General protocol histology
Leonardo Galindo

1. Fixation:
    a. Rinse tissue to be sectioned (e.g. roots) with 10% hypochlorite and 3 times with water (use four beakers for solutions), and cut with a clean blade (sterilize every time with 70% ethanol) in sections of 3 to 5mm.
    b. Place sections in FAA (see below) for a minimum of two days.
2. Dehydration
    a. Load tissues into cassettes. Use the cassettes that have a smaller pore than the sections.
    b. Place immediately in 50% ethanol for 1to 4 hour.
    c. Transfer to 70% ethanol and load onto the tissue processor: Leica TP-1020.
    d. Tissues can sit in 70% ethanol for a minimum of 2 hours.
    e. On the Leica TP1020 the tissues are further dehydrated through 70% Ethanol, 90% Ethanol, 100% Ethanol, Toluene, and then into paraffin wax.
3. Embedding
    a. Tissues are embedded into blocks using a TISSUE TEK II embedding center.
    b. Place cassettes with tissue into a warm wax bath (60°C).
    c. Pre-warm metal block molds by placing them at the metal edge of the wax bath (this will allow the wax to stay liquid for longer once it is poured in).
    d. Withdraw one cassette outside the bath and open carefully making sure the tissue stays inside. Place cassette with tissue back in the wax bath slowly to avoid sections floating out (a couple of cassettes can be used to sit the opened one).
    e. Fill a block mold half way with hot wax and quickly take it back to the edge of the wax bath.
    f. Take 2 to 3 pieces of tissue and place them touching the bottom and standing up to achieve the transversal sections (alternatively lay down flat for longitudinal sections). Change tweezers often when wax polymerizes to ease tissue manipulation (tweezers are stored in a hot holders to melt the wax).
    g. Wait 10 to 20 secs for some polymerization and while waiting place the plastic holder that matches the mold on top.
    h. Fill the assembly to the top and place on cool table to dry.
    i. Wax blocks will be ready to withdraw from metal wax holder in 10 minutes. Wax blocks can stay like this at room temperature until sectioned.
4. Sectioning
    a. Once the paraffin blocks are cooled they are sectioned on the microtome (Leica RM2125). Section thickness is 5 to 15 micrometers.

b. Set microtome to the desired section thickness (e.g. 8µm for pilot experiment). Make sure blade safe is on place and the movement of the block holder is locked.
c. Place block in the microtome holder and adjust the distance of the block away from the blade.
d. Start moving block up and down and closer to the blade using a quarter of a turn of the coarse crank (left handside) each time. Make sure to start using the blade on the left and move the blocks to the right as blade deterioration is seen on the sections.
e. Once the block touches the blade use the fine crank (right handside) to start doing the sections until you reach the tissue.
f. When you reach the sections move the fine crank slowly and hold down the section with a brush. Then move the crank fast to get consecutive bound sections.
g. When you achieve 4 to 5 bound sections use the finger and brush to pick up the sections and place them in a water bath (46°C) face up.
h. Immerse a glass slide diagonally bellow the sections and direct the sections to the slide using the bush. When the sections are on top of the slide withdraw the slide from the water so the wax sections stick on top.
i. View sections on a microscope to confirm quality.
j. If sections are satisfactory withdraw block from microtome (remember to lock the crank and place the blade safe before moving the block), and make 45 degree cuts around the tissue (this allows to have more sections in the final slide).
k. Replace block on microtome and get sections as described before but place final sections in quality labeled slides and leave to dry on slide holder.
l. Take slides to a 37°C at least over night to completely dry.

FAA fixative for microscopy preparations in histology and SEM of plant material

<u>To make 50mL in a falcon tube:</u>

25ml of 100% ethanol (from BioStores)
17.5mL of distilled water
5mL formaldehyde fisher F-79 (37%)
2.5mL acetic acid

Fix for a minimum of 2 days (under vacuum is ideal)