GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR11696 4930523C07Rik 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: **Electrophoresis Protocol:**

Name	Nucleotide Sequence (5' - 3')
1. CR_4930523C07Rik_comF	GTCTCAGACAAGGGGCTACTATATGG
2. CR_4930523C07Rik_wtR*	GATCGTCAATTATCTAACAAGCCCTCG
3. CR_4930523C07Rik_mutR	TGTAGGAATAGAACACACCCATCTCC

Agar	**1.5%**	**V:**	**90**	
Estimated Running		**90**	min.	
Primer		**Band** (bp)	**Genotype**	
1 & 2,1 & 3		497, 1347	wildtype	
1 & 3		570	mutant	

Allele Description: Exon 3 ENSMUSE00000881091 constitutively deleted from the 5'UTR through the 3'UTR from the 4930523C07Rik Gene ENSMUSG00000090394 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 777bp deletion from Chr 1: 159902396 - 159903172 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