GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11421 A830018L16Rik 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_A830018L16Rik_comF	GTTTCTAATGGGATTCCTTGAAAGAG
2. CR_A830018L16Rik_wtR*	GGTCAGGTGTCTCAGTTATTAACTT
3. CR_A830018L16Rik_mutR	CCACAGGACTGACCACAAGC

Electrophoresis Protocol:

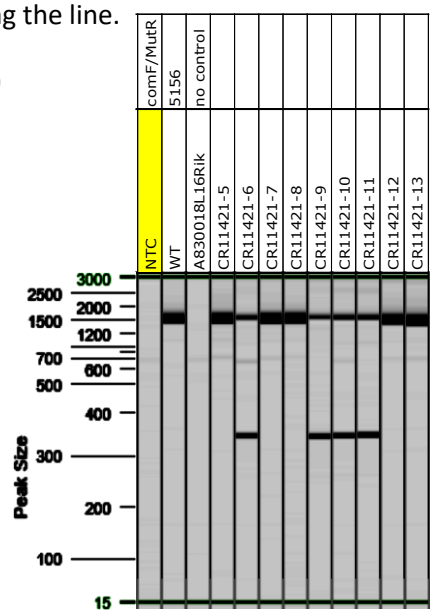
Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	356,1407	wildtype
1 & 3	342	mutant

Allele Description: Exon 2 ENSMUSE00001321665 and flanking splicing regions were constitutively deleted from the A830018L16Rik Gene ENSMUSG00000057715 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1065bp deletion from Chr 1: 11,582,018 – 11,583,082 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)



PCR protocol developed by MMRRC at University of California, Davis