PHALLOIDIN STAINING OF TARDIGRADE EMBRYOS

By Willow Gabriel, for Hypsibius dujardini embryos

- use siliconized eppendorf tubes for all steps
- wash by centrifugation at 4500 rpm for 1.5 min, followed by aspiration with a Pasteur pipet
- carry out all steps with agitation

1. collect embryos (cut out of moults) and adult tardigrades in water - keep amount of liquid to a minimum
2. fix for 20 min in 4% PFA in 0.5X PBT (w/ 0.05% Triton, not Tween)
   = 250µl 16% EM grade PFA + 750µl 0.5X PBT
   (Cat. # 15710, EM Sciences, www.emsdiasum.com)
3. sonicate on a Branson 250 sonifier for 20 seconds (5 sec pulses, 15 sec pause between pulses) at an amplitude of ~2.2 with a constant duty cycle.
4. allow to recover for 15 min on ice - during this time, put phalloidin (which is in alcohol) on speedvac for ~15 min (3µl phalloidin, reconstitute in 150µl PBT)
5. rinse 2X in 0.5X PBT
6. block 10 min in 5% DMSO, 1% BSA in 0.5X PBT
7. stain with phalloidin for 30 min at RT.
   *all remaining steps, including #7, to be carried out in the dark
8. wash 5X – 5 min in 0.5X PBT → add DAPI to second to last wash (10 min)
9. mount on subbed slide using mounting media; seal with fingernail polish