

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11692 Xrra1 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
<b>TOTAL VOLUME OF REACTION:</b>	<b>15.00 µL</b>

### Comments on protocol:

- Protocol may work with other DNA extraction methods.

### Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	2:00	<b>1x</b>
2. Denaturation	94	0:10	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	65 (↓1°C/cycle)	0:30	<b>10x</b>
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing <span style="float: right;">steps 5-6-7 cycle in sequence</span>	55	0:30	<b>25x</b>
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

### Primers:

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. CR_Xrra1_comF	GCCCTCAGTTCTACGCTTCTTCC	Agarose: 1.5%	V: 90	
2. CR_Xrra1_wtR*	CCAATTCAAACAGTCTTCATTCAGC	Estimated Running Time: 90 min.		
3. CR_Xrra1_mutR	GGTCTTTGAAAACCGTAGGGGAAGG	<b>Primer Combination</b>	<b>Band (bp)</b>	<b>Genotype</b>
		1 & 2, 1 & 3	347, 1006	wildtype
		1 & 3	376	mutant

**Allele Description:** Exon 3 ENSMUSE00001492067 and flanking splicing regions were constitutively deleted from the Xrra1 Gene ENSMUSG00000035211 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 630bp deletion from Chr 7: 99516165 - 99516794 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)

