Tomato Root Analysis Protocol
December 2010

Rehydration of Embedded Samples

Preparation:

- Forceps
- Latex gloves
- 5-15 small glass vials (5 per plug)
- 70%, 50%, 30%, and 10% EtOH
- ddH2O (MilliQ water)
- Agarose- embedded samples in FAA (plugs)

1) Select a maximum of 3 IL embedded root samples to analyze per day
2) Prepare the rehydration series, 70% EtOH, 50% EtOH, 30% EtOH, 10% EtOH and ddH2O, for <u>each</u> sample (plug) using small glass vials and label each vial in pencil
3) Carefully place the embedded samples into the vial with 70% EtOH using a pair of forceps and leave for 30 minutes
    a. Be sure to keep track of which IL is in which vial!
4) Repeat step 3 with the each solution in the series
5) The embedded samples can be left in ddH20 for up to a week if necessary

Preparing the Vibratome and Sectioning the Samples

Preparation:

- Ice bucket with ice water
- Fine- tipped paintbrush
- Sectioning block
- 12-well cell culture plate
- Krazy Glue (in vibratome room)
- Double- edged razor blade (in vibratome room)
- Agarose- embedded sample in ddH2O (plugs)

1) Label the wells in the cell culture plate with the genotypes of the samples
2) Cut a 1 cm section off the embedded sample with razor blade and trim the ends so the sample is perpendicular to each end of the section
3) Attach the agarose- section to the sectioning block with krazy glue, ensuring that the least damaged part of the roots are facing upwards
4) Let the krazy glue dry while completing steps 4-5 (needs several minutes to dry completely)
5) Break the double-edged razor blade in half and place one of the halves securely into the vibratome by pushing downward on the thin lever to open the blade clamp
6) Adjust the blade angle so the block holding the blade is perpendicular to the sample
    a. Loosen or tighten the large screw at the top of the block
7) Place the sectioning block with the attached sample into the vibratome and tightly screw into place with the large screw on the right

a. Be sure that the sample is well- secured to the sectioning block before placing in the vibratome

8) Fill the vibratome with ice water (avoid ice!) using the turkey baster until the sample and the edge of the razor blade are completely covered by the water
9) Turn the main dial counter-clockwise to raise the sectioning blade until it appears to be above the sample
    a. Clockwise- lowers the blade
    b. Counter-clockwise- raises the blade
10) Turn the vibratome on with the switch to the right and the lamp will automatically turn on
11) Use the switch on the left to control the blade movement and make certain that the blade is slightly above the sample. If it is not above the sample, adjust blade height with the main dial
    a. Up- Blade forward
    b. Holding Up- Blade fast forward (If you hold the switch up and then release, it will procedure forward slowly and will not automatically reverse until past the sample!)
    c. Down- Reverse
12) Make a thin sample cut by pressing up on the lever to move the blade quickly forward and then releasing just before the blade reaches the sample- the blade will continue forward and section the sample
13) Carefully capture the thin sample section floating in the water with the fine-tipped paintbrush and discard
14) Turn the main dial clockwise to cut a 120um section (1 full turn = 100um)
15) Capture the section and transfer to the appropriate well in the cell culture plate

Staining the Sections for Microscopy

Preparation:

- Toluidene blue stain with pipette
- ddH2O
- Pipettor and pipette tips
- Glass jar and pipette for discard stain
- 50% glycerol and pipette
- Globe scientific slides
- Glass cover slips
- 12 well cell culture plate with sections

1) After collecting 10-20 sections from a sample, remove the water from the well using a pipette
    a. Avoid damage to the sections by pressing the pipette into the bottom edge of the well
2) Fill the well half way with toluidene blue stain and gently swirl for 5 seconds
3) Pipette ddH2O into the well until it is ¾ of the way full and gently swirl for 5 seconds
4) Immediately remove the stain with the stain removal pipette and discard into the glass jar
5) Pipette ddH2O into the well until it is ¾ of the way full and gently swirl for 10 seconds
6) Immediately remove the ddH2O from the well and discard into the glass jar
7) Repeat steps 5-6 two more times

8) Carefully transfer the stained sections onto a labeled slide with the paintbrush and dab off excess H2O with a kimwipe
    a. Maximum of 8 sections per a slide
    b. Roots can fall out of the agarose sections if left in the ddH2O for too long or handled roughly
9) Add 2-3 drops of 50% glycerol to the sections with the glycerol pipette and cover with a glass cover slip
10) Check the quality of the sections under the microscope and capture images under 20x magnification
11) Label the images in the following way: *Genotype_Plug#_Root#_Initials*
    a. Sample image name: IL1-1_PG1_**Root1A**_MM
    b. Image of the same root in another section: IL1-1_PG1_**Root1B**_MM
    c. Image of a different root in the same or another section: IL1-1_PG1_**Root2A**_MM

Mallorie's Tips!

If most of the roots are popping out of the agarose sections...

- Change the blade angle (move the block holding the blade slightly more towards you)
- Try slightly thicker agarose sections (150um)
- Do not leave the 120 um sections in ddH2O for longer than ½ hour