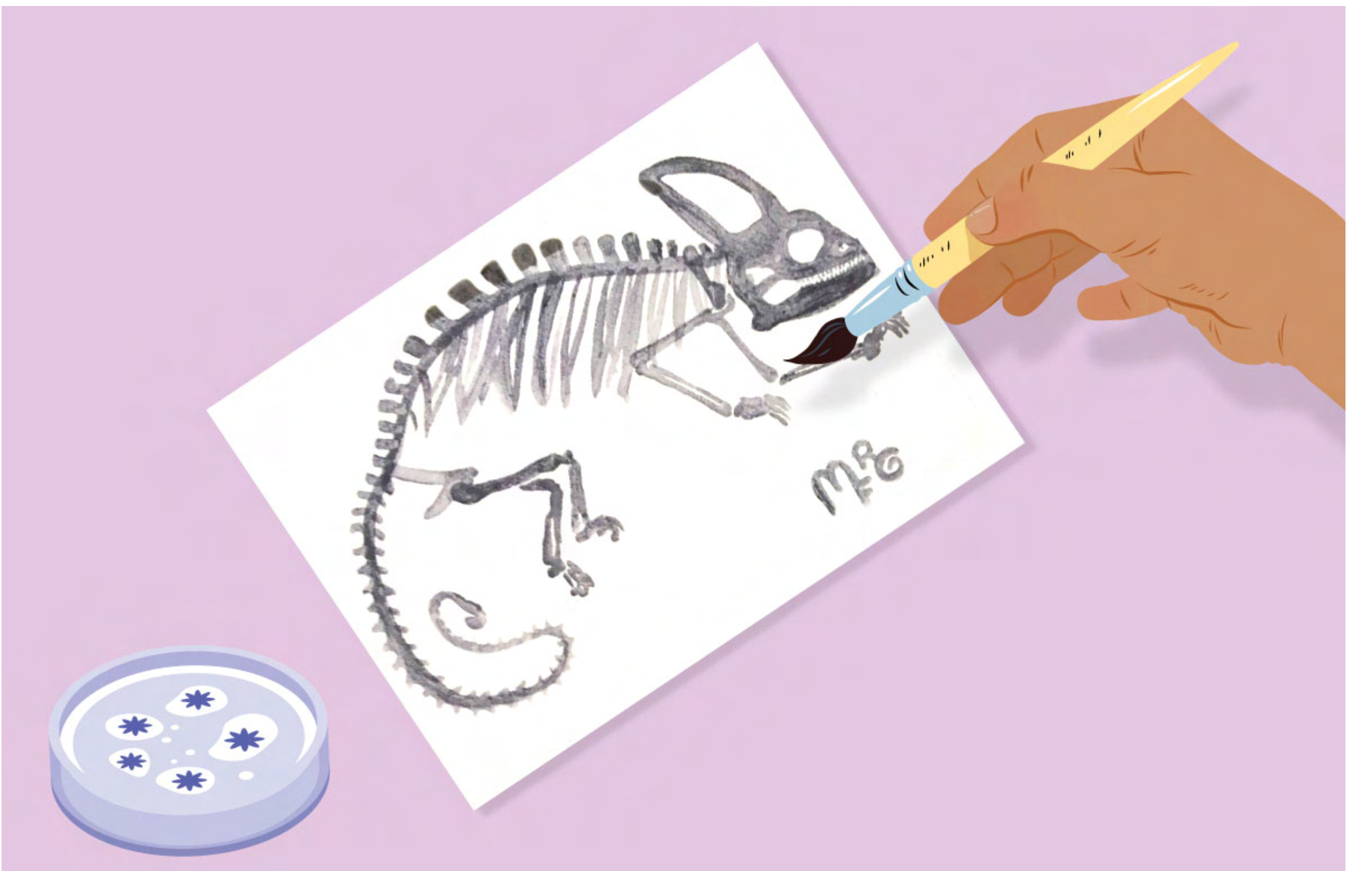
The Tech Interactive

Biotinkering Programs for Science Centers

Bacterial Inks



SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
SUPPORTED BY THE NATIONAL INSTITUTES OF HEALTH

Stanford MEDICINE | Department of Genetics

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Bacterial Inks Overview

Science Center Experience

Streptomyces violaceoruber is a naturally occurring soil bacteria that produces and releases a pH-sensitive pigment molecule. In this activity, visitors are challenged to figure out how to extract this interesting biological pigment molecule from the agar the bacteria was grown on in the lab. Visitors have access to water and a curated set of tools for exploration and problem solving. Once extracted, chemistry can be used to tinker with the pigment in their test tubes to create colors that they can contribute to communal collection bins. Finally, they get to paint their own artwork with watercolor paints made from visitor-harvested bacterial inks.

Subject
Biodesign

Ages
10+

Duration
30-40 min

Key Concepts
Bacteria, biological pigments, pH, acids and bases, paints

Activity Goals

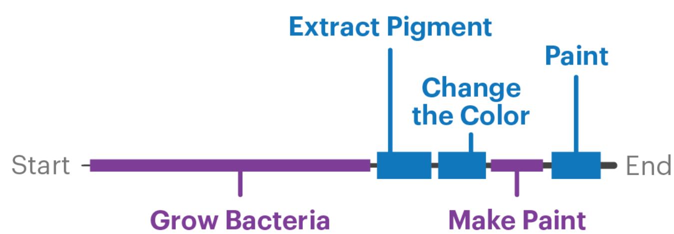
- Enable visitors to harvest, tinker, and create art with a color-changing pigment made by living bacteria.
- Support visitor agency with science through self-directed problem solving with tools and open-ended color exploration.
- Show that biology can be used as a sustainable manufacturing platform to create things that are relevant to everyday life.

Operational Considerations

Base Biology	Format Complexity	Lab Requirements	Cycle Length	Cost
Soil bacteria (*S. violaceoruber*)	High: Living Organism	Fridge, incubator, autoclave, laminar flow hood, sterile technique	2-3 weeks	$$$

Cycle Details

A full Bacterial Inks cycle takes a minimum of several weeks due to mandatory wait times for organism growth and paint drying (shown in purple). Using a cooking-show approach, however, the visitor-facing activity can be consolidated into a single ~30 min experience (shown in blue).



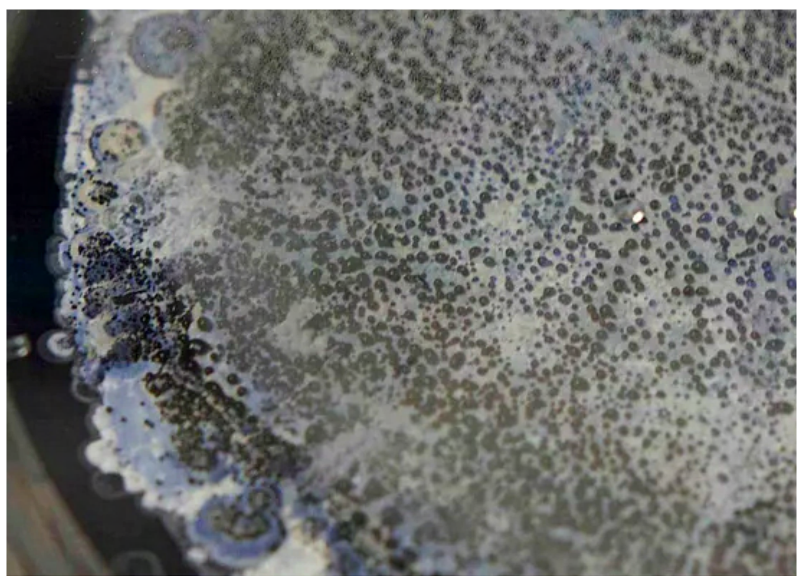
Background Information

Streptomyces Bacteria

Bacteria are a vast group of tiny, single-celled microorganisms that live all around us and have a wide variety of cell shapes, functions, and behaviors. They are typically just a few micrometers long and most are not harmful to people.

The bacteria used in this activity come from the *Streptomyces* genus, which is a large group of naturally occurring soil bacteria containing over 500 different species. *Streptomyces* are found around the world and play an important role in the environment and the global carbon cycle because they can break down dead organic material in soil to reclaim nutrients. This genus of bacteria may actually be somewhat familiar to people (or at least their noses) because they contribute to petrichor, which is the distinctive smell of soil after a rain. They produce the organic molecule geosmin, which has a strong "earthy" flavor and aroma. Geosmin is also found in beets! The production of this molecule is why *Streptomyces* bacteria grown in a sterile environment in a lab can smell a bit like dirt.

Streptomyces species have very complex life cycles which includes both mycelium and spores, meaning they have some similarities with fungi even though they are bacteria. They all have thread-like cell structures, but at the macroscopic level individual species can look very different (left image) because many of them make and release colorful molecules. This activity uses a specific species called *Streptomyces violaceoruber* (right image).

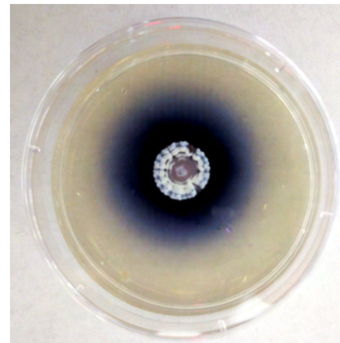


Do The Activity Bacteria Come From Soil?

Although *S. violaceoruber* were originally isolated from a soil sample, it is no longer necessary to go through that long, difficult process to obtain them. A sample of the strain is saved in a bio-repository called the American Type Culture Collection (ATCC) so that other scientists can purchase it for experiments. This is both much easier and safer because we can be 100% sure of what's growing.

How Does the Ink Get Its Color?

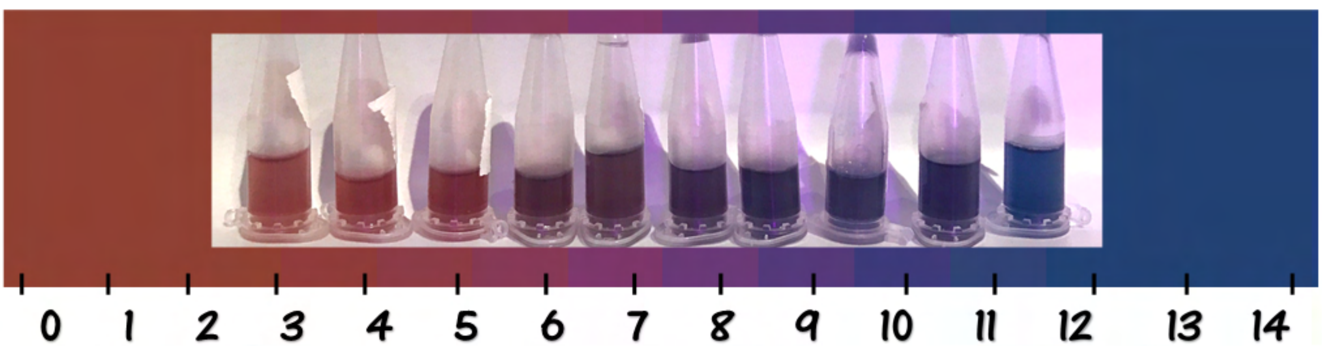
Pigment Production



S. violaceoruber bacteria make and then secrete a naturally-colored molecule called actinorhodin. When these bacteria are grown on an agar-filled Petri dish in a lab, the actinorhodin is easily visible because it creates a distinct colored halo surrounding the white bacterial colonies as it diffuses into the agar. Over time, the pigment will build up in the agar and can eventually saturate the entire Petri dish. This biologically manufactured pigment can then be harvested, purified, and concentrated, allowing it to be used in paint or ink formulations as a colorant.

Color-Changing Properties

The chemical structure of actinorhodin gives it the properties of a halochromic compound, meaning that it changes color depending on pH. When put into an acidic or basic environment, halochromic molecules bind to the hydrogen (H+) and hydroxide (OH-) ions in solution. This chemical interaction changes how electrons are localized in the molecule. Changes in electron localization alter how the molecule absorbs visible light, thereby changing the color that our eyes perceive. Due to these types of structural changes, actinorhodin looks blue in more alkali (basic) conditions and red in more acidic conditions, allowing us to create a variety of shades of paint with just one molecule.



Real-World Connections

Pigments Throughout History

Pigments are the basis of paint and have been used for millennia. Prehistoric humans were using basic pigments in cave paintings as far back as 30,000 - 40,000 BCE. These early pigments were usually as simple as ground earth or clay and were made into paint with spit or fat. Other natural pigments may come from plants, animals, or minerals. Some interesting examples of natural pigments that have been used by humans are:

- Indigo: A blue-ish pigment isolated from plants and commonly used for dying jeans.
- Ochre: Yellow or red clay that can be made into a pigment by grinding up earth.
- Carminic acid: A deep red anthraquinone produced by scaled insects.

- Copper: Various copper-containing compounds can be processed into green pigments.
- Tyrian purple: A reddish-purple pigment harvested from sea snails in the Mediterranean.

Today, there are also many synthetic pigments available. These are often sophisticated masterpieces of chemical engineering that are synthesized in a laboratory.

Streptomyces in Medicine

Streptomyces bacteria have a fascinating and impactful history in the field of medicine. This is due to the fact that they naturally make numerous different kinds of life-saving drugs, from antibiotics and antifungals to antiparasitics and immunosuppressants. In fact, the 1952 Nobel Prize in Physiology or Medicine was awarded to Selman Waksman for his discovery of streptomycin, the first antibiotic used to cure tuberculosis, which he isolated from (and named after) the bacteria *Streptomyces griseus*. Over the years, more than 50 different antibiotics have been isolated from various *Streptomyces* species, many of which are still used in medicine today.

Commercial Applications of Biological Ink

Biomanufacturing utilizes biological systems to produce commercially important biomaterials and biomolecules. A familiar example of biomanufacturing is industrial fermentation, in which living microorganisms such as bacteria and fungi are used to make products useful to humans. This includes lots of very familiar food items like bread, beer, cheese, and kimchi. In recent years, startups and companies have begun to explore possible applications for biological colors, both from bacteria as in this activity or from other organisms such as algae. For example, Pili Bio is a startup that is looking to commercialize the pigments produced by various *Streptomyces* species and other bacteria. Similarly, Living Ink has been exploring making biological inks but from algae pigments. Some of their prototype inks have been used on cardboard to make fully biodegradable packaging.

Environmental Impacts

Using a living system (in this case *Streptomyces* bacteria) as a manufacturing platform is a natural process which can produce fewer harmful chemical byproducts than many current methods of chemically synthesizing pigment molecules at a commercial scale. New sources of these kinds of pigments can also reduce the need to harvest pigments from more delicate or non-renewable natural sources, such as rare minerals or plants found in fragile ecosystems that may be vulnerable to overharvesting. Best of all, the end ink product is fully biodegradable.

Useful Vocabulary

Term	Definition
Acid	A substance with a pH of less than 7.
Actinorhodin	The pH-sensitive pigment secreted by *S. violaceoruber.*

Agar	A jello-like substance obtained from seaweed that is commonly used in Petri dishes as a substrate for growing microorganisms.
Bacteria	A large group of single-celled organisms that have cell walls but no membrane-bound organelles.
Base	A substance with a pH of greater than 7.
Fermentation	A metabolic process that converts sugar to acids and gasses or alcohol.
Geosmin	An organic molecule ($C_{12}H_{22}O$) with a distinct earthy flavor and aroma.
Genus	The second-from-lowest taxonomic classification in biology.
Halochromic	A compound that changes color depending on pH.
Logarithmic	A scale on which every number is 10 times stronger than the previous one.
pH scale	A logarithmic scale used to describe the pH of a solution that goes from 0 to 14. Solutions below 7 are acids, ones above 7 are bases, and 7 is neutral.
pH	Stands for “Potential of Hydrogen” and measures the concentration of hydrogen ions (H+) in water to determine whether a solution is acidic, basic, or neutral.
Pigment	A substance that imparts a color to other materials.
Petrichor	The strong earthy scent associated with rain falling on dry soil.
Species	The most basic (lowest) taxonomic classification of organisms in biology.

Visitor Experience

Operational Summary

Context

Bacterial Inks uses a staff-supported cooking show approach to consolidate a 2-3 week biological process into a single 30-40 minute experience for visitors. This activity was originally designed to run on a daily basis. While daily operations are not required, this schedule does allow for supply chain efficiency, as the materials made by past visitors can be used to support future visitors in a reliable and consistent fashion and bacterial growth timelines can be more easily managed by staff in backend labs.

This activity was created as a semi-facilitated experience organized around three hands-on engagement stations: pigment extraction, color-changing, and painting. Each station is designed to be largely self-guided, with the facilitator providing initial onboarding and support as needed to answer questions and encourage creativity and confidence. A facilitator-led introduction and individual supply distribution can happen at any time and location between when visitors enter and receive their challenge at the first station, depending on what works best for a given space and staffing model. The remainder of the experience can be fairly self-paced, with visitors progressing between stations when ready.

Activity Outline

1. Visitor Prep and Introduction
    - Visitors put on gloves.
    - Facilitator gives an overview of the activity.
    - Facilitator briefly introduces pigments, bacteria, *Streptomyces*, and agar.
2. Pigment Extraction Station
    - Facilitator explains the challenge: extract the bacteria pigment from the agar.
    - Visitors get individual supplies: pigmented agar, test tubes, and tube holders.
    - Visitors explore the available shared supplies and consider possible ways to use them.
    - Visitors experiment with water and tools to figure out how to get pure pigment liquid.
3. Color Changing Station
    - Facilitator (or instructional graphics) introduces the pH-sensitivity of the pigment.
    - Visitors experiment with adding acids and bases to their test tubes to alter the color.
    - Visitors contribute their final pigment colors to communal collection bins.
4. Painting Station
    - Visitors use paints made from pigment harvested by previous visitors to create art.

Visitor Prep and Introduction

Overview

Provide a brief overview of the activity to orient visitors to the nature of the experience and have all participants put on gloves. The facilitator should introduce visitors to pigments, bacteria, *Streptomyces,* and agar. Tailor the focus and depth of the background information shared to the target audience and local community being served.

Essential Materials	Individual • Gloves (all sizes) *Optional:* Physical examples to support the introduction (e.g., other natural pigments, Petri dish with pigment and grown *Streptomyces,* images of bacteria)
Example Setup	 

Engagement Strategies and Tips

Cultivate Confidence and Agency

- Successfully putting on gloves can be challenging and frustrating. Supports such as a hand measuring diagram can help young visitors navigate this step more independently.

Make Community-Relevant Connections

- Draw connections to historical and culturally-relevant examples of natural pigments and dyes that most connect with your audience.

Foster Scientific Curiosity

- Encourage visitors to bring their senses into the scientific process by having them try to describe or identify the smell associated with the pigmented agar (wet soil).

Pigment Extraction Station

Overview

This station is the core challenge of the activity. Visitors are challenged to come up with a way to extract the bacterial pigment from the agar that *S. violaceoruber* was grown on. They will need several individual activity supplies, such as pre-portioned agar and test tubes to collect their extracted liquid in, as well as access to a variety of shared extraction tools and water. After being given the challenge, visitors can be turned loose to explore and choose from an array of provided tools as they strategize and test out possible extraction methods. Once visitors have relatively concentrated and pure (no agar chunks) pigment liquid in their test tubes, they can move to the Color Changing Station.

<table>
<tr>
<td>Essential Materials</td>
<td>
<u>Individual</u>

Test tubes
Tube holders
Pigment-saturated agar (see <u>Backend Preparations</u> for details)

<u>Shared</u>

Water in tabletop bowls
Pipettes
Extraction tools with different functions such as:

Stirring (spoons, chopsticks, whisks, lab scoops, etc.)
Mashing (muddlers, tea bag presses, mashers, etc.)
Straining (tea strainers, cell strainers, slotted spoons, etc.)
Measuring (beakers, graduated cylinders, etc.)
Pouring (funnels, small pitchers, cups etc.)
Soaking (sponges, cotton balls, etc.)

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<td>Example Setup</td>
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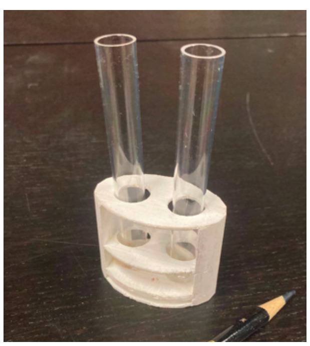

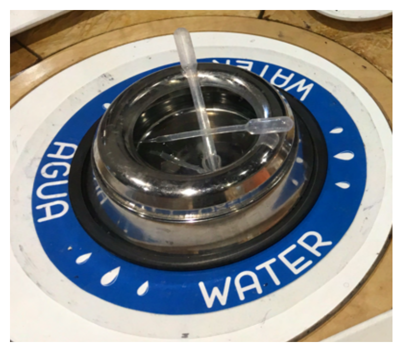

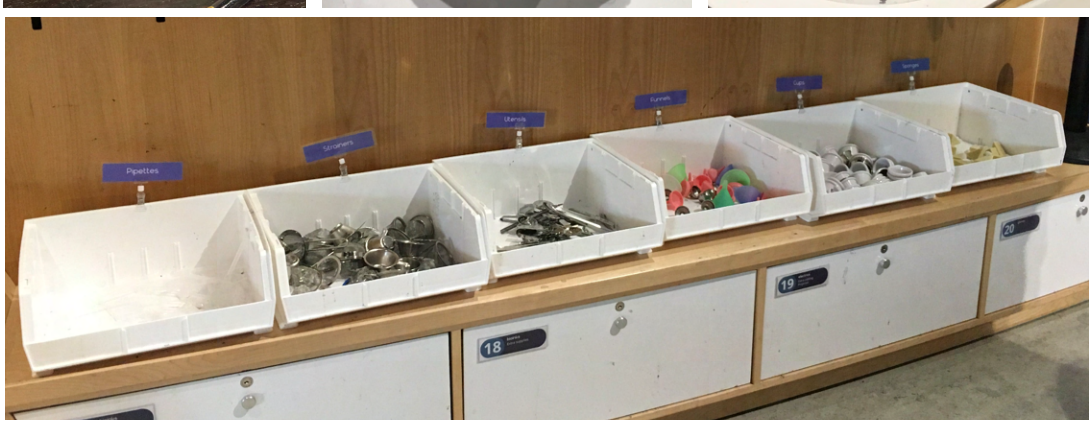
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Key Visitor Steps	1. Gather individual supplies. 2. Choose extraction tools. 3. Use water to extract pigment from the agar. 4. Collect concentrated pigment liquid (no agar) in test tubes. *Optional: Select new or additional tools as needed throughout the process.*

Engagement Strategies and Tips

Facilitate Constructive Problem Solving

- For visitors who are uncomfortable with the open-ended framing of the challenge and want a bit more guidance, a simple hint that the pigment is water soluble can be offered to get them started without prescribing what they should do.
- Use questions instead of direct suggestions to engage with visitors and encourage them to share their extraction ideas or what they have already tried, try new tools, or iterate on their approach.
- Intentional sizing of containers, such as keeping test tube volumes small, can increase visitor success by bounding solutions to those that produce more concentrated pigment liquids in a way that is not facilitator-prescribed.

Cultivate Confidence and Agency

- Providing tube holders that exactly match the number of test tubes used can be more welcoming and reduce confusion for young visitors.
- Mixing familiar kitchen tools and unfamiliar lab tools can help the science feel more welcoming and support confidence in a lab setting.

Encourage Experimentation and Open-Ended Exploration

- Having a tool station of freely available tools that visitors can go back to throughout their experimentation can encourage them to change directions and explore.

Highlight Authentic Science Practices: Collaboration

- Encourage visitors to work with and get inspiration from their neighbors. Table arrangements that create clusters of visitors can also encourage this.

Color Changing Station

Overview

Visitors experience the pH sensitivity of the bacterial pigment they just extracted by adding various acids and bases to their test tubes to see how the color changes. Let them freely explore the color changing using shared reagents until they're happy with the pigment liquid colors in their test tubes. At this point they can contribute their solutions to color-separated communal collection bins (blue, purple, pink) and move to the Watercolor Painting Station.

Essential Materials	<u>Shared</u> ● Acids and bases in dropper bottles (see [Backend Preparations]() for details) ● Pigment liquid collection containers (e.g., large beakers, filter flasks, etc.)
Example Setup	 
Key Visitor Steps	1. Add an acid or base to test tubes of extracted pigment liquid. 2. Observe how the color changes. 3. Choose a new acid or base and repeat. 4. Contribute final colors to communal collection bins.

Engagement Strategies

Cultivate Confidence and Agency

- Providing acids and bases in dropper bottles can support visitor confidence and agency because it makes the materials easier to use independently and control the volume added.
- Tabletop information and instructional graphics can be used to remove the need for a facilitator to introduce this step, allowing visitors to progress through the activity at their own pace.

Encourage Experimentation and Open-ended Exploration

- Diluting the acids and bases slows down the reaction and gives visitors more useful visual feedback. This can help visitors not overshoot a desired color, especially in the purple range.
- Have visitors only fill their test tubes halfway or have a way for them to remove some of the liquid at the start of this station so that volume limits don't hinder their experimentation.

Foster Scientific Curiosity

- Consider having a tabletop graphic with more info about pH, including the location on the pH scale of some familiar household acids and bases.
- Facilitators can help guide in-depth conversations about acid and base chemistry and halochromic compounds for visitors who are curious to understand more.

Watercolor Painting Station

Overview

Visitors use watercolor paints made from bacterial pigment harvested by visitors to create artwork of their own. The process of turning pigment liquid into watercolor paints takes a bit of time, so it is done behind-the-scenes by staff. This station should be set up with a variety of painting supplies for visitors to choose from. Paints of each color possible from this pigment (blue, purple, pink) should be set out to allow everyone access to the color(s) that they contributed to the communal collection bins.

Essential Materials	<u>Shared</u> • Bacterial watercolor paints (see Backend Preparations for details) • Paint brushes • Paper • Water in tabletop containers *Optional: Stamps, acids and bases*
Example Setup	
Key Visitor Steps	1. Dip a paintbrush in water. 2. Rub it on dried watercolor paint to activate it. 3. Apply paint to paper.

Engagement Strategies

Support Creativity

- Having brushes as well as some scaffolded tools (like stamps) can create a wider variety of engagement pathways for visitors with varying levels of artistic confidence.
- Providing acids and bases for visitors to use while painting allows them to change the color of paint once it is already on paper, bringing a whole new creative dimension to this art.
- Consider creating a location where visitors can leave behind their artwork to share with the community and serve as inspiration for future visitors.

Cultivate Confidence and Agency

- Instructional graphics can be used to help visitors who are less familiar with using watercolor paint to get started on their own.

Common Visitor Questions

Visitors often ask unpredictable or incredibly specific questions about the content or process of an activity while they are participating in the experience. Every audience will have different interests or prior knowledge that they bring to the experience. Below are examples of the most common questions we hear from visitors and the types of answers we aim to provide.

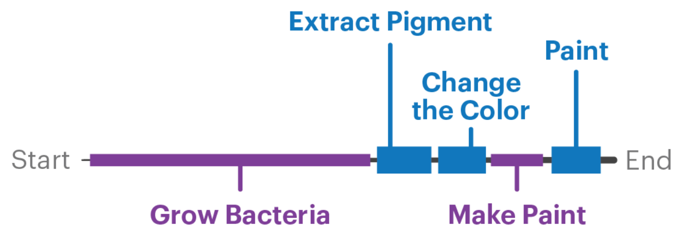
Question	Information
Is this bacteria dangerous?	*Nope!* *Streptomyces violaceoruber* is a naturally-occurring soil microorganism that has been well-studied and is known to be non-pathogenic to humans. On top of that, when the bacteria are grown in the lab to produce pigment, every step is done in a sterile environment to ensure that these harmless pals are the only microorganisms growing in the Petri dish.
Could this bacteria give me strep throat?	*Again, nope!* Although the name *Streptomyces* shares some similar sounds with the illness, they are not related in any way and there is no chance you could catch strep from these bacteria. The bacteria that could give you strep throat actually come from an entirely different genus called *Streptococcus*.
Why is making purple so difficult?	*Because the pigment is only that color in a narrow range of pH values.* Purple happens at the inflection point between pink and blue (around pH 9), so it can be hard to get the pH to stop right there. A good strategy to keep from flying right past purple is to add acids and bases in small increments with gentle mixing in between to ensure that the color has stabilized before adding more.
Can I paint with the pigment I extracted today?	*Unfortunately, no.* Because it takes time to turn the pigment liquid into watercolor paint, we have to collaborate with past visitors to have paint that is ready today. Future visitors will get to use paint made from your pigment! If you're interested in making biological pigment paints at home, however, a similar activity is possible using red cabbage in your own kitchen! Find our Cabbage Inks at-home activity guide on The Tech Interactive website.
If this bacteria is naturally in soil, why doesn't soil look blue?	*In nature, the bacteria don't grow as densely or purely as in the lab and soil is composed of many other things that add color.* Soil is a complex and diverse ecosystem that contains a variety of living microorganisms as well as lots of decaying organic matter. Also, there are many inorganic colored things in soil, such as minerals. Think about how rocks from various places can look very different! All of these things impact soil color.

Can bacteria produce other colors? Why can't we have those too?	*Yes!* Even just within the *Streptomyces* genus there are many different species that make different colors including purples, reds, yellows, oranges, greens, pinks, and more! There is a whole rainbow of possibilities, but growing each strain takes time to optimize, so we work with one that is well characterized.
Are there other pH-sensitive organisms in nature?	*Indeed there are.* One fun example is hydrangea flowers. The color of many hydrangea blooms acts as a natural pH indicator for the soil in which the plant grows. The blooms have blue flowers when the shrub grows in acidic soil, but develop red or pink flowers when grown in neutral or basic soils.

Backend Preparations

Overview of Components

The biological base of *Bacterial Inks* is living bacteria (*S. violaceoruber)*, so fairly intensive ongoing backend support is needed to operate this activity. A full activity cycle, from start to finish, takes a minimum of several weeks due to mandatory wait times (shown in purple) for organism growth and paint drying.



A staff-supported cooking-show approach can be used to consolidate the visitor-facing activity into a single floor experience (shown in blue). To achieve this, there are some backend operational tasks that need to be done by staff to support the purple phases of the cycle. Keep in mind that some of the initial preparation steps take significant time and must be done prior to beginning activity operations. Each staff-supported phase has been carefully designed to create final products that are in a format which is stable enough to be stored for weeks or even months (pigment-saturated Petri dishes, pigment liquid, and watercolor paints). This introduces some flexibility into the inherent biological system timeline, making it possible to support operational formats other than daily programming, if desired. Additionally, these points can be used to stabilize and buffer the cooking-show approach against supply chain disruptions and visitor number variability.

Backend preparations for this activity include:

- Growing Bacteria for Pigment Production
- Selecting and Preparing Acids and Bases
- Making Watercolor Paints

Institutional Note: Microbiology Skills Required

Culturing *S. violaceoruber* requires staff with a baseline level of comfort and skill with common microbiology equipment and practices, such as laminar flow hoods and sterile technique, to ensure safe and reliable growth of the organism.

Growing Bacteria for Pigment Production

Operating *Bacterial Inks* requires having a staff-supported backend system for growing and maintaining *S. violaceoruber* bacteria. This must be done at a scale that is sufficient to support the number of visitors participating in the activity, as the backend *S. violaceoruber* cultures are the source of bacterial pigment for all visitors. Getting a robust and thriving stock of bacteria established can take several weeks, but is critical for successfully running the activity.

Materials

Reusable Equipment	
Item	**Notes**
Autoclave	To sterilize tools
Incubator	Set to 28°C
Fridge	
Biosafety cabinet	Laminar flow hood can also be used, with precautions
Autoclave-safe containers	Appropriately sized for items being sterilized
Tweezers	Must be sterilizable

Consumable Supplies	
Item	**Notes**
S. violaceoruber bacteria	Available from American Type Culture Collection (ATCC)
Petri dishes	Must be sterile but any size can work
Yeast malt agar	To grow bacteria on
Filter papers	Sized to fit in Petri dishes
Aluminum foil	For autoclaving
Cotton swabs	Must be sterile
Isopropanol	In a spray bottle

Procedures

1. Get a bacteria stock growing following instruction from ATCC.
    - Let grow for 1-3 weeks to create a stock of dense, pigment-producing colonies.
2. Prepare materials needed to grow bacteria for pigment production.
    - Make Petri dishes with agar.
        - Sterilize yeast malt agar medium in an autoclave (15 minutes at 121°C).
        - Dispense into plates in a sterile environment and use sterile technique.
            - We add 150 ml to a 15 cm Petri dish, but any size/volume can be used.
        - Leave plates on the counter to solidify, then store upside down in a fridge.
    - Sterilize filter papers and tools.
        - Autoclave filter papers in an autoclave safe container wrapped in aluminum foil.
            - We use 12.5 cm diameter papers to fit in our 15 cm Petri dishes.
        - Autoclave tweezers in a metal autoclave box with tips facing down.
3. Start bacteria for pigment production in a sterile environment using sterile technique.
    - Use sterile tweezers to place a sterile filter paper on top of agar in Petri dishes.

- Streak out bacteria on filter papers.
  - Collect bacteria from a stock dish (step 1) on a sterile cotton swab.
  - Streak in a dense zig-zag onto the filter paper.
- Invert the dish and put it into a 28°C incubator.
  - Let it grow until the agar is saturated with dark blue pigment, usually 1-2 weeks.

4. Collect pigment-saturated dishes in a sterile environment using sterile technique.
   - Remove filter paper and spray agar with isopropanol (see time-saving tip below).
5. Store pigment-saturated dishes (if not using immediately).
   - Petri dishes with pigment-saturated agar can be stored in the fridge for up to several months. This can be valuable for helping to stabilize and buffer supply chain disruptions and visitor number variability.
6. Make individual portions of pigmented agar for visitors to use in the activity.
   - Exact portion size depends on the tools and containers being provided for the visitor experience and the concentration of pigment in the agar.

Time-Saving Tip: Reuse Mature Bacteria Filter Papers

If you're very careful with sterile technique at all stages of the growing process (including this one), time can be saved by reusing filter papers of mature bacteria. After removing the paper from a fully pigment-saturated Petri dish, place it in a fresh dish and press down gently. Filter papers with dense, mature bacteria can repeatedly saturate Petri dishes with pigment at a very fast rate!

Selecting and Preparing Acids and Bases

Operating *Bacterial Inks* requires having a staff-supported backend system for growing and maintaining *S. violaceoruber* bacteria. This must be done at a scale that is sufficient to support the number of visitors participating in the experience each day.

Materials

Reusable Equipment	
Item	**Notes**
Dropper bottles	For dispensing acids and bases

Consumable Supplies	
Item	**Notes**
Acids	Provide several options - lemon juice and vinegar both work well
Bases	Provide several options - laundry booster and baking soda both work well
Water	If needed

Procedures

1. Select ingredients for the visitor-facing activity.
    - Choose acids and bases from across the pH scale. Make sure to have some on both sides of pH 9 (the red-blue inflection point for actinorhodin).
        - Common household acids (e.g., lemon juice and vinegar) and bases (e.g., laundry booster and baking soda) work well.
        - Stay away from acids or bases that are too strong for your operational format, institutional preferences, and specific audience.
2. Prepare ingredients for visitor use.
    - Dissolve powdered items (such as baking soda) in water to create a solution.
    - Dilute acids or bases that are very concentrated with water.
        - This helps visitors not accidentally add too much.
    - Put final acid and base solutions into dispensing bottles or containers.
        - Dropwise dispensing bottles are helpful for controlling the mess, but any container and transfer method could work.

Making Watercolor Paints

Materials

Equipment	
Item	**Notes**
Coarse strainer	Mesh ones work well
Fine filter	Pore size 50-200 μm
Vacuum flask and pump	Hand or motorized pumps can both work
Mortar & pestle	
Measuring spoons	
Heat source	Needs a low setting to not burn the pigment

Supplies	
Item	**Notes**
Extracted pigment liquid	From visitors doing the activity
Diatomaceous Earth	
Watercolor medium	Can be either purchased or homemade
Water	

Procedures

1. Filter the pigment liquid.
    - Collect the various colors (blue, purple, red) from the activity communal collection bins.
    - Run the pigment liquids through a coarse strainer to remove larger chunks of agar.
    - Run them through a fine filter using a vacuum pump to remove smaller contaminants.
        - Filtered pigment liquid can be stored in the fridge for days to weeks if needed.

2. Prepare and concentrate the pigment.
    - Combine diatomaceous earth and filtered pigment liquid in mortars.
        - We use ¾ tsp of diatomaceous earth per 125 ml of liquid, but this ratio can vary depending on your pigment liquid concentration and desired final paint strength.
        - If desired, acids or bases can be used to further adjust the color at this point.
    - Evaporate the water to concentrate the pigment.
        - Set on a warming tray or use another gentle heat source to speed this process up and reduce chances of contamination growth.
        - Remove the mortar from the gentle heat source when dry.
    - After evaporation, there should be a completely dry layer of colorful pigment powder coating the bottom of the mortar.

Technical Note: How Hot is Too Hot?

We say to use a gentle heat source for evaporating the pigment liquid for a reason: too much heat for too long can actually burn the pigment, leading to ugly burnt brown colors instead of pretty blue, red, and purple hues! So if your paints look less colorful than you think they should based on the starting color of the pigment liquid, try a lower heat or less time on the heat source.

3. Make the watercolor paints.
    - Add 3 ml of watercolor medium and 1 ml of water to a mortar with a dried pigment layer.
    - Grind with a pestle until everything is combined as well as smooth and consistent.
    - Let watercolor paints dry out fully.
4. Store paints for future visitor use.
    - The paint is very stable and can be stored long-term (years even!) at room temperature. This can be valuable for helping to stabilize and buffer supply chain disruptions and visitor number variability.

Common Backend Questions

Standard operating procedures for this activity will vary based on the unique context of a given institution. Factors such as physical spaces, programming frequency, equipment availability, staffing models, and audience characteristics will introduce constraints and preferences that the general procedures above can be adapted to accommodate. Below are answers to the most common operational questions and insights from our experience running the activity in the BioTinkering Lab.

Question	Information
How long does it take for the bacteria to turn agar blue?	*Generally about a week. But it's biology, so it can vary.* A living system is very dependent on environmental parameters, so exactly how long will vary depending on your institutional growing conditions. We use large filter papers (12.5 cm diameter) and large Petri dishes (15 cm diameter) filled with 150 ml of agar to support the high volume of starting material we need for daily operations of the activity. With this set-up in our lab environment, mature filter papers of bacteria can pump out saturated plates in a week or less.
What are the best acids and bases to use?	*Any could work! Just provide a wide range (of safe materials).* We used two acids (lemon juice and vinegar) and two bases (dissolved baking soda and laundry booster) and tried to pick fairly familiar household ingredients. Additionally, we made sure to have ones that covered the full range of possible colors from this pigment molecule (red, purple, blue). Although any solution that changes the pH could work, we recommend avoiding very strong acids or bases that could be more dangerous than you are set up to manage.
How important is the filtering step?	*It is very important to make quality paints.* If too much agar is left in the pigment liquid, the paints that are produced will be very sticky, chunky, and hard to use. The finer the filter used, the better the quality of the paint that is produced. We filter all the way down to a fritted glass disc with a pore size of about 60-90 µm. It is also possible to fully sterilize the pigment liquid after visitors have engaged with it, but it will take several rounds of incremental prefiltering to get to an appropriately small pore size.
What is in the watercolor medium you use?	*A combination of gum arabic, honey, and glycerin.* Premixed, ready-to-use watercolor mediums can be purchased (we use one from Kremers). Alternatively, create your own medium from some or all of the ingredients listed above to save on costs. DIY recipes can work great but might take a bit of optimizing to create the most usable paint products.
Are other types of paint possible with bacterial pigment?	*Yes! Watercolors are just great for simplifying storage and setup.* We designed the activity to use watercolor paints because they are easy to store long-term and can be used directly from the mortar they were dried in, which makes daily operations dramatically easier for staff. However, it is of course possible to use the bacterial pigment to produce other types of paints (or even dyes) with different properties, if preferred. This is entirely based on what works best with operational logistics and the desired final artistic project.

Supplemental Resources

Full Materials List and Recommendations from The Tech

Reusable Equipment		
Item	**Notes**	**Specific Recommendations**
Autoclave	To sterilize tools	
Incubator	Set to 28°C	We use a small benchtop incubator.
Fridge		
Biosafety cabinet	Laminar flow hood can also be used, with precautions	
Autoclave-safe containers	Appropriately sized for items being sterilized	We use metal boxes for tweezers and large pyrex Petri dishes for filter papers.
Tweezers	Must be sterilizable	We use metal stamp tweezers to minimize filter paper rips.
Coarse strainer	Mesh ones work well	We use calculi strainers.
Fine filter	Pore size 50-200 μm	We use glass Buchner Filter Funnels with a coarse frit from Chemglass.
Vacuum flask and pump	Hand or motorized pumps can both work	We use a motorized pump for backend prep to reduce time and effort.
Mortar & pestle	To make watercolor paint in	We use ones that are approximately 4 inches wide and 3 inches deep.
Measuring spoons	For making watercolor paints	We use an adjustable spoon to achieve intermediate measurements in one scoop.
Heat source	Needs a low setting to not burn the pigment	We use a food warming tray.
Test tubes	For visitor-extracted pigment	We use 10 ml volume tubes.
Tube holders	For visitor rest tubes	We 3D-printed holders with only 2 openings.
Extraction tools	Provide a variety that supports visitor stirring, mashing, straining, measuring, pouring, and soaking.	We use lab spatulas and scoops, mini whisks, pitchers, beakers, mashers, tea strainers, sponges, cell strainers, graduated cylinders, portion cups, funnels, and tea bag presses.
Water bowls	Something stable to hold water for shared use during extraction	We use dog water bowls.
Pipettes	For visitors to move water with	We use 10 ml volume plastic pipettes.

Dropper bottles	For dispensing acids and bases	We use hard plastic droppers to discourage squeezing large volumes out at once.
Pigment liquid collection containers	Any container with enough volume can work	We use a custom filter flask assembly because it is sturdy enough to not get knocked over and allows us to filter the pigment liquid right away.
Paint brushes		
Water containers	For visitors to wash their brushes	We use no-spill paint cups to minimize mess when they are knocked over.
Stamps	Optional but recommended	

Consumable Supplies		
Item	**Notes**	**Specific Recommendations**
Gloves	All sizes	
S. violaceoruber bacteria	Available from American Type Culture Collection (ATCC)	Make stable stocks from the original purchase so they can be used whenever the bacteria need to be refreshed.
Petri dishes	Must be sterile but any size can work	We use 150 x 20 mm dishes from Celltreat.
Yeast malt agar	To grow bacteria on	We use HiMedia ISP Medium No. 2.
Filter papers	Sized to fit in Petri dishes	We use 12.5 cm diameter papers.
Aluminum foil	For autoclaving	
Cotton swabs	Must be sterile	We use 6” sterile cotton tipped applicators.
Isopropanol	In a spray bottle	
Acids	Provide several options	We use lemon juice and vinegar.
Bases	Provide several options	We use standard baking soda and Arm & Hammer laundry booster.
Water		
Diatomaceous earth	For making watercolor paints	
Watercolor medium	Can be either purchased or homemade	We use Kremer Watercolor Medium which contains gum arabic, honey and glycerin.
Paper	Watercolor paper is ideal because it is very absorbent	We use watercolor paper cut into small rectangles so visitors can use several.