GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11694 1110032F04Rik 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_1110032F04Rik_comF	ACACCTACGCTAGTGAGAGAGC
2. CR_1110032F04Rik_wtR*	AGGTACTTGACCTCCTCGTAGC
3. CR_1110032F04Rik_mutR	CAGGCACTTACAGTGAACACAAGG

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	1092, 1551	wildtype
1 & 3	738	mutant

Allele Description: Exon 1 ENSMUSE00000396332 and flanking splicing regions were constitutively deleted from the 1110032F04Rik Gene ENSMUSG00000046999 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 813bp deletion from Chr 3: 68777009 - 68777821 GRCm39 was sequence verified. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

Note: Non-coding exon 1 ENSMUSE00000898859 of Gm17641 gene is also deleted.

*wtR primer untested (ePCR verified**)**