Standard Operating Procedure (SOP) for Paraffin Slides and Scrolls

I. SCOPE AND PURPOSE

This procedure details the steps needed to prepare paraffin slides or scrolls (paraffin sections allowed to curl up) to maximize the ribonucleic acid (RNA) quality and quantity on extraction. RNA is susceptible to degradation from Rnases and other enzymes. Fixation and processing of tissues also damages RNA quality and quantity. Keeping samples cold and working quickly helps to maximize recovery.

II. PROCEDURE

A. Safety Procedures – Use appropriate knife safety procedures. Remove blades after use and dispose of in sharps container, put blade safety cover on when not sectioning.

Blades are very sharp! Always use care when handling. Use cut resistant gloves under latex gloves. Always remove knife or place knife guard on when not sectioning! Always lock hand wheel when not in use!

B. Quality Control

1. To avoid sample contamination, change blades between specimens
2. Change gloves between each sample
3. Wipe microtome paten (stage) with gauze soaked in RNase Away or RNase Zap.
4. Wipe again with gauze soaked in diethylpyrocarbonate (DEPC) treated water
5. Wipe all tools (brushes, forceps) with RNase Away and then with DEPC treated water
6. Clean glass dishes with RNase Away or RNase Zap and rinse with DEPC treated water
7. Wipe surface of water bath between samples to avoid cross contamination
8. Daily cleaning at end of the day
    a. Lock down microtome
    b. Remove blade from holder
    c. Dispose of blade in sharps container
    d. Wipe up all paraffin shavings for disposal in biohazard bag

C. Treating membrane slides

1. Prior to use, membrane slides must be cleaned and exposed to UV light
2. Place slides, membrane side up, on paper towels and spray with RNase Away/RNase Zap or place in coplin jar of RNase Zap/RNase Away. Allow to sit at least 5 minutes
3. Rinse well with DEPC treated water 3x.
4. Shake off excess water and lay slides membrane up on paper towel
5. Dry overnight in biosafety cabinet with UV light on
6. Store treated slides in nuclease free box at Room Temperature

D. Slides

1. Trim off any excess paraffin if necessary. Block face should be square or trapezoidal
2. Chill block on chill plate using small amount of DEPC treated water in clean glass dishes. Once well chilled, place block holder on microtome and orient so block face is

parallel to knife edge. Optimum angle will vary for each microtome (usually between 5 and 15 degrees)
3. Trim into block face until you get a complete section of the face of the tissue. Trim away at least 3-5 sections and discard
4. Carefully advance block with flywheel to start taking sections at the requested thickness (4-5 micron is typical, but can range from 2 micron up.)
5. Take one 4 micron section and float on room temperature DEPC treated water bath to expand section and remove wrinkles. Using an RNase free uncharged slide, transfer to 40-45° C DEPC treated water bath briefly for final expansion of sections.
6. Pick up expanded section on properly labeled, cleaned membrane slide. Center section on membrane, avoiding edges of the membrane. Place slide upright in ice cube tray to drain.
7. Once drained, store slides in nuclease free box at 2-6° C until needed

E. Scrolls

1. Trim off any excess paraffin, if necessary. Nucleotide extraction is best if paraffin is minimized. Leave only a small amount of paraffin surrounding the tissue, no more than 5mm
2. Chill block on chill plate using small amount of DEPC treated water in clean glass dishes. Once well chilled, place block holder on microtome and orient so block face is parallel to knife edge. Optimum angle will vary for each microtome (usually between 5 and 15 degrees)
3. Trim into block face until you get a complete section of the face of the tissue.
4. Carefully advance block with flywheel to start taking scrolls.
5. Take one 10 -15 micron scroll (or appropriate thickness, as requested)
6. Pick up scroll with clean forceps and place in properly labeled cryotube. Continue until all scrolls are collected
7. Store cryovials at 2-6° C until needed

III. REFERENCES

A. Micro-dissection Project Design, Golubeva, Y. and Sternberg, L. NIH BioTrac29 Course; Summer Session 2011.

B. Laser Capture Microdissection of Human Pancreatic B-Cells and RNA Preparation for Gene Expression Profiling. Lorella Marselli, Dennis C. Sgroi, Susan Bonner-Weir, and Gordon C. Weir; C. Stocker (ed.), Type 2 Diabetes, Methods in Molecular Biology, vol. 560 Humana Press, 2009.

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 2, Effective Date 8/27/2015

1. Minor formatting
2. Added “an RNase free”
3. Modified language for cut-resistant gloves to be worn under latex gloves

B. Version #1, Effective Date 1/3/2014 - New

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Signatures

Approved By: **Signature on file**

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