



Technical tips for analyzing gene expression in the pupal abdomen of *Drosophila*.

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The adult abdomen of *Drosophila* is a useful model for the study of pattern formation, cell migration, and ectoderm-mesoderm communication. Abdominal epidermis is formed during the first two days of prepupal/pupal development (Madhavan and Madhavan, 1980). Unfortunately, this is the least accessible, and therefore the least studied, stage of the fly life cycle. Thus, despite some recent successes (Shirras and Couso, 1996; Lawrence *et al.*, 1999; Kopp *et al.*, 1999), little remains known about abdominal development and patterning. Here, we describe some technical tips for analyzing gene expression in the pupal abdomen.

1. Allow larvae to pupate on a Kimwipe or a piece of paper towel. Select pupae at the appropriate stage of development (description of pupal stages can be found in Ashburner, 1989) and attach them to a piece of sticky tape (3M packing tape or FedEx address stickers offer the best grip). Cut pupae longitudinally along the dorsoventral or lateral midline with a razor blade. This is a very critical step. Use gentle sawing motion, with as little downward pressure as possible, to avoid disturbing the internal contents of the pupa; a properly cut pupa will look almost intact. We recommend using high-quality shaving (not industrial) razor blades.
2. Remove pupae from the pupal cases and place them in an Eppendorf tube containing 1 ml of the appropriate buffer. For antibody staining and RNA *in situ* hybridization, keep the tube on ice and do not cut more than 10-15 pupae at a time before placing them in buffer.
3. Once enough pupae are accumulated, rock the tube forcefully but smoothly by hand for 30-60 seconds. This step removes most internal organs and prevents them from being attached to the epidermis during fixation. It is important to rock the tube "just right": too much force will destroy the epidermis, while too little force will leave it obscured by the fat body, which will harden during fixation and become impossible to remove.
4. Let the pupal body walls (which should become mostly transparent) settle to the bottom of the tube, change the buffer, and fix the pupae as required. We recommend 30 minutes in 5% formaldehyde for RNA hybridization; 10-30 minutes in 2-4% formaldehyde for antibody staining (depending on the particular antibody); and 10 minutes in 1% glutar aldehyde for X-Gal staining. X-Gal staining may be performed without further dissection.
5. For antibody staining or RNA *in situ* hybridization, stain the pupae in 0.5% Eosin Y in the appropriate buffer for several minutes to make pupal tissues visible. If fluorescent microscopy is to follow, use 0.02% Methylene Blue or Toluidine Blue instead, as these dyes do not fluoresce on any of the three standard channels. Wash the pupae briefly and place them in a depression glass under a dissecting microscope to assess the condition of the abdominal epidermis. Select the pupae in which the epidermis is intact, and remove the remainder of the fat body and muscles with fine forceps.
6. Proceed with the staining. For RNA *in situ* hybridization, use the lowest temperature possible; harsh hybridization regime may destroy the fragile epidermis. Proteinase treatment should be kept to a minimum, as it tends to strip the epidermis off the basal membrane.

7. Stained material may be dehydrated in an ethanol series and stored indefinitely in 100% ethanol at -20°C. For mounting, rehydrate the pupae gradually in PBS, excise the appropriate part of the abdomen with microsurgical scissors (Storz Ophthalmic Instruments), and mount in 80% glycerol/20% PBS for transmission microscopy, or Vectashield (Vector) for fluorescent microscopy. Nomarski optics is useful for visualizing the outlines of individual cells.

References: Ashburner, M., 1989, *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory Press; Kopp, A.K., R.K. Blackman, and I. Duncan 1999, *Development* 126: 3495-3507; Lawrence, P.A., J. Casal, and G. Struhl 1999, *Development* 126: 2441-2449; Madhavan, M.M., and K. Madhavan 1980, *J. Embryol. Exp. Morph.* 60: 1-31; Shirras, A.D., and J.P. Couso 1996, *Dev. Biol.* 175: 24-36.