Protocol – Making Agarose Plugs for Sectioning

Gather roots 7 days after germination.

Prepare 2% agarose gel in a flask.

Distribute gel into several Eppendorf tubes while gel is still in liquid state.

Keep Eppendorf tubes on a heat block at a high enough temperature to keep the gel in liquid state.

Cut out 1-2cm section of the root near the MIDDLE of the root. (where you cut depends on what developmental zone you are looking at, but we are generally focused on the maturation zone)

Gather up to five of these 1-2cm root cuttings into a bundle.

Using forceps, pick up the bundle (being careful to only grab one end of the roots) and insert the bundle into one of your Eppendorf tubes containing agarose.

Try as best you can to keep the bundle of roots standing up straight in the tube.

Wait for agarose tubes to solidify.

Once tubes are solidified, you will remove the root-containing gel plug to a vial containing formalin acetic acid alcohol (FAA) **[50% ethanol(95%), 5% glacial acetic acid, 10% Formalin, 35% water]** to preserve the plugs.

Heat up a scalpel using a Bunsen burner and quickly cut off the bottom (tapered) end of the Eppendorf tube. You can then use any thin device (a pencil perhaps) to push the plug out from the cut end through the lid into your vial containing FAA.

The plugs can stay in FAA for up to two weeks before rehydration and sectioning, but I wouldn't wait too long. The protocol for rehydration and sectioning is on a different document.