GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: STOCK Tg(Ure2-cre)1Jlr/Mmcd **MMRRC #** 031699-UCD

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

Protocol:

Reagent/ Constituent	Volume (µL)
DNA Sample	1.0
10x Buffer (contains 15mM $MgCl_2$)	1.5
dNTPs (stock concentration is 25mM)	0.65
Primer 1 (stock concentration is 20µM) Cre484	0.65
Primer 2 (stock concentration is 20µM) Cre834	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	
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:30	} **35x**
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: Cre484	5'- GCA TTT CTG GGG ATT GCT TA -3'
2: Cre834	5'- GTC ATC CTT AGC GCCGTA AA -3'

Electrophoresis Protocol:

% Agarose: 1 **V:** 120

Estimated Running Time (min): 45

Primer Combination	Band	Genotype
1 and 2	~400 bp	Cre (+)

Ure2-Cre PCR