

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

**NAME OF PCR:** STOCK Tg(D430039N05Rik-EGFP)HX157Gsat/Mmucd **MMRRC #** 030209-UCD

### Protocol:

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M)	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	0.325
Primer 1 (stock concentration is 20µM)	0.5
Primer 2 (stock concentration is 20µM)	0.5
Taq Polymerase (5Units/µL)	0.2
DNA extracted with <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25µL</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. 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 <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	5:00	1
2. Denaturation	94	0:15	} 40x
3. Annealing } steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation	72	0:40	
5. Amplification	72	5:00	
6. Finish	4	Hold	n/a

### Primers:

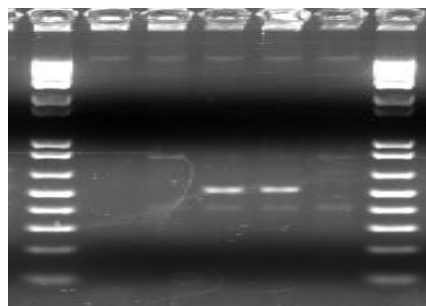
Name	Nucleotide Sequence (5' - 3')
1. D430039N05Rik (30209) F1	CAGCATCACCTTGCAGAGCACTT
2. GFP R2	TAGCGGCTGAAGCACTGCA

### Electrophoresis Protocol:

**Agarose:** 1.5% **V:** 90

**Estimated Running Time:** 90 min.

Primer Combination	Band	Genotype
1 and 2	520 bp	transgenic



**Lanes**  
 1 & 7. 1 kb+ ladder (Invitrogen, Cat. #10787-026)  
 2. Non-template control  
 3. Wild-type Control  
 4 & 5. D430039N05Rik tg/+  
 6. Other GENSAT line

1 2 3 4 5 6 7