Gladfelter Lab Protocols

Immunofluorescent Staining in *Ashbya*

- [ ] Grow a 50 mL culture of *Ashbya* at 30°C for 15 hours. Add 5.5 mL 3.7% formaldehyde and shake at 30°C for 1 hour.

- [ ] Prepare polylysine slides: Add 10 µL polylysine to each well for 5 minutes (aliquots stored in common stock -20°C), aspirate and let air dry, wash with 10 µL water, aspirate and let air dry.

- [ ] Divide culture into 15 mL conicals (cells do not pellet well in 50 mL conicals) and spin in benchtop centrifuge at 3000 rpm for 5 minutes.

- [ ] Remove supernatant (discard in formaldehyde waste in fume hood). Pool cells into one 15 mL conical and add 10 mL PBS. Spin at 3000 rpm for 5 minutes and remove supernatant.

- [ ] Add 1 mL Solution A and transfer cells to a 1.5 mL Eppendorf tube.
    - If you have many cells, you can add 2 mL Solution A and reserve 1 mL of cells at 4°C for up to a week.

- [ ] Add 100 µL of 15 mg/mL Zymolyase (aliquots stored in common stock -20°C). Incubate at 37°C, gently inverting every 5 minutes. Check cells under microscope every 10 minutes until 70-80% of cells are phase dark. Wild type typically takes 30-40 minutes, but strains vary considerably.
    - Note: Zymolyase is a fragile enzyme, when thawing do not vigorously shake or vortex the tube.

- [ ] Centrifuge at 2600 rpm for 2 minutes, remove supernatant. Wash 2 times with 1 mL solution A and resuspend in 500 µL Solution A.
    - Digested cells are fragile, take care while washing or they explode.
    - Final resuspension volume can be adjusted depending on cell density.

- [ ] Apply 20 µL cells onto polylysine treated well. Allow cells to settle for 15 minutes and aspirate. Let air dry completely.

- [ ] Wash cells 2X with 20 µL PBS.

- [ ] Block with 10 µL PBS + 1 mg/mL BSA (IgG free, stored at 4°C) for 30 minutes.

- [ ] Wash 2X with 20 µL PBS.

- [ ] Add 10 µL primary antibody diluted in PBS+BSA and place slide in humid chamber. Incubate at 4°C overnight. (antibodies stored in common stock -20°C)
    - Rat α-alpha tubulin use 1:50
    - Rabbit α-Cdc11 use 1:100

- [ ] Wash 5X with 20 μL PBS+BSA

- [ ] Incubate 1 hour in 1:200 secondary antibody + Hoechst (1:500) in PBS+BSA
    - AlexaFluor 488 or 568 goat α-rat and AlexaFluor 568 rat α-rabbit use 1:200

- [ ] Wash 5X with 20 μL PBS+BSA

- [ ] Add 7 μL Prolong Gold mounting medium (aliquots stored in common stock -20°C). Apply coverslip, let settle for 30 minutes with a kimwipe on top and weight applied, and seal edges with nail polish.

Solution A (100 mL)
10 mL 1 M Potassium phosphate buffer pH 7.5
60 mL 2 M Sorbitol
30 mL H_2O

filter sterilize