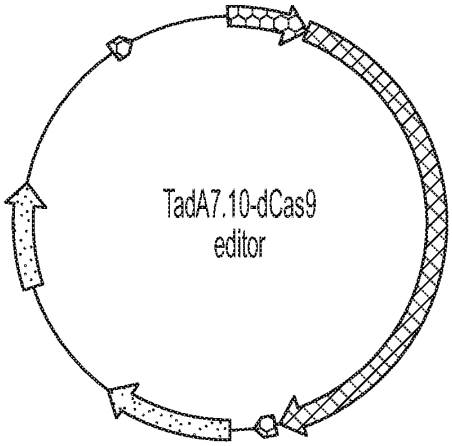


FIG. 1A

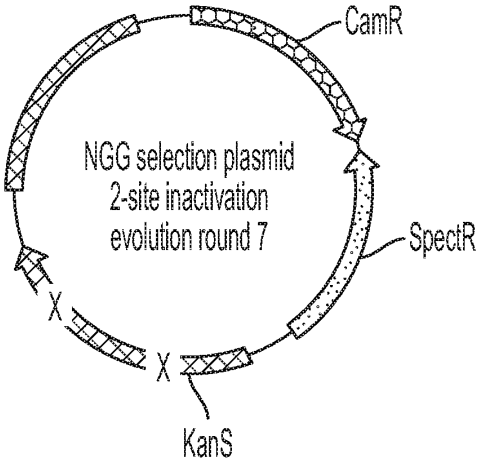


FIG. 1B

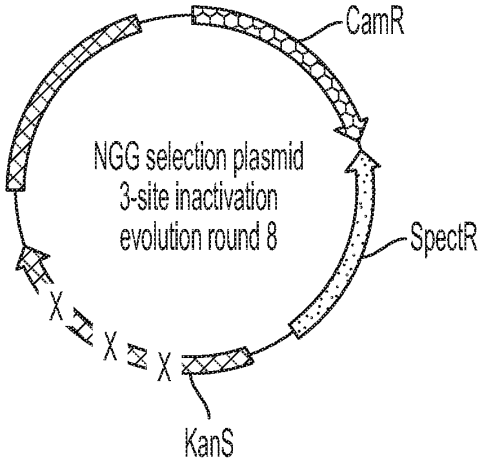


FIG. 1C

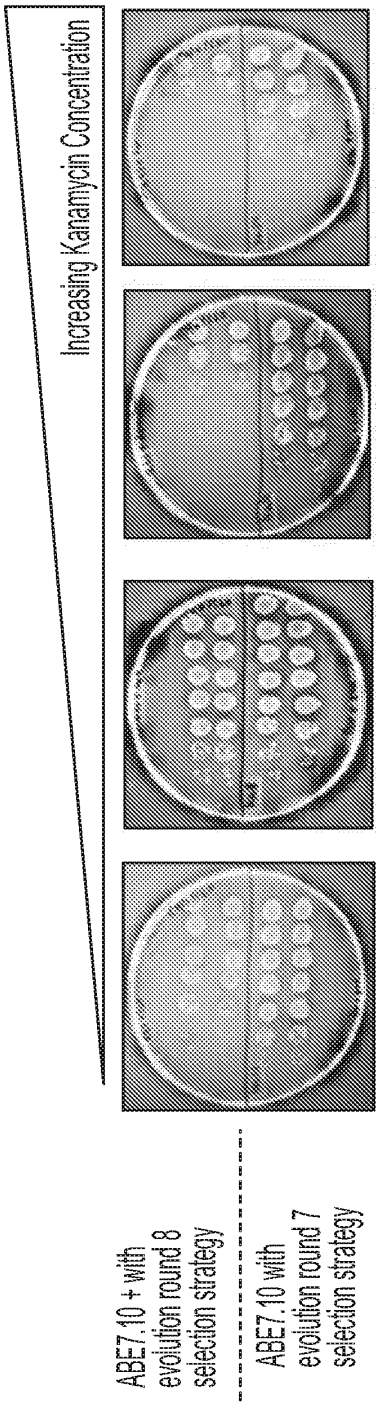

Increasing Kanamycin Concentration
ABE7.10 + with evolution round 8 selection strategy
ABE7.10 with evolution round 7 selection strategy

FIG. 2

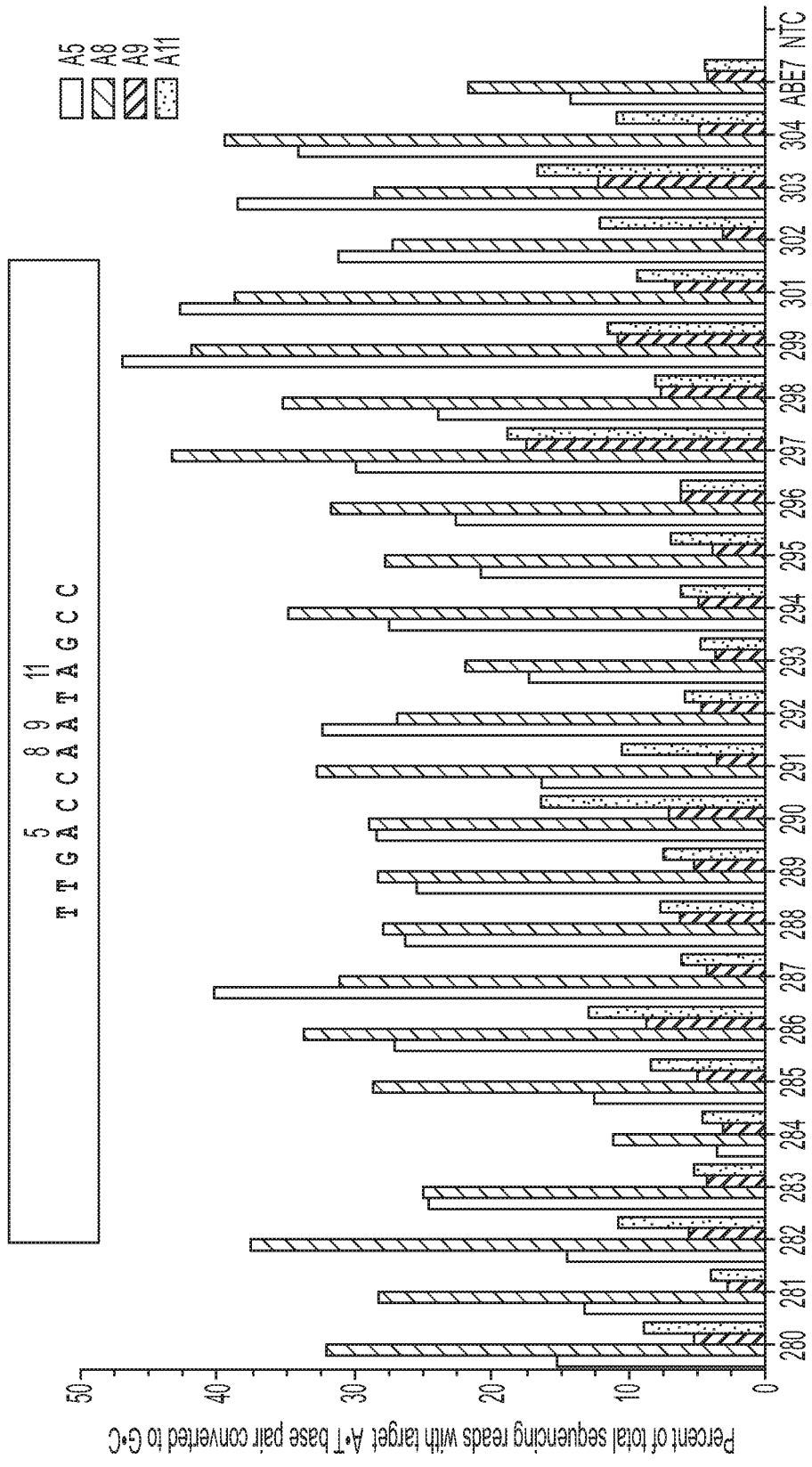

A5
A8
A9
A11
5 8 9 11
T T G A C C C A A T A G C C C
Percent of total sequencing reads with target A•T base pair converted to G•C
0
10
20
30
40
50
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
301
302
303
304
ABE7
NTC
ABE8 construct

FIG. 3A

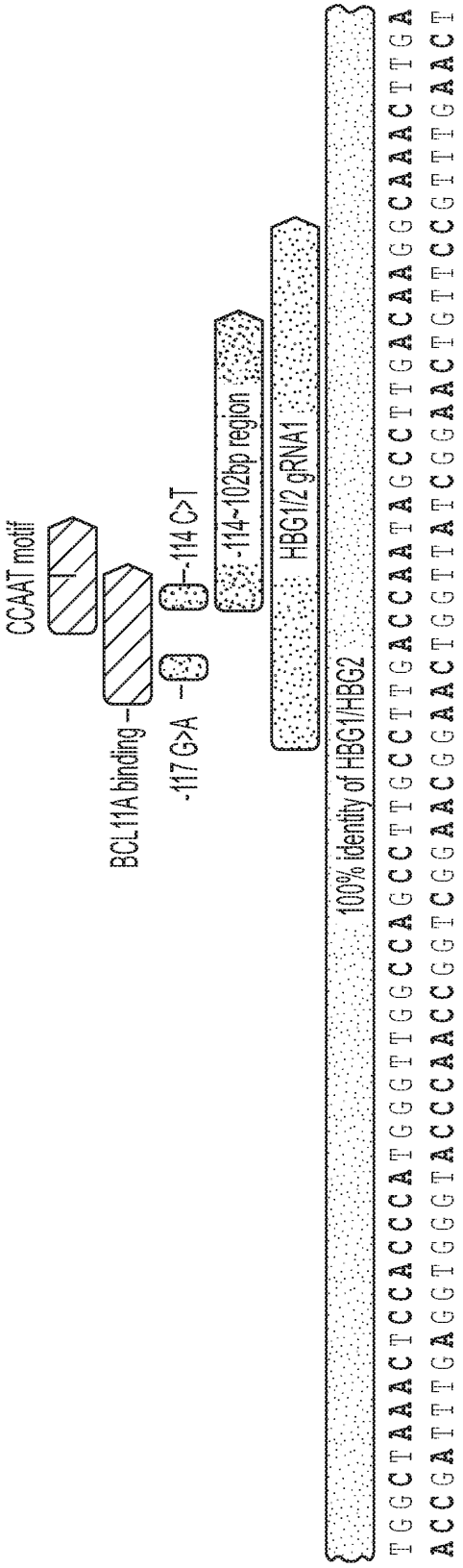

CCAAT motif
BCL11A binding
-117 G>A
-114 C>T
-114~102bp region
HBG1/2 gRNA1
100% identity of HBG1/HBG2
TGGCCTAAAACTCCACCCCATGGGTTGGCCAGCCCTTGCCTTGACCAAATAGCCCTTGACAAGGCCAAACTTGA
ACCGATTTGAGGGTGGGGTACCCCAACCCGGGTCGGAACCGGAACTGGGTTATCCGGAACTGGTTCCGTTTGAACT

FIG. 3B

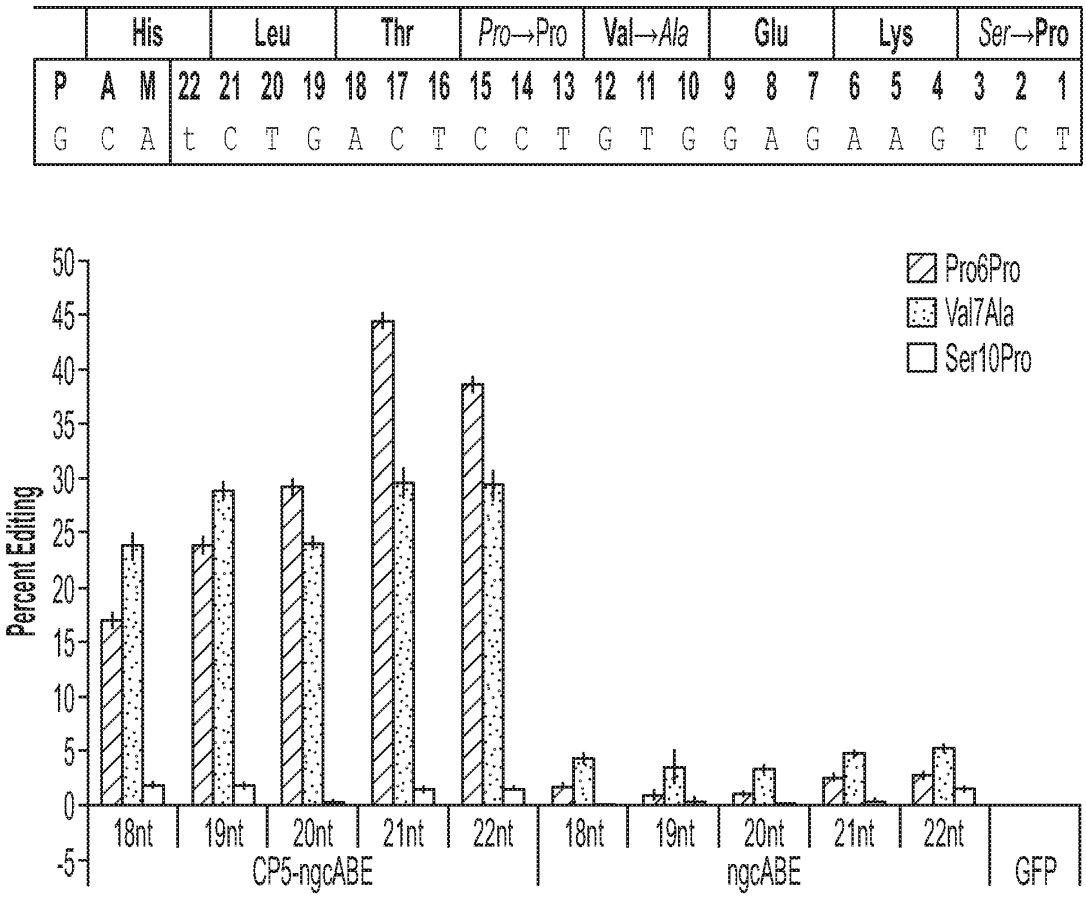

His
Leu
Thr
Pro→Pro
Val→Ala
Glu
Lys
Ser→Pro
P A M 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
G C A t C T G A C T C C T G T G G A G A A G T C T
Pro6Pro
Val7Ala
Ser10Pro
Percent Editing
50
45
40
35
30
25
20
15
10
5
0
-5
18nt
19nt
20nt
21nt
22nt
CP5-ngcABE
ngcABE
GFP

FIG. 4

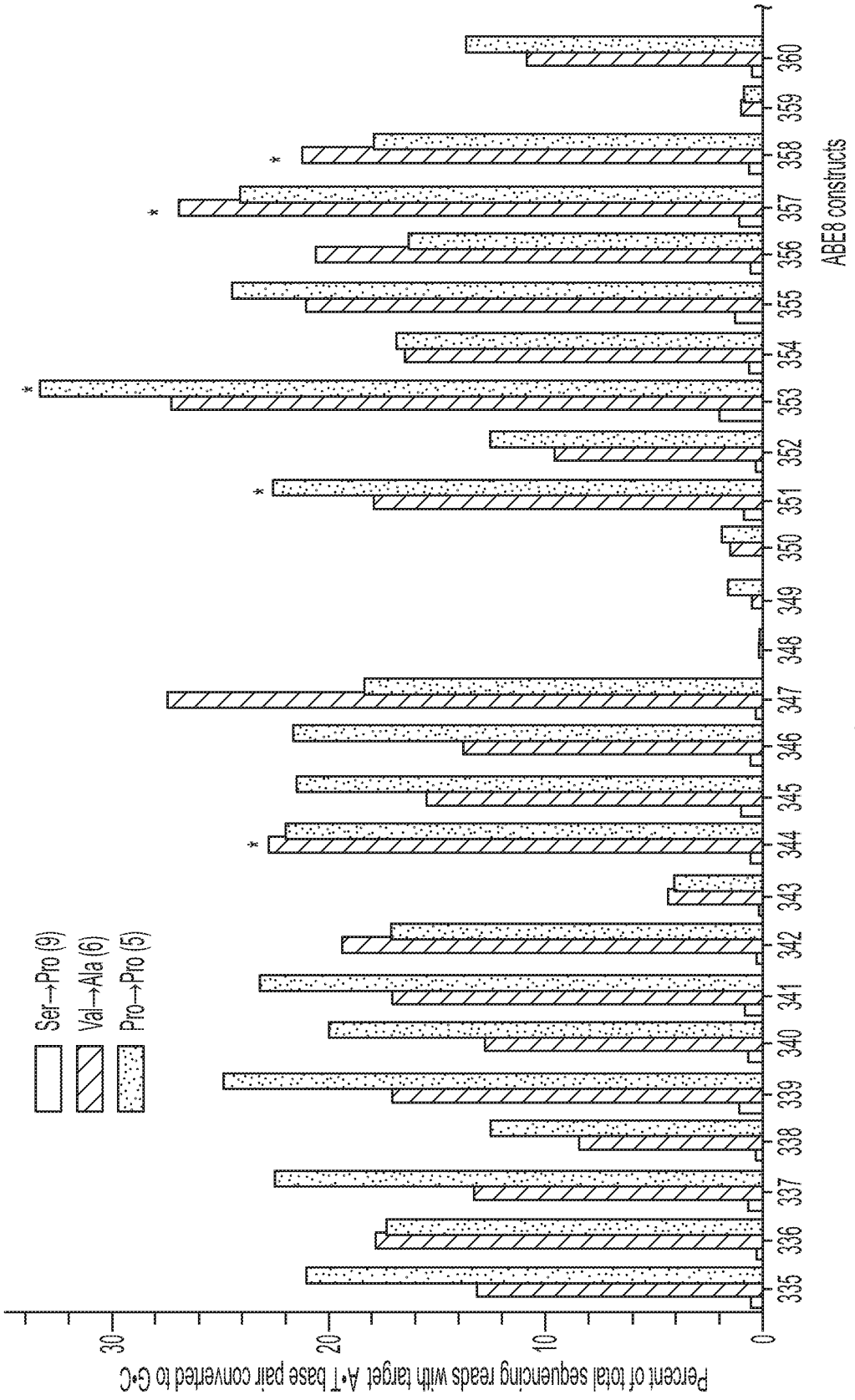

Percent of total sequencing reads with target A•T base pair converted to G•C
0
10
20
30
ABE8 constructs
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
*
*
*
*
Ser→Pro (9)
Val→Ala (6)
Pro→Pro (5)

FIG. 5

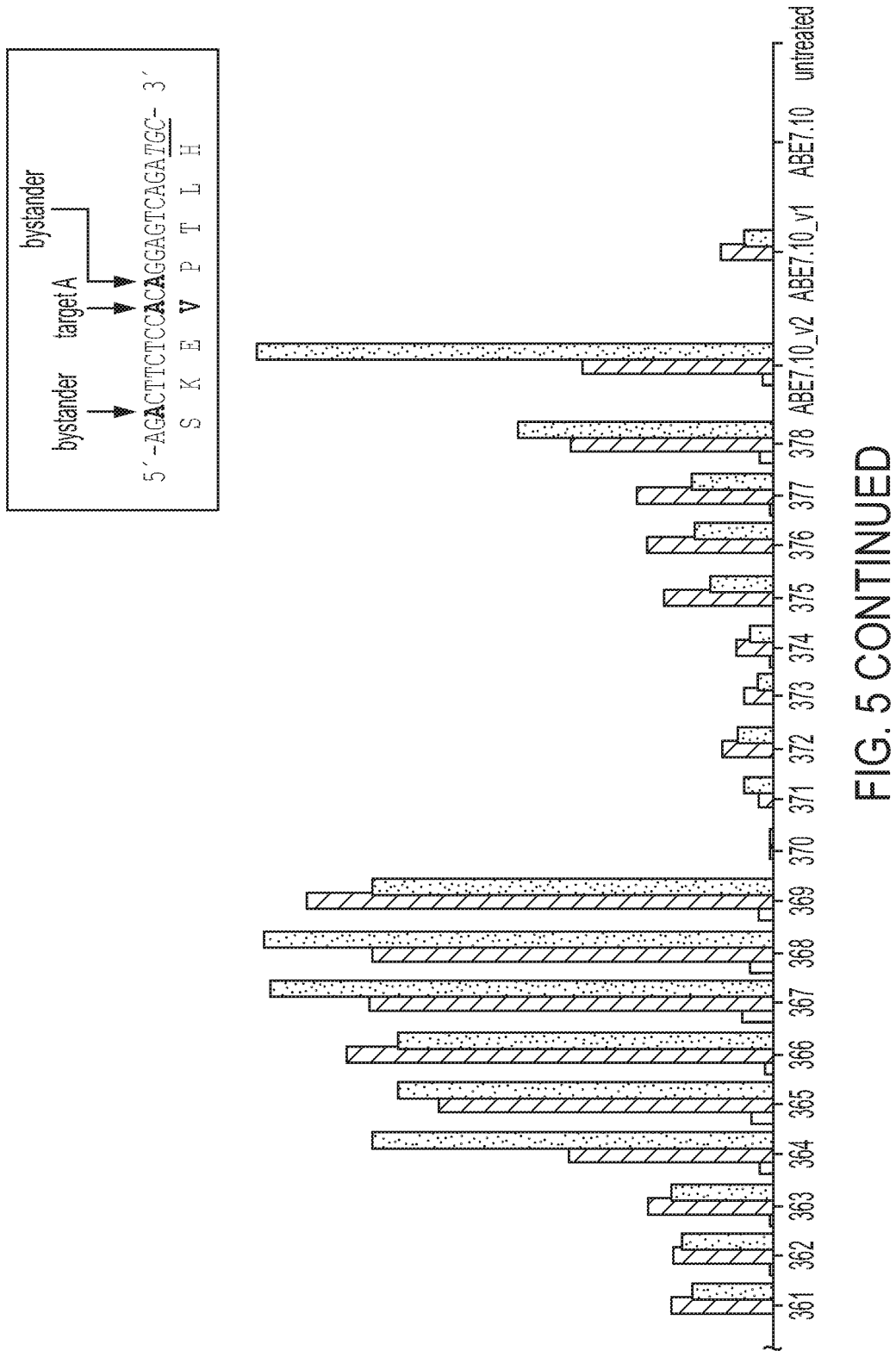


FIG. 5 CONTINUED

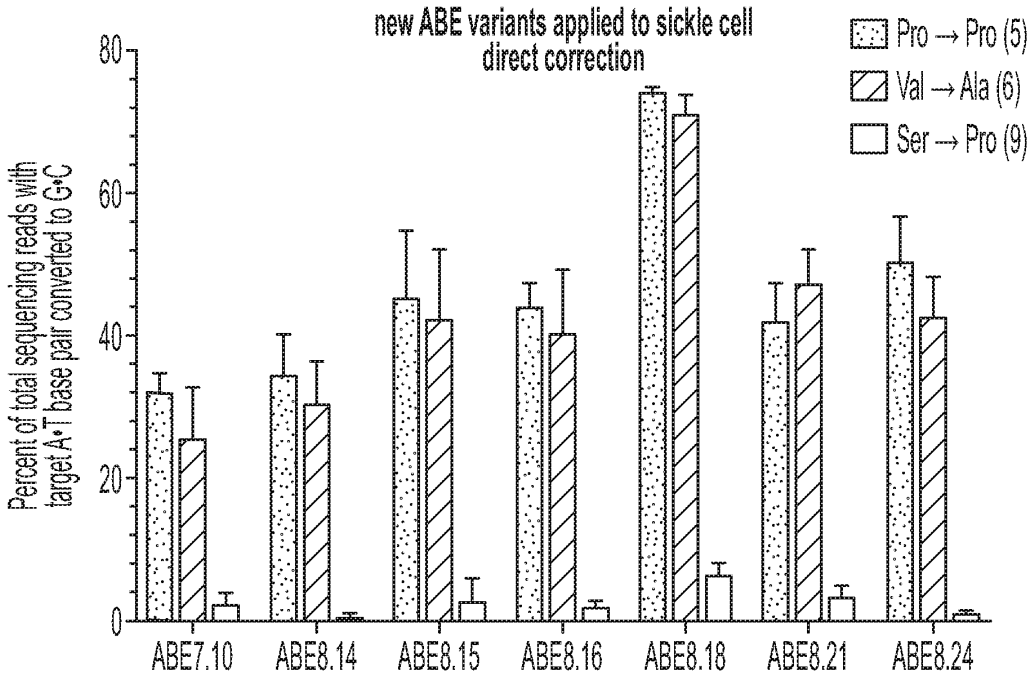

new ABE variants applied to sickle cell direct correction
Pro → Pro (5)
Val → Ala (6)
Ser → Pro (9)
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
ABE7.10
ABE8.14
ABE8.15
ABE8.16
ABE8.18
ABE8.21
ABE8.24

FIG. 6

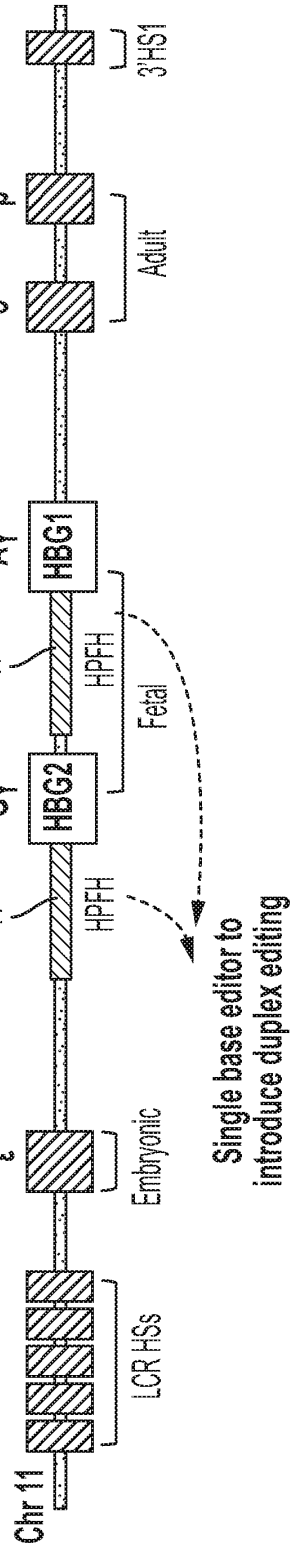

3'HS1
β
δ
Adult
Aγ
HBG1
A
HPFH
Fetal
Gγ
HBG2
A
HPFH
Single base editor to
introduce duplex editing
ε
Embryonic
LCR HSs
Chr 11

FIG. 7A

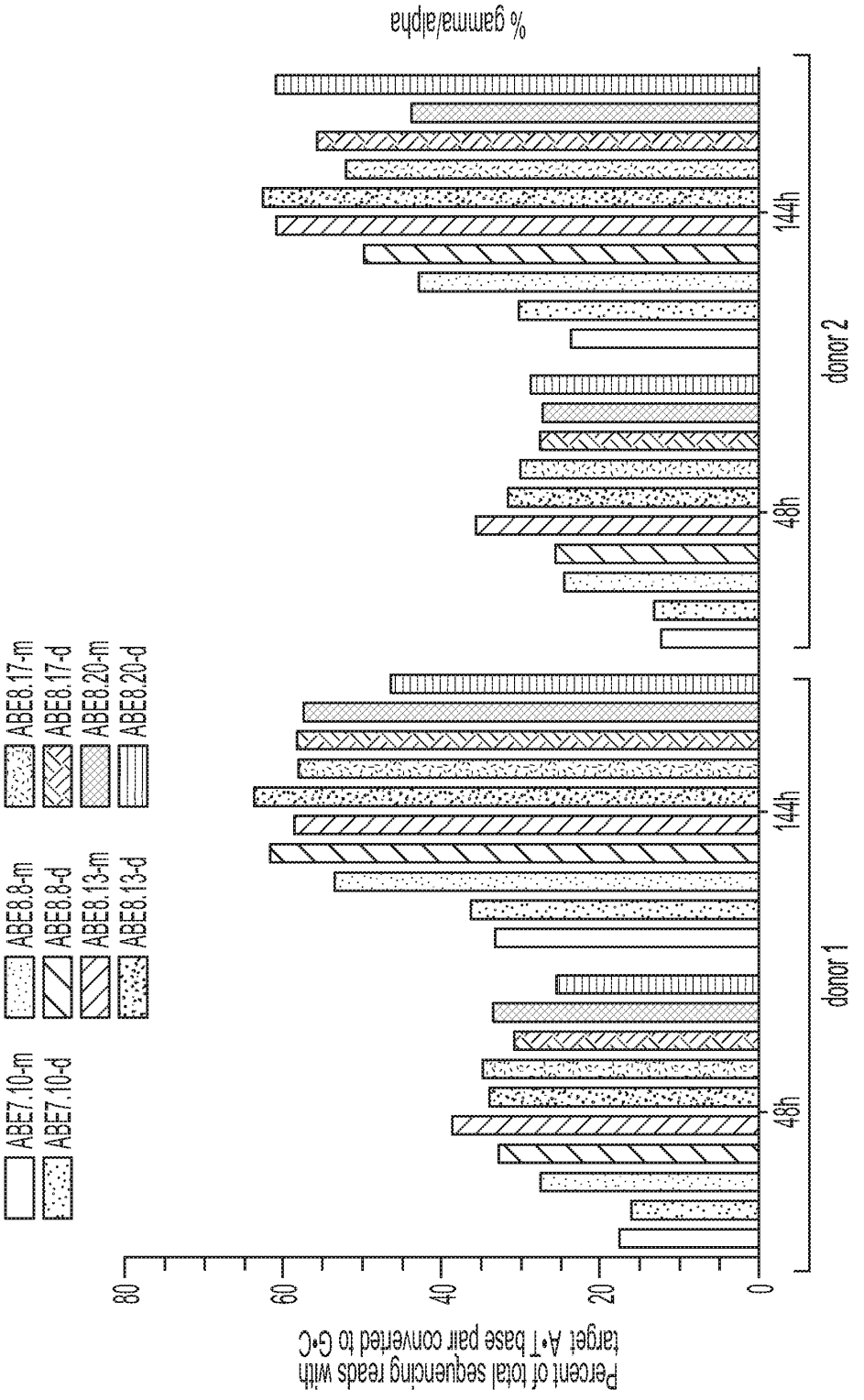

% gamma/alpha
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
48h
144h
48h
144h
donor 1
donor 2
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d

FIG. 7B

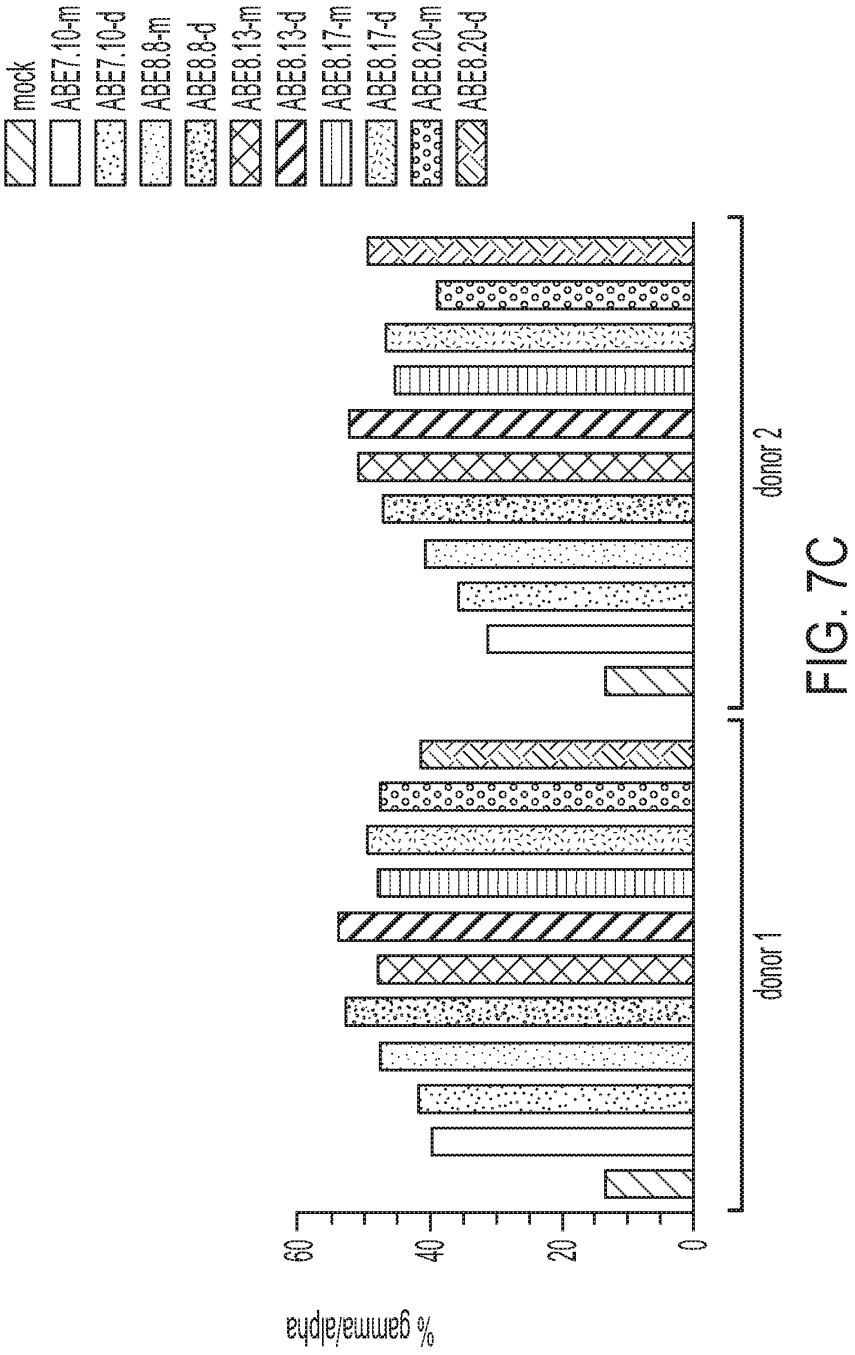

mock
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
% gamma/alpha
0
20
40
60
donor 1
donor 2

FIG. 7C

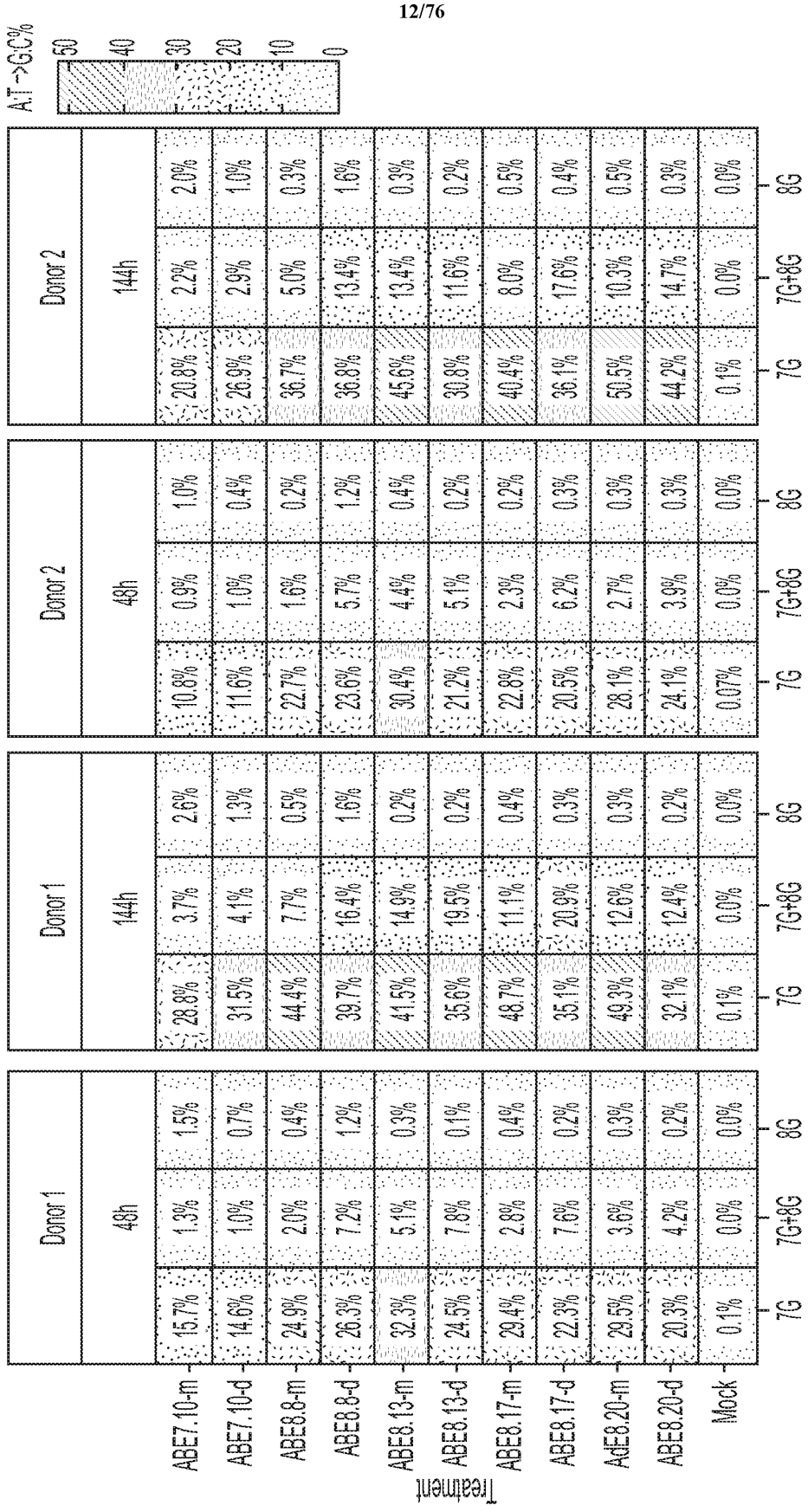

A:T ->G:C%
60
40
30
20
10
0
Donor 1
48h
144h
Donor 2
48h
144h
Donor 1
48h
144h
Donor 2
48h
144h
7G
7G+8G
8G
Target site editi
Treatment
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
AdE8.20-m
ABE8.20-d
Mock
15.7% 1.3% 1.5% 28.8% 3.7% 2.6% 10.8% 0.9% 1.0% 20.8% 2.2% 2.0%
14.6% 1.0% 0.7% 31.5% 4.1% 1.3% 11.6% 1.0% 0.4% 26.9% 2.9% 1.0%
24.9% 2.0% 0.4% 44.4% 7.7% 0.5% 22.7% 1.6% 0.2% 36.7% 5.0% 0.3%
26.3% 7.2% 1.2% 39.7% 16.4% 1.6% 23.6% 5.7% 1.2% 36.8% 13.4% 1.6%
32.3% 5.1% 0.3% 41.5% 14.9% 0.2% 30.4% 4.4% 0.4% 45.6% 13.4% 0.3%
24.5% 7.8% 0.1% 35.6% 19.5% 0.2% 21.2% 5.1% 0.2% 30.8% 11.6% 0.2%
29.4% 2.8% 0.4% 48.7% 11.1% 0.4% 22.8% 2.3% 0.2% 40.4% 8.0% 0.5%
22.3% 7.6% 0.2% 35.1% 20.9% 0.3% 20.5% 6.2% 0.3% 36.1% 17.6% 0.4%
29.5% 3.6% 0.3% 49.3% 12.6% 0.3% 28.1% 2.7% 0.3% 50.5% 10.3% 0.5%
20.3% 4.2% 0.2% 32.1% 12.4% 0.2% 24.1% 3.9% 0.3% 44.2% 14.7% 0.3%
0.1% 0.0% 0.0% 0.1% 0.0% 0.0% 0.07% 0.0% 0.0% 0.1% 0.0% 0.0%

FIG. 8A

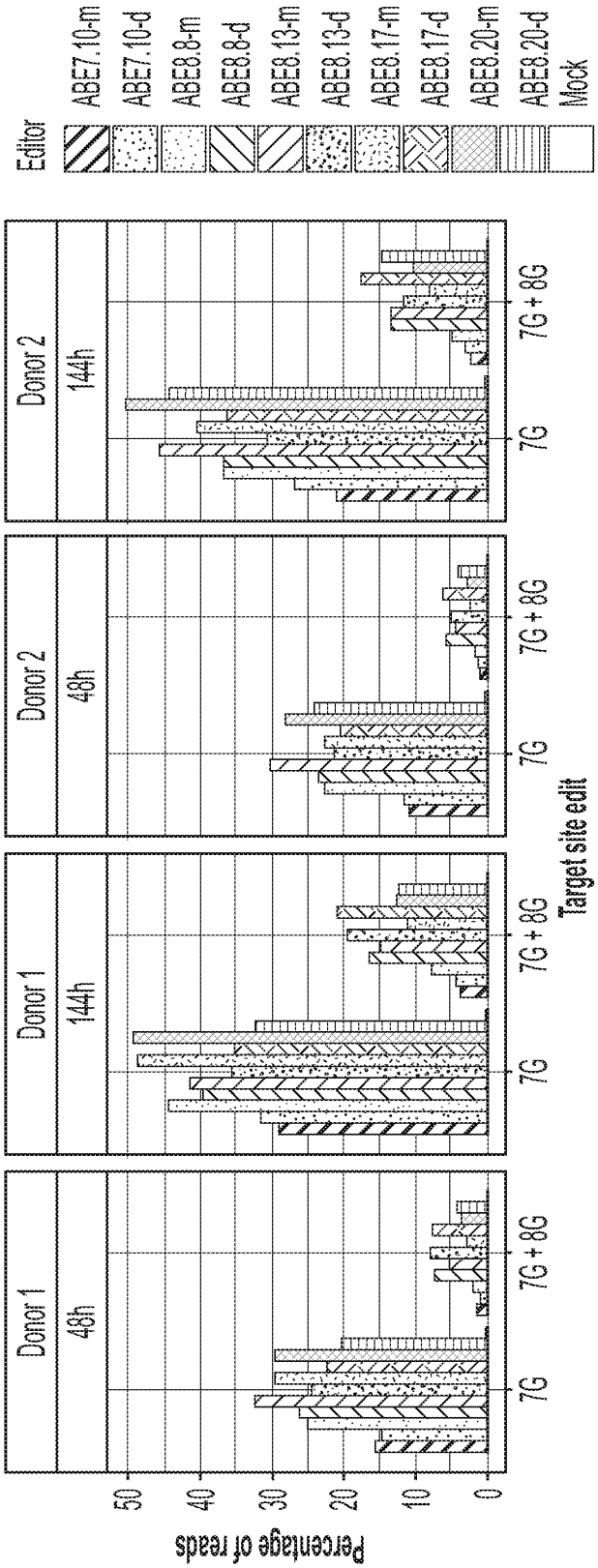

Editor
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
Mock
Donor 2
144h
Donor 2
48h
Donor 1
144h
Donor 1
48h
7G
7G + 8G
Target site edit
Percentage of reads
0
10
20
30
40
50

FIG. 8B

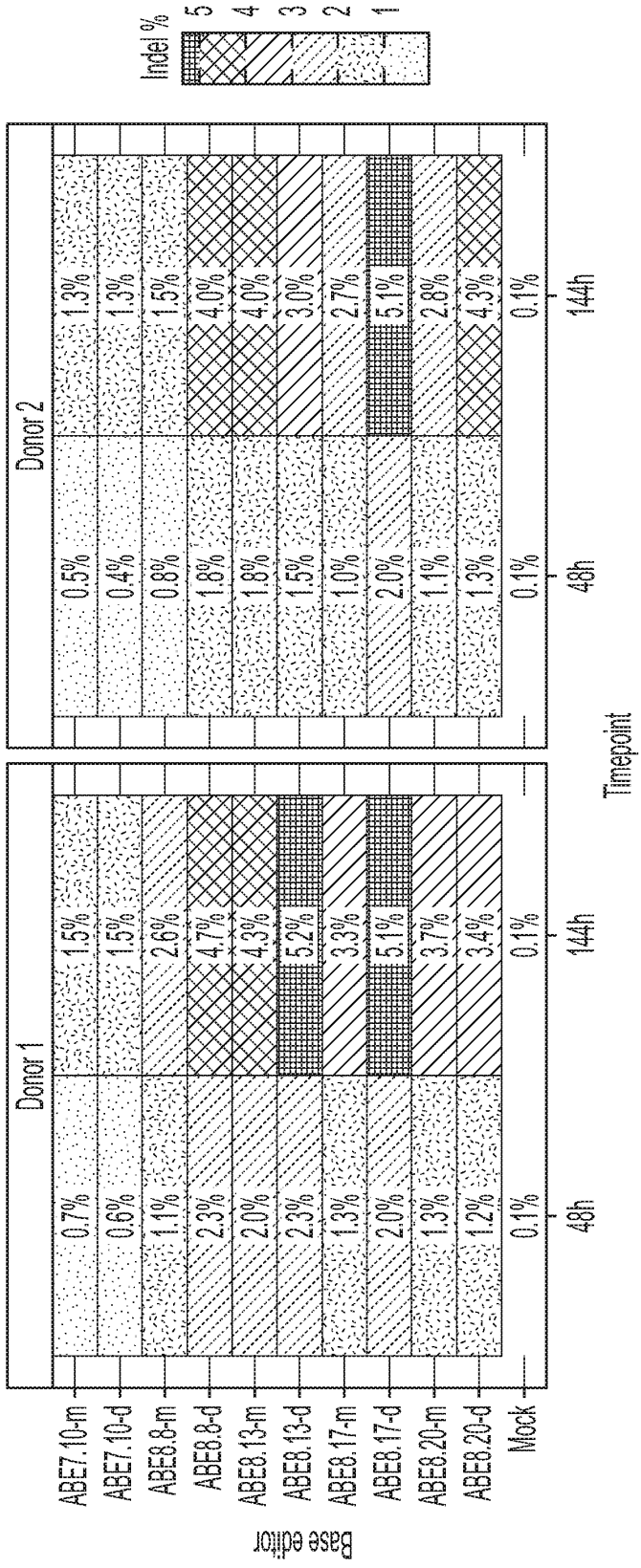
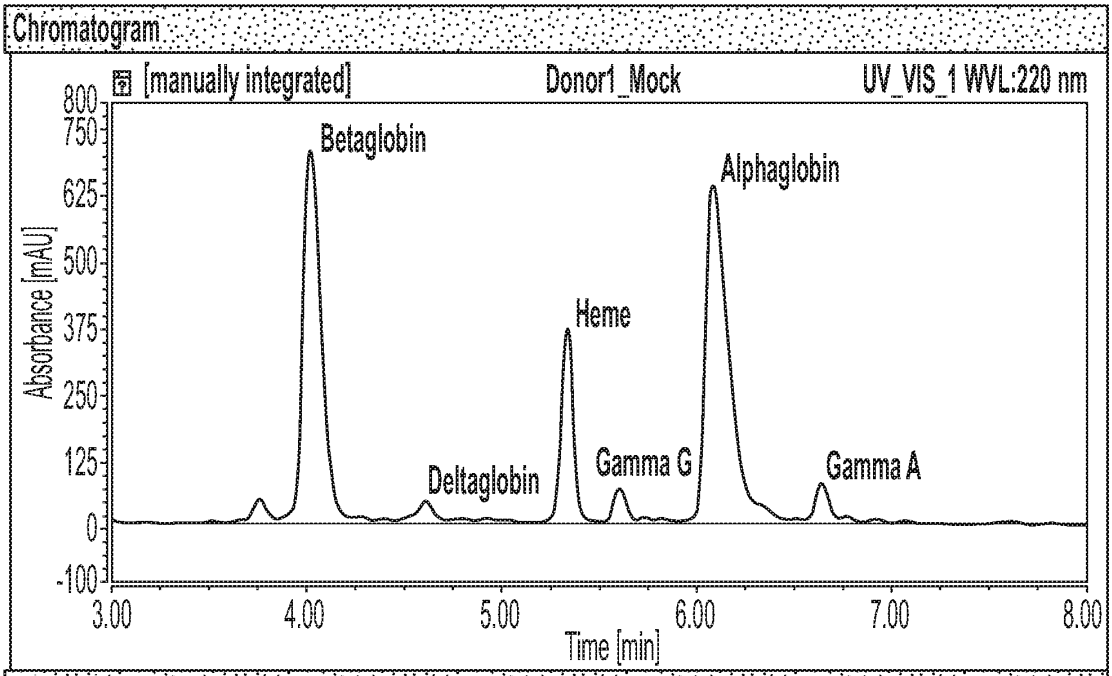

Indel %
5
4
3
2
1
Donor 1
Donor 2
0.7%
0.6%
1.1%
2.3%
2.0%
2.3%
1.3%
2.0%
1.3%
1.2%
1.5%
1.5%
2.6%
4.7%
4.3%
5.2%
3.3%
5.1%
3.7%
3.4%
0.1%
0.5%
0.4%
0.8%
1.8%
1.8%
1.5%
1.0%
2.0%
1.1%
1.3%
1.3%
1.3%
1.5%
4.0%
4.0%
3.0%
2.7%
5.1%
2.8%
4.3%
0.1%
48h
144h
Timepoint
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
Mock
Base editor

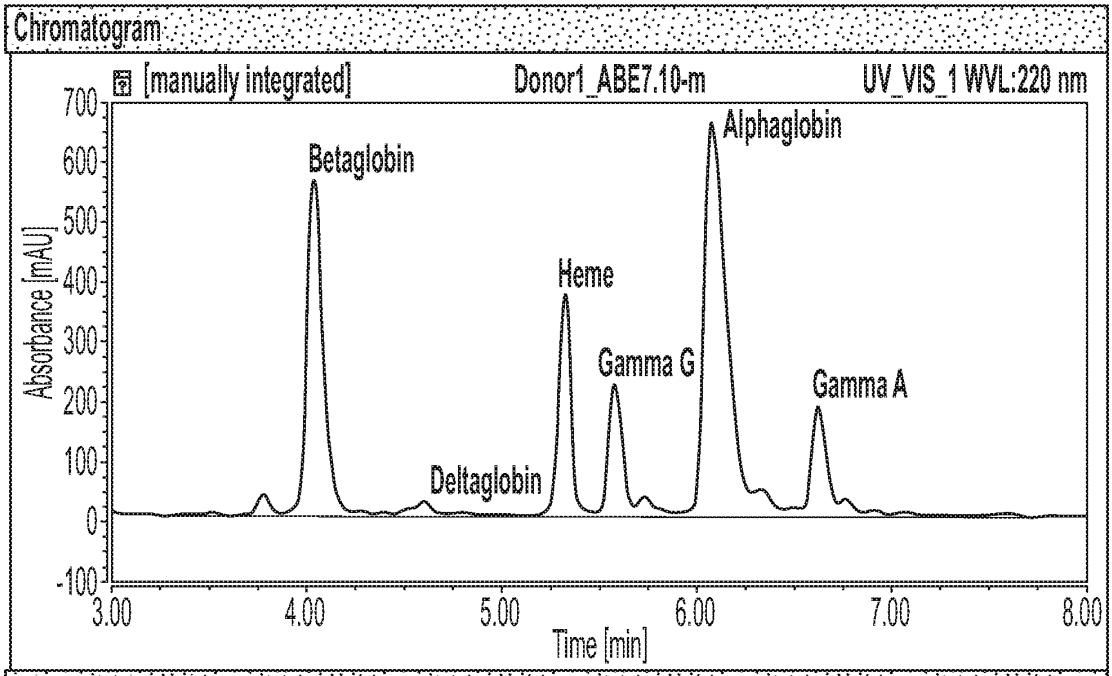
FIG. 9



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.020	77.631	701.634	33.61	32.98
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.610	3.780	42.943	1.64	2.02
14	Heme	5.337	27.191	366.542	11.77	17.23
15	Gamma G	5.603	5.248	66.622	2.27	3.13
18	Alphaglobin	6.083	88.477	636.811	38.31	29.94
21	Gamma A	6.637	6.751	77.427	2.92	3.64

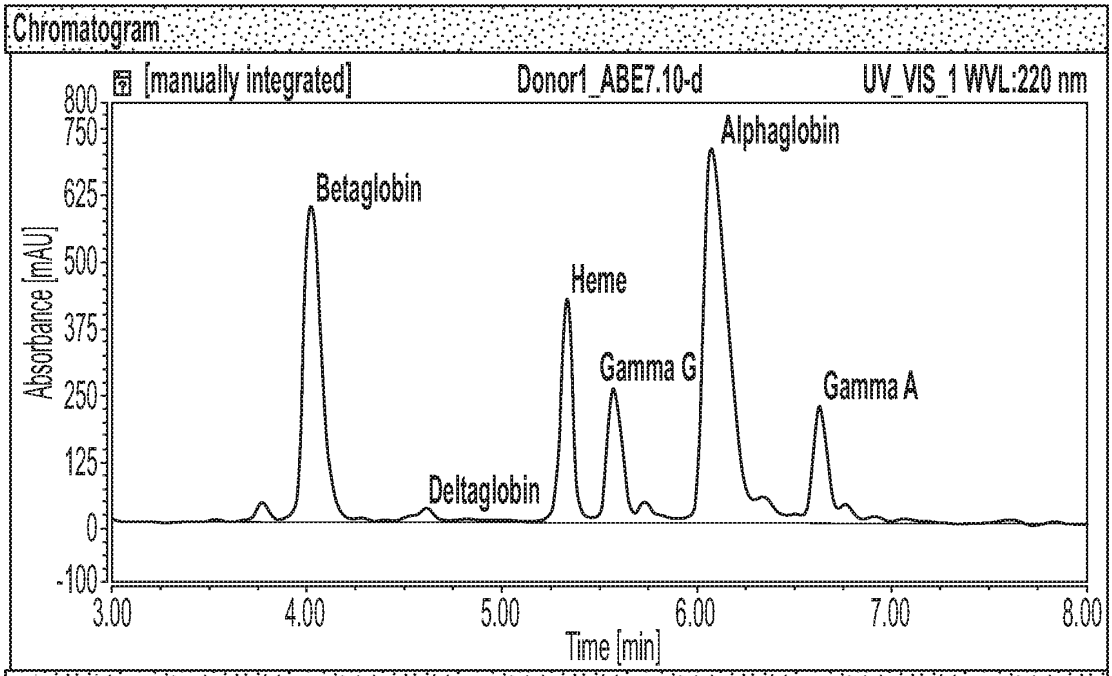
FIG. 10



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.043	56.404	561.657	24.21	24.80
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.607	2.463	24.382	1.06	1.08
13	Heme	5.330	27.334	371.802	11.73	16.42
14	Gamma G	5.583	18.764	222.410	8.06	9.82
16	Alphaglobin	6.080	88.236	657.695	37.88	29.04
19	Gamma A	6.627	16.470	185.055	7.07	8.17

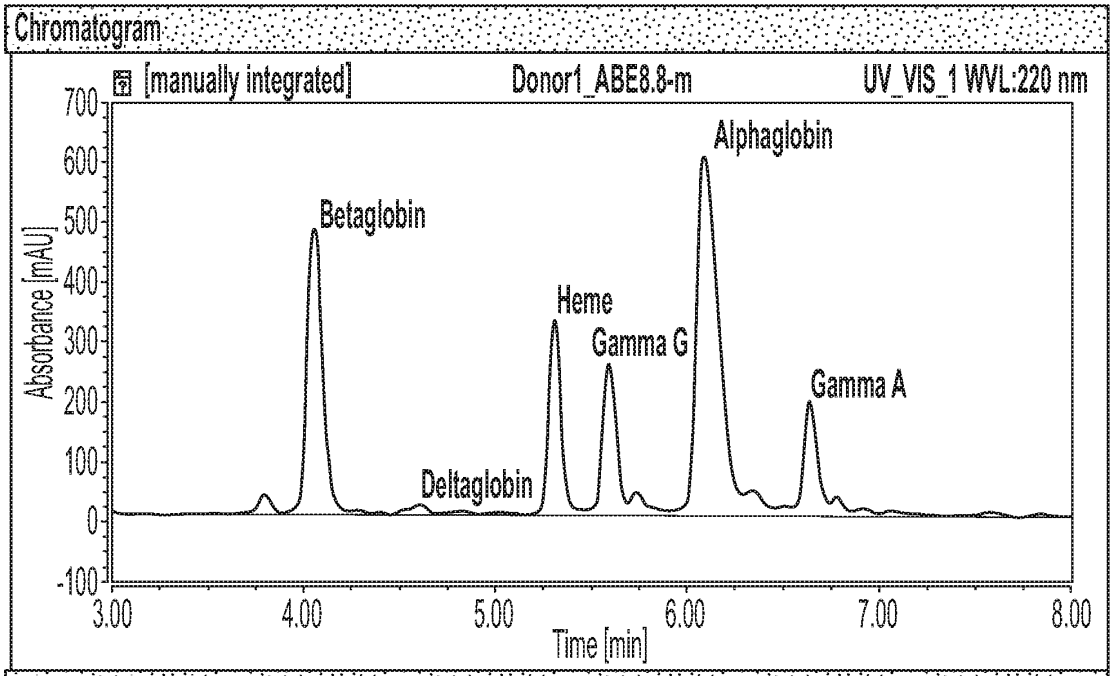
FIG. 11



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.020	63.540	596.818	23.88	23.74
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.613	2.822	27.354	1.06	1.09
14	Heme	5.333	31.163	421.735	11.71	16.77
15	Gamma G	5.570	22.558	253.399	8.48	10.08
18	Alphaglobin	6.070	100.612	704.910	37.81	28.03
21	Gamma A	6.623	19.220	220.846	7.22	8.78

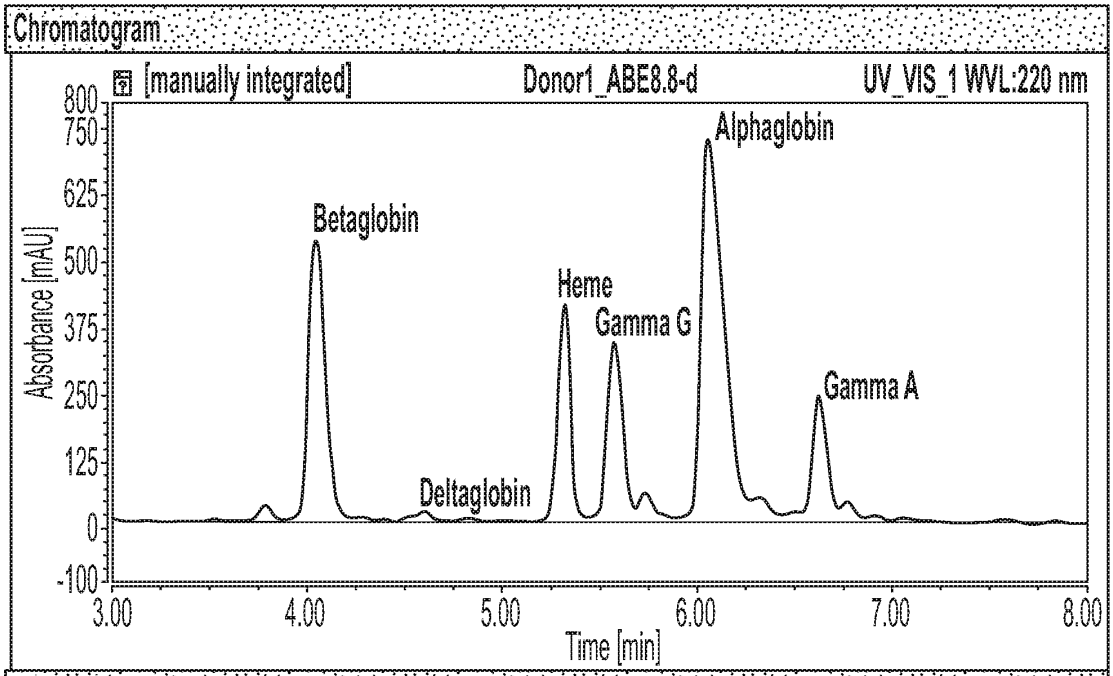
FIG. 12



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.053	44.227	479.648	21.03	22.78
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.607	1.704	17.056	0.81	0.81
13	Heme	5.310	24.221	326.068	11.52	15.48
14	Gamma G	5.590	21.346	256.462	10.15	12.18
16	Alphaglobin	6.087	79.706	602.792	37.90	28.62
19	Gamma A	6.640	16.631	192.016	7.91	9.12

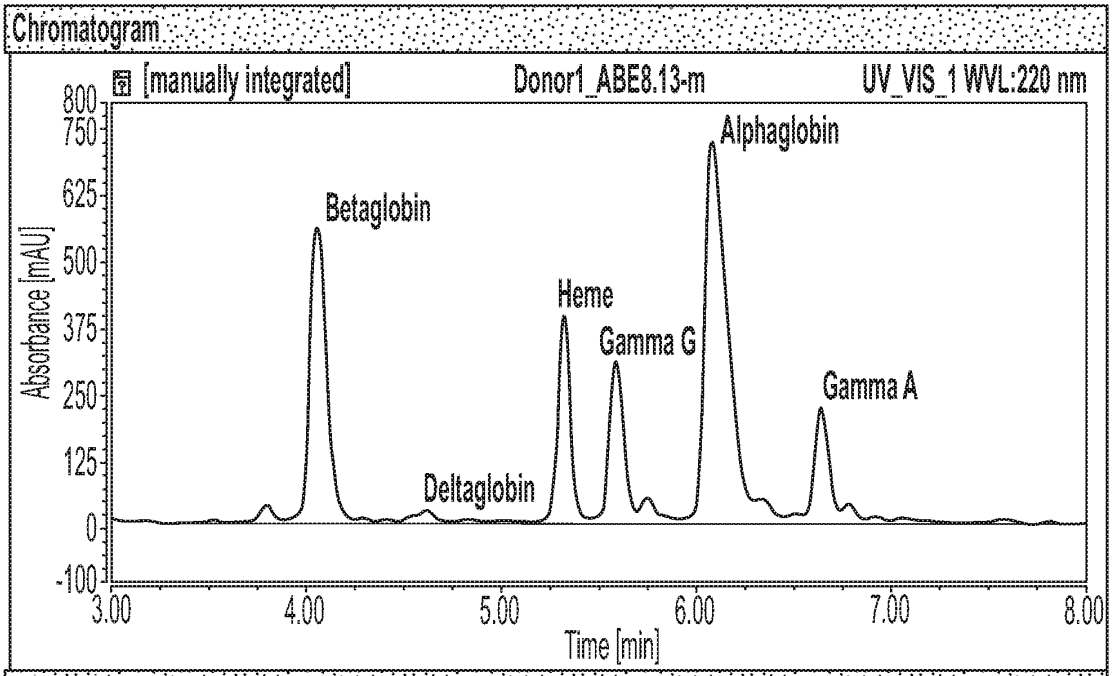
FIG. 13



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.043	51.802	530.669	19.60	20.80
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.597	2.269	21.040	0.86	0.82
13	Heme	5.323	30.637	410.481	11.59	16.09
14	Gamma G	5.573	30.747	340.883	11.63	13.36
16	Alphaglobin	6.060	98.417	721.350	37.24	28.28
19	Gamma A	6.627	21.595	239.647	8.17	9.39

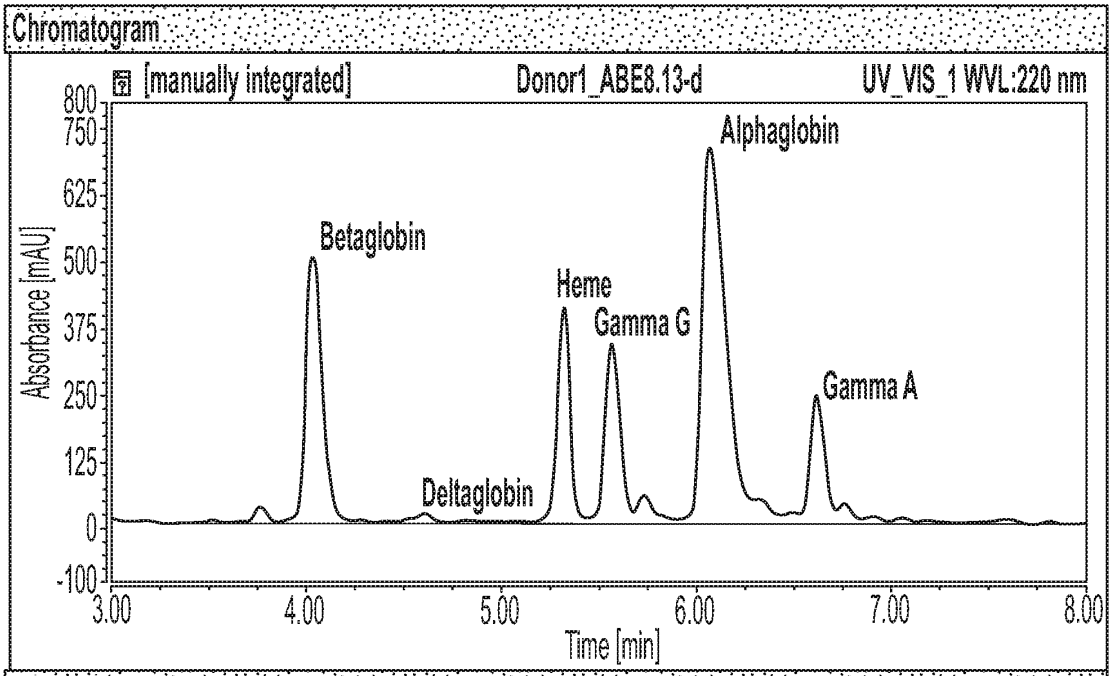
FIG. 14



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.047	55.084	554.598	21.23	22.22
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.613	2.417	24.372	0.93	0.98
13	Heme	5.320	29.500	393.066	11.37	15.75
14	Gamma G	5.580	27.177	306.655	10.48	12.29
16	Alphaglobin	6.073	98.048	719.982	37.79	28.85
19	Gamma A	6.630	19.949	219.869	7.69	8.81

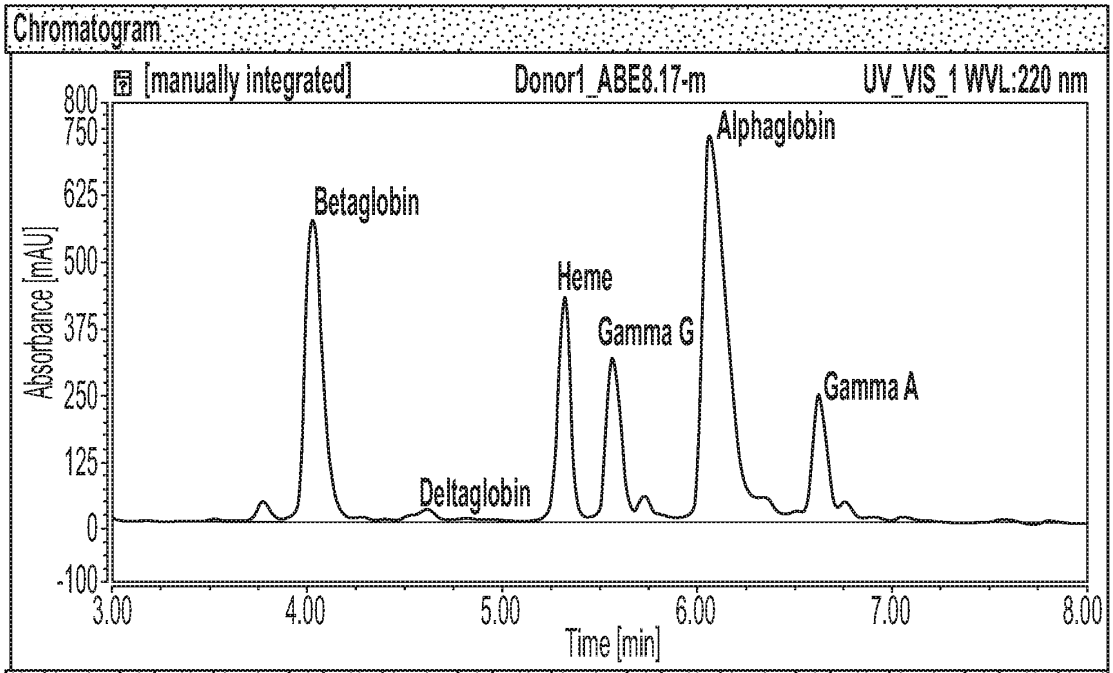
FIG. 15



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.037	49.284	501.306	19.04	20.14
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.610	1.960	18.855	0.76	0.76
13	Heme	5.327	30.274	407.530	11.69	16.37
14	Gamma G	5.570	31.059	339.163	12.00	13.63
16	Alphaglobin	6.070	98.109	707.464	37.89	28.42
19	Gamma A	6.617	21.869	243.715	8.45	9.79

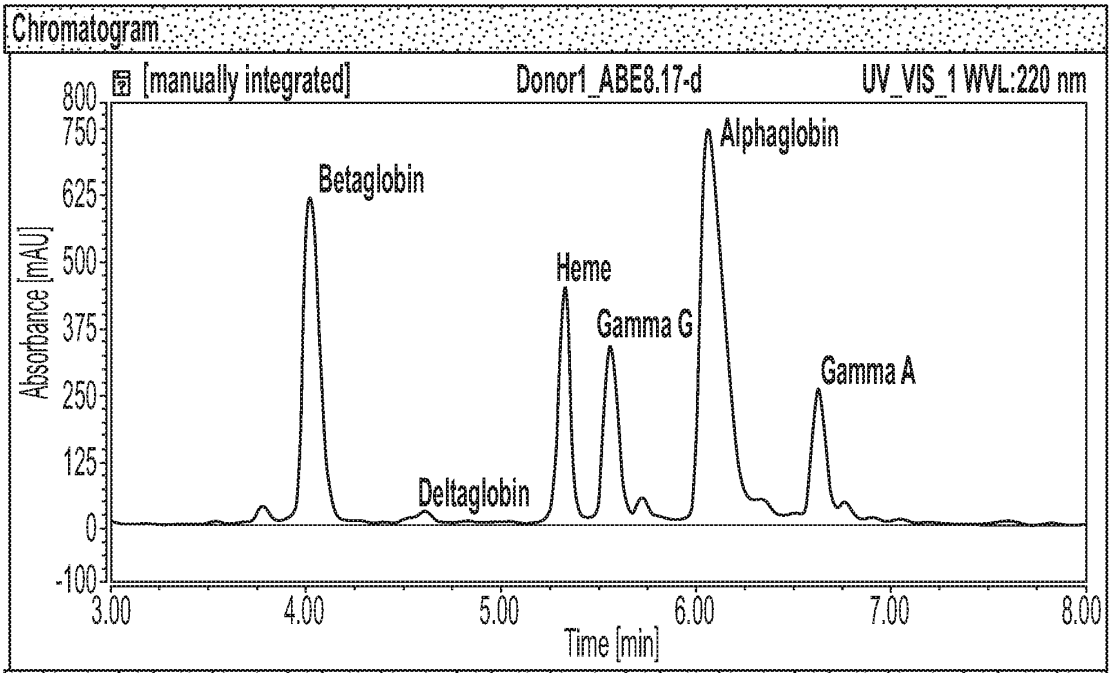
FIG. 16



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.030	59.000	569.475	21.35	21.91
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.617	2.483	24.660	0.90	0.95
13	Heme	5.323	31.792	426.360	11.50	16.40
14	Gamma G	5.567	28.258	310.274	10.22	11.94
16	Alphaglobin	6.067	104.586	728.120	37.84	28.01
19	Gamma A	6.627	21.512	243.710	7.78	9.37

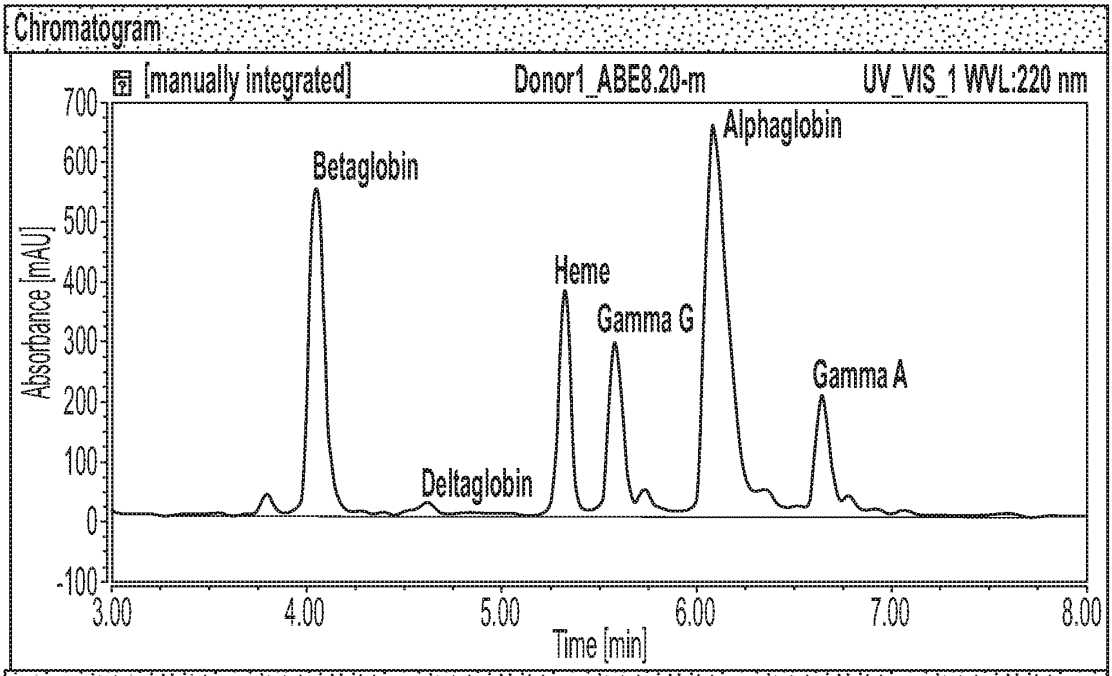
FIG. 17



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.023	60.046	613.639	21.18	22.63
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.610	2.491	24.450	0.88	0.90
14	Heme	5.330	33.236	446.899	11.72	16.48
15	Gamma G	5.560	29.840	335.709	10.52	12.38
17	Alphaglobin	6.063	106.077	742.712	37.41	27.38
20	Gamma A	6.627	22.570	257.472	7.96	9.49

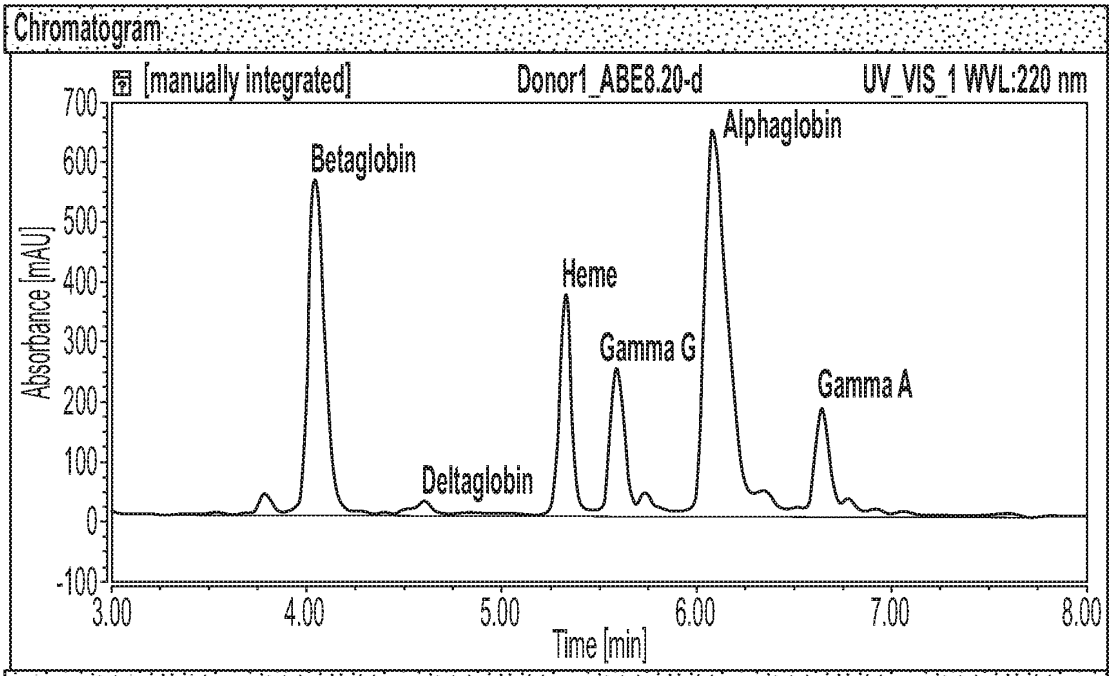
FIG. 18



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.040	51.610	548.050	21.57	23.31
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.610	2.233	21.329	0.93	0.91
13	Heme	5.320	27.916	377.668	11.67	16.06
14	Gamma G	5.573	24.931	291.767	10.42	12.41
16	Alphaglobin	6.077	90.099	653.942	37.66	27.81
19	Gamma A	6.637	17.798	203.835	7.44	8.67

FIG. 19

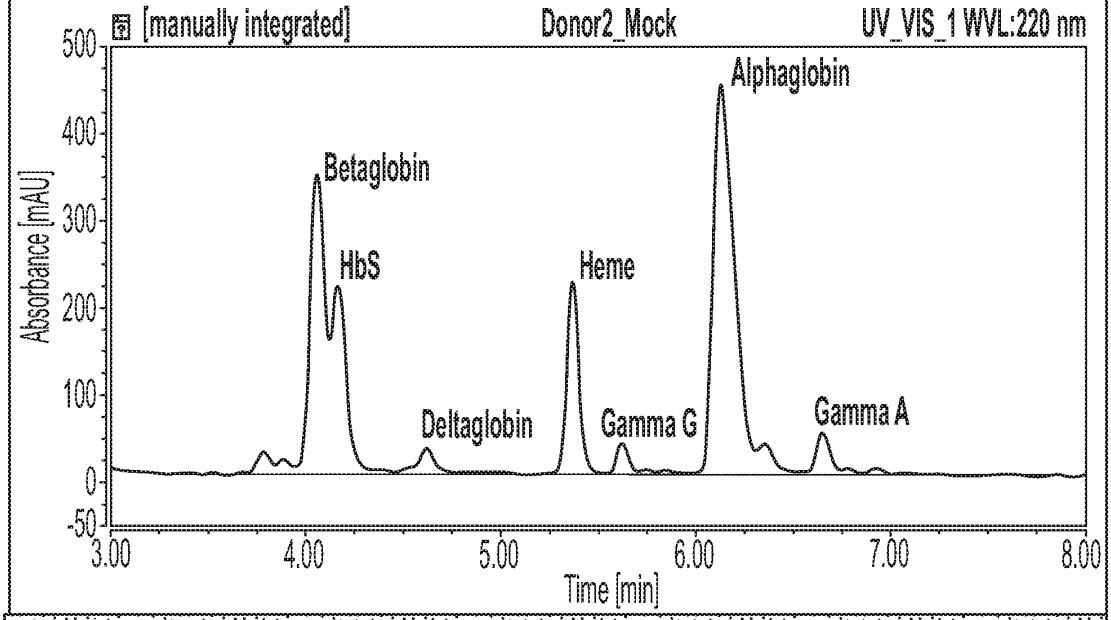


Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.040	55.490	562.369	23.82	24.71
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
10	Deltaglobin	4.600	2.399	23.713	1.03	1.04
14	Heme	5.327	27.308	371.229	11.72	16.31
15	Gamma G	5.587	20.808	248.715	8.93	10.93
17	Alphaglobin	6.073	87.517	645.669	37.56	28.38
20	Gamma A	6.637	15.700	180.637	6.74	7.94

FIG. 20

Chromatogram

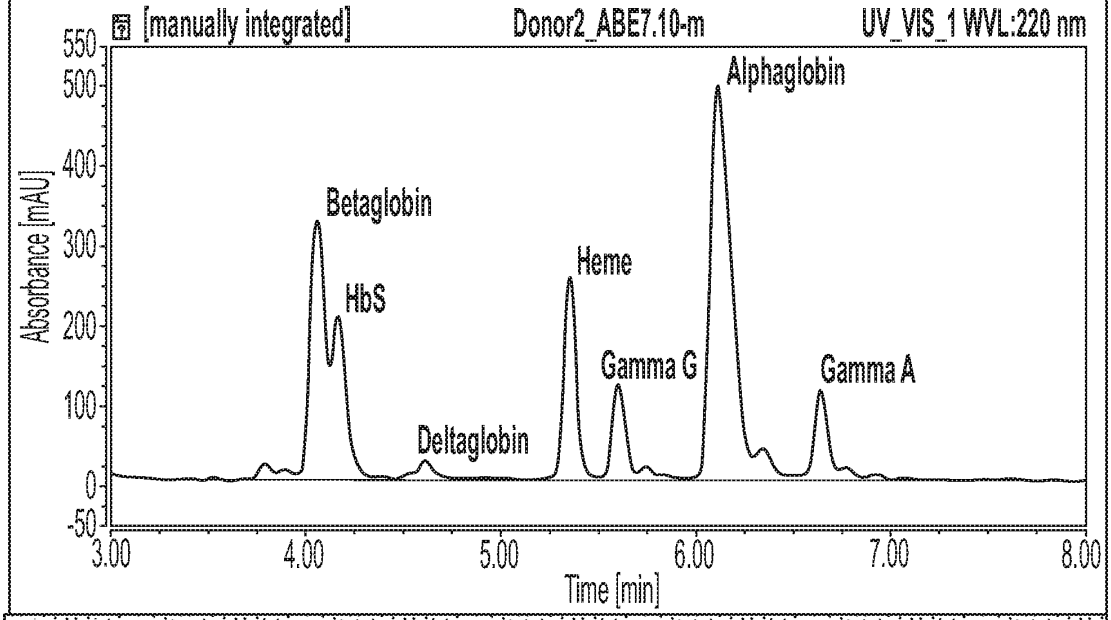


Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	28.943	345.410	20.25	23.19
6	HbS	4.160	19.794	216.879	13.85	14.56
9	Deltaglobin	4.617	3.170	30.593	2.22	2.05
11	Heme	5.367	16.498	222.155	11.54	14.91
12	Gamma G	5.620	2.805	37.002	1.96	2.48
15	Alphaglobin	6.127	54.386	448.385	38.05	30.10
18	Gamma A	6.647	4.170	49.520	2.92	3.32

FIG. 21

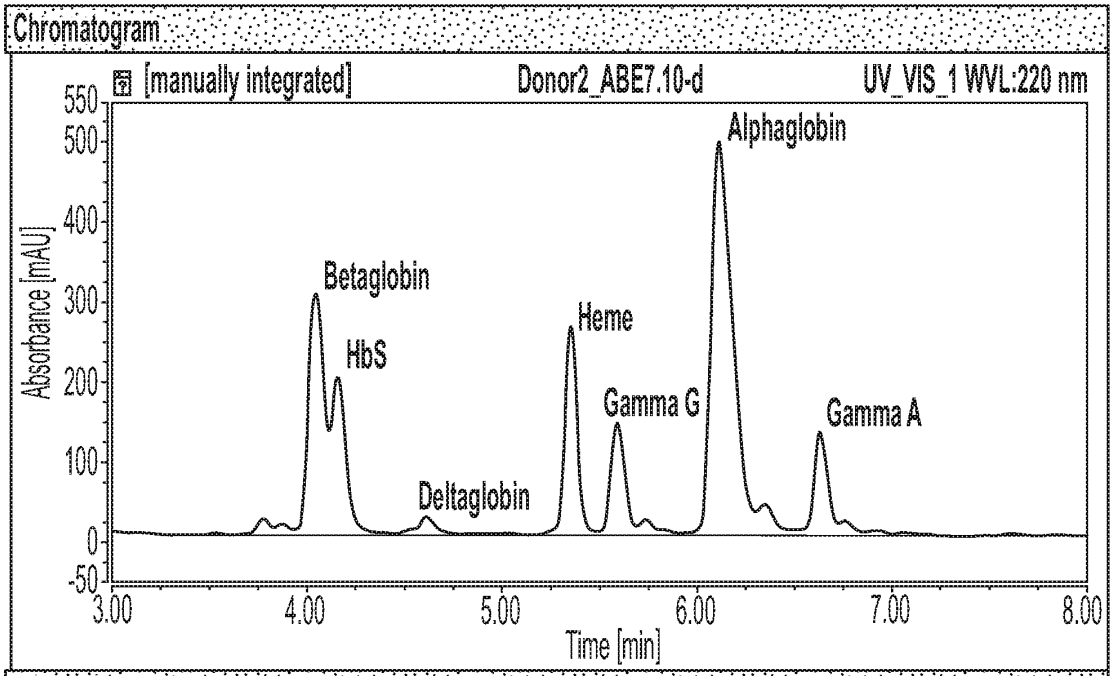
Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	27.210	325.305	16.96	19.17
6	HbS	4.163	18.038	204.594	11.24	12.05
9	Deltaglobin	4.610	2.458	24.147	1.53	1.42
14	Heme	5.353	18.788	253.663	11.71	14.94
15	Gamma G	5.600	9.475	120.558	5.91	7.10
18	Alphaglobin	6.110	60.688	493.180	37.83	29.06
21	Gamma A	6.637	9.526	113.714	5.94	6.70

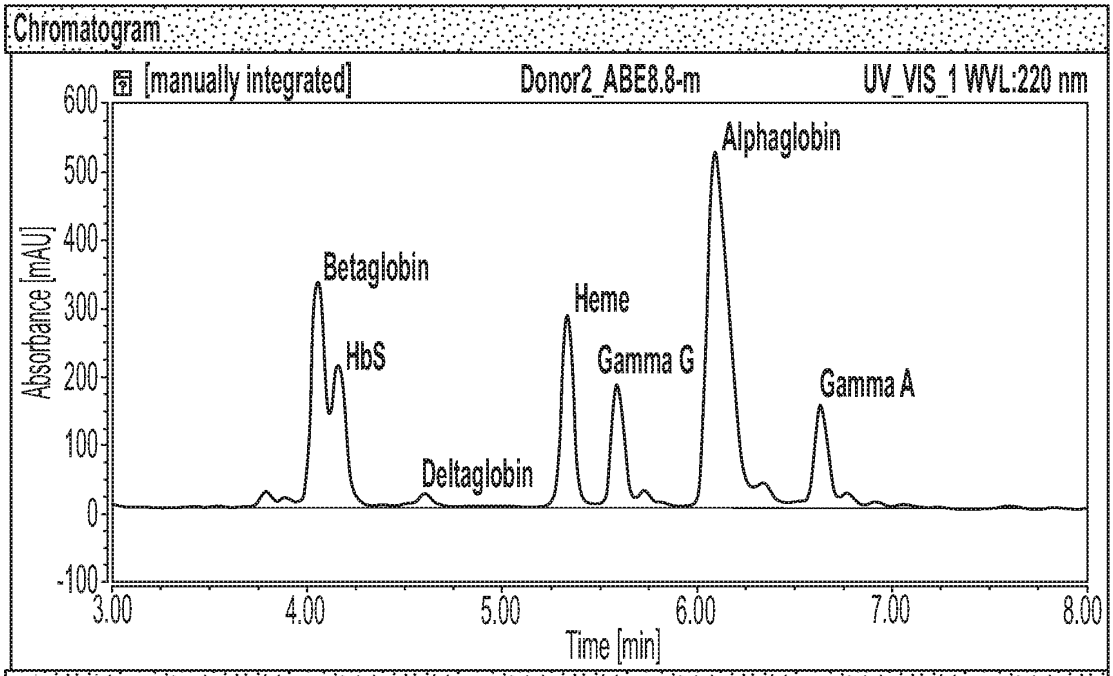
FIG. 22



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.050	26.525	303.461	15.99	17.61
6	HbS	4.160	17.741	200.547	10.70	11.64
9	Deltaglobin	4.620	2.209	22.450	1.33	1.30
14	Heme	5.357	19.493	262.669	11.75	15.24
15	Gamma G	5.593	11.528	141.729	6.95	8.23
18	Alphaglobin	6.113	62.859	497.019	37.90	28.85
21	Gamma A	6.630	10.827	131.062	6.53	7.61

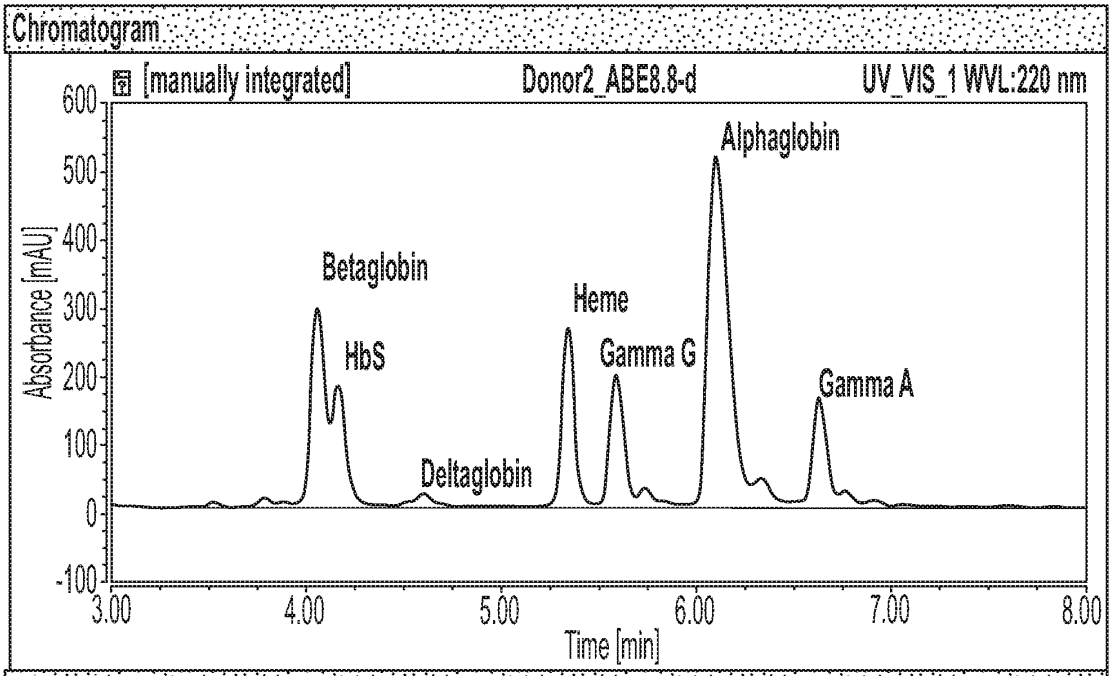
FIG. 23



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	26.492	330.353	14.76	17.48
6	HbS	4.160	17.650	209.154	9.84	11.07
9	Deltaglobin	4.603	2.190	21.674	1.22	1.15
14	Heme	5.340	20.930	280.930	11.66	14.87
15	Gamma G	5.593	14.357	180.573	8.00	9.56
18	Alphaglobin	6.097	67.616	524.013	37.68	27.73
21	Gamma A	6.637	12.935	152.286	7.21	8.06

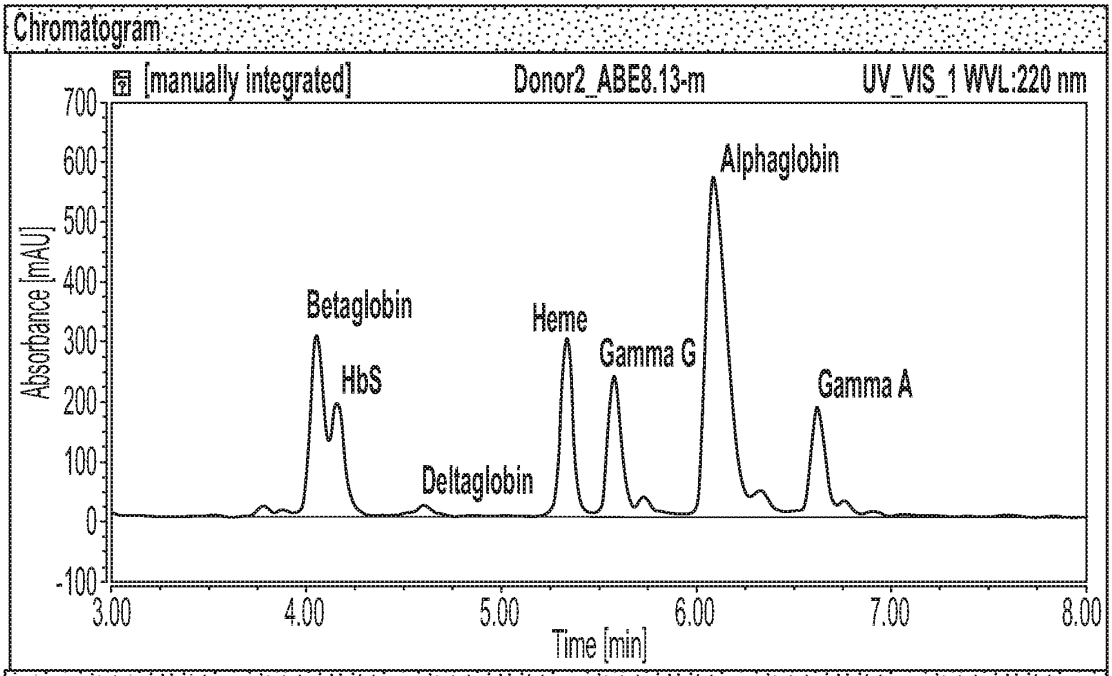
FIG. 24



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.063	23.888	291.371	13.90	16.06
7	HbS	4.167	15.512	178.139	9.02	9.82
10	Deltaglobin	4.607	2.249	20.296	1.31	1.12
15	Heme	5.343	19.534	262.940	11.36	14.49
16	Gamma G	5.593	16.034	195.308	9.33	10.77
19	Alphaglobin	6.103	63.055	512.839	36.68	28.27
22	Gamma A	6.633	13.860	161.449	8.06	8.90

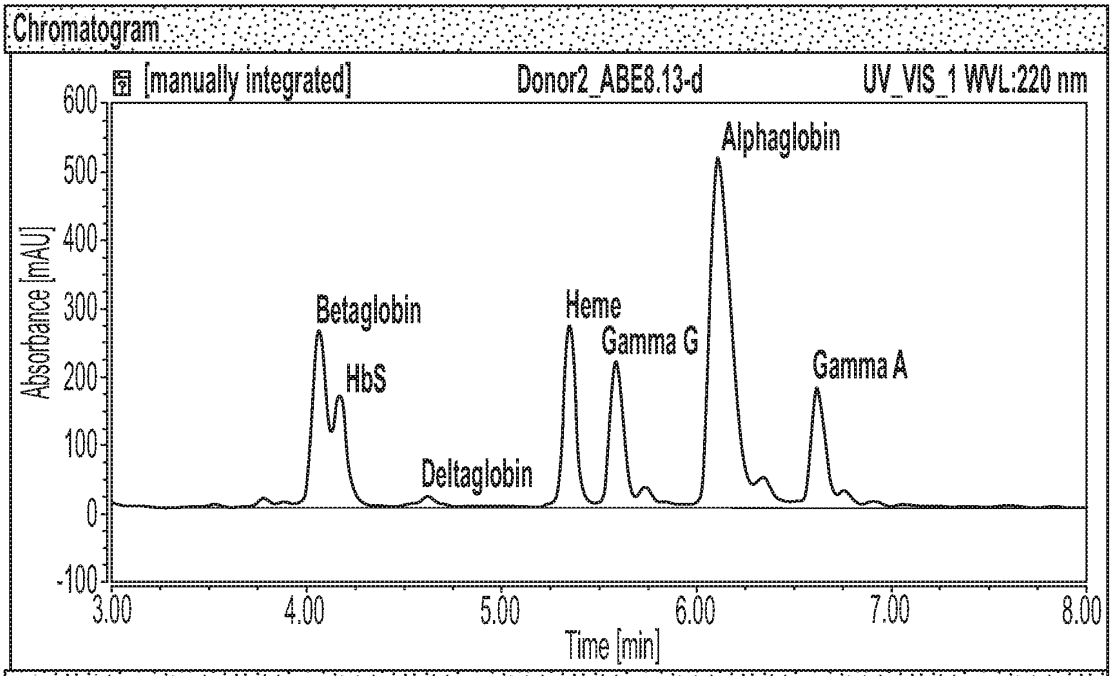
FIG. 25



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.057	24.649	303.680	12.77	15.05
7	HbS	4.163	16.705	190.174	8.65	9.43
10	Deltaglobin	4.607	2.114	20.478	1.10	1.01
15	Heme	5.340	22.158	298.385	11.48	14.79
16	Gamma G	5.583	20.107	236.242	10.41	11.71
19	Alphaglobin	6.093	71.587	568.640	37.08	28.18
22	Gamma A	6.623	16.259	184.774	8.42	9.16

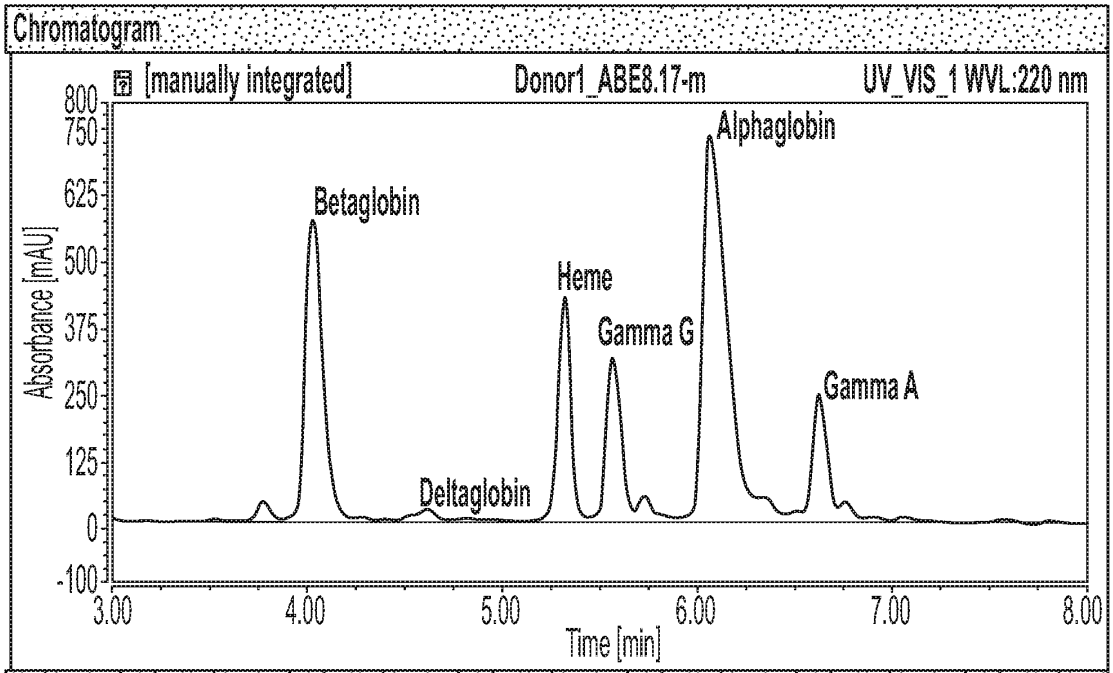
FIG. 26



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.057	21.912	260.274	12.79	14.49
6	HbS	4.163	13.772	163.531	8.04	9.10
9	Deltaglobin	4.617	1.827	16.318	1.07	0.91
13	Heme	5.347	19.932	268.078	11.64	14.92
14	Gamma G	5.587	18.026	215.798	10.52	12.01
17	Alphaglobin	6.110	64.064	513.833	37.40	28.61
20	Gamma A	6.620	15.335	177.074	8.95	9.86

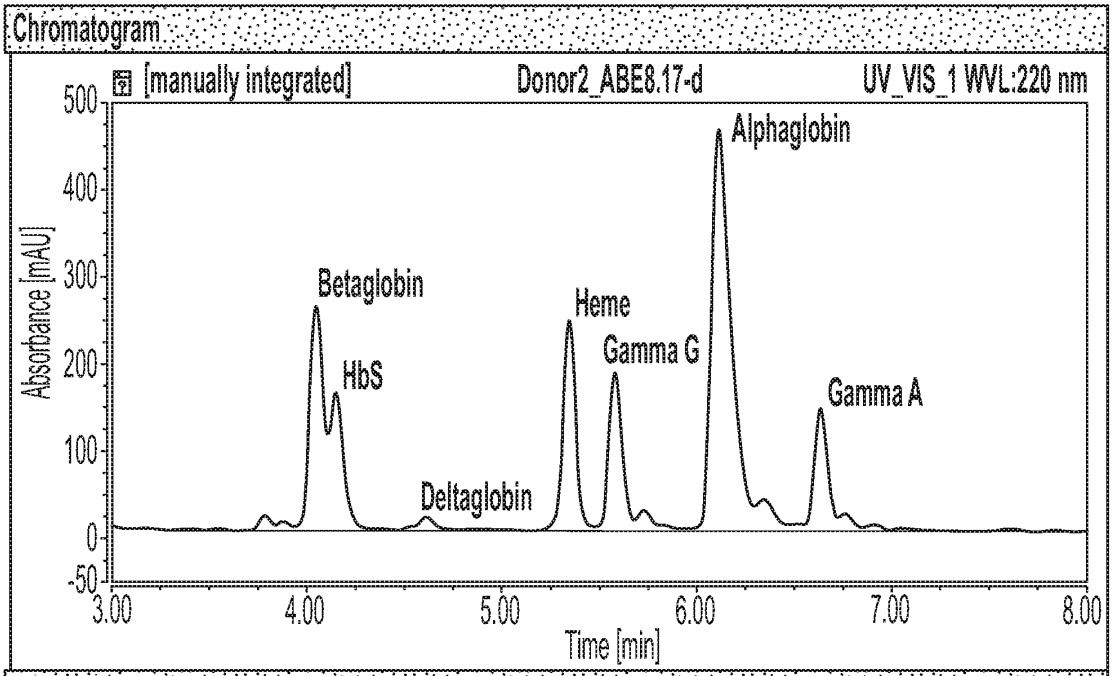
FIG. 27



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.030	59.000	569.475	21.35	21.91
n.a.	HbS	n.a.	n.a.	n.a.	n.a.	n.a.
9	Deltaglobin	4.617	2.483	24.660	0.90	0.95
13	Heme	5.323	31.792	426.360	11.50	16.40
14	Gamma G	5.567	28.258	310.274	10.22	11.94
16	Alphaglobin	6.067	104.586	728.120	37.84	28.01
19	Gamma A	6.627	21.512	243.710	7.78	9.37

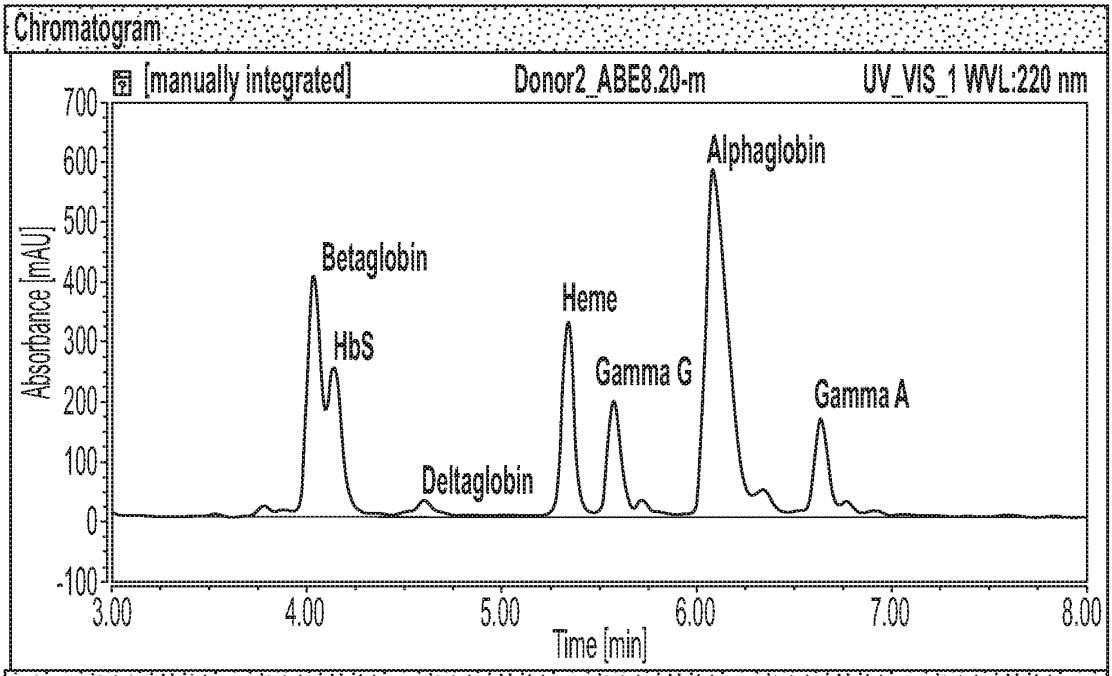
FIG. 28



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.050	20.688	260.391	13.65	15.97
6	HbS	4.150	13.530	159.994	8.93	9.81
9	Deltaglobin	4.617	1.590	15.794	1.05	0.97
14	Heme	5.350	18.069	242.095	11.92	14.85
15	Gamma G	5.583	14.825	182.475	9.78	11.19
18	Alphaglobin	6.117	57.055	464.210	37.65	28.48
21	Gamma A	6.637	11.691	142.851	7.72	8.76

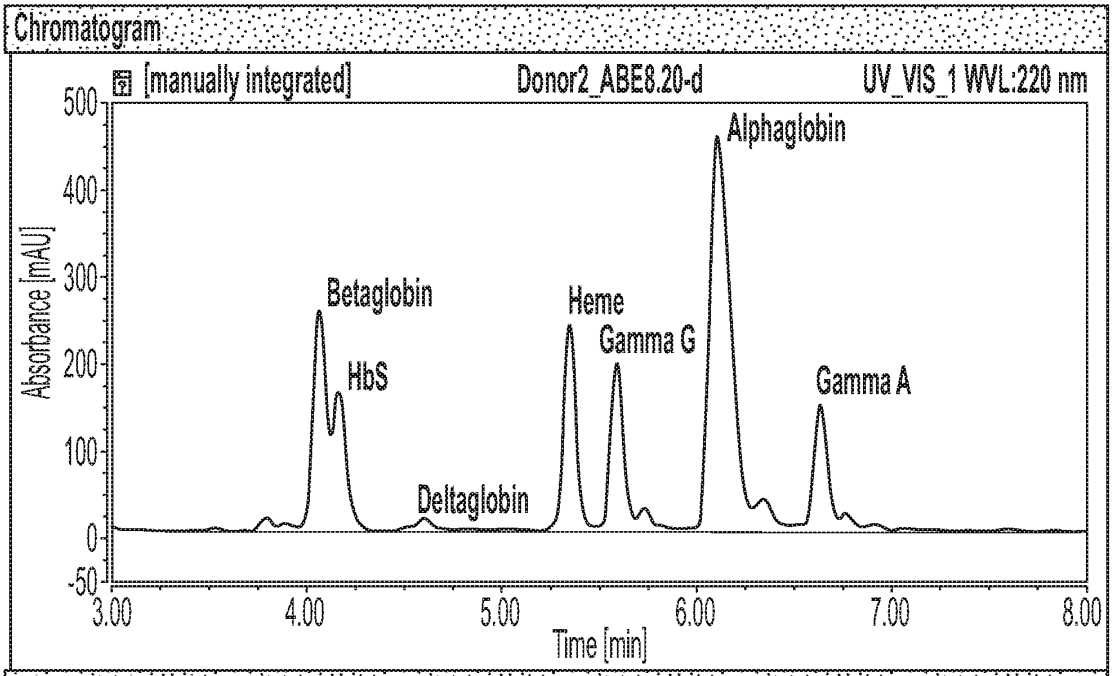
FIG. 29



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
6	Betaglobin	4.040	33.339	402.775	15.75	18.52
7	HbS	4.143	22.030	252.466	10.41	11.61
10	Deltaglobin	4.607	3.256	29.080	1.54	1.34
14	Heme	5.343	24.399	328.365	11.53	15.10
15	Gamma G	5.580	15.959	197.001	7.54	9.06
18	Alphaglobin	6.087	78.149	581.487	36.93	26.74
21	Gamma A	6.637	14.315	166.369	6.76	7.65

FIG. 30A



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
5	Betaglobin	4.067	20.307	253.509	13.46	15.57
6	HbS	4.163	12.923	160.772	8.57	9.87
9	Deltaglobin	4.607	1.534	14.720	1.02	0.90
14	Heme	5.350	17.781	237.713	11.79	14.60
15	Gamma G	5.593	15.582	195.753	10.33	12.02
18	Alphaglobin	6.107	56.188	456.808	37.25	28.05
21	Gamma A	6.633	12.248	146.288	8.12	8.98

FIG. 30B

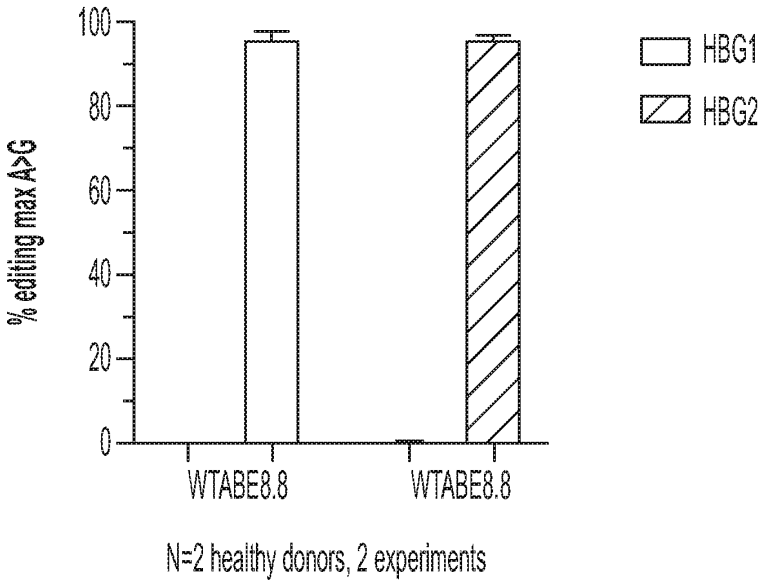


FIG. 31A

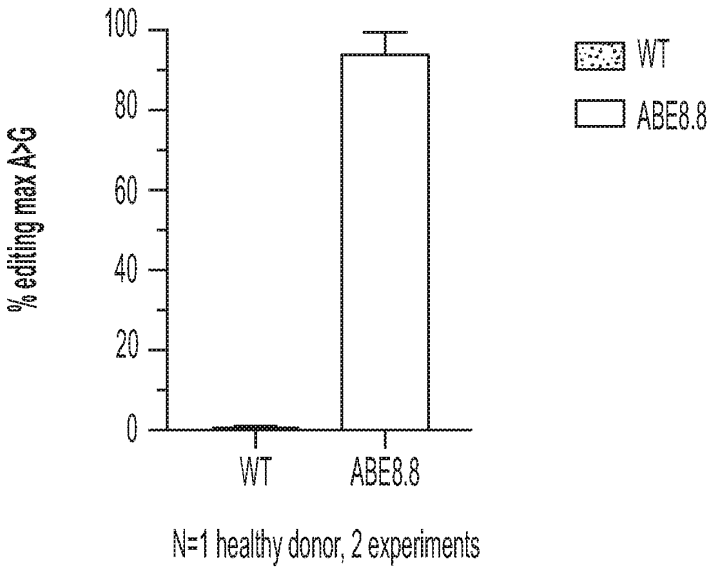


FIG. 31B

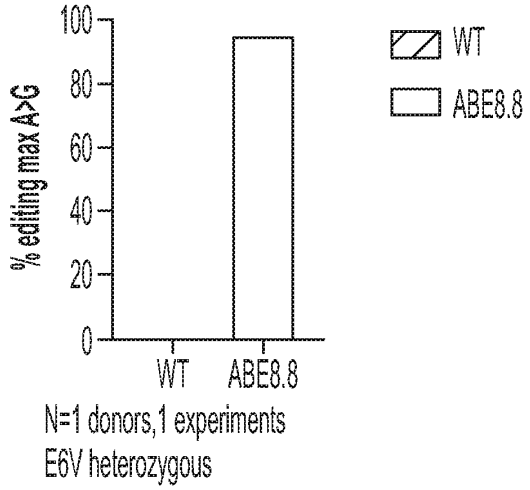


FIG. 31C

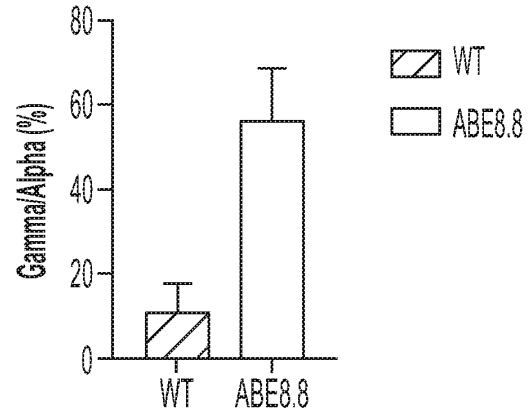


FIG. 31D

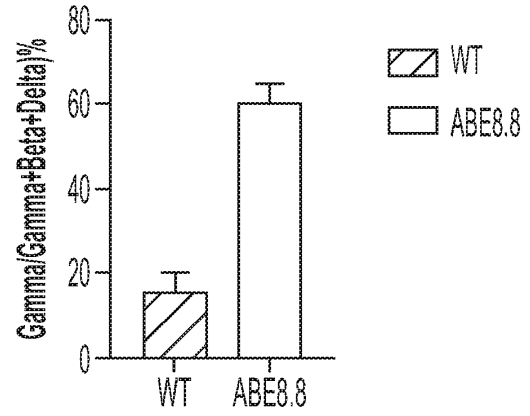


FIG. 31E

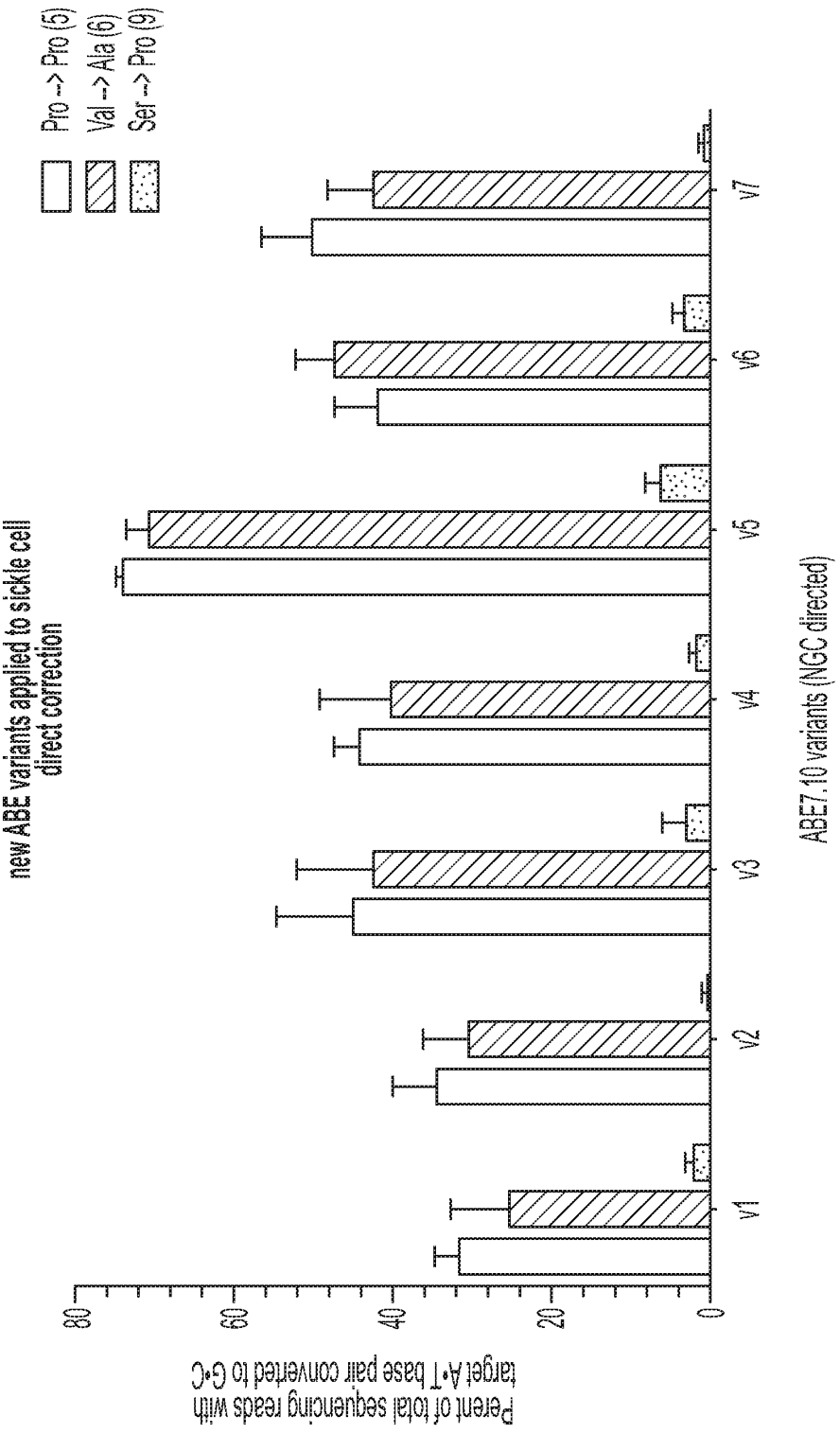

Pro --> Pro (5)
Val --> Ala (6)
Ser --> Pro (9)
new ABE variants applied to sickle cell direct correction
Perent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
v1
v2
v3
v4
v5
v6
v7
ABE7.10 variants (NGC directed)

FIG. 32A

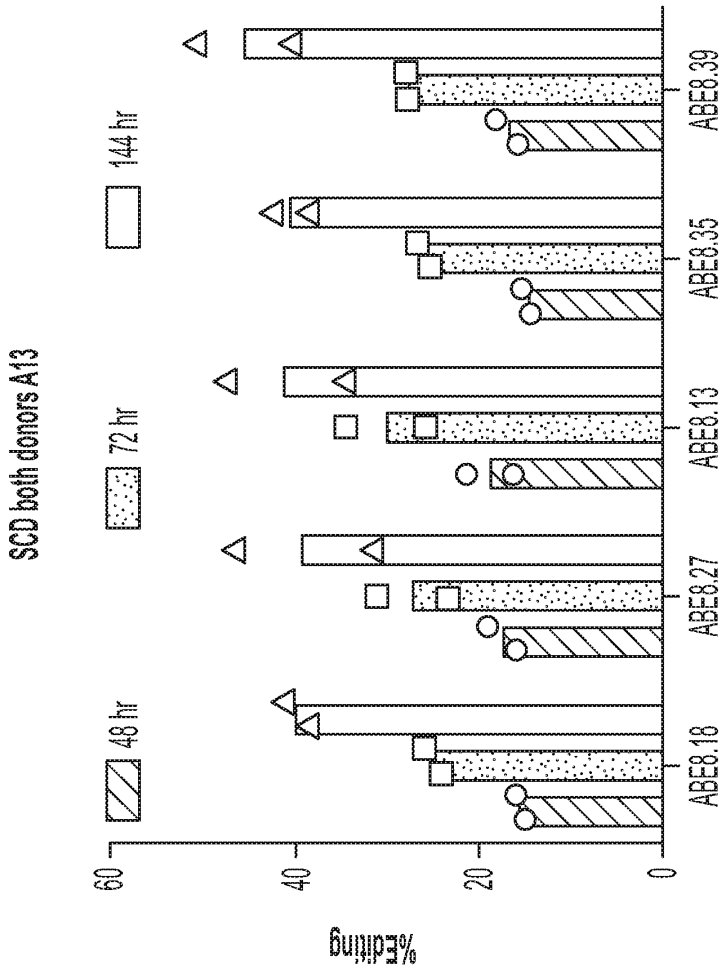

SCD both donors A13
48 hr
72 hr
144 hr
%editing
60
40
20
0
ABE8.18
ABE8.27
ABE8.13
ABE8.35
ABE8.39

FIG. 32B

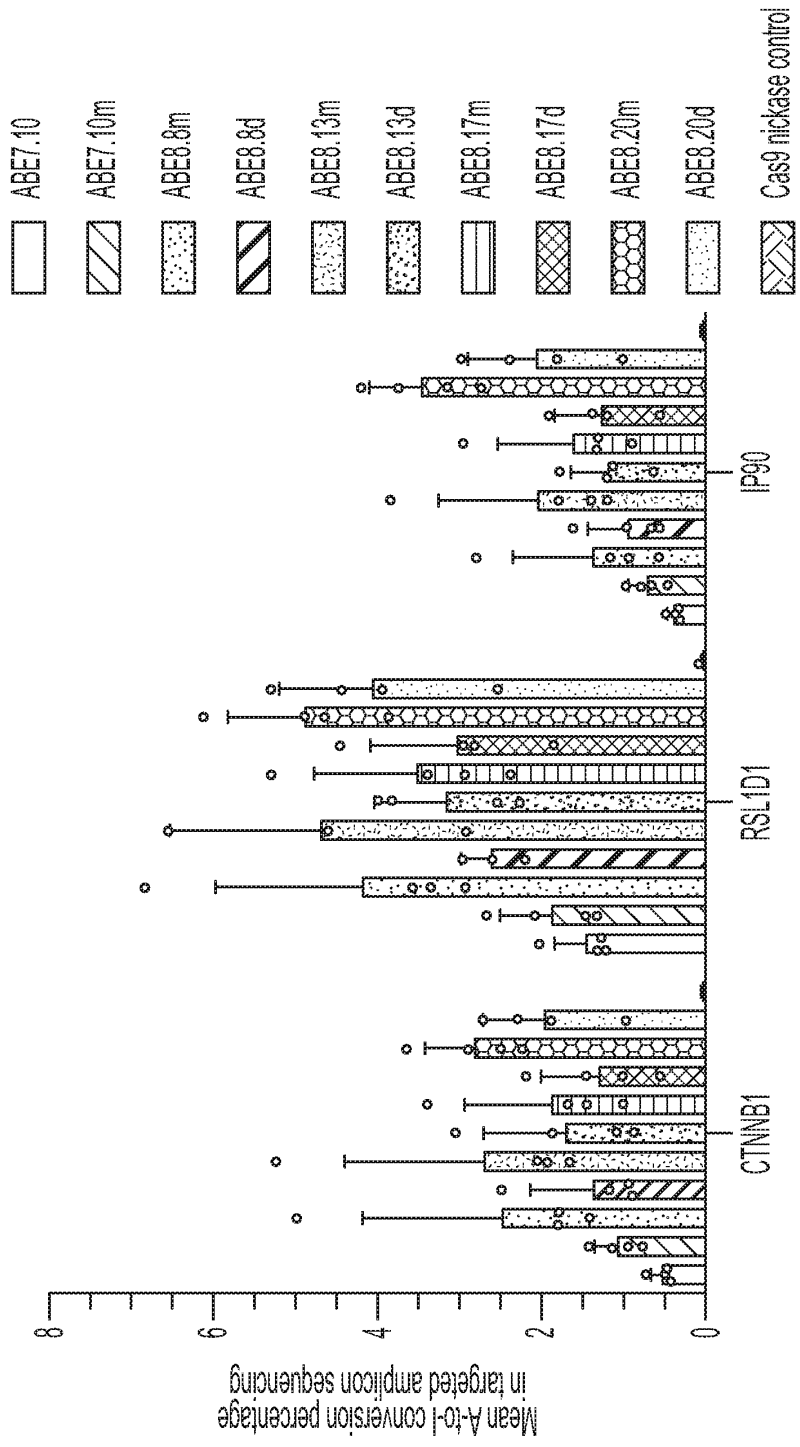

ABE7.10
ABE7.10m
ABE8.8m
ABE8.8d
ABE8.13m
ABE8.13d
ABE8.17m
ABE8.17d
ABE8.20m
ABE8.20d
Cas9 nickase control
IP90
RSL1D1
CTNNB1
0
2
4
6
8
Mean A-to-I conversion percentage
in targeted amplicon sequencing

FIG. 33A

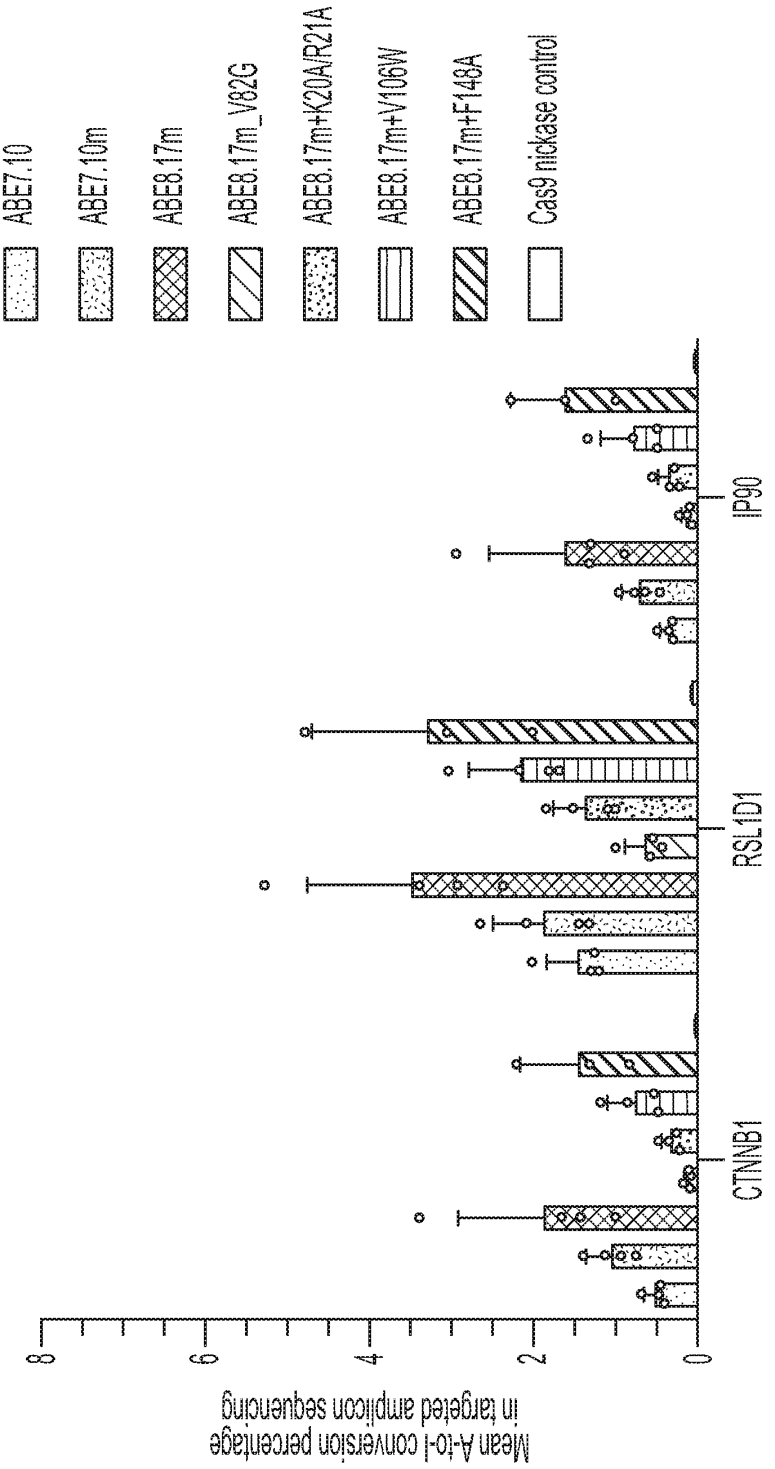

ABE7.10
ABE7.10m
ABE8.17m
ABE8.17m_V82G
ABE8.17m+K20A/R21A
ABE8.17m+V106W
ABE8.17m+F148A
Cas9 nickase control
Mean A-to-I conversion percentage in targeted amplicon sequencing
0
2
4
6
8
CTNNB1
RSL1D1
IP90

FIG. 33B

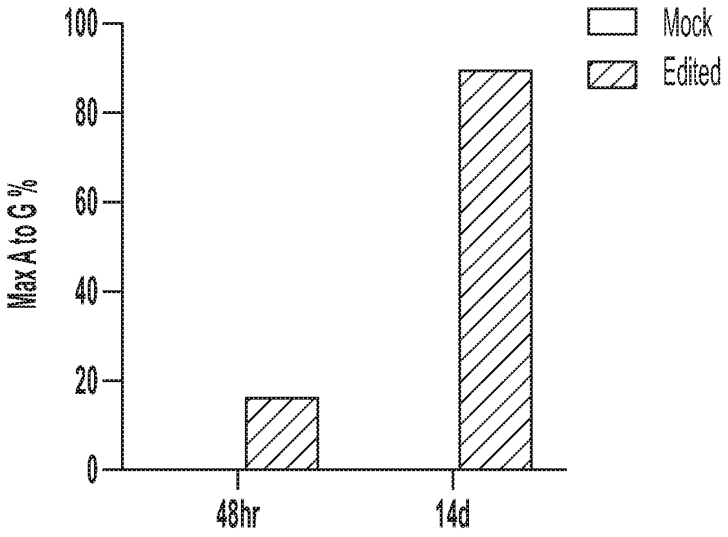


FIG. 34A

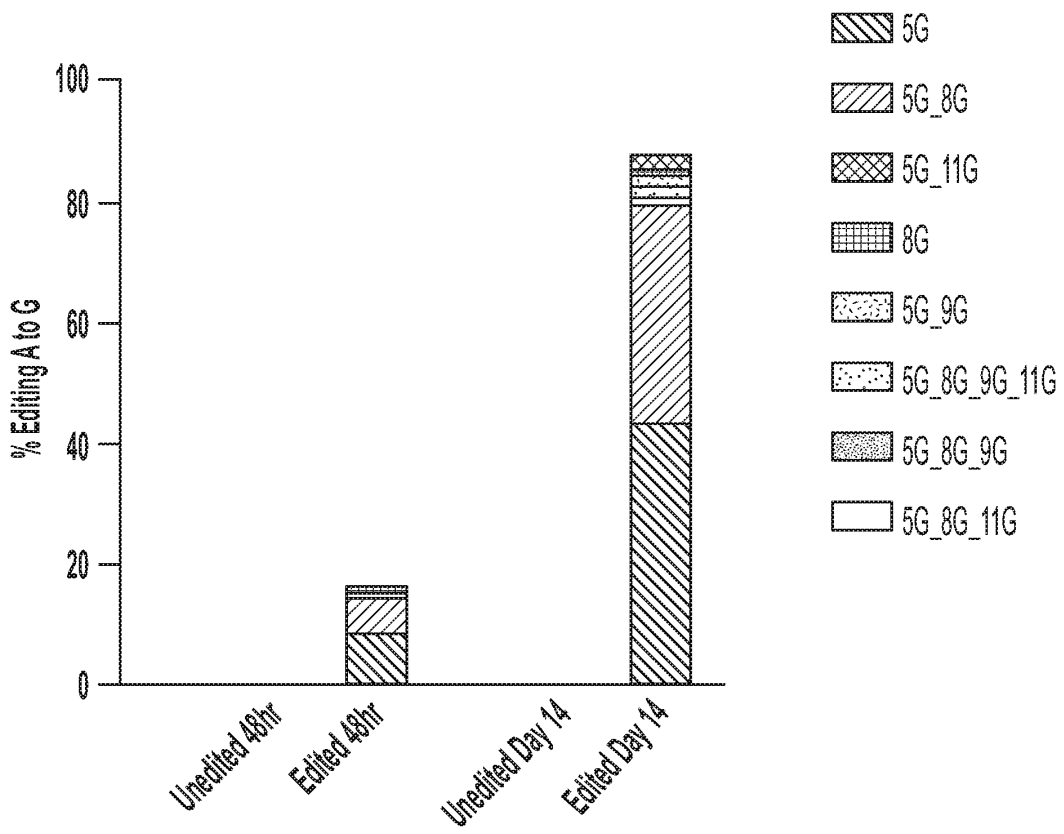

% Editing A to G
100
80
60
40
20
0
Unedited 48hr
Edited 48hr
Unedited Day 14
Edited Day 14
5G
5G_8G
5G_11G
8G
5G_9G
5G_8G_9G_11G
5G_8G_9G
5G_8G_11G

FIG. 34B

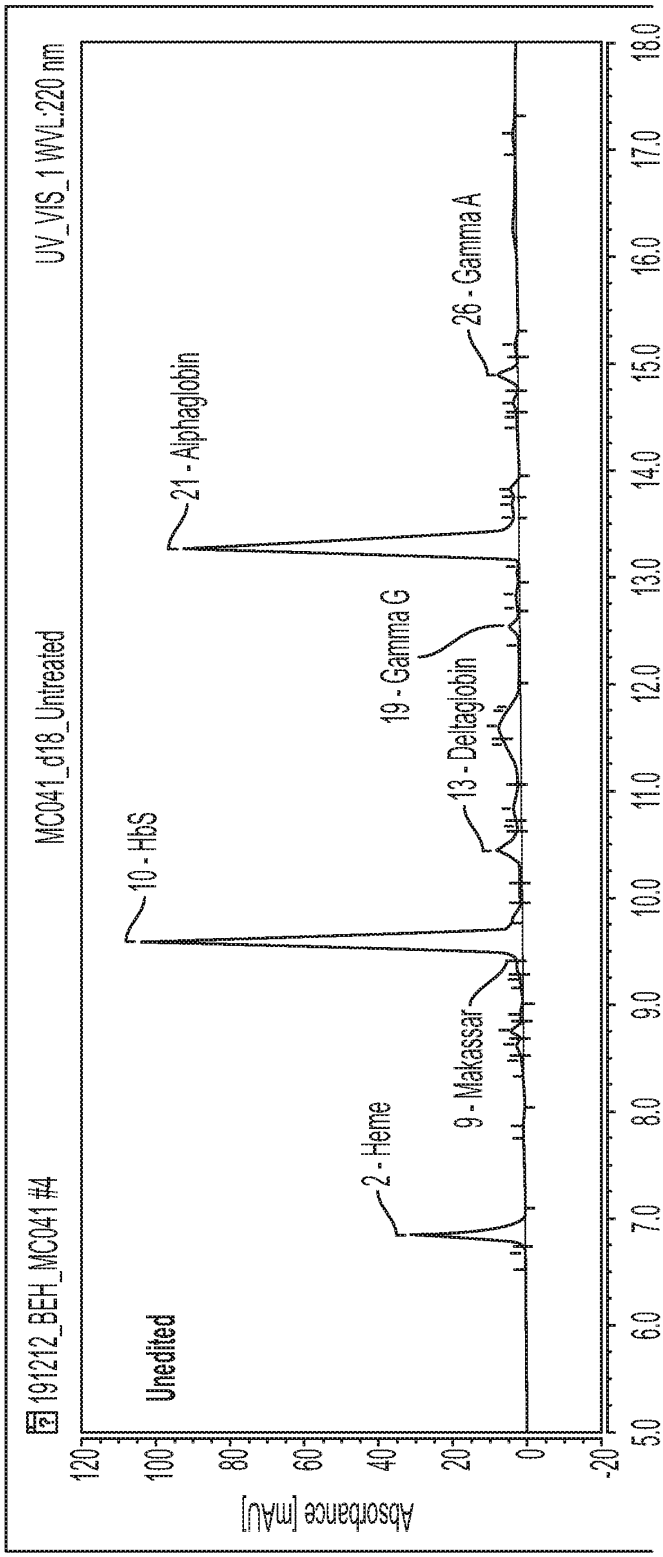

191212_BEH_MC041 #4
MC041_d18_Untreated
UV_VIS_1 WVL:220 nm
Unedited
Absorbance [mAU]
120
100
80
60
40
20
0
-20
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
2 - Heme
9 - Makassar
10 - HbS
13 - Deltaglobin
19 - Gamma G
21 - Alphaglobin
26 - Gamma A

FIG. 35A

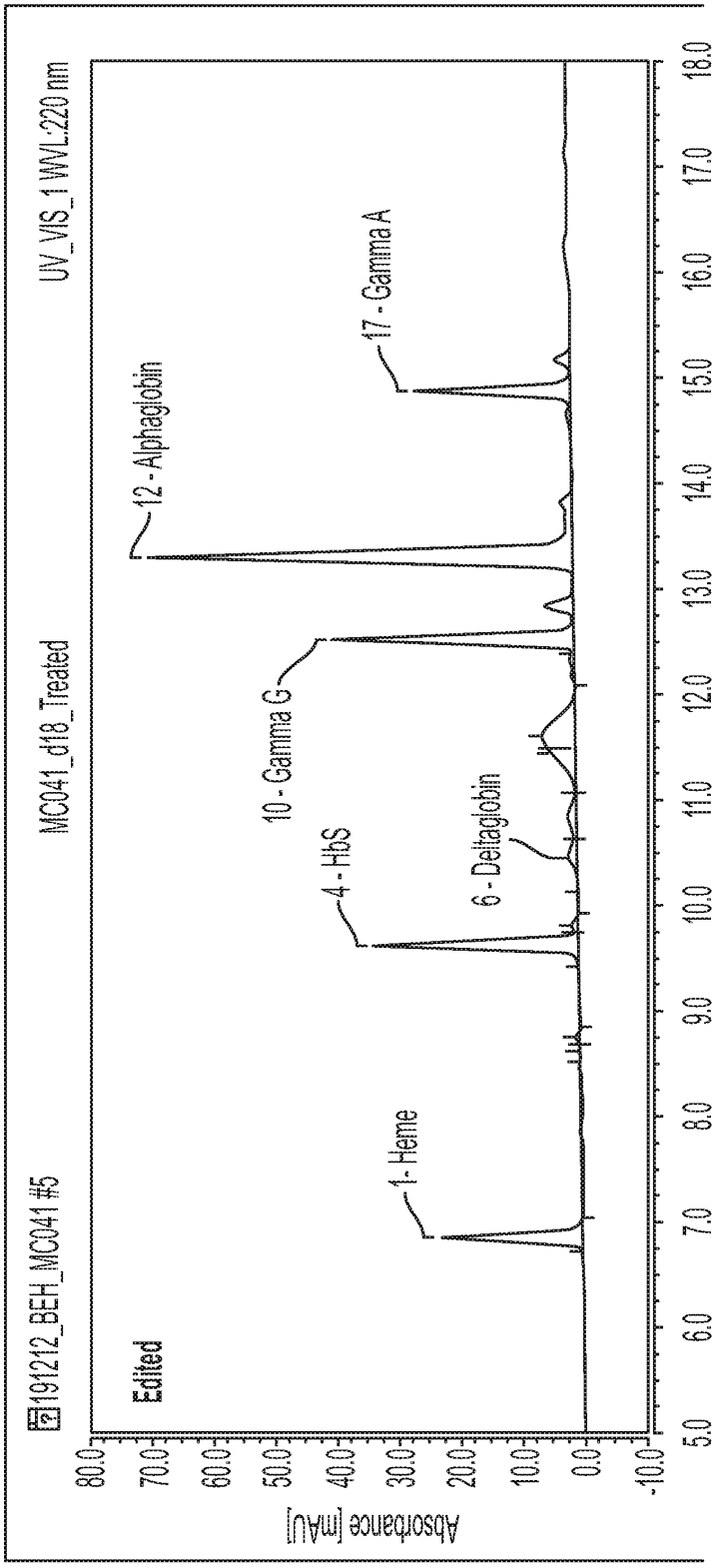

191212_BEH_MC041 #5
MC041_d18_Treated
UV_VIS_1 WVL:220 nm
Edited
Absorbance [mAU]
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
1 - Heme
4 - HbS
6 - Deltaglobin
10 - Gamma G
12 - Alphaglobin
17 - Gamma A

FIG. 35B

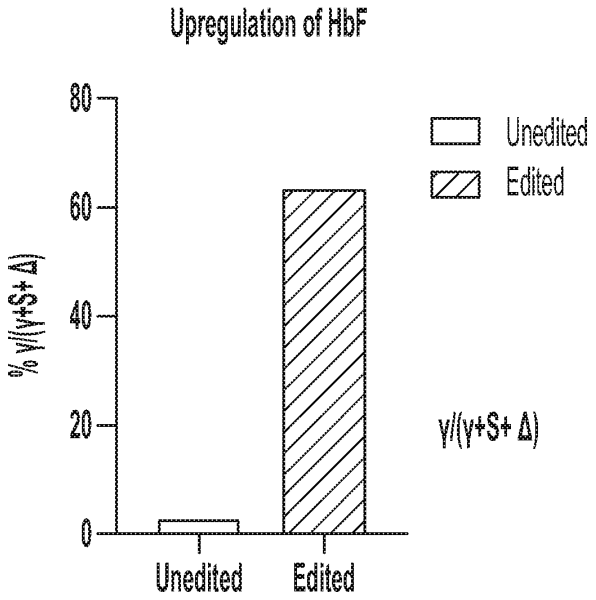


FIG. 35C

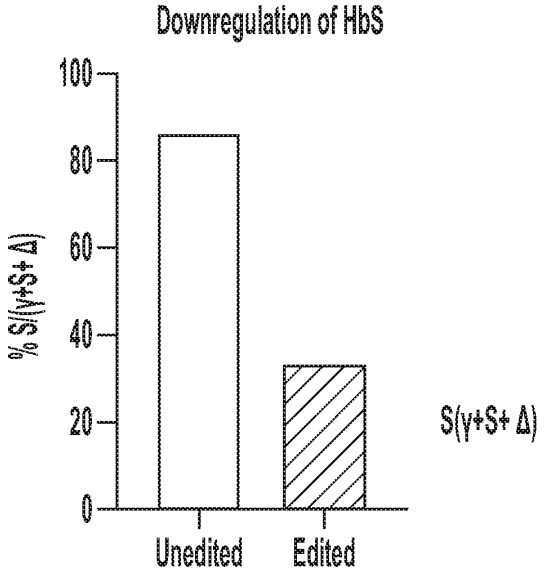


FIG. 35D

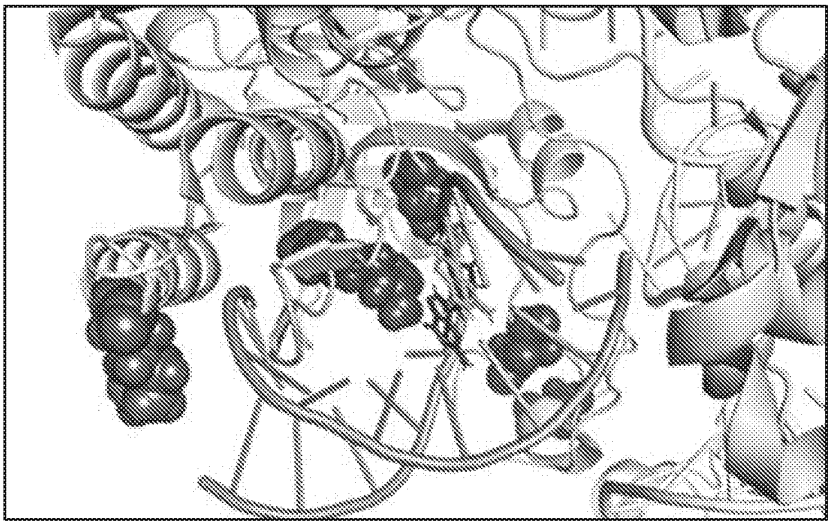
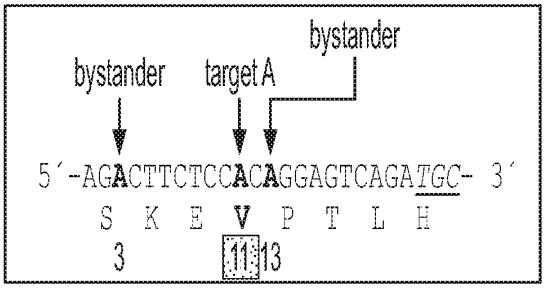


FIG. 36A



PAM	L1111	D1135	S1136	S1216	G1218	E1219	A1322	R1335	T1337
NGC		M	Q		K	S		E	R
NGA		V			R			Q	R
NGCG		V			R			E	R
NGN	R	V			R	F	R	A	R
NGC		G		G				Q	
						V			

FIG. 36B

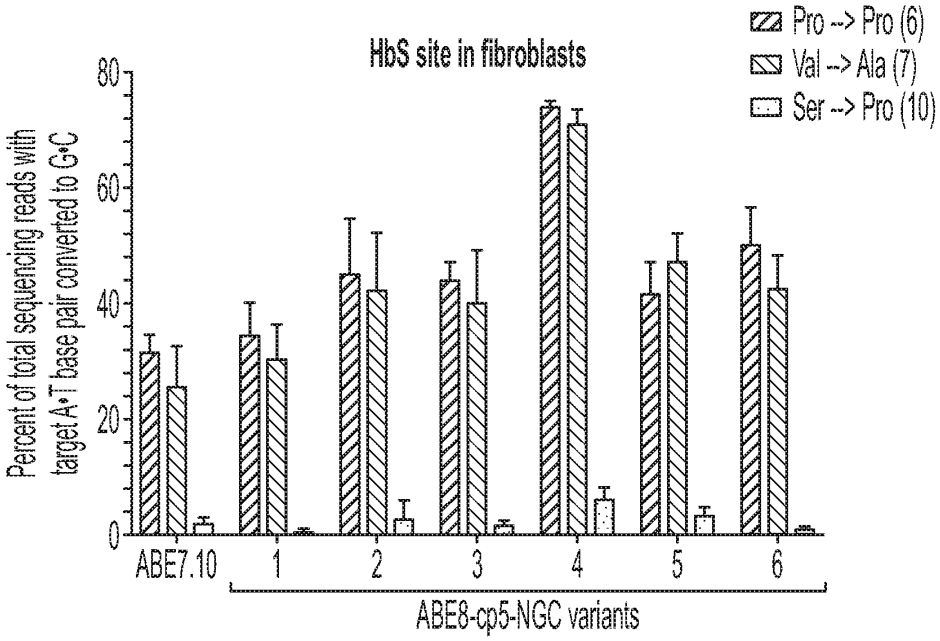

HbS site in fibroblasts
Pro --> Pro (6)
Val --> Ala (7)
Ser --> Pro (10)
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
ABE7.10
1
2
3
4
5
6
ABE8-cp5-NGC variants

FIG. 36C

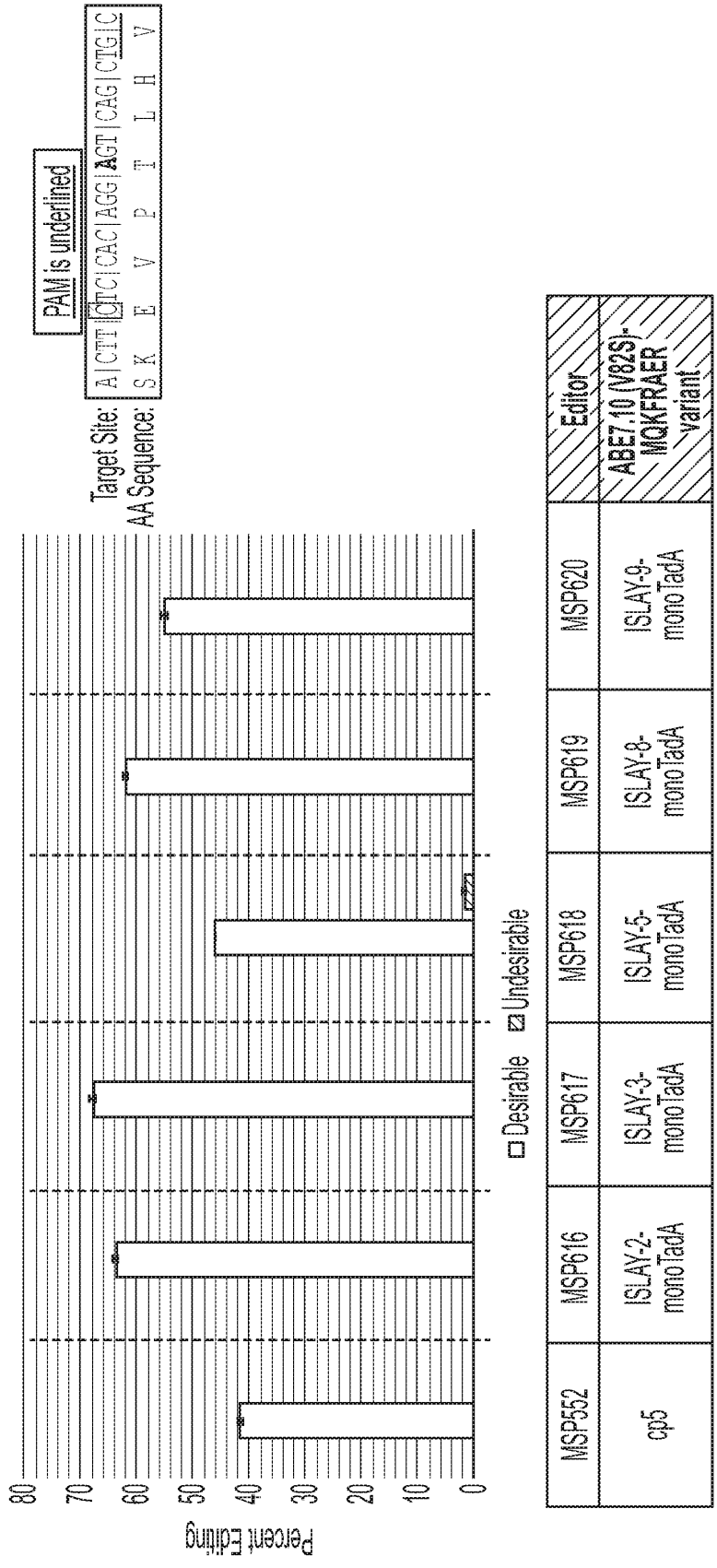

PAM is underlined
Target Site: A | CTT | CT | C | CAC | AGG | AGT | CAG | CTG | C
AA Sequence: S K E V P T L H V
Percent Editing
0
10
20
30
40
50
60
70
80
Desirable
Undesirable
MSP552
MSP616
MSP617
MSP618
MSP619
MSP620
Editor
cp5
ISLAY-2-monoTadA
ISLAY-3-monoTadA
ISLAY-5-monoTadA
ISLAY-8-monoTadA
ISLAY-9-monoTadA
ABE7.10 (V82S)-MQKFRAER variant

FIG. 37

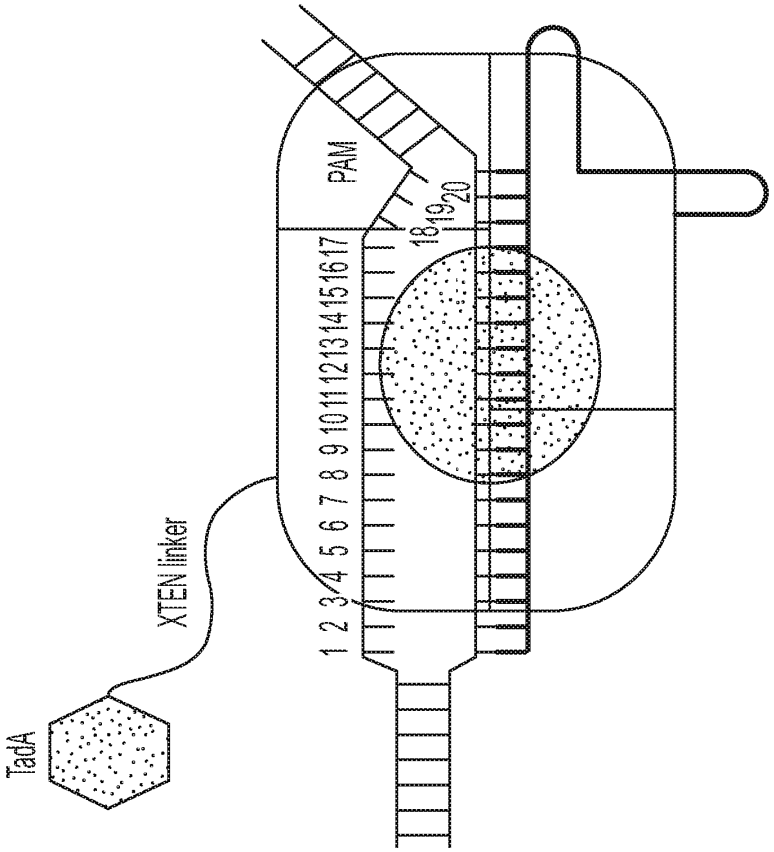


FIG. 38

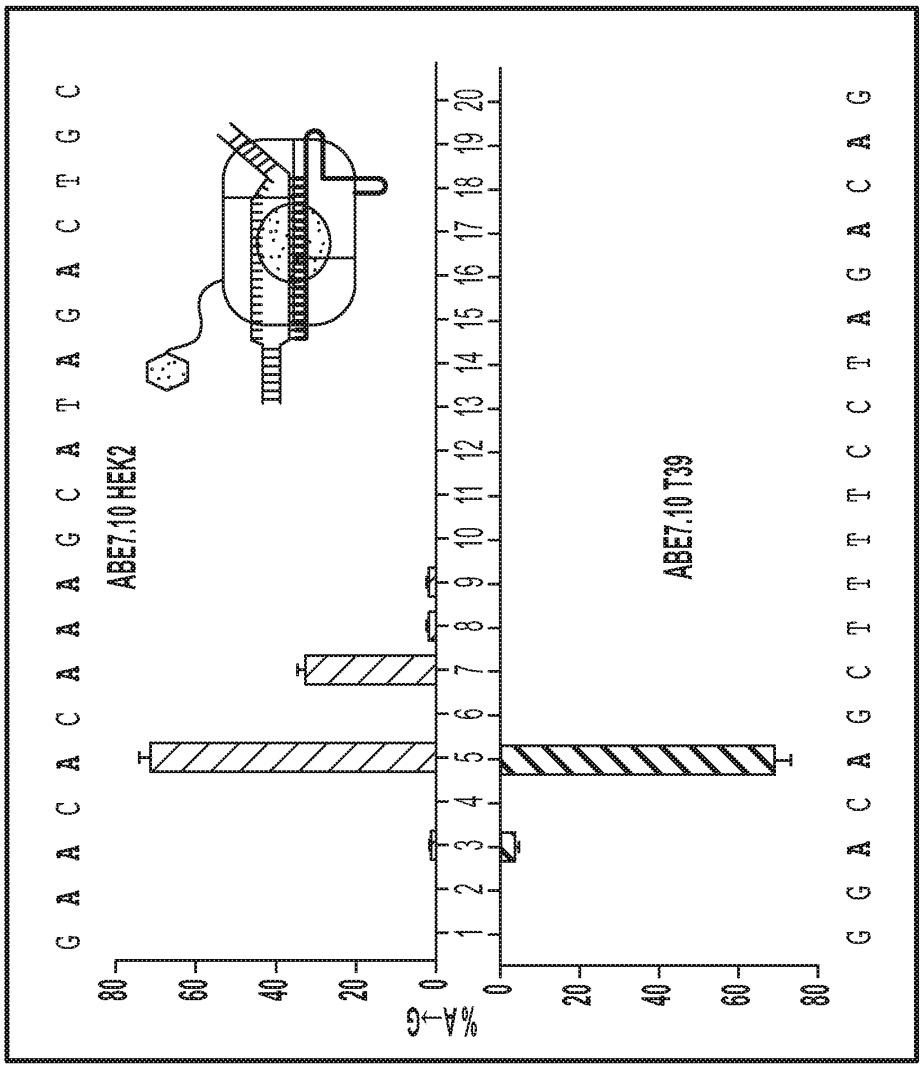


FIG. 38 CONTINUED

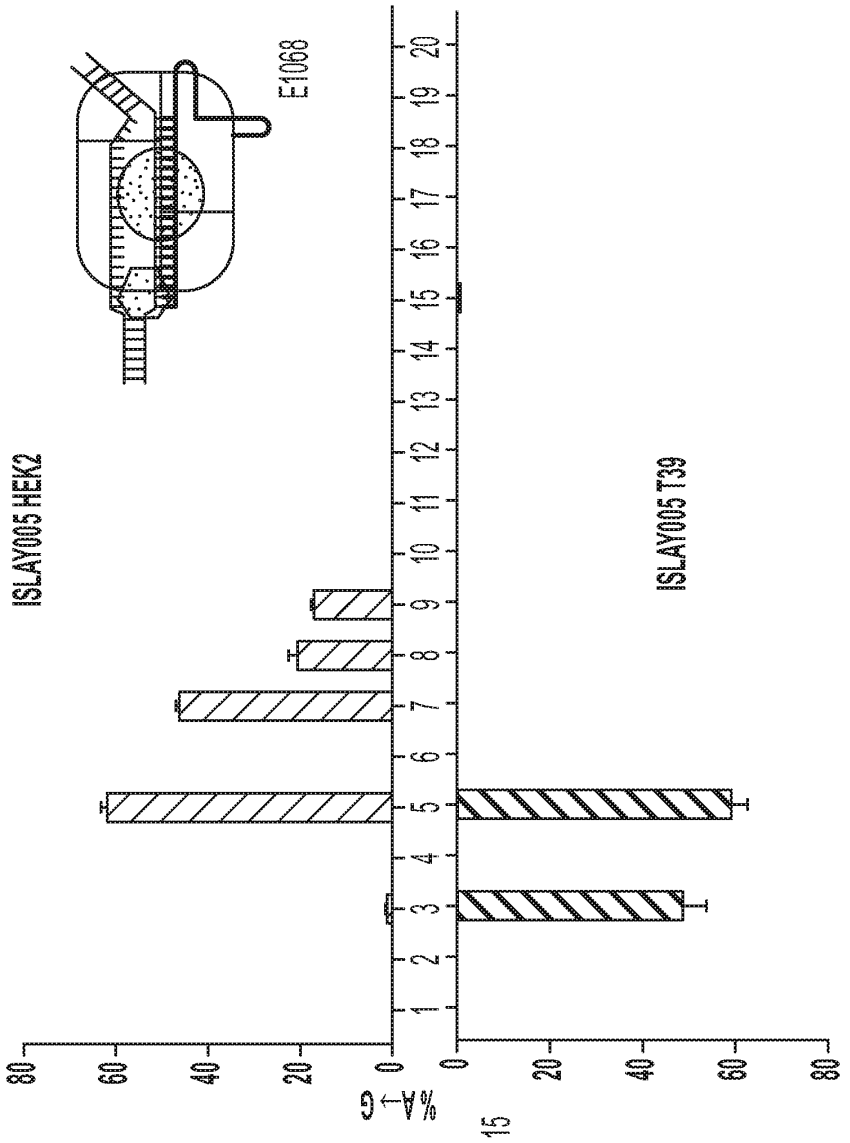


FIG. 38 CONTINUED

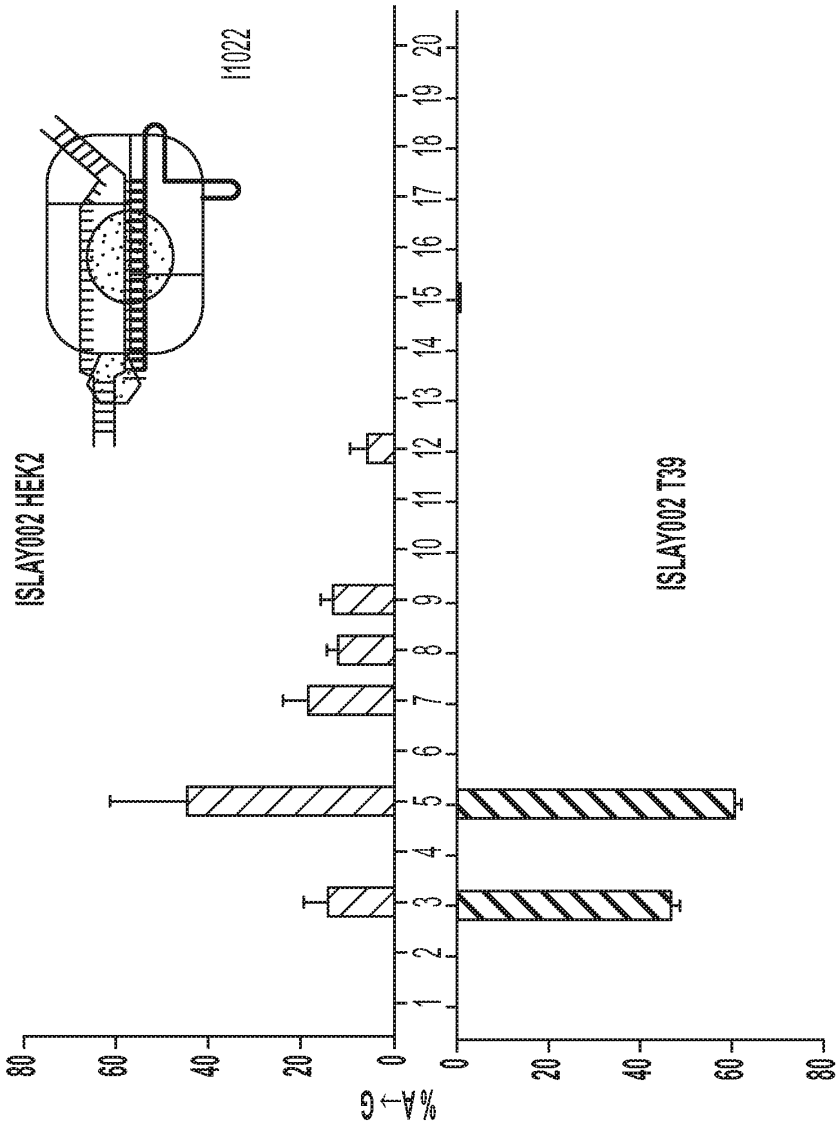


FIG. 38 CONTINUED

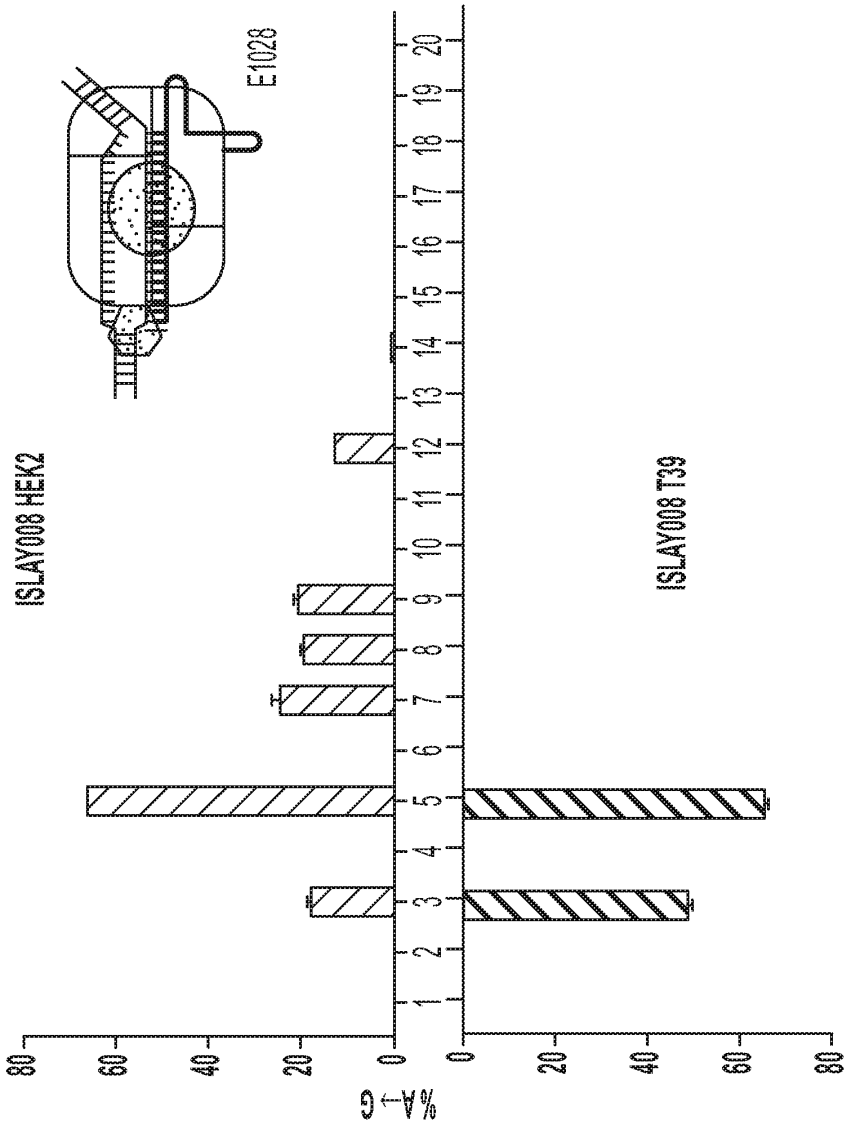


FIG. 38 CONTINUED

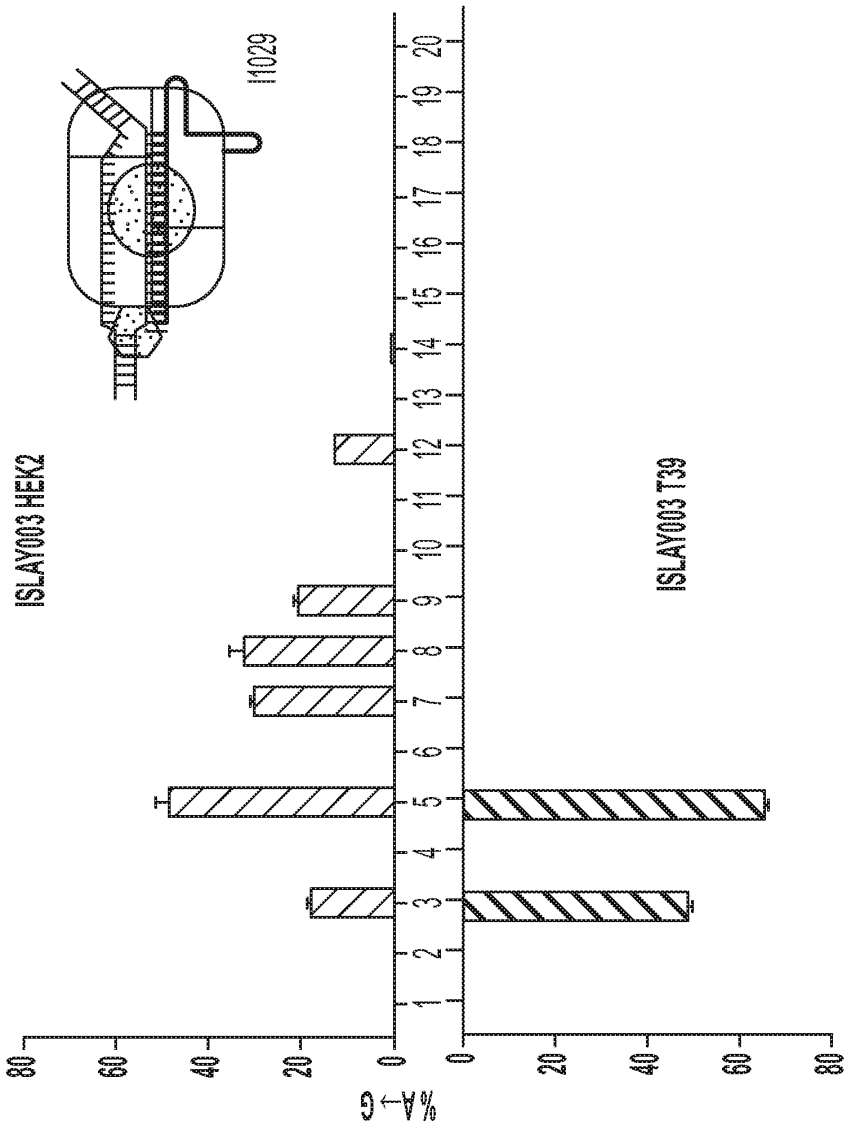


FIG. 38 CONTINUED

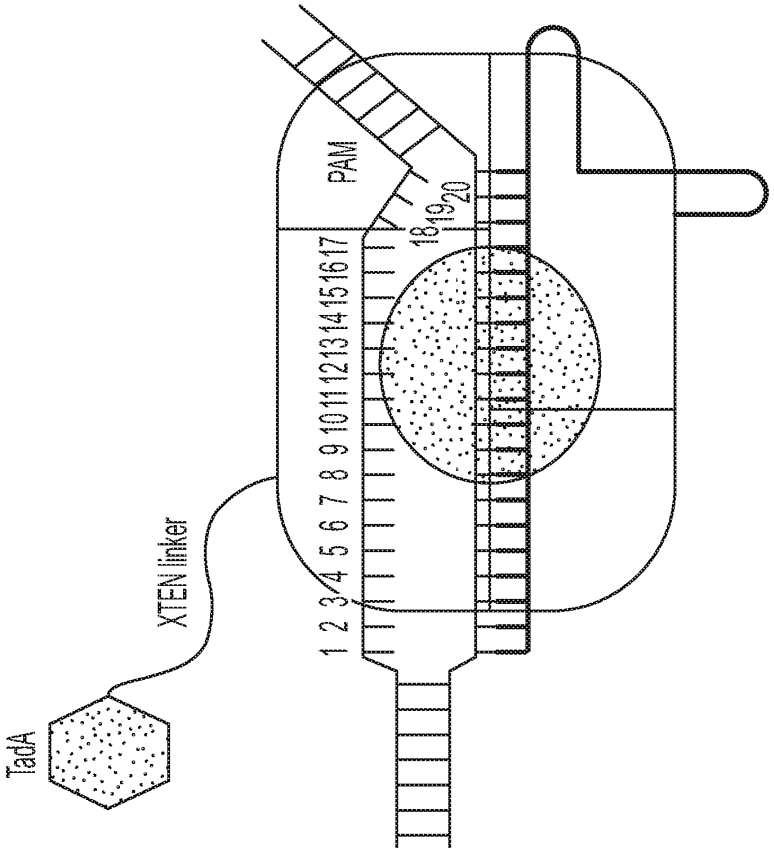


FIG. 39

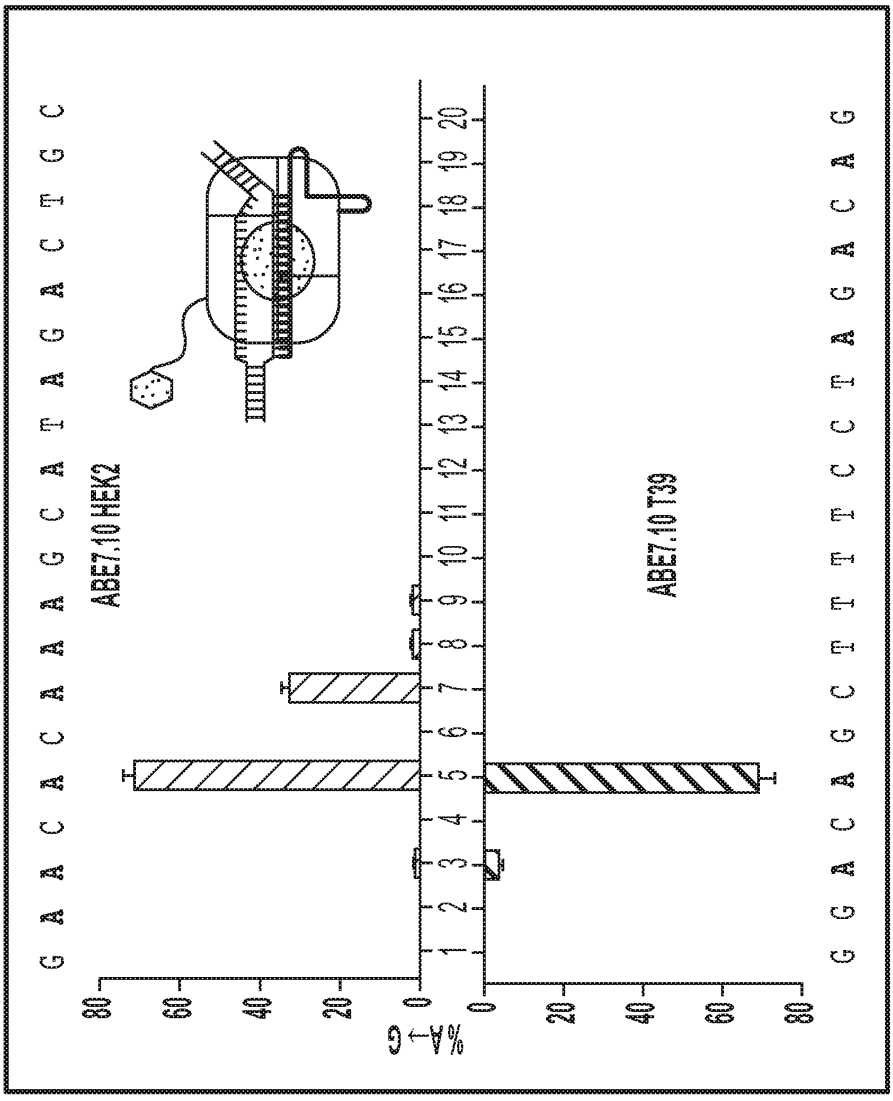


FIG. 39 CONTINUED

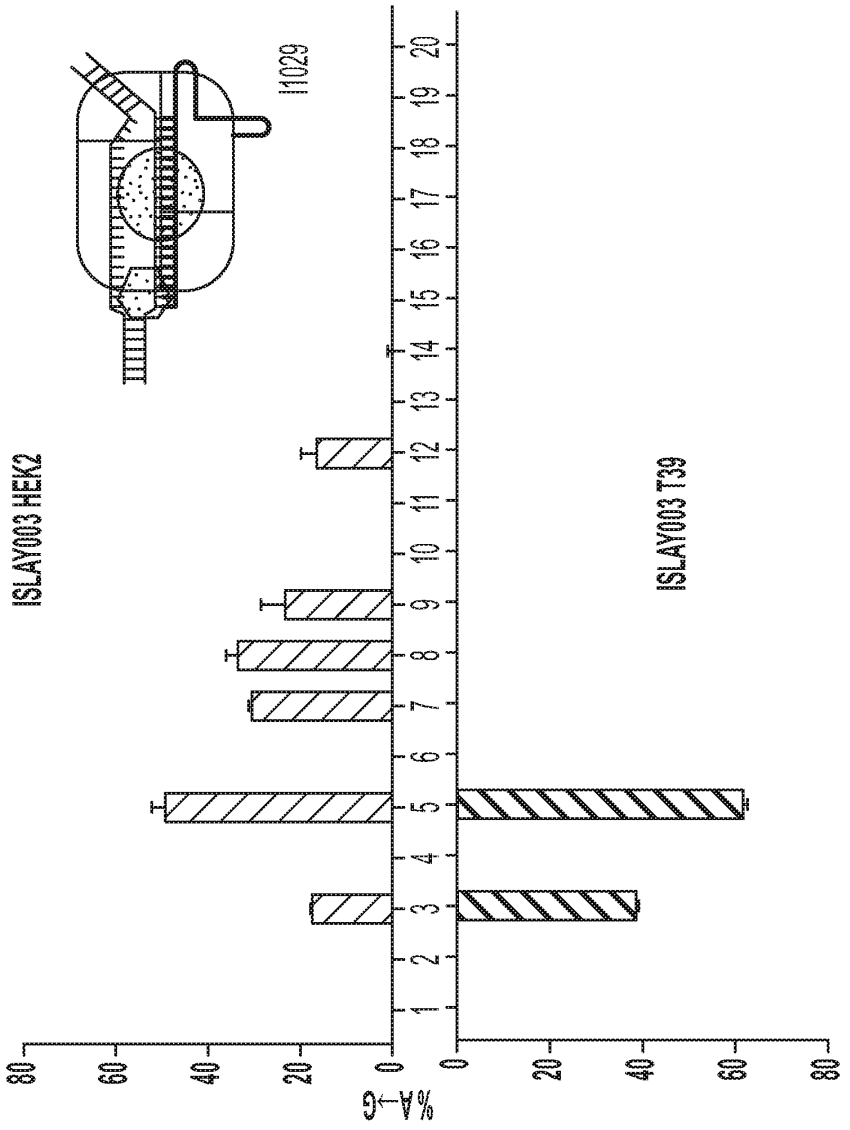


FIG. 39 CONTINUED

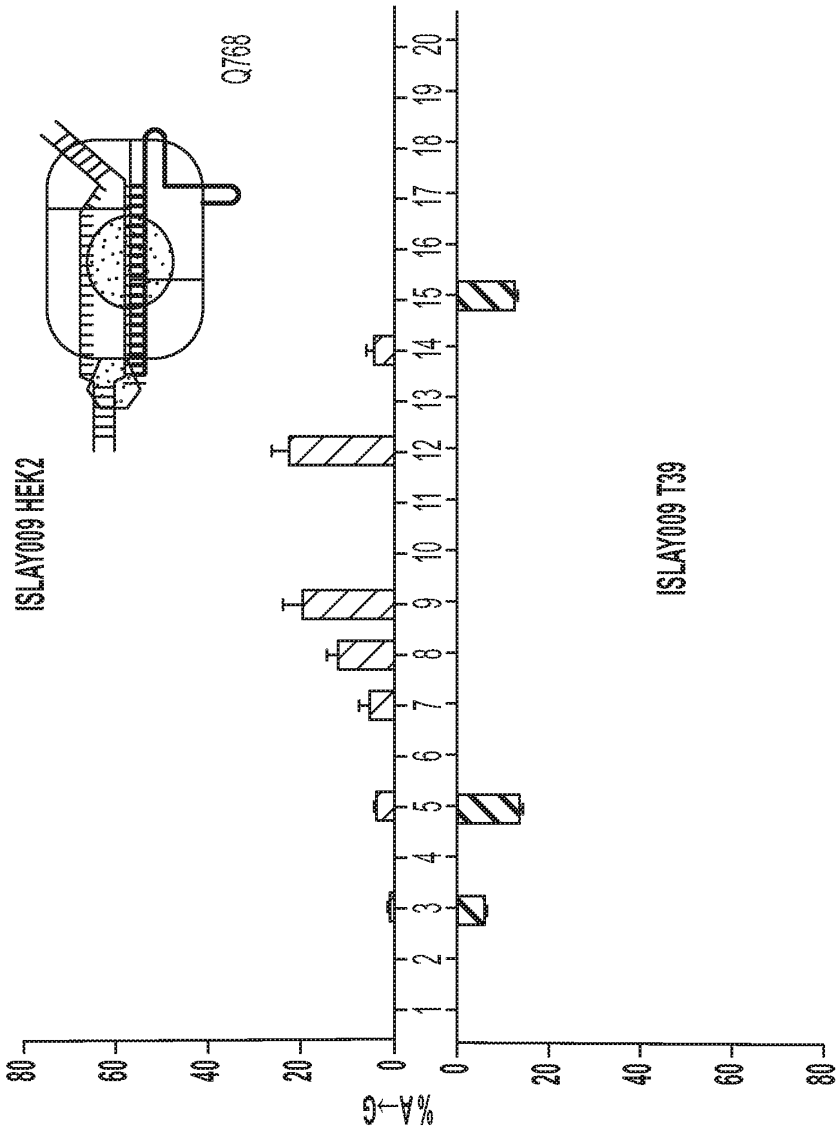


FIG. 39 CONTINUED

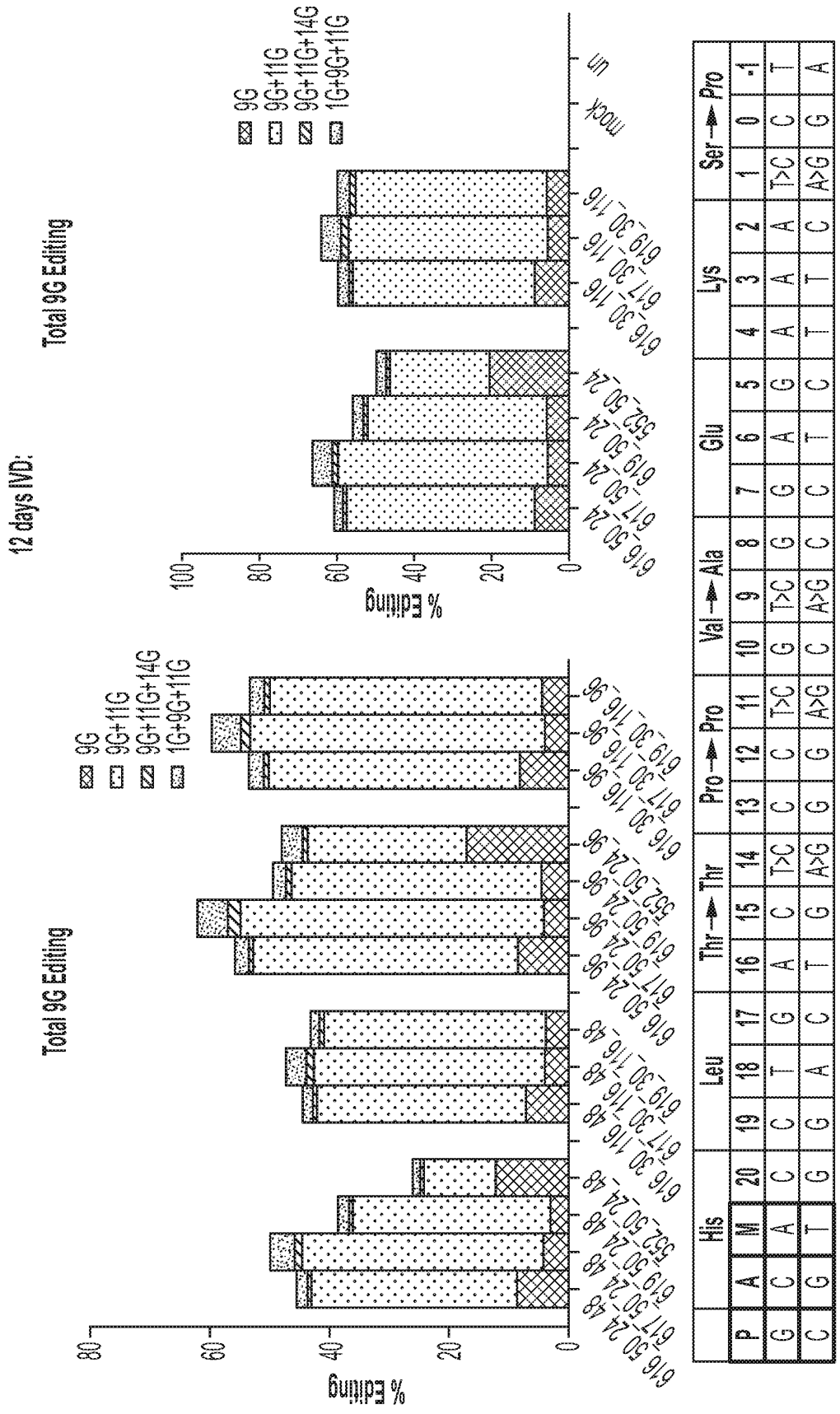

12 days IVD:
Total 9G Editing
% Editing
9G
9G+11G
9G+11G+14G
1G+9G+11G
mock
un
6/16_30_116
6/17_30_116
6/19_30_116
552_50_24
6/19_50_24
6/17_50_24
6/16_50_24
6/16_30_116_96
6/17_30_116_96
6/19_30_116_96
6/16_50_24_96
6/17_50_24_96
6/19_50_24_96
552_50_24_96
6/16_30_116_48
6/17_30_116_48
6/19_30_116_48
552_50_24_48
6/19_50_24_48
6/17_50_24_48
6/16_50_24_48
His
Leu
Thr → Thr
Pro → Pro
Val → Ala
Glu
Lys
Ser → Pro

FIG. 40

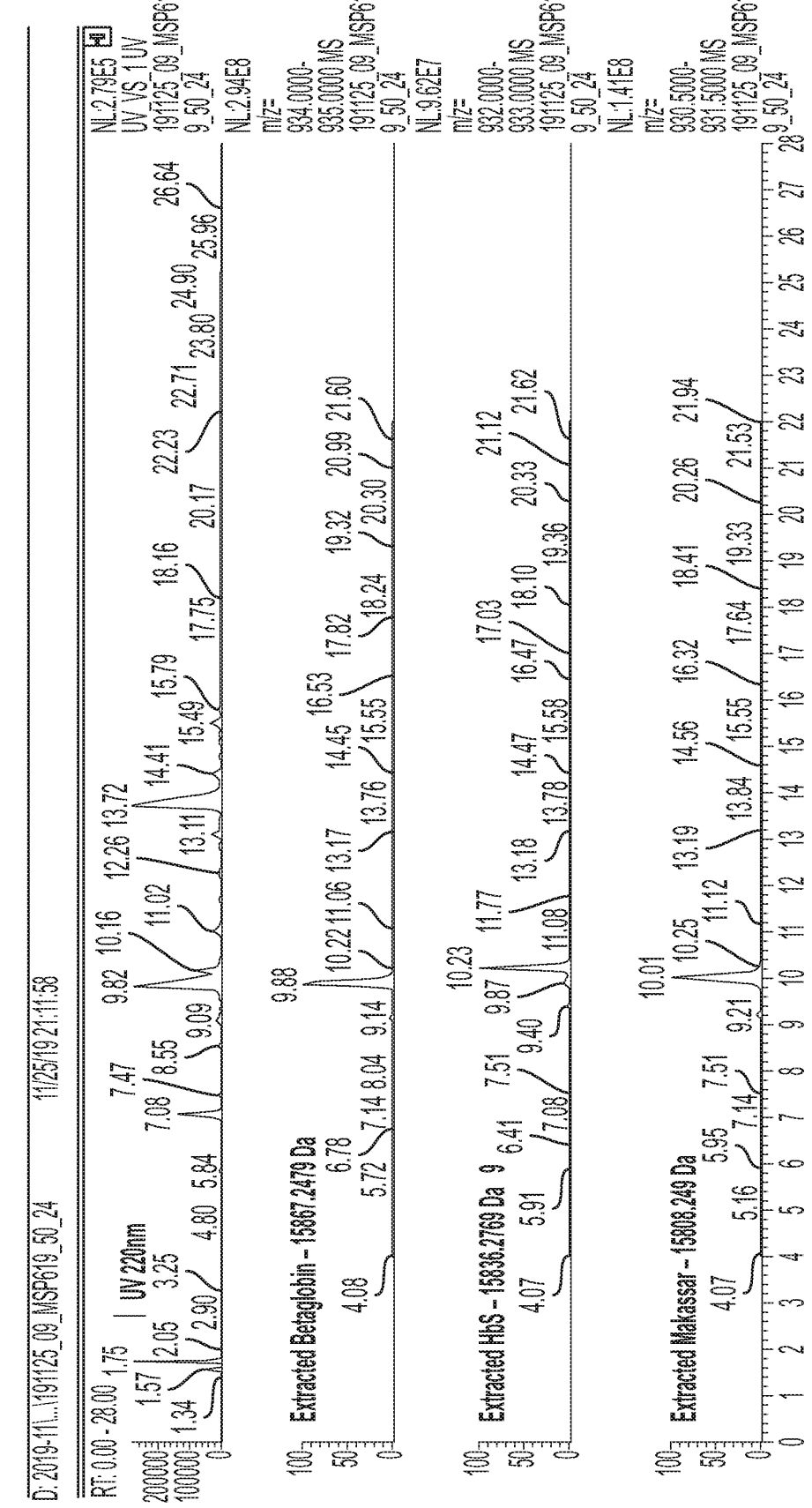

D:\2019-11\...\191125_09_MSP619_50_24
11/25/19 21:11:58
RT: 0.00 - 28.00
UV 220nm
NL:2.79E5
UV_VS_1 UV
191125_09_MSP61
9_50_24
NL:2.94E8
m/z=
934.0000-
935.0000 MS
NL:9.62E7
932.0000-
933.0000 MS
NL:1.41E8
930.5000-
931.5000 MS
Extracted Betaglobin – 15887.2479 Da
Extracted HbS – 15836.2769 Da
Extracted Makassar – 15808.249 Da
Time (min)

FIG. 41A

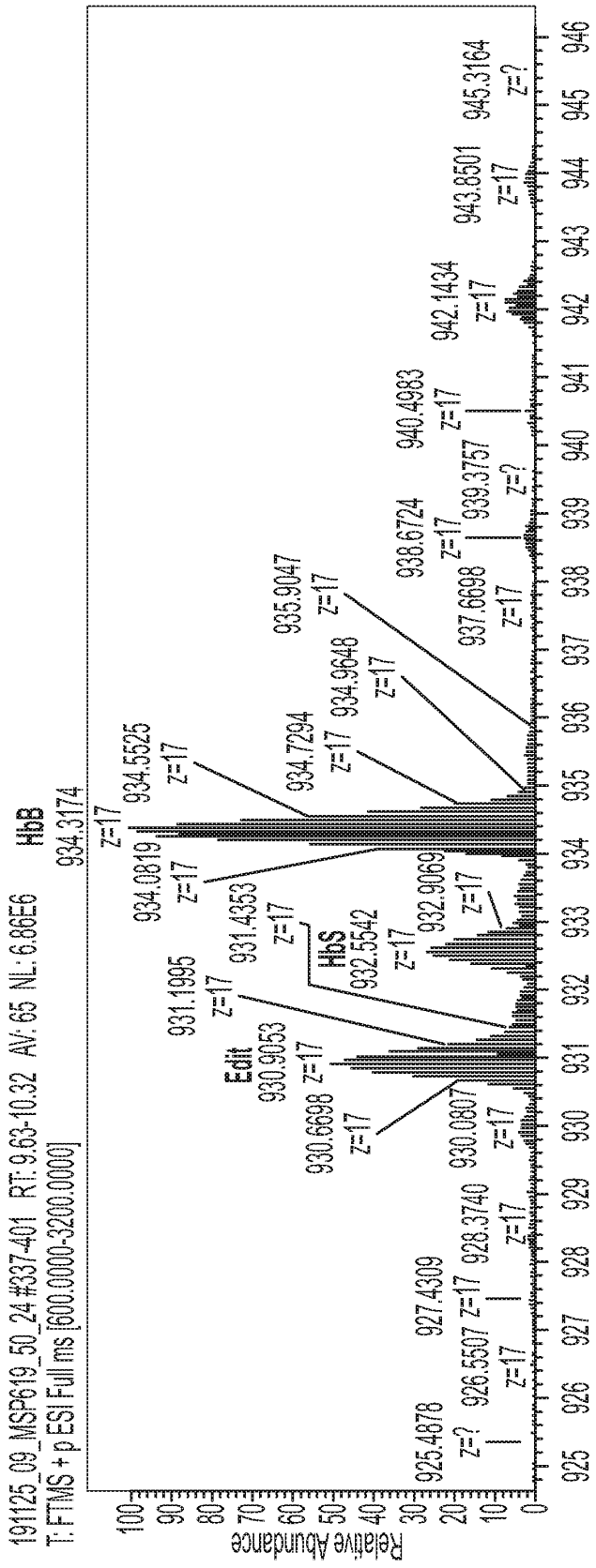

191125_09_MSP619_50_24 #337-401 RT: 9.63-10.32 AV: 65 NL: 6.86E6
T: FTMS + p ESI Full ms [600.0000-3200.0000]
Relative Abundance
HbB
934.3174
HbS
932.5542
Edit
930.9053
925.4878
926.5507
927.4309
928.3740
930.0807
930.6698
931.1995
931.4353
932.9069
934.0819
934.5525
934.7294
934.9648
935.9047
937.6698
938.6724
939.3757
940.4983
942.1434
943.8501
945.3164

FIG. 41B

MSP616	pMRNA-Trilink-ISLAY2-monoTadA-ABE7.10(V82S)-MQKFRAER 120A BbsI
MSP617	pMRNA-Trilink-ISLAY3-monoTadA-ABE7.10(V82S)-MQKFRAER 120A BbsI
MSP684	pMRNA-Trilink-ISLAY3-ABE7.10(V82S, Y147T, Q154S)-MQKFRAER 120A BbsI
MSP686	pMRNA-Trilink-ISLAY3-ABE7.10(V82T, Y147T, Q154S)-MQKFRAER 120A BbsI

21nt protospacer:

5′-*gsascsUUCUCCA*CAGGAGUCAGG *GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUsususu*-3′

20nt protospacer:

5′-ascsusUCUCCACAGGAGUCAGG GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUsususu-3′

19nt protospacer:

5′-csusuCUCCACAGGAGUCAGG GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUsususu-3′

FIG. 42

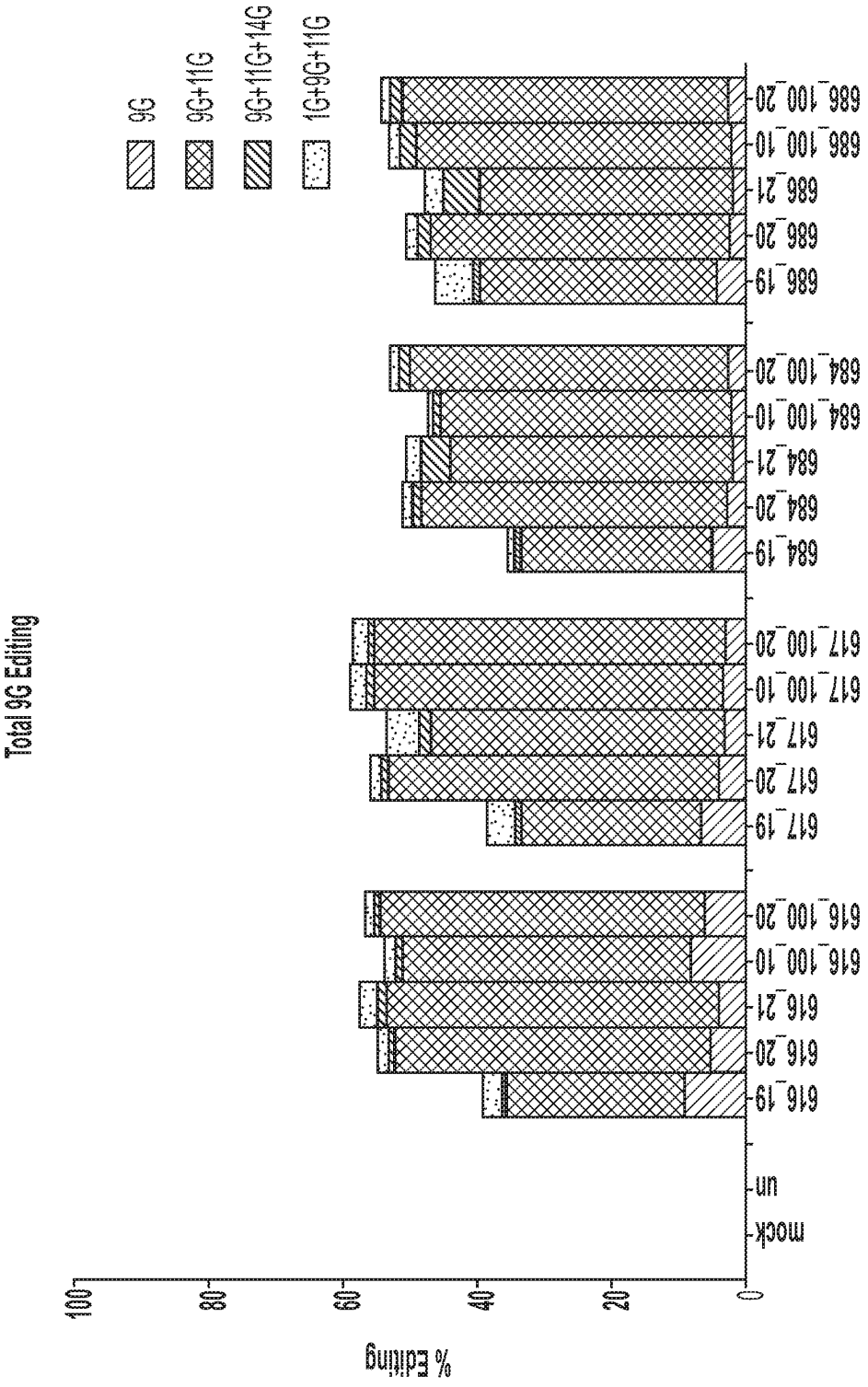

9G
9G+11G
9G+11G+14G
1G+9G+11G
Total 9G Editing
% editing
100
80
60
40
20
0
mock
un
616_19
616_20
616_21
616_100_10
616_100_20
617_19
617_20
617_21
617_100_10
617_100_20
684_19
684_20
684_21
684_100_10
684_100_20
686_19
686_20
686_21
686_100_10
686_100_20

FIG. 43A

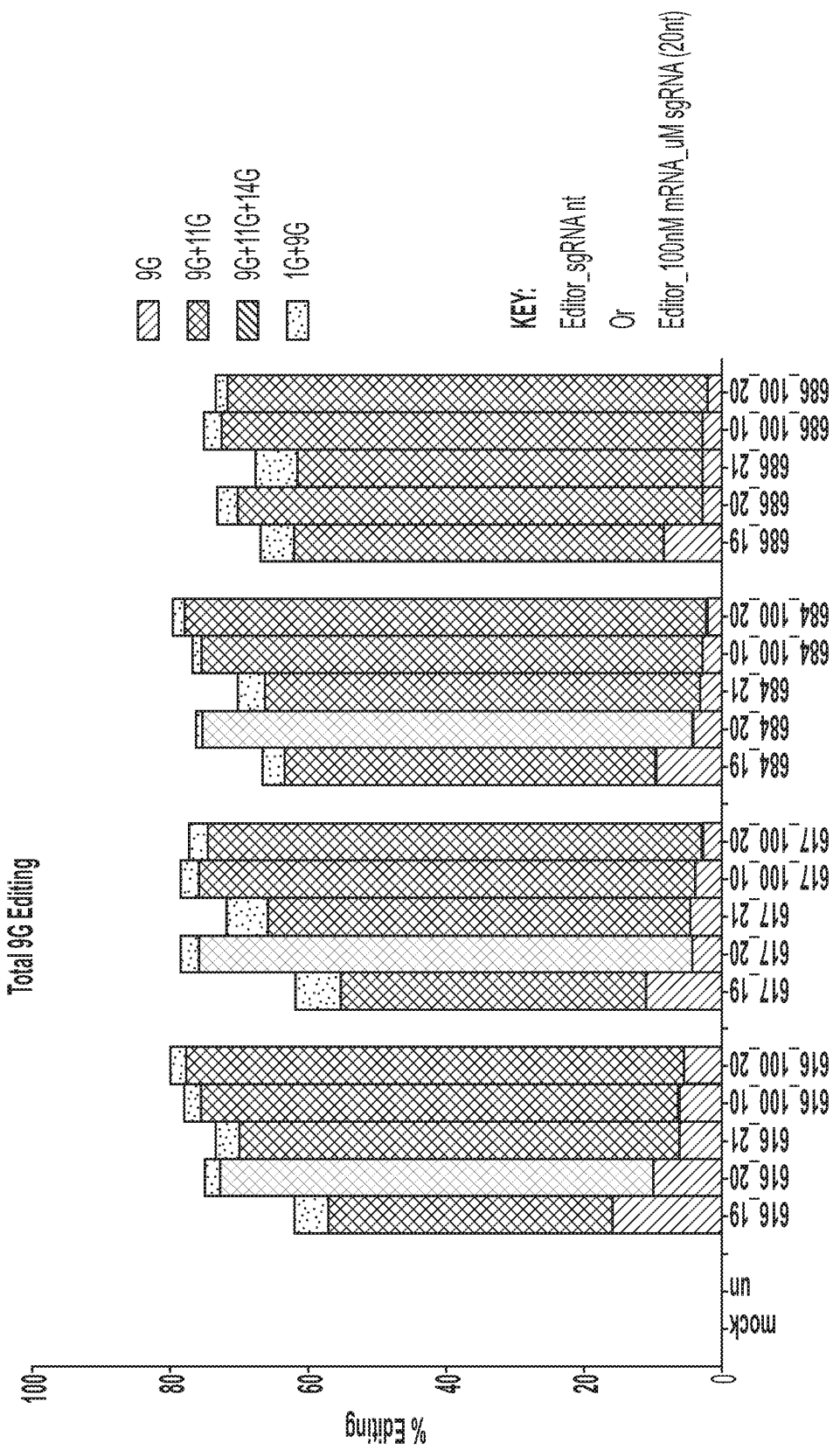

9G
9G+11G
9G+11G+14G
1G+9G
KEY:
Editor_sgRNA nt
Or
Editor_100nM mRNA_uM sgRNA (20nt)
Total 9G Editing
% Editing
100
80
60
40
20
0
mock
un
616_19
616_20
616_21
616_100_10
616_100_20
617_19
617_20
617_21
617_100_10
617_100_20
684_19
684_20
684_21
684_100_10
684_100_20
686_19
686_20
686_21
686_100_10
686_100_20

FIG. 43B

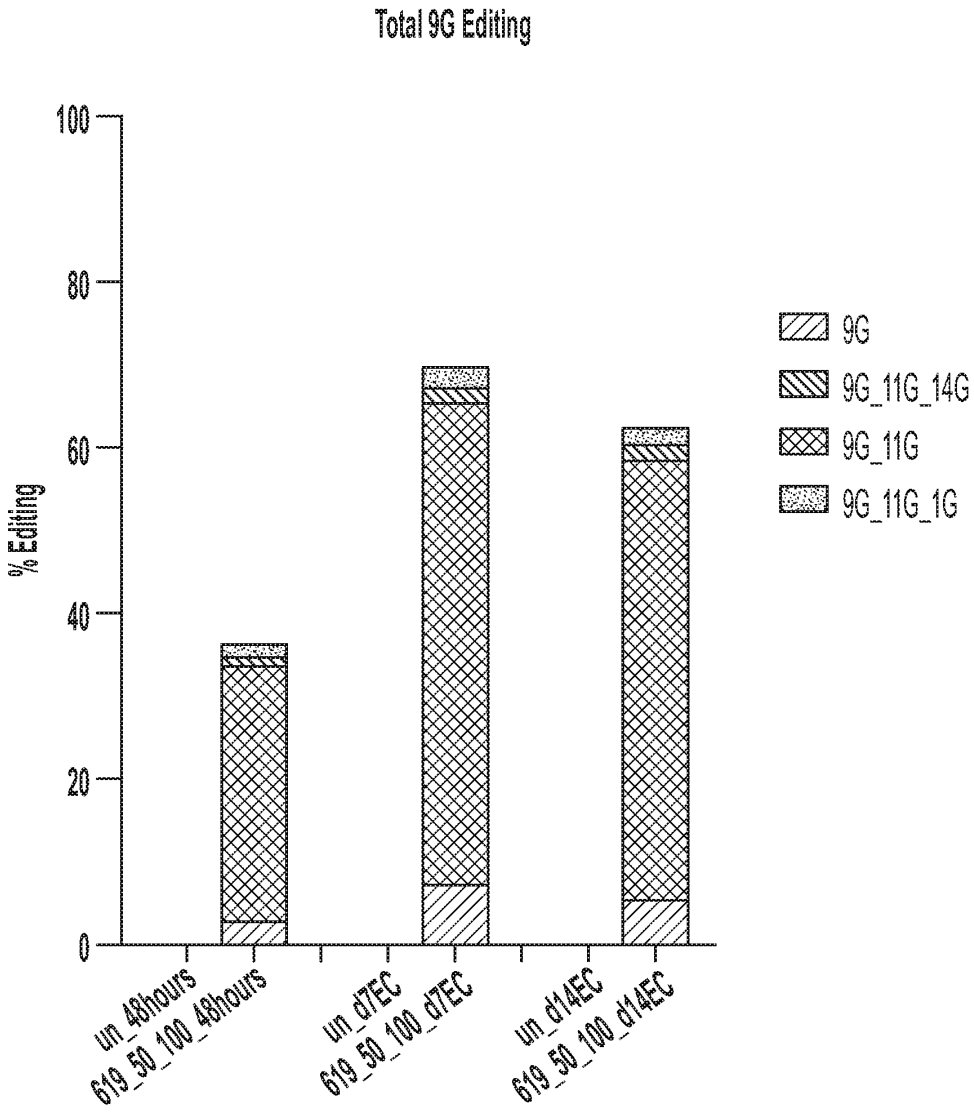
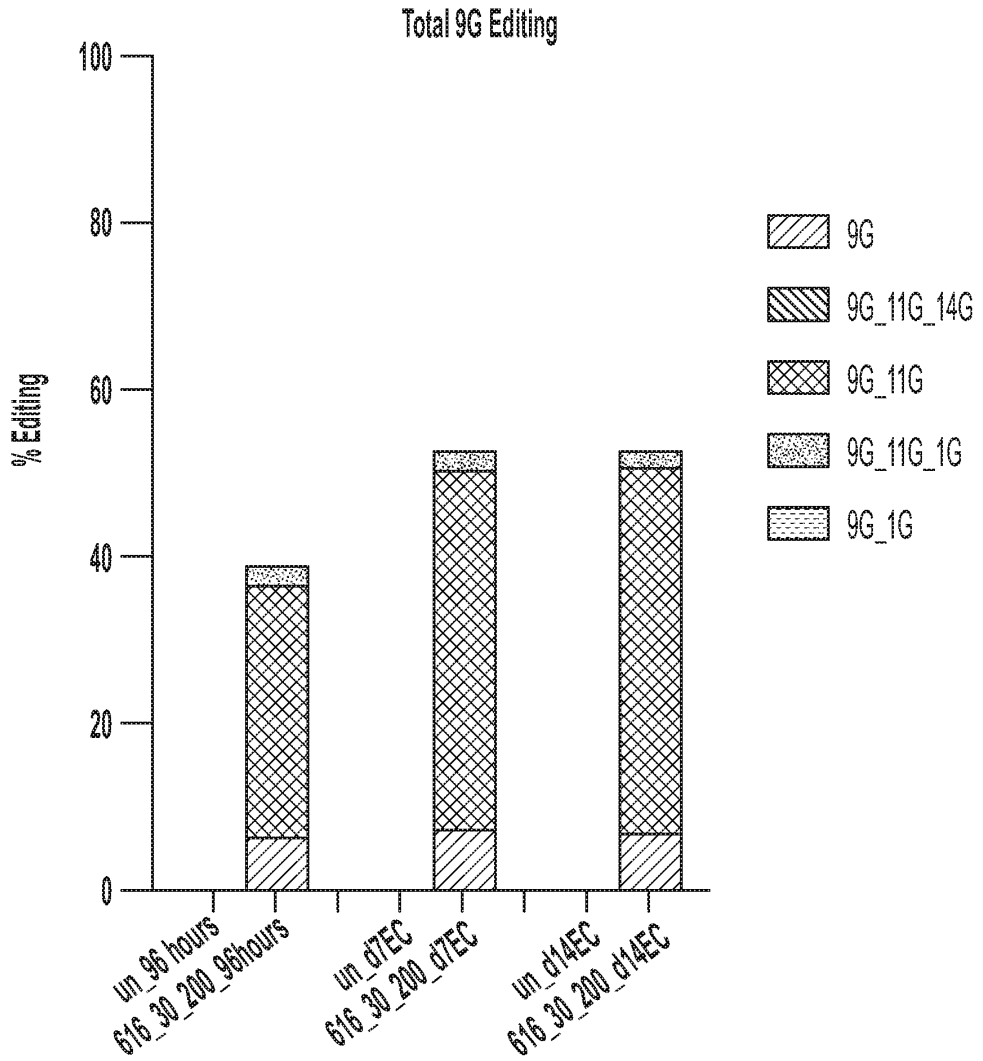

Total 9G Editing
% Editing
100
80
60
40
20
0
9G
9G_11G_14G
9G_11G
9G_11G_1G
un_48hours
619_50_100_48hours
un_d7EC
619_50_100_d7EC
un_d14EC
619_50_100_d14EC

FIG. 44A



	His			Leu			Thr→Thr			Pro→Pro			Val→Ala			Glu			Lys			Ser→*Pro*		
P	**A**	**M**	**20**	**19**	**18**	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**10**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**0**	**-1**
G	C	A	C	C	T	G	A	C	T>C	C	C	T>C	G	T>C	G	G	A	G	A	A	G	T>C	C	T
C	G	T	G	G	A	C	T	G	A>G	G	G	A>G	C	A>G	C	C	T	C	T	T	C	A>G	G	A

FIG. 44B

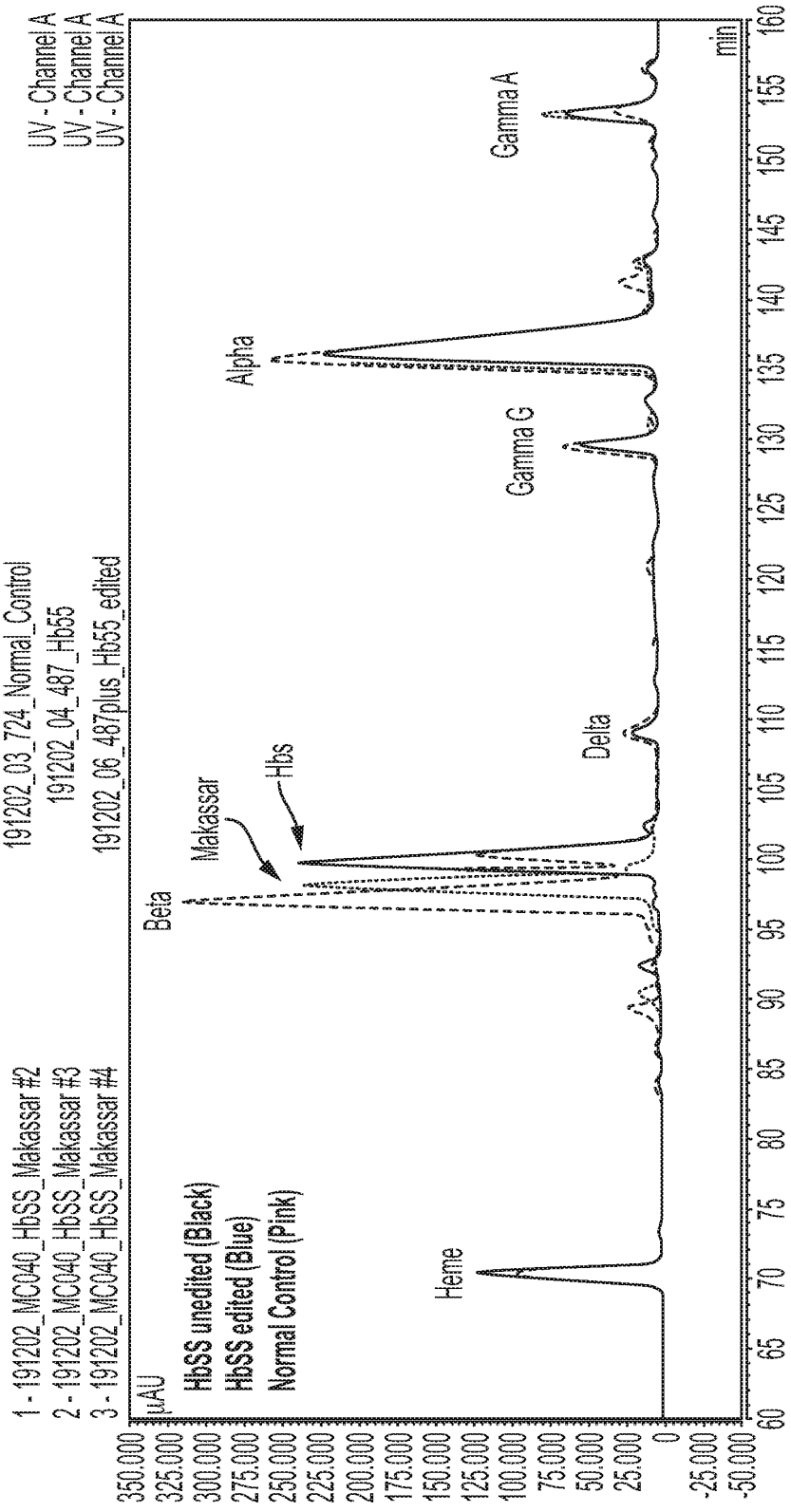

1 - 191202_MC040_HbSS_Makassar #2
2 - 191202_MC040_HbSS_Makassar #3
3 - 191202_MC040_HbSS_Makassar #4
191202_03_724_Normal_Control
191202_04_487_Hb55
191202_06_487plus_Hb55_edited
UV - Channel A
UV - Channel A
UV - Channel A
uAU
HbSS unedited (Black)
HbSS edited (Blue)
Normal Control (Pink)
Heme
Beta
Makassar
Hbs
Delta
Gamma G
Alpha
Gamma A
min

FIG. 45

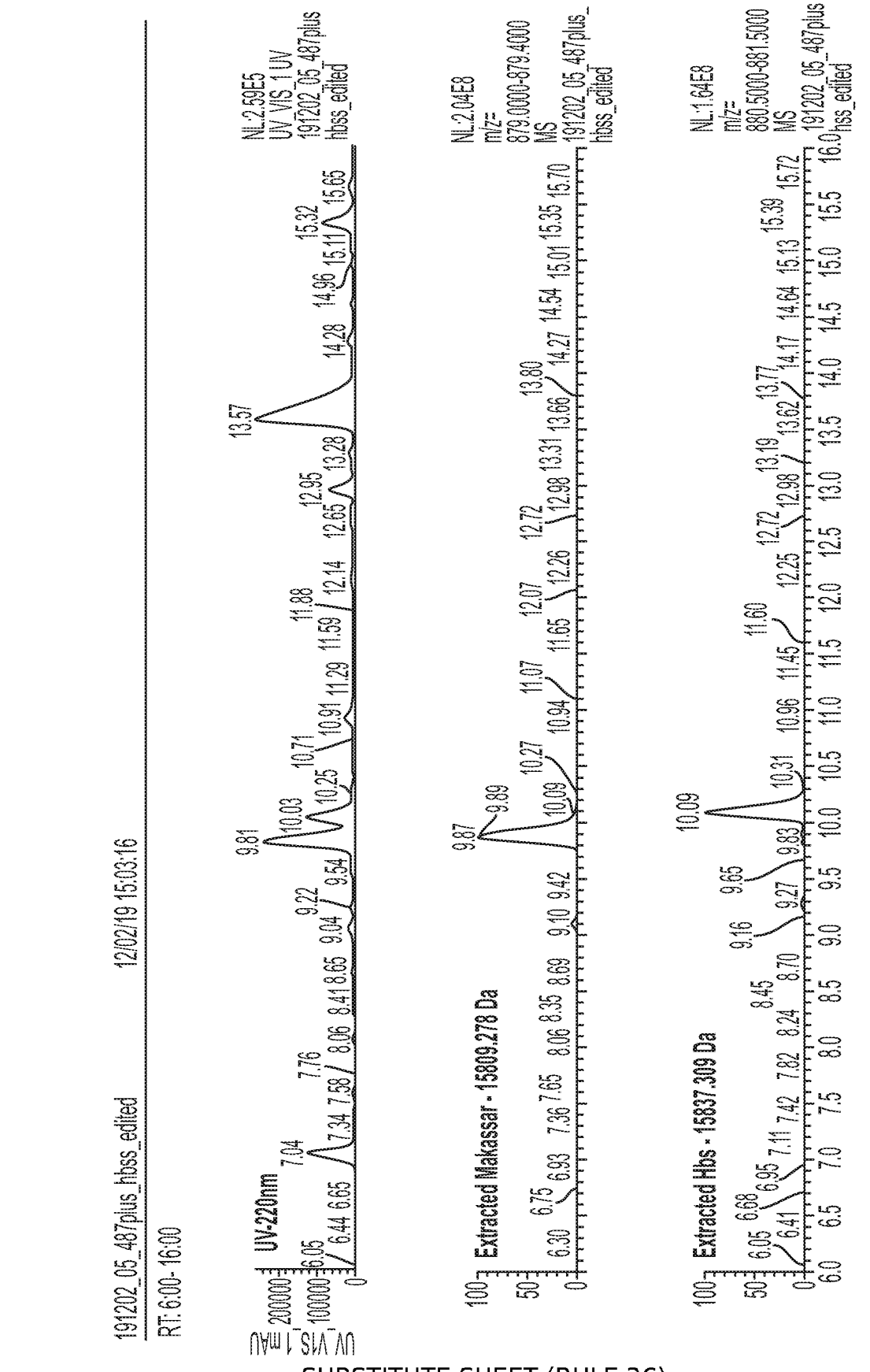

191202_05_487plus_hbss_edited
12/02/19 15:03:16
RT: 6.00 - 16.00
UV-220nm
UV_VIS_1 mAU
NL:2.59E5
UV VIS 1 UV
191202_05_487plus
hbss_edited
Extracted Makassar - 15809.278 Da
NL:2.04E8
m/z=
879.0000-879.4000
MS
191202_05_487plus_
hbss_edited
Extracted Hbs - 15837.309 Da
NL:1.64E8
m/z=
880.5000-881.5000
MS
191202_05_487plus
hbss_edited

FIG. 46A

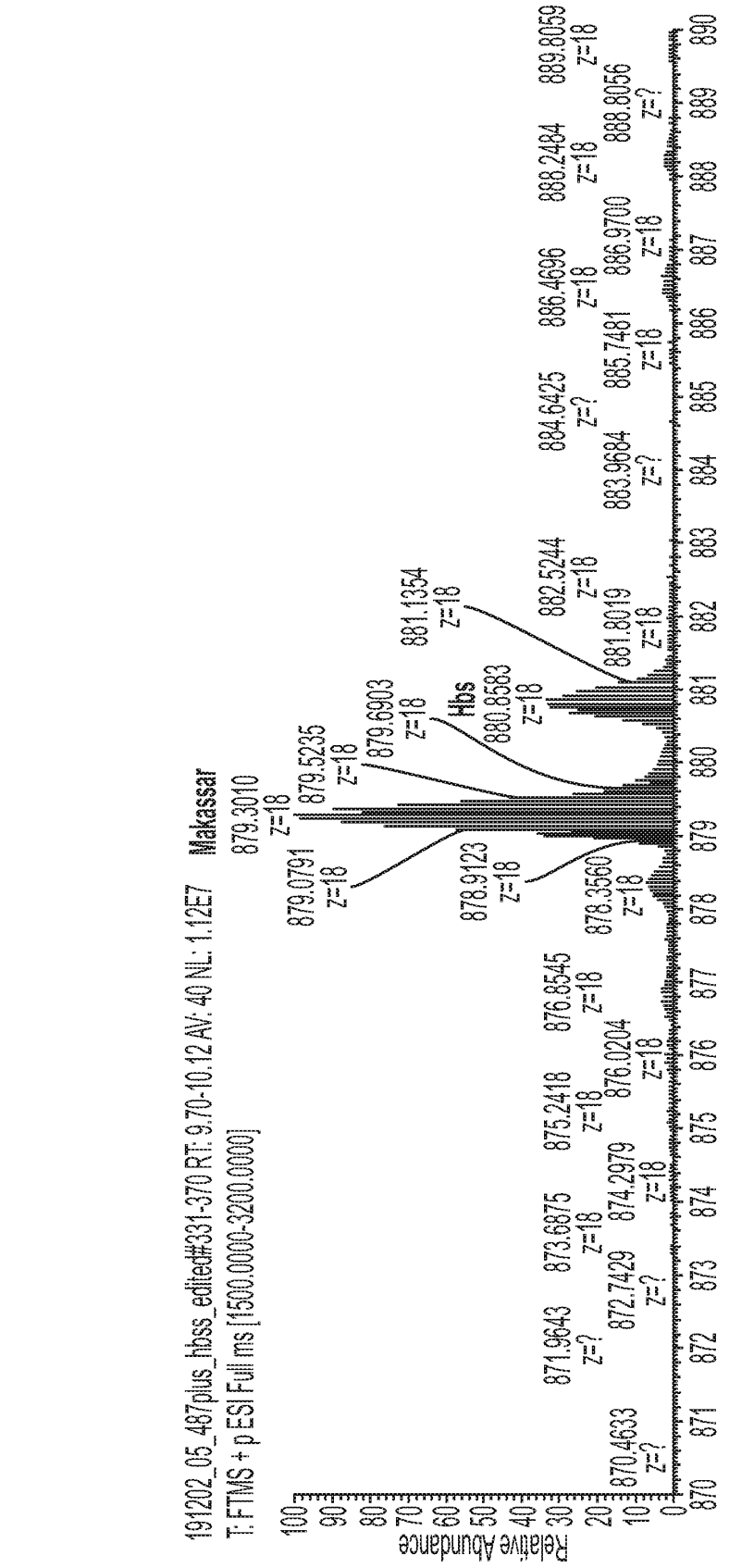

191202_05_487plus_hbss_edited#331-370 RT: 9.70-10.12 AV: 40 NL: 1.12E7 Makassar
T: FTMS + p ESI Full ms [1500.0000-3200.0000]
Relative Abundance
m/z
Hbs
870.4633 z=?
871.9643 z=?
872.7429 z=?
873.6875 z=18
874.2979 z=18
875.2418 z=18
876.0204 z=18
876.8545 z=18
878.3560 z=18
878.9123 z=18
879.0791 z=18
879.3010 z=18
879.5235 z=18
879.6903 z=18
880.8583 z=18
881.1354 z=18
881.8019 z=18
882.5244 z=18
883.9684 z=?
884.6425 z=?
885.7481 z=18
886.4696 z=18
886.9700 z=18
888.2484 z=18
888.8056 z=?
889.8059 z=18

FIG. 46B

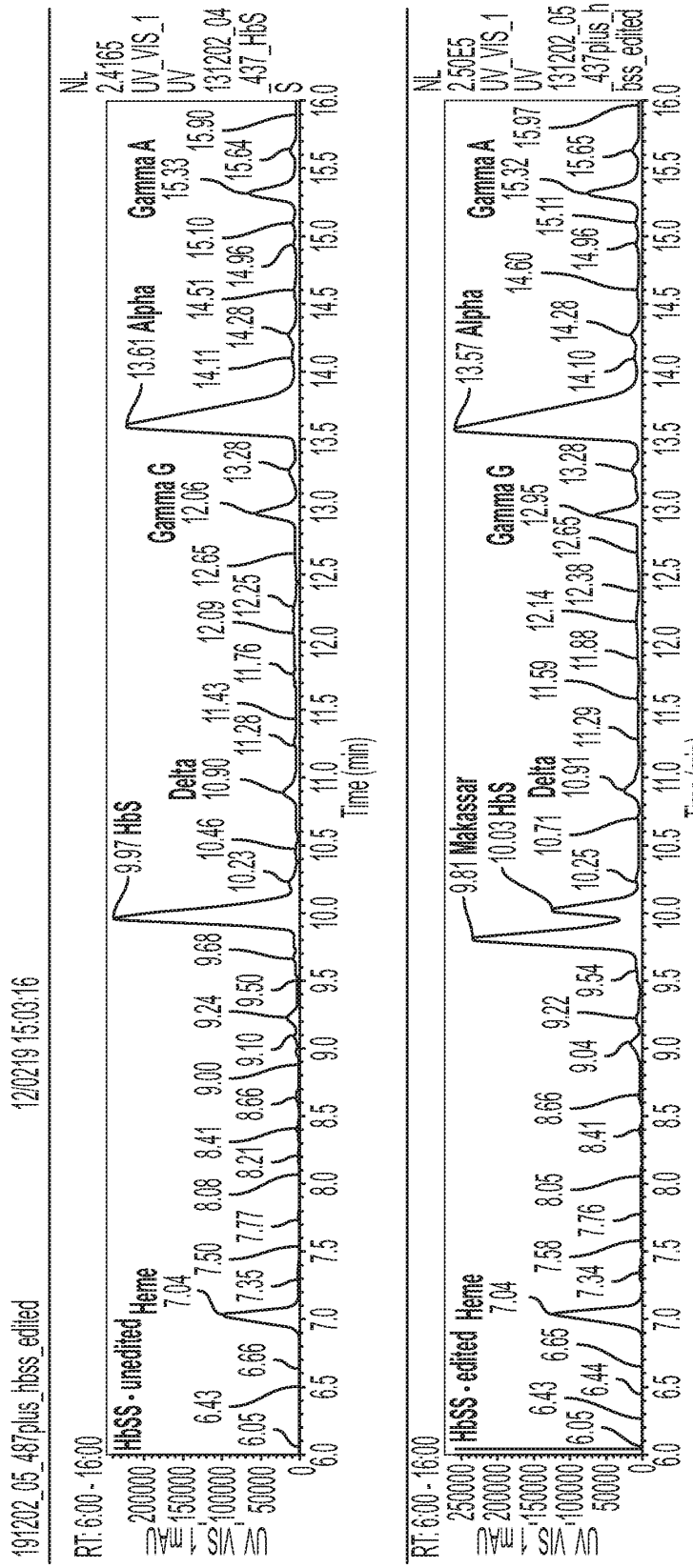

191202_05_487plus_hbss_edited
12/02/19 15:03:16
RT: 6.00 - 16.00
HbSS - unedited
Heme
HbS
Delta
Gamma G
Alpha
Gamma A
Time (min)
HbSS - edited
Makassar
NL
2.4165
2.50E5
UV_VIS_1
131202_04_437_HbS
131202_05_437plus_hbss_edited

FIG. 47

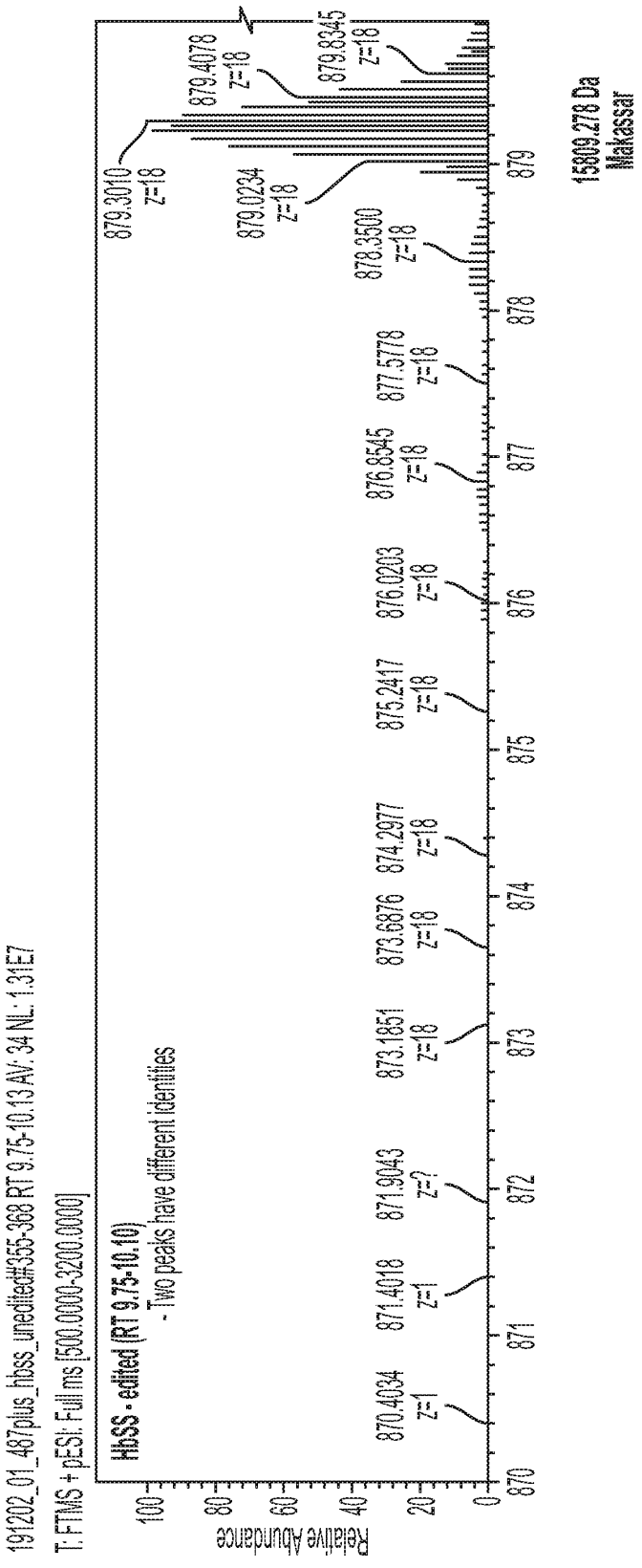


FIG. 47 CONTINUED

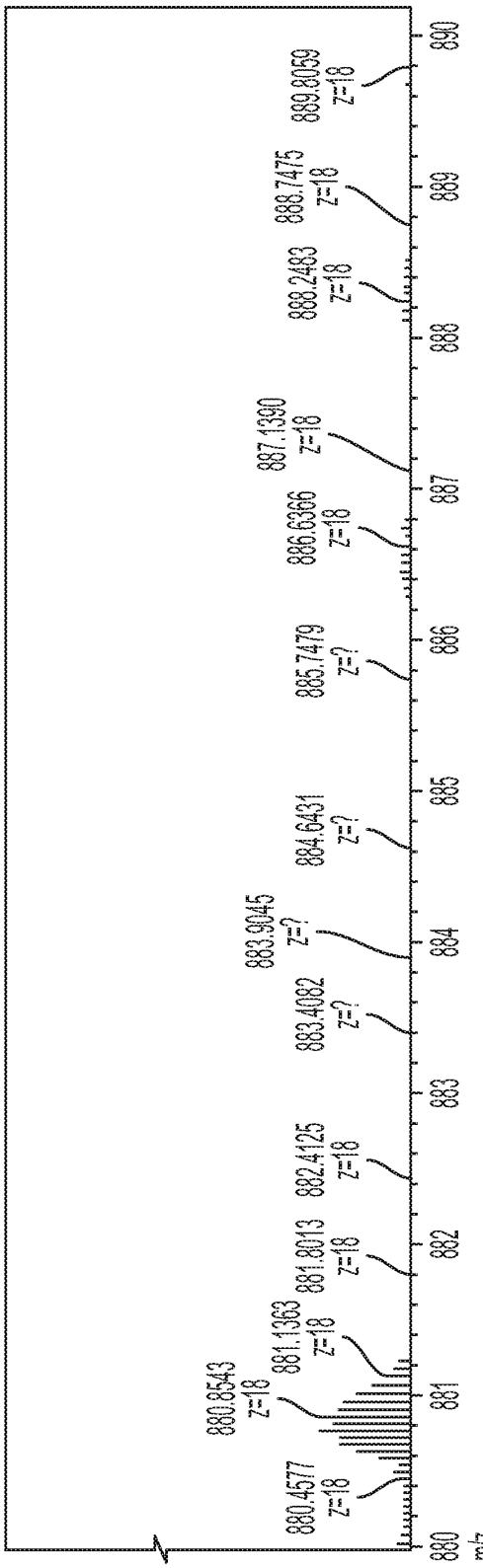


FIG. 47 CONTINUED

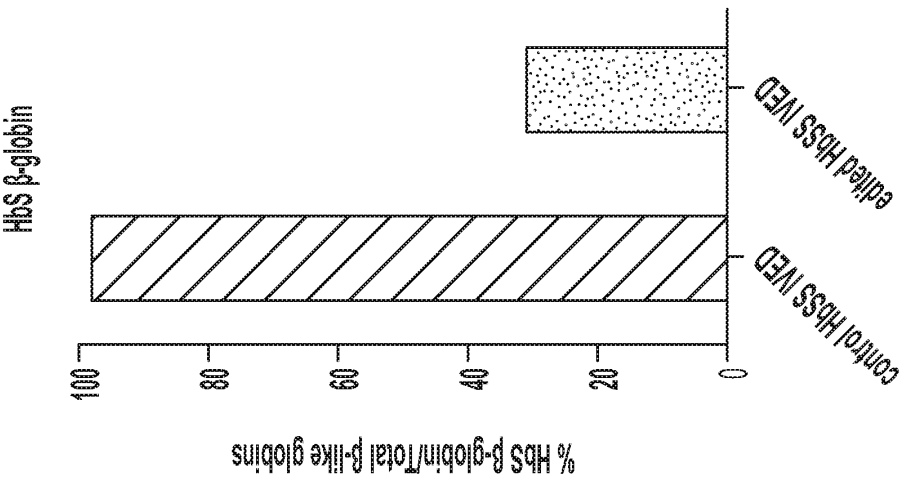


FIG. 48C

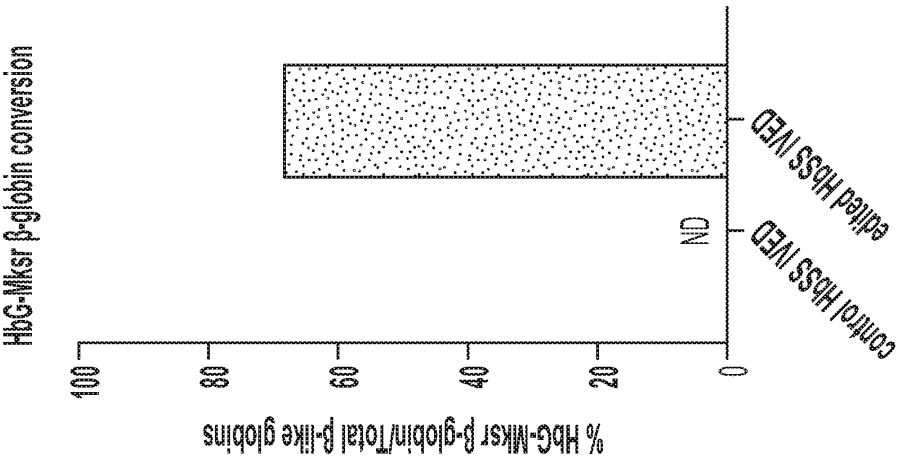


FIG. 48B

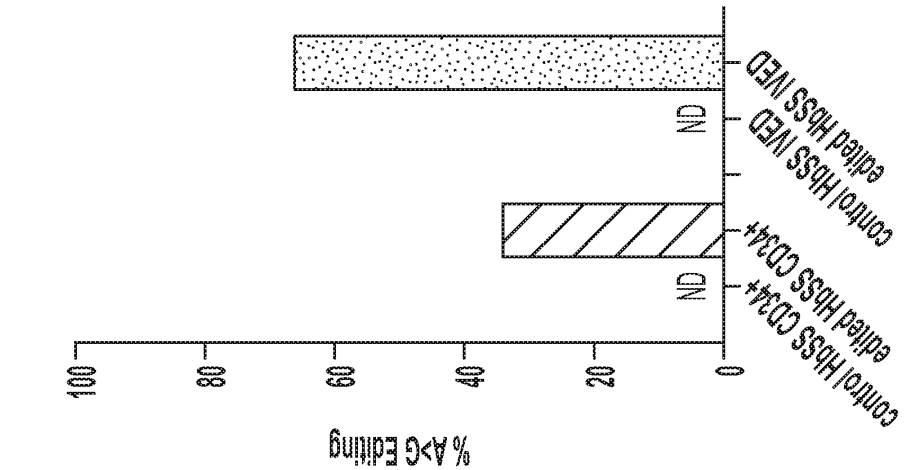


FIG. 48A

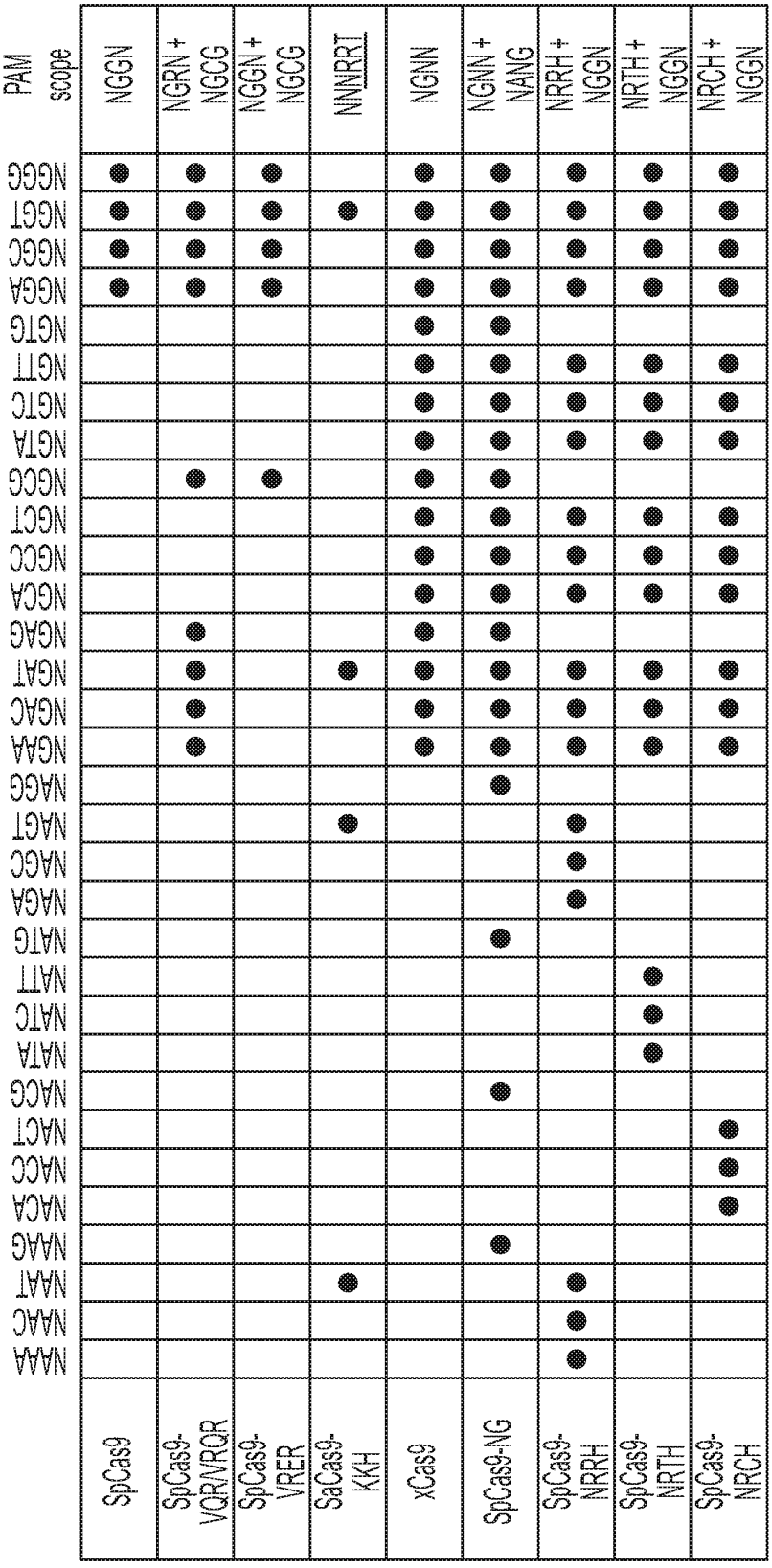

PAM scope
NGGN
NGRN + NGCG
NGGN + NGCG
NNNRRT
NGNN
NGNN + NAAG
NRRH + NGGN
NRTH + NGGN
NRCH + NGGN
NGGG
NGGT
NGGC
NGGA
NGTG
NGTT
NGTC
NGTA
NGCG
NGCT
NGCC
NGCA
NGAG
NGAT
NGAC
NGAA
NAGG
NAGT
NAGC
NAGA
NATG
NATT
NATC
NATA
NACG
NACT
NACC
NACA
NAAG
NAAT
NAAC
NAAA
SpCas9
SpCas9-VQR/VQR
SpCas9-VRER
SaCas9-KKH
xCas9
SpCas9-NG
SpCas9-NRRH
SpCas9-NRTH
SpCas9-NRCH

FIG. 49