GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11409 2200002D01Rik 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_2200002D01Rik_comF	TAACAGCCCAAATTAAGACACCTG
2. CR_2200002D01Rik_wtR*	GCTGTAGGTGTCTGGGAGAAAG
3. CR_2200002D01Rik_mutR	GAGGTAGCTATTCAAAAGACCCT

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	337, 1795	wildtype
1 & 3	416	mutant

Allele Description: Exon 1 to 4 (ENSMUSE00000455801, ENSMUSE00000200783, ENSMUSE00001209591, ENSMUSE00000495051) were constitutively deleted from the 5'UTR through the 3' UTR from the 2200002D01Rik Gene ENSMUSG00000030587 using CRISPR Cas9 gene editing technology in mouse zygotes. Please note that YIF1B 3'UTR was also deleted. The subsequent 1379bp deletion from chr7:28,946,811-28,948,189 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**)**