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 paraformaldehyde in 50 mL ddH₂O 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 ddH₂O.

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

Updated 10/03/13