Harvesting and DNA Preparation

Materials & Reagents required:

- 8M Ammonium Acetate Solution
- ES Cell Medium:
  - DMEM (Cat. No. SLM-220-B)
  - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
  - 1% Nucleosides,100x (Cat. No. ES-008-D)
  - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
  - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
  - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
  - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
  - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- Ice cold 70% Ethanol
- 100% Ethanol
- Lysis Buffer
- Microcentrifuge tubes
- Phenol/Chloroform/Isoamyl Alcohol (24:24:1)
- Pipette
- TE buffer

Procedure:

1. Label an appropriate number of microcentrifuge tubes to identify each well (eg. 1.7A1 – electroporation 1, plate 7, well A1).
2. Resuspend the ES cell cultures using a pipette set to 400 µL. Transfer 400 µL of the resultant cell suspension (total volume 500 µL) to each microcentrifuge tube.
3. After all wells have been harvested, add 500 µL of ES cell medium to each well containing ~100 µL of cell suspension, and return to incubator for 3–5 days. Change the cell media as required.
4. Collect the ES cells that have been transferred to the microcentrifuge tubes by centrifugation (30 seconds). Aspirate and discard the media.
5. Add 300 µL of fresh Lysis Buffer to each tube (there is no need to resuspend cells). Incubate at 37 °C overnight (not in a water bath).

6. The next day, add 37.5 µL of 8M Ammonium Acetate to each tube, then 350 µL of Phenol/Chloroform/Isoamyl Alcohol (it is recommended to do this in a fume hood). Mix by inversion approximately 5 times. DO NOT VORTEX! Centrifuge for 5 minutes.
7. Remove the upper aqueous layer, leaving any interface behind. Transfer to 750 µL (3 volumes) of 100% Ethanol and mix well. Often a precipitate is immediately visible.
8. Facilitate DNA precipitation by incubating each tube at -20 °C for 1 hour. Following this period, collect the DNA pellet by centrifugation for 10 minutes. Wash the pellet with 300 µL ice-cold 70% Ethanol and repeat the centrifugation for 5 minutes. Remove the liquid carefully, taking care not to disturb the pellet. Air-dry the pellet.
9. Redissolve the DNA pellet in 100 µL of TE (less if pellet is very small). Allow the pellet to completely dissolve for 2 hours at 65 °C then overnight at 4 °C.
10. Prior to restriction enzyme digestion, heat the DNA solution to 65 °C for 10 minutes. If pipetting is very difficult, pipette the solution straight from the 65 °C block. Depending upon the size of the original DNA pellet, between 10–30 µL of DNA should be used for Southern blot analysis (for Southern blot protocol, please see www.chemicon.com/techsupp/southern.asp).