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DNA Preparation from Fresh/Frozen Tissue

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National Institutes of Health

Reagents

Chloroform
EDTA, 0.5 M
Ethanol, absolute
Isoamyl alcohol
Sigma, Cat. I-3643
Phenol
Phosphate Buffered Saline (PBS), 1X
Proteinase K
EM Science, Gibbstown, WV Cat. 24568-2 (100 mg)
RNase A
Boehringer Mannheim, Cat. 109 169
Sodium dodecyl sulfate (SDS) solution, 10%
Digene Diagnostics, Beltsville, MD, Cat. 3400-1016

Preparation

DNA buffer (Tris-EDTA)

1 M Tris pH 8.0	20 ml
0.5 M EDTA	20 ml
Sterile water	100 ml

Proteinase K (10mg/ml)
Dissolve 100 mg Proteinase K in 10 ml TE for 30 min at room temperature (RT)
Aliquot and store at –20˚C

RNase A (20 mg/ml)
Dissolve 200 mg RNase A in 10 ml sterile water, boil for 15 min, and cool to RT.
Aliquot and store at –20˚C

Procedure

1. Put 60-80 mg of tissue in a petri dish with culture media and divide the tissue into two pieces.

2. Put the tissue into two sterile 15 ml tubes and centrifuge for 2 min at 4°C at 1500 rpm.

3. Remove the supernatant, and wash twice with 1 ml 1X PBS or DNA-buffer. (It is possible to store the pellet at -80°C; in that case, add 1 ml 1X PBS and resuspend the pellet. Use a cryo-tube and centrifuge at 1500 rpm for 2 min at 4°C. Remove the supernatant, and freeze the pellet.)

4. Remove supernatant and resuspend the pellet in 2.06 ml DNA-buffer.

5. Add 100 µl proteinase K (10 mg/ml) and 240 µl 10% SDS, shake gently, and incubate overnight at 45°C in a waterbath.

6. If there are still some tissue pieces visible, add proteinase K again, shake gently, and incubate for another 5 hr at 45˚C.

7. Add 2.4 ml of phenol, shake by hand for 5-10 min, and centrifuge at 3000 rpm for 5 min at 10°C.

8. Pipette the supernatant into a new tube, add 1.2 ml phenol, and 1.2 ml chloroform/isoamyl alcohol (24:1); shake by hand for 5-10 min, and centrifuge at 3000 rpm for 5 min at 10°C.

9. Pipette the supernatant into a new tube, add 2.4 ml chloroform/isoamyl alcohol (24:1), shake by hand for 5-10 min, and centrifuge at 3000 rpm for 5 min at 10°C.

10. Pipette the supernatant into a new tube, add 25 µl 3 M sodium acetate (pH 5.2) and 5 ml ethanol, shake gently until the DNA precipitates.

11. Take a glass pipette, heat it over a gas burner, and bend the end to a hook. Fish the DNA thread out of the solution using the hook and transfer DNA to a new tube.

12. Wash the DNA in 70% ethanol and dry it in the speed vac.

13. Dissolve the DNA in 0.5-1 ml sterile water overnight (or longer if necessary) at 4°C on a rotating shaker.

14. Measure the DNA concentration in a spectrophotometer and run 200 ng on a 1% agarose gel.

Tissue (mg)	5	10	15	20	40	60	80	100
Volume in µl								
Total	400	800	1200	1800	3200	4800	6400	8000
DNA buffer	360	680	1020	1360	2720	4080	5440	6800
Proteinase	20	40	60	80	160	240	320	400
10% SDS	40	80	120	160	320	480	640	800