GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: 129S6.Cg-Tg(KRT14-TFRC)#Lmil/Mmucd **MMRRC #** 036745-UCD

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	14.375
10x Buffer	2.5
dNTPs (stock concentration is 10mM)	2.0
Primer 1 (stock concentration is 3µM) TFR F	2.5
Primer 2 (stock concentration is 3µM) TFR R	2.5
Taq Polymerase 5Units/µL	0.125
DNA extracted w/ " ☐ NaOH ☒ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**25.000 µL**

Comments on protocol:

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☒	95	5:00	**1**
2. Denaturation		95	1:00	
3. Annealing	steps 2-3-4 cycle in sequence	55	1:00	**30x**
4. Elongation		72	1:00	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. TFR F	5'-CTG CTA TGG GAC TAT TGC TGT G-3'
2. TFR R	5'-CCG ACA ACT TTC TCT TCA GGT C-3'

Electrophoresis Protocol:

Agarose: 2% **V:** _____

Estimated Running Time: 30 **min**

Expected Band	Genotype
200 bp	Tg
No band	WT