

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

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**PCR Protocol: STOCK Tg(Klk8-EGFP)KD59Gsat MMRRC:031174-UCD**

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
25 mM MgCl <sub>2</sub>	1.7
5 M Betaine	6.5
10 mM dNTPs	0.5
DMSO	0.325
Primer 1: (20uM)	0.5
Primer 2: (20uM)	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 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 and protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non specific amplifications.

**Strategy:**

Steps	Temp (°C )	Time (m:ss)	# of Cycles
1. Initiation/Melting	94	5:00	<b>1</b>
2. Denaturation	94	0:15	\
3. Annealing steps 2-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	> <b>x 40</b>
4. Extension	72	0:40	/
5. Final Extension	72	5:00	<b>1</b>
6. Finish	4	Hold	--

**Primers:**

Primer Name	Nucleotide sequence (5'– 3')
<b>1: Klk8 (31174) F</b>	<b>CCCCAGTCCCCAGACAACAAG</b>
<b>2: GS eGFP R3</b>	<b>GGTCGGGGTAGCGGCTGAA</b>

**Electrophoresis Protocol:**

**% Agarose: 1.5 Volts : 90**

**Estimated Running Time (min): 90**

Primer Combinations	Band size (bp)	genotype
1 & 2	350	transgenic
<b>Tg copy # ~ 2 copy/genome</b>		

