

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

**Protocol Name:** FVB/NCrI-*Poln*<sup>em1Rwd</sup>/Mmucd **MMRRC: 043557-UCD**

**Protocol:**

Reagent/Constituent	Volume (μL)
Water	11.275
10x Buffer	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25 μL</b>

**Comments on protocol:**

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non-specific amplifications.

**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	65 to 55 (↓1°C/cycle)	0:30	<b>40x</b>
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

**Primers:**

**Electrophoresis Protocol:**

Name	Nucleotide Sequence (5' - 3')	Agarose: 2%	V: 90
1.43557-F	CCCTGGGAATACTTGGGACT	Estimated Running Time: 90 min.	
2.43557-R	ACTACCAGGCAGGACAGGTG	<b>Primer Combination</b>	<b>Band (bp)</b>
		1 & 2	376
		1 & 2	372
			<b>Genotype</b>
			Wt
			Poln(del4)

**Protocol / Gel Comments:** After the PCR, set up the digestion using NEB.com: buffer3 3 μl, ApaLI 1 μl, PCR product 16μl, dH<sub>2</sub>O 10 μl, for a total of 30 μl. Put the tube on the 75C heating block for 1 to 2 hrs. Spin down and add loading dye. Run 2% agarose gel for 60 min or longer. ApaLI will only digest KI DNA.

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