GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH X Proteinase K _____ Other_____

Protocol: **NAME OF PCR: FgfR1CFP**

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 2.5mM)	2
Primer 1 (stock concentration is 3 uM)	0.44
Primer 2 (stock concentration is 3 uM)	0.87
Primer 3 (stock concentration is 3 uM)	0.44
Taq Polymerase	0.25
Additives if applicable 50% Glycercol	5
TOTAL VOLUME OF REACTION:	25 ul

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	2	1
2. Denaturation } steps 2-3-4 will cycle in sequence	94	0.5	32
3. Annealing	55	0.5	
4. Elongation	72	0.5	
5. Amplification (i.e., 72°C, 10 min)	72	5	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: CL5	5'GAGCCAAGAGGCAGGTG3'
2: CL8	5'GCCTCCCTGTCTTCTGGTA3'
3: CL9	5'CCACTACCTGAGCACCCAGT3'
4:	

Electrophoresis Protocol:

% Agarose: 2 V : 10ul

Estimated Running Time (min): 45

Primer combination	Band (bp)	genotype
1&2&3	300	Wild Type
1&2&3	500	CFP