

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11705 Cxcl11 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:

### Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Cxcl11_comF	CACGATTATCACAGCAGACCTGG	Estimated Running Time: 90 min.	
2. CR_Cxcl11_wtR*	CTTGCCATTATCCTACCTCACTCAGC	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Cxcl11_mutR	GTTCTGGCACAGAGTTCTTATTGG	1 & 2, 1 & 3	462, 1370
		1 & 3	471
			mutant

**Allele Description:** Exon 2-3 (ENSMUSE00001253503, ENSMUSE00001225888) and flanking splicing regions were constitutively deleted from the Cxcl11 Gene ENSMUSG00000060183 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 899bp deletion from Chr 5: 92508777 – 92509675 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)

