GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: FVB/NJ-Tg(Chrna9-EGFP)1Jnz/Mmcd **MMRRC #** 010571-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	
10x Buffer (contains / without 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM)	0.5
Primer 2 (stock concentration is 20µM)	0.5
Primer 3 (stock concentration is 20µM)	0.5
Primer 4 (stock concentration is 20µM)	0.5
Taq Polymerase 5Units/µL	0.2
DNA extracted with "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- 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 and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ 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	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**40x**
4. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: BAC-1F	TAACTATGCGGCATCAGAGC
2: BAC-1R	GCCTGCAGGTCGACTCTAGAG
3: EGFP1–1	CCGGGATCACTCTCGGCATGGAC
4: α9B3	GGCTAGTCTAGACTAGGAATACACTGTGCTTTGTTG

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90 **Estimated Running Time:** 90 **min.**

Primer Combinations	Expected Bands	Genotype
1 and 2	330 bp	transgenic
3 and 4	430 bp	