

Site 3

Line #		
WT	GCCCTCTGCATCATCTCCG GAGGGGACGTTGGTCATAGC CAAAACTCAATC	
2	GCCCTCTGCATCATCTCCG-----TTGGTCATAGCCAAACTCAATC	9 bp deletion
3, 22	GCCCTCTGCATCATCTCCGGA-----CGTTGGTCATAGCCAAACTCAATC	5 bp deletion
13	GCCCTCTGCATCATCTCCGG---GGACGTTGGTCATAGCCAAACTCAATC	3 bp deletion
23	GCCCTCTGCATCATCTCCGGA----ACGTTGGTCATAGCCAAACTCAATC	4 bp deletion

Site 4

Line #		
WT	CGGTAATGGATTACCC TCG ----- GCATTACGAGGGCTGTC CTGTTTTATC	
2, 23	CGGTAATGGATTACCC TCGTAAT GCATTACGAGGGCTGTCCTGTTTTATC	4 bp insertion
3	CGGTAATGGATTACCC TCG ---- G TAT GAC -AGGGCTGTCCTGTTTTATC	1 bp deletion, 2 bp substitution
13	CGGTAAT A -----GCATTACGAGGGCTGTCCTGTTTTATC	11 bp deletion, 1 bp substitution
20	CGGTAATGGATTACCC-----CATTACGAGGGCTGTCCTGTTTTATC	4 bp deletion
22	CGGTAATGGATTACCC TCG -----CATTACGAGGGCTGTCCTGTTTTATC	1 bp deletion

Site 5

Line #		
WT	CTCAACAGGAGAGTCCA CACACGGGACTGCACCTGTG AAGGCAGATGATG	
2	CTCAACAGGAGAGTC--CACACGGGACTGCACCTGTGAAGGCAGATGATG	2 bp deletion
3, 22	CTCAACAGGA-----CTGCACCTGTGAAGGCAGATGATG	16 bp deletion
13, 23	CTCAACAGGAGAGTCCACAC-----CTGTGAAGGCAGATGATG	12 bp deletion
20	CTCAACAGGAGAGTCCACAC--GGGACTGCACCTGTGAAGGCAGATGATG	2 bp deletion

Site 6

Line #		
WT	CTTCACTCTCT CTGCGGGAGAACAGACAGAA TGGATTTCGTGCTTT	
2	CTTCACTCTCTCTGCGGGAGAACAGACAGAA T GGATTTCGTGCTTT	no edits
3	CTTCACTCTCTCTGCGGGAGAAC AAC GAATGGATTTCGTGCTTT	2 bp substitution
13, 20, 22, 23	CTTCACTCTCTCTGCGGGAGAACAG A ----ATGGATTTCGTGCTTT	4 bp deletion

Supplemental Figure 2

Sequence data displaying the results of the multiplex targeting experiment. The highlighted red text represents the protospacer and the black arrow represents the expected Cas9 cleavage site. Dashed lines indicate deletions and/or insertions. A summary of the genotyping data is shown in Figure 3B.