JB4 Embedding Protocol

1. **Materials (only essential ones)**

Day 1:

100% ETOH (under fume hood)

JB4 Solution A (under fume hood)

JB4 Catalyst (under fume hood)

PBS

Day 2:

JB4 Solution A

JB4 Catalyst

JB4 Solution B

Paraffin wax (in cabinet next to freezer)

Glass beaker to heat wax with (in the fridge)

Mold (in the fridge)

1. **Dehydration (day 1)**

\*samples should be fixed with 4% PFA prior to beginning the embedding process.

Dehydrate the sample by mixing the following concentrations of ethanol and PBS in a conical tube, then adding the sample. If the sample is already in the tube, add PBS first then ethanol.

1x50% ETOH in PBS (15 mins)

1x70% ETOH in PBS (15 mins)

1x90% ETOH in PBS (15 mins)

1x100% ETOH in PBS (15 mins)

Cover the outside of the conical tube with foil and mix on a rotary plate for the designated amount of time. The volume of the solution should be about 6-8 times the volume of the tissue.

1. **Infiltration (day 1)**

Infiltration solution (for 10ml JB4)

10mL solution A

0.125 g catalyst

(You can adjust the amount by how much solution you need. For example, 5mL solution A + 0.0625g catalyst)

Weigh out the catalyst and add to an appropriately sized conical tube.

Measure out the desired amount of solution A using a graduated cylinder.

Add solution A to the conical tube.

Invert a few times to mix ,and place the JB4 solution on a rotary plate for 20 minutes or until all of the catalyst is dissolved.

\*Infiltration Solution can be kept for up to two weeks at 4°C

Add the same volume of solution used in the dehydration step to the tube containing the sample according to the concentration and times below

3x100% JB4 solution (30 minutes, overnight, 30 minutes)

-----------------------------------------End of day 1--------------------------------------------

(Make sure to finish up the last 30 minutes of infiltration, using the infiltration solution from day 1)

1. **Polymerization (Day 2)**
   1. Make fresh JB4 solution (solution A + catalyst) as instructed in the infiltration step. The amount depends on the number of samples. For the smallest molds, use 1 mL of JB4 for each sample; for the largest molds, use 3 mL of JB4 infiltration solution (step II) for each sample.

\*Make sure the JB4 used for polymerization is made fresh that day. If you use JB4 that has been in the refrigerator it will not polymerize. Unless you need more for infiltration solution, only make enough for the samples you need to embed that day to avoid waste. If using colored JB4, see step IV.

* 1. Heat up paraffin wax in glass beaker (there’s a beaker that’s been used for wax in the fridge with the SETI sample mold etc).
  2. Place prepared samples in the desired mold.
  3. Add solution B (activator) to the tube containing JB4 and mix well. For each 1mL of JB4 solution, use 0.04mL of solution B (eg. 5mL of JB4 ------ 0.2mL of solution B). (If you are adding fluorescent beads to the sample, add them now).
  4. Pipet JB4 (with solution B) into the molds containing samples. Wait for 5-10 minutes until the solution starts to gel, before putting the plastic caps on the molds.
  5. Using a transfer pipet and hot paraffin wax, seal the gap around the cap.
  6. Add a blob of wax on the hollow core of the plastic cap to seal as well
  7. Place in samples in the fridge and allow to polymerize overnight

Complete polymerization takes 12-24 hours

\*JB4 absorbs moisture in the air, making it softer, so store samples should be stored in a low humidity environment such as a refrigerator or desiccator

1. **Coloring JB4 (optional step)**

Eosin- weigh out 8 mg/mL of JB4 solution. Mix well by shaking or using the vortexer.