Analysis of ES Cell Clones by Mini-Southern

Preparation of Genomic DNA

1. When the cells are ready, wash the plates twice with PBS and aspirate. Using the multichannel pipettor, add 50 µl of Lysis Buffer [10 mM Tris pH 7.5, 10 mM EDTA pH 8.0, 10 mM NaCl, 0.5% Sarcosyl, and 1 mg/ml Proteinase K (added fresh)] per well. *Note: From this point on, all manipulations are to be performed **outside** the tissue culture facilities.*

2. Incubate the plates overnight @ 60^{O} C in a humidified chamber (such as a plastic container with a wet sponge on the inside, or a seal-a-meal bag with a damp paper towel).

3. Next day, prepare a fresh solution of 75 mM NaCl in ethanol (add 150 µl of 5 M NaCl per 10 ml of cold absolute ethanol and mix well; the salt will precipitate, but this is of little consequence).

4. Using the multichannel pipettor, add 100 µl of the NaCl/ethanol solution per well. Allow the plate to rest on the bench @ room temperature for 15-30 minutes, or until the precipitated DNA is clearly visible under low-power magnification. The DNA adheres to the plastic, so look at the perimeter of each well to see the precipitated DNA.

5. Invert the plate to discard the solution (the DNA will remain adhered to the plate). Using the multichannel pipettor, add 70% ethanol to wash each well. Alternatively, a squirt bottle may be used, but a strong stream could detach the DNA from the plate. Invert the plate to discard the 70% ethanol and repeat the wash 2-3 times.

6. After the final wash, invert the plate, discard the 70% ethanol, and allow the plate to air-dry a few minutes. **At this point, the dried plates may be frozen at -80 ^{O} C**.

==========

MOST CUSTOMERS OF THE GENE TARGETING CORE WILL RECEIVE THEIR 3-4 PLATES OF CLONES FROZEN AT THIS STAGE OF THE PROTOCOL. MINI-SOUTHERN OR PCR ANALYSIS WILL BE PERFORMED BY USERS OF THE CORE IN THEIR OWN LABORATORY USING THEIR OWN EQUIPMENT AND REAGENTS.

Mini-Southern Analysis

1. While the plate are thawing, prepare the Restriction Enzyme Cocktail (1X Restriction Buffer specified for the enzyme being used, 1 mM Spermidine, 100 µg/ml Bovine Serum Albumin, and 10-15 units of enzyme).

2. Using the multichannel pipettor, add 30 µl of Restriction Enzyme Cocktail to each well and mix by pipetting up-and-down (especially if the DNA had been frozen down). **Change tips between one row and the next.**

3. Once the cocktail has been added to all the wells, incubate the plates overnight @ **37^{O} C** (assuming that this is the temperature favored by the restriction endonuclease) in a humidified chamber [place a wet paper towel along with the taped plate in a seal-a-meal bag].

4. Next day, prepare the agarose gel(s) for electrophoresis. Prepare a large gel tray (15 cm x 24 cm) with three 33-teeth combs (add 3 extra teeth per comb, secured with tape) evenly distributed along the length of the tray. Pour 300 ml of molten agarose into the tray; remove any bubbles with a needle. Allow the gel to solidify for about 30 minutes. This size of gel (3 lanes with 99 wells total) will

accommodate one 96-well mini-Southern digest plate at 32 samples per lane plus one well per lane for molecular weight markers. Alternatively, the investigator can run the DNA samples on any agarose gel or gels, as long as the comb well size can accommodate the 35 ul volume, without being too wide- as the average yield of DNA is 2-3 ug per sample.

5. Remove the 96-well mini-Southern digest plate from the incubator and add 4-5 µl of loading buffer to each well. Load the gel (30-35 µl per well) and run @ 80 V for approximately 4-6 hours. When deciding how far to allow the samples to migrate, take into account the size of the fragment(s) being distinguished. The gel can be run further as long as the bands from one lane of samples being detected with a probe do not overlap the bands from the next lane of samples.

6. After the electrophoresis is complete, transfer the DNA to GeneScreen *Plus* or Amersham Hybond N+ membranes according to the protocol s provided by the manufacturer, [or refer to the protocol "Genomic DNA: Restriction Enzyme Digests, Agarose Gel Electrophoresis, and Southern Transfer (Blotting)"].

NOTES: *This protocol may not work efficiently for all restriction enzymes. Therefore, make a pilot experiment to test the desired enzyme(s) before proceeding to the large experiment.*

The following is a list of performance of restriction enzymes in mini-Southern DNA digestions. It is culled from many sources- some hearsay.

==========

Performance of Restriction Endonucleases in Mini-Southern Digests

Good	**Poor**
Asp718	BspD1
Avr2	Cla1
BamH1	Hind2
Bgl1	Not1
Bgl2	Sac1
EcoR1	Sal1
EcoRV	Sma1
Hind3	Xba1
Kpn1 (okay)	Xho1
Nco1	Xmn1
Pst1	
Pvu2	
Sca1	
Stu1	
Sst1	

This list has been compiled from several different labs. Therefore, enzymes rated as poor performers may be a reflection of enzyme stability, a paucity of sites in the mouse genome, or simple operator error. We are interested in adding to this list and ask that you let the Core know of any recent experience with different enzymes in mini-Southern analysis.