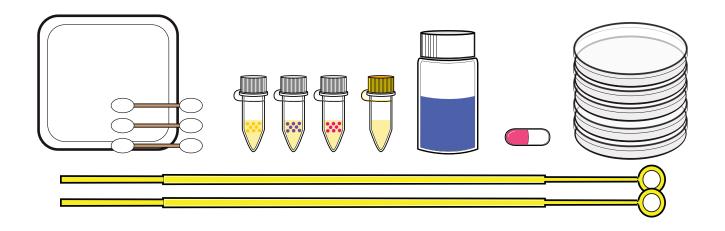
MAKING WITH BIOLOGY WITH

BIO PRINT-IT KIT™

User Manual —





BIO PRINT-IT KIT™

— User Manual ———

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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, store, use and get the most out of your Kit and importantly, 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 Engineer-it Kit™, Canvas Kit™, Print-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 ingredients, no special containment or training is required in North America*. However, 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+ unsupervised.

We recommend that the discard container be emptied by an adult and that the cleaning instructions be strictly followed for safety and experiment success. Make sure to store the ingredients in accordance with the instructions found in this booklet. Eye-wear is not provided but can be worn.

- Do not eat or drink near your experiments. Keep your experimentatleast 10 feet from food, drinks, etc. Undernocircumstances should you consume any of the ingredients.
- 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 wear extra protection, such as long sleeves and a face mask, to ensure no contact with the ingredients.

- Wash your hands before and after manipulating your experiment, the ingredients, or the hardware.
- Wear gloves, even when cleaning your station or handling the consumables (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. Also, after you put your gloves on, be aware of what you touch. Try not to touch your face, scratch itches with your gloved fingers!
- 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 consumables. An old 1L yogurt container, large plastic cup or the like will do. Used consumables will be loops, any tube or used petri dish.

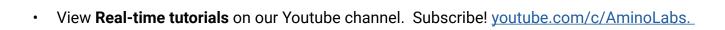
If you would like to do a short Online lab safety course for your edification, we recommend this Government of Canada course: https://training-formation.phac-aspc.gc.ca/course/index.php?categoryid=7

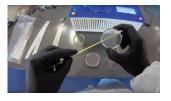
How will I learn?

Learning and prototyping with genetic engineering and cells is becoming accessible to newcomers of all ages and backgrounds 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. and our Amino Labs Kits make it easy to add a DNA program into living cells by following the instructions in this booklet. Everything you need to complete the science is included; each ingredient in the kit is pre-measured and labeled for a stress-free experience. Our all-in-one stations decrease 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 your reference, we have added a glossary at the end. We also have additional resources to help you go further in your learning:

• An essential addition to our ecosystem is the free Virtual Bioengineer™ simulation developed with the educators at the Biobuilder Educational Foundation. A 20 minutes guided experience that makes it easy to practice using a DNA Playground™ and kits beforehand. While the main simulator focusses on the Engineer-it kit™ experience, many manipulations you will be doing are very similar. It also includes additional information on the manipulations and a more in-depth look into DNA and genetic engineering. We recommend it strongly! Complete it online at www.amino.bio/vbioengineer.



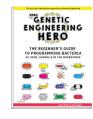




Would you like for an Amino Labs team member to tutor you through your journey? Try the **Cyber Workshop & Tutoring**, a 3-day+ experience completed via video conferencing. www.amino.bio/products/cyberworkshop.



 Are you interested in the theory behind the experiment? In going deeper on the science, learning protips and eventually completing more advanced genetic engineering? The Zero to Genetic Engineering



Why make Art with biology?

A word by BioArtist Marcus Banks

I use biologicals in my art for a few reasons: the relationships between organisms can be so complex they become the only analogies suitable for certain art pieces, bacteria are so interesting they automatically bring an additional layer of meaning to my art and, most simply, BIOLOGY IS COOL!

Through safe practices, you can easily create and wear or display your bacterial images. And as you paint it's fun to see what has worked and what hasn't, what is different from your original idea and how the bacterial growth can provide an element of randomness to certain images.

Art and Science are connecting in ways like never before and with this kit, we hope you are able to continue exploring microbiology, molecular biology, and art practices. I

We encourage you to take notes on your process, make some predictions, and record your results to further explore your predictions! But, most of all, have fun!

-Marcus

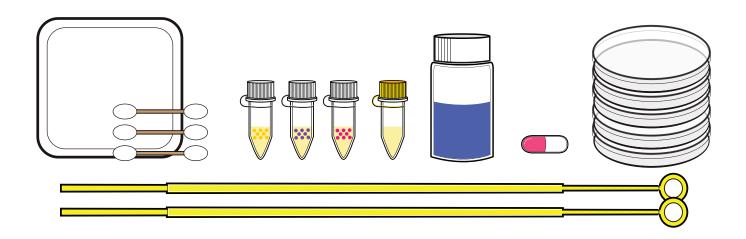
Marcus Banks can be reached through email at marcusbioart@gmail.com





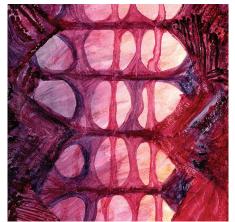
Art by Marcus Banks

Discover your Biolnk Print Kit™



The BioInk Print Kit™ lets you use the provided colored bacteria or/and your previously engineered bacteria made with an Engineer-it Kit to create prints! Simply create selective agar petri dishes following these instructions, use the bacteria "paintbrushes" to create your art on the agar, incubate and transfer the designs, patterns, paintings to the provided fabric, some papers or any porous surface. Let your creativity grow with bacterial art!

Art by Marcus Banks









Kit Components



Agar Powder: This LB agar powder is industry standard. Each tube of LB agar powder can make 45 mL of molten LB agar (3.5% w/v). Agar is both the surface the bacteria grow on and the food they eat to grow.¹ *Optional, as you may have a kit with pre-made agar plates



Sterile Water: Sterility is critical when you're bioengineering. This is distilled water that has been sterilized with an autoclave in order to ensure there are no contaminating organisms present. This 50 mL volume, when used with LB agar powder is enough to make 5 LB agar plates. *Optional, as you may have a kit with pre-made agar plates



Yellow Loops / Large: Large inoculating loops are used for transferring 10 uL of liquid and/or other tasks. Yellow loops are great for spreading out bacteria after a transformation.



Petri Dish / Plate: 6cm petri dishes are large enough for typical lab experiments and help save on cost of reagents as well as reduce waste. *Optional, as you may have a kit with pre-made agar plates. In this case, the petri dishes/plates are filled with agar, a jelly-like substance bacteria eat and grow on.



Inactivation Bag: A heavy duty bag to put all of the kit waste in. After your experiment, add bleach and water to the bag to inactivate all the samples and practice safe science.



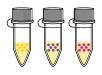
2 types of Paintbrushes: Cotton/rubber swabs and traditional art paintbrushes to help you paint your bacteria on the agar.



Fabric / Bandana: Use this specially chosen fabric as your final canvas. Wear it as a bandana, headband, tie it around your backpack, display it in a frame... discover the possibilities¹



Fixing Medium: Always use the fixing medium on top of your bacteria print on fabric or paper to help "fix" the print and make it safe for touching and wearing ¹



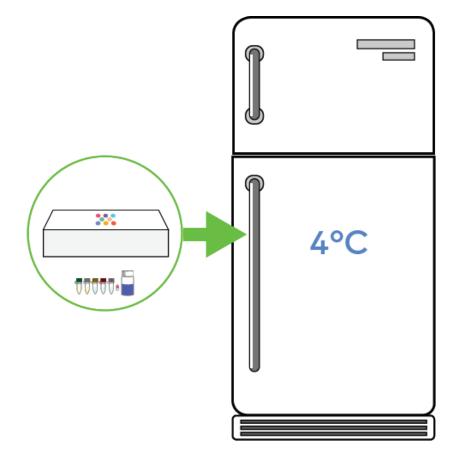
Colored Bacteria (variable): These safe and friendly bacteria are naturally-occurring or engineered to be colorful so that you may paint in multiple colors. ¹

¹ For education purposes only.

Unpacking and Storing your kit

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

Do Not Freeze your kit!



Technical Specs

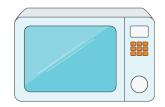
Growth plates: 6 cm petri dishes Selection/Antibiotic: variable LB agar powder (1.75 g) 50 mL sterile water

Necessary Equipment

For Best results:

- DNA Playground[™] or BioExplorer[™]
- Microwave





Alternative solution:







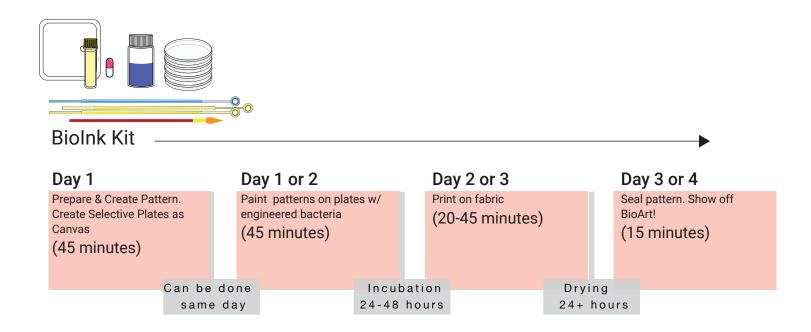
- Microwave
- Timer
- Incubator or warm environment and thermometer (for 37°C): This will replace the Incubator set to "37". If you do not have an incubator (biology or egg one, as long as they set to 37°C) you can create one using an online tutorial (ex: www.instructables.com/id/Low-cost-and-ac-curate-incubator-for-DIY-biology/.) If you have neither incubator or DIY version, you can try incubating the cells in a resealable bag in a warm environment. Your yield won't be as good as with an incubator, but should work. Note that it will take a few more days to see results.

Necessary Safety Supplies

- Disposable container 500ml-1L to hold inactivation bag (e.g. yogurt container, plastic cup)
- Latex, nitrile, or similar gloves like the ones found at a pharmacy. (At least 3 pairs/person)
- Chlorinated bleach (mix a 10% solution: 1 part bleach to 9 parts water)



Timeline



The Print-it Kit[™] takes 4 days of hands-on activity to complete. 6 main "activities" make up the Kit experiment:

- 1. Make selective plates Day 1, 20-35 minutes
- 2. Streak your colored cells to make paint Day 1, 20 minutes, incubate 16-24 hrs
- 3. Stencil your art on paper Day 2, variable time

- 4. Paint with your Bacteria
 Day 2, 20+ minutes , 24-72 hrs incubation
- Print your art on fabric
 Day 3, 20+ minutes , 24 hrs drying
- 6. Seal your art on fabric

 Day 4, 10 minutes, 24 hrs drying

Experiment Protocol

1. Creating LB Agar Plates Day 1, 25 minutes

Goal Create non-selective and selective LB agar plates.

Materials from your kit

(2) LB agar powder

(2) 50 mL sterile water

(2) antibiotic pill

(8) 6 cm petri dishes

(1) Sharpie marker



Prepare

1.1 Using a sharpie-type pen, label the bottom of the petri dishes as follows: **8x** S. (for selective) + Add [your initials] if doing this in groups with multiple kits. (*The bottom is the side with little tabs*)

Note You will be making 2 batches of agar with this kit. Each batch is made with 1 sterile water, 1 tube of agar, 1 antibiotic pill and 4 petri dishes. Steps 1.2 to 1.7 instruct you on how to make your first batch of agar. Follow the directions closely, using only 1 bottle of water, 1 tube of agar, 1 antibiotics pill and 4 plates at a time.



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 should see a rolling boil where many bubbles are rising constantly. Careful, the bottle will be hot!
- 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 agar tube. This is due to the water evaporation coming into contact with the agar powder as you pour it in. This is okay, we have accounted for this loss of powder.
- 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 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.





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, which means don't swirl too vigorously. The gelatin capsule of the pill 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 agar into 4 petri dishes. Place their lids on. Let solidify.
- 1.8 Repeat steps 1.2 to 1.7 with the second bottle of water, agar powder, antibiotics and 4 petri dishes.
- 1.9 Let the LB agar harden. You will use 1 plate in the next step. You can store the remaining 7 plates in the zip-



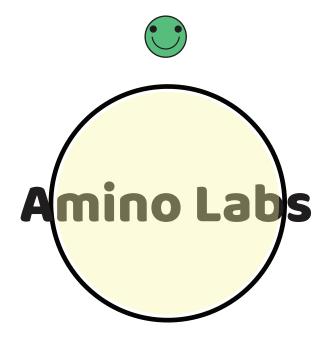






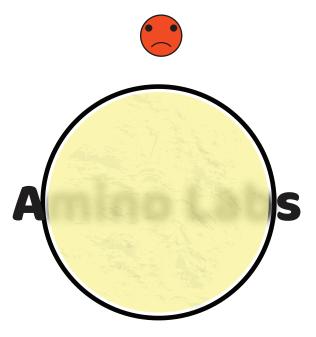
Checkpoint - Agar Plates

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.

Unfortunately, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

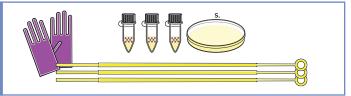
2. Grow your biopaint Day 1, 25-45 minutes + 24 hours wait time

Goal Create living paintings

Materials from your kit (1) Selective Agar plates

(1-4)* Tubes of Bacteria Paint

(1-4)* Yellow Loops



*Depending on the kit you have, you will find 1, 2, 3 or 4 tubes of "bacteria paint".

Prepare

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

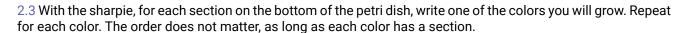


1 color



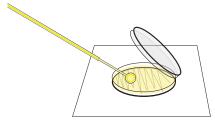
Streak

2.2 Take your tube(s) of colored bacteria. Mark up the bottom of your petri dish in sections using a sharpie, like you would divide a pie: Divide your petri dish into as many sections as colors you have, for example, 3 sections if you have 3 colors.



- 2.4 Place you petri dish on top of the zigzag pattern on the corresponding stencil on the next page. The right stencil will be the one that is divided in the same amount of sections as your petri dish.
- 2.5 Open one of the yellow loop by holding the stick end of it, not the loop end.
- 2.6 Dip the loop end of the yellow loop into one stab of colored bacteria paint.
- 2.7 On your petri dish, find the section that you reserved for this color and, using the end of the loop you dipped in the colored bacteria, trace the zigzag line of the stencil.
- 2.8 Discard the Loop in your inactivation bag.
- 2.9 Using a new yellow loop each time, repeat steps 2.5 2.8 for each colored bacteria tube you have.
- 2.10 Close your tube(s) of bacteria. Return it/them to a refrigerator a ziplock bag or sealed container.

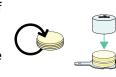




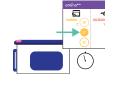
Incubate Overnight

2.11 Incubate your streaked plate upside down at ~37°C for 16 to 24 hours ((it may take longer if you do not have an incubator): You must flip your plates upside down so that the agar is up and the lid down. (Place it on top of the incubator paddle if you are using Amino Lab's hardware, and place the incubator humidity chamber on top)

This will be your biopaint to create your art tomorrow. If you have Amino Labs' minilab, remember to close the incubator door and lock it using the incubator kev.



















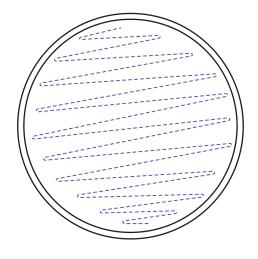




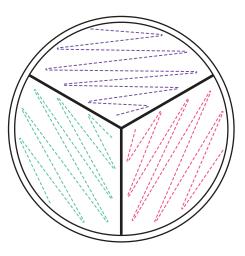


2. Stencils

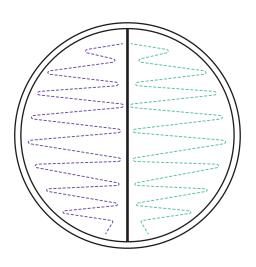
1 color



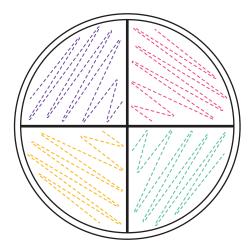
3 colors



2 colors

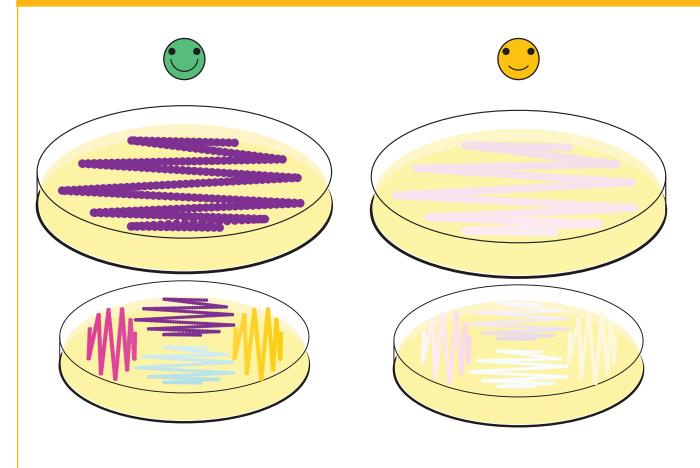


4 colors



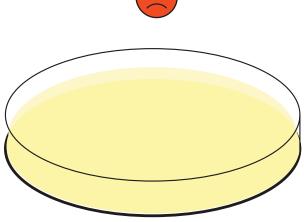
Checkpoint - Bacteria Paint

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



A perfect bacteria plate has lots of brightly colored bacteria after incubation. Proceed to the next page.

A bacteria plate with somewhat colored bacteria after incubation needs more time to grow. Incubate longer and verify at 12hr intervals until the colors are bright.



If you see no growth on your plate:

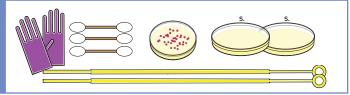
- 1. If your incubator was not at 37°C or is homemade, incubate for another 24hrs.
- 2. If you are certain you incubated at 37°C, or incubated for 48hrs and still have no colonies, you might not have had cells on your loop when you streaked. Repeat Step 2: Grow your bio paint on the plate.
- 3. If you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

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

Goal Create living paintings

Materials from your kit (2-3) Selective Agar petri dish Biopaint plate from yesterday

Loops
"Paintbrushes"



Prepare

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





Paint!

- 3.2 Using the blank stencils in your kit, sketch your art piece for each canvas you would like to paint. You can also use the pre-made canvas included.
- 3.3 Make sure you have colored bacteria on your biopaint petri dish from the prior day. If colors have not appeared yet, wait longer, up to 48 hours. If you have colors, take your petri dish of biopaint from the incubator (and freshly engineered bacteria if you completed an Engineer-it Kit previously).
- 3.4 Set one of your selective petri dish canvas on top of one of your sketched stencil or the image stencil from the kit.
- 3.5 Using the yellow loops, the blue loops and the bacteria "paintbrushes" paint your art onto the agar by dipping into the colored bacteria from the biopaint petri dish and tracing your image, gliding on top of the agar. The agar is like Jell-O, be careful not to puncture it as you paint.

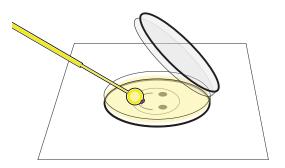
Note that you will not see the bacteria appear right away, but you may be able to see a "wet" trace where you have painted on top of the agar. You only need to dip into the colored bacteria on the petri dish to collect biopaint.

Note that you can mix colors as you wish. If you have art paintbrushes at home, you can dip them in diluted bleach, rinse them thoroughly and use them to paint. Clean them by soaking them in bleach overnight.

3.6 You do not need to paint all canvases at the same time. In fact, we recommend painting 1 or 2 the first day and seeing how the image develops while it incubates. Bacterial painting is an art that surprises!

Return the unused canvas to the ziplock bag and refrigerate. Add the biopaint petri dish to the ziplock bag and refrigerate as well.





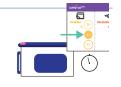
Incubate

3.7 Incubate your art canvases **upside down** at ~37°C for 16 to 24 hours (it may take longer if you do not have an incubator). You must flip your plates upside down so that the agar is up and the lid down. (Place it on top of the incubator paddle if you are using Amino Lab's hardware, and place the incubator humidity chamber on top) Note that you can only incubate 2 petri dish canvases at a time in a DNA Playground. Plan accordingly!



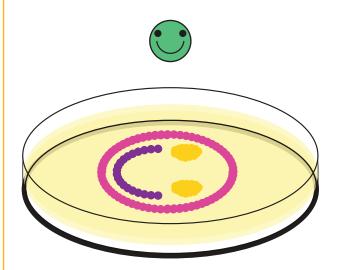


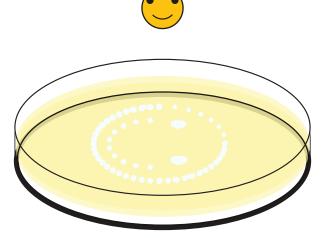


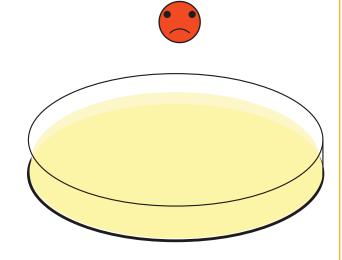


Checkpoint - Did your living art grow?

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







Your living painting appears over the next 16-48 hours of incubation. Your painting is ready when it is brightly colored, or as brightly colored as you like.

Proceed to the next step

A bacteria plate with somewhat colored bacteria after incubation needs more time to grow. Incubate longer and verify at 12hr intervals until the colors are as bright as you want.

If you see no growth on your plate:

- 1. If your incubator was not at 37°C or is homemade, incubate for another 24 hrs.
- 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 3: Paint with bacteria on the plates.
- 3. If you still have no colonies after repeating Step 3, contact us at help@amino. bio and we will help you succeed.

4. Prepare to print! Day 3 or 4, 5-10 minutes, 2 hrs + of drying time

Goal Prepare your living paintings so that stick on fabric



You will see your living paiting appear over 16 to 48 hours!

4.1 Once satisfied with the colors that have developed in your art, leave the plates at room temperature with the lids partially open so that all the excess water in the agar evaporates.

This will help your art "stick" to the fabric. Leave them to dry over a few hours or overnight.

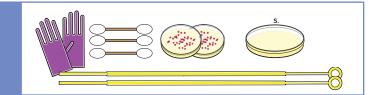
4.2 If you have unused canvas petri dishes, you can repeat steps 3.1 - 3.7 for those canvases at any time. Note that you can only incubate 2 petri dish canvases at a time in a DNA Playground. Plan accordingly!

5. Print! Day 3 or 4, 20-60 minutes + 24 hours wait time

Goal Transfer your art to fabric

Materials from your kit Your art (1) Fabric

Loops
"Paintbrushes"



Prepare

5.1 Plan where you want to print your agar art onto the fabric... once it is pressed, the original disappears.

Note:

If you still have biopaint left on the original biopaint petri dish, you can use it to add details and patterns to your fabric. Do this either through the pressing technique below or by dipping a clean paintbrush in the bacteria and painting directly on the fabric.

Print!

- 5.2 Open the first art canvas you want to print. Make sure it has been "air dried" at least 2 hours, with the lid partially open (*Checkpoint step 2*).
- 5.3 Lay the fabric on top of the agar, at the location where you want the art to be transferred onto the fabric.
- 5.4 Press down on the fabric using gloved fingers and the printing press disk in your kit. Make sure that all the fabric touches the art.
- 5.5 Leave the fabric inside the petri dish for a minute to ensure good contact and transfer.
- 5.6 Remove the fabric gently and, **with gloved hands**, remove any pieces of agar that may have become stuck on the fabric (it happens!). Throw these in the inactivation bag.
- 5.7 If you have other canvas art plates, or some remaining art on the first agar art plate, repeat steps 4.3 to 4.6. This can be done over a few days as your canvases get incubated. Repeat for any patterns or art you wish to transfer.

Tips:

Try layering your art by pressing the same fabric spot in different agar art canvases! Add details with a paintbrush dipped directly in colored bacteria.

Air dry

5.8 Let your printed fabric air dry for 12-24 hours away from sunlight.

6. Seal your print Day4+

Goal Seal in, or "fix" your print on fabric for safety and preservation

Materials from your kit Your printed fabric

(1) Ink Sealer / Fixing medium tube

(1) Sealer paintbrush



Seal

6.1 Using a large paintbrush or your gloved fingers, spread the fixing medium from your kit over the entire print surface on your fabric. This will make sure you can safely wear and touch your fabric prints.

Note:

If you are printing your fabric over a few days, wait until your printing is complete before sealing the art.

Air Dry

6.2 The fixing medium will dry over another 24 hours. Once dry gently handwash it with soapy water and rinse.

Congratulations!



You have now joined the global community of bioartists! You have now made a wearable art piece using biology, and only a few people in the world can say that.

Share your results with your friends and our growing community and visit our website to see what's next on your journey! © 💆 @aminobiolab

Now, let's make sure you dispose of and store your remaining material correctly.

Storage, Disposal, Clean Up

After you see your results, you may have blank cells, transformed cells, various plates and ingredients in your inactivation bag, reusable or even unused components. Disposing of them responsibly is an integral part of your experiment:

If you would like to preserve your living painting or experiment results in their Petri dishes instead of disposing of it/them, use a Keep-it Kit from our online store which will help you preserve up to 2 plates of bacteria. If you do not have a Keep-it Kit on hand but will be getting one in the near future, keep the petri dish you wish to preserve in a zip-lock bag in a cool area and out of sunlight in the meantime. You can even refrigerate it to keep it "fresh" for a month or 2.

- 1. Reusable materials: DNA and engineered cells (either on a petri dish or from stabs) can last up to 6 months when stored in a refrigerator. If you wish to keep them, close them tightly and store them in a zip-lock bag, inside a sealed plastic container in a refrigerator away from food items. If not, add them to the inactivation bag. Make sure the lids are off the tubes so that the inactivating liquid you will add can get inside.
- 2. Unused ingredients: If you did not use all the agar plates in your kit, you can store these for later use. Store them in their zip-lock bag, within a sealed container in the refrigerator for a few months. Keep away from food items.
- 3. Inactivation: Dispose of the rest of your material by adding all of it to the inactivation bag, including any petri dish with bacteria you are not keeping for a Keep it, and any tubes, gloves, and loops. Any paper packaging like loop packages and bags can go in the regular garbage.

Add a solution of bleach water to the bag by following the instructions on the inactivation bag. You can also find these instructions with videos on our Youtube channel youtube.com/c/AminoLabs

4. Clean your workspace by using a solution of 10% chlorinated bleach or spray cleaner to wipe down your work area and equipment. Do not use rubbing alcohol on the Minilabs. A solution of 10% chlorinated bleach can be made with 1 part bleach for 9 parts water.

More Information





All Amino Labs products, from the hardware to the DNA, are invented, designed, manufactured and shipped by us, in our laboratory- workshop in Canada and we'd love to hear your feedback and suggestions to continue to make our products better and 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: j@amino.bio

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".

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 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

like mini-factories. In this case, we have a plasmid that encodes for the creation of colorful pigments.

Heatshock: When the cells are moved from ice-cold to warm temperature, typically 42C, in order to take in DNA plasmids more efficiently.

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

Inoculating Loops: 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

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

Here are some possible common issues:

Your agar is too wet/ doesn't solidify: The agar, if done correctly, will be the consistency of Jell-O. If not:

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

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 not expressing your DNA program / There is mold on your petri plate: Danger! If at the end of 24-48 hours your resulting bacteria/plate is: i) not the right color; ii) not colorful at all; iii) is black when it shouldn't be, then this is a sign that your culture is NOT YOUR ENGINEERED BACTERIA. You should immediately inactivate it and clean your space and unit.

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. *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.*

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



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