AMERICAN SOCIETY FOR MICROBIOLOGY agar art®

Edvo-Kit # 228

Edvo-Kit #228

Agar Art: Creating Masterpieces with Microbes

Experiment Objective:

In this experiment, students will harness the color producing power of chromogenic bacteria to create works of living microbial art.

See page 3 for storage instructions.

Version 228.200918

EDVOTEK®

Table of Contents

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

Experiment Components

Components	Storage	Check (√)
• Ampicillin	Freezer	❑
• IPTG	Freezer	❑
• BactoBeads™ transformed with GFP Plasmid	4 °C (with desiccant)	❑
• BactoBeads™ transformed with Blue Plasmid	4 °C (with desiccant)	❑
• BactoBeads™ transformed with Purple Plasmid	4 °C (with desiccant)	❑
• BactoBeads™ transformed with Pink Plasmid	4 °C (with desiccant)	❑

This experiment is designed to create 25 pieces of microbial art.

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.

Reagents and Supplies

Store all supplies below at room temperature.

- ReadyPour™ Luria Broth Agar, sterile ❑
- Luria Broth Liquid Media ❑
- Inoculating loops, sterile ❑
- Plastic loops, non-sterile ❑
- Toothpicks ❑
- Petri plates ❑
- 1.5 mL Microcentrifuge tubes ❑
- 10 mL pipet, sterile ❑

Requirements

- Distilled water
- Gloves
- UV light source such as EDVOTEK® Cat. #969, Cat. #557, or Cat. #558 (If using fluorescing bacteria)
- Microwave
- Incubator or water bath
- One adjustable pipette and tips (20-200 µL)
- Pipet pump (optional)
- Vortexer (optional)
- Fine tip paint brushes (optional)

Background Information

Art guides, challenges, and inspires the scientific process in complex, powerful and often unpredictable ways. Take these three true stories:

- In 1837, Charles Darwin presented a collection of specimens from his voyage to John Gould, the curator at the Zoological Society of London. The latter - an ornithologist and artist - identified several birds that had been misidentified as wrens but were, in fact, new finch species. This reclassification inspired Darwin to investigate the island origin of these finches and to begin to formulate his theory of evolution by natural selection. In turn, Gould helped illustrate and edit the bird section for the book "Zoology of the Voyage of H.M.S. Beagle" (Figure 1a) along with several other bird monographs and today is known as the English Audubon.

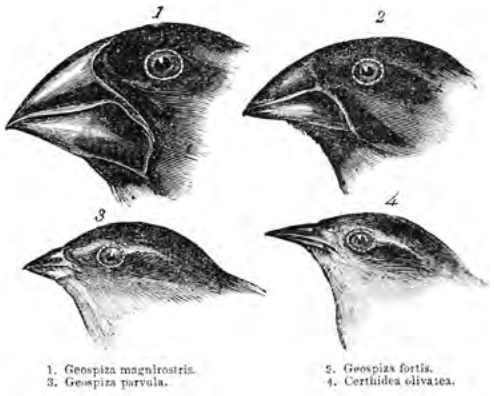


Figure 1a: Darwin's Finches by Gould.[1]

- Alexander Fleming is famous for his accidental discovery of penicillin in 1928. After returning from holiday, he found that a fungal contamination in his Petri plates had killed much of the disease-causing bacteria *staphylococci*. He is less famous for his bacterial paintings (Figure 1b) which he also created on Petri plates and at one point presented as a small exhibit to the queen of England! It's speculated that his search for colorful microorganisms to use as "paints" may have been the original source of the *Penicillium* fungus.

- For over a decade *Science Magazine* and the American Association for the Advancement of Science (AAAS) have been hosting an annual "Dance Your Ph.D." competition where Ph.D. students are challenged to reinterpret their thesis as a dance. The results are approachable, fun, and informative videos - many with over a hundred thousand YouTube views. While one winner receives a cash prize, most participants cite inspiration and new perspectives as the biggest rewards.

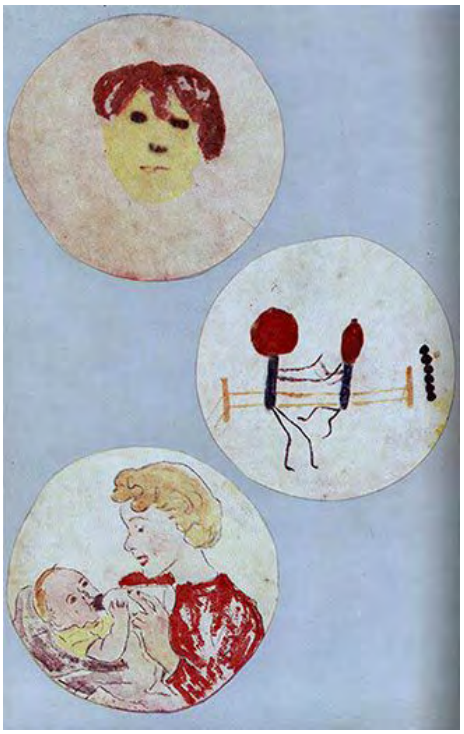


Figure 1b:
Sir Alexander Fleming's microbial art.[2]

In turn, science has contributed to countless artists and artistic works. Many artists draw inspiration from scientific studies that give a deeper and often unexpected view of the world. Individuals from both the arts and sciences have contributed to, and benefited from, the development of technologies like the camera, microscope, and computer. In addition, science has led to the creation of new media. For instance, some of the most vivid pigments used in medieval illustrations were the result of early alchemy experiments. Similarly, in the emerging field of Bio Art, artists used biotechnology to try to integrate actual life into their creations.

Bio Art

At the intersection of art, science, technology, and medicine is Bio Art. A crazy, broad, sometimes funny, and sometimes disturbing field that includes glow in the dark rabbits, ears grafted onto artists arms, computers that translate poetry into DNA sequences and then insert them into bacteria, and floating snowflakes made out of silicon and sperm cells. The key-qualifying agent is that the work contains some type of living matter such as genes, cells, tissues or animals.

Working with life introduces both challenges and controversies. For instance, working with living organisms requires special animal welfare considerations as well as the inevitable logistical hurdles of keeping the art alive and safe for viewing for as long as possible. Other works raise even more complex questions about genetic privacy, the use of cells and organisms for art or science, and even who gets to create and modify life. In many of these cases, the work is intentionally controversial in order to prompt discussion about the promises and risks of new scientific technologies. This can be particularly powerful when a piece does not promote a single side or viewpoint but rather sparks open-ended questioning.

Bio Art is extremely effective at drawing attention to the beautiful, strange, and even grotesque details of life that can often go unseen and can also promote meaningful discussions. It also engages individuals who might otherwise be unaware or uninterested in new developments and questions in this field. A solid understanding of the science behind the artwork facilitates all these objectives. It can also power the creation process itself and, more practically, help the artist to optimally use their tools of the trade.

The Science of Agar Art

In this lab, you will be working with colorful bacteria to create works of agar art (much like Alexander Fleming). Pigmented bacteria are known as chromo-bacteria. Most bacteria are naturally white, cream or yellow although some are purple, pink, orange, green, and brown. Pigments are generally contained in the periplasm or wall of the cell and serve a variety of purposes ranging from photosynthesis to protection from UV radiation or oxidative stress.

Bacteria can also be engineered to express a color using recombinant DNA. One application of such bacteria is as biosensors. In these cases, the modified bacteria are programmed to express color in the presence of a specific chemical. This allows dangerous pollutants to be detected and their sources traced. Recombinant chromo bacteria are great for microbial art because they are colorful, safe, and easy to grow and maintain. For information about how these strains are created, review the background in your transformation lab.

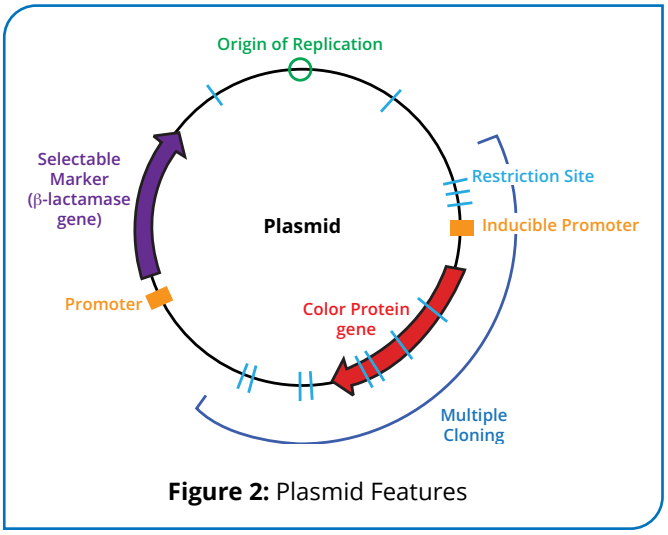


Figure 2: Plasmid Features

For your art, you will be using a benign strain of *E. coli* that has been utilized extensively in microbiology. This strain lacks a restriction system and consequently easily accepts and maintains foreign DNA. The foreign DNA, in this case, is a specialized plasmid (Figure 2) which contains the following features:

1. Origin of Replication: a DNA sequence that allows bacteria to copy the plasmid.
2. Multiple Cloning Site: a short DNA sequence that contains many unique restriction enzyme sites that allow scientists to introduce additional genes.
3. Promoter: a DNA sequence that recruits RNA polymerase to the beginning of the gene sequences, where it can begin transcription.
4. Selectable marker: a gene that codes for ß-lactamase, an enzyme that degrades the antibiotic ampicillin and allows the bacteria to grow even in the presence of this antibiotic.
5. Inducible promoter: a cellular "on/off" switch that controls the expression of the color pigment so that it is only produced when a small molecule known as isopropyl- ß-D-thiogalactopyranoside (IPTG) is present in the environment.
6. New gene sequence: the DNA sequence coding for the specific color protein.

One of the most popular colored proteins is the Green Fluorescent Protein, or GFP (Figure 3). This small protein (approximately 27 kilodaltons) possesses the ability to absorb blue light and emit green light in response. This activity, known as fluorescence, does not require any additional special substrates, gene products or cofactors to produce light. GFP was first isolated from the jellyfish *Aequorea victoria* in the 1970s. Once scientists identified its DNA sequence, they were able to

modify the behavior of GFP by rewriting small segments of DNA. The resulting structural changes cause the protein to absorb and emit different wavelengths of light. This has been used to create a variety of different fluorescent colors and even colors that can be seen under non-UV light (Figure 4).

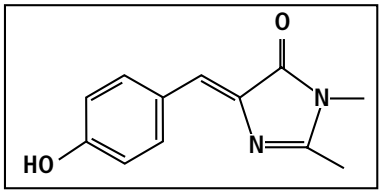


Figure 3: GFP Chromophore

As long as they are grown in the right environment, bacteria with the recombinant plasmid will express the specified color and will pass on this ability to subsequent offspring. Bacteria grow as colonies. Each colony represents an individual bacteria cell or group that has divided repeatedly to form a visible patch. These colonies can also merge to form a field or mat of bacteria. Consequently, these bacteria can be used to create a continuous line, larger circles, or really any other shape.

Combining LB Agar, Ampicillin, and IPTG creates an optimal environment. The LB agar contains the necessary sugars and nutrients for general bacteria growth as well as a firm and stable (non-degradable) surface. However, it does not preferentially grow one kind of bacteria over another. In order to avoid contamination from outside bacteria, ampicillin is added to the plates. This also serves to maintain selective pressure on the transformed plasmid. Also added is IPTG which signals the cell to begin producing the color pigment.

REFERENCES:
(1) Image, https://commons.wikimedia.org/wiki/File:Darwin%27s_finches_by_Gould.jpg, John Gould (14.Sep.1804 - 3.Feb.1881) [Public domain]
(2) Image, http://www.microbialart.com/galleries/fleming/, Sir Alexander Fleming, used with permission from the Alexander Fleming Laboratory Museum.

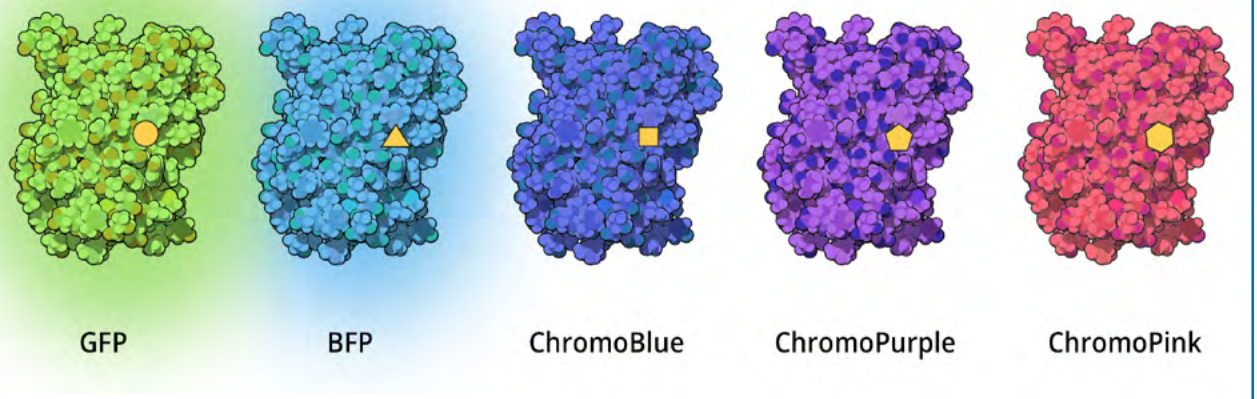


Figure 4: Structural changes can create different colored proteins.

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will harness the color producing power of chromogenic bacteria to create works of living microbial art. To do this you will first create a bacterial paint (Bio-Paint) by culturing transformed *E. coli*, which contain a GFP, or GFP derivative gene, in a solution of Luria broth, IPTG, and ampicillin. While these bio-paints incubate, you may choose to plan out your work of art. Following incubation, you will transfer these color producing microbes onto an agar plate canvas which also contains Luria broth, IPTG, and ampicillin. Following a second incubation, you will inspect, admire, and hopefully share your living art.

LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. They also use their notebooks to plan new experiments, to dream up new questions and designs, and, occasionally, to doodle. Today, you can use your notebook to sketch the art you will be creating.

LABORATORY SAFETY

IMPORTANT NOTE: Both bio-paints and plates contain ampicillin. Students who have allergies to antibiotics (penicillin, ampicillin, kanamycin, or tetracycline) should not participate in this experiment.

1. Wear gloves when working with bacteria and wash your hands following each module.
2. Always wash hands thoroughly with soap and water after working in the laboratory.
3. If you are unsure of something, ASK YOUR INSTRUCTOR!

Wear gloves and safety goggles

MODULE OVERVIEW

Module I
15 min. with overnight incubation OR 45 min. (includes a 30 min. incubation)

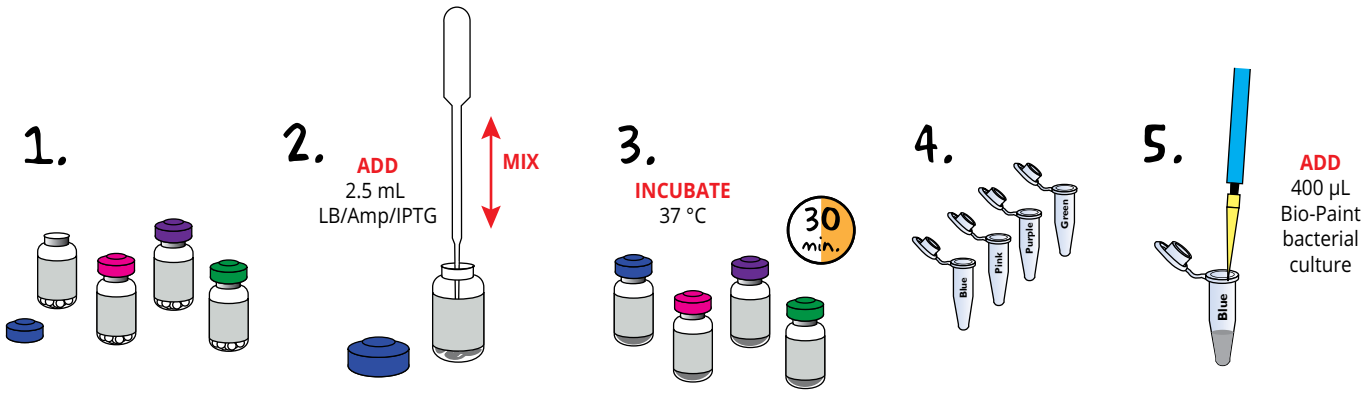
↓

Module II
15 - 30 min. with overnight incubation.

↓

Module III
At least 10 min.

Module I: Prepare Your Bio-Paint



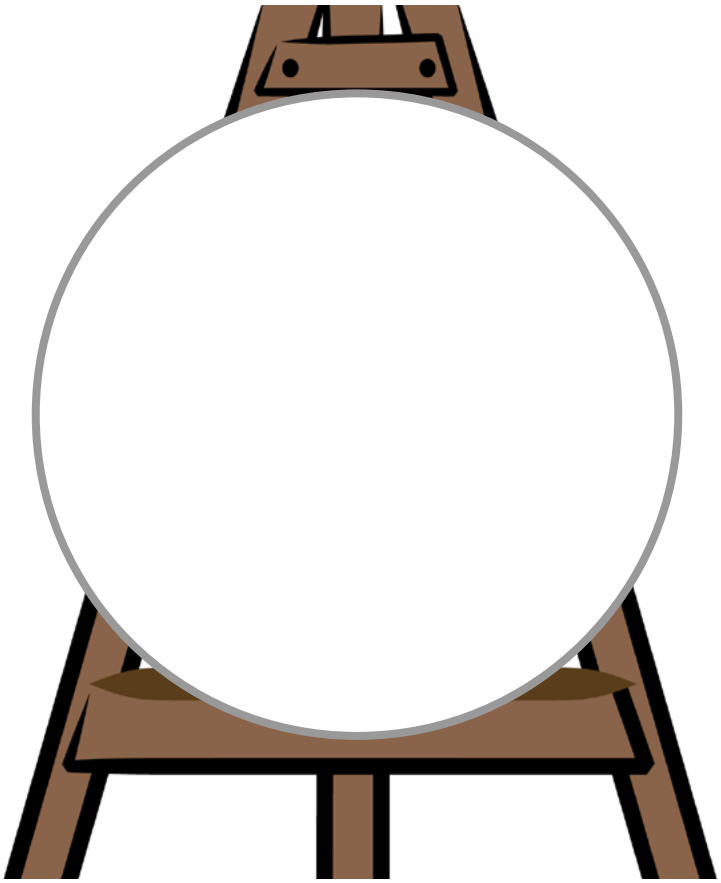
Each kit contains four separate types of BactoBeads™ that produce the colors Blue, Pink, Purple, and Fluorescent Green (GFP). Take care to keep the cultures separate so that the 'paint' colors are bright and strong.

1. Carefully **OPEN** a vial of BactoBeads™ and **SAVE** the stopper.
2. **ADD** 2.5 mL of LB/Amp/IPTG broth to the vial of BactoBeads™ and **MIX** by gently pipetting up and down. The broth will be cloudy, but there should be no large pieces of bead in the vial. **REPEAT** with the additional vials making sure to use a fresh pipet for each vial.
3. **CAP** and **INCUBATE** at room temperature or at 37 °C for 30 minutes.
4. **LABEL** five 1.5 mL microcentrifuge tubes with each color.
5. **ALIQUOT** 400 µL of the Bio-Paint bacterial culture to each tube. Tubes will need to be shared between 2-3 individuals.

OPTIONAL STOPPING POINT:
Following incubation, paint can be stored in the refrigerator for one week. Allow refrigerated Bio-Paints to return to room temperature before using them. You should invert the vial several times to ensure an even distribution of cells.

Module II: Create Your Agar Art

In this module, you will use agar plates as canvases, toothpicks, and loops as paintbrushes, and bacteria as paints to **CREATE** a work of Agar Art. Before you begin, you may want to **READ** the Tips and Tricks on page 10, **REVIEW** the art examples on page 11, or **SEARCH** online for "Agar Art". You may also want to use the agar plate template (below) to **CREATE** a sketch or more detailed drawing of your work. Once you have finished painting your Agar Art, **INCUBATE** your plate at 37 °C for 16-24 hours or at room temperature for 24-48 hours.



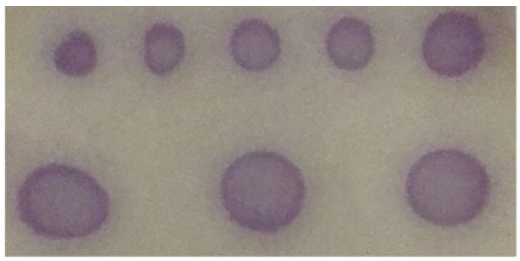
Module II: Create Your Agar Art, continued

TIPS AND TRICKS FOR PAINTING WITH BIO-PAINTS

- The color in Bio-Paint can take several hours to develop on the plate. However, paint strokes can still be observed as wet trails or drops on the agar.
- Planning ahead helps. Consider sketching your Bio Art beforehand using the agar plate template on page 9. You can also make a more detailed drawing, overlay your plate, and then trace your work directly onto the agar.
- Use the blunt end of your toothpick brush to avoid gouging the agar as the transformed bacteria will not grow as well on broken agar. If you are uncertain about painting on agar for the first time, use the loop which is a less precise but more forgiving tool.
- Traditional brush strokes work well with this strain of bacteria. However, you can also use pointillism - a technique of painting where small distinct dots are applied in a pattern to create an image.
- If pipets are available, use these to create circles of highly concentrated color bacteria. We had great results using volumes between 2- 20 µL (see examples on page 11).
- If using multiple colors of bacteria, color code your brushes using colored sharpies or lab tape. Do not cross contaminate paints.
- For best results, allow your canvas to dry for 10 minutes. Then flip the plate upside down before incubating (lid should be on the bottom and agar on the top).

EXAMPLE BRUSH STROKES

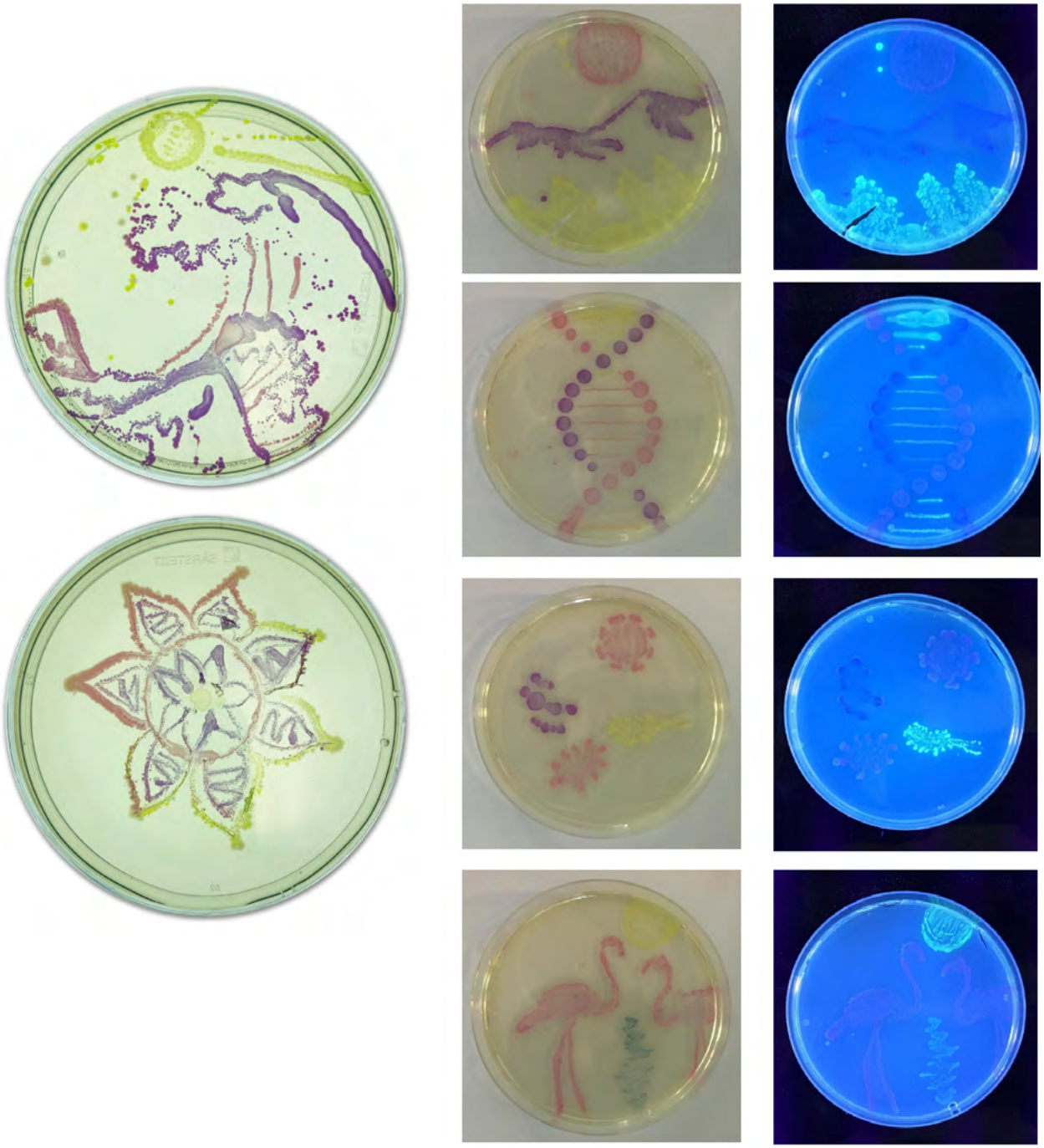
Students can use micropipettes to create dots of various sizes. The first row below shows dots created by pipetting 1 µL, 2 µL, 3 µL, 4 µL, and 5 µL volumes and the second row shows dots created by pipetting 10 µL, 15 µL, and 20 µL volumes.



Module II: Create Your Agar Art, continued

EXAMPLE ARTWORK

These plates were created using bio-paints included with this experiment. Please note, a UV light or transilluminator will be needed to view the GFP. For best results, we recommend the UV Transilluminator (Cat. #558) or the TruBlu™ 2 Transilluminator (Cat. #557).



Left: Artwork under normal lighting conditions. Right: Same artwork under UV light.

Module III: Exhibit Your Agar Art

Interested in showing off your bio-art skills? Consider submitting your entry to the **American Society for Microbiology's Agar Art contest**! Each year, hundreds of scientists and artists of all ages from around the world use chromogenic bacteria to create a wide variety of artistic works. Contestants are eligible to win prizes and a chance to have their art displayed for public audiences. Past winning contest submissions have been featured at the **ASM Microbe** annual meeting, at the **"Microbes Rule!"** exhibit at the Liberty Science Center in Jersey City, N.J., and at the **AMR Challenge celebration** in New York during the 2019 United Nations General Assembly.

Visit https://asm.org/agarart for more information.

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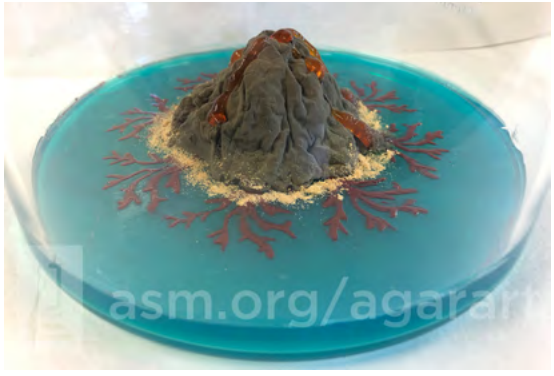


CREDITS:
"Hungarian Folk Art," Zita Pösténvi, Microbiologist, SYNLAB Hungary Ltd., Budapest, Hungary. (Top, left)

"The battle of winter and spring," Ana Tsitsishvili, Undergraduate Student, Agricultural University of Georgia, Tbilisi, Georgia. (Top, right)

"Fu(n)ji-san," Isabel Franco Castillo, Ph.D. Student, Instituto de Ciencia de Materiales de Aragón (ICMA-CSIC/UNIZAR & CIBER-BBN), Zaragoza, Spain. (Bottom)

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Draw and label the plasmid that enables the bacteria in this lab to become paint.

2. Many BioArtists praise both the limits and serendipitous outcomes of working with living organisms - although they also agree it can be frustrating and demanding too! What were some of the limits, challenges and unknowns that using Bio-Paints (as opposed to traditional paints) introduced? How did these affect your painting approach?

3. Painting with your transformed colonies may give you a different perspective of the bacteria you are working with. Brainstorm and list at least three bacterial traits that make them easy to transform. Next, brainstorm and list traits that make them easy to paint with.

4. Research and present on an individual who worked or is working at the intersection of art and science.

Instructor's Guide

NOTES TO THE INSTRUCTOR

Preparation For:	What to do:	When:	Time Required:
Module I: Growing Bio-Paint	Locate transformation plates	Anytime before Module I	10 min.
	Prepare and aliquot liquid LB/Amp/IPTG solution	Day of Module I	10 min.
	Equilibrate incubator or waterbath at 37 °C	Day of Module I	5 min.
Module II: Create Your Agar Art	Prepare agar plates	Before Module II	20 min.
	Prepare control Bio-Paint (Optional)	Day before or day of Module II	10 min.
	Equilibrate incubator at 37 °C	Day of Module II	5 min.
Module III: Exhibit Your Agar Art	Set up UV light box or white light box	Day of Module III	5 min.
Results and Clean Up	Soak and discard contaminated materials	After Module III	15 min.

PreLab Preparations - Module I

IMPORTANT READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicillin, ampicillin, kanamycin or tetracycline should not participate in this experiment.

PREPARE LB/AMP/IPTG BROTH

1. **ADD** 200 µL of distilled water to the ampicillin tube and **MIX** by pipetting up and down. Once hydrated, the ampicillin should be **STORED** at 4 °C.

2. **PIPET** 15 µL of the hydrated ampicillin and 15 µL of IPTG into the Luria Broth liquid media. **MIX** by pipetting up and down or by shaking the tube several times. ***NOTE: Store the remaining hydrated ampicillin and IPTG in the refrigerator to be used in the agar plates (Module II).***

FOR MODULE I:

- *4 BactoBead™ vials*
- *Transfer pipets*
- *4 microcentrifuge tubes*
- *10 mL LB/Amp/IPTG broth*

EQUILIBRATE WATER BATH OR INCUBATOR

- **EQUILIBRATE** water bath or incubator at 37 °C. ***NOTE: If you do not have an incubator, locate a warm spot (sunny window, heating vent etc.) for the students to keep their prepared paints. Bio-Paints prepared without an incubator or water bath will take longer to incubate (between 1-3 days). Mix these tubes daily.***

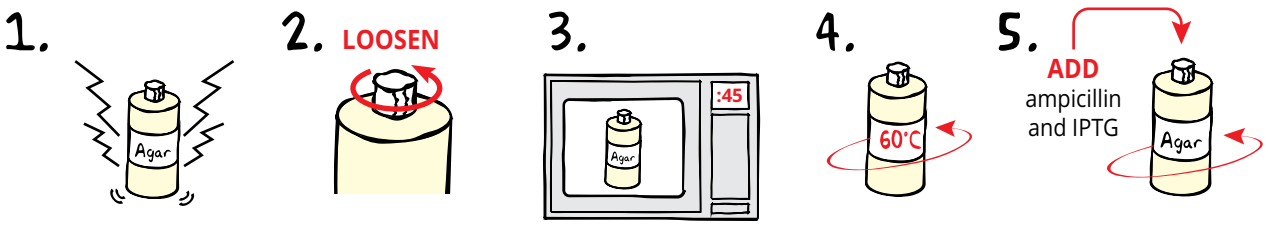
CLEAN UP INSTRUCTIONS

BactoBeads™ used in this experiment is not considered pathogenic. Regardless, it is good practice to follow simple safety guidelines in the handling and disposal of material contaminated with bacteria.

1. Following each module wipe down the lab benches with a 10% bleach solution or laboratory disinfectant.
2. Always wash hands thoroughly with soap and water after working in the laboratory.
3. Materials that come in contact with the bacteria (petri plates, pipet tips, loops, tubes etc.) should be disinfected before disposal in the garbage by either (a) soaking in a 10% bleach solution overnight or (b) autoclaving at 121 °C for 20 minutes.

PreLab Preparations - Module II

PREPARE THE AGAR MEDIUM



1. **BREAK** solid ReadyPour™ LB Agar into small chunks by vigorously squeezing and shaking the plastic bottle.

2. **LOOSEN**, but DO NOT REMOVE, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. ***CAUTION: Failure to loosen the cap prior to heating may cause the bottle to break or explode.***

3. **MICROWAVE** the ReadyPour™ Agar on high for 45 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and **MIX** by swirling the bottle. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).

4. **COOL** the ReadyPour™ Agar to 60 °C with careful swirling to promote even dissipation of heat.

NOTE for Step 3:
Use extra care and make sure the agar does not boil out of the bottle. Pay close attention and stop the heating if it starts to bubble up.

5. **ADD** 180 µL of ampicillin and 150 µL of IPTG to the cooled ReadyPour™ Agar. **RECAP** the bottle and **SWIRL** to mix the reagents. ONLY ADD REAGENTS TO COOLED AGAR as they can degrade at high temperatures. ***NOTE: To ensure that all the IPTG and ampicillin is used, add a small amount of sterile water of molten agar to each tube. Shake and then empty the tube.***

POUR THE AGAR PLATES

6. Using a fresh 10 mL pipet, **PIPET** 10 mL of the LB/AMP/IPTG medium into 25 large petri plates. If you do not have a pipet pump, this can be accomplished by pouring to a .5 cm height. Make sure agar completely and smoothly covers each plate.

7. **COVER** and **WAIT** at least twenty minutes for the plates to solidify. For best results, leave plates at room temperature overnight.

8. **STORE** plates at room temperature for no more than two days. If plates are prepared more than two days before use, they should be inverted in a plastic bag and placed in the refrigerator. Refrigerated plates must be brought to room temperature before painting.

FOR MODULE II
Each Student Should Receive:

- *Agar plate*
- *Non-sterile loop*
- *Toothpicks*
- *Bio-paint tubes (can be shared by 2-3 students, depending on class size)*

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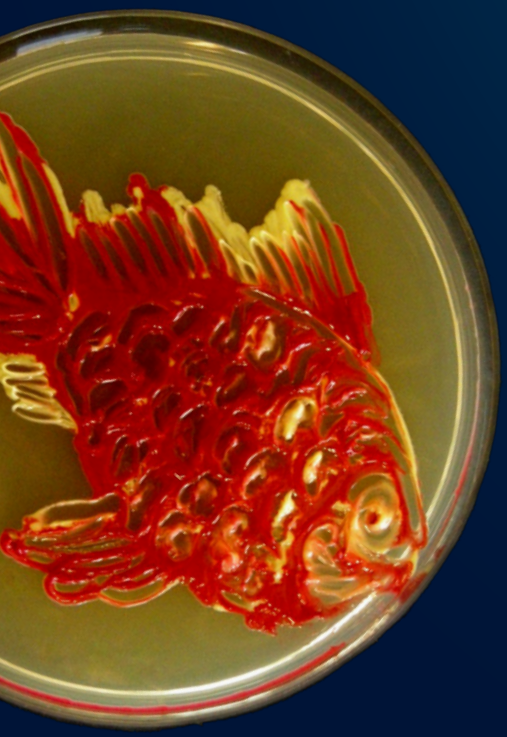
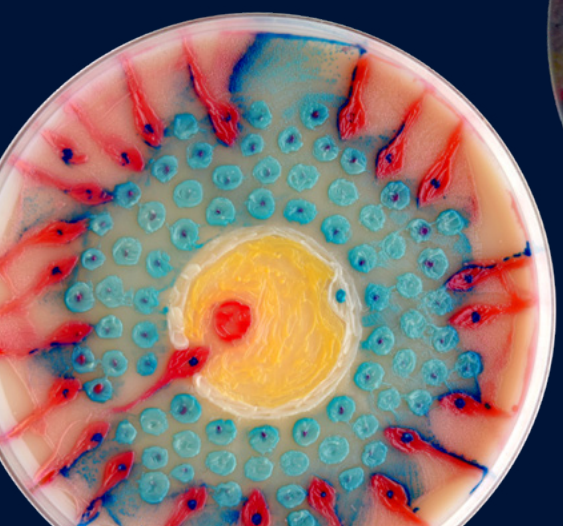
agar art®

Show off your work by entering the 2023 Agar Art contest, sponsored by the American Society for Microbiology (ASM)! Participants have the chance to win up to $200 in prizes and have their work displayed publicly by the ASM. **Contest deadline is October 27, 2023.**

More information can be found at **asm.org/agarart.**





Please refer to the kit insert for the Answers to Study Questions