



## INTRODUCTION

Wouldn't it be great if we could regrow any part of our bodies? That way, we could recover from any injury like Deadpool and other superheroes can. Although superheroes are made up, animals with amazing regeneration abilities do exist. Understanding how these animals, including starfish, salamanders, and planarians, regenerate lost tissues could someday help us improve our own abilities to recover from injury.

Planarians are simple, multicellular flatworms found in freshwater streams and ponds. They are an ideal animal for studying regeneration because they are small, grow fast, and are easy to take care of. In this activity, you will observe your own planarian and design an experiment to better understand how planarians regenerate.

## MATERIALS

- up to 3 planarians
- Petri dishes
- small pieces of hard-boiled egg yolk or liver (cut to the size of a grain of rice or small pea)
- bottled water
- plastic pipette
- clear ruler
- piece of dark paper
- concave microscope slide
- dissecting microscope or magnifying glass
- small soft brush
- paper towels
- permanent marker

For Part 2 of the activity, you may also need the following:

- plain glass microscope slide
- lens paper
- coverslip or scalpel
- ice (optional)

## PROCEDURES

### Part 1: Getting to Know Your Planarian

In this first part of the activity, you will observe and interact with your very own planarian.

1. Fill a Petri dish about halfway with bottled water.
2. Use a plastic pipette to transfer a planarian to the Petri dish.
3. Observe your planarian. Record your initial observations in the space below.

4. Measure the length and width of your planarian. This could be tricky! Hint: Cold water may make the planarian move more slowly.
  - a. Record your planarian's measurements.  
Length \_\_\_\_\_ Width \_\_\_\_\_
  - b. Describe the technique you used to measure your planarian below, so that others will be able to repeat the process and obtain similar data.
  
5. Observe your planarian as it moves around in the Petri dish. If your planarian is not moving, try touching it gently with your pipette, stirring the water gently with the pipette, or removing most of the water.
  - a. Describe how the planarian moves. For example, does it lead with only one end or both ends? Does it wiggle from side to side, squeeze and stretch its body, or both?
  
  - b. Do you think the planarian uses muscles for these movements? Explain your reasoning using evidence from your observations.
  
6. Cover half of the Petri dish's top with a piece of dark paper, then observe your planarian's behavior for several minutes. Do you think that planarians can detect light? Do planarians prefer light or dark? Explain your reasoning using evidence from your observations.
  
7. Add a small piece of hard-boiled egg yolk or liver to the Petri dish. Again, observe your planarian for several minutes. Do you think that planarians can detect food in their environment? How might they do this? Explain your reasoning using evidence from your observations.
  
8. Use a pipette to gently transfer your planarian from the Petri dish to a concave microscope slide. Be sure to place a drop of water on the slide along with the planarian.
  - a. If the planarian has attached itself to the side of the Petri dish, use a soft brush to nudge it off.
  - b. Transfer the planarian to the slide quickly, so that it won't attach itself to the inside of the pipette. If the planarian gets stuck, flush it back into the Petri dish and try again.
  
9. Observe the planarian under a dissecting microscope or with a magnifying glass. Sketch your planarian in the box below.

- a. In your sketch, label the anterior (head) and posterior (tail) ends. The anterior is the end with what appears to be two eyes.
- b. Label any other features you observe. These can be identified and relabeled later during your investigation.



10. While the planarian is still on the slide, pour the water and food out of the Petri dish and wipe the dish clean with a paper towel. Use a permanent marker to label the side or bottom of the dish with your name or group.
11. Add new water and return your planarian to the Petri dish. This can be done by gently flushing the planarian off the slide with water from your pipette.

## Part 2: Designing an Experiment to Investigate Planarian Regeneration

In this part of the activity, you will design an experiment to investigate the regenerative abilities of planarians. During the experiment, you should try cutting your planarians into two or more pieces. Don't worry about hurting the planarians. They do not feel pain when cut, only pressure.

You will start by formulating a hypothesis and then developing a research plan. **Read all of the information in Part 2 before writing down any answers.**

1. Come up with a **question** about planarian regeneration that your group wants to investigate and describe it in the space below. Some ideas include:
  - Do different planarian pieces regenerate at different rates?
  - Do the regenerating pieces have the same types of tissues as the original planarian did?
  - Do all of the regenerating pieces respond to stimuli such as light or food?
  - Are there any changes in the movements or behavior of the regenerating pieces over time?
2. Describe a **hypothesis** that your group will test to investigate your question. Remember that a hypothesis has to be testable. In this case, pick a hypothesis that you will be able to test in two to three weeks with up to three planarians, using materials approved by your teacher.
3. Develop a research plan for testing your hypothesis. Your plan should include a description of the independent variable(s), dependent variable(s), control(s), materials needed, and a detailed procedure.

- a. Describe your **independent variable(s)**. Possible independent variables include the type of cut (completely severing the planarian or partially cutting through it) and the location of the cut.
  
- b. Describe your **dependent variable(s)**. Possible dependent variables include the planarian's rate of regrowth or the response to a stimulus.
  
- c. Describe your **control(s)**.
  
- d. List all the **materials** you will need for your experiment. Include how many planarians you will use (maximum of three planarians per group, or as allowed by your teacher).
  
- e. Describe the **procedure** for your experiment in detail. Make sure to include the following information:
  - how each planarian will be treated (types and locations of cuts, etc.)
  - what types of data you will collect (drawings, size measurements, color changes, behavioral observations, etc.), and how often you will collect this data
  - how long the experiment will take (usually two to three weeks, or as allowed by your teacher)
  
- f. Predict what you will observe if the results of your experiment support your hypothesis.

Use these tips to help design your research plan:

- You will be able to cut one or more of your planarians. Planarians do not feel pain when cut, and they don't bleed.
- Use the following steps to cut your planarians:
  - Prepare a cutting surface by wrapping a folded piece of lens paper around a plain glass microscope slide. You may want to place the slide on ice, because cooling planarians will help keep them still and make cutting them easier.
  - Pipette your planarian onto the lens paper and wait for the planarian to stretch out.

- Use a coverslip or scalpel to cut the planarian. It may be helpful to use a magnifying glass or a dissecting microscope when cutting.
- You might want to cut your planarian into two sections or make a partial cut either across, up and down, or diagonally. If you make a partial cut, leave the coverslip or scalpel in the cut for about 10 seconds to prevent the cut from sealing back up.
- If you decide to cut your planarians into two or more pieces, the different pieces should be kept in separate Petri dishes.
  - Once the planarians are cut, transfer each piece to a different dish with bottled water inside. Label each dish with your name or group, as well as identifying information about the sample (planarian number, piece number, etc.).
  - Be sure to label the side or bottom of each dish with a permanent marker. This will keep the dishes from getting mixed up if the lids are accidentally swapped.
- Make sure to carefully record where you cut each planarian. You can sketch your planarians to show the cuts you made using a table like the one shown below. In your sketches, label anterior and posterior ends, and number each piece.

Planarian 1	Planarian 2	Planarian 3

4. Submit your research plan to your teacher for approval. If your plan is not approved, revise it and resubmit it. After receiving approval, set up your experiment.
5. Record the data you decided to collect in a notebook, spreadsheet, or other data table.
6. Take care of your planarians as you carry out your experiment. Try to disturb the planarians as little as possible during this time, but make sure to **change their water two to three times a week and feed them once a week.**
  - To change the water, first make sure there are no planarians floating on the surface. If any planarians are floating, carefully make them sink with a plastic pipette. Next, carefully remove most of the old water using the pipette, leaving the planarians in place. You can also slowly pour off the water, as the planarians typically stick to the bottom of the dish. Finally, gently add new bottled water.
  - To feed the planarians, give them small pieces of hard-boiled egg yolk or liver, cut to the size of a grain of rice or small pea (one piece of food per dish). Let them eat undisturbed for an hour, or until they stop feeding and swim away from the food. After feeding is complete, remove the leftover food and change the water using the method described above. **Do not leave the food in the water for longer than four hours, and do not feed planarians for three or four days after cutting them.**

7. At the end of your experiment, answer the following questions.
  - a. Did your experiment support or refute your hypothesis, or were your results inconclusive? In the space below, justify your reasoning using data from your experiment.
  
  
  
  
  
  
  
  
  
  
  - b. What are two things you have learned about planarian regeneration? Use the results of your experiment to provide evidence for your claims.
  
  
  
  
  
  
  
  
  
  
  - c. What is one question you still have about planarian regeneration?

### Part 3: Watching a Film about Planarian Regeneration

In this final part of the activity, you will watch a video to learn more about planarian regeneration.

1. Watch the short film [Identifying the Key Genes for Regeneration](#). As you watch, list any information that supports or refutes the hypothesis from your experiment (Part 2 of the activity).
2. Planarians served as model organisms in both your experiment and in the film. Answer the following questions based on what you learned from the film.
  - a. Which cells are responsible for regeneration in planarians?
  
  
  
  
  
  
  
  
  
  
  - b. Why are planarians' regeneration abilities greater than those of humans?
  
  
  
  
  
  
  
  
  
  
  - c. How can scientists identify genes important in regeneration?
  
  
  
  
  
  
  
  
  
  
  - d. How does a planarian "know" whether to regenerate a head or a tail? What might cause the planarian to regenerate the wrong body part?
3. Explain how understanding regeneration in planarians might help improve human health.