

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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 530-754-MMRRC

NAME OF PCR: C57Bl/6J-Atp8b1^{tm1Nbf}/Mmcd MMRRC # 036310-UCD

Protocol: (PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (μL)
Water	9.8
10x Buffer (without 15mM MgCl ₂)	2.0
dNTPs (stock concentration is 2mM)	2.0
Primer 1 (stock concentration is 50μM)	0.2
Primer 2 (stock concentration is 50μM)	0.2
Primer 3 (stock concentration is μM)	
Primer 4 (stock concentration is μM)	
Taq Polymerase	0.2
Additives / Other (if applicable):	
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	
TOTAL VOLUME OF REACTION:	
	14.4μL

Comments on protocol:

- 25mM MgCl₂ (1.6), 5X dilution for 50μM stock primer

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	10	1
2. Denaturation	94	30	} 35
3. Annealing	50	30	
4. Elongation	72	1	
5. Amplification	72	10	1
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: MUS-IR-5	GGTAACTAATACACTAGATAC
2: MUS-IF-1	AATACCCCAAGCCTAACG
3: MUS-IR-8	ACATCCATCCCGTTAGGC
4: G923T-WT-F1	CACCGATGTCTGCCATGG
5: G923T-MUT-F1	CACCGATGTCTGCCATGT

Electrophoresis Protocol:

Agarose: 2% V: 100
 Estimated Running Time: 70 min.

Master Mix 1: MUS-IF-1 ~ MUS-IR-5 (should give you product for all three wt, +/- & -/-)

Master Mix 2: G923Twt ~ IR-8 (should give you product if wildtype allele is present (wt or +/-)

Master Mix 3: G923T-MUT-F1 ~ IR-8 (should give you product if mutant allele is present (-/- or +/-)

