

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 <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> 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? <input type="checkbox"/>	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? <input type="checkbox"/>	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' GTTGACCACAGATGAAACG
3: . β-globinF	5'CCAATCTGCTCACACAGGATAGAG AGGGCAGG
4: . β-globinR	5'CCTTGAGGCTGTCCAAG TGATTCAGGCCATCG

Electrophoresis Protocol:

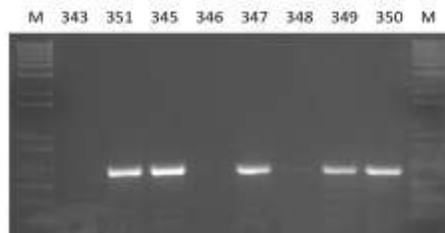
Agarose: 1% 120V:

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.

Enam 5.2kb Tg



Genotyping with primers 5'-AAGTTTTGGGATTTGGCTCA-3' 5'-GTTGACCACAGATGAAACG-3' and 35 cycle reaction.