Appendix 9.c
Standard Operating Procedure (SOP) for Samples Preparation and Processing: Tissue and slides

Version 4.2 – April 2024

Change history

Version	Date	Changes
V4.2.	April 2024	Adapting Mutographs SOP for preparation of sections from Formalin-Fixed Paraffin-Embedded (FFPE) tissue
V4.1.	March 2021	Rewording for clarification in: 2.2.1/3 2.3/notes 2.4
V4.0.	July 2020	Overall reorganisation of protocol, now appendices with individual versioning. Revised Appendix 9 Sample Processing, formerly Appendix 6

1 Paraffin embedded tissue and histological/cytological slides

1.1 Materials used

- 1.8 ml cryotubes (no colour tops) using the provided labels with the correct HEADSpAcE IDs. (Please ensure to affix the HDS label on the front of the slide.)

1.2 FFPE sample type

1. A whole FFPE block
2. Part of a FFPE block
3. FFPE Scrolls

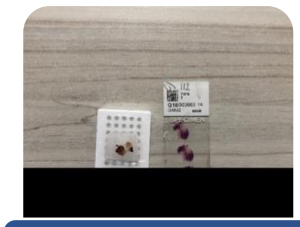
1.3 FFPE sample collection and preparation

1.3.1 FFPE Block

In collaboration with the pathologist who conducted the diagnosis of the tumour:

Identify the formalin-fixed paraffin-embedded tissue blocks (FFPE blocks) which best represent the tumour. Select one block for the purpose of the research biorepository and collect, with the following priority order:

If possible, obtain from the pathological department a representative H&E stained slide that served for the diagnosis. Representativeness of the slide (majority of the tumour, worst of the tumour, both) should be noted in the clinical and pathological data form.



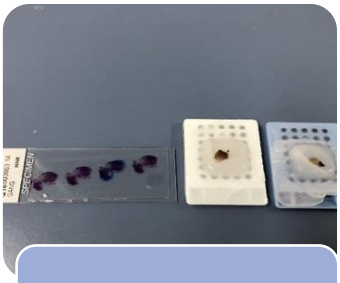
1. Select block to divide

2. Match slide with tissue and mark

3. Cut paraffin embedded block

4. Embed the paraffined tissue maintaining orientation and fill in the cubet with paraffin

5.Place the cassette on top until tissue block is ready



6.The result is two blocks

1.3.2 Scroll preparation

Tissue scrolls must be prepared from FFPE block with at least 40% of viable tumour cells with no necrosis or haemorrhage, confirmed by pathologist.

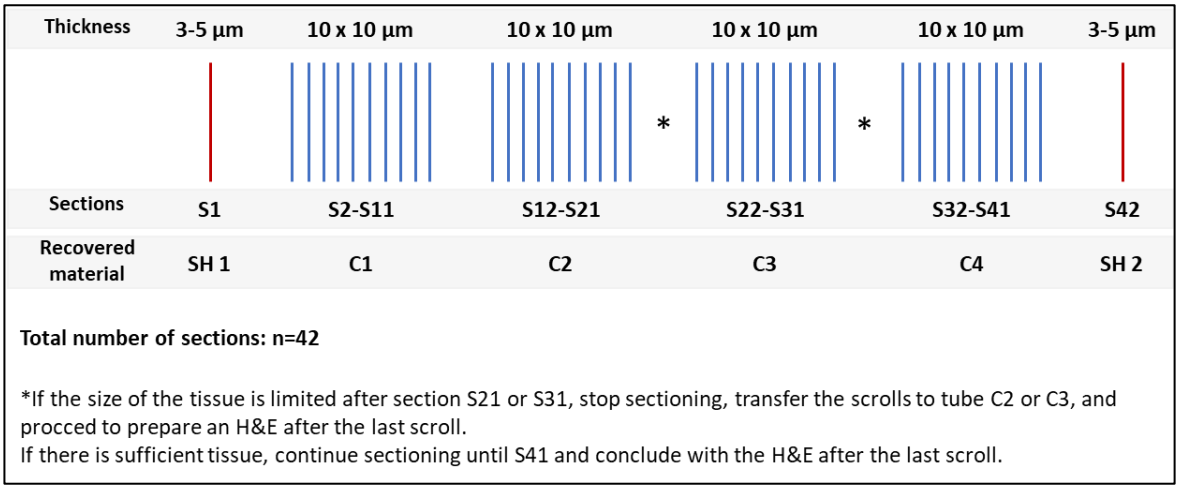


Fig 1. Schematic representation of the sectioning procedures.

Step 1: Prepare the slides and 1.5mL tubes using the provided labels with the correct HEADSpAcE IDs. (Please ensure to affix the HDS label on the front of the slide.)

Step 2: Wear disposable gloves, remove the used blade, and clean the microtome (especially the surface where the slides are collected and the holder - forceps), and the bench with DNA cleaner and 70% ethanol using disposable tissue papers.

Discard cleaning tissue papers and gloves.

Step 3: Wear new disposable gloves and insert a new microtome blade.

Step 4: Cool down the paraffin blocks by putting them over ice.

Step 5: Mount tumour paraffin block.

Step 6: Trim the block until you achieve an even cutting surface. Ensure that the entire embedded piece reaches the level of cutting. Avoid over-trimming, especially if the tissue size is small.

Step 7: Generate one section of 3-5µm thick on a coated slide (*Superfrost* is recommended) for H&E staining (**labelled 'SH1'**). See below for desiccation, deparaffinization, and H&E staining.

Step 8: Generate 10 consecutive scrolls of 10µm thick for DNA extraction.

Step 9: Clean carefully the tweezers with DNA cleaner followed by 70% ethanol and

collect the scrolls in a new sterile 1.5mL tube (**labelled 'C1'**).

Warning, it is likely that after every ~10 sections the block needs to be cooled down on -20◦C plate.

Step 10: Generate 10 new consecutive scrolls of 10µm thick for DNA extraction.

Step 11: Collect with clean tweezers (extensively washed with DNA cleaner, followed by 70% ethanol) the scrolls in a new sterile 1.5mL tube (**labelled 'C2'**).

In case the specimen size is limited, please skip to step 16.

Step 12: Generate 10 consecutive sections of 10µm thick for DNA extraction.

Step 13: Collect with clean tweezers (extensively washed with DNA cleaner, followed by 70% ethanol) the sections in a new sterile 1.5mL tube (**labelled 'C3'**).

In case the specimen size is limited, please skip to step 16.

Step 14: Generate 10 new consecutive scrolls of 10µm thick for DNA extraction.

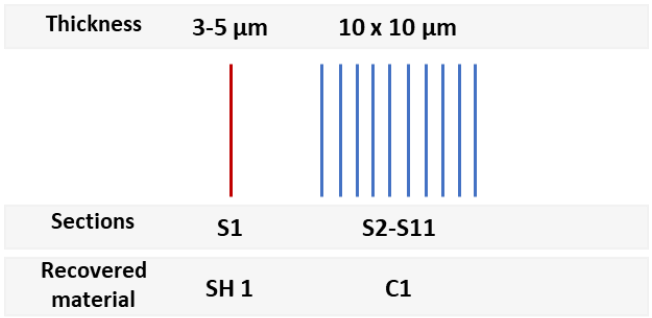
Step 15: Collect with clean tweezers (extensively washed with DNA cleaner, followed by 70% ethanol) the sections in a new sterile 1.5mL tube (**labelled 'C4'**).

Step 16: Generate one final section of 3-5µm thick and mount it on a coated slide (*Superfrost* is recommended) for H&E staining (**labelled 'SH 2'**).

Step 17: Return to step 2 and process a new specimen.

1.4 Adjacent normal (if available) (separate block, no tumor as confirmed by pathologist)

Sections of adjacent normal tissue must come from a block that has been pathologically verified to be tumour free. Tissue next to the tumour on the tumour block cannot be considered adjacent normal and macro-dissected at a later time.



Total number of sections: n=11

****Sections for methylation should only be collected if both FFPE tumour and FFPE Adjacent normal are available

1.5 Storage

- Tubes with FFPE scrolls and H&E slides can be stored at room temperature. Prevent exposure to sun or extreme temperature variance.
- Store tubes and slides in moisture resistant cardboard boxes or plastic storage boxes.