

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: C57BL/6J-Myd88^{kd}/Mmcd, (*lackadaisical*) **MMRRC #** 010473-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) 10473 PCR F1	0.5
Primer 2 (stock concentration is 20μM) 10473 PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with <input type="checkbox"/> NaOH <input type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> 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? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	} 10x
3. Annealing } steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation	72	0:40	
5. Denaturation	94	0:15	} 30x
6. Annealing } steps 5-6-7 will cycle in sequence	55	0:30	
7. Elongation	72	0:40	
8. Amplification	72	5:00	1
9. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 10473 PCR F1	GCA GTC AGT GCT CTT ACC GGC TGA G
2: 10473 PCR R1	AAT GAG CAG CTT GCC CAA GGT CCC
3: 10473 _Seq (F)	TCT TAC CGG CTG AGC CAT CTC
4: 10473 _Seq (R)	AAG GTC CCA GGT CCA TCC ATC

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	426 bp	<i>lackadaisical</i>

Mutation site (red) and flanking sequence:

WT aaat**A**ctta
lackadaisical aaat**G**ctta