

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: STOCK Tg(Rasgrp1-cre)PO1Gsat/Mmucd MMRRC # 034811-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	11.275
10x Buffer	2.5
MgCl ₂ (25mM)	1.7
Betaine (5M)	6.5
dNTPs (10mM)	0.5
DMSO	0.325
Primer 1 (20μM)	0.5
Primer 2 (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	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

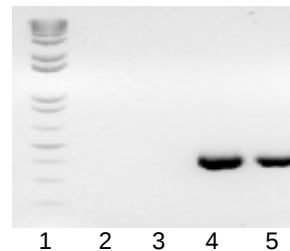
Name	Nucleotide Sequence (5' - 3')
1: Rasgrp1 (34811) F1	CATCAACgCCTgTCAggTggTT
2: CreGS R1	CGGCAAACGGACAGAAGCATT

Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	300 bp	transgenic



Lanes

1: 1kb+ ladder (Invitrogen, Cat. #10787-026)

2: ntc

3: wild-type & Cre

4-5: Rasgrp1+