THE UNIVERSITY OF CHICAGO
HUMAN TISSUE RESOURCE CENTER

LCM Preparation for Frozen Sections

1. Sample Freezing in OCT

1.1. Prepare your 2-methylbutane solution.

1.2. Pour 2-methylbutane in a metal bowl or plastic beaker. Add small pieces of dry ice into the solution to cool the solution down to -40°C to -50°C.

1.3. Collect dissected tissue specimen. Place tissue on paper towel to get rid of excess liquid before you put the tissue into the O.C.T.

1.4. Choose proper size of cryomold for tissue specimen.

1.5. Add a thin layer of O.C.T. compound (Optimal Cutting Temperature) to cryomold and place tissue specimen in desired orientation. Completely fill the mold with OCT. **There should be no bubbles and the tissue should be completely immersed in OCT.**

1.6. Hold the mold with hemostat and let the bottom fo the mold touch the cold 2-methylbutane.

1.7. Place cryomold in cold (-40°C) 2-methylbutane solution when it is completely white and let it stay in the cold bath for another 5 minutes.

1.8. Wrap the frozen block with labeled foil. Store frozen sample in the cryomold in a –80°C freezer or temporarily on dry ice.

1.9. Repeat these steps if you have more than one sample.

2. Slide Preparation

2.1. Pre-cool the cryostat to the temperature recommended by the manufacturer for the specimen you are preparing (**-20°C** for most of tissues or **-30°C to -35°C** for lipid rich tissues).

2.2. Remove and discard old microtome blade. Wipe down the knife holder and anti-roll plate in the cryostat with 100% ethanol to avoid sample cross-contamination.

2.3. Install a new disposable microtome blade in the cryostat.

2.4. Set cutting thickness to desired thickness (**4-10 µm**). Set cutting angle to **10** degrees (HM505EVP).

2.5. Transfer the cryomold containing the specimen from the –80°C freezer to the cryostat, transporting on dry ice if necessary.

2.6. Wait a minimum of **10 minutes** for the specimen to equilibrate with the temperature of the cryostat.

2.7. Mount specimen to the specimen holder (chuck) with OCT. After frozen specimen trimming cut **4-10 µm** sections.

2.8. Sections should be mounted within the membrane of the PEN-Membrane slides. Place slide immediately into slide box on dry ice (or -20°C or -80°C freezer). Do not allow slide to dry at room temperature.

2.9. If cutting more than one specimen, use a new disposable microtome blade for each one. In addition, wipe down knife holder and anti-roll plate with **100% ethanol** in between each specimen to avoid cross-contamination.

2.10. Proceed immediately to the "Staining and Dehydration" segment of the protocol or store at **–80°C** for up to two months.

Note: *Frequent cycling of the tissue block from –80°C to -20°C for cryosectioning may accelerate RNA degradation. For best results, cut and mount a sufficient number of sections for two months' use during one cryosectioning session. Store the mounted sections at –80°C until needed.*

3. Alternative Protocol for H&E Staining and Dehydration

Note: Carry out the "H&E Staining and Dehydration" protocol with only one slide at a time. Change all solutions in the plastic slide jars between each case (maximum of 5 slides) to avoid cross contamination. Do not reuse solutions. Do not transfer solutions back into their original bottles. If you plan to reuse jars, discard all water-based solutions upon completion of staining. Clean them with 100% ethanol, followed by distilled water, RNase AWAY or RNase ZAP, then distilled water and allow drying completely in the hood).

3.1. Label and fill seven slide coplin jars as follows:

	Coplin jar (glass or plastic)	Time
a	70% Ethanol (made with DEPC water)	minimum of 30 seconds @ room temp (-20°C for long term storage)
b	DEPC water	30 seconds
c	Mayer's Hematoxylin Gill II or III Methylene Blue (better for DNA)	2 minutes 10-30 seconds 2 minutes
d	DEPC water	30 seconds
e	Alcoholic Eosin	10 seconds
f	95% Ethanol	30 seconds
g	100% Ethanol	30 seconds
h	Air dry	1-5 minutes

3.2. Fill the labeled coplin slide jars with **50 ml** of the appropriate solution (see table).

3.3. Remove one slide at a time from the slide box on dry ice (or -20°C or –80°C freezer), and place it in slide jar "a" containing **70% ethanol** for **30 seconds**. Use cover glass forceps to transfer slides from jar to jar.

3.4. Transfer the slide to jar "b" containing **DEPC water** for **30 seconds**.

3.5. Transfer the slide to jar "c" containing hematoxylin solution for appropriate time **(see table)**.

3.6. Place the slide in jar "d" containing **DEPC water** for **30 seconds**.

3.7. Transfer the slide to jar "e" containing **alcoholic eosin** solution for **10 seconds**.

3.8. Transfer the slide to jar "f" containing **95%** ethanol for **30 seconds**.

3.9. Transfer the slide to jar "g" containing **100%** ethanol for **30 seconds**.

3.10. Air-dry slide for Leica AS LMD for **1-2 minutes** and start laser microdissection immediately.

3.11. Discard all used staining and dehydration solutions according to your procedures.