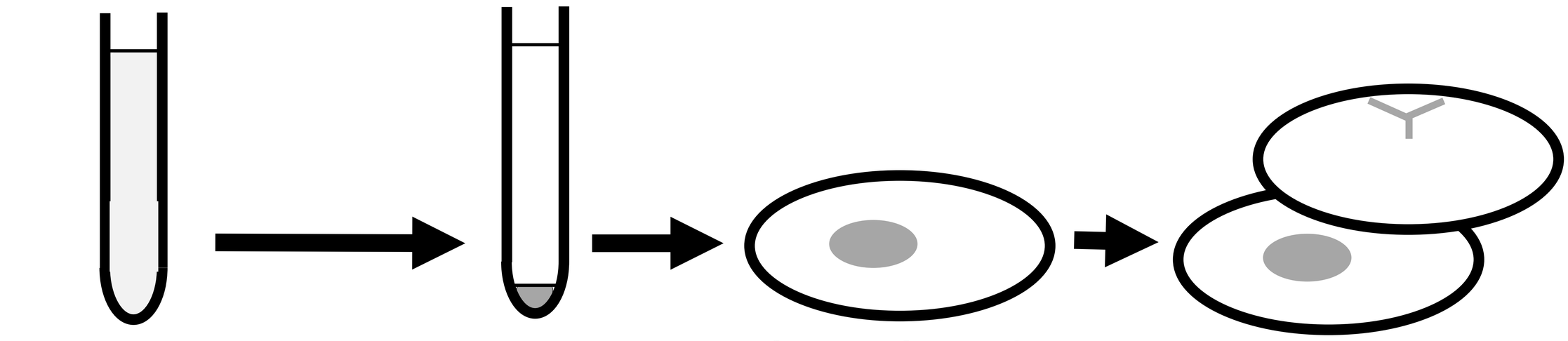
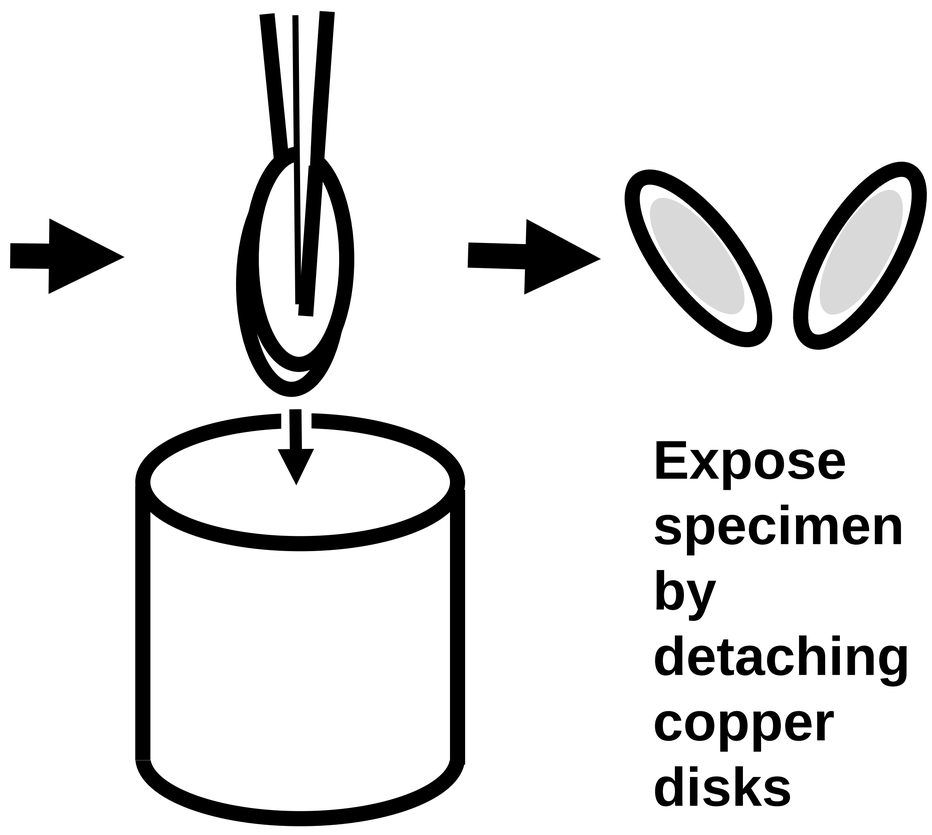


Liquid culture of cells

Collect by centrifugation

Place a drop of specimen onto a copper disk

Cover with another copper disk



Plunge-freeze in liquid propane

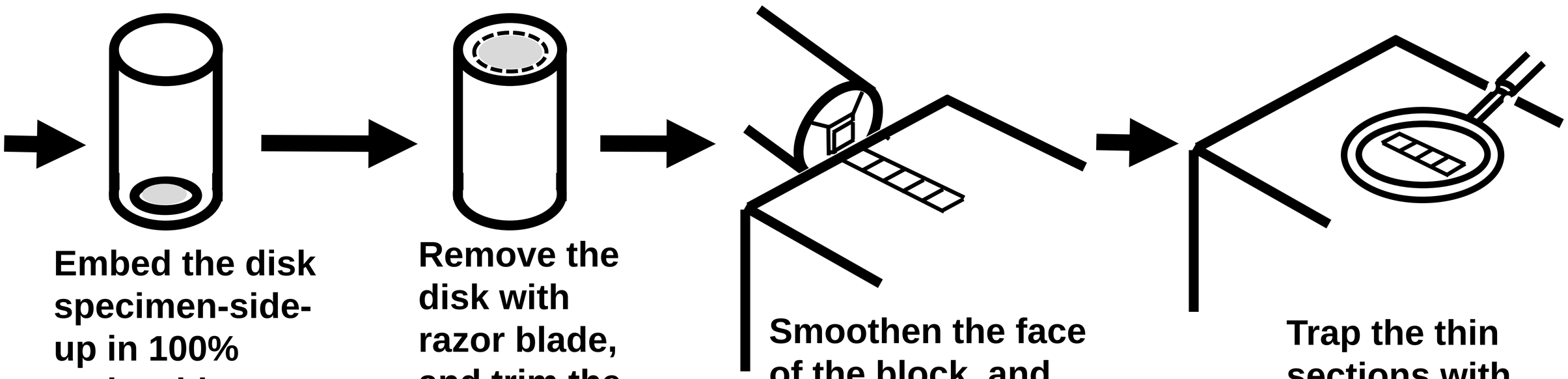
Expose specimen by detaching copper disks

Put specimen in acetone containing 2% OsO4 in a glass vial and place them in
- 80 ºC freezer for 2 days

2 h at - 20 ℃, 2 h at 4 ℃, and 15 min at r. t.

Transfer the specimen in acetone in plastic tube, 5 min at room temperature

30% epoxy resin (without accelerator) (in acetone), 60 min at r. t. in 60% resin, 60 min at r. t. in 90% resin, 60 min at r. t. in 100% resin, overnight at 37 ℃

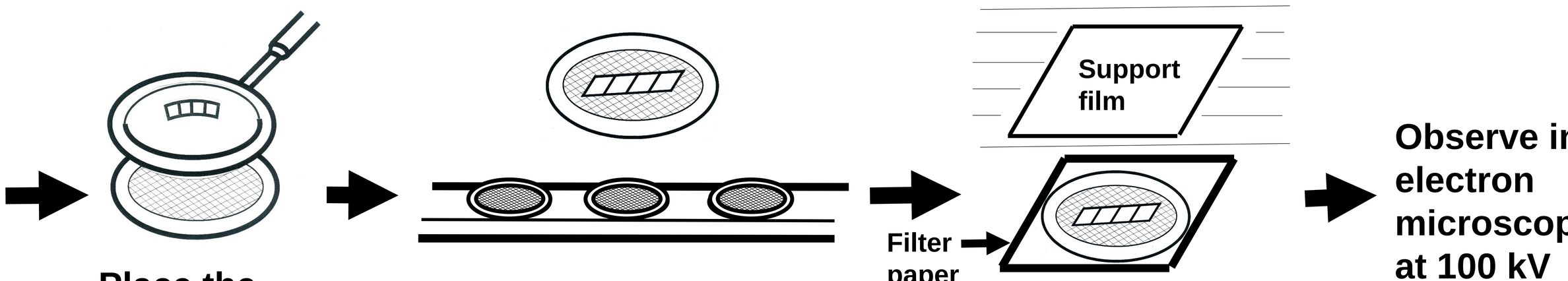


Embed the disk specimen-side-up in 100% resin with accelerator, and polymerize at 60 ℃ for 24 h

Remove the disk with razor blade, and trim the block

Smoothen the face of the block, and make ultrathin sections with diamond knife

Trap the thin sections with a loop



Place the loop over a 300-mesh-grid, and dry

Stain sections with uranyl acetate and lead citrate for 3 min each using silicone half tube

Filter paper

Cover the grids with plasma-polymerized naphthalene support film

Observe in electron microscope at 100 kV