GENOTYPING BY PCR PROTOCOL FORM

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DNA Extraction Method: NaOH __X__ Proteinase K _____ Other_____

Protocol: **NAME OF PCR:** MMRRC Strain #30919, B6;129X1-*Fgfr2*^{tm1Moon}, (FgfR2YFP)

Reagent/ Constituent	Volume (uL)
DNA Sample	2
10x Buffer (contains 15mM MgCl2)	2.2
dNTPs (stock concentration is 2.5mM)	1.32
Primer **1** (stock concentration is 3 uM)	0.83
Primer **2** (stock concentration is 3 uM)	1.64
Primer **3** (stock concentration is 3 uM)	0.83
Taq Polymerase	0.2
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**22 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	94	0.5	32
3. Annealing } steps 2-3-4 will cycle in sequence	60	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: AM246	**5 ' CTGTCTGCCTCAGTATCCACACATAAA3 '**
2: AM266	**5 ' CTGGTTAGGTAACATGTTTCCAAC3 '**
3: AM270	**5 ' TGTACAGATATCGTCGAGCTC3 '**
4:	

Electrophoresis Protocol:

% Agarose: 2 **V : 10ul**

Estimated Running Time (min): 45

Primer combination	Band (kB)	genotype
1&2&3	238	Wild Type
1&2&3	155	YFP

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES