

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

Investigator/PI: MMRRC at UC Davis Address: _____ Contact: Renee Araiza

Telephone: (530) 754-8686 FAX: (530) 752-2993 email: mmrcc@ucdavis.edu

Protocol:

NAME OF PCR Cre

Reagent/ Constituent	Volume (uL)
Water	15.2
10x Buffer w/ 15 mM MgCl ₂	2
10 mM dNTP mix	0.5
Primer 1: Cre Up2 -20 uM	0.5
Primer 2: Cre Dn2 -20 uM	0.5
Taq Polymerase, 5 Units/uL	0.5
DNA Sample	1
TOTAL VOLUME OF REACTION:	20 ul

Comments on protocol: Works well on template samples processed with NaOH DNA Extraction protocol.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE []	95	1:00	1
2. Denaturation	94	1:00	\
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:30	> 30
4. Elongation	72	1:00	/
5. Amplification (i.e., 72°C, 10 min)	72	5:00	1
6. Finish (i.e., 4°C, indefinite)	25	hold	--

Primers:

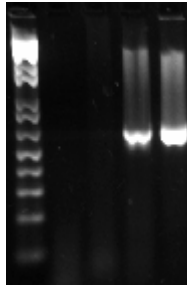
Primer Name	Nucleotide sequence (5' - 3')																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1: Cre Up 2	g	A	T	C	T	C	C	g	g	T	A	T	T	g	A	A	A	C	T	C	C	A	g	C											
2: Cre Dn 2	g	C	T	A	A	A	C	A	T	g	C	T	T	C	A	T	C	g	T	C	g	g													

Electrophoresis Protocol:

% Agarose: 2 mV : 80

Estimated Running Time (min): 90

Number	Band	Genotype
1	No band	wild type
2	650 bp	Cre present
3		



Left to Right:
 1 Kb+ Ladder,
 Water, B6,
 Cre positive control,
 Cre positive sample