

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

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Strain Name a4(L9A)-YFP		MMRRC Stock Number 41836

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NAME OF PCR: a4(L9A)-YFP **MMRRC:** 0-CTR

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

Reagent/Constituent	Volume (µL)
Water	21
10x Buffer	2.5
MgCl ₂ (stock concentration is mM)	
Betaine (stock concentration is 5M) <i>Optional</i>	
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	
Primer Mix. (stock concentration is 33µM)	0.75
Primer 2. (stock concentration is 20µM)	
Primer 3. (stock concentration is 20µM)	
Primer 4. (stock concentration is 20µM)	
Taq Polymerase 5Units/µL	0.125
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:	25 µL

Comments on protocol:

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Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input checked="" type="checkbox"/>	94	3 min	1
2. Denaturation	94	1 min	
3. Annealing steps 2-3-4 cycle in sequence	56	1 min	35 x
4. Elongation	72	2 min	
5. Amplification	72	10min	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1%	V: 65
1. <u>A4XFPE</u>	CAACCGCATGGACACAGCAGTCGAGA	Estimated Running:Time: 60 min.	
2. <u>A4XFPM</u>		Primer Combination	Band
3. <u>A4XFP2R</u>		mutant band	1036 bp
4.		mutant band	542 bp
5.		WT band	284 bp

Please size gel images to fit in this space

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Protocol / Gel Comments:

Primers used are:

1) A4XFPE

5'CAACCGCATGGACACAGCAGTCGAGAC 3'

2) A4XFPM

5' GCACAAGCTGGAGTACAACACTACAACAGC 3'

3) A4XFP2R

5'CTCAGTCAGGGAAGCAGCTCCATCTTG3'

Gel pictures:

