Fixing fly pupae for antibody staining of legs

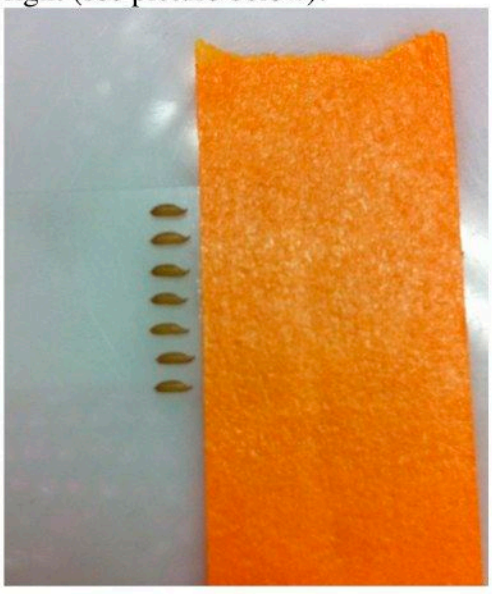
1. Collect white prepupae (if possible) and sex them. Put pieces of wet paper towel or Kimwipes in petri dishes. Put sexed pupae into corresponding dishes and incubate them for 5-7hr (for prepupal legs) or 16-48 hr (for pupal legs) at 25 ºC. Note: these times are for *D. melanogaster*; many other species develop more slowly. For example, 48 hours in *D. immigrans* would be the developmental equivalent of 24 hours in *D. melanogaster*.

2. Collect aged pupae and put them on dry Kimwipe for a couple of minutes to dry (otherwise they will not stick to the tape). Do not dry too long or the pupal case will be too brittle.

Alternative: if collecting and aging white prepupae is not practical, select young unstaged pupae and hope that the mix will contain the ages you are interested in. Use only the pupae that have no visible eye pigment. If the eyes are starting to turn orange, red, or brown, this pupae is too old for antibody staining.

3. Attach a piece of sticky tape (I like packaging tape) to a white background, sticky side UP.

For 24-28 hr dissection arrange pupae on sticky tape on their side, with anterior facing right (see picture below).



For 5-7 hr dissection arrange pupae on sticky tape with their dorsal side on the tape and anterior facing up (see picture below).