

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

NAME OF PCR: C57BL/6J-Tlr6^{int}/Mmcd, (insouciant) MMRRC # 010474-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	12.675
10x Buffer (contains 15mM MgCl ₂)	2.5
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 25mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM) 10474 PCR F1	0.5
Primer 2 (stock concentration is 20μM) 10474 PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

- PCR products are verified to contain the correct amplicon size by running ~10μl of the reaction on a gel and the remaining 15μl purified via column based PCR purification method for sequencing.
- 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 MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	} 10x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Denaturation	94	0:15	} 30x
6. Annealing	55	0:30	
7. Elongation		0:40	
8. Amplification	72	5:00	1
9. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 10474 PCR F1	CTT CAG ATT CCC AAT ACC ACC GTT CTC
2: 10474 PCR R1	GCA TCT TGA TGT TCA TCT CAG CAA ACA C
3: 10474_Seq (F)	CCA ATA CCA CCG TTC TCC ATT TG
4: 10474_Seq (R)	ATG TTC ATC TCA GCA AAC ACC GAG

Electrophoresis Protocol:

Agarose: 2% mV: 80 Estimated Running Time: 90 min

Primer Combination	Band	Genotype
1 and 2	436 bp	<i>insouciant</i>

Mutation site (red) and flanking sequence:

WT caagTgttc
insouciant caagCgttc