GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: C57BL/6-Tg(Prph1-cre)35Dmd/Mmucd **MMRRC #** 000120-UCD

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

Protocol: (Cre)

Reagent/ Constituent	Volume (µL)
Water	18.1
10x Buffer (contains 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
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 (5Units/µL)	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

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

Strategy:

Steps		Temp (^oC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

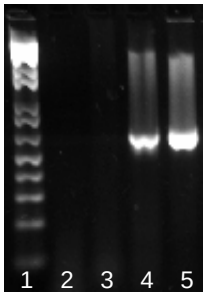
Primer Name	Nucleotide Sequence (5' - 3')
1: Cre Up 2	GAT CTC CGG TAT TGA AAC TCC AGC
2: Cre Dn 2	GCT AAA CAT GCT TCA TCG TCG G

Electrophoresis Protocol:

% Agarose: 2 **mV:** 100

Estimated Running Time (min): 60

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



Lanes:
1. 1 Kb+ Ladder
2. H_2OWater
3. B6
4. Cre positive control
5. Cre positive sample