Plastic Embedding of GUS - Stained Arabidopsis Seedling Root

1. Prepare 20 ml of 1% agarose in 0.1 M sodium phosphate buffer (pH 6.5 or 6.8) and dissolve it by heating.

2. Pour the agarose solution into suitable mold and place mold on top of a heat block adjusted to 45 degrees. We use the lid part of a square petri dish as a mold, and set the heat block to 65 degrees because there is a gap between the surface of the heat block and the mold.

3. Let the agarose cool (to the point when you cannot feel heat from it).

4. Pick up the seedling using forceps and embed it into the agarose solution quickly. About 20 seedlings can be set in a square plate. They don't need to be kept very straight, but they should be well separated. You don't have to take too much care to keep the seedlings straight.

5. Turn off the heat block and let the agarose solidify.

6. Cut out the agarose block. The width of the block is not important (usually 2-3 mm), but the length should be approximately the length of the mold.

7. Do the GUS staining of the roots within this agarose block. We usually use 6 to 7 blocks in a 1.5 ml eppendorf tube. Make the GUS staining solution assuming that 1 gram of agarose gel equals 1 ml of volume. At this time, don't forget to account for the fact that the agarose block is sodium phosphate buffer.

8. After staining, move the agarose blocks into a 20 ml scintillation vial which already has 5-10 ml fixation solution. Place the vial at 4 degrees for 3-5 hours, then change the fixation solution and put it at 4 degrees again overnight. We typically put 12-14 blocks into a vial and fix them with 4% paraformaldehyde in PBS.

9. Do the dehydration series: 15%, 30%, 50%, 75%, 95%, 100% ethanol, each step for 1 hour. Change the 100% ethanol with new 100% ethanol and place them at 4 degrees overnight.

10. Infiltrate the agarose block with infiltration resin under vacuum for 6 hours, then replace with fresh resin and put them back under the vacuum. At this step, we wrap the scintillation vial with aluminum foil.

11. Embed the agarose block, seal it to make an anaerobic condition, and place at 4 degrees overnight.

12. Do sectioning as usual.