

**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	
Y240F-BL6	43549	

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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:



