

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: Dr. Barbara Kream Address: UCONN Health Center Contact: Nicole Gilbert

Telephone: 860-679-3849 FAX: 860-679-1258

email: bkream@nso1.uchc.edu or ngilbert@neuron.uchc.edu

DNA Extraction Method: NaOH _____ Proteinase K X Other _____

Protocol: NAME OF PCR: CRE

Reagent/ Constituent	Volume (uL)
DNA Sample	5.0
10x Buffer	5.0
dNTPs (stock concentration is 10mM)	1.0
Primer 1 (stock concentration is 5 uM) working stock is 0.5ug/uL	0.5
Primer 2 (stock concentration is 5 uM) working stock is 0.5ug/uL	0.5
50mM MgCl ₂	1.5
H ₂ O	36.25
Taq Polymerase	0.25
Additives if applicable:	
TOTAL VOLUME OF REACTION:	
	50 ul

Comments on protocol (e.g., different concentration of MgCl₂, etc): We usually make the PCR mix then add the TAQ before we add the mix to the PCR tube. We usually add 5uL of our DNA to each PCR tube and 45uL of the PCR mix for a total reaction volume of 50ul per sample.

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE []	94	5:00 min	1
2. Denaturation	94	0:30 sec	
3. Annealing	58.7	0:30 sec	35x
4. Elongation	72	0:30 sec	
5. Amplification (i.e., 72°C, 10 min)	72	2:00 min	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: CRE 1	GATGGATTTCGCTCTCTGGTGTAG
2: CRE 2	AGCTTG CATGATCTCCGGTATTG
3:	
4:	

Electrophoresis Protocol:

% Agarose: 1% V : 150

Estimated Running Time (min): ~45-60 min

Primer combination	Band (kB)	genotype
(i.e. 1&2)	268	TG/+

In the gel picture, # 9 is negative, #10-14 are positive, and #15 is undertermined.

QuickTime™ and a PDF Layer (uncompressed) decompressor are needed to see this picture.

