

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 each 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 ddH₂O 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

