Shuaibin (Stephan) Chang’s brain embedding protocol

Wear double layers of mask, wear the white coat and gloves

Prepare a set of embedding mold and base

1. Removing dura
   1. Please refer to the video on basecamp: <https://3.basecamp.com/3596813/buckets/2063119/todos/4366703696>
   2. Use sharp but round tweezer, perform inside the fume hood for safety
   3. Gently open every sulcus to remove the dura inside, in the meantime if sample is dehydrated, put it back to PBS for hydration, and then continue.
   4. After fully removing dura, put sample back to PBS for hydration
2. Cook agar
   1. Use 4.5% agarose in 1X PBS(2.25g per 50mL, 4.5g per 100mL)
   2. Use large (500mL) beaker to avoid overflow when cooking
   3. Use another large container (large enough to hold the 500mL beaker) to cook DI water to about 90 degree
   4. Let the DI water cool in air, then cook 4.5% agar. When cooking, cover the beaker with paper or tissue to reduce water evaporation
   5. When agar is boiling, take it out of the microwave oven and stir with a spoon for better mixing water with agar
   6. Cook for another 30-60 seconds, then take it out to cool in air. Bubble will disappear when cooling.
3. Embedding
   1. Use an ice box or any water-proof container (or one of the bath), get some ice from the ice maker next to the lab
   2. When agar is cooled to 70 degree, check if DI water is between 60-70 degree. If not, cook more or add cold water
   3. Immerse sample in DI water to warm up
   4. When agar is cooled to 65 degree, take out the sample, dry it using paper
   5. Use tweezer or other tool to remove the superficial layer of agar, take it out or push it to the side of the beaker, then immerse the sample in agar
   6. Cook the DI water for about 30 seconds, then immerse the whole beaker in the DI water, so that agar will solidify slowly
   7. Take the whole thing inside the fume hood, use spoon to gently squeeze the sample to remove the air bubble in sulcus. Repeat a couple times until there’s no air bubble in each sulcus
   8. Take the beaker out of fume hood, use spoon to take the sample out and put inside the embedding mold, with the flat surface of the sample facing downward.
   9. Pour more agar to fill the mold, when the mold is almost full, put on the embedding base. Gently adjust the base to make sure it’s fully covering the mold **without tilting**.
   10. Put the mold inside the ice box, surrounding it with ice. Pour more agar onto the base until there’s a convex surface. When cooling, the agar will shrink and leak, so more agar on top is better. Use tweezer to remove air bubble if there’s any in the holes of the base.
   11. You should sit down and monitor when the agar solidifies. If the embedding base was not tilted, there shouldn’t be much leak, and the agar surface will stay convex when it’s fully solid.
4. Retrieve the sample
   1. Follow the “nature crack” of the mold that comes from 3D printing, use the knife (you can find one in the wet lab) to cut an opening.
   2. Gently peel off the wall of the mold follow that opening, when the contact is loose, gently take out the sample.
   3. Use the large knife to cut off extra agar so the base is flat.