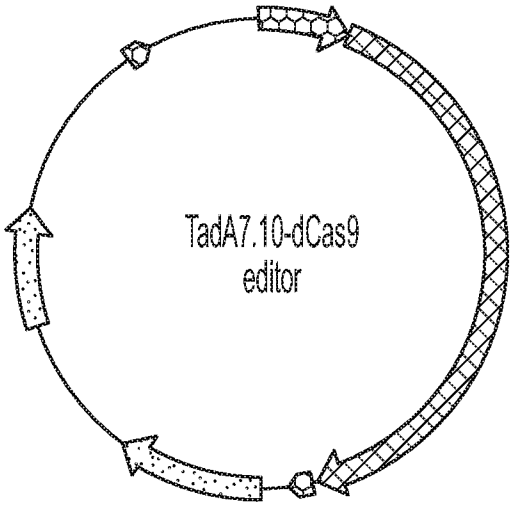



FIG. 1A

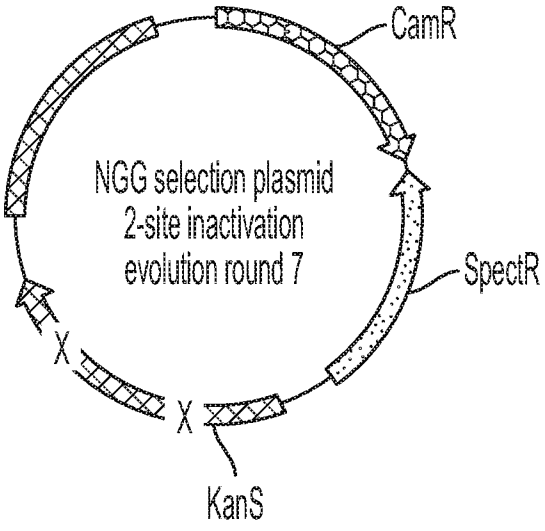



FIG. 1B

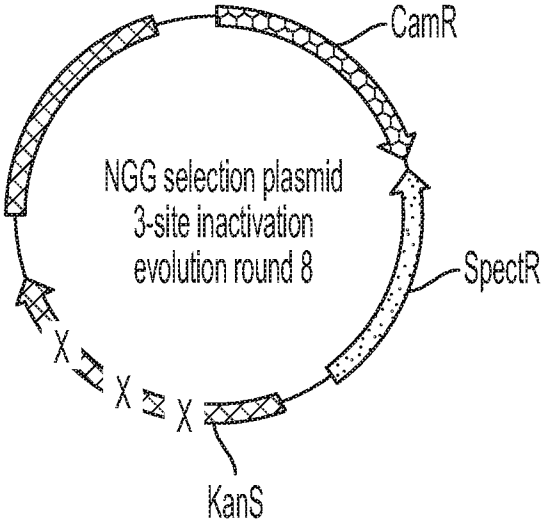



FIG. 1C

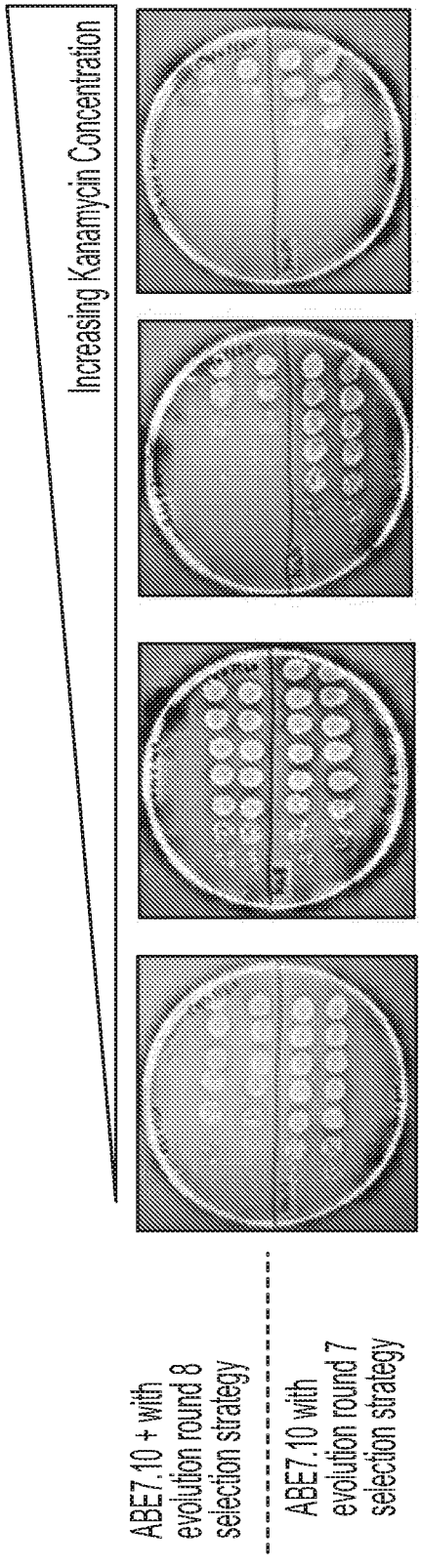

Increasing Kanamycin Concentration
ABE7.10 + with evolution round 8 selection strategy
ABE7.10 with evolution round 7 selection strategy

FIG. 2

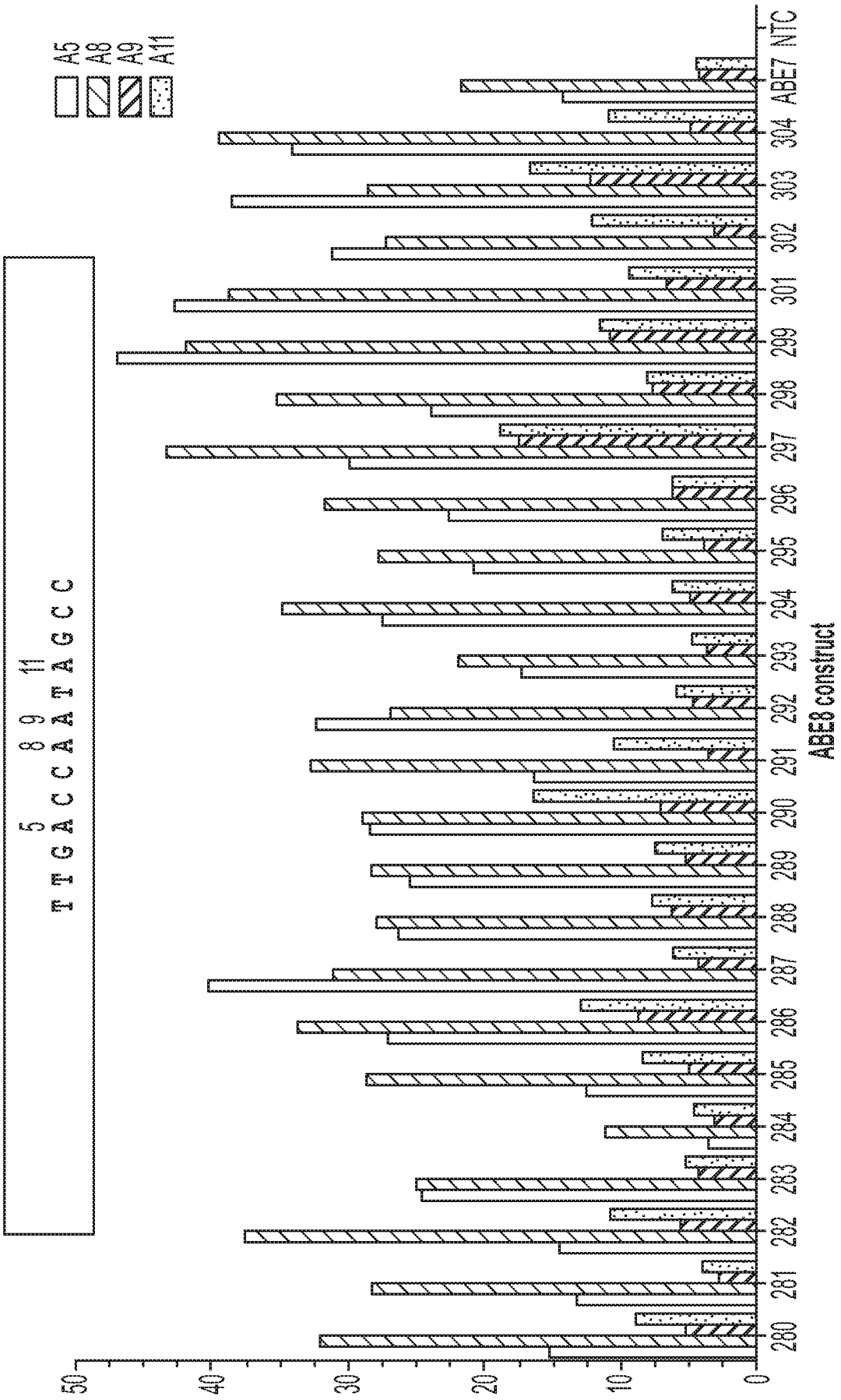

A5
A8
A9
A11
5 8 9 11
T T G A C C A A T A G C C C
Percent of total sequencing reads with target A•T base pair converted to G•C
0
10
20
30
40
50
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
301
302
303
304
ABE7
NTC
ABE8 construct

FIG. 3A

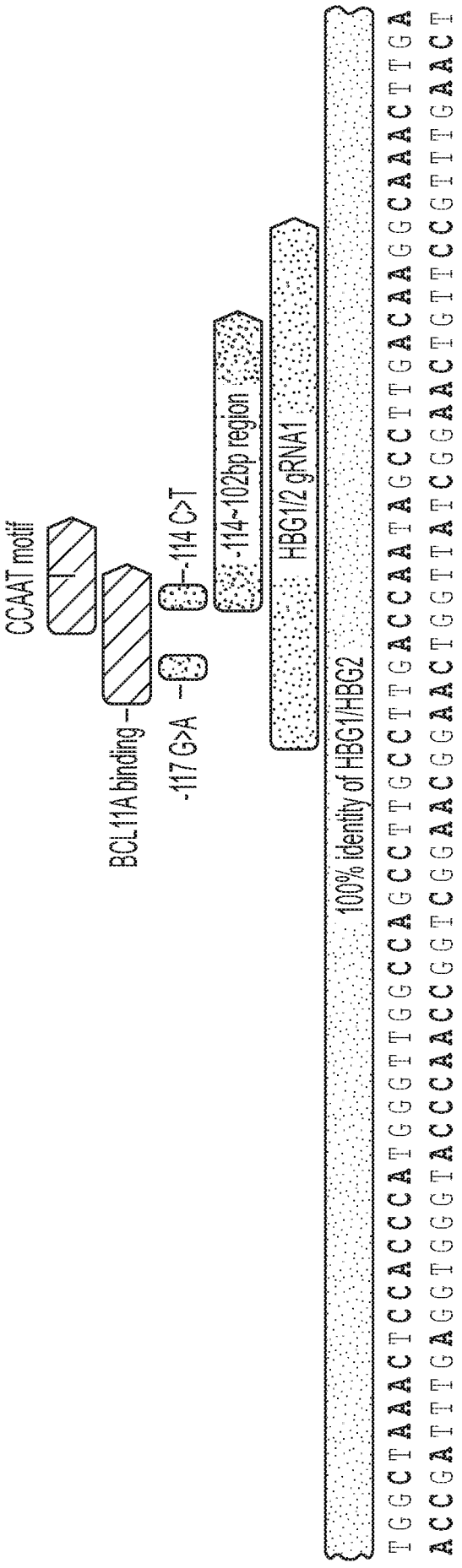

CCAAT motif
BCL11A binding
-117 G>A
-114 C>T
-114~102bp region
HBG1/2 gRNA1
100% identity of HBG1/HBG2
TGGCCTAAAACTCCACCCCATGGGTTGGCCAGCCTTGCCTTGACCAATAGCCTTGACAAGGCAAACTTGA
ACCGATTTTGAGGTGGGTACCCCAACCCGGTCGGAACGGAACTGGTTATCGGAACTGTTCCGTTTGAACT

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 |

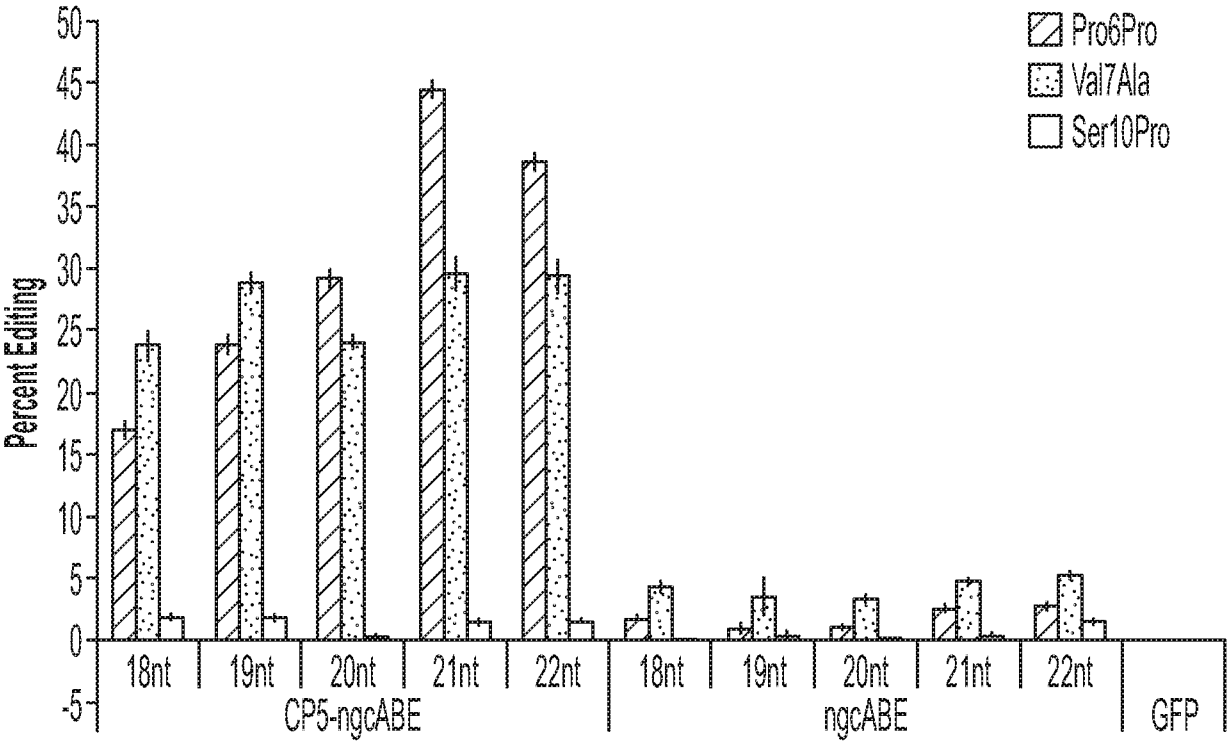



FIG. 4

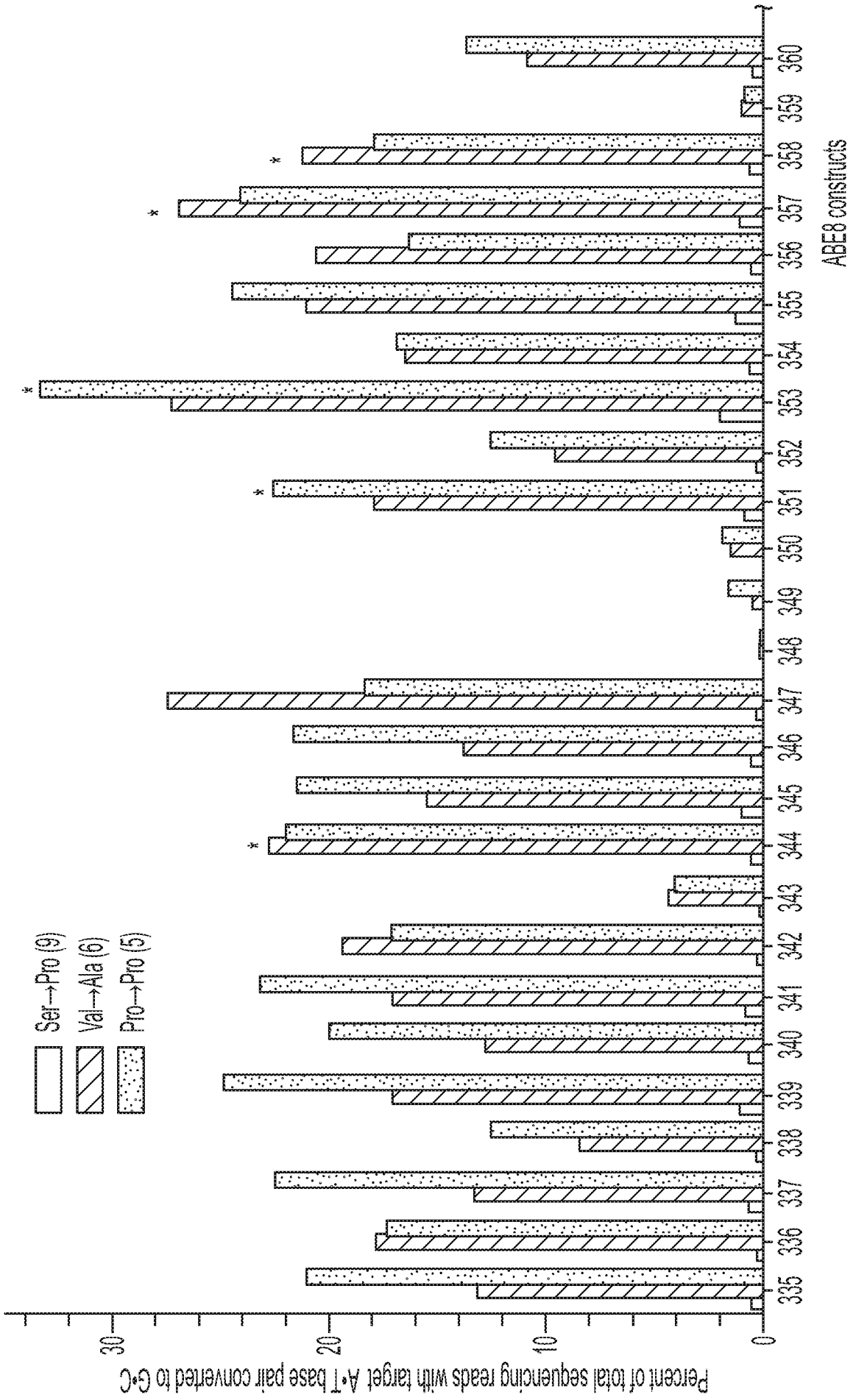

Percent of total sequencing reads with target A•T base pair converted to G•C
0
10
20
30
ABE8 constructs
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
*
*
*
*
Ser→Pro (9)
Val→Ala (6)
Pro→Pro (5)

FIG. 5

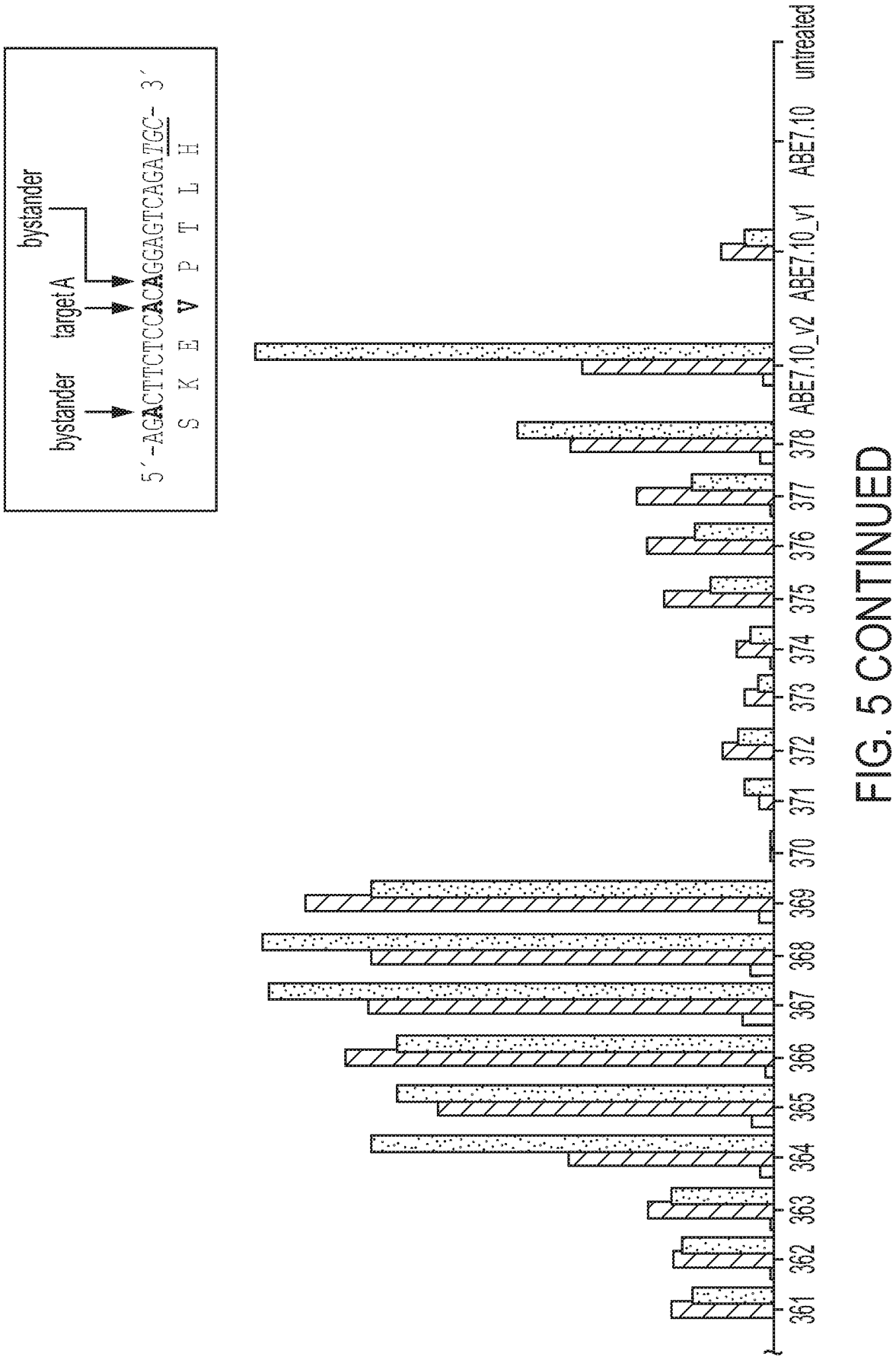



FIG. 5 CONTINUED

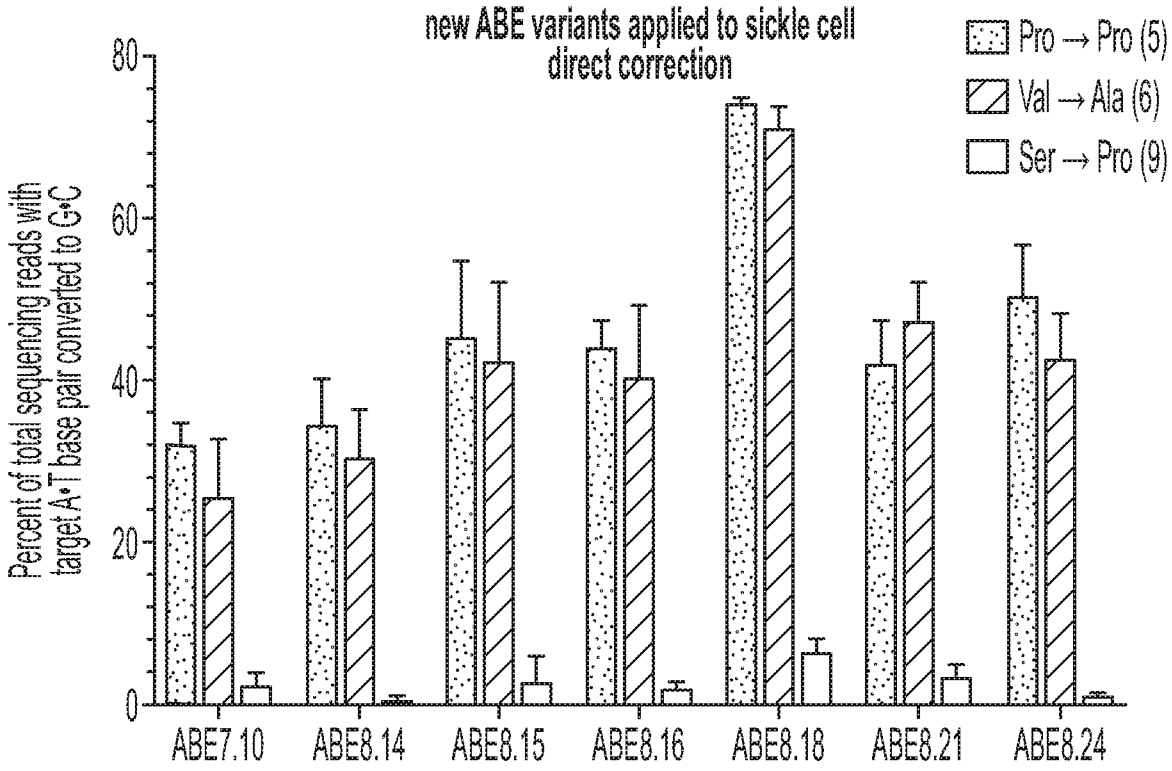

new ABE variants applied to sickle cell direct correction
Pro → Pro (5)
Val → Ala (6)
Ser → Pro (9)
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
ABE7.10
ABE8.14
ABE8.15
ABE8.16
ABE8.18
ABE8.21
ABE8.24

FIG. 6

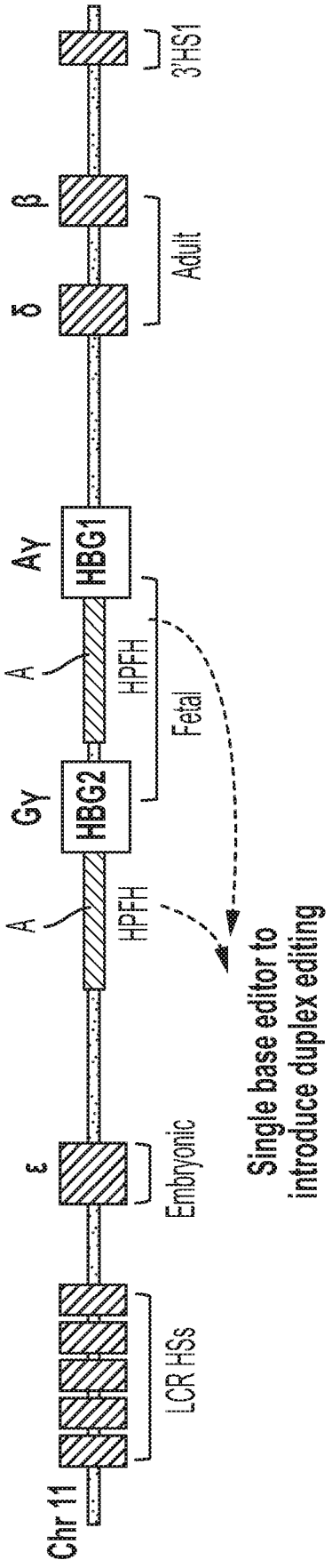

3'HS1
β
δ
Adult
Aγ
HBG1
A
HPFH
Fetal
Gγ
HBG2
A
HPFH
ε
Embryonic
Single base editor to
introduce duplex editing
LCR HSs
Chr 11

FIG. 7A

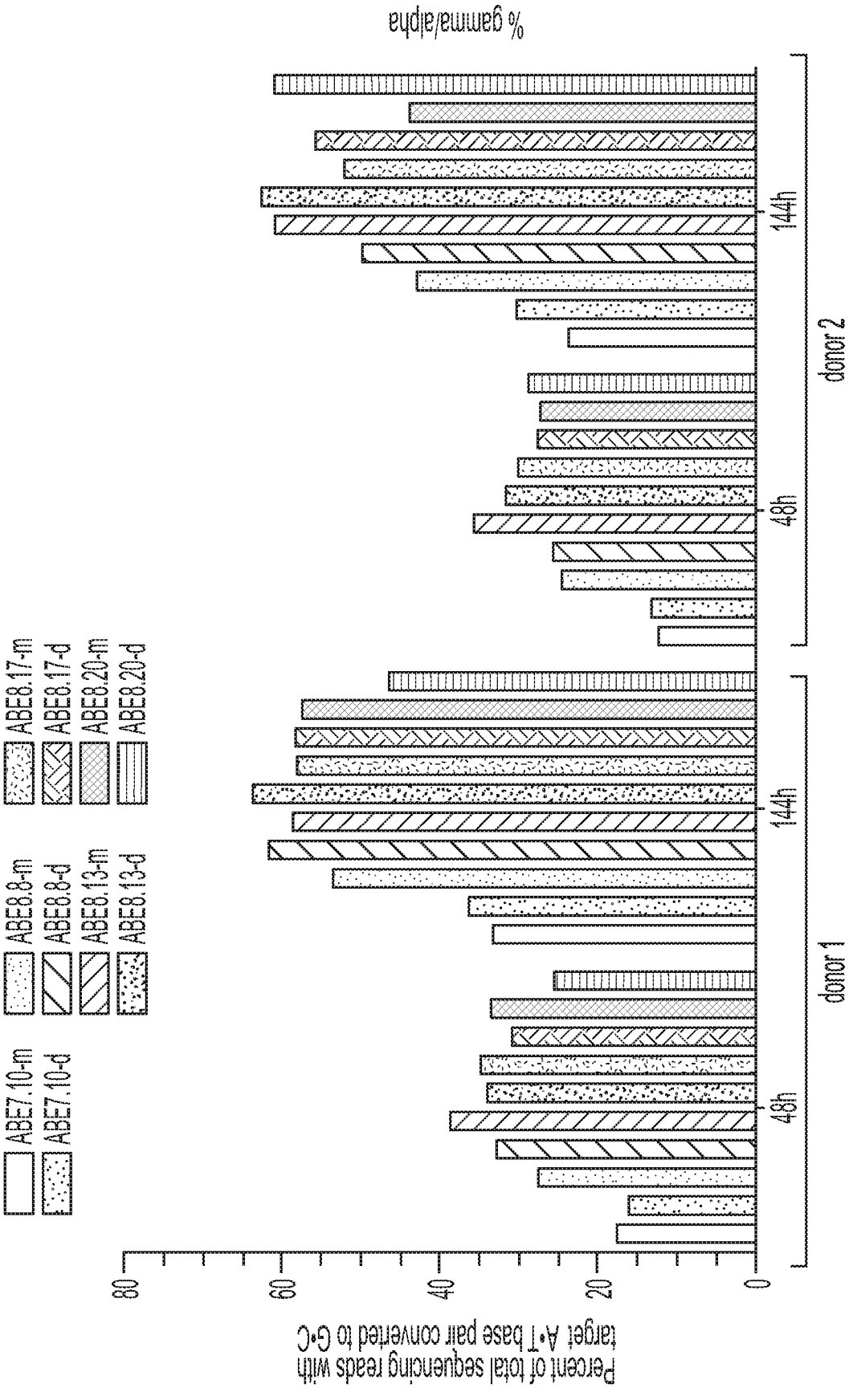

% gamma/alpha
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
48h
144h
donor 1
48h
144h
donor 2
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d

FIG. 7B

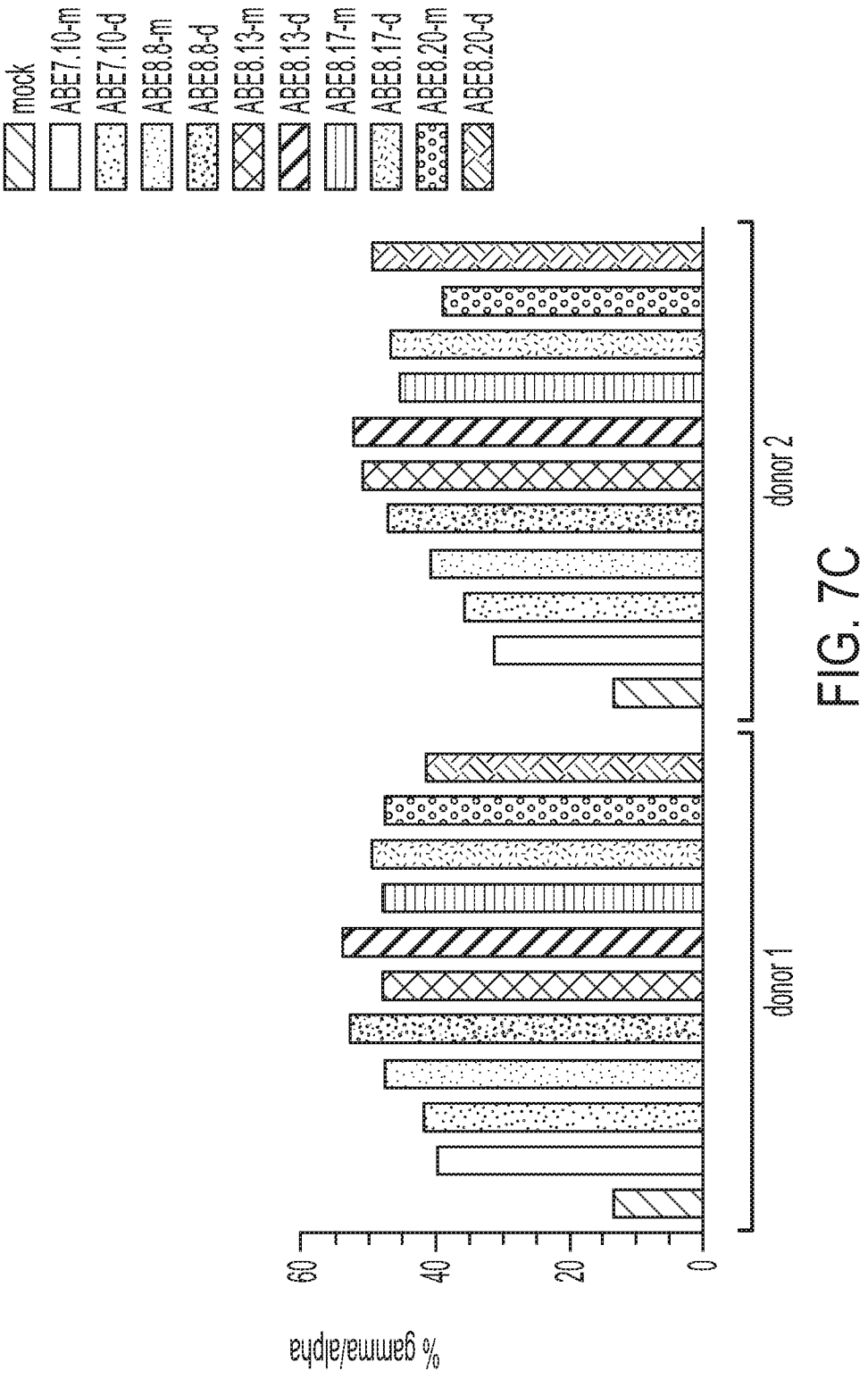

mock
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
donor 1
donor 2
0
20
40
60
% gamma/alpha

FIG. 7C

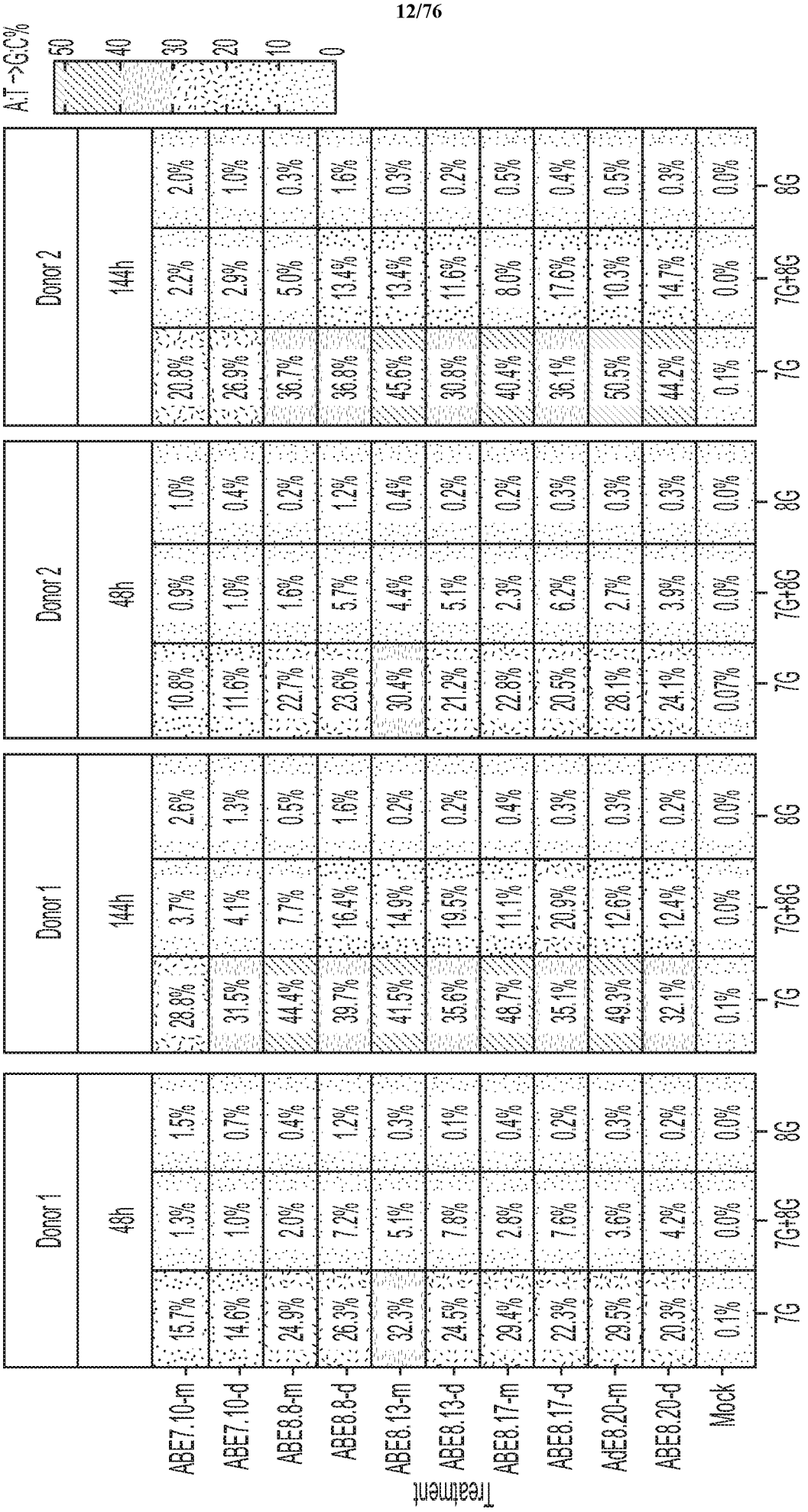

A:T ->G:C%
50
40
30
20
10
0
Donor 1
48h
144h
Donor 2
48h
144h
Donor 1
48h
144h
Donor 2
48h
144h
7G
7G+8G
8G
Target site edit
Treatment
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
AdE8.20-m
ABE8.20-d
Mock

FIG. 8A

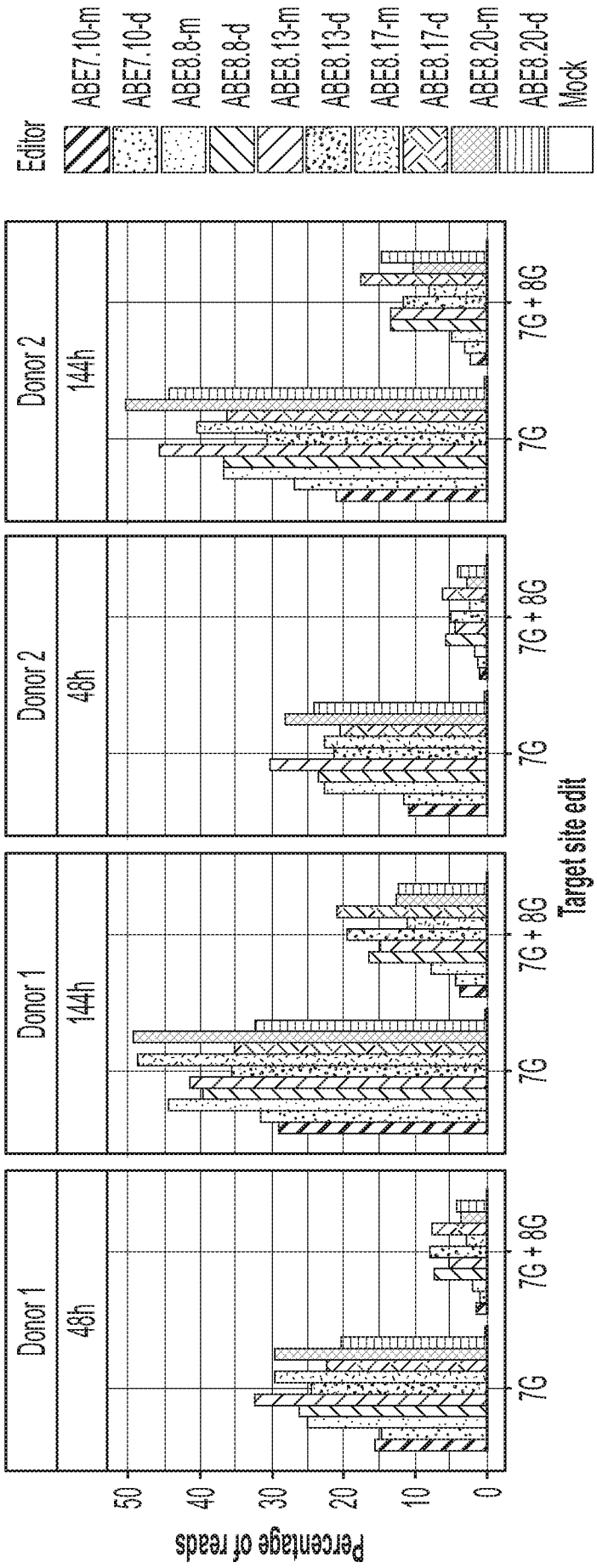

Editor
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
Mock
Donor 1
48h
Donor 1
144h
Donor 2
48h
Donor 2
144h
7G
7G + 8G
Target site edit
Percentage of reads
0
10
20
30
40
50

FIG. 8B

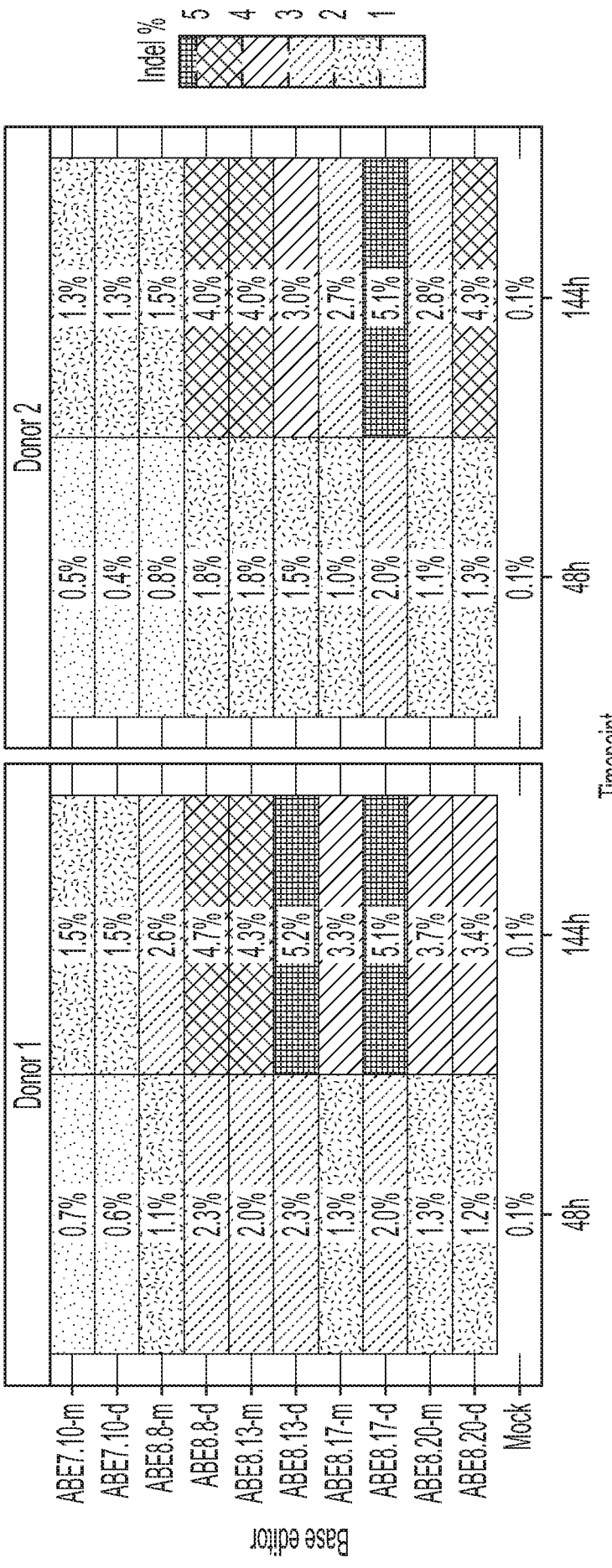
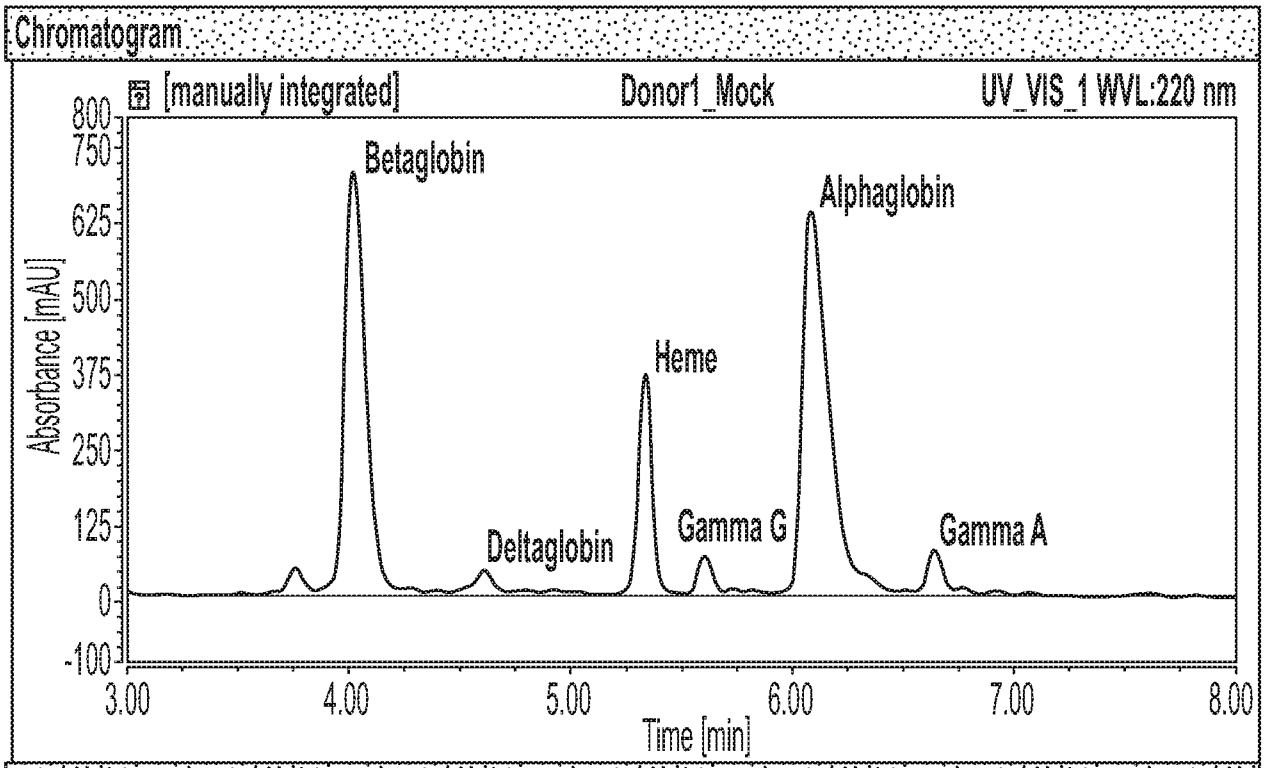

Indel %
5
4
3
2
1
Donor 1
Donor 2
ABE7.10-m
ABE7.10-d
ABE8.8-m
ABE8.8-d
ABE8.13-m
ABE8.13-d
ABE8.17-m
ABE8.17-d
ABE8.20-m
ABE8.20-d
Mock
Base editor
48h
144h
Timepoint
0.7%
1.5%
0.6%
1.5%
1.1%
2.6%
2.3%
4.7%
2.0%
4.3%
2.3%
5.2%
1.3%
3.3%
2.0%
5.1%
1.3%
3.7%
1.2%
3.4%
0.1%
0.1%
0.5%
1.3%
0.4%
1.3%
0.8%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

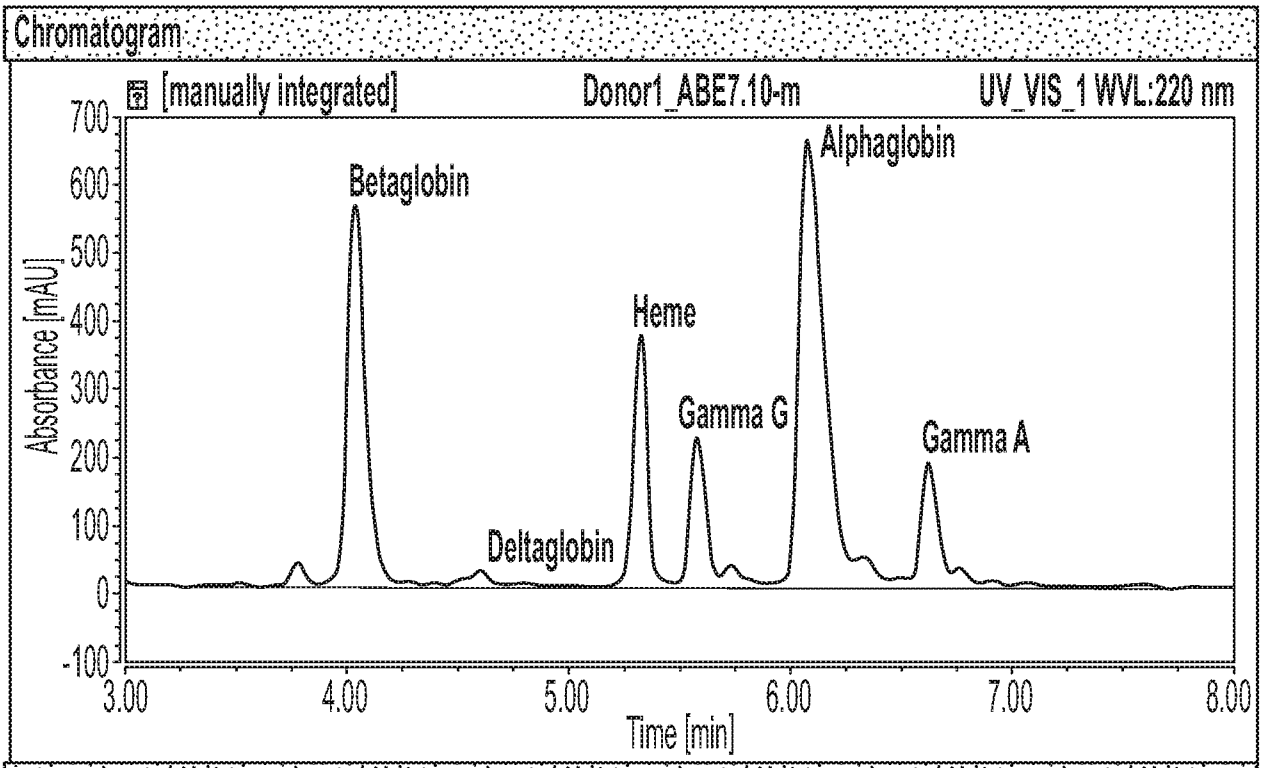
FIG. 9




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 |

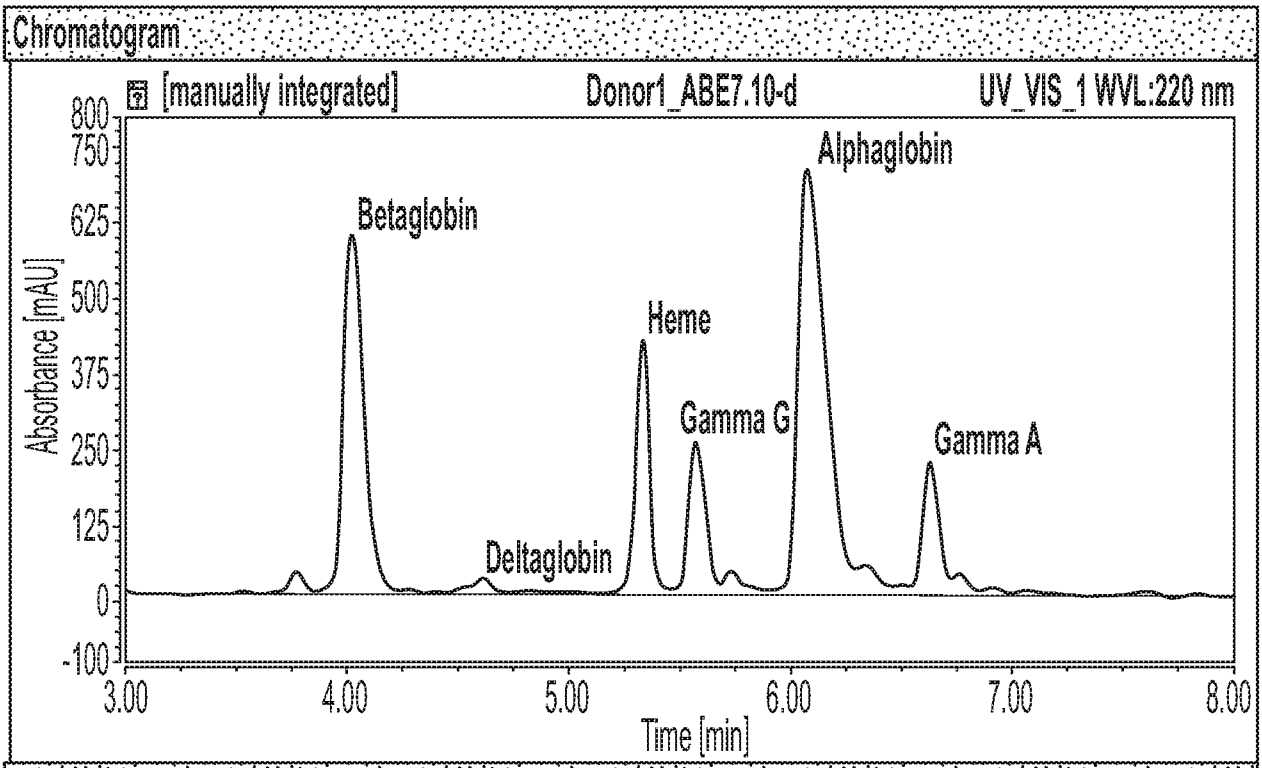
FIG. 10




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 |

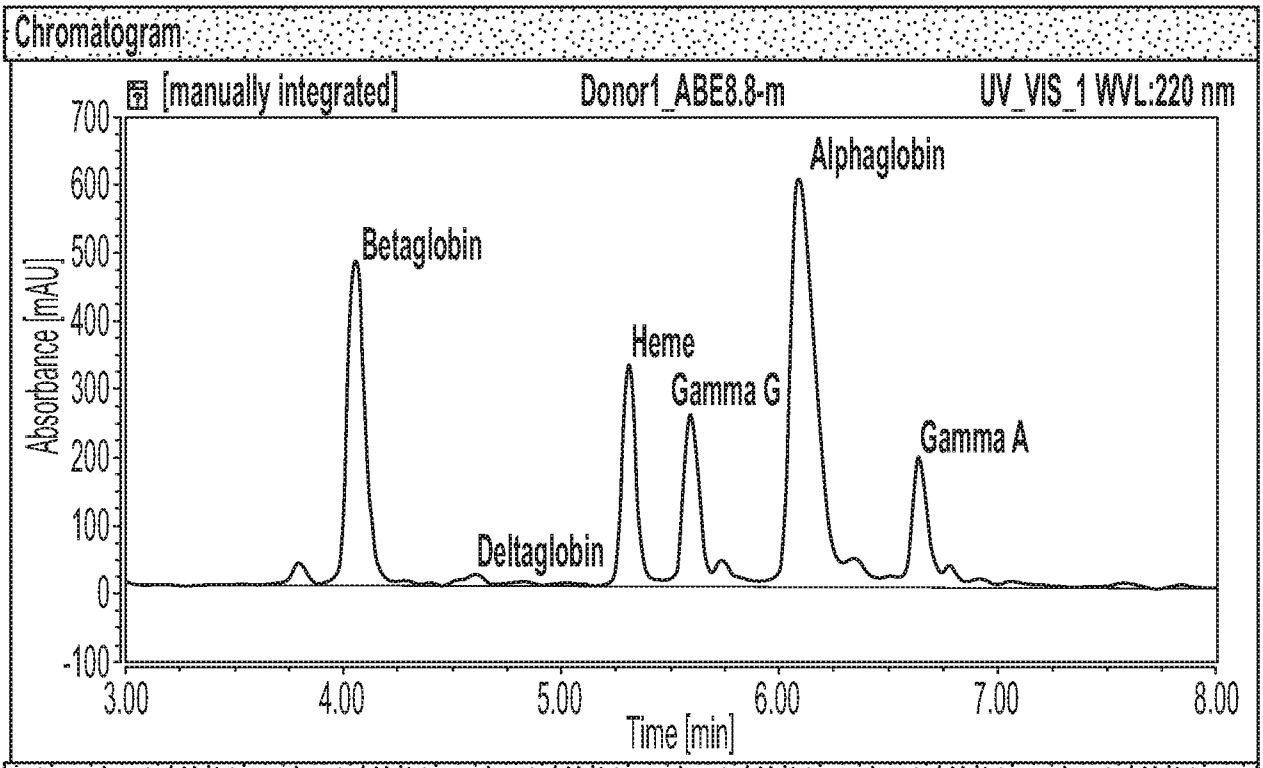
FIG. 11




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 |

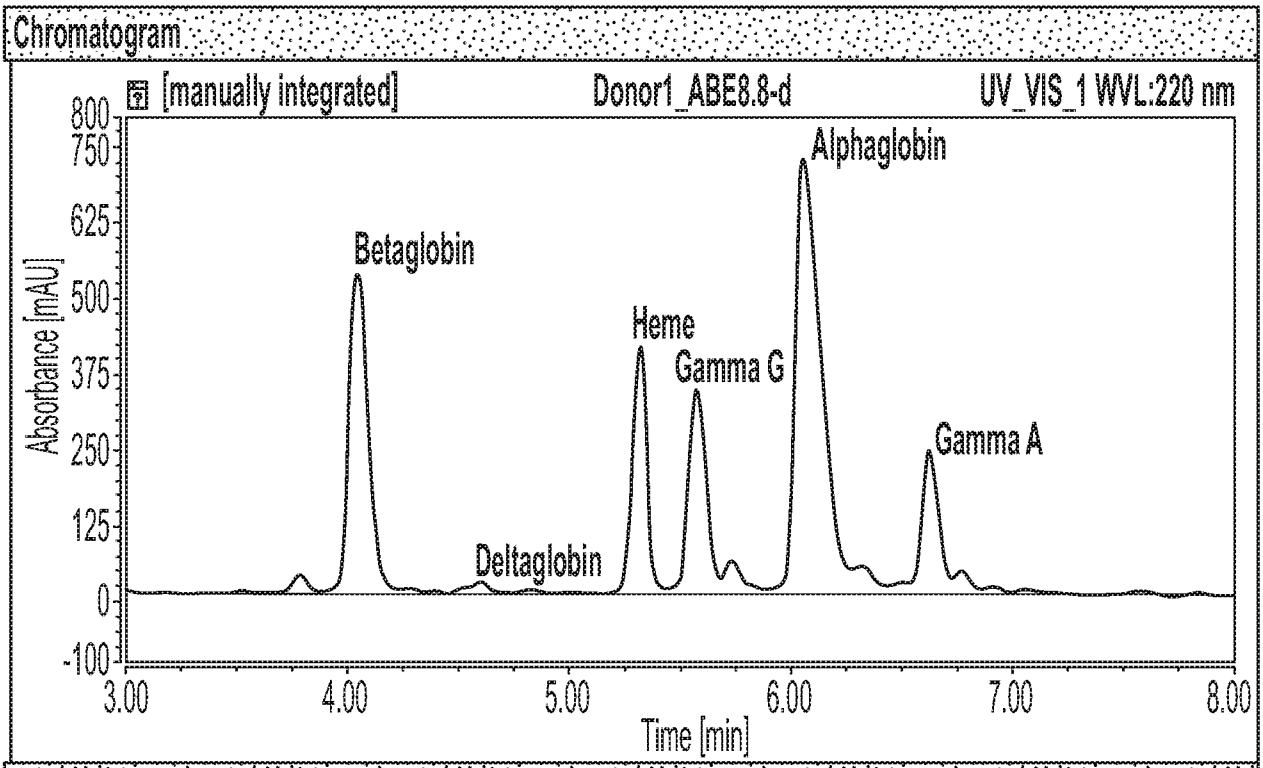
FIG. 12




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 |

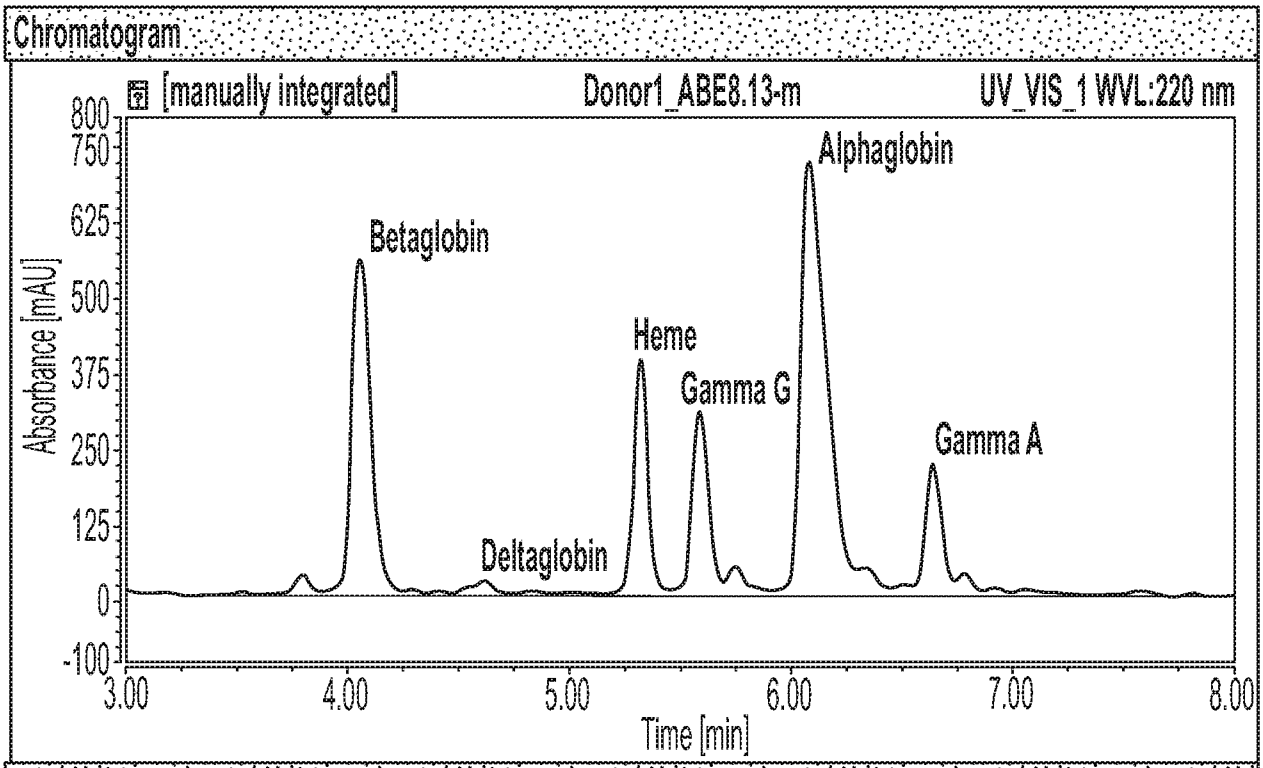
FIG. 13




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 |

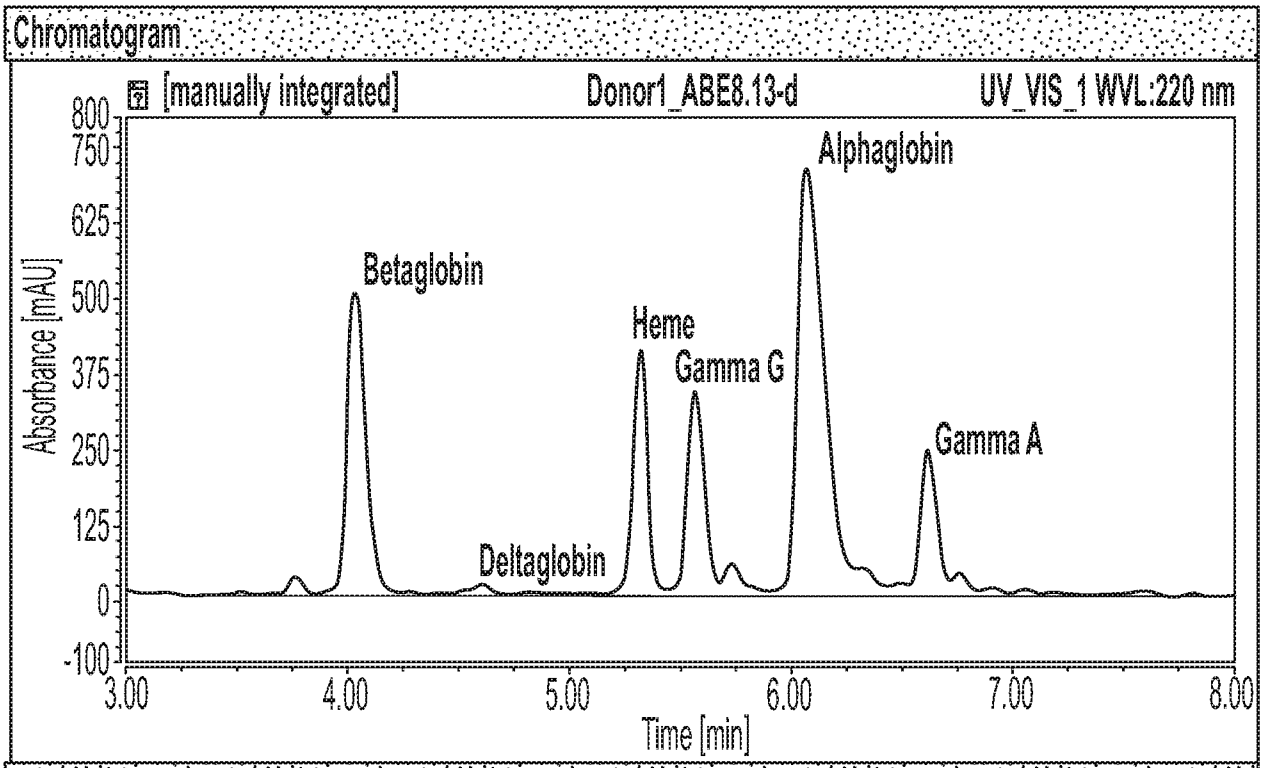
FIG. 14




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 |

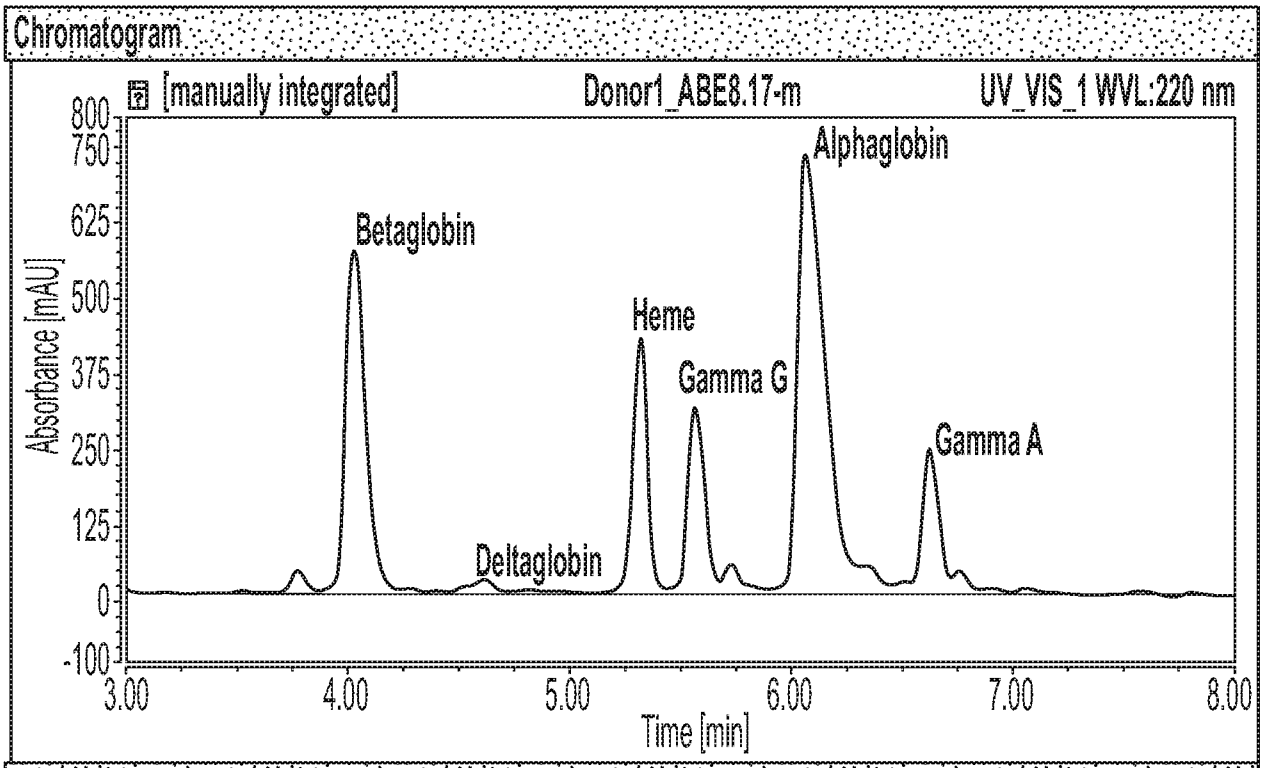
FIG. 15




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 |

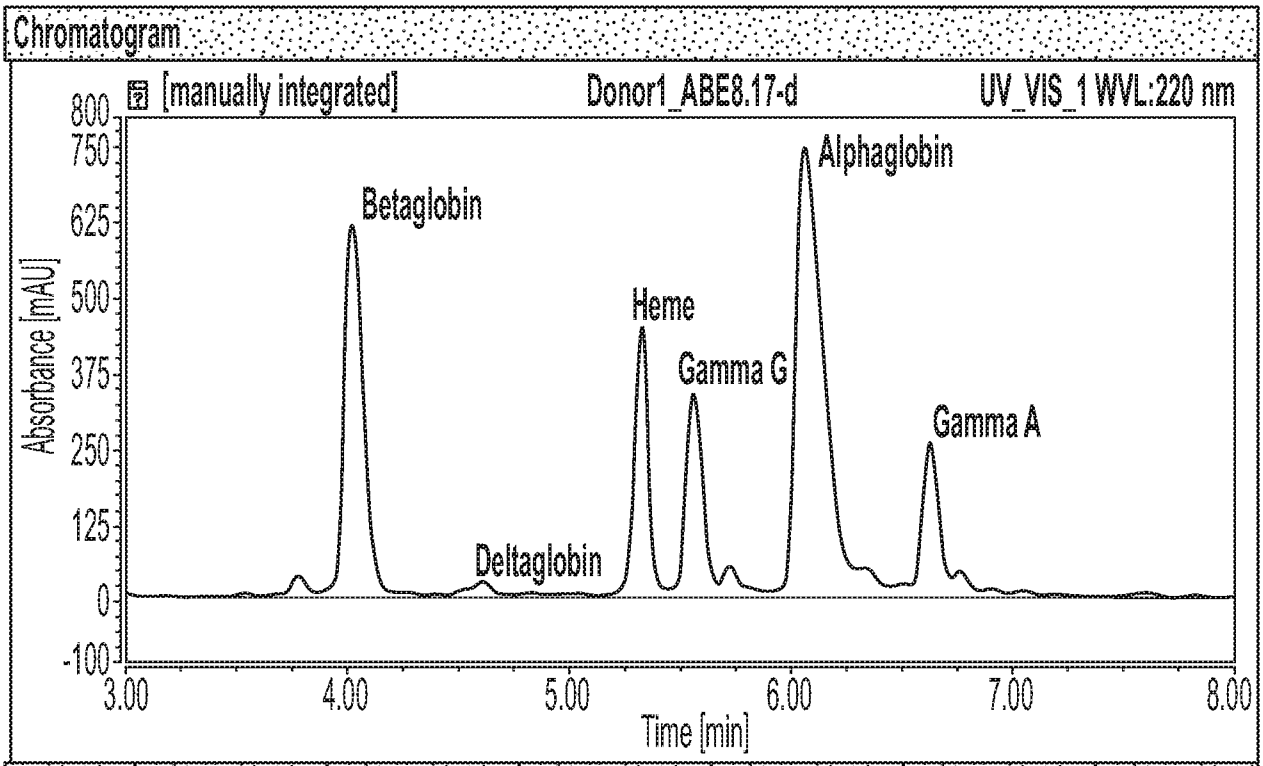
FIG. 16




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 |

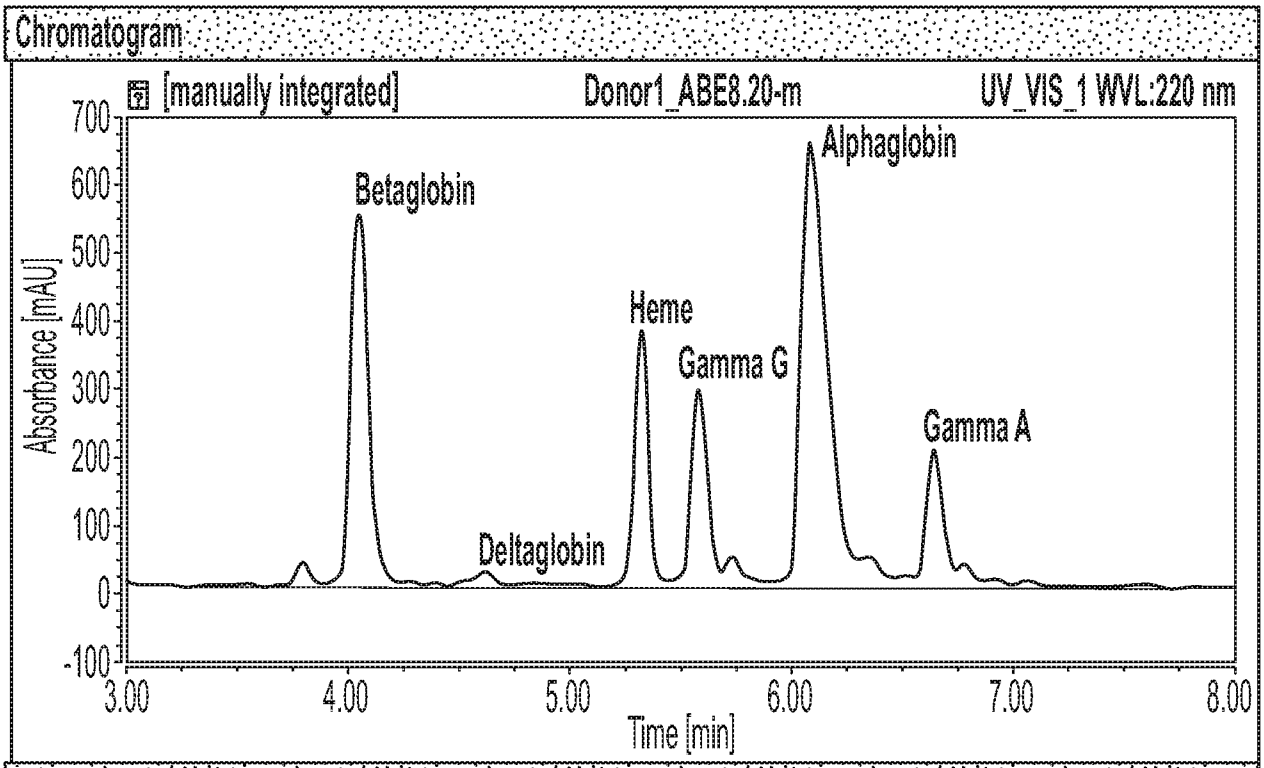
FIG. 17




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 |

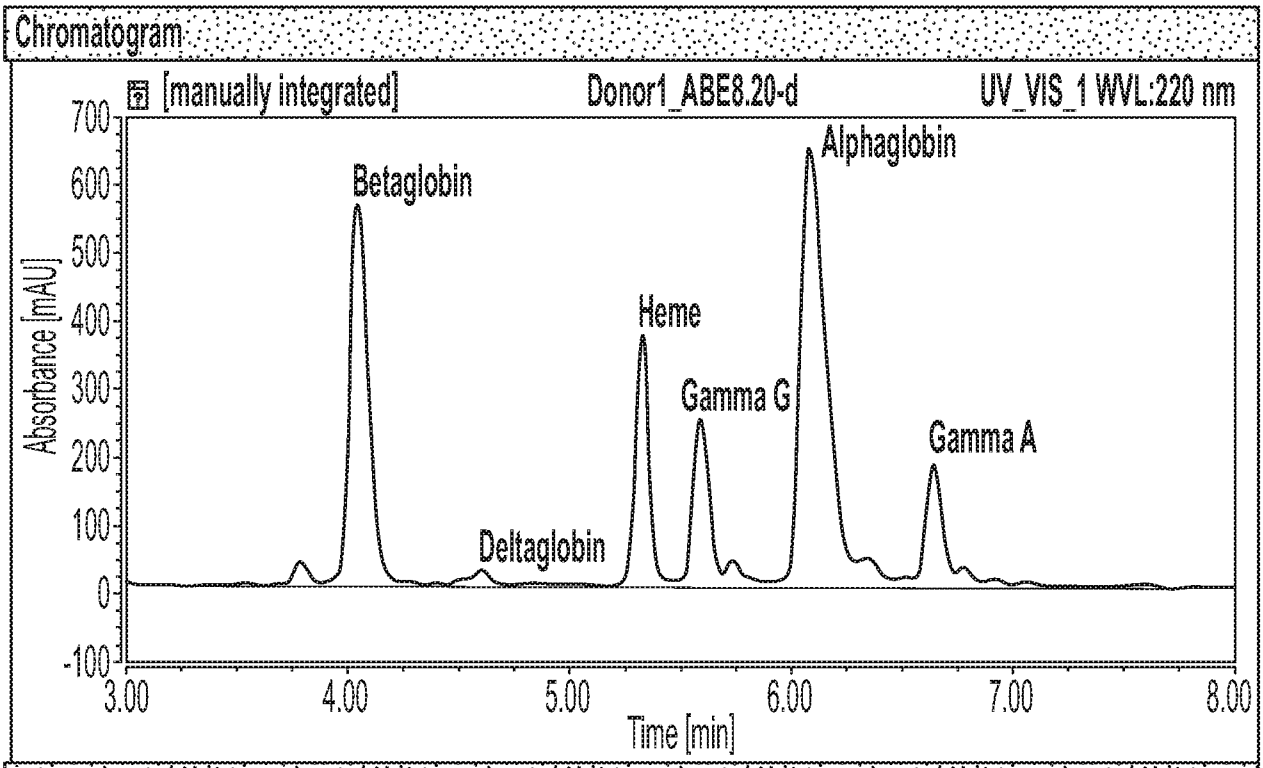
FIG. 18




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

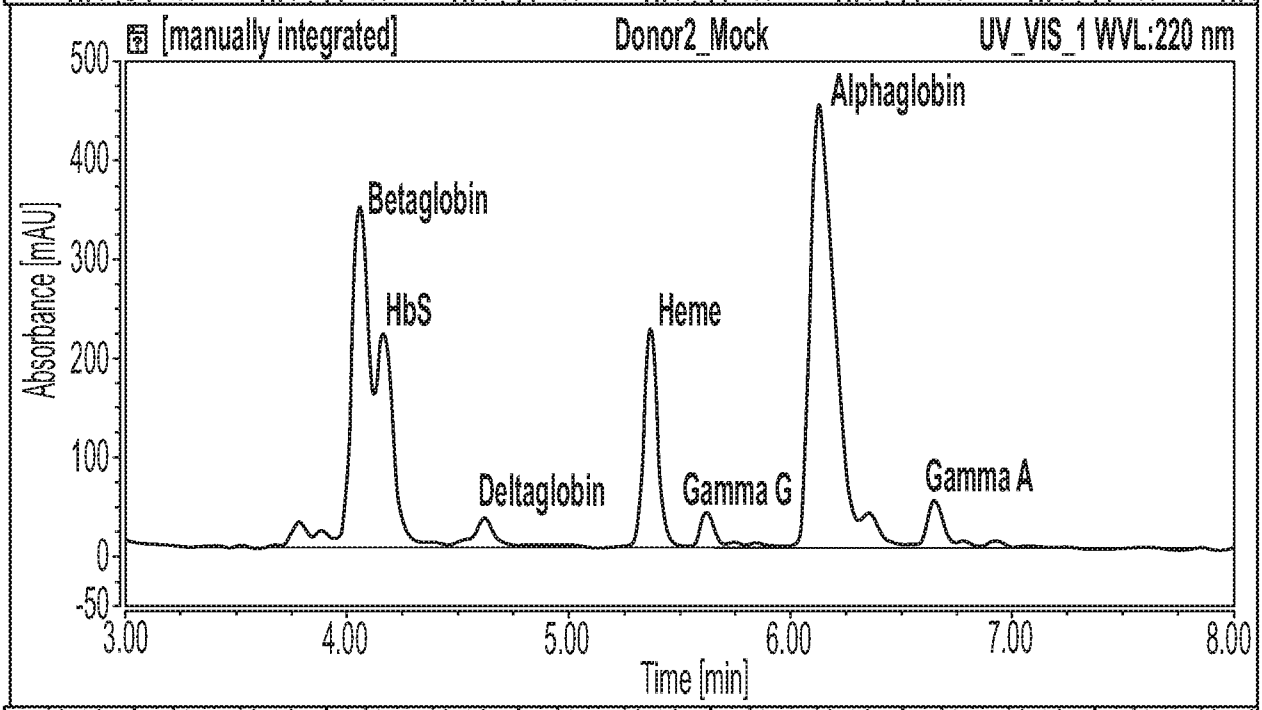



Integration Results

| 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

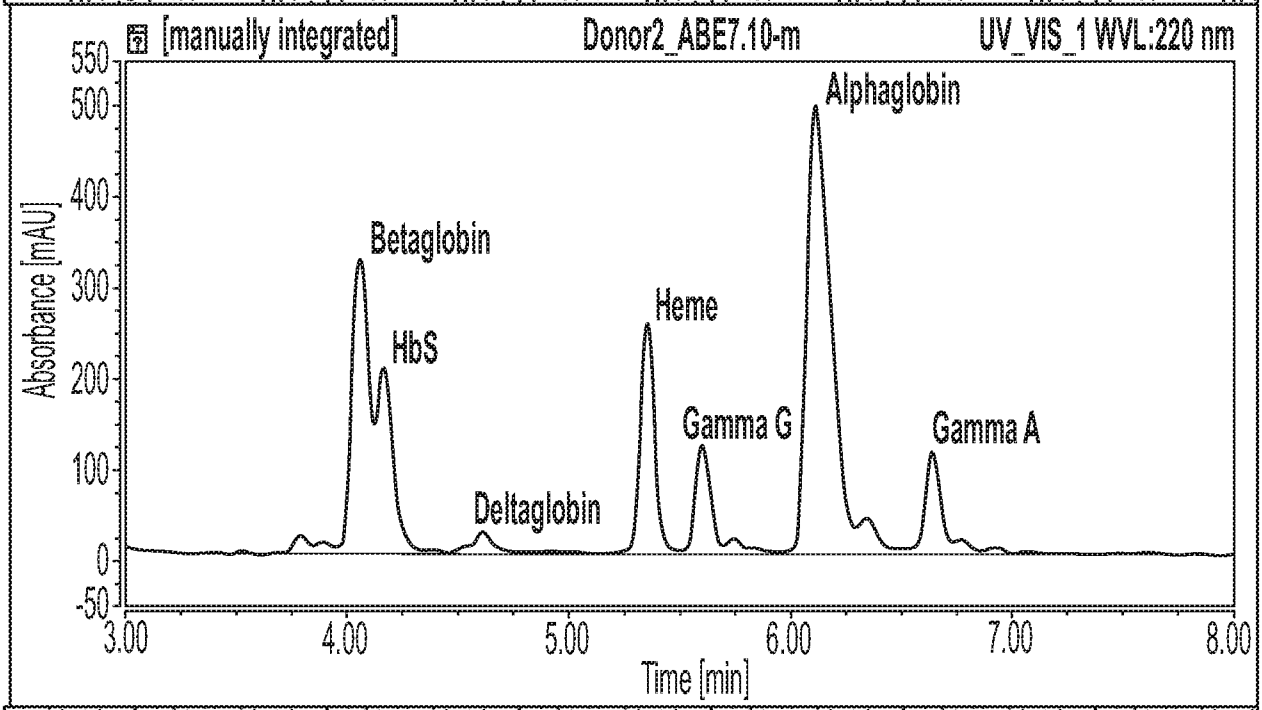



Integration Results

| 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

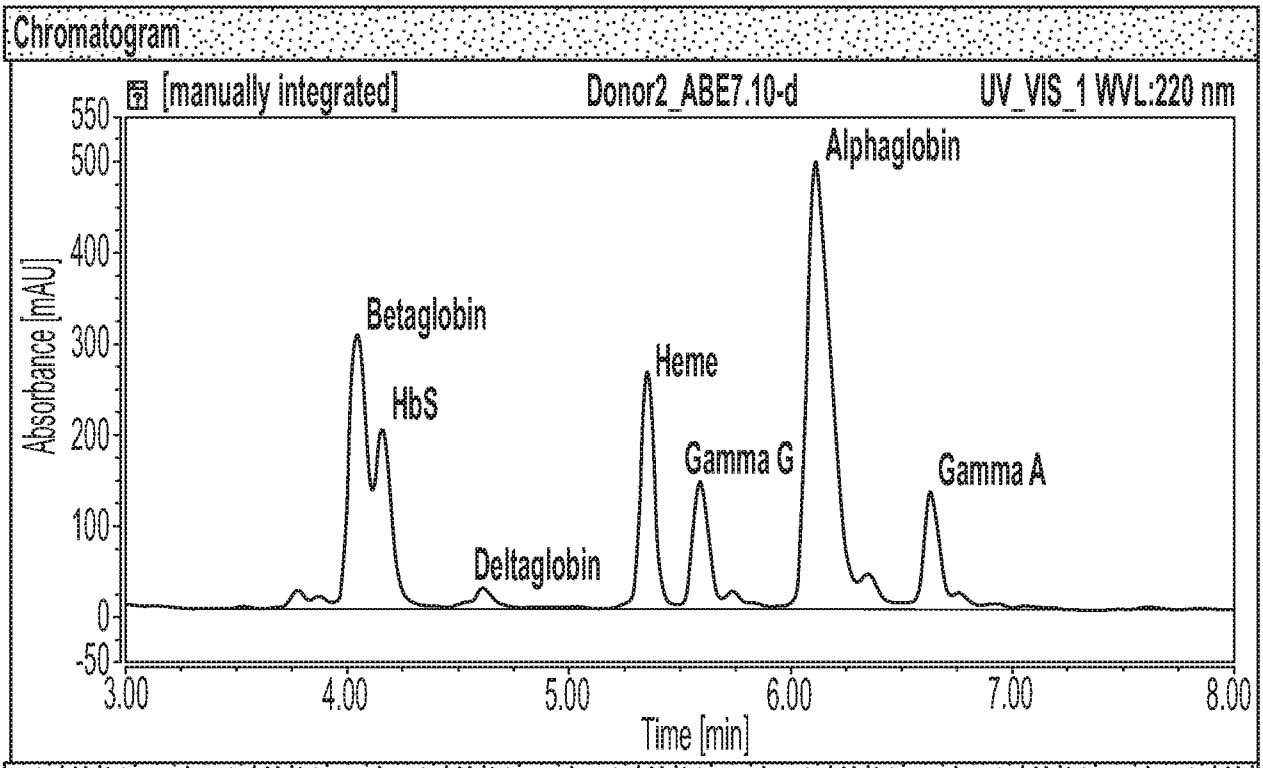
Chromatogram




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

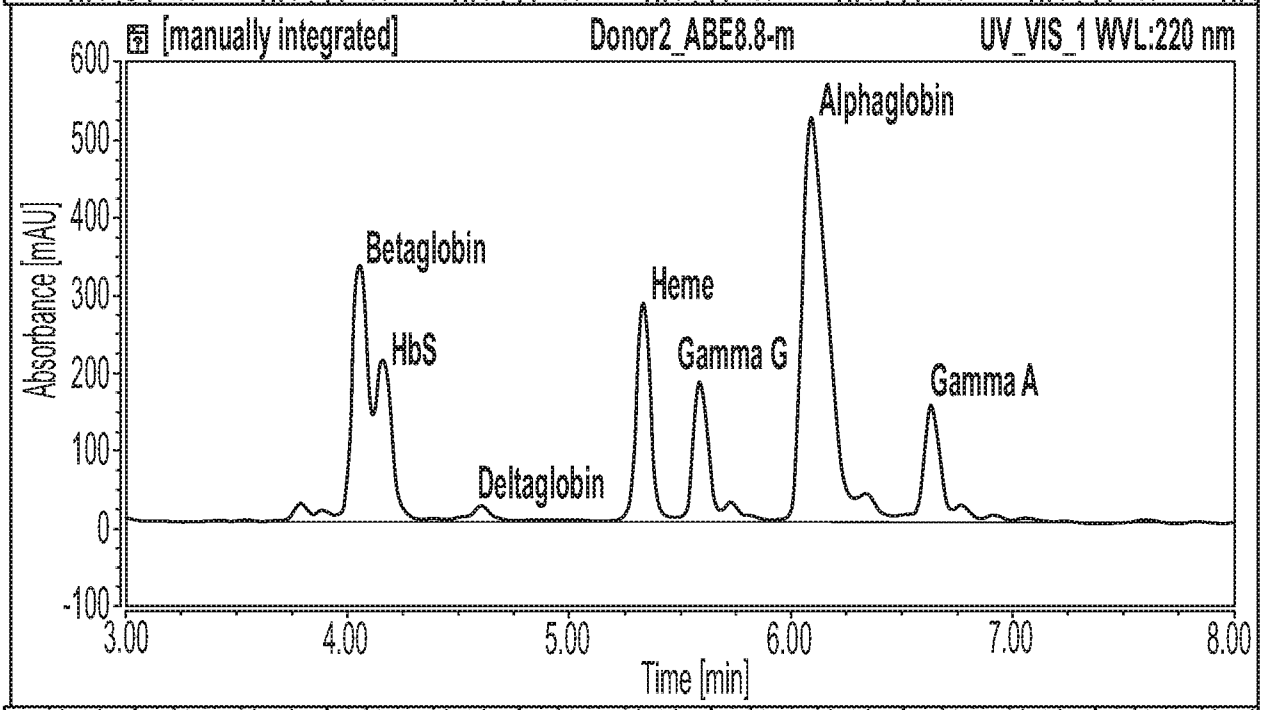



Integration Results

| 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

Chromatogram

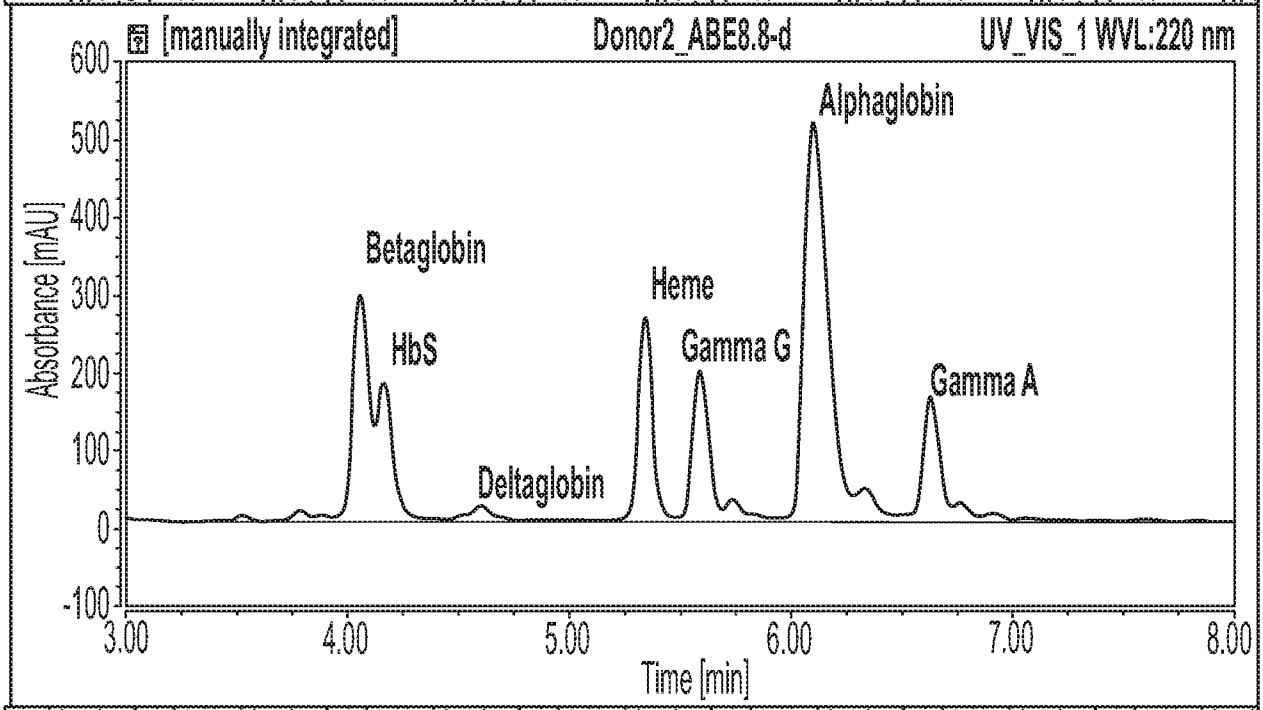



Integration Results

| 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

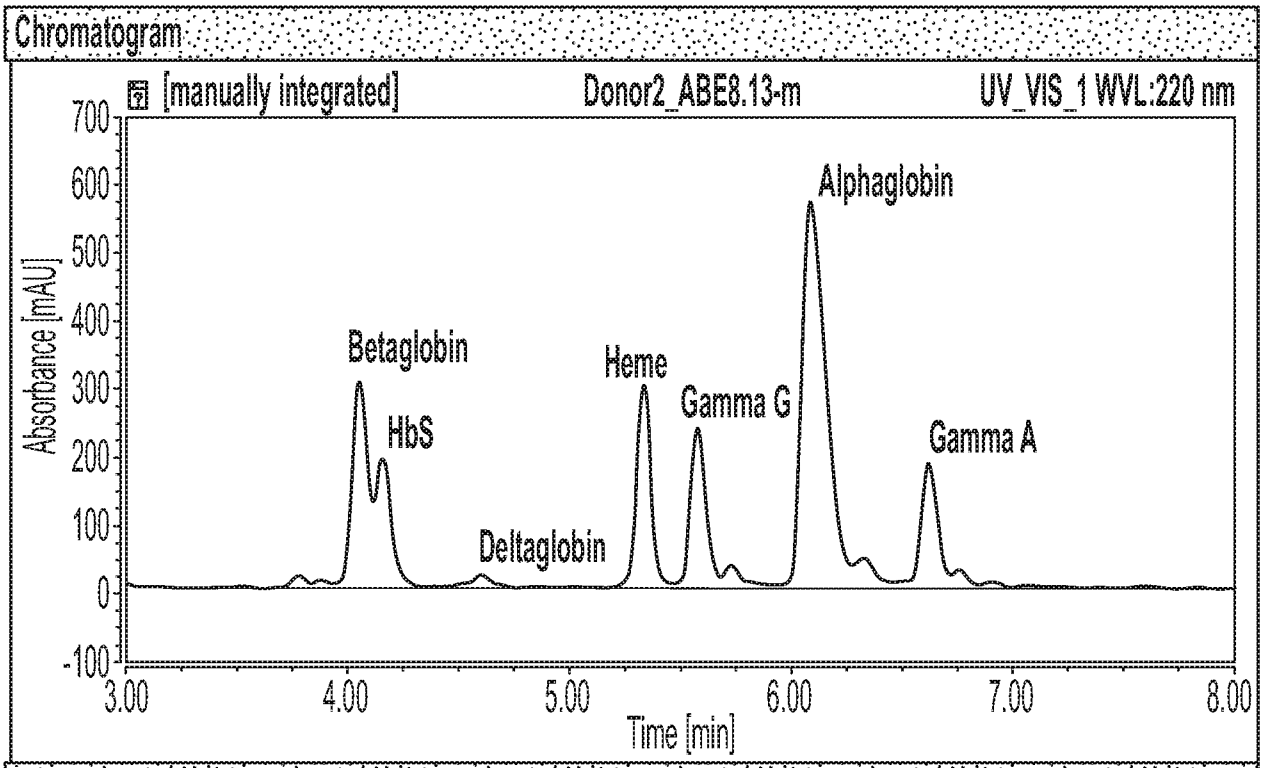
Chromatogram




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 |

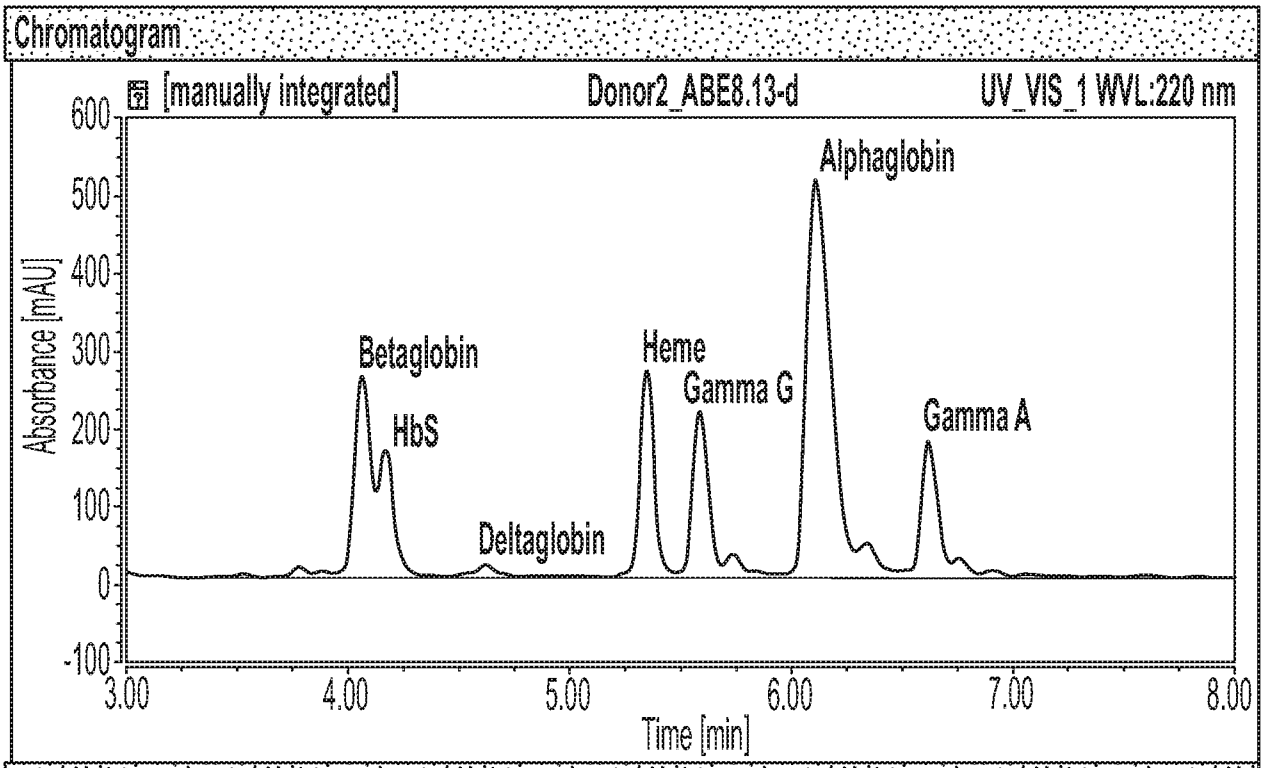
FIG. 25




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 |

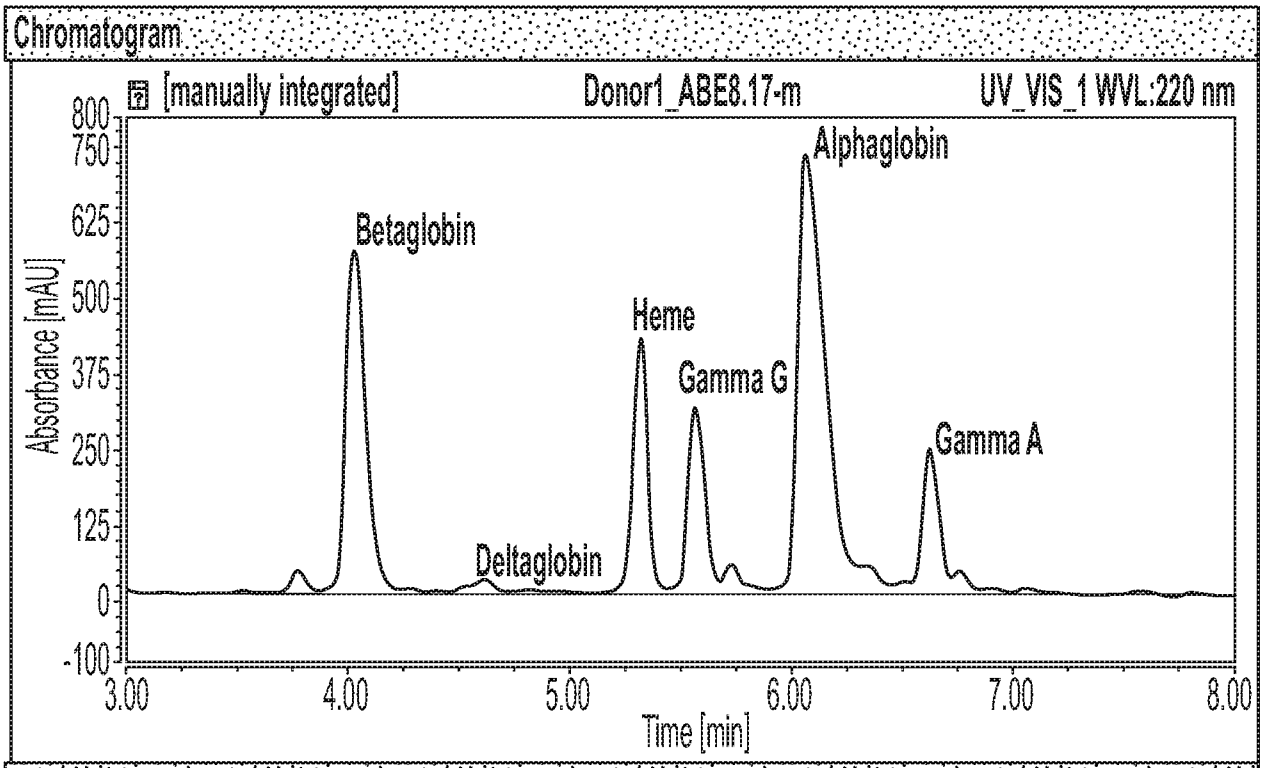
FIG. 26




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 |

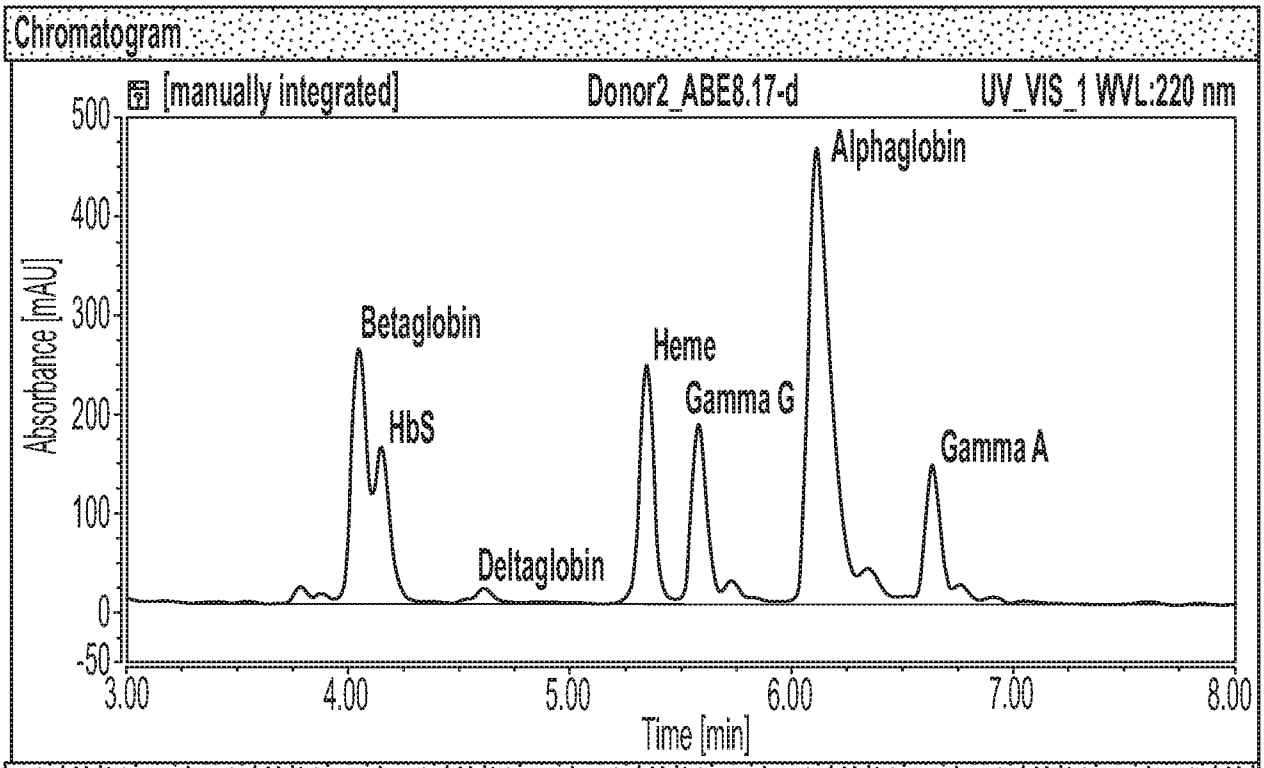
FIG. 27




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 |

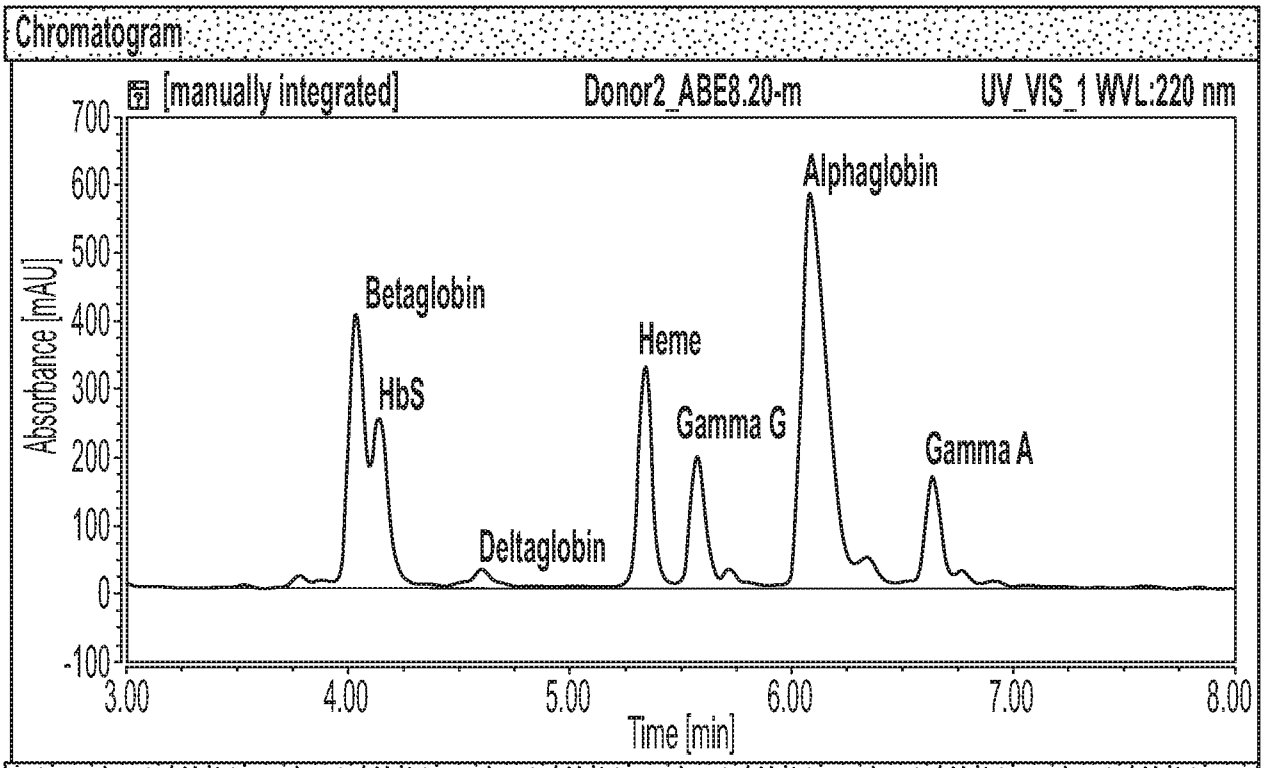
FIG. 28




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 |

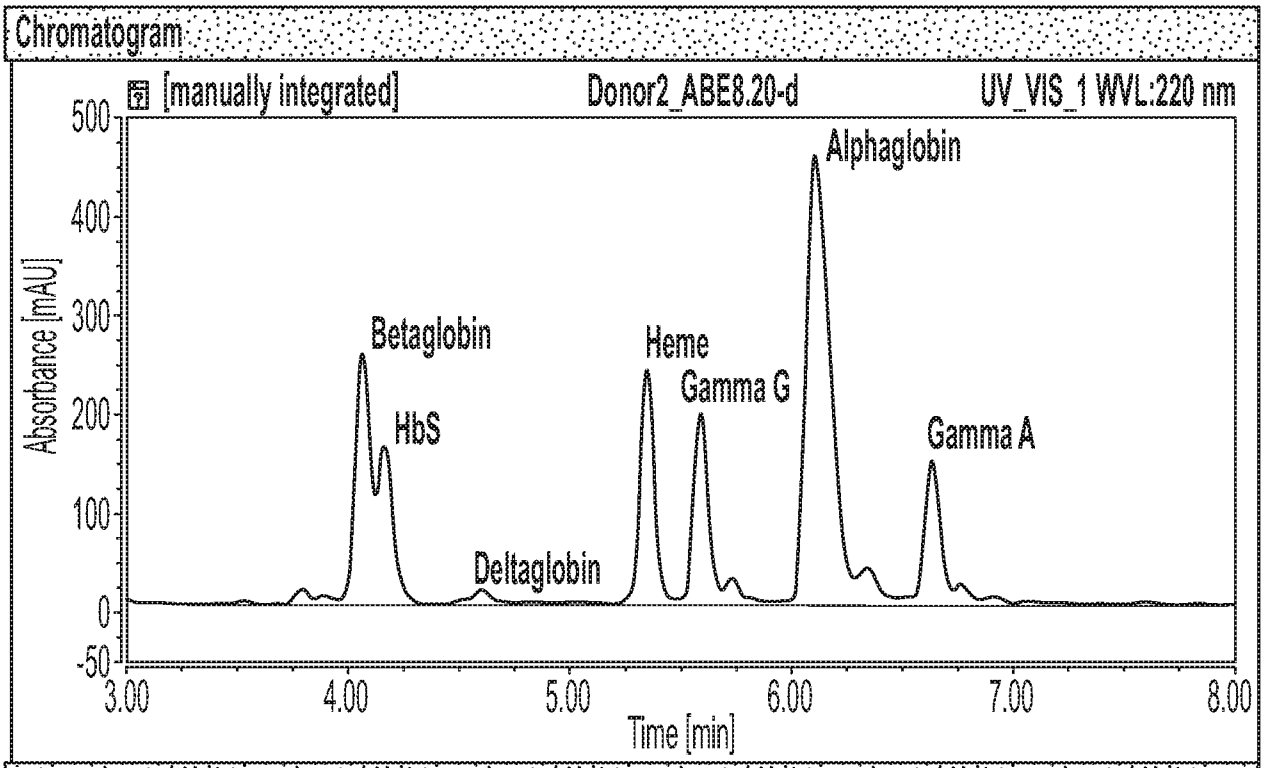
FIG. 29




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

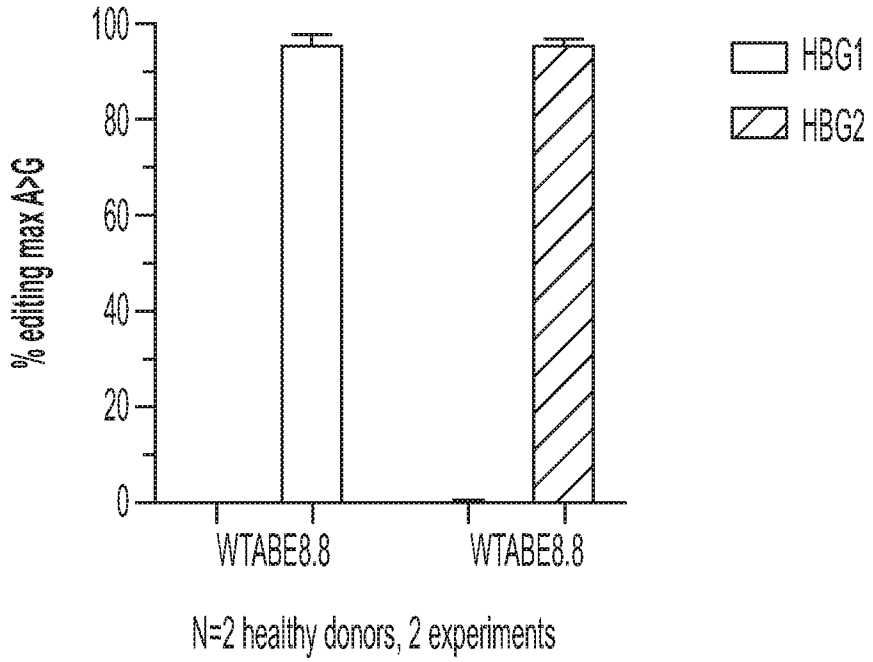



FIG. 31A

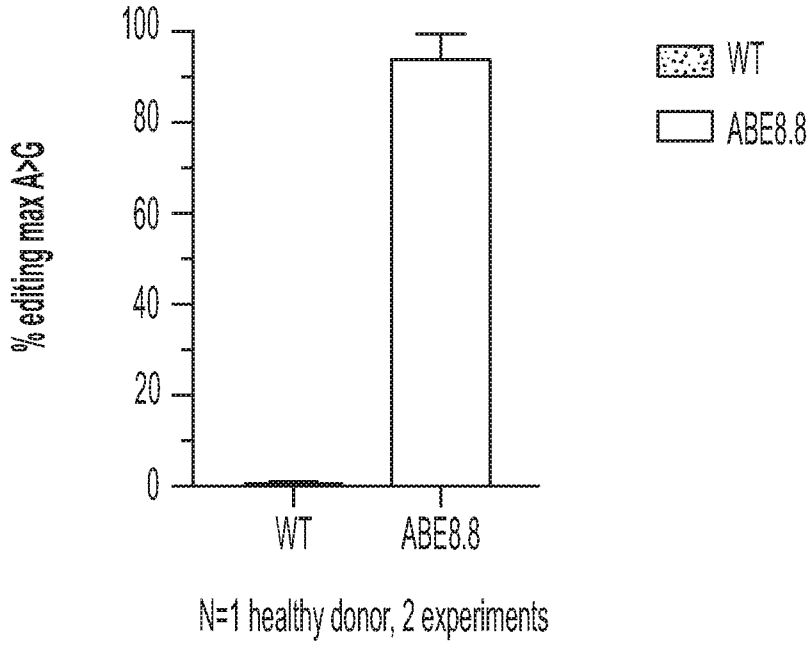



FIG. 31B

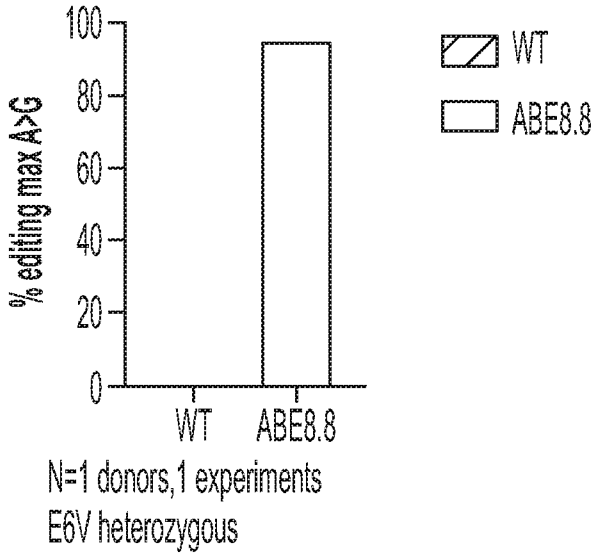



FIG. 31C

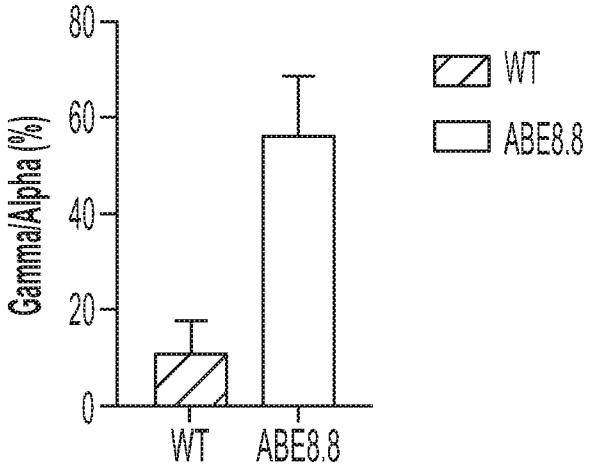



FIG. 31D

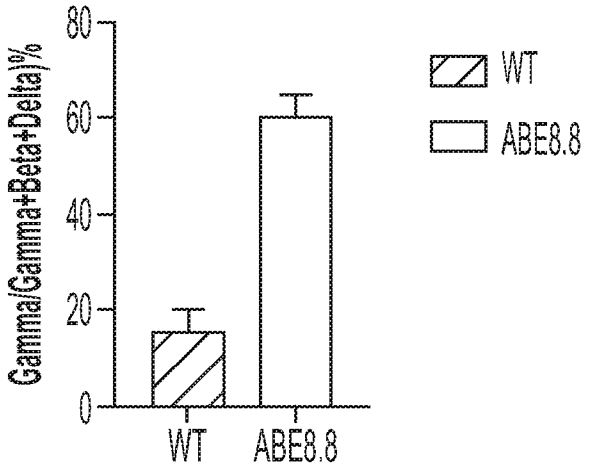



FIG. 31E

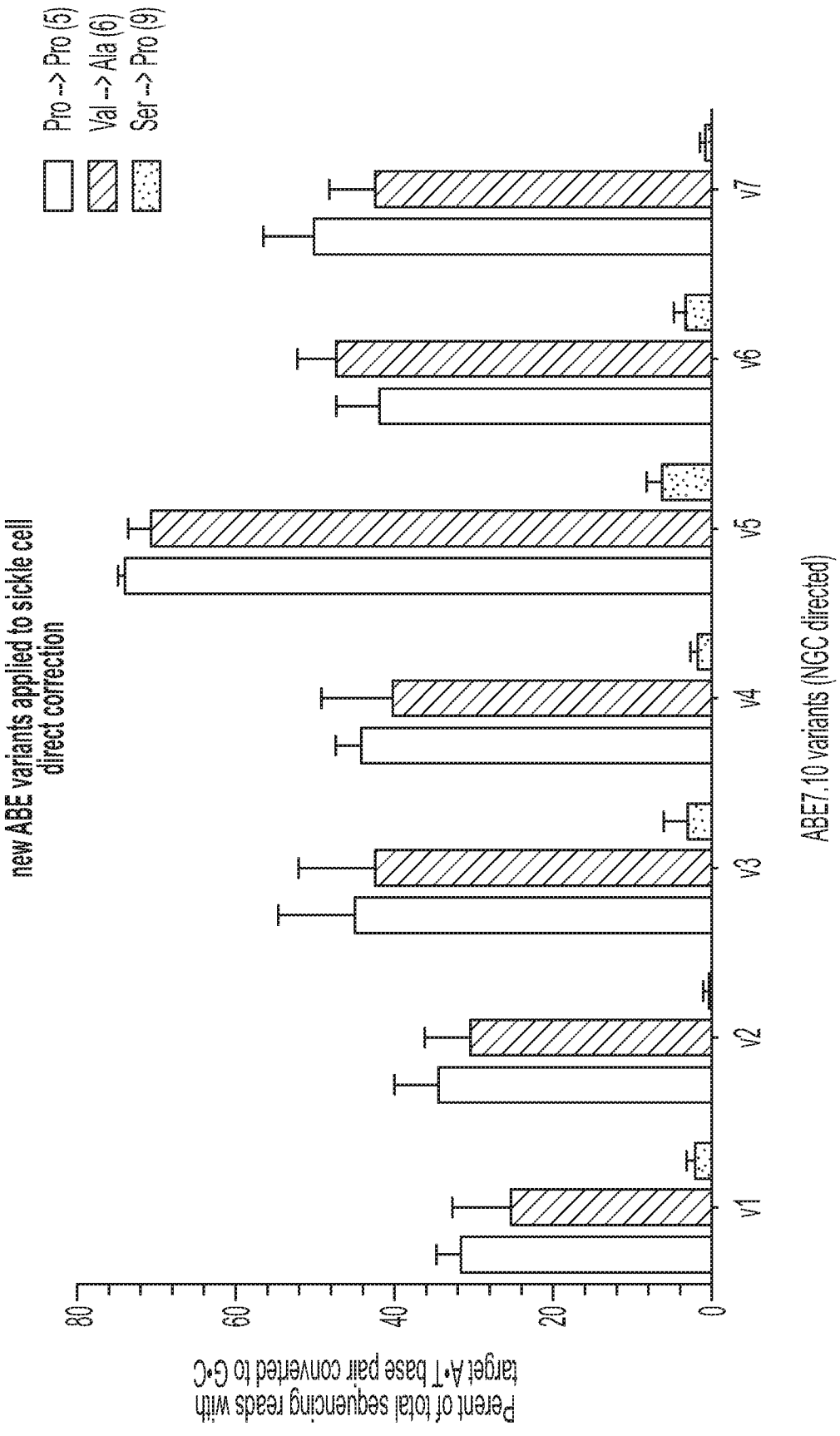

Pro --> Pro (5)
Val --> Ala (6)
Ser --> Pro (9)
new ABE variants applied to sickle cell direct correction
Perent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
v1
v2
v3
v4
v5
v6
v7
ABE7.10 variants (NGG directed)

FIG. 32A

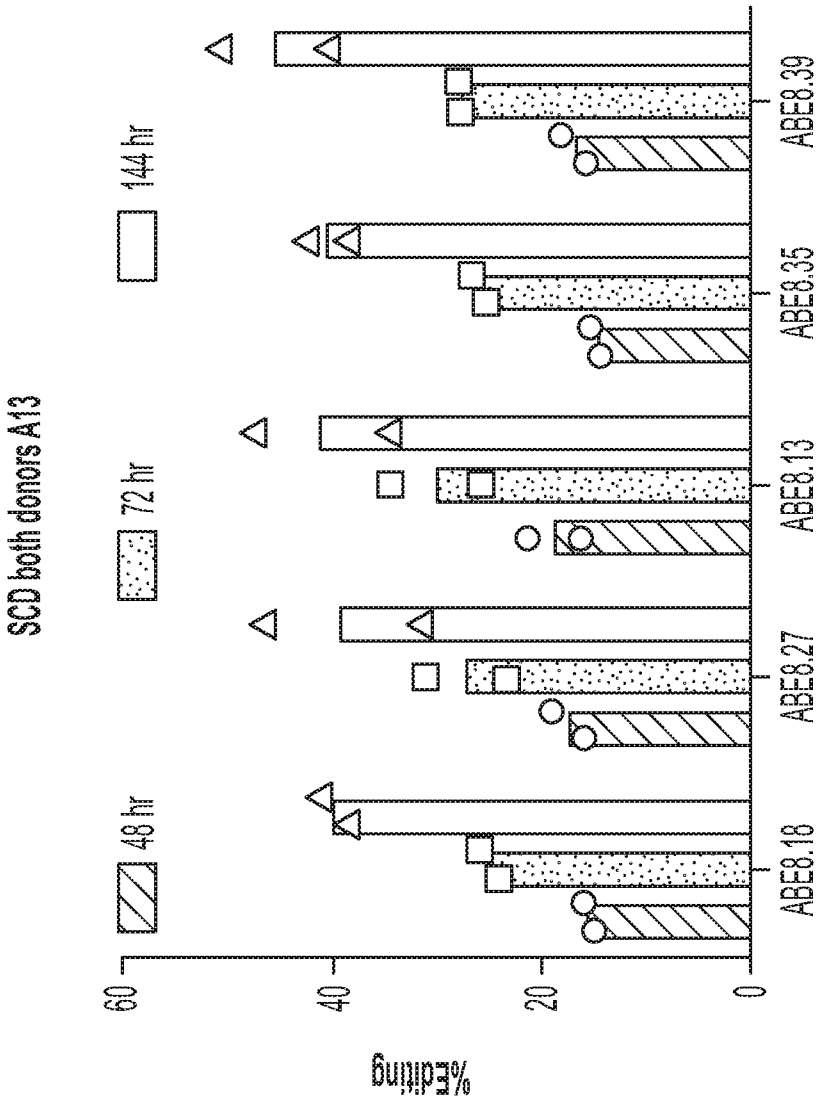

SCD both donors A13
48 hr
72 hr
144 hr
%editing
60
40
20
0
ABE8.18
ABE8.27
ABE8.13
ABE8.35
ABE8.39

FIG. 32B

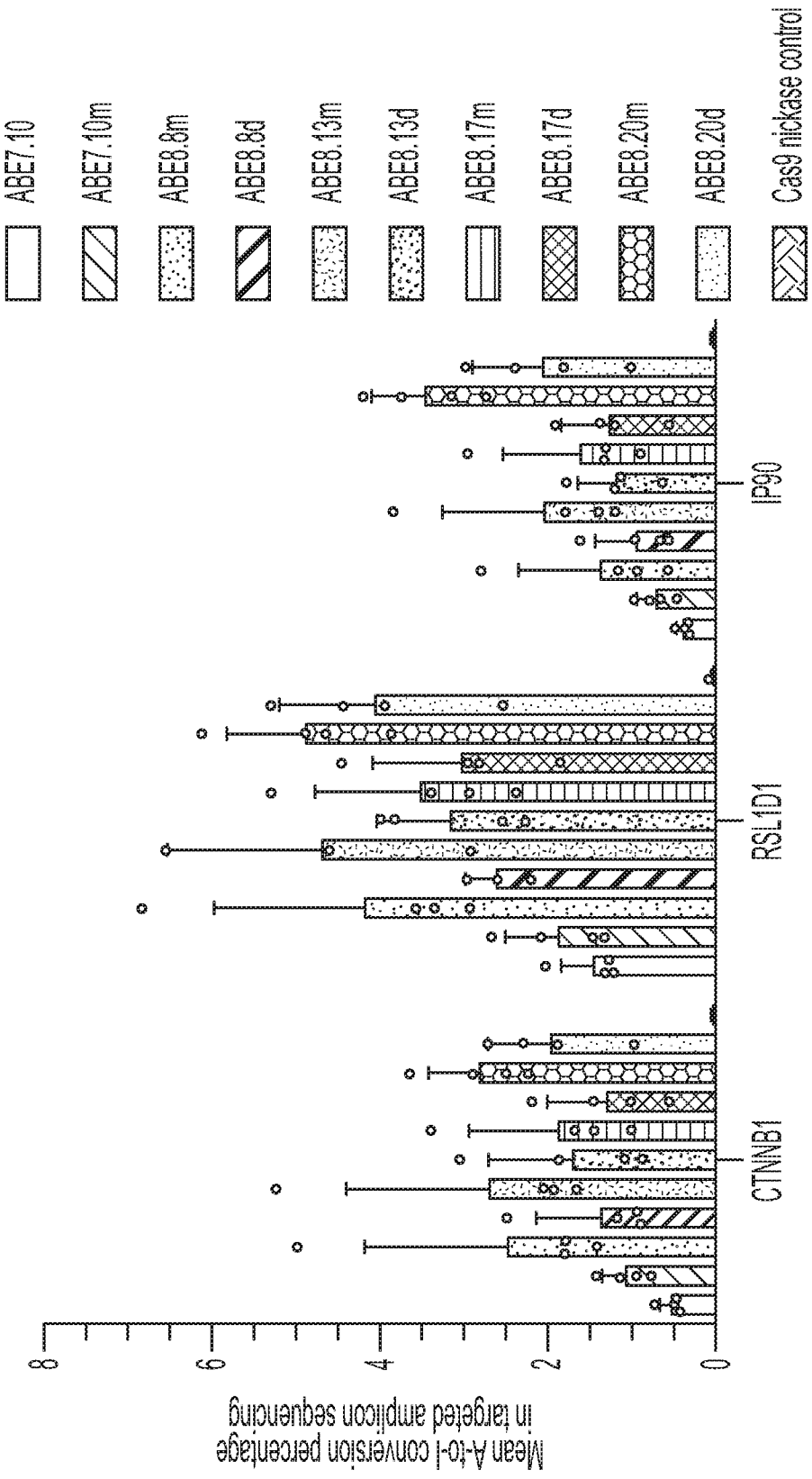

ABE7.10
ABE7.10m
ABE8.8m
ABE8.8d
ABE8.13m
ABE8.13d
ABE8.17m
ABE8.17d
ABE8.20m
ABE8.20d
Cas9 nickase control
IP90
RSL1D1
CTNNB1
0
2
4
6
8
Mean A-to-I conversion percentage
in targeted amplicon sequencing

FIG. 33A

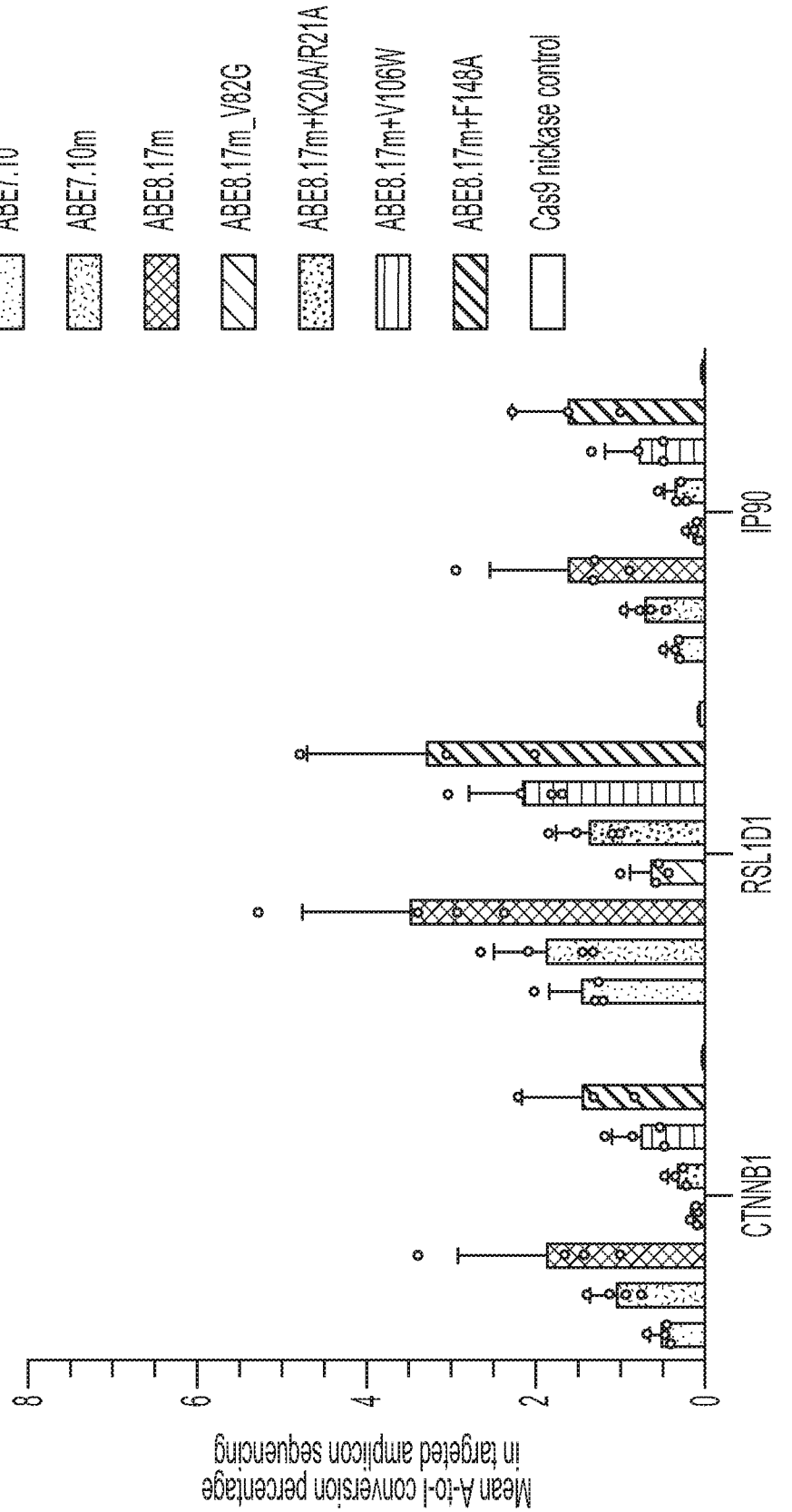

ABE7.10
ABE7.10m
ABE8.17m
ABE8.17m_V82G
ABE8.17m+K20A/R21A
ABE8.17m+V106W
ABE8.17m+F148A
Cas9 nickase control
Mean A-to-I conversion percentage in targeted amplicon sequencing
0
2
4
6
8
CTNNB1
RSL1D1
IP90

FIG. 33B

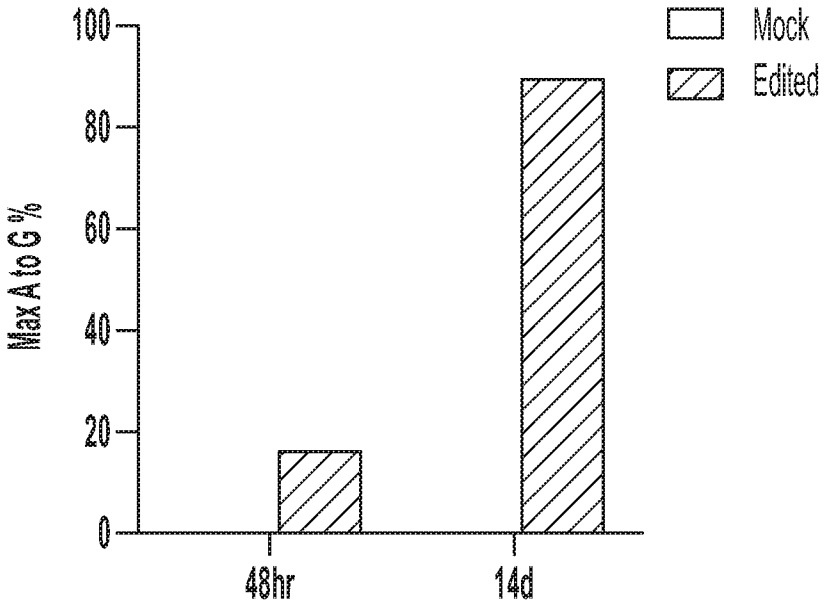



FIG. 34A

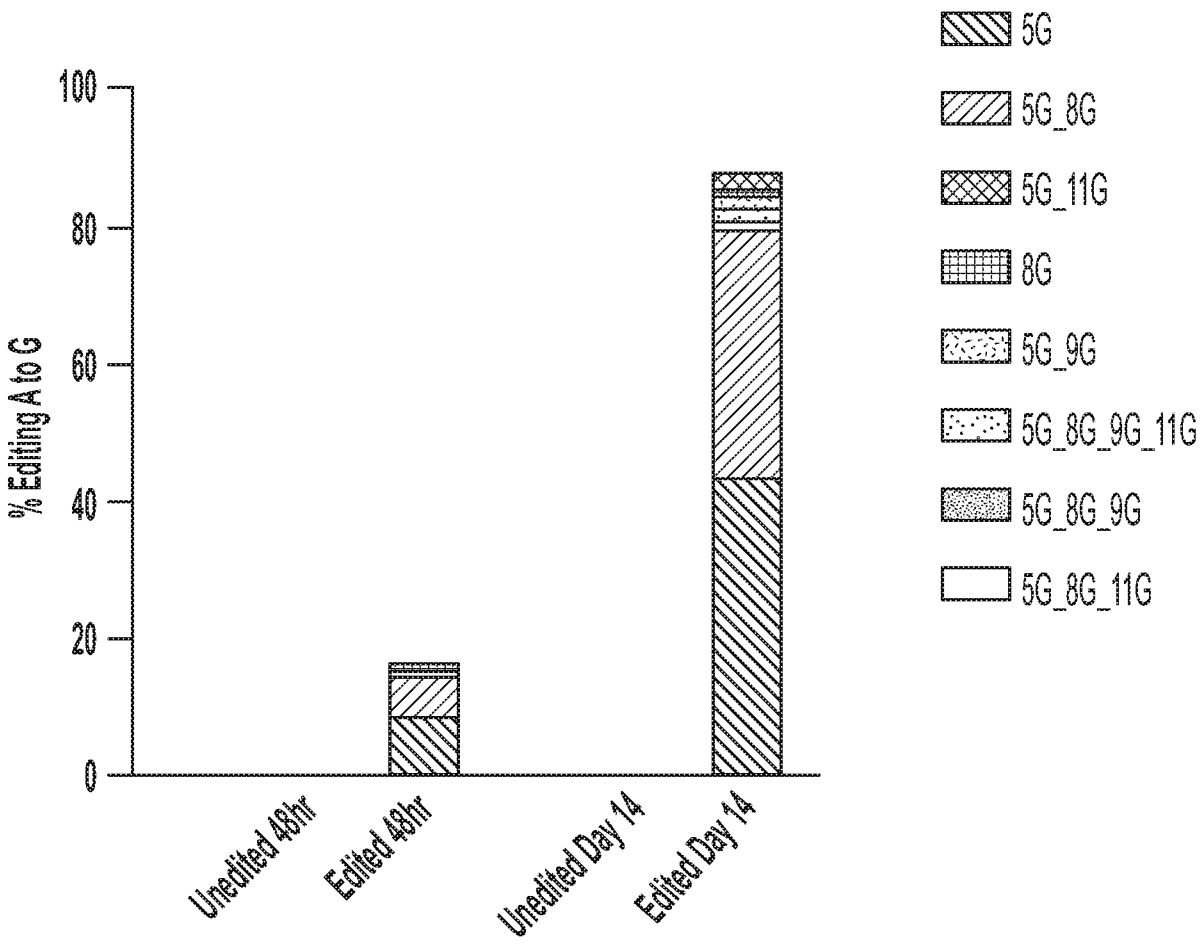

% Editing A to G
100
80
60
40
20
0
Unedited 48hr
Edited 48hr
Unedited Day 14
Edited Day 14
5G
5G_8G
5G_11G
8G
5G_9G
5G_8G_9G_11G
5G_8G_9G
5G_8G_11G

FIG. 34B

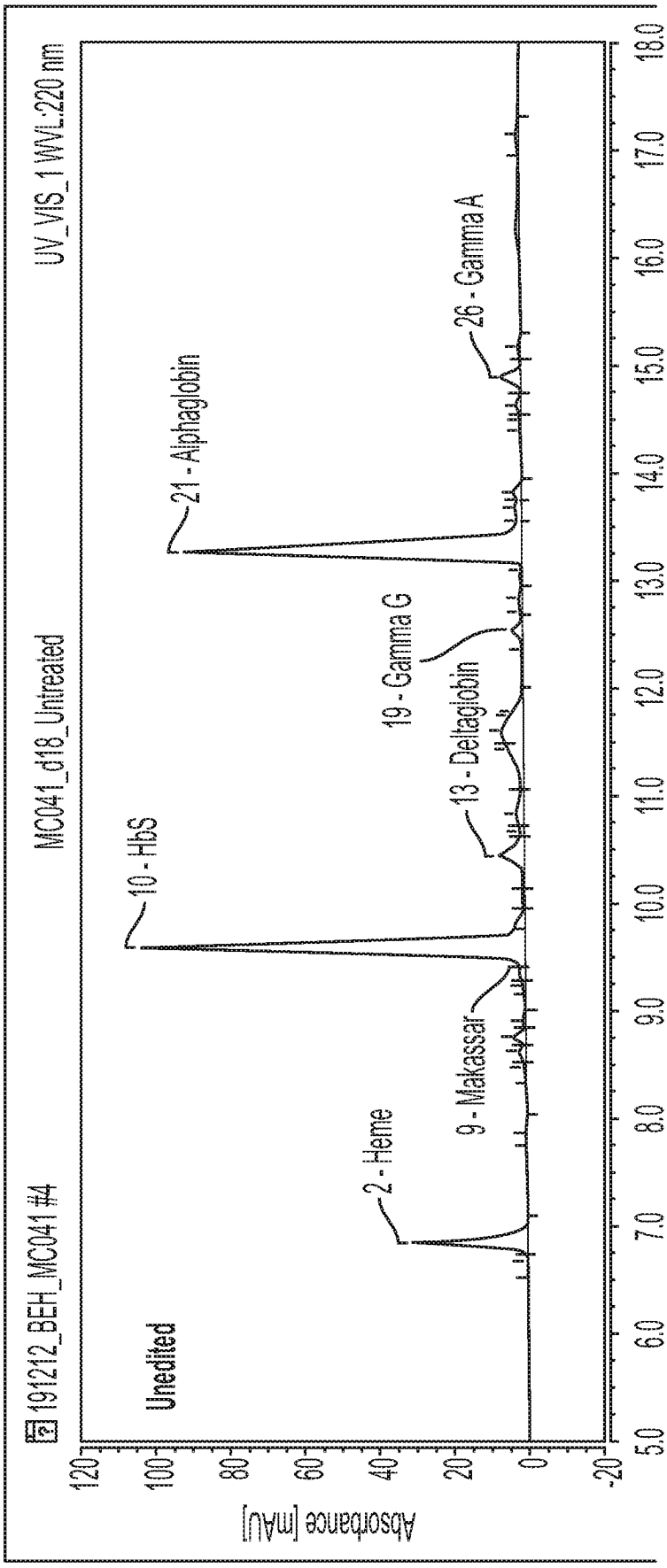

191212_BEH_MC041 #4
MC041_d18_Untreated
UV_VIS_1 WVL:220 nm
Unedited
Absorbance [mAU]
120
100
80
60
40
20
0
-20
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
2 - Heme
9 - Makassar
10 - HbS
13 - Deltaglobin
19 - Gamma G
21 - Alphaglobin
26 - Gamma A

FIG. 35A

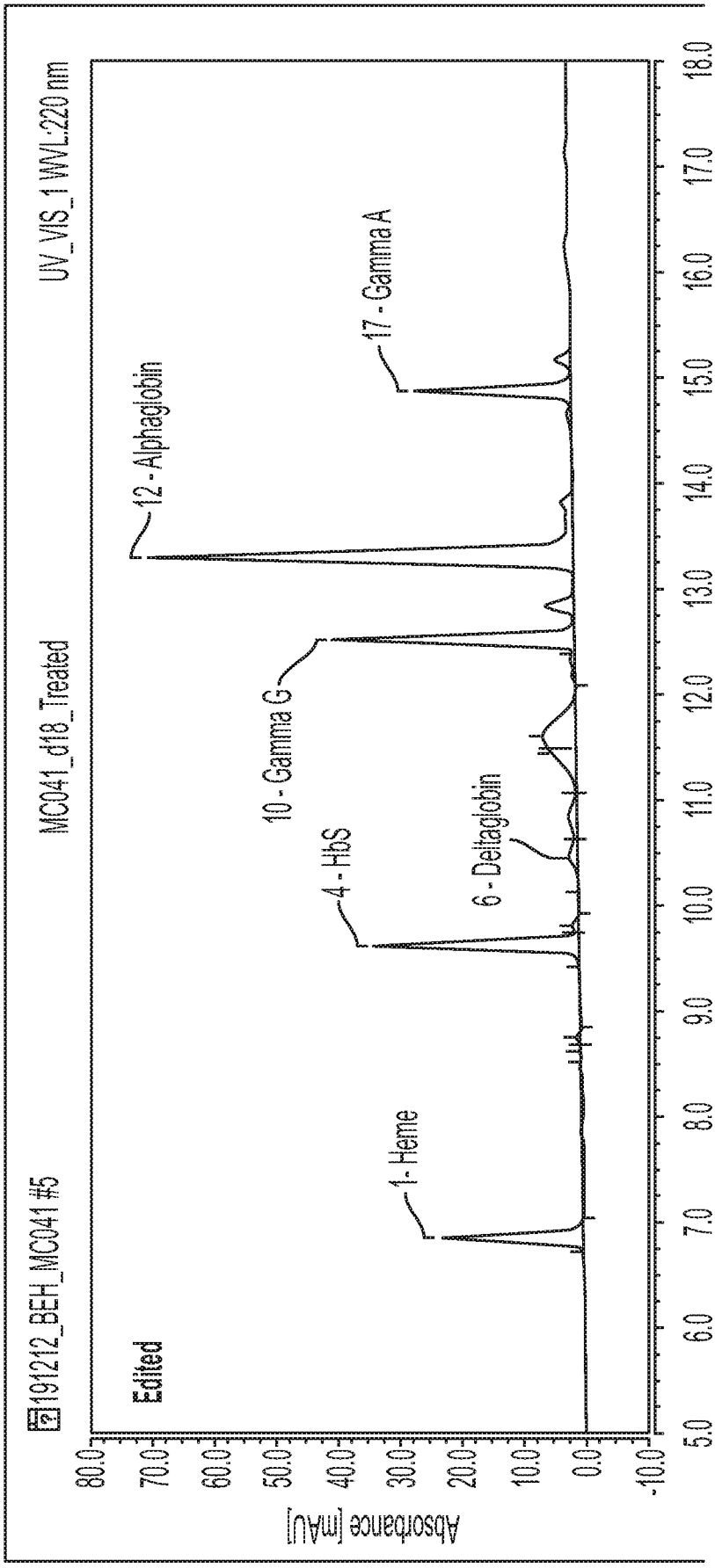

191212_BEH_MC041 #5
Edited
MC041_d18_Treated
UV_VIS_1 WVL:220 nm
1 - Heme
4 - HbS
6 - Deltaglobin
10 - Gamma G
12 - Alphaglobin
17 - Gamma A
Absorbance [mAU]
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0

FIG. 35B

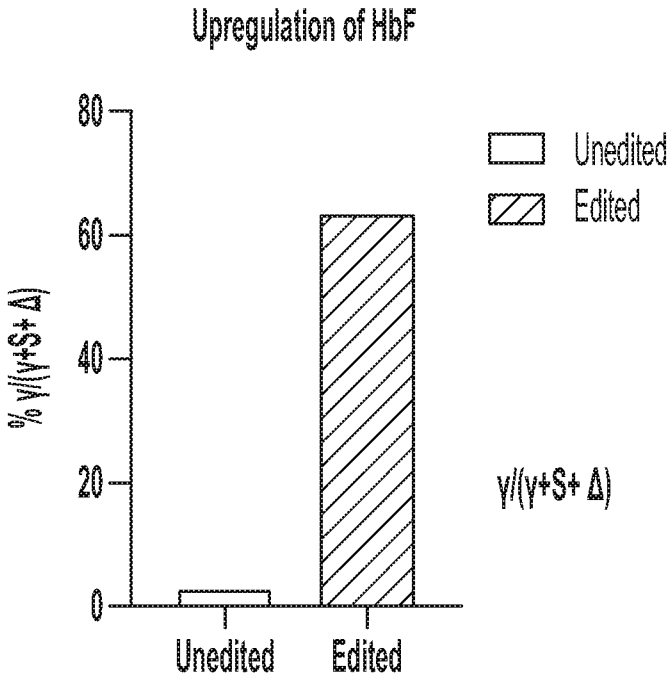



FIG. 35C

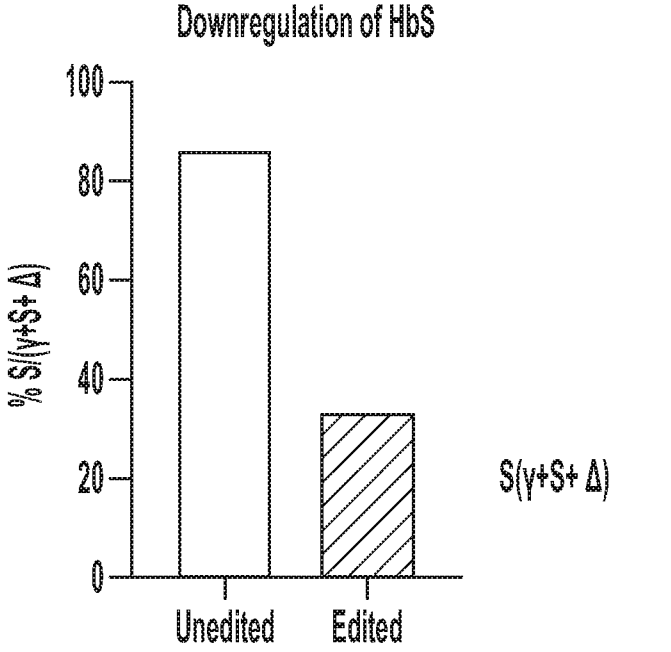



FIG. 35D

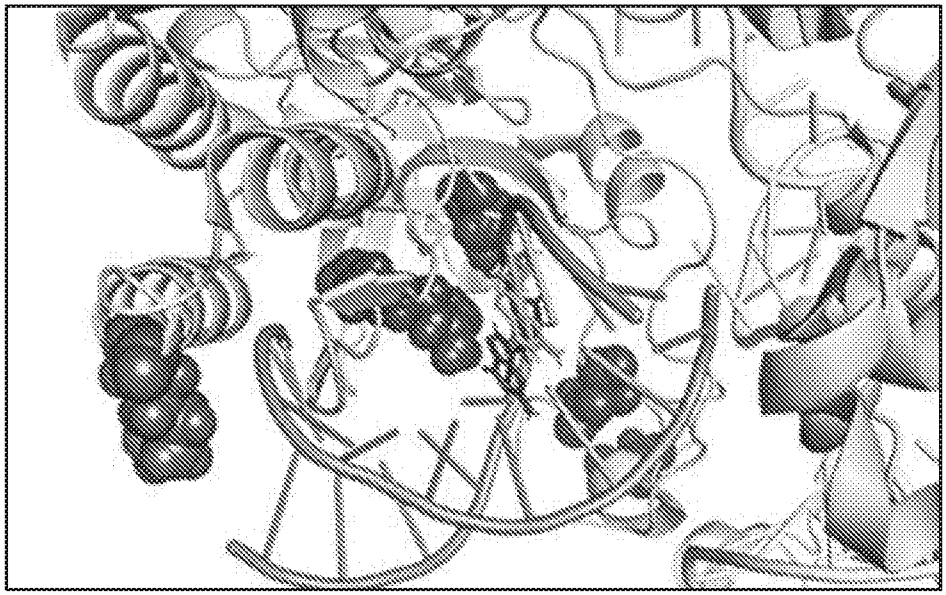
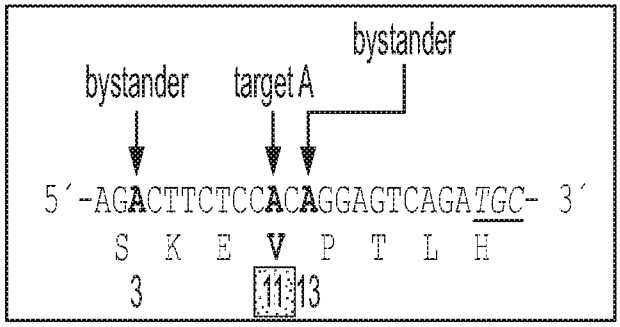


FIG. 36A




| 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

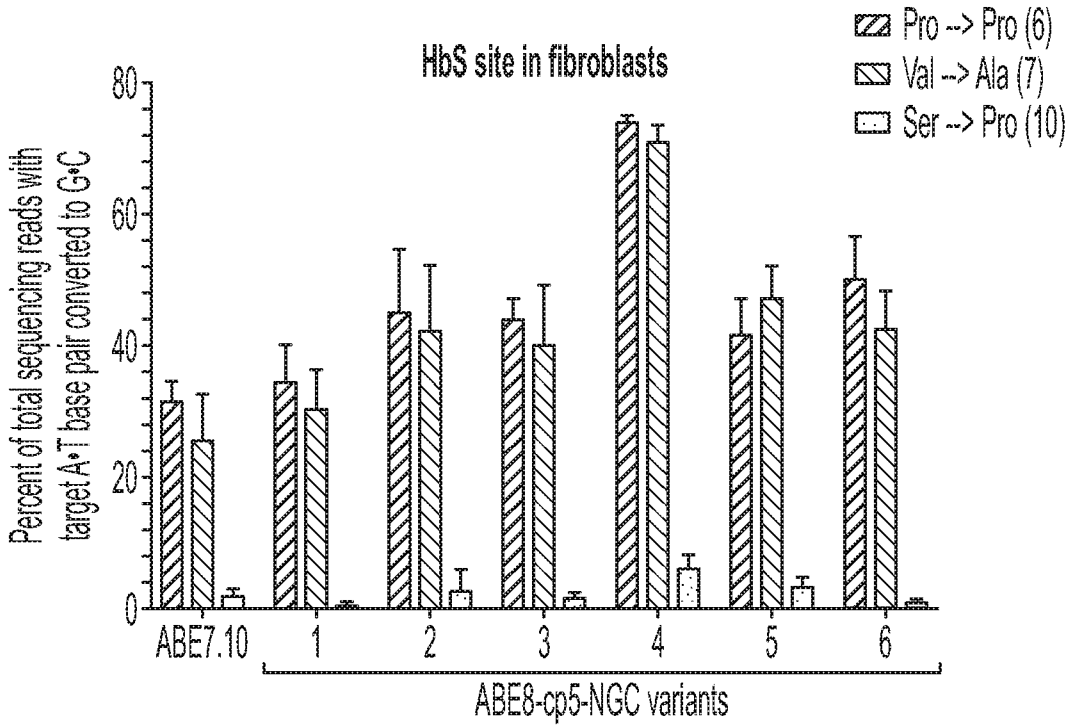

HbS site in fibroblasts
Pro --> Pro (6)
Val --> Ala (7)
Ser --> Pro (10)
Percent of total sequencing reads with target A•T base pair converted to G•C
0
20
40
60
80
ABE7.10
1
2
3
4
5
6
ABE8-cp5-NGC variants

FIG. 36C

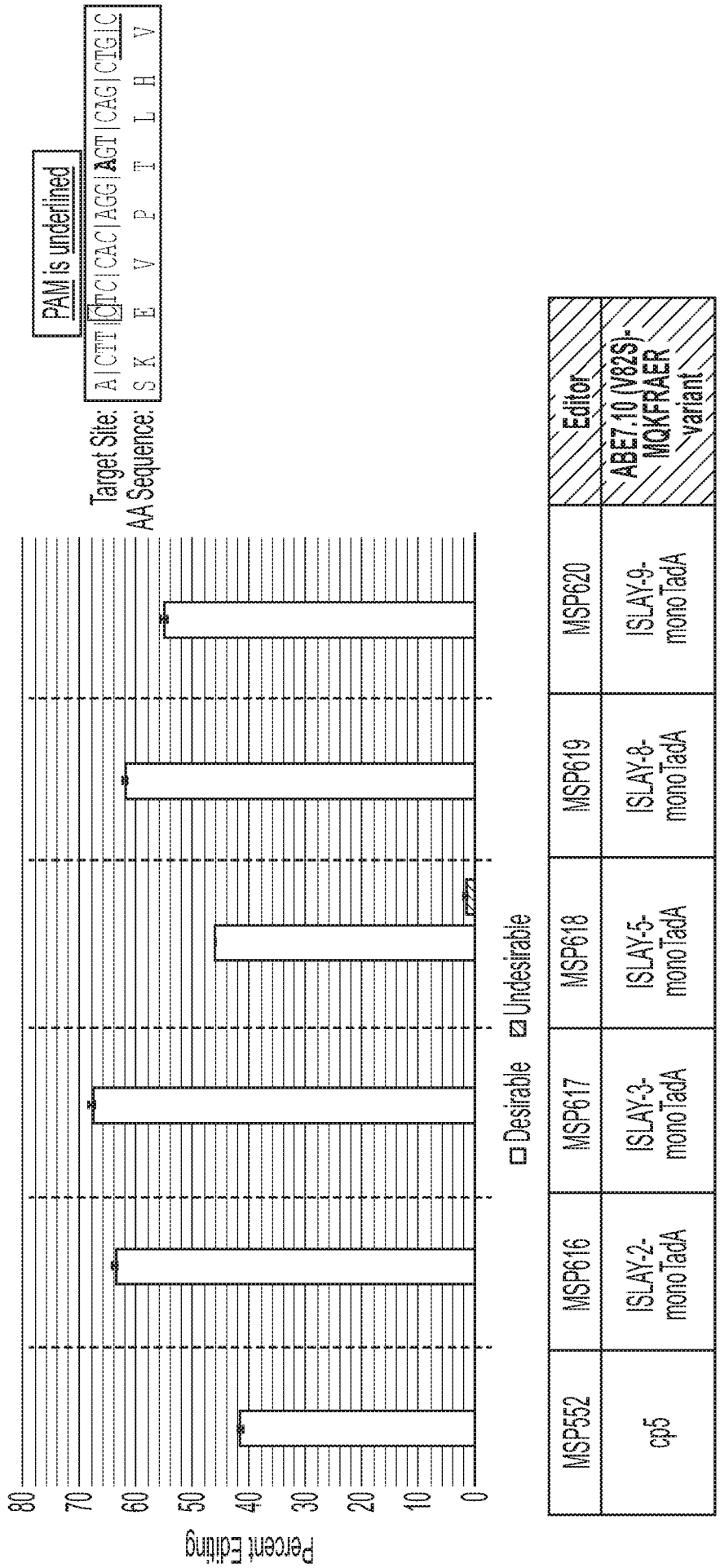

PAM is underlined
Target Site: A CTT CT C CAC AGG AGT CAG CTG C
AA Sequence: S K E V P T L H V
Desirable
Undesirable
Percent Editing
0
10
20
30
40
50
60
70
80
MSP552
MSP616
MSP617
MSP618
MSP619
MSP620
Editor
cp5
ISLAY-2-monoTadA
ISLAY-3-monoTadA
ISLAY-5-monoTadA
ISLAY-8-monoTadA
ISLAY-9-monoTadA
ABE7.10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

FIG. 37

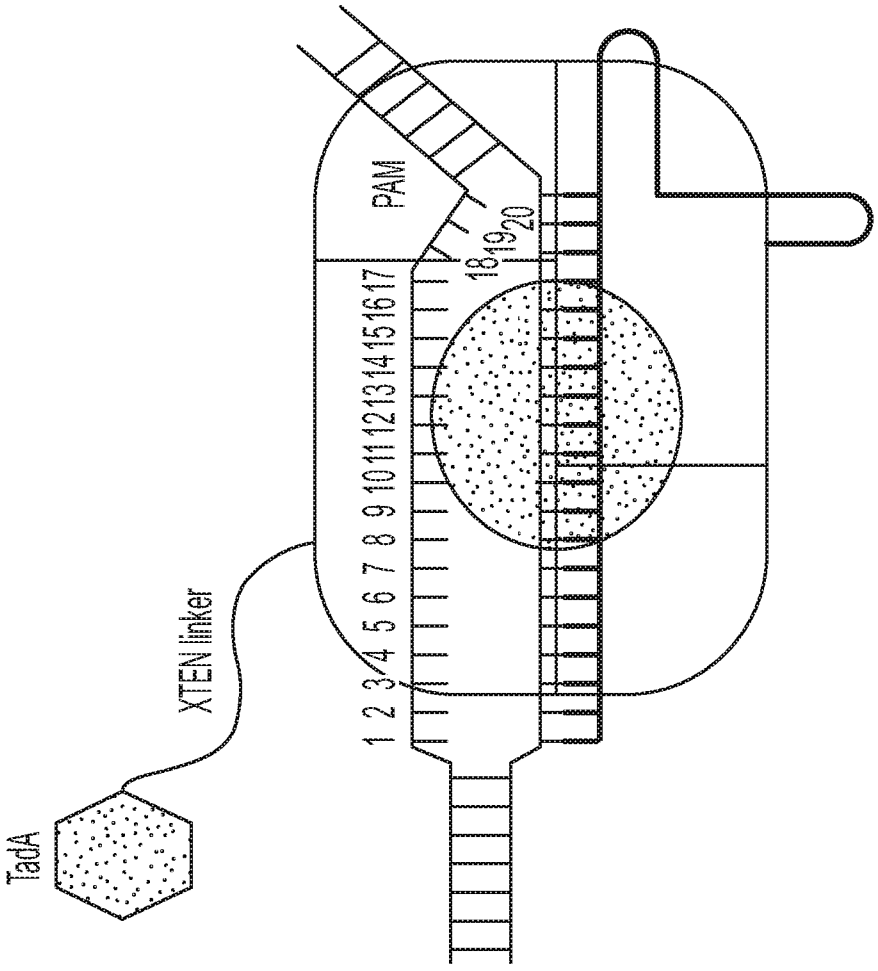



FIG. 38

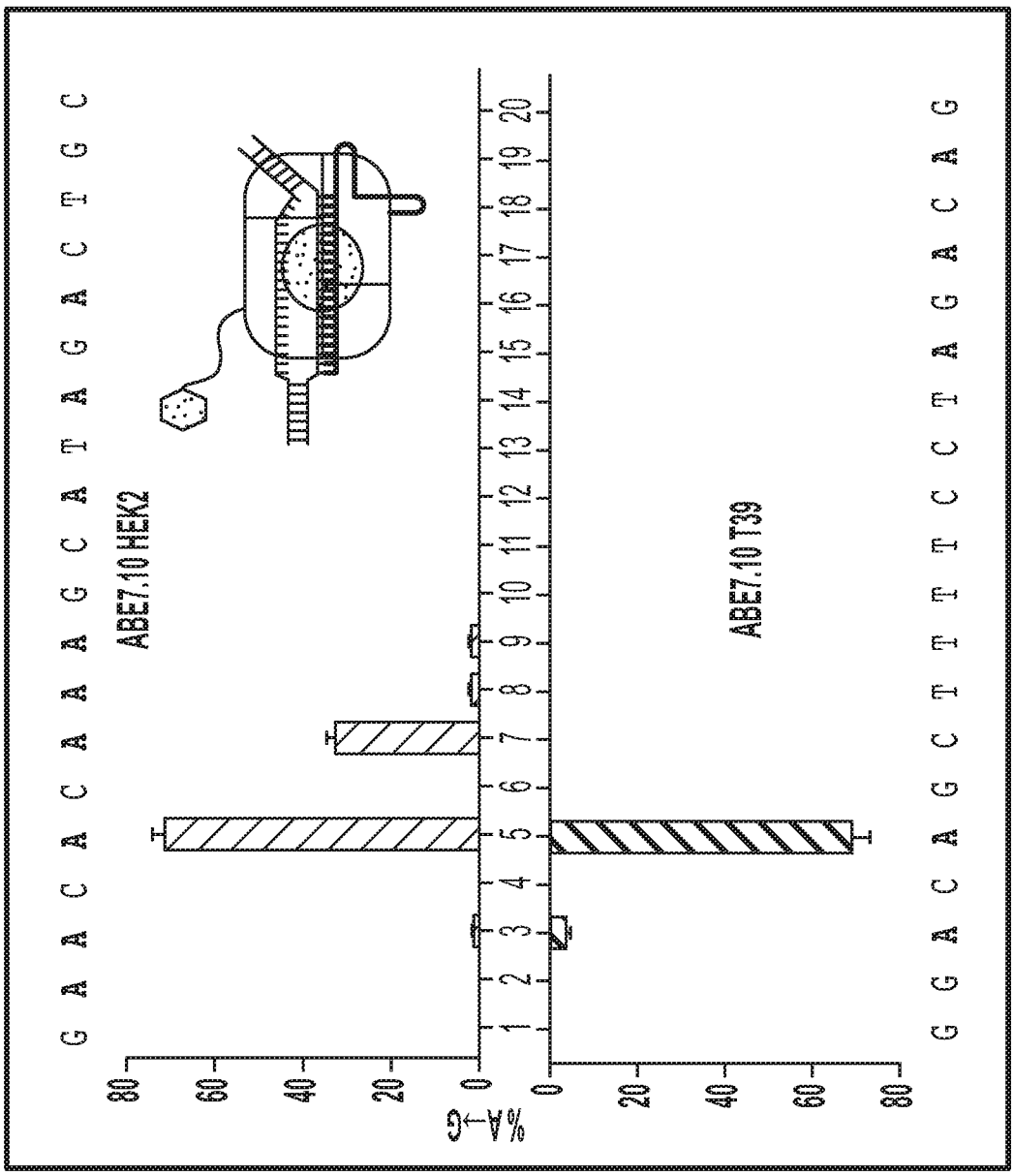



FIG. 38 CONTINUED

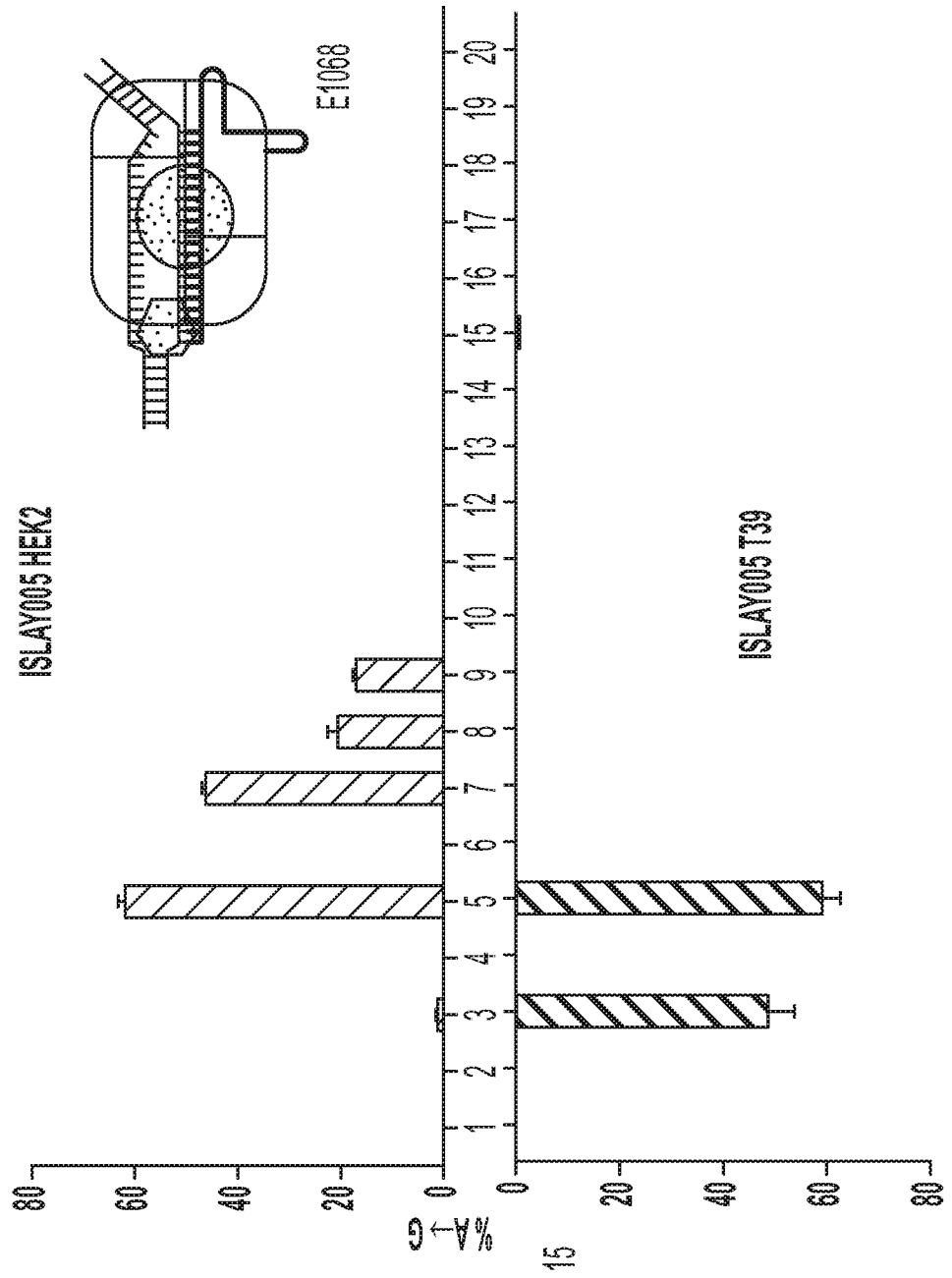



FIG. 38 CONTINUED

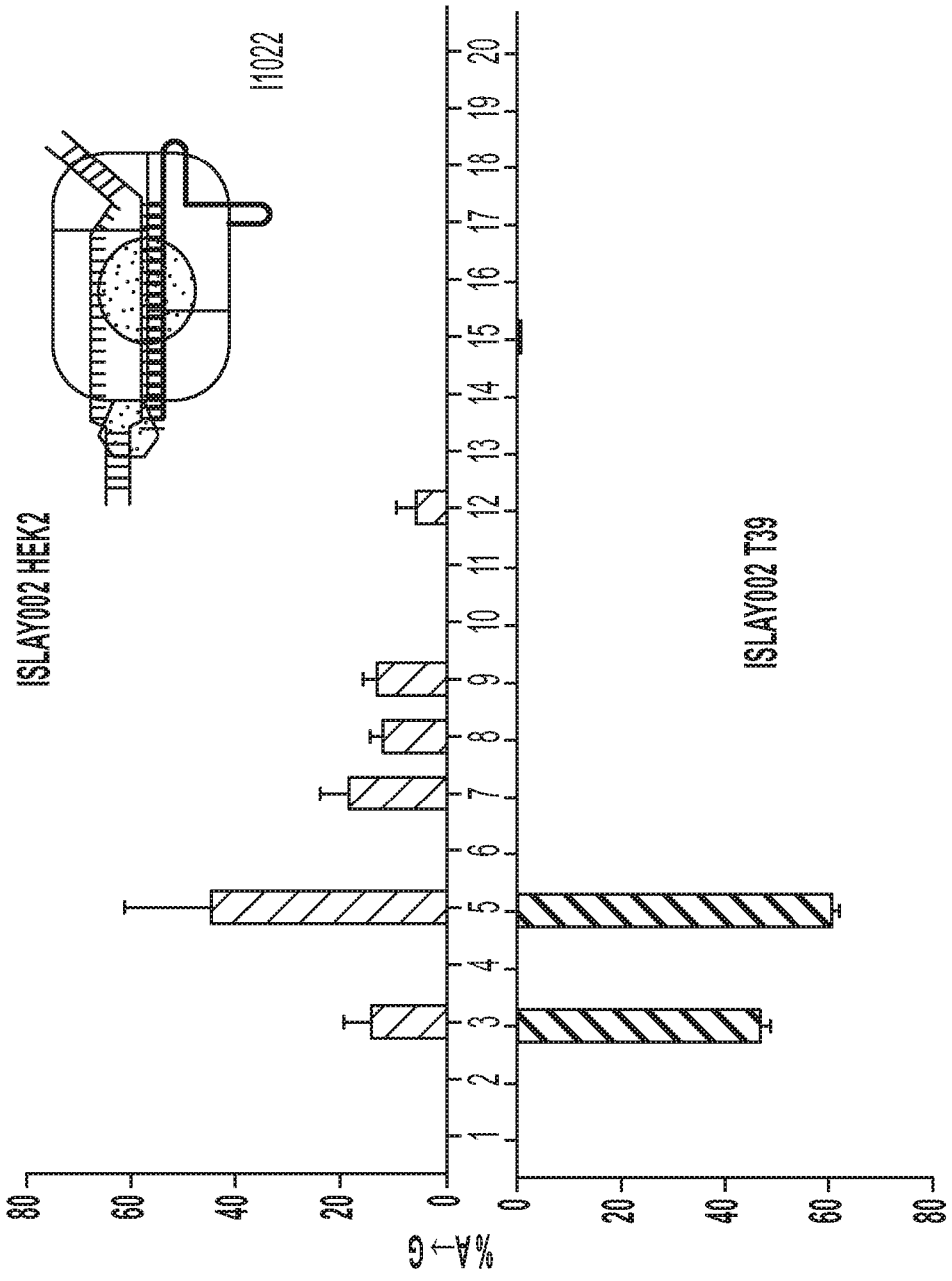



FIG. 38 CONTINUED

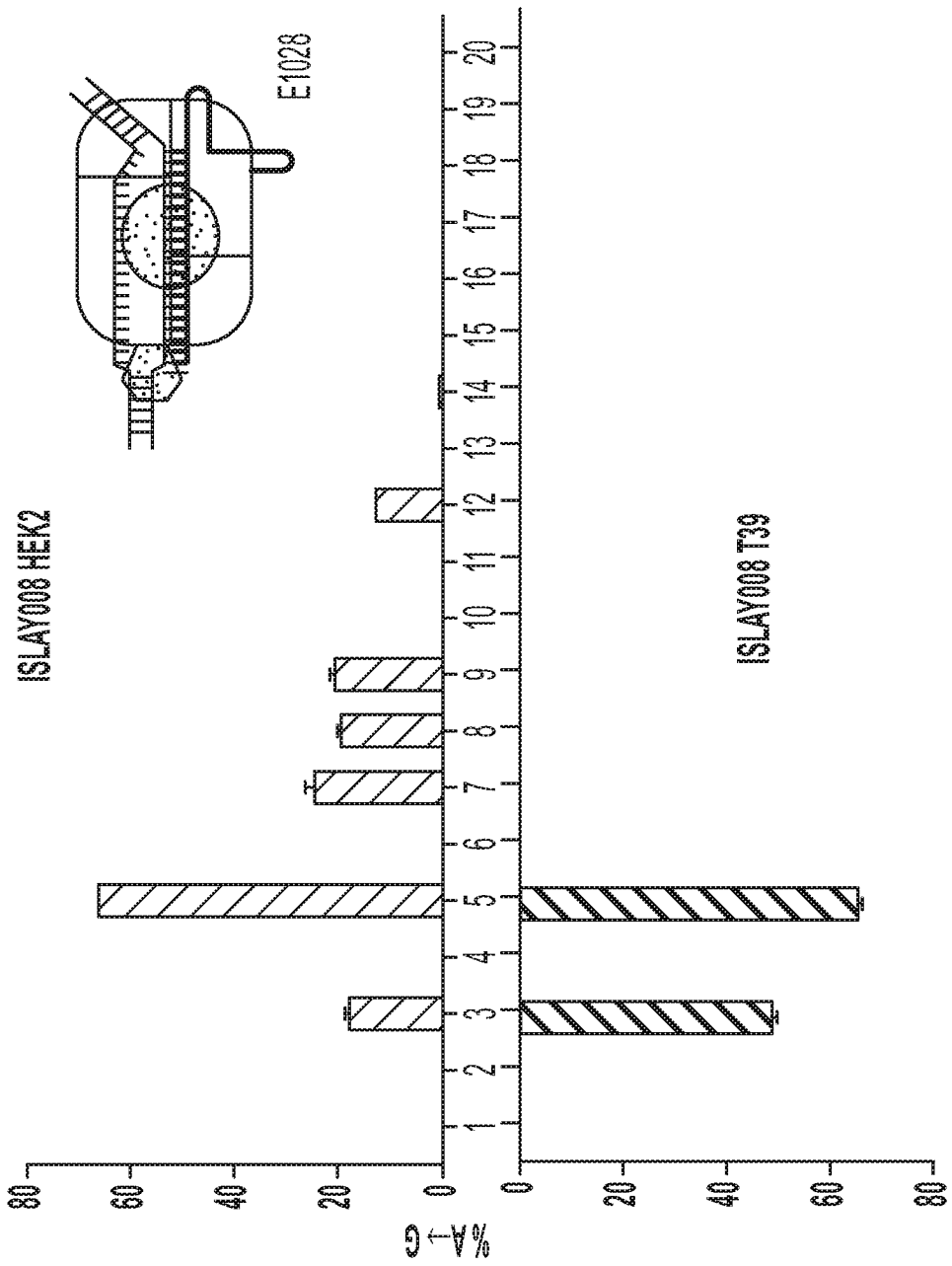



FIG. 38 CONTINUED

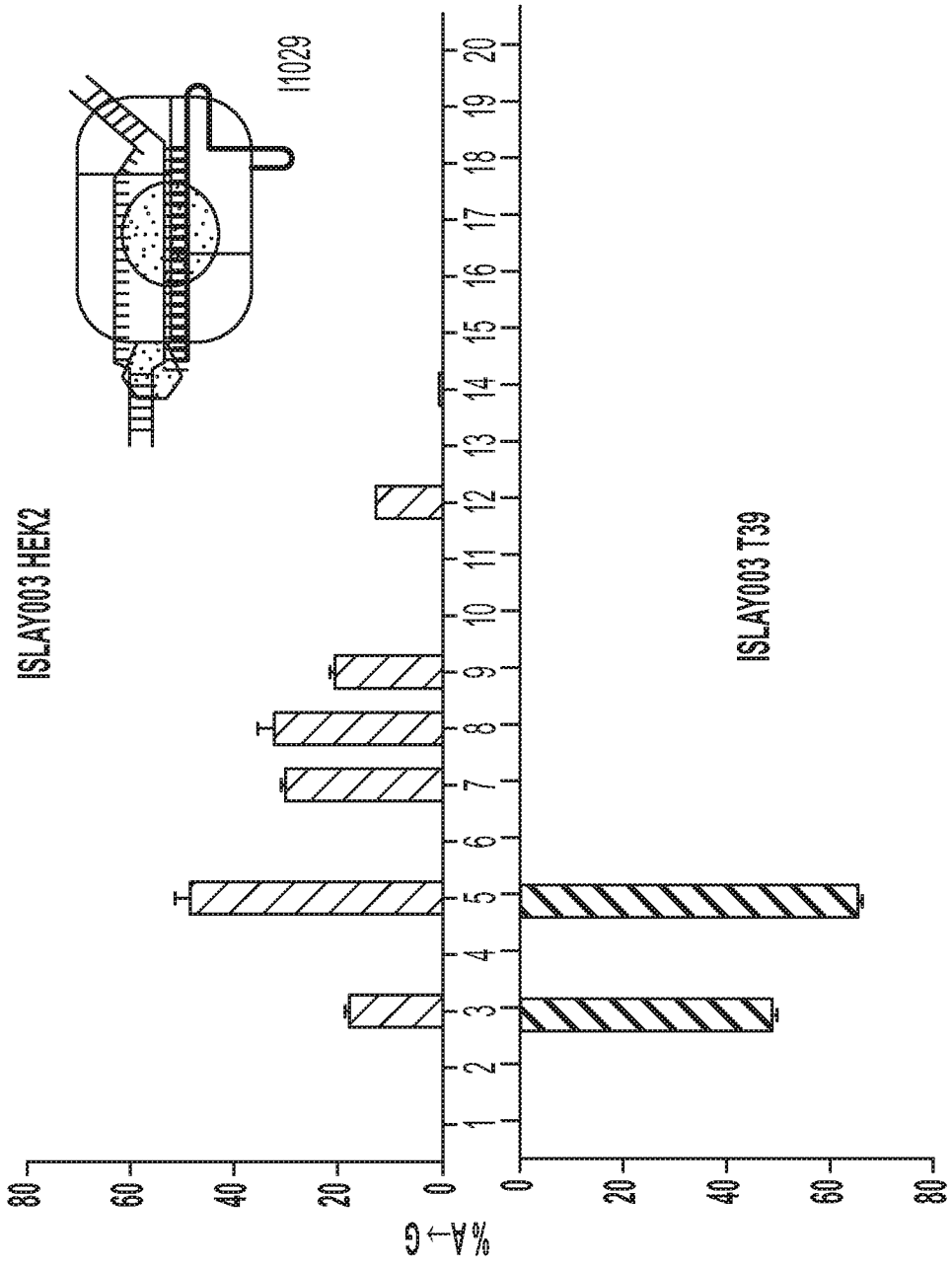



FIG. 38 CONTINUED

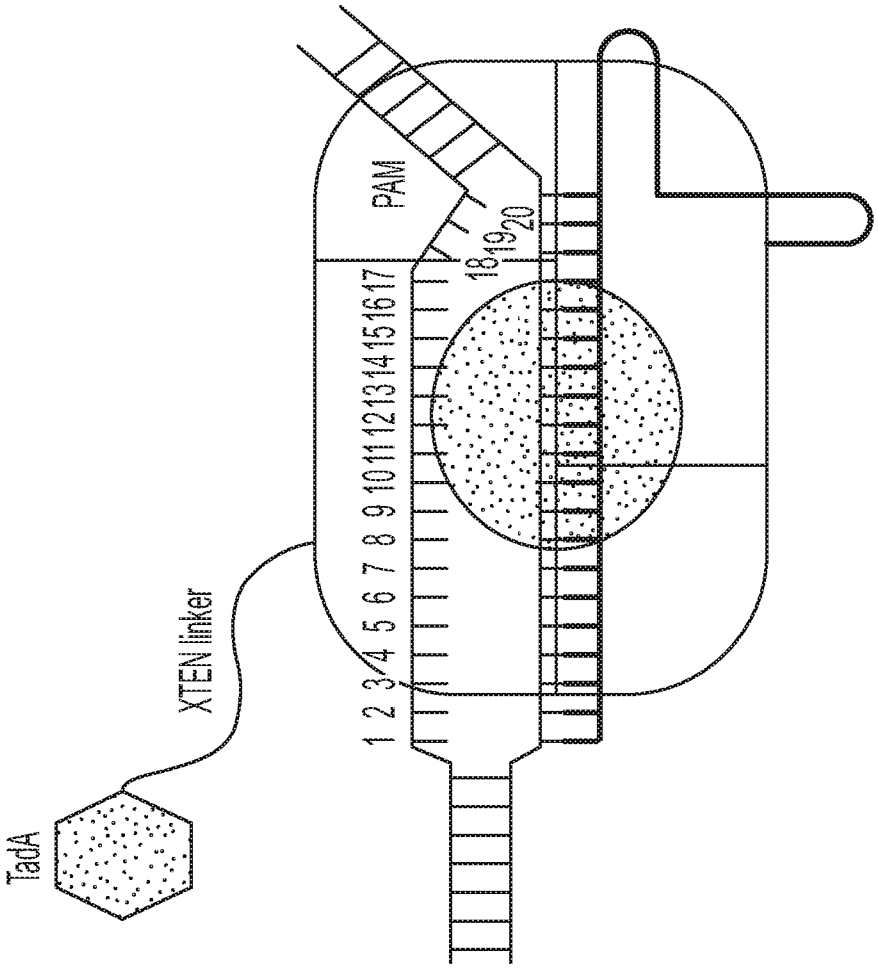



FIG. 39

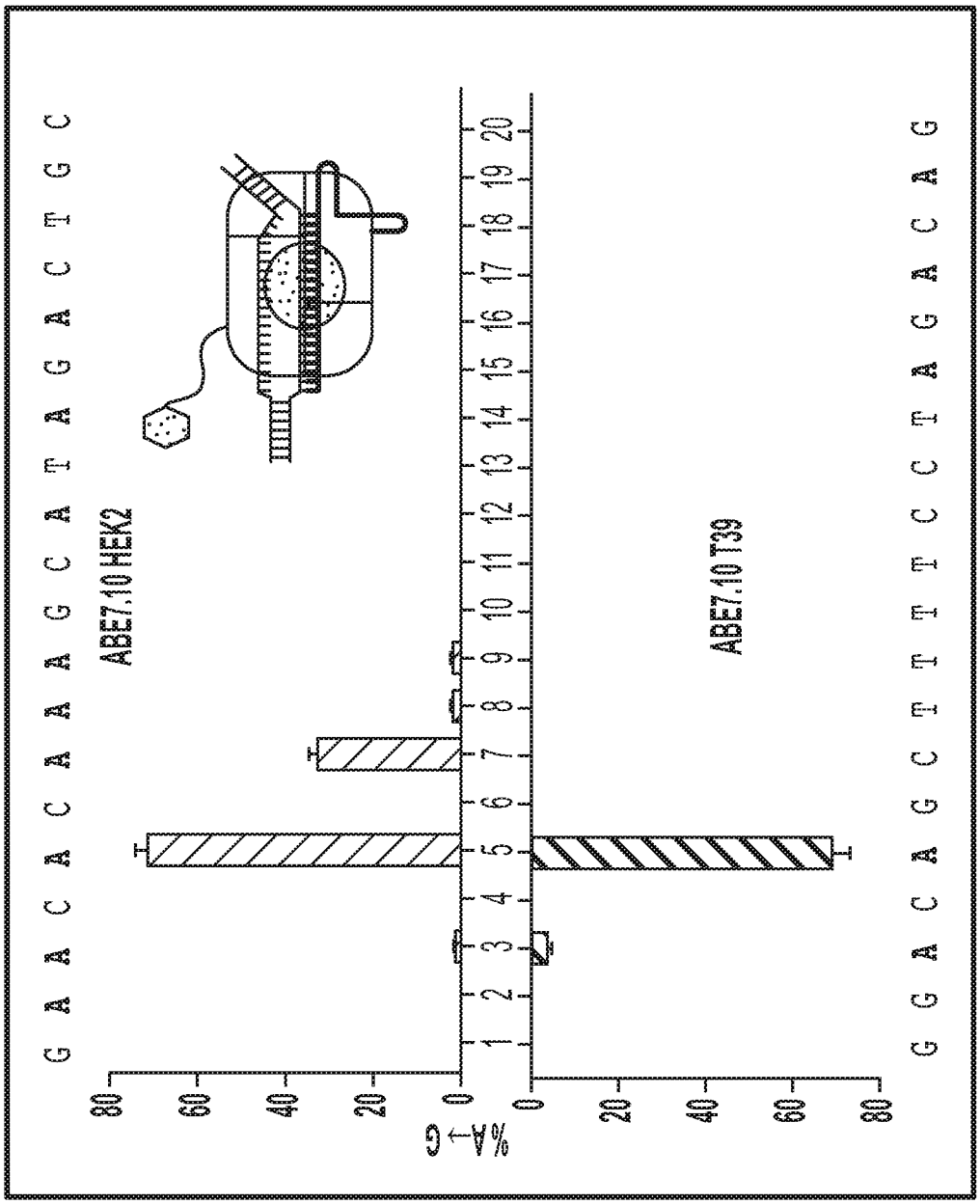



FIG. 39 CONTINUED

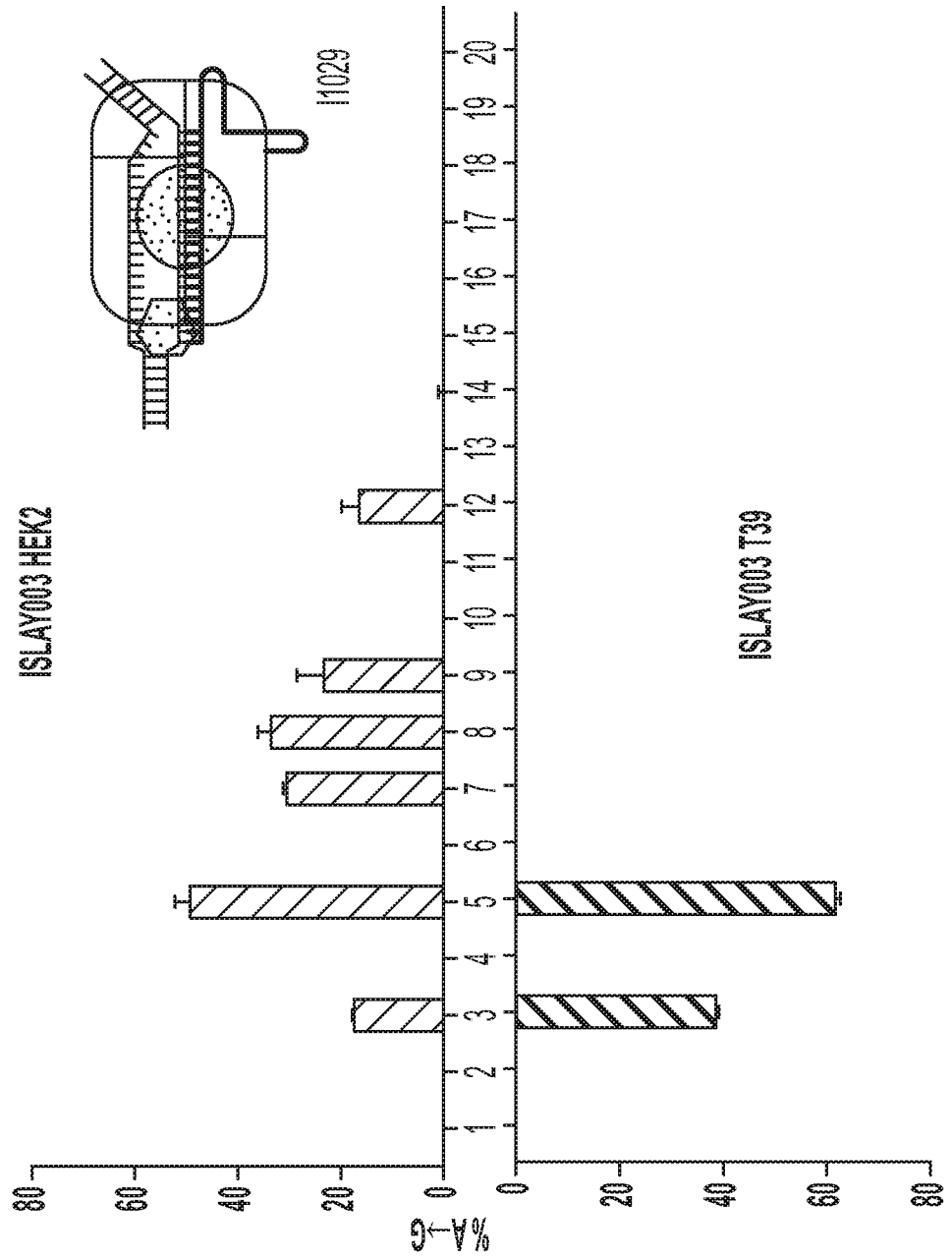



FIG. 39 CONTINUED

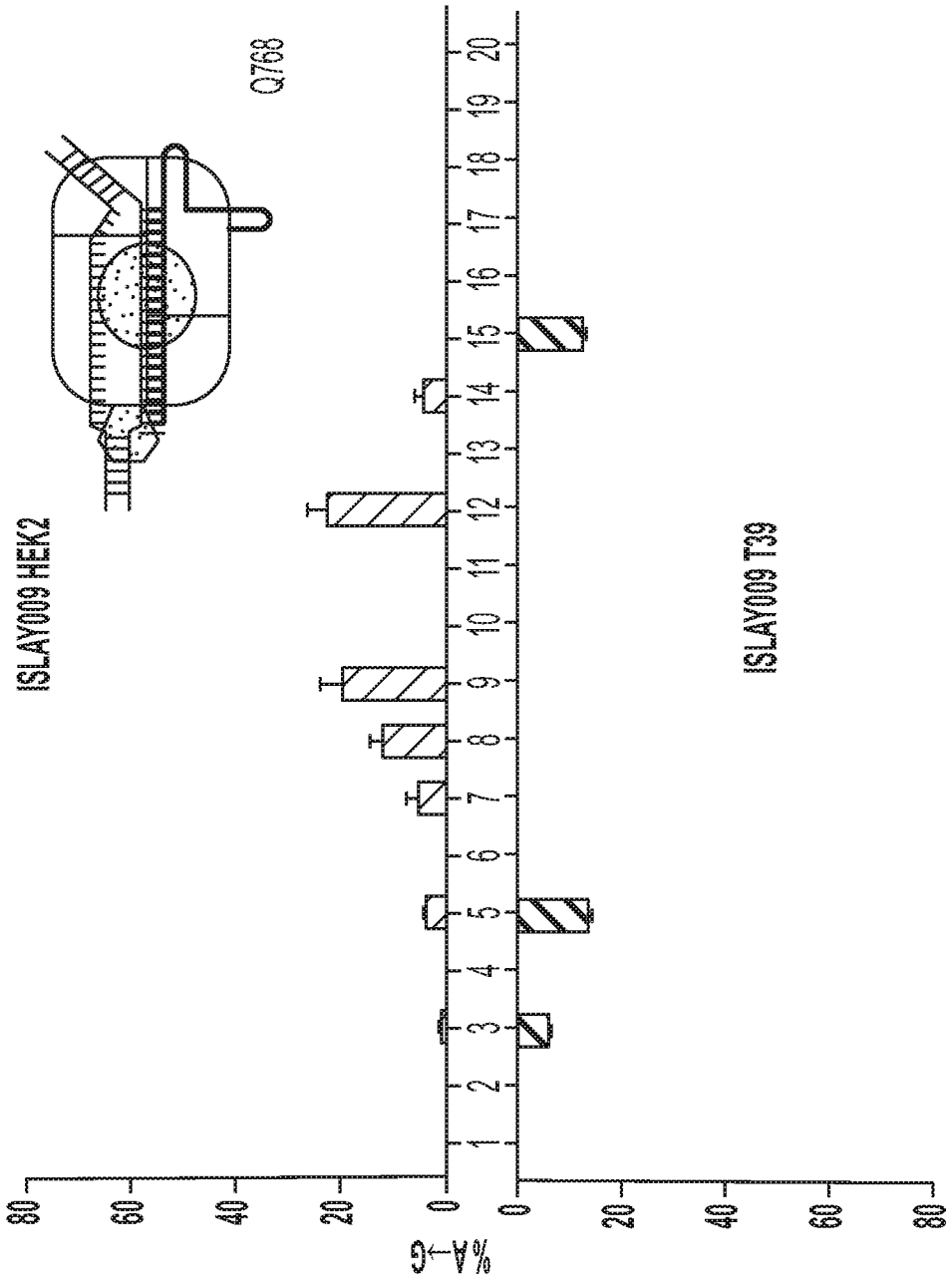



FIG. 39 CONTINUED

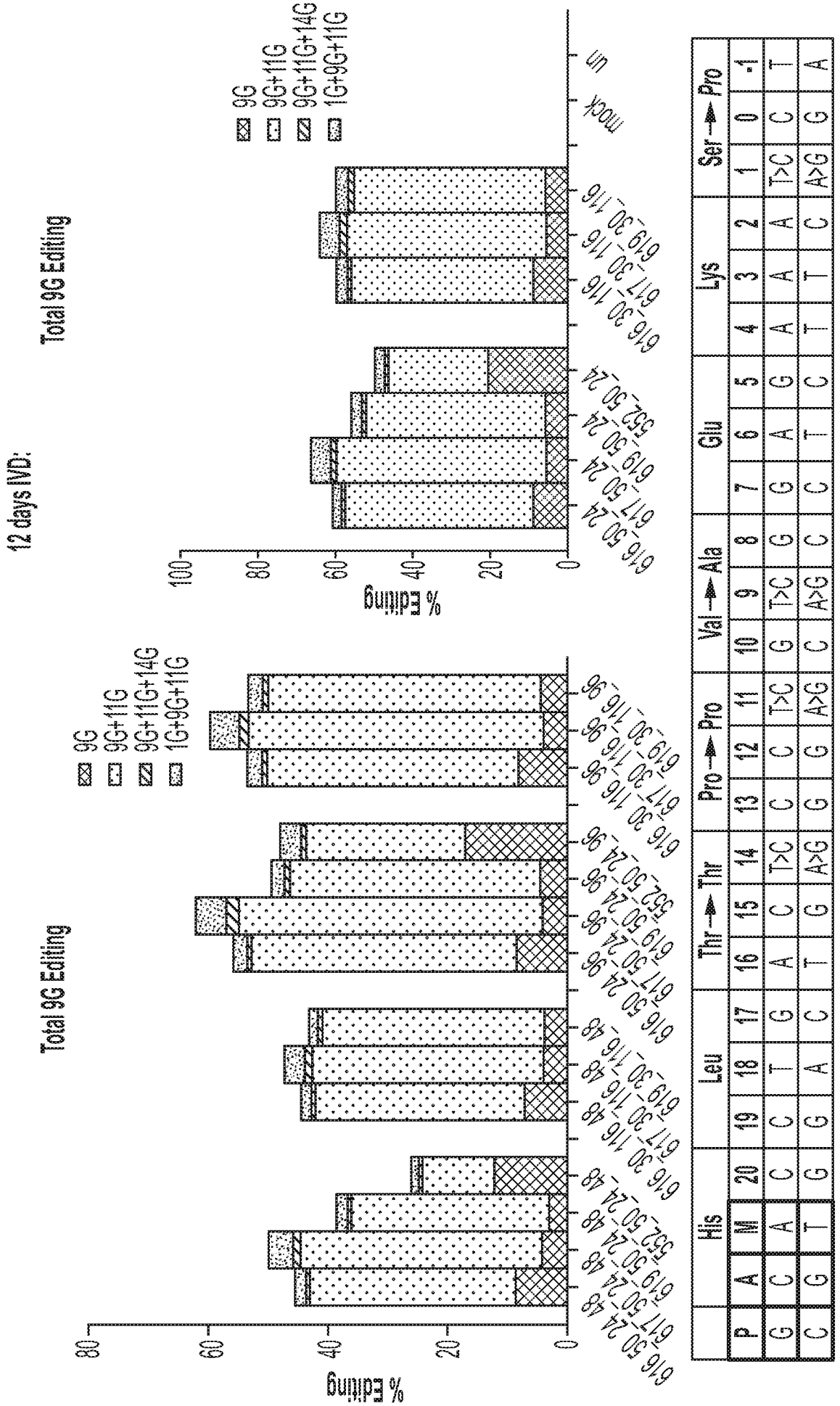

12 days IVD:
Total 9G Editing
9G
9G+11G
9G+11G+14G
1G+9G+11G
% Editing
0
20
40
60
80
100
mock
un
6\16 30 116
6\17 30 116
6\19 30 116
6\16 50 24
6\17 50 24
6\19 50 24
552 50 24
6\16 30 116 96
6\17 30 116 96
6\19 30 116 96
6\16 50 24 96
6\17 50 24 96
6\19 50 24 96
552 50 24 96
6\16 30 116 48
6\17 30 116 48
6\19 30 116 48
6\16 50 24 48
6\17 50 24 48
6\19 50 24 48
552 50 24 48
His
Leu
Thr→Thr
Pro→Pro
Val→Ala
Glu
Lys
Ser→Pro
P G C C
A C C G
M A C G
20 C G A
19 C C G
18 T G A
17 G C T
16 A T G
15 C G A
14 T>C A>G
13 C G G
12 C G G
11 T>C A>G
10 G C C
9 T>C A>G
8 G C C
7 G C G
6 A T A
5 G C G
4 A T A
3 A T A
2 A C A
1 T>C A>G
0 C G C
-1 T A

FIG. 40

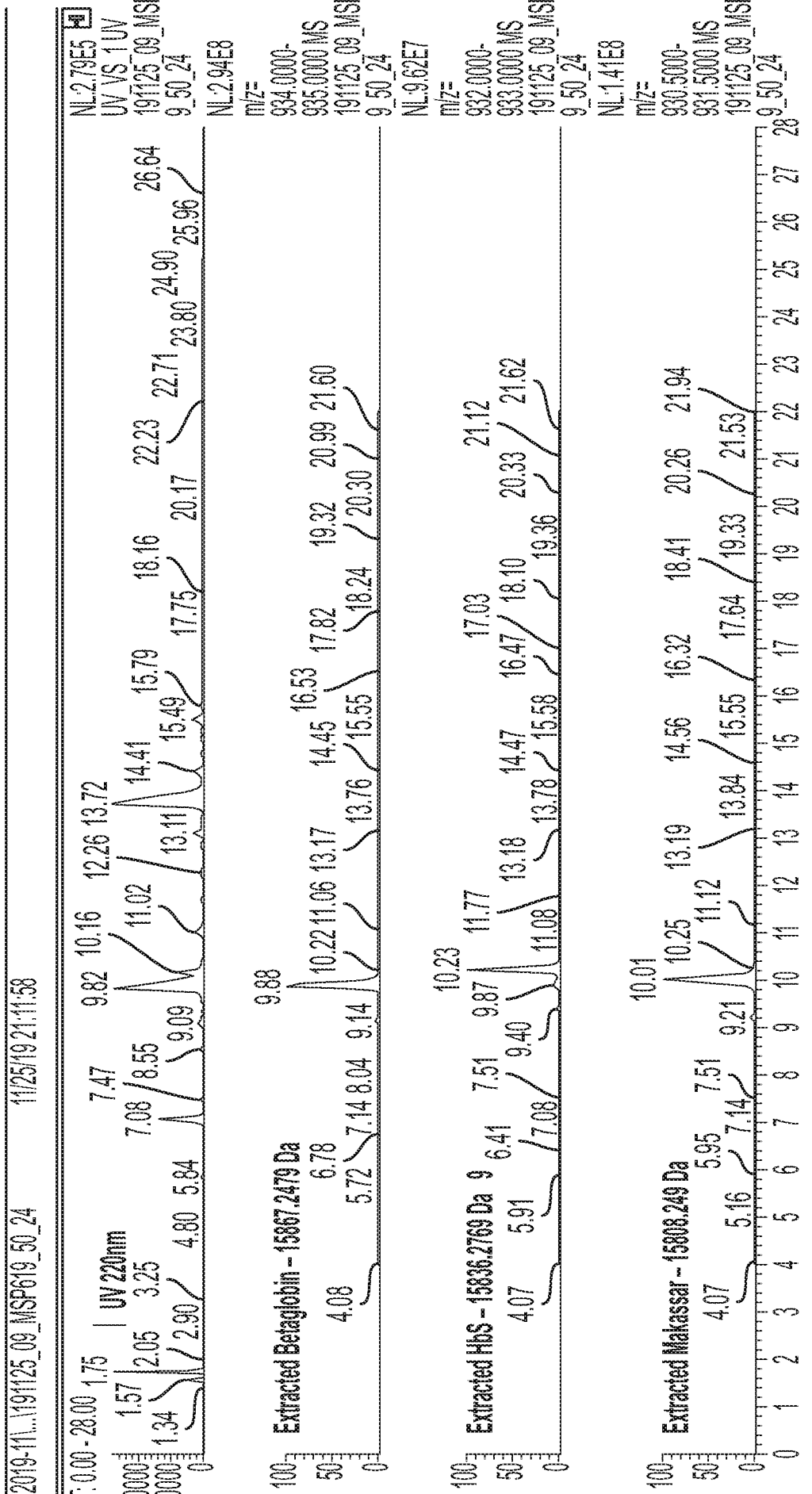

D:\2019-11\...\191125_09_MSP619_50_24
11/25/19 21:11:58
RT: 0.00 - 28.00
UV 220nm
UV VS 1 UV
191125_09_MSP61
9_50_24
NL:2.79E5
NL:2.94E8
m/z=
934.0000-
935.0000 MS
NL:9.62E7
932.0000-
933.0000 MS
NL:1.41E8
930.5000-
931.5000 MS
Extracted Betaglobin - 15867.2479 Da
Extracted HbS - 15836.2769 Da 9
Extracted Makassar - 15808.249 Da
Time (min)

FIG. 41A

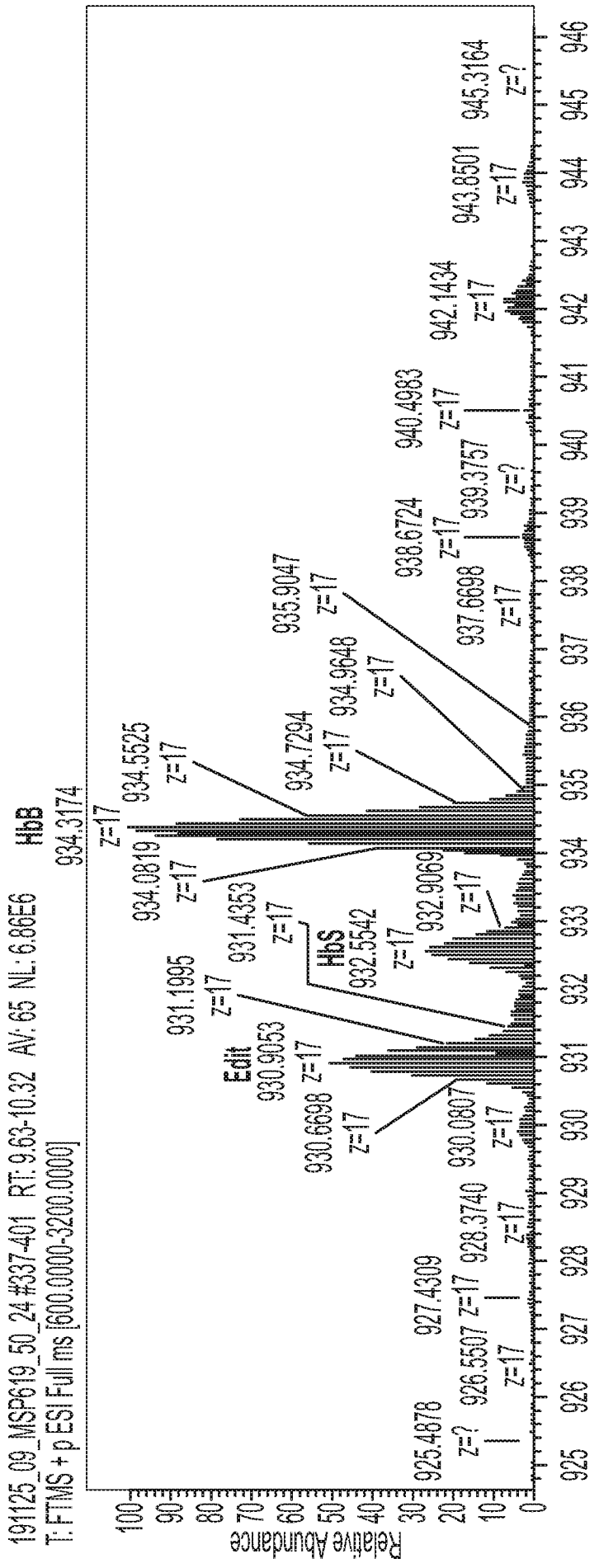

191125_09_MSP619_50_24 #337-401 RT: 9.63-10.32 AV: 65 NL: 6.86E6
T: FTMS + p ESI Full ms [600.0000-3200.0000]
HbB
934.3174
HbS
932.5542
Edit
930.9053
Relative Abundance
925.4878
926.5507
927.4309
928.3740
930.0807
930.6698
931.1995
931.4353
932.9069
934.0819
934.5525
934.7294
934.9648
935.9047
937.6698
938.6724
939.3757
940.4983
942.1434
943.8501
945.3164
z=17
z=?

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′-gsascsUUCUCCACAGGAGUCAGG 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′-csususCUCCACAGGAGUCAGG GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUsususu-3′

FIG. 42

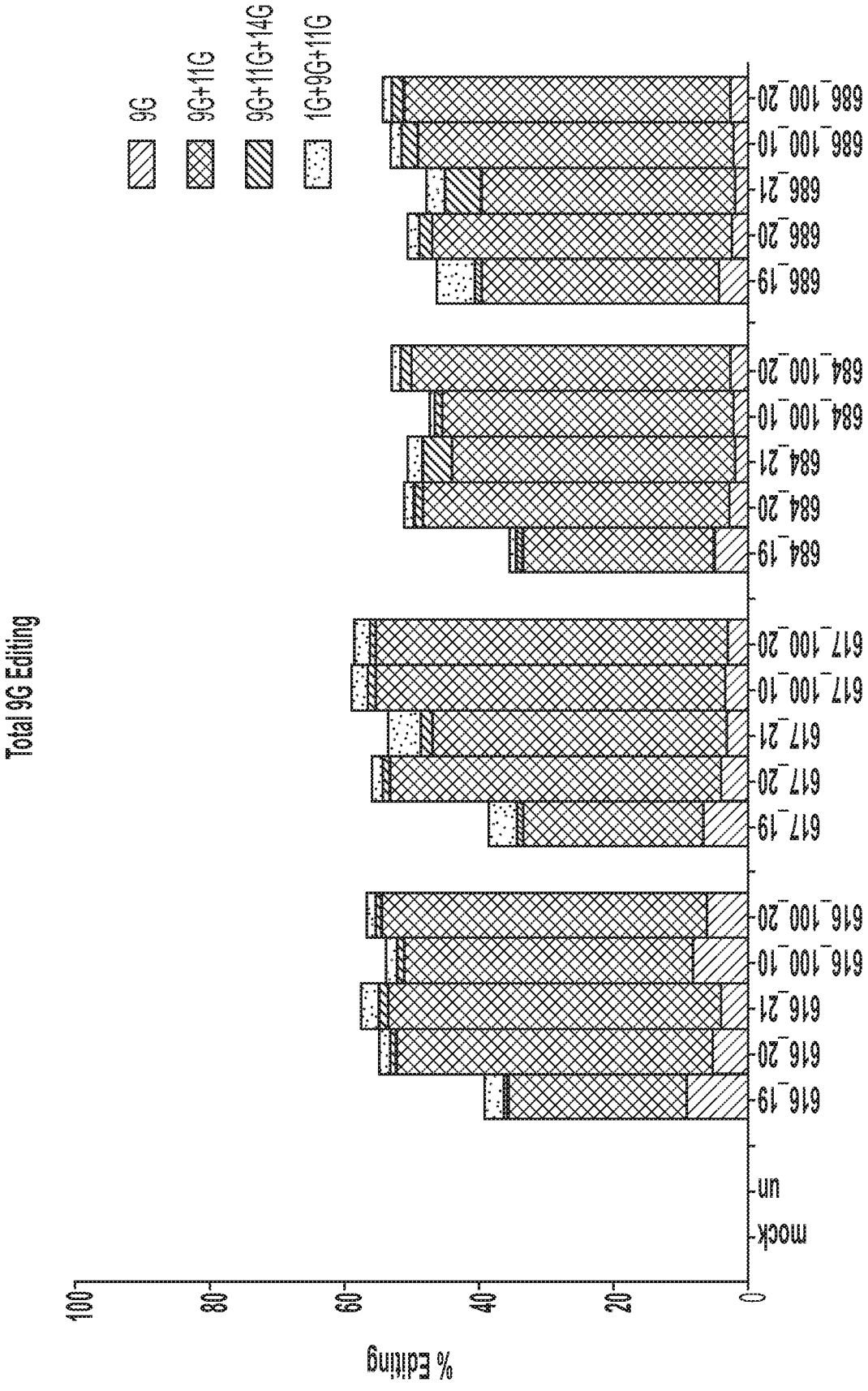

9G
9G+11G
9G+11G+14G
1G+9G+11G
Total 9G Editing
% Editing
100
80
60
40
20
0
mock
un
616_19
616_20
616_21
616_100_10
616_100_20
617_19
617_20
617_21
617_100_10
617_100_20
684_19
684_20
684_21
684_100_10
684_100_20
686_19
686_20
686_21
686_100_10
686_100_20

FIG. 43A

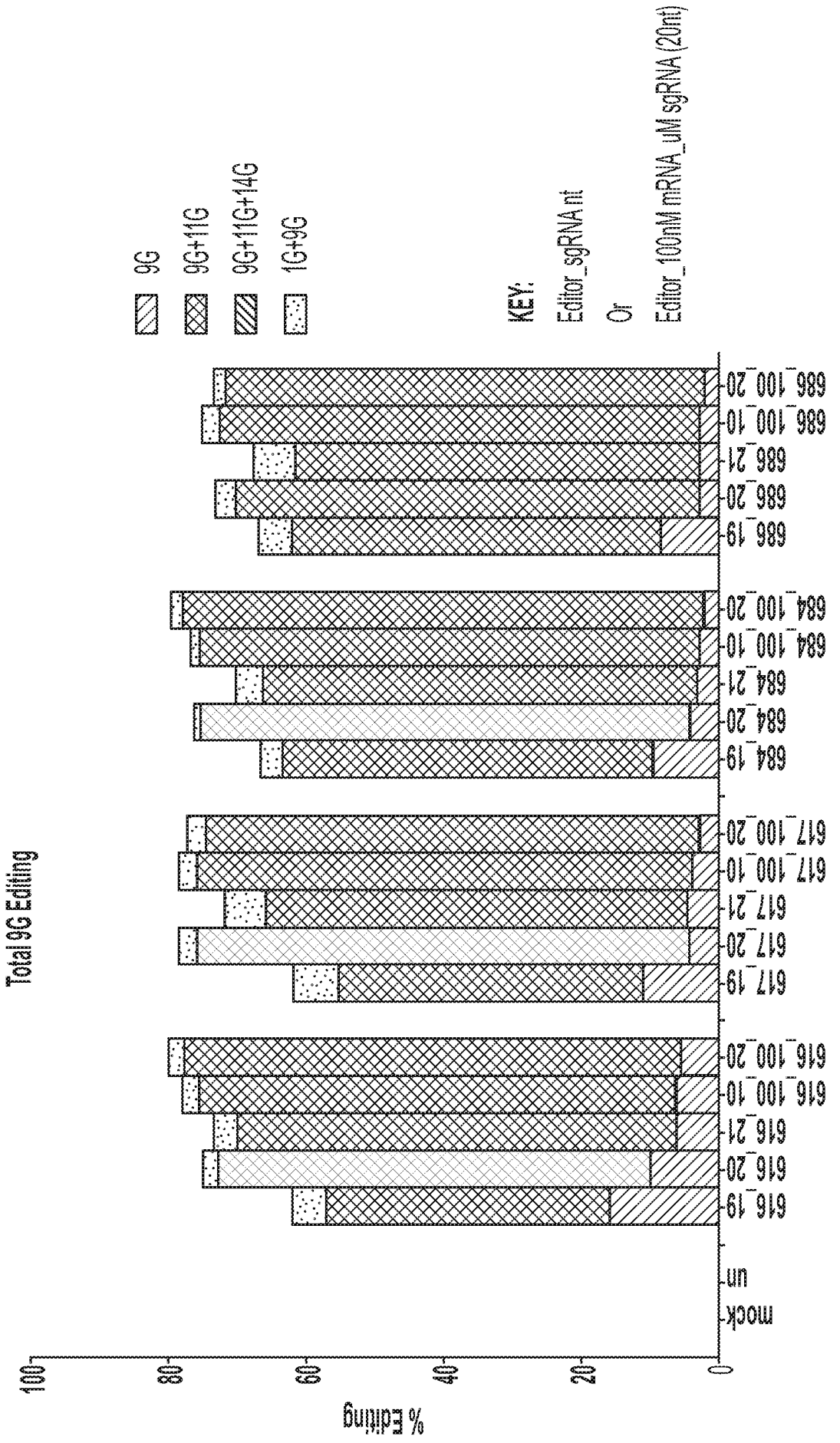

9G
9G+11G
9G+11G+14G
1G+9G
KEY:
Editor_sgRNA nt
Or
Editor_100nM mRNA_uM sgRNA (20nt)
Total 9G Editing
% Editing
100
80
60
40
20
0
mock
un
616_19
616_20
616_21
616_100_10
616_100_20
617_19
617_20
617_21
617_100_10
617_100_20
684_19
684_20
684_21
684_100_10
684_100_20
686_19
686_20
686_21
686_100_10
686_100_20

FIG. 43B

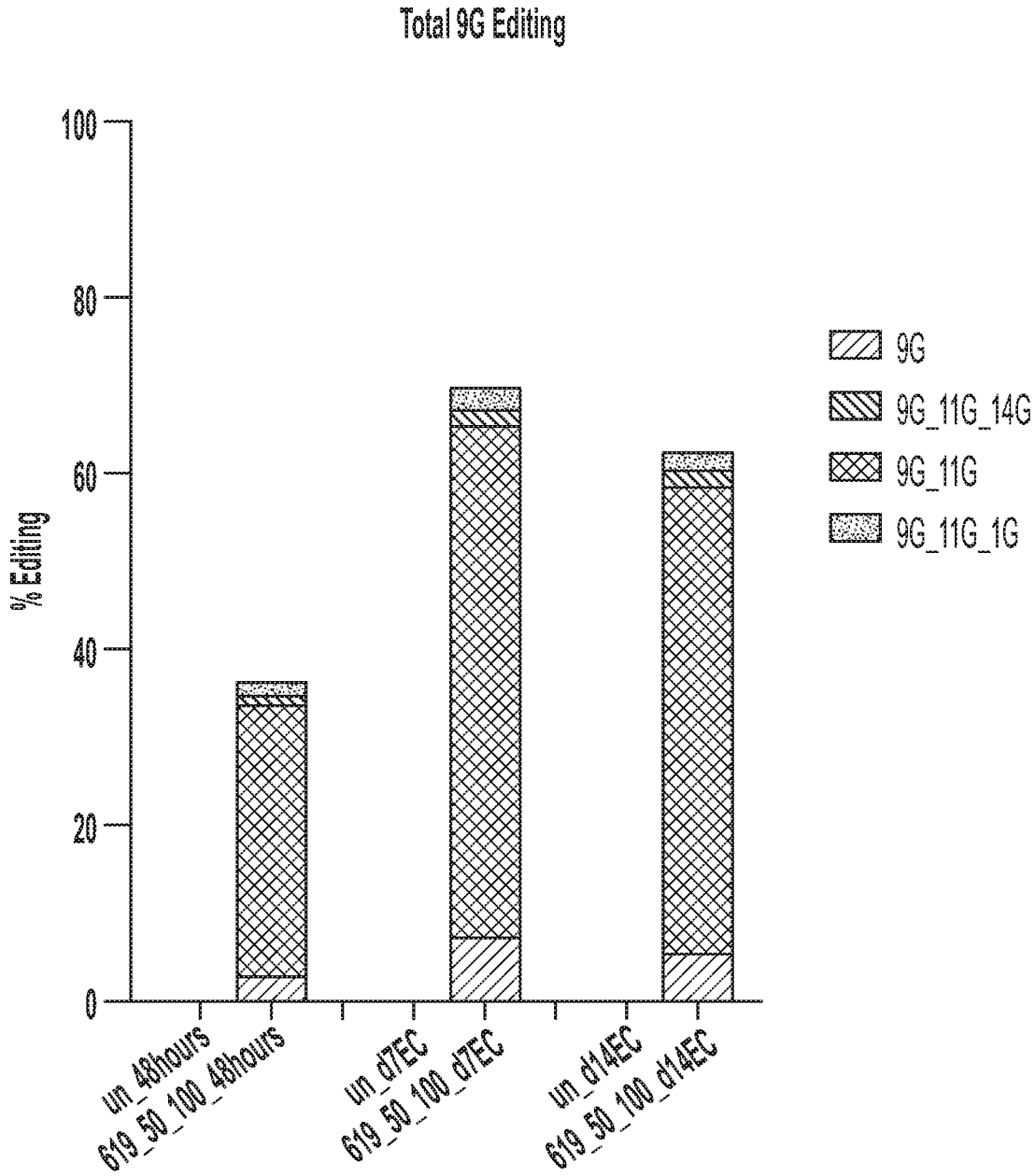
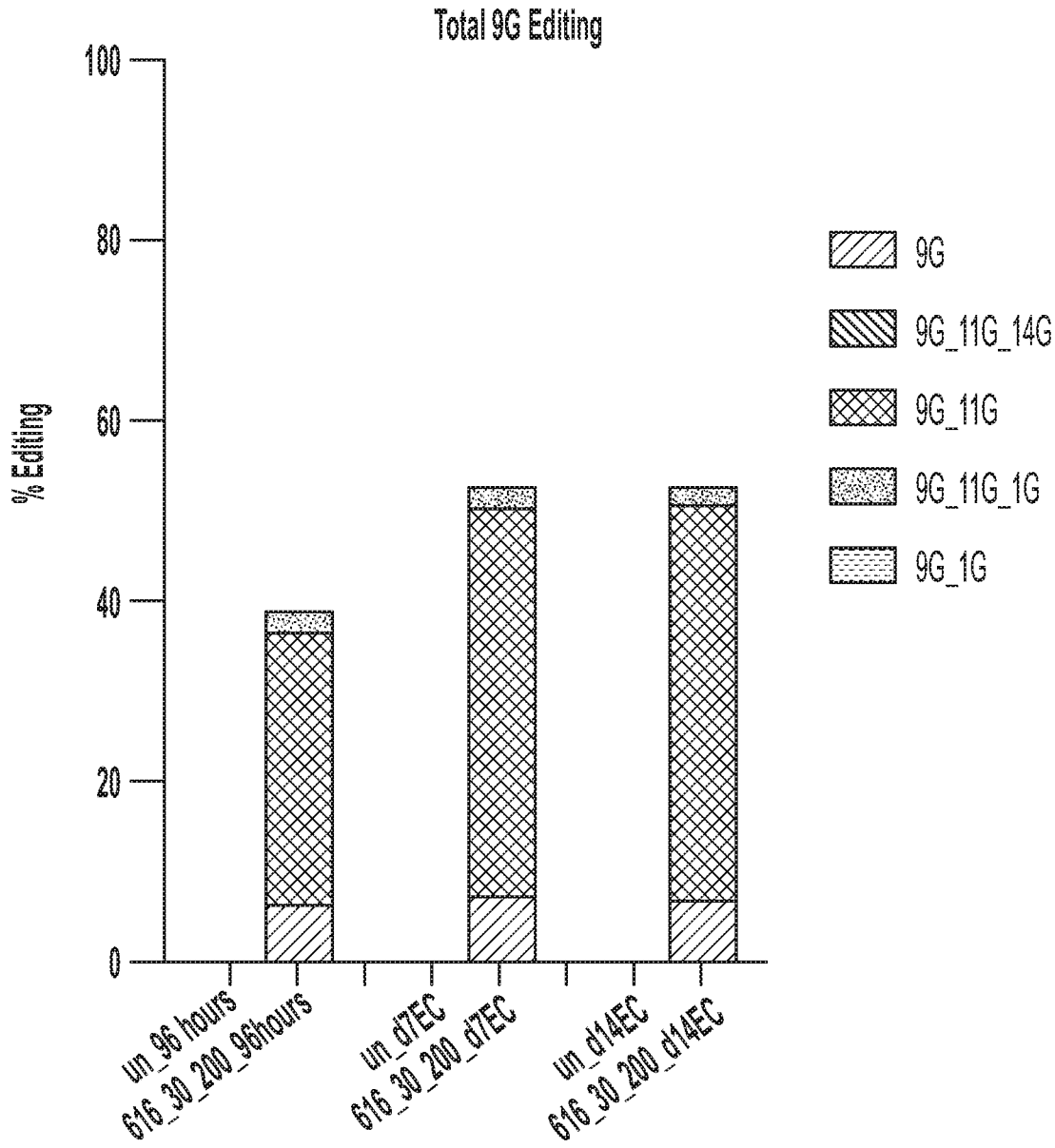

Total 9G Editing
% Editing
100
80
60
40
20
0
9G
9G_11G_14G
9G_11G
9G_11G_1G
un_48hours
619_50_100_48hours
un_d7EC
619_50_100_d7EC
un_d14EC
619_50_100_d14EC

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

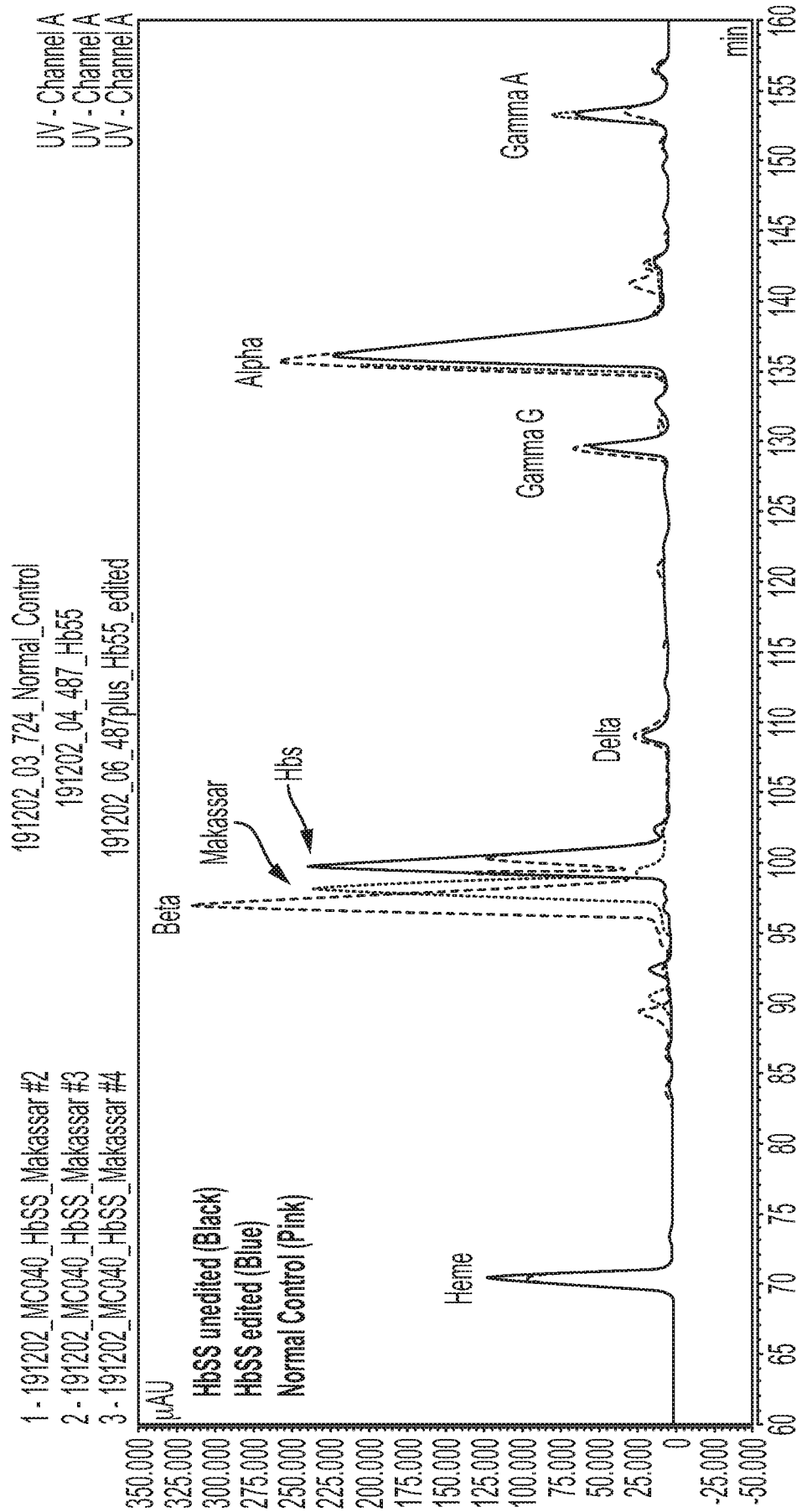

1 - 191202_MC040_HbSS_Makassar #2
2 - 191202_MC040_HbSS_Makassar #3
3 - 191202_MC040_HbSS_Makassar #4
191202_03_724_Normal_Control
191202_04_487_Hb55
191202_06_487plus_Hb55_edited
UV - Channel A
UV - Channel A
UV - Channel A
µAU
HbSS unedited (Black)
HbSS edited (Blue)
Normal Control (Pink)
Beta
Makassar
Hbs
Alpha
Heme
Delta
Gamma G
Gamma A
min
350.000
325.000
300.000
275.000
250.000
225.000
200.000
175.000
150.000
125.000
100.000
75.000
50.000
25.000
0
-25.000
-50.000
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160

FIG. 45

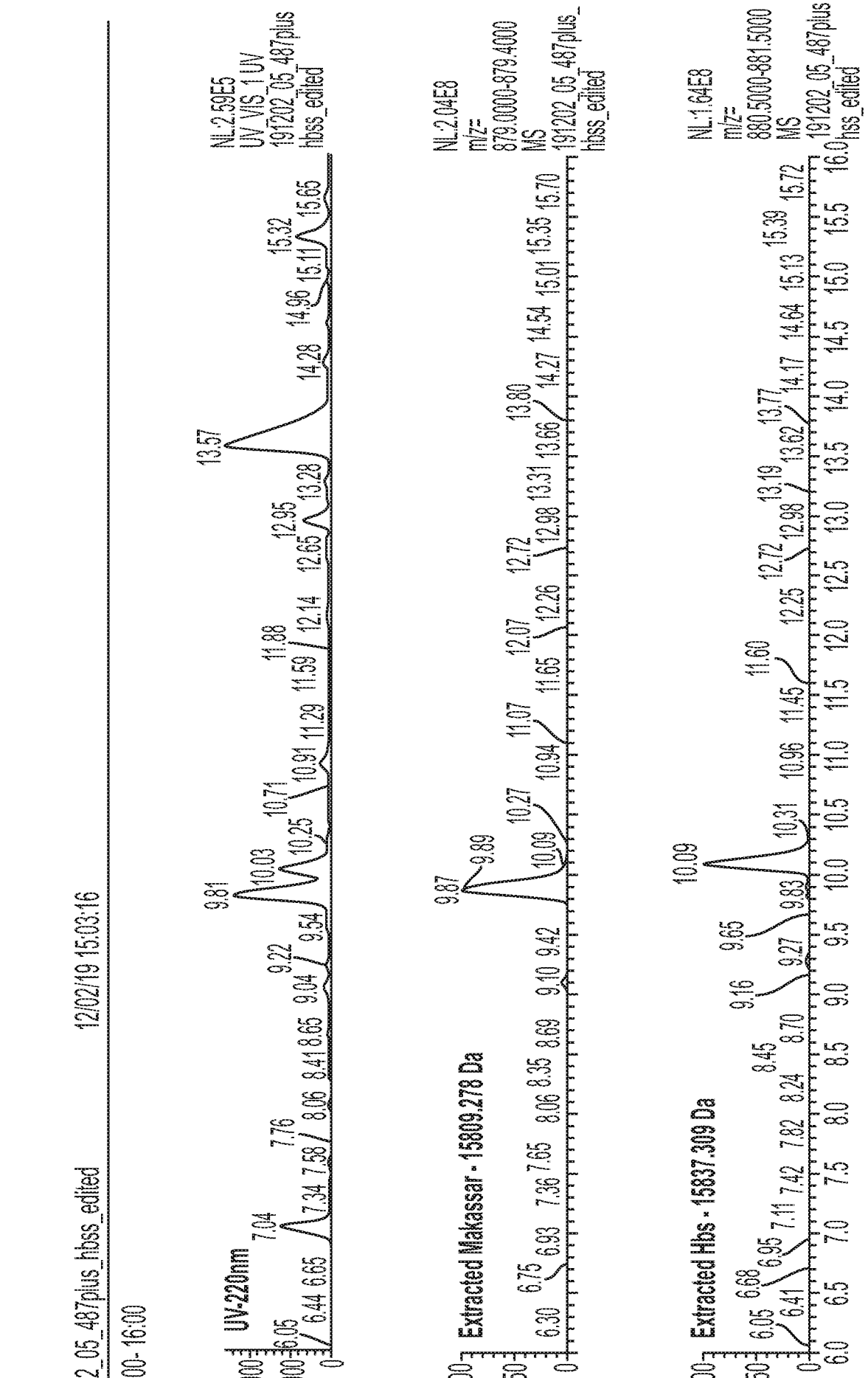

191202_05_487plus_hbss_edited
12/02/19 15:03:16
RT: 6:00 - 16:00
UV-220nm
UV_VIS_1 mAU
NL:2.59E5
UV VIS 1 UV
191202_05_487plus
hbss_edited
Extracted Makassar - 15809.278 Da
NL:2.04E8
m/z=
879.0000-879.4000
MS
191202_05_487plus_
hbss_edited
Extracted Hbs - 15837.309 Da
NL:1.64E8
m/z=
880.5000-881.5000
MS
191202_05_487plus
hbss_edited

FIG. 46A

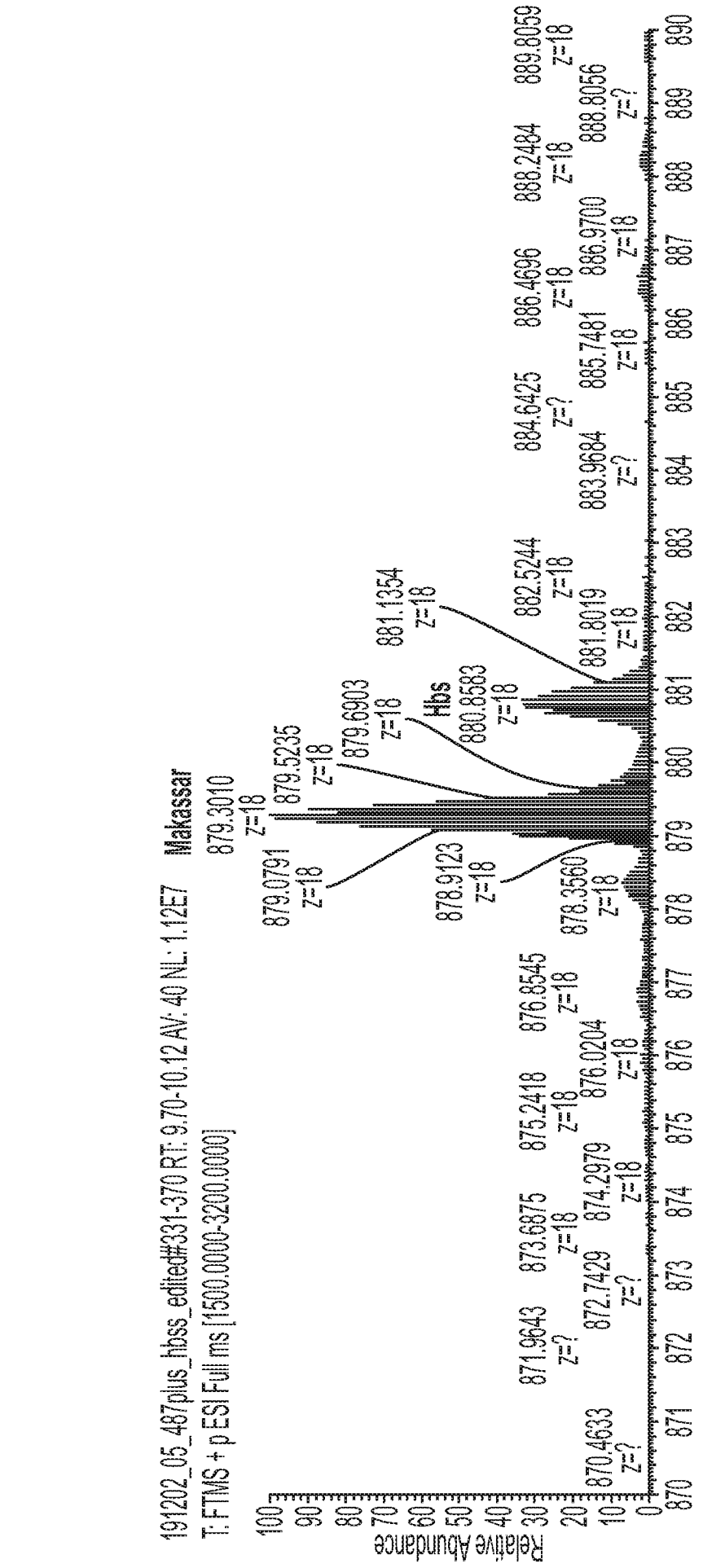

191202_05_487plus_hbss_edited#331-370 RT: 9.70-10.12 AV: 40 NL: 1.12E7 Makassar
T: FTMS + p ESI Full ms [1500.0000-3200.0000]
Relative Abundance
m/z
Hbs
870.4633 z=?
871.9643 z=?
872.7429 z=?
873.6875 z=18
874.2979 z=18
875.2418 z=18
876.0204 z=18
876.8545 z=18
878.3560 z=18
878.9123 z=18
879.0791 z=18
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

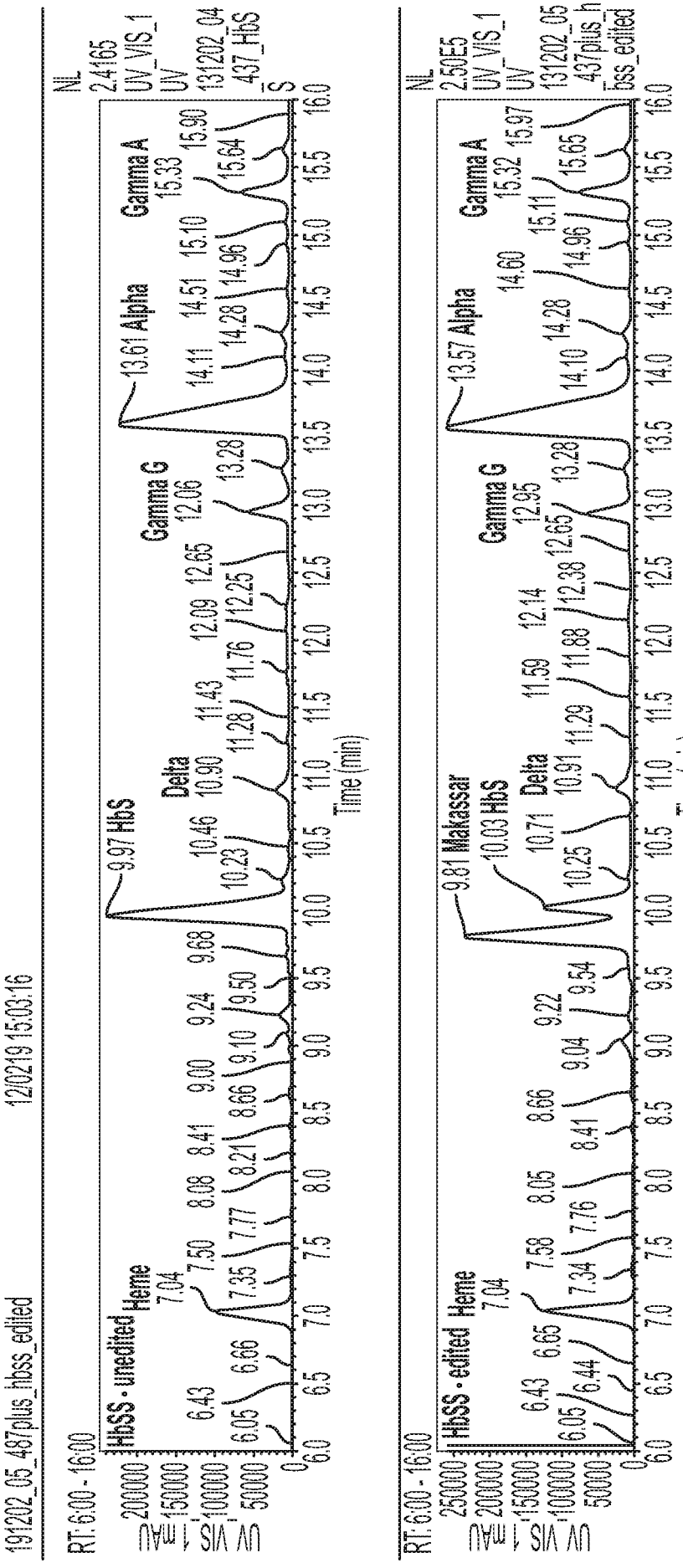

191202_05_487plus_hbss_edited
12/02/19 15:03:16
RT: 6.00 - 16.00
HbSS - unedited
Heme
9.97 HbS
Delta
Gamma G
13.61 Alpha
Gamma A
NL
2.41E5
UV_VIS_1
UV
131202_04
437_HbS
S
HbSS - edited
Heme
9.81 Makassar
10.03 HbS
Delta
Gamma G
13.57 Alpha
Gamma A
NL
2.50E5
UV_VIS_1
UV
131202_05
437plus_h
bss_edited
mAU
UV_VIS_1
Time (min)

FIG. 47

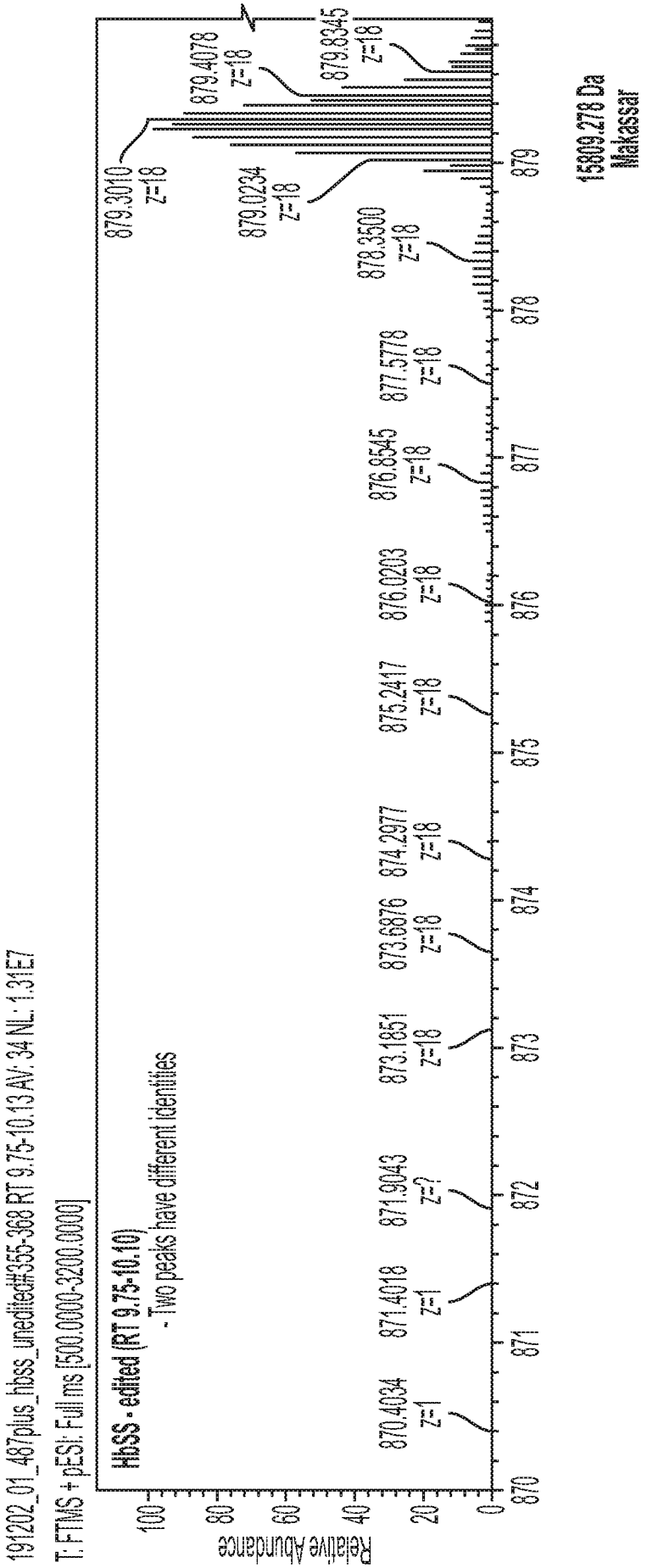



FIG. 47 CONTINUED

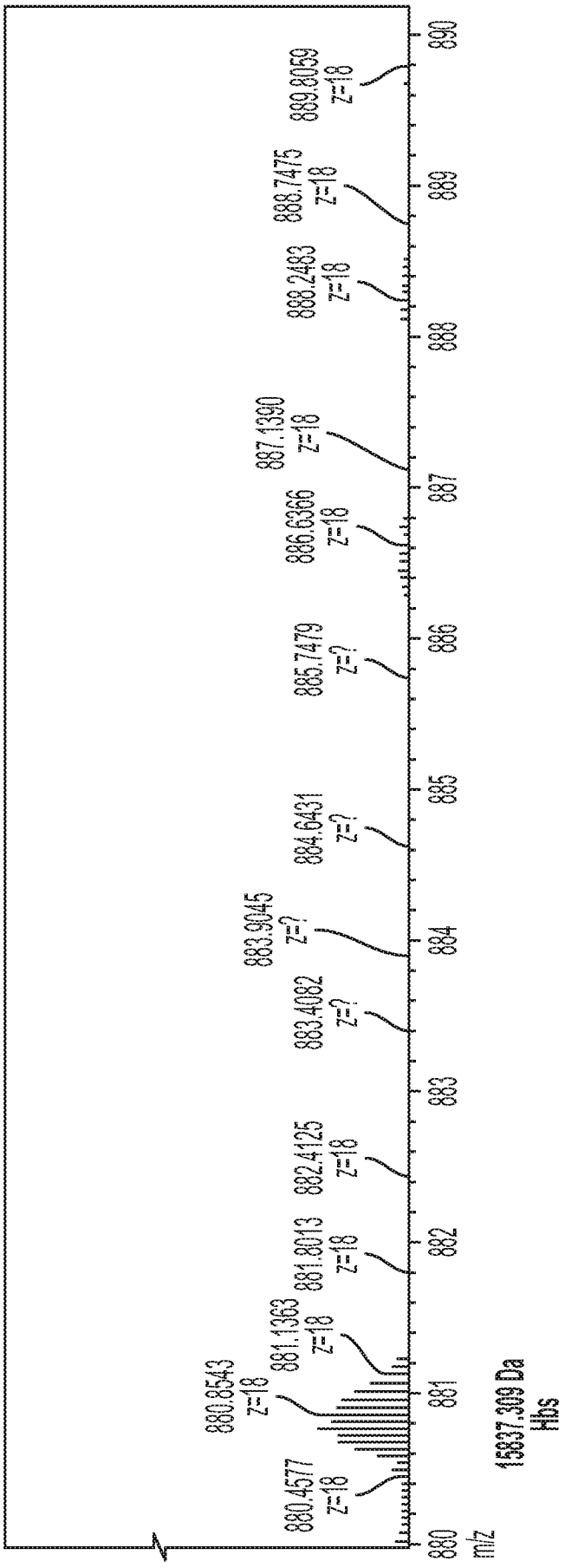



FIG. 47 CONTINUED

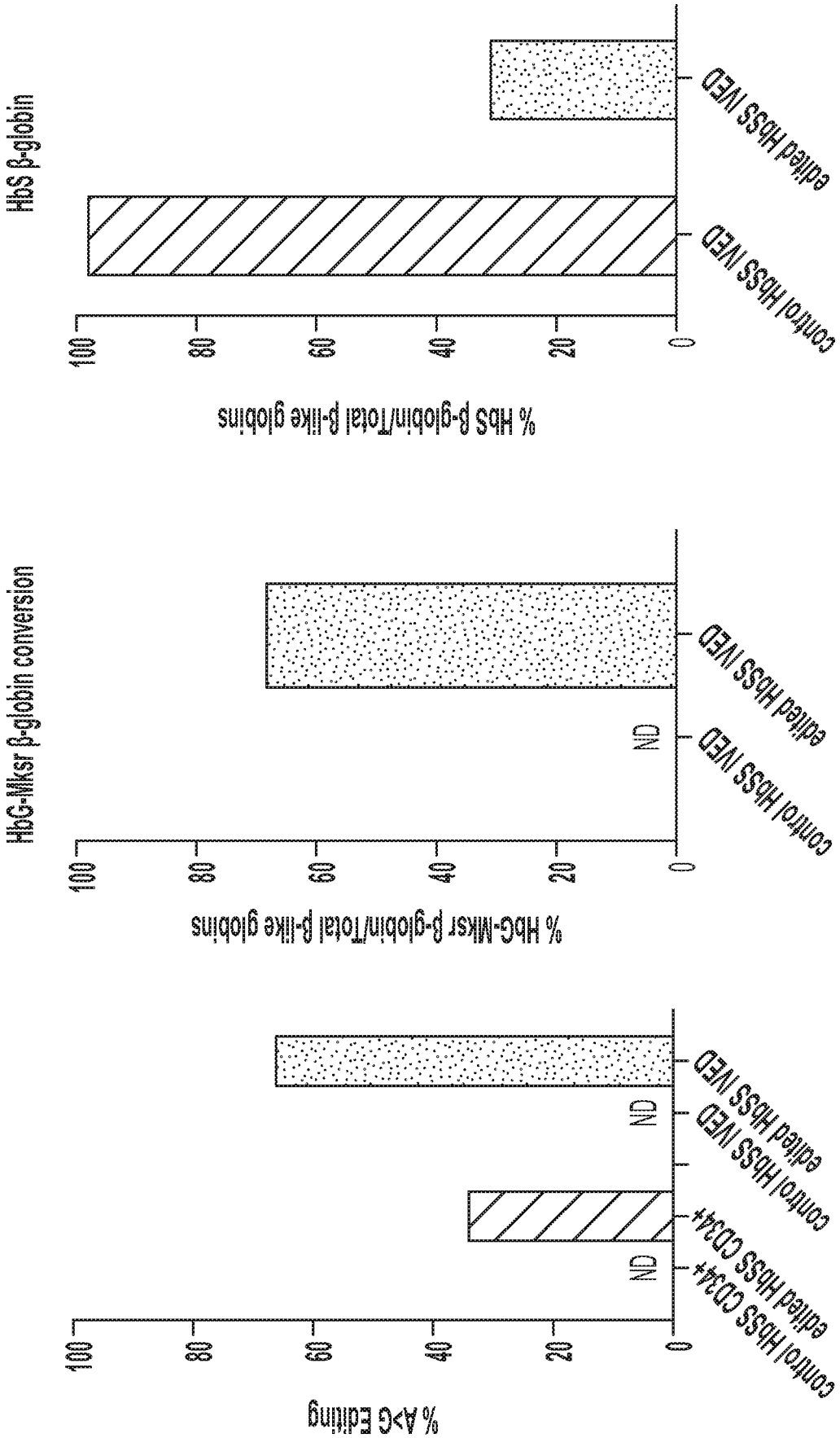



FIG. 48A




FIG. 48B




FIG. 48C

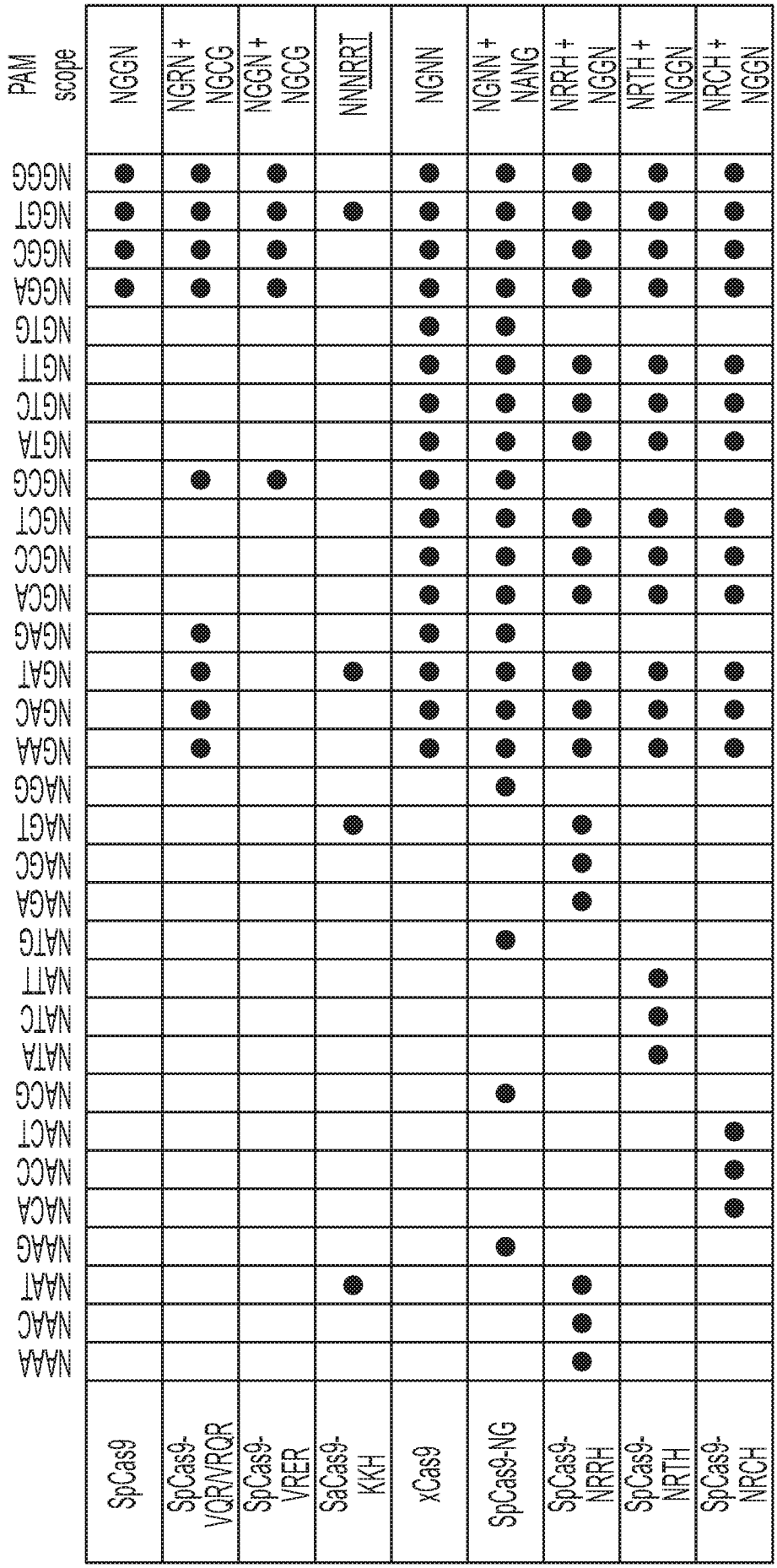

PAM scope
NGGN
NGRN + NGCG
NGGN + NGCG
NNNRRT
NGNN
NGNN + NANG
NRRH + NGGN
NRTH + NGGN
NRCH + NGGN
NGGG
NGGT
NGGC
NGGA
NGTG
NGTT
NGTC
NGTA
NGCG
NGCT
NGCC
NGCA
NGAG
NGAT
NGAC
NGAA
NAGG
NAGT
NAGC
NAGA
NATG
NATT
NATC
NATA
NACG
NACT
NACC
NACA
NAAG
NAAT
NAAC
NAAA
SpCas9
SpCas9-VQR/VRQR
SpCas9-VRER
SaCas9-KKH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

FIG. 49