

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Protocol:

NAME OF PCR STOCK Tg(Gli1-EGFP)197Gsat #10441-UCD

Reagent/ Constituent	Volume (uL)
Water	18.5
10x Buffer	2.5
25 mM MgCl ₂	1.0
10 mM dNTPs	0.5
Primer 1: 10441 F1 -10 uM	0.5
Primer 2: GFP R2 -10 uM	0.5
Taq Polymerase-5 Units/ul	0.5
DNA Sample	1
TOTAL VOLUME OF REACTION:	25 ul

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 MgCl₂ 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	94	0:20	\
3. Annealing } steps 2-3-4 will cycle in sequence	60 to 50	0:30	> x 35
4. Elongation	72	0:30	/
5. Final Extension	72	5:00	1
6. Finish	25	Hold	--

Primers:

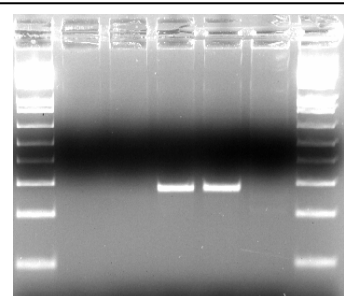
Primer Name	Nucleotide sequence (5' - 3')																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1: 10441 F1	C	C	C	T	C	A	T	C	C	T	T	C	C	C	T	g	A	g	A	C															
2: GFP R2	T	A	g	C	g	g	C	T	g	A	A	g	C	A	C	T	g	C	A																

Electrophoresis Protocol:

% Agarose: 2 Volts : 90

Estimated Running Time (min): 90

Number	Band (bp)	Genotype
1	None	Wild type
2	~300	Transgene present



Lanes
 1 & 7: 1 kb+ ladder (Invitrogen, Cat. #10787-026)
 2: H₂O
 3: Wild Type Control
 4 & 5: Gli1 tg/+
 6: Other gensat line