

**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.*
- *Save as JPG or PNG or similar format that's compatible with both PC and Mac Word versions.*
- *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	
Y240F-4B2-FVB	43550	

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NAME OF PCR: Y240F

MMRRC: 0-CTR

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

Reagent/Constituent	Volume (µL)
Water	28.5
10x Buffer	5
MgCl ₂ (stock concentration is mM)	
Betaine (stock concentration is 5M) <i>Optional</i>	
dNTPs (stock concentration is 10mM)	1
DMSO <i>Optional</i>	
Primer 1. (stock concentration is 20µM)	5
Primer 2. (stock concentration is 20µM)	5
Primer 3. (stock concentration is 20µM)	
Primer 4. (stock concentration is 20µM)	
Taq Polymerase 5Units/µL	.5
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	5
<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 µL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	3	1
2. Denaturation	94	2	
3. Annealing steps 2-3-4 cycle in sequence	51	:45	40x
4. Elongation	72	1	
5. Amplification	72	5	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.2%	V: 70
1. Exon 7F	CAG ATC CTC AGT TTG TGG TCT	Estimated Running:Time: 120 min.	
2. Exon7R	CAG GTG AGT CTG CTT ACA TG	Primer Combination	Band
3.		Exon7F + Exon7R	350 bp
4.			bp
5.			bp
		Genotype	Sequence

Please size gel images to fit in this space

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Protocol / Gel Comments:

After running the PCR, purify, and send to sequencing with Exon7F and see if wildtype, homo, or het.

Gel pictures:



