

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Trpm2<sup>tm1Lex</sup>/Mmucd

MMRRC: 032691-UCD

**Protocol:**

Reagent/Constituent	Volume (µL)
Water	10.275
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
Primer 3. (stock concentration is 20µM)	0.5
Primer 4. (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	40x
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: 1.5%	V: 90
1. DNA083-4	CCTGCTCTTGCAAAGGCTGC	Estimated Running: Time: 90 min.	
2. DNA083-10	GCAGAACTGCAACCCATGTG	<b>Primer Combination</b>	<b>Band (bp)</b>
3. DNA083-18	GCTACTGTTATCAGAGAACAG	1 & 2	558
4. IRESUP	GCTAGACTAGTCTAGCTAGAGCGG	3 & 4	480
5. UNQ5070-R	AGCCTGAAGATAGCCACTGAGAATGG	5 & 6	402
6. Neo3a	GCAGCGCATCGCCTTCTATC		<b>Genotype</b>

