

GENOTYPING BY PCR PROTOCOL
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NAME OF PCR: STOCK Tg(Lrrn3-EGFP)DX105Gsat/Mmucd MMRRC # 010593-UCD

Protocol:

Reagent/ Constituent	Volume (μL)	
Water	11.275	
10x Buffer	2.5	
MgCl ₂ (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 ☐ NaOH ☒ Proteinase K ☐ Other:	1.0	
TOTAL VOLUME OF REACTION:		25μl

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 MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	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. Lrrn3 (10593) F2	GTCAGCTCCTACTGAACTTACTGGC
2. GFP R3	GGTCGGGGTAGCGGCTGAA

Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	320 bp	transgenic



Lanes
 1. 1 kb+ ladder
 (Invitrogen, Cat. #10787-026)
 2. Non-template control
 3. Wild-type & eGfp
 4. Lrrn3 tg/+