

## Cryosectioning

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Take to room 3021:

- the petri dish with the frozen block of tentacles
- TFM
- a gasket slide
- a box of polylysine slides
- a PAP pen
- paraformaldehyde in a Coplin jar
- gloves
- a razor blade

1. Immediately place the tentacle block into the cryostat as well as a razor blade
2. Get the Taylor lab chuck and place on platform on left side and place a big blob of TFM on it and allow to freeze
3. Meanwhile, use the diamond pen to number 4 to 6 polylysine slides and then use the PAP pen to trace the outline of the gasket twice onto each slide
4. Using gloves, get the knife out of the Taylor lab box and slide into the slot so that the "Leica" faces to the left and finger tighten the black screws -keep the knife frost free with a brush
5. Find a nick-free part of the knife by brushing it with one of the stiff brushes and looking for nicks
6. Adjust the cover slip so that it just barely sticks out over the knife
7. Place a drop of TFM (make it a little bigger than the trimmed block) onto the big frozen blob of TFM on the chuck and let it freeze
8. Trim the block with tentacles in it: hold the block down with a probe and use a razor blade to cut off the edges to make a square, then cut off the edges of the square to make an octagon
9. Put the chuck on -push back on the lever, line up the red dots and turn clockwise
10. Advance the platform "quickly" (by turning on the orange light and then pressing the lowest white button) until the platform gets close to the knife
11. Then, start turning the crank in 50 um increments until a section or two are cut

12. Take off the chuck, put a small drop of TFM on it and then quickly, using the probe, pick up our trimmed block and stick it on the dot of TFM so that it sticks to the platform
13. Re-trim to remake the octagon -cut all the way down to the big blob, but don't cut through the big blob
14. Back the platform away from the knife (orange button and upper white button)
15. Put the chuck back on such that the flat side of the octagon is parallel to the knife
16. Bring the platform back forward (orange button and lower white button) until the block gets near the knife
17. Hold down cover slip and start making 50um sections until until you start seeing tentacle tissue
18. Place a polylysine slide in the cryostat for a minute or so to cool it and then place it on the cover of the cryostat
19. Adjust the knife to 20um and start making real sections
  - Hold down the coverslip as you are making each section.
  - To pick up each section, use a little TFM to make a sticky point on one of the fine brushes, then touch the brush lightly to the section to pick it up
  - Pick up the polylysine slide and bring it into the cryostat chamber
  - Place the section on the slide by touching the section to the slide -the section should come off the brush and melt onto the slide
  - Then put the slide back onto the cover, make another section, bring the slide into the cryostat chamber, place the new section on the slide, etc. -add as many sections as will fit within the PAP outline
20. When the slide is full of sections, slide it into the Coplin jar with paraformaldehyde. (If you need to wait to proceed, do not put in paraform. Instead put in dessicant in -80 freezer)

Troubleshooting:

Problem: Block too big: Make sure tentacles are close together with no space between them.