Preparation of FFPE sections for RNA isolation

Prepared by: OHSU Gene Profiling Shared Resource (GPSR)
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Adapted from QIAGEN miRNeasy FFPE Handbook, ver. May 2018

Starting material recommendations:

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation and crosslinking of nucleic acids. To limit the extent of nucleic acid fragmentation and crosslinking be sure to:

- Use tissue samples less than 5 mm thick to allow complete penetration by formalin
- Fixate tissue samples in 4–10% neutral-buffered formalin as quickly as possible after surgical removal
- Use a maximum fixation time of 24 hours (longer fixation times lead to over-fixation and more severe nucleic acid fragmentation, resulting in poor performance in downstream assays)
- Thoroughly dehydrate samples prior to embedding
- Use low-melting paraffin for embedding
- If possible, store blocks at 4°C

Preparation of sections:

General Information: The starting material for RNA purification should be **freshly cut sections** of FFPE tissue, each with a thickness of up to 20 µm. Thicker sections may result in lower nucleic acid yields, even after prolonged incubation with proteinase K. RNA yields will depend on tissue type and size of specimen within block.

- **Recommended: One to 2 sections, each with a thickness of 10 µm or less and a surface area of up to 250 mm^2 combined in one nuclease-free tube for RNA extraction.** Up to 4 - 10 µm sections can be combined if required for adequate RNA recover**y.** One or 2 sections, each with a thickness of 20 µm are also acceptable, however, **t**hinner sections are preferred. More than 4 sections can be combined if the **total sum of the thickness of the sections is 40 µm or less** (e.g., eight 5 µm thick sections).
- If less than 30% of the surface area consists of tissue, more than 40 µm of sectioned material can be combined if the excess paraffin is removed using a scalpel prior to starting the sectioning procedure or if scraping from slide.
- The first section or two from the specimen surface should be discarded.

Sectioned sample collection, storage and shipment

Based on GPSR experience and vendor recommendations:

1. Minimize sources of RNase-contamination while sectioning the sample blocks.
2. Transfer freshly cut specimen sections (up to 40 µm per sample) directly into nuclease-free microfuge tubes.
3. Seal each tube immediately after all sections are added and transfer to 4 ^{o}C or freeze.
4. Ship samples or transfer to core laboratory on ice or dry ice as soon as possible after sectioning.
5. If samples are being delivered to another lab at OHSU, the receiving laboratory should store samples at 4 ^{o}C or freeze and deliver to the core laboratory ASAP.

Consider sending test sections to the core lab for an initial extraction to determine how many sections are required for adequate RNA recovery for downstream applications

For further information, please contact:
Kristina Vartanian, Research Associate, Gene Profiling Shared Resource – vartania@ohsu.edu
Chris Harrington, Director, Gene Profiling Shared Resource – harringc@ohsu.edu