

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: 129-Fgf15^{tm1Sms}/Mmcd **MMRRC #** 032840-UCD

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

Protocol:

Reagent/ Constituent	Volume (µL)
DNA Sample	1.0
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer 1 (stock concentration is 20µM) Neo	0.33
Primer 2 (stock concentration is 20µM) FGF15 F	0.33
Primer 3 (stock concentration is 20µM) FGF15 R	0.33
Taq Polymerase	0.25
Additives / Other, if applicable: Q Reagent (qiagen)	5.0
Water	14.75
TOTAL VOLUME OF REACTION:	25 µL

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

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	} 30x
3. Annealing	60	0:30	
4. Elongation	72	0:45	
5. Amplification (i.e., 72°C, 10 min)	72	10:00	1
6. Finish (i.e., 4°C, indefinite)	16	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Neo	CGC CTT CTT GAC GAG TTC TT
2: FGF15 F	CAA GGG AAT ATG GCT CCA GTC TG
3: FGF15 R	ATA TCC ACG CAG AAG CTG GCA GTG

Electrophoresis Protocol:

% Agarose: 2 **V:** 120

Estimated Running Time (min): 30

Primer Combination	Band	Genotype
1 and 3	370	KO
2 and 3	500	WT
1 and 2 and 3	370, 500	Het

