**Genomic DNA preparation from ES clones**

Many good protocols exist for DNA preparations. This is an excellent, but somewhat

labor-intensive protocol that we routinely use in our lab. 1/2 well of a 6 well plate yields about 100 ug of DNA that "cuts like butter." You are ready to extract the DNA from your ES cells when the cells are nearly confluent in a 6 well plate; media should appear yellow.

1. From a 6-well plate, harvest the clones in each well by trituration and add to numbered eppendorf tubes.
2. Gently centrifuge at 4000 RPM for 5 minutes.
3. Aspirate Trypsin / EDTA being careful to leave the pellet.
4. Add 200 ul 1 X PBS and gently bump each tube with the vortex mixer to disperse the pellet.
5. Add 200 ul 2X Lysis buffer and 5 ul Proteinase K (@20 mg / ml) to each sample. Mix well by rocking tubes back and forth.
6. Incubate at 52oC overnight.
7. Pour supernatant into a phase loc gel tube (5’ Prime Cat#2302800)
8. Add 400 ul phenol (equilibrated with Tris pH 8.0) to each sample. Vortex gently (3 short pulses) and centrifuge at 15,000 RPM for 2 minutes
9. Add 400 ul chloroform / isoamyl alcohol (49:1). Vortex gently (3 short pulses) and centrifuge at 15,000 RPM for 2 minutes.
10. Pour aqueous phase into a new eppie tube.
11. Add 200 ul 7.5 M NH4OAc and precipitate with 600 ul Isopropanol. Invert and rock until phases are well mixed (give it about 15-20 min). Centrifuge at 15,000 RPM for 10 minutes. Gently aspirate supernatant.
12. Wash pellet with cold 70% ethanol. Centrifuge at 15,000 RPM for 5 minutes. Aspirate supernatant.
13. Resuspend pellet in 400 ul TE buffer and 3 ul RNAse A (@10 mg / ml).
14. Incubate => 2 hrs at 37oC.
15. Pour supernatant into a phase loc gel tube (5’ Prime Cat#2302800)
16. Add 400 ul phenol (equilibrated with Tris pH 8.0) to each sample. Vortex gently (3 short pulses) and centrifuge at 15,000 RPM for 2 minutes
17. Add 400 ul chloroform / isoamyl alcohol (49:1). Vortex gently (3 short pulses) and centrifuge at 15,000 RPM for 2 minutes.
18. Pour aqueous phase into a new eppie tube.
19. Precipitate with 40ul 3 M NaOAc pH 5 and 1ml ethanol. Invert and rock gently until phases are well mixed (about 15-20 min). Centrifuge at 15,000 RPM for 10 minutes at 4oC. Aspirate supernatant carefully.
20. Wash pellet with cold 70% ethanol. Centrifuge at 15,000 RPM for 1 minute. Aspirate supernatant.
21. Resuspend pellet to a final concentration of about 500 ng/ul with TE buffer and allow 12 hrs for DNA to resuspend.
22. Use 15 ul of gDNA in a 30 ul Southern digest.

Reagents:

Lysis Buffer (2X) Proteinase K

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4 ml 25% SDS 250 ul 1 M Tris pH 8

8 ml 0.25 M EDTA 5 ul 1 M CaCl2

2 ml 1 M Tris pH 7.5 4.75 ml d H 2 0

86 ml d H 2 0 100mg Proteinase K (FC=20mg/ml)