GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: B6.Cg-Tg(CD68-Tnfrsf13c)MB21Nemz/Mmucd **MMRRC #** 036508-UCD

Protocol: MB21 PCR

(PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (µL)
Water	12.5
10x Buffer*	2.5
dNTPs (stock concentration is 2.5mM)	2.5
Primer 1 (stock concentration is 10µM)	1.0
Primer 2 (stock concentration is 10µM)	1.0
Taq Polymerase	0.5
DNA sample extracted ☒ NaOH ☐ Proteinase K ☐ Other:	5.0
TOTAL VOLUME OF REACTION:	**25.00µL**

Comments on protocol:

Note: DNA is from a crude lysis method involving a 1-2 mm tail sample, protocol below.
*10X PCR buffer: 100mM tris HCl (pH 8.0)
500mM KCl
15mM $MgCl_2$

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	**1**
2. Denaturation	94	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:30	} **32x**
4. Elongation	72	0:40	
5. Amplification	72	7:00	**1**
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. #179 (mb/dbf common)	GAGCAACTGGTGCAGACAG
2. #180 (myc as)	CTTCAGAGATGAGTTTCTGCTC

Electrophoresis Protocol:

Agarose: 1.5% **V:** 115

Estimated Running Time: 60 **min.**

Primer Combination	Expected Bands	Genotype
1 and 2	200 bp	Tg+

DNA ISOLATION FROM MOUSE TAIL SAMPLES

- ADD 100ul Lysis Solution per 1-2 mm tail sample
- Heat at 95°C for 60 minutes
- Cool to 4°C for 5 minutes
- Immediately add 100 ul Neutralization Solution
- Pipette up and down to mix the buffers
- Store at 4°C

- Use 10ul of crude DNA per 30ul PCR reaction

1 Liter of Lysis Solution

- 1 g NaOH pellets
- 400ul of 0.5 M EDTA pH 8.0

1 Liter of Neutralization Solution

- 6.3 g Tris HCl (Tris Hydroxymethyl Aminomethane Hydrochloride)