Electron Microscopy Shared Resource
Manager: Caleigh Azumaya
Version 1.3, 19-Apr-22

emsr@fredhutch.org
206-667-3176
DE-780 (Thomas Building)

Negative stain bench protocol

Location:
DE-797 (EM sample prep room)

General rules:

- Do not use the negative stain bench unless you have been trained by EM staff
- Use tools only for their intended purposes
- Clean up after yourself in DE-797
- Report any missing tools to Caleigh ASAP
- Log use in the physical logbook by the NS bench

Sign-up rules:

- Use iLab to reserve NS bench (base rate, $6/hr) for the **entire time** you will need to be in the EM prep room
- Users must wear gloves during entire procedure
- Cancel at least 30 min prior to reservation or you will be charged
- Door access can be requested from Caleigh (cazumaya@fredhutch.org)

Training plan:

- Please contact Caleigh at cazumaya@fredhutch.org for 1:1 training upon request
    - Two 1-hour training sessions and qualification test required before independent use approval can be granted
    - If returning >90 days after last session, please contact Caleigh (phone or email) for refresher walk-through and quiz

Training objectives:

- Explain applications, limitations, and common roadblocks in NS and cryoEM
- Evenly carbon coat negative stain grids
- Make uranyl formate stain and effectively generate contrast on a standard protein sample

User provided materials:

- Negative stain grids (after initial 2 training sessions)
- Sample and buffer

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Shared resource tools list: if anything is missing/broken, please contact Caleigh ASAP

- Bench: P10, P100, P1000, parafilm, milliQ water, gloves, tips, kim wipes, reverse action tweezers, sharp tweezers, slides, filter paper,
- Drawer: uranyl formate, beakers, stir bars, scoopula, syringes, filters, permanent marker, eppendorfs

Startup checklist:

- Glow discharge grids you will use
- Inventory tools and make sure you have everything needed for staining
- Make stain or bring frozen aliquots (protocol below)
- Set up stain bench
    - Lay down parafilm and pipet 30 uL drops of milliQ (x2), stain (x2) for each sample (only do ~6 samples at a time or they will evaporate!)
    - Fold enough filter paper for blotting and cut piece for final blot
    - Lay out a labeled large sheet of filter paper for grid drying

Staining protocol:

- Pick up grid with reverse action tweezers
- Pipet 4 uL of sample on carbon side of grid and wait 30 seconds
- Flip grid over and blot side against filter paper until droplet is completely absorbed
- Touch carbon to surface of first water droplet
- Side blot against filter paper until droplet is completely absorbed
- Touch carbon side to surface of second water droplet
- Side blot against filter paper until droplet is completely absorbed
- Touch carbon side to surface of first stain droplet
- Side blot against filter paper until droplet is completely absorbed
- Touch carbon side to surface of second stain droplet and leave attached for 30 seconds
- Side blot against filter paper until droplet is completely absorbed
- Blot off remaining stain at interface of grid and tweezers w/ filter paper triangle
- Release carbon side up on labeled filter paper
- Move all finished grids to grid storage box

Shutdown checklist:

- Soak up any stain remaining on a filter paper
- Throw anything with UF contact into the UA waste container (fume hood)
- Clean up bench area, take anything you brought into the room out

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- Sign logbook with all session details
- Report anything wrong to Caleigh via email

Stain making protocol

- Weight out ~38mg uranyl formate powder (directly into 10mL beaker using an analytical balance)
- Drop in small stir bar and cover on stir plate
- Boil 5 mL of milliQ water and add to UF, stir for 5min (timing is important)
- Add ~6.5uL of 5M NaOH (stock in chemical hood), stir for 5min (timing is important)
- Filter stain through 0.2um filter into round bottom tube
- Date tub, cap, and cover in aluminum foil
- Wait for stain to cool while you are setting up your NS bench for use
- Stain can be snap frozen and stored at -80°C, thaw immediately before use

Grid making protocol

- Buy from Ted Pella -- 01754-F suggested

OR

- Buy from Ted Pella – G400 suggested
- Fill large dish with milliQ water, cover and allow to sit until bubbles settle out
- Add 2 drops of collodion to the center of the dish from ~6 inches above the surface
- Do NOT disturb dish/water from here on
- Pour grids out in a petri dish lined with filter paper and tap to spread out
- Place grids very gently, darker side down on the film across the water's surface
    - Lay out in ~4 rows no wider than the width of glass slide
    - Do not overlap grids
    - Do not wrinkle film
    - Do not pierce film
- Cut a piece of filter paper large enough to cover the grids you have laid out
- Gently lay/drop on top of grids and let sit until water is absorbed through the paper
- Pierce the film around the piece of filter paper with two pipet tips and use them to collect excess collodion
- Prepare a petri dish lined with a dry piece of filter paper
- Carefully grab a corner of the filter paper and flip over into petri dish so it is film side up
- Allow to dry half-covered overnight
- Stick filter paper to a slide using double sided tape
- Carbon coat using 208C carbon coater on settings XXX
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