

## DAPI Staining of *Hypsibius dujardini* embryos

From Gabriel et al., 2007 in *Developmental Biology*

- Remove embryos from the parental exuvia by slicing with 25 gauge hypodermic needles.
- Pipet embryos into eppendorf tubes, and replace liquid with absolute methanol at 4°C, incubate for 20 minutes
- Followed with a 90%-70%-50% methanol series at room temperature (RT).
- Post-fix in 4% paraformaldehyde in 0.5X PBT (0.5X phosphate buffered saline with 0.05% Triton X-100) for 10 minutes at RT.
- Sonicate in this fixative on a Branson 250 sonifier at an amplitude of 2.2 with a constant duty cycle for 4 pulses of 5 seconds each, with 15 seconds recovery on ice between each pulse.
- Recover for 15 minutes on ice followed by 5 washes of 5 minutes each in 0.5X PBT.
- Add DAPI (5µg/µl) to the next wash, incubate for 20 minutes
- Wash twice more in 0.5X PBT for 5 minutes each.
- Mount on slides coated in 0.2% gelatin, 0.02% chrome alum, 0.1% polylysine, 1mM azide.