PCR-based genotyping protocol for KOMP-Regeneron derived mice & ES cell clones

January 9th 2009

I DNA extraction from mouse tissues (following manufacturers suggestions)

Materials

DNeasy® Tissue Kit 250 (Qiagen 69506)
100% Ethanol (Gold Shield Chemical Company DSP-CA-151)

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

II PCR strategy and reaction

Oligo design

Design a “common” forward direct oligo in the upstream arm of homology ~150bp from non-genomic selection cassette.

Design a reverse compliment oligo in the non-genomic selection cassette ~150bp from upstream junction to arm of homolgy

Design a reverse compliment oligo to the “common” forward direct oligo that will give ~400-500bp amplicon. Design this of published wild type contig and be sure wild type and mutant amplicons will differ in size.

Existing Oligos (clone identification)

5’ Universal LacZ-Rev: GCTGGCTTGGTCTGTCTGTCCTA

3’ Universal Neo-Fwd: GCAGCCTCTGTTCCACATACACTTCA

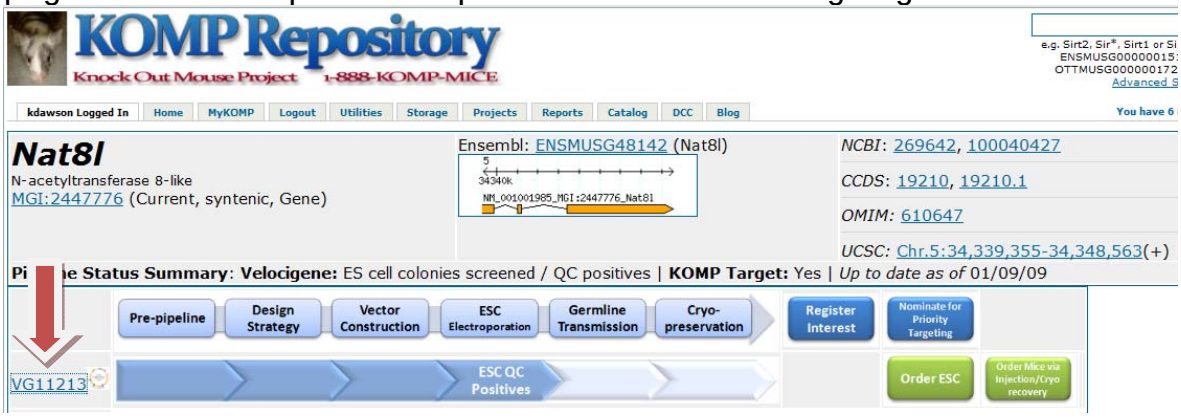
Use the 5’ Universal paired with “SU” gene specific oligo

Use the 3’ Universal paired with “SD” gene specific oligo

Access to vector data

1. From http://www.KOMP.org type official gene symbol into search bar and click on correct gene symbol when list populates.

2. Click on the 7 digit project ID “VGXXXXX” relating to your order. This page will show expected amplicon size from existing oligo PCR.



3. Blast this sequence and align against genomic contig to characterize wildtype sequence that would be used for the wild type oligo design.

Materials and equipment

AmpliTaq® DNA Polymerase w/ 10X Buffer II (ABI N808-0156)
dNTP set (Fisher BP2564-4)
Peltier Tetrad2 thermal cycler (BioRad).
Oligos (Sigma Genosis)

Procedure

1. Prepare the following master mix on ice:

Components	Volume per rxn
Molecular grade H20	18.6 ul
10 X PCR buffer II	2.5 ul
25mM MgCl	1.7 ul
dNTP (10mM each)	0.5 ul
Oligo's (20uM each)	0.5 ul
Amplitaq® Polymerase (5U/uL)	0.2 ul
total master mix	24 ul

2. Briefly vortex master mix and transfer 24uL of to each 200ul thin walled reaction tube on ice.

3. Briefly vortex DNA and input 1uL 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	30X
55 °C	30 sec	
72 °C	40 sec	
72 °C	5 min	1x
15 °C	forever	

III Data Analysis

Materials and Equipment

Loading Dye (15ml glycerol; 35ml H20, 125mM each Bromophenol Blue/Xylene Cyanol).
1 Kb plus DNA Ladder (Invitrogen 10787-026)
GenePure LE Agatose (ISCBioExpress E-3120-500)
Gel Logic Imaging System 100 (Kodak)

Procedure

1. Prepare a 1.5% TBE Agarose gel with 0.25ug Ethidium Bromide per ml agarose (non toxic comparable would be SYBRsafe, Invitrogen S33102).

2. Line pipette tips with Loading Dye and mix with finished PCR reaction.

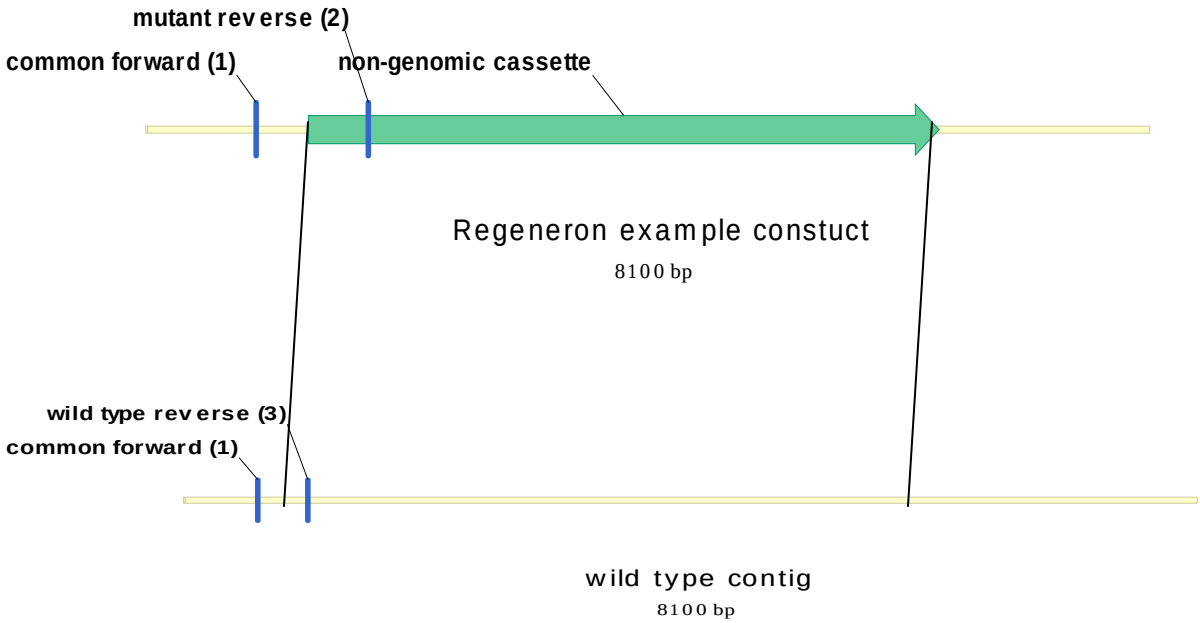
3. Inject 50% reaction volume into well (12.5 ul).

4. Run gel at 120 volts for 1.5 hours in TBE.

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).

PCR Strategy example



Example: primer combinations for Regeneron-targeted gene

test	common forward (primer 1)	mutant reverse (primer 2)	wildtype reverse (primer 3)	expected amplicon size
Wild type	x		x	~300
Clone ID	x	x		~500