

Science Education Collection

Drosophila melanogaster Embryo and Larva Harvesting and Preparation

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Abstract

Drosophila melanogaster embryos and larvae are easy to manipulate and develop rapidly by mechanisms that are analogous to other organisms, including mammals. For these reasons, many researchers utilize fly embryos and larvae to answer questions in diverse fields ranging from behavioral to developmental biology. Prior to experimentation, however, the embryos and larvae must first be collected.

This video will first demonstrate how "egg-laying cups" are used to collect *Drosophila* embryos on agar plates. The harvest and dechoriation of embryos will then be described. Next, the video will demonstrate how to identify and manipulate *Drosophila* in one of the three larval stages that follow the embryo stage. Finally, examples of some of the ways in which fly embryos and larvae are used in biological research are provided.

Transcript

Drosophila melanogaster embryos and larvae are easy to manipulate and their development is guided by mechanisms that exist in other organisms, including mammals. Learning to harvest and prepare embryos and larvae is a preliminary step in many experimental processes from behavioral to developmental biology. This video will cover the standard methods for collecting and harvesting *Drosophila* embryos and larvae, essential procedures in the use of this versatile model organism.

The study of the *Drosophila* embryo has provided great insight into the manner by which genes regulate development, from the mRNA that is expressed as a gradient in the oocyte, to the genes that form the anterior-to-posterior segmented body plan. Some of these genes, like the homeobox genes are highly conserved between this insect and mammals.

The hearty nature of *Drosophila* embryos allows them to withstand exposure to harsh environments and chemicals, which makes them extremely practical to study.

After fertilization one female fly can lay up to 100 embryos per day, which will hatch into larvae after 12-15 hours.

In order to manipulate *Drosophila* embryos, they must first be collected.

Embryos are collected in egg-laying chambers often referred to as "egg-laying cups".

To assemble the laying cup, first poke holes or cut out part of a container and cover it with porous material to allow for ventilation. Then obtain an apple or grape juice agar plate, streak it with yeast paste, and scratch the plates in the center. The presence of yeast paste will induce egg laying.

Quickly add flies to the egg-laying chamber, invert the chamber so the plate is at the bottom, and allow it to incubate. After the desired incubation time invert the egg chamber and bang it on the bench top a few times. The flies fall to the bottom and are briefly disoriented. Quickly replace the old agar plate with the fresh plate layered with the yeast paste. To acquire the best-aged embryos change the plates every 1-3 hours.

Plates will have hundreds of embryos, especially near the scratches and yeast.

20 flies of each sex should produce 100-200 embryos per hour. Now the embryos can be harvested.

The tools needed for embryo harvesting are a strainer or sieve, which can vary greatly in size and complexity, a paintbrush, and distilled water.

First, loosen embryos by immersing the plate with distilled water, gently brushing the surface with a paintbrush. Next, filter out liquid by pouring the mixture into the sieve. Rinse the embryos with water.

Rinsing is often followed by dechoriation — the removal of the hard outer membrane of the embryo, or chorion.

Dechoriation can be done manually via a dissection of the embryo out of the chorionic sheath. Alternatively, embryos can be placed into 50% bleach. It takes 2-10 minutes for the chorion to dissolve, as noted by the disappearance of the dorsal appendages. Rinse thoroughly with distilled water to make sure the naked embryo is undamaged by bleach. Dechoriation is a prerequisite to techniques like microinjection and live cell imaging.

Now that you have gotten a sense of embryo biology, collection, and harvesting, let's move on to the next stage in the *Drosophila* life cycle: larvae.

Drosophila larvae have three "instar," or molting, stages. The first instar lasts one day, the second another day, and the third two more days. First and second instar larvae are found in the food of the vial 1-3 days after setting up a cross. On the 4th day, third instar larvae migrate up, or "wander" up the sides of the container, where they will ultimately form cocoons called pupae.

Larvae are often used for experimentation because of their imaginal discs. Imaginal discs are partially developed organs that are known to become whole parts of the adult fly. For example, an imaginal eye disc will become an adult eye, antennal discs will become antennae, and imaginal wing discs will become wings. The study of imaginal discs has led to important discoveries in *Drosophila*, such as the role of homeobox genes in pattern formation.

Larvae collection is simpler than embryo collection, because it does not require the transfer of flies into special housing.

Individual larva can be removed with tweezers or a spatula. When collecting a large number of early stage larvae you can employ an alternative collection method using a sucrose solution, which is more dense than the larvae and causes them to float.

Add sucrose solution to the vial, which buoys larvae to the top. Dislodge the food by placing the vial on a rotator. Then remove larvae with a brush or pipette and harvest for experiments.

Now that we've covered collection and harvesting techniques for embryos and larvae, now let's see how to apply them to experimentation.

Since they are mobile, *Drosophila* larvae can be used for behavioral experiments.

Here you see a "crawling assay", which is used to evaluate *Drosophila* locomotor behavior under specific conditions. The crawling assay measures the distance the larva travels, in order to observe the effects of a drug on motor function. Collected larvae are immersed in a drugged sucrose solution that is predicted to interfere with motility.

Microinjection is a procedure for creating genetically modified fruit flies, known as transgenic mutants, by inserting customized genetic material in the form of a circular DNA plasmid.

These embryos are dechorionated by physically rolling them on double-sided tape so chemicals won't damage them. Embryos can then be injected with plasmids that encode proteins, like tubulin, fused with fluorescent reporter proteins, like GFP. Experiments can then be performed to visualize cellular processes like mitosis from established transgenic fly lines.

Embryos can be used to visualize the presence of transcripts through fluorescent in situ hybridization.

In this experiment, whole embryos are observed for the presence of a desired mRNA transcript via fluorescence microscopy. The embryos are fixed using a biphasic solution that dechorionates, and therefore prepares the embryo for staining. The naked embryos are on the bottom layer. After immunofluorescent staining, the presence of the desired protein can be seen using fluorescence microscopy.

You've just watched JoVE's embryo and larva harvesting and preparation video. We reviewed the collection, harvesting, and preparation of embryos and larvae and some important applications applied to the early stage organisms. Thanks for watching!