GENOTYPING BY PCR PROTOCOL FORM

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NAME OF PCR: **B6(D2)-Tg(CSN2-fat-1)1Vane/Mmcd** **MMRRC #** **031129-UCD**

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

Protocol:

Reagent/ Constituent	Volume (µL)
DNA Sample	2.5
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 2.5 mM)	1.25
Primer **1** (stock concentration is 10 µM)	1.25
Primer **2** (stock concentration is 10 µM)	1.25
Primer **3** (stock concentration is µM)	
Primer **4** (stock concentration is µM)	
Taq Polymerase	0.125
Water	16.125
Additives, if applicable:	
TOTAL VOLUME OF REACTION:	**25 µL**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc):

_____

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE ☐	94	5	**1**
2. Denaturation	94	1	
3. Annealing } steps 2-3-4 will cycle in sequence	60	2	} **35x**
4. Elongation	72	2	
5. Amplification (i.e., 72°C, 10 min)	72	8	**1**
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: SalNcoOm3F	GTCGACCATGGTCGCTCATTCCTCAG
2: SalNcoOm3R	GTCGACTTACTTGGCCTTTGCCTTC
3:	
4:	

Electrophoresis Protocol:

% Agarose: _____ **V:** _____

Estimated Running Time (min):

Primer combination	Band (kB)	Genotype
1 and 2	1.221	Fat1 positive