DAPI instructions [ Final concentration = 0.5ug/mL ]

* STOCK: Dissolve 5mg bottle into 1mL to make 5mg/mL stock solution. Store at -20C
* INTERMEDIATE: Mix 2uL of STOCK into 100uL diH2O to make a 100ug/mL intermediate solution. Store at 2-4C
* 2x WORKING: Mix 100uL INTERMEDIATE into 10mL diH2O to make 1ug/mL working solution. Store at 2-4C
* 1x WORKING: For final concentration, mix 2x WORKING in 1:1 ration with 2x PBS to get a final working concentration of 0.5ug/mL.
* Incubate sample for 20 minutes at room temp

Calcofluor white instructions [ Final concentration = 6.25uM ]

* STOCK: Comes in a 5mM solution. Store at -20C
* INTERMEDIATE: Dilution 1:10 to get 0.5mM intermediate solution. Store aliquots at -20C
  + E.g. 1mL into 9mL water—then aliquot into 1mL samples and freeze
* 2x WORKING: Thaw intermediate solution and dilute 1:10 again; 1mL intermediate into 9mL diH2O to get a 50uM solution. Store at 2-4C.
* 1x WORKING: Mix 2x working solution 1:1 with 2x PBS for a final concentration of 25uM
* Incubate sample for 20 minutes at room temp
* \*\*\*\* As of 4feb2020, I think I will actually use a final concentration of 6.25uM to match PI, and mimics protocols in Deborah 1987, *Cytometry*. (they use a 10uM, but said concentration didn’t seem to effect CFW)
* 4x WORKING: Thaw intermediate solution and dilute 1:20; 0.5mL intermediate into 9.5mL diH2O to get a 25uM solution. Store at 2-4C.
* 1x WORKING: Mix 4x working solution 1:1:2 (CFW, 4xPI, 2xPBS) to get final working concentration of 6.25uM. Incubate for 15 minutes at room temp.

Propidium iodide instructions [ Final concentration = 6 uM ]

* STOCK: Dissolve 10mg in 6.2339mL to get stock concentration of 2.4mM
* INTERMEDIATE: Mix 1mL into 9mL to get intermediate concentration of 240uM (a 20x working solution, according to Tim’s protocols, but actually a 80x for me).
* 4x WORKING: Thaw intermediate solution and dilute 1:10 again; 1mL intermediate into 9mL diH2O to get a 24uM solution. Store at 2-4C.
* 1x WORKING: Mix 4x working solution 1:1:2 (CFW, 4xPI, 2xPBS) to get final working concentration of 6uM. Incubate for 20 minutes at room temp.
* \*\*\*\*\* After messing up dilution on 4feb2020, we can actually dilute out PI even further
* 4x WORKING: Thaw intermediate solution and dilute 1:20; 0.5mL intermediate in 9.5 mL diH2O to get a 12uM 4x working solution.
* 1x WORKING: Mix 4x working solution 1:1:2 (CFM, 3xPI, 2xPBS) to get final working concentration of 3uM. Incubate samples for 20 minutes at room temp.

Tim’s Propidium iodide instructions

* STOCK: Already in 2.4mM stock
* 4x WORKING: Mix 10uL of stock into 990uL diH2O to get 1mL of 24uM working solution
* 1x WORKING: Mix 4x working solution 1:1:2 (CFW, 4xPI, 2xPBS) to get final working concentration of 6uM. Incubate for 20 minutes at room temp.

Temporary CFW dilution from 8x stock

* Currently, I have a 50uM solution in the fridge. To get 1x, I need to:
  + Dilute 1:2 with water (500uL stock into 500uL diH2O) to get 4x
  + Working solution mix with 1:1:2 (CFW, 4xPI, 2xPBS)

Final CFW and CV staining:

Calcofluor white instructions [ Final concentration = 50uM ]

* STOCK: Comes in a 5mM solution. Store at -20C
* INTERMEDIATE: Dilution 1:10 to get 0.5mM intermediate solution. Store aliquots at -20C
  + E.g. 1mL into 9mL water—then aliquot into 1mL samples and freeze
* 1x WORKING: Thaw intermediate solution and dilute 1:10 again; 1mL intermediate into 9mL diH2O to get a 50uM solution. Store at 2-4C.
* Fix membranes in 1mL 100% ice cold methanol over ice for 15 minutes.
* Soak in 1mL diH2O for 5 minutes to remove methanol
* Place membrane in 250uL of 50uM solution, let sit for 15 minutes at room temperature.
* Transfer to 1mL diH2O and set for 1 minute before transferring to slide and viewing

CV instructions [ Final concentration = 0.1% ]

* Prepare 0.1% solution, autoclave, and aliquot into 50mL tubes
* Fix membranes in 1mL 100% ice cold methanol over ice for 15 minutes.
* Soak in 1mL diH2O for 5 minutes to remove methanol
* Drop 500uL of 0.1% CV and let sit for 10 minutes at room temperature.
* Transfer to 1mL diH2O and set for 5 minutes at room temperature
* Briefly dip in fresh 1mL diH2O to get rid of excess dye; then set out to dry complete.
* Punch with ¼” hole puncher and transfer disk into a 96-well plate for later.

CV extraction

* Add 200uL 30% Acetic acid to each well, and let incubate for 20 minutes.
* Pipette up and down 5 times and transfer 100uL into fresh 96-well plate
* Read at 590nm