

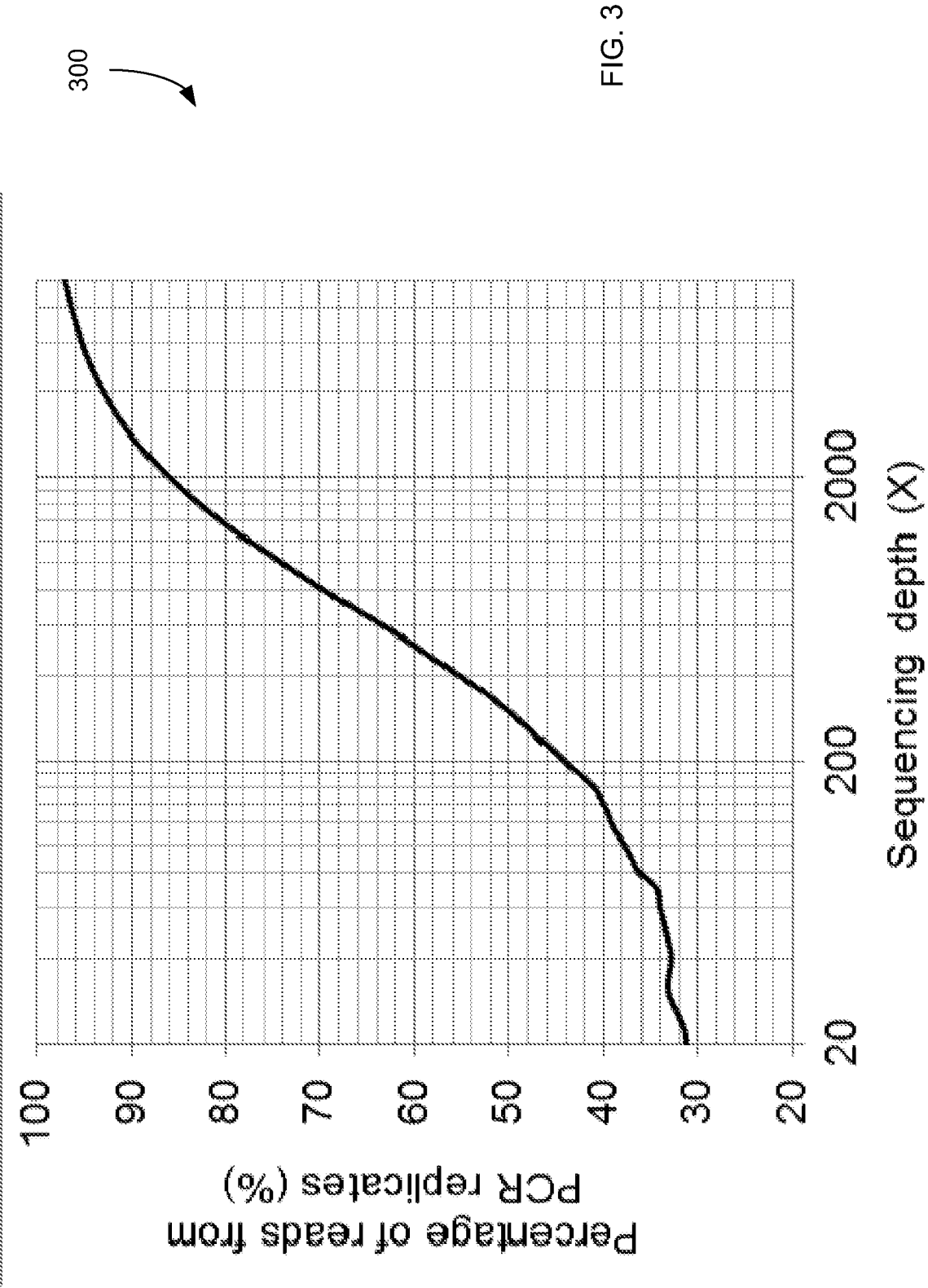


	Breast	Kidney	Colon	Liver	Lung	Prostate	Cervix	Stomach	Skin	Endometrium	Thyroid	Ovary
TP53	23%	6%	44%	27%	34%	13%	5%	33%	24%	18%	6%	46%
APC	2%	1%	45%	3%	2%	2%	2%	8%	6%	5%	3%	2%
BRAF	1%	2%	12%	1%	2%	1%	1%	1%	42%	2%	41%	7%
PTEN	4%	3%	5%	2%	2%	7%	4%	4%	8%	38%	2%	3%
VHL	0%	36%	1%	0%	0%	0%	0%	0%	1%	1%	0%	0%
KRAS	2%	0%	33%	2%	16%	4%	6%	6%	2%	15%	2%	12%
EGFR	1%	1%	2%	1%	29%	3%	1%	4%	3%	4%	1%	2%
PIK3CA	27%	2%	13%	3%	4%	2%	14%	10%	7%	23%	3%	9%
RET	1%	1%	4%	1%	2%	0%	1%	2%	3%	2%	26%	1%
ARID1A	4%	2%	11%	8%	4%	1%	4%	15%	6%	24%	0%	9%
PBRM1	1%	24%	4%	1%	2%	0%	1%	3%	4%	2%	0%	0%
CTNNB1	1%	7%	5%	23%	2%	3%	3%	8%	6%	18%	3%	6%
FOXJ2	0%	0%	1%	0%	1%	0%	0%	1%	0%	0%	0%	21%
TSHR	1%	0%	2%	0%	2%	0%	0%	2%	4%	1%	21%	0%
TERT	3%	2%	3%	15%	1%	0%	1%	1%	20%	2%	11%	4%
CDKN2A	2%	2%	1%	5%	8%	1%	5%	4%	18%	2%	3%	5%
GRIN2A	2%	1%	8%	2%	5%	1%	2%	6%	17%	4%	1%	1%
PIK3R1	2%	0%	4%	1%	1%	0%	2%	1%	2%	16%	0%	1%
PTCH1	1%	1%	6%	1%	2%	0%	1%	5%	16%	3%	1%	1%
NRAS	1%	0%	4%	0%	1%	1%	1%	1%	16%	2%	7%	1%
ROS1	1%	1%	5%	1%	4%	1%	2%	5%	14%	4%	1%	2%
CDH1	11%	0%	3%	1%	1%	1%	1%	13%	2%	3%	0%	0%
FGFR3	0%	0%	2%	0%	0%	1%	1%	1%	13%	1%	0%	0%
KMT2C	9%	3%	11%	4%	8%	4%	8%	11%	12%	8%	3%	3%
NF1	3%	1%	7%	2%	6%	0%	3%	6%	12%	5%	1%	4%
HRAS	0%	0%	0%	0%	0%	2%	4%	1%	11%	0%	4%	0%
GATA3	11%	0%	3%	0%	2%	0%	0%	3%	2%	1%	1%	1%
KMT2D	3%	3%	10%	3%	6%	2%	8%	9%	11%	7%	0%	1%

FIG. 1

			Tumor DNA fraction									
			10%	5%	2%	10%	5%	2%	10%	5%	2%	2%
			Sequencing depth									
			100	100	100	150	150	150	200	200	200	200
			Fraction of genome searched									
No of mutations per genome	300	1/5	34	7	0	52	19	1	58	34	3	3
		1/10	17	3	0	26	10	1	29	17	2	2
		1/20	8	2	0	13	5	0	15	8	1	1
	1000	1/5	112	22	1	174	64	4	194	112	11	11
		1/10	56	11	0	87	32	2	97	56	5	5
		1/20	28	5	0	43	16	1	49	28	3	3
	3000	1/5	336	65	2	521	193	11	582	336	32	32
		1/10	168	33	1	260	97	6	291	168	16	16
		1/20	84	16	1	130	48	3	146	84	8	8
	10000	1/5	1119	218	7	1736	645	37	1941	1119	105	105
		1/10	560	109	4	868	322	19	971	560	53	53
		1/20	280	54	2	434	161	9	485	280	26	26
	30000	1/5	3357	653	22	5208	1935	111	5824	3357	316	316
		1/10	1679	326	11	2604	967	56	2912	1679	158	158
		1/20	839	163	5	1302	484	28	1456	839	79	79

FIG. 2



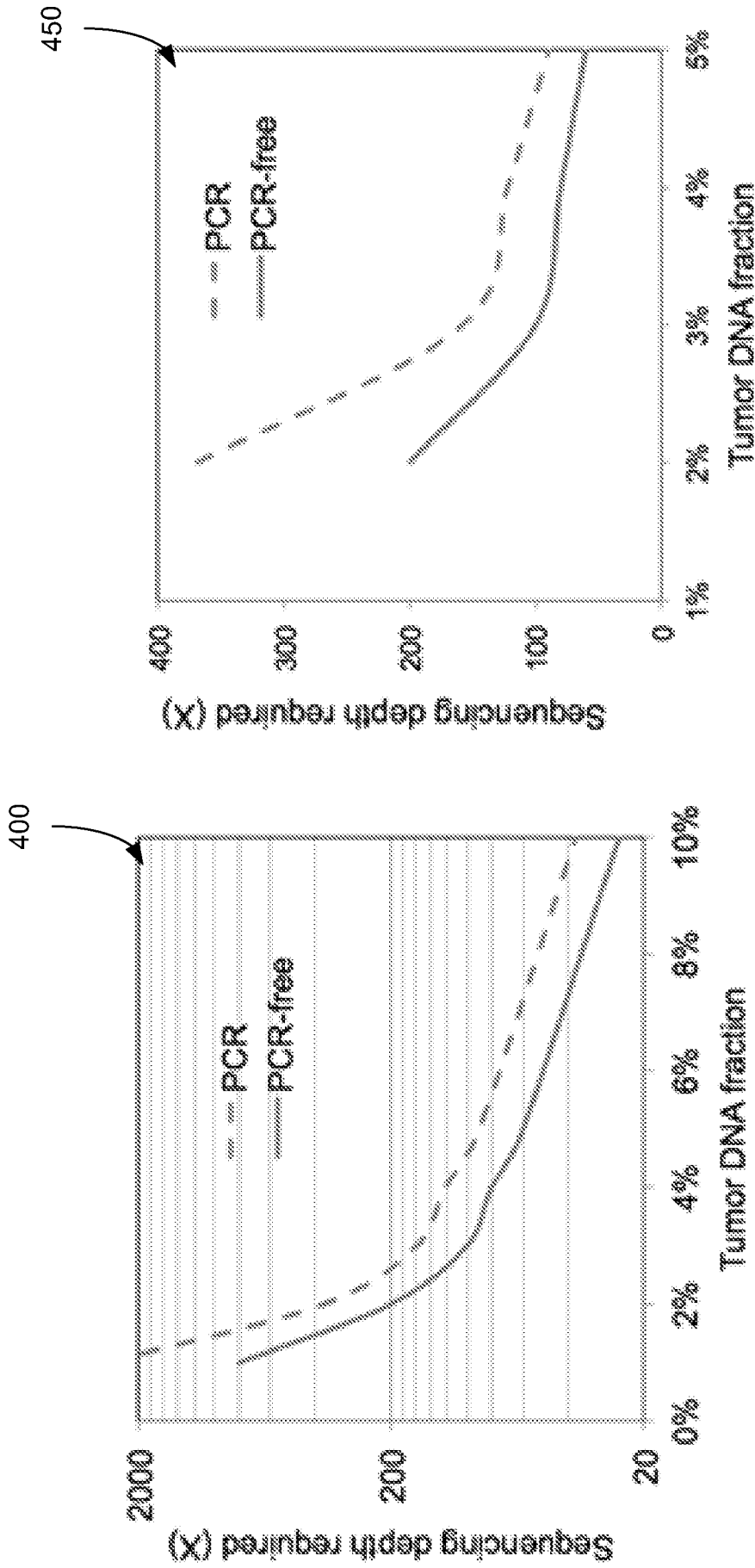


FIG. 4A

FIG. 4B

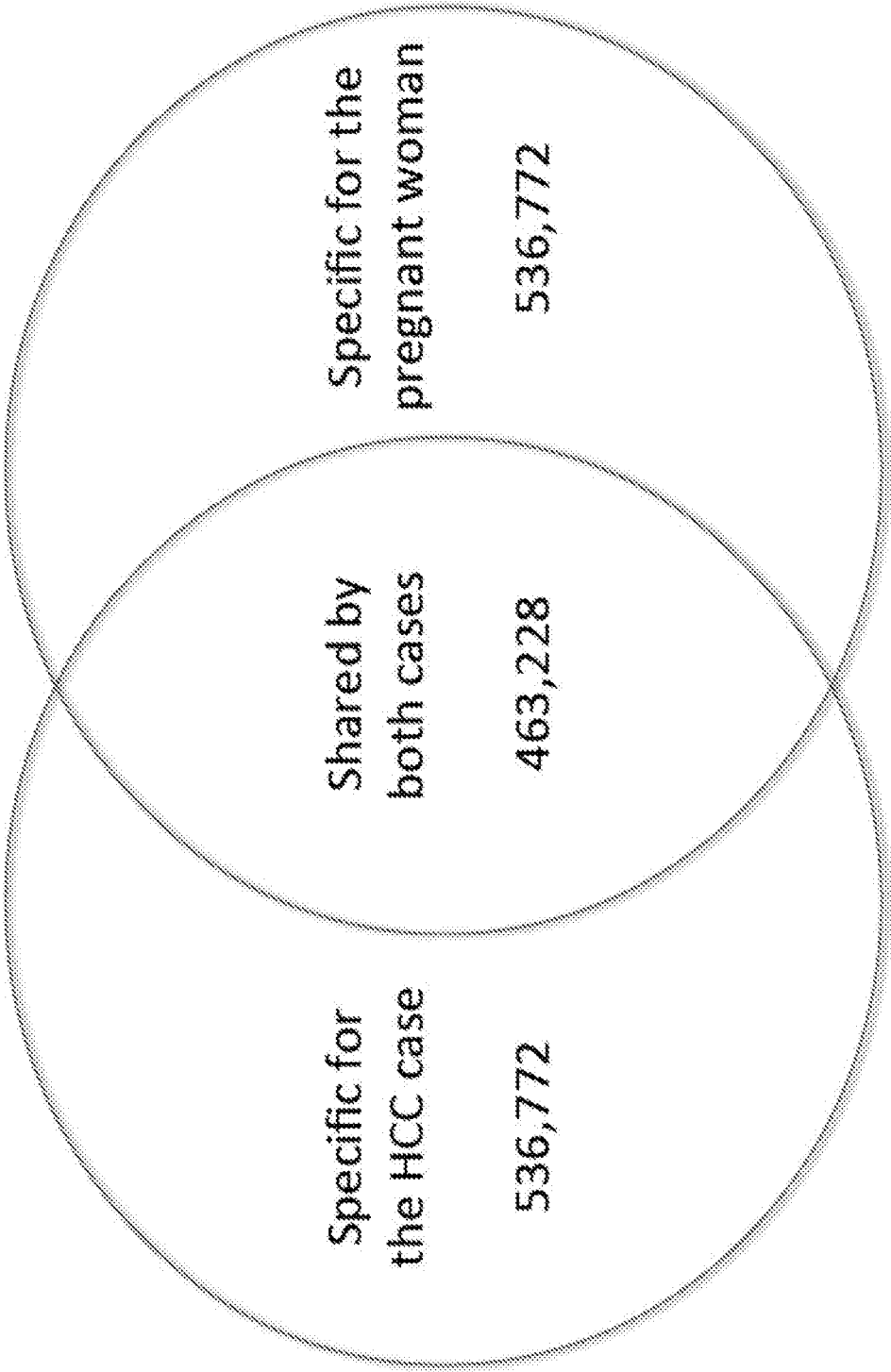


FIG. 5

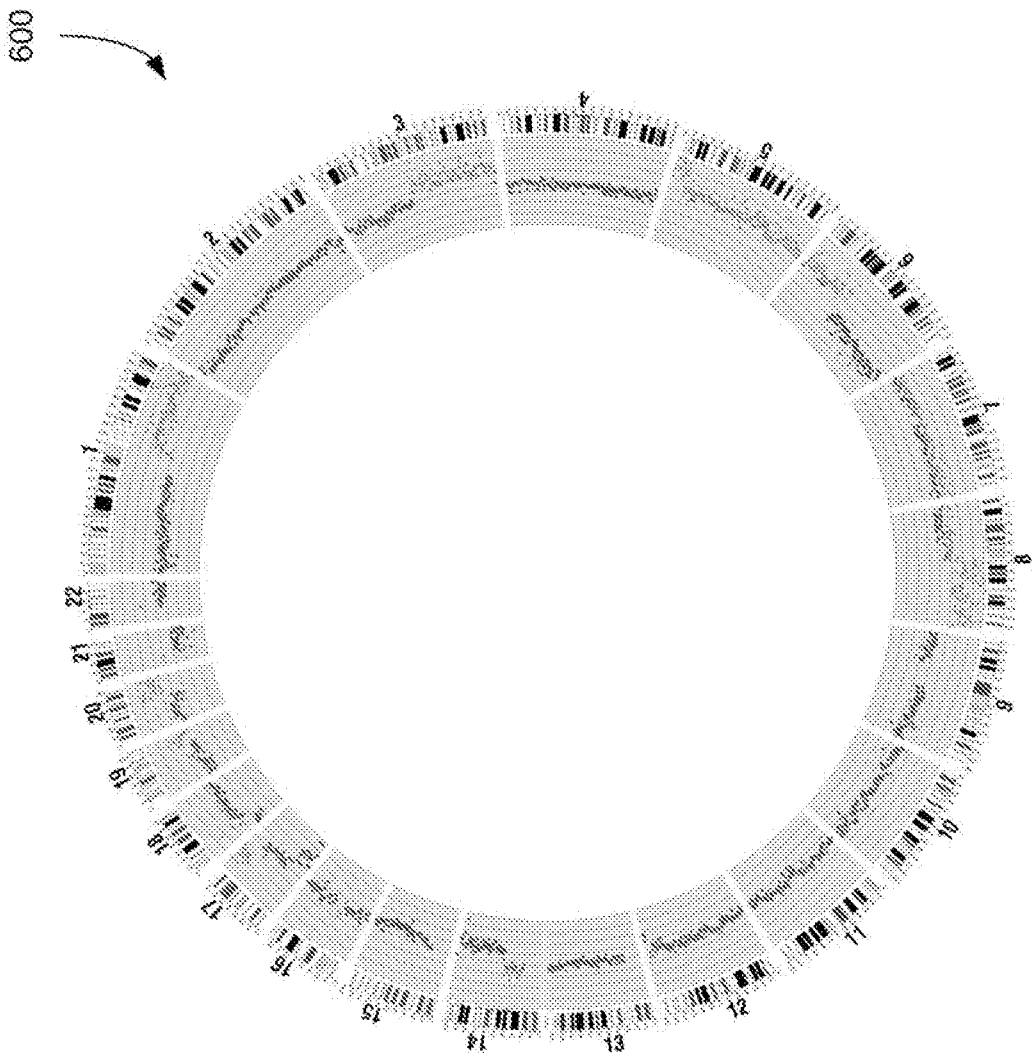


FIG. 6

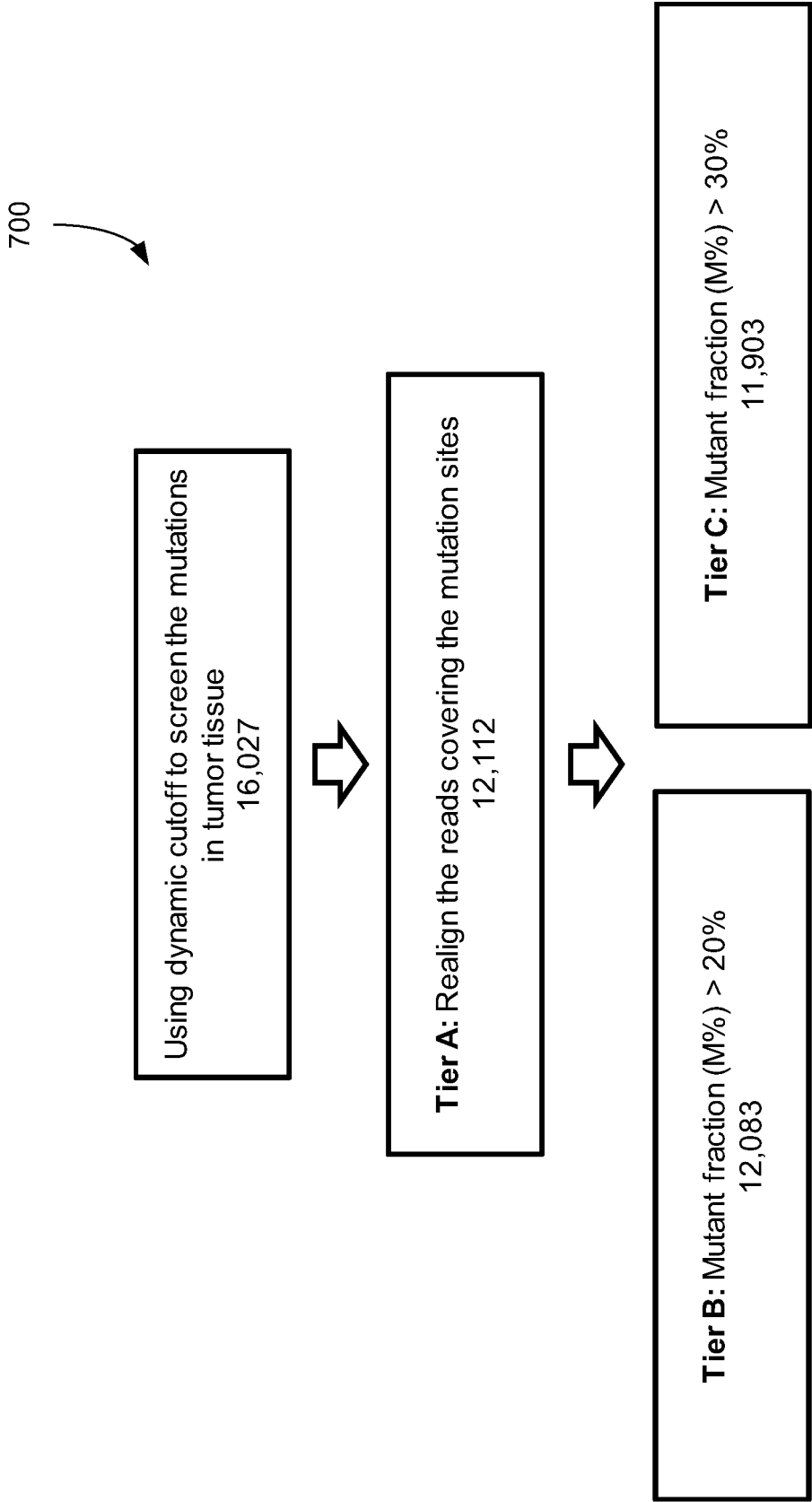


FIG. 7

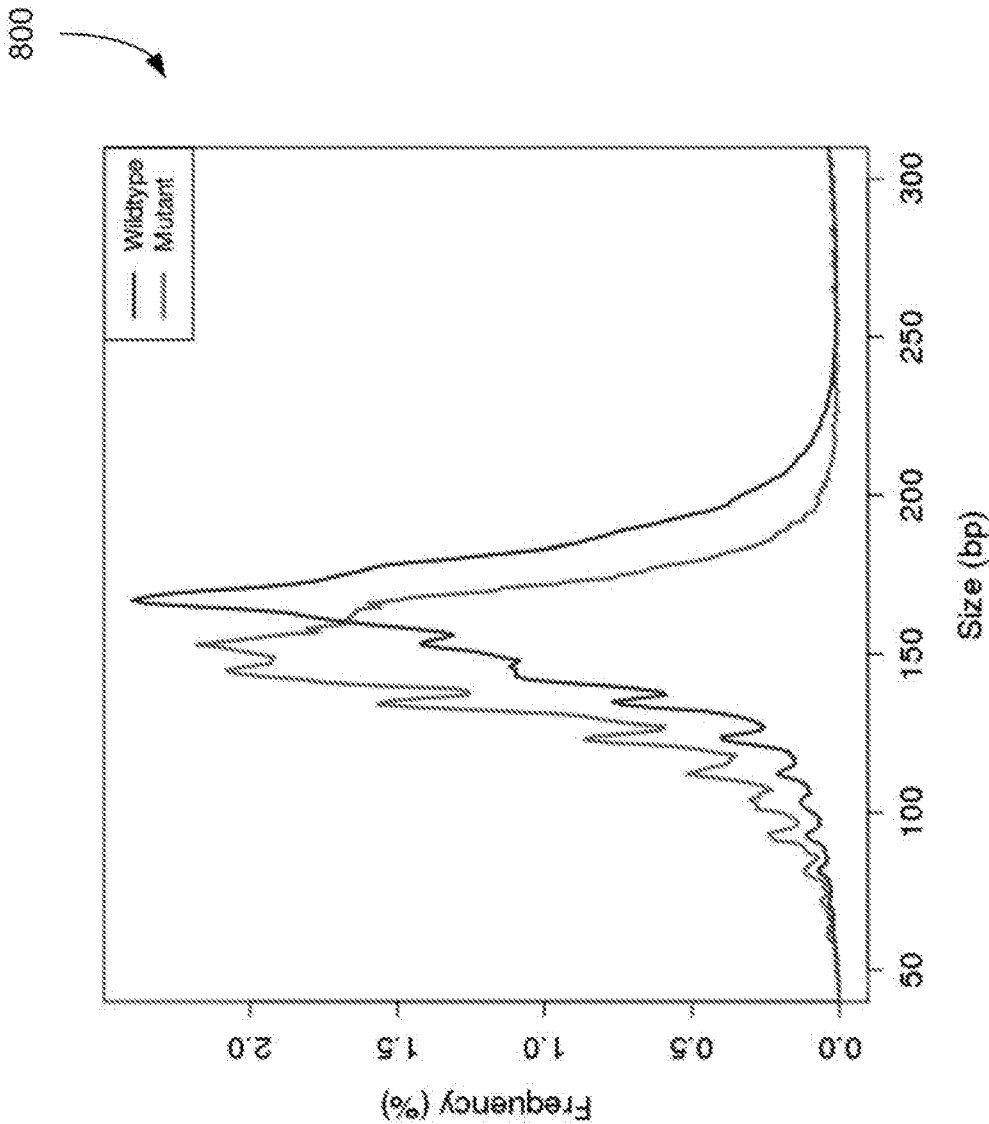


FIG. 8

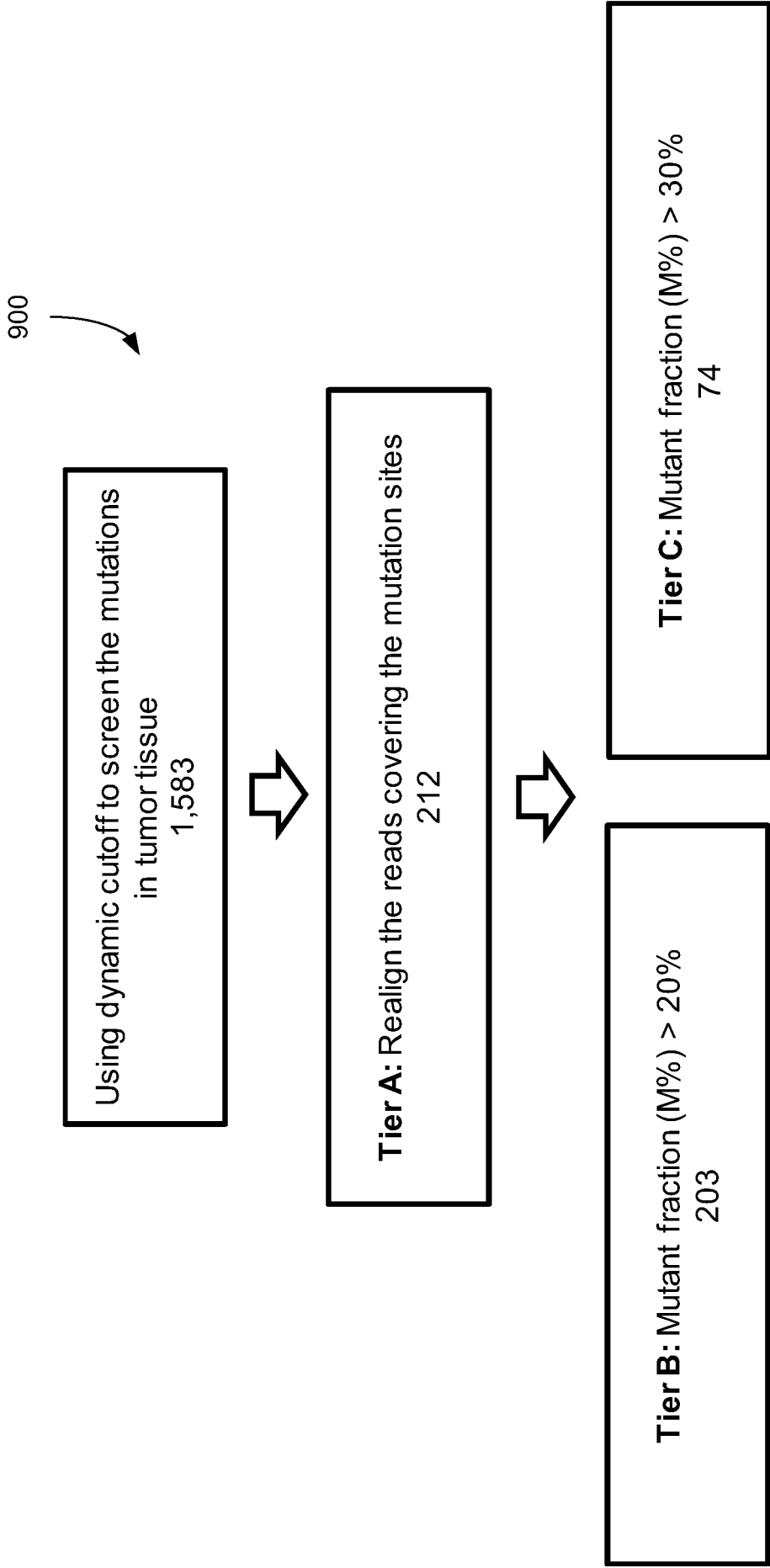


FIG. 9

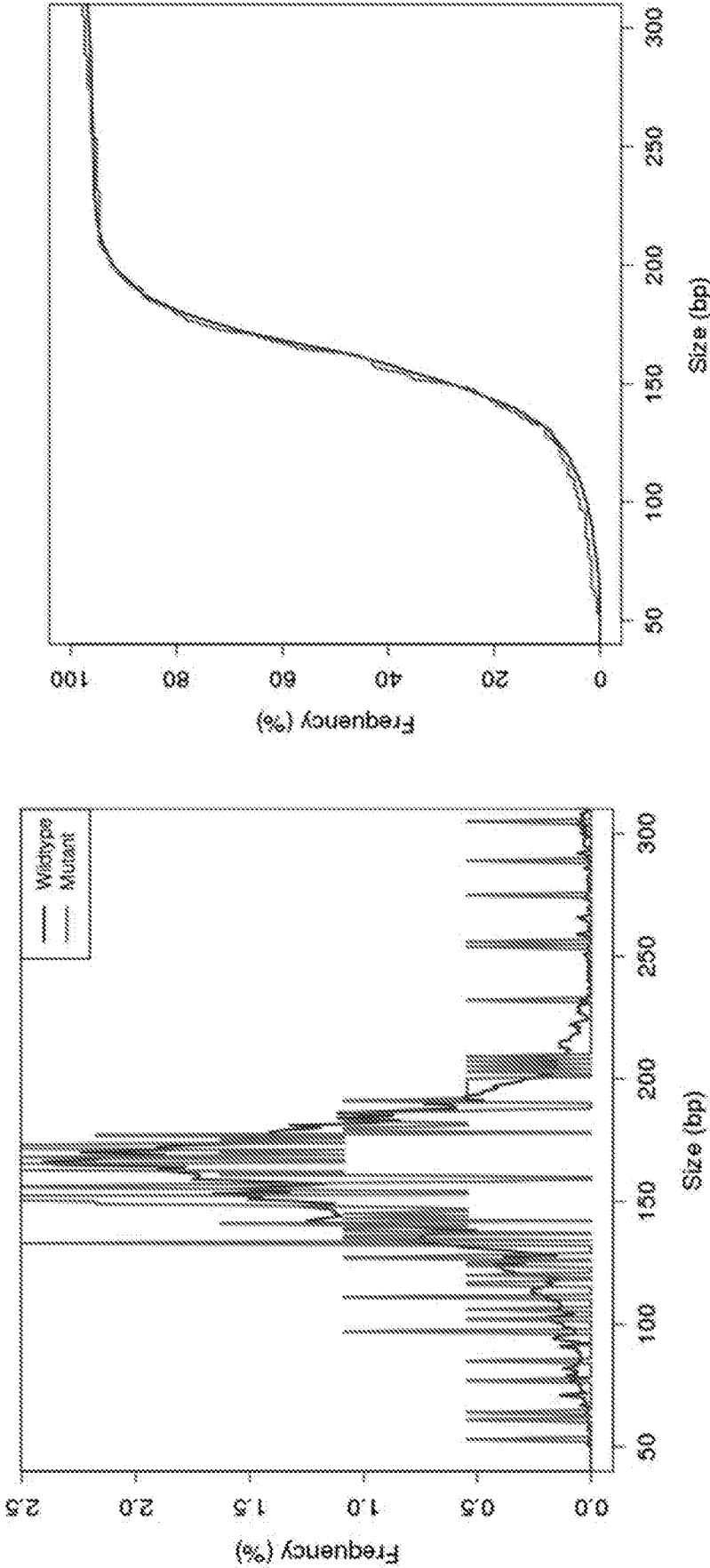


FIG. 10B

FIG. 10A

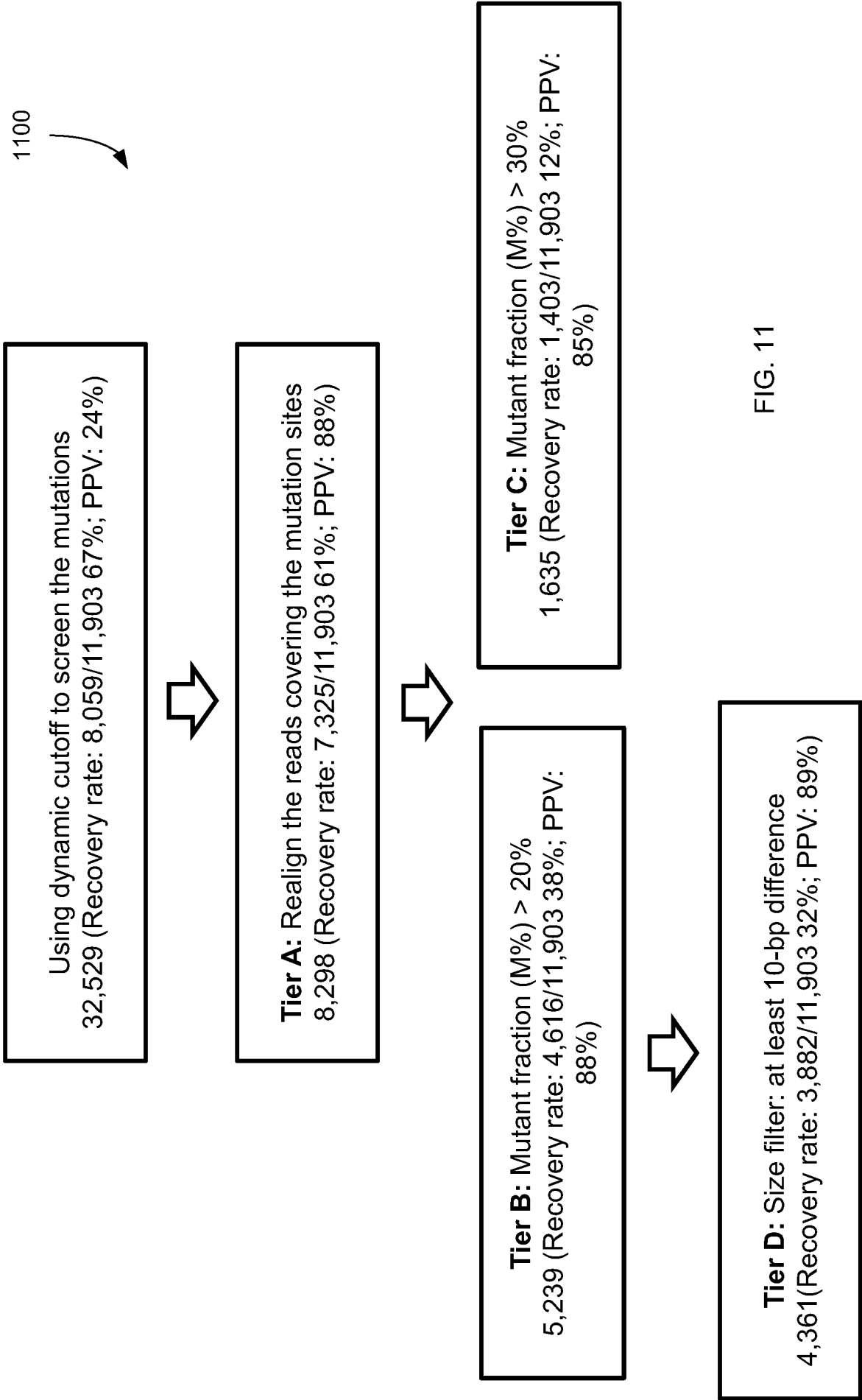


FIG. 11

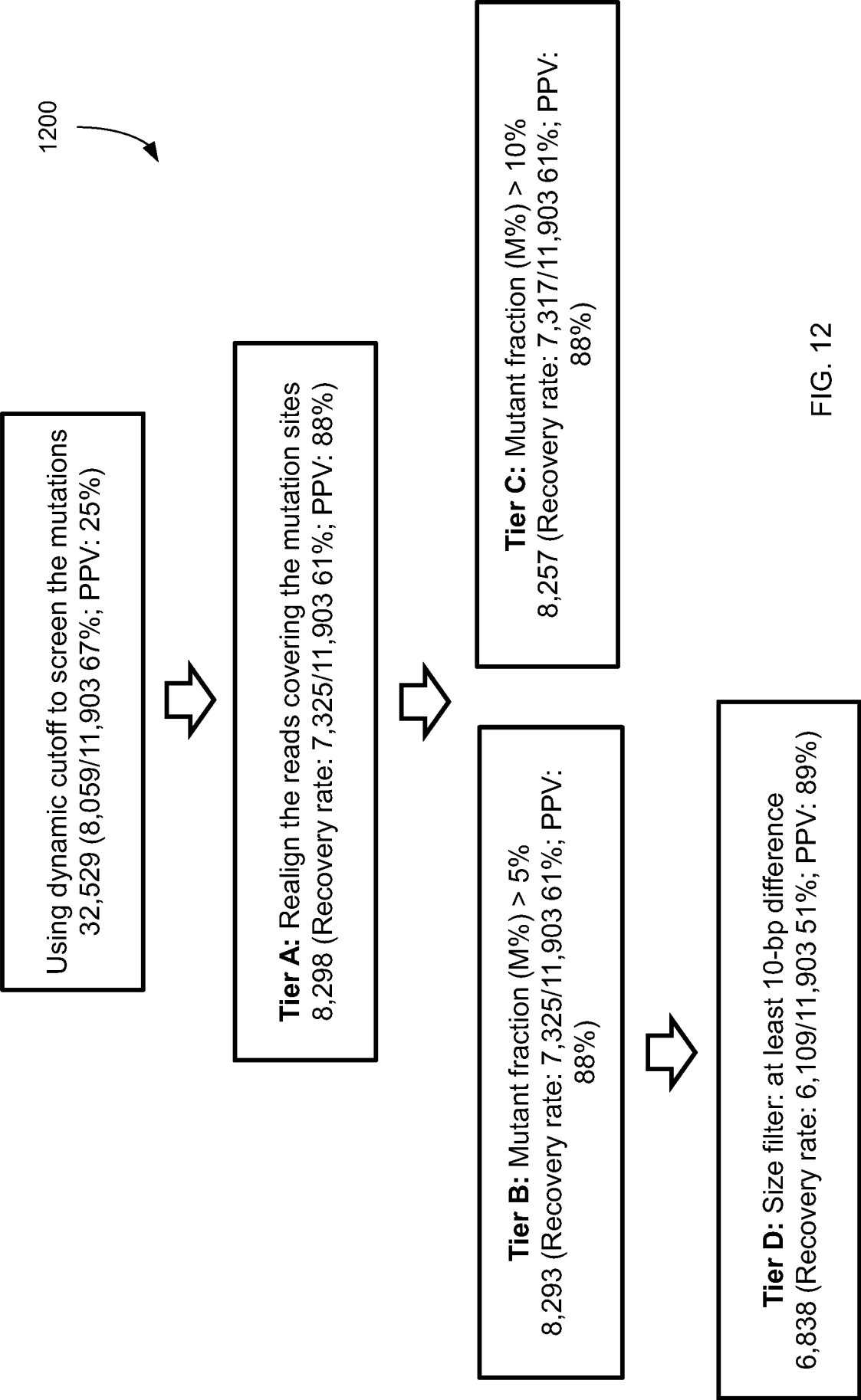


FIG. 12

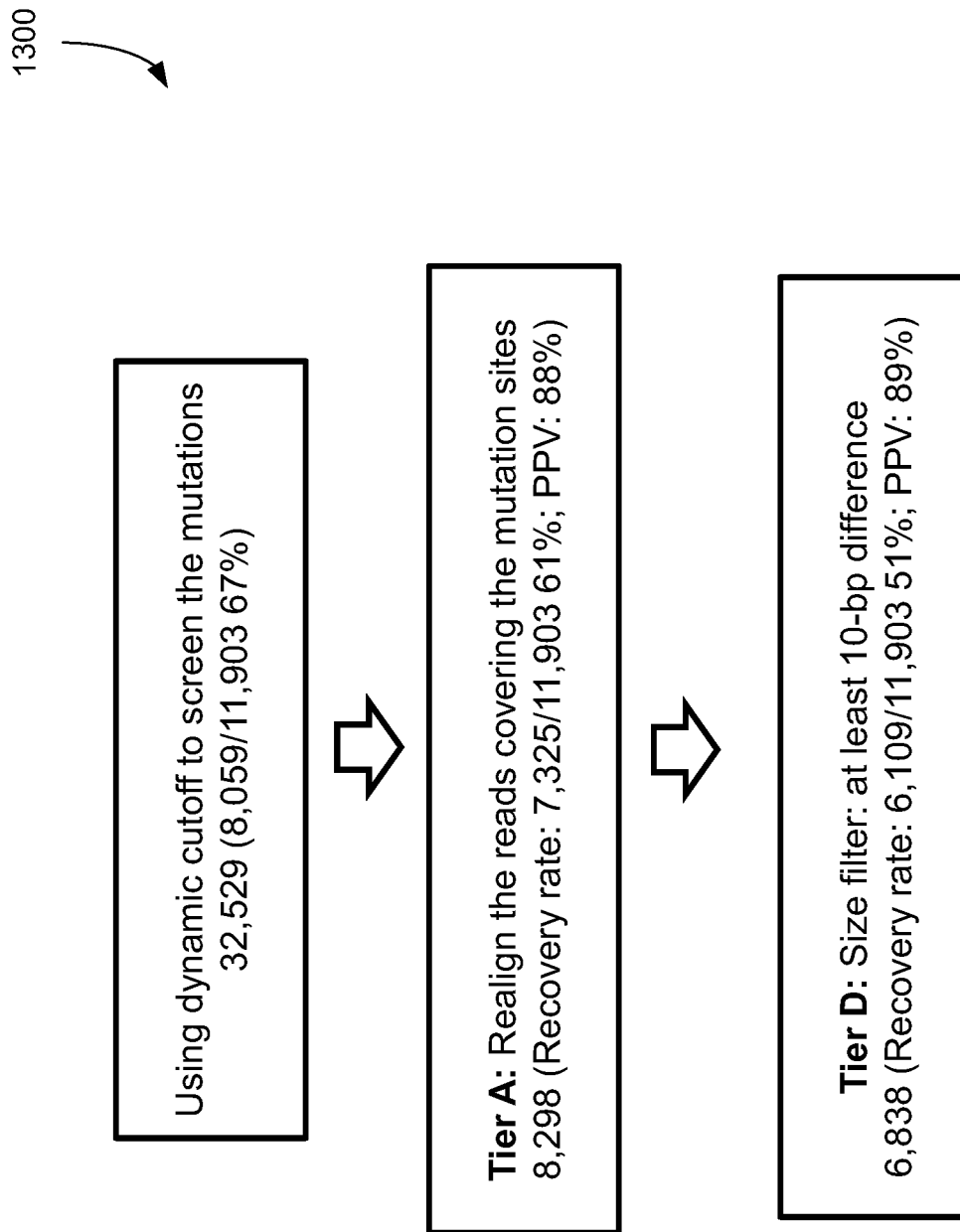


FIG. 13

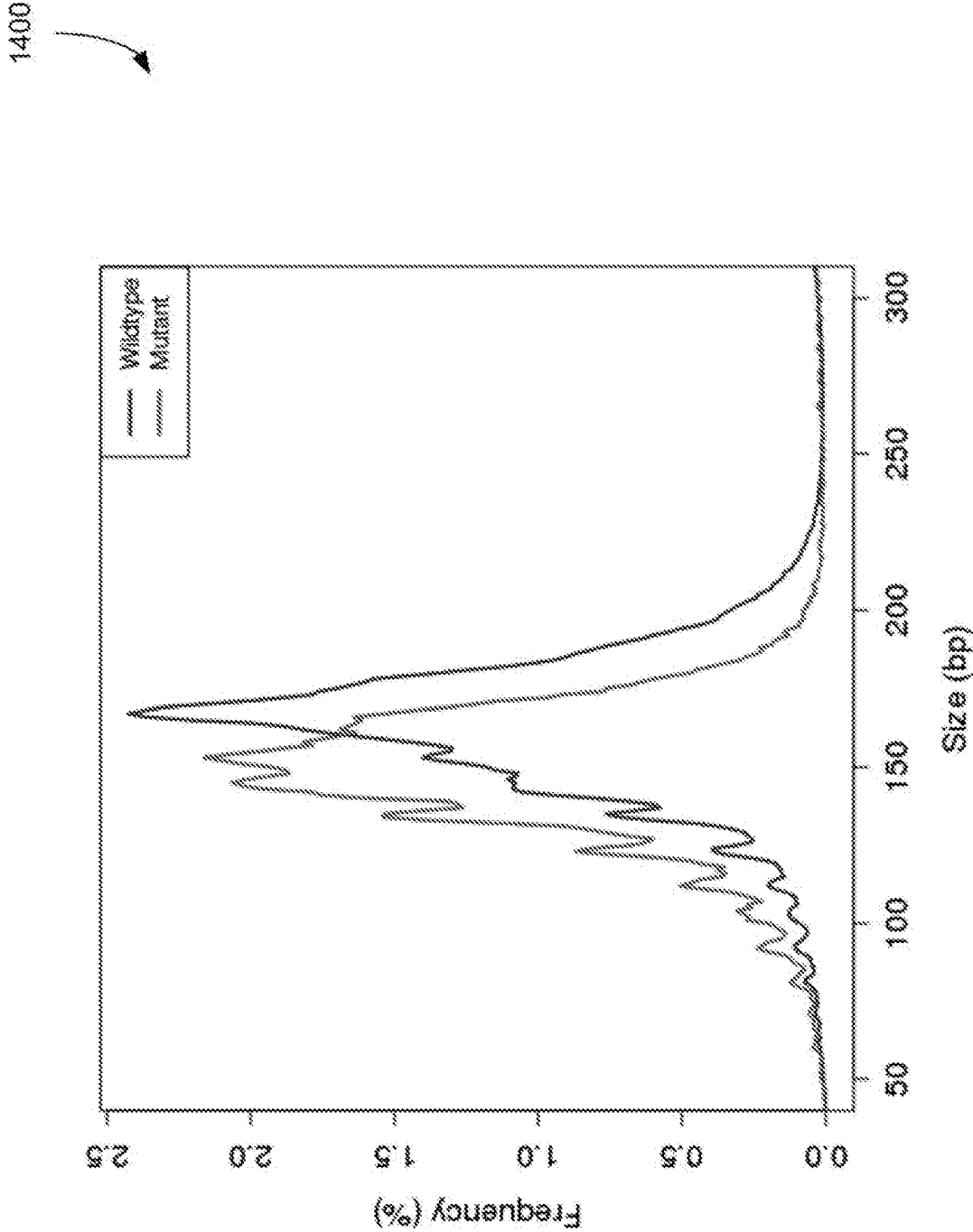


FIG. 14

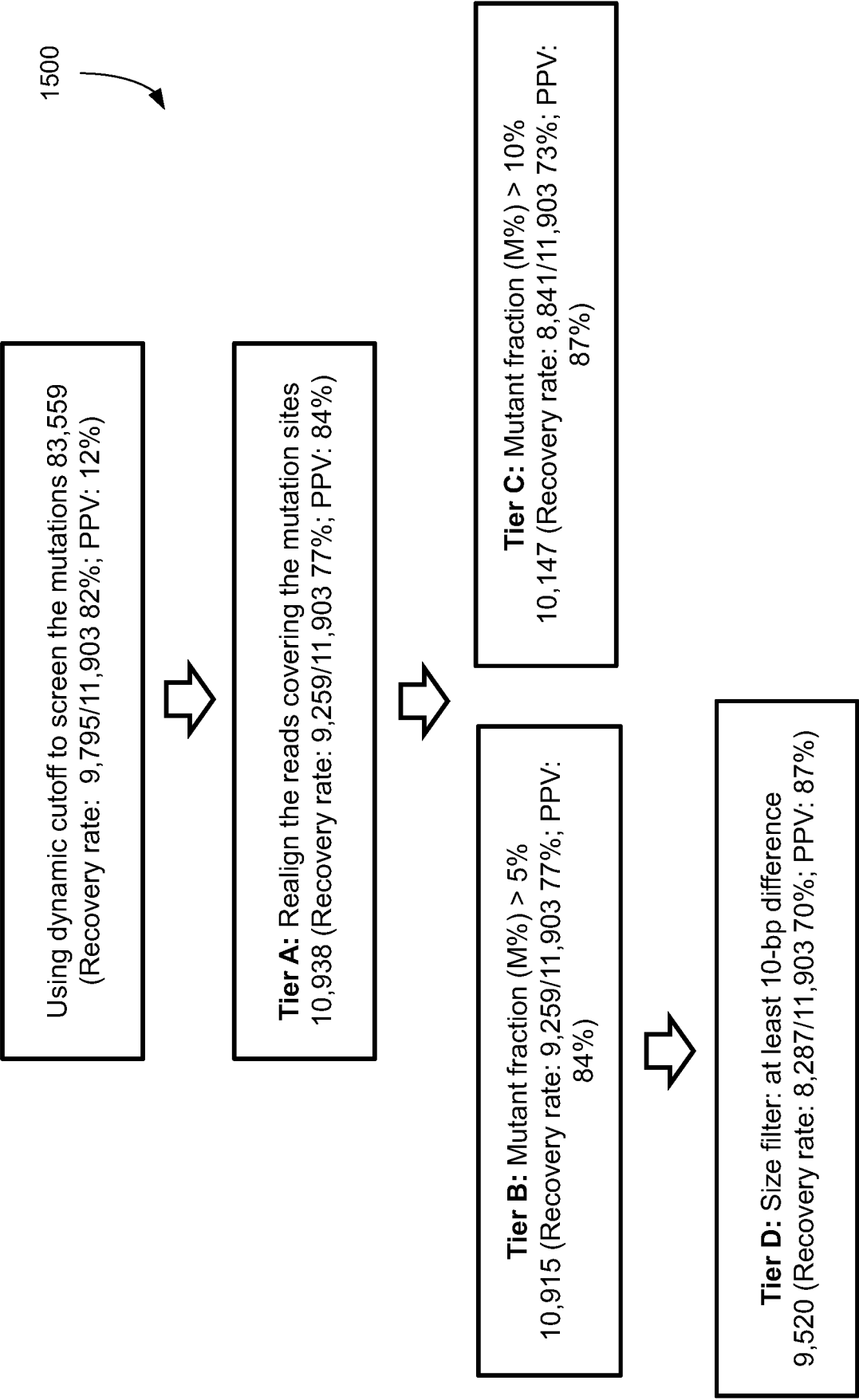


FIG. 15

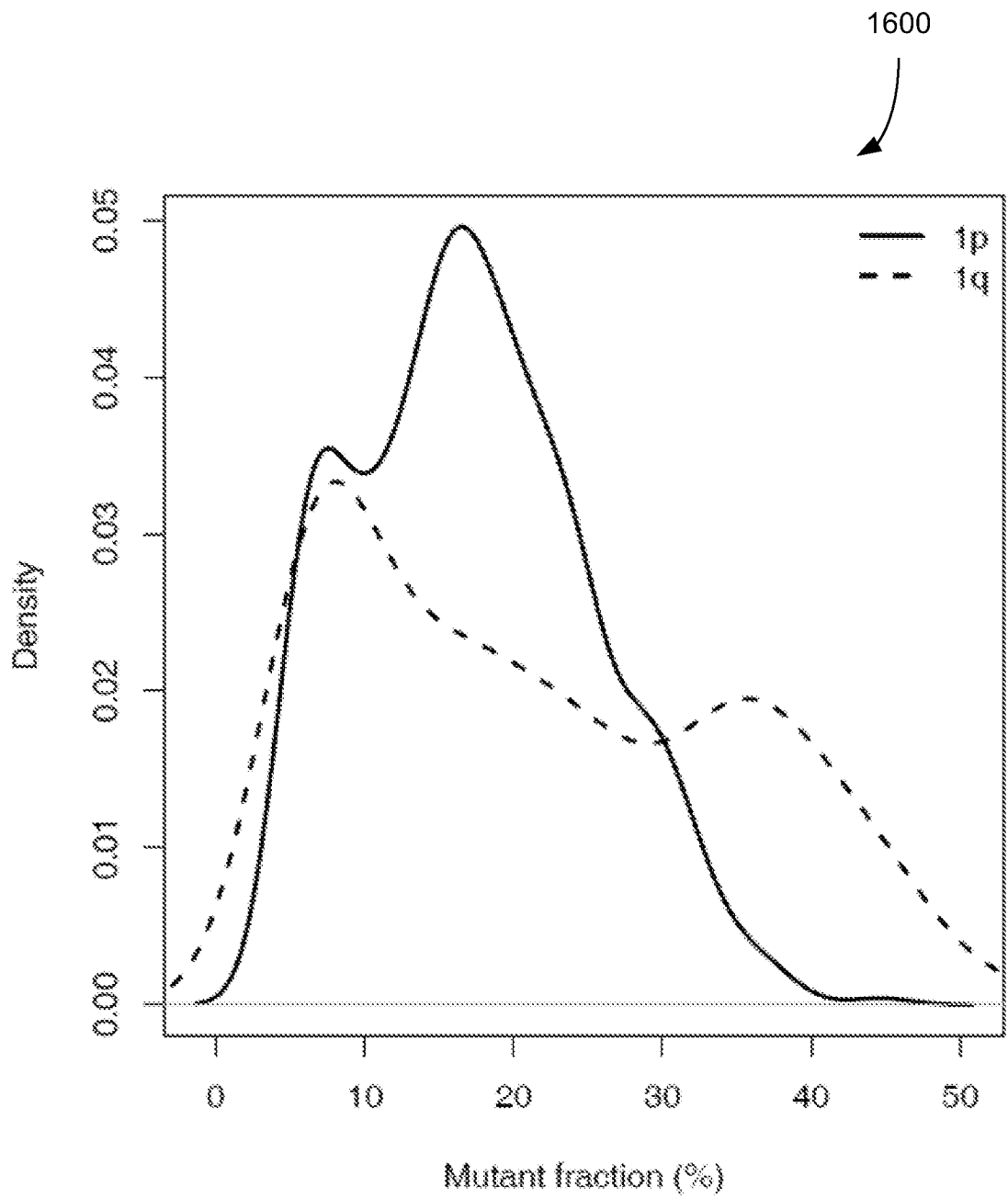


FIG. 16

17/50

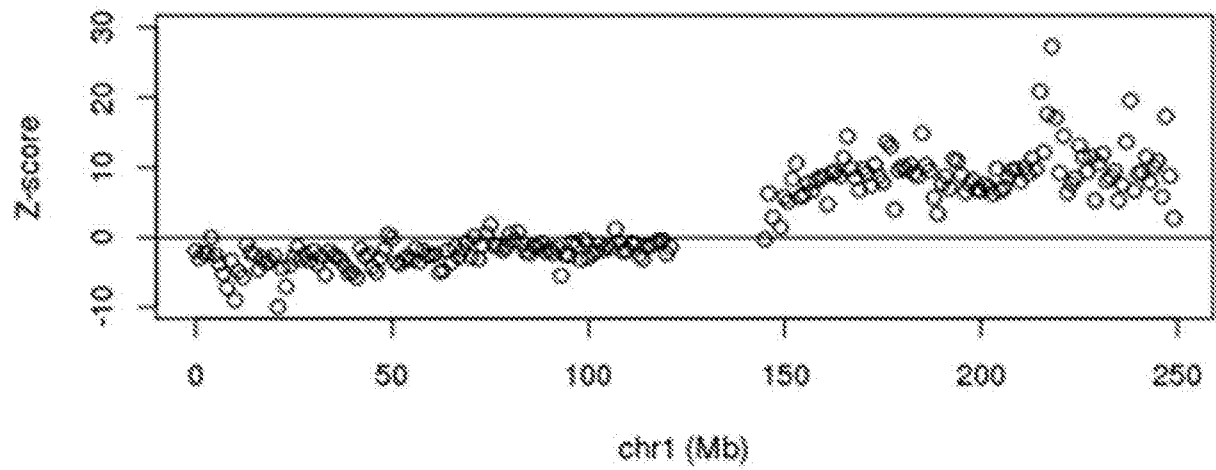


FIG. 17A

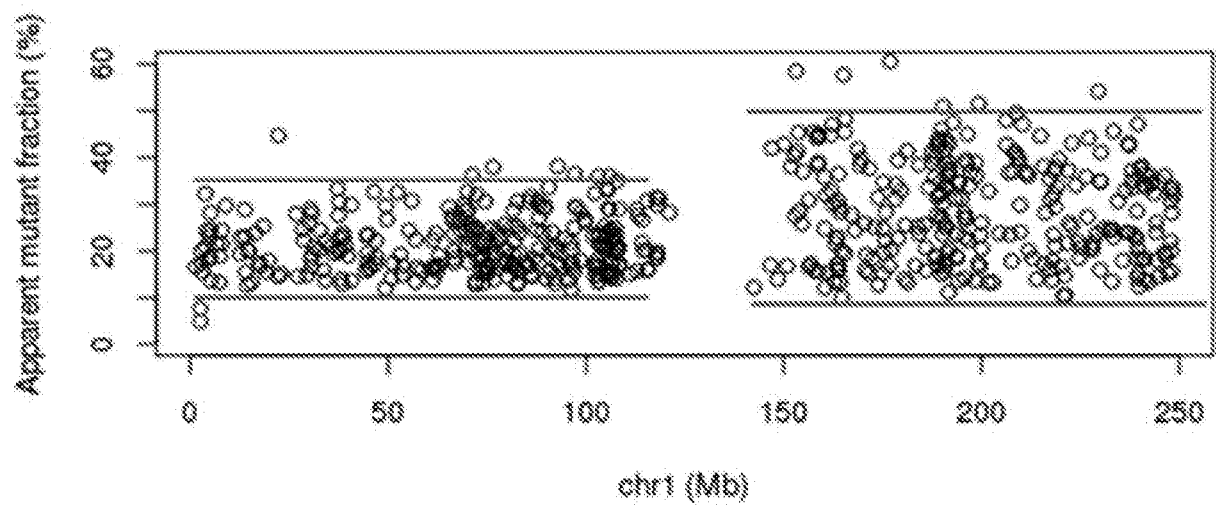


FIG. 17B

1800



FPR<1e-10 and score1 > 0.01			Predicted sensitivity of mutation detection in plasma (%)					
Sequencing depth	Cutoff for mutant alleles		1%	2%	5%	10%	15%	20%
	Buffy coat	Plasma						
50	<=1	>=6	0.0001	0.0	1.4	23.8	62.2	87.0
100	<=1	>=7	0.0010	0.1	13.3	78.0	98.2	99.9
150	<=2	>=7	0.0170	1.2	47.5	98.2	100	100
200	<=2	>=8	0.0237	2.1	66.7	99.8	100	100
250	<=2	>=9	0.0277	3.2	79.9	100	100	100
500	<=3	>=10	1.3695	41.7	99.9	100	100	100
600	<=4	>=11	2.0092	53.8	100	100	100	100
1000	<=6	>=12	41.6960	98.9	100	100	100	100

FIG. 18

1900

FPR<0.1% and score1 >0.01			Predicted sensitivity of mutation detection in plasma (%)					
	Cutoff for mutant alleles		1%	2%	5%	10%	15%	20%
Sequencing depth	Buffy coat	Plasma						
50	<=1	>=3	0.175	1.899	24.242	73.497	94.085	98.966
100	<=1	>=3	1.90	14.29	73.50	98.97	99.98	100
150	<=2	>=4	1.86	18.47	86.79	99.91	100	100
200	<=2	>=4	5.27	37.12	97.07	100	100	100
250	<=2	>=4	10.9	55.95	99.47	100	100	100
500	<=3	>=5	38.40	93.29	100	100	100	100
600	<=4	>=6	39.37	95.42	100	100	100	100
1000	<=6	>=7	77.98	99.92	100	100	100	100

FIG. 19

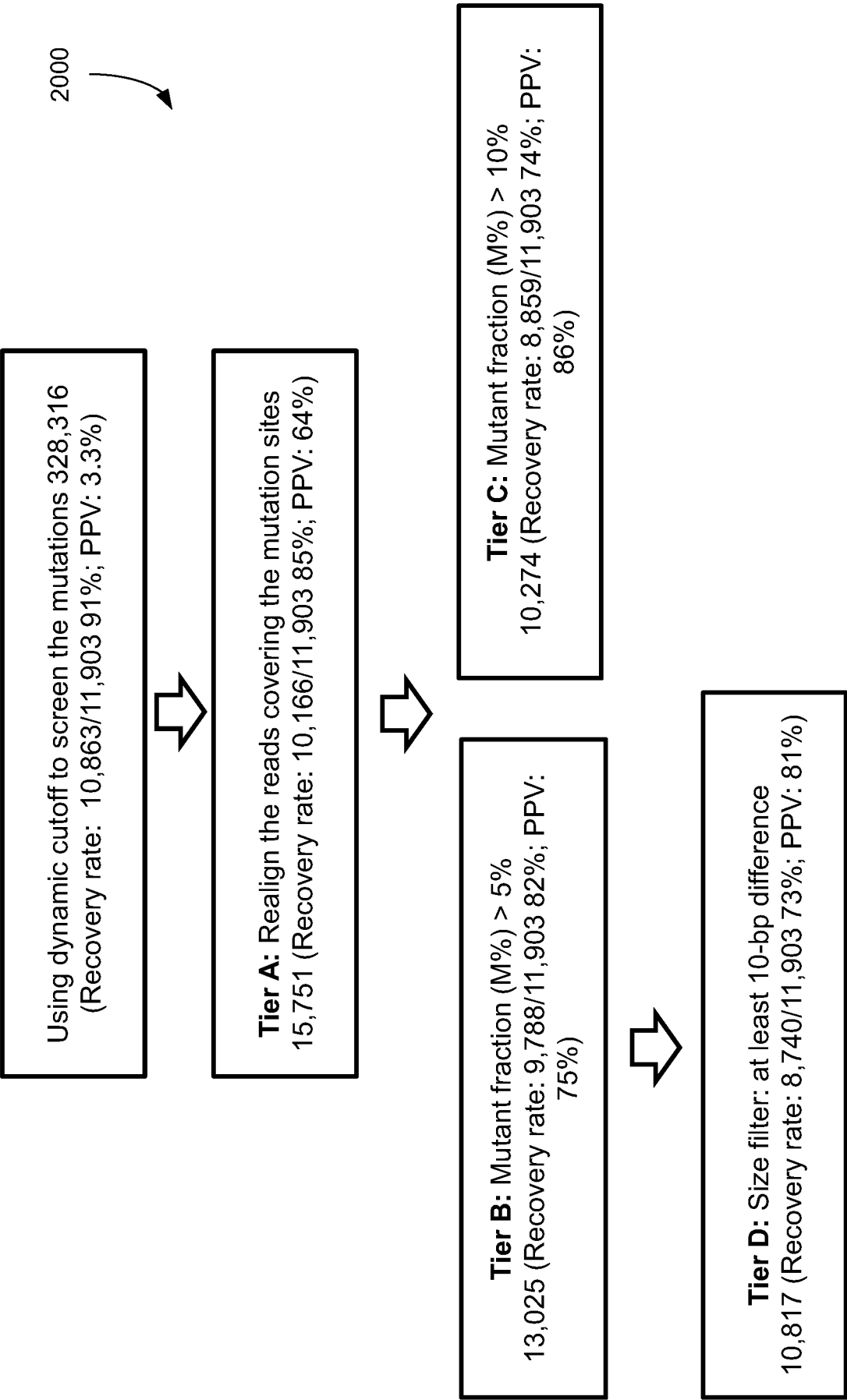


FIG. 20

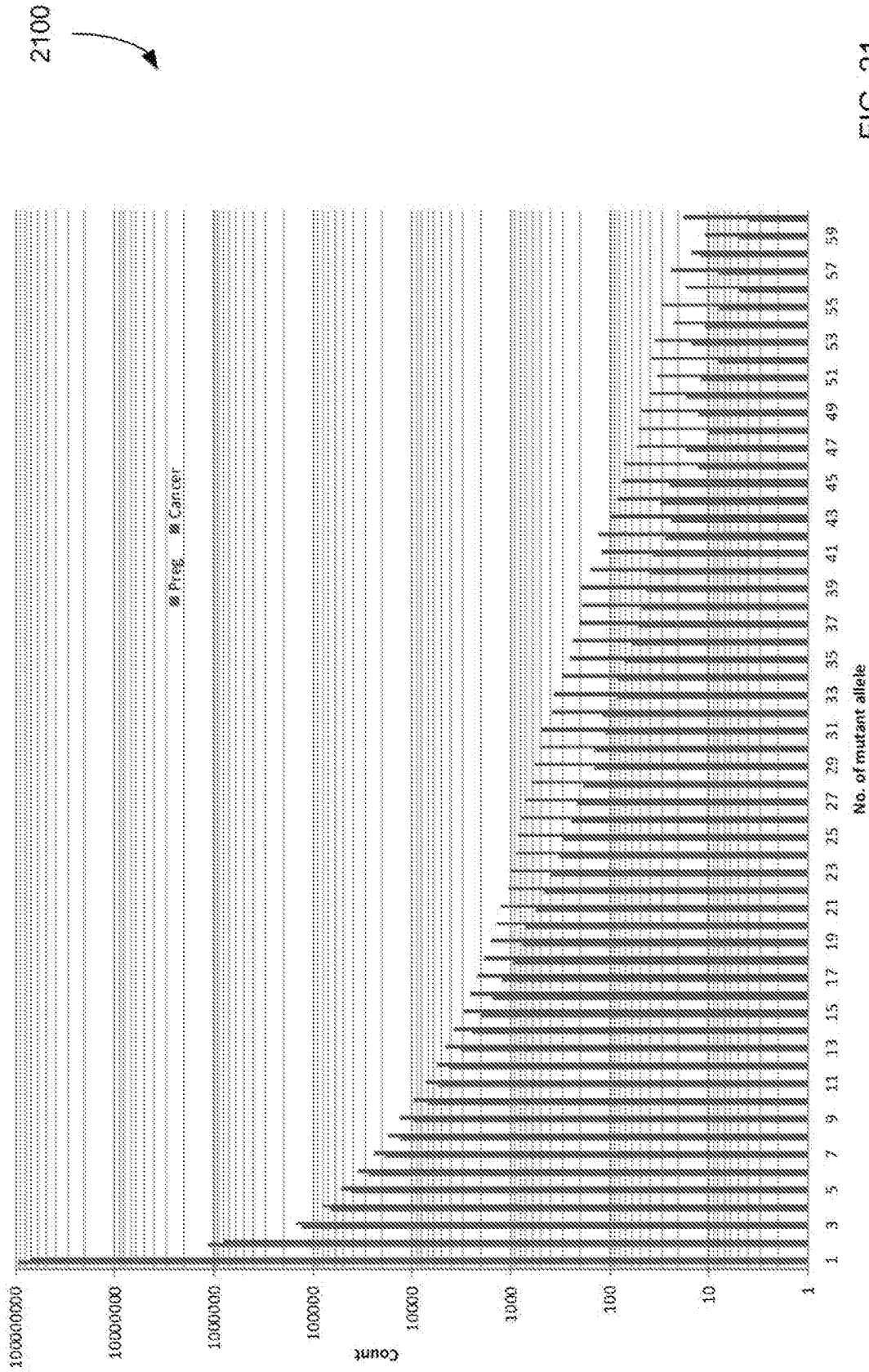


FIG. 21

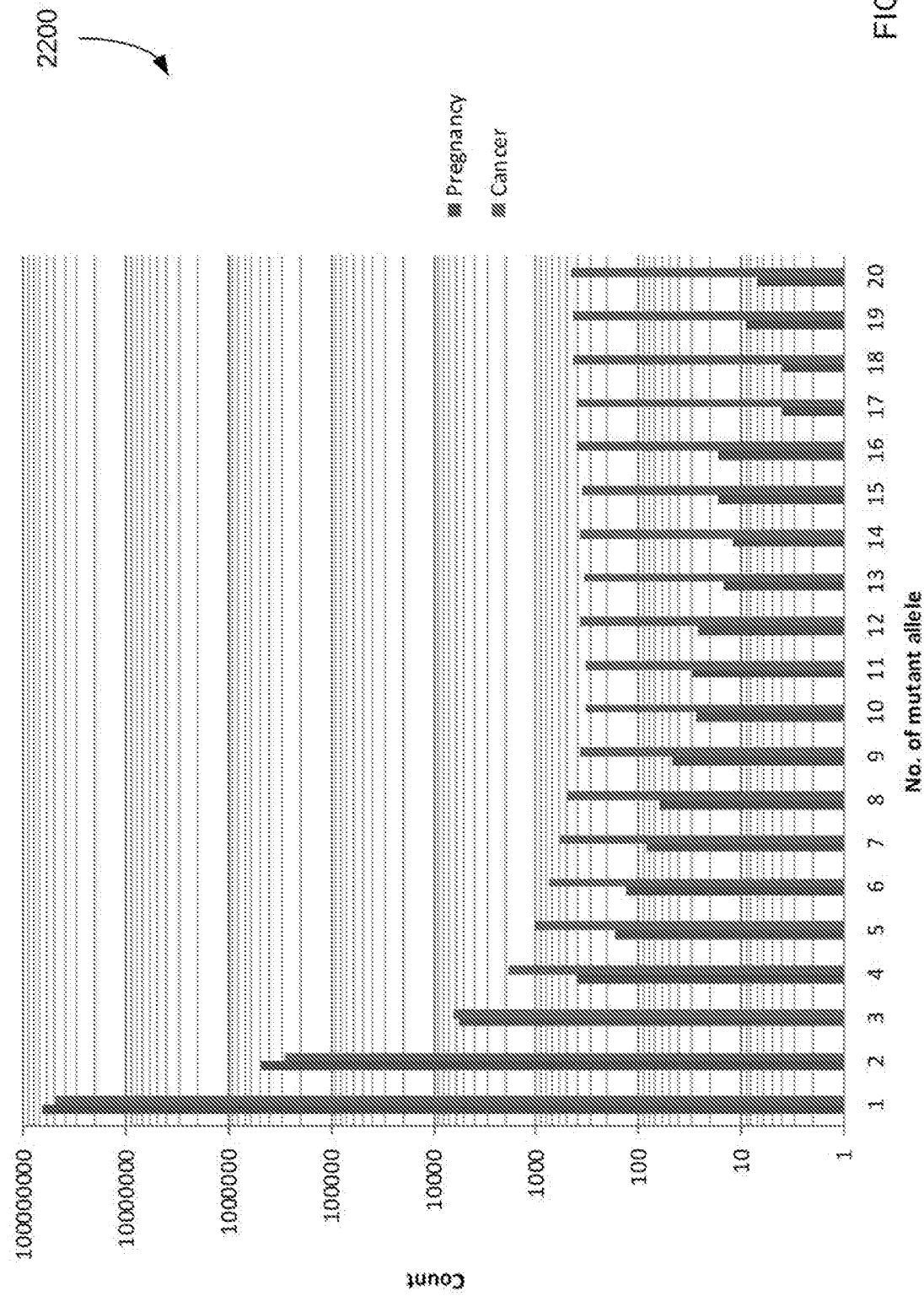
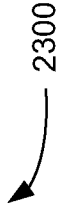


FIG. 22

Fixed cutoff					Size filter				
Cutoff used for mutation identification (at least N mutant alleles seen in plasma)	The number of putative mutations identified in plasma	The number of somatic mutations verified in tumor tissue	PPV%	Recovery rate%	Size cutoff of at least X bp difference	The number of putative mutations identified in human genome	The number of somatic mutations verified in tumor tissue	PPV%	Recovery rate%
2	1238513	11395	0.92	95.73	5	616199	10822	1.76	90.9
					10	500002	10117	2.02	85.0
					15	393749	8583	2.18	72.1
					20	302989	6176	2.04	51.9
3	423581	11114	2.62	93.37	5	238275	10595	4.45	89.0
					10	186803	9901	5.30	83.2
					15	140332	8404	5.99	70.6
					20	100861	6031	5.98	50.7
4	278504	10847	3.89	91.13	5	149269	10358	6.94	87.0
					10	115078	9680	8.41	81.3
					15	84331	8215	9.74	69.0
					20	58226	5878	10.10	49.4
5	197631	10590	5.36	88.97	5	106435	10133	9.52	85.1
					10	81133	9475	11.68	79.6
					15	58571	8037	13.72	67.5
					20	39388	5730	14.55	48.1
6	145772	10375	7.12	87.16	5	79705	9930	12.46	83.4
					10	60411	9285	15.37	78.0
					15	43200	7872	18.22	66.1
					20	28653	5600	19.54	47.0

FIG. 23


 2300

Cutoff used for mutation identification (at least N mutant alleles seen in plasma)	Fixed cutoff				Size filter			
	The number of putative mutations identified in plasma	The number of somatic mutations verified in tumor tissue	PPV%	Recovery rate%	Size cutoff of at least X bp difference	The number of putative mutations identified in human genome	The number of somatic mutations verified in tumor tissue	PPV%
2	303265	10587	3.49	88.94	5	156732	10072	6.43
					10	127277	9417	7.40
					15	99588	7986	8.02
					20	75324	5712	7.58
3	21837	10388	47.57	87.27	5	28643	9921	34.64
					10	24250	9273	38.24
					15	19336	7868	40.69
					20	14012	5619	40.10
4	15493	10152	65.53	85.29	5	14896	9712	65.20
					10	13382	9077	67.83
					15	11115	7702	69.29
					20	7949	5486	69.01
5	13629	9936	72.90	83.47	5	13121	9519	72.55
					10	11896	8904	74.85
					15	9925	7556	76.13
					20	7061	5367	76.01
6	12596	9765	77.52	82.04	5	12277	9359	76.23
					10	11143	8754	78.56
					15	9283	7425	79.98
					20	6574	5266	80.10
								44.2

2400

FIG. 24

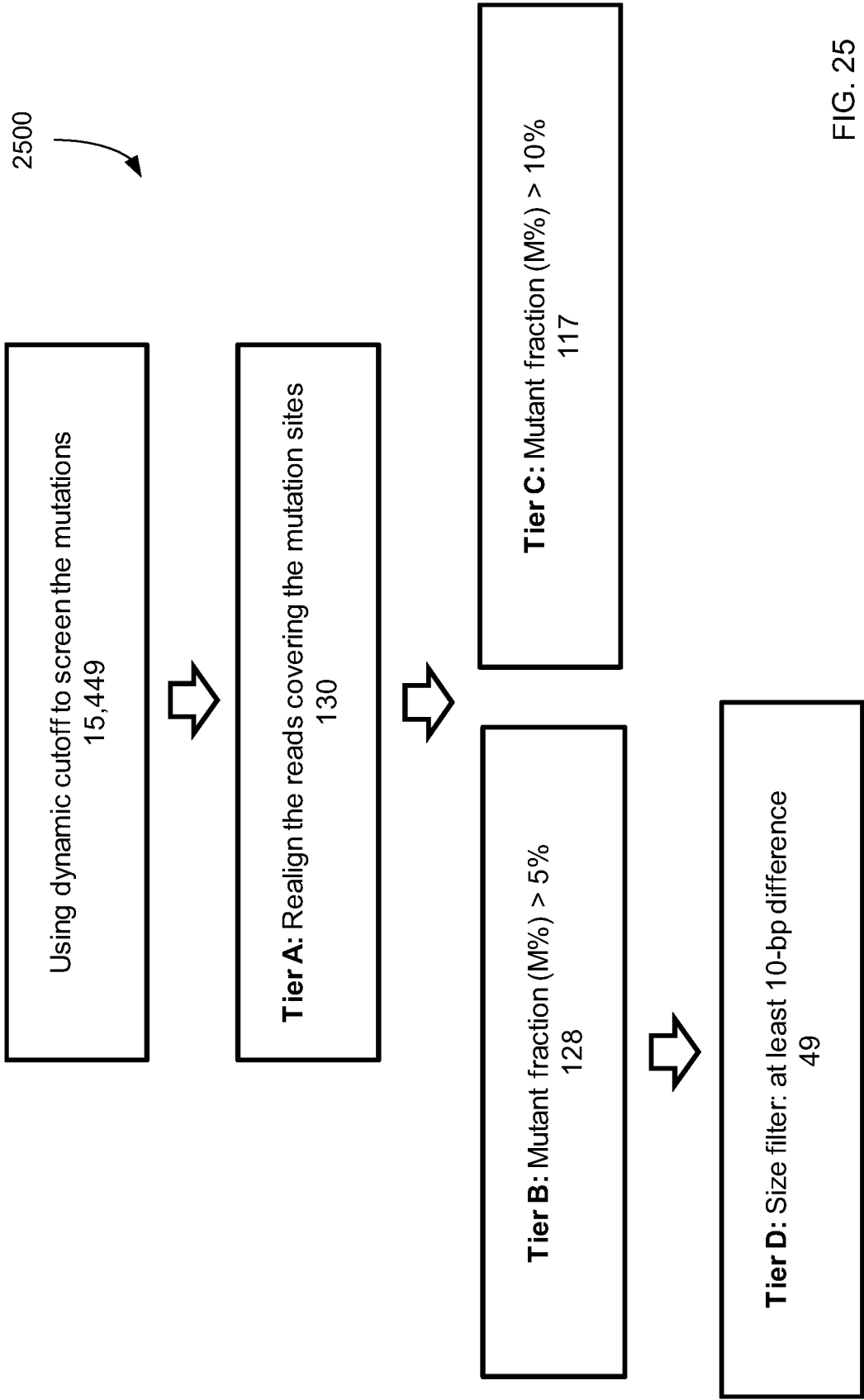


FIG. 25

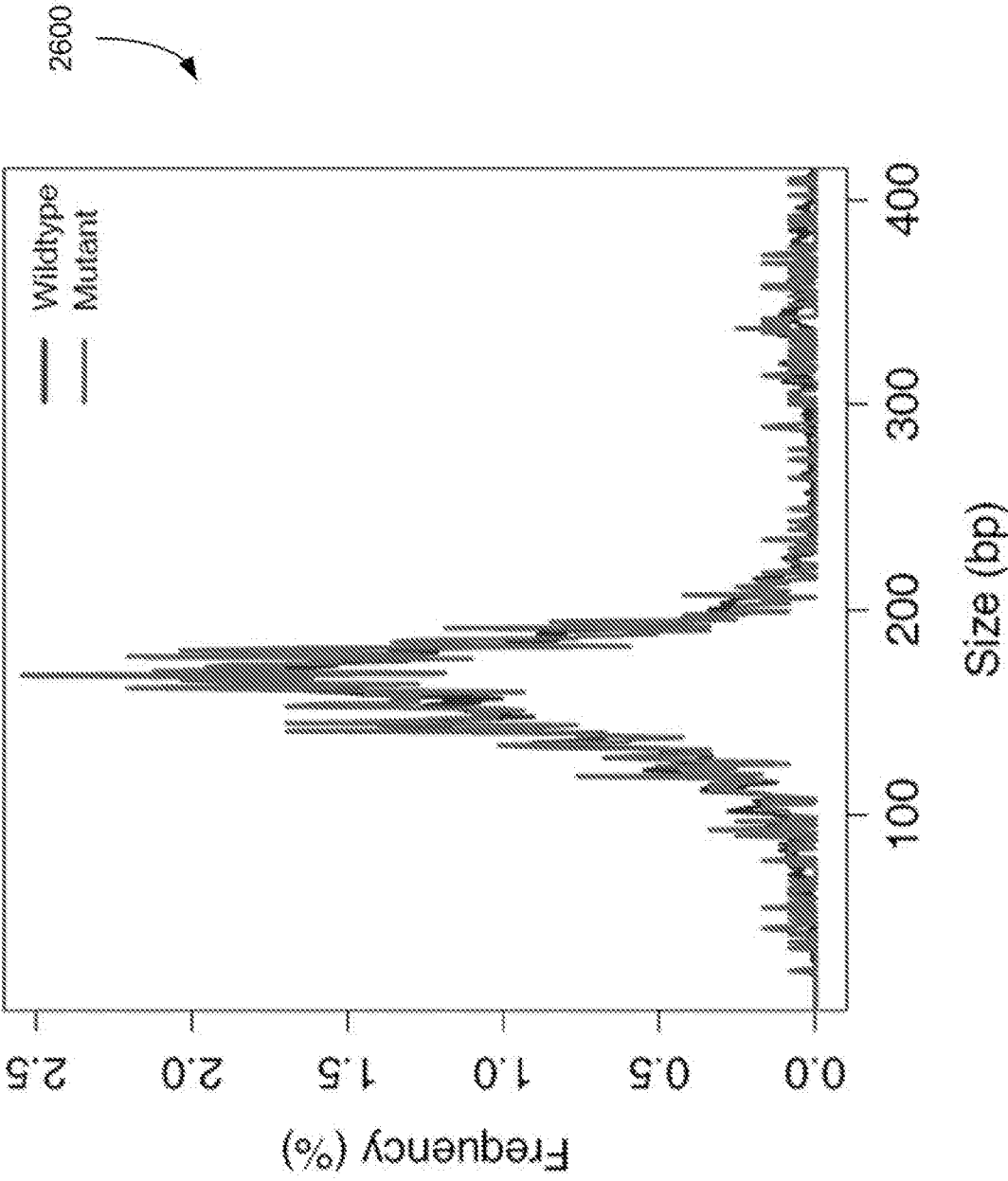
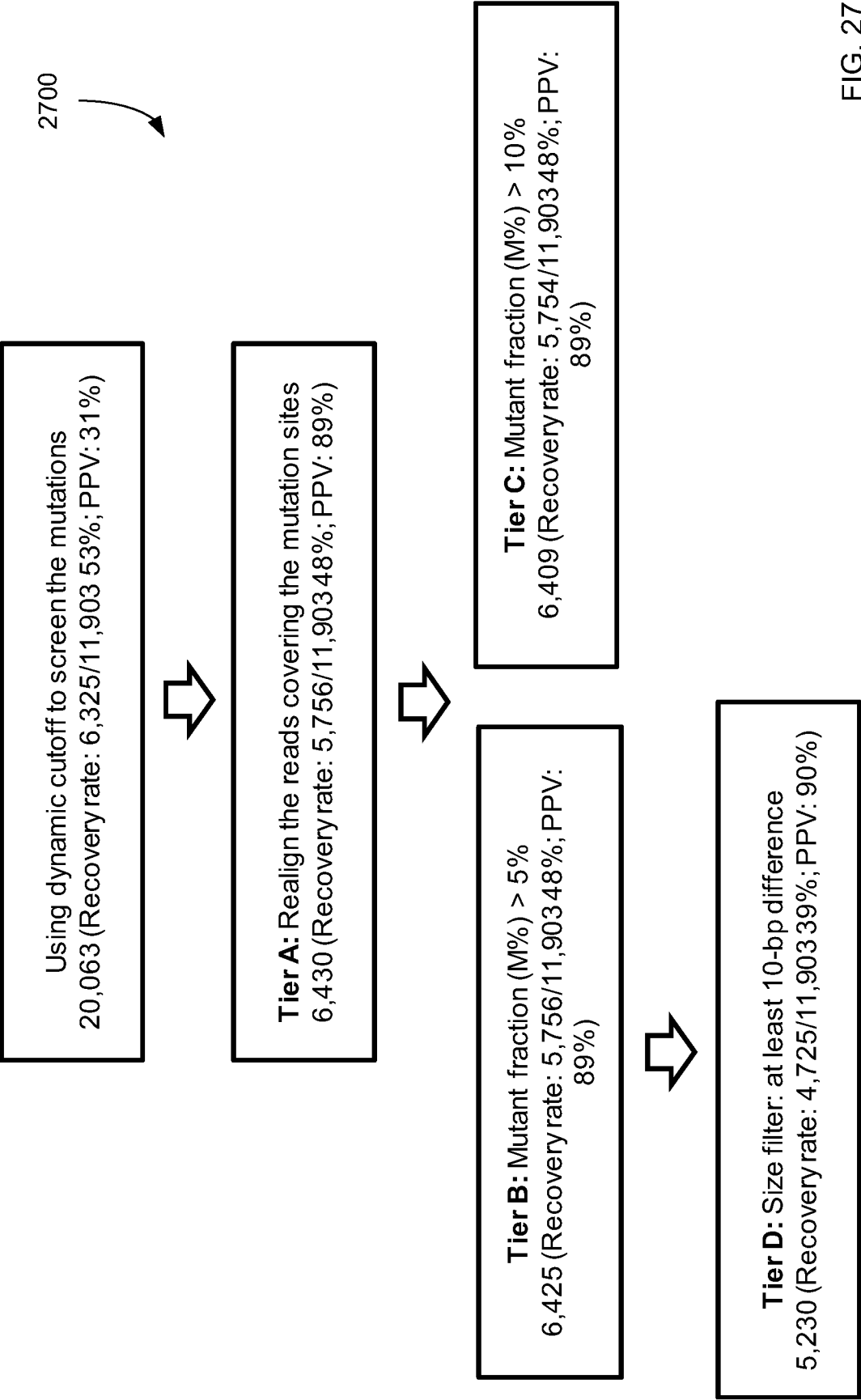
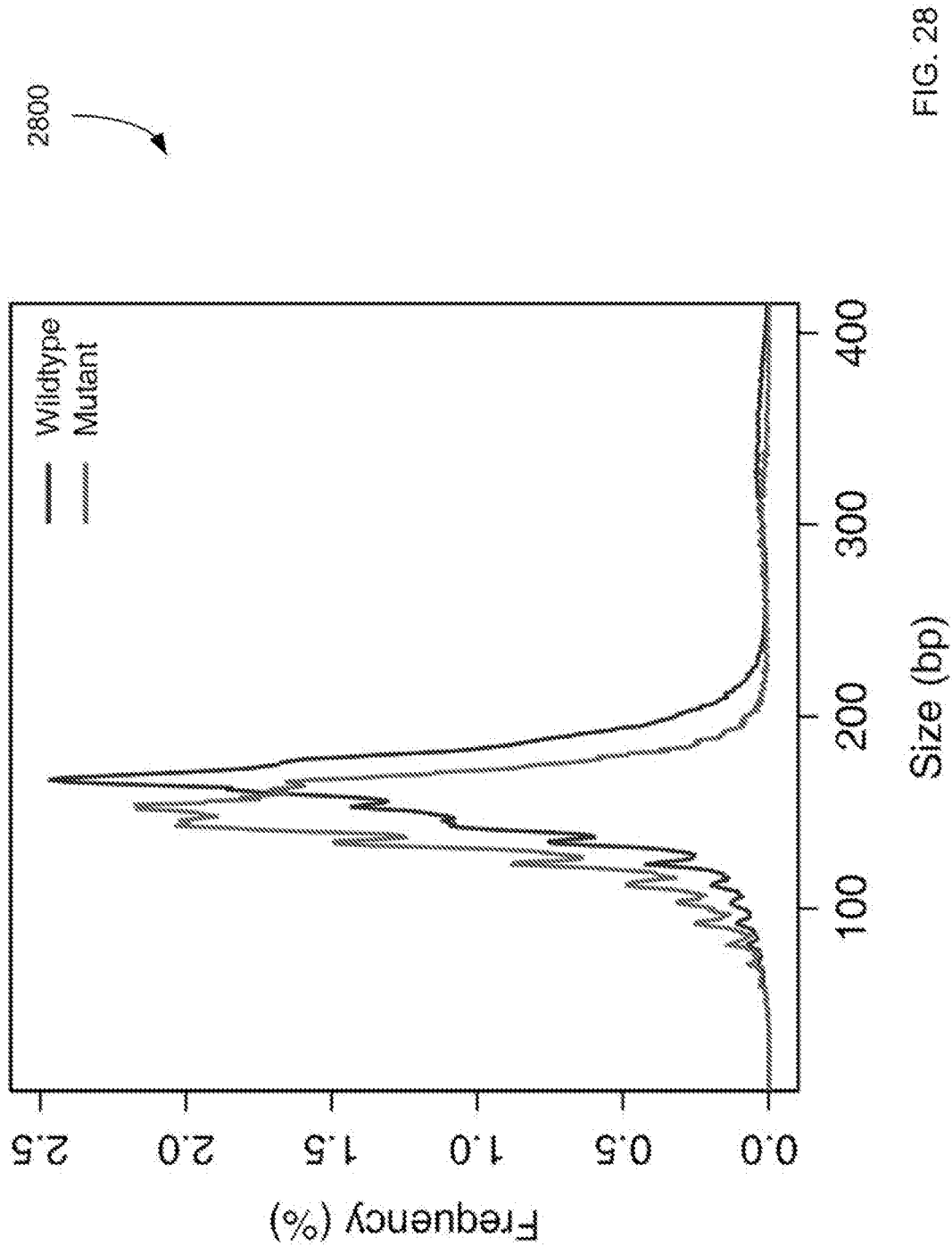


FIG. 26





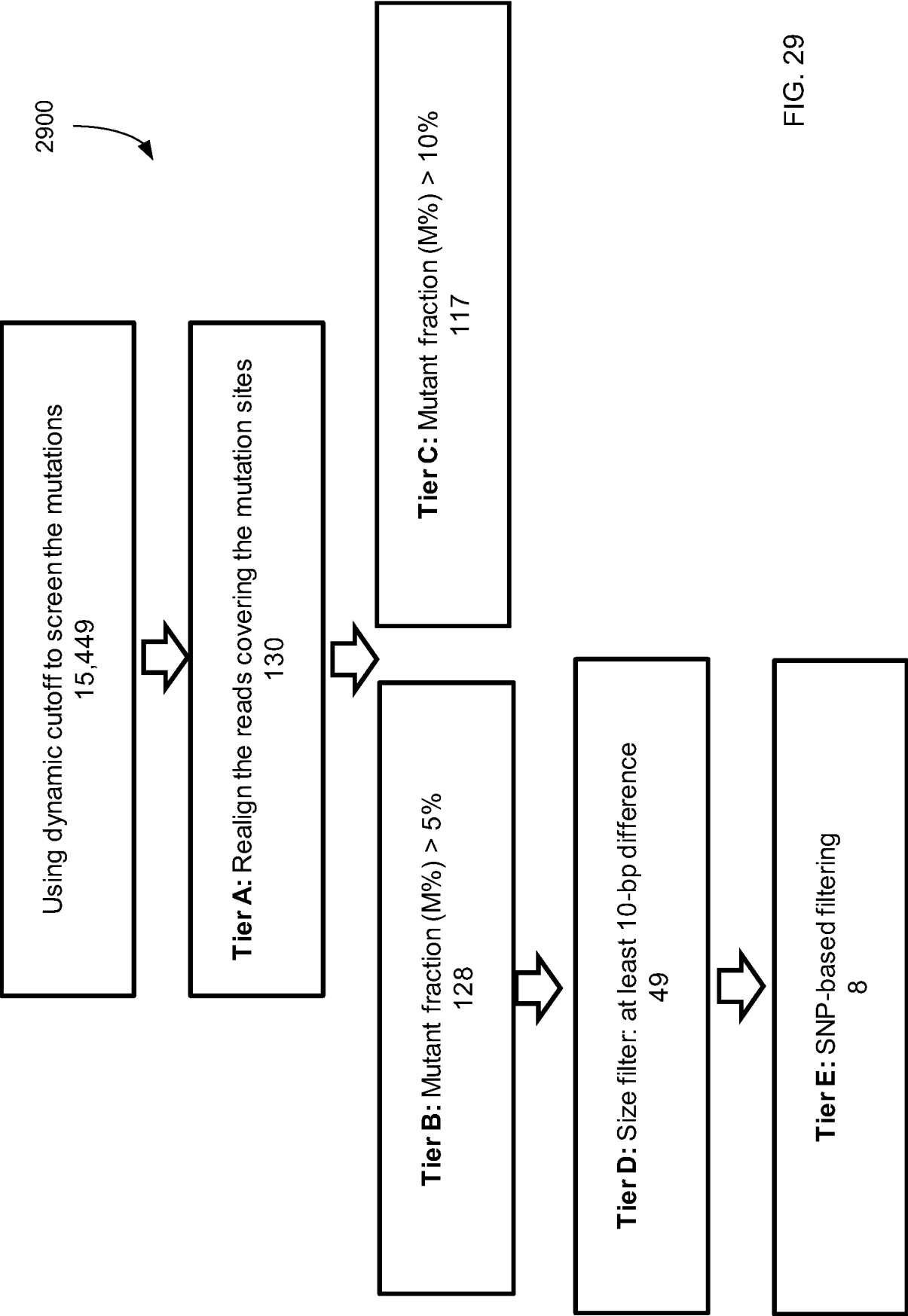


FIG. 29

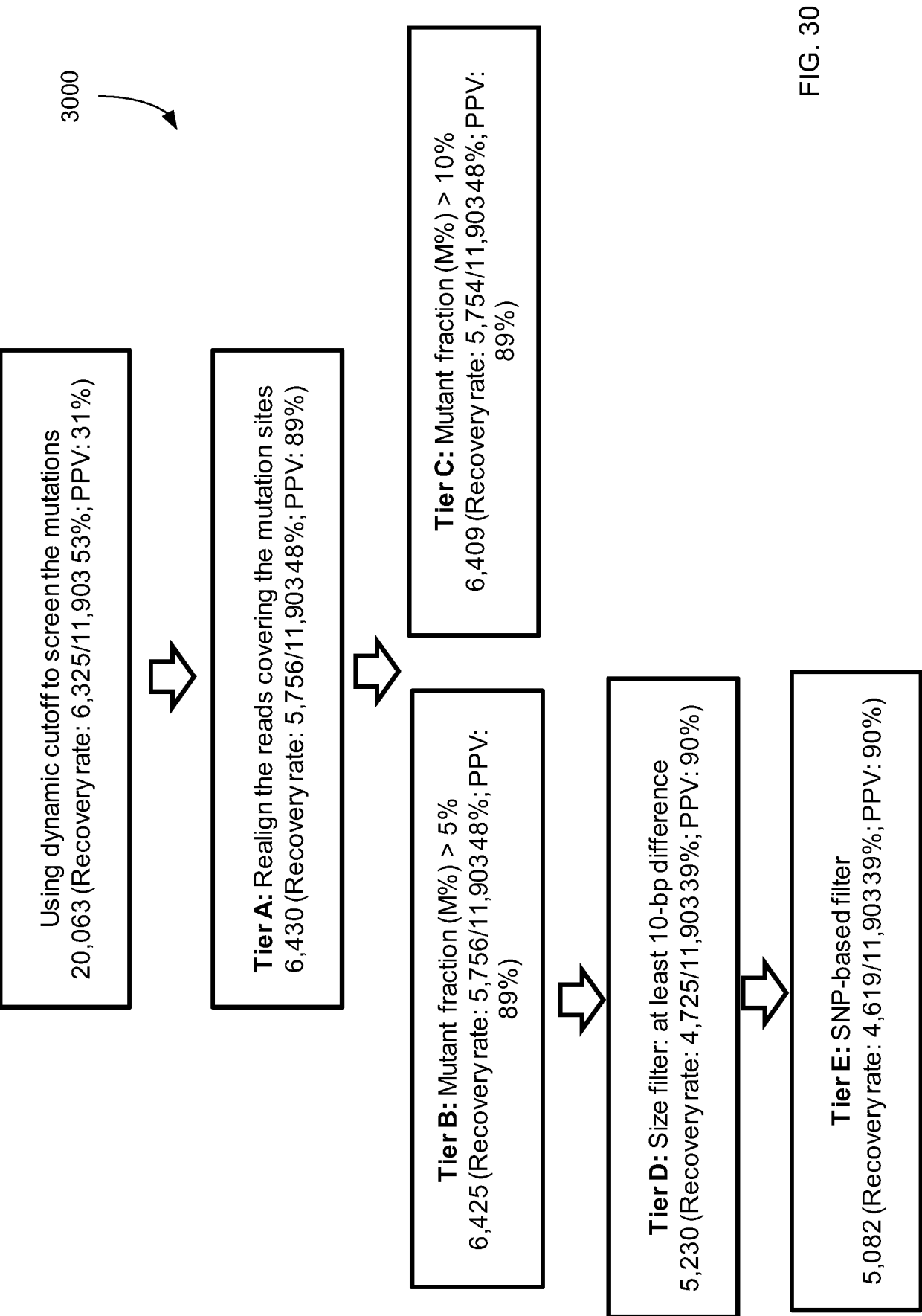


FIG. 30

3100

Source	ID	Correlation between plasma mutation and H3K4me1	Correlation between plasma mutation and H3K27ac	Correlation between plasma mutation and H3K9me3
Breast	E027	-0.415	NA	0.352
Monocytes	E029	-0.421	-0.38	0.444
blood	E030	-0.400	NA	0.482
liver	E066	-0.553	-0.50	0.570
brain	E071	-0.410	-0.42	0.366
colon	E076	-0.401	-0.39	0.134
Gastric	E094	-0.459	-0.48	0.318
lung	E096	-0.460	-0.47	0.016
Ovary	E097	-0.391	-0.42	0.080
pancreas	E098	-0.463	-0.47	0.301
Sigmoid colon	E106	-0.464	-0.44	0.455
Small intestine	E109	-0.472	-0.42	0.356

FIG. 31

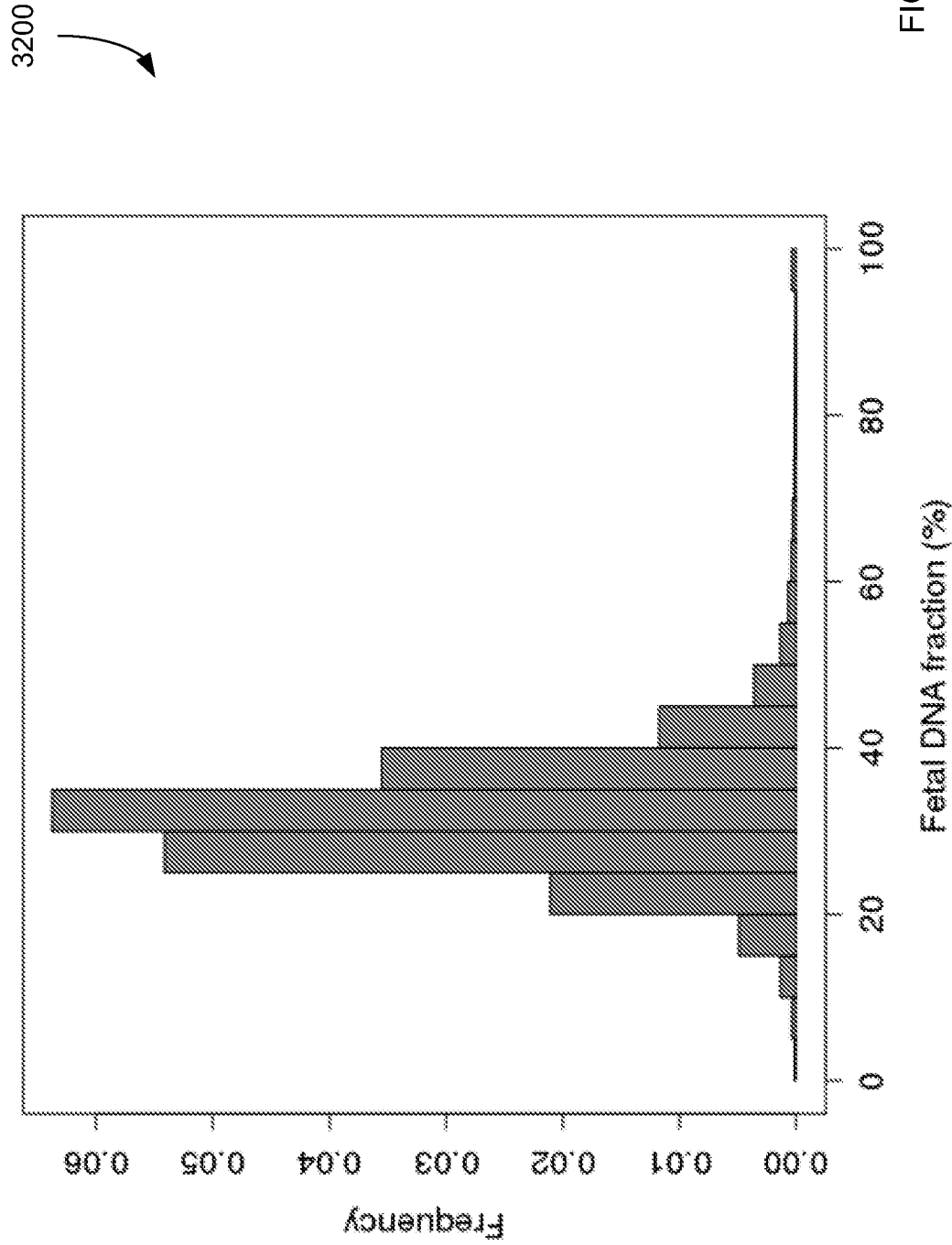


FIG. 32

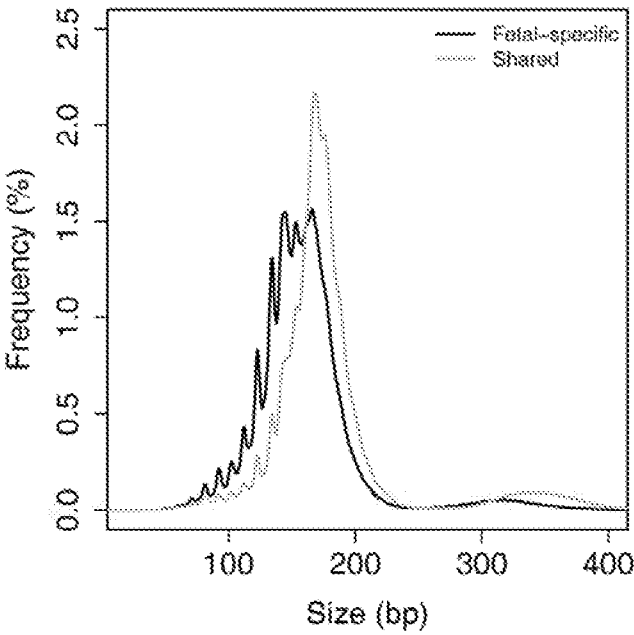


FIG. 33A

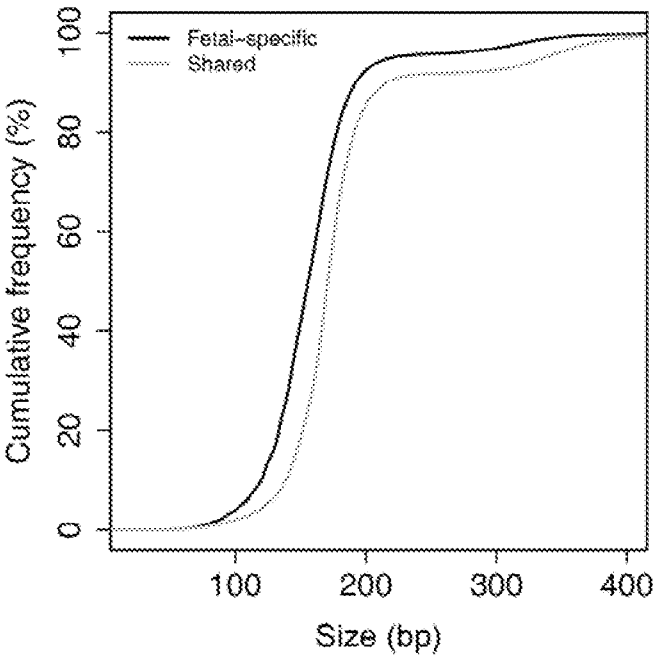


FIG. 33B

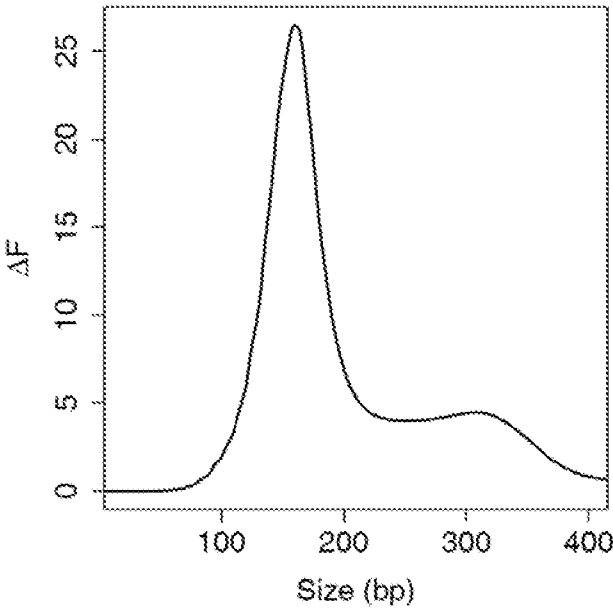


FIG. 33C

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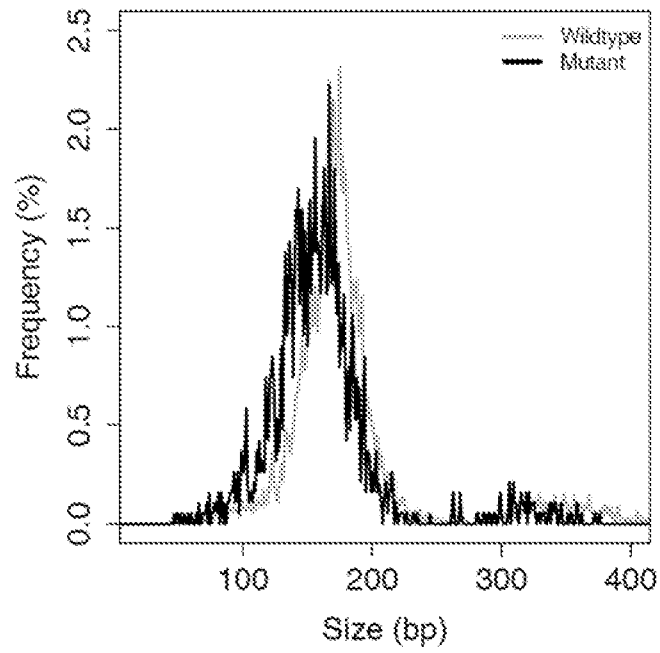


FIG. 34A

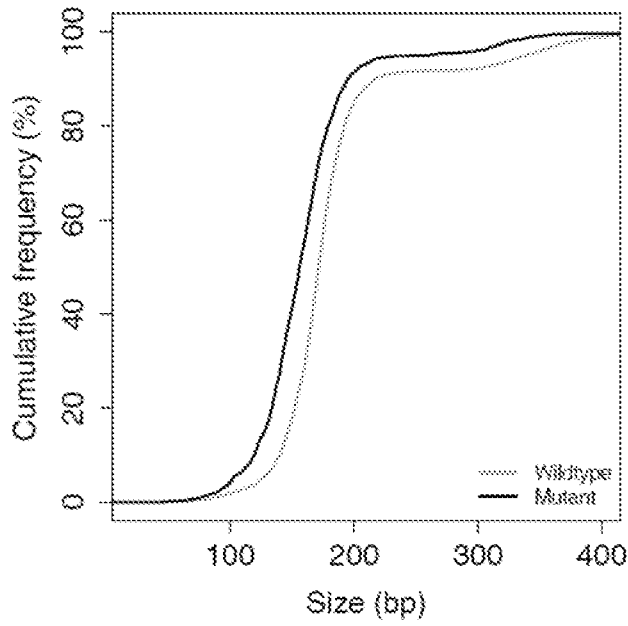


FIG. 34B

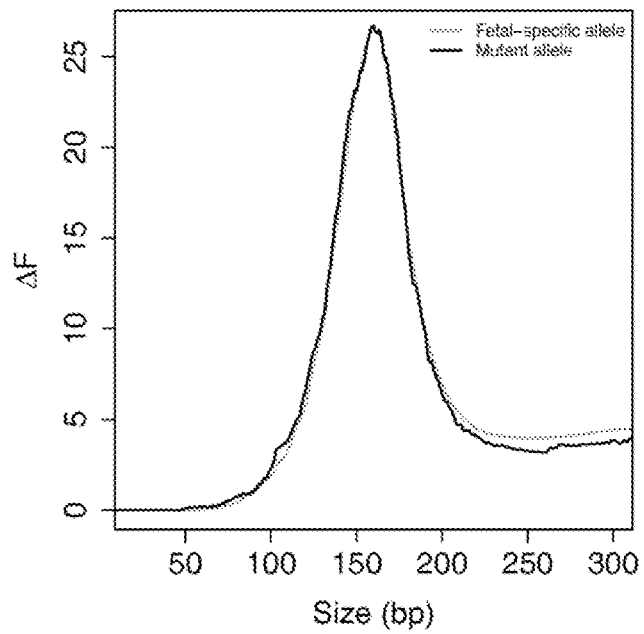


FIG. 34C

De novo mutation identification in plasma

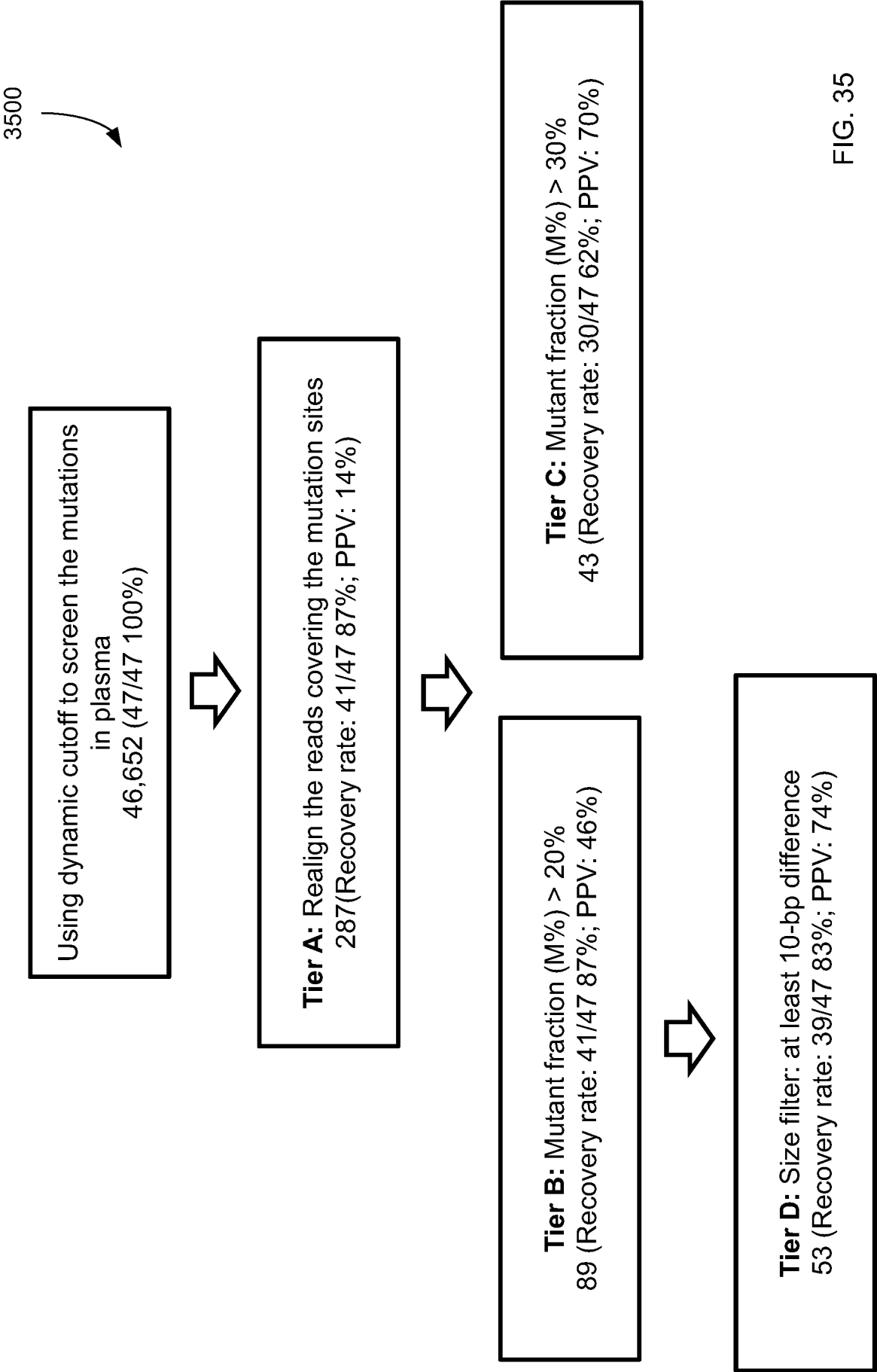


FIG. 36B

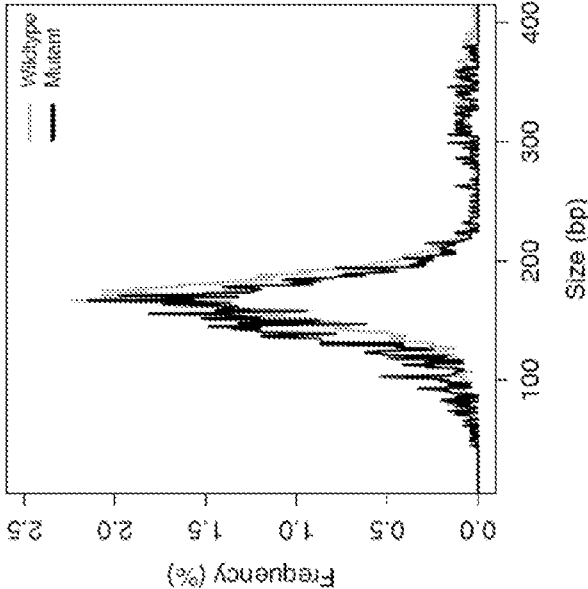


FIG. 36D

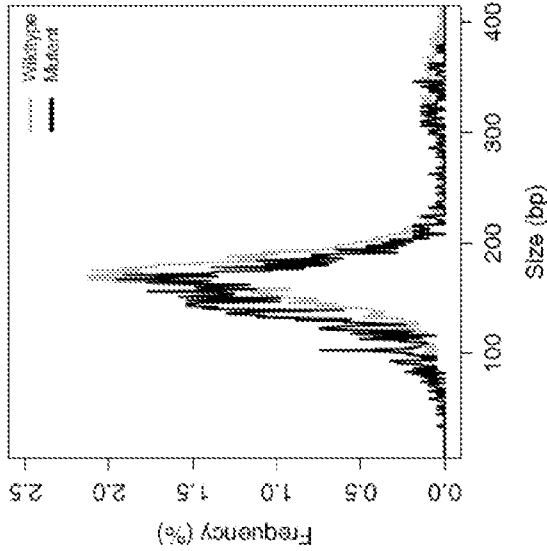


FIG. 36A

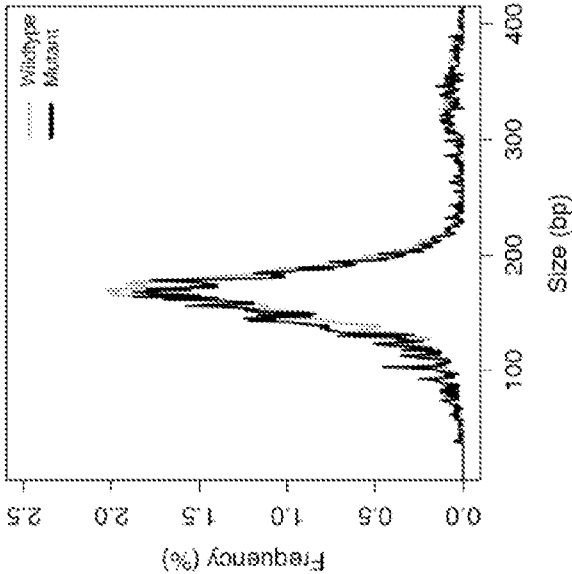
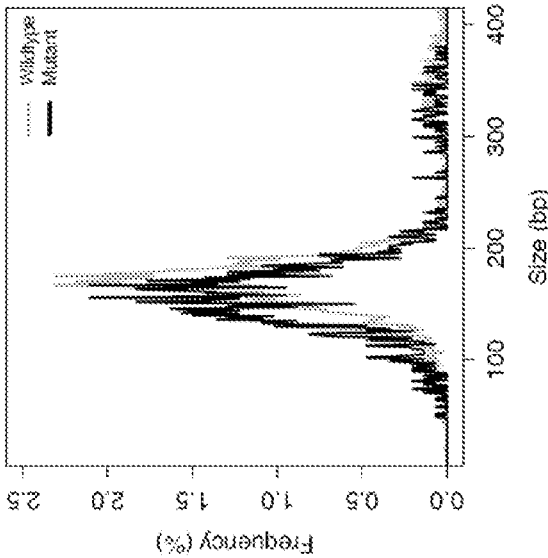


FIG. 36C



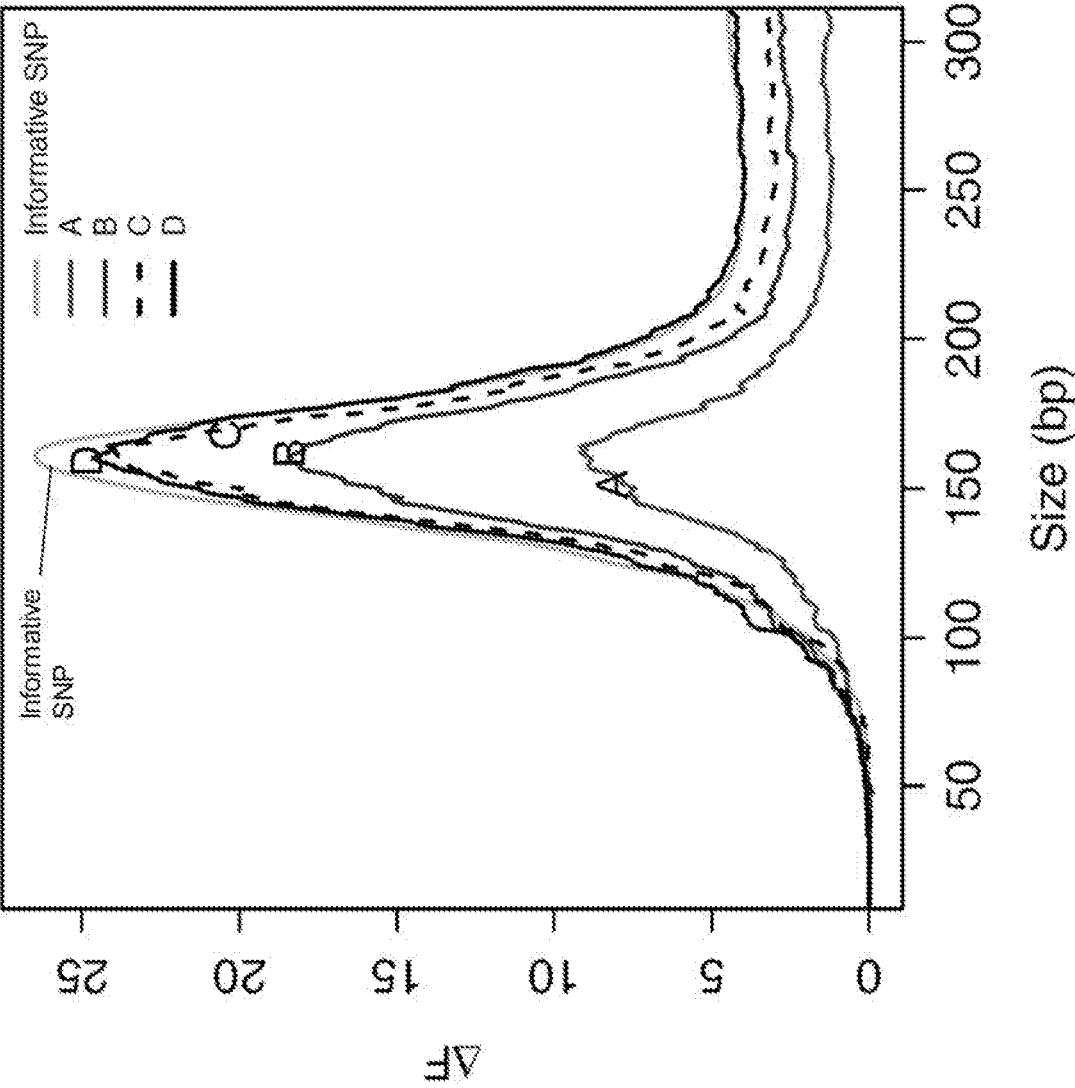


FIG. 37

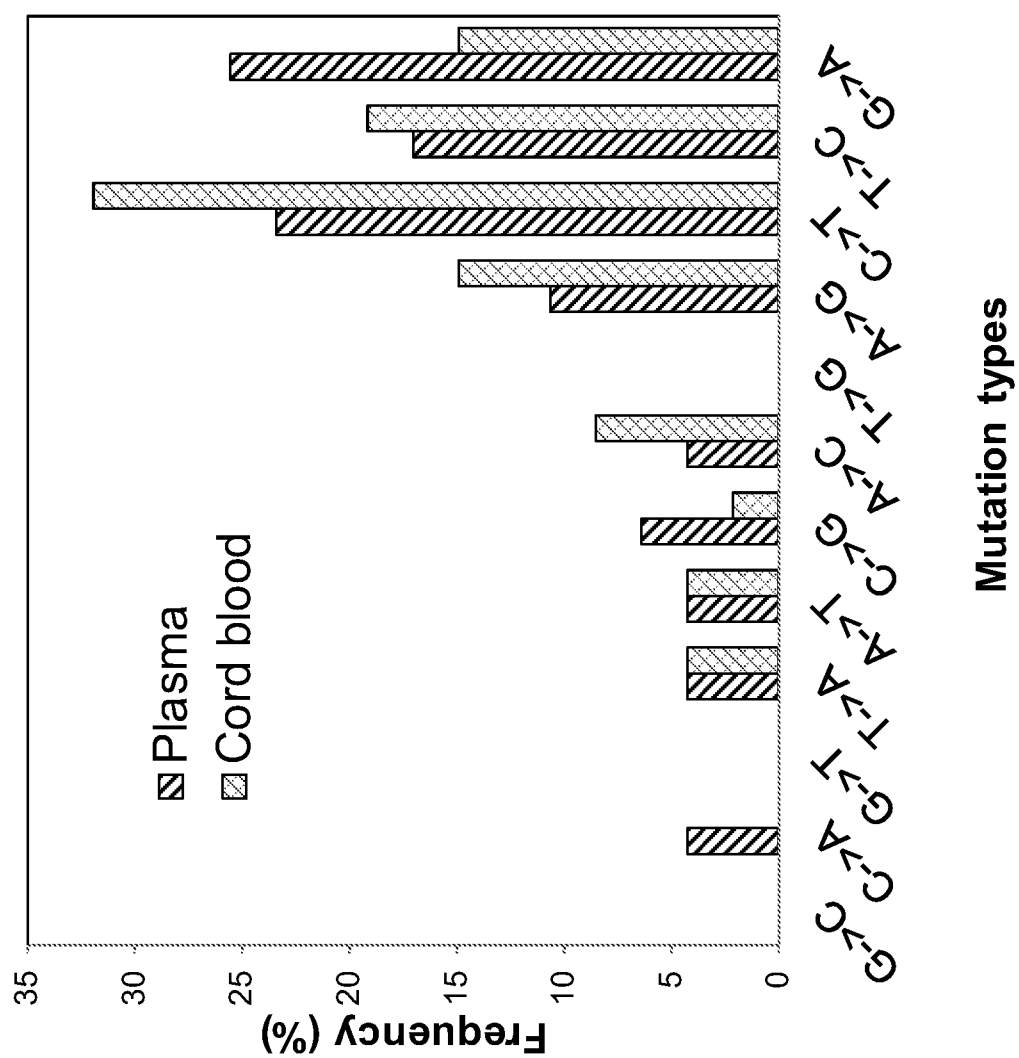


FIG. 38

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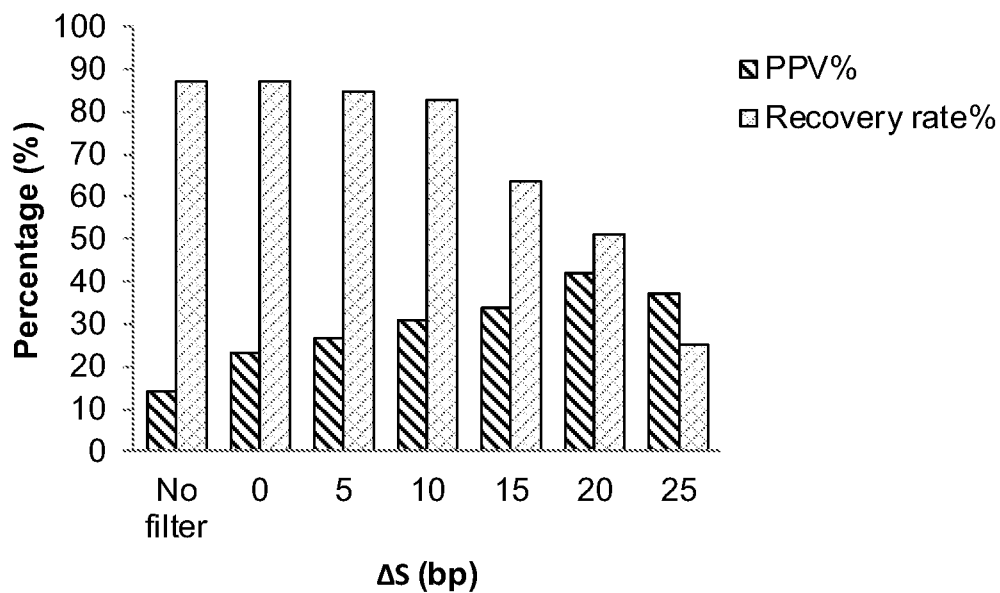


FIG. 39A

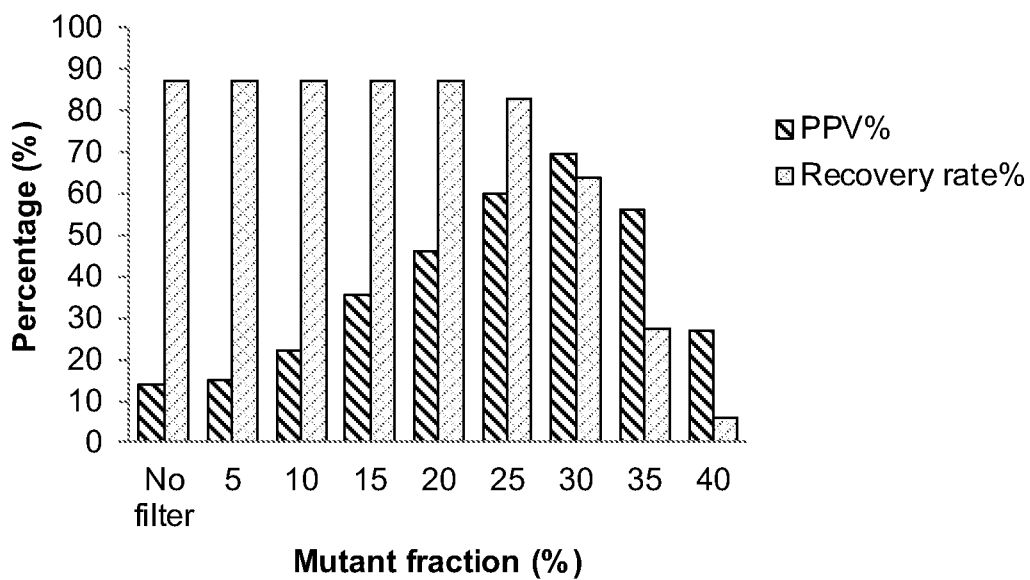
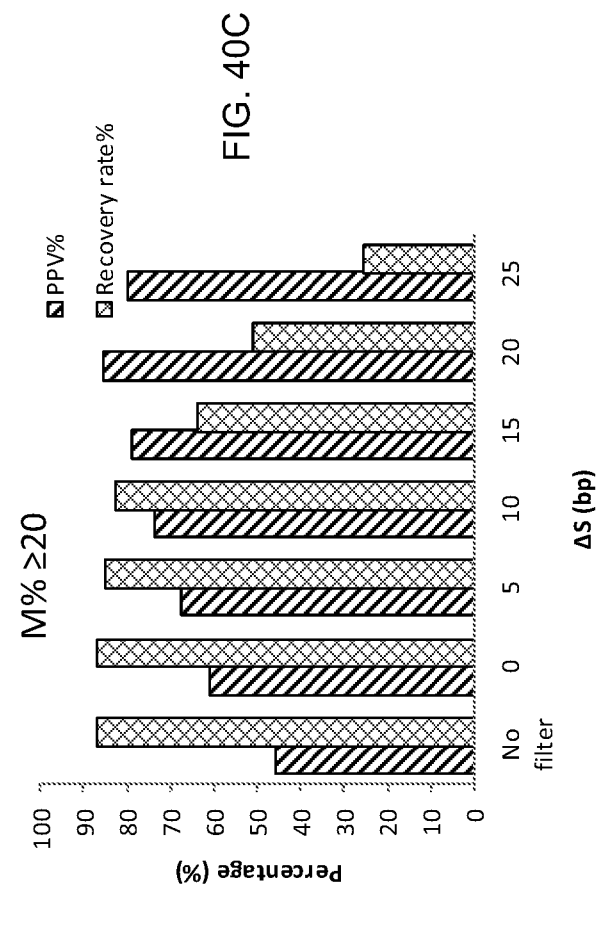
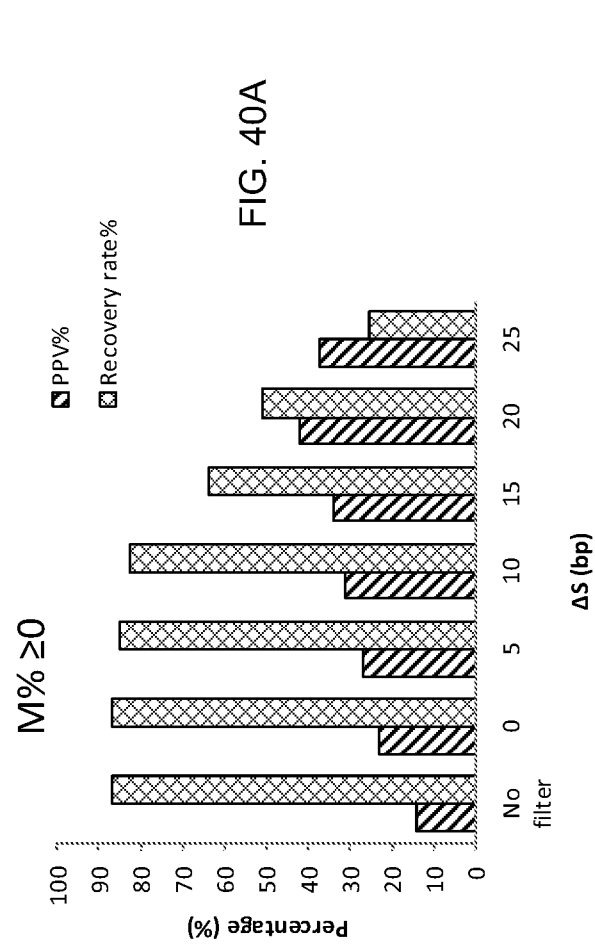
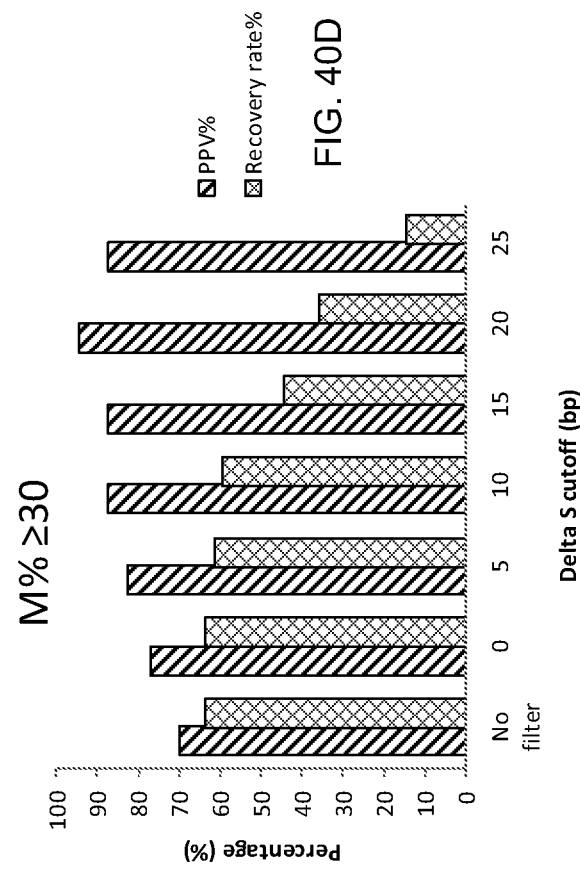
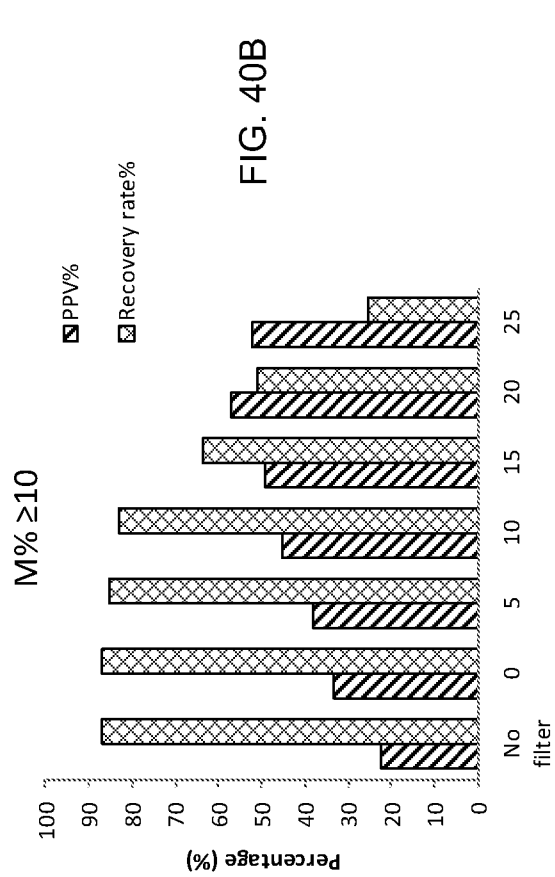


FIG. 39B



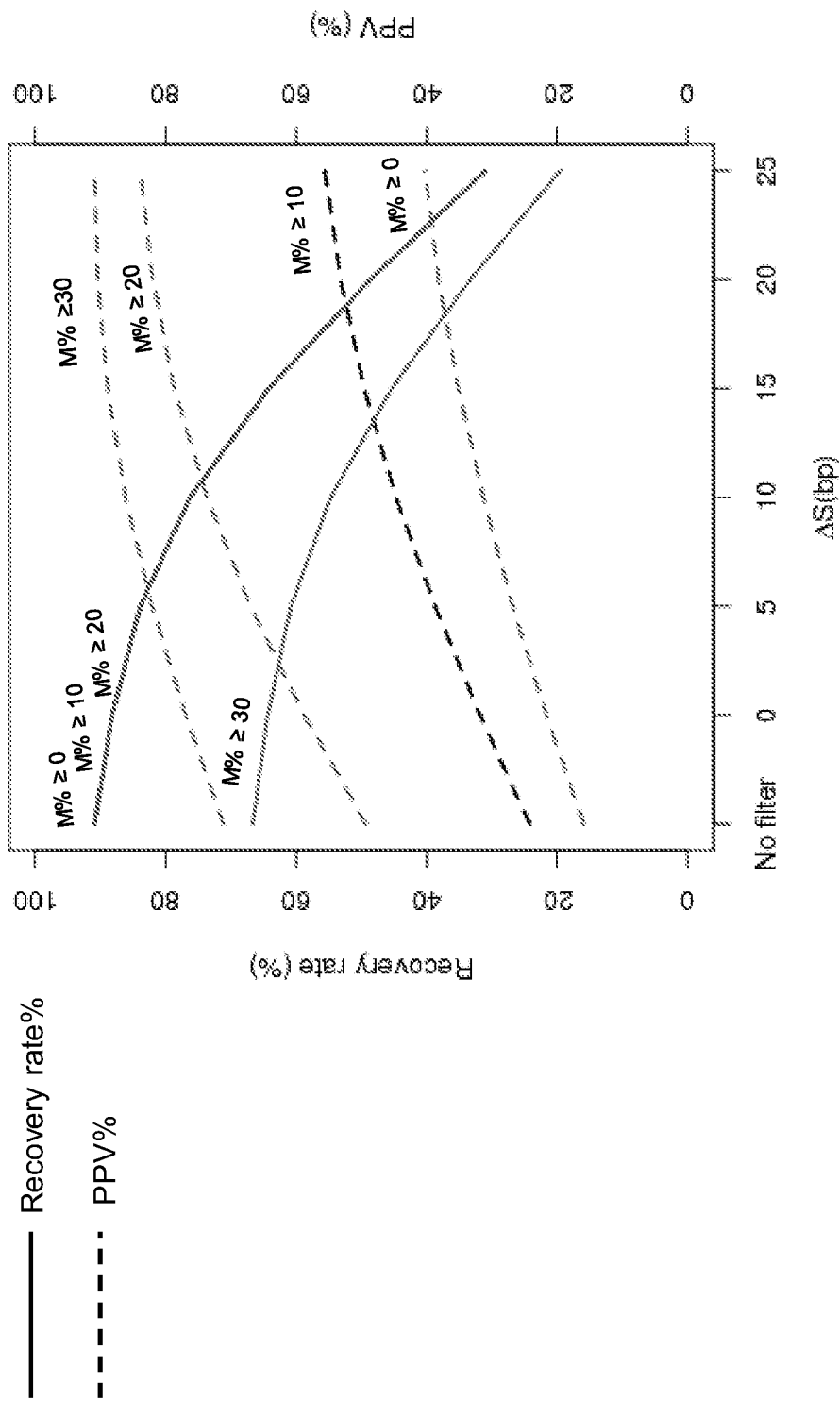


FIG. 41

Name	Chr position	Maternal plasma (NGS)		Father		Mother		Cord blood		Placenta		Remark
		Genotype	Count	NGS	Sanger seq	NGS	Sanger seq	NGS	Sanger seq	NGS	Sanger seq	
TP1	chr1:66987866	CT	259:44	CC	CC	CC	CC	CT	CT	CT	CT	
TP2	chr2:231414780	CT	239:53	CC	CC	CC	CC	CT	CT	CT	CT	
TP3	chr3:126060289	GA	246:50	GG	GG	GG	GG	GA	GA	GA	GA	
TP4	chr4:73534843	AC	204:45	AA	AA	AA	AA	AC	AC	AC	AC	
TP5	chr4:75070975	CC	294:0	CC	CC	CC	CC	CT	CT	CC	CC	Cord blood-specific Placenta-specific
TP6	chr4:95295449	CA	261:35	CC	CC	CC	CC	CC	CC	CA	CA	
TP7	chr5:83895390	GA	253:42	GG	GG	GG	GG	GA	GA	GA	GA	
TP8	chr6:54202751	CA	232:38	CC	CC	CC	CC	CA	CA	CA	CA	
TP9	chr7:41399864	TA	282:59	TT	TT	TT	TT	TA	TA	TA	TA	
TP10	chr8:117728725	CT	235:43	CC	CC	CC	CC	CT	CT	CT	CT	
TP11	chr10:22255065	AG	207:48	AA	AA	AA	AA	AG	AG	AG	AG	
TP12	chr11:76736964	TC	234:58	TT	TT	TT	TT	TC	TC	TC	TC	
TP13	chr12:106632793	GA	230:44	GG	GG	GG	GG	GA	GA	GA	GA	
TP14	chr14:57083665	TC	205:52	TT	TT	TT	TT	TC	TC	TC	TC	
TP15	chr17:66731200	CT	241:44	CC	CC	CC	CC	CT	CT	CT	CT	
TP16	chr19:33302746	GA	248:32	GG	GG	GG	GG	GG	GG	GA	GA	Placenta-specific
TP17	chr20:41416859	CT	165:49	CC	CC	CC	CC	CT	CT	CT	CT	
TP18	chr21:26925757	CT	220:47	CC	CC	CC	CC	CT	CT	CT	CT	

FIG. 42

Name	Chr position	Maternal plasma (NGS)		Father		Mother		Cord blood		Placenta		Remark
		Genotype	Count	NGS	Sanger seq	NGS	Sanger seq	NGS	Sanger seq	NGS	Sanger seq	
TP19	chr1:2815785	GA	216/38	GG	GG	GG	GG	AG	AG	GA	GA	
TP20	chr2:60295478	CT	310/50	CC	CC	CC	CC	CT	CT	CT	CT	
TP21	chr3:75762728	AG	331/39	AG (58.1)	AG	AG (62.1)	AG	AG (66.3)	AG	AG (27.1)	AG	All heterozygous
TP22	chr3:77051026	CT	264/53	CC	CC	CC	CC	CT	CT	CT	CT	
TP23	chr4:34755555	CG	262/49	CC	CC	CC	CC	CG	CG	CG	CG	
TP24	chr4:54613139	CT	168/28	CC	CC	CC	CC	CT	CT	CT	CT	
TP25	chr4:68332287	AG	182/41	AA	AA	AA	AA	AG	AG	AG	AG	
TP26	chr4:113248366	AT	225/51	AA	AA	AA	AA	AT	AT	AT	AT	
TP27	chr4:181441802	TA	206/41	TT	TT	TT	TT	TA	TA	TA	TA	
TP28	chr5:2366786	CT	233/43	CC	CC	CC	CC	CT	CT	CT	CT	
TP29	chr5:146803590	CG	270/44	CC	CC	CC	CC	CG	CG	CG	CG	
TP30	chr6:32603242	GA	133/16	GG (36)	GG	GG (24)	GG	GG (36)	GG	GA (21.3)	GG	Placenta-specific, HLA region
TP31	chr6:55920652	TC	271/54	TT	TT	TT	TT	TC	TC	TC	TC	
TP32	chr6:106112398	GA	277/57	GG	GG	GG	GG	GA	GA	GA	GA	
TP33	chr6:169849375	TC	182/32	TT	TT	TT	TT	TC	TC	TC	TC	
TP34	chr6:169897294	AG	224/47	AA	AA	AA	AA	AG	AG	AG	AG	
TP35	chr8:173340569	CG	281/38	CC	CC	CC	CC	CG	CG	CG	CG	
TP36	chr8:107961680	AC	309/49	AA	AA	AA	AA	AC	AC	AC	AC	
TP37	chr9:36612823	TC	290/35	TT	TT	TT	TT	TC	TC	TC	TC	
TP38	chr10:50117839	AT	247/65	AA	AA	AA	AA	AT	AT	AT	AT	
TP39	chr10:121730789	GA	204/41	GG	GG	GG	GG	GA	GA	GA	GA	
TP40	chr12:57200848	TC	270/57	TT	TT	TT	TT	TC	TC	TC	TC	
TP41	chr12:130838521	CT	159/31	CC	CC	CC	CC	CT	CT	CT	CT	
TP42	chr14:33427740	GA	240/44	GG	GG	GG	GG	GA	GA	GA	GA	
TP43	chr15:27113786	TC	239/43	TT	TT	TT	TT	TC	TC	TC	TC	
TP44	chr15:82768263	GA	132/16	GG (45)	GG	GG (22)	GA	GG (37)	GA	GA (11.1)	GA	Placenta-specific, inconsistent NGS and Sanger seq results
TP45	chr17:2434676	TC	276/47	TT	POR failed	TT	POR failed	TC	POR failed	TC	POR failed	Re-sponse POR
TP46	chr17:78880681	GA	223/39	GG	GG	GG	GG	GA	GA	GA	GA	
TP47	chr18:10102981	GA	156/23	GG	GG	GG	GG	GA	GA	GA	GA	
TP48	chr20:44745129	AG	191/34	AA	AA	AA	AA	GA	GA	GA	GA	

FIG. 43

Down-sampling analysis

Simulated mutations: 3000

De novo mutations: 47

Fetal DNA fraction (%): 32%

Seq depth (X)	Pass dynamic cutoff	Pass realignment	Pass size filter	Recovered from 47 mutations	False positives	Recovered from 3000 simulated mutations	PPV%	Recovery rate (%)
25	834	482	476	10	4	462	99.16	15.49
50	3496	1522	1499	28	10	1461	99.33	48.87
100	10132	2072	2021	35	32	1954	98.42	65.28

FIG. 44

FIG. 45A

The no. of mutations = 3000											
Depth (X)	The number of true mutations detected at different tumor DNA fractions (%)										
	1%	2%	3%	4%	5%	10%	15%	20%	30%	FP	
25	0	0	1	4	10	102	340	709	1587	4	
50	0	0	3	10	24	311	961	1706	2664	10	
60	0	1	6	21	51	541	1415	2187	2873	12	
80	0	4	22	67	151	1113	2176	2736	2986	18	
90	0	7	35	104	227	1411	2441	2860	2996	25	
100	0	10	53	152	319	1692	2633	2929	2999	32	
200	11	155	550	1113	1685	2921	2998	3000	3000	45	
400	49	642	1667	2435	2806	3000	3000	3000	3000	na	
600	250	1665	2658	2942	2992	3000	3000	3000	3000	na	
800	643	2430	2941	2996	3000	3000	3000	3000	3000	na	

FIG. 45B

The no. of mutations = 6000											
Depth (X)	The number of true mutations detected at different tumor DNA fractions (%)										
	1%	2%	3%	4%	5%	10%	15%	20%	30%	FP	
25	0	1	3	9	19	205	681	1418	3173	4	
50	0	1	6	19	49	622	1923	3413	5327	10	
60	0	2	13	42	103	1082	2829	4374	5746	12	
80	0	8	43	134	302	2227	4352	5472	5972	18	
90	1	13	70	209	453	2822	4882	5721	5991	25	
100	1	21	106	305	638	3384	5266	5858	5997	32	
200	21	310	1101	2227	3370	5841	5996	6000	6000	45	
400	98	1284	3333	4869	5612	6000	6000	6000	6000	na	
600	500	3331	5317	5883	5985	6000	6000	6000	6000	na	
800	1287	4861	5882	5992	6000	6000	6000	6000	6000	na	

FIG. 45C

The no. of mutations = 9000											
Depth (X)	The number of true mutations detected at different tumor DNA fractions (%)										
	1%	2%	3%	4%	5%	10%	15%	20%	30%	FP	
25	0	1	4	13	29	307	1021	2128	4760	4	
50	0	1	8	29	73	933	2884	5119	7991	10	
60	0	3	19	63	154	1623	4244	6561	8619	12	
80	0	12	65	201	453	3340	6528	8208	8957	18	
90	1	20	105	313	680	4233	7324	8581	8987	25	
100	1	31	159	457	956	5076	7899	8787	8996	32	
200	32	466	1651	3340	5056	8762	8994	9000	9000	45	
400	147	1927	5000	7304	8418	9000	9000	9000	9000	na	
600	751	4996	7975	8825	8977	9000	9000	9000	9000	na	
800	1930	7291	8823	8988	8999	9000	9000	9000	9000	na	

FIG. 46A

The no. of mutations = 10000												
Depth (X)	The number of true mutations detected at different tumor DNA fractions (%)											
	1%	2%	3%	4%	5%	10%	15%	20%	30%	FP		
25	0	1	5	14	32	341	1135	2364	5289	4		
50	0	1	9	32	81	1036	3204	5688	8879	10		
60	0	3	21	70	171	1803	4716	7290	9576	12		
80	1	13	72	224	504	3711	7254	9120	9953	18		
90	1	22	117	348	755	4703	8137	9535	9985	25		
100	2	34	177	508	1063	5640	8777	9763	9996	32		
200	35	517	1835	3712	5617	9736	9994	10000	10000	45		
400	163	2141	5555	8115	9353	9999	10000	10000	10000	na		
600	834	5551	8861	9805	9974	10000	10000	10000	10000	na		
800	2145	8101	9803	9987	9999	10000	10000	10000	10000	na		

FIG. 46B

The no. of mutations= 20000												
Depth (X)	The number of true mutations detected at different tumor DNA fractions (%)											
	1%	2%	3%	4%	5%	10%	15%	20%	30%	FP		
25	0	2	10	29	65	682	2269	4728	10578	4		
50	0	3	18	64	163	2072	6408	11376	17758	10		
60	0	7	42	140	342	3607	9432	14581	19153	12		
80	1	26	144	447	1007	7422	14508	18241	19905	18		
90	2	43	233	696	1510	9406	16275	19069	19971	25		
100	3	69	354	1017	2125	11280	17554	19526	19991	32		
200	71	1035	3669	7423	11235	19471	19988	20000	20000	45		
400	326	4282	11111	16231	18706	19999	20000	20000	20000	na		
600	1668	11103	17723	19611	19949	20000	20000	20000	20000	na		
800	4290	16203	19606	19974	19999	20000	20000	20000	20000	na		

FIG. 46C

The no. of mutations = 30000												
Depth (X)	The number of true mutations detected at different tumor DNA fractions (%)											
	1%	2%	3%	4%	5%	10%	15%	20%	30%	FP		
25	0	3	15	43	97	1023	3404	7092	15866	4		
50	0	4	28	96	244	3109	9613	17064	26637	10		
60	0	10	63	211	513	5410	14147	21871	28729	12		
80	2	39	216	671	1511	11134	21761	27361	29858	18		
90	3	65	350	1044	2265	14110	24412	28604	29956	25		
100	5	103	531	1525	3188	16921	26331	29289	29987	32		
200	106	1552	5504	11135	16852	29207	29982	30000	30000	45		
400	489	6422	16666	24346	28058	29998	30000	30000	30000	na		
600	2502	16654	26584	29416	29923	30000	30000	30000	30000	na		
800	6434	24304	29410	29962	29998	30000	30000	30000	30000	na		

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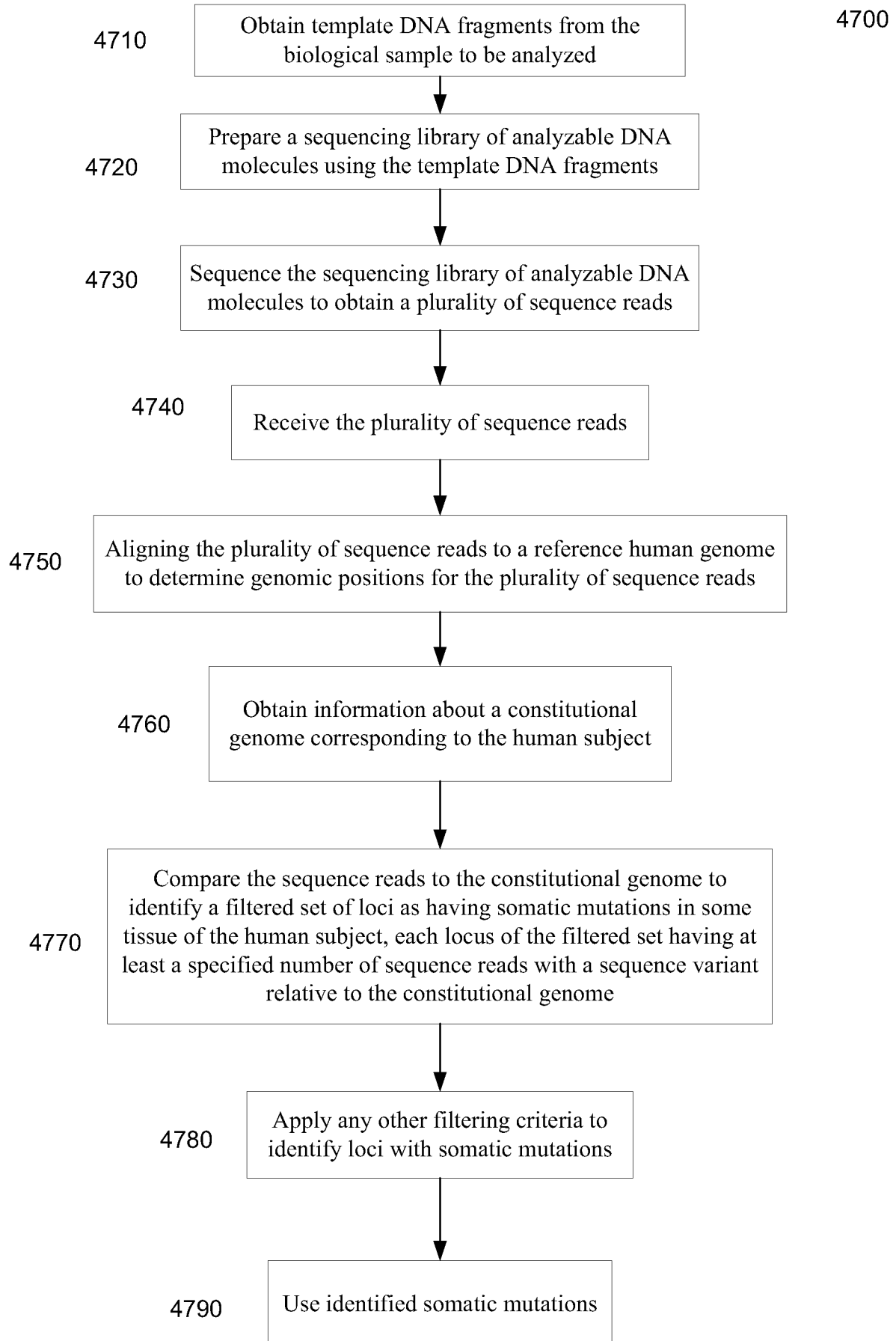


FIG. 47

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4800

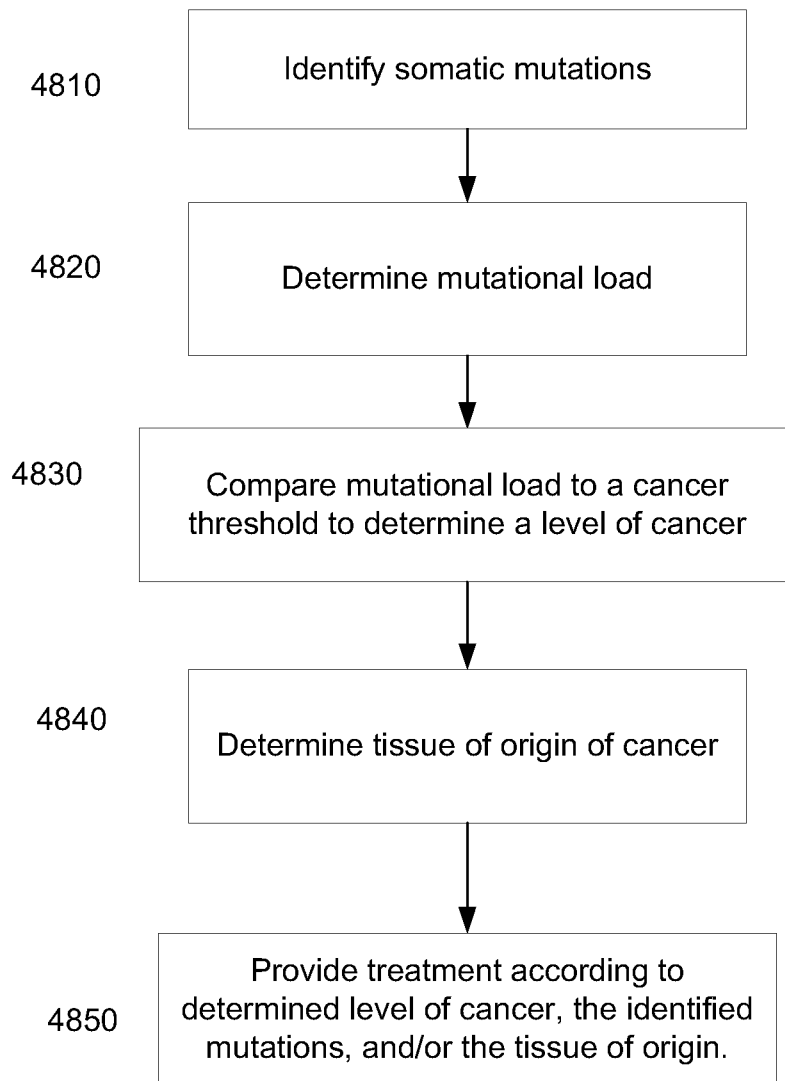


FIG. 48

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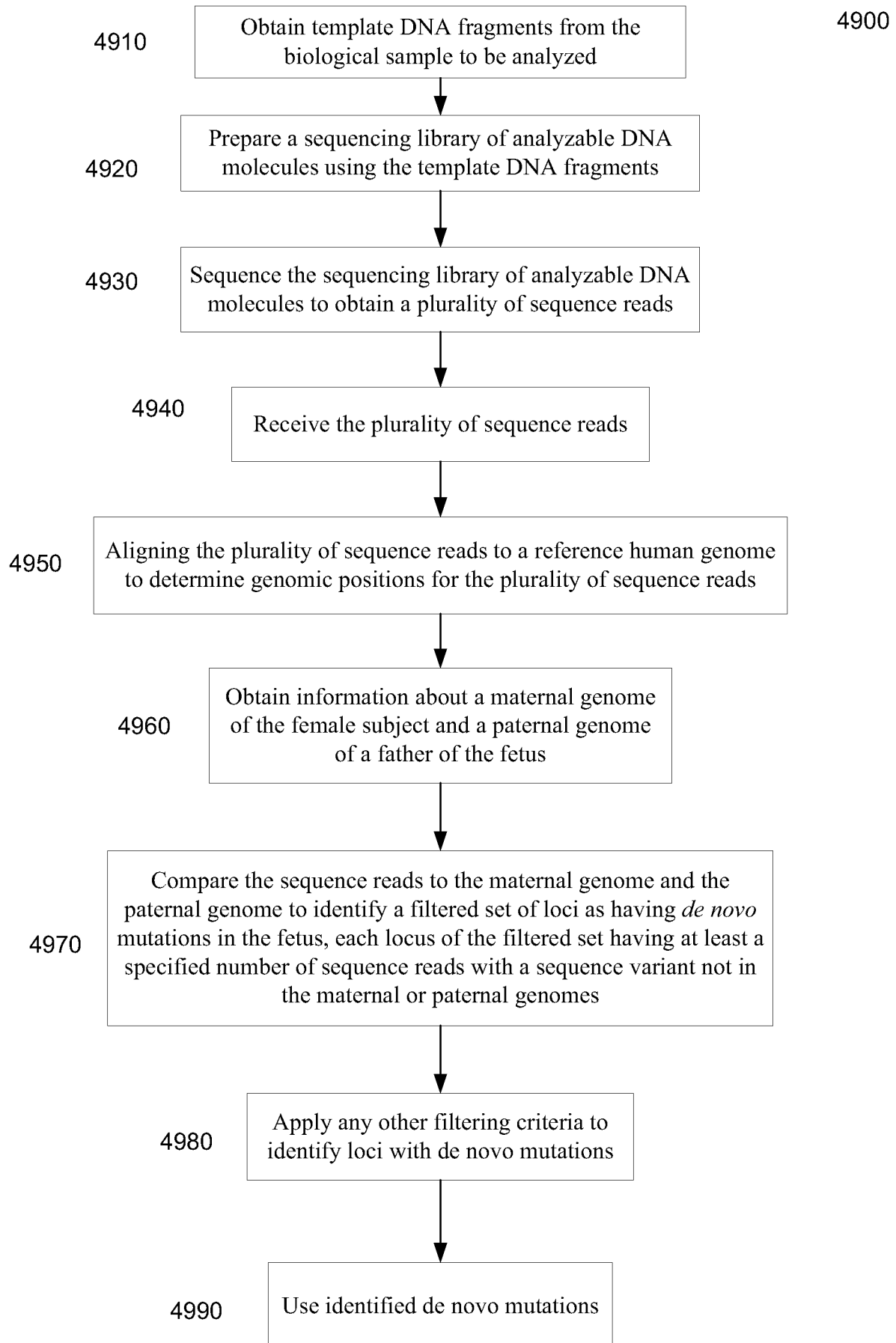


FIG. 49

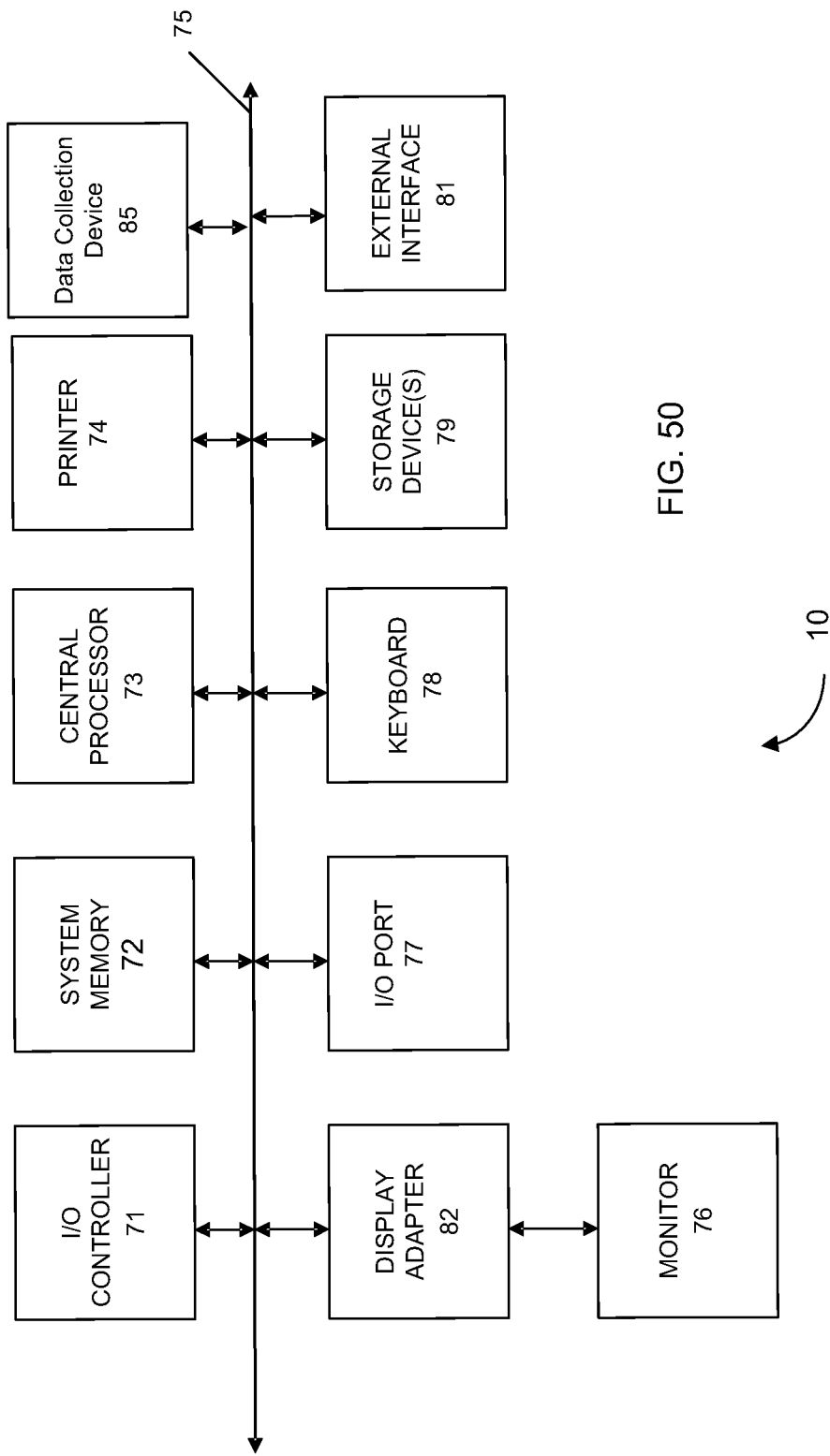


FIG. 50