

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: C57BL/6JApb-Stat1^{fae}/Mmcd (Face) **MMRRC#** 031690-UCD

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

Protocol:

Reagent/ Constituent	Volume (μL)
Water	20.0
10x Buffer (contains 15mM MgCl ₂)	2.0
dNTPs (stock concentration is 25mM)	0.5
Primer 1 (stock concentration is 10μM)	0.5
Primer 2 (stock concentration is 10μM)	0.35
Primer 3 (stock concentration is 10μM)	0.6
Taq Polymerase	0.05
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μl

Comments on protocol:

DNA isolated from ear punch.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START?.. ☐	94	2:00	1
2. Denaturation	94	0:33	
3. Annealing steps 2-3-4 will cycle in sequence	62	0:33	35x
4. Elongation	72	0:30	
5. Amplification	72	0:30	1
6. Finish	25	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Face Fw	TGGACCTCACTGGTTTCCTC
2: Face Rev WT	GGATTCTCTGGTATGTTCTCGGCAGTCA
3: Face Rev Mut	TTGGGGTACAGATACTTCAGGATATTCTCTGGTATGTTCTCGGCATCCT

Electrophoresis Protocol:

% Agarose: 2 V: 350
 Estimated Running Time (min): 20

Expected Bands	Genotype
162 bp	Mut
141 bp	WT
162 / 141 bp	Het