Gladfelter Lab Protocols

Fixation for Preservation of GFP Signals

- [ ] Thaw 4% paraformaldehyde. This takes a while, so take it out early.
    - Aliquots are stored on the door of the -20°C freezer.

- [ ] Add 4% paraformaldehyde 1:1 to *Ashbya* culture (final concentration 2%) and shake at 30°C for 20 minutes. Generally, a 10 mL culture is appropriate.
    - Fixation time may need to be adjusted for specific signals.

- [ ] Spin down cells at 300 rpm for 5 minutes in the benchtop centrifuge in a 15 mL conical(s). Remove supernatant by pipetting and dispose of in hazardous waste container in fume hood.

- [ ] Wash cells twice with 10 mL 1X PBS.

- [ ] For Hoechst stain, resuspend cells in 0.5 mL 1X PBS, transfer to 1.5 mL Eppendorf tube, and add Hoechst 1:200 (2.5 µL). Incubate in the dark at room temperature for 30 minutes.
    - Hoechst aliquots are stored in the common stock -20°C freezer.

- [ ] Spin at 9 rpm in microfuge, remove supernatant. Wash twice with 1 mL 1X PBS, remove supernatant.

- [ ] Resuspend in 10 µL mounting medium, apply 10-15 µL of cells to a glass slide and cover with a long (22 x 50 mm) coverslip.
    - Prolong mounting medium aliquots are in the stock -20°C freezer.

- [ ] Remove excess medium by applying a Kimwipe to the edge of the coverslip, seal with nailpolish. Store slides at -20°C.

Making paraformaldehyde:

- [ ] Dissolve 4 g parafomaldehyde in 50 mL ddH_2O and add 1 mL 1M NaOH.

- [ ] Stir at 65°C in the fume hood until dissolved.

- [ ] Add 10 mL 10X PBS and allow to cool.

- [ ] Adjust pH to 7.4 using 1 M HCl (approximately 1 mL).

- [ ] Adjust final volume to 100 mL with ddH_2O.

- [ ] Store in 10 mL aliquots on door of -20°C.

Updated 10/03/13