GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR10767 Gnptg exdel**

Protocol: ***GoTaq® G2 Colorless Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.5
Primer 2. (stock concentration is 20µM) wtR	0.5
Primer 3. (stock concentration is 20µM) mutR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		68	2:00	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**25x**
7. Elongation		68	2:00 (↑20sec/cycle)	
8. Finish		4	∞	n/a

Primers:

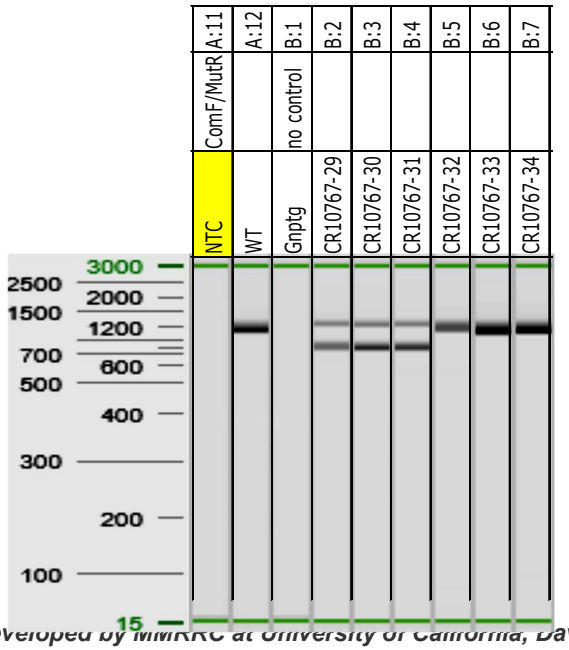
Name	Nucleotide Sequence (5' - 3')
1. CR_Gnptg_comF	AGCTCCAGCGTTTGTTCAAGAGC
2. CR_Gnptg_wtR	CGAGTACATTATGAGTCCTTCATCCAAG
3. CR_Gnptg_mutR	CACCTGGAGTGTTGAGTCTTCATACC

Electrophoresis Protocol:

Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	557, 1019	wildtype
1 & 3	687	mutant

Allele Description: Exon 3 ENSMUSE00000294657 and flanking splicing regions were constitutively deleted from the Gnptg gene ENSMUST00000038973.6 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)