

# **NANOGOLD PROTOCOL (Uli Tepass from Burry et al., 1992) for electron microscopy**

**On discs:**

## **DAY1**

- 1) Dissect out eye discs fresh in 1X PBS**
- 2) Fix in PEMS fix (see notes below) with 0.25% glu. for 5' then 20'**
- 3) Return discs to 1X PBS and remove peripodial membrane.**
- 4) Wash discs 2X 10' in 1X PBS**
- 5) Permeabilize in 1X PBS, 10% goat serum, 0.02% saponin (from 1% filter-sterilized stock soln. kept in fridge), 1mg/ml BSA, 50mM glycine (2mg/ml) 5X 10'**
- 6) Incubate O/N at 4°C in primary ab in permeabilization buffer.**

## **DAY2**

- 7) Wash 7X 10' in permeabilization buffer.**
- 8) Incubate in 1 in 400 (or as appropriate) secondary coupled w/ 1.4nm gold particles for 3hrs at room temp.**
- 9) Wash 2X 10' in perm. buffer.**
- 10) Wash 7X 10' in 1X PBS.**
- 11) Post fix in 4% fresh formaldehyde and 2% glu. in 1X PBS O/N @ 4**

## **DAY3**

- 12) Wash in 50mM HEPES buffer w/ 200mM sucrose pH 5.8 for 6X 5' at RT.**
- 13) Enhance with NPG (see below) for 20' in the dark.**
- 14) Rinse in neutral fixer soln. of 250mM sodium thiosulphate and 20mM HEPES (pH 7.4) 3X 10' at RT.**
- 15) Wash in 0.1M sodium phosphate (pH 7.2) 3X 10'**
- 16) Wash in 0.1M sodium cacodylate (pH 7.2) 3X 10'**
- 17) Incubate in 0.1% OsO<sub>4</sub> in 1X PBS for 30'**
- 18) Rinse in 0.1M sodium cacodylate (pH 7.2) for 3X 10'**
- 19) Dehydrate in 30%, 50%, 70%, 80%, 95%, 3X 100% etOH for 10' each**
- 20) Transition to propylene oxide for 3X 10'**
- 21) Then 1:1 propylene oxide and pure plastic O/N**

## **DAY4**

- 22) Transfer to pure plastic for 4hrs under vacuum**

23) Embed, bake for 2 days at 60°C and section.

**NOTES:**

- i) All solutions should be made fresh using sterilized ddH<sub>2</sub>O. Bacteria appear as large black structures with the electron microscope.
- ii) 10ml total PEMS-buffer= 1ml PIPES (1M, pH 6.9), 2.5ml EGTA (200mM, pH 8), 20 MgSO<sub>4</sub> (1M) and 6.48ml ddH<sub>2</sub>O. For 10ml total PEMS fix add 1.8ml of 37% (fresh) formaldehyde to 8.2ml of PEMS-buffer. You can also use PLP fix in this protocol, but still add the glutaraldehyde.
- iii) The secondary antibody that I used was
- iv) The NPG-silver enhancement solution was made as described in Burry et al. 1992 (Journal of histochemistry and Cytochemistry, 40, (12): 1849-1856). It requires four components:
  - a) 10ml 1M HEPES buffer (add 12ul of 10N NaOH to give pH6.8). Make on the day of use.
  - b) Gum arabic stock: agitate 50g of gum arabic in 100ml of ddH<sub>2</sub>O for several days until dissolved. Filter through 6 layers of coarse gauze and store in aliquots at -20°C. Thaw on day of use. (Check big freezer for my stash of this).
  - c) NPG stock: bring 10mg of N-propyl gallate (Sigma) dissolved in 250ul of 100% ethanol to 5ml with ddH<sub>2</sub>O. Make day of use.
  - d) Silver lactate stock: dissolve 36mg of silver lactate (Fluka) in 5ml of distilled water, just before use and store IN THE DARK.For 10ml of NPG Enhancement solution: 30 minutes before use, combine 5ml of gum arabic stock with 2ml of 1M HEPES buffer: agitate. About 3 minutes before use, mix in 1.5ml of NPG stock. One minute before use, add 1.5ml of silver lactate stock THEN MIX IN THE DARK.
- v) TO BE COMPLETED