ALKALINE PHOSPHATASE STAINING
OF TARDIGRADE EMBRYOS

Staining adapted from an ascidian protocol of Imai, el al. (*Development*, 127: 3009-3020) 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 3 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 bottled spring water (we have used Deer Park and Crystal Geyser, successfully) - keep amount of liquid to a minimum
2. fix for 20 min in 4% PFA in 0.375X PBT (PBS with w/ 0.05% Triton, not Tween) = 250µl 16% EM grade PFA + 750µl 0.5X PBT
   (We use 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
5. rinse 2X in AP staining buffer (100mM NaCl, 50 mM $MgCl_2$, 100mM Tris-HCl, pH 9.5).
7. add NBT/BCIP (3µl each per 1 mL of staining buffer) for 3-5 hours.
   * this and all subsequent steps 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