PCR genotyping

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Summary:

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

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Molecular grade H20	Sigma-Aldrich	W4502
*GoTaq® G2 Colorless Master Mix	Promega	M7833
DNeasy® Tissue Kit	Qiagen	69506
Agarose	Sigma-Aldrich	A9414
1kb+ DNA ladder	Life Technologies	10787-018
SYBR SAFE DNA stain	Life Technologies	S33102
100% EtOH	Gold Shield Chemicals	DSP-CA-151

* GoTaq® G2 Colorless Master Mix is a premixed ready-to-use solution containing GoTaq® G2 DNA Polymerase, dNTPs, MgCl2 and reaction buffers at optimal concentrations for efficient amplification of a wide range of DNA templates by PCR. GoTaq® G2 DNA Polymerase exhibits 5′→3′ exonuclease activity.

GoTaq® G2 Colorless Master Mix, 2X: GoTaq® G2 DNA Polymerase is supplied in 2X Colorless GoTaq® G2 Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM MgCl2.

Protocol:

1. DNA Extraction using DNEasy kit. Warning: use appropriate PPE at all times including lab coat, gloves, and eye protection.

- **a.** Make a cocktail of ATL with 180uL buffer ATL and 20uL Proteinase K. Add 200uL to each tube. Be sure tail snip is submerged.
- **b.** Incubate at 55°C for 6-12 hours in heat block or water bath.
- **c.** Remove tubes from heat source and vortex.
- **d.** Add 400 ul of AL/Ethanol mixture to each tube. Vortex.

e. 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.

f. 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.

g. 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.

h. *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!***

i. 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.

j. 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 4^{o}C refrigerator for short term and -20^{o}C for long term.

2. PCR:

a. Prepare the following master mix on ice:

GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	**Volume (µL) per**
Water	5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar	1.5
TOTAL VOLUME	**15.00 µL**

b. Briefly vortex master mix and transfer 13.5 uL of to each 200ul thin-walled reaction tube on ice.

c. Briefly vortex DNA and input 1.5 uL of ~100 ng DNA into reaction tube.

d. PCR with the following thermal conditions

Steps	**Temp (^{o}C)**	**Time**	**# of**
1. Initiation/Melting HOT START?	94	5:00	**1**
2. Denaturation	94	0:15	
3. Annealing (steps 2-3-4 cycle in sequence)	65 to 55	0:30	**40x**
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	15	∞	n/a

3. Data Analysis. Warning* wear UV protection during imaging.

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

Reagent Preparation:

Reagent 1: loading dye (15ml glycerol; 35ml H20, 125mM each Bromophenol Blue/Xylene Cyanol).