FITC-ConA Pulse Chase Growth Assay

- 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.

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