SOUTHWESTERN
THE UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER
AT DALLAS

TRANSGENIC TECHNOLOGY CENTER

Isolation of ES Cell DNA From One Well of a 6-well Plate
3-5-12

1. You have just been given 6-well plates containing your putative targeted ES cell clones and you will need to confirm the clones, indeed, carry your targeted mutation.

2. If you do not have time to deal with the plates immediately, remove the media, cover the top of the plate with an adhesive foil sheet and place it in a -80^{o}C freezer until you can begin the analysis.

3. If you want to proceed immediately to the analysis, remove the media and wash the well twice with 1ml of PBS minus Ca/Mg.

4. Add .25% trypsin/EDTA and let the plate sit in an incubator at 37^{o}C, 5 min.

5. Transfer the contents of the plate to an eppendorf tube and spin at 2000 rpm for 2 min.

6. Discard the supernatant and resuspend the cells in 2 ml of SNET (SDS, NaCl, EDTA and Tris, pH 8) lysis buffer with proteinase K (125ug/ml). Transfer the contents of the tube to a 14 ml Falcon tube and place the tube in a shaker at 55^{o}C for several hours. When the sample is fully digested it should appear homogenous without any noticeable clumps of cell debris.

7. Perform a phenol/chloroform extraction of the lysis solution, and precipitate the DNA with cold ethanol. The sample can be held in ethanol at -80^{o}C indefinitely. Resuspend the DNA in 300ul of TE. (You can use whatever method you prefer to rid the solution of protein, clean up the prep and precipitate the DNA.) Quantify the DNA concentration using a spectrophotometer, nanodrop or your favorite method.

8. The DNA can be used for restriction digestion with the appropriate enzymes and Southern blotting or for diagnostic PCR.

9. If you elected to store the plate at -80C until you have time, thaw the plate and add 2ml of SNET lysis buffer with PK to the well. Transfer the contents of the well to a 14 ml Falcon tube and place the tube in a shaker at 55^{o}C for several hours.

10. Extract the sample with phenol/chloroform or use you favorite DNA extraction method, ppt the DNA with cold ethanol and resuspend in 300ul TE. Quantify the DNA concentration.

11. Once you have confirmed which clones contain your targeted mutation, please fill out the requisite form detailing which 3 clones should be injected into blastocysts to generate chimeras and send it to Robin Nguyen. Please fill out a People Soft IDR to cover the cost of the injection and send it to Robin Nguyen at the Transgenic Technology Center.