Penn Center for Musculoskeletal Disorders Histology Core

Protocol: Cryofilm Technique for Sectioning Calcified Tissue

Materials

- Cryofilm 2C(10), 3.5 cm, Part# CFS 105 (Section-Lab, Japan)
- Cryomolds
- Dry ice
- OCT embedding medium
- Forceps
- Aluminium foil
- 10% neutral buffered formalin (NBF)
- Dry ice
- Blades
- Plastic slides
- Slide box
- Pencil
- Large and small paint brushes
- 70% ethanol
- Gloves
- Glass slides (prefer poly-L-lysine-coated)
- Chitosan
- Acetic acid
- dH_2O

Fixation

1. Fix the tissue with 10% NBF at 4°C until properly fixed (~1 hour per mm tissue thickness).
2. Perfuse the tissue with 30% sucrose solution (in 1x PBS) overnight at 4°C.

Embedding

1. Place a labelled empty cryomold on dry ice in a container for 1 min. Keep on dry ice during the entire embedding procedure.
2. Cover the bottom of the cryomold with ~2-3 mm OCT.
3. Remove excess tissue and place the specimen to be frozen against the bottom of the cryomold in the OCT before it hardens.
4. Fill the cryomold containing the base of OCT and frozen tissue with more OCT. Cover the dry ice container and allow the OCT to harden.
5. Wrap the block in aluminium foil and keep in -80ºC until cryosectioning.

Tape (Cryofilm)-Stabilized Sectioning

1. Put on gloves
2. Chill tools in cryostat chamber
3. Wipe chamber, platform and blade with 70% ethanol
4. Set chamber to -22ºC, let cool down for about 10mins
5. Turn on light
6. Acclimatise blocks in recessed area
7. Put paintbrushes into cab
8. Squeeze block of OCT/tissue out of plastic mould
9. Put OCT on chuck
10. Press OCT/tissue block onto the chuck rough side down before the OCT hardens completely
11. Freeze OCT to equilibrate to the cryostat temperature (~15 min)
12. Chill slides in cryostat
13. Move glass safety and insert blade
14. Insert chuck, orient and tighten bolt
15. Trim the block by moving the stage

a. Anti-clockwise = closer

b. Clockwise = further away

16. Set cutting thickness to 20 µm
17. Trim by turning wheel away to expose tissue
18. Use big paintbrush to clean block
19. Use small paintbrush to check section
20. Adjust cutting thickness to 5-8 µm
21. Turn wheel 1-2 times to change thickness
22. Cut a piece of cryofilm large enough to cover the region of interest and pre-chill in the cryostat (multiple pieces can be cut and store in the cryostat)
23. Remove non-adherent backing from the cryofilm by grapsing the tape by the non-sticky silver/gold tabs using forceps then place the tape on to the block sticky-side down
24. Apply pressure to the cryofilm using the roller.
25. Cut section and collect by gently pulling them out with forceps
26. Place the section tissue side up on a plastic slide within the cryostat

27. Remove the plastic slide from the cryostat and allow OCT to melt such that the section adheres to the surface of the slide
28. Store slide in pre-cooled slide container
29. Repeat sectioning for serial sections or other regions of interest
30. When finished cover exposed tissue with a drop of OCT to prevent freeze-drying and store the rest of block at -80ºC

Transferring to Glass Slides

1. Prepare a 1% w/v chitosan adhesive by dissolving 1 g of chitosan powder in 100mL of acetic acid solution (0.25% v/v) and stir the solution overnight. Store at room temperature.
2. Deposit a drop of chitosan solution on the slide for each section that will be transferred
3. Cut off the silver/gold tab of the tape and place each taped section tissue side up onto the adhesive. Avoid bubbles
4. Drag excessive chitosan to the bottom edge using forceps
5. Place slides on top of a paper towel in a slide box. Gravity will then drag the excess chitosan to fall down onto the towel
6. Place the slide box with its lid propped open in the refrigerator overnight to allow the chitosan to dry

References

- Dyment, N.A., Jiang, X., Chen, L., Hong, S.H., Adams, D.J., Ackert-Bicknell, C., Shin, D.G., Rowe, D.W. (2016) *High-Throughput, Multi-Image Cryohistology of Mineralized Tissues*. J Vis Exp, (115) e54468.
- Kawamoto, T. (2003) *Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants*. Arch Histol Cytol, 66(2):123-4.