

DNA Extraction from Paraffin Blocks v1.0

Protocol

- ❑ Cut 10-20X 10 um sections of formalin fixed paraffin samples into eppendorf tubes.
- ❑ Add 1 ml xylene, mix, incubate at 55 C for 15 mins. Release pressure, spin down for 2 minutes in eppendorf ultramicrofuge, pipet off & discard supernatant.
- ❑ Repeat Step 2.
- ❑ Add 100% ethanol, incubate for 15 mins. Spin down.
- ❑ Remove ethanol, Repeat Step 4, allow pellet to air dry.
- ❑ Add up to 1 ml of Proteinase K in digestion buffer to a final concentration of 0.3-0.5 mg/ml (less buffer for smaller tumors).
- ❑ Incubate overnight, shaking, at 55 C.
- ❑ Add fresh concentrated PK (same amount as day 1 from 20 mg/ml stock).
- ❑ Incubate overnight at 55 C.
- ❑ Repeat step 8 & 9.
- ❑ Add equal volume phenol/chloroform/isoamyl alcohol to the PK digested aqueous solution, spin 2 mins, remove upper aqueous phase to new tube. (repeat PCI extraction if necessary -- it usually is).
- ❑ Take 330 ul aqueous phase per eppendorf tube, add 1/2 original volume (165ul) ammonium acetate (7.5M) [can add 1-3 ul Glycogen here to preserve low amounts of DNA. Glycogen makes a nice visible pellet] and 2-2.5X volume with 100% ethanol. Let sit at RT for ~2 hours or overnight at -20 C.(better O.N.).
- ❑ Spin for 20 minutes at 4 C, decant ethanol (rinse pellet with etoh if it doesn't move), blot dry, air dry.
- ❑ Carefully resuspend the pellets in small volumes of TE buffer (~15-20 ul) and let dissolve at RT overnight (or 55 C for 2 hours). Combine tubes from the same sample.
- ❑ Measure DNA concentration on the Fluorometer. Ideal DNA concentration is from 200-500 ug/ml.
- ❑ Denature the DNA at 70 C before running 0.2 ug on 1% gel to check the size. Size should range from 100bp-3Kb.

Solutions

- ❑ **Digestion buffer:** 100 mM NaCl/10mM Tris-HCl, pH 8.0 and 25 mM EDTA, pH 8.0/0.5% SDS. Store at RT.

- **Proteinase K:** Stored as 20 mg/ml aliquots at -20 C; Can be refrozen a few times.
- Use Proteinase K at 0.3-0.5 mg/ml in digestion buffer
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