

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: STOCK Tg(Nefh-cre)12Kul/Mmucd MMRRC # 000049-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	15.2
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20μM) Cre Up 2	0.5
Primer 2 (stock concentration is 20μM) Cre Dn 2	0.5
Taq Polymerase 5 Units/μL	0.5
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	25μL

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 } steps 2-3-4 will cycle in sequence	60	0:30	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	25	∞	n/a

Primers:

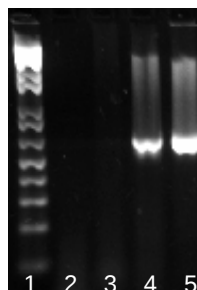
Name	Nucleotide Sequence (5' - 3')
1: Cre Up 2	GAT CTC CGG TAT TGA AAC TCC AGC
2: Cre Dn 2	GCT AAA CAT GCT TCA TCG TCG G

Electrophoresis Protocol:

% Agarose: 2 mV: 80

Estimated Running Time (min): 90

Expected Bands	Genotype
No band	WT +/+
650 bp	Cre +



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