

SECTIONING PROCEDURE FOR LASER CAPTURE MICRODISSECTION

(Procedures for Soybean)
(Min Chen, August 2009)

Slides needed: PEN-Membrane Slides (Leica)

There are 2 different PEN-Membrane slides.

1. Regular slide (Cat No. 11505158). See the next section about how we need treat them with DEPC.
2. Nuclease and Human nucleic acid free slide (Cat No. 11505189).

Preparations of RNase-free PEN membrane slides:

Following steps are done in a fume hood. Wear gloves.

1. Place a NEW piece of bench paper on the bench of a fume hood.
2. Place in the fume hood following items: (a) **two** DEPC-treated and baked glass staining dishes, (b) **five** DEPC-treated and baked glass slide racks and their spring wire handles, (c) a box of PEN-membrane slides.
3. Transfer 10 PEN-membrane slides from their slide box onto each of five DEPC-treated and baked glass slide racks.
4. Prepare ~500 mL of ~0.1% FRESH **DEPC water** in a DEPC-treated and baked glass bottle by adding 500 mL of DEPC stock solution (D-5758, Sigma) to Milli-Q water and shaking the bottle vigorously to dissolve DEPC droplets.
5. Pour ~200 mL of freshly prepared DEPC water into each of two DEPC-treated and baked glass staining dishes.
6. Dip each rack of slides in the dish of DEPC water for 1-2 minutes. So, two racks are processed at each time. *Note: slides can be not treated for more than 2 minutes.*
7. Remove the racks of slides from DEPC water using spring wire handles and rattle the racks to shake excess water off the slides.
8. Air-dry the racks of slides on a NEW piece of aluminum foil wiped with DEPC water for overnight.
9. Repeat steps 6-8 for the remaining PEN-membrane slides.
10. Wipe the inside of the box of PEN-membrane slides with a piece of Kimwipes wetted with DEPC water.
11. Air-dry the box on another piece of aluminum foil wiped with DEPC water for overnight.
12. Next morning, prepare a FRESH DEPC water as in step 1 to clean gloves for handling DEPC-treated slides.
13. Put on rubber gloves and pour a small amount of Freshly prepared DEPC water on the gloves, rub them together, and dry gloves with Kimwipes.
14. Transfer DEPC-treated slides from the slide racks into the DEPC-treated slide box.
15. Put a piece of white tape on the slide box and write on the tape "DEPC-treated slides, Date, and your initial."
16. Store the DEPC-treated slides in a drawer until needed.

Treating All Equipments with Fresh DEPC'd Water Before Mounting Tissues onto Plastic Blocks:

1. Make a fresh DEPC'd water by adding 0.5ml DEPC into 1000ml ddH₂O and shaking well.
2. Take several sheets of KimWipe and wet with fresh DEPC water made at step 1.
Wipe all equipments:
 - A Razor blade
 - A piece of aluminum foil (for cutting the block)
 - Ethanol lamp
 - Spatula
 - Marker
 - Plastic blocks

Mounting Tissues onto Plastic Blocks:

1. Light up the ethanol lamp.
2. Cut the paraffin block from the aluminum boat. Clear as much paraffin as you can.
3. Heat up one end of the spatula under the ethanol lamp.
4. Apply heated end of the spatula under the paraffin block to melt paraffin and quickly apply to plastic block. Repeat to ensure that the block is steady and secure on the plastic block. Add extra paraffin on the side, if needed, to secure paraffin block to plastic block.
5. Place the plastic block in the refrigerator to harden the paraffin. Incubate for several hours.
6. Before sectioning, trim off part of the paraffin from the tissue. This will reduce the size of your section and will enable you to have more sections on each slide. Don't trim too close to the tissue, as that will prevent the sections from forming a ribbon.

Preparations:

Thickness of sections: 3-10 um depending on the tissues you collect. It is easy to identify the tissues with thinner sections. But in order to get same amount of RNA/DNA, it will require more work for sectioning and LCM capturing from thinner sections than from thicker sections. Basically, as long as you can clearly identify the tissues, you can try thicker sections.

Treating All Equipments with Fresh DEPC'd Water Before Sectioning:

1. Turn on a slide incubator and set to 42°C first.
2. Make a fresh DEPC'd water by adding 0,5ml DEPC into 1000ml ddH₂O and

shaking well.

3. Take several sheets of KimWipe and wet with fresh DEPC water made at step 1. Wipe all equipments:
 - Slide incubator
 - Microtome
 - Microscope
 - Brushes
 - A regular DECP treated PEN membrane slide (for examination)
 - Ribbon holder (wrapped with aluminum foil)
 - Pipetman
 - Surgery blade and handle
 - Pen and Pencil
 - Tip box
 - Section Blade
 - A piece of aluminum foil (for cutting the block)
 - The box of PEN membrane slides
 - Screw driver/handle
 - 50ml centrifuge tube with DECP'd water

Sectioning on the Microtome:

1. Trim the block. When trimming a paraffin block make sure that the upper and lower lines of the edge of the block is parallel as it is facing you in the microtome. This will ensure that your ribbon will be straight, and not bend. When sectioning many loose little items pooled together like tobacco or Arabidopsis seeds, make sure that -when trimming- to leave a little area of paraffin around the specimens. This will help in getting the sections stick together and form a long ribbon.
2. Make sure all the handles are locked before placing the section block on the microtome.
3. Place the block on the microtome and fasten the block.

Note: At this step, remember to place the block so that the widest end of the section block is at the bottom. This will prevent the ribbons from curling up when sectioning.

4. Release the blade apparatus so that the angle is now at zero.
5. Line the paraffin section against the blade apparatus so that the section is parallel to the blade apparatus. Adjust the paraffin section using the adjustment knobs holding the block in place. There are two knobs, one for adjusting right to left and one for adjusting up and down. This step will help ensure that the blade lines up with the section block and hopefully will give us better section.

Note: This does not mean that you will always get good sections. It depends on the orientation of the seed that was embedded and the orientation of the embryo.

However, most of the time this step will help ensure that you have a few good sections.

6. Once the section block is lined up with the blade apparatus, move the blade apparatus back ~ .1mm from the section block.

Note: Use the different section of blade for different blocks.

7. Adjust the angle of the blade to 2-3. The measurement of the angle is on the right side of the blade apparatus.

8. Make sure that the angle of the blade is locked and the blade apparatus is also locked.

9. Make sure the apparatus holding the section block is locked.

10. Adjust the thickness of the sections appropriately.

11. Start sectioning.

Note: The first 20-30 sections are probably not going to contain any part of the embryo. With experience, you will know how many sections you must do in order to get to the embryo. However, to ensure that you will get good sections, try to keep the ribbon as long as possible. Use a brush and needle to slowly guide the ribbon so that it will stay intact. Longer ribbons are easier to work with than shorter ones. However, you may not get long ribbons at all. Sometimes, this could be due to the position on the blade you are sectioning. If that's the case, move the blade apparatus so that the section will be in a new position on the blade.

12. Sectioning until you can see the embryo, then sectioning until the ribbon has eight to ten sections.

Note: If your sections curl up, preventing the formation of a ribbon, try sectioning the block with the knife set at a different angle.

13. Place the ribbon in order onto the regular DEPC treated PEN slide using brush and surgery blade.

14. Examine the sections under the microscope. You want to see sections containing an intact embryo with both the suspensor and embryo proper shown.

Note: Examine the position of the sections in the ribbon. For different stage seed, the sections mounted onto the slide are different

15. If you find that you have good sections you want to keep, place those sections on the ribbon holder and discard the ribbons you don't want to keep.

Note: I usually section a ribbon with eight to ten sections, then transfer it onto the regular DEPC treated PEN slide. After I have six to eight ribbons, I will examine them under the microscope. The number of section per ribbon depends on the size

of the block. Basically, the length of the ribbon is a little shorter than the height of the rectangle area on the PEN slide. We try to put as many section as possible. Since the sections will expand, we need to leave some space for expansion.

16. Get a RNA-free PEN membrane slide and put it on the slide incubator. Make sure you put the side with membrane up. Place about 1.5ml of DEPC'd water on the slide.

Note: As you can see, there is a rectangle area on the slide, where the membrane is not attached to the glass slide. Try to put water only cover that area, so you can maintain the position of ribbons on the slide.

17. Gently place the ribbons onto the water. DO NOT touch the surface of the membrane. Arrange the ribbons in order. Usually, 13-15 ribbons are enough to cover the rectangle area.

18. Leave the slide on the slide incubator about 15-30 minutes. Remove the extra water using pipetman. Let the slide dry for about 1 hour, then save the slide in a DECP treated slide box and store at room temperature until next step.

19. A day before LCM capturing, de-paraffin the slides by dipping the slides in 100% xylene for 2 min twice. Let slides air-dry in the hood for overnight. Make sure briefly cover the slides with DEPC-treated aluminum foil. Put the de-paraffined slides back to DEPC treated slide box and store at room temperature until LCM capturing.

Note: After embedding seeds in paraffin, section and capturing tissues ASAP to get best quality of RNA/DNA. For RNA isolation, it would be better to finish sectioning and capturing in a month. For DNA isolation, it would be better to finish sectioning and capturing in 3 months.