

Scott's simplified squashing protocol

Squashing:

1. Dissect salivary glands from wandering 3rd instar in 1xPBS+0.1% Triton X-100 in a depression slide, or whatever you wish. Remove as much of the fat body as is possible (this shouldn't hurt anything, though).
2. Using a pipetman, place salivary glands in acetic acid fixative. Incubate glands in fixative for EXACTLY 2 minutes (longer fixation may adversely affect the squash quality).
3. After fixation, move glands to 45% acetic acid. The glands can be left in this solution for up to an hour (but squash the glands ASAP).
4. To squash, place 1-2 glands in drop of 45% acetic acid on subbed slide.
5. Cover with siliconized coverslip.
6. Using a pencil, hammer (order them from Carolina Biological Supply, cat #WW-69-6437), or other similar object begin tapping on the coverslip, starting at the center and spiraling outwards. Then, spiral inward toward the center. The squashing can be checked under a phase contrast microscope during this process, to make sure that the spreading is good. During squashing, remove excess acetic acid from edges of coverslip with a Kimwipe.
7. Flip the slide over onto a paper towel, and press firmly with your thumb on the back of the coverslip (be careful, the slide can be cracked at this step).
8. Mark the edges of the coverslip on the back of the slide, and label the slide.
9. Freeze slide by placing it on a metal block surrounded with liquid N₂ (I've found that using the metal temperature blocks works best. Place them in a Styrofoam container, with a lid, and add enough liquid N₂ until the level of liquid is just below the top of the thermal block). Multiple slides can be stored in the liquid N₂ until ready to use. Submerging the slides in liquid nitrogen is also fine.
10. Before use, pop off the coverslip using a razor blade. If you prefer, the slides can be stored (minus coverslips) dry at -70°C for up to a month before use.

If you are going to incubate salivary glands with drugs, hormones, or other fun things, then:

1. Dissect salivary glands from 3rd instar larvae in Grace's medium (I order the powder mix from Sigma, and supplement it with L-methionine, L-leucine, and sodium bicarbonate as indicated on the product insert). Make sure to adjust the Grace's medium to pH of 6.6-6.8. Multiple salivary glands can be accumulated in Grace's medium before drug treatment. Other tissue culture media can be used, but I've found that the salivary glands give the best "response" in Grace's.
2. For drug treatment, simply add the compound to the Grace's medium.
3. Fix as above, and proceed per the above protocol.

Staining:

1. Place slides in a coplin jar containing antibody dilution buffer. Incubate three times, 30 minutes each wash.
2. Remove slides from coplin jar, blot edges on a paper towel to get rid of the excess buffer.
3. Add 35µL of primary antibody diluted in antibody dilution buffer. Cover with a piece of parafilm trimmed to the width and length of the slide.
4. Incubate overnight at 4°C, in a humidified chamber sealed with parafilm.
5. Carefully remove parafilm and wash slides three times in antibody dilution buffer, fifteen minutes each wash.
6. After washes are complete, remove slides from coplin jar, blot edges on a paper towel to get rid of the excess buffer.
7. In the dark (or at least out of direct light), add 50µL of fluorophore-conjugated secondary antibody diluted 1:250 in antibody dilution buffer. Cover with a piece of trimmed parafilm (as before, see above). All steps should be done in the dark after this point to prevent photobleaching of secondary antibody!

8. Incubate 30 minutes at 37°C in a humidified chamber (wrap in foil to keep light out).
9. Carefully remove parafilm and wash slides three times in antibody dilution buffer, fifteen minutes each wash.
10. Rinse slides in 1xPBS.
11. Stain slides with DAPI, or Hoechst, as appropriate (DAPI staining works best, just pour on DAPI solution for 1 minute).
12. Rinse slides in 1xPBS. Blot edge on paper towel to remove excess liquid.
13. Add a drop of Vectashield to slide, and cover with a coverslip. Keep slides in dark chamber until ready to view.

Good Luck!

Solutions:

Antibody dilution buffer

1xPBS
0.1% Triton X-100
1% BSA

Acetic Acid Fixative

50% glacial acetic acid
0.1% Triton X-100
3.7% Formaldehyde (I use the liquid stuff)

Subbed Microscope slides

Dip slides in 10% poly-L-lysine, allow to drip dry. Subbed slides can be stored at room temperature. Remove dust prior to using by dipping them in absolute ethanol and wiping dry with Kimwipe.

Siliconized Coverslips

Dip coverslips in Sigmacote, and allow to drip dry. Siliconized coverslips should be rinsed in absolute ethanol and wiped dry using a Kimwipe before using.