

## Extraction and Isolation of DNA from Paraffin-Embedded Tissue

### Extraction of core

1. If you intend to isolate DNA from both normal and tumor tissue, one must first prepare an H&E stain from a top slide of the tissue in the block. This slide should be read by an experienced pathologist, and the normal/tumor regions marked accordingly. Once this is done, you can use the slide to identify the corresponding regions in the block. Depending on the aim of the project, it may also be acceptable to use any or all parts of the tissue in the block.
2. Using a 14G needle as your cutting tool, pierce the block in the region of interest, and cut out a 1 to 3 mm core by turning the needle in the block. The depth of the cut should be sufficient to completely pierce the tissue. If you are unable to use the coring method, and can use DNA extracted from any part of the tissue, you can cut 6-8 10 um sections on the microtome and use for DNA extraction. Remember to wipe the stage with alcohol and replace the blade after each block, in order to avoid cross contamination.
3. Carefully transfer the newly cut core or shavings into either a 2 ml polypropylene microcentrifuge tube, or a 15 ml polypropylene centrifuge tube. Label the tube appropriately.
4. Repeat the coring or cutting process as necessary. Place each core or shaving into a separate tube.
5. At a later time, it is recommended that you seal the block with melted paraffin.

### Removal of Paraffin

*(Note: Xylene is a hazardous chemical. The step 6 must be done in a fume hood, and the resulting waste handled appropriately.)*

6. Treat the core via the following steps in 2.0 ml polypropylene microcentrifuge tubes (15 ml polypropylene centrifuge tubes work as well – adjust centrifugation accordingly):
  - a. Xylene, 1 ml for 10 minutes – pellet at 14,000 rpm for 10 min – aspirate supt.
  - b. Repeat
  - c. 100% Ethanol, 1 ml for 10 minutes – pellet at 14,000 rpm for 10 min – aspirate supt.
  - d. Repeat
  - e. 80% Ethanol, 1 ml for 10 minutes – pellet at 14,000 rpm for 10 min – aspirate supt.
  - f. Repeat
  - g. 50% Ethanol, 1 ml for 10 minutes – pellet at 14,000 rpm for 10 min – aspirate supt.
  - h. Repeat
7. Add 1 ml H<sub>2</sub>O and incubate at 4°C overnight.

### Digestion of Protein

8. Pellet sample at 14,000 rpm for 10 minutes – aspirate supt.
9. Add 700 ul of Nucleic Acid Lysis buffer (NALB).
10. Add 50 ul of Proteinase K (@ 30 mg/ml).
11. Incubate for 24 hours at 65°C.
12. Add an additional 50 ul of Proteinase K (@ 30 mg/ml).

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13. Incubate for an additional 24 hours at 65°C.

**Precipitation & Isolation of DNA**

14. If the sample requires the elimination of endogenous RNA (recommended), add 10 ul of RNase A (10mg/ml) and incubate at 37°C for 30 minutes.
15. Add 250 ul of 6 M NaCl (saturated).
16. Let stand at room temp for 10 minutes.
17. Pellet sample at 14,000 rpm for 10 minutes.
18. Carefully transfer supernatant to a clean microcentrifuge tube.
19. Add 1 ml of ice-cold (or -20°C) 100% Ethanol.
20. Carefully mix and place at -20°C for 20 minutes.
21. Pellet sample at 14,000 rpm for 10 minutes.
22. Carefully discard the supernatant.
23. Wash pellet with 1.5 ml of 70% Ethanol.
24. Pellet sample at 14,000 rpm for 10 minutes.
25. Carefully discard the supernatant.
26. Allow pellet to air dry on the benchtop for 10 to 15 minutes.
27. Add 30 to 80 ul of TE Buffer.

Nucleic Acid Lysis Buffer

10 mM Tris Base (1.21 g/L)

400 mM NaCl (32.4 g/L)

2 mM Na<sub>2</sub>EDTA (0.75 g/L)

0.7% SDS (7.0 g/L)