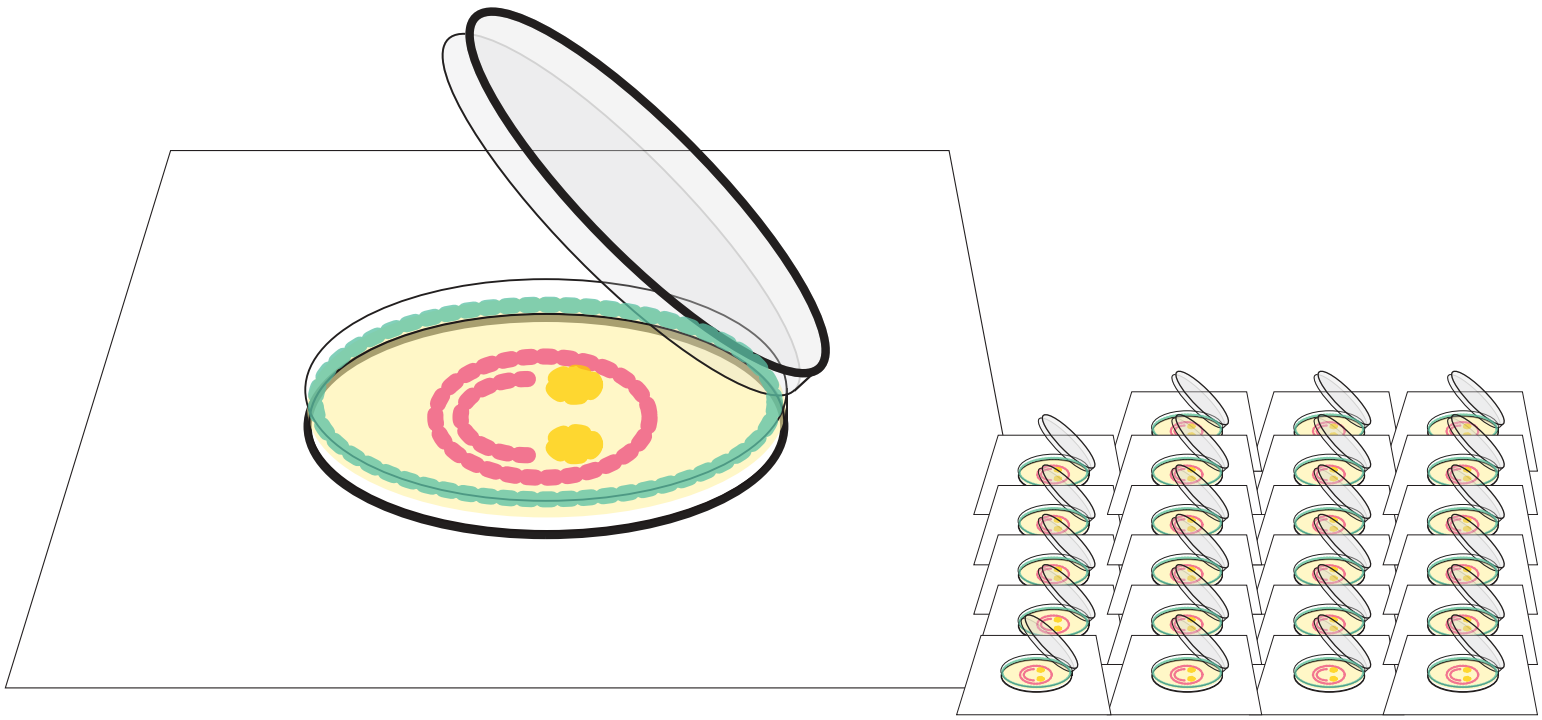
CANVAS KIT™

CLASSROOM & GROUP MANUAL

For use with the **group** kit size

a (2x) 45-minute class + (1x) 15-minute class periods experiment procedure



www.amino.bio

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CANVAS KIT™

CLASSROOM & GROUP MANUAL

Getting Ready - for educators

The Experiment - for educators & students

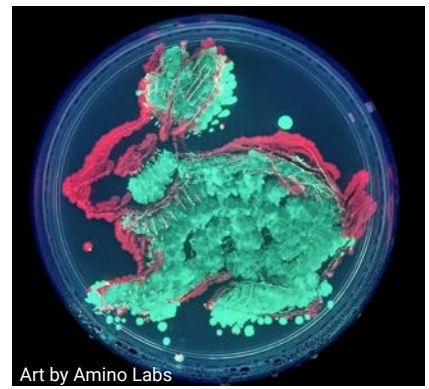
For educators

The student experiment protocol:

For educators and students

More information - for educators & students

Welcome! Let's get started



This user guide was created to help you get the most out of your Amino Labs experience. Even if you are familiar with genetic engineering, science or other Amino Labs™ products, please take the necessary time to read through this guide. This will ensure you practice safe science as well as store, use, and get the most out of your kit. It will also let you know what to do in case of a spill or accident.

In the first section, you will learn about your kit's components, how to store them before and during your experiment, as well as a few tips on activities to complete before you get your hands wet. The second section is procedural -- these are the step by step instructions on how to run your experiment. Make sure to follow our tips to ensure your best success! The third section covers "what's next"; how to keep your creations, store or dispose of any leftover ingredients and general clean up instructions. The final section is there to help you -- a glossary, troubleshooting, and our contact information.

Amino Labs is excited to welcome you to the world of the genetic engineering with the, Canvas Kit™, Engineer-it Kit™ and our entire ecosystem of easy-to-use, easy-to-succeed at products!

Following this guide will help ensure that you are getting the most out of your current and future experiences to keep on making new creations with DNA. Have fun!

Practicing safe science

Genetic engineering and life sciences are safe activities when you follow simple guidelines. Read on to ensure you adopt safe practices.

The kit in your hands contains only non-pathogenic ingredients. These are part of the biosafety Risk Group 1 (RG1) (Biosafety Level 1). This is the most benign level and therefore the safest: with these kits, no special containment or training is required in North America. But you must follow these safety guidelines for your safety and the success of your experiment(s)!

We recommend the system and kits for ages 12+, under adult supervision, and 14+ with or without supervision. We recommend that an adult empties the discard container. The cleaning instructions must be strictly followed for safety and experiment success. Make sure to store the kit per the instructions found in this booklet.

- Do not eat or drink near your experiments. Keep your experiment at least 10 feet from food, drinks, *etc*. Under no circumstances should you eat any of the kit's content.
- Immunocompromised persons: While the ingredients in these kits are non-pathogenic, some persons, such as immunocompromised persons, can be affected by large numbers of bacteria and should talk to their doctor before doing any experiment.
- Wash your hands before and after manipulating your experiment, or the hardware.
- Wear gloves, even when cleaning your station or handling the kit contents (petri plates, loops, etc). This will protect you from your experiment, and your experiment from you. Any latex, nitrile, or general purpose gloves you can find at the pharmacy will do. After you put your gloves on, be aware of what you touch. Try not to touch your face or scratch itches with your gloved hands!
- If using the DNA Playground™ or BioExplorer™ place it on a stable work surface. Keep it level at all times.
- Clean up your station, spills and work surface before and after use. Use a 10% solution of chlorinated bleach generously sprayed onto a paper towel and rub onto any contaminated surfaces. (Careful! This can discolor your clothes). A chlorinated spray cleaner also works.
- Find a container to hold the inactivation bag where you will discard used items. An old 1L yogurt container, large plastic cup or the like will do. Used items (in science, these are often called consumables) will be loops, tubes or used petri dish.
- Eye-wear is not provided but can be worn.

You can download a biosafety poster for your space from www.amino.bio/biosafetyinaction and complete a short safety quiz at www.amino.bio/biosafety-quiz

If you would like to do a short Online lab safety course for your edification, we recommend a Government of Canada course: www.amino.bio/biosafety

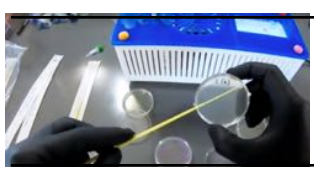
How will students learn?

Learning and prototyping with genetic engineering and cells is becoming accessible to newcomers ages 12+ thanks to dedicated scientists and kits such as the one you are about to use!

One of the easiest ways to learn a new science, hobby or topic is by trying it hands-on. Amino Labs kits make it easy to do science by following the instructions in this booklet. Everything you need is included; each ingredient in the kit is pre-measured and labeled for a beginner-friendly experience. Our all-in-one DNA Playground minilab (mini-laboratory) decreases setup time, mess, guesswork and the need to collect and calibrate multiple machines. The included instructions should be easy-to-follow for everyone but may contain some new terms for which we have added a glossary at the end. Don't hesistate to have students flip to it during or before your experiment.

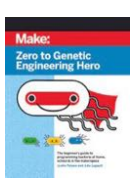
We also have additional resources to help students and teachers go further:

An essential addition to our ecosystem are the free **Virtual Bioengineer™ simulations** developed with the educators at the Biobuilder Educational Foundation. These simulations are 20 minutes guided experiences that make it easy to practice using a DNA Playground™ and experiment kits beforehand. The simulations includes additional information on the manipulations and a more in-depth look into the kit components. We recommend it strongly! Complete online at [www.amino.bio/vbioengineer.](www.amino.bio/vbioengineer)



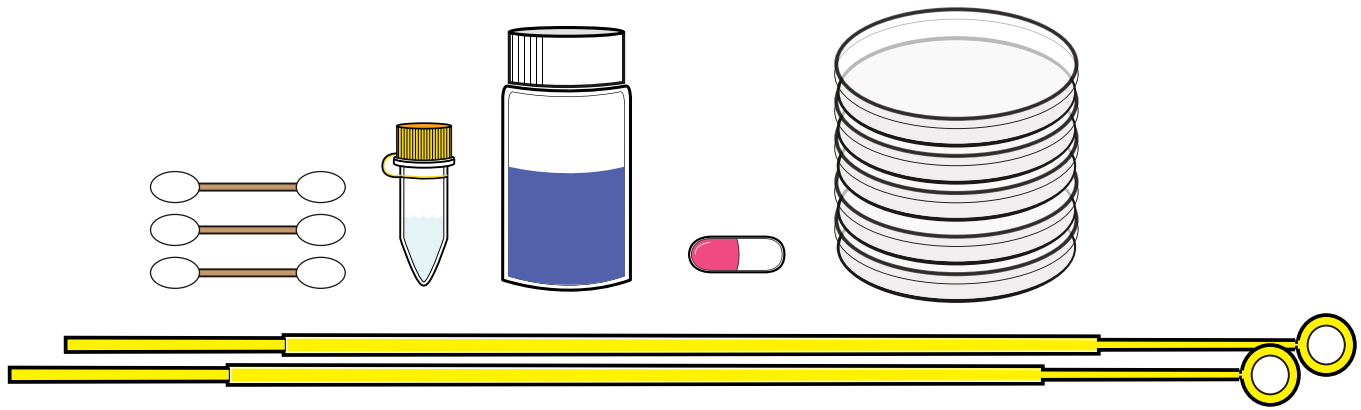
View **video tutorials** at youtube.com/Aminolabs

Would you like for an Amino Labs team member to guide you through your journey? Try the Virtual **Teacher Professional Development or Student Tutoring**, a multi-day+ experience completed via video conferencing. https://amino.bio/collections/virtual-sessions



Are you interested in teaching the theory behind the experiment? In going deeper on the science, learning pro-tips and eventually moving onto advanced genetic engineering? The ***Zero to Genetic Engineering Hero*** **book** is for you. Find out more at www.amino.bio/book

Discover your Canvas Kit™



The Canvas Kit™ lets you use your colored engineered bacteria to create living paintings! By following the experiment instructions on the next pages, you will create selective agar petri dish “canvases”, use the bacteria “paintbrushes” to create your living art on the agar surface and incubate over 24 to 48 hours to let your creativity grow!

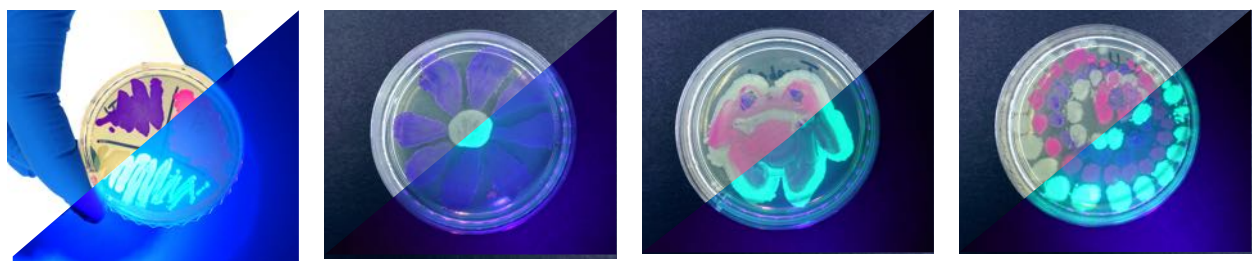
While the Canvas Kit comes in Individual Size or Group Size, these contain the same ingredients, in different quantities. This classroom-specific manual is aimed at teachers using the group size of the Canvas kit. The group kit is perfect for a class of 24 divided into groups of 3. Diagrams on page 12 show how the kit breaks down into student packs and how to divide your students into groups.

If you are teaching or doing the exercise as a small group or alone, we have a manual available for you. Visit www.amino.bio/instructions to download the INDIVIDUAL version of the manual for the Canvas kit.

Group size - Who gets what?

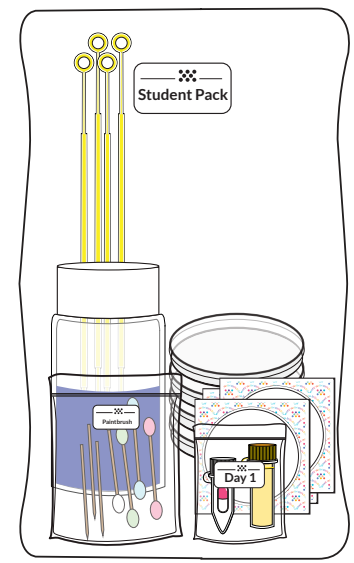
The group-size kit contains 8 student packs and 1 bag of Shared Materials:

- Each “student pack” allows for three pieces of art, and one painting palette of bacteria paint. Divide your class into 8 teams and give one student pack per team. If you have more than 3 students per team, see the note below.
- The Shared materials bag contains 2 tubes of each bacterial paint color (pink, purple, fluorescent cyan), 2 inactivation bags to safely dispose of the science waste, and 2 pre-drawn stencils in case some students need a bit of inspiration.



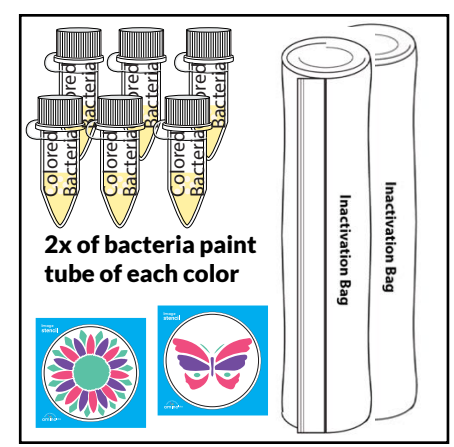
Each “Student Pack” allows you to create a painting palette and 3 pieces of art and the group Canvas Kit contains 8 of these student packs. The Canvas kit comes with 3 colors of bacterial paint: pink, purple and fluorescent cyan. Above, on the left, is the paint palette, and on the right are 3 pieces of art. Each is photographed under natural light (top segment) and blacklight (bottom segment).

Student pack



x 8

Shared materials



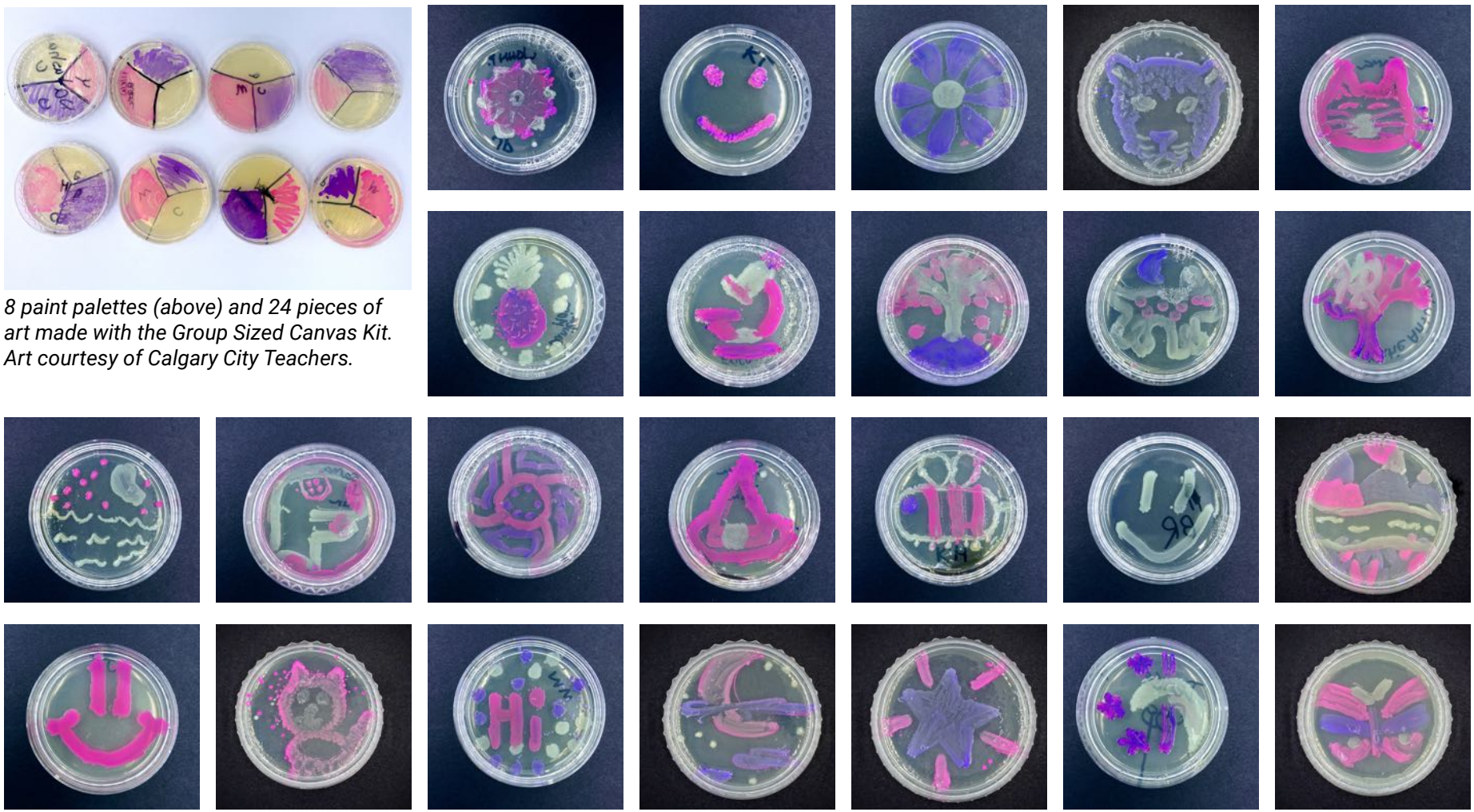
x 1

How many living paintings will be created?

Using the instructions as is, your class can make 24 art pieces on their petir dishes. BUT you can modify the instructions to suit the needs of your class quite easily. In total, this kit allows you to create 32 agar Petri dishes. By default, the instructions show how to turn 8 of these into painting palettes, which are used to grow the bacteria paint from the tubes included in the kit. The idea behind this is to have each student group have their own painting palette from which to get bacteria paint from when making their art, and for the group to learn + practice steaking bacteria with an inoculation loop. Once 8 of the petri dishes are used as painting palette, you are left with 24 petri dishes for art. However, not every student group needs to create their own painting palette since there will be sufficient bacteria paint to share

the painting palettes between student tems. So if you have 25-30 students in your class, you can simply keep more Petri dishes to create the art, by having teams share the painting palettes between themselves. For a whole class, 2 painting palettes can be enough since the paintbrushes only need to be dipped once in the bacterial paint per art piece. To use this method, instruct only certain teams to make the paint palettes. For example, if you have 28 students, you can have 4 teams of students create the painting palettes, keeping the remaining 28 petri dishes for art creation.

Another option is to have your students create their art as a team instead of having one piece of art per student. It is also possible to purchase the Canvas “Refill” kit which contains the same materials as the standard kit (petri dish making supplies, stencils, sterile paintbrushes) but no bacteria. With the refill, you get more Petri dishes that can be used to create art alongside the regular Canvas Kit.



8 paint palettes (above) and 24 pieces of art made with the Group Sized Canvas Kit. Art courtesy of Calgary City Teachers.

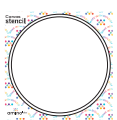
Kit components

In each student pack:

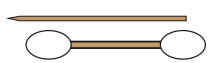
Sterile Water: Sterility is critical when genetic engineering. This sterile water bottle contains distilled water sterilized in an autoclave to ensure there are no contaminating organisms present. This 50 mL volume, when used with LB agar powder is enough to make 4 LB agar plates.[1]

Inoculating Loops: Inoculating loops can be used to streak (spread) cells onto the surface of the agar. They can also be used for transferring liquids or cells from tube to tube or tube to petri dish. In this way, they replace costly traditional pipettes. Different loop colors will hold different quantities of liquid.

Petri Dish / Plate: A Petri dish is a transparent lidded dish that scientist and students use to grow cells in. Petri dish will be filled with a solid media that the cells can eat and grow on. The container is named after its inventor, German bacteriologist Julius Richard Petri. 6cm Petri dishes are large enough for this lab experiment and help save on the cost of reagents and reduce waste.



Blank stencil: These stencils can be used to draw your bioart image before tracing it on the agar. Place the stencil under the petri dish to trace.



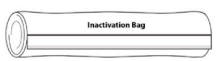
Paintbrushes: Sterile swabs and picks to help you paint your bacteria on the agar.

Day 1 bag

Agar Powder: This LB agar powder is industry standard. Each tube of LB agar powder can make 50 mL of molten LB agar (3.5% weight/volume). Agar is both the surface the bacteria grow on and the food they eat to grow.[1]

Antibiotics/Selection Marker: Amino Labs' proprietary antibiotic delivery system helps stabilize antibiotics for shipping and long-term storage. These capsules have a measured amount of antibiotics for 50 mL of molten LB agar. The amount of antibiotics included in the capsule is 1000 less than a standard dose for a toddler.[1]

In the shared resources bag:



Inactivation Bag: A heavy duty bag to put opened tubes, used loops and petri dishes in. After the experiment is completed, simply add bleach and water to the bag to inactivate all the material and practice safe science as per *Storage, disposal & clean up* Instructions.

Image stencil: These stencils can be used as your bioart image. Place the stencil under the petri dish to trace the image.

Bacteria paint bag

Colored bacteria paint: These bacteria are engineered to be colorful and are non-pathogenic.

[1] For education purposes only.

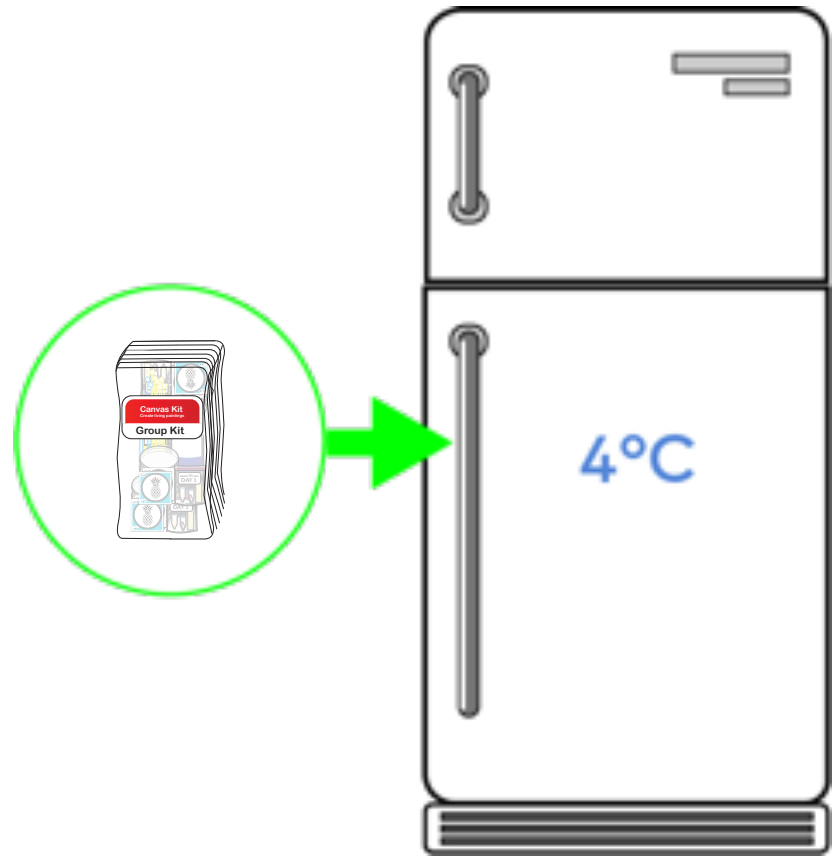
Unpacking and storing kits

For a better shelf life and successful experiments, place your Canvas Kit™ in a standard refrigerator at around 4°C.

Once refrigerated upon arrival, your kit will be best by the date found on the 'Best by' sticker on the outer packaging of the kit. The Date on the sticker is in Month/Year format. After the month on your sticker is over, the bacteria may not grow as well, as fast.

If your refrigerator is not a science-only refrigerator, we recommend placing your science experiments inside a sealed plastic container before placing them in the refrigerator, especially once your kit is open.

Do Not Freeze your kit!



Technical specs

Growth plates: 6 cm petri dishes
Selection/Antibiotic: variable

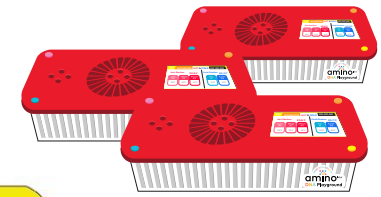
Bacteria paint: engineered bacteria stab

Solid growth media:
LB agar powder (1.6 g)
50 mL sterile water

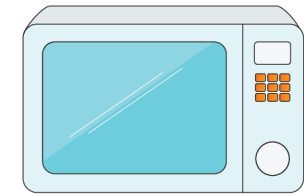
Necessary equipment

For Best results:

- 3x DNA Playground™ or Classroom Incubator (at 37°C)
- Microwave



or



Alternative solution:

- **Microwave**
- **DIY incubator or room temperature**: This will replace the **Incubator** set to **"37"**. If you do not have an incubator, you can create one using our online tutorial https://www.youtube.com/watch?v=LEsv0Qvbczs. If you have neither incubator or DIY version, you can incubate the petri dishes in a resealable bag in a warm environment. Note that it will take a few more days to see results at room temperature and the bacteria colors will not be as bright.

Necessary safety supplies

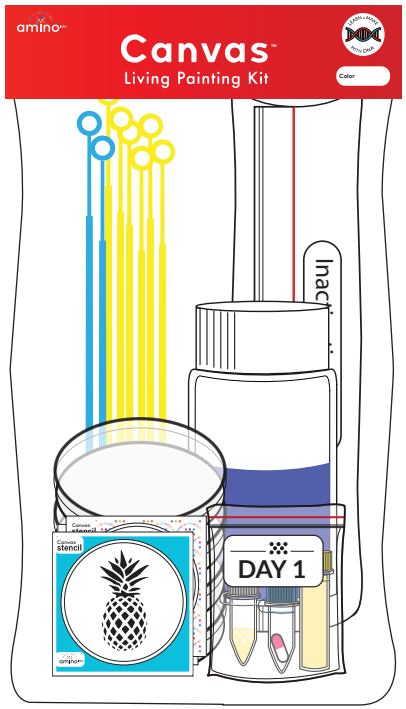
Disposable container 500ml-1L
to hold tubes, loops and other contaminated waste (e.g., yogurt container, plastic cup). 1 per station

Latex or nitrile gloves
like the ones found at a pharmacy. 1 pairs/student if students keep & reused each day, or 3 pairs/student if not saved & reused.

Chlorinated bleach spray
1 to share in the classroom (or you can mix a 10% solution: 1 part bleach to 9 parts water in a spray bottle)

Bleach ~500 mL
to inactivate all the experiment materials at the end of the experiment.

3-class period timeline



Canvas Kit

Day 1

Create painting palette(s)
Each student group or individual makes LB agar plates with antibiotics (selective plates) and streaks each of the colored bacteria paints from the tubes included in the kit onto one of these selective plates. This amplifies the colorful bacteria so there is enough to paint with. Incubate it so that it becomes the painting palette.
(60 minutes)

Day 2

Paint your bioart
Each student or individual can use a blank stencil to draw their picture that will become the bioart. Set the stencil under the petri dish in order to trace it using the bacteria paintbrushes and the painting palette. Incubate the petri dish(es).
(30+ minutes)

Day 3

See your bioart
View the living art grow and change color over the next 24-72 hours. Use natural and UV light to see the different colors and photographs!
(10+ minutes)

The Canvas Kit™ takes 2 days of hands-on activity to complete, and 24 to 72 hours to see results.
5 activities make up the Canvas Kit experiment:

1. **Make selective LB agar plates**
   Day 1, 20-35 minutes

2. **Streak colored cells to make enough paint**
   Day 1, 20 minutes, incubate 24 to 48 hrs*

   *If you need to incubate your bacteria paint for longer than 48 hrs (ex: over the weekend) you can incubate it at 30℃ instead of 37℃. This is only okay in the Canvas kit!

3. **Stencil art on paper**
   Day 2, variable time

4. **Paint with Bacteria**
   Day 2, 20+ minutes , 24-72 hrs incubation

5. **View results**
   Day 3, 20 minutes

Recommended pre-labs

Amino Labs has some resources that should be used by your students before they complete the hands-on experiment to maximize their understanding and success. These pre-labs are meant to ensure your students know, understand, and complete all the experiment steps. Completing the pre-labs also minimizes the number of questions your students will have during the hands-on experiment.

1. Virtual Bioengineer Simulator - Canvas Kit Edition

www.amino.bio/pages/vbioengineer_canvas

This free simulator walks your students through the entire Engineer-it Kit's materials and procedure. The students can complete the simulator as homework or in class with the use of the school's computer lab or the student's laptop computers. The simulator takes approximately 25 minutes to complete. It is also common to project the simulator and complete it as a group during class or as a review if the students have completed it as a homework assignment.

2. A short experiment-readiness quiz

www.amino.bio/canvas-pre-test

A short activity to test whether your students read the experiment procedures and are ready to start the experiment. The students can complete the test online as a google form on a computer or mobile device, or you can print it out.

2 key pitfalls to avoid!

In the next pages are detailed, step-by-step instructions to complete the experiment. These include instructions to prepare the classroom and the students' instructions. **Please make sure the students read all the steps before starting the hands-on manipulation;** some steps will be done in rapid sequence. The best way to ensure students success is by having students complete the recommended pre-labs on the previous page.

While all the steps outlined in the experiment protocol are important and should be followed as described, the MOST IMPORTANT considerations for success are:

1. In Step 1: When making the LB agar, make sure that the water is boiling before adding the agar powder. **Students have to see the water bubbling!** Caution, the bottles will be hot!

2. In Step 3: **Before painting the art or flipping the painted petri dish upside down for incubation, make sure the agar is dry and there is no condensation.** Otherwise, the bacteria paint may touch the condensation/water on the surface of the agar and spread around the agar. This means the painting will turn out very blury!

Teacher Experiment Setup

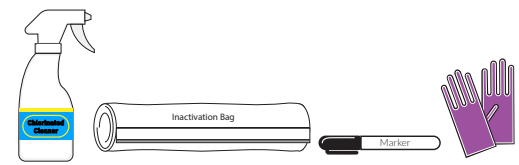
0. Prepare your classroom space

Goal Set yourself up for sucess.

Materials from your kit
(1 per group) Student packs
Shared materials bag

Materials not in your kit
(1 per table) Discard container
Chlorinated bleach spray or wipes
(1 per table) Permanent marker

(1) Paper towels
(1 pair per student) Gloves



Make sure the class has access to a microwave before starting!

0.0 Have students download/print the manual and read *Practicing safe science*, the *2 pitfalls to avoid*, the *Student experiment protocol* (including the *Checkpoints)*, and the *Glossary* pages.

0.1 Set down the DNA Playground(s), or other lab equipment (37°C incubator) on or near the students work stations. Make sure the equipment is level and on a stable surface. Refer to the instruction manual to make sure you know how to use your equipment safely. For this kit, you can also incubate at room temperature - no equipment needed. Note that room temperature incubation will add more incubation time to your experiment.

0.2 Set one discard container per work station (as per the *Necessary safety supplies* page).

0.3 Set one Student Pack and one permanent marker (sharpie) per student-group area. Keep the shared materials in a common area so all students can access them on step 2.

0.4 Ask the student to use the discard container to dispose of:

- any used inactivation loops, paintbrushes
- empty tubes (agar, antibiotics tubes, etc...)
- any gloves that have touched bacteria.

Paper, plastic packaging and gloves that have not come into contact with bacteria should be disposed in the regular garbage or recycling bin. After each day's experiment or at the end of the entire experiment, have students pour the content of their discard container into an inactivation bag. Follow the instructions at the end of the experiment to inactivate the contents.

0.5 Ask the students to put on their gloves.

0.6 Have the students wipe down their work surface with chlorinated bleach spray, wipes or 10% bleach solution. Do not have them spray bleach solution directly on the DNA Playground.

0.7 After the students complete the experiment, follow the *Storage, discard & clean up* procedures with them.

If you are saving the tubes of cells for a future experiment, place back in their ziplock bag after use and refrigerate. We recommend you use a sealed plastic container to store all your experiment materials inside a refrigerator if you also use this refrigerator to store food or drinks. If you are not saving them, place the open tubes in a discard container and dispose of them after all the student-groups have used them.

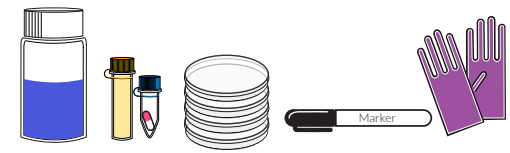
Student Experiment Protocol

1. Creating selective LB Agar Plates Day 1, 25 minutes

Goal Create selective LB agar plates.

Materials from your kit
(1) 50 mL sterile water
(1) LB agar powder
(1) antibiotic pill
(4) 6 cm petri dishes

Materials not in your kit
(1) Sharpie marker

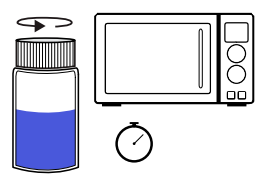


Prepare

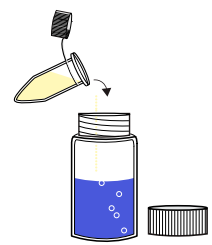
1.1 Label each petri dish with a sharpie-type pen. Make sure to label the bottom of the petri dishes (*the bottom is the half with the smaller diameter of the two. The bottom fits inside the lid and it may have star-shaped ring around it)*. Label **4x** S. (for selective) + Add [your initials] if doing this in groups.

Mix the Agar

1.2 Unscrew the lid from the sterile water bottle and keep it loosely on top of the bottle to prevent any contaminants from entering the water, but allowing air to escape. This will prevent pressure build-ups.

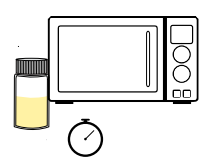


1.3 Place the bottle in the microwave and heat the water **until you see it boil**. You can use 45 seconds as your starting time but you have to see a rolling boil where many bubbles are rising constantly before you continue to the next step. Careful, the bottle will be hot! **!! If the water does not boil, the agar powder will not dissolve and your plates will not solidfy !!**



1.4 Add the tube of agar powder to the boiling water. Careful, the water is hot! Some agar powder may "clump" around the lip of the tube due to the water evaporation. This is okay, we have accounted for this possible loss.

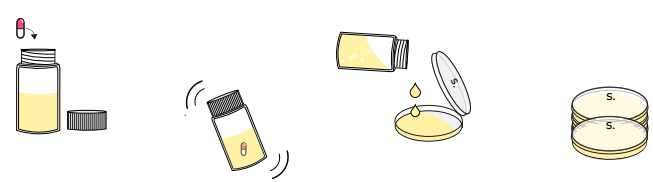
1.5 Microwave the water and agar powder in 4 seconds intervals until you see it boil again. Instead of a rolling boil, you will see more of a foam forming above the molten LB agar liquid. ***Careful, the liquid will boil over if you microwave in more than 4 sec. increments.*** After you see the liquid foaming, swirl to mix for 10 seconds. Try not to shake vigorously as this will create bubbles in your agar and make the surface of your agar uneven.



Note: If you've done the Engineer-it Kit before, note that you will not be making a non-selective plate. All 4 plates will be selective agar.

Make selective (S.) plates

1.6 Add the antibiotic pill to the bottle of agar and gently swirl for a few minutes until the contents of the pill have dissolved. Do not introduce bubbles into the LB agar: don't swirl too vigorously. The gelatin capsule may not fully dissolve. The important thing is that the contents of the capsule do dissolve.



1.7 Once the antibiotic pill is dissolved, pour the molten LB agar into the bottom half of the 4 petri dishes. Place

the lids 3/4 of the way back on so that the agar can cool and dry (solidify).

Pro-tip: If there are water droplets on the surface of the LB agar, this can disrupt your art. Bacteria that you will be painting with can enter a droplet and spread throughout the droplet therefore ‘smudging’ your art. To avoid this make sure the lid is partially over top to allow for evaporation and a dry LB agar surface.

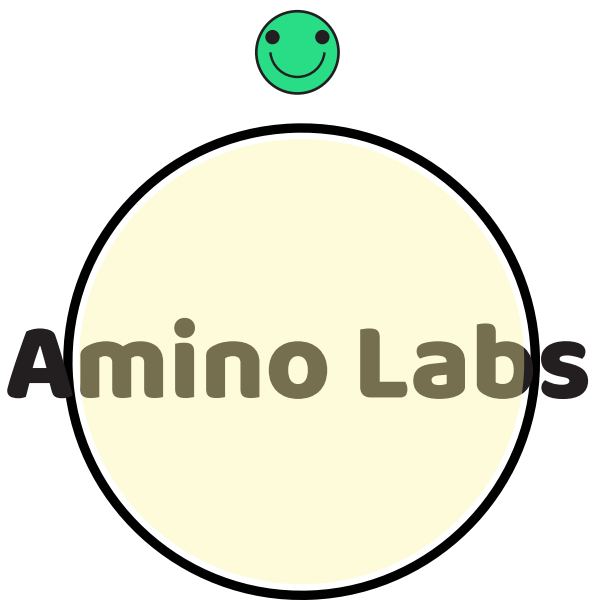
1.8 Let the LB agar harden. This can take up to 20 minutes depending on how warm and humid your environment is. You will use 1 plate in the next step. You can store the remaining 3 plates in the ziploc bag in the refrigerator for day 2.

Troubleshooting tip

If your plates do not solidify after 30 minutes it is very likely that the water was not boiled enough to dissolve the agar powder. As a ‘hack’, you can pour all of the petri dish content back into the water bottle and microwave until you see it boil. Swirl to mix and re-pour your plates.

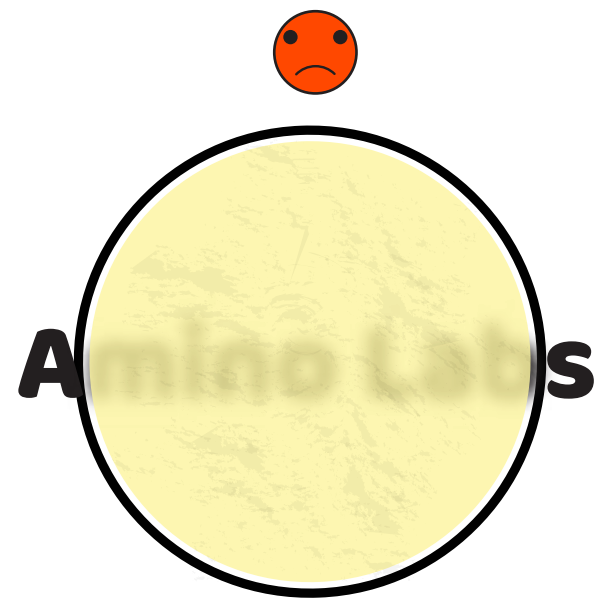
Checkpoint - Agar Plates

Use this guide to check if you are ready to move onto the next step.



A perfect Agar plate is completely clear and solid - if you set it 4” above some image or text, you should be able to read it / see it clearly.

Move on to the next step!



An agar plate that is cloudy and/or bumpy and/or soft is not ideal - if you set your plate 4” above some text or image and cannot see clearly through it, it means you needed more boiling or mixing.

Troubleshooting tip

If your plates do not solidify after 30 minutes it is very likely that the water was not boiled enough to dissolve the agar powder. As a ‘hack’, you can pour all of the petri dish content back into the water bottle and microwave until you see it boil. Swirl to mix and re-pour your plates.

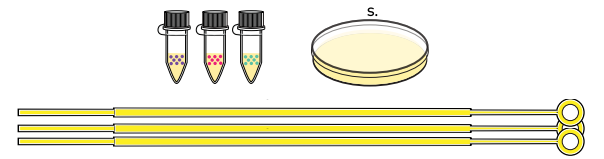
Unfortunately, if the agar does not solidify, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

2. Create your painting palette Day 1, 25-45 minutes + 24 hours wait time

Goal **Create bacteria paint painting palettes.**

Materials from your kit
(1) Selective agar plates
(3) Yellow loops

Shared resources
Tubes of bacteria paint

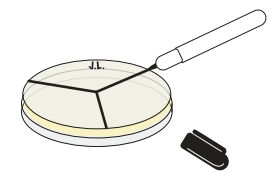


Prepare

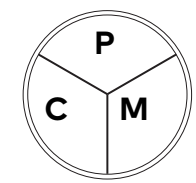
2.1 If you have an incubator, turn it on to 37°C.

Streak

2.2 Using a permanent marker like a sharpie, divide up the bottom of your petri dish into 3 sections since you have 3 bacteria paint colors: Magenta, Cyan and Purple. Divide the petri dish like you would divide a pie.



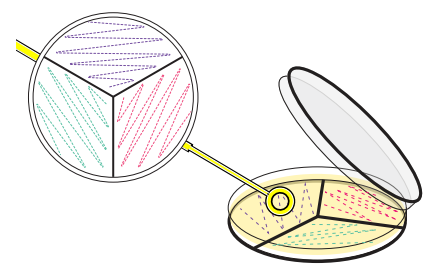
2.3 Use the marker to assign one paint color per section. The order does not matter, as long as each color has a section.



2.4 Open one of the yellow loop by holding the straight end of it, not the loop end. Remove from the packaging. Don't let the loop end touch anything yet!

2.5 Open one of your colored bacteria tube and dip the circular end of the yellow loop into the stab of colored bacteria.

2.6 Open your petri dish, and find the section assigned to this color. Using the end of the loop you dipped in the colored bacteria, trace a zigzag line across the section. You can print the stencil on the next page and place your petri dish on top to trace it if you want.



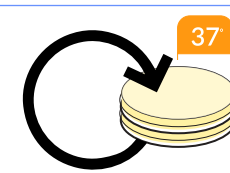
2.7 Discard the loop in your discard container.

2.8 Using a new yellow loop each time, repeat steps 2.5 - 2.8 for the other 2 colors of paint.

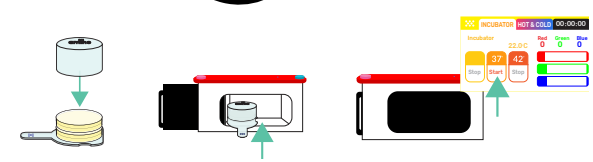
2.9 Close your tubes of bacteria and return them to the group sharing area.

Incubate Overnight

2.10 Flip your petri dish upside down and incubate it **upside down** at ~37°C for 24 to 48 hours* . This will be the painting palette of bacteria paint you will use to create your living art. If you have Amino Labs' minilab, remember to put the humidity chamber on top of your plate and to close and lock the incubator door.

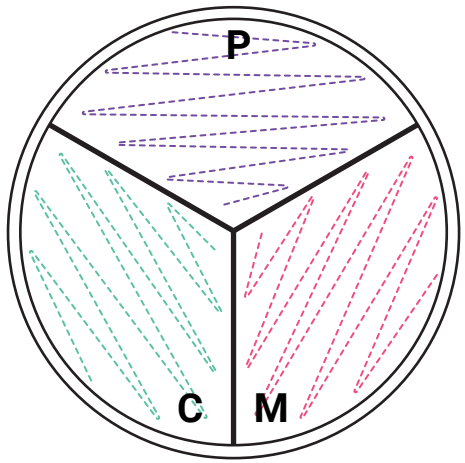


If you don't have an incubator, it can take up to 3 days for you to see the paint grow and color. Note that the colors will be more pastel if you are incubating at room temperature.



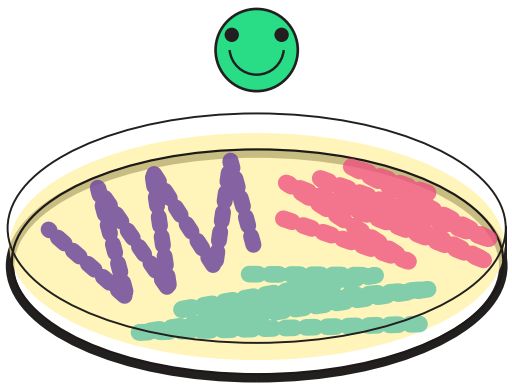
*If you need to incubate your bacteria paint for longer than 48 hrs in an incubator (ex: over the weekend) you can incubate it at 30°C instead of 37°C.

Stencil



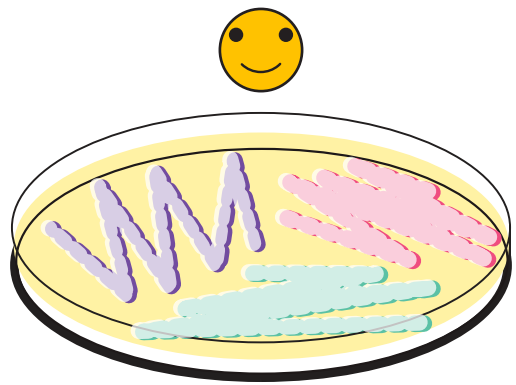
Checkpoint - Bacteria Paint

Use this guide to check if you are ready to move onto the next step.



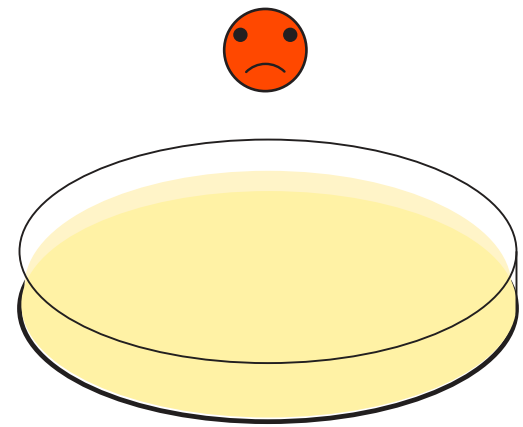
A perfect painting palette has lots of brightly colored bacteria after incubation. This suggests that you have made your LB agar petri dishes properly and have the right amount of antibiotics.

If you are incubating at room temperature, your bacteria will not become as bright as the examples below That's ok! Proceed to the next page.



A painting palette showing lightly colored bacteria after incubation requires more time to grow if you are using at 37°C incubator. At room temperature, the bacteria will stay pastel colors. You can continue incubating, checking every 12hrs, until the colors are bright or if you are short on time, you can continue ahead with the experiment.

If your bacteria are not changing color you might have forgotten to add the antibiotics, or you had to re- microwave your agar once you added the antibiotics. This could have which degraded the antibiotics. Contact help@amino.bio



If you see no growth on your plate:

1. If your incubator was not at 37°C or is homemade, incubate for another 24hrs.
2. If you are certain you incubated at 37°C, or incubated for 48hrs and still have no colonies, you might not have had cells on your loop when you streaked. Repeat Step 2: Grow your bio paint on the plate.
3. If you still have no colonies after repeating Step 2, contact us at help@amino.bio, and we will help you succeed.

What can you expect the colors to look like?

Under regular light

Purple

Magenta

Cyan

Under UV/blacklight

Purple

Magenta

Cyan

3. Paint with bacteria! Day 2, 30-60 minutes + 24+ hours wait time

Goal **Create living paintings**

Materials from your kit

Incubated painting palette | Sterile Bacteria Paintbrushes bag | Selective Agar petri dish (1/student)

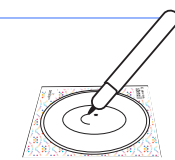
Prepare

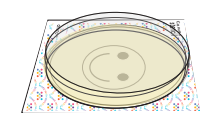
3.1 Make sure you have colored bacteria on your painting palette petri dish from the prior day. If colors have not appeared yet, wait longer, up to 48 hours. Once you have colors, take your painting palette petri dish with your colored bacteria paint from the incubator.

3.2 If you have an incubator, turn it on to 37°C.

Paint!

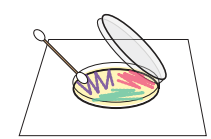
3.3 Using the blank stencils in your kit, sketch an art piece for each petri dish your are painting. You can also use the canvas stencil that already has an image included in the kit.

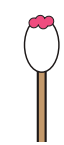


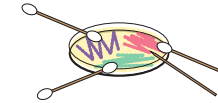


3.4 Set one of your selective petri dish canvas on top of your sketched stencil or the image stencil from the kit and open the lid of the petri dish.

3.5 Take your sterile bacteria paintbrushes and open the painting palette lid. You'll paint your art by dipping the end of the paintbrush in the colored bacteria of the painting palette and gently tracing your image, gliding on top of the agar on the selective petri dish. The agar is like a soft Jell-O, be careful not to puncture it as you paint.

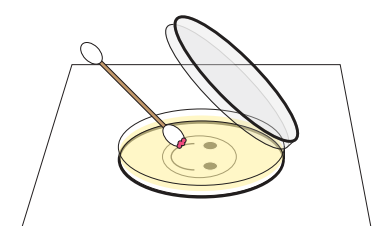






Note: Assign a bacteria color to each paintbrush as you only have a few of these. Set them down on the edge of the bacteria painting palette when you are not using them until you have completed your art.

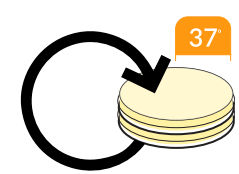
You will not see the bacteria appear right away, but you may be able to see a "wet" trace where you have painted on the agar. **You only need to dip into the colored bacteria on the painting palette once to collect bacteria paint.**



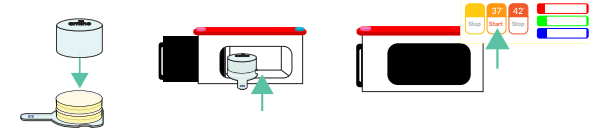
3.6 If you still have any unused petri dishes, keep them and the painting palette in a ziplock bag and refrigerate until you are ready to make more art.

Incubate

3.7 Flip your petri dish upside down and incubate it **upside down** at ~37°C for 24 to 48 hours* . If you have Amino Labs' minilab, remember to put the humidity chamber on top of your plate and close and lock the incubator door.



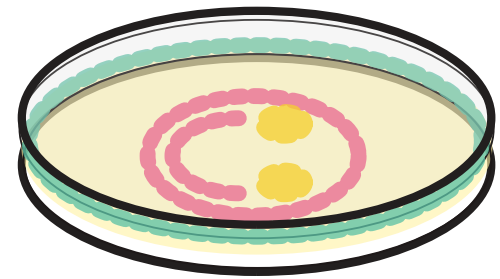
If you don't have an incubator, it can take up to 3 days for you to see the paint grow and color. Note that the colors will be more pastel if you are incubating at room temperature.



*If you need to incubate your bacteria paint for longer than 48 hrs in an incubator (ex: over the weekend) you can incubate it at 30°C instead of 37°C..

4. Did your living art grow? Day3+

Goal **Verify if your bacteria paintings grew**	



You should see your living painting appear over the next 24 to 72 hours! Keep an eye out, and your camera ready to document. Congratulations!

If there are any unused petri dishes left you can repeat steps 3.2 - 3.7 for those canvases or keep them in a bag in the reefrigerator for up to a month. If you see any unexpected growth on these, follow the inactivation instructions.

Note:
If you cannot see any growing cells at all after 48 hours of incubation at 37C, your experiment may have failed. See our troubleshooting guide at the end of the manual, compare results with your group, if applicable, or contact us with photos of your result and any documentation of your process so that we can help you succeed in the future. Make sure, if possible, to also review the video tutorials on the youtube channel (youtube.com/c/aminolabs) to see if you missed any steps!

Don't forget to look at your art under blacklight or UV light to see it fluoresce!

CONGRATULATIONS

Wolf, Amino Labs

Crane by FirstSTEAM attendee

Bacteria by Amino Labs

Darwin
Dr. T. Ryan Gregory
Guelph University

You have now joined the global community of bioartists! Happy with your artwork? There are many opportunities to share it online, exhibit it in your community and even participates in contests and artist communities on the web!

Share your results with friends and our growing community. Find us on Instagram, Twitter, and Facebook @ aminobiolab

Don't forget, you can preserve your bioart with our *Keep-it Kit*™ . For now, let's make sure you dispose of and store your remaining material correctly.

Storage, Disposal, Clean Up

After everyone sees their results, all experiment Petri dishes, tubes of cells, loops should be in the discard containers. Disposing of experiment materials is an integral part of the experiment. **Always wear gloves for cleanup!**

A. Preserving Petri dishes: If you want to preserve the living paintings or experiment results in Petri dishes instead of disposing of them, use one of our Keep-it Kits. This will help you maintain the petri dish by pouring a special resin on top. If you do not have Keep-it Kits on hand but will be getting one soon, keep the Petri dishes you want to preserve in a ziploc bag in a cool area and out of sunlight in the meantime. You can refrigerate it to keep it "fresh" for up to a month.

B. Reusable materials: If you have DNA in your kit, it can last up to 6 months when stored in a refrigerator. If you wish to keep it, store it in a ziploc bag inside a sealed plastic container in a refrigerator away from food items. If you do not wish to keep it, add to an inactivation bag. Make sure the lids are separate from the tubes so that the inactivating liquid can get inside. If you see any mold or unknown bacteria growing on any material at any point, immediately inactivate them by using a solution of bleach water. Follow the inactivation instructions below. If you are out of inactivation bags, use a sturdy ziploc type bag or disposable container with a lid. Always wear gloves when handling experiment materials and cleaners!

C. Unused ingredients: If you did not use all the agar Petri dishes you poured, store these for later use. Store them in their ziploc bag within a sealed container in the refrigerator for up to a few months. Keep them away from food items. If you see any mold or unknown bacteria growing inside, then you should always immediately inactivate the Petri dishes.

D. Inactivation: Dispose of bacteria, agar, tubes, loops, paintbrushes, Petri dishes, contaminated gloves, and other non-paper material from the discard containers by having the students transfer it to an inactivation bag. Remind students that any paper packaging like loop wrappers, plastic bags, and gloves that have not touched bacteria go in the regular garbage or recycling.

Make sure all the tubes have their lids off once in the inactivation bag and add a solution of 1 part bleach to 4 to 6 parts water to the inactivation bag. Close the bag and let sit for 24 to 48 hours before discarding the liquid in the toilet and the solids & bags in the garbage. Step-by-step instructions are on the inactivation bag and in an Inactivation video on youtube; youtube.com/c/AminoLabs.

Spray some chlorinated bleach cleaner in the discard container(s) once emptied. Let it sit for an hour before wiping down. You can wait to wipe it down until you empty out your inactivation bags the next day.

E. Clean your workspace: Use a chlorinated spray cleaner, wipes, or a solution of 1 part chlorinated bleach to 9 parts water to wipe down your work area and equipment. You can wipe down the minilabs with this solution and follow it with an eyeglass or window cleaner to remove the inevitable streaking from the bleach cleaner. Never use rubbing alcohol (isopropyl alcohol) on the DNA Playgrounds.

Glossary

Agar: is a Jello-like substance that serves as a growth media for bacteria. It is mixed with our bacteria's favorite food: Lysogeny broth (LB). LB is made up of yeast, vitamins, and minerals. LB can also be found liquid-form.

Antibiotics: When you transform bacteria, they will become resistant to a type of antibiotics no longer used in hospitals. This antibiotic will be mixed in with the agar and LB so that, as you incubate your culture, only transformed bacteria will grow. This is called a "selection marker".

Autoclave: An autoclave is a machine used to carry out industrial and scientific processes requiring elevated temperature and pressure in relation to ambient pressure/temperature. In life science, autoclaves are used to sterilize equipment and supplies by subjecting them to pressurized saturated steam at high temperatures (around 250 °F) for several minutes, up to an hour. Autoclaves are similar to some baby bottle sterilizers which you might be familiar with.

Buffers: Buffers are saline solutions that help, in this case, open up the cell membranes so that they may take up new DNA.

Cells: Cells are tiny, living units that function like mini-factories. Bacteria are single-celled organisms (unicellular) microorganisms. They are different from plant and animal cells because they don't have a distinct, membrane-enclosed nucleus containing genetic material. Instead, their DNA floats in a tangle inside the cell. Individual bacteria can only be seen with a microscope, but they reproduce so rapidly that they often form colonies that we can see. Bacteria reproduce when one cell splits into two cells through a process called binary fission. Fission occurs rapidly, in as little as 20 minutes.

Competent Cells: Since DNA is a very hydrophilic molecule, it won't normally pass through a bacterial cell's membrane. In order to make bacteria take in the DNA plasmid, the cells must first be made "competent" to take up DNA. This is done by creating small holes in the bacterial cells by suspending them in a solution with a high concentration of calcium (the transformation buffer). DNA can then be forced into the cells by incubating the cells and the DNA together on ice, placing them briefly at 42°C (heat shock), and then putting them back on ice. This causes the bacteria to take in the DNA and is called "Transformation".

DNA: The DNA is the set of instructions that tell the cell how to function like a computer program tells your computer what to do. DNA stands for **D**eoxyribo**n**ucleic **a**cid.

DNA plasmid: A plasmid is a small circular piece of DNA (about 2,000 to 10,000 base pairs) that contains essential genetic information for the growth of bacteria. Bacteria share vital information by passing it among themselves in the form of genes in plasmids. By inserting a new plasmid in our bacteria, we can get them to produce things for us,can get them to produce things for us, ike mini-factories. In this case, we have a plasmid that encodes for the creation of colorful pigments.

Genome: a genome is all genetic material of an organism. It consists of DNA. Learn more about genomes in the *What is DNA?* simulator on amino.bios

Heatshock: is when the cells are moved from ice-cold to warm temperature, typically 42℃, to take in DNA plasmids more efficiently.

Inoculation: is when you introduce bacteria into a medium suitable for its growth.

Inoculating Loops: are used to transfer liquids, cells, and DNA from one vial to the next instead of traditional lab pipettes, making your job easier, and less costly. They come in different pre-calibrated sizes, so you do not need to worry about minuscule liquid volumes. They are also used to spread bacteria on an agar surface without puncturing the soft agar.

Non-Selective: A non-selective plate means that any cells/bacteria put on this agar will grow as long as they are oxygen-loving organisms (called aerobic bacteria).

Plates (or Petri dish): A petri dish is a small plastic container used to culture (grow) bacteria in a controlled environment.

Recovery period: is the period after the heat shock in which the cells develop their antibiotics resistance and start dividing.

Selective: A selective plate means it contains antibiotics. When you insert a new DNA program into cells to make them create pigments, or anything else, you also put a “selective marker” (antibiotics resistance) inside the code. This means that only the cells that have taken up the new program will be able to grow on a plate that has the antibiotics mixed in. You only get the cells you transformed!

Transformation: See competent cells.

Troubleshooting

Here are some possible common issues:

Your agar is too wet/ doesn't solidify:

When done correctly, the agar will be the consistency of Jell-O. If it is not:

1. You likely did not heat (boil) the water before, or after adding the LB agar powder

2. You might not have added all the powder from the tube, resulting in too much water vs. LB agar powder.

3. You may not have fully dissolved the powder, meaning it cannot turn into a gel and will look cloudy. You can practice by making Jell-O! Next time heat and swirl longer to ensure the powder is fully dissolved.

You don't have any colonies and its been 24+ hours:

Don't worry, every scientist has experienced this, and it can take some practice before success.

1. Double check that your incubator is on at 37°C. If it is not, or if you are growing at room temperature, then it can take much longer to see the bacteria colonies. Keep waiting!

If you kept the second half of your recovered cells, you can pour them on your plate after 48 hours of seeing no engineered colonies grow and keep incubating.

2. You may need to try again to hone your skills. See our Youtube videos for tips and tricks on how to improve your chances of success.

Your colonies of bacteria grew, but they are the wrong color or there is mold on your petri dish:

Danger! If at the end of, or during, the incubation period your resulting bacteria/plate is: a)not the right color; b)is black when it shouldn't be, this is a sign that your culture is NOT YOUR ENGINEERED BACTERIA. You should immediately inactivate it and clean your space and unit.

To inactivate it, either add it to the inactivation bag or pour 100% chlorinated bleach into the dish, put the lid on and let it sit for 24 hours before throwing it out: The strong oxidizing environment degrades any living organisms. After 24 hours, if there are still organisms present add more concentrated bleach until it is almost full, and let stand for a further 24 hours.

There may be mold in your environment. We recommend, getting a small air purifier with a HEPA filter for the room.

Always be aware that concentrated bleach is a strong oxidizing agent and if poured on the skin can cause irritation, and on clothes remove color. Follow the safety and handling protocol on the manufacturer's label.

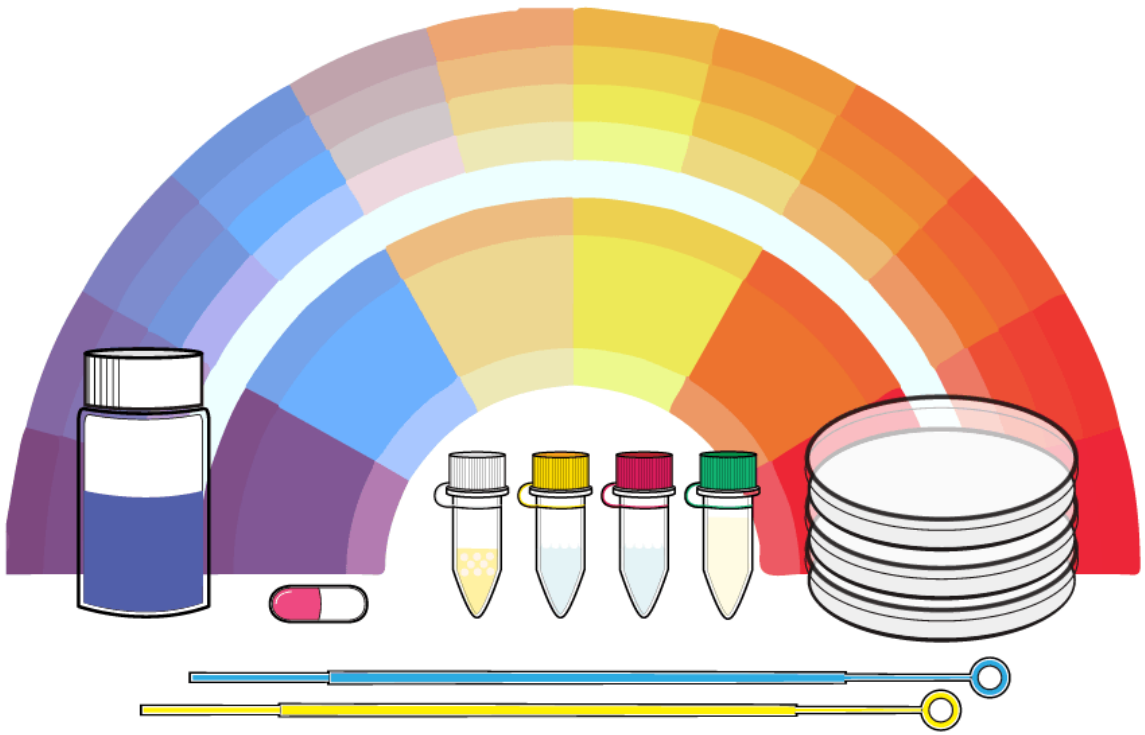
Find an interactive troubleshooter online at amino.bio/troubleshoot. We recommend using it for tips, tricks and to claim your Success Guarantee Kit if you need of one.

If anything else causes you issues, please contact us : help@amino.bio

Made in Canada

All Amino Labs products, from the hardware to DNA, are invented, designed, manufactured in and shipped from our laboratory-workshop in Canada. We'd love to hear your feedback and suggestions so that we can continue to make our products better and more fitting to your needs. Answers to your questions and help are also just an email away.

Help and General inquiries: help@amino.bio
Feedback, Suggestions, Comments: info@amino.bio



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