

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		MMRRC Stock Number
SR3_Twist1		To be assigned

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NAME OF PCR: _____ MMRRC: 0-UCD

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

Reagent/Constituent	Volume (µL)
Water	15.12
10x Buffer (Platinum Taq PCR buffer)	2
MgCl ₂ (stock concentration is 50 mM)	0.6
Betaine (stock concentration is 5M) <i>Optional</i>	
dNTPs (stock concentration is 10mM)	0.4
DMSO <i>Optional</i>	
Primer 1. (stock concentration is 20µM)	0.24
Primer 2. (stock concentration is 20µM)	0.24
Primer 3. (stock concentration is 20µM)	0.16
Primer 4. (stock concentration is 20µM)	0.16
Taq Polymerase 5Units/µL (5U/µl Platinum Taq DNA Polymerase)	0.08
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: 20.000 µL

Comments on protocol:

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Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	1 min	1
2. Denaturation	94	20 sec	
3. Annealing steps 2-3-4 cycle in sequence	58	20 sec	35x
4. Elongation	72	40 sec	
5. Amplification	72	7 min	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. SR89F	CACTTTGCACATACCACCTGT	Estimated Running Time: 90 min.		
2. SR89R	GTGGAGGGCACAGAAACAAA	Primer Combination	Band (bp)	Genotype
3. SR77F	TTACAAGCAGGGTGGAGTC	1 + 2	548 bp	Wild-type
4. SR77R	GGCTCAGGCGTAACTCATGT	3 + 4	425 bp	KO deletion
5.				
6.				
7.				

*Please size gel images and comments
to fit within this space*

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Protocol / Gel Comments:

In a PCR reaction, Primer 1 : Primer 2: Primer 3 : Primer 4 = 3 : 3 : 2 : 2

Gel pictures:

