

**GENOTYPING BY PCR PROTOCOL FORM**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**

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**Protocol:**

**NAME OF PCR** STOCK Tg(Syt7-EGFP)197Gsat #30063-UCD

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
25 mM MgCl <sub>2</sub>	1.7
5M Betaine	6.5
10 mM dNTPs	0.5
DMSO	0.325
Primer 1: 30063 F1 –10 µM	0.5
Primer 2: GFP R2 –10 µM	0.5
Taq Polymerase-5 Units/µl	0.2
DNA Sample	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25 µl</b>

**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<sub>2</sub> may be adjusted to reduce non-specific banding. \*\*Betaine and DMSO are absolutely required for this PCR to work.

**Strategy:**

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

**Primers:**

Primer Name	Nucleotide sequence (5'– 3')
1 Syt7 (30063) F1	gCAgCggCggCAgAgAA
2 Gfp R2	TAgCggCTCAAgCACTgCA

**Electrophoresis Protocol:**

% Agarose: 1.5 Volts : 90

Estimated Running Time (min): 90

Primer Combinations	Band size (bp)	genotype
1 & 2	340	transgenic

