

Start video:

An ideal dissection specimen is an animal that has been fasted for at least 1 day prior to dissection, so that the gut is empty.

Weigh the animal and prepare a syringe and 23-gauge needle containing 1/6 body mass by volume of isotonic magnesium chloride (see below for MgCl<sub>2</sub> procedure).

Grasp the animal so that the rear sinus on the right posterior side of the animal is exposed.

Squeeze gently so that the sinus appears as a slight bump under the skin.

Using a very shallow angle of approach, inject anesthetic into the posterior sinus.

Rest the animal in air for approximately 5 minutes, and check level of anesthesia by pinching the tail; the goal is an absence of response or only a very weak, slow tail withdrawal response.

Pin out the animal foot side down, taking care to avoid the head and the tail. Roll back the parapodial flaps for mounting through them, and place additional pins through the skin thickness, avoiding piercing internal organs.

The goal is to cut open the dorsal surface, from the base of the genital groove, anteriorly through the midline between the rhinophores.

Locate the initial incision site just to the left of the base of the genital groove. This is anterior to the opening of the ink gland. Using forceps to stabilize the skin, press the lower blade of the scissors through the skin, and either cut or pull the blade through the skin in the anterior direction, while pulling up on the blade, to avoid nicking the gut.

Push internal organs to the side, and adjust pins if necessary.

The head- or ring ganglia should be visible, arranged in a ring around the buccal mass.

Locate the bright yellow abdominal ganglia posterior to the main head ganglia, and separated from them by a pair of long connectives.

Remove the abdominal ganglia completely (as illustrated), or if it is desired to leave them adhered to the head ganglia, cut the connectives serving the lower viscera but leave connectives to the head ganglia intact.

The head ganglia are arranged loosely connected to the buccal mass by many small connections, which can be cut to free them. These are the cerebral ganglia, located most anterior and in the center, the paired left and right pedal-pleural ganglia, and the buccal ganglia. The tiny paired buccal ganglia are more tightly attached to the ventral buccal musculature, and will often require special attention to free them. After freeing the ganglia from the buccal mass, remove the head ganglia from the animal either by cutting the nerve connectives between one pair of pedal-pleural ganglia and the cerebral ganglia (as shown), or by cutting the esophagus, which keeps the main connectives between ganglia intact.

Place the ganglia in 2 rinses of artificial seawater plus penicillin-streptomycin. A dissection done with clean instruments and without either cutting the esophagus or nicking the gut will yield ganglia suitable for digestion for short-term tissue culture.

**Isotonic MgCl<sub>2</sub>:** Dissolve 108 g of MgCl<sub>2</sub> · 6H<sub>2</sub>O in 1450 ml high quality water; will yield a solution of approximately 960 mosm. Chill to 15°C in refrigerator in glass media jars.