Gladfelter Lab Protocols

Isolation of Spores with Zymolyase ("Dirty" Spore Prep)

- ☐ Inoculate an AFM plate with mycelium in the middle of the plate. Grow for ~10 days, or until the mycelium grows to the edges of the plate.

- ☐ Strip off mycelium from the plate with a spatula or toothpick and suspend in 10 mL sdd$H_2O$ in a 15 mL Sarstedt tube. Shake vigorously/vortex to disrupt mycelium.

- ☐ Add 200 μL 15 mg/mL Zymolyase and incubate in roller drum at 37°C for 1-2 hours. Solution will look more homogeneous.
    - Zymolyase aliquots are stored in the stock -20°C freezer
    - Thaw Zymolyase gently, as the enzyme will be destroyed if you vigorously shake or vortex it.
    - Do not vortex tube after addition of Zymolyase.

- ☐ Spin down spores in benchtop centrifuge at 3000 rpm for 5 minutes, pour off supernatant

- ☐ Wash three times with 5 mL 0.03% v/v Triton X-100.
    - The pellet is difficult to resuspend via vortexing, so it is easiest to add 1 mL 0.03% Triton by pipetting and resuspend pellet with the pipette tip before adding additional 4 mL 0.03% Triton.

- ☐ Resuspend spores in 1 mL 0.03% Triton + 1 mL 50% glycerol. Aliquot into cryo-tubes and store at -80°C.
    - If spores are going to be used within 5 days, they can be stored at 4°C.