

PCRC SOP 4: Tissue Block Sectioning

Preparation of tissue blocks for sectioning and use in laser-capture microdissection

Tissue Sectioning:

1. Clean down work area and monochrome with: clini-wipes, xylene, RNA-se away-wipes (in that order).
2. Clean out water-bath with clini-wipes, then RNA-se away-wipes, then fill up and rinse well with distilled water. Fill with distilled water. Ensure there is no dust in work area or on waterbath/lid.
3. Heat water-bath to 42°C
4. Before cutting blocks, place them face-down on a clean ice block for 5 mins
5. Place tissue block in holder in monochrome, and insert new blade
6. Cut 10µm-thick sections
7. Allow sections to flatten on the warm water surface for 2 mins before lifting onto membrane-coated slides (at a 45° angle)
8. Allow mounted sections to dry at room temperature for 10 mins then melt onto the membrane surface in a hot airstream for 30 seconds.

Removing Paraffin:

1. Dewax sections in xylene for 2 x 1 mins, then rehydrate through graded ethanol solutions (100%, 95%, 70%, in DEPC-treated water).

Hematoxylin and Eosin Staining:

Allow the slide containing the tissue section to remain in the following solutions for the specified times in the specified sequence

Solution	Time
Rnase free water	10 seconds
Mayer's Hematoxylin	10 seconds
Rnase free water	10 seconds
70% alcohol	15 seconds
Eosin	15 seconds
95% alcohol	30 seconds
95% alcohol	30 seconds
100% alcohol	30 seconds
100% alcohol	30 seconds-1 minute
Xylene	1 minute

Allow mounted sections to dry at room temperature for 10 mins

Perform microdissection immediately thereafter