

PHALLOIDIN STAINING OF TARDIGRADE EMBRYOS

By Willow Gabriel, for *Hypsibius dujardini* embryos

From Gabriel, W.N. and B. Goldstein. (2007) Segmental Expression of Pax3/7 and Engrailed Homologs in Tardigrade Development. *Development Genes and Evolution* 217: 421-433.

- 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)
 - = 250 μ l 16% EM grade PFA + 750 μ l 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 \sim 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 \sim 15 min (3 μ l phalloidin, reconstitute in 150 μ l 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 \rightarrow add DAPI to second to last wash (10 min)
 9. mount on subbed slide using mounting media; seal with fingernail polish