2004

DNA Preparation from Cell Lines, High Salt Method

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

EDTA
Ethanol, absolute
Isopropanol
Phosphate Buffered Saline (PBS), 1X
Proteinase K
EM Science, Cat. 24568-2
Sodium Acetate, 3 M pH 5.2 (Molecular Biology Grade)
Quality Biological, Inc., Cat. 351-035-060
Sodium chloride (NaCl)
Mallinckrodt, Cat. 7581
Sodium Dodecyl Sulfate (SDS), 10%
Digent Diagnostics, Inc., Cat. 3400-1016
Tris EDTA (TE), pH 7.4
Quality Biological, Inc., Cat. 351-010-130
Tris EDTA (TE), pH 8.0
Quality Biological, Inc., Cat. 351-011-131
Tris HCl, pH 8.0
Quality Biological, Inc, Cat. 351-007-100

Preparation

Proteinase K solution

Proteinase K	100 mg
Tris EDTA, pH 7.4	10 ml

Nuclei lysis buffer

Tris EDTA, pH 8.0	1 ml
NaCl, 5 M	8 ml
EDTA, 0.5 M	0.4 ml
Add dH_2O to	100 ml

6 M Sodium chloride

Sodium chloride	3.5 g
dH_2O	10 ml

Procedure

A. Cell Collection and Lysis

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 and resuspend cell pellet twice with 10 ml 1X PBS, centrifuging between washes.

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

3. Add 3 ml nuclei lysis buffer, resuspend the pellet, add 100 μl Proteinase K (10 mg/ml) and add 400 μl of 10% SDS, shake gently, and incubate at 45°C overnight.

B. Precipitation in High Salt Concentration

1. To lysate, add 1 ml of 6 M NaCl**.**

2. Shake tubes vigorously by hand for 15 sec.

3. Centifuge at 3000 rpm for 15 min.

4. Transfer supernatant into a new tube and centrifuge at 3000 rpm for 15 min.

5. Repeat steps 3 and 4 until tube is clear of salt (at least 3-4 times).

C. Precipitation with Ethanol

1. Transfer supernatant into a new tube; measure the volume of the supernatant.

2. Add 1/10 the total volume 3 M sodium acetate (pH 5.2) and 2.5-3 times total volume cold 100% isopropanol; shake gently until the DNA is precipitated.

3. Using the hook, transfer the DNA into a new tube containing 13 ml of 70% ethanol.

4. Place on inverting rack and invert for 2 hr to thoroughly rinse.

5. Transfer DNA into new Eppendorf tube (1.5 ml) and centrifuge for 30 min at 14,000 rpm.

6. Dry pellet by inverting on paper, and speed vac for 5 min.
7. Add 200 µl dH20 and resuspend at 37˚C overnight in Thermomixer.
8. Measure the DNA concentration and run 1-5 µl (approximately 200 ng) for gel electrophoresis on agarose gel (1%) in 1X TAE buffer. Also, measure the DNA with NanoDrop and print out results for future reference.