TO KEEP CONSTANT ACROSS ALL EXPERIMENTS:

* Stock 1% tryptone broth for feeding, aliquoted into 30mL samples, kept in fridge.
* Stock Bd culture in 1% T broth for culture. Need at least 25mL. Aliquoted into 3mL cultures, each—use test tubes I think.
* Stock isolate microbes, in plates.
* \*\* Enough plates to do nutrient tube streaks to see if contaminated \*\*

DAY 1

* Autoclave 40 tubes full of 8mL in 16mm test tubes 1% tryptone media for culturing
  + Transfer liquid to 15mL microcentrifuge tubes
  + If each culture was in EVERY single jar, you’d need ~ 3.71mL for just inhibitory. Multiple by 2 for everything; ~ 8mL.
* Glue all jars with silicon caulking
* Autoclave 75 mL media to make x5 1% T plates (0.75g Tryptone; 0.75 agar) for Bd culturing
* Pour plates, cool.
* When cool, inoculate XX cultures into broth and incubate in shaker--?
* Ensure there is a 30mL tryptone broth for feeding for the duration of the experiment
* Plan out all culture orders, etc. Print out sheet. [1/2]

DAY 2

* Put 100mL spring water in all jars and autoclave.
* Autoclave 40 clean 16mm test tubes for mixing cultures in for tomorrow
* Ensure there is ~ 350mL autoclaved spring water available for dilutions tomorrow
* Put 500uL/plate of Bd stock culture on each of 5 Bd plates. Let dry, and then wrap up with parafilm.
* Plan out all culture orders, etc. Print out sheet. [2/2]

DAY 3

* Arrive at 7:00am to prepare cultures
* Spin down all 40 tubes in centrifuge @ 180g (rpm??) for 5 minutes
  + Decant supernatant
  + Re-suspend with 4mL spring water
* Put 100uL of each culture in a 96-well plate to measure OD
* Dilute each isolate to 0.1 OD in clean 16mm test tubes with spring water
* Create mixtures of bacteria in sterile 16mm test tubes
* Inoculate all cultures. Do it “by culture” to save pipette tips. Hopefully do this by 9am

DAY 4

* Arrive at 9:00am to feed cultures; 100uL each
* Autoclave remaining jars for Bd transfer [1/3]
* Ensure there is ~50mL spring water, autoclaved [1/3]

DAY 5

* Arrive at 9:00am to feed cultures; 100uL each
* Autoclave remaining jars for Bd transfer [2/3]
* Ensure there is ~50mL spring water, autoclaved [2/3]

DAY 6

* Arrive at 9:00am to feed cultures; 100uL each
* Ensure there is ~50mL spring water, autoclaved [3/3]
* Autoclave remaining jars for Bd transfer [3/3; early]
* Plate wash Bd. Add 4mL spring water to each plate; let sit for 30 min; pipette off into sterile falcon tube.
* Count zoospore concentration with haemocytometer; dilute with sterile spring water until concentration is 1x10^5 zoospores/mL
* @8:00pm
* Add 1mL (100,000 zoospores) into each CLEAN jar
* Transfer each biofilm matrix into new jar
  + Try swabbing stainless steel head with sterile cotton swab soaked in 70% ethanol, then dry with a dry cotton swab to remove as much ethanol as possible.
* Put back in shaker; finish around 9-9:30pm
* Autoclave old jars overnight

DAY 7

* Arrive at 9:00am to feed cultures; 100ul each
* Retrieve autoclaved jars. Wash all jars and re-fill with 100mL spring water
* Autoclave fresh jars
* Ensure there is at least 30mL of ice-cold methanol \* [1/3]
* Ensure there is 60mL of 1x PBS, 10mL 2x PBS \* [1/3]
* @ 9pm, change jars, put back in shaker
* Cell-scrape all jars and filter all water through 0.22um filters; freeze filters for later processing. Count zoosporangia?? Store some sampled water??
* Autoclave old jars overnight

DAY 8

* Arrive at 9:00am to feed cultures; 100uL each
* Retrieve autoclaved jars. Wash all jars and re-fill with 100mL spring water
* Ensure there is at least 30mL of ice-cold methanol \* [2/3]
* Ensure there is 60mL of 1x PBS, 10mL 2x PBS \* [2/3]
* @ 9pm, change jars, put back in shaker
* Cell-scrape all jars and filter all water through 0.22um filters; freeze filters for later processing. Count zoosporangia?? Store some sampled water??
* Autoclave old jars overnight

DAY 9

* Arrive at 9:00am to feed cultures; 100uL each
* Retrieve autoclaved jars. Wash all jars and re-fill with 100mL spring water
* Ensure there is at least 30mL of ice-cold methanol \* [3/3]
* Ensure there is 60mL of 1x PBS, 10mL 2x PBS \* [3/3]
* @ 9pm, change jars, put back in shaker
* Cell-scrape all jars and filter all water through 0.22um filters; freeze filters for later processing. Count zoosporangia?? Store some sampled water??
* Autoclave old jars overnight

DAY 9

* Arrive at 9:00am to sample!
* Retrieve autoclaved jars. Wash all jars and fill with
* Fill one test plate with 1x PBS
* Have empty waste jars where liquid will “dump out”
* For each membrane:
  + Cut into weighted thirds
  + Put medium sized quarter into foil packet for DNA processing
  + Dip largest quarter in 1x PBS, and transfer to “empty” test plate
  + Dip other medium sized quarter into PBS, then into second “empty” test plate
  + Cover original jar to process liquid later
  + Add 500uL 0.1%CV/well to first test plate. Set aside for 10 minutes.
  + Add 500uL ice-cold 100% methanol to second test plate. Put in fridge for 20 minutes.
  + After CV is done, transfer to original “dirty” PBS
  + Peel all membranes off and fix in 100% methanol for 20 minutes at 4C or on ice
  + Rinse membranes in 1x PBS for ~ 5 minutes while I dispose of methanol and rinse off plate
  + Fill 2nd plate with CFW/PI/PBS mixture
* For each membrane [ option 2]:
  + Cut into weighted thirds.
  + Put medium sized quarter into foil packet for DNA processing
  + Dip largest quarter and other medium sized quarter in test plate A with 1x PBS, and transfer to test plate B
  + Add 500uL ice-cold 100% methanol to test plate B. Put in fridge for 20 minutes.
  + With test plate A, put liquid in autoclave waste jar and thoroughly spray down with 70% ethanol and then put under UV for 15 minutes. Mean while, make fresh ~20mL of CFW/PI/PBS (Fl) stain.
  + In test plate C, fill with 1x PBS.
  + Rinse methanol-soaked membranes in 1xPBS thoroughly; ~ 5 minutes.
    - Discard methanol and 1xPBS when done. Both should be sterile.
    - Re-fill test plate C with diH2O
  + In test plate B, transfer large portion. Add 500uL 0.1% CV for 10 minutes.
    - After done, rinse with diH2O, and set out to dry.
    - Rinse off plate into CV waste and re-fill with diH2O
  + In test plate A, transfer small portion. Add 500uL FL stain for 20 minutes.
    - Label some microscope slides
    - After done, rinse with diH2O, and transfer to labelled microscope slides
    - Add antifade reagent, if required, or seal with clear nail polish.
  + Visualize and photograph slides.
    - Store in fridge for later vizualization
  + Put liquid from test plate A into DAPI/CFW/PBS waste. Put all plates in autoclave.
* Cell-scrape all jars and filter all water through 0.22um filters; freeze filters for later processing. Count zoosporangia?? Store some sampled water??
* Autoclave EVERYTHING.

*Quantifying zoospores*

* Cell-scrape the walls of each jar
* Take 100uL and plate onto 1% Tryptone plate; I want to see how many zoosporangia end up growing? Can do plate count estimates using dissecting scope
* Spin down 25mL at a time in a centrifuge at 180rpm for 10 minutes. Can probably do this in C335 entirely, since there is a flame up there.
* Re-suspend pellet in 2mL of diH2O (50x concentrated) 🡪
* Put 1mL of this in a microcentrifuge tube. Spin down at max speed for 2 minutes. Freeze and save for DNA extraction later.
  + rRNA copy number in this 1mL 🡪 x2 = total rRNA copy number in entire jar
  + Other 1mL will be used for plate counts and microscopy.