THE CHILDREN'S HOSPITAL AT WESTMEAD

TUMOUR BANK

STANDARD OPERATING PROCEDURE

CONSTRUCTING TISSUE MICROARRAYS	
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1. PURPOSE

The purpose of this document is to outline standardised procedures to be followed when locating, collecting and sampling tissue; and annealing and sectioning a constructed tissue microarray (TMA).

2. SCOPE

This protocol covers all TMA's constructed by the Tumour Bank (TB). Prior to constructing/planning a TMA, every core of tissue should be accessed and approved for use by a member of the Histopathology Department.

3. RESPONSIBILITIES

TMAs will be constructed by the TB's Histopathology Research Assistant (RA) under guidance by the TB Project Officer.

4. MATERIALS, EQUIPMENT AND FORMS

- Diagnostic/donor tissue blocks
- Paraffin wax recipient block
- Large embedding (eyeball) mould
- Superfrost plus slides
- MTA-1 Beecher Manual tissue arrayer
- Automated H&E stainer
- Microtome
- Water bath
- Incubator
- Automated Immunohistochemical stainer (Biosystems BOND-max)
- Block screening form/approval worksheet for pathologist to sign

5. METHOD

5.1 Making the Tissue Microarray

a. Sampling the correct site from donor blocks is critically important for constructing tissue arrays.
b. Obtain a fresh H&E slide from each block and use as a guide to select regions for sampling.
c. Circle or mark the sampling site on the corresponding H&E slide before starting the array process.
d. A blank paraffin block is the recipient for tissue samples.
e. Prepare the block by melting paraplast plus wax and dispensing it into a deep eyeball size mould.
f. Place a cassette on top of the melted paraffin until the wax is cooled and the mould is ready to be removed.
g. Check block for any holes or cracks that may have risen during the block preparation.
h. Ensure that the block surface is flat and parallel to the underside of the cassette, by facing off or trimming the block surface on a rotary microtome.
i. Take care not to introduce scores or nicks in the paraffin recipient block.
j. Tissue cores are to be deposited in a grid pattern to form a "tissue microarray".
k. Up to a 1000 patient samples can be put onto one recipient block depending on the size of the cores.
l. Place the donor paraffin blocks under a low wattage lamp before coring. This makes the donor blocks softer, less likely to crack and easier to punch.

5.2 Annealing of the Tissue Microarray Block

The tissue microarray block must be smoothed and levelled before sectioning.

Annealing or warming up of the paraffin promotes adherence of the tissue biopsies (cores) to the walls of the holes in the array block and makes the wax flexible to handle.

This is important because levelling maximizes the number of sections containing all the samples in the array.

5.2.1 Procedure for Annealing

a. Place block facing upward in slide oven at 64°C for 10 minutes (1st Round).).
b. Use a clean glass microscope slide to level the face of the block. Place the glass slide on top of the block, applying even pressure to push all the cores on the array to the same level.
c. Let block cool at room temperature for 10 minutes.
d. Place block facing upward in slide oven at 64°C for 10 minutes (2nd Round).).
e. Use a clean glass microscope slide to level the face of the block. Place the glass slide on top of the block, applying even pressure to push all the cores on the array to the same level.
f. Let block cool at room temperature for 10 minutes.
g. Place block facing upward in slide oven at 64°C for 10 minutes (3rd Round).).
h. Use a clean glass microscope slide to level the face of the block. Place the glass slide on top of the block, applying even pressure to push all the cores on the array to the same level.
i. Let block cool at room temperature for 10 minutes.
j. Place block facing upward in slide oven at 64°C for 10 minutes (4th Round)
k. Use a clean glass microscope slide to level the face of the block. Place the glass slide on top of the block, applying even pressure to push all the cores on the array to the same level.
l. Let block cool at room temperature for 10 minutes.
m. After annealing the block, allow block to settle overnight before sectioning.
n. Take a digital photo of the tissue microarray block before sectioning.

5.3 Sectioning of the Array Block

The sectioning of the TMA block can be done using standard microtome sectioning techniques for cutting the slides. One TMA can produce up to 150 standard sections.

a. Note the depth of the block and adjust the microtome distance accordingly.
b. Use a new disposable blade. Section a blank wax TMA block to blunt the knife slightly before trimming the TMA.
c. Trim block
d. Put on ice for 20 minutes
e. Serial Section Block
f. Float out ribbon on 42°C water bath
g. Use Superfrost Plus slides to pick up sections
h. Don’t waste any sections
i. Pick up every section including those with folds or bubbles.

5.4 Sectioning Problems

- When moving the knife to new part always blunt blade slightly on a new blank wax block to avoid discs (cores) rolling.
- If discs are rolling onto themselves then the blade is probably too sharp or wax needs to re-anneal around the cores.
- Put in slide oven (64°C) for 1 minute (max), then let block cool on ice.

5.5 Please refer to the following manuals for further technical instructions

- Manual Tissue Arrayer Technical Manual Version 1
- Manual Tissue Arrayer MTA-1 Beecher Instruments Instruction Manual.

6. SAFETY

- All local chemical and sharps policies must be adhered to.
- Safety equipment required includes latex gloves, lab gown, safety glasses, oven mitt.