GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Protocol:

NAME OF PCR: STOCK Tg(Pcdh21-cre)66Gsat/Mmcd MMRRC# 030952-UCD

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
25 mM $MgCl_2$	1.7
5M Betaine	6.5
10 mM dNTPs	0.5
DMSO	0.325
Primer **1:** Pcdh21 F1 –10 µM	0.5
Primer **2:** CreGS R2 –10 µM	0.5
Taq Polymerase-5 Units/µl	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25 µl**

Comments on protocol: Use Touch-Down cycling protocol-first 10 cycles anneal at 60° C decreasing in temperature by 0.5° C; next 10 cycles anneal at 55° C; final 15 cycles anneal at 50° C. *Concentration of $MgCl_2$ may be adjusted to reduce non-specific banding.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	1:00	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94	0:20	\
3. Annealing	60 to 50	0:30	> **x 35**
4. Elongation	72	0:30	/
5. Final Extension	72	5:00	**1**
6. Finish	25	Hold	--

Primers:

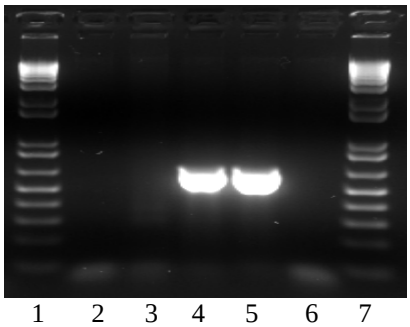
Primer Name	Nucleotide sequence (5'– 3')
1 Pcdh21 F1	**CgCCgCAgTCACCCACAA**
2 CreGS R2	**CCCCAgAAATgCCAgATTACgTAT**

Electrophoresis Protocol:

% Agarose: 1.5 Volts : 90

Estimated Running Time (min): 90

Primer Combinations	Band size (bp)	genotype
1 & 2	550	transgenic



1 2 3 4 5 6 7

Lanes
1 & 7: 1 kb+ ladder (Invitrogen, Cat. #10787-026)
2: H_20
3: Wild Type Control
4 & 5: Pcdh21 tg/+
6: Other gensat line