



PLTW

**STUDENT
VERSION**

EDVOTEK & PLTW Experiment #401

Under the Sea (PBS 4.2.2)

Experiment Objective:

In this experiment, students will examine the presence of a bioactive compound in samples collected from sea sponges. Students will first screen multiple sponges to determine which samples are worth in-depth analysis. Next, positive samples will be tested to quantify the concentration of the compound and determine which sponges are the best candidates for further study.

See page 3 for storage instructions.

Version 401.220314

PROTOCOL HAS BEEN UPDATED!
Please review before beginning experiment!

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Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets



Experiment Components

COMPONENTS

	Storage	Check ✓
A Sponge Solution 1	Refrigerator	<input type="checkbox"/>
B Sponge Solution 2	Refrigerator	<input type="checkbox"/>
C Sponge Solution 3	Refrigerator	<input type="checkbox"/>
D Sponge Solution 4	Refrigerator	<input type="checkbox"/>
E Sponge Solution 5	Refrigerator	<input type="checkbox"/>
F Screening Reagent	Refrigerator	<input type="checkbox"/>
G Lysis Buffer	Refrigerator	<input type="checkbox"/>
H 10 mg/mL Standard Solution	Refrigerator	<input type="checkbox"/>
I Quantitative Testing Reagent	Refrigerator	<input type="checkbox"/>
J NaOH Solution	Refrigerator	<input type="checkbox"/>

This experiment
is designed for
10 groups.

REAGENTS AND SUPPLIES

Store all components below at room temperature.

- Sponge fragments ☐
- 5 cm Petri plates ☐
- 2 mL Screw top tubes ☐
- 1.5 mL Snap top tubes ☐
- 5 mL Snap top tubes ☐

Experiment Requirements *(NOT included with this experiment)*

- Adjustable volume micropipettes
- Water bath(s) and/or hot plate (37°C and 99°C)
- Forceps (Optional)

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Background Information

For centuries, humans have ventured into distant, unexplored, and inhospitable environments in search of new discoveries. While some of these expeditions sought to increase our understanding of the world, many others hoped to expand empires, uncover new resources, and enrich stakeholders. Occasionally the expedition would collect exotic plant and animal samples with unique medicinal properties. Modern scientists have continued this search for useful compounds in nature in a process known as bioprospecting.

Bioprospecting has become a major component of modern biotechnology, agriculture, chemical engineering, and medicine. For example, the thermophile bacteria *Thermus aquaticus* was discovered in a geyser at Yellowstone National Park in 1966. This bacteria produces an enzyme, *Taq* polymerase, that can survive in the extremely high temperatures found in geysers and hot springs. Scientists were able to isolate *Taq* polymerase and use it to amplify DNA through a process known as the polymerase chain reaction (PCR).

The pharmaceutical industry also depends on bioprospecting for the development of novel medications. According to the World Health Organization, approximately 25% of medicines used worldwide are plant-derived or modified from naturally occurring compounds. Compounds that are able to produce a biological response in cells and tissues are referred to as bioactive (Figure 1). Pharmaceuticals derived from naturally occurring bioactive compounds include common medications like aspirin (pain relief and fever reduction), quinine (malaria), and taxol (cancer). Many other plants and animals contain active compounds, with new discoveries happening every year.

The search for novel bioactive compounds starts with the collection of samples from the environment, including plants, animals, bacteria, and fungi. The samples are returned to the laboratory where they are identified, cataloged, and prepared for testing. Initial tests often seek to detect any bioactivity within a sample, while later tests might identify the specific compound producing a response.

High-Throughput Screening Assays

Modern labs utilize high-throughput screening assays to streamline the initial tests, allowing for hundreds or thousands of samples to be rapidly tested. Scientists will design each experiment to test a specific hypothesis, which could involve either quantitative or qualitative assays. A qualitative experiment collects data that is observable but not measured. This type of experiment often provides “yes or no” experimental results. For example, a qualitative high-throughput screen might indicate if a compound is present in a sample, but will not provide data on the concentration. On the other hand, a quantitative experiment provides measurable, numerical results. Quantitative screens might determine size, mass, concentration, and many other measurable variables.

Polymerase Chain Reaction (PCR)

To perform PCR, purified double-stranded DNA is mixed with short DNA primers, *Taq* polymerase, and nucleotides. The mixture is heated to 94°C to “denature” the sample, or unzip the template DNA into single strands.

Next, the sample is cooled to 45°C-60°C, allowing the primers to base pair with their target DNA sequences (a step known as “annealing”). Finally, the temperature is raised to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer and synthesize a new strand of DNA. Each cycle of denaturation, annealing, and extension doubles the amount of DNA.



Figure 1: White willow (*Salix alba*) bark has been used since the 18th century as a treatment for fever and pain due to the presence of multiple bioactive compounds.

All high-throughput screens must balance the speed to test each sample, the cost of the experiment and materials, and the quality of the results. Often, multiple rounds of high-throughput experiments will be used to provide the best combination of these criteria. For example, if a pharmaceutical company is searching for an anti-cancer compound they might first use a high-throughput growth assay in cultured cells to determine if any compounds can inhibit cell division. The results from this first assay might be a simple yes-or-no qualitative answer. Once drug candidates have been selected, in this case the compounds that inhibit cell division, they would undergo an additional rounds of more intensive, and expensive, testing. The second round of tests might use quantitative assays to assess the level of activity, identify which components in a mixture are bioactive, or determine concentration of a compound.

Large high-throughput screens can potentially compare tens of thousands of specimens at a time. The US National Cancer Institute alone has amassed a collection of over 50,000 different plants, microorganisms, and marine organisms to test for cancer therapeutic properties. Automation and robotics have therefore become a significant part of high-throughput screens (Figure 2). Modern automated setups can prepare samples, perform the experiment, gather data, and analyze results!

Despite the potential for major discoveries in genetics, novel therapeutics, and chemical synthesis, there are potential drawbacks to bioprospecting. One major concern involves the destruction of the environment as samples are collected. Additionally, if a screen discovers a promising result it can lead to overharvesting and the removal of key species from the environment. Researchers must keep in mind the balance between identifying new samples from nature and the impact from harvesting.

An additional consideration during bioprospecting involves the use of traditional knowledge about a plant or animal without permission from a local people, known as biopiracy. Biopiracy often occurs when resources are taken from less affluent countries, but it can also occur by exploiting subpopulations within well developed countries. These resources are converted into patented products, with little to no wealth being transferred back to the native populations who originally discovered their usefulness. Many countries are now requiring contracts before researchers are allowed to collect samples, and the World Trade Organization has helped to develop a legal framework to protect plant and animal resources.

In this experiment, you will simulate the high-throughput screening of a bioactive compound within sea sponges. Five different species of sea sponge have been sustainably harvested from the Cayman Islands and returned to the laboratory for processing (Figure 3). First, you will use a simple qualitative test to identify sponges that contain the compound. Next, you will use a quantitative assay to determine the concentration of the bioactive compound in the positive sponges. Together, these results will help to guide the development of a novel therapeutic treatment!

Example of a Multi-step Bioprospecting Screen

- Step 1: Collect samples from the environment
- Step 2: Catalog and culture organisms
- Step 3: Screen for potential bioactive compounds and specific uses
- Step 4: Product development (testing, clinical trials, sales)



Figure 2: An example of an automated pipetting station.



Figure 3: Examples of different sea sponges in the Cayman Islands.

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, you will perform two assays on samples of sponge collected from a natural environment in the Cayman Islands. In Module I, a qualitative screen will be used to identify sponges that are positive for an important bioactive compound. Sponges that test positive in Module I will be further analyzed in Module II, where you will identify the concentration of the compound.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

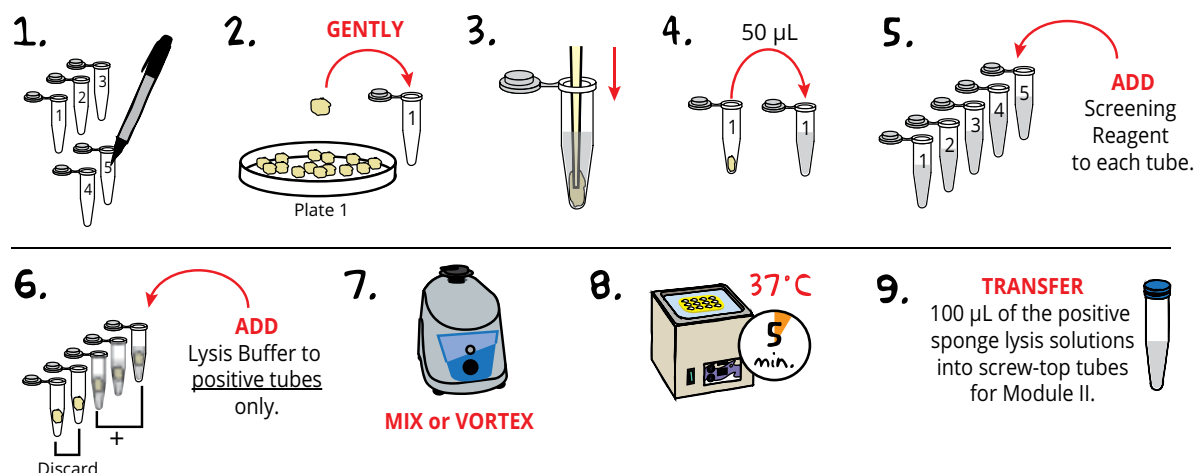
- Record (draw) your observations, or photograph the results.

After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Module I: Preparing Sponge Samples and Running Qualitative Screen

In this experiment you will perform two assays on sponge samples collected from a natural reef environment in the Cayman Islands. In Module I, a qualitative screen will be used to identify sponges that are positive for an important bioactive compound. You will use forceps or gloved fingers to transfer sponges into tubes and then use a testing reagent to determine if the sponge contains the compound. Sponges that test positive in this qualitative test will undergo a brief lysis to ensure that the bioactive compound is extracted. These samples will then be further analyzed in Module II, where you will identify the concentration of the compound.



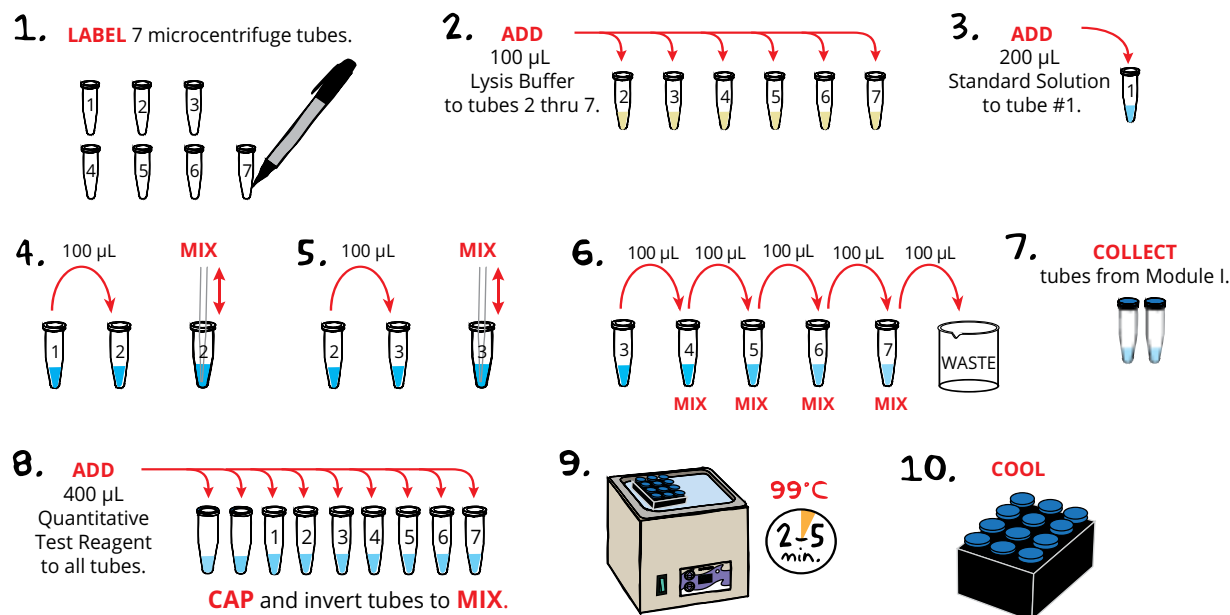
1. **LABEL** 5 snap top microcentrifuge tubes as #1-5. These will correspond to sponge samples that your teacher has prepared.
2. Using forceps or gloved fingers, carefully **TAKE** one piece of sponge from a petri dish and **PLACE** into the matching snap top tube. **Be careful not to squeeze the sponge while transferring to the tube.** **REPEAT** the same procedure with the remaining 4 samples.
3. Using a pipet tip, **PRESS DOWN** on the sponge until it is at the bottom of the tube. **SQUEEZE** the sponge against the bottom of the tube 3-4 times to release bioactive compounds that might be present. Gently **PIPET** up and down to mix the liquid.
4. **TRANSFER** 50 µL of the liquid to a fresh tube and **LABEL** it with the number of the sponge. **SAVE** the sponges and remaining liquid for step 6.
5. **ADD** 10 µL of Screening Reagent to each tube and **FLICK** the tubes to mix. A color change indicates that a bioactive compound can be found in the sponge. **RECORD** your observations in Table 1 and **IDENTIFY** sponges for further study (the ones testing positive in the qualitative screen). **Note: Unlike this qualitative screening test, these sponges will need a more complete lysis for the quantitative assay in Module II.**
6. Retrieve the tubes containing sponges from step 3. **ADD** 100 µL of Lysis Buffer to the tubes matching the positive Qualitative Screen results. The sponges that produced negative results will not be used in Module II and may be discarded at this time. **SAVE** the remaining lysis buffer for use in Module II.
7. **VORTEX** or pipet up and down to mix.
8. **INCUBATE** the tubes in a water bath for 5 minutes at 37°C.
9. **TRANSFER** 100 µL of the lysed sponge solutions into screw-top microcentrifuge tubes, then proceed to Module II.



OPTIONAL STOPPING POINT: Samples can be stored at 4°C for up to 1 week.

Module II: Quantifying Bioactivity in Sponge Samples

In Module II, you will test the positive samples from Module I to determine the concentration of bioactive compound in each sponge. You will first prepare a standard curve which will be compared to the unknown sponges. Next, samples are mixed with a quantitative test reagent and incubated in a 99°C water bath or boiling water on a hot plate. A color change reaction will be used to determine the concentration of the bioactive compound and select which species of sponge should be studied further.



- 1. LABEL** 7 screw top microcentrifuge tubes #1-7.
- 2. ADD** 100 µL of Lysis Buffer to tubes #2 through 7.
- 3. ADD** 200 µL of the 10 mg/mL Standard Solution (Std) to tube #1.
- 4. PIPET** 100 µL from tube #1 into tube #2. **MIX** the sample by gently pipetting up and down 5 times.
- With a new pipet tip, **TRANSFER** 100 µL from tube #2 into tube #3 and **MIX** as in step 4
- Continue to serially **DILUTE** the remaining samples through tube #7. **DISCARD** 100 µL from tube #7 into a waste beaker.
- 7. COLLECT** your experimental samples from Module I.
- 8. ADD** 400 µL Quantitative Test Reagent to each screw top tube. **CAP** and **INVERT** each tube several times to **MIX**.
- 9. INCUBATE** the samples in a 99°C water bath for 2-5 minutes or until tube #1 is red in color.
- Carefully **REMOVE** the samples from the water bath and place them on your bench to cool.
- 11. EXAMINE** the samples and **RECORD** your results in Table 2. **COMPARE** the experimental results with the standard curve to determine the concentration of bioactive compound in your sponge samples.

NOTE: A yellowish precipitate may appear in the samples. The tubes can be inverted to resuspend the precipitate while comparing colors to the standard curve.

Student Worksheet

TABLE 1

Sponge Number	Results of Qualitative Screen	Concentration of Bioactive Compound

TABLE 2

Sample	Concentration	Color
Std. Curve #1	10 mg/mL	
Std. Curve #2		
Std. Curve #3		
Std. Curve #4		
Std. Curve #5		
Std. Curve #6		
Std. Curve #7		

Study Questions

1. What is bioprospecting?
2. How has bioprospecting been traditionally used to benefit mankind?
3. Describe any potential drawbacks to bioprospecting.
4. What is a high-throughput screen? How does it differ from a traditional experiment?
5. What criteria must a scientist balance when designing a high-throughput screen?
6. Describe the difference between a qualitative and quantitative screen.