GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **B6.129-*Hapln*^{1tm1Nid}/Mmucd** **MMRRC #** **015959-UCD**

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

Protocol:

Reagent/ Constituent	Volume (µL)
Water	19.0
10x Buffer (contains 15mM $MgCl_2$)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20µM) Crtl1 Up	0.5
Primer 2 (stock concentration is 20µM) Crtl1 Dn	0.5
Primer 3 (stock concentration is 20µM) Neo TD F	0.5
Primer 4 (stock concentration is 20µM) Neo TD R	0.5
Taq Polymerase 5 Units/µL	0.5
DNA Sample	0.5
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

Note: *Crtl1* is an older synonym of the *Hapln1* gene. Works well on NaOH extracted DNA, but artifacts may be seen from the *Crtl1* amplicon.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	2:30	**1**
2. Denaturation	95	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	62	0:30	} **25x**
4. Elongation	72	0:30	
5. Amplification	72	3:00	**1**
6. Finish	20	∞	n/a

Primers:

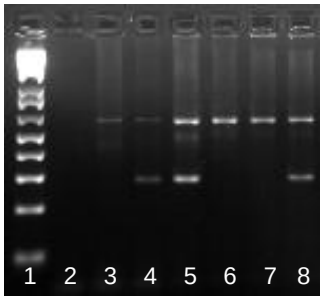
Primer Name	Nucleotide Sequence (5' - 3')
1: Crtl1 Up	GCA CTT TTG TTT TGG CTC CT
2: Crtl1 Dn	CCC CTA TTT TAG AAT GCC TT
3: Neo TD F	CTT GGG TGG AGA GGC TAT TC
4: Neo TD R	AGG TGA GAT GAC AGG AGA TC

Electrophoresis Protocol:

% Agarose: 2 **mV:** 80

Estimated Running Time (min): 90

Expected Bands	Genotype
613 bp	Wild-type (+/+)
280 / 613 bp	Het (+/-)
290 bp	KO Neo (-/-)



Lanes:
1. 1Kb+ ladder
2. H_2O
3. B6
4. *Crtl1* +/-
5. +/- sample
6-7. +/+ samples
8. +/- sample