GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11463 3110009E18Rik 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_3110009E18Rik_comF	GTGCTTTAGCTTCACTTTGTCTG
2. CR_3110009E18Rik_wtR*	CCATACTGATGTGGAAGCTATGC
3. CR_3110009E18Rik_mutR	CCTGTAAGGTAAACACTATTGACTACAC

Electrophoresis Protocol:

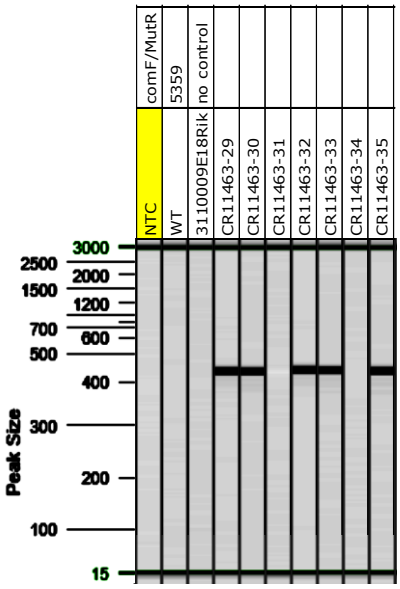
Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	531, 6165	wildtype
1 & 3	430	mutant

Allele Description: Exon 3-4 (ENSMUSE00000158636, ENSMUSE00001208891) and flanking splicing regions were constitutively deleted from the 3110009E18Rik Gene ENSMUSG00000026388 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 5735bp deletion from Chr 1: 120,088,193 – 120,093,927 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