Materials List for:

Preparation of Adult *Drosophila* Eyes for Thin Sectioning and Microscopic Analysis

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Materials

General equipment:

- For general fly husbandry see [10]
- Standard dissection microscope (e.g. Nikon SMZ-645 Stereo Zoom Microscope), preferably with a ring light source to prevent casting a shadow of your hands.
- CO_2 Fly pad, covered with 3MM Whatman paper to provide protection against dirt during dissection.
- Gelatin coated slides: dissolve 10g gelatin and 1g $CrKSO_4$x 12 H_2O in 1L water on a heating plate (bring almost to a boil, takes approximately 2 hrs). Dip well washed (soap) and rinsed (dH_2O) slides in holders into gelatin solution and air-dry covered with foil overnight.
- Heating oven (70°C; old vacuum ovens that were used for nitrocellulose filter drying are well suited for this purpose).
- Microtome capable of ultrathin sectioning (0.5-1 µm thick sections), e.g. those used by electron microscopy facilities. We use a Sorvall MT5000 Ultramicrotome with a Histo quality diamond knife. Glass knives also work.

Fixation solutions:

- Prepare a stock solution of 0.2 M Sodium Phosphate buffer, pH 7.2.
- Prepare 2% glutaraldehyde in 0.1M Sodium Phosphate buffer, pH 7.2 (can be stored at 4°C for 4 weeks). Make at least enough to have 200µl per genotype you want to embed. Keep on ice.
- Osmium solution: 2% OsO_4 in 0.1M Sodium Phosphate buffer, pH 7.2. Make only enough to have 200µl per genotype you want to embed, since the solution cannot be stored. Keep on ice.

Glutaraldehyde and OsO_4 are highly toxic and should be handled with extreme care in a well-ventilated hood. Use filter tips when handling OsO_4 to prevent blackening of your pipette.

Dehydration: Make 30%, 50%, 70%, (80%), 90%, and 100% ethanol solutions. Preferably cool 30%, 50%, 70% ethanol on ice before use.

Staining solution:

1% Toluidine-blue in 1% Borax.

	Soft	Hard
Resin A	54g	50g
Hardener B	44.5g	50g
Accelerator C	2.5g	1.75g
Plasticizer D	10g	0.75g

Table 1. Resin preparation

To prepare the resin, carefully, but thoroughly mix all ingredients in a plastic beaker using a magnetic stirrer with a large stir bar. Avoid bubbles. After mixing, aliquot in standard scintillation vials or similar containers and freeze at -20°C. Use soft resin for all applications unless your EM facility asks for the harder formulation. Use gloves to handle resin (carcinogenic in its unpolymerized form). To polymerize resin on equipment and waste, bake at 70°C overnight. Waste may then be safely discarded and equipment cleaned and reused. Spilled unpolymerized resin can be cleaned with isopropanol.

Reagent	Supplier	Catalog number
Fly pad	e.g. Genesee	59-119
Glutaraldehyde	Sigma	G7526-10 X 10 ML
OsO_4	Polysciences,Inc.	0972A-20
Propyleneoxide	Fisher	04332-1
Scalpel handles, for #3	Fisher	22080046

Scalpel blades #11	Fisher	08-916-5B
Transfer pipettes	Fisher	13-711-7M
Durcupan (R) ACM resin	Sigma (Fluka)	44610-1EA
Steel dissecting needle	Fisher	S17346
BEEM flat embedding mold	Electron Microscopy Sci.	70904-12
Teflon coated razor blades	Electron Microscopy Sci.	71970
Q-tip (sterile swabs)	Fisher	14-959-81
Glass slides	Fisher	12-550-143
Cover slips No 1, 22X60 mm	Fisher	12-531K
Gelatin	Fisher	ICN96010280
Cr(III) K SO_4 dodecahydrate	Sigma	243361
Diamond Histo knife, 6mm	Diatome US	60-His
Toluidine Blue O	Fisher	BP107-10
Borax (Na-Tetraborate)	Fisher	AC20629-1000
DPX mounting medium	Sigma	44581-100ML

Table 2. Materials