Gladfelter Lab Protocols

FITC-ConA Pulse Chase Growth Assay

FITC-ConA pulse

- [ ] Grow a 10 mL *Ashbya* culture 13-14 hours.
- [ ] Spin down culture for 5 minutes at 300 rpm.
    - If spin is too fast, cells will get stressed.
- [ ] Remove supernatant and resuspend cells in 10 mL 50 mM Tris pH 7.5,150 mM NaCl.
- [ ] Wash cells 2X with 50 mM Tris pH 7.5, 150 mM NaCl.
- [ ] Resuspend cells in 10 mL 50 mM Tris pH 7.5, 150 mM NaCl.
- [ ] Add 250 µL of 1 mg/mL FITC-ConA stock and shake at room temperature for 10 minutes.
    - FITC-ConA stock is stored in common -20°C freezer.
    - Wrap tube in foil to minimize light exposure.
    - Tube can be taped to western blot shaker.
- [ ] Spin cells at 300 rpm for 5 minutes and wash 2X with 10 mL AFM.
- [ ] Resuspend cells in 10 mL AFM + Amp and appropriate selection. Let grow in baffled flask at 40 rpm and 30°C for 1 hour.

Formaldehyde fixation and Hoechst stain

- [ ] Add 1.1 mL 37% formaldehyde (final concentration 3.7%) to culture.
    - 37% formaldehyde is stored in the flammables cabinet under the chemical fume hood.
- [ ] Return cells to shaker at 30°C and 40 rpm for 1 hour.
- [ ] Spin cells down at 1000 rpm for 5 minutes. Remove supernatant, dispose of in formaldehyde waste in chemical hood.
- [ ] Wash cells 2X with 1X PBS. Resuspend in 500 µL 1X PBS in Eppendorf tube.
- [ ] Add 1 µL Hoechst. Incubate at room temperature in the dark for 10 minutes.
- [ ] Spin cells at 13k rpm in microfuge and remove supernatant. Wash once with 1X PBS. Remove as much supernatant as possible.
- [ ] Add 10 µL mounting medium and mix with pipette tip. Pipette cells onto a glass slide and cover with coverslip.
- [ ] Remove excess liquid with a kimwipe and seal with nailpolish.

Updated 10/09/13