

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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PCR Protocol: STOCK Tg(Vegfa-EGFP)KR374Gsat/Mmcd MMRRC:031739-UCD

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
25 mM MgCl ₂	1.7
5 M Betaine	6.5
10 mM dNTPs	0.5
DMSO	0.325
Primer 1: (20uM)	0.5
Primer 2: (20uM)	0.5
Taq Polymerase-5 Units/µl	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25 µl

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	94	5:00	1
2. Denaturation	94	0:15	\
3. Annealing steps 2-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	> x 40
4. Extension	72	0:40	/
5. Final Extension	72	5:00	1
6. Finish	4	Hold	--

Primers:

Primer Name	Nucleotide sequence (5'– 3')
1: Vegfa (31739) F	GGGAGGAAGAGAAGGAAGAGGAGAG
2: GS eGFP R3	GGTCGGGGTAGCGGCTGAA

Electrophoresis Protocol:

% Agarose: 1.5 Volts : 90

Estimated Running Time (min): 90

Primer Combinations	Band size (bp)	genotype
1 & 2	480	transgenic
Tg copy # ~ 48 copy/genome		

