GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11472 Ghdc 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_Ghdc_comF	ATCTTCCACTGACCCAAACCAG
2. CR_Ghdc_wtR*	GGTCACACAGGCAGTACTTCC
3. CR_Ghdc_mutR	CCAGTTAGTAGGAGAATCACCTGG

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	305,1392	wildtype
1 & 3	454	mutant

Allele Description: Exon 4 ENSMUSE00000112789 and flanking splicing regions were constitutively deleted from the Ghdc Gene ENSMUSG00000017747 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 938bp deletion from Chr 11: 100,659,470 – 100,660,407 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)