

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: B6.129(FVB)-Tg(Nefh-cre)1Kul/Mmcd **MMRRC #** 015960-UCD

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

Protocol:

Reagent/ Constituent	Volume (µL)
DNA Sample	1.0
10x Buffer (contains 15mM MgCl ₂)	2.0
dNTPs (stock concentration is 10 mM)	0.5
Primer 1 (stock concentration is 20 µM) Cre Up2	0.5
Primer 2 (stock concentration is 20 µM) Cre Dn2	0.5
Taq Polymerase, 5 Units/µL	0.5
Water	15.2
TOTAL VOLUME OF REACTION:	20µL

Comments on protocol:

Works well on template samples processed with NaOH DNA Extraction protocol.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	1:00	1
2. Denaturation	94	1:00	} 30x
3. Annealing	60	0:30	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	25	hold	n/a

Primers:

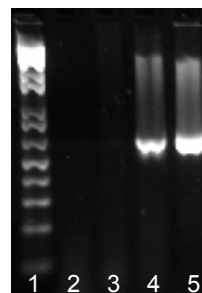
Primer Name	Nucleotide Sequence (5' - 3')
1: Cre Up 2	GAT CTC CGG TAT TGA AAC TCC AGC
2: Cre Dn 2	GCT AAA CAT GCT TCA TCG TCG G

Electrophoresis Protocol:

% Agarose: 2 mV: 80

Estimated Running Time (min): 90

Expected Band	Genotype
No band	wild-type
650 bp	Cre present



Lanes:
 1. 1 Kb+ Ladder
 2. H₂O
 3. B6
 4. Cre positive control
 5. Cre positive sample