PREPARATION OF HIGH MOLECULAR WEIGHT DNA FROM CULTURED CELLS

This is a general method for preparing high molecular weight DNA from cultured cells.

A. Solutions and Reagents:

-20x PBS: 160 g NaCl
4 g KCl
25 g Na_2HPO_4
4 g KH_2PO_4
dH_2O to 1 liter

-RNase stock: Dissolve pancreatic ribonuclease at 1 mg/ml in 0.02 M sodium acetate pH 5.2. Place 5 min in a boiling water bath and store frozen at - 20°C divided into aliquots.

B. Procedure:

1. Confluent, healthy cells are washed once on the dish with 1x PBS. One confluent 10 cm plate contains 10^7 cells.

2. Cells are scraped into 1x PBS and pelleted by centrifugation at 2000 RPM for 5 minutes.

3. Decant PBS, resuspend pellet in 10 mM Tris, 10 mM EDTA, 100 µg/ml proteinase K (1.0ml/10^7 cells). After re suspension add SDS to 0.5% as quickly as possible. A very viscous solution should form. (In some cases, if cells have high levels of DNase, heat solution to 70°C for 15 minutes. Then add more proteinase K to 100 µg/ml). Let incubate for 6-12 hours at 37°C.

4. Add NaCl to 150 mM, and gently extract twice with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and twice with chloroform/ isoamyl alcohol (24:1).

5. Add twice the volume of room temperature EtOH to the aqueous phase, mix gently by inversion. A thick fibrous clot will form. Pluck clot and transfer into 70% EtOH/water and then into 100% EtOH. Spin out DNA at 4000 rpm for 10 minutes, pour off EtOH, invert tube and allow pellet to dry.

6. Redissolve pellet with 10 mM Tris, 10 mM EDTA (0.5-1.0ml/10^7cells). If DNA does no immediately dissolve, incubate at 37°C with gentle rocking until it does. (This may take as long as two days).

7. RNase with heat treated RNase A at 20 ug/ml and RNase Tl at 1 unit/ml for 2-4 hours at 37°C. Bring to 0.2 M NaCl and 0.1% SDS.

8. Add proteinase K, 100 µg/ml and incubate for additional 2 hours.

9. Extract once with phenol/chloroform/isoamyl alcohol and once with chloroform/isoamyl alcohol.

10. EtOH precipitate as before (step 5), taking sterile precautions after 100% EtOH wash. Resuspend DNA as before in sterile 1 mM Tris 1 mM EDTA, 1.0 ml/3 x 10^7 cells. Solution should be viscous, with a concentration of about 1 mg/ml.

C. Comments:

1. Do not dry the DNA pellet too much in step 5, as it will be difficult to resuspend if you do.

2. The first six steps of purification are enough for most purposes (e.g., the preparation of carrier DNA for transformation, restriction analysis of the DNA). Nevertheless, at this point the DNA is contaminated with some RNA, and the subsequent steps are necessary to make an accurate measurement of the DNA concentration.

3. RNase T1 in step 7 is not generally necessary, but be aware that RNase A does not cut poly A tracks.

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