Inside a Developing Fruit Fly Embryo*

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Laboratory experience strengthens theoretical concepts and is undoubtedly more engaging than plain textbook content. Developmental biology remains one of the less explored disciplines of biology in colleges and universities due to the unavailability of resources and infrastructure. Developmental biology focuses on how the fertilized egg develops into a multicellular organism that performs complex tasks. Thus, understanding embryogenesis is at the core of this subject. Can we see what is happening inside a developing embryo without high-end or fluorescence microscopy or having to deal with ethical issues? Yes, if we step on the shoulders of giants who worked on fruit flies before the emergence of high-end techniques! If you have wild-type fruit flies, a few chemicals and a regular light microscope in your college biology laboratory, we describe here a modified protocol to help you get an inside view of a gastrulating Drosophila embryo.

Introduction

Developmental processes are complex. Understanding the physiological, molecular and genetic aspects of development and integrating all information is crucial if we want to better understand this crucial stage of the life-cycle of any multicellular organism. Drosophila (fruit fly) is one of the model organisms to study developmental biology. The 1995 Nobel Prize in Physiology/Medicine was awarded to Edward B. Lewis, Christiane Nüsslein-Volhard and Eric Wieschaus for their discoveries concerning "the genetic control of early embryonic development" in Drosophila melanogaster. Most biology students study develop-

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**Figure 1.** Schematic of the early stages of *Drosophila* embryogenesis.

*Drosophila* embryos develop from blastula stage to final larval morphology in about 22 hours. Simply by counting hours since egg-laying, we can estimate the age of an embryo.

Drosophila embryos undergo rounds of cell division to form a blastula. The blastula consists of a single layer of epithelial cells encasing a cavity termed the blastocoel (Figure 1). *Drosophila* embryos develop from blastula stage to final larval morphology in about 22 hours. Simply by counting hours since egg-laying, we can estimate the age of an embryo. When we keep flies in a fresh bottle only for 17 hours, we know that the youngest embryo in the bottle could be 0 hours old, and the oldest could be
17 hours old. In the next section, we will discuss how we can collect precisely aged embryos.

**Ageing and Collection of Embryos**

At first, we need to obtain wild type flies. In fruit fly genetics, a normal fly that does not carry any mutation or that does not have any transgene inserted is called the wild type (WT). If you capture a fruit fly using banana as bait at home, the population you would end up growing could be different from the wild type flies maintained as the standard normal population used in fruit fly genetics. The unidentified insect you captured could also be a pest for the normal population or carrier of a disease of other plants and animals. Growing it could be hazardous. We would strongly suggest you obtain flies from a recognized laboratory if your college or university does not maintain wild type fruit flies. You can request a nearby research institute/university faculty with a *Drosophila* lab. Or you may reach out to us. Next, we will prepare the bottle (as shown in Figure 2).

**Figure 2.** How you can turn a plastic bottle into a device for collection of *Drosophila* eggs. (1) Take any small narrow-mouthed (about 200 ml) soft plastic bottle and clean thoroughly. (2) Cut it using a hot kitchen knife at around 1 inch from the bottom, as shown by the dashed line. (3) This separates the bottom chamber. (4) Seal the two parts of the bottle together with cello tape. (5) Pour fly media in the bottle to a level below knife cut and plug the bottle with sterile cotton.

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Box 1. Composition of Fly Medium

For 250 ml of fly medium, weigh 20 g of corn flour, 5 g of D-glucose/dextrose, 10 g of sucrose, 2 g of agar, and 3.75 g of yeast extract powder. Make up the volume to 250 ml. Stir thoroughly, autoclave at 1200°C for 25 minutes, cool the media up to 55°C and then add 1 ml propionic acid and 0.15 ml of orthophosphoric acid. Weigh 250 mg of Tego(methylparahydroxy benzoate). Dissolve Tego in 1 ml of ethanol and add dissolved Tego to the media prepared.

Figure 3. How to transfer flies. The bottle has flies hovering over the whole bottle as shown by black dotted lines in (1). (2) Bottle A is banged over a hard sponge to make the flies settle at the bottom. (3) The flies are quickly transferred to another bottle by inverting the bottle with flies over the bottle with fresh food and banging both the connected bottles on a sponge.

Fill the media (see Box 1) up to a level below the knife cut of the bottle. Apply soaked yeast granules paste on the walls of the bottle (the yeast granules improve the chances of egg lying). Transfer wild type flies to this bottle (as shown in Figure 3). Incubate the bottle overnight (~17 hrs) at 25°C or room temperature, away from direct sunlight, heat or chemicals.

On the second day, transfer the flies incubated overnight into another bottle. Now, all the embryos seen on the food medium in the original bottle are aged between 0 to 17 hours. Remove the cello tape from the incubated bottle. Use a ‘wet’ paintbrush to collect embryos (Figure 4) from the bottom half of the bottle.
Now that we have collected embryos, we need to break open the eggshell. We use freshly diluted household bleach for this purpose.

**Dechorionating the Embryos**

Transfer the embryos to a microcentrifuge tube containing 1 ml of water. Now, gently invert the tube ~10 times to wash. Then put the tube in the rack and allow the embryos to settle to the bottom. Remove the water from the tube using a plastic dropper/glass Pasteur pipette (*Figure 5*).

Repeat the wash with water. Now 'bleach' the embryos with 1 ml of 50% bleach and incubate for 2 to 3 minutes. Bleach re-
Figure 6. The *Drosophila* embryo. Notice that the ventral surface is more curved than the dorsal. You should not see dorsal horns in dechorionated embryos.

![Diagram of Drosophila embryo](image)

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1Composition of PBS: 60 mM \( \text{K}_2\text{HPO}_4 \) 40 mM \( \text{KH}_2\text{PO}_4 \) 1.4 M \( \text{NaCl} \).

Fixing preserves tissue and prevents its degradation. Once the *Drosophila* embryos are fixed, the vitelline membrane needs to get permeabilized so that the dye—toluidine blue (TB)—can enter the embryo later. We will use formaldehyde for fixation and heptane for permeabilization.

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Fixing and Permeabilizing Embryos

To fix the embryos, add 70 \( \mu \text{l} \) of 37% formaldehyde and 700 \( \mu \text{l} \) heptane to the microcentrifuge tube containing the embryos. Both heptane and formaldehyde are toxic, so avoid inhaling or getting them on your skin. Perform this step in a fume hood. Gently invert the tube several times to mix. Incubate the microcentrifuge tube with gentle shaking for 15 minutes at room temperature. Embryos are fixed. Remove the embryos from the shaker and let them settle in the rack for 2 minutes. They will
aggregate at the interphase between the two liquids. Remove the fixative/heptane solution above and below your embryos using a Pasteur pipette/plastic dropper. Quickly add 1 ml PBST. Continue with two washes of PBST.

Seeing morphogenetic movements of *Drosophila* embryo is difficult for an untrained eye. Next, we will stain the embryos with toluidine blue to increase contrast, ensuring better visualization.

**Staining With Toluidine Blue**

Remove PBST and add 1 ml of TB² similarly, as described in *Figure 5*. Incubate for 1 to 5 minutes (or till the embryos are visibly stained in light blue). Give a single wash with 1 ml of PBS. Once PBS is removed, dehydrate embryos in grades of ethanol (this removes excess stain). (35%, 50%, 75%, 95% of ethanol). Incubate embryos in the ascending grades of 1 ml ethanol for 2 minutes each at room temperature (*Figure 5*). For observing all stages, particularly the early cellularization stage, use very lightly stained embryos. After the germ band begins to extend, the cells of the germ band stain blue. The region near the anterior side of the cephalic furrow stains purple or dark blue, and the amnioserosa looks light blue or yellow (*see Figure 7*).

You may also wish to go through ‘The Interactive Fly’, a website maintained by the Society of Developmental Biology, to view beautiful videos and demonstrations of *Drosophila* gastrulation. The link is mentioned in the suggested readings at the end of the article. You will also find reference images and diagrams for identifying stages of *Drosophila* embryogenesis on the website.

Mount embryos in glycerol and observe under 10x and 40x objectives of an upright microscope. While mounting, put a tape/glue additional coverslips on both sides of the sample. This ensures that the embryos do not get squashed while mounting. You should be able to observe most of the stages of a developing *Drosophila* embryo using TB staining (*Figure 7*).

Once you have mastered the technique and are confident about it,
Figure 7. Toluidine blue staining of gastrulating *Drosophila* embryo as viewed under 10x (A, B, C) and 40x (A', B', C') objectives of upright light microscope. (A, A'): Cellularization. The stained embryo is at stage 5 of *Drosophila* embryogenesis. Cellular blastoderm (single layer of embryonic epithelial cells) can be seen in lightly stained embryo. (B, B'): Germ band elongation. Embryo stained is at stage 10 of embryogenesis. Posterior midgut (black dashed line-B'), stomodeum (red arrow-B), germ band (red dash-B), and amnioserosa (yellow dots-B) are marked. The stomodeum is a depression between the brain and the pericardium in an embryo and is the precursor to the mouth. The amnioserosa is a short-lived extra embryonic epithelial tissue that plays a crucial role in the embryo development. (C, C'): Germ band retraction. The embryo stained is at stage 13 of embryogenesis. Cyan dots in C outline protruberances of rudimentary head segments of the embryo, and yellow dots mark amnioserosa. Other abdominal segments can also be seen.

you can answer many questions. For example, would you like to explore if certain environmental conditions or exposure to a putative teratogen is arresting/slowing the development of fly embryos?

Do let us know at the email address mentioned in the author information when you perform this experiment in your lab, of the difficulties you faced, and the results you observed. We will be happy to help and aid you to improvise.

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Suggested Reading


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