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100

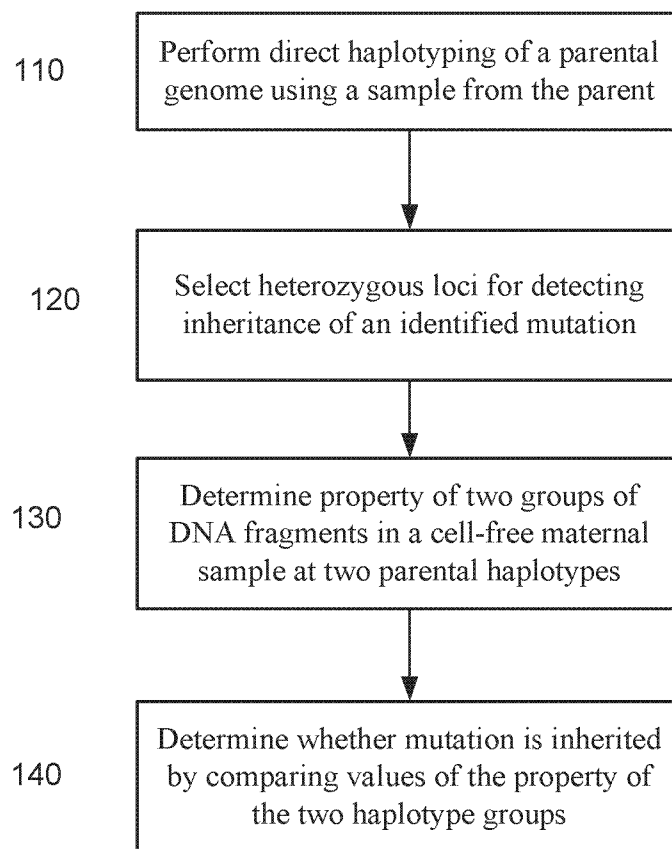


FIG. 1

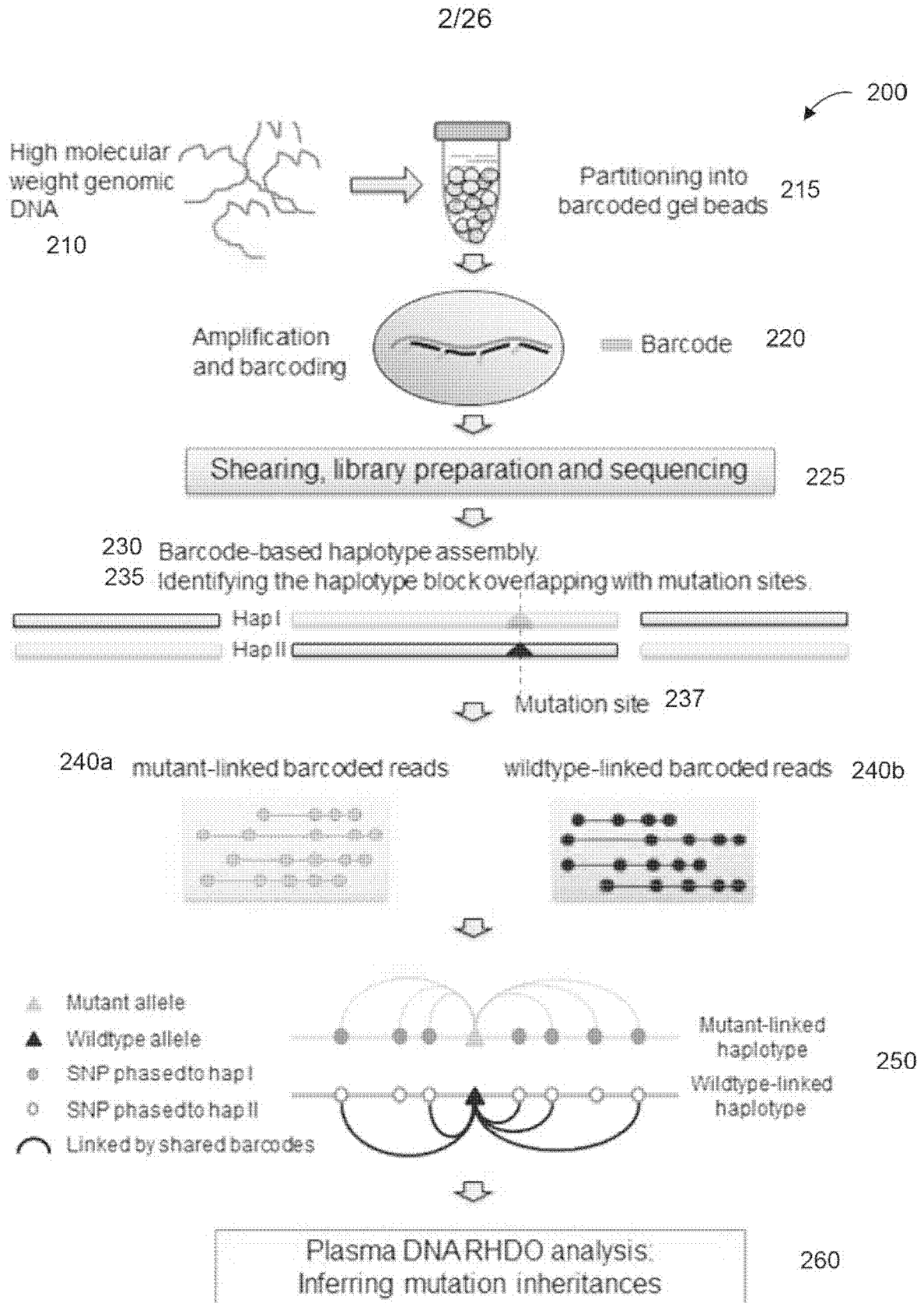


FIG. 2

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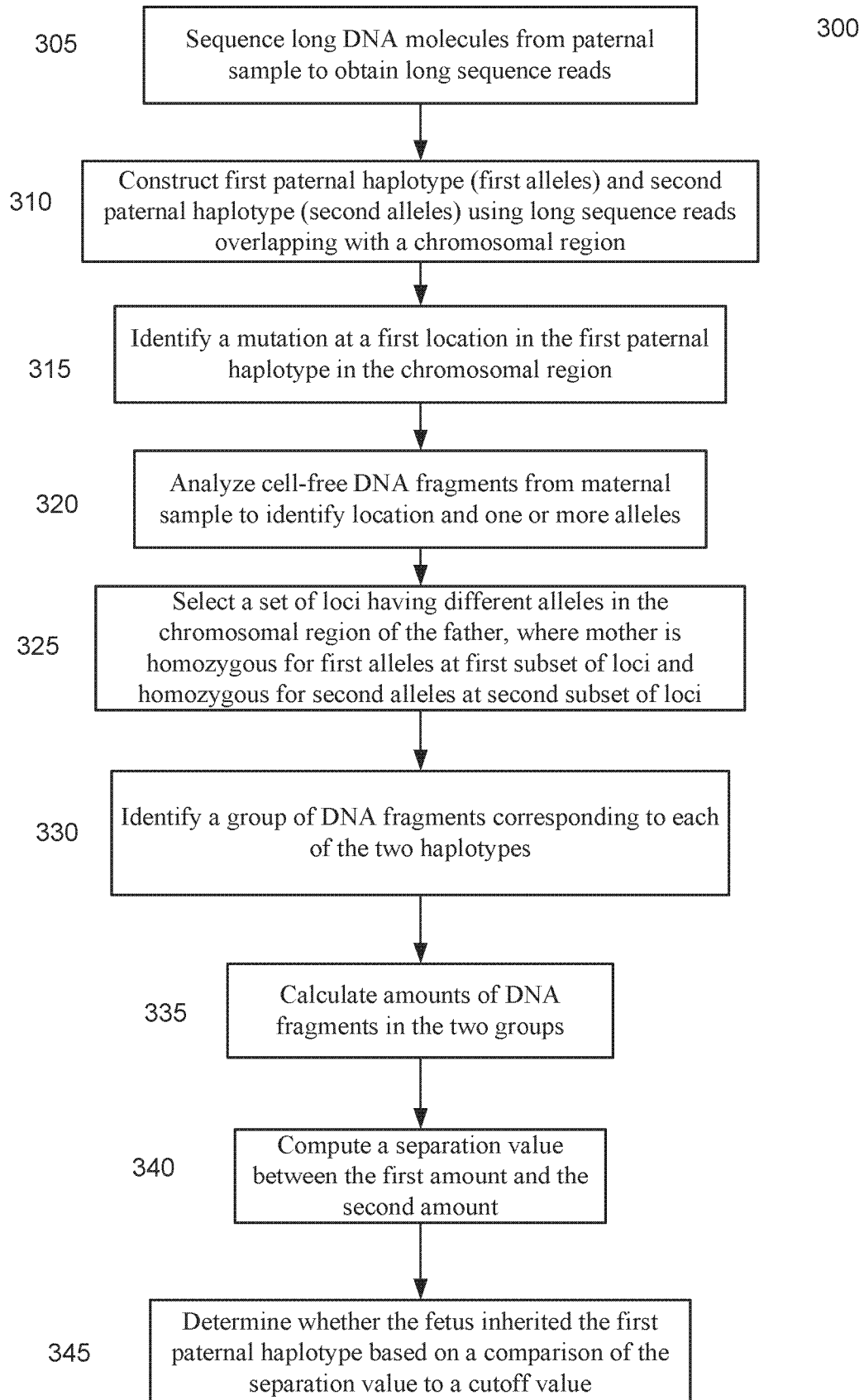


FIG. 3

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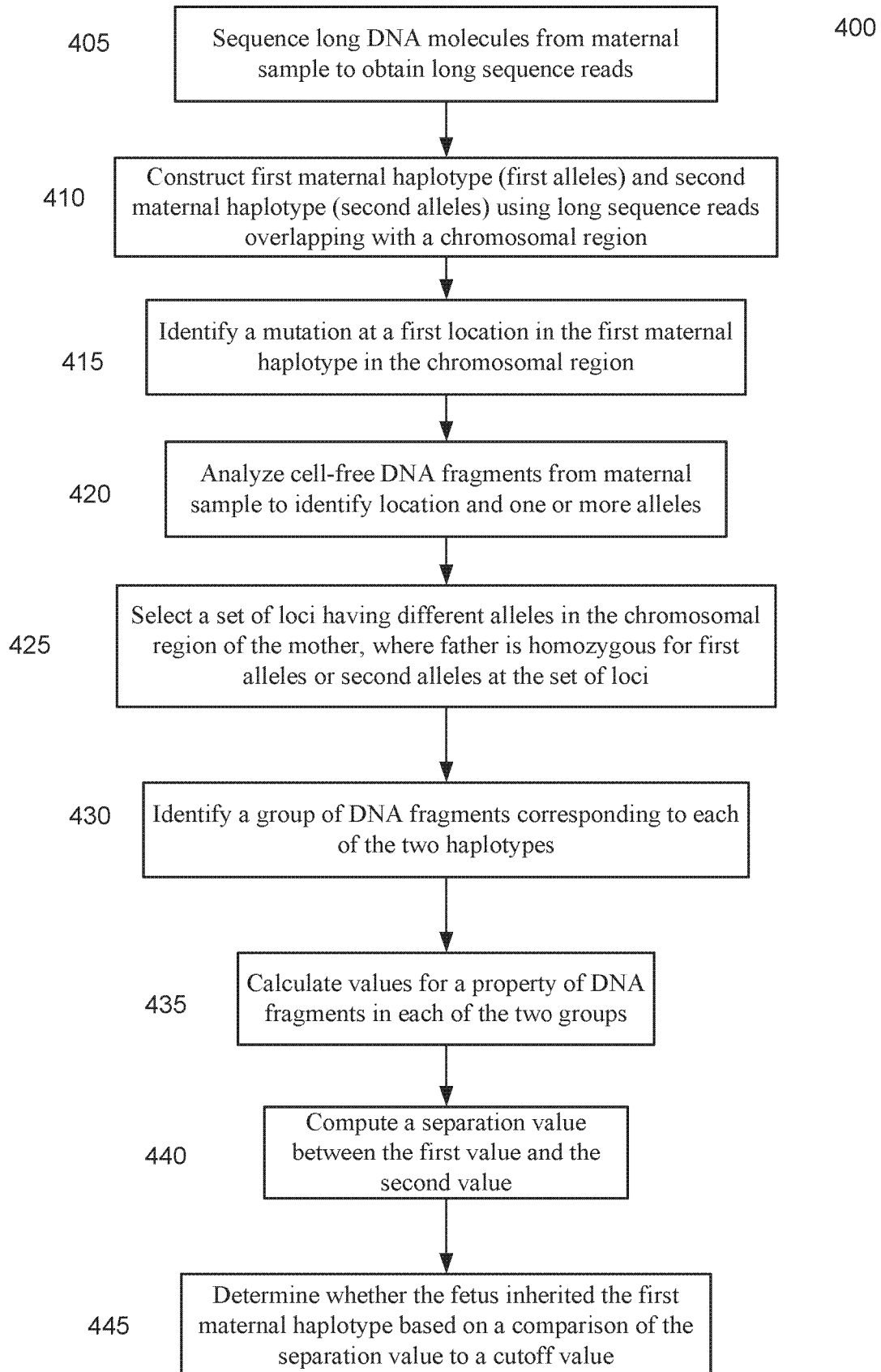


FIG. 4

500

Family	510 Diseases	515 Gene	Location of gene	Genotypes			Fetal sex	Gestation age (week)**	Fetal DNA concentration
				520	525	530			
				Mother	Father	Fetus			
A	CAH	CYP21A2	chr6	del/nl	Int2/nl	nl/nl	F	8 1/7	5.4
B	CAH	CYP21A2	chr6	Int2/nl	Ex3/nl	Ex3/Int2	M	11 1/7	8.8
C	CAH	CYP21A2	chr6	del/nl	del/nl	nl/nl	F	9 6/7	8.5
D	CAH	CYP21A2	chr6	del/nl	L307frameshift/ R356W	L307fs/del	F	8 2/7	15.3
E	Beta thalassaemia	HBB	chr11	c.316-197C>T/nl	c.126_129delC TTT/nl	c.316-197C>T/nl	F	17 3/7	4.7
F	EVC	EVC and EVC2	chr4	c.1006-12A>G/nl in EVC2 and c.83T>C/nl in EVC	c.871- 2_894del/nl in EVC2	c.1006- 12A>G/c.871- 2_894del in EVC2 and c.83T>C/nl in EVC	F	20 3/7	14.7
G	Hemophilia A	F8	chrX	c.1898T>G/nl	N/A	nl	M	21 4/7	23.1
H	Hemophilia A	F8	chrX	c.71A>G/nl	N/A	nl	M	34	11.8
I	Hemophilia A	F8	chrX	c.1292T>C/nl	N/A	c.1292T>C	M	31	12.3
J	Hemophilia B	F9	chrX	c.802T>A/nl	N/A	c.802T>A	M	21 2/7	22.2
K	Hemophilia B	F9	chrX	c.509G>A/nl	N/A	nl	M	30 6/7	10.1
L	Hemophilia B	F9	chrX	c.391+10T>G/nl	N/A	c.391+10T>G	M	2nd trimester	21.1
M	Hunter syndrome	IDS and IDS2	chrX	heterozygous IDS/IDS2 rearrangement	N/A	hemizygous IDS/IDS2 rearrangement	M	13 1/7	12.6

Abbreviations: del, 30-kb large gene deletion; int2, intron 2, c.293-13A/C>G; ex3, exon3, c.332_339del; nl, normal allele; CAH, congenital adrenal hyperplasia.

**At the time of blood sampling for analysis

FIG. 5

600

Family	Sample	N50 phase block (bases)	Mean molecular length (bases)	Multiplex	No. of reads	Mapped rate %	PCR dup %	On target %	Depth
A	Mother	2,880,842	60,895	8-plex	64,863,804	99.0	41.8	75.5	622
	Father	7,470,465	106,595	8-plex	63,281,470	99.1	29.4	75.1	602
B	Mother	7,459,275	102,611	8-plex	70,566,296	99.1	39.8	76.2	683
	Father	7,455,661	123,928	8-plex	65,683,570	99.1	42.6	76.4	638
C	Mother	3,138,312	81,422	8-plex	76,310,502	99.1	32.9	76.7	743
	Father	13,963,723	105,338	8-plex	67,566,714	99.0	48.5	76.7	660
D	Mother	7,500,966	121,627	8-plex	67,269,244	99.0	41.6	74.9	641
	Father	7,453,927	165,121	8-plex	60,721,154	99.0	48.2	75.0	580
E	Mother	6,878,088	116,477	1-plex	782,212,352	88.3	4.5	N/A	30
	Father	5,705,746	137,142	1-plex	878,723,404	82.1	15.8	N/A	30
F	Mother	5,145,348	110,618	1-plex	737,850,636	85.1	4.1	N/A	26
	Father	4,694,185	106,575	1-plex	1,123,528,532	86.2	23.9	N/A	42
G	Mother	7,121,693	138,606	1-plex	949,207,436	84.1	16.7	N/A	33
H	Mother	9,320,574	137,888	1-plex	839,556,770	84.6	6.1	N/A	30
I	Mother	6,006,613	126,482	1-plex	1,118,205,288	85.3	30.9	N/A	41
J	Mother	8,049,279	132,255	1-plex	1,107,144,338	85.6	16.1	N/A	41
K	Mother	11,443,836	143,945	1-plex	740,594,254	88.9	5.0	N/A	28
L	Mother	11,505,095	128,427	1-plex	942,377,736	88.0	5.8	N/A	36
M	Mother	7,030,562	110,503	1-plex	964,114,164	92.2	5.5	N/A	39

FIG. 6

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700

Family	Mapped reads	Nonduplicated reads	PCR dup %	Target region coverage (%)	On-target rate (%)	Average depth
A	59,841,409	44,880,799	25.0	89.4	69.2	528
B	37,612,242	27,842,484	26.0	98.8	68.2	314
C	54,943,416	40,050,033	27.1	91.3	65.7	441
D	46,246,188	32,079,412	30.6	97.6	67.9	356
E	235,265,694	147,172,043	37.4	98.5	73.7	404
F	215,802,580	149,984,115	30.5	95.4	79.4	130
G	195,908,989	85,876,725	56.2	100.0	75.3	208
H	152,375,354	84,375,318	44.6	100.0	71.4	196
I	88,575,975	56,744,814	35.9	100.0	77.0	145
J	176,991,146	84,324,564	52.4	100.0	75.4	217
K	140,555,505	90,453,108	35.7	100.0	71.4	225
L	161,995,418	104,159,888	35.7	100.0	71.3	260
M	68,173,450	55,720,431	18.3	99.9	71.3	151

FIG. 7

800				
Family	Sample	Phase block across target region	Length of phase block across target region (bases)	No of SNPs across region
A	Mother	chr6:27979631-32679591	4,699,960	4519
	Father	chr6:24958611-32429077	7,470,466	4631
B	Mother	chr6:24958611-32414273	7,455,662	4079
	Father	chr6:24985475-32414273	7,428,798	3595
C	Mother	chr6:24957224-38920455	13,963,231	9106
	Father	chr6:29293472-32431785	3,138,313	2774
D	Mother	chr6:24958611-32412539	7,453,928	4152
	Father	chr6:24957224-32456672	7,499,448	3891
E	Mother	chr11:3628799-12689249	9,060,450	9468
	Father	chr11:1868168-22588088	20,719,920	17163
F	Mother	chr4:3570130-8737592	5,167,462	5292
	Father	chr4:3671398-7773524	4,102,126	4274
G	Mother	chrX:153020246-154404181	1,383,935	448
H	Mother	chrX:154210627-155173951	963,324	23
I	Mother	chrX:147798372-155025884	7,227,512	3188
J	Mother	chrX:135540724-139774089	4,233,365	1163
K	Mother	chrX:134856212-153728057	18,871,845	8767
L	Mother	chrX:130630587-146943274	16,312,687	7354
M	Mother	chrX:147662589-152231103	4,568,514	1235

FIG. 8

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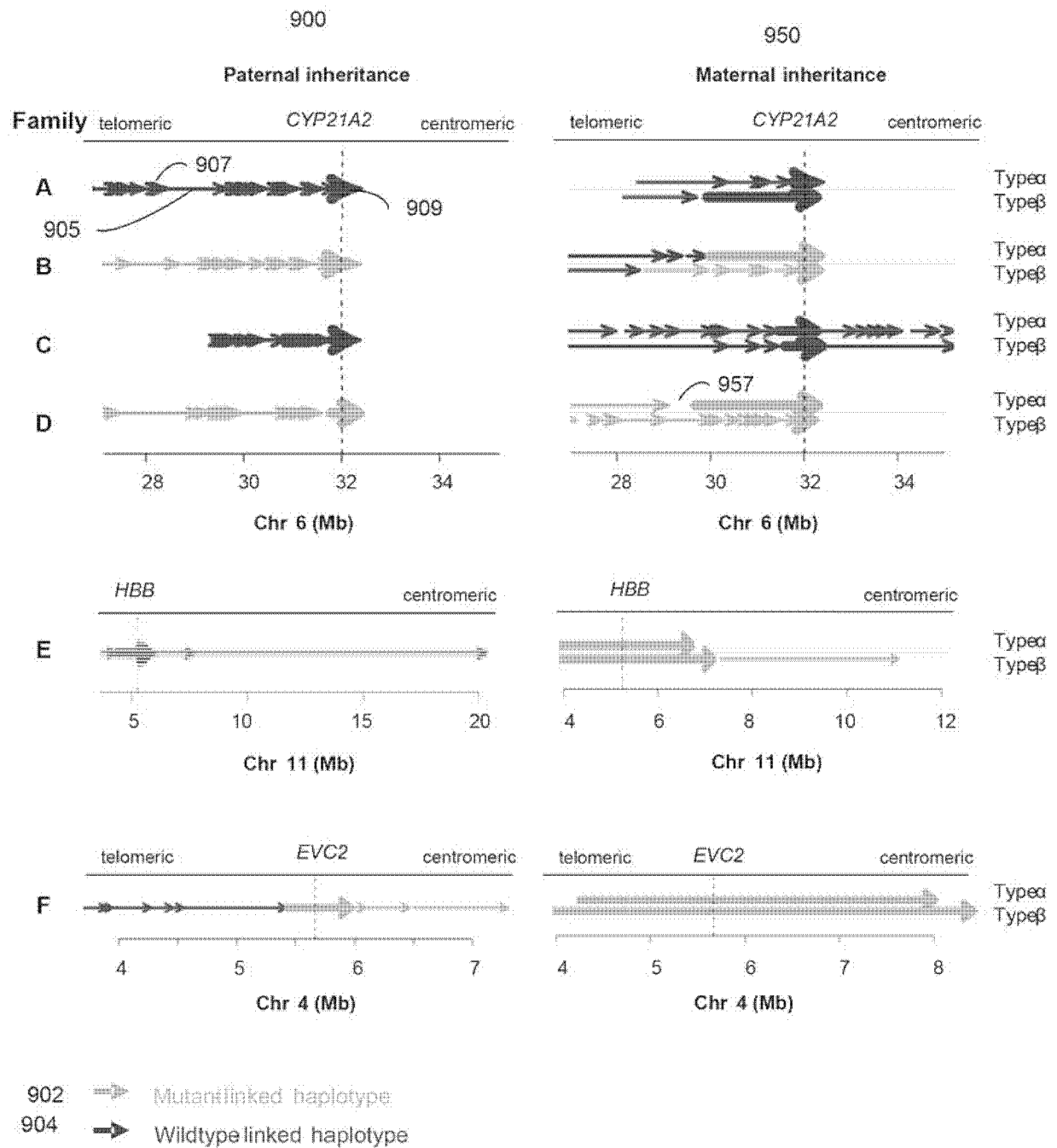


FIG. 9

FIG. 10A

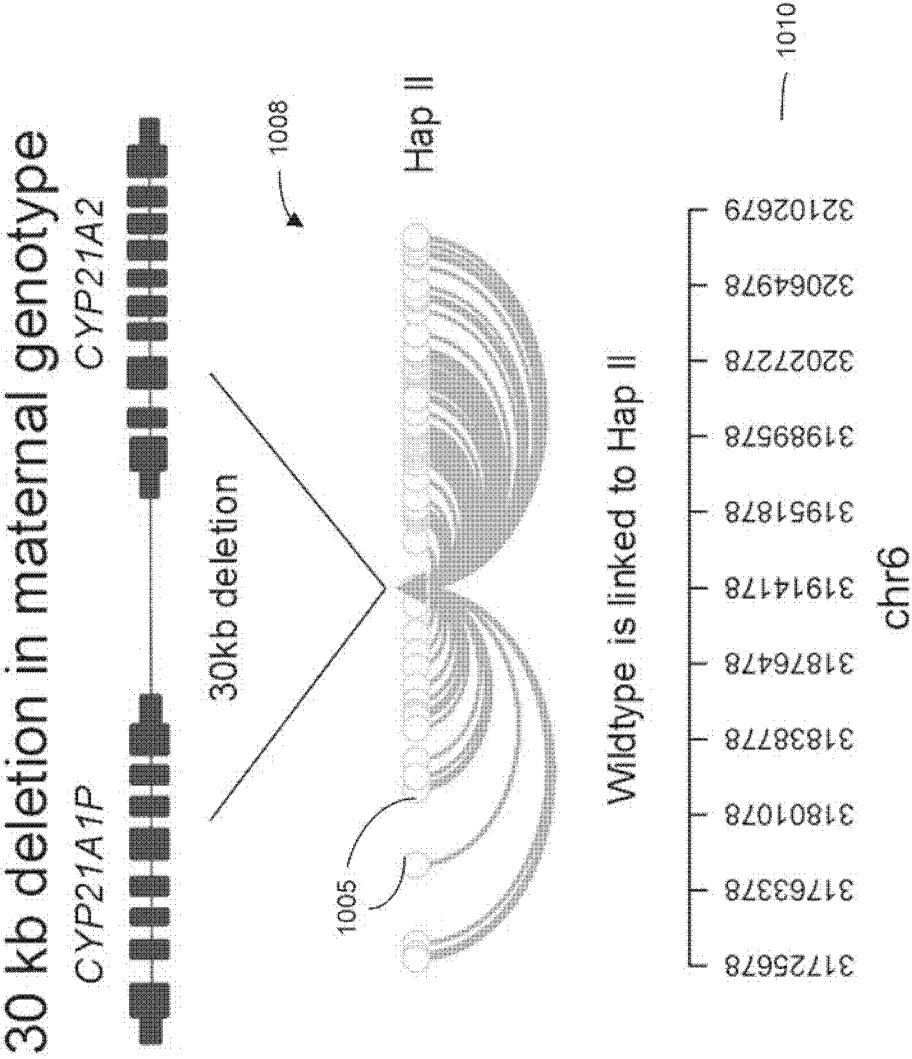
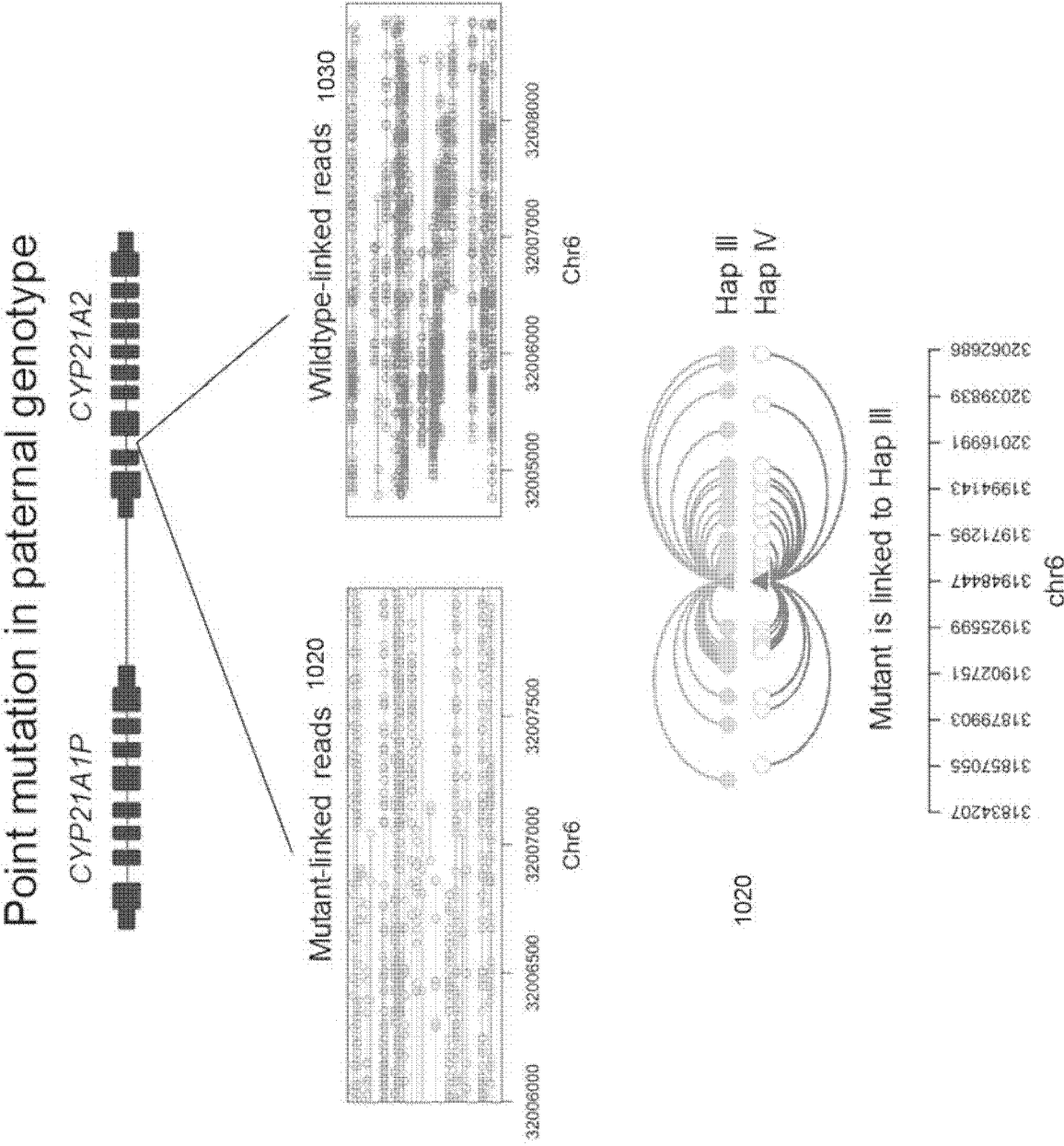


FIG. 10B



1100

Family	Gestation age (Week)**	Sequencing depth	Fetal DNA%	Paternal inheritance		Maternal inheritance			
				No. of paternal- specific SNPs used	No. of KS test***	Type α SNPs		Type β SNPs	
						No. of SNPs used	No. of SPRT classifications	No. of SNPs used	No. of SPRT classifications
A	8 1/7	528	5.4	2863	65	108	6	92	2
B	11 1/7	314	8.8	2152	35	197	6	123	9
C	9 6/7	441	8.5	1769	49	364	28	192	6
D	8 2/7	356	15.3	2211	51	49	3	221	18
E	17 3/7	404	4.7	6542	7	146	1	128	2
F	20 3/7	130	14.7	1667	9	23	1	40	2
G	21 4/7	208	23.1	N/A	N/A	6	1	N/A	N/A
H	34	196	11.8	N/A	N/A	Not enough read counts for classification		N/A	N/A
I	31	145	12.3	N/A	N/A	76	7	N/A	N/A
J	21 2/7	217	22.2	N/A	N/A	39	4	N/A	N/A
K	30 6/7	225	10.1	N/A	N/A	112	8	N/A	N/A
L	2nd trimester	260	21.1	N/A	N/A	222	22	N/A	N/A
M	13 1/7	151	12.6	N/A	N/A	42	2	N/A	N/A

**At the time of blood sampling for analysis

***The haplotype block was required to fit these criteria: the number of SNPs in test region ≥ 25 ; the cumulative difference between two haplotypes $>0.53\%$; and the p-value of the KS test <0.05 .

FIG. 11

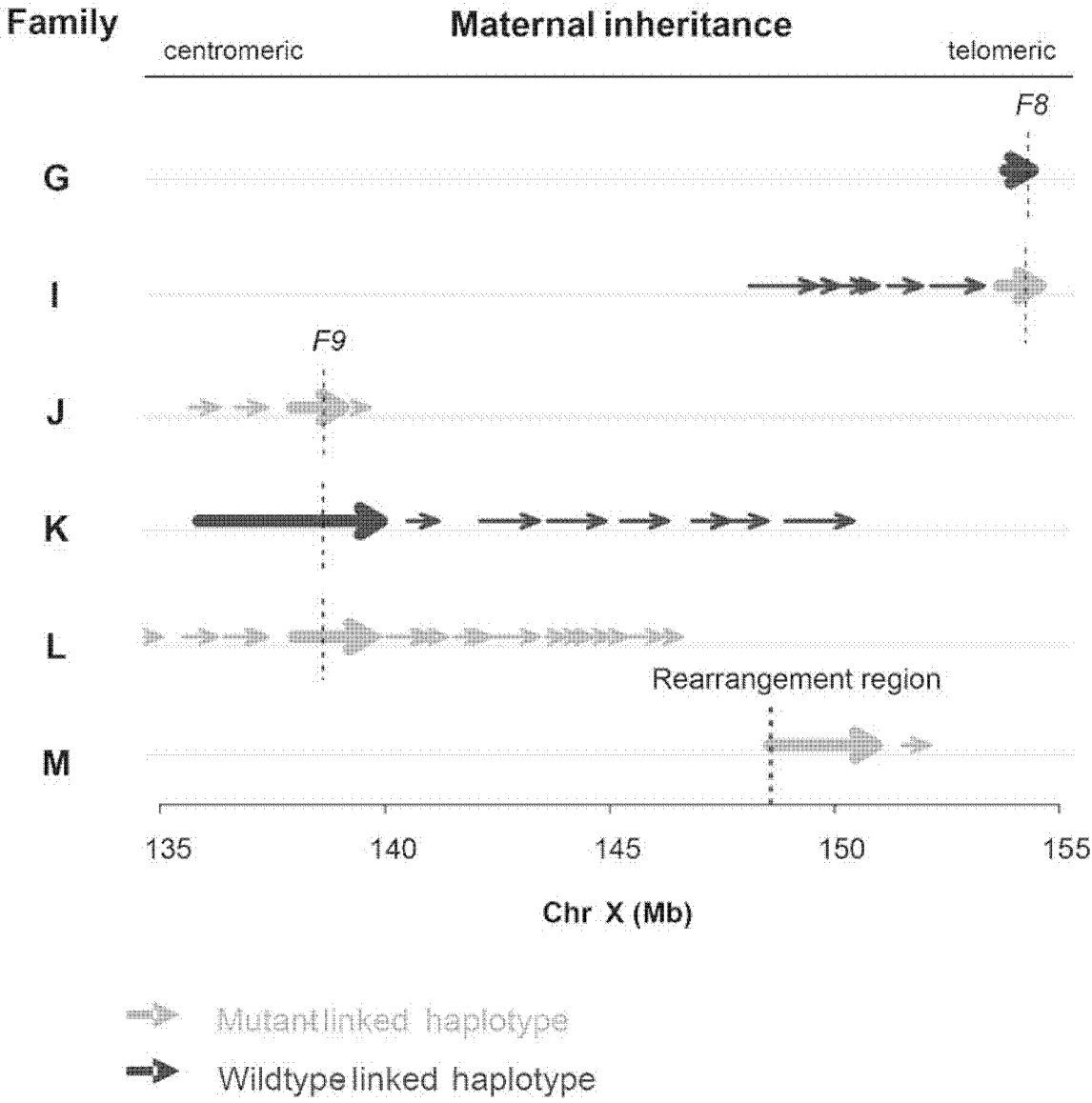
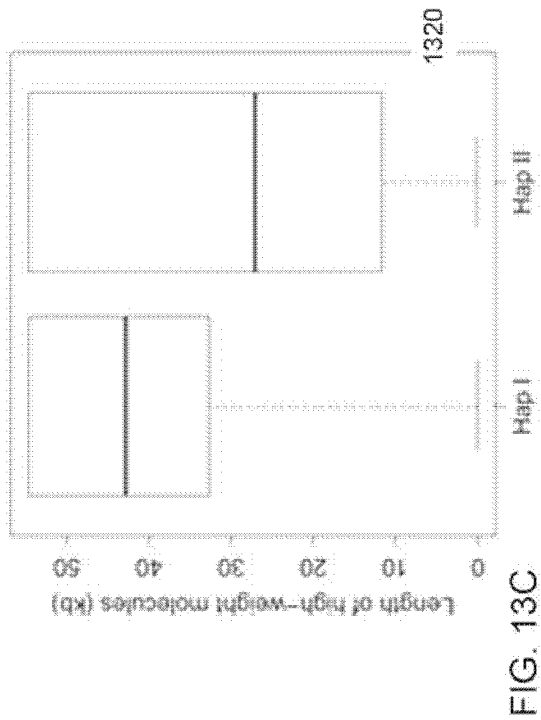
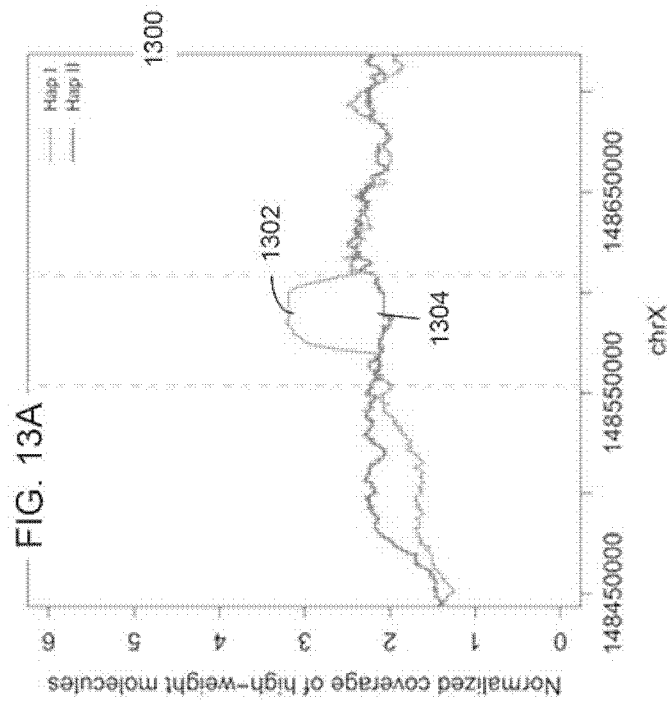
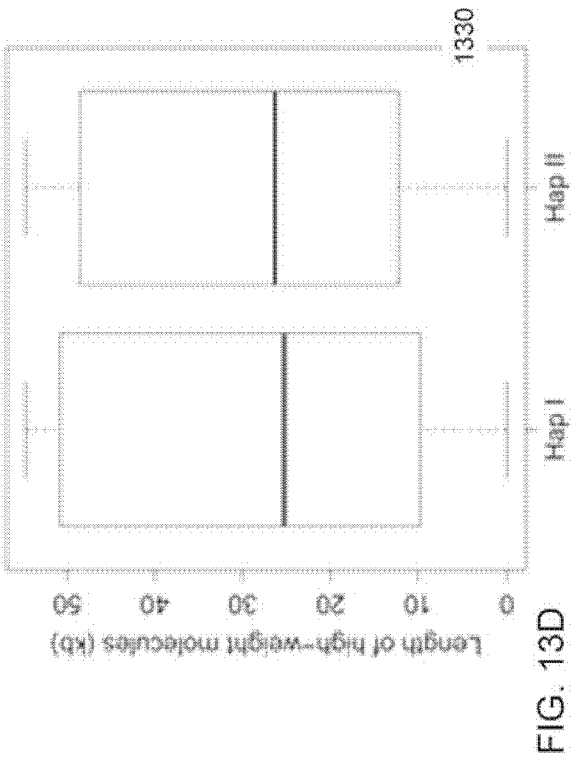
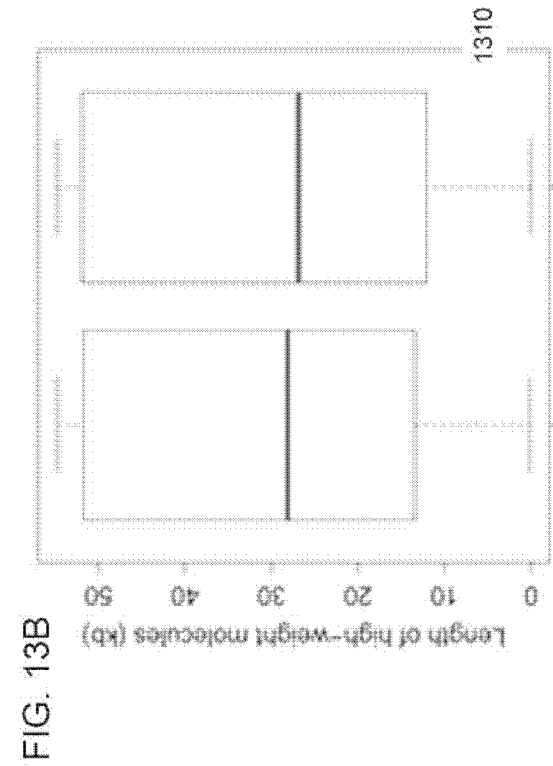


FIG. 12



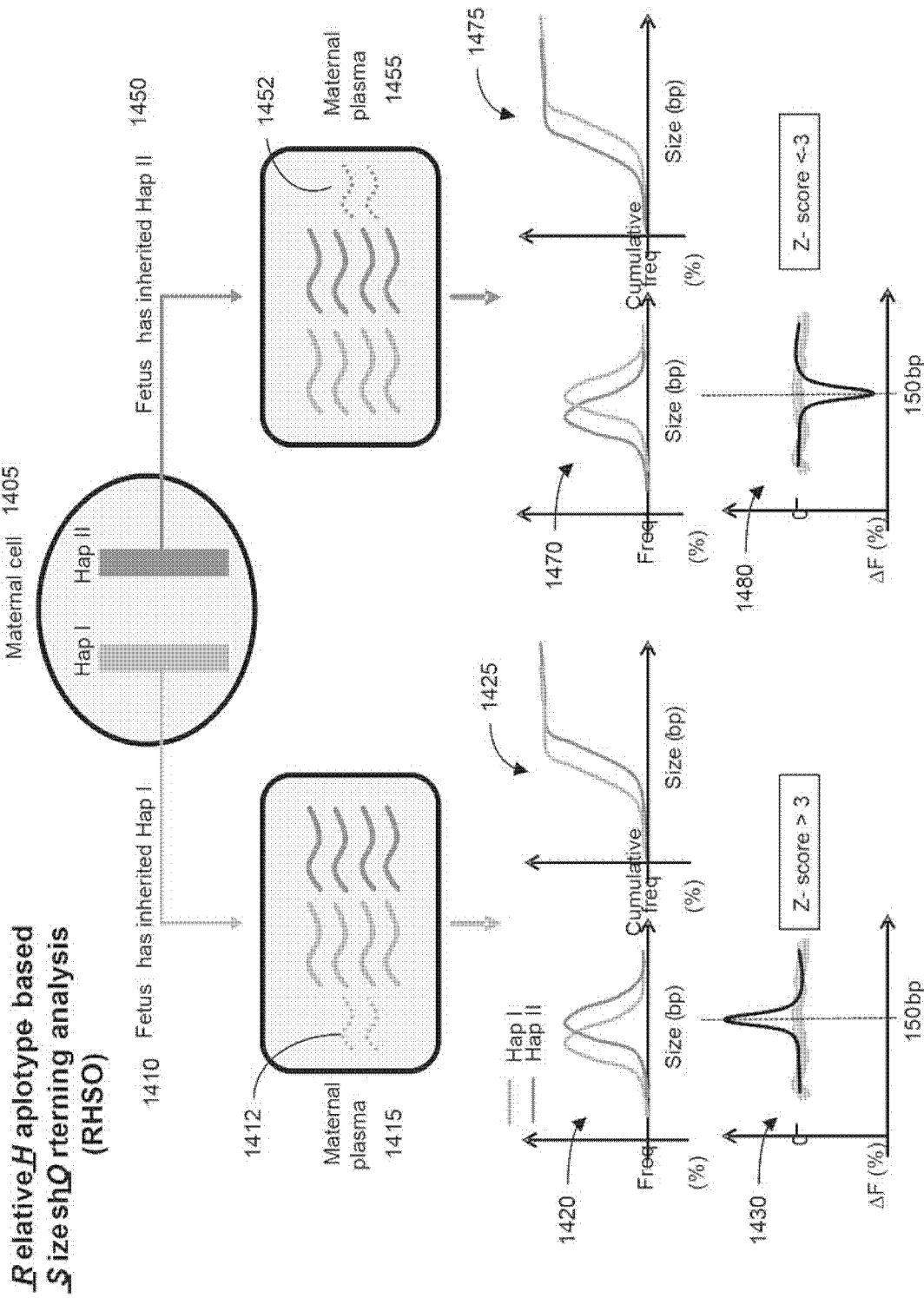


FIG. 14

FIG. 15A

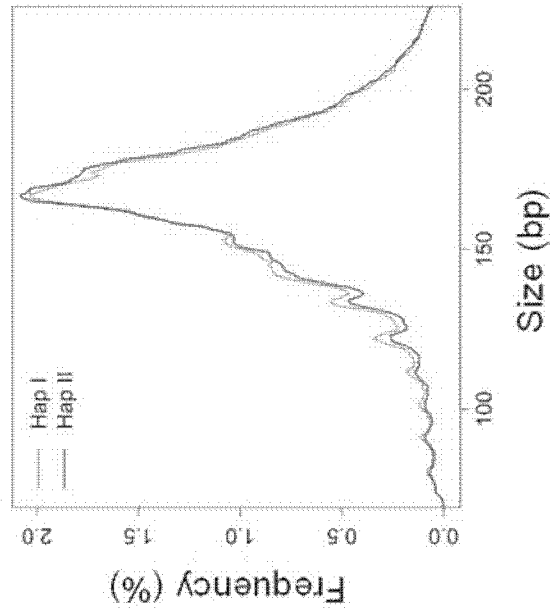


FIG. 15B

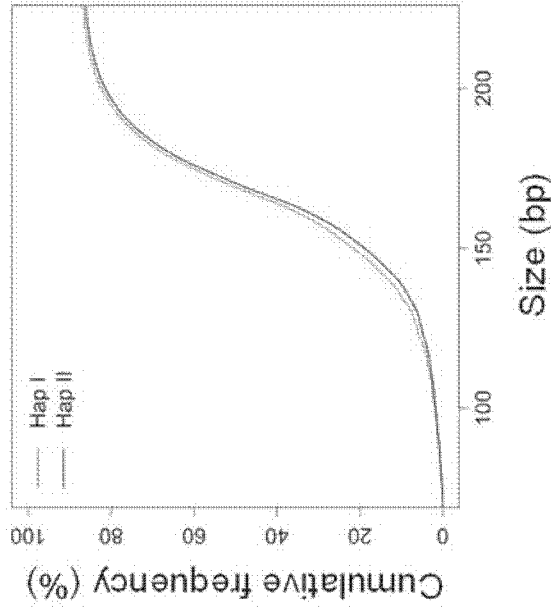
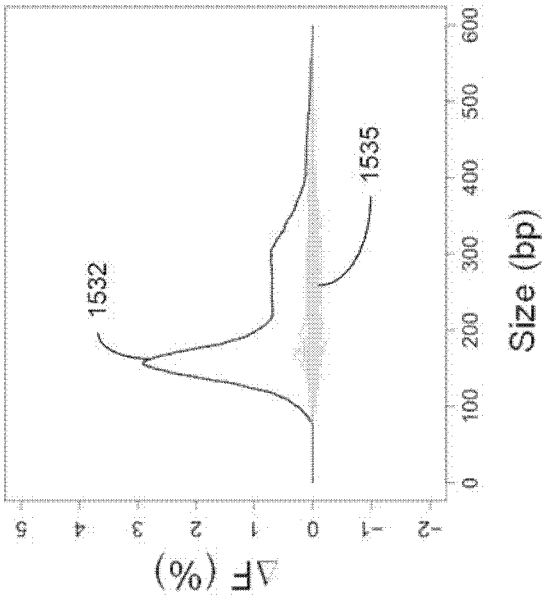


FIG. 15C



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1600

Sample	Diseases	Gene	Fetal sex	Fetal DNA fraction (%)	Maternal inheritance	No. of informative SNPs	No. of plasma DNA molecules	PRHDO ^(a) (z-score Zc)	RHSO ^(b) (z-score Zs)
HA1	Hemophilia A	<i>F8</i>	M	23.1	Wildtype	14	2063	Hap II (-3.68)	Hap II (-0.93) ^(c)
HA4	Hemophilia A	<i>F8</i>	M	11.8	Wildtype	1	176	Hap II (-0.75)	Hap II (-1.6)
HB1	Hemophilia B	<i>F9</i>	M	22.2	Mutant	71	12896	Hap I (7.43)	Hap I (7.43)
HB2	Hemophilia A	<i>F8</i>	M	12.3	Mutant	17	1347	Hap I (2.97)	Hap I (0.85)
HB3	Hemophilia B	<i>F9</i>	M	10.1	Wildtype	229	26234	Hap II (-16.44)	Hap II (-9.69)
HB4	Hemophilia B	<i>F9</i>	M	21.1	Mutant	332	54709	Hap I (25.68)	Hap I (8.72)
M12418	Hunter syndrome	<i>IDS</i> and <i>IDS2</i>	M	12.6	Mutant	56	7941	Hap I (3.98)	Hap I (1.88)
M11678	EVC	<i>EVC</i> and <i>EVC2</i>	F	14.7	Mutant	247	24151	Hap I (5.57)	Hap I (7.02)
M12426	Beta thalassemia	<i>HBB</i>	F	4.7	Mutant	1002	258112	Hap I (4.83)	Hap I (7.15)
M10623	CAH	<i>CYP21A2</i>	F	5.4	Wildtype	1808	1083765	Hap II (-29.92)	Hap II (-8.16)
MP21	CAH	<i>CYP21A2</i>	M	8.8	Mutant	2351	834123	Hap I (13.14)	Hap I (7.46)
MP29	CAH	<i>CYP21A2</i>	F	8.5	Wildtype	4238	2016139	Hap II (-10.01)	Hap II (-22.15)
MP4	CAH	<i>CYP21A2</i>	F	15.3	Mutant	2239	894660	Hap I (11.03)	Hap II (-0.82)
MP1	CAH	<i>CYP21A2</i>	M	4.8	Wildtype	883	65787	Hap I (0.03)	Hap II (-0.39)
MP5	CAH	<i>CYP21A2</i>	F	15.6	Wildtype	1086	139305	Hap II (-6.49)	Hap II (-4.52)
MP10	CAH	<i>CYP21A2</i>	F	13.5	Wildtype	1124	65739	Hap II (-7.18)	Hap II (-7.33)
MP14	CAH	<i>CYP21A2</i>	M	7.8	Mutant	1518	37782	Hap I (5.81)	Hap I (6.1)
MP16	CAH	<i>CYP21A2</i>	F	22.3	Mutant	2113	187945	Hap I (17.74)	Hap I (12)
MP24	CAH	<i>CYP21A2</i>	M	7.7	Mutant	2622	937556	Hap I (26.99)	Hap I (19.45)
MP26	CAH	<i>CYP21A2</i>	F	17.6	Mutant	3239	1183693	Hap I (49.24)	Hap I (26.07)
MP27	CAH	<i>CYP21A2</i>	F	3.8	Mutant	3666	1134355	Hap I (14.82)	Hap I (5.31)
MP31	CAH	<i>CYP21A2</i>	F	11.5	Mutant	3713	1165731	Hap I (39.5)	Hap I (39.44)
MP9	CAH	<i>CYP21A2</i>	M	3.4	Wildtype	3072	908740	Hap II (-6.65)	Hap II (-3.95)
MP17	CAH	<i>CYP21A2</i>	F	3.7	Mutant	3287	486513	Hap I (12.72)	Hap I (10.71)
MP32	CAH	<i>CYP21A2</i>	M	8.6	Mutant	3799	385605	Hap I (25.4)	Hap I (21.12)
MP3	CAH	<i>CYP21A2</i>	F	1.4	Wildtype	1051	48149	Hap I (0.47)	Hap I (0.95)
MP30	CAH	<i>CYP21A2</i>	F	9	Mutant	2265	803944	Hap I (13.25)	Hap I (11.03)

(a) PRHDO: Poisson-based RHDO analysis without the paternal genotypes where RHDO stands for relative haplotype dosage analysis.

(b) RHSO: Relative haplotype-based size shortening analysis.

(c) The cells in shaded yellow represents those samples whose maternal inheritance are not able to be determined by PRHDO or RHSO.

FIG. 16

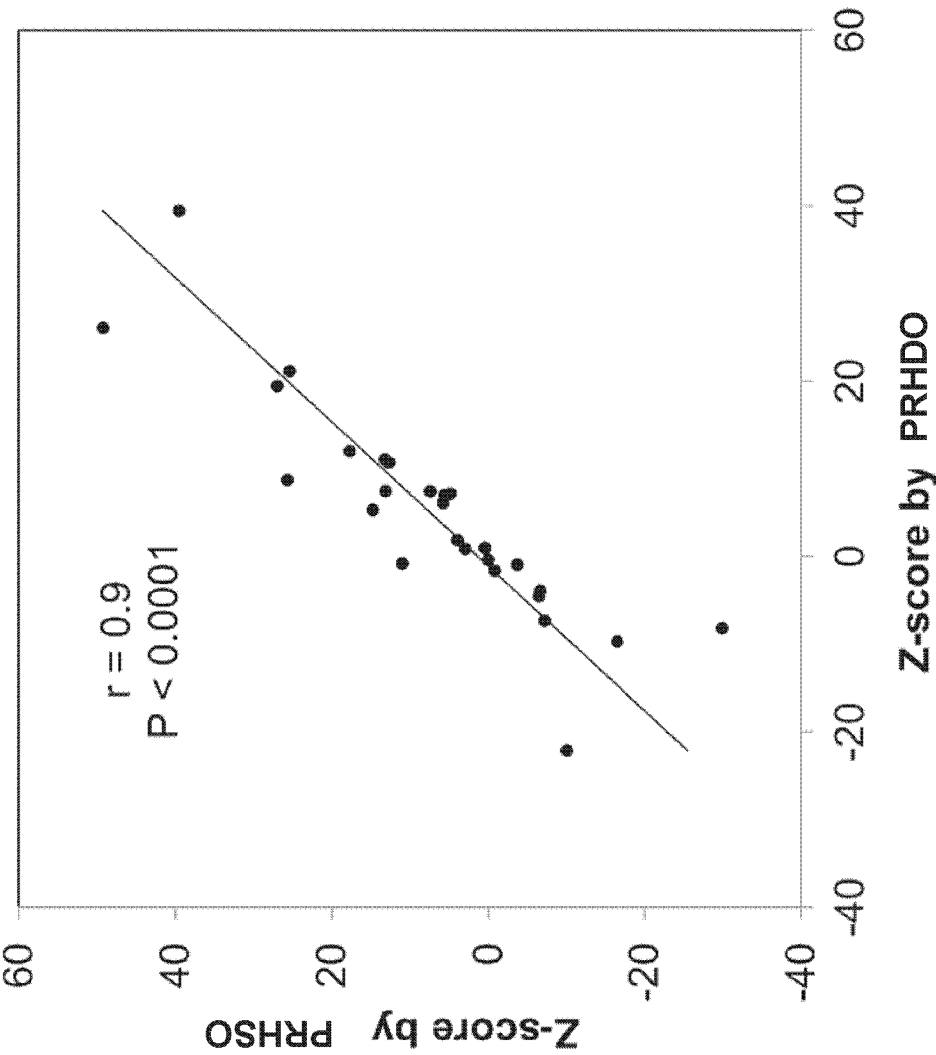


FIG. 17

FIG. 18B

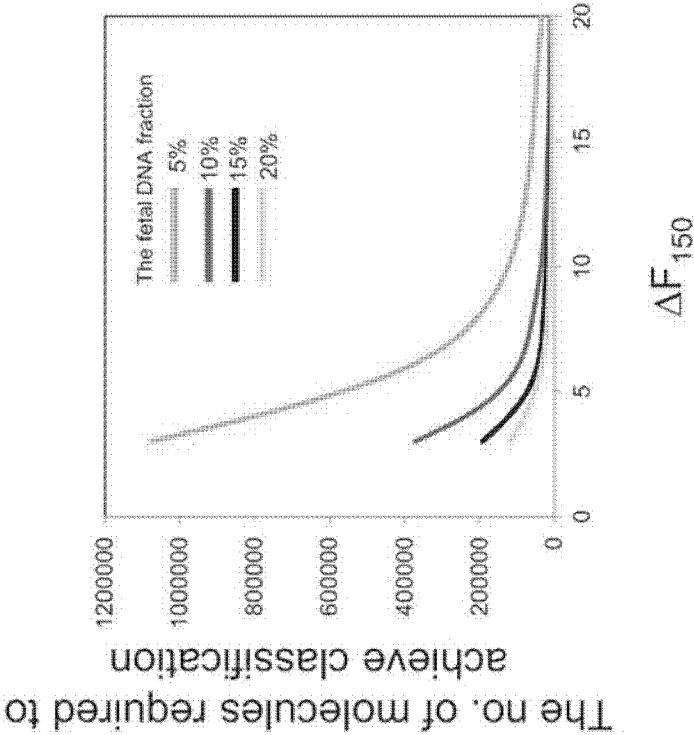
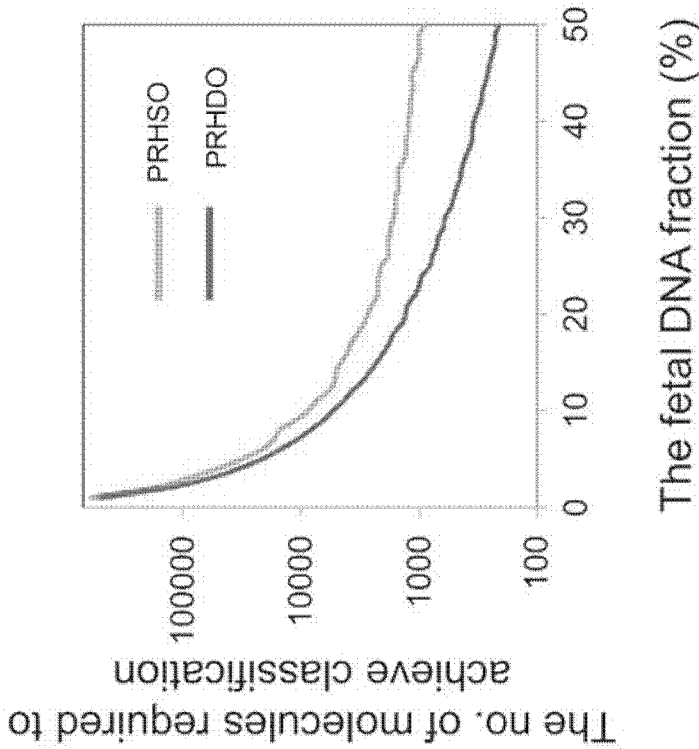
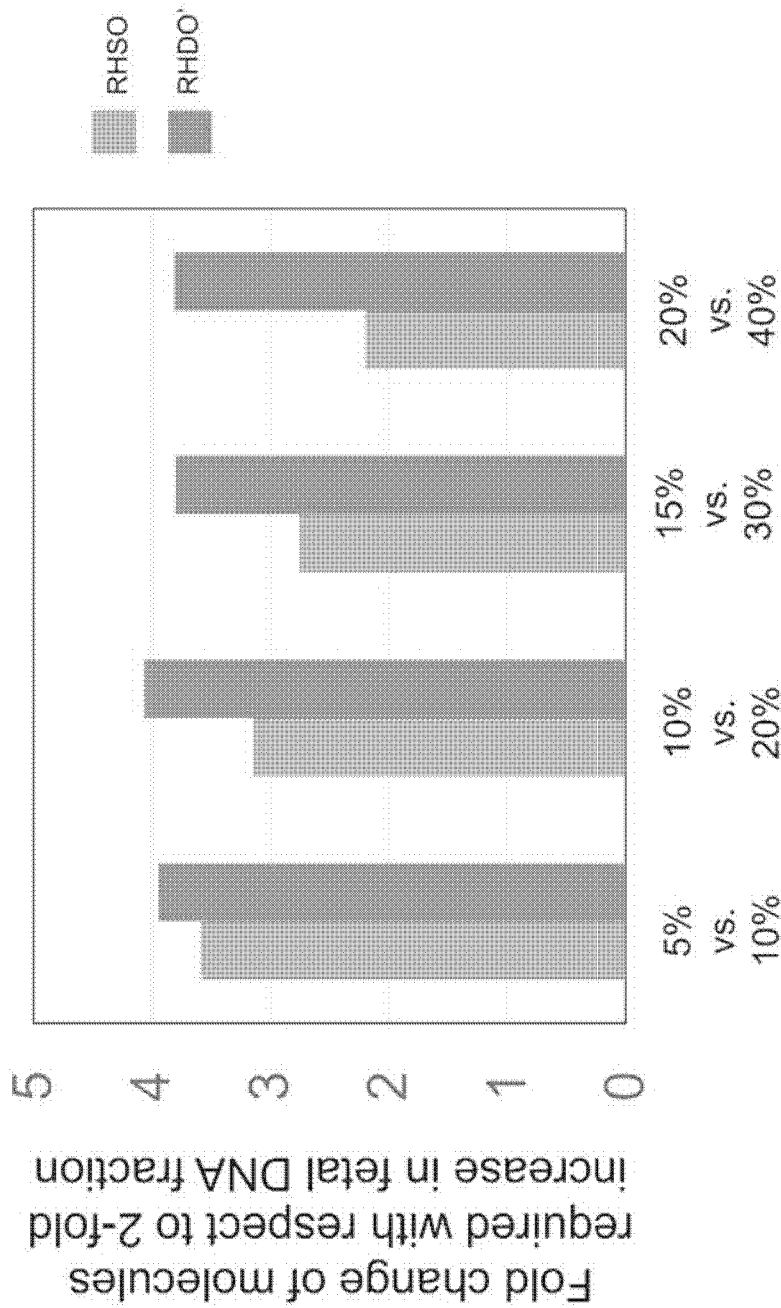


FIG. 18A





Fetal DNA fraction changes

FIG. 19

Sample	Diseases	Gene	Fetal sex	Fetal DNA fraction (%)	Maternal inheritance	No. of informative SNPs	No. of plasma DNA molecules	PRHDO ^(a) (z-score)	PRHSO ^(b) (z-score)	No. of molecules required for RHDO* by simulation	No. of molecules required for RHDO by simulation
HA1	Hemophilia A	F8	M	23.1	Wildtype	14	2063	Hap II (-3.68)	Hap II (-0.93) ^(c)	1001	5200
HA4	Hemophilia A	F8	M	11.8	Wildtype	1	176	Hap II (-0.75)	Hap II (-1.6)	3744	19000
HB1	Hemophilia B	F9	M	22.2	Mutant	71	12896	Hap I (7.43)	Hap I (7.43)	1082	6000
HB2	Hemophilia A	F8	M	12.3	Mutant	17	1347	Hap I (2.97)	Hap I (0.85)	3744	18000
HB3	Hemophilia B	F9	M	10.1	Wildtype	229	26234	Hap II (-16.44)	Hap II (-9.69)	5312	26000
HB4	Hemophilia B	F9	M	21.1	Mutant	332	54709	Hap I (25.68)	Hap I (8.72)	1245	6400
M12418	Hunter syndrome	IDS and IDS2	M	12.6	Mutant	56	7941	Hap I (3.98)	Hap I (1.88)	3744	17500
M11678	FVC	FVC and EVC2	F	14.7	Mutant	247	24151	Hap I (5.57)	Hap I (7.02)	2634	5050
M12426	Beta thalassemia	HBB	F	4.7	Mutant	1002	258112	Hap I (4.83)	Hap I (7.15)	20958	31000
M10623	CAH	CYP21A2	F	5.4	Wildtype	1808	1083765	Hap II (-29.92)	Hap II (-8.16)	20958	31000
MP21	CAH	CYP21A2	M	8.8	Mutant	2351	834123	Hap I (13.14)	Hap I (7.46)	6463	11000
MP29	CAH	CYP21A2	F	8.5	Wildtype	4238	2016139	Hap II (-10.01)	Hap II (-22.15)	6463	11000
MP4	CAH	CYP21A2	F	15.3	Mutant	2239	894660	Hap I (11.03)	Hap I (-0.82)	2300	1700000
MP1	CAH	CYP21A2	M	4.8	Wildtype	883	65787	Hap I (0.03)	Hap II (-0.89)	20958	31000
MP5	CAH	CYP21A2	F	15.6	Wildtype	1086	139305	Hap II (-6.49)	Hap II (-4.52)	14803	21000
MP10	CAH	CYP21A2	F	13.5	Wildtype	1124	65739	Hap II (-7.18)	Hap II (-7.33)	3074	5150
MP14	CAH	CYP21A2	M	7.8	Mutant	1518	37782	Hap I (5.81)	Hap I (6.1)	10698	17000
MP16	CAH	CYP21A2	F	22.3	Mutant	2113	187945	Hap I (17.74)	Hap I (12)	1082	2250
MP24	CAH	CYP21A2	M	7.7	Mutant	2622	937556	Hap I (26.99)	Hap I (19.45)	10698	17000
MP26	CAH	CYP21A2	F	17.6	Mutant	3239	1183693	Hap I (49.24)	Hap I (26.07)	1788	3750
MP27	CAH	CYP21A2	F	3.8	Mutant	3666	1134355	Hap I (14.82)	Hap I (5.31)	58178	90000
MP31	CAH	CYP21A2	F	11.5	Mutant	3713	1165731	Hap I (39.5)	Hap I (39.44)	4308	7350
MP9	CAH	CYP21A2	M	3.4	Wildtype	3072	908740	Hap II (-6.65)	Hap II (-3.95)	58178	90000
MP17	CAH	CYP21A2	F	3.7	Mutant	3287	486513	Hap I (12.72)	Hap I (10.71)	58178	90000
MP32	CAH	CYP21A2	M	8.6	Mutant	3799	385605	Hap I (25.4)	Hap I (21.12)	8223	15000
MP3	CAH	CYP21A2	F	1.4	Wildtype	1051	48149	Hap I (0.47)	Hap I (0.95)	129028	170000
MP30	CAH	CYP21A2	F	9	Mutant	2265	803944	Hap I (13.25)	Hap I (11.03)	6463	11000

(a) PRHDO: Paternal-free RHDO analysis without the paternal genotypes where RHDO stands for relative haplotype dosage analysis.

(b) PRHSO: paternal-free relative haplotype-based size shortening analysis.

(c) The cells in shaded yellow represents those samples whose maternal inheritance are not able to be determined by PRHDO or PRHSO.

2000

FIG. 20

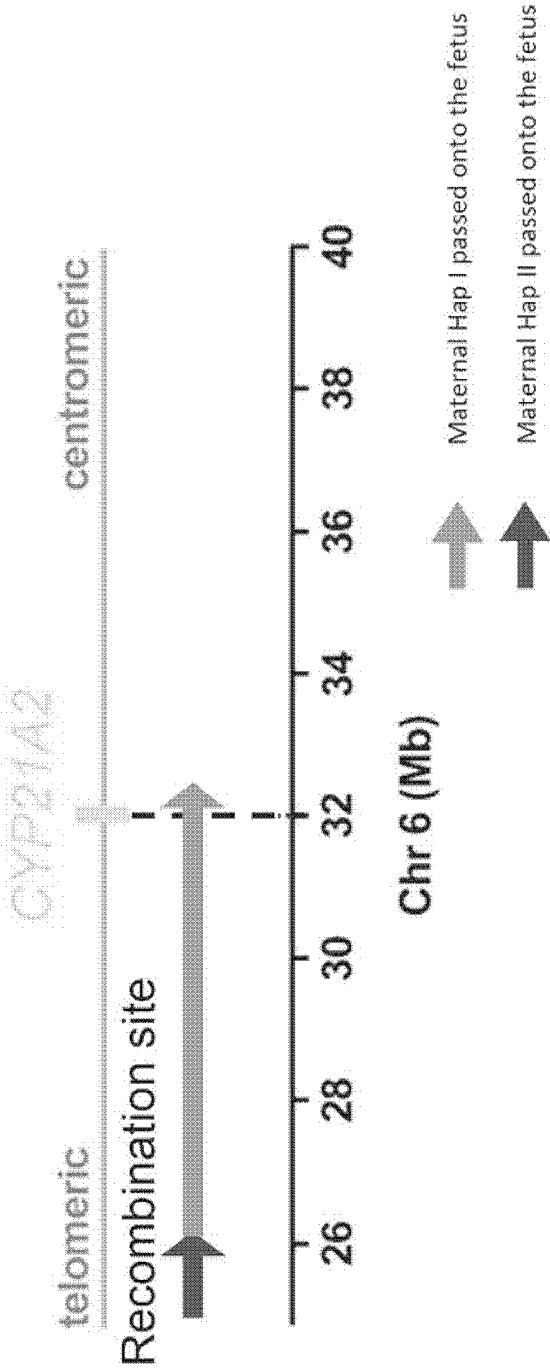


FIG. 21

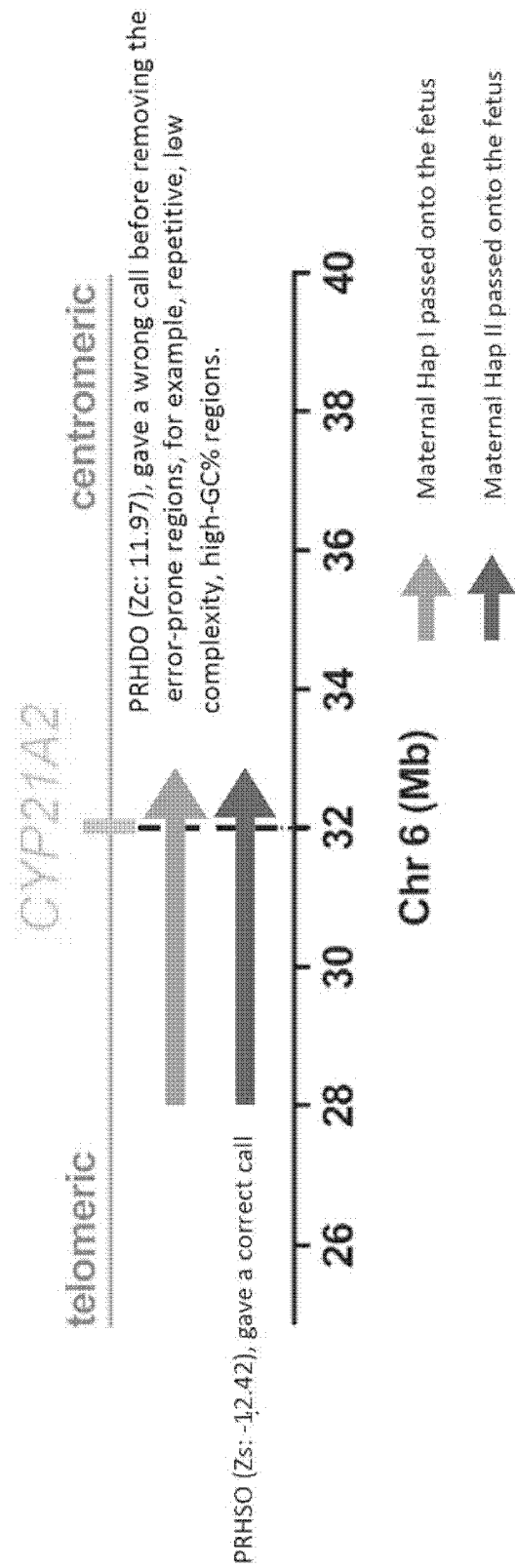


FIG. 22

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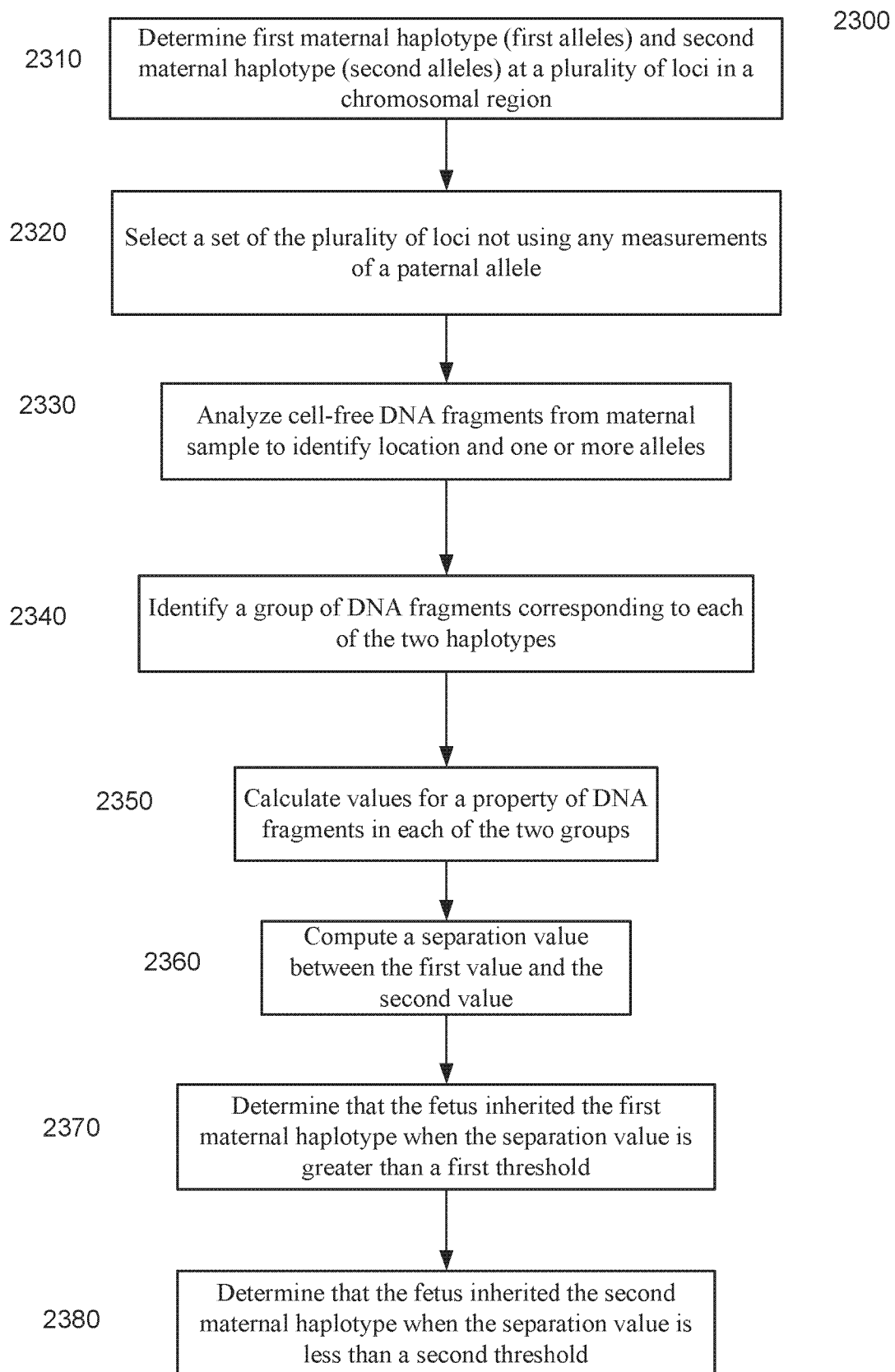


FIG. 23

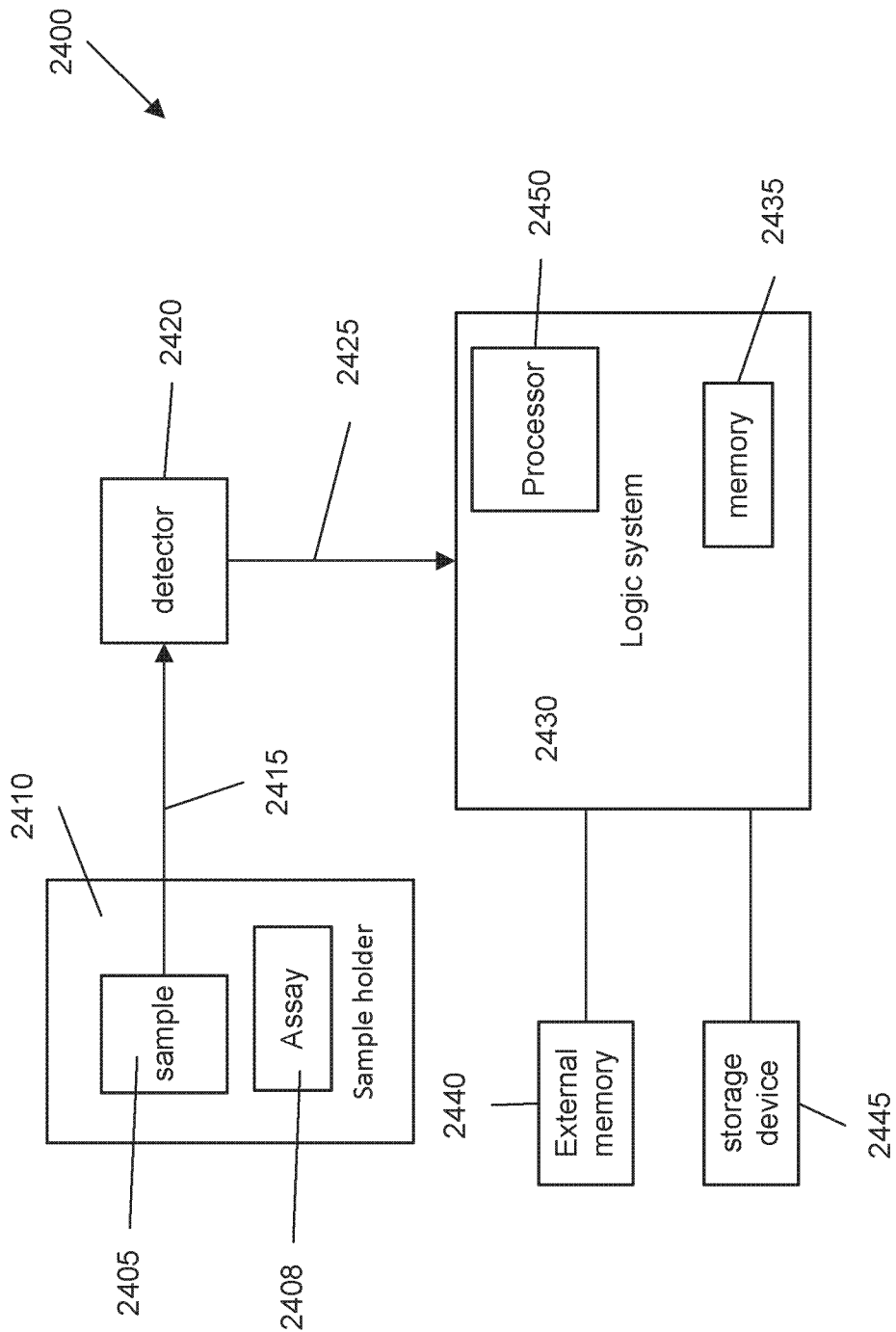


FIG. 24

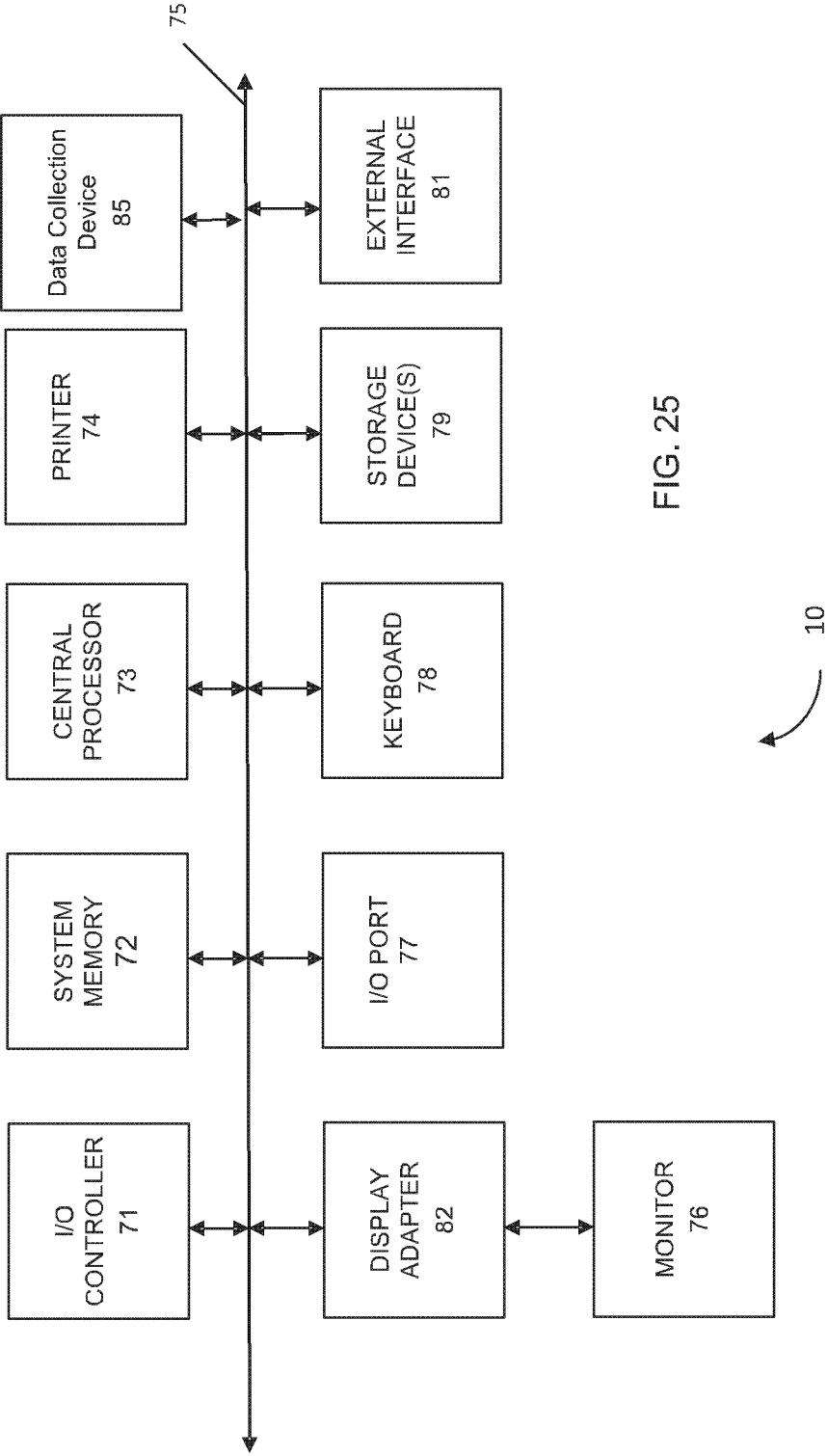


FIG. 25