

Collection and Dissection of *Drosophila* pupal genitalia

Reagents

Nuclease Free H2O
GE SH30538.02

Ethanol 200 proof
Decon Laboratories #2716

Methanol
Fisher A412P-4

16% Paraformaldehyde (PFA)
EMS #15710

Collect Prepupae

Search vials for white prepupae and move to a clean glass viewing dish.

Sort males and females to separate wells by looking for larval testis, two clear circles on the ventral/lateral sides of the pupa, which are only present in males.

Place all samples you want to dissect later on a Kimwipe in a petri dish and incubate at 25°C before dissection.

Dissection of pupal genitalia

Fill a micro-centrifuge tube at least half way with 4% paraformaldehyde in PBT, and place on ice.

Move pupae from incubator to a clean glass viewing well.

Fill 3 other glass viewing wells with PBS.

Impale a pupa in the anterior half with the a microforcep and move to a well with PBS. Using spring scissors to cut the bottom 2/5ths of the pupae off. Discard anterior 3/5s of the pupae.

Move the posterior tip of the pupa to a clean well.

Using a 200µL tip gently flush most of the fat bodies, but try not to wash out the pupal genitalia. *Some lab members wash out the genitalia at this step, but it can be difficult to locate the sample amid all the fat bodies, especially after several dissections.*

Transfer pupal genitalia to a clean well with PBS and gently wash the sample until you flush the pupal genitalia from the sample.

Transfer the pupal genitalia to the third clean well. *This serves as a final wash before fixation.*

Using a 20µL pipet transfer the sample in ~2ul of PBS to the 4% PFA solution and place on ice. *We collect samples in 4% PFA on ice, but we do not consider fixation to begin until the sample is placed at room temperature.*

Repeat previous steps until all pupae are dissected or 1 hour has past.

Fix samples

Incubate samples in 4% PFA at room temperature for 30 minutes.

Wash 2X quickly with methanol.

Wash 2X quickly with ethanol.

Store samples in ethanol at -20°C indefinitely.