

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11710 Gm17455 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')	Agarose: 1.5%	V: 90
1. CR_Gm17455_comF	GGGCTTTTAAGTTTACGACTGTCTGG	Estimated Running Time: 90 min.	
2. CR_Gm17455_wtR*	GGCTTCAGTCCAGTCATAGTCACC	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Gm17455_mutR	GTGCCTCAAGTTACACCTCAGAGC	1 & 2, 1 & 3	1013, 1287
		1 & 3	710
			wildtype
			mutant

**Electrophoresis Protocol:**

**Allele Description:** Exon 2 ENSMUSE00001406351 and flanking splicing regions were constitutively deleted from the 5'UTR through the 3'UTR from the Gm17455 Gene ENSMUSG00000090439 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 577bp deletion from Chr 10: 60238645 - 60239221 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.

\*wtR primer untested (ePCR verified)

