

GENOTYPING BY PCR PROTOCOL
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NAME OF PCR: B6;129P2-Tk1^{tm1Vnd}/Mmucd MMRRC # 000014-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	19.375
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20μM) Agl2F	0.625
Primer 2 (stock concentration is 20μM) Agl2R	0.625
Taq Polymerase 5 Units/μL	0.25
5x Q-Solution	0.125
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	15:00	1
2. Denaturation	94	0:45	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	60	1:00	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish		∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Agl2F	AGG GAG GTG CCT GGC TAA CTG ACC GCA
2: Agl2R	GCG GCA CAC GGA GTG ATA CTT GTC GGC

Electrophoresis Protocol:

% Agarose: _____ V: _____

Estimated Running Time (min): _____

Expected Bands	Genotype
430 bp	KO -/-
530 / 430 bp	Het +/-
530 bp	WT +/+