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DNA Preparation from Adherent Cells

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Reagents

Chloroform
Mallinckrodt, cat. 4440
EDTA, 0.5 M
Ethanol, 100%
Ethanol, 70%
Isoamyl Alcohol
Sigma, cat. I-3643
Phenol
Gibco BRL, Cat. 15513-039
Phosphate Buffered Saline (PBS), 10X and 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%
Tris-HCl, 1 M, pH 8.0
TAE buffer (Tris acetate/disodium EDTA), 1X
Bio Whittaker, Cat. 16-011V
Trypsin
Gibco BRL, Cat. 25200-056
Distilled Water
Gibco BRL, Cat. 15230-170

Preparation

DNA buffer

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

Chloroform/Isoamyl alcohol 24:1

Chloroform	24 ml
Isoamyl alcohol	1 ml

Procedure

1. Use trypsin or cell scraper to remove cells from tissue culture flask (T-75). Centrifuge cultured cells for 10 min at 10°C (1200 rpm). Remove supernatant and re-suspend cell pellet in 1X PBS and wash twice with 10 ml 1X PBS, centrifuging between washes.

2. Resuspend pellet in 10 ml DNA buffer. Centrifuge cells for 10 min at 10 °C (1200 rpm). Remove supernatant.

3. Add 3 ml DNA-buffer, re-suspend the pellet, add 125 ml Proteinase K (10 mg/ml) and 400 ml 10% SDS; shake gently and incubate overnight at 45°C.

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

5. Transfer the supernatant into a new tube (15 ml); measure the volume. Add 1.8 ml phenol and 1.8 ml chloroform/isoamylalcohol (24:1) or a total amount equal to the volume of the supernatant. Shake by hand for 10 min (RT); centrifuge for 10 min at 10°C (3000 rpm).

6. Transfer the supernatant into a new tube (15 ml); measure the volume. Add 3.6 ml chloroform/isoamylalcohol (24:1) or an amount equal to the volume of the supernatant. Shake by hand for10 min (RT); centrifuge for 10 min at 10°C (3000 rpm).

7. Transfer the supernatant into new tube, measure the volume. Add 1/10 volume 3 M sodium acetate (pH 5.2) and 3 x the volume 100% isopropanol (2-propanol); shake gently until the DNA is precipitated.

8. Use a sterile glass pipette to transfer the precipitated DNA into a tube with 30 ml of 70% ethanol 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 SpeedVac for 5 min. Dissolve the DNA in 300-500 μl sterile water and place in an eppendorf thermomixer shaker overnight at 37°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.