



# The Science and Ethics of Stem Cell Research

## Plenty of Planaria Teacher Overview

---

### Objectives

Students will be able to:

- Distinguish between types of stem cell potency (totipotent, pluripotent, and multipotent).
- Compare planaria stem cells to human stem cells.
- Design unique questions to test planaria regeneration, and analyze their data in support of a conclusion.
- Synthesize and evaluate trends in class data.

### Class Time

- 1 class period for initial laboratory.
- 10-15 minutes each day over the course of the next week or two to record observations.
- 1 class period for data results and analysis.

### Prior Knowledge Needed

Basic lab techniques, inquiry skills, basic understanding of cell biology.

### Common Misconceptions

- Planaria will die when you cut them up.
- All stem cells are the same.

### Purpose

The purpose of this lesson is to introduce students to fundamental stem cell concepts using brown planaria (*Dugesia tigrina*) as a model organism. This model works well for demonstrating stem cell function, development, and the complexity of tissue regeneration. This lesson also functions as a starting point for students to begin thinking about the concept of regeneration and stem cells in other organisms.

### Key Concepts

- Stem cells are undifferentiated cells that can make more of themselves (self-renew) and can develop into specific cell types (differentiate).
- TOTIPOTENT stem cells are capable of regenerating ALL cells present in the organism, in contrast to PLURIPOTENT stem cells (which can make most cells) and MULTIPOTENT stem cells (which can make cells within a tissue type).
- Totipotent cells begin as non-differentiated cells and then commit to a developmental pathway to become differentiated. Alternatively, they can divide to make more totipotent cells.
- Planaria are capable of regeneration of a wide range of tissue structures due to the presence of totipotent cells – the ‘neoblasts’ – which divide by mitosis.
- Human totipotent cells are present only in the early divisions of the embryo (before 3 days). Human totipotent cells are not referred to as neoblasts.
- Pluripotent cells are used to create human embryonic stem cell lines.

## Materials

### Planaria

The brown planaria, *Dugesia tigrina*, and black planaria, *Dugesia dorotocephala*, can be purchased from commercial supply houses, such as **WARDS** and **Boreal/Science Kits**.

<http://www.wardsci.com>

<http://www.sciencekit.com>

The brown planaria are smaller than the black planaria, but they are usually able to regenerate fully in about two weeks. Although small, they can still be seen without magnification. The black planaria are heartier and larger than the brown planaria, but may take up to four weeks to fully regenerate. Teachers experience a lot of variability in the time it takes for full regeneration.

### Planaria lab materials (per lab group)

- enough small Petri dishes and planaria for each student in class (three planaria per lab group)
- microscope slides
- lens paper
- 1 scalpel
- 1 pipet
- camels hair brush or small paint brush
- dissecting microscope or magnifying glass
- wax pencil or sharpie
- rulers (clear)

### Teacher Resource Sheets

- Planaria Illustrations
- Care and Feeding of Planaria
- Animals in Research
- Potential Extensions

### Student Handouts

- Handout 1—*Student Background*
- Handout 2—*Animals in Research*
- Handout 3—*Research Proposal Form*
- Handout 4—*Investigation*

An accompanying PowerPoint presentation on Planaria is available from the Northwest Association for Biomedical Research website ([nwabr.org](http://nwabr.org)).

A free, helpful video from the Howard Hughes Medical Institute (HHMI) about stem cells and regeneration can be found at: <http://www.hhmi.org/biointeractive/stemcells/index.html>. Included on the video is a short interview with Dr. Sánchez Alvarado and his lab members about planaria, their ability to regenerate, and their ties to stem cell research.

---

## Timeline

Prep Time:

**Two to three weeks prior to beginning the lab, order planaria and supplies**

**Read over ‘The Care and Feeding of Planaria’**

Planaria do better when they have been fed/acclimated before cutting so give yourself enough time to do that!

**This lab is designed to be finished before the stem cell unit begins.** This allows students to develop concepts of totipotency, pluripotency, and multipotency before the names for these concepts are introduced. However, some teachers begin the lab right before the first day of the unit, do the unit while the planaria are growing, and then conclude the unit and the lab at the same time. If you choose this latter option, develop the ideas of totipotency, multipotency and pluripotency at the beginning of the lab.

Arrange for use of microscopes, if available

Prepare food for planaria

1-2 hours to read through lesson plan, make copies, and prepare lab materials.

---

## CLASSROOM IMPLEMENTATION

### A. Invitation to Learn

Ask students, “**How do identical twins form? Why can’t that same process occur later in development?**”

Students will realize that the early embryo is capable of splitting into two genetically identical individuals. However, once differentiation of cells has occurred the cells ordinarily ‘commit’ to a fate.

Stress to students that some cells are capable of making all the cells in the human body and the placenta. In humans, they occur only in the first few cell divisions (before 3 days). [While these are the TOTIPOTENT cells, it is best to develop the concept first and provide the vocabulary later on in the lesson].

After the first few divisions, some human cells still retain the ability to make a great variety of cell types, but they cannot regenerate the whole human organism in the uterus [PLURIPOTENT cells, which can make most cell types, and MULTIPOTENT cells, which can make cells of a specific tissue type such as blood].

This can be demonstrated to students by asking them, “**If red blood cells last only four months and white blood cells only a few days, how can your circulatory system keep making all the blood you need? How can providing new bone marrow to a person with leukemia help cure them?**”

---

*Develop the idea of stem cells as undifferentiated cells that can make more of themselves as well as develop into a variety of different cell types.*

**Some organisms, such as planaria, have tremendous flexibility in regeneration. A planaria fragment 1/279th the size of the fully grown planaria can regenerate into a new planaria!**

Planaria are used as a model organism in this lab, and by researchers studying stem cells, because of their ability to regenerate. These seemingly simple organisms are actually quite complex—they are capable of regenerating a wide range of tissue structures which make up different organ systems. The only dividing cell in the planaria is the neoblast. This means the neoblast is capable of differentiating into any cell type the planaria requires for regeneration, whether it be a flame cell, photoreceptor cell, nerve cell, or excretory cell. For this reason, the neoblast is considered TOTIPOTENT.

In the wild, planaria reproduce both sexually and asexually. When they reproduce asexually, the bottom portion will attach to a rock or solid surface, and the top will pull away. Hence, the cutting of planaria will be similar to a process that occurs naturally. Still, as with any animal used in laboratories, respect and careful handling must be stressed.

## **B. Planaria Inquiry Lab**

### **Day One**

Introduce the Lab.

Let students become more familiar with the planaria through observation, research and/or the PowerPoint presentation available at [nwabr.org](http://nwabr.org).

Have student teams complete the 'Research Proposal' and 'Animals in Research' forms and submit them for approval.

### **Day Two**

1. Lab teams receive three planaria.  
For one, they should make a horizontal cut.  
For the second, they should make a cut of their choosing, predicting the results. The third will be a control.
2. Over the course of the next 7-14 days, students should record data. Students draw their planaria, attempt to measure them, and make behavioral observations (light responses, eating, touch responses). It might be hard to measure the planaria – use this as an opportunity to discuss how challenging it sometimes is to make scientific measurements. They can decide, for example, to measure the planaria only when fully extended, or they can try to make several individual measurements and then average them.
3. In the final debriefing, student groups provide their summaries to the class. Each student will record the class data.
4. As a large group, the students will discuss trends and conclusions. Direct the discussion towards the idea of POTENCY.

---

### **Address key concepts in the debriefing:**

Planaria represent regeneration “experts.” Very small segments from vertical, horizontal or diagonal cuts can regenerate complete organisms within 7-14 days. (There are two exceptions – the tip of the nose and the pharynx cannot regenerate a new organism.) When a horizontal cut is made, each cut end knows whether to become a head or tail.

Stem cells are undifferentiated cells that can make more of themselves (self-renew) and can develop into specific cell types (differentiate).

TOTIPOTENT cells are capable of regenerating ALL cells present in the organism, and of making an entire organism.

PLURIPOTENT cells can make most cells, except placenta (in mammals), and thus cannot begin a new organism.

MULTIPOTENT cells can make cells within a tissue type, such as blood.

Planaria have totipotent cells – the ‘neoblasts’ – which divide by mitosis. Human totipotent cells are present *only* in the early divisions of the embryo (before 3 days). Human totipotent cells are not referred to as neoblasts.

*Pluripotent* cells are used to create human embryonic stem cell lines.

### **Adaptations**

Simplify/modify written responses.

Have students only conduct the cutting in half experiment.

Conduct the experiment as a demonstration.

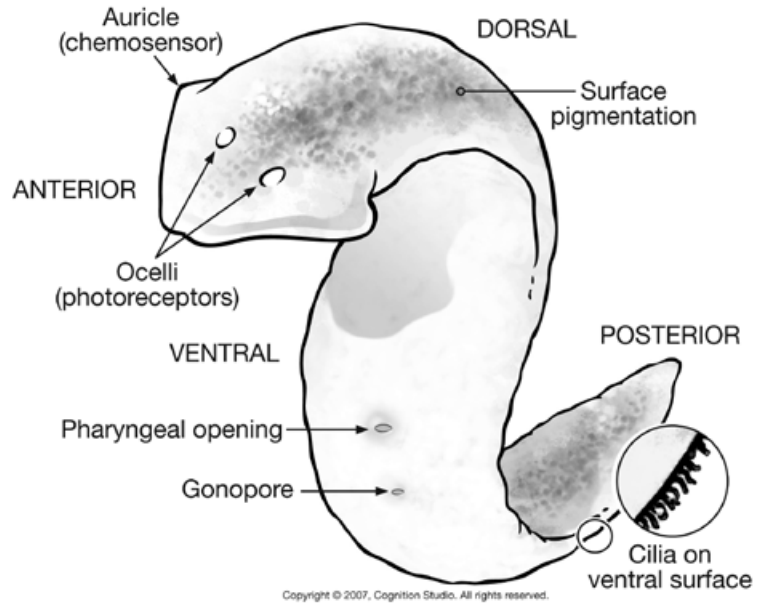
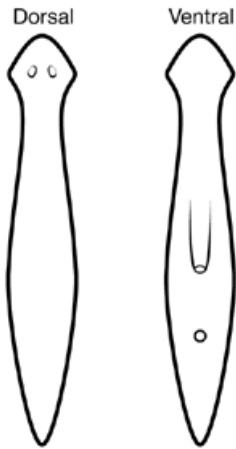
### **Assessment Suggestions**

Monitor discussions and review written responses.

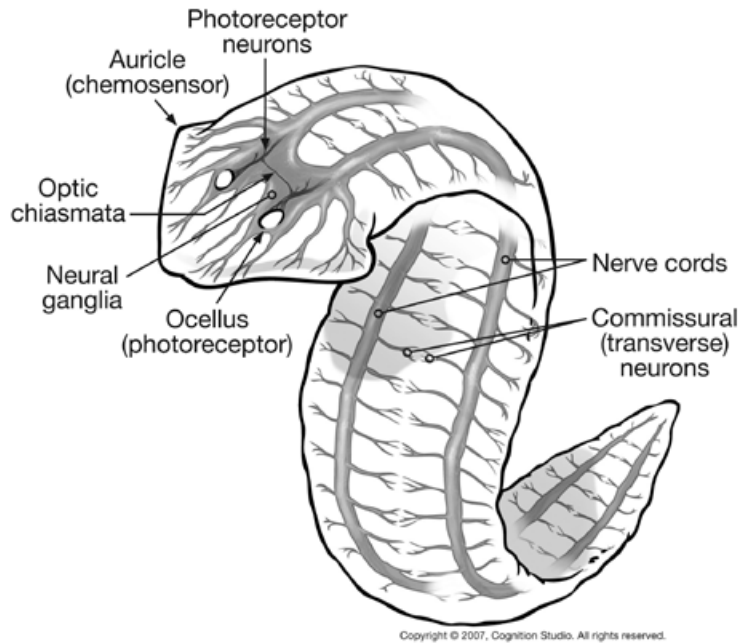
### **References**

1. Newmark, Phillip, and A. Sánchez Alvarado, Not Your Father’s Planarian: A Classic Model Enters the Era of Functional Genomics, 3, 210-220 (2002).
2. Davenport, J, What Controls Organ Regeneration, from Science, 309, 84 (2005).

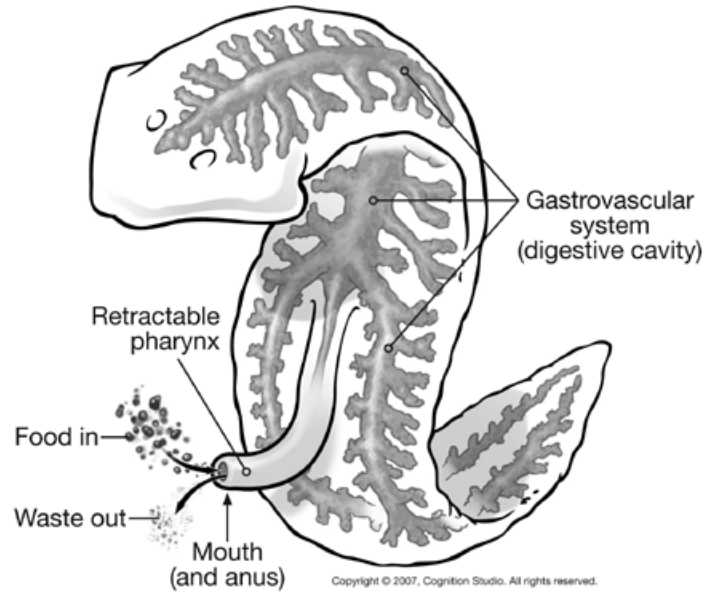
Planaria Overview



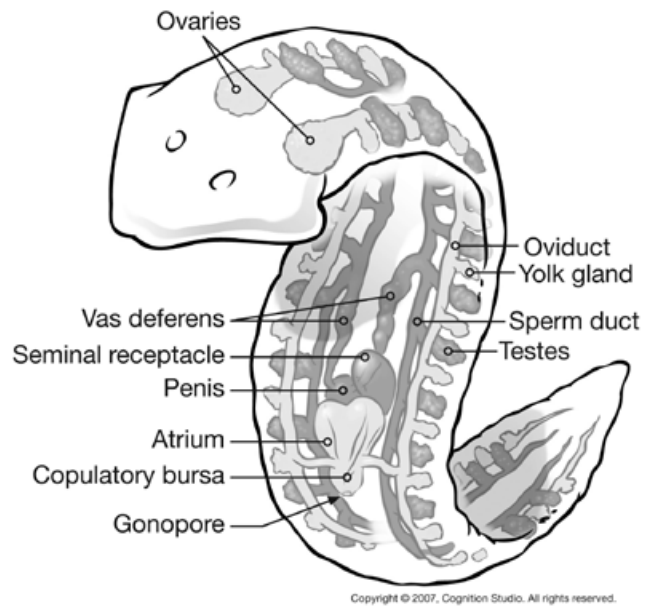
Nervous System



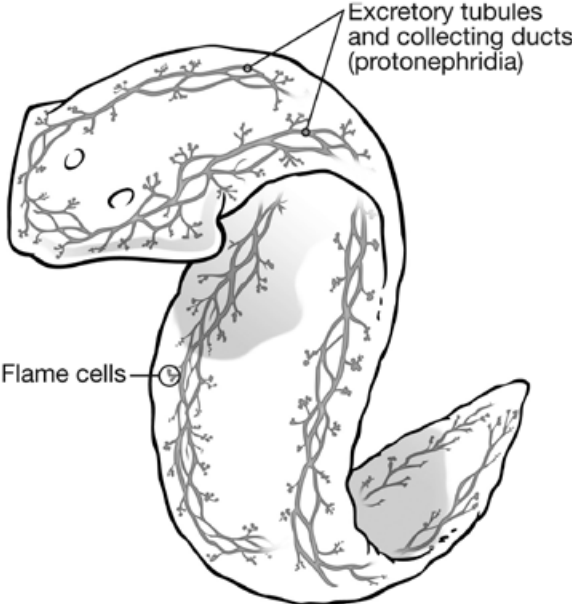
### Gastrovascular System



### Reproductive System



**Excretory/  
Osmoregulatory  
System**



Copyright © 2007, Cognition Studio. All rights reserved.



**1. The WATER is MOST important!**

Planaria survive best in unpolluted pond, stream or lake freshwater (not saltwater). Bottled spring water also works for culturing.

Fresh tap water is not recommended, and distilled water lacks the minerals and nutrients that planaria need to survive.

**2. Acclimation Period**

Before using planaria in a dissection experiment, we recommended that they have 1-2 weeks of acclimation in your classroom. This will give you time to be feed them and test your water. A container with shallow water having a large surface area (better oxygen exchange) is better than a container of deep water with little surface area. During this period, observations and functional experiments can be done (e.g., movement in response to touch or light; feeding observations). Students may take turns doing the feeding and changing the water.

**3. Water Changing**

The water should be changed at least twice a week. It must be changed 1-4 hours after feeding to prevent the growth of bacteria. There are three methods of changing the water.

- a. The old water can be carefully poured off the planaria. Some planaria may be floating on or near the surface of the water. Be sure that they are not poured out!
- b. Using an eyedropper, the planaria may be individually transferred to containers of aged tap water or spring water. You must transfer them quickly so that they don't attach to the inside of the dropper.
- c. Use an eye dropper or pipette to remove old water, being careful not to remove any planaria. Refill with aged tap water or spring water.

Never use a hard or sharp instrument to scrape the planaria into a container.

**4. Feeding**

Feed planaria small bits of liver once a week.. Chop the liver into small pieces and freeze. Planaria may also eat egg yolk. Some teachers have had success with Beta fish food.

Let them eat for up to 4 hours (until no longer feeding).

Change the water after feeding to prevent uneaten food from decaying in the water. Pouring off the water afterwards is usually easiest. A paper towel may be used to clean the bottom of the container before adding in more water.

**5. Living Conditions**

Planaria are sensitive to extremes of light, temperature, and pH.

Keep planaria at a reasonable room temperature (68-72 degrees F). Do not refrigerate them.

Do not expose to harsh light. Keep them in the dark most of the time – in a closed cupboard or drawer.

Lids on containers should be loosely closed.

### 6. Dissection Suggestions

Because of the small size of the planaria, vertical cuts can be difficult.

To slow down or immobilize planaria, try putting them on wet lens paper wrapped around a glass slide, or try a glass slide placed over ice. Water that has been frozen in a Petri dish can make a good platform for cutting planaria.

When making a partial cut that does not completely separate a body part, hold the blade in the cut for at least five seconds to prevent the pieces from fusing back together. (The first 30 minutes may be critical for keeping the “parts” separated so that they don’t fuse back together.)

### 7. After Dissection

The planaria will show regeneration after 1-2 weeks. It may take 4 weeks to get complete regeneration. During that time, do not feed the planaria. Disturb them as little as possible – for water changes, it is best to pour off water, rather than move them with an eye dropper.

After horizontal cuts, the “head portions” may move normally. The “tail portions” will adhere to the container.

After partial vertical cuts, the cuts should be observed daily and may require re-cutting during the first three days.

In addition to observing the regeneration of the flatworm **appearance**, students can better appreciate the regeneration of internal organs and neural connections by observing the regeneration of **function**.

### 1. Regeneration of Photoreceptor Function

Planaria will avoid or swim away from light. After cutting the planaria, students should record their observations of appearance AND test for the re-establishment of neural connections to the photoreceptors (“eye”). If the planaria containers can be kept in dim light, a flashlight can be used to direct intense light onto the planaria. Students should record the numbers of days of growth after cutting that is required before they see an avoidance response to the light. The distance that the planaria moves from the light will be greatest when the photoreceptors are functioning. Students should note the difference in time between when they see the photoreceptors and when the photoreceptors appear to be functioning.

### 2. Faster Regeneration near the Head

It has been observed that there is faster regeneration if a cut is made nearer the head because of a higher concentration of growth factors near the head. Have students make horizontal cuts on three different planaria. The cuts should be at different distances from the head, such as  $\frac{1}{4}$ ,  $\frac{1}{2}$ , and  $\frac{3}{4}$  body length from the head. Students should observe regeneration and record the difference in speed of regeneration.

### 3. Digestive Track Function

The digestive system can be studied by feeding planaria a colored substance such as carmine powder or carbon black. It may be possible, but more difficult, to see “yellow” in the gut after feeding them cooked egg yolk. After cutting planaria, students can observe regeneration and note when the re-generated flatworms begin to eat and have a functioning gut.

### 4. “Wild” vs. “Purchased” Planaria

“Wild” planaria can be collected by dangling cooked meat or egg yolk in a cheesecloth bag at the edge of a freshwater stream or pond for 15-30 minutes. The planaria can then be carefully removed from the side of the cheesecloth and grown in the lab in the usual growing conditions. It could be beneficial to grow the wild planaria in water from their original source.

Students should observe the differences in appearance, feeding behavior, and regeneration time between “wild” and “purchased” lab planaria. There are many different families of planaria and it is unknown if there will be observable differences.

