

Planaria Regeneration Activity

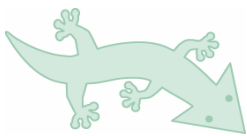


Guide to This Activity

This activity was created in support of the 2006 Holiday Lectures on Science on Stem Cells.

Planaria, petri dishes, and other equipment are available from biological supply companies at modest cost.

In most cases, regeneration should be complete within a week to ten days.



Visit the website:

www.hhmi.org/biointeractive/stemcells

- Video interviews with scientists working on stem cells and planaria.

www.hhmi.org/biointeractive/activities

- Downloadable version of this document.
- Other classroom activities to supplement the Holiday Lectures on Science DVDs.

What Are Planaria?

Planaria are a type of flatworm belonging to the phylum Platyhelminthes. They are one of the simplest types of multicellular animals.

For over 100 years, scientists have known that planaria can regenerate. Early pioneers of planaria biology noticed different regeneration abilities in different species. Moreover, they also noticed that in some species, different parts of the body had different regeneration capacities. In this

experiment, we will investigate the capability of different body sections to regenerate.



Aims of the Planaria Regeneration Activity

When a planaria's head is cut off, the remaining tail section will first regenerate a head. Even if the cut is made very close to the tail, the small tail section first regenerates the head and then continues to regenerate the rest of the tissue between the head and the tail.

We will use this property and compare how long it takes for worms cut in different places to regenerate a head. If different parts of the planaria body have equal ability to regenerate, they should all regenerate the

head in the same amount of time. If not, they should regenerate the head in different amounts of time.

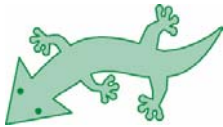
The regenerative capacity of different body sections may be an indicator of the location of stem cells called *neoblasts*. For instance, if one body segment has a low capacity to regenerate, perhaps only a few neoblasts exist in the area around the cut. Additional neoblasts may need to migrate to the area or be created by cell division, slowing down the rate of regeneration.

What You Need for This Activity

- 9 planaria
- 3 small plastic petri dishes
- 1 large plastic petri dish
- 1 plastic pipette
- 1 magnifying glass
- 1 plastic coverslips

- spring water
- paper towels
- thermometer
- permanent marker





Caring for the Planaria

The planaria shipped from suppliers should be fine for more than a week without changing their water. However, if you can change the water every few days, they may be able to stay healthy longer. If you change water, be sure to only use spring water. Poland Springs works best on the East Coast.

The worms prefer to be kept in the dark, but they will be fine under normal room light for the length of the experiment. A bright flashlight or other strong light

source can be used to demonstrate the worm's aversion to light. This can also be done by shading half of their dish with a piece of thick paper or dark plastic.

Some worms will likely fission during the experiment. They do this by sticking their tail end to the bottom of the dish and swimming away until a small tail section breaks from the rest of the worm. This is how some species of planaria reproduce. Fission shouldn't affect the outcome of the experiment, but it is an interesting process that you may be lucky enough to observe.

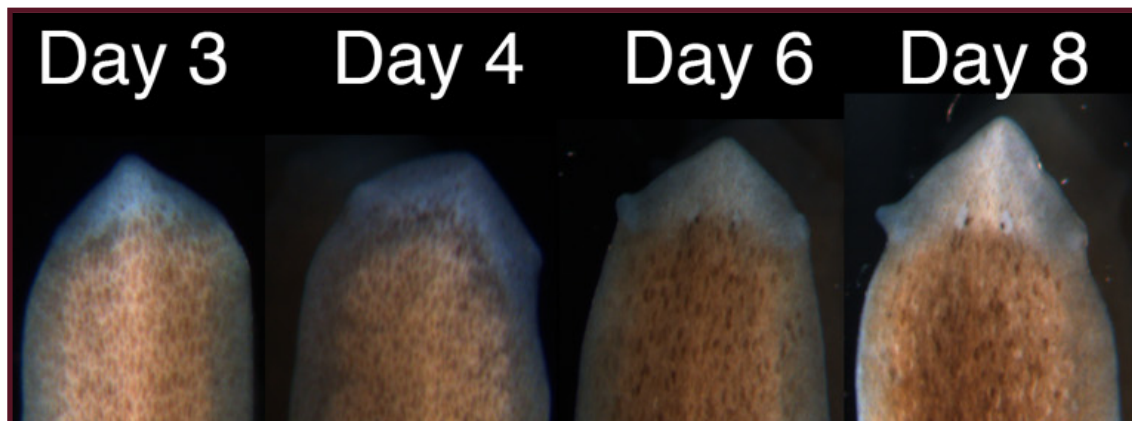
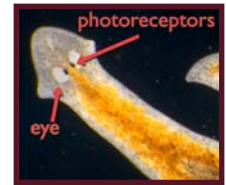
Procedure

1. Number the bottoms of three of the petri dishes 1 through 3, fill halfway with spring water, and set aside. (Marking the bottoms will prevent confusion by accidental swapping of lids.)
2. Using a plastic transfer pipette, transfer a planaria into one of the remaining unlabeled empty plastic petri dish.
3. Optional: Soak up excess water with a paper towel. Limiting the amount of water can reduce the mobility of the planaria and facilitate the next step. Be careful not to touch the planaria—they will stick to the paper and could then die.
4. Using the plastic coverslip, cut the planaria into two pieces at one of the three spots indicated on the diagram on the right. *Planaria can move rapidly. Cutting them in a precise location can be difficult. Some people find it useful to try to cut using a magnifying glass or, if available, a dissecting microscope. Cooling the worm water and the cutting dish with ice can help slow the worms down.*
5. Using a transfer pipette, gently transfer the tail fragment into the corresponding numbered petri dish (i.e., if you cut at spot 1, then transfer the tail fragment into dish 1).
6. Repeat steps 2 through 5 with the remaining worms until you have at least three or four tail fragments of each of the three cutting positions.
7. Save all the head fragments in the cutting dish with some spring water.
8. On the data form or your notebook, record the time and room temperature when you finished cutting all the planaria.



Procedure (continued)

9. Make any initial observations about the head fragments and each size of tail fragment and record them in the areas indicated on the data form or your notebook.
10. Answer the “day zero” questions in the following section.
11. Regeneration will be assessed by monitoring how long it takes for the photoreceptors to appear. Photoreceptors appear as black dots located inside the pale round eye (see the photograph on the right). In the example of a regenerating planaria shown below, the eyes appear around day 4 and the photoreceptors on day 6. You would score this animal as having regenerated on day 6. Every day, count the number of tail fragments in each dish that have regenerated photoreceptors and record the numbers and any other observations on the data form or your notebook.



12. Days 3 to 5 will be critical in early detection of the regenerated photoreceptors. If possible, you may want to observe the worms twice during these days—once in the morning and once at the end of the day.
13. Continue until all the fragments have regenerated or died, or 14 days have passed. At the end of the experiment answer the “final day” questions in the following section.



Questions

Day Zero

What environmental variables do you think may influence regeneration?

After you cut the planaria, how does the mobility of the tail fragments compare with the mobility of the head fragments? Do they move the same or differently? If they move differently, why do you think that is?

Do you think the head fragments prefer light or shade? How could you test this?

Final Day

Did all the tail fragments regenerate photoreceptors? If not, which fragments did not?

Did all the tail fragments regenerate photoreceptors at the same rate? If not, which were slower and which were faster?

What does the relative rate of appearance of photoreceptors in the different tail fragments tell you about the regeneration ability of different sections of the worm?

Did you notice a change in mobility in the tail fragments over time? What about the head fragments?

How could you test whether the regenerated photoreceptors are functional? Do you think photoreceptors are functional before, as soon as, or sometime after they become visible? Why?

How could testing photoreceptor function be useful in measuring the rate of head regeneration in tail fragments?

As the days went by, what did you notice about the color of the regenerating tissue?



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**Cleaning Up**

After the experiment is over, you will need to dispose of your worms. Do not release them into the environment.

The animals could be maintained in the classroom, donated to another classroom or science department, or kept at home with parental permission. As a last resort, they could be humanely disposed of. Ask your teacher for advice on what should be done.

Reporting Your Results

When you have finished collecting data, prepare your result in the following format:

Position 1: 4, 4, 5, 5

Position 2: 3, 3, 4, 4

Position 3: 4, 4, 5, -

Each number denotes the day that the photoreceptors appeared on a particular worm. If a worm dies, do not include a number for that worm. In the above example, out of four worms cut at position 3, two regenerated the photoreceptors on day 4, one on day 5, and one did not survive. If a worm does not regenerate after 14 days, do not include a number for that worm.

If you are doing this as a classroom activity, your teacher will collate the data and discuss the overall result.

Some students who attended the 2006 Holiday Lectures on Science participated in this activity. Their results are discussed in the first half of lecture 2. You can view it on a free DVD available from <http://catalog.hhmi.org> or by webcast from <http://www.hhmi.org/biointeractive/stemcells/lectures.html>

About This Activity

This activity was originally developed by the laboratory of Alejandro Sánchez Alvarado, HHMI, University of Utah. It was modified by Otto Guedelhofer and Satoshi Amagai.

About the Holiday Lectures on Science and BioInteractive

As part of its mission to strengthen science education, HHMI presents the Holiday Lectures on Science, an annual series that brings the latest developments in a rapidly moving field of research into the classroom. The lectures are given by HHMI investigators and other leading scientists. The 2006 Holiday Lectures, *Potent Biology: Stem Cells, Cloning, and Regeneration*, are the fourteenth in the series, which began in 1993.

biographies of the lecturers, and other resources are at www.holidaylectures.org. DVDs and CD-ROMs can be ordered through HHMI's Online Catalog at <http://catalog.hhmi.org>.

The BioInteractive website (www.biointeractive.org) features virtual labs, animations, and other engaging instructional materials. They can be used to supplement the lecture topics or help learn about important concepts in the biomedical sciences.

To complement the Holiday Lectures and enhance their usefulness in the classroom, HHMI produces a variety of free science education materials. Lecture summaries,

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