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.

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Strain Name		MM	MMRRC Stock Number	
Col8a2 L450W		Sub	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) Optional	0
dNTPs (stock concentration is 10mM)	1
DMSO Optional	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
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.	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?	95	3min	1
2. Denaturation		95	15s	
3. Annealing	steps 2-3-4 cycle in sequence	60	30s	30 x
4. Elongation		68	30s	
5. Amplification		68	5min	1
6. Finish		4	8	n/a

Primers:

Electrophoresis Protocol:

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

Please size gel images to fit in this space

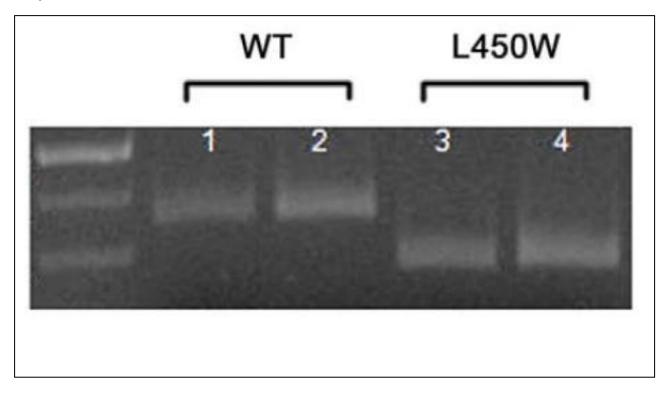
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Protocol / Gel Comments:

Use Bsrl 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:



PCR protocol provided by Donating Investigator