

GENOTYPING BY PCR PROTOCOL
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NAME OF PCR: STOCK Tg(Thbs3-cre)SW52Gsat/Mmucd MMRRC # 036633-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 and protocol may be tested without. 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 } 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	1
6. Finish	4	∞	n/a

Primers:

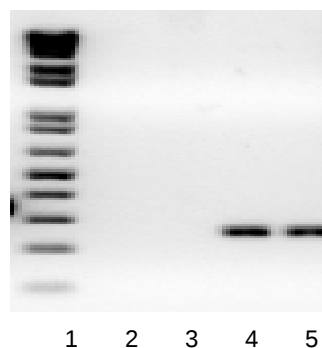
Name	Nucleotide Sequence (5' - 3')
1: Thbs3 (36633) F2	CCTGCTCTCCGCCTAGTCTTTTC
2: CreGS R1	CGGCAAACGGACAGAAGCATT

Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	260	transgenic



Lanes
1. 1 kb+ ladder
(Invitrogen, Cat. #10787-026)
2. Non-template control
3. Wild-type & Cre
4-5. Thbs3