Protocol

Collection and Mounting of *Drosophila* Embryos for Imaging

Richard M. Parton, Ana Maria Vallés, Ian M. Dobbie, and Ilan Davis

INTRODUCTION

The fruit fly *Drosophila melanogaster* is an important model for basic research into the molecular mechanisms underlying cell function and development, as well as a major biomedical research tool. A significant advantage of *Drosophila* is the ability to apply live cell imaging to a variety of living tissues that can be dissected and imaged in vivo, ex vivo, or in vitro. For example, such imaging can be used for visual genetic screens such as analysis of morphological characteristics or of the distribution of fluorescently tagged proteins in living embryos. Indeed, *Drosophila* embryos have proven to be a useful model system for studying a variety of cellular processes such as asymmetric division, migration, wound healing, apoptosis, and fasciculation, as well as for tracking lipid droplet motility, protein recycling, fast mRNA transport, and the movement of chromosomal loci within individual cells. A crucial first step before imaging is preparation of the experimental material to ensure physiological relevance and to achieve the best conditions for image quality. Because it contributes to autofluorescence and lack of transparency, it is useful to remove the chorion before imaging by treating the embryos with bleach. This protocol describes the collection and mounting of *Drosophila* embryos for live cell imaging.

RELATED INFORMATION

The procedure presented here is summarized and illustrated in Figure 1. General information is available about **Live Cell Imaging in *Drosophila melanogaster*** (Parton et al. 2010). Information is also available on **Maintenance of a *Drosophila* Laboratory: General Procedures** (Ashburner and Roote 2007).

In principle, the simplest method of introducing membrane-permeable reagents into *Drosophila* embryos is to soak the tissue in the reagent. However, in practice the waxy vitelline membrane surrounding embryos prevents membrane-permeable reagents from accessing the plasma membrane. The solution to this problem comes at a cost: Embryonic vitelline membranes can be permeabilized with heptane or octane before adding the membrane-permeable solutions, but this treatment yields embryos with very fragile membranes that exhibit nonspecific lethality and sickness. With practice and suitable equipment, injection is the fastest, most reliable, and quantitatively reproducible technique to introduce fluorescent labels into embryos.

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Drosophila melanogaster

For culture of Drosophila*, see* ***Maintenance of a* Drosophila *Laboratory: General Procedures*** *(Ashburner and Roote 2007).*

Adapted from *Live Cell Imaging*, 2nd edition (ed. Goldman et al.).
CSHL Press, Cold Spring Harbor, NY, USA, 2010.
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Halocarbon oil (series 700; Halocarbon Products Corporation)

With a refractive index similar to glycerol, Halocarbon oil also has good optical properties for imaging; Halocarbon oil also provides a good environment for injection and is particularly useful for long-term imaging of embryos. However, unlike with aqueous solutions, changes in the medium are not possible.

<R>Heptane glue

<!>Sodium hypochlorite (7%, w/v)

Prepare fresh before use. Alternatively, use commercial bleach diluted 1:1 with water.

Water agar plates (i.e., 1.5%-2.0% agar)

<R>Wine agar plates

Yeast powder or paste

Equipment

Coverslips, glass

Drying chamber

<!>*Prepare a small plastic container with a tight-fitting lid, containing dry silica gel underneath a metal mesh.*

Egg-laying cage

Use a plastic cylinder with a nylon mesh top.

Filter tube

Use a plastic tube (2- to 5-cm diam) with nylon mesh fixed to the bottom.

Microscope, dissecting

Paintbrush, small, soft-bristled

Scalpel

Slides

Tape

Teflon membrane (e.g., standard membrane kit; YSI Life Sciences 5793) (optional; see Step 13)

Weigh boat, plastic

METHOD

1. Place flies (5-12 d after hatching) in the egg-laying cage. Tape a wine agar plate sprinkled with yeast powder or smeared with yeast paste to the bottom of the cage.
2. After the desired time, exchange the plate.

   Age as required to obtain the appropriate stage (e.g., for blastoderm embryos, age for 2 h at 25°C).
3. Add ~5 mL water to the plate that has been removed. Use a soft-bristled paintbrush to loosen the embryos from the agar surface.
4. Pour the suspended embryos into the filter tube. Using a plastic weigh boat as a washing basin, rinse the embryos twice with water at room temperature to clean off yeast and debris.
5. Dechorionate the embryos (i.e., remove the egg shell or chorion) by incubating with gentle agitation in a freshly prepared solution of ~7% sodium hypochlorite for 1-2 min.

   When dechorionation is complete, the embryos will tend to float.
6. Wash the embryos twice with water at room temperature.
7. Blot excess water from the filter tube. Use a fine brush (e.g. small paintbrush) to transfer the dechorionated embryos to a water agar plate for arrangement.
8. View the embryos under a dissecting microscope. Space them at least one embryo-width apart to avoid problems of anoxia.

   Arrangement in rows facilitates injection.

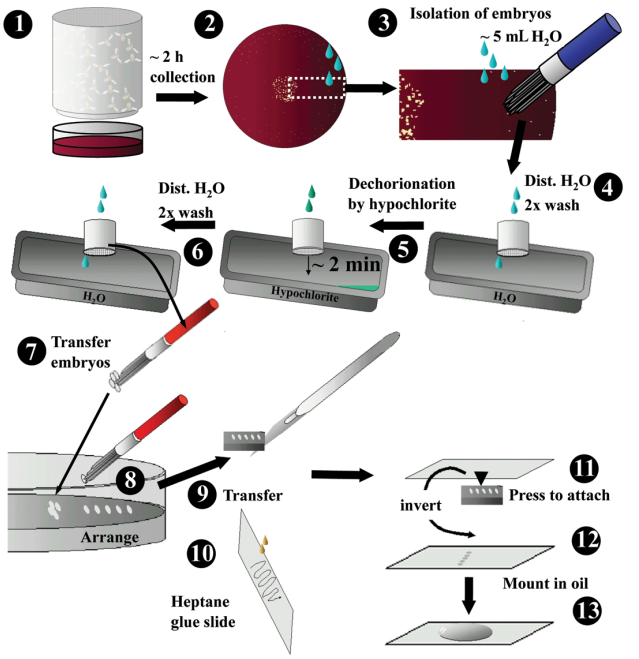

FIGURE 1. Collection and mounting of *Drosophila* embryos.

9. Cut out an agar block under the aligned embryos. Use the block to convey the embryos to a slide.
10. Apply ~20 µL of heptane glue at an angle to a glass coverslip over a 1.5- × 3-cm area. Streak from left to right to coat the coverslip with the glue. Allow to dry.

    The dried glue should be only just visible on the slide.
11. Press the coverslip firmly against the agar block to transfer the embryos.

    The agar block prevents crushing and desiccation.
12. Gently dry the embryos in a chamber containing a small amount of silica gel for 4-10 min.

    Embryos should be neither too dehydrated nor too turgid. Slight dehydration removes surface water and allows the embryos to be flattened or injected more easily. Avoid excessive dehydration; it leads to flaccid, defective embryos. The right extent of dehydration can be judged with experience.
13. Cover the embryos with Halocarbon oil.

    If access to the embryos is not required, a Teflon membrane helps to maintain viability for prolonged observations and improves optical clarity. The preparation is now ready to view under the microscope.

REFERENCES

Ashburner M, Roote J. 2007. Maintenance of a *Drosophila* laboratory: General procedures. *Cold Spring Harb Protoc* doi: 10.1101/pdb.ip35.

Parton RM, Vallés AM, Dobbie IM, Davis I. 2010. Live cell imaging in *Drosophila melanogaster*. *Cold Spring Harb Protoc* (this issue). doi: 10.1101/pdb.top75.

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