

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: STOCK Tg(l12b-cre)1Jlr/Mmcd **MMRRC #** 031698-UCD

DNA Extraction Method: ☐ NaOH ☒ Proteinase K ☐ Other: _____

Protocol:

Reagent/ Constituent	Volume (µL)
DNA Sample	1.0
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 25mM)	0.65
Primer 1 (stock concentration is 20µM) l12bCre-406F	0.65
Primer 2 (stock concentration is 20µM) l12bCre-848R	0.65
Q	1.25
MQ Water	17.3
Taq Polymerase	1.0
TOTAL VOLUME OF REACTION:	25µL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	95	5:00	1
2. Denaturation	95	0:30	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:30	
4. Elongation	72	0:45	
5. Amplification (i.e., 72°C, 10 min)	72	10:00	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: l12bCre-406F	5'- AGC AAA TGC ACA GCA GCG AAG T -3'
2: l12bCre-848R	5'- TTA TTC AAC TTG CAC CAT GCC GCC -3'

Electrophoresis Protocol:

% Agarose: 1 **V:** 120

Estimated Running Time (min): 45

Primer Combination	Band	Genotype
1 and 2	~440 bp	Dlxi12bcre(+/-)

Dlxi12b-Cre PCR

