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DNA Preparation from Paraffin Tissue

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Reagents

Chloroform
Mallinckrodt, Cat. 4440
EDTA, 0.5 M
Ethanol, absolute
Isoamyl alcohol
Sigma, Cat. I-3643
Phenol
Proteinase K
RNase A
Boehringer, Cat. 109 169
Sodium acetate, pH 5.2
Sodium chloride, 5 M
Sodium thiocyanate (NaSCN), 1 M
Sigma, Cat. S 7757, 250 g)
TE Buffer (Tris-EDTA), pH 7.4
Tween 20
Xylene

Preparation

Chloroform/Isoamyl alcohol 24:1

Chloroform	24 ml
Isoamyl alcohol	1 ml

DNA extraction buffer
1.5 ml 5M NaCL
5.0 ml 0.5M EDTA
0.5 ml Tween 20
Fill up to 100 ml with sterile water.

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

RNase A (20 mg/ml)
Dissolve 200 mg RNase A in 5 ml sterile water.
Boil for 15 min.
Cool to room temperature
Aliquot and store at –20˚.

Procedure

1. Cut 50 μm slices of formalin-fixed and paraffin-embedded tumor samples. (Note: Cut 4 μm slices before and after each 50 μm slice for Haematoxylin-Eosin staining to insure that the tissue is still representative.)

2. Incubate in xylene at 45°C for 15 min.

3. Centrifuge 10 min at 14,000 rpm.

4. Pipet off supernatant.

5. Repeat steps 2-4.

6. Add 1 ml 100% ethanol to tissue pellet, vortex, centrifuge for 10 min at 14,000 rpm, and pipet off supernatant.

7. Add 1 ml 90% ethanol to tissue pellet, vortex, centrifuge for 10 min at 14,000 rpm, and pipet off supernatant.

8. Add 1 ml 70% ethanol to tissue pellet, vortex, centrifuge for 10 min at 14,000 rpm, pipet off supernatant, and dry pellet in speed vac.

9. Resuspend pellet in l ml NaSCN (1 M) and incubate at 37°C overnight.

10. Centrifuge for 10 min at 14,000 rpm; pipet off supernatant.

11. Resuspend pellet in 400 μl of DNA extraction buffer.

12. Add 5 μl RNase (20 mg/ml) and incubate for 1 hr at 37˚C (RNase treatment is optional, paraffin material often does not contain large amounts of RNA).

13. Add 40 μl of Proteinase K (10 mg/ml), vortex briefly, and incubate at 55°C overnight (if tissue is not completely dissolved, add additional proteinase K and continue incubating; tissue should be dissolved).

14. Add 440 µl of phenol, shake vigorously by hand for 5 min, and centrifuge for 5 min at 8000 rpm.

15. Pipet supernatant into a new tube, add a solution of 220 µl phenol plus 220 µl chloroform/isoamyl alcohol (24:1), shake vigorously by hand for 5 min, and centrifuge for 5 min at 8000 rpm.

16. Pipet supernatant into a new tube, add 440 µl chloroform/isoamyl alcohol (24:1), shake vigorously by hand for 5 min, and centrifuge for 5 min at 8000 rpm.

17. Pipet supernatant into a new tube (2 ml eppendorf tube), add 1/10 volume of sodium acetate (pH 5.2), add 3 volumes of ice cold 100 % ethanol, and keep tube for 1 hr at –80˚C or overnight at –20˚C.

18. Centrifuge for 30 min at 4˚C.

19. Remove and save supernatant (optional, in case you are not sure of the precipitation).

20. Dry pellet in speed vac.

21. Add 20-50 µl sterile H_20 (depending on the amount of DNA you expect, which is subjective to experience).

22. Shake gently in thermomixer at 37°C for 2 hours (DNA should be dissolved, but if you have doubts put on a rotating shaker in the cold room overnight).

23. Measure DNA concentration with a spectrophotometer and run around 200 ng on a 1% agarose gel.