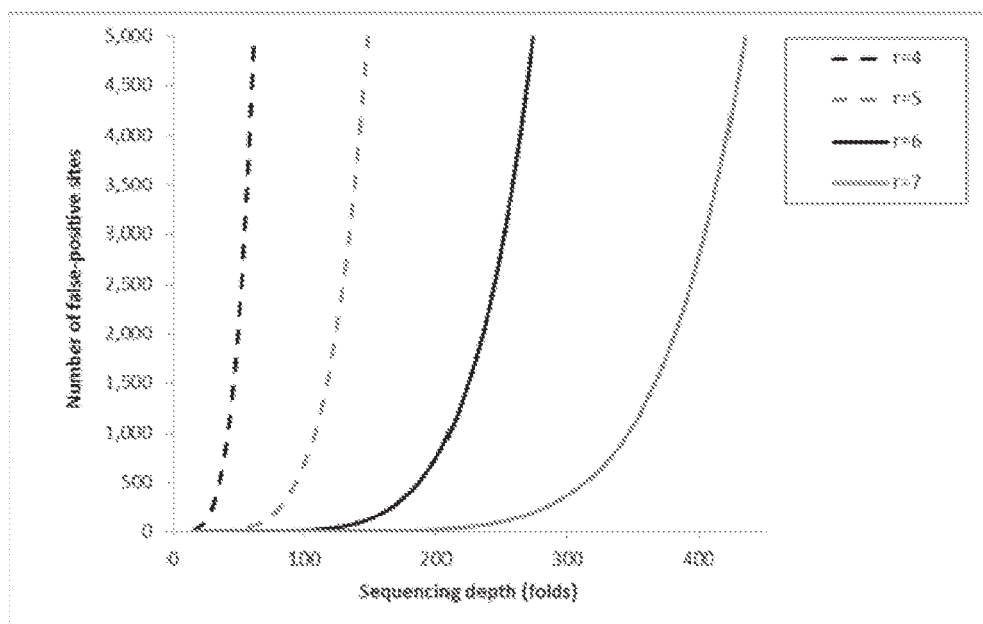
500

FIG. 5



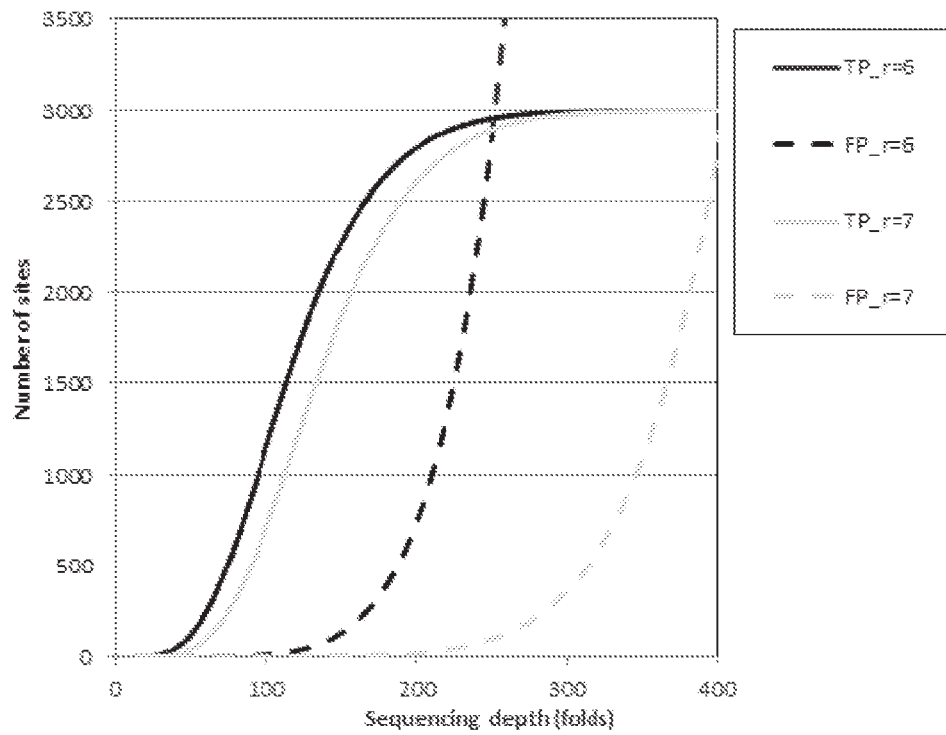
600

FIG. 6A



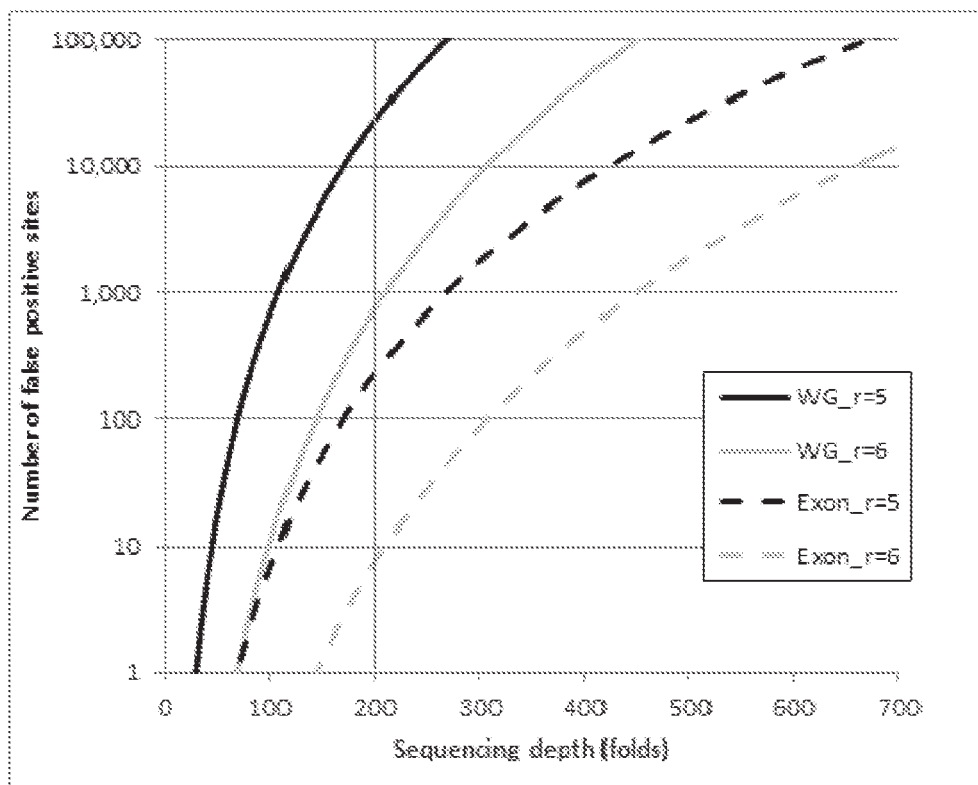
650

FIG. 6B



700

FIG. 7A



750

FIG. 7B

Case	No. of SNVs detected in the tumor tissue	Time point	No. of tumor-associated SNVs sequenced from plasma (% of SNVs seen in tumor tissue)	No. of plasma DNA sequence reads showing SNVs (p)	No. of plasma DNA sequence reads showing wildtype sequence (q)	Deduced fractional concentration of tumor-derived DNA by SNV analysis ($\frac{2p}{p+q}$)	Deduced fractional concentration of tumor-derived DNA by GAAL analysis
HCC1	2,840	Pre-Tx	2,569 (94%)	11,389	31,602	53%	52%
		Post-Tx	44 (1.5%)	91	46,898	0.4%	1.4%
HCC2	3,105	Pre-Tx	1,097 (35%)	1,490	57,865	5.0%	4.3%
		Post-Tx	72 (2.3%)	206	66,692	0.6%	0.9%
HCC3	3,171	Pre-Tx	461 (15%)	525	48,886	2.1%	5.6%
		Post-Tx	31 (1%)	67	58,862	0.2%	0.9%
HCC4	1,334	Pre-Tx	201 (15%)	248	18,527	2.6%	7.6%
		Post-Tx	74 (5.5%)	149	22,144	1.3%	2.7%

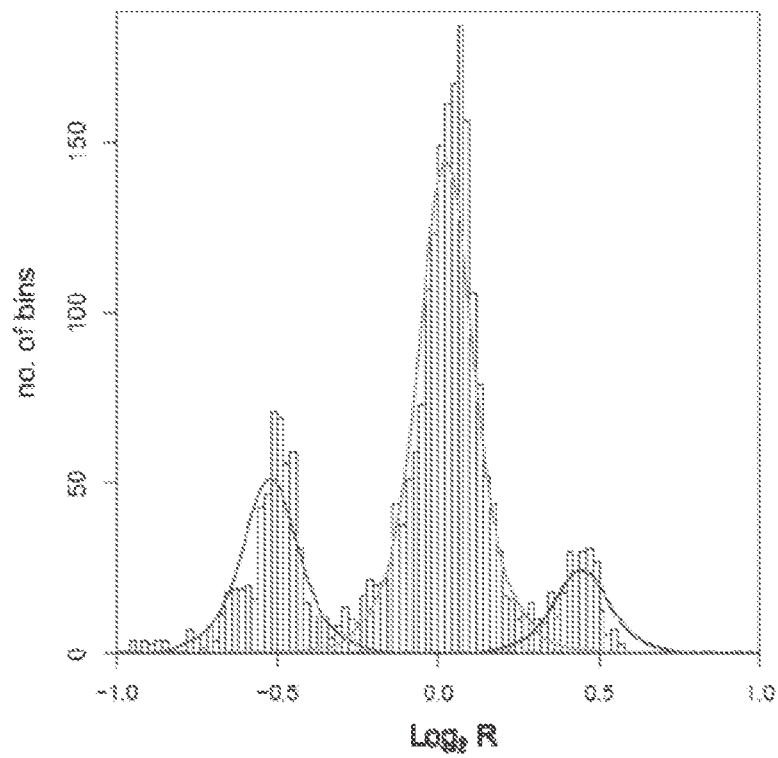
FIG. 8

	HCC1 SNVs		HCC2 SNVs		HCC3 SNVs		HCC4 SNVs		All SNVs	
	Plasma DNA sequence reads showing tumor-associated SNVs	Plasma DNA sequence reads showing wildtype sequence	Plasma DNA sequence reads showing tumor-associated SNVs	Plasma DNA sequence reads showing wildtype sequence	Plasma DNA sequence reads showing tumor-associated SNVs	Plasma DNA sequence reads showing wildtype sequence	Plasma DNA sequence reads showing tumor-associated SNVs	Plasma DNA sequence reads showing wildtype sequence	Plasma DNA sequence reads showing wildtype sequence (q)	Fractional concentration of all tumor-associated SNVs in plasma ($\frac{2p}{p+q}$)
Controls										
C01	40	31,261	66	38,172	30	33,823	69	14,389	117,645	0.35%
C02	60	34,368	112	42,660	37	37,462	58	16,097	130,587	0.41%
C03	55	37,931	100	46,297	50	41,181	74	17,039	142,448	0.39%
C04	41	40,277	87	48,441	39	43,541	87	17,358	149,617	0.34%
C05	42	31,655	71	38,787	46	34,566	70	14,821	119,829	0.38%
C06	41	34,604	97	43,999	26	37,477	79	16,762	132,842	0.37%
C07	52	38,664	118	47,200	61	41,840	72	17,601	145,305	0.42%
C08	57	38,514	131	46,464	42	41,662	81	17,275	143,915	0.43%
C09	41	34,945	115	43,760	33	38,039	81	16,503	133,247	0.40%
C10	44	35,197	89	43,622	51	38,370	65	16,009	133,198	0.37%
C11	41	36,693	95	45,108	39	39,732	72	16,801	138,334	0.36%
C12	45	39,506	104	50,085	46	42,536	91	18,598	150,725	0.38%
C13	36	33,799	71	40,309	39	36,241	70	14,450	124,799	0.35%
C14	42	38,199	104	47,832	42	41,245	91	17,807	145,083	0.38%
C15	49	36,106	90	43,129	41	38,432	73	16,154	133,821	0.38%
C16	38	33,633	66	40,023	41	36,431	52	14,502	124,589	0.32%
									Mean	0.38%

FIG. 9

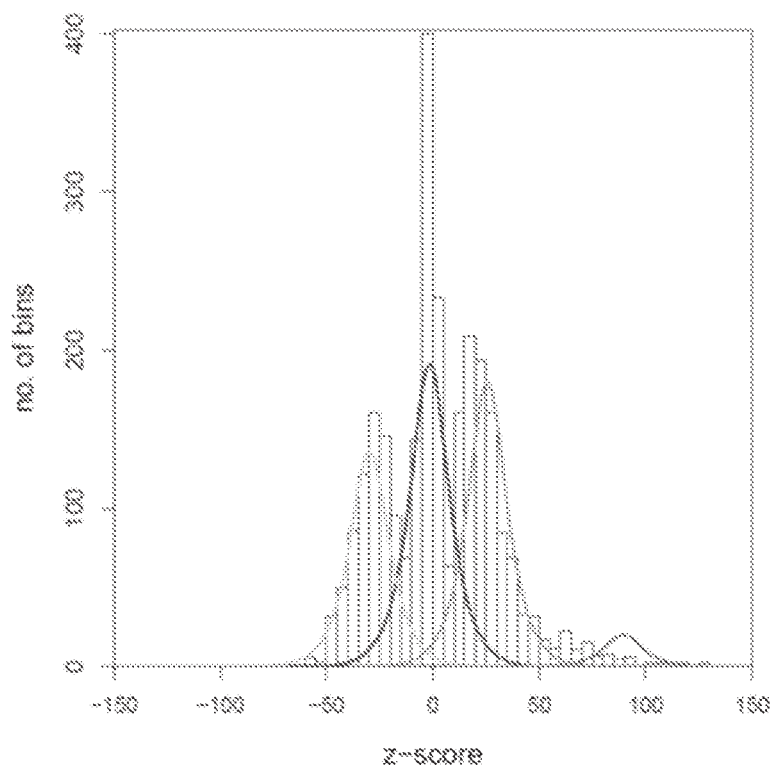
900

10/26



1000

FIG. 10A



1050

FIG. 10B

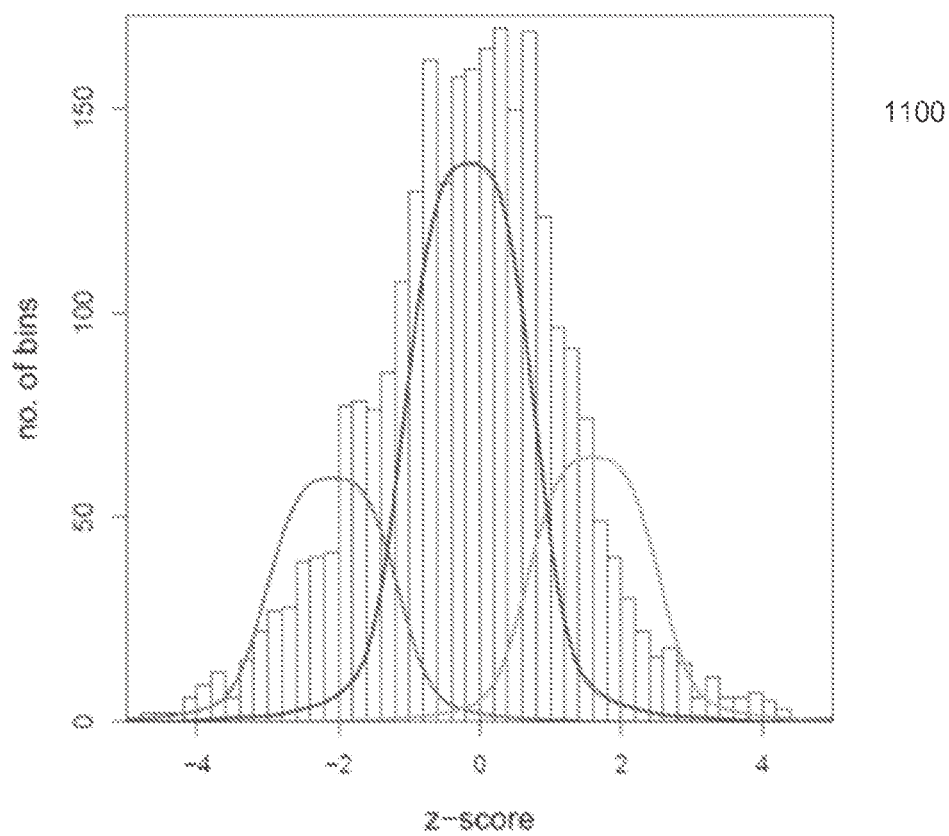


FIG. 11

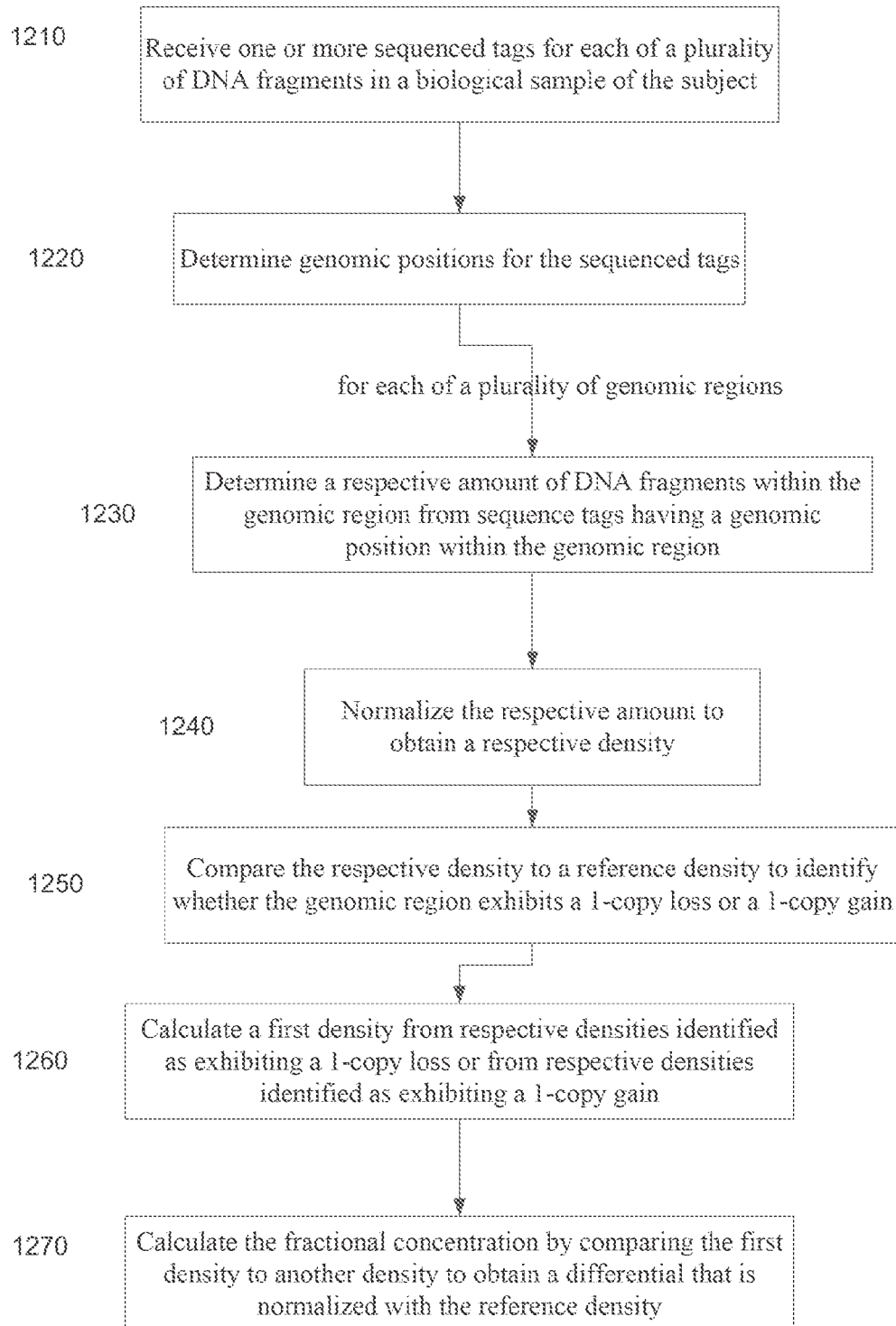


FIG. 12

No. of times detected in plasma (N)	No. of potential mutations detected N times in plasma	No. of potential mutations detected $\geq N$ times in plasma	Tumor mutations detected N times in plasma	Tumor mutations detected $\geq N$ times in plasma	Percentage of mutations detected compared with N=1	Positive predictive value
1	3,605,085	3,623,970	61	2,064	100%	0.06%
2	13,518	18,885	35	2,003	97%	11%
3	2,029	5,367	71	1,968	95%	37%
4	766	3,338	83	1,897	92%	57%
5	437	2,572	140	1,814	88%	71%
6	313	2,135	161	1,674	81%	78%
7	265	1,822	165	1,513	73%	83%
8	261	1,557	192	1,348	65%	87%
9	231	1,296	185	1,156	56%	89%
10	191	1,065	160	971	47%	91%
11	179	874	165	811	39%	93%
12	148	695	137	646	31%	93%
13	122	547	109	509	25%	93%
14	99	425	92	400	19%	94%
15	74	326	71	308	15%	94%
16	54	252	49	237	11%	94%
17	39	198	39	188	9%	95%
18	35	159	33	149	7%	94%
19	24	124	23	116	6%	94%
20	20	100	20	93	5%	93%
21	18	80	17	73	4%	91%
22	11	62	10	56	3%	90%
23	9	51	9	46	2%	90%
24	8	42	5	37	2%	88%
25	6	34	6	32	2%	94%
26	7	28	5	26	1%	93%
27	8	21	8	21	1%	100%
28	1	13	1	13	1%	100%
29	4	12	4	12	1%	100%
30	8	8	8	8	0%	100%

FIG. 13A

No. of times detected in plasma (N)	No. of potential mutations detected N times in plasma	No. of potential mutations detected $\geq N$ times in plasma	Tumor mutations detected N times in plasma	Tumor mutations detected $\geq N$ times in plasma	Percentage of mutations detected compared with N=1	Positive predictive value
1	3,155,634	3,167,144	51	54	100%	<0.01%
2	9,835	11,510	2	3	6%	0.03%
3	1,123	1,675	1	1	2%	0.06%
4	314	552	-	-	-	-
5	132	238	-	-	-	-
6	49	106	-	-	-	-
7	22	57	-	-	-	-
8	11	35	-	-	-	-
9	6	24	-	-	-	-
10	5	18	-	-	-	-
11	4	13	-	-	-	-
12	2	9	-	-	-	-
13	2	7	-	-	-	-
14	3	5	-	-	-	-
15	-	2	-	-	-	-
16	-	2	-	-	-	-
17	1	2	-	-	-	-
18	-	1	-	-	-	-
19	1	1	-	-	-	-
20	-	-	-	-	-	-
21	-	-	-	-	-	-
22	-	-	-	-	-	-
23	-	-	-	-	-	-
24	-	-	-	-	-	-
25	-	-	-	-	-	-
26	-	-	-	-	-	-
27	-	-	-	-	-	-
28	-	-	-	-	-	-
29	-	-	-	-	-	-
30	-	-	-	-	-	-

FIG. 13B

FIG. 14A

1400

Sequencing depth	Pre-treatment plasma			Post-treatment plasma		
	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor
<50	36.3%	9	8	30.5%	0	0
50 – 125	29.9%	15	15	31.2%	0	0
126 – 235	18.6%	21	21	20.8%	0	0
236 – 380	9.4%	7	6	10.9%	0	0
381 – 560	4.2%	2	2	4.8%	0	0
561 – 760	1.6%	3	3	1.7%	0	0
Total		57	55		0	0

FIG. 14B

1450

Sequencing depth	Pre-treatment plasma			Post-treatment plasma		
	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor
<50	30.0%	0	0	27.1%	0	0
50 – 125	33.6%	2	2	32.5%	0	0
126 – 235	21.6%	6	6	22.2%	0	0
236 – 380	10.2%	5	5	11.5%	0	0
381 – 560	3.7%	4	4	4.9%	0	0
561 – 760	0.9%	1	1	1.7%	0	0
Total		18	18		0	0

FIG. 15A

1500

Sequencing depth	Pre-treatment plasma			Post-treatment plasma		
	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor
<50	38.8%	0	0	18.5%	0	0
50 – 125	45.7%	0	0	28.6%	0	0
126 – 235	15.2%	0	0	25.1%	0	0
236 – 380	0.2%	0	0	16.2%	0	0
381 – 560	<0.1%	0	0	8.3%	0	0
561 – 760	<0.1%	0	0	3.4%	0	0
Total		0	0		0	0

FIG. 15B

1550

Sequencing depth	Pre-treatment plasma			Post-treatment plasma		
	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor
<50	26.7%	0	0	24.4%	0	0
50 – 125	35.9%	1	1	31.2%	1	1
126 – 235	24.7%	2	2	23.1%	0	0
236 – 380	10.4%	0	0	13.1%	0	0
381 – 560	2.2%	0	0	6.0%	0	0
561 – 760	0.1%	0	0	2.2%	0	0
Total		3	3		1	1

Sequencing depth	Pre-treatment plasma			Post-treatment plasma		
	percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor	Percentage of nucleotides with the specified sequencing depth	Total no. nucleotide positions with single nucleotide variations detected in plasma	No. of sites with corresponding changes in tumor
<50	31.1%	7	7	30.8%	0	0
50 – 125	30.3%	14	14	33.2%	1	0
126 – 235	20.5%	19	19	21.6%	0	0
236 – 380	11.3%	13	13	10.3%	0	0
381 – 560	5.2%	7	4	3.5%	0	0
561 – 760	1.8%	4	2	0.7%	0	0
Total		64	59		1	0

FIG. 16

1600

Sequencing depth (folds)	Sensitivity when mutant is defined as being seen in the tumor tissue sequencing data for at least n times, where n=					
	4	5	6	7	8	9
20	73%	56%	38%	24%	13%	7%
21	77%	60%	43%	28%	16%	9%
22	80%	64%	47%	31%	19%	11%
23	83%	68%	51%	35%	22%	13%
24	85%	71%	55%	39%	26%	15%
25	87%	75%	59%	43%	29%	18%
26	89%	78%	63%	47%	33%	21%
27	90%	80%	67%	51%	36%	24%
28	92%	83%	70%	55%	40%	27%
29	93%	85%	73%	59%	44%	30%
30	94%	87%	76%	62%	48%	34%
31	95%	89%	78%	66%	51%	37%
32	96%	90%	81%	69%	55%	41%
33	96%	91%	83%	72%	58%	44%
34	97%	93%	85%	74%	61%	48%
35	97%	94%	87%	77%	65%	51%
36	98%	95%	88%	79%	68%	54%
37	98%	95%	90%	82%	70%	58%
38	99%	96%	91%	84%	73%	61%
39	99%	97%	92%	85%	76%	64%
40	99%	97%	93%	87%	78%	67%
41	99%	98%	94%	88%	80%	69%
42	99%	98%	95%	90%	82%	72%
43	99%	98%	96%	91%	84%	75%
44	100%	98%	96%	92%	86%	77%
45	100%	99%	97%	93%	87%	79%
46	100%	99%	97%	94%	89%	81%
47	100%	99%	98%	95%	90%	83%
48	100%	99%	98%	95%	91%	84%
49	100%	99%	98%	96%	92%	86%
50	100%	99%	99%	97%	93%	88%
51	100%	100%	99%	97%	94%	89%
52	100%	100%	99%	97%	95%	90%
53	100%	100%	99%	98%	95%	91%
54	100%	100%	99%	98%	96%	92%
55	100%	100%	99%	98%	96%	93%
56	100%	100%	99%	99%	97%	94%
57	100%	100%	100%	99%	97%	95%
58	100%	100%	100%	99%	98%	95%
59	100%	100%	100%	99%	98%	96%
60	100%	100%	100%	99%	98%	96%
61	100%	100%	100%	99%	98%	97%
62	100%	100%	100%	99%	99%	97%
63	100%	100%	100%	100%	99%	97%
64	100%	100%	100%	100%	99%	98%
65	100%	100%	100%	100%	99%	98%
66	100%	100%	100%	100%	99%	98%
67	100%	100%	100%	100%	99%	99%
68	100%	100%	100%	100%	99%	99%
69	100%	100%	100%	100%	100%	99%
70	100%	100%	100%	100%	100%	99%

1700

FIG. 17

Sequencing depth (folds)	No. of false positives in the whole genome when a mutant is defined as being seen in the tumor sequencing data for at least n times, where n=					
	4	5	6	7	8	9
20	1	0	0	0	0	0
21	1	0	0	0	0	0
22	1	0	0	0	0	0
23	1	0	0	0	0	0
24	1	0	0	0	0	0
25	1	0	0	0	0	0
26	2	0	0	0	0	0
27	2	0	0	0	0	0
28	2	0	0	0	0	0
29	3	0	0	0	0	0
30	3	0	0	0	0	0
31	3	0	0	0	0	0
32	4	0	0	0	0	0
33	5	0	0	0	0	0
34	5	0	0	0	0	0
35	6	0	0	0	0	0
36	7	0	0	0	0	0
37	7	0	0	0	0	0
38	8	0	0	0	0	0
39	9	0	0	0	0	0
40	10	0	0	0	0	0
41	11	0	0	0	0	0
42	12	0	0	0	0	0
43	14	0	0	0	0	0
44	15	0	0	0	0	0
45	17	0	0	0	0	0
46	18	0	0	0	0	0
47	20	0	0	0	0	0
48	22	0	0	0	0	0
49	24	0	0	0	0	0
50	26	0	0	0	0	0
51	28	0	0	0	0	0
52	30	0	0	0	0	0
53	33	0	0	0	0	0
54	35	0	0	0	0	0
55	38	0	0	0	0	0
56	41	0	0	0	0	0
57	44	0	0	0	0	0
58	47	0	0	0	0	0
59	51	0	0	0	0	0
60	54	0	0	0	0	0
61	58	0	0	0	0	0
62	62	0	0	0	0	0
63	66	0	0	0	0	0
64	71	0	0	0	0	0
65	75	0	0	0	0	0
66	80	0	0	0	0	0
67	85	0	0	0	0	0
68	90	0	0	0	0	0
69	96	0	0	0	0	0
70	102	0	0	0	0	0

FIG. 18

1800

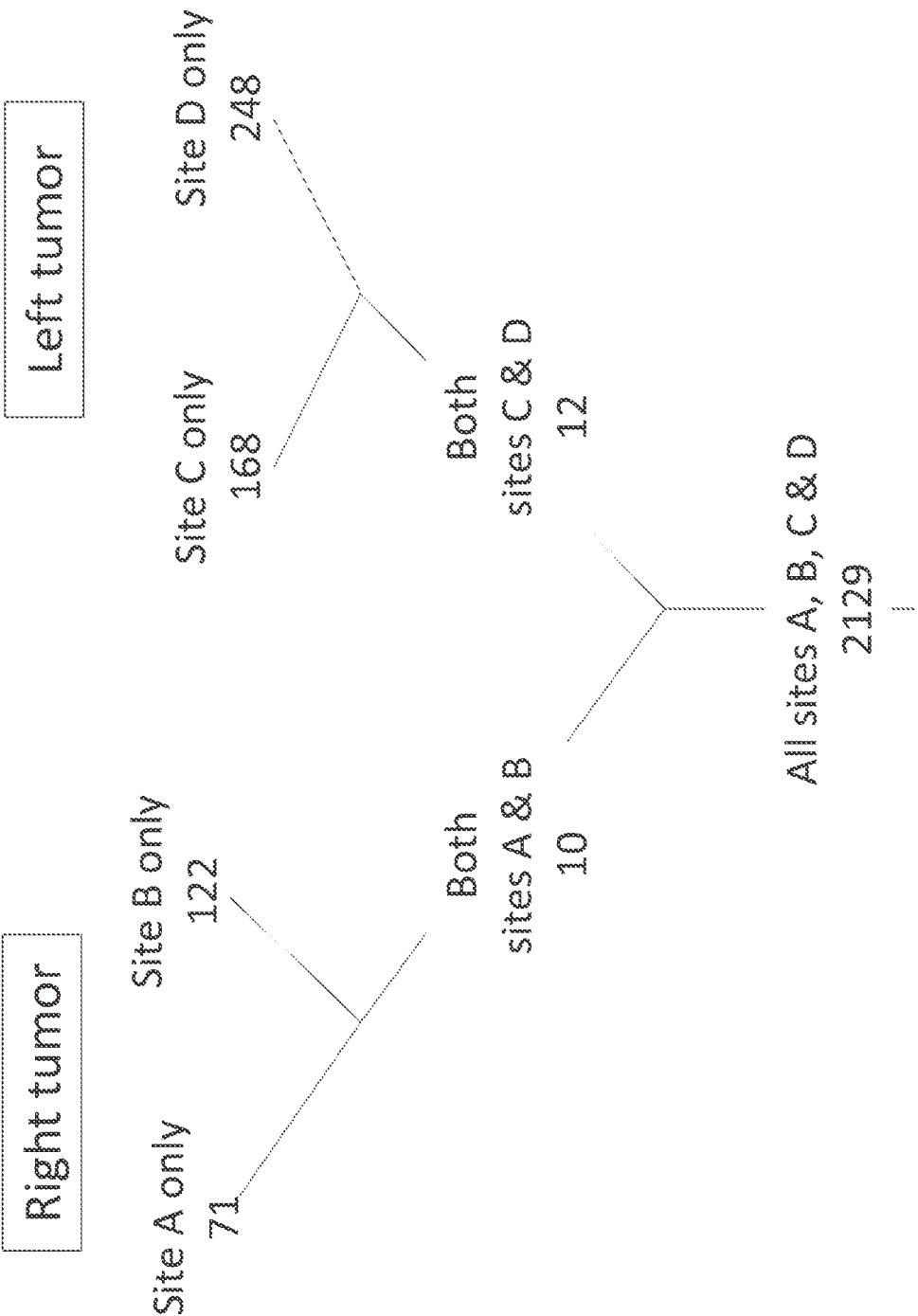


FIG. 19

Category	No. of loci with a mutation	Pre-treatment plasma			Post-treatment plasma		
		No. of DNA fragments carrying wildtype alleles	No. of DNA fragments carrying mutant alleles	Fractional concentration of tumor-derived DNA	No. of DNA fragments carrying wildtype alleles	No. of DNA fragments carrying mutant alleles	Fractional concentration of tumor-derived DNA
A	71	3,321	37	2.20%	2,149	5	0.46%
B	122	5,633	8	0.28%	3,516	1	0.06%
C	168	8,507	51	1.19%	5,438	2	0.07%
D	248	10,880	26	0.48%	7,060	2	0.06%
AB	10	423	21	9.46%	297	0	0.00%
CD	12	569	3	1.05%	333	0	0.00%
ABCD	2129	70,940	21,344	46.26%	61,417	54	0.18%

FIG. 20 2000

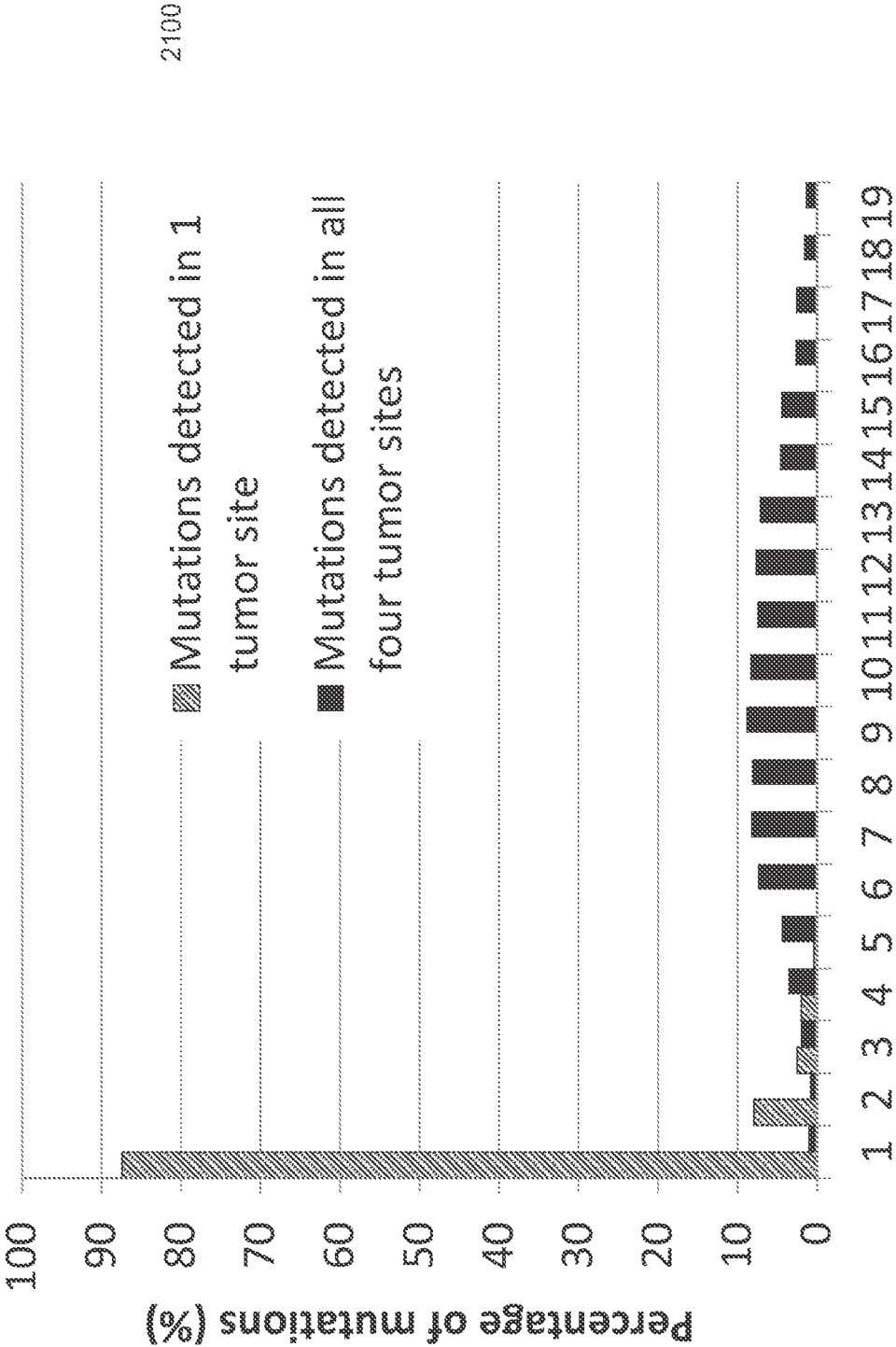


FIG. 21

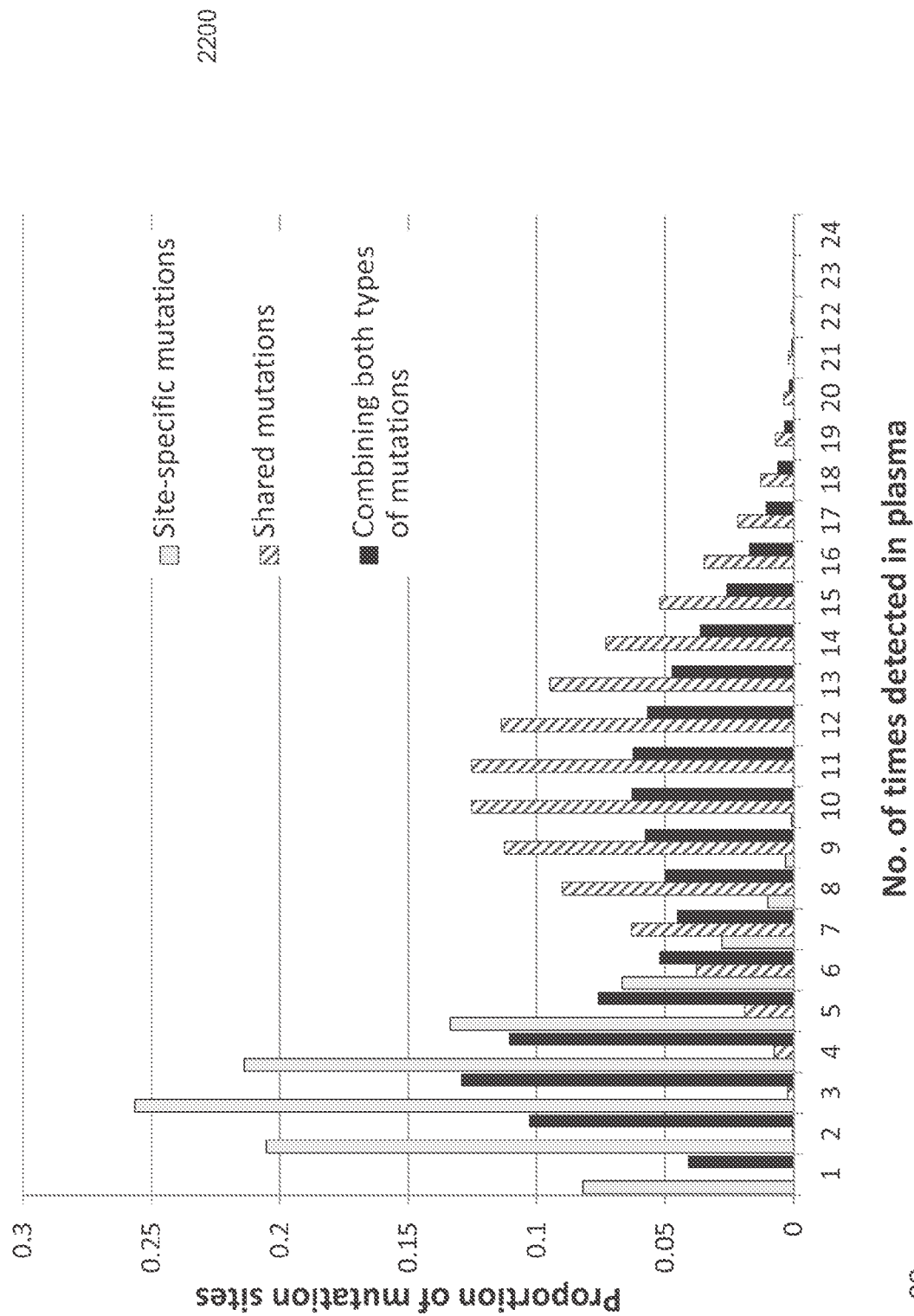


FIG. 22

Chr.	No. of loci	Healthy control subject																TC16
		TC1	TC2	TC3	TC4	TC5	TC6	TC7	TC8	TC9	TC10	TC11	TC12	TC13	TC14	TC15		
		No (fractional concentration) of mutant alleles in plasma																
A	71	0	1 (0.17%)	1 (0.18%)	0	1 (0.17%)	1 (0.07%)	1 (0.20%)	0	6 (0.17%)	0	0	0	1 (0.17%)	2 (0.38%)	0	2 (0.38%)	
B	122	0	0	0	0	0	1 (0.04%)	0	1 (0.11%)	3 (0.05%)	1 (0.10%)	1 (0.12%)	0	0	0	0	1 (0.11%)	
C	168	0	0	0	0	0	2 (0.06%)	0	2 (0.16%)	0	0	0	0	1 (0.07%)	0	0	0	
D	248	0	0	1 (0.06%)	0	0	0	0	0	1 (0.01%)	0	0	0	1 (0.05%)	0	0	0	
AB	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
CD	12	0	0	0	0	0	0	0	0	1 (0.16%)	0	0	0	1 (0.92%)	0	0	0	
ABCD	2129	11 (0.07%)	3 (0.02%)	2 (0.01%)	3 (0.06%)	5 (0.03%)	10 (0.22%)	4 (0.03%)	4 (0.03%)	19 (0.02%)	3 (0.02%)	0	0	1 (0.01%)	5 (0.03%)	2 (0.01%)	4 (0.03%)	

FIG. 23

2300

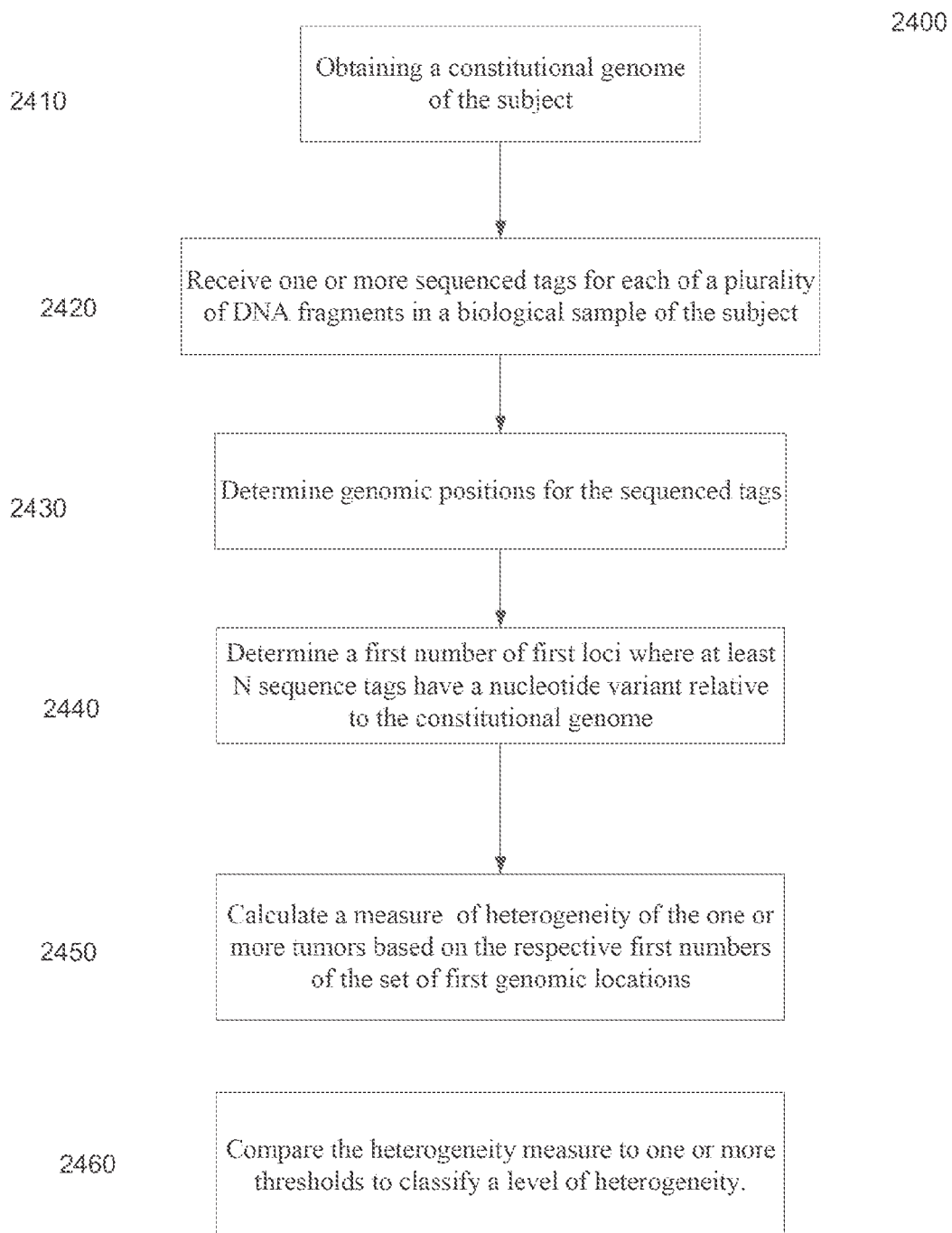


FIG. 24

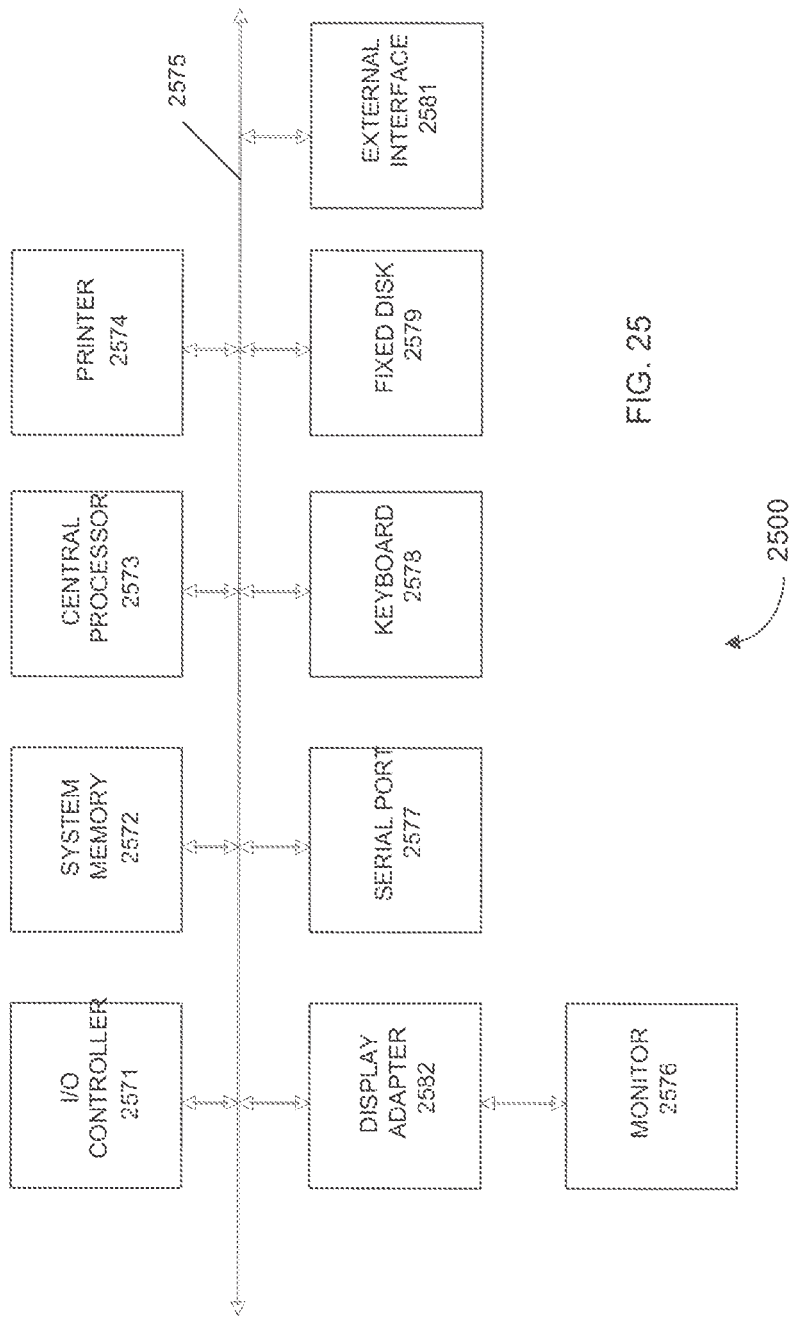


FIG. 25