GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: C57BL/6J-*Lck*^{m1Btlr}/Mmucd, ***(iconoclast)*** **MMRRC #** **030958-UCD**

Protocol: ***Genotyping Protocol provided by Donating Investigator***

Reagent/ Constituent	Volume (µL)
Water	37.0
10x Buffer	5.0
dNTPs (stock concentration is 25mM)	2.5
Primer 1 (stock concentration is 20µM) PCR F	1.0
Primer 2 (stock concentration is 20µM) PCR R	1.0
Taq Polymerase	2.5
gDNA template (50-100ng/µl) extracted with ☐ NaOH ☒ Proteinase K ☐ Other: Any	1.0
TOTAL VOLUME OF REACTION:	**50.0µL**

Comments on protocol:

- *Iconoclast* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change.
- lab uses JumpStart®REDTaq®ReadyMix® (P1107- Sigma), 12.5 µl in a 25 µl reaction (includes Taq, buffer, dNTPs).
- Confirm the size and presence of the amplicon on a 1X TAE gel. Purify using spin dialysis or the QIAGEN QIAquick PCR purification kit. Sequence the purified DNA fragments utilizing the Sequencing Primers listed.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	**1**
2. Denaturation	94	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:30	} **29x**
4. Elongation	68	1:00	
5. Amplification	68	7:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Lck_icono_F	ACGATCTAGTCCGCCATTACACCAG
2. Lck_icono_R	AGGAACTGCTCTTCCATCCCCATAG
3. Lck_icono_seqF	CCCTCGGGACTGATTGGAAAG
4. Lck_icono_seqR	TAGCTCAGCGTTTGAGAGCAC
Use primers 1 & 2 for amplification and 3 & 4 for sequencing.	

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	1527 bp	***iconoclast***
SNP found at position ~ of sequencing		

Mutation site (red) and flanking sequence:

WT: acccgcggc**T**agtccggct

iconoclast: acccgcggc**C**agtccggct