High MW Genomic DNA Isolation

Lysis Buffer:
100 mM NaCl
10 mM Tris pH 8.0
25 mM EDTA pH 8.0
0.5% SDS

Lyse cells

Tissue: Cut frozen tissues into small pieces with a razor blade, and grind into powder with a mortar and pestle in liquid N_2. Transfer pulverized tissue into microcentrifuge tube. Resuspend in 0.4 ml lysis buffer, supplemented with 0.1 mg/ml proteinase K (optional). Lyse O/N at 55°C.

Cells: Wash cells with PBS. Pellet cells and resuspend in 0.4 ml lysis buffer, supplemented with 0.1 mg/ml proteinase K (optional). Lyse O/N at 55°C.

Phenol-choloroform extraction & Ethanol precipitation

1. Add 0.4 ml Phenol:Chloroform:Isoamyl alcohol and gently rotate tubes 3-4 hours at room temp.

2. Spin max. speed in microcentrifuge for 5 minutes.

3. Remove 0.3 ml of aqueous (top) layer and transfer to new tube.

4. Add 30 μl 3 M NaOAc and 600 μl 100% EtOH, gently mix.

5. Spin max. speed in microcentrifuge for 10 minutes.

6. Remove supernatant and add 1.0 ml 70% EtOH, invert tube several times.

7. Spin max. speed in microcentrifuge for 2 minutes.

8. Remove supernatant, spin max. speed in microcentrifuge for 10 seconds.

9. Pipet off remaining 70% EtOH, and immediately resuspend (DO NOT VORTEX) in 20-200 μl of sterile H_2O or TE or 10 mM Tris pH 7.5-8.0. Place in 37°C incubator or < 65°C heat block, with caps open for 5-30 minutes to evaporate any residual EtOH.

10. Store at 4°C.

Alternative high-throughput DNA isolation

Lysis Buffer:
10 mM Tris pH 7.4
10 mM EDTA pH 8.0
10 mM NaCl
0.5% Sarcosyl
1 mg/ml Proteinase K

Precipitation Solution:
75 mM NaCl in 100% EtOH

1. Obtain cells in a 96 well plate.

2. Aspirate off media, and gently wash with PBS.

3. Add 50 μl lysis buffer to each well.

4. Place the plate in a humidified chamber (tuperware with a wet paper towel in it) to prevent drying out the sample, and incubate at 65°C O/N.

5. Add 100 μl of Preciptation solution per well, precipitate at room temp. for 30 minutes.

6. Prepare restriction enzyme mix.

7. Invert and blot on paper towels to drain supernatant.

8. Carefully pipet 70% EtOH into the wells. Invert and drain.

9. Repeat two more times for a total of three washes.

10. Air dry for 10-15 minutes.

11. Resuspend precipitated DNA in Restriction digest mix. Incubate in a humidified chamber O/N.

Southern Blot

20X SSC:
3 M NaCl
0.3 M $Na_3Citrate•2H_2O$

10X SSPE:
1.5 M NaCl
100 mM NaH2PO4
10mM EDTA
pH 7.4 with 10 M NaOH (approx. 8 ml for 1 L)

FBI Buffer:
10% PEG 8000
7% SDS
1.5X SSPE

Restriction digest and Transfer

1. Prepare 0.5 L of 0.25 M HCl in ddH_2O. Prepare 1 L of 0.4M NaOH in ddH_2O.

2. Prepare 5X SSC in 0.2 M Tris pH 7.4.

3. Prepare 0.6% agarose gel in 0.5X TAE with Ethidium bromide.

4. Digest gDNA with preferred restriction enzyme O/N. If using a 96 well plate, place the plate in a humidified chamber (tuperware with a wet paper towel in it) to prevent drying out the sample. Consider the use of Spermidine (1 mM final concentration) to enhance the efficiency of the restriction digest.

5. Add 6X sample buffer/dyes to digest and load 5-10 μg per well. Load appropriate molecular weight marker. Run at 33 V for 14 hours.

6. Prepare filter paper (Whatman 3MM or similar), cut to approx. 1 mm shorter than the gel in each dimension. A stack of at least ½ inch will suffice. Cut the nylon membrane to the same dimensions as the filter paper.

7. Photograph gel using a UV fluorescent ruler with the “0” placed at the bottom of the well.

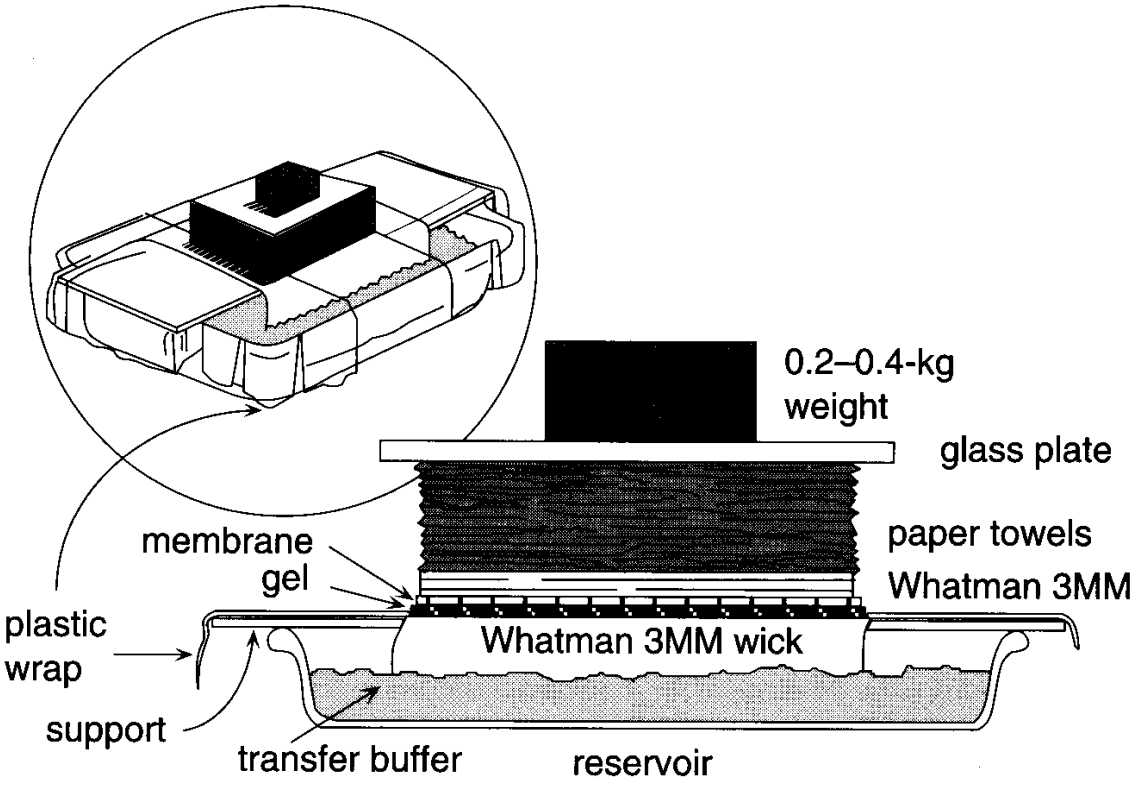
8. Rinse gel in ddH_2O.

9. Soak gel in 0.25 M HCl for 15 minutes.

10. Rinse gel in ddH_2O.

11. Soak gel in 0.4 M NaOH for 5 minutes.

12. Assemble capillary transfer apparatus:



taken from *Current Protocols in Molecular Biology*

Transfer buffer = 0.4 M NaOH.

13. Allow transfer to proceed for 3 hours.

14. Before separating gel and nylon membrane, mark wells with pencil.

15. Remove marked membrane and neutralize in 5X SSC in 0.2 M Tris pH 7.4 for 5 minutes.

16. UV crosslink membrane. Store dry at room temperature.

Hybridization

1. Pre-hyb. Membrane in FBI buffer (10-20 ml) supplemented with 0.1 mg/ml sonicated salmon sperm DNA. Rotate at 65°C for at least 1 hour.

2. Label probe using a commercial kit. Denature labeled probe at 100°C for 5 minutes. Pipet probe directly into Pre-hyb., avoid getting any of the probe on the membrane before it is diluted in the Pre-hyb.

3. Hybridize O/N at 65°C.

4. Wash with a large volume of 2X SSC + 0.1% SDS (fill hyb. bottle halfway) at 65°C for 30 minutes.

5. Repeat wash.

6. Wrap membrane in plastic wrap and obtain exposure.

7. If background is too great, the blot may be washed additionally with decreasing salt concentrations (e.g. 0.5X SSC + 0.1% SDS, 0.1X SSC + 0.1% SDS etc.).