Protocol 2: Observing Planarian Regeneration

This experiment is suitable for students at any level of education. The aim of this experiment is to observe the regeneration of worms following amputation to identify: blastema formation, antero-posterior (A/P) and dorsal-ventral (D/V) axes conservation, eye regrowth, re-pigmentation of the regenerated tissues, and worm behavior. In Section 2.1, three different amputation paradigms are suggested, but the student should feel free to experiment also different strategies.

2.1. Planarian amputations

Put the desired number of 7-day starved worms in a Petri dish of adequate size as reported in Section 1.1 on the basis of the final number of fragments to be obtained.

1. Wipe the blade with ethanol

   Note: the edge of a coverslip works as well as blade

2. Cut the worms according to the intended amputation plan (A, B or C, described in the following paragraph or any desired amputation paradigms). If available, this step is easier and more precise if performed under a lens magnification or dissecting microscope.
3. Wipe the blade after every 2-3 cuts to remove planarian mucus.
4. On 1, 2, 4, 6, and 7 day post amputation, change the water as described in Section 1.2.
5. Start to feed the planarians again 2 weeks after the amputation, as described in the Section 1.3.

The suggested amputation strategies are:

A. Two cuts perpendicular to the A/P axis: the first cut is made between the eyes and the pharynx, the second cut between the pharynx and the tip of the tail. Three fragments are obtained: head, trunk and tail.

B. One cut parallel to the A/P axis: the cut is made along the animal's midline between the two eyes, along the pharynx and the tail. Two fragments are obtained, right and left fragments.

C. One cut oblique to the A/P axis: the cut is made from the right side close to the head, across the pharynx, to the left side close to the tail. Two fragments are obtained, the head with a partial left side and the tail with partial right side.

REQUIRED MATERIALS 2.1:

Plastic Pasteur pipettes
Petri dishes
Ethanol
Blade or coverslip
Paper towels or lab wiper KimWipes™
Optional: Lens magnification or dissecting microscope or smartphone
Squeeze bottle or standard bottle and plastic Pasteur pipettes
Instant Ocean® Sea Salt or 1x Montjuïc water
Waste container

2.2. Observation of the regeneration and image acquisition

During amputation students should pay attention to the "escape" behavior of the worms, mucous production, and body contraction. During the days after the amputation, the

students can examine wound closure by muscle contraction, blastema formation, swimming behavior, conservation of the body axes, tissue remodeling to re-establish proper body proportions, eye formation, and pigmentation of the regenerated tissues that represents the end of the process.

A detailed description of the regeneration time course of each planarian species after each amputation paradigm are available in the paper “Hands-on, classroom studies of regeneration, and stem cell biology using freshwater planarians”.

The specific features of each observed time point can be documented with picture acquisition and movie recording as described in the Section 1.5.

REQUIRED MATERIALS 2.2:

Lens magnification or dissecting microscope or smartphone
Optional: Camera
Plastic Pasteur pipettes
Petri dishes
Squeeze bottle or standard bottle and plastic Pasteur pipettes
Cold Instant Ocean® Sea Salt or 1x Montjuïc water
Waste container