

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
 2795 2nd Street, Suite 400, Davis, CA 95618  
[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)  
 530-754-MMRRC

**NAME OF PCR:** B6;129S6-Gt(ROSA)26Sor<sup>tm2(mCherry)Mgn</sup>/Mmucd **MMRRC #** 036286-UCD

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

Reagent/ Constituent	Volume (µL)
Water	15.8
10x Buffer (contains / without 15mM MgCl <sub>2</sub> )	2.5
dNTPs (stock concentration is 1.25mM)	4
Primer 1 (stock concentration is 20µM)	0.75
Primer 2 (stock concentration is 20µM)	0.75
Taq Polymerase Amplitaq Gold 1000units	0.2
DNA sample extracted <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	1
<b>TOTAL VOLUME OF REACTION:</b>	
	<b>25µL</b>

**Comments on protocol:**

**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	6:00	1
2. Denaturation	94	1:00	} 40
3. Annealing } steps 2-3-4 will cycle in sequence	56	1:00	
4. Elongation	72	1:00	
5. Amplification	72	7	1
6. Finish	4	n/a	n/a

**Primers:**

Name	Nucleotide Sequence (5' - 3')
1: Rosa26.S1	AGACTTATCTACCTCATAGGTG
2: pRosaR1	GGTCTTACAGTAATCTCTG

**Electrophoresis Protocol:**

**Agarose:** 1% **V:** 50ml

**Estimated Running Time:** 20 min.

Primer Combination	Band	Genoty
S1 + R1	537 bp	mut
	503 bp	wt

This photo shows genotyping that includes a second screen. The double banded lanes show the amplicons of interest for MMRRC purposes; primers R26.S1 and pRosaR1.

