

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: STOCK Tg(Tmem90a-EGFP)LE39Gsat/Mmcd MMRRC # 033092-UCD

DNA Extraction Method: ☐ NaOH ☒ Proteinase K ☐ Other: _____

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 (5 Units/μL)	0.2
DNA Sample	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	Hold	n/a

Primers:

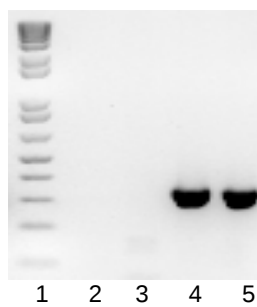
Name	Nucleotide Sequence (5' - 3')
1: Tmem90a (033092) F	CTCCTGACAGGCTCCGTGAAGAC
2: GS eGFP R3	GGTCGGGGTAGCGGCTGAA

Electrophoresis Protocol:

% Agarose: 1.5 V: 90

Estimated Running Time (min): 90

Primer Combination	Band	Genotype
1 and 2	325 bp	transgenic
Tg copy # ~ 8 copies/genome		



Lanes
 1: 1kb+ ladder (Invitrogen, Cat. #10787-026)
 2: ntc
 3: wild-type & eGFP
 4-5: Tmem90a+