Gladfelter Lab Protocols

Ashbya Actin Staining (Phalloidin) Protocol

- [ ] Inoculate a 10 mL AFM + Amp culture (and appropriate selection) with *Ashbya* clean spores (10-30 µL depending on strain and clean prep density).
- [ ] Incubate 15-16 hours at 30°C.
- [ ] Add 1.1 mL 37% formaldehyde (3.7% final concentration) and shake at 30°C for 1 hour.
- [ ] Spin down at 1000 rpm for 5 minutes in benchtop centrifuge. Dispose of supernatant in hazardous waste.
- [ ] Wash twice with 10 mL 1X PBS, spinning at 1000 rpm for 5 minutes in benchtop centrifuge.
- [ ] Resuspend pellet in 100 µL PBS and transfer to 1.5 mL eppendorf tube.
- [ ] Add 10 µL *Alexa Fluor*^{TM} Phalloidin (6.6 µM), mix gently by pipetting.
- [ ] OPTIONAL DNA STAINING STEP: Add 0.2 µL Hoechst, mix gently by pipetting.
- [ ] Incubate at room temperature for 1 hour in the dark.
- [ ] Spin at 10,000 rpm for 2 minutes in microfuge.
- [ ] Wash twice with 1 mL 1X PBS. Remove as much supernatant as possible.
- [ ] Add 10 µL *Prolong Gold*^{TM} mounting medium. Spot 15 µL cells in small drops over slide. Cover with 24 x 50 mm coverslip.
- [ ] Compress to flatten and press out excess liquid for at least 30 minutes. Seal with nailpolish and store at -20°C.

Updated 03/31/15