

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: Universal Cre MMRRC # -UCD

Protocol: (Cre)

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer (contains / without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20µM) Cre Up 2	0.5
Primer 2 (stock concentration is 20µM) Cre Dn 2	0.5
Taq Polymerase 5Units/µL	0.2
DNA extracted with <input checked="" type="checkbox"/> NaOH <input type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	1.0
TOTAL VOLUME OF REACTION:	25µL

Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	} 10x
3. Annealing } steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation	72	0:40	
5. Denaturation	94	0:15	} 30x
6. Annealing } steps 5-6-7 will cycle in sequence	55	0:30	
7. Elongation	72	0:40	
8. Amplification	72	5:00	1
9. Finish	15	∞	n/a

Primers:

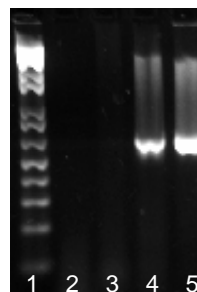
Name	Nucleotide Sequence (5' - 3')
1: Cre Up 2	GAT CTC CGG TAT TGA AAC TCC AGC
2: Cre Dn 2	GCT AAA CAT GCT TCA TCG TCG G

Electrophoresis Protocol:

Agarose: 2% mV: 100

Estimated Running Time: 60 min.

Expected Bands	Genotype
No band	WT -/-
650 bp	Cre present



Lanes:

1. 1 Kb+ Ladder
2. H₂O
3. B6
4. Cre positive control
5. Cre positive sample