

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

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)  
530-754-MMRRC

Please provide the following information required for genetic analysis of your mutant mice.

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Strain Name		MMRRC Stock Number
<b>SR14_Tbx3</b>		<b>46271</b>

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NAME OF PCR: \_\_\_\_\_ MMRRC: 0-UCD

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

Reagent/Constituent	Volume (µL)
Water	14.92
10x Buffer (10 x Platinum Taq High Fidelity buffer)	2
MgCl <sub>2</sub> (stock concentration is mM) (50 mM MgSO <sub>4</sub> )	0.8
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)	0.2
Primer 2. (stock concentration is 20µM)	0.2
Primer 3. (stock concentration is 20µM)	0.2
Primer 4. (stock concentration is 20µM)	0.2
Taq Polymerase 5Units/µL (5U/µl Platinum Taq DNA Polymerase High Fidelity)	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>	<b>TOTAL VOLUME OF REACTION: 20.000 µL</b>

Comments on protocol:

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Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	1 min	1
2. Denaturation	94	15 sec	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	58	30 sec	<b>35x</b>
4. Elongation	68	1 min	
5. Amplification	68	7 min	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. SR95F	CACGGATCTCAAATAGCCAGG	Estimated Running Time: 90 min.		
2. SR95R	AAGTGTGAAGGCTGTCTCCA	Primer Combination	Band (bp)	Genotype
3. SR67F	CGTCTCCCTCTCAGCTTCAG	1 + 2	887 bp	Wild-type
4. SR67R	GATATCCCTGGCCTCCATT	3 + 4	700 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 = 1 : 1 : 1 : 1

**Gel pictures:**

