Sample fixation and vibratome sectioning

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Recipes

- **Prepare 7% agar (for 100mL)**

7g	TC agar
0.15g	KCl
0.15g	CaCl2
100mL	water

- **FAA**

		Final conc.
Ethanol	50 ml	50%
Glacial acetic acid	5 ml	5%
Formaldehyde (37-40 %)	10 ml	4%
Distilled H20	35 ml	

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100 ml

Protocols

- **Fixation**

1. Cut a piece of stem with haustorium.
2. Melt 7% TC agar by using Microwave (soften in about 2 mins).
3. Pipette 1.2 mL agar in 2mL tubes.
4. Embed samples in 7% agar (push to near bottom but not to the end).
5. Put the tube on rack until agar become solid.
6. Heat the knife and cut the bottom of the tubes, then open the lid and push out the gel block.
7. Add 13mL FAA in in cylinder bottles.
8. Put the gel blocks in cylinder bottles, put in 4C overnight.

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1. Transfer gel blocks to 50% EtOH.
2. After 1hr, transfer gel blocks to 70% EtOH.
3. Samples can be preserved in 70% EtOH for a few months.

- **Vibratome Sectioning**

1. Put all the tools on ice (blades, brushes, small petri dishes), and prepare cold water.
2. Glue sample on the block with superglue, and put on ice. Make sure to wait until the glue become solid before you start sectioning.
3. Put cold water in vibratome sink.
4. Lower the stage (counter clock wise) put the sample on stage.

5. Put the blade on clip (switch up to lock the blade on the vibratome.)
6. Bring water to the blade level.
7. Rise the sample to the water level.
8. Adjust parameter: Speed -> 4; amplitude-> 4~4.2 (This can be adjust based on samples.)
9. Clockwise 1 cycle -> rise 100um (This can be adjust based on samples.)
10. Fishing the sections and put them in water in small petri dishes.

(Modified by Min-Yao Jhu)
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