

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

2795 2nd Street, Suite 400, Davis, CA 95618

mmrrc@ucdavis.edu

530-754-MMRRC

NAME OF PCR: STOCK Tg(Hcrtr1-EGFP)KP68Gsat/Mmcd **MMRRC #** 030803-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
MgCl ₂ (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
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. 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	} 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	Hold	n/a

Primers:

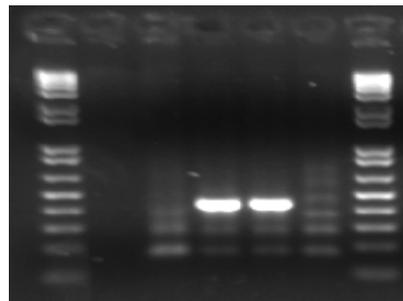
Name	Nucleotide Sequence (5' - 3')
1. Hcrtr1 (30803) F1	CTCCTTTCTTCCCTCCTTCCTTTCTT
2. GFP R2	TAGCGGCTGAAGCACTGCA

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	450 bp	transgenic



Lanes
 1 & 7. 1 kb+ ladder (Invitrogen, Cat. #10787-026)
 2. Non-template control
 3. Wild-type Control
 4 & 5. Hcrtr1 tg/+
 6. Other GENSAT line

1 2 3 4 5 6 7