

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: STOCK Tg(Fezf1-EGFP)LK300Gsat/Mmucd MMRRC # 031010-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>25.000μL</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 HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	} 40x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Amplification	72	5:00	1
6. Finish	4	Hold	n/a

### Primers:

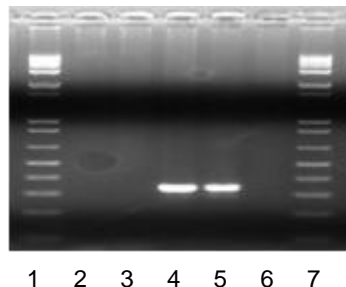
Name	Nucleotide Sequence (5' - 3')
1. Fezf1 (31010) F1	GACAAGCAGGGGACAAGGCAA
2. GS eGFP R3	GGTCGGGGTAGCGGCTGAA

### Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	310 bp	transgenic



Lanes  
 1 & 7: 1 kb+ ladder  
 (Invitrogen, Cat. #10787-026)  
 2: H<sub>2</sub>O  
 3: Wild-type Control  
 4 & 5: Fezf1 tg/+  
 6: Other GENSAT line