Vezina Lab Vibratome Sectioning

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1 *Works for me* This protocol is published without a DOI.

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PROTOCOL CITATION

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At least one day prior to starting

1 Make molds and vibratome blades

2 Soak forceps & molds in RNase inhibitor soln

3 Prep low-melt agarose

Prepare sample tissues

4 Remove dehydrated tissue from –20°C storage

5 Rehydrate through series of MeOH / PBSTw washes at room temperature with rocking
a. 1 x 10 min with 75% MeOH / 25% PBSTw
b. 1 x 10 min with 50% MeOH / 50% PBSTw
c. 1 x 10 min with 25% MeOH / 75% PBSTw
d. 2 x 10 min with 100% PBSTw

Embed tissue in agarose

6 Transfer one UGS into petri dish of PBSTw, use forceps to remove loose tissue and debris from around UGS, trim off approximately two-thirds of end of bladder

7 Prepare tissue and mold for embedding a. place a single mold, flat surface down, on plain glass slide b. set a timer for 2 min, remove agarose from drying oven c. use plastic transfer pipet to add enough agarose to fill mold (~8 – 10 drops) d. start timer, return agarose to oven

8 Embed tissue
a. when 1 min remains on timer, pluck cleaned tissue sample out of PBSTw & blot dry on Kimwipe (note: it is important to dry tissue as completely as possible to allow agarose to bind directly to tissue)
b. when agarose has cooled for 1 min 50 sec, transfer tissue from Kimwipe to mold, gently poke tissue into agarose with forceps
c. for saggital sections, orient sample on side, parallel to slide & in middle of mold
d. after sample is oriented, place mold in refrigerator to set agarose
e. Note: adjust cooling time based on how first sample sinks in agarose. If sample rapidly sinks to bottom, increase cooling time by 10 sec increments; if sample will not sink because agarose is thickening, decrease cooling time
f. Note: if sample is not oriented properly, carefully peel out of agarose & re-embed; for very minor adjustments, try trimming flat surface of agarose with razor blade

Vibratome sectioning

9 Prep vibratome
a. mount specimen bath inside vibratome bath using set screws
b. insert blade into blade holder, be sure not to push blade too far back into holder or blade angle will be incorrect
c. use blade angle indicator to set blade angle at 35°, tighten screw
d. fill specimen bath with 1X PBS e. pack wet ice around specimen bath

10 Mount sample
a. pop solidified agarose plug out of mold, blot flat bottom surface dry with Kimwipe
b. if necessary for orientation, cut round border of plug on one side to create a flat edge, then cut a small bevel in that edge
c. put drop of superglue onto round specimen mounting disk
d. place plug, flat surface down, into glue & allow glue to dry
e. slide mounting disk into specimen bath, taking care not to cut plug on blade
f. use screwdriver to tighten mounting screw to hold specimen disk in place

11 Section sample
a. advance blade into plug by 100 – 300 µm cuts at high speed (setting = 6)
b. as blade approaches tissue but before entering it, adjust settings to speed = 2, amplitude = 4, section thickness = 55 µm
c. use blunt forceps to collect each slice as it comes off of blade
d. transfer slice into individual well of 24-well culture dish containing 0.5 mL PBSTw, keep slices in order for serial staining, keep plate on ice
e. Note: watch ice surrounding specimen bath throughout sectioning process, replace ice as needed & remove excess water so specimen bath doesn't flood

12 Examine slices under scope, discard those that will not be useful

13 If desired, photograph ea sample slice to serve as reference for planning experiment

14 Store sections at 4°C for use 8on following day

Solutions

15 **4% agarose:** 2 g low-melt agarose in 50 mL PBS

16 **PBSTw:** 1X PBS + 0.1% Tween 20, add 1 µL of 0.2 M sodium azide per 1 mL PBSTw to prevent contaminating growth, sterile filter to remove insolubles/contaminants

17 **0.2 M sodium azide:** Dissolve 1.3 g sodium azide in 100 mL double-distilled H2O, pH to 7.6 (note: quite sensitive to pH change so need very little NaOH to adjust)

18 **Agarose:** Make 4% low-melt agarose in PBS (measure 2 g agarose and add to 50 mL PBS)
Microwave briefly to get agarose into solution, prep for embedding by cooling to 62° - 65° in drying oven
Agarose soln may be stored at RT, then melted & reused for up to 6 – 8 weeks