Preparation of formalin-fixed paraffin-embedded (FFPE) tissues for RNA extraction

Protocol For:

Methods comparison for high-resolution transcriptional analysis of archival material on Affymetrix Plus 2.0 and Exon 1.0 microarrays

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Read the protocol online: www.BioTechniques.com/protocols/113261

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Protocol overview

• This protocol covers tissue sectioning and macrodissection steps in preparation for RNA extraction from FFPE tissues. Although originally developed for RNA extraction using the Optimum FFPE RNA Isolation Kit (Ambion, UK), the protocol is relevant for FFPE tissue preparation for RNA extraction using any extraction method

• Macrodissection is carried out at 2× magnification using a dissecting microscope with external fiber-optic illumination

• Adopt strict RNase-free handling conditions throughout, including the use and frequent changing of powder-free latex gloves. Clean work surfaces with disinfectant or 70% alcohol.

• Use disposable microtome knives and disposable, single-use flat scalpel blades (for macrodissection)

Procedure

1. Decontaminate all equipment (including microtome and knife, forceps, scalpel blade holder, tissue storage containers, and macrodissecting cutting surface) before preparation of each sample, using RNaseZAP on non-metallic and ElectroZAP on metallic equipment.
2. Wipe equipment clean with xylene (in addition to RNase decontamination) and dry with blue towels (repeat between samples to minimize carry-over of wax and cross-contamination of tissue).
3. Commence sectioning at 5-µm thickness and discard top few whole sections (to avoid using the oxidized/contaminated surface of tissue block).
4. Set to microtome to cut 10-µm sections and cut a number of sections equivalent to ~1–2 cm^2 tissue area from each block (this is sufficient for three extraction experiments/reactions; more may be needed if the tissue is hypocellular).
5. For RNA extraction from whole sections, place sections immediately into a labeled RNase-free microcentrifuge tube, close cap and proceed to step 8.
6. For macrodissection, place sections/ribbons into covered RNase-free containers (to minimize drying out and exogenous RNase contamination) and proceed immediately to step 7.
7. Macrodissect tissue of interest using a new, sterile, and disposable scalpel blade and immediately place dissected tissue (≤4 cm^2) into a labeled RNase-free microcentrifuge tube and close cap (work quickly and trim excess wax where possible).
8. Repeat steps 2–7 until all samples are prepared.
9. Proceed to the deparaffinization step of RNA extraction protocol, or freeze sections at -20°C until RNA extraction can be carried out.

Reagents

• RNaseZAP (Ambion, Huntingdon, Cambridgeshire, UK)
• ElectroZAP (Ambion)
• Xylene (AnalaR) (BDH Laboratory Supplies, Poole, Dorset, UK)
• RNase-free water (Ambion)

Equipment

• Dissecting microscope with fiber-optic illumination
• Disposable scalpel blades
• Microtome
• Tissue storage containers
• Scalpel blade holder
• Forceps
• Blue towels
• Microcentrifuge tubes

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