STANDARD OPERATING PROCEDURE	**SOP No. 012** **Cutting of Formalin Fixed, Paraffin Embedded (FFPE) Samples- ICR**
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1. PURPOSE

To describe the procedures for cutting Formalin Fixed, Paraffin Embedded (FFPE) samples by the Cancer Biomarker (CB) Team at the Institute of Cancer Research. This procedure applies to all FFPE samples (biopsies and archival) received by the CB team for further processing. Note: This SOP describes the generic procedures for cutting FFPE samples using a microtome or a Manual Tissue Arrayer (MTA). The purpose is to ensure that all the SSPE samples are cut in uniform manner and in accordance with the principles of GCP and other applicable regulations.

The following SOP for Blood and Cytology Sample processing is followed by the Institute of Cancer Research Network Site of the PCBN ONLY.

2. RESPONSIBILITIES

Authorized personnel cutting FFPE samples are responsible for the following:

- Comply with the procedure
- Follow all other precautions required for the handling of human blood and tissue samples
- Obey Team Leader who has overall responsibility of the procedure
- All FFPE samples received are logged into the Histopathology Sample Entry (HSE) and on the Progeny.

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

Equipment	Materials
Semi-automated rotary microtome or Sledge microtome Manual Tissue Arrayer Water Bath Hot Plate	Disposable microtome blades 20% Ethanol or 20% Industrial Methylated Spirit (IMS) Distilled water Ice Tray Plain slides or Charged slides Forceps and brush

5. PROCEDURES

5.1 Cutting FFPE Samples Using a Microtome

- Notes:
  - Microtome blades are extremely sharp, take a great deal of care when handling, and dispose of them in a sharps bin.
  - The blade in the holder must not be left unattended for a long period of time whilst not being used. Use the knife guard to shield and protect it.
  - Take care when using the hot plate, as the surface could cause burns if touched for a long period of time.
- Prepare the microtome according to EQU/088 or EQU/089.
- Switch the hot plate; this should be set to approximately 60°C.
- Fill the water bath with distilled water and heat to the required temperature (48-53°C).
- Cool the blocks by placing on an ice tray. They can be put in the freezer for a few minutes to accelerate the process.
- Adjust the thickness for the required one:
  - Cut at 1-3 µm for Hematoxylin and Eosin (H&E) sections.
  - Cut at 3-4 µm for unstained sections (U/s) used for Immunohistochemistry (IHC), Florescence in Situ Hybridization (FISH) or Immunofluorescence (IF) techniques.
  - Cut at 5-10 µm (thick sections) for DNA/RNA extraction. Make sure blade, blade holder and forceps are cleaned with RNase zap in between samples to avoid contamination. Use gloves when preparing these samples.
- Cut sections as below:
  - Note: when enough tissue is not available, cut as many sections as possible.
  - For Molecular Characterization (M/C) archival blocks, cut H&E and 3-7 u/s if needed for further analyses (e.g. Sequencing, IHC, etc.). Make sure there are at least 2 u/s stored before returning the blocks returning the blocks to the CTOs or data manager.
  - For fresh FFPE biopsies and 2472 archival blocks cut a number of u/s slides being at least 6 where possible, and an H&E on either sides.
  - For DNA/RNA extraction cut at least 60 microns of tissue when possible. The sections are either put in 1.5 mL microcentrifuge tubes or mounted on charged slides for Nuclear Fast Red (NFR) staining but other instructions might be given.
  - For FFPE biopsies that need to be reported by the RMH, cut an H&E and a number of u/s as required (6-20 normally),
- The sections can either be floated out directly (with the shiny side down) on to the surface of a warm water bath (48-53°C), a cold water bath or carefully placed on to a slide with 20% IMS or 20% ethanol on the surface, and then floated out on to the water bath, taking care to avoid the sections moving too quickly.
- The water bath temperature may be adjusted depending on the wax used in the block being cut.
- The ribbon may be split in to individual sections by carefully using the tips of forceps or a slide, and the sections carefully picked up on to the middle of labelled glass slides whenever possible.
  - Use plain slides for H&E sections (charged slides may be used if sections need to be stained on the same day).
  - Use charged slides for u/s sections.

- If not labelled yet, label slides with trial number, section sequence number and H&E when for H&E staining.
    - M/C samples: use progeny number as the trial number.
    - 2472 samples: use the trial number.
    - All other trials: use specific trial requirements or in its absence use the trial number.
- The slides are left to drain standing up against the side of the water bath or hotplate until the sections are completely dry, and then placed on to the hot plate (60°C) to melt the wax if required.
- The sections can then be placed in to a rack and dried overnight in the 37°C oven, or in the 60°C oven for up to 1 hour before staining.
    - If slides are not sued within 2 weeks, dip them in wax as described below.
    - Fill water bath with distilled water and heat 60°C.
    - Warm empty glass beaker in water bath, placing a weight on top to prevent it from floating. Do not allow any water to spill in.
    - Fill beaker with wax from the embedding center, which is also heated to 60°C, and place carefully into the water bath ensuring that no water is allowed to flow in.
    - Place slides back to back in pairs, so that the sections face outwards.
    - Holding the slides carefully together at the top, quickly dip them in the molten wax, so that the level of the wax is higher than the section, but does not cover the pencil label.
    - Leave the slides to dry, by standing them upright.
    - Repeat for each pair of slides, topping up the wax when the level gets too low to cover the sections adequately.
    - Cool the slides completely to allow the wax to harden, before scraping off any excess wax, and storing the slides.
    - Pour the unused molten wax back into the embedding center or discard in the yellow bin, and allow the glass beaker to cool down. Switch off water bath.
    - Store slides in the respective storage files. Slides must be filed under trial name, then under trial number and finally under slide number.
- Record all the work done in the respective spreadsheets and/or Progeny as per SOP/57/.

5.2 Cutting FFPE Samples using a MTA

- Whenever coring is better than thick sections for sequencing, prepare the MTA according to EQU/091/.
- A specialist research scientist (pathology) needs to circle the area of interest on the H&E slide.
- Circle the same area on the block using a marker.
- Core the area identified and release the core in to a 1.5 mL microcentrifuge tube previously labelled with the sample identifier (progeny number for M/C samples, trial number for other trials). Note: To avoid contamination, make sure needle is properly clean before coring a block.
- Give the samples to the sequencing team and record all the work done in the respective spreadsheets and/or Progeny as per SOP/57/.
- Record the information required by each trial or study (e.g. total fixation time, processing schedule, core length, etc) in the respective spread sheets and/or Progeny.

5.3 Related Documents (please contact query@prostatebiorepository.org for information regarding these additional documents)

- EQU/088/ (Operation and Maintenance of a Sledge Microtome)
- EQU/089/ (Operation and Maintenance of Semi-automated Rotary Microtome)
- EQU/091/ (Use and Maintenance of the Manual Tissue Arrayer)
- MET/157/ (Fixing, Processing and Embedding of Tissue Samples in the Cancer Biomarkers Team)
- SOP/057/ (Receipt, Handling, Storage and Return of Clinical Tissue Samples in the CB Laboratory Using Progeny LIMS)
- MET/178/ (Nuclear Fast Red (NRF) Staining of Tissue Samples)