

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: C57BL/6N-*Ispd*<sup>em2Mbp</sup>/Mmucd MMRRC: 037583-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>25.000 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:**

Name	Nucleotide Sequence (5' - 3')
1. 37583-comF2	CCAGAAGTGGTGATTATCCATGACG
2. 37583-wtR2	GACAGCCCTAAAGAAATGTCTTCAGG
3. 37583-MutR	ATGACAGCCCTAAATAACTTCGTATAG

**Electrophoresis Protocol:**

Argarose: 1.5%    V: 90		
Estimated Running Time: 90 min.		
Primer Combination	Band (bp)	Genotype
1 & 2	217	Wild-type
1 & 3	235	Floxed

