GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **STOCK Tg(Neto2-EGFP)PS83Gsat/Mmucd** **MMRRC #** **036133-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 $MgCl_2$ 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	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		4	Hold	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Neto2 (36133) F1	GAGACCCATACCATGAGAGAGAGCC
2: GFP R3	GGTCGGGGTAGCGGCTGAA

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90

Estimated Running Time: 90 **min.**

Primer Combination	Band	Genotype
1 and 2	350 bp	transgenic

1 2 3 4 5

Lanes
1. 1 kb+ ladder
(Invitrogen, Cat. #10787-026)
2. Non-template control
3. Wild-type & eGFP
4-5. Neto2 tg/+