

**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER**

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- Open the original graphic in the program that created it
- Choose File, Save As
- Select No Compression in the save options.
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- Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.

These instructions are very generic. The menu options for your graphics program may be different.

Donating Investigator/PI		
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Strain Name		MMRRC Stock Number
Col8a2 L450W		Submission ID: 42276

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NAME OF PCR: Col8a2 L450W Genotyping **MMRRC:** 0-CTR

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/Constituent	Volume (µL)
Water	40.75
10x Buffer	5
MgCl ₂ (stock concentration is mM)	0
Betaine (stock concentration is 5M) <i>Optional</i>	0
dNTPs (stock concentration is 10mM)	1
DMSO <i>Optional</i>	0
Primer 1. (stock concentration is 20µM)	1
Primer 2. (stock concentration is 20µM)	1
Primer 3. (stock concentration is 20µM)	0
Primer 4. (stock concentration is 20µM)	0
Taq Polymerase 5Units/µL	0.25
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1
<i>The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.</i>	TOTAL VOLUME OF REACTION: 50.000 µL

Comments on protocol:

- Mg⁺⁺ is included in 10X PCR Buffer. The final Mg⁺⁺ concentration in PCR Reaction Buffer is 1.5 mM.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	95	3min	1
2. Denaturation	95	15s	
3. Annealing steps 2-3-4 cycle in sequence	60	30s	30x
4. Elongation	68	30s	
5. Amplification	68	5min	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose:	V:
1. Forward	ATTCGAGGAGACCAAGGGCCTAAT	Estimated Running:Time: min.	
2. Reverse	AAGTGAGCACTGCAGTAAAGGCTG	Primer Combination	Band
3.		1+2	269 bp
4.		1+2	348 bp
5.			bp
			Genotype
			homozygous
			WT

Please size gel images to fit in this space

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Protocol / Gel Comments:

Use BsrI restriction enzyme to digest PCR product, the fragment reveals a 348 bp band in WT mice (lanes 1, 2) and a 269 bp band in mice homozygous for the L450W mutation (lanes 3, 4).

Gel pictures:

