

Preparation of Tissue for Larval In Situ Hybridization (Frozen Sections)

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Although overall reactions are identical to the protocol for adults, preparation of samples needs some considerations. Therefore I will describe here how to dissect larvae, embed in the frozen compound and section them.

A. Dissection of Larvae

1. Pick up third instar larvae from vials, and place them in 1xPBS to get rid of debris attached to their bodies.

2. Place larvae in a small dish filled with 1xPBS. Pinch neck and posterior end of a larva with sharp forceps under the microscope, then pull it apart. The head will dissociate from the rest of the body (Figure (1)).

Although the definition of neck is intuitive, junction between 4th and 5th segment will be recommended. A larva has a pair of characteristic fat tissues around the neck, apart from the rest of the fat tissues in more posterior part. It is apparent when a larva fully stretches its body and can be served as a landmark.

3. Usually you can see two hemispheres of brain and ventral ganglion, which consist of the larval central nervous system, extending from the open end of dissociated head. It is saved as a landmark, while all the fat tissues, digestive tubes and salivary glands are removed by using sharp forceps (Figure (2)). All the imaginal discs do not need to be removed. After this procedure, a head will look like a picture of Figure (3).

4. Transfer larval heads into OCT in plastic boat. Let OCT penetrate in heads by mixing OCT gently with forceps (Figure (4)). Do not leave heads in OCT for more than 10 minutes at room temperature, for OCT gets dry and hardened.

5. Transfer larval heads to new plastic boat filled with fresh OCT, 14 heads per boat. Send all the heads to the bottom of the boat, and align them in 7 x ~ 2 rows (Figure (5)). It is recommended that you place heads so that ventral surfaces are up toward experimenter, while dorsal surfaces come down, facing the bottom plate of the plastic boat (Figure (6)). This configuration allows you to make fewer sections before you reach dorsal organ ganglions which are located closer to dorsal surface.

6. Freeze them on dry ice. These frozen blocks can be saved in -80°C freezer for later use. I validate that blocks can be saved up to a week, but longer preservation may be possible.

B. Sectioning of blocks

Although there is no special protocol for sectioning, you have to be well trained to use it. Black mouse hooks will be a good indicator for slices containing dorsal organs, but dorsal organ ganglion will be present in only 1 or 2 slices per larva. Therefore, it is required that as many slices (at least containing mouse hooks) as possible should be collected. If examination on terminal organs or ventral organs is necessary, much more

attention has to be paid. My experiences have indicated that 1 to 6 samples with signals on dorsal organ ganglions can be collected from on slide (containing 14 heads).

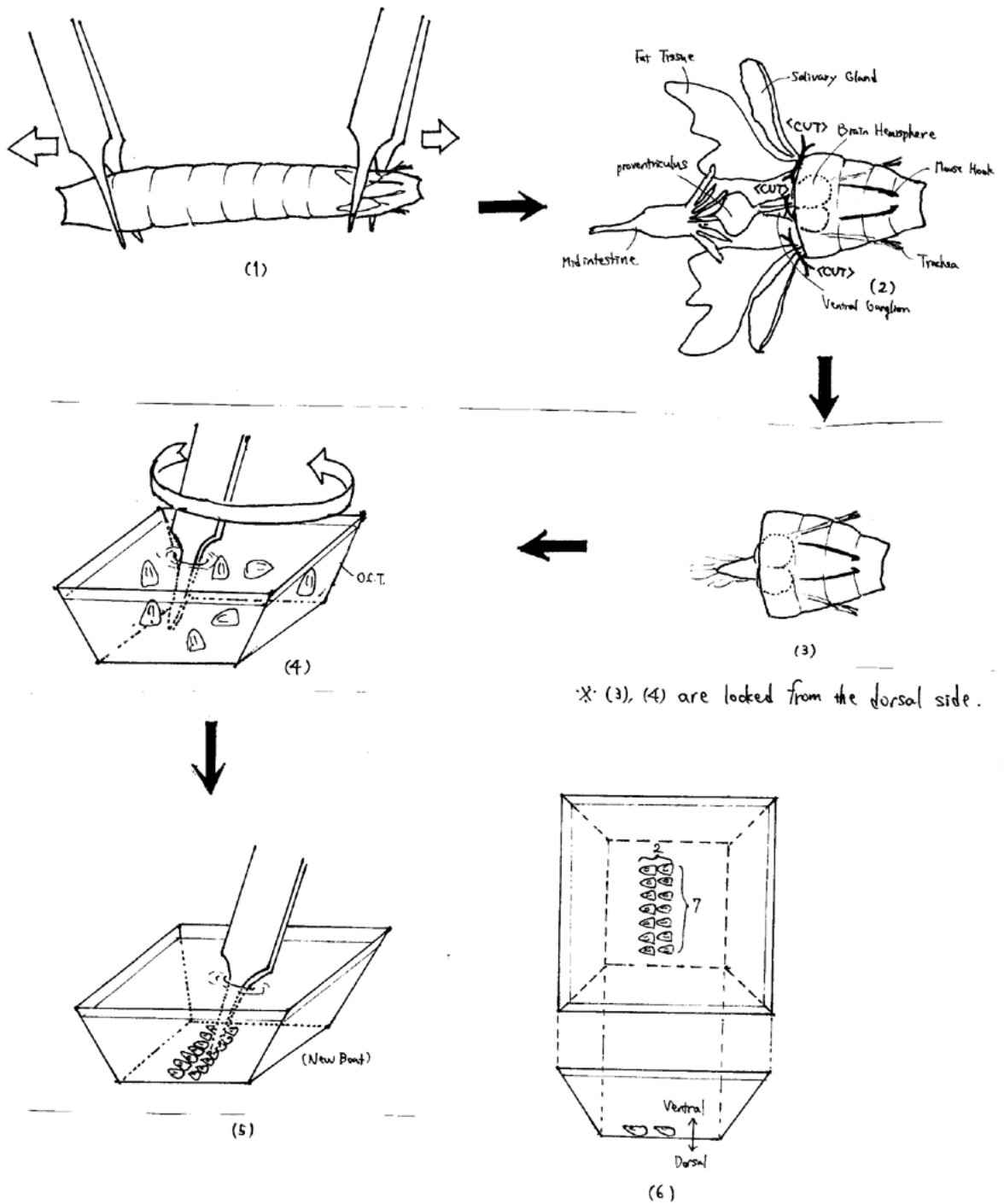
12 μm is shown to be good thickness for larval in situ hybridization. Sometimes I found that 14 μm , the conventional thickness, was not as good. The reason is so far unknown, but I would just remind you that 12 μm is working well.

Before starting collecting slices, the angle of collars should be adjusted so that the bottom plane of the block becomes parallel to the plane of the knife. This is important in two senses; it would make the shape of slices better, and it would reduce the number of slices you have to make.

Usually sectioning one block will take you 45 to 60 minutes. Considering the time for the rest of the in situ hybridization protocol, 6 would be the maximum number you can handle at once. All the conventional steps are applied thereafter.

Sectioning part is critical for getting successful result. It would be a good idea to train yourself with dummy frozen blocks until you feel comfortable in using the cryostat.

The sample preparation and sectioning procedures are still under development. Any improvement on this protocol is highly welcome.



* (3), (4) are looked from the dorsal side.

FIGURES: Sample preparation.