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DNA Preparation from Lymphoblastoid Cells

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Reagents

Chloroform
Mallinckrodt, Cat. 4440
Ethanol, absolute
Isoamyl Alcohol
Sigma, Cat. I-3643
Phenol buffer
Gibco BRL, Cat. 15513-039
Phosphate Buffered Saline (PBS), 1X
Gibco BRL, Cat. 10010-023
Proteinase K
Gibco BRL, Cat. 24568-2
Sodium acetate, 3 M, pH 5.2
Sodium dodecyl sulfate (SDS), 10%
TAE buffer
Bio Whittaker, Cat. 16-011V
Tris HCl, pH 8.0
Trypsin
Gibco BRL, Cat. 25200-056
Distilled Water
Gibco BRL, Cat. 15230-170

Preparation

DNA buffer

1M Tris HCl, pH 8.0	100 ml
0.5 M EDTA	100 ml
dH_2O water	300 ml

Chloroform/Isoamylacohol 24:1

Chloroform	24 ml
Isoamylacohol	1 ml

Procedure

1. Tap cell culture flask by hand and transfer contents to 15 or 50 ml centrifuge tube. Centrifuge cultured cells for 10 min at 1200 rpm. Wash cell pellet twice with 10 ml 1X PBS, centrifuging between washes.

2. Remove supernatant, wash cells twice with 10 ml DNA buffer and re-suspend in 10 ml DNA buffer; incubate for 10 min on ice.

3. Centrifuge for 10 min at 1200 rpm and remove supernatant. Add 3 ml DNA buffer; re-suspend the pellet. Add 100 µl Proteinase K (10 mg/ml) and 400 µl of 10% SDS. Shake gently and incubate in 45˚C waterbath overnight.

4. Add 3.6 ml of phenol. Shake by hand for 10 min (RT); centrifuge for 10 min at 10°C at 3000 rpm.

5. Transfer supernatant into a new 50 ml tube. Add 1.8 ml phenol and 1.8 ml chloroform/isoamylalcohol (24:1), shake by hand for 10 min (RT); centrifuge for 5 min at 10°C (3000 rpm).

6. Transfer supernatant into a new 50 ml tube. Add 3.6 ml chloroform/isoamylalcohol and shake by hand for 10 min (RT); centrifuge for 5 min at 10°C (3000 rpm).

7. Transfer supernatant into a new 50 tube. Add 36 µl (1/10) 3 M sodium acetate (pH 5.2), mix gently, and add 3 volumes of 100% ethanol; shake gently until the DNA has precipitated.

8. Use a sterile glass pipette to transfer the precipitated DNA into 30 ml of 70% tube. Place on inverting rack and invert for 2 hr to thoroughly rinse. Transfer DNA into a sterile eppendorf tube.

9. Centrifuge for 20 min at 14,000 rpm. Dry pellet in a speed vac for 5 min. Dissolve the DNA in 300 µl sterile water and place on a rotating shaker overnight at 4°C.

10. Measure the DNA concentration and run 1-5 µl (approximately 200 ng) for gel electrophoresis on agarose gel (1%) in 1X TAE buffer.