

# Cuticle Preparations

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Adapted from Wieschaus and Nüsslein-Volhard (2003)

- Collect larvae 24 hrs after egg laying
- Dechorionate in 50% bleach for 2 minutes
- Rinse in distilled H<sub>2</sub>O
- Place into a glass scintillation vial containing methanol/heptane (1:1). Shake the glass vial for 30 seconds
- Remove the upper phase of heptane and wash larvae with 100% methanol 3x
- Removed methanol and transfer embryos into an acetic acid/glycerol (3:1) solution, and incubate for at least one hour at 70 °C
- After incubation, remove the acidic acid/glycerol mix completely
- Add 100 µl of mounting medium (Hoyer's medium) allowing the larvae to soak for 15-30 minutes
- Carefully drop the larvae onto a glass slide and coverslip
- Place 10 g weights on the coverslip to flatten the cuticles for one day in a 70C oven
- Cuticle preps are visualized using dark field microscopy

## Hoyer's medium:

gum arabic 30 g  
glycerol 16.0 ml  
chloral hydrate 200 g  
distilled water 50.0 ml

Dissolve gum in water, a little heat helps; add chloral hydrate, then glycerol