TEM TISSUE SAMPLE PREPARATION

Fixation

1. Fix tissue quickly by dipping small pieces (~ 1 mm-thick in at least one direction) into 2.5% gluteraldehyde and 2% paraformaldehyde in 0.1M sodium cacodylate buffer (pH 7.4). Chop into small 1 mm^3 cubes in the hood and leave to fix for 1 hour @ RT. These buffers can be obtained at the Yale EM facility.
2. Wash with 0.1M sodium cacodylate buffer 3 x 20 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

1. Postfix 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 15 minutes.
2. Stain in 2% uranyl acetate in H_2O for 1 hour @ RT in the dark.

Dehydration

1. Wash in water 3 x 5 minutes.
2. Dehydrate 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
4. Leave in fresh propylene oxide for 10 minutes with the lid closed.

Infiltration & Embedding

1. Replace with 50% propylene oxide / 50% Epon mix. Leave on the wheel for 2 hours with the lid closed.
2. Replace with pure Epon and leave on the wheel for 2 hours with lid open. Repeat once.
3. Transfer samples to fresh Epon in moulds or Beem capsules (remove air bubbles). Add computer-printed labels. Cure in the oven overnight @ $60^{o}C$.