

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6.SJL-Tg(Enam-lacZ)1Jcch/Mmucd MMRRC # 036488-UCD

Protocol: (PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (μL)
Primer 1 (stock concentration is 100μM) 10pM	1
Primer 2 (stock concentration is 100μM) 10pM	1
Primer 3 (stock concentration is 100μM) 10pM	
Primer 4 (stock concentration is 100μM) 10pM	
Supermix from Invitrogen	17
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	1 (50 ng)
TOTAL VOLUME OF REACTION:	20μL

Comments on protocol:

- Run separate reactions for primers 1 and 2, and primer 3 and 4.

Strategy:

Steps for primers 5' and 3'	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5	1
2. Denaturation	58	30s	} 30
3. Annealing } steps 2-3-4 will cycle in sequence	72	1 m	
4. Elongation	94	30s	
5. Amplification	72	1 m	1
6. Finish	4	n/a	n/a

Steps for primers 3 and 4	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5	1
2. Denaturation	60	30s	} 30
3. Annealing } steps 2-3-4 will cycle in sequence	72	1 m	
4. Elongation	94	30s	
5. Amplification	72	1 m	1
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 5' primer	5' AAGTTTTGGGATTTGGCTCA
2: 3' primer	5' GTTGCACCACAGATGAAACG
3: . β-globinF	5'CCAATCTGCTCACACAGGATAGAG AGGGCAGG
4: . β-globinR	5'CCTTGAGGCTGTCCAAG TGATTCAGGCCATCG

Electrophoresis Protocol:

Agarose: 1% 120V:

Estimated Running Time: 35 min

Primer Combination	Band	Genotype
5'+3'	600 bp	+
Primer 3+4	494 bp	+

The gel image shows the anticipated PCR results of DNA sample from Enam5.2kbTg mice. The PCR amplification using the globin primer set was also conducted but not shown.

