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## A simple axial grafting method for Hydra

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USD Hydra



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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:** Hydra, grafting, regeneration, simple axial grafting method for hydra, tissue grafting in hydra, threading hydra tissue, hydra tissues onto fishing line, hydra tissue ring, method for axial grafting, simple method for axial grafting, axial grafting, simple axial grafting method, lateral grafting experiment, contribution of lateral grafting experiment, tissue grafting, lateral grafting method, separate hydra polyp, other during grafting, reliable creation of multiple graft, multiple graft, grafting, tissue ring, hydra, pieces of polyethylene tubing, polyethylene tubing, secondary body axis, tissue, tissues in close contact, immobilizing tissue, body axis patterning, body axis, understanding body axis, cell migration, exploring body axis patterning

## Abstract

This protocol is a low cost and easy to implement method for axial grafting of Hydra. In 1742, Trembley first observed that tissues from two separate Hydra polyps can join when the cut edges are placed in contact (Lenhoff and Lenhoff, 1984). Tissue grafting in Hydra has remained a valuable tool for exploring body axis patterning and cell migration in this anatomically simple system. Ethyl Browne observed that grafting of a single tentacle onto the body column induced the development of a secondary body axis, the first demonstration of a developmental organizer in animals (Browne, 1909). Shimizu (2012) has reviewed the contribution of lateral grafting experiments to understanding body axis patterning in Hydra.

Various techniques have been developed for immobilizing tissues in close contact with each other during grafting. MacWilliams (1983) describes a method where Hydra and Hydra tissue rings are threaded onto fine glass needles held in place by wax. Other published methods describe threading Hydra tissues onto fishing line (Sugiyama and Fujisawa, 1978) and holding them in place with either pieces of Parafilm (Shimizu and Sawada, 1987) or pieces of polyethylene tubing (Forman and Javois, 1999).

Here we describe a simple method for axial grafting which allows rapid and reliable creation of multiple grafts. This method uses fishing line "skewers" attached to a microscope slide to align tissue rings cut from the body column. The slide holder petri dish allows the slide to be removed for cleaning or replaced. Attaching the skewers to a microscope slide was inspired by the lateral grafting method described by Wang and Collins (2020).

Thank you to Rob Steele for assistance with this protocol.

## Image Attribution

Callen Hyland



## Guidelines

For general guidelines for maintaining, working with and disposing of Hydra, please see [Low Cost Methods for Hydra Care](#).

Hydra should be starved at least 48 hours before starting this experiment.

## Materials

### Materials for making grafting slides:

- Glass microscope slides
- Monofilament fishing line (any brand, 0.2-0.6 mm diameter)
- Two-part epoxy
- Toothpick or pipette tip
- Fine forceps
- Scalpel or razor blade
- Stereo dissecting microscope

### Materials for making slide holder:

- 100 mm diameter petri dish
- Two-part epoxy
- Toothpick or pipette tip
- Three or four 0.65 mL microcentrifuge tubes
- Razor blade

### Materials for grafting protocol:

- Grafting slides
- Slide holder
- Petri dish for dissections
- Glass Pasteur pipette and bulb
- Scalpel
- Fine forceps
- Filter paper, Kimwipe, or Parafilm
- Hydra and Hydra medium
- Slide holder or 100 mm diameter petri dish
- Stereo dissecting microscope
- Double-sided tape (optional)

## Troubleshooting



## Safety warnings

- ⚠ Use caution when working with glass Pasteur pipettes and sharp items like razor blades and scalpels. Dispose of broken glass, used Pasteur pipettes, razor blades and scalpels in a sharps container.

## Before start

Collect all materials and review protocol. Grafting slides and slide holder should be made the day before starting the protocol.

## Prepare grafting slides

- 1 Working under the dissecting microscope, cut the fishing line into 10 mm segments using the razor blade or scalpel. Cut the fishing line at an angle to create a sharp point (Figure 1). We usually make slides with 10-12 fishing line "skewers" each.

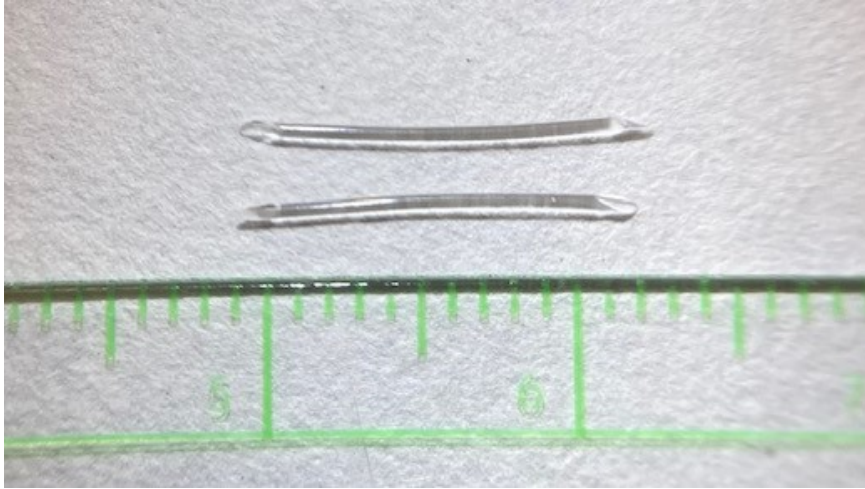


Figure 1. Fishing line skewers cut at an angle.

- 2 Prepare the two-part epoxy according to the manufacturer's instructions. Do the next two steps quickly before the epoxy dries.
- 3 Use a micropipette tip or wooden toothpick to smear a stripe of epoxy on the edge of a microscope slide (Figure 2).

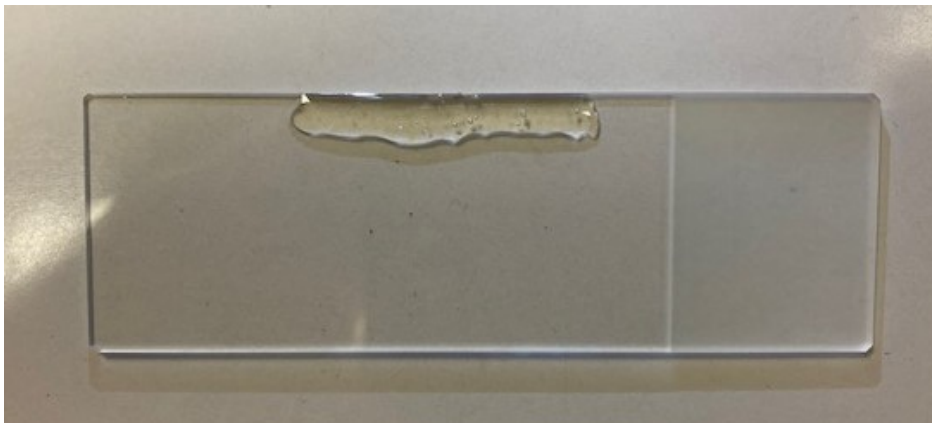


Figure 2. Stripe of epoxy on microscope slide.

- 4 Use forceps to place the fishing line skewers in the wet epoxy so that the sharp points are oriented perpendicular to the edge of the slide and parallel to each other (Figure 3). The pieces of fishing line can move around while in the epoxy is still wet and might need to be adjusted.

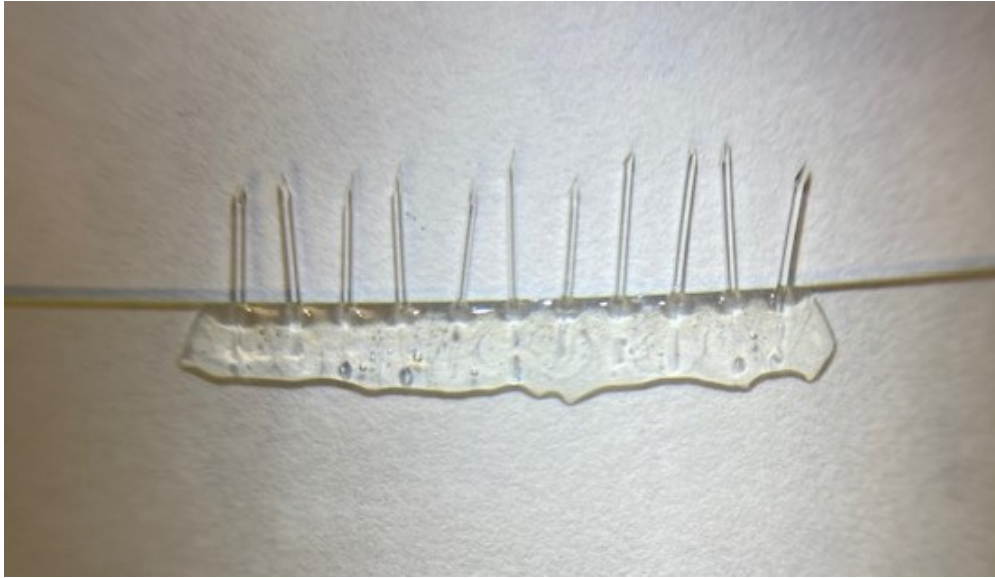


Figure 3. Fishing line skewers embedded in epoxy.

- 5 Allow the epoxy to cure overnight.

## Prepare slide holder

- 6 Use a razor blade to cut off lids of 0.65 mL microcentrifuge tubes. The slide holder in the figure is made with three microcentrifuge tube lids, but you could also use four to make the slide holder more secure.
- 7 Use a razor blade to cut off half of the inside part of the lid (Figure 4).

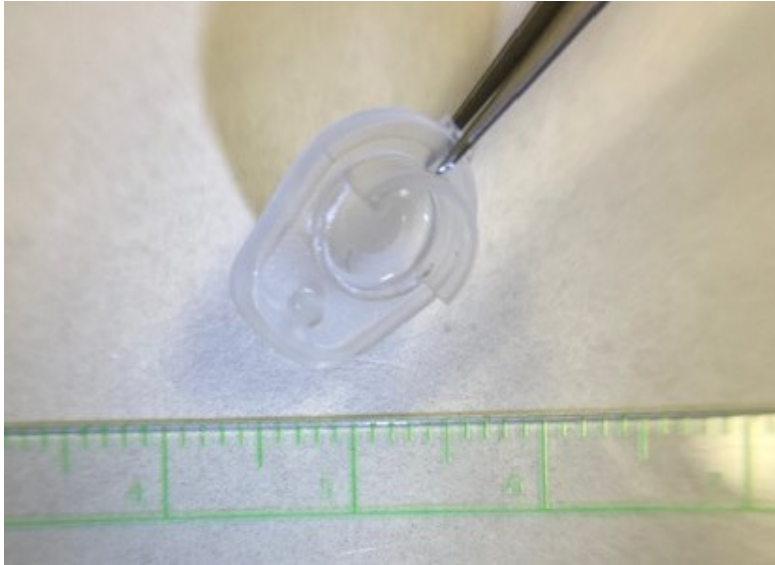


Figure 4. microcentrifuge tube cap cut for the slide holder.

- 8 Prepare the two-part epoxy according to the manufacturer's instructions. Do the next step quickly before the epoxy dries.
- 9 Use a pipette tip or toothpick to smear epoxy on the flat side of the microcentrifuge lids and position the tube lids in the 100 mm petri dish to secure your microscope slide (Figure 5). Use the same type of microscope slide to position the tube caps as you used to make the grafting slides. We have found that slide brands differ slightly in their dimensions.





Figure 5. Slide holder made of a petri dish and microcentrifuge tube lids holding the grafting slide in place.

- 10 Allow the epoxy to cure overnight.

## Hydra axial grafts

- 11 Place the grafting slide in your 100 mm petri dish. You can use either the custom slide holder or a piece of double-sided tape to hold the slide to the bottom of the dish. Fill the petri dish with Hydra medium so that the grafting slide is submerged.
- 12 Fill your other petri dish with Hydra medium. Working under the dissecting microscope, use the scalpel to cut the Hydra horizontally through the body column to make the pieces that will be grafted together. The figures demonstrate a simple graft with a head, foot, and body column from three different polyps.
- 13 Use the Pasteur pipette to transfer the tissue pieces to the petri dish with the grafting slide. Use forceps to slide each tissue piece onto the fishing line skewer.



**Note**

Linalool anesthetic can be used to immobilize Hydra prior to dissection. This may increase the success rate of grafting (Goel et al., 2019).

- 14 Use forceps to slide a small piece of filter paper or Kimwipe onto the end of the fishing line (Figure 6). This will keep the tissue pieces from coming off the end of the fishing line. Use the filter paper to gently press the tissue pieces together.

**Note**

A small piece of polyethylene tubing can also be used to hold the tissue pieces on the fishing line (Forman and Javois, 1999). We do not use this method, both to reduce cost and to make the protocol adaptable for any size fishing line.



Figure 6. Hydra tissue rings on skewers held on with small pieces of Kimwipe.

- 15 Allow at least 2 hours for the tissue pieces to form the graft.
- 16 Use forceps to remove the filter paper or Kimwipe, then gently slide the grafted Hydra off the end of the fishing line. Use the Pasteur pipette to transfer the grafts to another petri dish or plate with Hydra medium.

Figure 7 shows the result of grafting together a head and foot piece of a transgenic Hydra expressing DsRed in the ectoderm with a body column piece from a non-fluorescent AEP-HS Hydra three hours after the tissue pieces were placed on the fishing line.

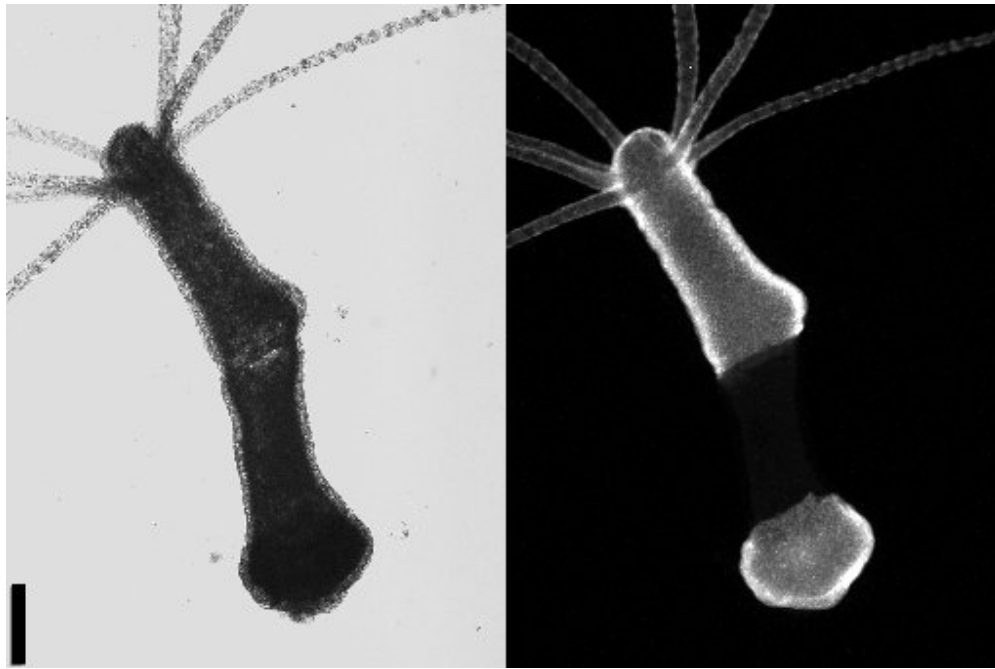


Figure 7. Graft between AEP-HS body column piece and DsRed-expressing head and foot. Scale bar is 50  $\mu$ m.

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