

Abdominal cuticle prep protocol

Fix flies for at least two days (or many years) in 3:1 ethanol:glycerol (glycerol prevents bristles from breaking off, not necessary otherwise)

Dump them in a depression glass and break the abdomen off from the rest of the body using two pairs of forceps.

Wrap abdomens in a Kimwipe and gently blot them off (otherwise glycerol makes them sticky)

Line them up on a piece of thick paper so that the anterior (broken) side of each abdomen faces to the left (if you are right-handed)

Have a needle in your left hand to control the abdomens. In your right hand, align a sharp razor blade so that it's angled about 20-30 degrees off horizontal, with the left corner down.

Align the razor blade along the dorsal midline of the abdomen.

Keeping this angle, place the left corner of the razor on the dorsal midline close to the anterior end

Slice with the razor blade to the left and downward until the corner cuts into the paper. Do not chop straight down, use a slicing motion. If you angle the razor just right, a single slicing cut will cut through the whole dorsal midline all the way to the posterior tip of the abdomen (genitalia) without damaging the ventral cuticle.

Option 1 (if you need good optics)

1. Once all the abdomens are cut, put them in 10% KOH for 15-20 minutes to begin dissolving soft tissues.
2. Using dull forceps, probe inside the cut to loosen up the soft tissues and open the abdominal body wall until it's flat.
3. Once all abdomens are open flip them with the cuticle side up. If KOH has dried out during this time, add a drop of water.
4. Put a cover slip on top so that the abdomens stay flat
5. Incubate at 65C for 3 hours to dissolve soft tissues
6. Wash off the cover slip and wash the cuticles well with water in a beaker or depression glass.
7. Dehydrate them in 100% n-propanol. They will become very brittle - be careful.
8. Using a cut-off pipette, suck up the cuticles and pipet them onto a slide.
9. Under the microscope, flip the cuticles so that the outside is facing up. Be careful not to touch them too much, they are very brittle now. Line them up as needed.
10. Remove excess propanol, leaving each cuticle in a small drop.
11. Mount in Euparal (I think you can get it from Carolina Biological).
12. Wait for 2 days for the Euparal to dry.

Option 2 (if you just want to see pigmentation)

1. Loosen up the abdomens in KOH as above.
2. Wash them in water and mount in Hoyer's medium.
3. Wait for 2-3 days for the soft tissues to clear.

Duncan, I.M. 1982. Polycomblike: a gene that appears to be required for the normal expression of the bithorax and antennapedia gene complexes of *Drosophila melanogaster*. *Genetics* **102**(1): 49-70.