

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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 530-754-MMRRC

NAME OF PCR: STOCK Tg(Cklfsf5-EGFP)DL108Gsat/Mmcd MMRRC # 010768-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	Hold	n/a

Primers:

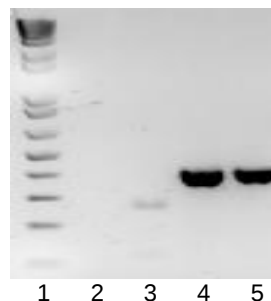
Name	Nucleotide Sequence (5' - 3')
1: Cklfsf5 (10768) F	CTGCCTTTCCCCAGAGTCCTACAG
2: GS eGFP R3	GGTCGGGGTAGCGGCTGAA

Electrophoresis

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	400 bp	transgenic
Tg copy # ~ 1 copy/genome		



Lanes
 1: 1kb+ ladder
 (Invitrogen, Cat. #10787-026)
 2: Non-template control
 3: Wild-type & eGFP
 4-5: Cklfsf5 +