

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

Protocol Name: CR10064 Trap1 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-Trap1_comF	TCAAGTTGCCCTTTCCCTATCCAG	Estimated Running Time: 90 min.	
2. CR-Trap1_wtR	GACCAATGATCTTGCTGCTGGTCT	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR-Trap1_mutR	CCACTGAGCTGGGTATGTCCTGC	1 & 2, 1 & 3	322, 979
		1 & 3	302
			wildtype
			mutant

### Electrophoresis Protocol:

**Allele Description:** Exon 6 [ENSMUSE00000127855](#) and flanking splicing regions were constitutively deleted from the Trap1 gene [ENSMUSG0000005981](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

