

# GENOTYPING BY PCR PROTOCOL FORM

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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**DNA Extraction Method:** NaOH \_\_\_\_\_ Proteinase K X Other \_\_\_\_\_

**Protocol:** NAME OF PCR: Frem<sup>bat</sup>

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl <sub>2</sub> )	5
dNTPs (stock concentration is 25mM)	0.4
Primer <b>1</b> (stock concentration is 20 uM)	1
Primer <b>2</b> (stock concentration is 20 uM)	1
Primer <b>3</b> (stock concentration is 20 uM)	
Primer <b>4</b> (stock concentration is 20 uM)	
Taq Polymerase	2.5
water	39.1
<b>TOTAL VOLUME OF REACTION:</b>	<b>50 ul</b>

**Comments on protocol** (e.g., different concentration of MgCl<sub>2</sub>, etc): using the same primer set for sequencing.

### Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	94	2	1
2. Denaturation	94	15 seconds	30
3. Annealing } steps 2-3-4 will cycle in sequence	60	15 seconds	
4. Elongation	72	30 seconds	
5. Amplification (i.e., 72°C, 10 min)	72	7	1
6. Finish (i.e., 4°C, indefinite)		n/a	n/a

### Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1:Frem_typeF	AACAAGACAGGCTTCGGCAGAAATC
2:Frem_typeR	AGCACTCACCAAACGGGCTACCTAC
3:	
4:	

### Electrophoresis Protocol:

% Agarose: \_\_\_\_\_ V : \_\_\_\_\_

Estimated Running Time (min): \_\_\_\_\_

Primer combination	Band (bp)	genotype
(i.e. 1&2)	479	
(i.e. 3&4)		
(i.e. 1&2&3)		

### The flanking sequences of mutation sites:

**Wt** ACAACAGG**T**ACTTTCC  
**Bat** ACAACAGG**C**ACTTTCC