TEM CELL SAMPLE PREPARATION

Fixation

1. Wash cells with PBS buffer 3 times at room temperature (optional).
2. Replace PBS buffer (medium) with 2.5% gluteraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) for 1 hour @ RT. These buffers can be obtained at the Yale EM facility.
3. Wash cells with 0.1M sodium cacodylate buffer 3 x 5 minutes.

At this point, Yale investigators should bring their samples to the Yale EM facility so they may complete the process for you which will take several days. The remaining steps involve extremely harzardous chemicals. Contact Xinran.Liu@yale.edu for more information.

Postfixation

Postfix cells in 1% osmium tetroxide in 0.1M cacodylate buffer for 1 hour @ RT in the hood.

En Bloc Staining

1. Wash in 50mM sodium maleate buffer (pH 5.2) 3 x 5 minutes.
2. Stain in 2% uranyl acetate in maleate buffer for 1 hour @ RT in the dark.

Dehydration & Infiltration

1. Wash in water 3 x 5 minutes.
2. Dehydrate cells in the following order:
   - 50% ethanol 2 x 5 minutes
   - 70% ethanol 2 x 5 minutes
   - 90% ethanol 2 x 5 minutes
   - 100% ethanol 3 x 10 minutes
3. Replace ethanol with propylene oxide

   Cells that have not been scraped from culture dishes will be removed from the plastic surface of the dish at this stage. The propylene oxide will dissolve the plastic and the cell layer will float off. Remove the cells quickly because the propylene oxide continues to dissolve the plastic. Transfer the cell layer, in propylene oxide, to Eppendorf tubes, making sure the cells do not dry at any stage.
4. Wash several times in propylene oxide (4-5 times) to remove plastic residues.
5. Replace with 50% propylene oxide / 50% Epon. Leave on the wheel for 2 hours with the lid closed.
6. Replace with pure Epon and leave on the wheel for 2 hours with lid open. Repeat once.
7. Transfer the cell pellets to fresh Epon in molds or Eppendorf tubes, add computer-printed labels. Cure in the oven overnight @ 60^{o}C.