

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
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 530-754-MMRRC

**NAME OF PCR:** STOCK Tg(Crh-cre)282Gsat/Mmcd **MMRRC #** 030850-UCD

**Protocol:**

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M)	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	0.325
Primer 1 (stock concentration is 20µM)	0.5
Primer 2 (stock concentration is 20µM)	0.5
Taq Polymerase (5Units/µL)	0.2
DNA extracted with <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	1.0
<b>TOTAL VOLUME OF REACTION:</b>	
	<b>25.00</b>

**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 and protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non specific amplifications.

**Strategy:**

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

**Primers:**

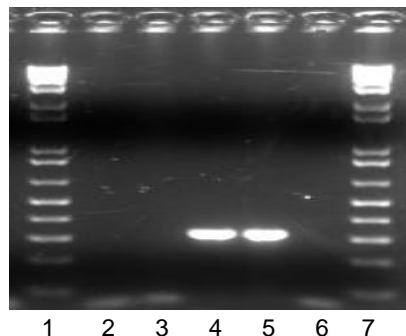
Name	Nucleotide Sequence (5' - 3')
1: Crh F1	CTGTCTTGTCTGTGGGTGTCCGAT
2: CreGS R1	CGGCAAACGGACAGAAGCATT

**Electrophoresis Protocol:**

**Agarose:** 1.5% **V:** 90

**Estimated Running Time:** 90 min.

Primer Combination	Band	Genotype
1 and 2	310 bp	transgenic



Lanes  
 1 & 7. 1 kb+ ladder (Invitrogen, Cat. #10787-026)  
 2. Non-Template Control  
 3. Wild-type & Cre  
 4 & 5. Crh tg/+  
 6. Other GENSAT Line