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Transmission electron microscopy

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Ching-Chieh Chou[1], Judith Frydman[1]

[1]Stanford University

Ching-Chieh Chou
Stanford University

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We use this protocol and it's working

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Abstract

Processing human cells for transmission electron microscopy analysis.

1 Cells are grown on Ibidi dishes: µ-Dish 35 mm, high Grid-50 Glass Bottom is a 35 mm.

2 Cells are fixed in Karnovsky's fixative: 2% Glutaraldehyde (EMS Cat# 16000) and 4% PFA (EMS Cat# 15700) in 0.1 M Sodium Cacodylate (EMS Cat# 12300) pH 7.4 for 1 hr at room temperature. After fixation samples are placed at 4°C.

3 Cells are then post-fixed in cold 1% Osmium tetroxide (EMS Cat# 19100) in water and allowed to warm for 2 hr in a hood, washed 3X with ultra-filtered water, then en bloc stained 2 hr in 1% Uranyl Acetate at room temperature.

4 Samples are dehydrated in a series of ethanol washes for 10 min each at room temperature beginning at 30%, 50%, 70%, 95%, changed to 100% 2X, then Propylene Oxide (PO) for 10 min.

5 Samples are infiltrated with EMbed-812 resin (EMS Cat#14120) mixed 1:1, and 2:1 with PO for 2 hr each.

6 Samples are placed into EMbed-812 for 2 hr opened then placed into flat molds w/labels and fresh resin and placed into 65°C oven overnight.

7 Cells of interest are located using the grid pattern and cut out with a gem saw and remounted on pre-labeled resin blocks with fresh resin and polymerized overnight again.

8 Once full polymerized the glass coverslip is etched away using hydrofluoric acid for 20 min.

9 Using the finder grid pattern left behind the block faces are trimmed down allowing for serial sectioning of the cells of interest.

10 Sections are taken around 90 nm, picked up on formvar/Carbon coated slot Cu grids, stained for 40seconds in 3.5% Uranyl Acetate in 50% Acetone followed by staining in 0.2% Lead Citrate for 6 min.

11 Observed in the JEOL JEM-1400 120kV and photos are taken using a Gatan Orius 2k X 2k digital camera.