

Antibody-Staining of Adult Heads (Cryostat Sections)—From Zipursky lab

- 1) Dissect heads and place in PBS. Then, cut off proboscis in PBS.
- 2) Fix in 4% paraformaldehyde in .1M phosphate buffer (pH 7.4) 4 hours on ice
- 3) Wash 3x in PB for 10 minutes each
- 4) Cryoprotection: 1x in 5% sucrose in PB 10min, 1x in 10% sucrose in PB 10min, overnight in 20% sucrose in PB at 4C
- 5) Preparation of cryostat sections (10um):
 - Make 20% Carboxymethylcellulose (CMC) the night before (to get rid of bubbles)
 - Embed the head on a peg covered with CMC
 - Dip the whole thing in liquid nitrogen until the bubbling stops
 - Place in cryostat (-21 object temp, -23 chamber temp)
- 6) Collect sections on Superfrost slides. Thaw sections by placing slide on top of cryostat for a couple of seconds. Dry the samples at room temperature for a couple of minutes. Reduce surface of samples by outlining with PAP pen.
- 7) Cover sections with 10% sucrose in PB for at least ten minutes (bring over 10% in slide containers to place samples in for duration of sectioning.)
- 8) Transfer to 5% sucrose in PB for 10min and then PB for 10 min and then PBS for 10 min in humid chamber.
- 9) Preincubate in 10% NGS in .5% PBT (PBS + Triton) 15min
- 10)