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Working

UC Davis - Genotyping

Brandon Willis¹

¹University of California, Davis

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Mouse Metabolic Phenotyping Centers
Tech. support email: info@mmpc.org

Lili Liang

ABSTRACT

Summary:

Standard PCR conditions for genotyping of mutant mouse DNA extracted from somatic (e.g., tail) tissue.

EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=260&docType=Protocol>

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Molecular grade H ₂ O	W4502	Sigma Aldrich		
Betaine 5M	B0300	Sigma Aldrich		
DMSO 99.9%	D8418	Sigma Aldrich		
ATAQ DNA Polymerase kit	N8080172	Life Technologies		
100 MM DNTP set	10297018	Life Technologies		
Associated Oligos (20uM)		Life Technologies		
DNeasy® Tissue Kit	69506	Qiagen		
Agarose	A9414	Sigma Aldrich		
1kb DNA ladder	10787-018	Life Technologies		
SYBR SAFE DNA stain	S33102	Life Technologies		
100% EtOH	DSP-CA-151	Gold Shield Chemicals		

MATERIALS TEXT

Reagent Preparation:

Reagent 1: loading dye (15ml glycerol; 35ml H₂O, 125mM each Bromophenol Blue/Xylene Cyanol).

Note:

Sigma-Aldrich, [RRID:SCR_008988](#)

Life Technologies, [RRID:SCR_008817](#)

QIAGEN, [RRID:SCR_008539](#)

SAFETY WARNINGS

Warning: use appropriate PPE at all times including lab coat, gloves, and eye protection.

1 I. DNA Extraction using DNEasy kit.

Procedure

1. Make a cocktail of ATL with 180uL buffer ATL and 20uL Proteinase K. Add 200uL to each tube. Be sure tail snip is submerged.

2. Incubate at 55°C for 6-12 hours in heat block or water bath.

3. Remove tubes from heat source and vortex.

4. Add 400 ul of AL/Ethanol mixture to each tube. Vortex.

5. Pour liquid into an appropriately labeled Qiagen spin column. Centrifuge at 8,000 rpm for 1 minute. Note AL/Ethanol mixture is 1 part AL buffer to an equal part of Ethanol. Usually made 50 ml at a time, with 25 ml of AL and 25 ml of 200 proof (100%) Ethanol.

6. Transfer spin column to a new collection tube and add 500 ul of AW1. Be sure ethanol (200 proof) has been added to AW1 buffer; if you add ethanol to AW buffer, be sure to mark the bottle. Spin at 8,000 rpm for 1 minute.

7. Transfer spin column to new collection tube. Add 500 ul of AW2. Again, be sure 200 proof ethanol has been added. Spin at max speed (14,000 rpm) for 3 minutes.

8. Carefully remove tubes from centrifuge. Transfer to a 1.5 ml microtube. Check each spin column for Ethanol before placing in the microtube! If ethanol is still on the spin column, pour off the fluid from the collection tube and spin again for 1 minute at 14,000 rpm.

Residual ethanol may inhibit PCR!

9. Add 200 ul of Buffer AE (elution buffer) to spin columns in microcentrifuge tubes. Incubate at room temp for 2 minutes. Spin at 8,000 rpm for 1 minute.

10. Once you have eluted twice (for a total of 200 ul in each tube), label each tube with the sample info that was put on the spin column. Be sure to date the first, last, and approximately every fifth tube for each batch of tails. Store in a cardboard box in the 4oC refrigerator for short term and -20oC for long term.

Warning: use appropriate PPE at all times including lab coat, gloves, and eye protection.

2 II. PCR

1. Prepare the following master mix on ice:

Components	Volume per rxn
Molecular grade H2O	14.38 ul
10 X PCR buffer II	2.0 ul
25mM MgCl	1.36 ul
dNTP (10mM each)	0.4ul
Oligo's (20mM each)	0.5 ul
Amplitaq® (5U/uL)	0.16 ul
total master mix	18.8 ul

2. Briefly vortex master mix and transfer 18.8 uL of to each 200ul thin walled reaction tube on ice.

3. Briefly vortex DNA and input 1.2 uL of ~100 ng DNA into reaction tube.

4. PCR with the following thermal conditions

Temp	Duration	Repetitions
94 °C	5 min	1x
94 °C	15 sec	10X (decrease 1C/cycle)
65 °C	30 sec	
72 °C	40 sec	
94 °C	15 sec	
55 °C	30 sec	30X
72 °C	40 sec	
72 °C	5 min	
15 °C	forever	1x

3 III. Data Analysis.

1. Prepare a 1.2% TBE Agarose gel (~1min per 100ml) with 10ul SyberSafe per 100ml agarose.
2. Line pipette tips with Loading Dye and mix with finished PCR reaction.
3. Inject 75% reaction volume into well (15 ul).
4. Run gel at 120 volts for 1.5 hours in TBE. TBE just covering the gel.
5. Image under UV and adjust and store image for record.
6. Controls: non-template control, isogenic wild type, 1kb plus ladder. (positive control if available).

Warning* where UV protection during imaging.



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