

## **Fixing organoids for Histology**

### **Embedding organoids in low melting point agar**

1. If not already aliquoted, prepare 2% low melting point agar in water and put into 1.5mL snap cap tubes. Unused aliquots of 2% agar can be kept in the fridge for years
2. Warm a heating block to **65°C**. Heat the agar in the block until liquefied. In the meantime, prepare labelled snap cap tubes for all samples.
3. Once organoids are settled to the bottom of their tubes (in 70% EtOH), pipette excess ethanol away, leaving the organoids in **approximately 100µL volume**.
4. Transfer organoids in ethanol to corresponding labeled snap cap tubes.
5. QUICKLY add **300µL agar** per tube & mix carefully.
  - a. Avoid bubbles but mix thoroughly.
  - b. Work quickly as agar polymerizes/solidifies rapidly.
6. Centrifuge tubes for **5 minutes at 4°C at 500G** to aggregate the organoids and allow the agar to polymerize around them.
  - a. If unsatisfied w/ the organoids' location within the agar, put the snap cap tube back on the heating block for ~10 minutes and centrifuge again.
7. Label histology cassettes for each sample using **pencil**
8. Add small amount of ethanol to each tube (containing organoids + agar) and flick the tube very hard to dislodge the contents.
9. Remove agar w/ organoids embedded from tubes.
  - a. Gently grab the agar puck with forceps so as not to damage/break the agar; or
  - b. tip the whole agar puck out of the tube once it is dislodged.

10. Make a **transverse** cut of the agar puck with bias towards the tip of the cone where the organoids are.
  - a. Do not cut the portion of the puck containing the organoids too thin or it could curl once embedded in paraffin, leading to them occupying different planes within the block.
11. Place both halves of the cut agar puck inside the labelled cassette for the sample. Add a **foam insert** to the cassette if you think the sample could fall out of the cassette.
12. Place the entire cassette into a bucket of **70% ethanol** and submit for paraffin embedding.