GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

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

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Strain Name B6.Cg-Aldh1a2tm1Jln/Mmucd		MMRRC Stock Number 041423

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NAME OF PCR: **Raldh2 Floxed Genotyping** **MMRRC:** **041423**

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

Reagent/Constituent	Volume (µL)
Water	14.80
10x Buffer	2.00
$MgCl_2$ (stock concentration is 50 mM)	0.60
Betaine (stock concentration is 5M) *Optional*	0
dNTPs (stock concentration is 10mM)	0.40
DMSO *Optional*	0
Primer 1. (stock concentration is 20µM)	0.40
Primer 2. (stock concentration is 20µM)	0.40
Primer 3. (stock concentration is 20µM)	
Primer 4. (stock concentration is 20µM)	
Taq Polymerase 5Units/µL	0.20
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.2
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**20.0 µL**

Comments on protocol:

Cut off ~2mm of tail specimen into 1.5mL capped tube; add 500µL of 0.05M NaOH solution into tube.

- Place on heat block at 95°C for 20min. Add 50µL of 1M TRIS/EDTA solution. Vortex till disintegration.

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	**1**
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65	0:30	**36x**
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	4.0	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Common Rev	TGAGCTGGCTAAAGGCATTTGTAGTC
2. WT Forward	GACGGAGGACAGAGCCAAACTTACTC
3. Flox Forward	GGAATGTGGGACTCTGCCAGAAG
4. Del Forward	CGACTCACTATAGGGCGAATTGGG
5.	
6.	
7.	

Argarose: 2.5% V: 125 volts

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1&2	294	WT
1&3	419	Floxed
1&4	239	Deletion

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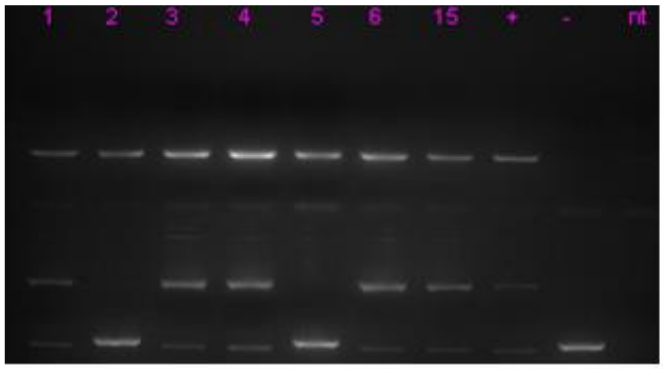

1 and 2

1 and 3

1 and 4

1Protocol / Gel Comments:

Mouse 1 is +/del; Mouse 2 and 5 are +/fl; Mouse 3, 4, 6 and 15 are +/+. + and – are WT and fl/fl controls.

Note: Flox primers don't identify deletion and vice versa.

WT mice using primers 1 and 3 yield a non-specific band.