Video Article

Preparation of embryos for Electron Microscopy of the *Drosophila* embryonic heart tube

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Abstract

The morphogenesis of the *Drosophila* embryonic heart tube has emerged as a valuable model system for studying cell migration, cell-cell adhesion and cell shape changes during embryonic development. One of the challenges faced in studying this structure is that the lumen of the heart tube, as well as the membrane features that are crucial to heart tube formation, are difficult to visualize in whole mount embryos, due to the small size of the heart tube and intra-lumenal space relative to the embryo. The use of transmission electron microscopy allows for higher magnification of these structures and gives the advantage of examining the embryos in cross section, which easily reveals the size and shape of the lumen. In this video, we detail the process for reliable fixation, embedding, and sectioning of late stage *Drosophila* embryos in order to visualize the heart tube lumen as well as important cellular structures including cell-cell junctions and the basement membrane.

Video Link

The video component of this article can be found at https://www.jove.com/video/1630/

Protocol

1. Freshly prepare 2ml of a fixative solution containing 12.5% Glutaraldehyde in 50mM Cacodylate buffer (pH7.4) in a 20 ml glass scintillation vial. Add 8ml of n-heptane and shake vigorously. Allow the two phases to separate. Remove upper phase containing n-heptane saturated with glutaraldehyde, place into a clean scintillation vial and set aside. This will be the fixative solution.
2. Collect 0-20 hour embryos using an embryo collection chamber, which is a small basket with a removable mesh insert. Remove the outer chorion membrane by soaking embryos in a 50% bleach solution for 2-3 minutes, or until embryos float to the surface of the bleach. Rinse thoroughly with ddH20 and blot embryos on a paper towel.
3. Using forceps, pick up mesh insert which is covered with dechorionated embryos and place into the fixative solution, allowing embryos to fall from the mesh. Allow embryos to fix for 1.5 hours at 4°C while mixing on a Nutator.
4. Prepare a Petri dish lined with double-sided tape. Remove embryos from the fixative using a Pasteur pipet and place them onto taped surface of Petri dish.Transfer the embryos in a minimal amount of fixative solution to prevent the heptane in the fixative from dissolving the glue of the tape. Shake the Petri dish to make a single layer of embryos. Place Petri dish in hood until heptane evaporates. Add enough PBS + 0.1% Tween20 to cover the embryos. Hand-devitellinize late stage 16 embryos under a dissecting microscope with a blunt tungsten needle. Recover embryos with a Pasteur pipet into a 1.5 ml microfuge tube. Steps 5-7 are carried out in this microfuge tube.
5. Rinse embryos in 0.1M Cacodylate buffer, pH 7.4 and then post-fix in a solution containing 1% osmium tetroxide in 0.1M Cacodylate Buffer, pH 7.4 for 1 hour at room temperature. Rinse embryos with 0.1M Cacodylate buffer, pH 7.4.
6. Dehydrate embryos in a graded series of ethanol and acetone. Dehydrate embryos with 50% ethanol for 10 minutes, followed by 70% ethanol for 10 minutes. Then dehydrate in 90% acetone for 10 minutes, and two 100% acetone steps of 10 minutes each.
7. Remove acetone and then begin the infiltration process by adding a 1:1 Epon-Spurr resin: acetone mixture to the embryos. Microwave for three minutes using a Pelco Biowave Pro microwave while keeping the sample under vacuum pressure. Apply vacuum pressure to the sample for an additional five minutes after the microwave has ended. Exchange the 1:1 resin, acetone mixture with 100% Epon-Spurr resin and microwave again under vacuum pressure. Exchange the 100% Epon-Spurr resin a final time, and microwave under vacuum pressure.
8. Using a Pasteur pipet, embryos are transferred from the microfuge tube to a silicone embedding mold. Align embryos into a row such that the posterior end of the embryo aligns with the tapered edge of the mold. Bake in a 70°C oven overnight.
9. Remove sample and prepare for sectioning.
10. Begin cutting 1µm sections using a Diamond Histo Knife and Richert Ultracut E Microtome. Take note of when the first embryo section is reached. From this point, cut 100µm into the sample. Remove a section using a loop, and place onto a glass slide. Briefly heat the section on a hot plate. Stain the section using a drop of Methylene Blue. Under a light microscope identify the heart lumen.
11. Cut 90nm sections using a Diamond Ultra 45° Knife and Richert Ultracut E Microtome. Pick up multiple sections on a copper grid.
12. Grids are stained with 3% uranyl acetate saturated in 50% ethanol for ten minutes and then rinsed three times in double distilled water. Then, the grids are stained with lead citrate (0.04gms dissolved in 10ml double distilled water and 100ml 10M NaOH) for 2.5 minutes in a chamber containing NaOH pellets to create a CO_2-free environment. The grids are rinsed three times in double distilled water.

13. Sections are examined with a JEOL 1200EX electron microscope at 80Kv, and photographed with an AMT digital camera.

Discussion

The morphogenesis of the *Drosophila* embryonic heart tube has emerged as a valuable model system for studying cell adhesion and cell shape changes during embryonic development. One of the challenges faced in studying this structure is that the lumen of the heart tube is difficult to visualize in whole mount embryos. The technique demonstrated in this video, which was adapted from previously described procedures [1-2], has proved to be successful in efficiently and reliably analyzing a substantial number of genotypes for heart tube and lumen formation[3].

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