

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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

530-754-MMRRC

Protocol Name: CR11701 Cd200r2 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_Cd200r2_comF	GAATGATGGAAAGACTGTGGCATTAGC	Estimated Running Time: 90 min.	
2. CR_Cd200r2_wtR*	GGAACCCTGTGAGTTAGGTCTAGC	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Cd200r2_mutR	GGTGTCTTCTCAGTTTTGTGAACC	1 & 2, 1 & 3	509, 1651
		1 & 3	576
			<b>Genotype</b>
			wildtype
			mutant

**Allele Description:** Exon 3-4 (ENSMUSE00000662667, ENSMUSE00000995170) and flanking splicing regions were constitutively deleted from the Cd200r2 Gene ENSMUSG0000090176 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1075bp deletion from Chr 16: 44729196 – 44730270 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)

