

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: C57BL/6N-Sirpa<sup>tm1a(EUCOMM)Hmgu/BcmMmucd</sup>

MMRRC: 037779-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. 37779-lacF	GCTACCATTACCAGTTGGTCTGGTGT
2. 37779-neoF	GGGATCTCATGCTGGAGTTCCTCG
3. 37779-loxF	GAGATGGCGCAACGCAATTAATG
4. 37779-TTR	CGAGGTCTAAATCTTCTCTGCTTCAGG
5. 37779-R	GCCACTAGGAGAAGGTATGAACAAGG
6. 37779-F	CTGTCAATCATCTGAAGCAGGAAGC

### Electrophoresis Protocol:

Argarose: 1.5% V: 90		
Estimated Running Time: 90 min.		
Primer Combination	Band (bp)	Genotype
3 & 5	249	Floxed
2 & 4	612	Pre-Cre
1 & 5	547	Post-Cre
4 & 6	283	Wild-type
4 & 6	485	Post-Flp
5 & 6	469	Post-Flp & Post-Cre

