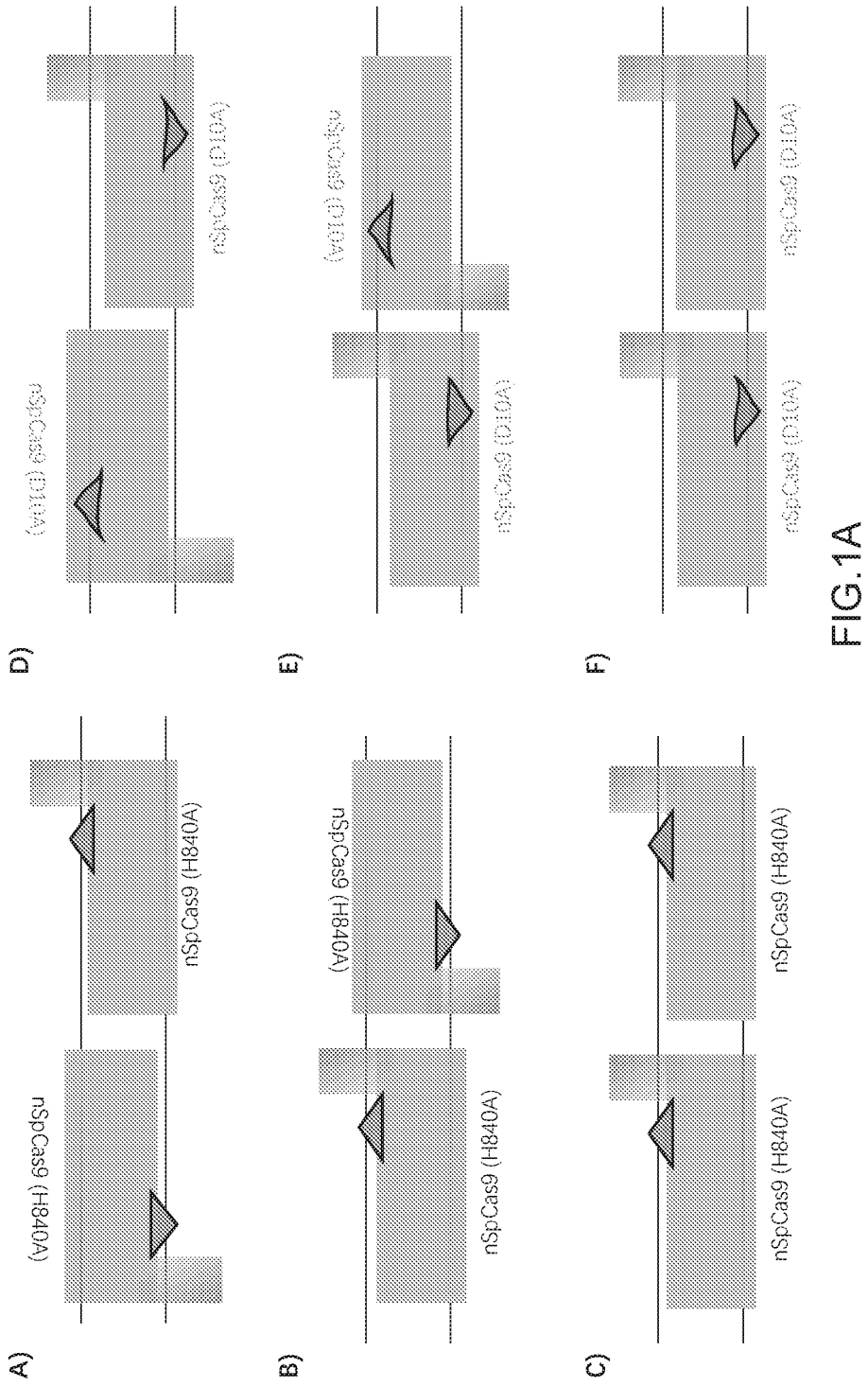

A)
nSpCas9 (H840A)
nSpCas9 (H840A)
B)
nSpCas9 (H840A)
nSpCas9 (H840A)
C)
nSpCas9 (H840A)
nSpCas9 (H840A)
D)
nSpCas9 (D10A)
nSpCas9 (D10A)
E)
nSpCas9 (D10A)
nSpCas9 (D10A)
F)
nSpCas9 (D10A)
nSpCas9 (D10A)

FIG.1A

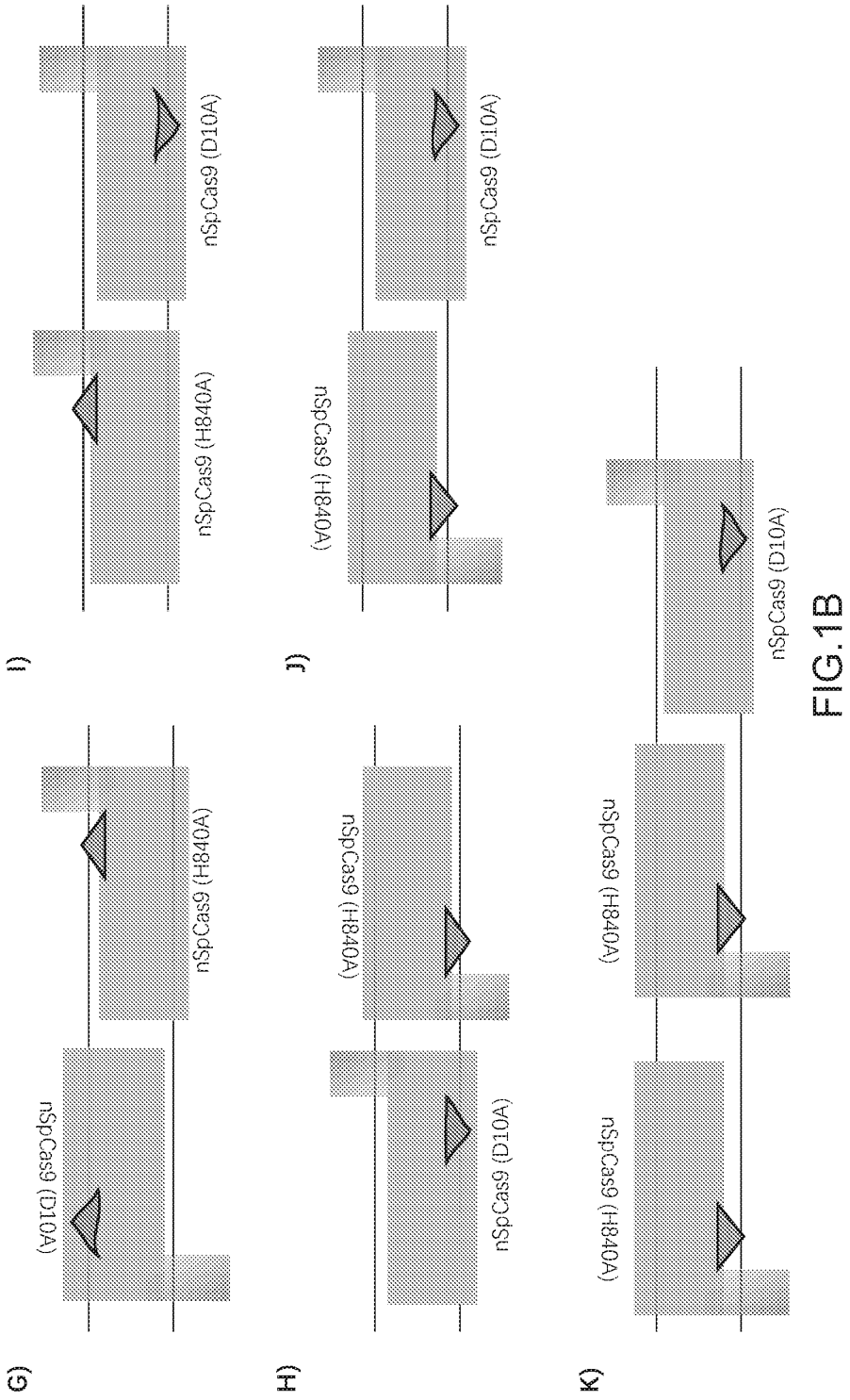

I)
nSpCas9 (D10A)
nSpCas9 (H840A)
J)
nSpCas9 (H840A)
nSpCas9 (D10A)
G)
nSpCas9 (H840A)
nSpCas9 (D10A)
H)
nSpCas9 (H840A)
nSpCas9 (D10A)
K)
nSpCas9 (H840A)
nSpCas9 (H840A)
nSpCas9 (D10A)

FIG.1B

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
guides	-108 / 0	-108 / 0	-38 / 0	-38 / 0	0 / +26	0 / +26	0 / +37	0 / +37	0 / +63	0 / +63	0 / +90	0 / +90	-108 / 0	-108 / 0	-38 / 0	-38 / 0	0 / +26	0 / +26
ODN	D36P36	D36P36	D36P36	D36P36	D36P36	D36P36	D36P36	D36P36	D36P36	D36P36	D36P36	D36P36	D20P36	D20P36	D20P36	D20P36	D20P36	D20P36

No.	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
guides	0 / +37	0 / +37	0 / +63	0 / +63	0 / +90	0 / +90	-108 / 0	-38 / 0	0 / +26	0 / +37	0 / +63	0 / +90	-108	-38	+26	+37	+63	+90	0	/
ODN	D20P36	D20P36	D20P36	D20P36	D20P36	D20P36	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN	no ODN

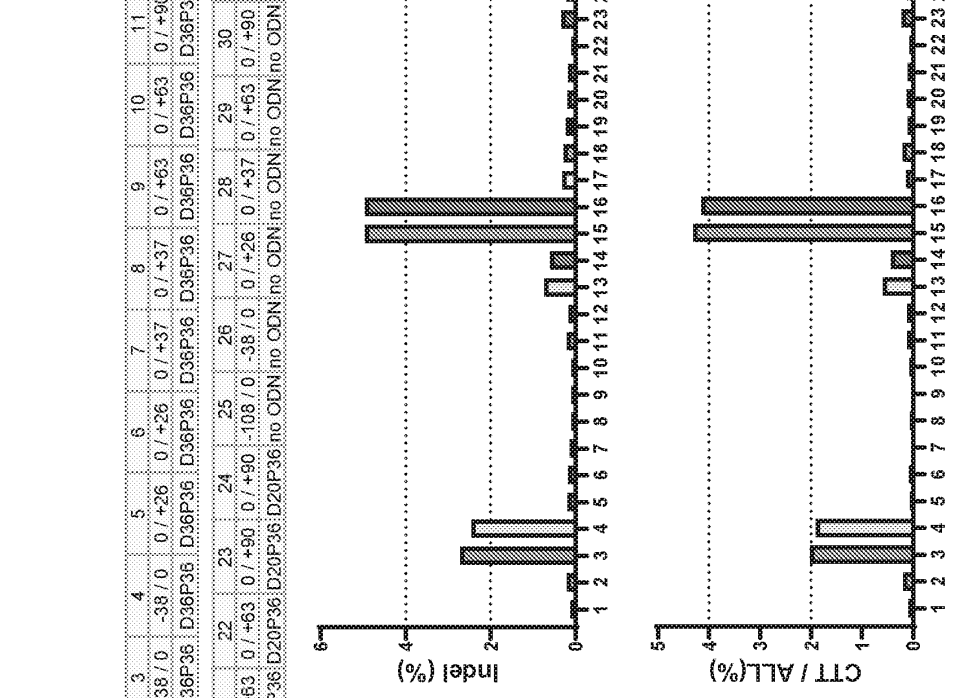


FIG. 2

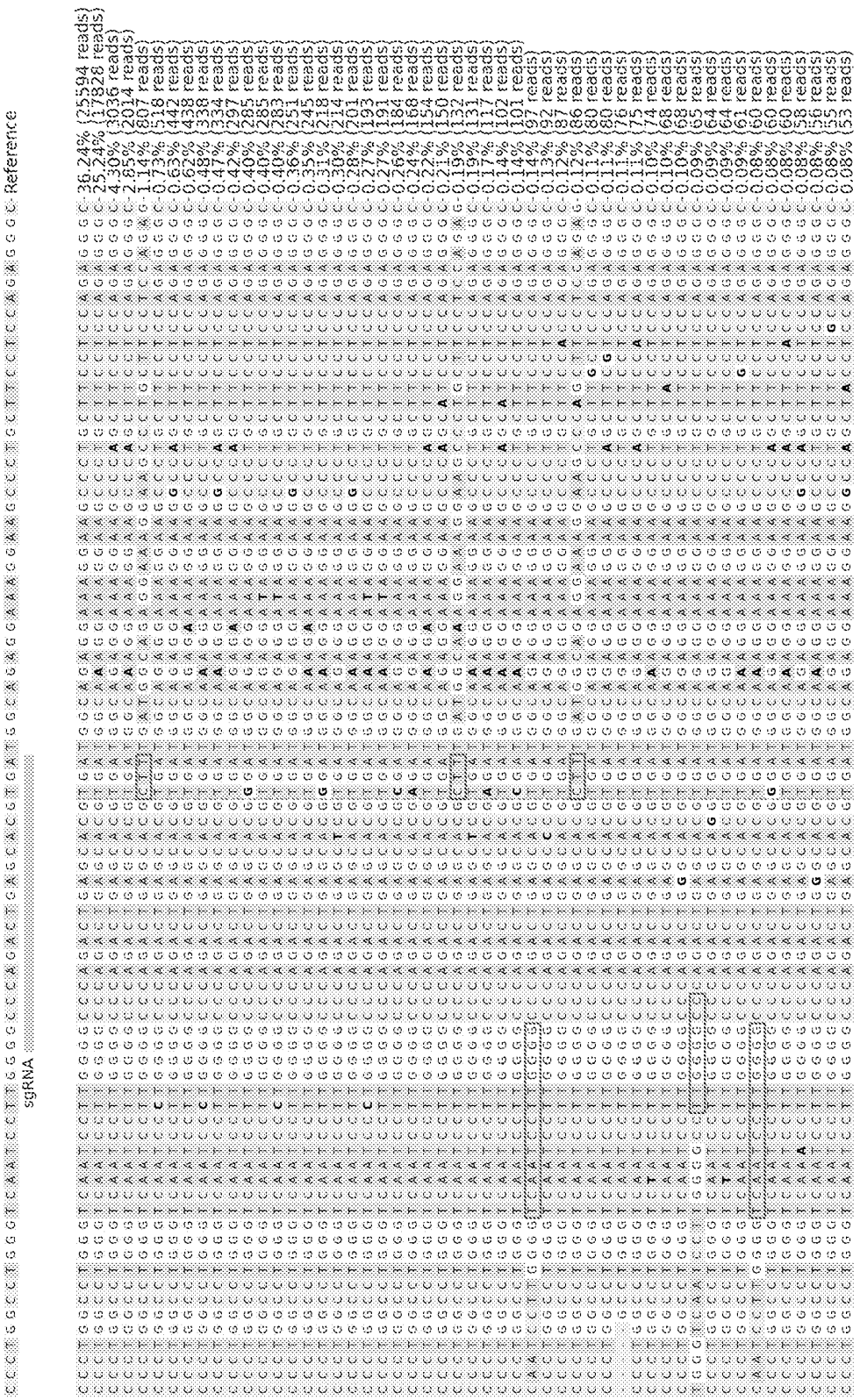

sgRNA
Reference
36.24% (25594 reads)
25.24% (17828 reads)
4.30% (3036 reads)
2.85% (2014 reads)
1.14% (807 reads)
0.73% (518 reads)
0.62% (442 reads)
0.49% (348 reads)
0.47% (334 reads)
0.42% (297 reads)
0.40% (285 reads)
0.40% (283 reads)
0.36% (251 reads)
0.35% (245 reads)
0.31% (218 reads)
0.30% (214 reads)
0.28% (201 reads)
0.27% (193 reads)
0.27% (191 reads)
0.26% (184 reads)
0.24% (168 reads)
0.22% (154 reads)
0.21% (150 reads)
0.19% (132 reads)
0.19% (131 reads)
0.17% (117 reads)
0.14% (102 reads)
0.14% (101 reads)
0.14% (97 reads)
0.13% (92 reads)
0.12% (87 reads)
0.12% (86 reads)
0.11% (80 reads)
0.11% (80 reads)
0.11% (76 reads)
0.11% (75 reads)
0.10% (74 reads)
0.10% (68 reads)
0.10% (68 reads)
0.09% (65 reads)
0.09% (64 reads)
0.09% (64 reads)
0.09% (61 reads)
0.08% (60 reads)
0.08% (60 reads)
0.08% (58 reads)
0.08% (56 reads)
0.08% (55 reads)
0.08% (53 reads)

FIG. 3

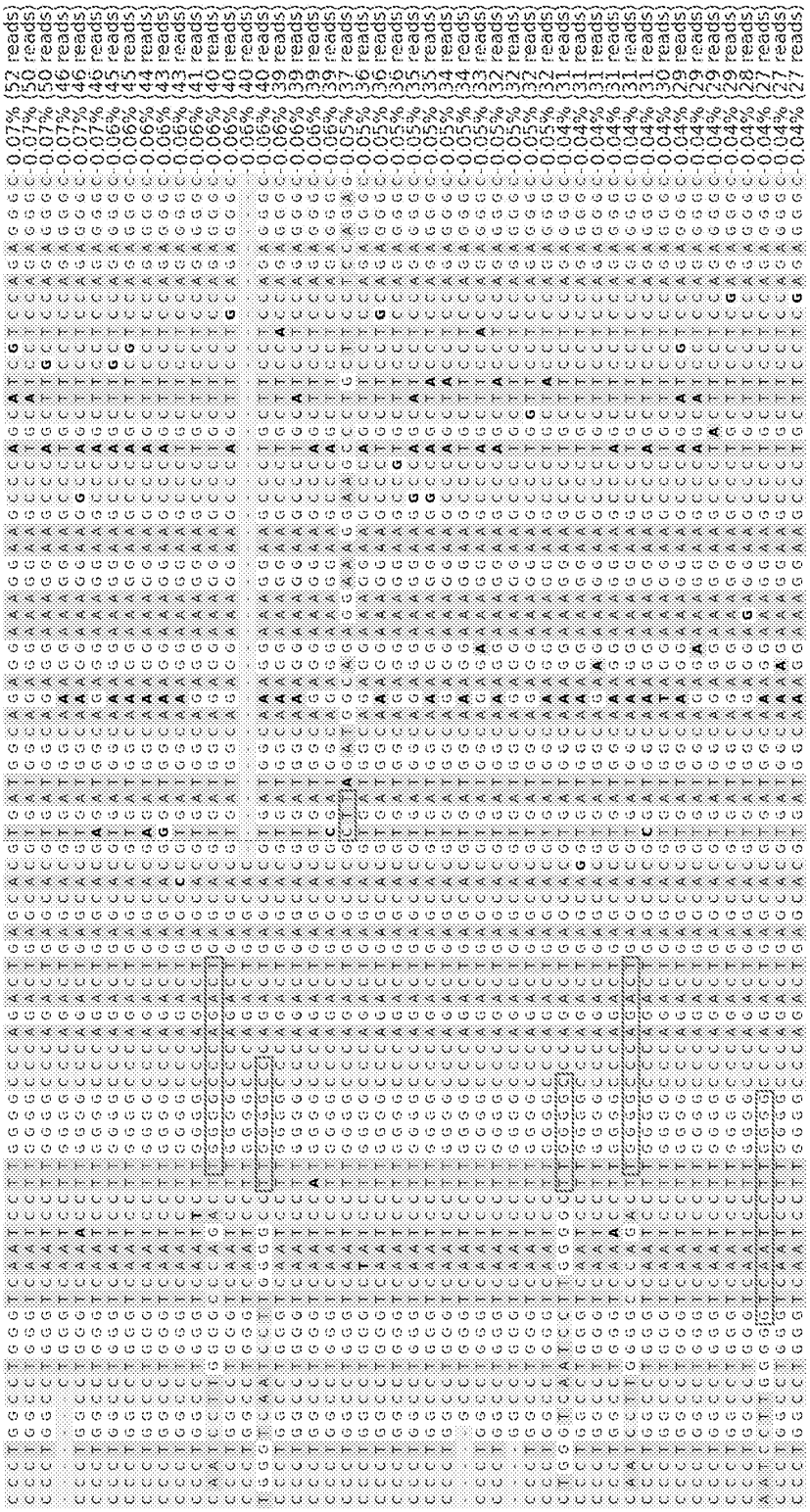


FIG. 3 (continued)

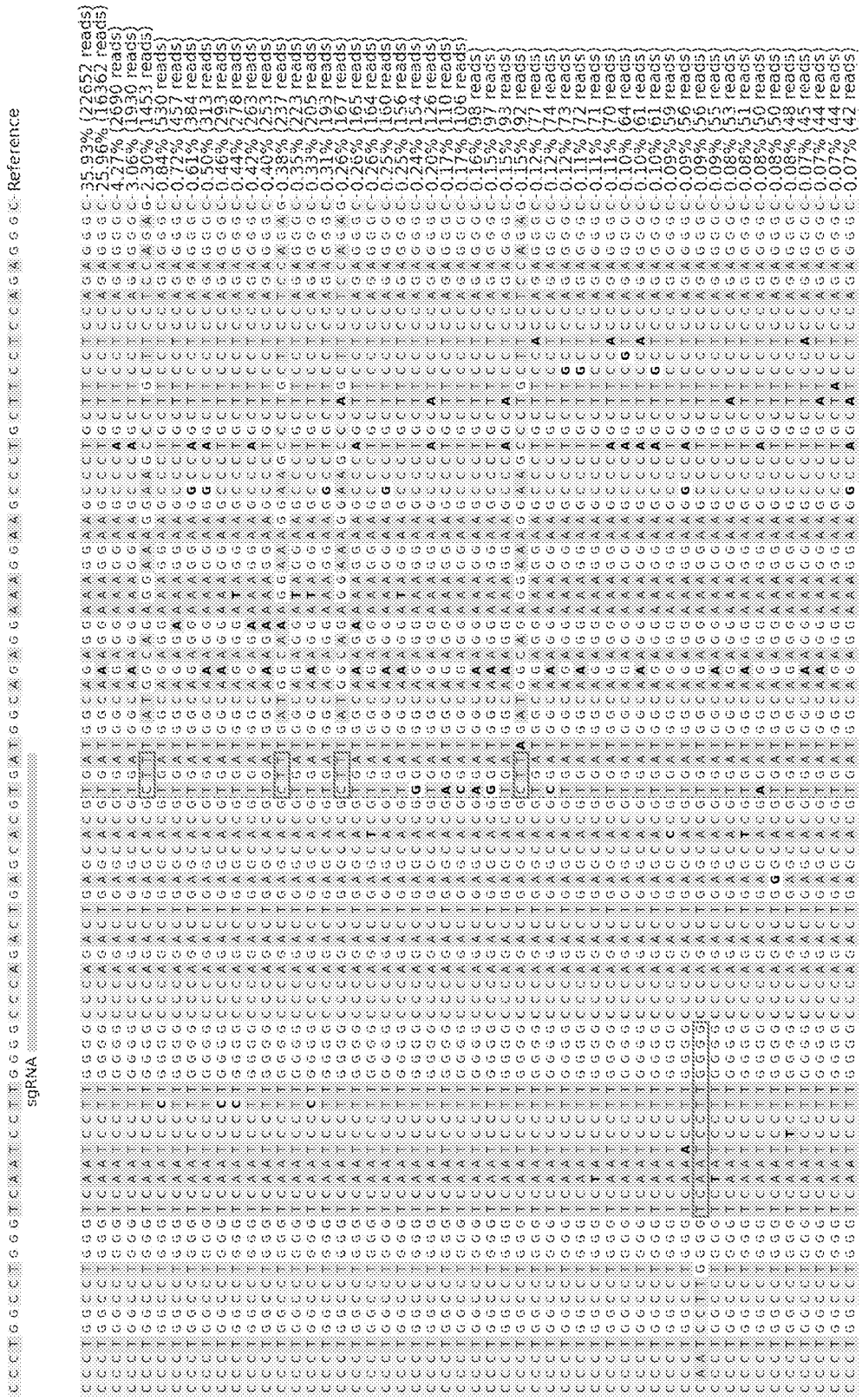

sgRNA
Reference
35.93% (22652 reads)
25.96% (16362 reads)
4.27% (2690 reads)
3.06% (1930 reads)
2.30% (1453 reads)
0.84% (530 reads)
0.72% (457 reads)
0.61% (384 reads)
0.50% (313 reads)
0.46% (293 reads)
0.44% (278 reads)
0.42% (263 reads)
0.40% (253 reads)
0.38% (237 reads)
0.35% (223 reads)
0.33% (205 reads)
0.31% (193 reads)
0.26% (165 reads)
0.26% (164 reads)
0.25% (160 reads)
0.25% (156 reads)
0.24% (154 reads)
0.20% (126 reads)
0.17% (110 reads)
0.17% (106 reads)
0.16% (98 reads)
0.15% (97 reads)
0.15% (93 reads)
0.13% (82 reads)
0.12% (77 reads)
0.12% (74 reads)
0.12% (73 reads)
0.11% (72 reads)
0.11% (71 reads)
0.11% (70 reads)
0.10% (64 reads)
0.10% (61 reads)
0.10% (61 reads)
0.09% (59 reads)
0.09% (56 reads)
0.09% (56 reads)
0.09% (55 reads)
0.08% (53 reads)
0.08% (51 reads)
0.08% (50 reads)
0.08% (50 reads)
0.08% (48 reads)
0.07% (45 reads)
0.07% (44 reads)
0.07% (44 reads)
0.07% (42 reads)

FIG. 4

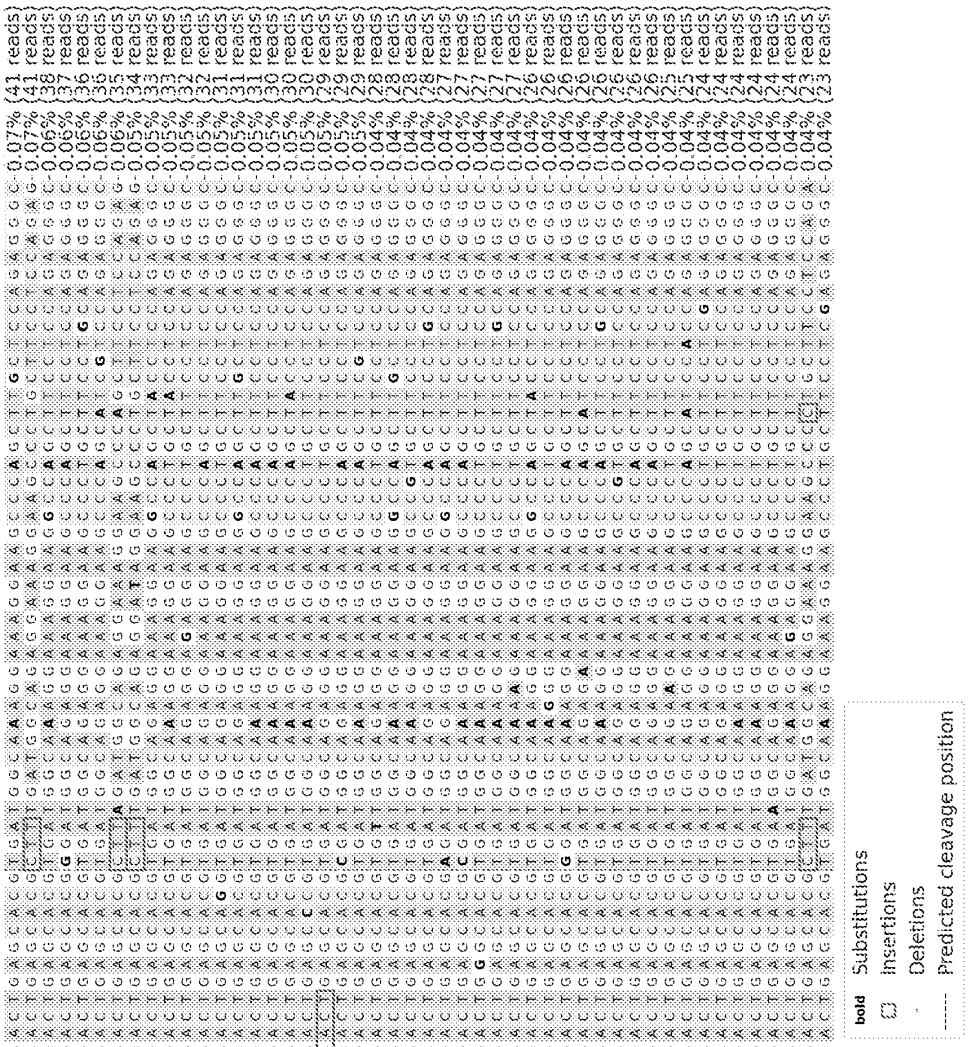


FIG. 4 (continued)

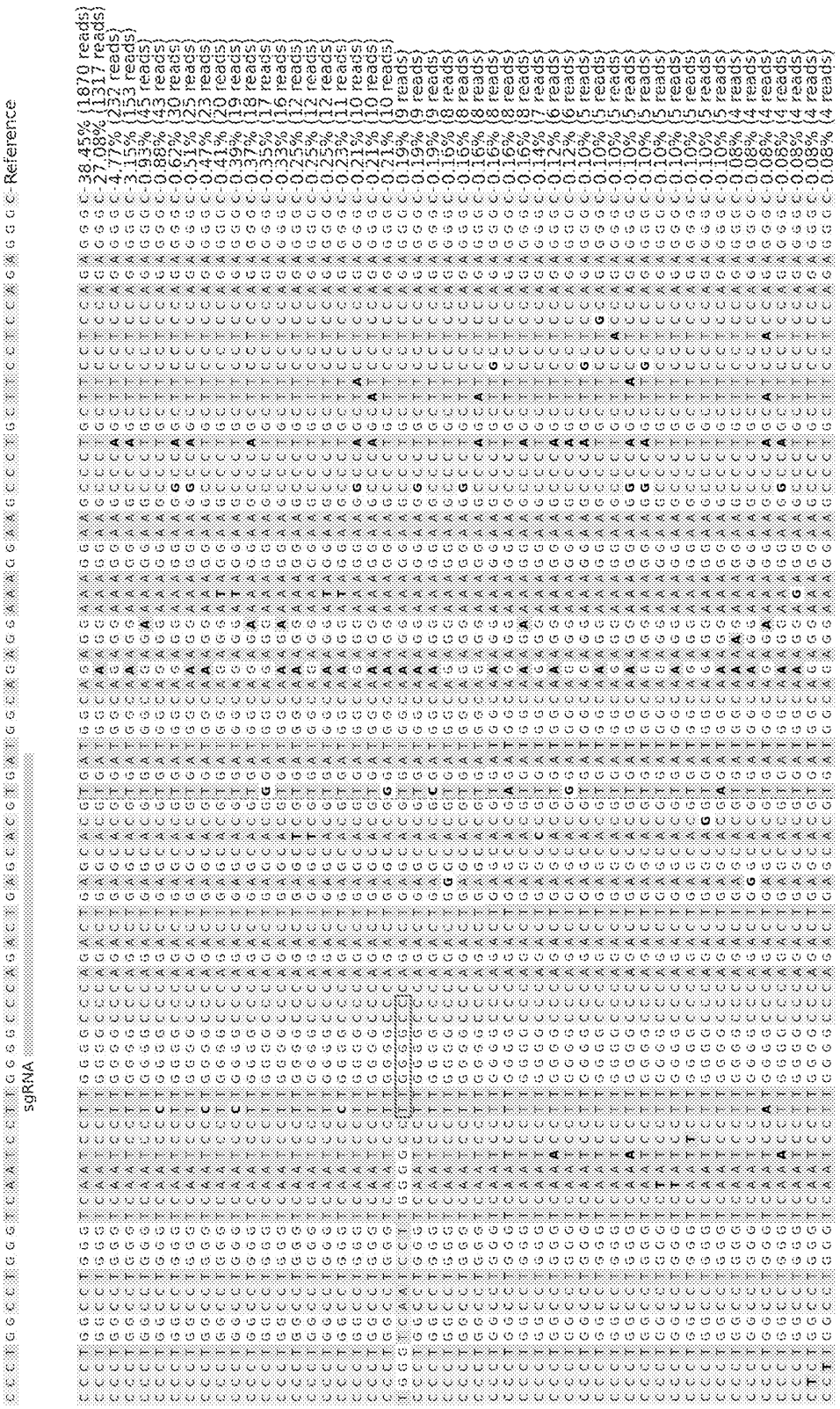


FIG. 5

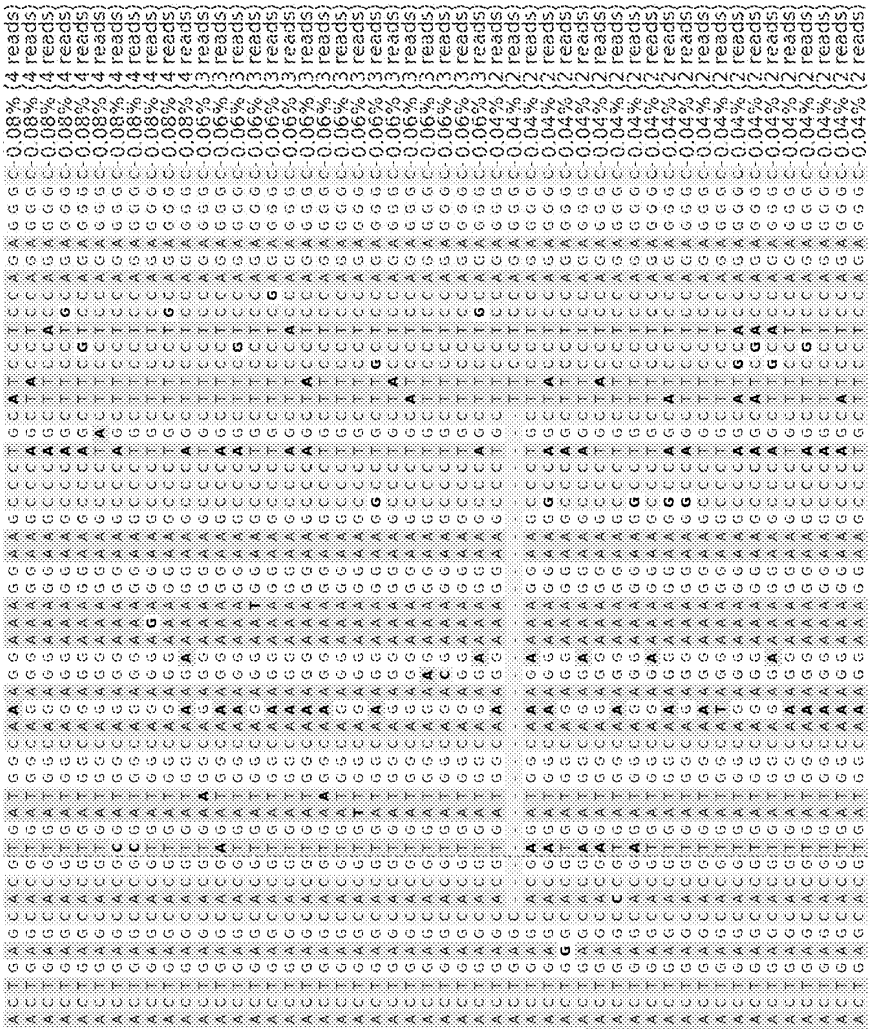


FIG. 5 (continued)

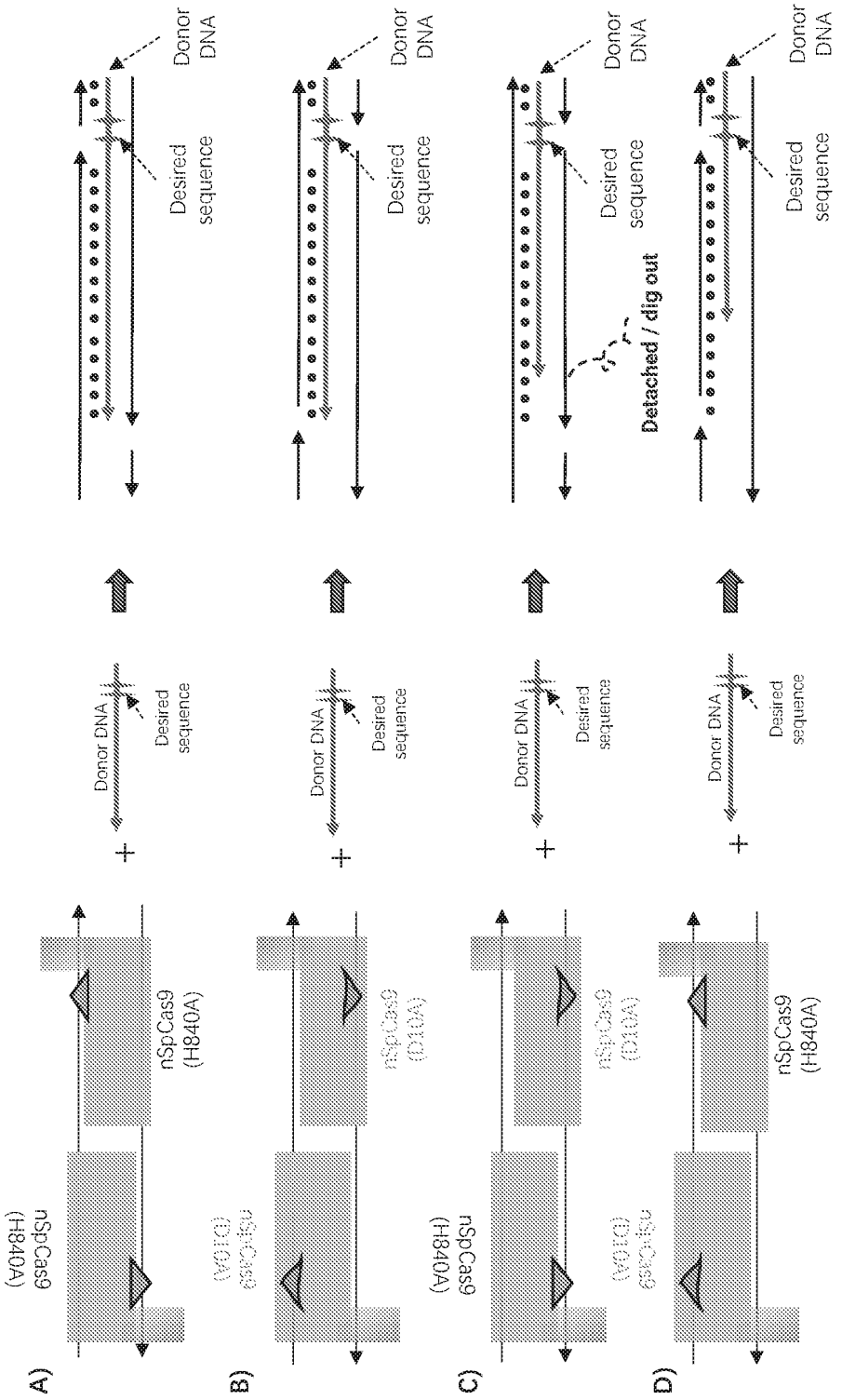
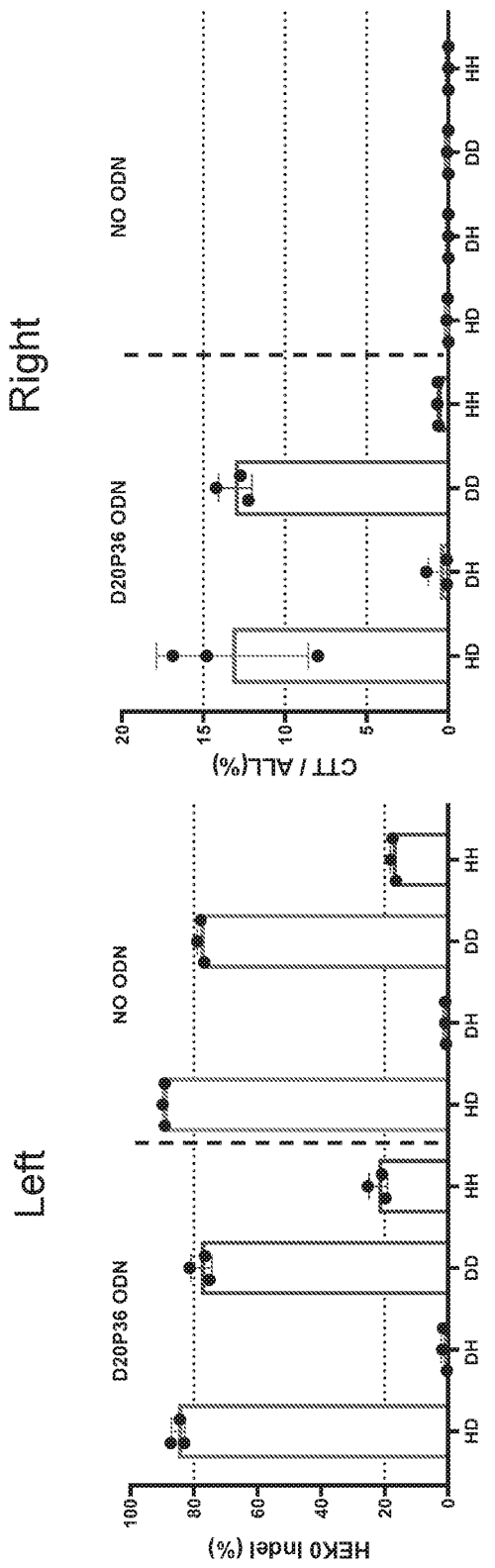

A)
B)
C)
D)
nSpCas9 (H840A)
nSpCas9 (D10A)
Donor DNA
Desired sequence
Detached / dig out

FIG.6



HD: H840A(HEK -38)+D10A(HEK 0)
DH: D10A(HEK -38)+H840A(HEK 0)
DD: D10A(HEK -38)+D10A(HEK 0)
HH: H840A(HEK -38)+H840A(HEK 0)

FIG.7

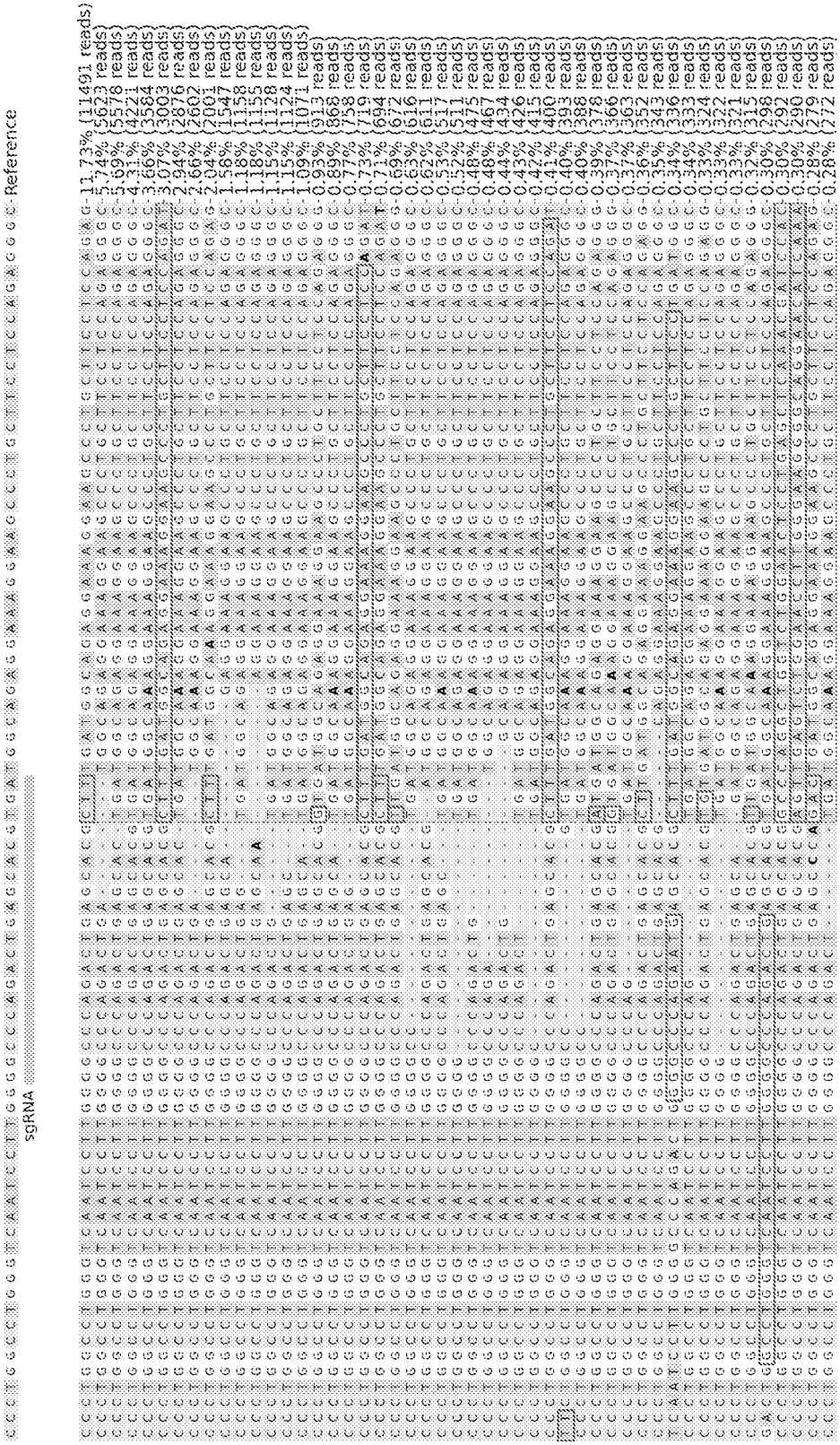

Reference
sgRNA
11.73% (11491 reads)
5.74% (5623 reads)
5.69% (5578 reads)
4.31% (4221 reads)
3.66% (3584 reads)
3.07% (3003 reads)
2.94% (2876 reads)
2.66% (2602 reads)
2.04% (2001 reads)
1.58% (1547 reads)
1.18% (1158 reads)
1.15% (1125 reads)
1.15% (1124 reads)
1.09% (1071 reads)
0.93% (913 reads)
0.89% (868 reads)
0.77% (758 reads)
0.73% (719 reads)
0.71% (694 reads)
0.69% (672 reads)
0.63% (616 reads)
0.62% (611 reads)
0.53% (517 reads)
0.52% (511 reads)
0.48% (475 reads)
0.48% (467 reads)
0.44% (434 reads)
0.43% (426 reads)
0.42% (415 reads)
0.41% (400 reads)
0.40% (393 reads)
0.40% (388 reads)
0.39% (378 reads)
0.37% (366 reads)
0.37% (363 reads)
0.36% (352 reads)
0.35% (343 reads)
0.34% (336 reads)
0.34% (333 reads)
0.33% (324 reads)
0.33% (322 reads)
0.33% (321 reads)
0.32% (315 reads)
0.30% (298 reads)
0.30% (292 reads)
0.30% (290 reads)
0.28% (279 reads)
0.28% (272 reads)

FIG. 8

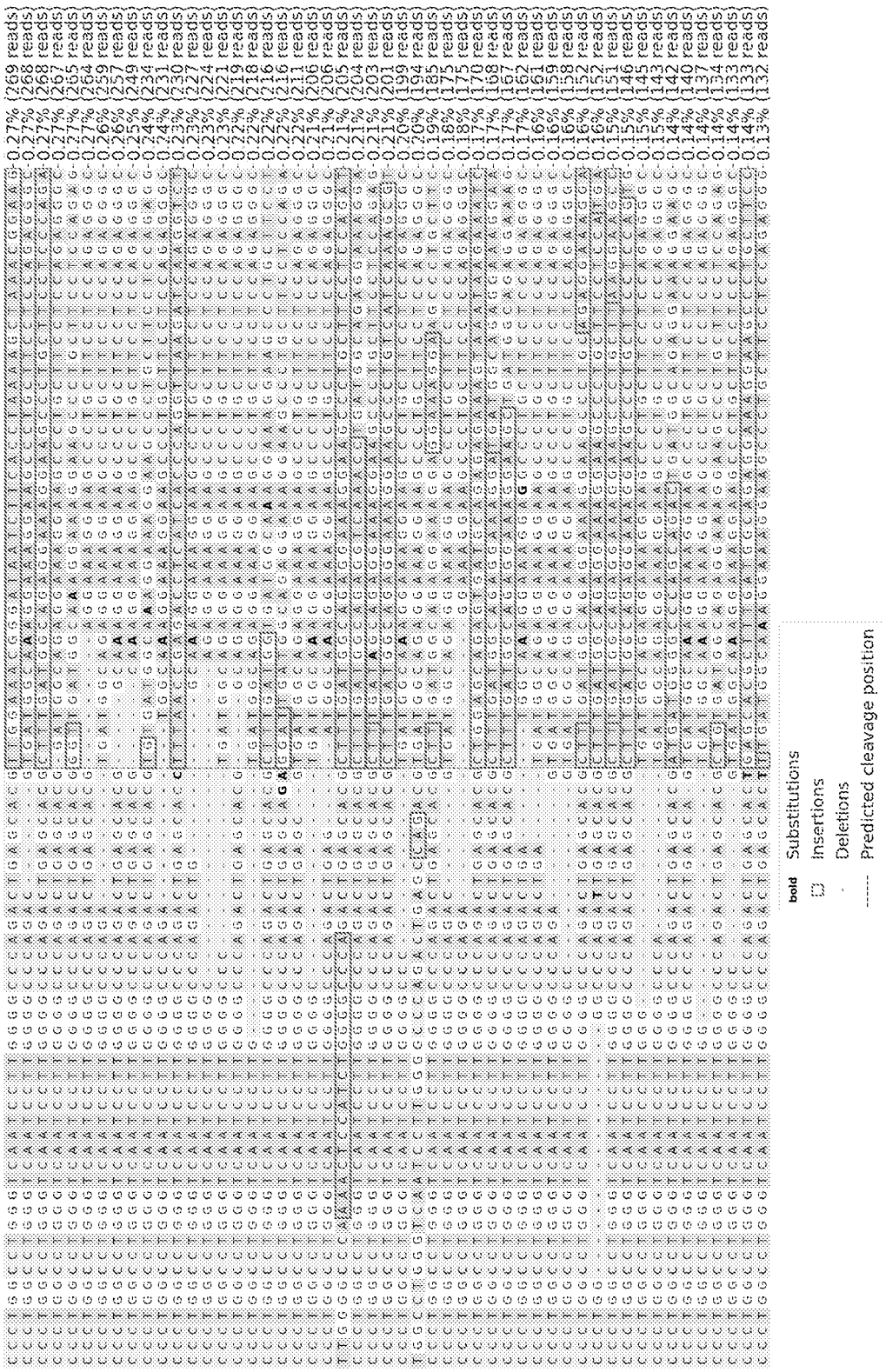


FIG. 8 (continued)

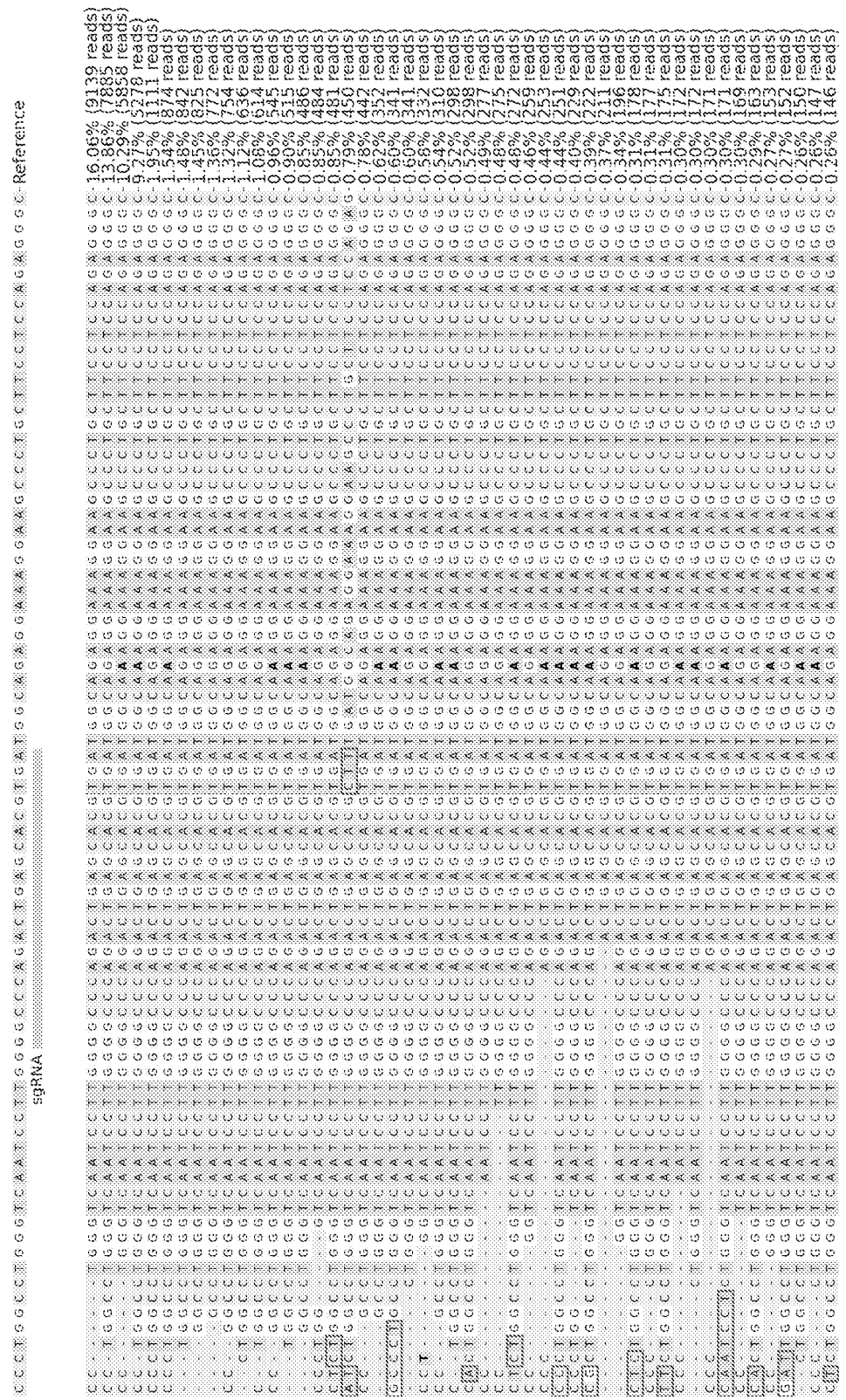

sgRNA
Reference
16.06% (9139 reads)
13.86% (7885 reads)
10.29% (5858 reads)
9.27% (5278 reads)
1.95% (1111 reads)
1.54% (874 reads)
1.48% (842 reads)
1.45% (825 reads)
1.36% (772 reads)
1.32% (754 reads)
1.12% (636 reads)
1.08% (614 reads)
0.96% (545 reads)
0.90% (515 reads)
0.85% (486 reads)
0.85% (484 reads)
0.85% (481 reads)
0.79% (450 reads)
0.78% (442 reads)
0.62% (352 reads)
0.60% (341 reads)
0.60% (341 reads)
0.58% (332 reads)
0.54% (310 reads)
0.52% (298 reads)
0.52% (298 reads)
0.49% (277 reads)
0.48% (275 reads)
0.46% (262 reads)
0.46% (259 reads)
0.44% (253 reads)
0.44% (251 reads)
0.40% (229 reads)
0.39% (222 reads)
0.37% (211 reads)
0.34% (196 reads)
0.31% (178 reads)
0.31% (177 reads)
0.31% (175 reads)
0.30% (172 reads)
0.30% (172 reads)
0.30% (171 reads)
0.30% (171 reads)
0.30% (169 reads)
0.29% (163 reads)
0.27% (153 reads)
0.27% (152 reads)
0.26% (150 reads)
0.26% (147 reads)
0.26% (146 reads)

FIG. 9

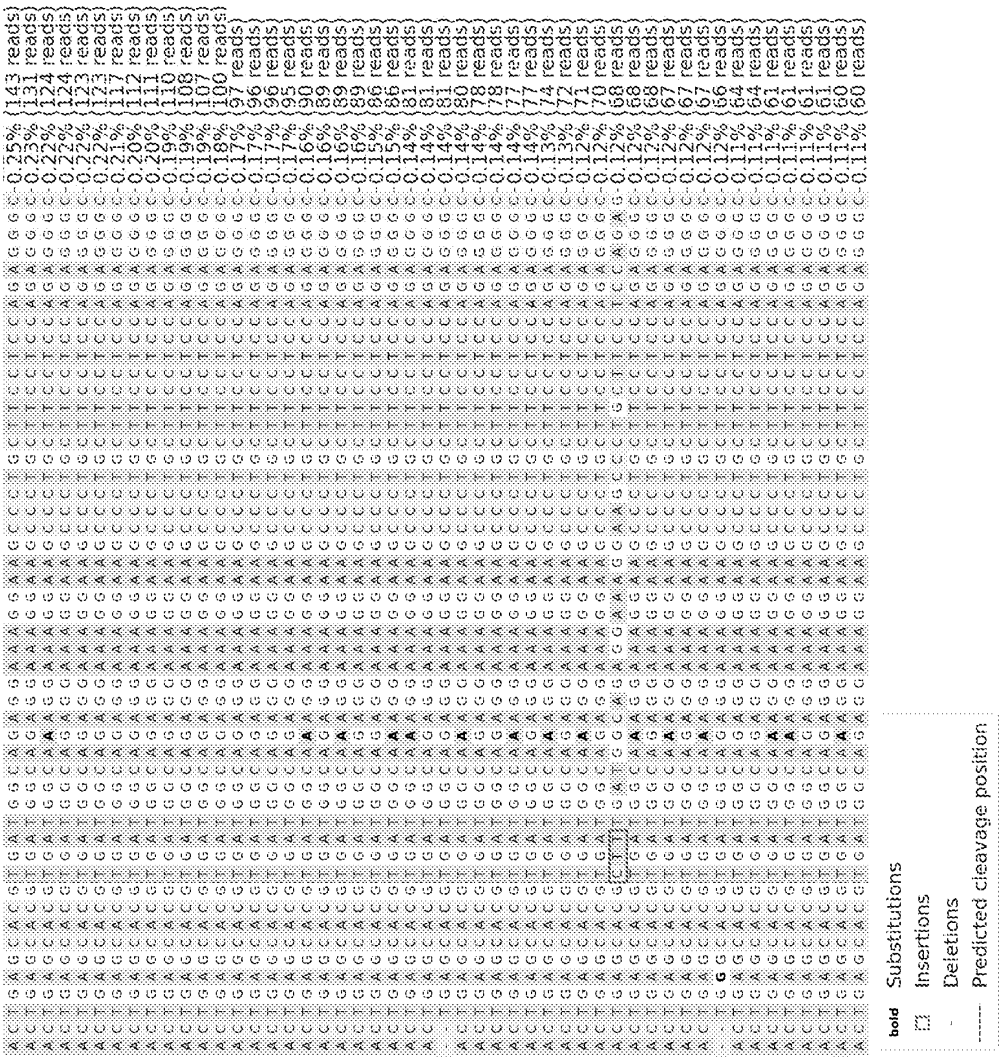


FIG. 9 (continued)

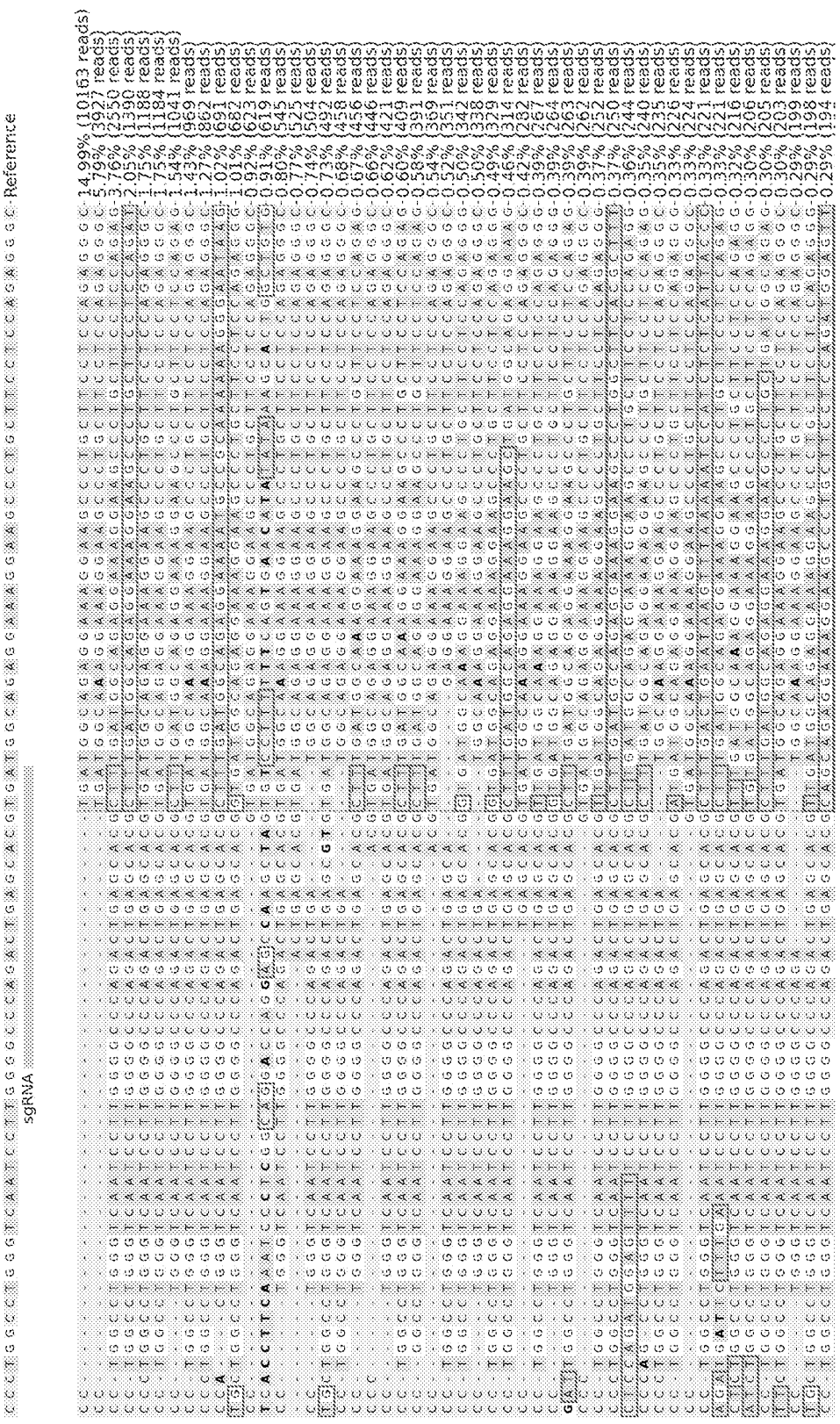

sgRNA
Reference
14.99% (10163 reads)
5.79% (3927 reads)
3.76% (2550 reads)
2.05% (1390 reads)
1.75% (1188 reads)
1.75% (1184 reads)
1.54% (1041 reads)
1.43% (969 reads)
1.27% (862 reads)
1.02% (691 reads)
1.01% (682 reads)
0.92% (623 reads)
0.91% (619 reads)
0.80% (545 reads)
0.77% (525 reads)
0.74% (504 reads)
0.73% (492 reads)
0.68% (458 reads)
0.67% (456 reads)
0.66% (446 reads)
0.62% (421 reads)
0.60% (409 reads)
0.58% (391 reads)
0.54% (369 reads)
0.52% (351 reads)
0.50% (342 reads)
0.50% (338 reads)
0.49% (329 reads)
0.46% (314 reads)
0.42% (282 reads)
0.39% (267 reads)
0.39% (264 reads)
0.39% (263 reads)
0.39% (262 reads)
0.37% (252 reads)
0.37% (250 reads)
0.36% (244 reads)
0.35% (240 reads)
0.35% (235 reads)
0.33% (226 reads)
0.33% (224 reads)
0.33% (221 reads)
0.33% (221 reads)
0.32% (216 reads)
0.30% (206 reads)
0.30% (205 reads)
0.30% (203 reads)
0.29% (199 reads)
0.29% (198 reads)
0.29% (194 reads)

FIG. 10

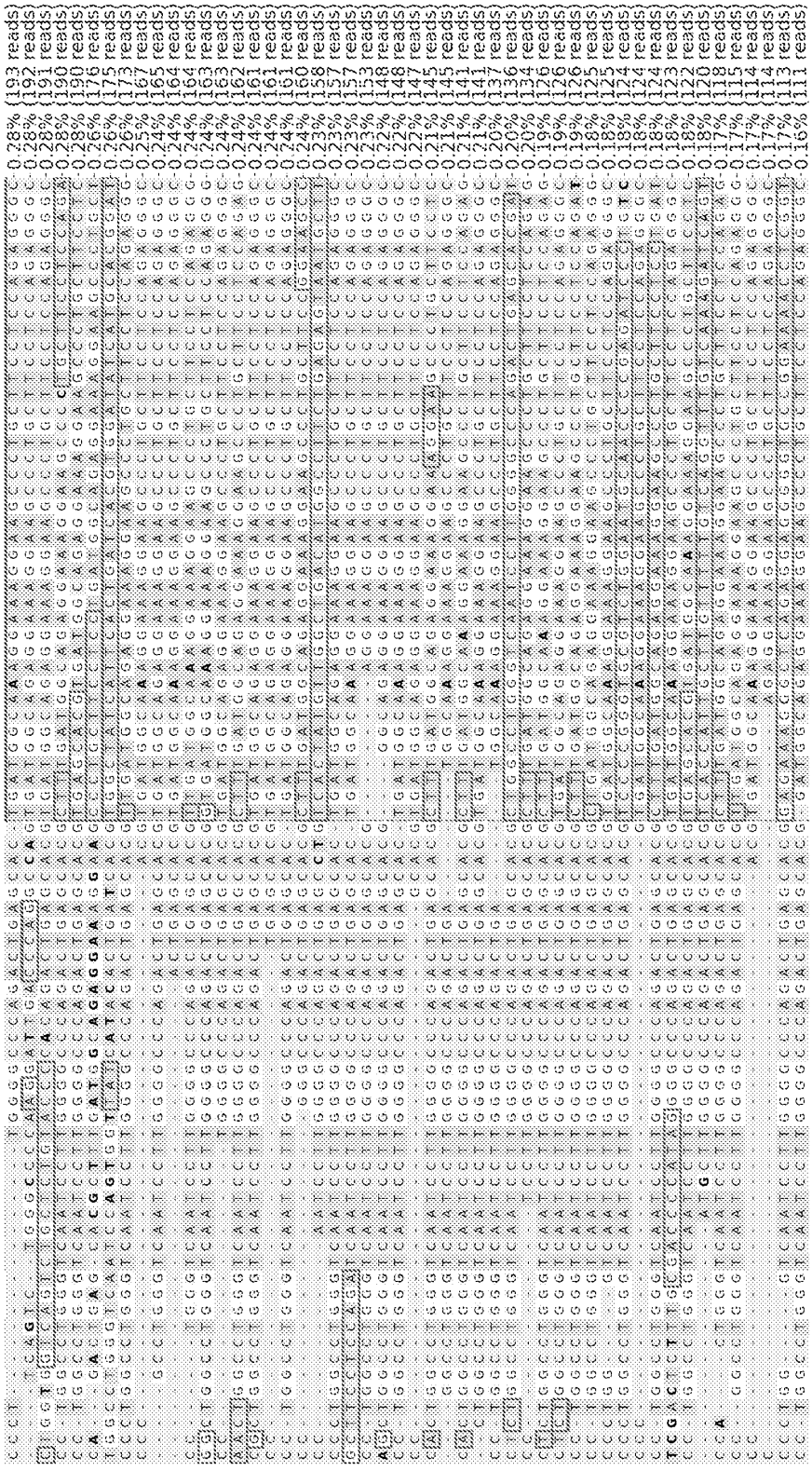


FIG. 10 (continued)

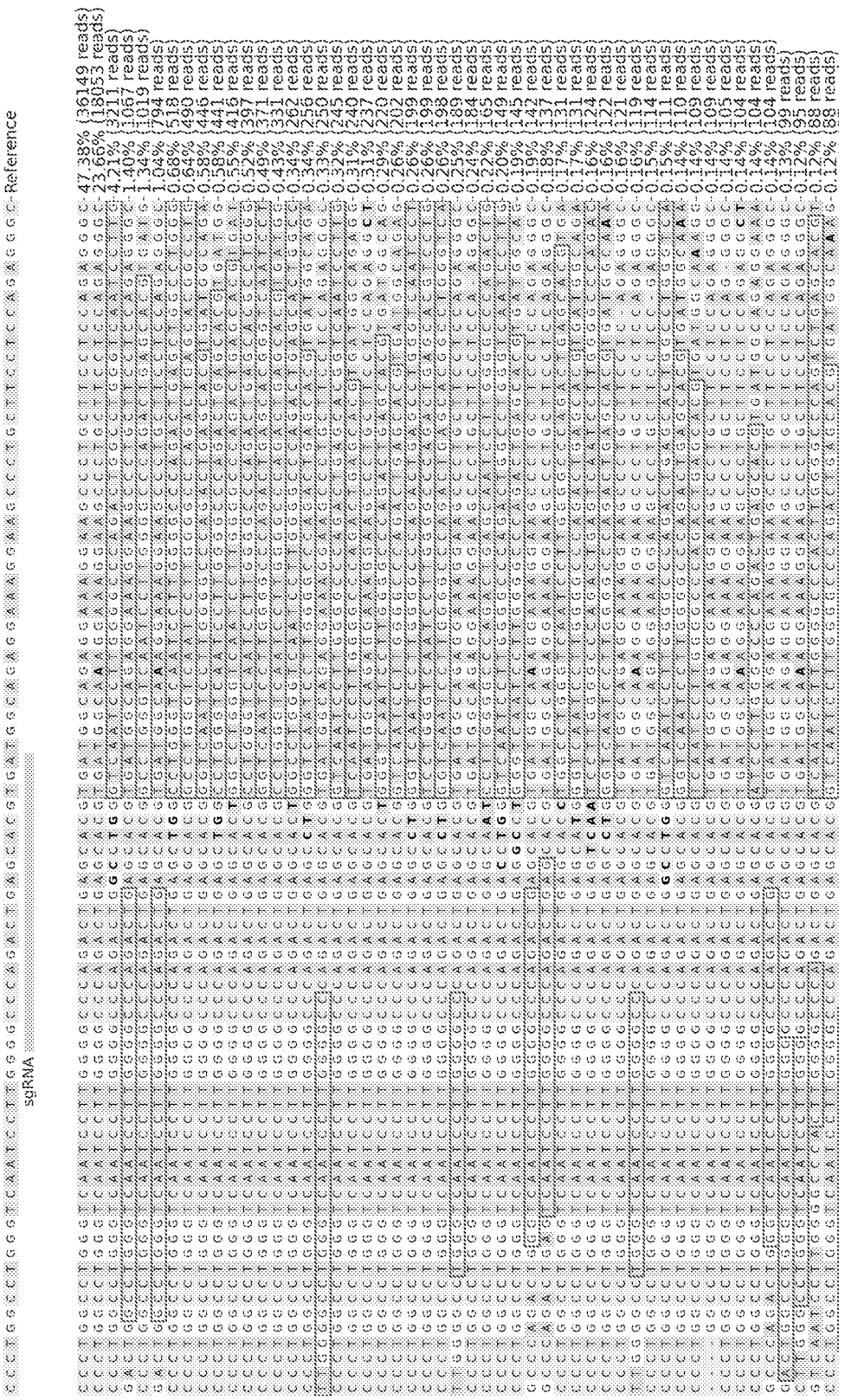

sgRNA
Reference
47.38% (36149 reads)
23.66% (18053 reads)
4.21% (3211 reads)
1.40% (1067 reads)
1.34% (1019 reads)
1.04% (794 reads)
0.68% (518 reads)
0.64% (490 reads)
0.58% (445 reads)
0.58% (441 reads)
0.55% (416 reads)
0.52% (397 reads)
0.49% (371 reads)
0.43% (331 reads)
0.34% (262 reads)
0.34% (256 reads)
0.33% (250 reads)
0.32% (245 reads)
0.31% (240 reads)
0.31% (237 reads)
0.29% (220 reads)
0.26% (202 reads)
0.26% (199 reads)
0.26% (198 reads)
0.25% (189 reads)
0.24% (184 reads)
0.22% (165 reads)
0.20% (149 reads)
0.19% (145 reads)
0.19% (142 reads)
0.18% (137 reads)
0.17% (131 reads)
0.16% (124 reads)
0.16% (122 reads)
0.16% (121 reads)
0.16% (119 reads)
0.15% (114 reads)
0.15% (111 reads)
0.14% (110 reads)
0.14% (109 reads)
0.14% (109 reads)
0.14% (105 reads)
0.14% (104 reads)
0.14% (104 reads)
0.13% (99 reads)
0.12% (95 reads)
0.12% (88 reads)
0.12% (88 reads)

FIG. 11

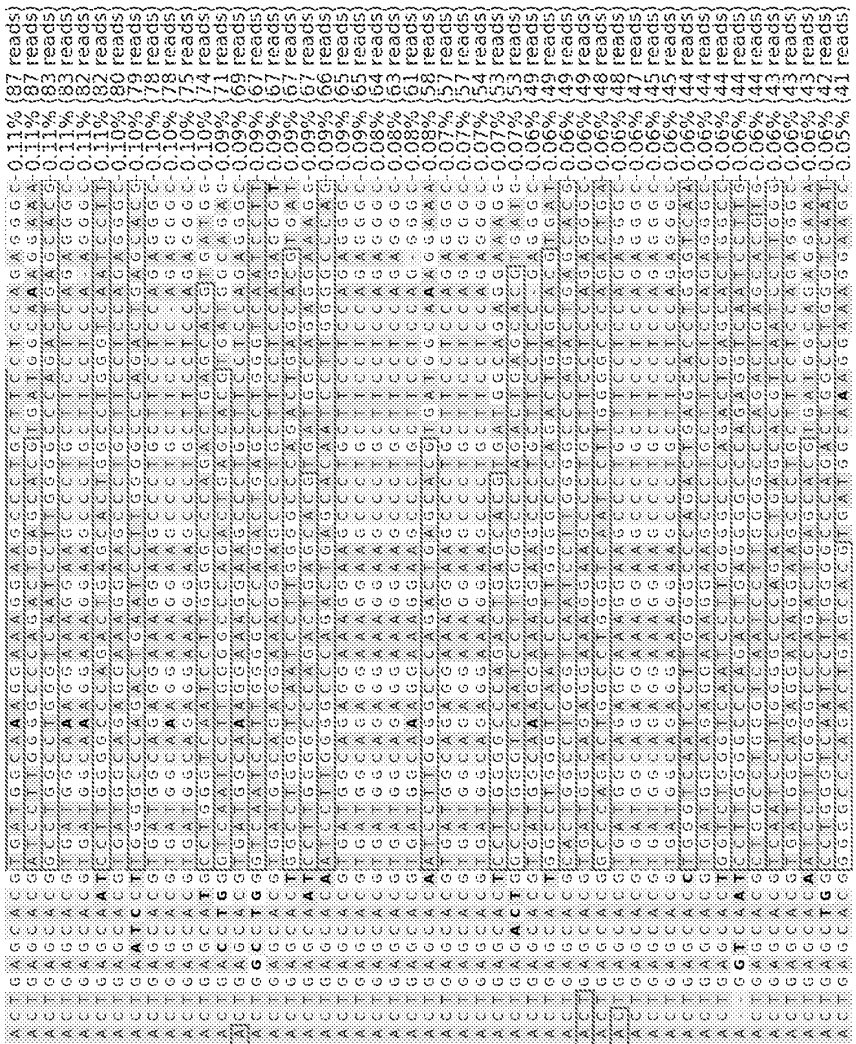


FIG. 11 (continued)

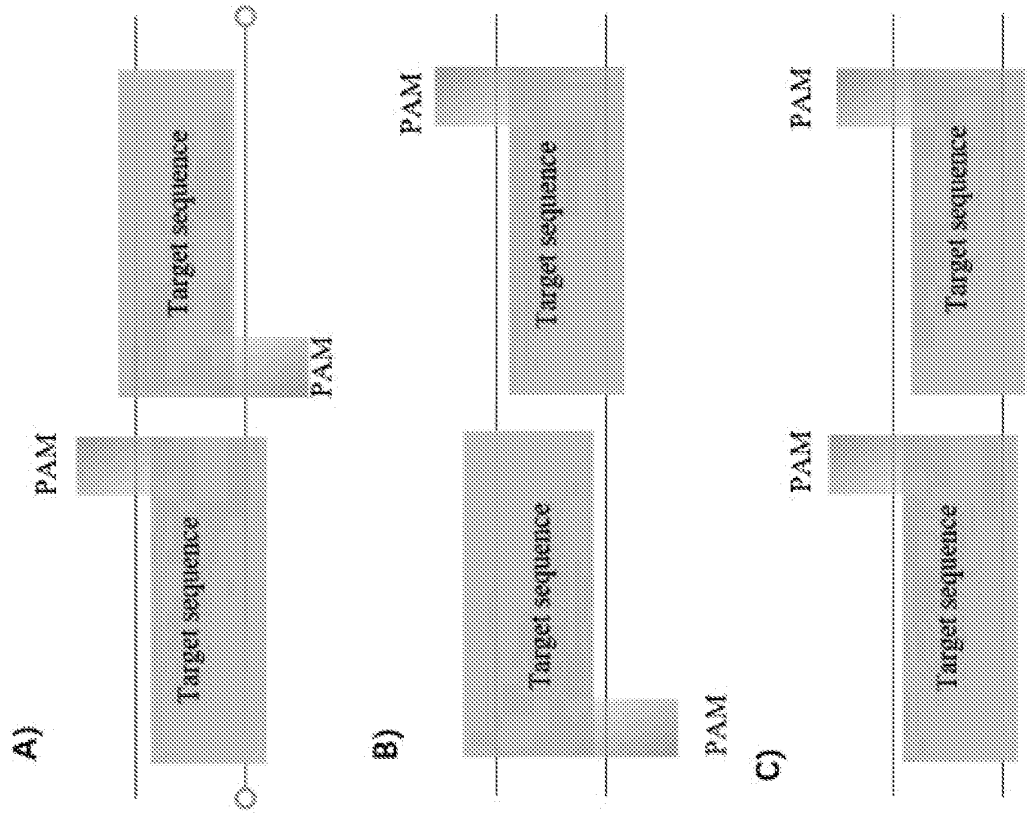

A)
PAM
Target sequence
Target sequence
PAM
B)
PAM
Target sequence
Target sequence
PAM
C)
PAM
Target sequence
PAM
Target sequence

FIG. 12