Protocol: Paraffin section of mosquito midgut

Day 1

- Make buffers and fixative
- A. 50mM potassium-phosphate (pH 7.0), make 10X stock
- B. Make 10% para-formaldehyde stock: Dissolve 5g para-formaldehyde powder (Sigma) in 50mL dH_2O by heating to 55-70^oC on stir plate in fume hood. Solution will be milky. Add 1M KOH dropwise to hot solution until it clears.s Cool. Stored in dark bottle in refrigerator for at least one week.
- C. Alternate fixative: FAA

45 ml 95% EtOH; 40ml dH_2O; 5ml glacial acetic acid; 10ml 37%(w/w) formaldehyde

- Make 4% para-formaldehyde: 4ml 10% para-formaldehyde, 1ml 10X PBS buffer, 5ml dH_2O. Fix the tissue in fresh made 4% para-formaldehyde, rolling at 4^oC for 5h to O/N.

Day 2

- Wash tissue with cold 1X PBS two times and dH_2O three times, 5min each.
- Dehydration: Pass tissue through an EtOH series, 25%, 50%, 70%, 30min each. Leave tissue in70% EtOH O/N or couple months at 4^oC. [It is important to make sure all the tissue are separated from each other.]

Day 3

- Continue dehydration with 100% EtOH twice, 30min each.
- Infiltrate gradually with xylene/HemoDe: 25%, 50%, 75% (in dry 100% EtOH), 30min each. Transfer tissue to glass vial with 100% HemoDe and incubate for 1hr, twice.
- Infiltration: Add 4-5 Paraffin chips (Paraplast Plus) to each vial containing 100% HemoDe. After 20-30 min, add 5 more chips. By the end of day, you should have added about 2 chips per ml HemoDe in your vial (like 20 chips/vial). Leave it O/N at RT. Fill a beaker full of chips and place in a 60^oC oven.

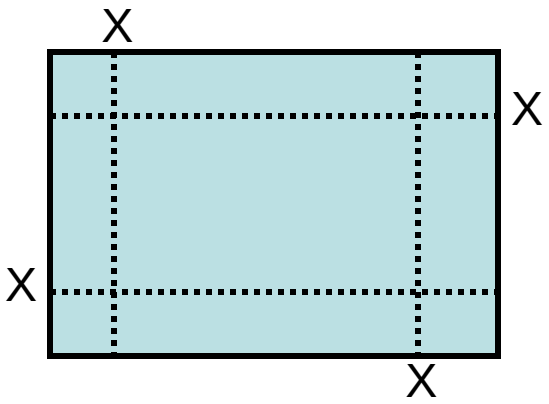
Day 4

- Check if the chips in the vials are at least partially solubilized. Incubate the vial at 42^oC to finish the solubilization process. Then take out 2ml HemoDe/wax mixture and add 5 chips, incubate at 42^oC for 20 min, change for 4-5 times till most solution contains wax (about 3 hr). Add 10 chips at the last change.
- Incubate vials at 60^oC till all wax melt. Pour out the old wax quickly, if necessary pipette out the leftover. Then add 60^oC preheat wax and incubate at 60^oC, total three times. [It is important to shake the vial to detach the tissue from the bottom.]

Following will be done in Plant science building

Things Needed: index card, vials with tissues, enough paraplast in 60^oC (In Dr. Perry's lab), key to main entrance or section room, or Perry's lab.

- Embedding: Turn on the water bath and hot plate. Warm up the vial in water bath.
- Make some boats as following. It has raised edges of 1 cm and enough area so that the samples will be well separated. Seal the edges using tape and label boats properly. Place boats in the hot plate and cover the edges with wax.



- Swirl the sample vial in the water bath until the tissue pieces move freely, quickly warm up the lip over a flame and pour the infiltrated tissue into the boat. Top off the boat with melted paraplast. Use a heated metal probe to arrange the tissue pieces so they are well separated and straightforward. Move the boat to the edge of hot plate (cooler). As soon as a slight cast has formed over the top, transfer the boat carefully to an ice bath. Use a heated metal probe to keep the top part of the block open until the bottom have solidified. Let the boat sit upright for 30 min or so. Then flip the boat over the ice and store at 4^{o}C O/N or until section.

Day 5

- Turn on the water bath and hot plate. Boiling the water with zeolite/ boiling cease, and keep it in water bath.
- Sectioning: Mark the good tissue and cut it as trapezia shape using heated knife, three from each boat. Then stick onto the wood block and label the block. Cut the wax block into small pyramid.
- Clean the section blade with HemoDe. Setup the blade and make the tissue facing you and straight by adjusting the position of wax block. Cut the sample block piece by piece (manually moving the blade toward sample slowly and carefully) until almost touch the tissue. Then cut tissue using 5-10 um and use a brush to hold the pieces.
- Put slide onto the hot plate ("+" side facing up) and add warm boiled and filtered water to cove the slide. Put wax pieces from three different tissue samples onto the slide row by row. Make sure the smooth side of wax pieces facing down and all the pieces floating on the water surface and no one attached the slide. Incubate on the hot plate for 10 min.
- Suck out the water with a glass pipette and remove the air bubble using filter paper. Incubate on the hot plate O/N to melt the wax. Then the slide can be stored at 4^{o}C.

==========**section stop here**==========

Day 6 (For DAPI staining)

- Deparaffinization: (warm up at 37^{o}C for 30min if slides are taken out from 4^{o}C.)
- Wash the slides with 100% HemoDe for three times, 5min each.
- Wash with series EtOH 1-3min each: 100%, 95%, 75%, 50%, 25%. Then wash with dH_2O, 1X PBS and 1X PBT(0.1% Triton X-100 in PBS).
- Clean the edge of slides and mark the edge using Hydrophobic maker, so solution won't come out. Add 100uL 1:1000 DAPI to the slide for 5 min, and wash with PBS. Then dry a little bit, and add 20ul glycerol and cover it.