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Detection of Environmental *Staphylococcus aureus* using Colorimetric Loop-Mediated Isothermal Amplification (LAMP)

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Abstract

Staphylococcus aureus (S. aureus) is a common bacterium that colonizes external mucous membranes in approximately one third of the population. *S. aureus* is also part of the natural skin flora, contributing to its abundance on commonly touched surfaces. In this series of laboratory experiments, students perform environmental swabs, plate and grow mixed bacterial cultures, perform clonal expansion of probable colonies, and then use colorimetric Loop-Mediated Isothermal Amplification (LAMP) as a final confirmation of the presence of *S. aureus*. Loop-Mediated Isothermal Amplification (LAMP) is a DNA amplification technique capable of providing visually interpretable color-based results within one hour using crude cell lysates. LAMP does not require a thermal cycler and performs optimally at a single temperature (60-65°C), requiring only a simple heating block or hot water bath. Learning objectives include isolating and culturing a target bacterial colony, bacterial lysis techniques, using LAMP to confirm the presence of a target organism.

Keywords: Loop-mediated isothermal amplification, *Staphylococcus aureus*, DNA amplification, microbiology

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INTRODUCTION

Herein, we describe a three-part laboratory progression in which students perform environmental sampling, clonal expansion, and loop-mediated isothermal amplification (LAMP) to test for the presence of *Staphylococcus aureus*. This progression spans the first three lab sessions of the semester, including the first two which are also spent performing safety training, syllabus discussion, and a basic introduction to laboratory skills. While we use this laboratory over the first three weeks of the semester in our 200-level Introduction to Cellular and Molecular Biology course, we believe it can be used, as-is, in other 100- or 200-level laboratory courses.

Student Interaction and Engagement

This module was constructed for use as a 200-level Biology laboratory. Traditionally, the course was designed such that nearly every week was a different experiment. Essentially, students would arrive, learn a new technique, and then leave. Almost none of the laboratories were connected together into cohesive modules. This created a disjointed laboratory experience for our students. This was especially true at the beginning of the semester.

In the past, the first laboratory of the semester was used to go through laboratory safety training, the syllabus, and laboratory notebook requirements. The second laboratory was used to perform a simple diffusion experiment, and a handful of activities that were designed to give students experience with basic benchtop skills. Students performed no hands-on activities the first week and did not interact much during the second. What these labs inadvertently achieved was setting the students up for a semester of experimental silos and a lack of engagement. To combat this, we sought to design a laboratory module that would complement the necessary instructions of week one, and the skill-building activities of week two while engaging our students in an actual laboratory experiment. In addition, we wanted students to begin the semester with a short experimental progression that would be simple, interesting, and promote student ownership of their science.

A large hurdle that needed to be addressed was how to implement a meaningful experience into the existing first two weeks of the laboratory. To achieve this, we constructed a progression that begins with two, short laboratory experiments, that could fit into the existing lessons. The first of which is an environmental sampling experiment and the second was a clonal expansion. These experiments effectively fit into existing laboratories as neither requires extensive lecturing or time for substantial hands-on activities. The clonal expansion experiment is performed the following week and provides a low-stakes use of sterile technique, a skill that will be used throughout the semester.

By the start of the third week of the progression, students have performed environmental swabs, and selected bacteria off of their growth plate that they believe to be *S. aureus.* The third week utilizes a DNA amplification technique, loop-mediated isothermal amplification (LAMP), to verify whether their selected colony truly is *S. aureus.* There are multiple benefits of this procedure over traditional polymerase chain reactions. The first is that the amplification is performed at a single temperature, therefore, a thermocycler is not required. Second, the amplification can be started and analyzed within a single laboratory period. Finally, the analysis of the reaction is very simple and accessible to undergraduate students.

Laboratory set-up and design

This module is a three-week progression, with two weeks of short experiments followed by a third week that fills an entire laboratory session. The first two weeks of the module fit into two pre-existing laboratories. This makes the most sense at the beginning of the semester where the first labs are usually filled with information regarding the course and skill-building exercises that will be used throughout the semester. We allocate 15-20 minutes of class time for week one of the module (environmental swabs), and for week two of the module (clonal expansion). The third week of the module is the LAMP procedure and analysis. This laboratory typically takes roughly 2-2.5 hours to complete for our students.

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In our experience, the preparation for the laboratory is in line with set-up for other 200-level courses. The environmental sampling requires that nutrient-agar plates are prepared in advance. However, these can also be purchased if time constraints exist. The second week requires the preparation of bacterial culture tubes with nutrient broth prior to the start of class. The third, and final week, is the most time intensive set-up, but is simple and relatively quick to accomplish. The detergent used to make the lysis solution for the DNA isolation must be prepared prior to class. We also aliquot all reagents for the LAMP reaction prior to class. This is done to reduce the likelihood of accidental reagent waste by our students.

STUDENT OUTLINE

Objectives

Practice and improve the skills required to perform basic molecular biology techniques.
Perform calculations and discuss concepts related to LAMP master mix preparation for DNA amplification.
Discuss experimental design, including positive and negative controls.
Differentiate the stages and components of DNA amplification.
Analyze experimental results.

Introduction

Bacteria are prokaryotic, single-celled, organisms lacking many of the organelles seen in eukaryotes. Bacterial DNA is free-floating in the cytoplasm, where it can easily associate with ribosomes (Rocha 2008) (figure 1). Due to the lack of a nucleus, lysing the bacterial cell wall is a primary goal when working with bacterial DNA.

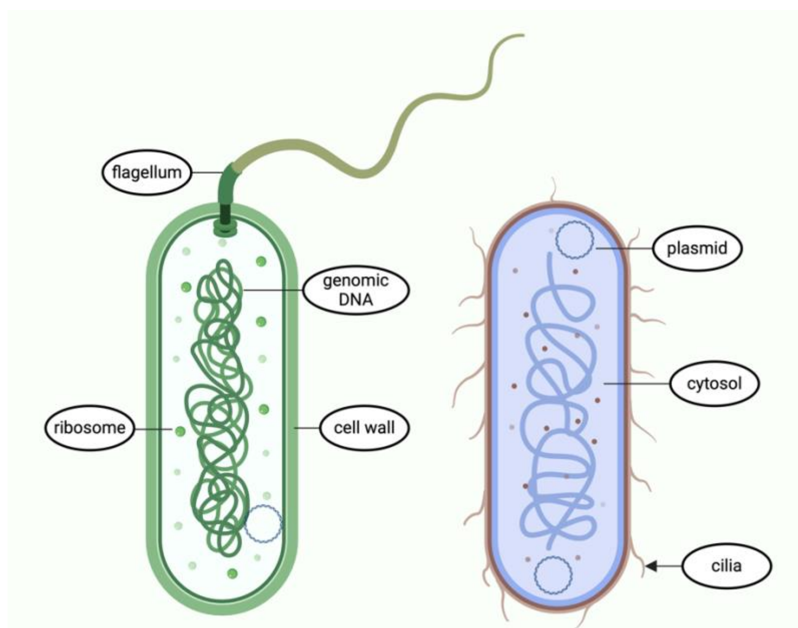

Figure 1. An outline of the basic morphology of bacteria. Notice that prokaryotic organisms do not have a nucleus or many of the other organelles seen in eukaryotes. Prokaryotic organisms also have a cell wall in addition to a simple plasma membrane. This figure was created with Biorender.com.

There are two types of bacterial cell walls, and bacteria may be classified based on these properties: Gram-positive or Gram-negative. Gram-negative bacteria have a thin layer of peptidoglycan surrounded by a lipid membrane (Kim et al. 2015). Peptidoglycan is a rigid substance composed of glycosaminoglycan chains interlinked with short peptides. Gram-positive bacteria lack an outer lipid membrane and possess a thick layer of peptidoglycan (figure 2) (Wientjes et al. 1991). Due to the thick and rigid peptidoglycan layer, gram-positive bacteria are often more challenging to lyse in experimental procedures.

In this lab module, we will be attempting to isolate and grow *S. aureus* a Gram-positive bacterium commonly found on shared surfaces, skin, and external mucous membranes like the inner nostril (Kluytmans et al. 1997). The first step in isolating a common bacterium is to perform environmental swabs. This is done by swabbing surfaces, then streaking an agar plate containing nutrients for the bacteria to

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grow on.

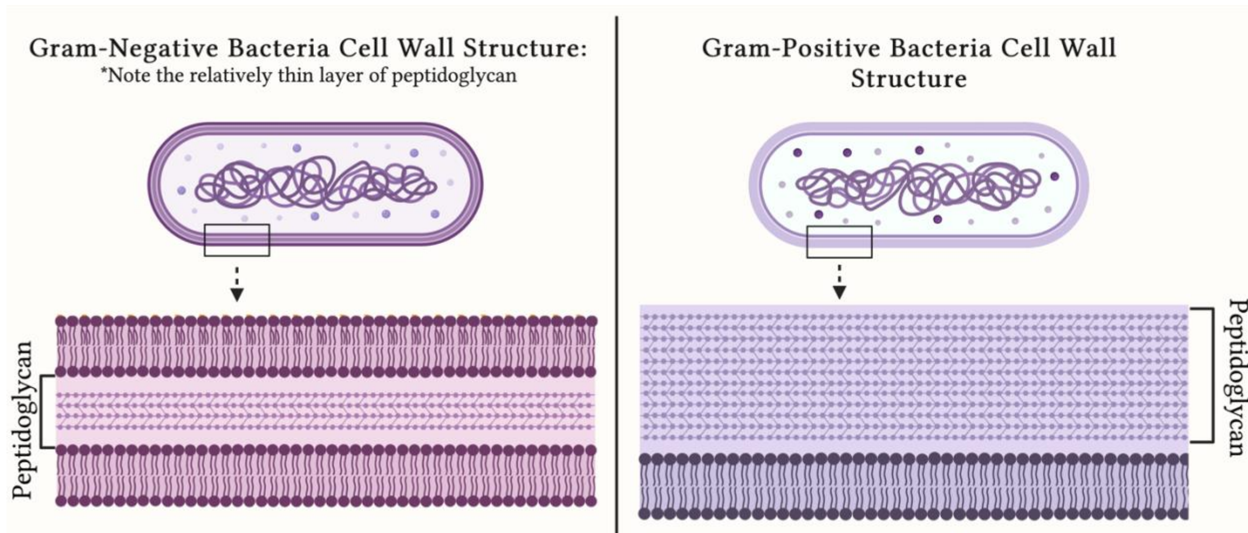

Figure 2. A comparison of gram-negative and gram-positive cell wall structure. Gram-negative bacteria have a thin layer of peptidoglycan between two lipid membranes, while gram-positive bacteria have a thick layer of peptidoglycan and one lipid membrane. This image was created using Biorender.com.

After keeping these plates at optimal conditions for bacterial growth (usually 37ºC, human body temperature), it is often possible to see a variety of distinct colonies (figure 3). You can then select a single colony to expand from this plate using another agar plate or a liquid nutrient broth. Since the appearance of *S. aureus* colonies on bacterial growth plates is well characterized, we will attempt to isolate and grow a colony that most closely resembles it. We can do this by selecting one candidate from the environmental plate, then inoculating it in nutrient broth to expand that colony, a process known as clonal expansion. The isolation and growth of a single colony is standard microbiology practice because it allows the analysis of a single type of bacteria.

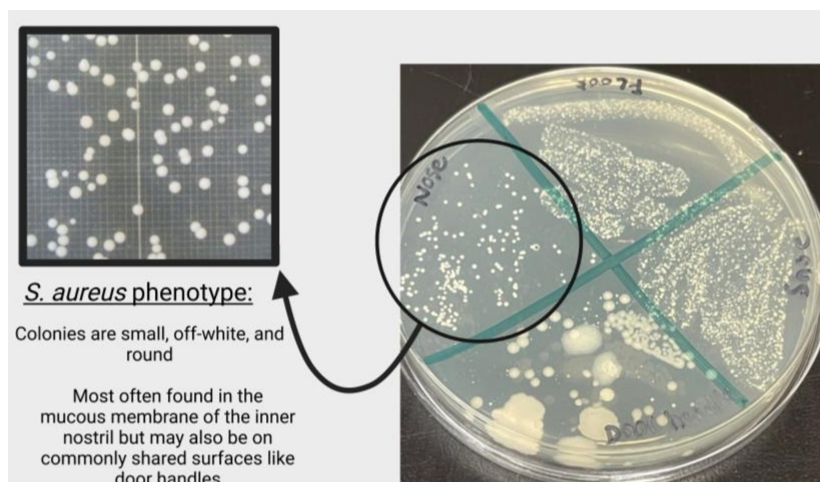

Figure 3. An example of *Staphylococcus aureus* on an environmental plate containing several species of bacteria. Notice that the bacteria in the quadrant labeled 'door handle' are large, slightly yellow, and have uneven edges. Alternatively, the bacteria in the quadrants labeled 'shoe' and 'floor' do not appear as distinct colonies, but instead are tightly packed and almost appear like a film on the plate. These example characteristics can be used to narrow down your selection to the correct bacteria.

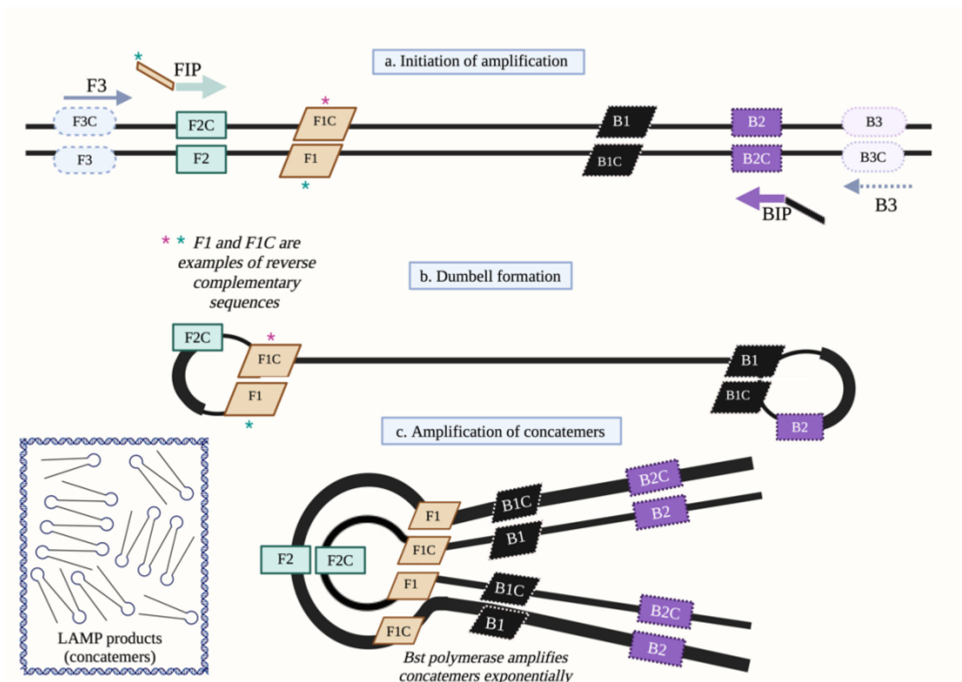

Figure 4. Schematic representation of the amplification of a DNA segment using Loop-Mediated Isothermal Amplification (LAMP). This image was created with Biorender.com.

Once a single colony has been expanded, samples will be tested for the presence of a *S. aureus* specific DNA sequence. To access the DNA, it is first necessary to lyse the bacteria by damaging the cell wall. To target the thick peptidoglycan wall of *S. aureus*, we will use a solution made with a standard household dish detergent. Once the cell walls have been degraded and DNA is accessible, lysed bacteria will be tested for the presence of *S. aureus* DNA using loop-mediated isothermal amplification (LAMP). LAMP is a DNA amplification technique capable of performing at a single temperature (between 60-65°C) due to the strand displacing activities of its F3 and B3 primers, as well as *Bst* polymerase (Notomi et al. 2000). *Bst* polymerase, the enzyme employed in LAMP, is derived from ***B**acillus **s**tearo**t**hermophilus*, a thermophile found naturally in hot springs, soil, and ocean sediment (Chander et al. 2014). The thermophilic nature of the polymerase allows it to function optimally at high temperatures, while strand-displacing activities of the primers and polymerase allow the reaction to proceed at a single temperature (isothermally). Once the F3 and B3 primers have displaced DNA strands to allow amplification, primers FIP and BIP amplify target sequences creating stem-loop structures and eventually concatemers due to reverse-complementarity sequences in the primers (Notomi et al. 2000). Concatemers (DNA molecules that contain several repeating copies of the same DNA sequence) act as seeds for exponential amplification, as each contains many new sites for amplification (figure 4).

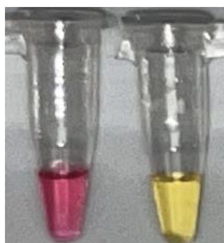

Figure 5. A colorimetric indicator causes reactions positive for DNA amplification to turn yellow, while negative reactions remain pink due to the phenol red pH indicator.

Due to the ability of LAMP to rapidly amplify DNA from crude lysates at a single temperature, if target DNA is present in the reaction, a positive or negative result can be detected between 30-60 minutes

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(Choate et al. 2023). LAMP can be paired with various indicators, and in this lab, a phenol-red-based colorimetric indicator will be employed. The phenol red indicator is pH-based, such that when the acidity of the reaction increases due to DNA amplification, the color of the LAMP reagent changes from pink to yellow (Tanner et al. 2015) (figure 5). The acidity of a DNA amplification reaction changes because a hydrogen ion is released when a new phosphodiester bond is formed at the 3' hydroxyl group (figure 6), which makes the reaction more acidic. Since DNA amplifies so efficiently with LAMP, the rapid synthesis of new bonds quickly impacts the pH within a small reaction (25 µL). This property allows us to use pH as an indicator of DNA replication, and consequently, the presence of our target organism, *S. aureus*, in the reaction.

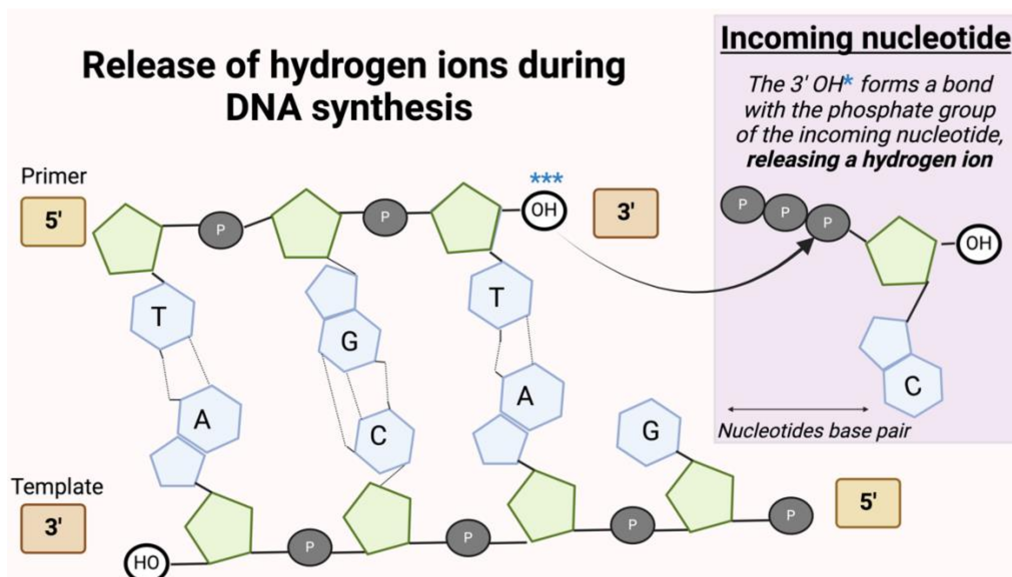

Figure 6. Amplification of DNA releases free H^+ ions into the reaction which causes the acidity of the reaction to increase. The addition of a pH indicator in the reaction mix will allow for the visualization of this process. This image was created with Biorender.com.

When performing any DNA amplification experiment, it is essential to include the appropriate control groups. A positive control consists of DNA that should amplify independently of the experimental variables. This is often a highly conserved gene like Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) known to be reliably present in the genome. A control group will have its own primers if the gene being amplified is different from the experimental gene. **A positive control aims to ensure your experimental conditions and reagents are functioning properly and that a lack of amplification is due to the absence of your target sequence; that shows it is not a faulty experiment.** Our positive control in this lab will be DNA derived from a pure culture of *S. aureus.* A positive reaction in this tube gives us confidence that our master mix and reactions were prepared correctly and that our conditions are optimal to amplify target DNA.

A negative control group is equally important when using amplification techniques. A negative control often consists of water, buffer, or saline in place of DNA, that is, typically the same reagent in which your primers and DNA were diluted. **The purpose of a negative control is to ensure reactions are not falsely positive due to a contaminated workspace or reagents, or poor technique.** A negative control showing the absence of DNA amplification gives confidence that experimental results are due to the presence of your target sequence, not other confounding variables. When performing DNA amplification techniques in the lab, unexpected positive or negative control results cause data obtained from experimental variables to be considered unreliable. The presence of appropriate controls is an essential part of every experiment and, when used correctly, will support your findings.

In this three-week laboratory progression, you will be performing environmental swabs, isolating and growing a single colony, lysing the bacterial colonies, then looking for the presence of *S. aureus* DNA in your sample using colorimetric LAMP (figure 7). These experiments will take place over the course of

three weeks to allow ample time for your bacterial colonies to grow.

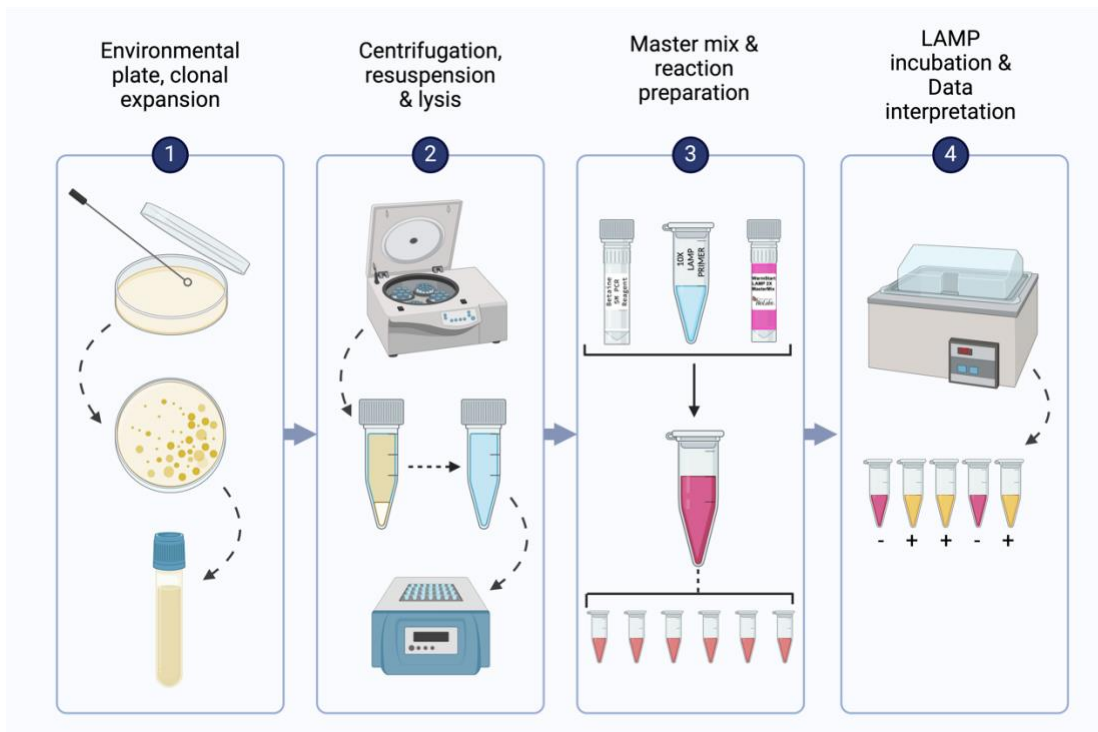

Figure 7. General workflow of the 3-week *Staphylococcus aureus* LAMP laboratory. The experiments in column 1, the production of an environmental plate and subsequent clonal expansion, will take place over the course of two weeks due to the time needed for bacteria to grow sufficiently. Columns 2-4 will be completed in a single lab period using bacterial samples from your clonal expansion. This image was created with Biorender.com.

Week 1: Perform environmental / self-swabs to acquire a colony of *S. aureus.*

Materials:
Distilled water
Prepared agar plate
Four sterile swabs

This week, you will swab your laboratory environment to begin a three-week module in which you will test for the presence of *S. aureus* (figure 8). This organism is a common type of bacteria found in the environment in soil and water. It often
resides on human skin and in the nose. Try swabbing some different objects, such as doorknobs, your phone, the inside of your nose, or your arm.

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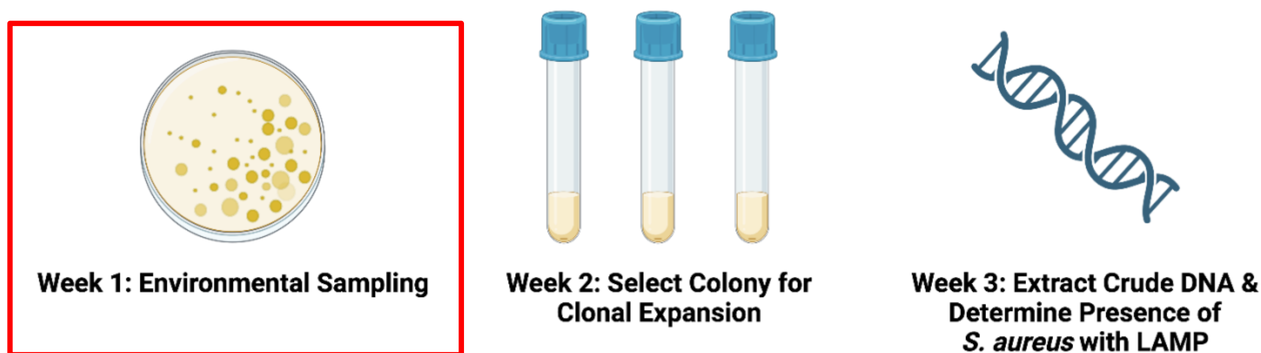

Figure 8. Week one of a three-week experiment testing for the presence of *Staphylococcus aureus* in the laboratory environment. A swabbing of the environment will be performed this week.

1. Each pair of students should swab 4 different surfaces and streak them onto an agar plate. Try to isolate each individual swab to ¼ of the plate (see figure 9 for an example). Wet the swab with distilled water before using it and **use a new swab for each surface**; try not to overlap where previously streaked. Label your plate with what was swabbed (example in the image below). Dispose of all sterile swabs in an orange biohazard bag.

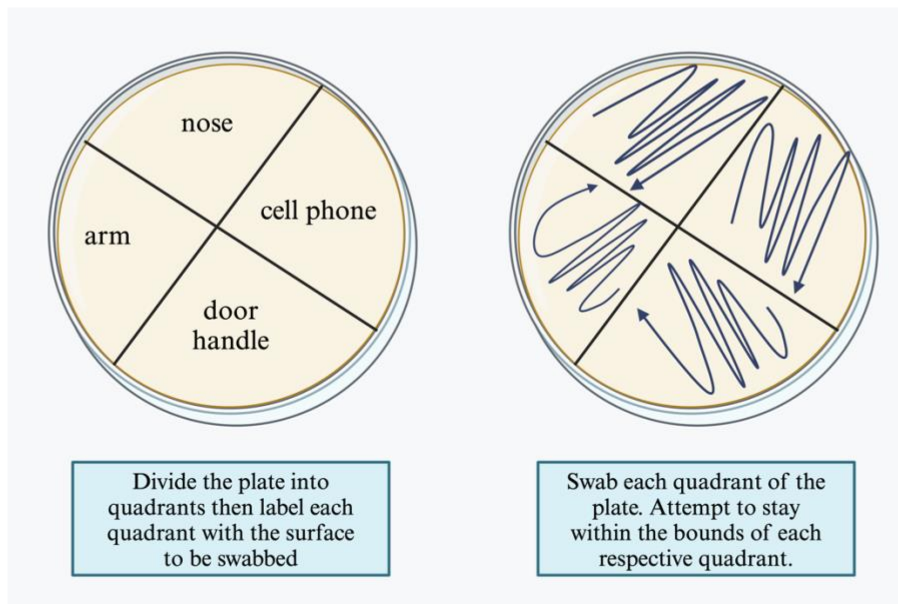

Figure 9. An example of the setup and swabbing of an environmental plate. This image was created using Biorender.com.

2. Label the plate with your initials and your instructor's initials, the date, incubation temperature, and growth medium. Plates will be stored inverted overnight at 37ºC; then refrigerated at 4ºC to avoid the overgrowth of bacteria.

Week 2: Isolate a colony of *S. aureus* from your environmental plate and inoculate a liquid culture.

Materials:
Environmental plate from last week
Pipet tip or inoculating loop
Culture tube containing nutrient broth

This week we will continue our three-week experiment in which we are testing for the presence of *S. aureus* (figure 10). Last week you swabbed surfaces in the laboratory and introduced them to bacterial growth plates. This week, you will obtain your plate from last week to determine if you have bacterial growth. Your plate may look like the one shown in figure 3 above. Your goal is to transfer one bacterial colony from your plate into bacterial culture tubes. This will create millions of copies of genetically identical bacteria through a process known as clonal expansion.

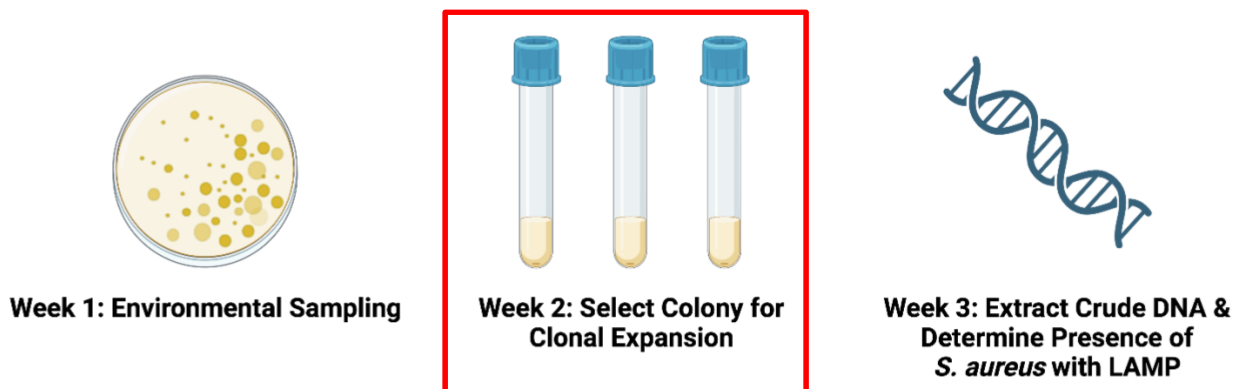

Figure 10. Week two of a three-week experiment testing for the presence of *Staphylococcus aureus* in the laboratory environment. This week, we will use growth morphology to identify a potential colony of *S. aureus* on the environmental plate from last week. The colony will be isolated and grown in liquid growth media.

Some criteria you can use when deciding which colony to select are colony size, proximity, appearance, and coloration. For example, notice in figure 3 that the bacteria in the quadrants labeled 'floor' and 'shoe' are numerous, small, tightly packed and surrounded by a thin opaque film, which may be difficult to see in the picture. These are likely colonies of *Mycobacterium smegmatis*. Contrast the morphology of these bacteria with *S. aureus*, which have distinct, round, generally uniformly sized white colonies. Another example on this plate is found at the outer edge of the quadrant labeled 'door handle'. Notice that these bacterial colonies are large and beige with somewhat puffy looking edges. These colonies are probably *Bacillus subtilis*. When contrasting the phenotype of *B. subtilis* with that of *S. aureus*, there is a noticeable difference between coloration and colony size. Learning to choose the right bacteria from a plate takes practice, and you may wish to ask your instructor to assist you in selecting the best choice from your plate.

1. Wearing gloves, locate your plate from last week. With the lid still on your plate, look at the colonies present and try to identify how many different ones you have. Note variations in colony color and size.
2. Attempt to locate a single colony of *S. aureus*. You are looking for an off-white, medium-sized circular colony. Refer to figure 3, and if you are unsure, ask your instructor for help. If you believe you do not have a *S. aureus* colony, see if a nearby group has one to spare.
3. Choose one colony that looks the most like *S. aureus* to use in the lab next week. One colony doesn't look like a lot, but there can be millions of bacteria present in a single colony, so you do not

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need to take more than one. Try to avoid touching surrounding colonies while collecting the one you've chosen.

4. Acquire one 5 mL aliquot of nutrient broth per group. Using a sterile spreader, gently scoop a single colony off the plate with an inoculating loop or pipet tip and place it into the nutrient broth tube. You may have to gently swirl the sterile spreader in the broth for a short time to ensure the whole colony comes off. Alternatively, the pipet tip with the bacterial colony can be ejected directly into the nutrient broth.
5. Dispose of your gloves, bacterial plate, and inoculating loop (if applicable) in a designated biohazard bag. Label your tube with your initials, lab section, the growth medium, incubation temperature, and the date, and give it to your instructor. Your tube will be kept overnight at 37ºC with gentle shaking, then moved to 4ºC until the next lab period.

Week 3: Verifying the presence of *S. aureus* using LAMP.

Materials:
Confluent culture from last week
Centrifuge
Lysis solution
Heat block
Pipette & pipette tips
NEB colorimetric master mix
Betaine
10X LAMP primers
Distilled water
PCR tubes (4 per group)
Locking (screw on cap) 1.5 mL tube (1 per person)
Hot water bath set to 65ºC
Transfer pipette

This week, we are performing the third and final experiment in our three-week laboratory progression (figure 11). The LAMP reaction will be used to verify the presence of *S. aureus* from the bacteria colony you selected last week. Be sure to review the background on the LAMP reaction in the introduction section above.

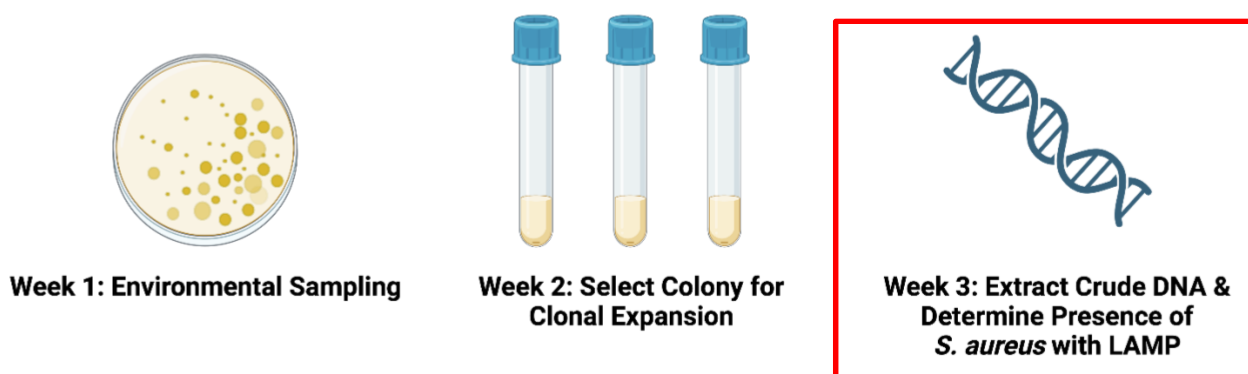

Figure 11. Week two of a three-week experiment testing for the presence of *Staphylococcus aureus* in the laboratory environment. DNA from Liquid bacterial colonies will be isolated followed by performing loop-mediated isothermal amplification to test for the presence of *S. aureus.*

1. Acquire your broth tube and note the difference in its appearance. The broth might appear somewhat cloudy and/or have debris visibly gathered at the bottom (see figure 12 for an example).

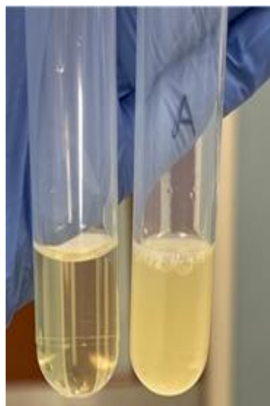

Figure 12. An example of a liquid culture before (left) and after (right) bacterial growth. Confluent cultures become opaque and may have visible debris gathered at the bottom.

2. Centrifuge the tube at 14,000xg for 10 minutes to pellet bacterial cells.
3. Using a transfer pipette, dispose of the supernatant (liquid above the cell pellet) into a designated biohazardous waste container. Try not to disturb the pellet at the bottom. The liquid can alternatively be decanted (poured) into a waste container- the bacterial pellet should remain adhered to the bottom of the culture tube.
4. Using a pipette, add 600 μL of the provided lysis solution, vortex well, then incubate at 100ºC for 10 minutes.
5. While samples are incubating, prepare the master mix for LAMP in a 1.5 mL Eppendorf tube using sterile technique.

Table 1. Reagent volumes used in the preparation of colorimetric LAMP master mix. Note that the value calculated for a 2-person group has an additional 0.5 sample volume added. This is common practice when preparing master mixes to account for the instrumental error associated to the pipette.

Component	Volume per reaction (μL)	Volume per 2-person group (μL, 4 samples total)
Colorimetric LAMP Master Mix (**Fully thaw and vortex prior to use)**	12.5	56.25
10X LAMP primer mix	2.5	11.25
Betaine	5	22.5

6. Acquire 4 PCR tubes and label them a-d. Add 20 μL of the master mix containing colorimetric reagent, primers, and betaine from table 1 to each tube.
7. Once your bacterial culture is finished incubating, vortex it thoroughly, then, wearing gloves, prepare your samples as follows:
   a. **Negative control** (Master mix and 5 μL of water)
   b. **Positive control** (Master mix and 5 μL of provided *S. aureus* DNA)
   c. **Student sample 1** (Master mix + 4 μL of lysed bacterial DNA)
   d. **Student sample 2** (Master mix + 4 μL of lysed bacterial DNA)

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8. Place the tubes in the warm water bath at 65ºC for 55 minutes.
9. Bring the tubes back to your table and place the tubes on ice for 30 seconds, then analyze the color changes. The samples should be briefly cooled down to assess the color changes properly. Record your results in table 2. Simply write yes or no in the appropriate columns.
10. Dispose of your LAMP reactions in a designated biohazard bag without opening the tubes.

Results

Table 2. *LAMP colorimetric results following a 55-minute incubation at 65ºC. Record which samples were positive or negative for amplification.*

Sample	Positive	Negative
Negative control (water)		
Positive control (*S. aureus* lysate)		
Student sample 1		
Student sample 2		

Note: *Recall that pink coloration indicates that no significant DNA amplification has taken place (negative) while orange or yellow coloration indicates that significant DNA amplification occurred (positive).*

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MATERIALS

Detailed preparation instructions are in Appendix A.

Week1:
90 mm petri dishes – 1 plate per group of two
Tryptic soy broth (TSB) or Lysogeny broth (LB)
Agar – combined with the TSB to produce 1 plate per group of two
Sterile swabs – 4 per group of 2
1.5 mL microcentrifuge tubes with sterile water – 1 per group of two

Week 2:
Bacterial culture tubes – 1 per person
LB or TSB broth – 5 mL per culture tube
Sterile inoculating loop – 1 per person

Week 3:
Centrifuge for 1.5 ml microcentrifuge tubes
Lysis solution – 600 µL per person
Heat block set to 100°C
P1000, P200, P20, and P10 pipettes
Sterile P1000, P200, P20, and P10 pipette tips
Transfer pipette – 1 per person
NEB colorimetric master mix – See table 1
Betaine – See table 1
10X LAMP primers – See table 1 and supplemental table 2A
PCR tubes – 2 per person
Locking (screw on cap) 1.5 mL tube (1 per person)
Hot water bath set to 65°C

NOTES FOR THE INSTRUCTOR

This laboratory experiment has been designed for introductory cellular and molecular biology students at the collegiate level. This experiment spans three weeks and is highly modifiable based on your semester schedule and your students' needs. Through this three-week experiment, students should be exposed to aseptic technique, basic bacterial culture techniques, DNA extraction, loop-mediated isothermal amplification (LAMP), and proper experimental design. The instructor notes below will give talking points for each portion of the module.

Week 1:

During Week 1, students will take environmental or self-swabs. In our labs, students work in pairs, with one plate broken into quadrants. Each student then does two environmental swabs. Remember, *S. aureus* is often found in the environment in soil and water, it often resides on human skin and in the nose. Suggesting students do one self-swab may increase their chance of positive results.

When teaching:

- Review proper aseptic technique and the importance of using it when working with environmental bacteria, including wearing gloves, lab coats, and goggles when necessary.
- Be sure to discuss bacterial growth plate components and why bacteria will grow on them.
- Demonstrate how to do an environmental swab and proper plating technique.
- Throw all waste into a proper biohazard container.
- Detail how students should label their plates.

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Week 2:
During Week 2 students will be inoculating single colonies from their environmental plates into nutrient broth to grow pure cultures for analysis through LAMP. Students should observe the different distinct colonies grown on their plates and record their observations. How many distinct colonies grew? What are their phenotypes? What swabbed surfaces were responsible for growth? Then, students should inoculate a colony that looks most like *S. aureus* into nutrient broth. In our labs, students work in pairs, but each student will isolate one colony to create their own liquid culture.

When teaching:
- Demonstrate the proper way to inoculate a single colony with proper aseptic technique, wearing gloves, lab goggles, and a lab coat when necessary.
- Identify *S. aureus* for students, an opaque, off-white colony.
- Define clonal expansion and why it is necessary to inoculate only one colony.
- Students should take a photo of their environmental plate to include in their laboratory notebooks prior to selecting. They should also clearly indicate the colony that they selected.
- Detail how students should label their culture tubes.
- Depose of all waste in a proper biohazard container.
- This is a lab in which sterile technique can be stressed as strongly as you wish. We do not have our students work near a flame due to safety concerns. However, if you want to stress sterile technique, feel free add a flame source to the required materials list.

Week 3:
During Week 3 students will be using LAMP to detect *S. aureus* from their bacterial cultures from the prior week. Each student will extract a crude DNA lysate from their bacterial culture from last week. It should be noted that the DNA purification is not necessary for LAMP to work. However, if you would like to add an additional laboratory session in which you compare LAMP to PCR, you will want to have purified DNA. If your students perform the purification, each pair of students will have six samples that undergo the LAMP reaction; positive control (*S. aureus* lysate stock prepared by the instructor), negative control (nuclease-free water), student 1 crude lysate, student 2 crude lysate, purified student 1 sample, and purified student 2 sample. Again, the DNA purification may be omitted for time or cost, as results between the crude and purified student samples should be consistent with each other.

Material Prep:
- *Staphylococcus aureus* lysate (positive control) – *S. aureus* can be obtained commercially, or prepared from a self-swab by the instructor prior to the start of the module.
- Colorimetric LAMP master mix (New England Biolabs, product number: M1800S).
- Primer stocks should be rehydrated in buffer or molecular grade water, prepared primers should be diluted in molecular grade water to avoid interference with the colorimetric indicator.
- 100 µL 10x primer aliquots should be prepared with 16 µL FIP, 16 µL BIP, 2 µL F3, 2 µL B3, and 64 µL of molecular grade water.

When teaching:
- Review proper aseptic technique and the consequences on contamination, reminding students to wear gloves, lab coats, and goggles when necessary.
- Demonstrate how to appropriately calculate reagent volumes for a master mix.
- Discuss the necessity of a positive and negative control in proper experimental design.
- Discuss LAMP, the reagents involved, and the mechanism of the reaction. This should be a main focus of the lecture. Depending on the level of your students, you will want to adjust how detailed you get with the primers and the products of amplification. Amplification is more complicated than PCR, and can be difficult for lower level undergraduate students to comprehend.
- Discuss possible sources of error in detail, making sure students know the purpose of each reagent and procedural step and how these connect to common errors.

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APPENDIX

Bacterial plates:

Agar dishes for environmental plates may be prepared with various growth mediums, including Lysogeny Broth agar. These solutions are typically autoclaved based on liquid volume prior to pouring plates, then plates are allowed to solidify overnight then maintained at 4°C until use.

Handling and storage of environmental plates

Once students have streaked their agar plates, they should be stored inverted at 37°C for approximately 24 hours. Prolonged growth periods may result in bacterial lawns and difficulty isolating a single colony from the plate.

Liquid cultures

Broth for liquid cultures should be prepared in sterile tubes, and a volume of anywhere between 3-5 mL will be sufficient for bacterial growth and isolation. Various broths can facilitate bacterial growth. Tubes should be kept at 4°C until use. Once students have inoculated the tubes, they should be kept overnight at 37°C with shaking then moved to 4°C until use.

Bacterial lysis solution

The bacterial lysis solution has been prepared in distilled water using either 40 mg/L powdered dish detergent (7th generation unscented) or 40 mg/L powdered laundry detergent (OxiClean^{TM}) with successful results. An excess of detergent over 40 mg/L will inhibit the colorimetric reagent and is not recommended. During the incubation period at 100°C, tubes with screw on caps are recommended, as the high temperature can cause pressure to build up in standard 1.5 mL tubes which causes them to open and lose contents.

Positive control lysate

Positive control lysate can be prepared by inoculating a 20 mL culture with a colony of *S. aureus*, growing overnight at 37°C with shaking, centrifuging at 14,000xg for 10 minutes, then decanting the growth medium. Add 2.5 mL of lysis solution, vortex, then incubate at 100°C for 10 minutes. Once complete, vortex and aliquot to 1.5 mL labeled tubes for student use.

Primer preparation:

S. aureus LAMP primers utilized in these experiments are found in supplemental table A1 and were sourced from (Tie et al. 2012). If desired, F3 and B3 may also be utilized as PCR primers for *S. aureus* and instructions for doing so are included in their manuscript.

Supplemental table A1. LAMP primer sequences specific to S*. aureus* were sourced from Tie et al. (2012) and should be rehydrated to 100 µM in TBE buffer prior to use.

Primer	Primer Sequences
F3	5′-GCATTTACGAAAAAAAATGGTAGA-3′
B3	5′-TGTTCATGTGTATTGTTAGGTT-3′
FIP	5′-GCCACGTCCATATTTATCAGTTCTAAATGCAAAGAAAATTGAAGTCG-3′
BIP	5′-TATGCTGATGGAAAAATGGTAAACGTAAACATAAGCAACTTTAGCCAAG-3′

Similar to PCR primers, LAMP primers will come as dehydrated oligonucleotides. Rehydrate the primers in a TBE buffer according to instructions to reach a concentration of 100 µM. From these stocks, prepare 10X stocks according to supplemental table A2.

Supplemental table A2. Primer volumes for the preparation of a 10X primer stock.

Reagent	Volume (µL)
Forward inner primer (FIP)	16.0
Backward inner primer (BIP)	16.0
Forward outer primer (F3)	2.0
Backward outer primer (B3)	2.0
Molecular grade water*	62.0

*It is critical to utilize water for the preparation of 10X primer stocks to avoid adding buffer to the pH-based colorimetric system. This is also the case for purified DNA (i.e., elution buffer) or other buffer-containing samples that you may wish to utilize with colorimetric LAMP. Addition of even small volumes of buffer to the reaction will inhibit colorimetric changes from occurring.

General notes regarding the colorimetric LAMP test:

LAMP can contribute to amplicon contamination, which may be avoided by advising students to refrain from opening reactions after the incubation period.

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