Alkaline Lysis of Mammalian Cells for Southern Blotting

Protocol adapted from JinDong Wang's protocol

1. Resuspend cells in 3 ml of 50 mM glucose, 25 mM Tris (pH 8.0), 10 mM EDTA (This is MaxiPrep Solution #1) per $1x10^7$ cells.
2. Spike in 10ng pPUR plasmid as a recovery control and a DpnI digestion-to-completion control.
3. Lyse cells in 3 ml 200 mM NaOH, 1% SDS (This is MaxiPrep Solution #2) per $1x10^7$ cells for 5 min on ice.
4. Neutralize cell lysate in 3 ml ice-cold 5 M KOAc + glacial acetic acid (This is MaxiPrep Solution #3) per $1x10^7$ cells for 10 min on ice.
5. Spin lysates at 15k rpm, 4C, 30min in a 50Ti rotor in the ultracentrifuge.
6. Transfer the supernatant to a fresh conical tube
7. Add an equal volume of 1:1 phenol:chloroform, vortex 30-45sec, spin at table top centrifuge at 3k rpm for 10 min, allow for phase separation. Transfer aqueous phase to fresh conical tube.
8. Add an equal volume of chloroform, vortex 30-45sec, spin at table top centrifuge at 3k rpm for 10 min, allow for phase separation. Transfer aqueous phase to fresh conical tube.
9. Add 1ul 20mg/ml glycogen and an equal volume of isopropanol. Incubate at -80C for >15min.
10. Spin precipitated DNA at 30k rpm, 4C, 20-30min in
11. Resuspend DNA pellet in 200ul 1xTE. Add RNase A to 100ug/ml final concentration. Incubate at 37C for >60min.
12. Add Proteinase K to 200-500ug/ml final concentration and SDS to 0.5% final. Incubate at 37C for 3hrs to o/n.
13. Add an equal volume of 1:1 phenol:chloroform, vortex 30-45sec, spin in mirocentrifuge at 15k for 5 min. Transfer aqueous phase to fresh micro tubes.
14. Add an equal volume of chloroform, vortex 30-45sec, allow for phase separation. Transfer aqueous phase to fresh conical tube.
15. Add 1ul 20mg/ml glycogen and an equal volume of isopropanol. Incubate at -80C for >15min.
16. Digest 1-1.5x10^7 cell equivalents for each Southern blot to be done as follows:
    10% of total: only linearize with appropriate RE (p3035: BssHII)
    90% of total: linearize with same RE and digest with DpnI*
    Incubate at 37C o/n

(*To monitor the completion of DpnI digestion, set up the digestion in 100~200 µl in total volume, after enzyme is added, take out 10 µl of the total reaction, mix with 1 µ (1µg) undigested plasmid (for example p3035), let the reaction go as long as the testing reaction goes. Run the gel to test if the control plasmid is digested completely before doing step 17.

17. Ethanol precipitate DNA and resuspend DNA (1 x 10^7 cells equivalent) in <20 µl TE. If it will be used in more than two times gel running, resuspend in ~20 µl TE.