

Negative Stain

Bench

~for proteins~

Fred Hutch

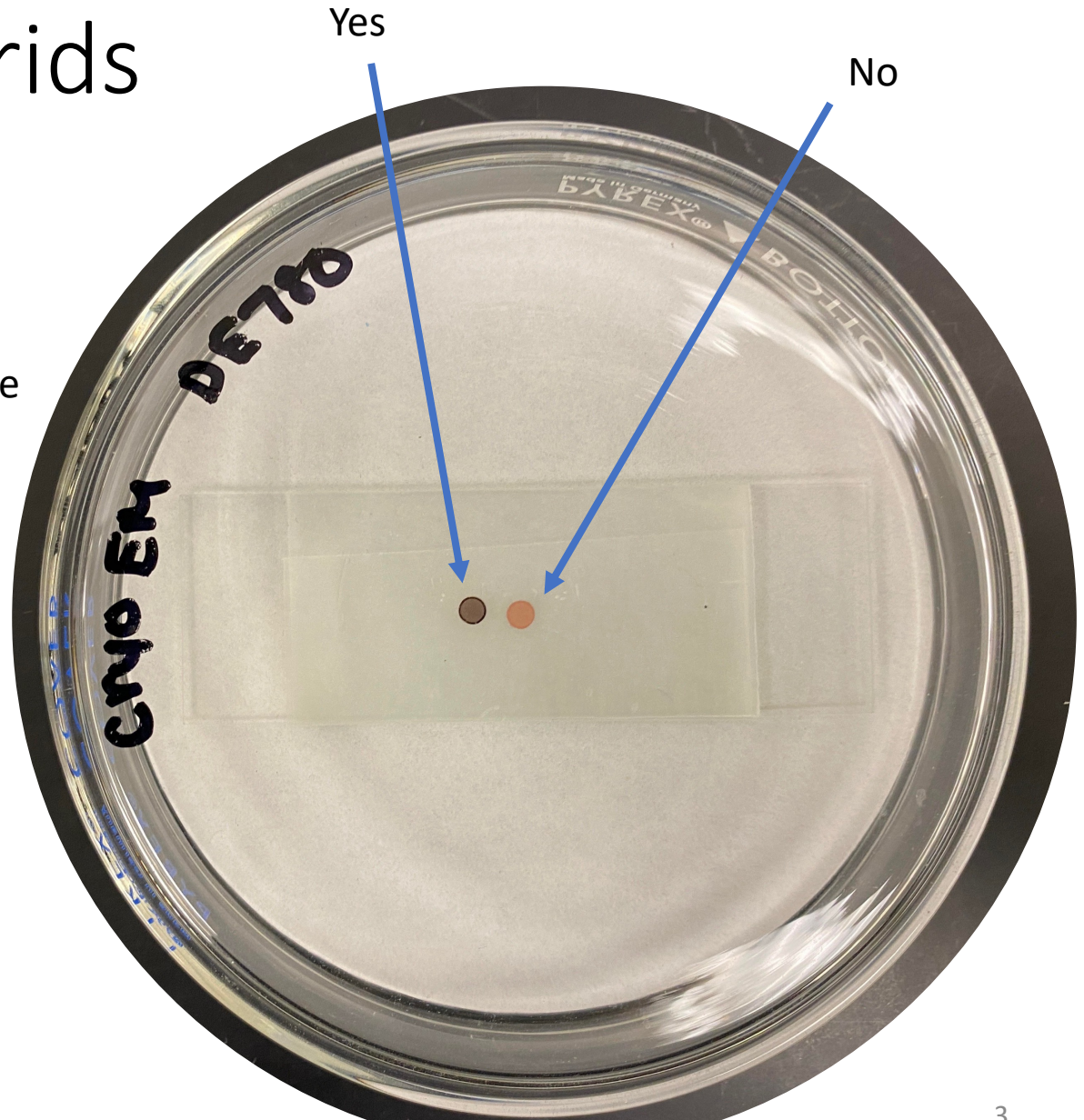
Inventory your tools

- Large, medium, and triangle filter paper
- Tweezers and reverse action tweezers
- Water and stain
- Glow discharge slide and grids
- Timer and parafilm

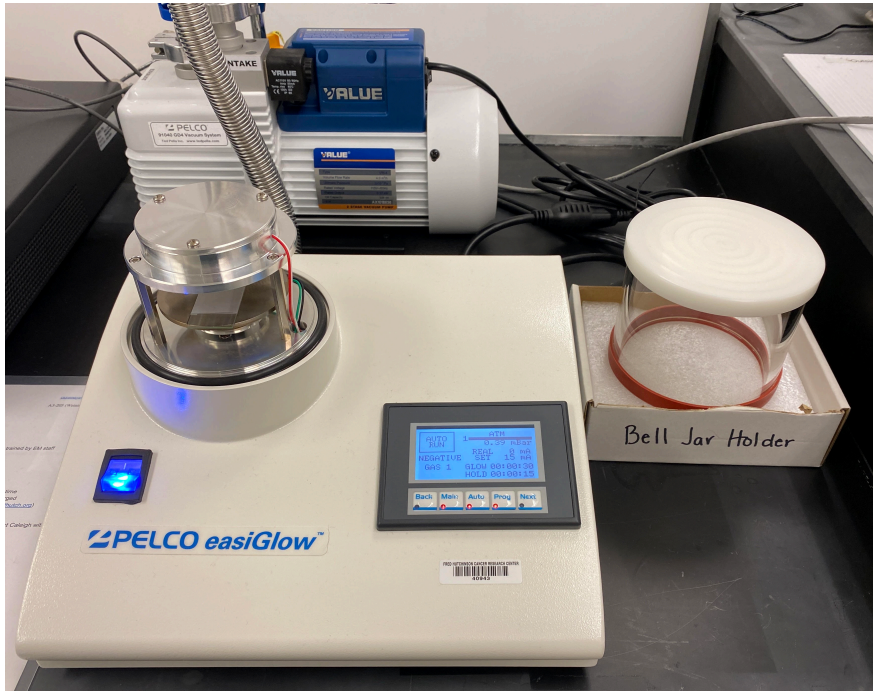


Get your grids

- Lay out all the grids you want to stain in the whole session
- Carbon side up (dark/shiny)



Glow discharge them



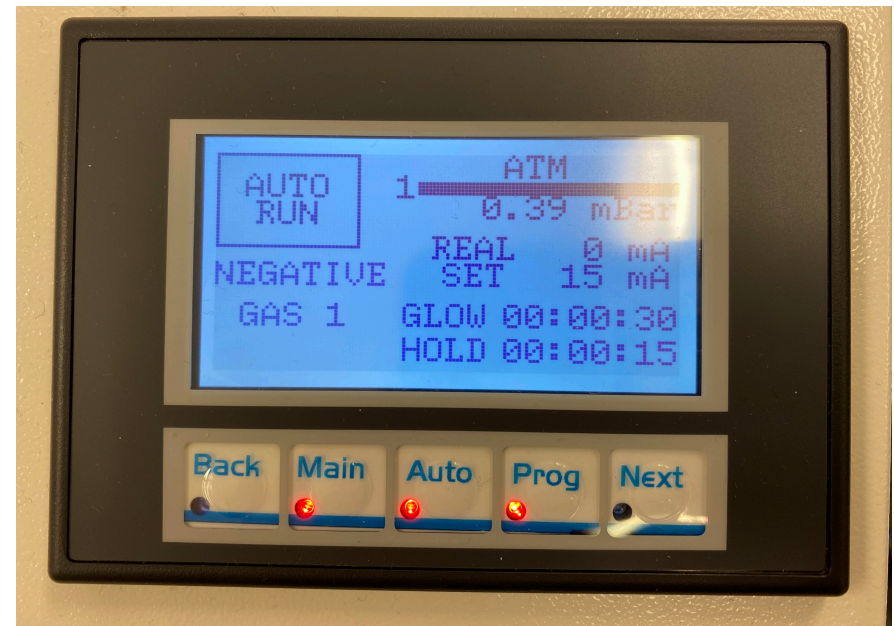
Standard settings should be fine for everyone's negative stain.

0.39 mbar

Glow = 15mA

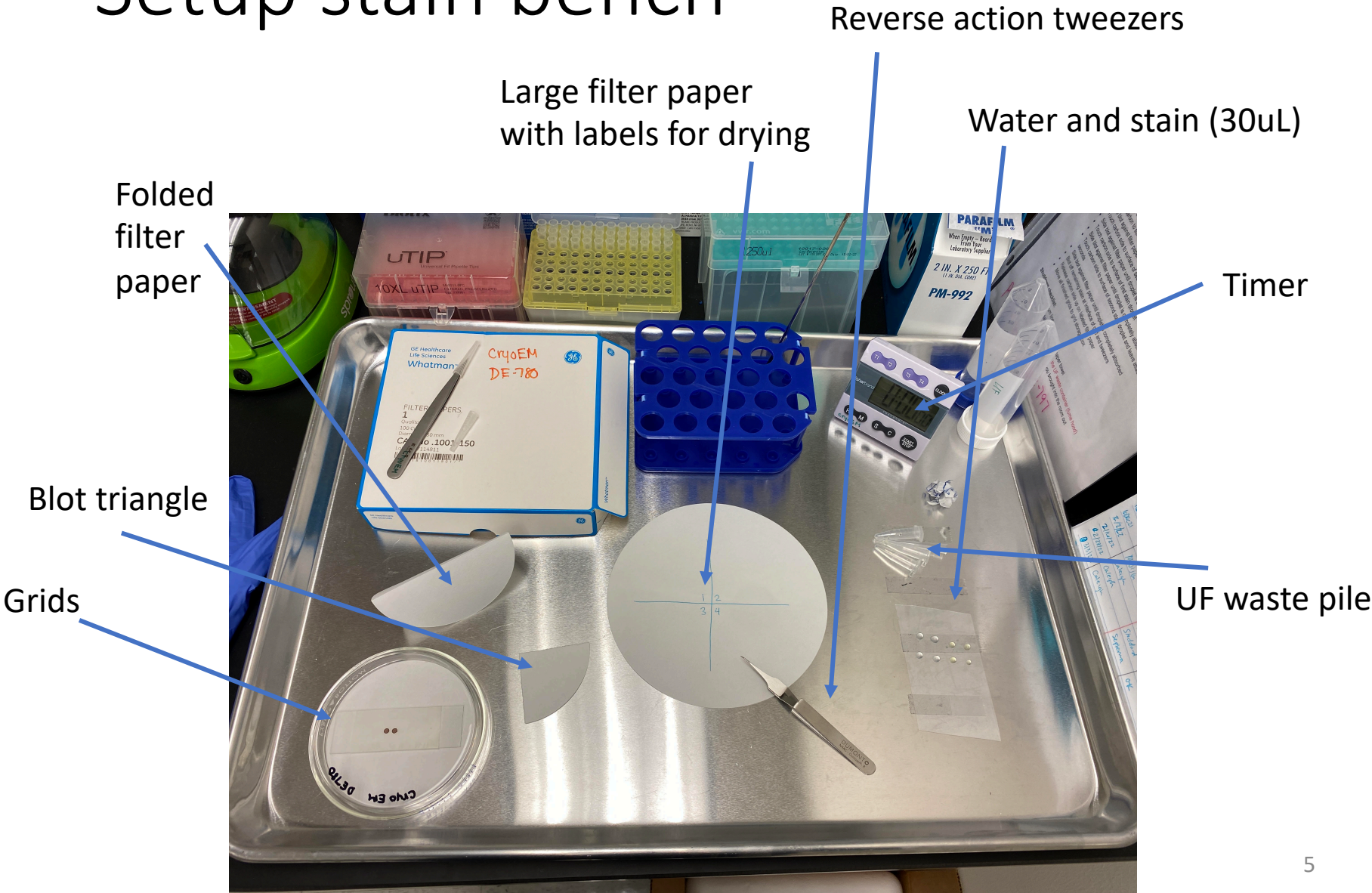
Glow = 30s

Hold = 15s



Turn on the easiGLOW and remove the bell jar carefully to insert slide with grids.

Setup stain bench



Protein (30s), water, water, stain, stain (30s)



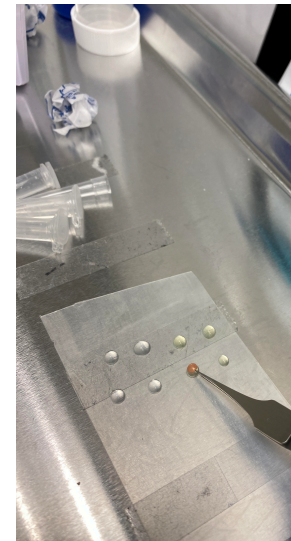
side blots
between each



water



water



stain



30s in stain

Final blot and drying time

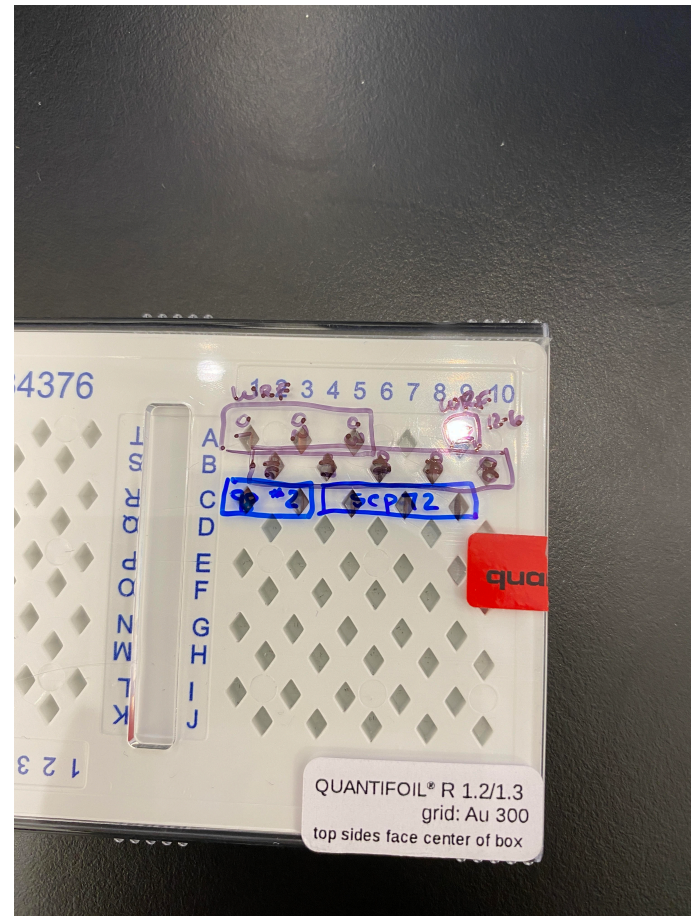
Run a small piece of filter paper between the tweezer points to absorb the last bit of stain at the point of the tweezers

Drop off, carbon side up, on filter paper until you're done with this batch



Store grids

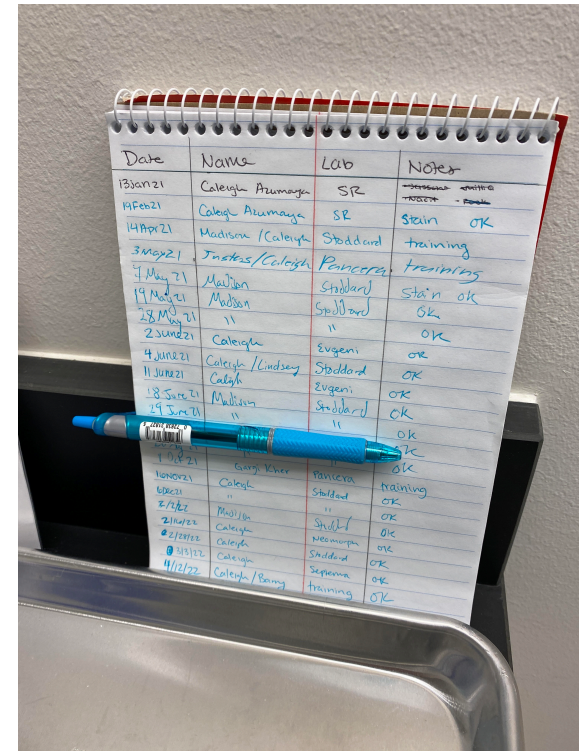
Store grids in a grid box with labels on the box or in a spreadsheet elsewhere



Clean up

Anything that touched UF, including gloves, goes into the UA waste in the hood – soak up liquid waste onto filter paper

Return all tools to bench/drawer and sign logbook before you leave



Do shutdown bench check list!

Clean up!

Sign logbook!