Embedding of cell monolayers

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.
3. Wash cells with 0.1M sodium cacodylate buffer 3 x 5 minutes.

POSTFIXATION
1. 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 AND 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 moulds or Eppendorf tubes, add computerprinted labels. Cure in the oven overnight @ 60°C.