2-feb-2020

Today I started Pilot 3. I’d grown four strains of bacteria (20D, BTB45, 24G, and burk). BTB45 is the OG culture from alex’s stash; 20D and 24G were both from my re-passaged from liquid batch. Burk is Tim’s.

I grew them from Friy6pm to Sunday 8am—burk was a little cloudy but the rest were pretty cloudy. I spun down all 2mL in the tubes into 1.5mL autoclaved centrifuge tubes. Discarded supernatant (repeated 2x for volume). Then, resuspended in 1mL spring water (autoclaved). I put it in sterilized test tubes to see turbidity. Diluted (by hand) with more sterile psring water. The btb45 and 20d still looked darker than burk, even after ~ 2x-3x volume increase. But oh well.

Diluted out to 10-3, 10-5, and 10-6; then plated 100uL of 10-5 and 10-6 to get 10-6 and 10-7plates. I put 100uL of OG culture (first test tube) into each jar for monoculutres; and 25uL of each for quad cultures.

I accidentally fed 14 on the inside, even though it was supposed to be fed on outside. Don’t think I will matter since I’m still 4 days away from exposing to bd.

I started the bd plates on Friday 6pm too; I put 500uL of Tim’s JEL 1/24 culture and spread around. I can see clumps of sporangia.

Finished around 10am, and put jars in shaker at 80rpm. I set it to 20C, but Id n’t think the AC is working, and the temp gage reads 24C. I might borrow a thermometer somewhere and actually record air temp.

3-feb-2020

Fed cultures at 8am; took until 8:30am. 100uL each, except 14 and 18 which were fed outside. Used a ethanol lamp to keep sterile area, and pour ~ 30mL culture into 50mL test tube to feed for rest of experiment. I fed 3 jars at once with same tip to conserve tips.

I also started a “maturity” assay today—

At 3:30pm, I ethanol’d, evaporated, then UV’d 6 membranes. Placed on 1% T plates.

I flooded one corner of a Bd plate with 500uL of spring water, rested for ~ 20-30 min, then put 50uL of this onto each membrane (and on the agar directly as a positive control). What I’m going to do is wait for them to grow over the course of a few days and destructively sample and stain each of them with DAPI, PI, and CFW.

4-feb-2020

Fed fultures at 8:am. Took until 8:30am again. 100uL each, except 14 and 18 which got it outside. Nothing exceptional. Need to remind myself to test temperature.

Going to stain first membrane of Bd maturity assay.

1. Fix with ice-cold methanol for 20 min on ice 🡪 it is sterile now!
2. Cut in half
3. Incubate half in DAPI (1:1 with 2x PBS from stock in fridge) for 20 min
4. Incubate other half in 1:1:2 (CFW, PI, 2x PBS) for 20 min
5. Visualize right away downstairs. Try to sneak in between classes.

4feb2020

Took one membrane off the T plate and put it in 100% ice cold methanol, put in fridge for 20 min.

All the methanol defs evaporated. Will need to find way to prevent this, both for safety and other reasons. Then, I rinsed in di H2O for 1-2 minutes and dipped several times. Finally, placed face-down in mixture of PI and CFW

I fucked up diluting Tim’s PI.I put 10uL of 24uM in 9.9mL water instead of 990uL. Oops. So I tried mixing my mistake (2.4uM) in with my CFW stock (50uM) with 450uL PI (@2.4uM) and 50uL CFW (@50uM). Then staining for 5-8 minutes because I ran out of time. Rinsed in diH2O for a few dips.

It worked!

In fact, PI was a little *too* bright. I may need to rinse better next time. CFW was perfect. Nice crisp outlines.

5feb2020

Fed at 8:10am; 100uL each except 14 and 18 which were fed outside.

Broth still clean.

Going to sample 2nd day bd, but going to wait til later so I can visualize alone (after class). Maybe start staining at 5pm and visualize at 6pm.

Also, need to transfer into Bd jars and initiate Bd colonization—since I’m doing comps tomorrow, no time to clean jars in morning so I’m going to try to do it a little early today and clean jars before I leave at 8pm. Maybe do it before Bd visualization so that I can autoclave before I visualize; and then clean jars after they are cool (after I vizualize). Then, I can fill them in and stick them to autoclave overnight, maybe leave around 7pm.

\*\* What I acutally did—

Started prepping Bd plates at 3:15pm. Let sit for 30 minutes. I added 5mL of sterile spring water to each plate, and got back 3.5 and 4.25 mL from each plate. Total ~ 9mL though? Not sure how that math added up, maybe I counted wrong. But total 9mL. Bd concentration was 3.7x10^6. Put 10uL on each side of haemocytometer. Counted 15, 17, 12, 13, 17 in 5 squares; multiplied by 25; then by 10,000.

Added 270 uL per fresh jar of water. Then, switched over apparatus at ~ 4:00pm. Autoclaved right away, along with remaining bd zoospores. Keeping Bd jars in lab now since I know the temperature is okay.

\*\* The membranes that I glued didn’t seem to have any water inside them. I wonder if that’s where the bacteria are getting in? And I need to manually add water into them then.

Also, jars 17, 18, 20 had unknonw amounts of Bd because I may have miscounted/mispipetted. Tracking to be on the safe side.

Going to stain at a slightly different concentration today:

* 400uL 2.4uM PI
* 100uL 50uM CFW

See if I can get a less bright red.

At 4:45pm I started fixing day 2 of bd growth assay membrane. Going to incubate on top of ice pack in 12-well plate in methanol. I should probably save methanol and not flush it down the sink—I’ll have a methano waste. Added 1mL ethanol for one membrane.

This membrane I will cut in half. Half will be stained with CFW+PI, the other DAPI. That way I know for sure if I am able to visualize nuclei, or if PI is staining too much RNA stuff.

6feb2020

Fed @ 7:30am today. 100uL each, except 14, 18 which is outside.

Changed jars and autoclaved at 4:00pm.

Didn’t scrub anything or sample bd since it was original inoculant bd. Tomorrow I should try to pellet down Bd.

Visualized; SO MANY NUCLEI!

I did 250ul water 250ul DAPI

400uL CFW, 100uL PI🡪 worked really well

Incubated ~ 15min on ice cold methanol

Rinsed/soakd in di H2O for 5 min + 3 dips

Incubated in dyes for 5 minutes

Rinsed and soaked for 2 min + 3 dips

🡪 visualization was great.

7feb2020

Fed at 8:10a

Stained with CFW (400uL) and 100ul PI; worked well.

Tried CFW + DAPI; didn’t work very well. (CFW was 50uL but stained really bright anyway)

Tried DAPI only; worked well.

Did jar change at 2:45. Tried spinning down to see if I can get pellet…

8Feb2020

Fed at 10am, but didn’t change jars bc got too much stuff to do

9Feb2020

Sampled at ~3pm. Took a total of 6 hours. (Approximately, but I also did it twice with the other membrane).

100uLPI and 200uL CFW + 200uL water--- not enough PI?? Can’t see it very well.

First:

* Cut vertical 2/3
* 1/3🡪 DNA; put in 1mL dih2o. Leave for now.
* 2/3 🡪 methanol for 15 min; then cut in 2/3 again. Larger goes into CV 0.1% (500uL) for 10 minutes; the other goes into PI+CFW (above) for 5 min.
* Soak in di H2O each for ~ 5 minutes.

CV worked well, but PI and CFW didn’t reallydo well—PI not visible, and there were no zoosporangia!!!!

Tried cutting the second membrane in half (half to CV and half to microscopy) but again; very few zoosporangia.

The CV on methanol’ed and non-methaol’ed look ~ the same, so I’ll check this. WAY easier if things are dead first.

Also, checked zoospore count in 2 jars (1 and 14); they were about the same; all “non-motile” zoospore looking things; around 13/square so quite a few?