



Experiment 1

Protocol: Investigating the Effects of Beta-Catenin or APC Knockdown on Planarian Regeneration

Objective: To examine the effects of RNA interference (RNAi) targeting beta-catenin or APC on the regeneration of planaria.

Materials:

- Petri dishes (at least 2 per experimental condition)
- RNAi-treated planaria (10 control + 10 RNAi of interest)
- Planarian water
- Cover slips (for cutting worms)
- Transfer pipettes
- Dissection microscope
- Permanent marker (for labeling dishes)
- Notebook or Excel document to record phenotypes

Procedure:

- 1. Preparation:**
 - Label the Petri dishes with the experimental conditions (Control, Beta-Catenin RNAi, or APC RNAi).
- 2. Collecting and Placing Planaria:**
 - Fill each Petri dish approximately halfway with planarian water.
 - Using a transfer pipette, gently transfer 10 planaria into each designated Petri dish.
 - Ensure planaria are healthy and actively moving before proceeding.
- 3. Amputation Procedure:**
 - Using a cover slip, carefully cut each planaria transversely in half or thirds, depending on the experimental design.
 - Ensure cuts are clean and consistent across all samples to maintain uniformity.
- 4. Incubation:**
 - Place Petri dishes in a dark environment at room temperature.
 - Minimize disturbance to the samples to allow proper regeneration.
- 5. Observation and Data Collection:**
 - Observe the planaria daily under a dissection microscope.
 - Record the regeneration progress, noting differences in head, tail, or midsection regeneration between control and RNAi-treated groups.
 - Remove any dead planarian or debris and add fresh planarian water as needed.
 - Take qualitative (how do they look?) and quantitative (how many?) notes on morphology and any abnormal regenerative patterns.
- 6. Analysis:**
 - At the end of the observation period (typically one - two weeks), compare regeneration success across conditions.
 - Take photographs, if possible, to document changes.
- 7. Summary Report:**
 - Each student must write a summary of their results.
 - The summary should include:
 - a) An Introduction explaining the purpose of the experiment.
 - b) A Hypothesis predicting the expected effects of beta-catenin or APC knockdown on regeneration.
 - c) A Results section summarizing observations, differences between control and experimental groups, and any unexpected findings.
 - The summary should be concise, well-organized, and clearly present the experimental outcomes.

Safety and Considerations:

- Handle planaria gently to avoid injury.
- After completing the experiment, dispose of planarian waste and water properly by adding to a container of 10% bleach.
 - Do not dispose of planarian down the drain.



Experiment 2

Protocol: Investigating the Effects of Glucose and Calcium Chloride on Planarian Regeneration

Objective: To examine the effects of different concentrations of glucose and calcium chloride on the regeneration of planaria.

Materials:

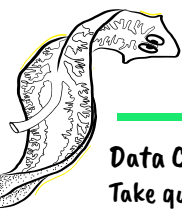
- Petri dishes (at least 3 per experimental condition)
- Planaria (10 per condition)
- Planarian water (control group)
- Calcium chloride (CaCl₂) solutions (0.1%, 0.5%, and 1%)
- Glucose solutions (1%, 5%, 10%)
- Cover slips (for cutting worms)
- Transfer Pipettes
- Dissection microscope
- Permanent marker (for labeling dishes)

Procedure:

- 1. Preparation:**
 - Label the Petri dishes with the experimental conditions
 - Option 1 – Planarian Water Control, 0.1% CaCl₂, 0.5% CaCl₂, 1% CaCl₂
 - Option 2 – Planarian Water Control, 1% Glucose, 5% Glucose, 10% Glucose
- 2. Collecting and Placing Planaria:**
 - Using a transfer pipette, gently transfer 10 planaria into each designated Petri dish.
 - Ensure planaria are healthy and actively moving before proceeding.
 - Fill each Petri dish halfway with the corresponding solution.
- 3. Amputation Procedure:**
 - Using a cover slip, carefully cut each planaria transversely in half or thirds, depending on the experimental design.
 - Ensure cuts are clean and consistent across all samples to maintain uniformity.
- 4. Incubation:**
 - Place Petri dishes in a dark environment at room temperature.
 - Minimize disturbance to the samples to allow proper regeneration.
- 5. Observation and Data Collection:**
 - Observe the planaria daily under a dissection microscope.
 - Record the regeneration progress, noting differences in head, tail, or midsection regeneration between control and RNAi-treated groups.
 - Remove any dead planarian or debris and add fresh planarian water as needed.
 - Take qualitative (how do they look?) and quantitative (how many?) notes on morphology and any abnormal regenerative patterns.
- 6. Analysis:**
 - At the end of the observation period (typically one – two weeks), compare regeneration success across conditions.
 - Take photographs, if possible, to document changes.
 - Discuss potential effects of calcium chloride and glucose on regeneration rates and morphology.
- 7. Summary Report:**
 - Each student must write a summary of their results.
 - The summary should include:
 - a) An Introduction explaining the purpose of the experiment.
 - b) A Hypothesis predicting the expected effects of beta-catenin or APC knockdown on regeneration.
 - c) A Results section summarizing observations, differences between control and experimental groups, and any unexpected findings.
 - The summary should be concise, well-organized, and clearly present the experimental outcomes.

Safety and Considerations:

- Handle planaria gently to avoid injury.
- After completing the experiment, dispose of planarian waste and water properly by adding to a container of 10% bleach.
 - Do not dispose of planarian down the drain.



PLANARIAN OUTREACH PROGRAM

Student Lab Handout

Name: _____ Class: _____ Period: _____

Data Collection:

Take qualitative (how do they look?) and quantitative (how many?) notes on morphology and any abnormal regenerative patterns.

days post amputation (dpa)	Qualitative notes: What features are regenerating? What new tissue has formed?	Quantitative notes: How many planarians have survived? How many planarians have lysed?

Critical Thinking Questions:

Using your online researching skills, answer these questions during each day of data collection.

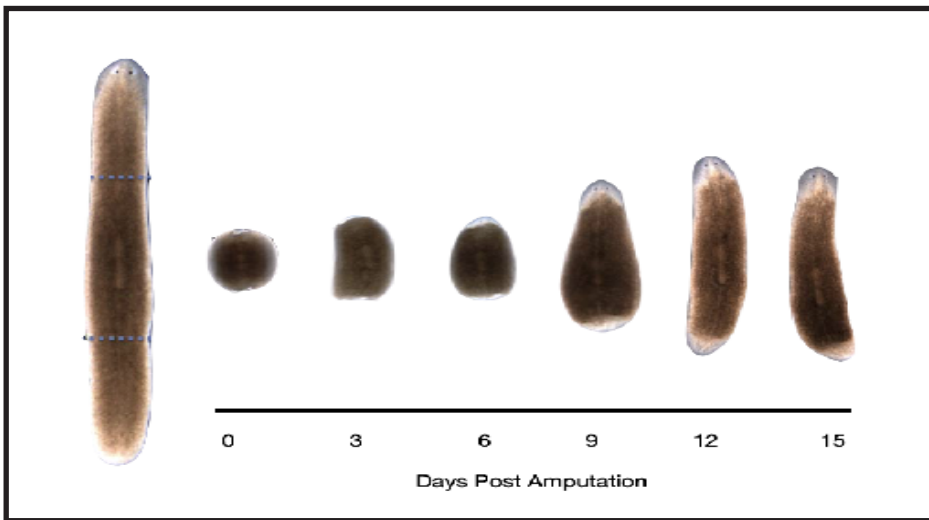
1. What role do neoblasts play in the regeneration process of planarians?
2. How do planarians detect and respond to light despite lacking complex eyes?
3. How do planarians reproduce asexually, and what advantages does this provide?
4. If a planarian is exposed to a toxin that specifically affects its neoblasts, what would be the likely consequences for its regeneration ability?
5. How could studying planarian regeneration contribute to advancements in human medicine, such as tissue engineering or wound healing?




Summary Report:

Write a Summary of your Results. The summary should be concise, well-organized, and clearly present the experimental outcomes.

Normal Regeneration Time Course:



Common Phenotypes of Healthy and Unhealthy Planaria:

Healthy	c 	Unhealthy
Phenotype: <ul style="list-style-type: none"> • Fully formed • Edges of animal appear smooth • Pigment: Brown Behavior: <ul style="list-style-type: none"> • Motility: Glides & elongates body 		Phenotype: <ul style="list-style-type: none"> • Regression • Lesions • Lysis Behavior: <ul style="list-style-type: none"> • Motility defects • No response to food • No response to light

Check out this youtube video!

