

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Protocol:

Strain name and stock number: Gensat Stringent PCR Protocol

Reagent/ Constituent	Volume (µL)
H2O	18.0
10x Buffer	2.5
25 mM MgCl ₂	1.5
10 mM dNTPs	0.5
Primer 1 : Gene Specific Fwd Primer (GSP) –10 µM	0.5
Primer 2 : GFP R2 –10 µM	0.5
Taq Polymerase-5 Units/µl	0.5
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25 µl

Comments on protocol: Use Touch-Down cycling protocol-Starting at 65° C decreasing in temperature by 0.5° C each cycle for 24 cycles; next 16 cycles anneal at 55° 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	5:00	1
2. Denaturation	95	0:20	\
3. Annealing	65 to 55	0:30	> x 40
4. Elongation			
5. Final Extension	72	5:00	1
6. Finish	4	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: GSP Fwd																																		
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

Example Gel Photo

1 2 3 4 5 6

1: 1 kb+ ladder (Invitrogen, Cat. #10787-026)
 2: water control
 3: wild type control
 4, 5: Emx1 tg/+
 6: other gensat line