GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR10808 Gapdh 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_Gapdh_comF	TGTAGCCTGAAGTCCAGCCATGC
2. CR_Gapdh_wtR	TCAGGTTGCACCATATCAAGGGTG
3. CR_Gapdh_mutR	CGAACCAAAGCATCGACCAGTG

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	889, 2561	wildtype
1 & 3	674	mutant

Allele Description: Exon 3 ENSMUSE00001220755, Exon 4 ENSMUSE00001237509, Exon 5 ENSMUSE00000472146, Exon 6 ENSMUSE00000491786, Exon 7 ENSMUSE00000487077 and flanking splicing regions were constitutively deleted from the Gapdh gene ENSMUST00000073605.14 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals. The Iffo1 Gene ENSMUSG00000038271 had ~89 bases deleted from the 3' UTR.

*wtR primer untested (ePCR verified)