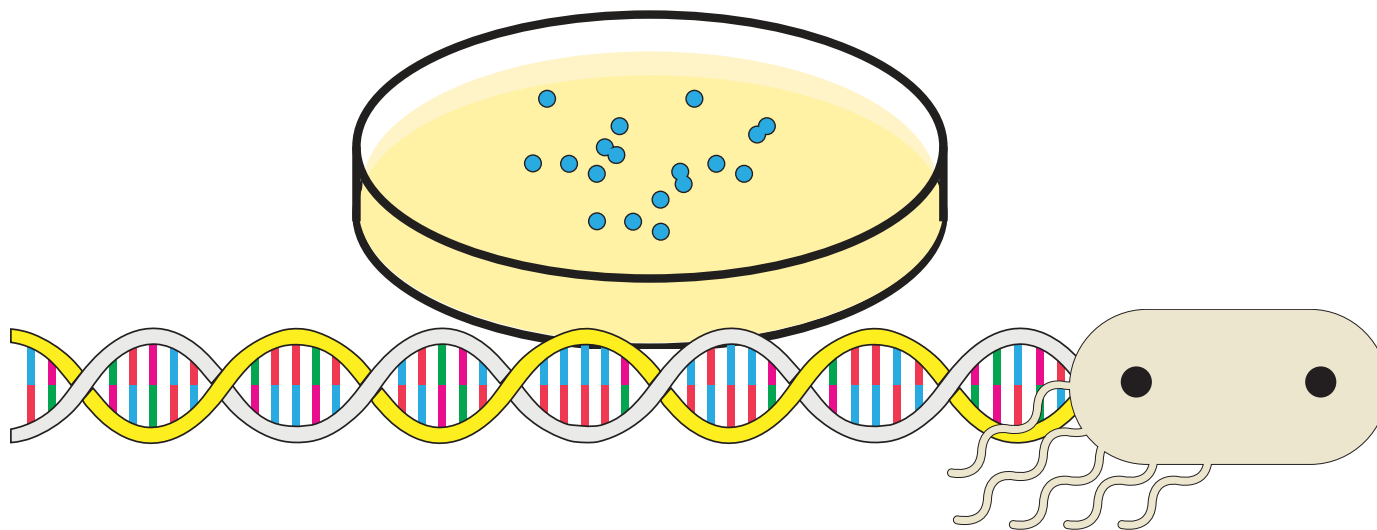


ENGINEER-IT KIT™

INDIVIDUAL KIT MANUAL

For use with individual kits, alone or in small groups



ENGINEER-IT KIT™

INDIVIDUAL KIT MANUAL

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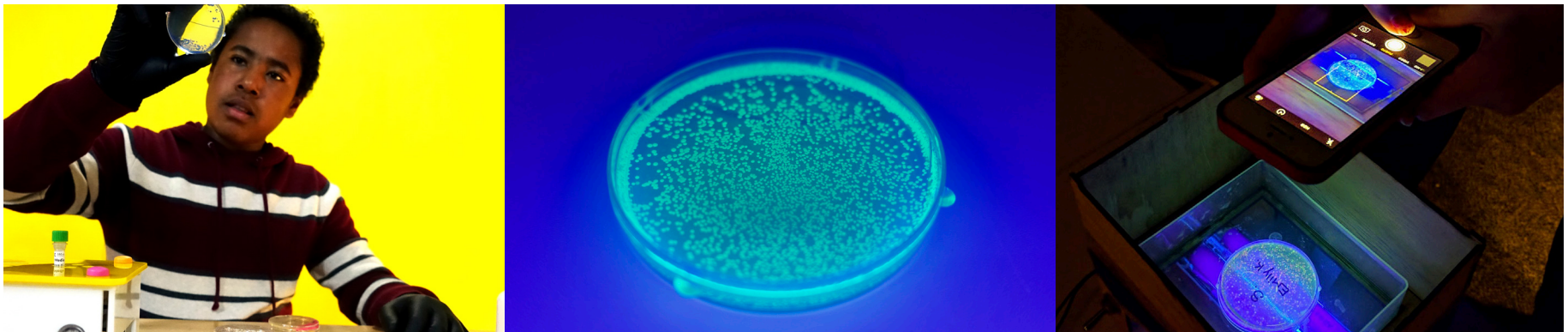
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Welcome! Let's get started

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 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 Engineer-it Kit™, Canvas 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!**



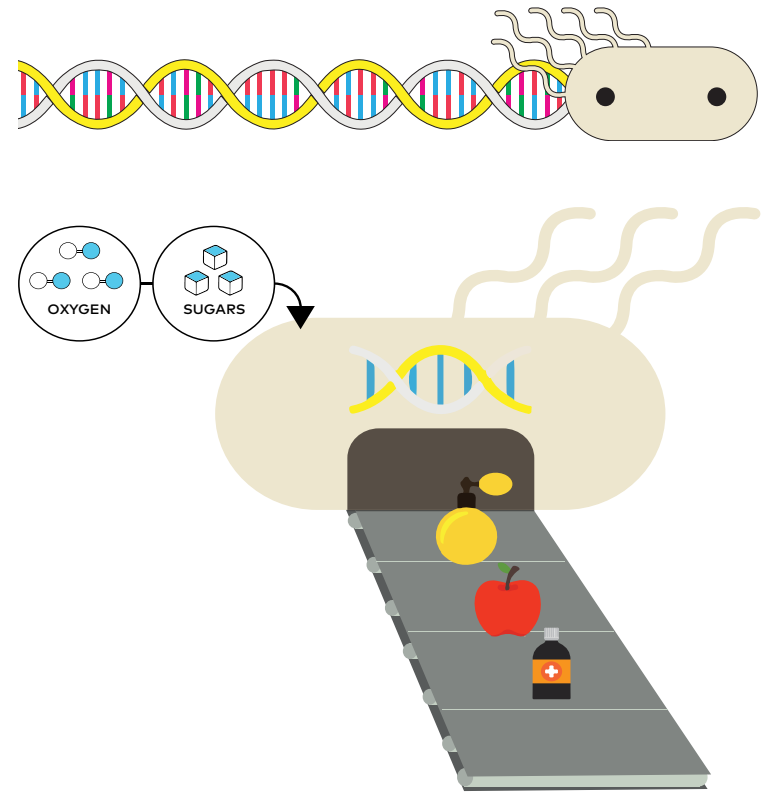
Why genetic engineering?

Bacteria are fascinating—and they are part of a revolution in sustainable advancements across food science, energy, health, and materials. Genetic engineers (or biological engineers) produce medicine, food, fuel, household products, and new materials by using bacteria to “read DNA” and create from these blueprints.

Similar to miniature factories, bacteria can follow DNA programming that has been inserted in them through genetic engineering and create products in response. Each bacterium produces a small quantity of the product which, when cultivated in large vessels, generate significant amounts of pigments, medicine, plastic compounds, and more. These can then be extracted and used by industries and individuals. Since bacteria multiply rapidly when they are fed the right sugars and amino acids and are kept in a controlled environment, creating products through genetic engineering can be sustainable and safe.

Thanks to the hard work of scientists around the world, programming cells is improving our quality of life and keeping diseases at bay. Already in the hands of hundreds of millions of people each day, the number of genetically engineered products will continue to rise in the future. And now, you too can program bacteria! Amino Labs kits have everything you need to engineer organisms whether for the first time or the hundredth!

By getting hands-on experience with genetic engineering and biotechnology, you will become immersed in some of the most cutting-edge science of the 21st century.



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 more safe science resources, including a fun video, go to <http://amino.bio/practicesafescience>

How will I learn?

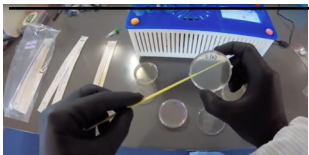
Learning and prototyping with genetic engineering and cells is becoming accessible to newcomers ages 12+ thanks to dedicated scientists and kits such as the one you are about to use!

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 hesitate to flip to it during or before your experiment.

We also have additional resources to help you go further:



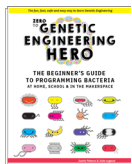
An essential addition to our ecosystem are the free **Virtual Bioengineer™ simulations** developed with the educators at the Biobuilder Educational Foundation. These simulations are 20 minutes guided experiences that make it easy to practice using a DNA Playground™ and experiment kits beforehand. The 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.



View **Real-time tutorials** videos at youtube.com/c/AminoLabs.

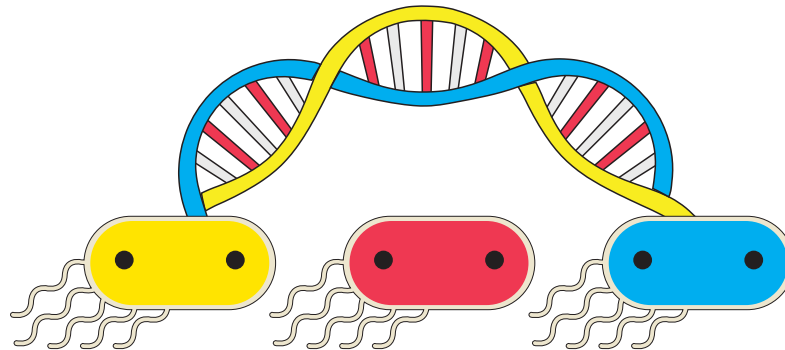


Would you like for an Amino Labs team member to guide you through your journey? Try the **Cyber Workshop & Tutoring**, a 3-day+ experience completed via video conferencing. www.amino.bio/products/cyberworkshop.



Are you interested in the theory behind the experiment? In going deeper on the science, learning 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 the Engineer-it Kit™



The Engineer-it Kit™ has everything you need to insert DNA programs into bacteria and have them produce what the DNA “tells” them. Note that the bacteria, the antibiotics, and the different buffers were made to fit perfectly with the way we created the DNA Program so make sure you keep kit components separate if you have more than one kit.

Speaking of DNA Programs, the DNA Program in your kit encodes the cells for a certain behavior. For example, with our Magenta DNA Program, your bacteria will produce a fluorescent magenta protein that you will see as colored bacteria on your petri dish! Amazing!

The Engineer-it Kit comes in individual size or group/classroom size. These contain the same ingredients, in different quantities. This individual or small group-specific manual is aimed at learners using the individual-sized version of the Engineer-it kit. If you are a small group or an individual using parts of the group-sized kit, or parts of the Full Rainbow version of the kit, that is ok too! Store the rest in the refrigerator to use at a later time.

If you are teaching or doing the exercise as a large group or classroom, we have a manual available for you. Visit www.amino.bio/instructions to download the CLASSROOM version of the manual for the Engineer-it kit.

What does your kit allow you to do?

As we saw earlier, cells are tiny living units that function like mini-factories. Bacteria are single-celled organisms. 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. Under perfect conditions, a single bacterium could grow into over one billion bacteria in only 10 hours!

Each bacteria, or cell, is told how to use its factory-like capabilities by its DNA. DNA is like a computer program; it is the set of instructions that tell the cell(s) how to function.

In this Engineer-it Kit, you get a DNA plasmid (sometimes refer to as a DNA Program since it is an easier language to grasp). DNA plasmids are also a set of instructions for the cells, but much smaller. A plasmid has only a few functions as opposed to the complete genome of the bacteria. By inserting new plasmids in the bacteria, we can get them to produce things for us. For example, with the magenta Engineer-it Kit, the plasmid encodes for the creation of magenta-colored proteins, which turn the bacteria magenta colored.

Since DNA is a very hydrophilic (water-loving) molecule, it won't normally pass through the bacteria's cell membrane. To make bacteria take in the plasmid, the bacteria must first be made "competent." This means 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-cold, putting them briefly at 42°C, and then back on ice. This causes the bacteria to take in DNA and is called "Transformation."

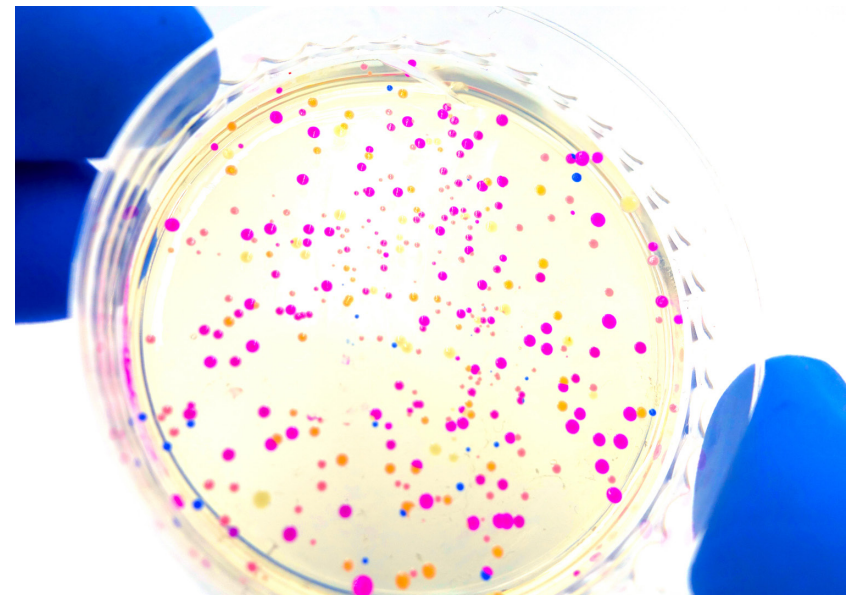
The bacteria you will find in your kit are standard lab bacteria commonly used to bioengineer, *E. coli* K-12. This bacteria is safe for use in your home, the classroom, a community, or maker space anywhere in North America.

You will need to grow your bacteria on the nutrient agar petri dish (plates) before you insert the DNA plasmid. Freshly grown bacteria take up DNA much better than older ones since they are still in a growth phase. Nutrient

agar is a Jell-O-like staple food source for the bacteria which you will pour into the provided plates (petri dish) in the first step. Two types of agar will be made: non-selective and selective. The non-selective agar allows any bacteria to grow, while the selective agar has an antibiotic mixed in which allows only the engineered bacteria to grow. The DNA plasmid you insert will make your bacteria resistant to the antibiotic added to the selective agar, and so only your engineered bacteria will grow.

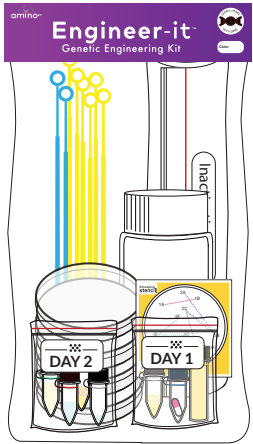
Specifically, your kit will allow you to complete the following hands-on steps to insert a DNA plasmid into bacteria, also called a “bacterial transformation”:

1. Make selective and non-selective agar plates for growing bacteria
2. Grow/streak blank (non-engineered) *E. coli*
3. Make *E. coli* cells chemically competent (able to take up DNA plasmids)
4. Transform the competent cells with DNA program
5. Recover the engineered cells
6. Grow the engineered cells on plates
7. View the results



Engineer-it kit results using the Mr. Sparkle DNA program

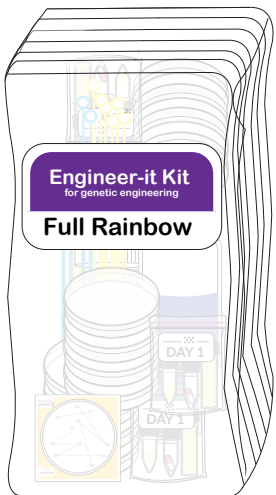
Using the Individual Kit Size



The Engineer-it Kit™ individual size lets you grow and engineer bacteria with the DNA Program provided once by yourself or with a small group. You notice you have 4 Petri dishes in your bag. That is because you will also complete to 2 control Petri dishes. Control Petri dishes are what allow scientists and scientific explorers to see if and where their experiments succeeded, failed, or both! These instructions will show you how to do a positive control and a negative control and explain in details how controls work.

This kit can be used alone or in a small group (with parent supervision if you are under 16). In the next pages, you will find descriptions of the kit's content.

Using the Full-Rainbow Kit



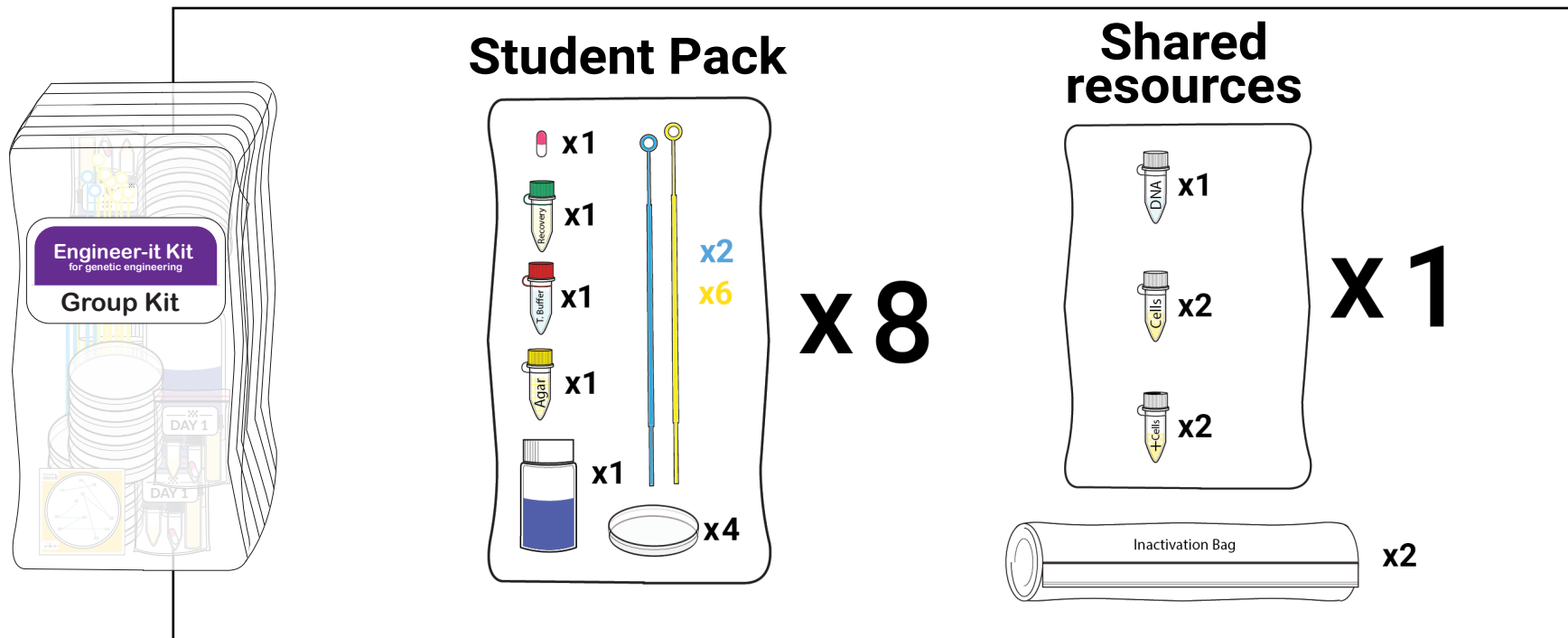
The Engineer-it Kit™ Full-Rainbow kit lets you grow and engineer bacteria with the DNA Programs provided 10 times by yourself or with in a group. Each kit has a different DNA program to produce different pigment and within the pack each kit is like an individual-size kit. Everything you need is in each kit.

This kit can be used alone or in a small group (with parent supervision if you are under 16). In the next pages, you will find descriptions of the each kit's content.

Using the Group kit individually or with a small group

The Engineer-it Kit™ for groups contains 8 individually-wrapped student packs and one shared resources bag containing the blank cells, the positive control cells, the DNA program, and the inactivation bags which are shared by everyone. With 8 student packs, you can run 8 engineering experiment using the same DNA. Take the number of student packs you need and refrigerate the rest.

Remember to keep your DNA and cells by closing the tubes and placing them back in a refrigerator after using them! In the next pages, you will find descriptions of the kit's content.



Kit components



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



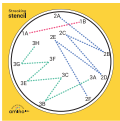
Blue Loops: Small inoculating loops are used for transferring 1 μ L of liquid and mixing. μ L stands for μ L which means microliter, so one-millionth of a liter. These replace costly traditional pipettes.



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



Petri Dish / Plate: 6cm Petri dishes are large enough for this lab experiment and help save on the cost of reagents and reduce waste.



Streaking Stencil: To grow the blank bacteria into separated, fast-growing colonies for engineering, a specific streaking pattern that dilutes the number of bacteria you initially have on your loop must be followed. This stencil will help you trace the pattern when you place it under a petri dish.



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.

Day 1 bag (used on the first day of the experiment)



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

Day 1 bag (cont.)

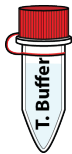


Antibiotics/Selection Marker: Amino Labs' proprietary antibiotic delivery system helps stabilize antibiotics for shipping and long-term storage. These capsules have a measured amount of antibiotics for 50 mL of molten LB agar. The amount of antibiotics included in the capsule is 1000 smaller than a standard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

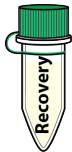


Blank Bacteria/cells: This safe, standard K12 lab strain of *E. coli* is non-pathogenic and is the strain used by thousands of labs around the world. This strain comes as a "stab," a small tube which contains agar in which the bacteria are 'stabbed'.¹

Day 2 bag (used on the second day of the experiment)



Transformation Buffer: Amino Labs' proprietary transformation buffer is used in a colony transformation procedure to yield high transformation efficiencies. When you adhere strictly to the transformation protocol, this buffer rivals other commercially available competent cells & procedures that are costly and require specialized laboratory equipment to store.¹



Recovery Media: Amino Labs' recovery media is used after the heat shock during the transformation protocol. This nutrient broth aids the cells in recovering and has a proprietary recipe that further boosts the cells ability to survive the transformation and begin dividing.¹



+ Cells: This stab of engineered *E. coli* K12 is non-pathogenic and allows you to create a positive control sample by growing it on a plate to test if your selective agar plates were successful.



DNA: This tube contains DNA plasmids which you will use to program your bacteria so that they create colorful pigments.

¹ For education purposes only.

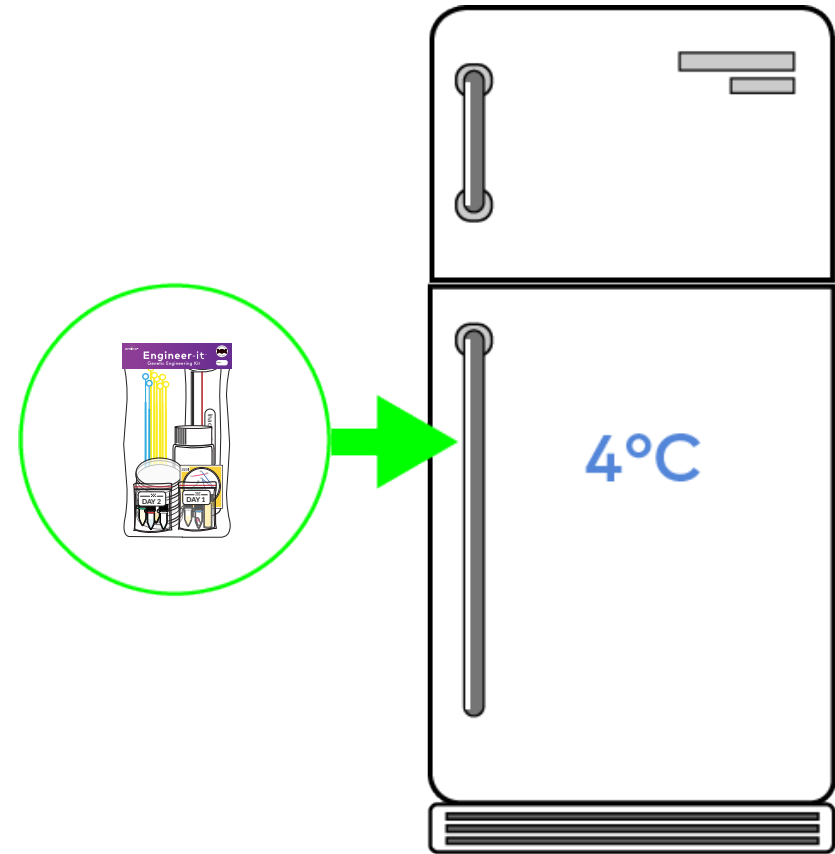
Unpacking and storing kits

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

If you can fit the whole pack, go ahead and store it all in the refrigerator. If you need to save space, please put the DAY 1 and DAY 2 bags in the refrigerator. The rest can stay at room temperature.

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
DNA plasmid 250 ng
Selection/Antibiotic: variable

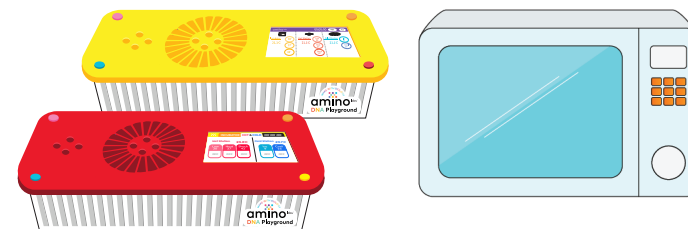
Transformation Buffer: 50 uL tubes
Recovery media: 350 uL tubes
Cells /+Cells: K12 *E. coli* Stab

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

Necessary equipment

For Best results:

- **DNA Playground:** One DNA Playground Classroom size per 4 experiments running at the same time or one DNA Playground Home size per experiment
- **Microwave**
- **1 Sharpie-type marker**



Alternative solution:

- **Microwave**
- **Ice bucket or bowl and ice:** This will become your "**Cold station**" "Ice" for the experiment. Make sure to keep the ice from melting too much during the experiment. You may need a fresh replacement during the experiment if the room you are in is warm.
- **Hot water bath or bowl with hot water:** This will become your **Hot station** set to "**Shock/42**" for the experiment. Heat the water to 42°C and try to keep it as stable as possible while you heatshock.
- **Thermometer** (for 42°C)
- **Timer**
- **Incubator** (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 Search for DIY incubator on our youtube chanel - [Youtube.com/aminolabs](https://www.youtube.com/watch?v=LEsv0Qvbczs) - or go to this direct link: <https://www.youtube.com/watch?v=LEsv0Qvbczs>



Necessary safety supplies



Disposable container 500ml-1L

to hold tubes, loops and other contaminated waste (e.g., yogurt container, plastic cup).

Latex or nitrile gloves

like the ones found at a pharmacy. 1 pairs/person if you will keep & reused each day, or 4 pairs/person if not saved & reused.

Chlorinated bleach spray

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

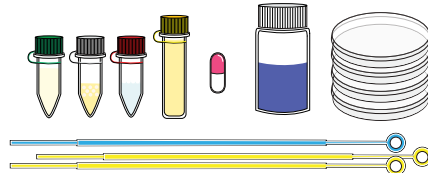
Bleach ~250 mL

to inactivate all the experiment materials at the end of the experiment.

4-day (period) timeline



Pre-labs



Engineer-it Kit

Pre-lab(s)

Use the Virtual Bioengineer to prepare for the hands-on experience with theory and procedures (30 minutes)

Day 1

Create petri dishes, LB agar, streak blank bacteria and incubate (45 minutes)

Incubation
12-24 hours

Day 2

Make cells chemically competent & transform with DNA plasmid. Start recovery (45 minutes)

Recovery
12-24 hours

Day 3

Plate transformed cells and incubate (45 minutes)

Incubation
24-48 hours

Day 4

View colonies and analyze results. (10-45 minutes)

The 4-day timeline of the Engineer-it Kit™ is made up of 4 days of hands-on activity and 24 to 72 hours before seeing results. *This does not include the time necessary to complete the pre-labs activities (on the next page).*

Six main steps make up the Engineer-it experiment:

1. Make selective and non-selective LB agar petri dishes
Day 1, 25 minutes
2. Grow blank cells
Day 1, 20 minutes + 12-24 hours incubation MAXIMUM
3. Make cells chemically competent
Day 2, 20 minutes
4. Engineer competent cells with DNA & start recovery
Day 2, 20 minutes + 12-24 hours recovery*
5. Plate recovered, engineered cells
Day 3, 20 minutes + 24-72 hr incubation
6. View results
Day 4, 20 minutes

* if you have to complete your experiment in 3 days instead of 4, you can shorten the recovery step from 24 hours to 1 hour before proceeding to step 5 on the same day.

Recommended pre-exercises

Amino Labs has many resources that you should use before they complete the hands-on experiment to maximize your understanding and success. These exercises are meant to ensure you know, understand, and complete all the experiment steps.

1. Virtual Bioengineer Simulator - Engineer-it Kit Edition

www.amino.bio/vbioengineer

This free simulator walks you through the entire Engineer-it Kit's materials and procedure. The simulator takes approximately 25 minutes to complete and is a fun, easy way to get ready for the real experiment!

2. Youtube follow along Engineer-it experiment video

<https://youtu.be/ZADzCQValI0>

The Engineer-it Kit 'follow along' video shows you the entire Engineer-it Kit's materials and procedure from the experimenter's point of view. You can view the video while answering the questions found at

www.amino.bio/engineer-it-youtube-prelab

3. Engineer-it Kit experiment procedure - a quick test

www.amino.bio/engineer-it-prelab-quiz

After you read through the manual steps to familiarize yourself with them, you can complete a short activity to see whether you are ready to start the experiment.

4 key pitfalls to avoid!

In the next pages are detailed, step-by-step instructions to complete the experiment and genetically engineer bacteria with DNA. **Please make sure you read all the steps before starting the hands-on manipulation;** some steps will be done in rapid sequence. The best way to ensure success is by completing 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. **You have to see the water bubbling!** Caution, the bottles will be hot!

2. In Step 2: **After streaking the blank cells, the colonies should be used no later than 24 hours after streaking.** Otherwise, your experiment will not work.

Note: If you can do the next session the next day but more than 24 hours from the previous session, here is a tip: you can start incubating the streaked petri dishes at 30°C overnight and turn the temperature up to 37°C at least 3 hours before you are ready to continue the experiment. This way, your cells will be optimal for the next steps. By growing cells initially at 30°C, they grow slowly, and then by changing to 37°C, they grow optimally.

3. In Step 3: When adding the Blank Cells to the Transformation Buffer and the DNA to the competent cells in Step 4, make sure you are doing this with the tubes on ice or in the cold startion of the DNA Playground set to 'Ice: 4°C' (not to 16°C.)

4. In Step 4: When adding the DNA to the tubes of competent cells, make sure you see liquid in the loop before adding it to their tube. Also make sure to twist the loop in the liquid for at least 5 seconds to ensure the DNA mixes in.

Experiment Protocol

0. Prepare your space

Goal Set yourself up for success.

Materials from the kit

(1) Inactivation bag

Materials not in your kit

(1) 1L discard container

Chlorinated bleach spray or wipes

Paper towels

(1/person) Pair of gloves



Make sure you have the necessary materials as explained on page 15, including gloves, microwave, and cleaner before you start and that you have read and understood the safety guidelines. You can download and print a safety checklist to complete before and after your experiment from www.amino.bio/checklist

0.1 Put on your gloves, and if you have one, your lab coat or apron.

0.2 Set your inactivation bag inside your disposable 1L yogurt-type container. You will use your inactivation bag to dispose of:

- your tubes of cells or DNA if you are not saving them for a future experiment*,
- any used inactivation loops,
- blank cell petri dish once they have been used to create competent cells
- any empty tubes like the agar, buffer and selection tubes,
- any gloves that have touched bacteria.

You can dispose of paper and plastic packaging in the regular garbage can, as well as gloves if you have not accidentally touched bacteria.

0.3 Wipe down your work surface with the chlorinated bleach spray or wipes.

0.4 Set down your DNA Playground, BioExplorer, or other personal lab equipment (you need an incubator for this experiment) on or near your work surface. Make sure it is level and on a stable surface. Refer to the instruction manual to make sure you know how to use your equipment safely.

* If you are saving the tubes of cells for a future experiment, place them back in their ziploc bag after use and store them in a refrigerator. We recommend you use a sealed plastic container to store all your experiment materials inside a refrigerator if you also use this to store food or drinks.*

Student's Experiment Protocol

1. Creating LB Agar Plates Day 1, 25 minutes

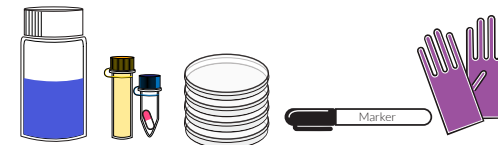
Goal Create non-selective and selective LB agar plates.

Materials from your kit - BAG 1

(1) 50 mL sterile water
(1) LB agar powder

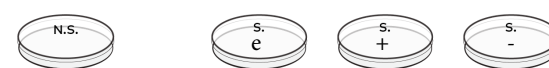
(1) antibiotic pill
(4) 6 cm petri dishes

(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 **1x** N.S. [your initials] **3x** S. [your initials]. Of these three, label one "+", one "-", and one with an "e".



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 start at 45 seconds but you have to see a rolling boil where many bubbles are constantly rising before you go to the next step. **!! If the water does not boil, the agar powder will not dissolve and your plates will not solidify !!** 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 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.



Make non-selective (N.S.) plate

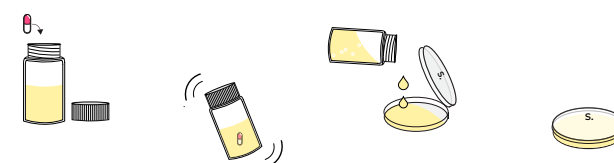
1.6 Pour molten LB agar in the **bottom half** of your N.S. Petri dish. Enough to fill the petri dish half-full. Swirl the plate to make sure the molten LB agar fills the bottom. If the agar does not cover all the bottom, gently tilt it. Place the lid 3/4 of the way back on so that the agar can cool and dry (solidify).



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

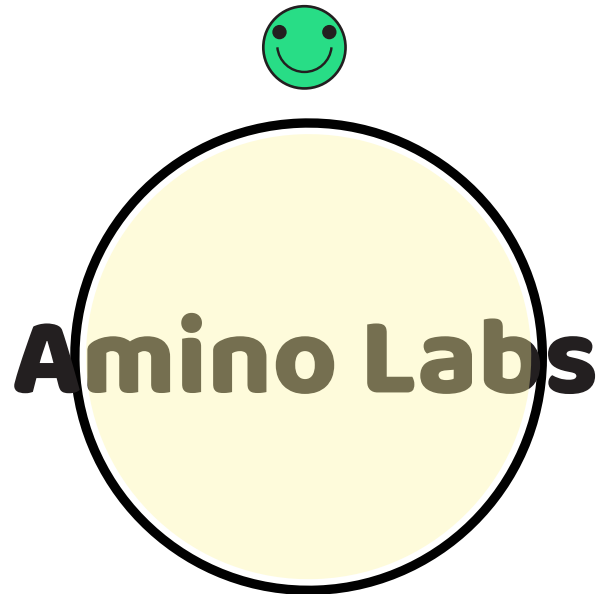


1.8 Let the LB agar harden. The non-selective plate and the "-" selective plate are used in the next step. Put the remaining selective plates in their original ziploc bag for later use, and store in a refrigerator.



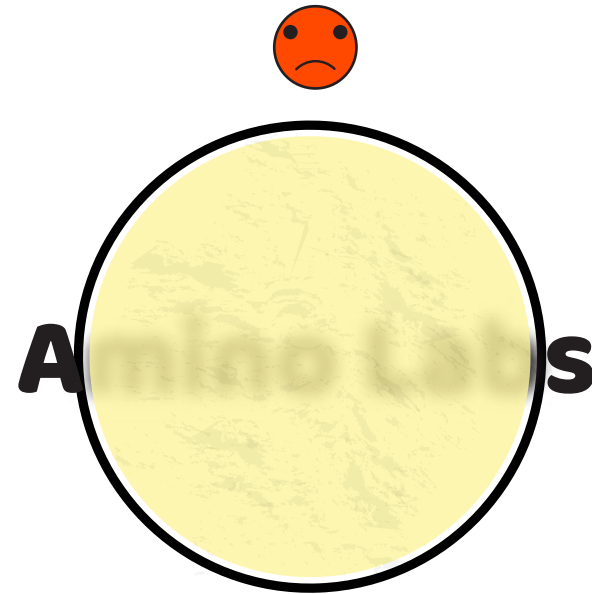
Checkpoint - Agar Plates

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.

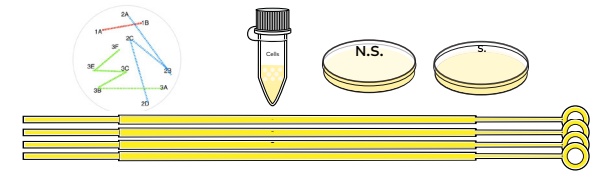
Unfortunately, if the agar does not solidify, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

2. Growing Blank Cells Day 1, 20-45 minutes + 12-24 hours wait time

Goal Streak petri dish with provided cells to get fast-growing, well-separated colonies that will be engineered. Fast growing cells take up DNA the best!

Materials from your kit

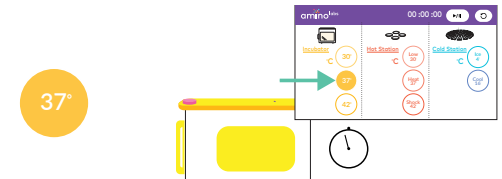
Non-selective & "-" selective plate	(4) Yellow Inoculation Loops
(1) Plate streaking stencil	(1) Stab of blank cells (Not Cells +)



Prepare

2.0 Inspect your N.S. and S.- plates for water droplets on the surface. If some are present, take the lid half-off and let the water evaporate before using.

2.1 Turn on your Incubator to 37°C



Streak your Plate

2.2 Place your N.S. Petri dish on top of the Streaking stencil. Take one yellow loop and dip it into the stab of blank cells. The stab will be jelly-like. Inspect your loop to make sure it looks wet to confirm you have collected cells.

2.3 Open your petri dish, and trace the line 1 of the stencil on the surface of the agar with the loop. Hold the loop at a 45° angle, similar to how you would hold a knife to butter a piece of bread. You can trace it back and forth a few times to deposit a lot of cells on this line. Discard the Loop in the discard container.

2.4 Using a new yellow loop, trace line 2 only once. Discard the loop.

2.5 Using a new yellow loop, trace line 3 only once. Discard the loop. Close your petri dish and set aside.

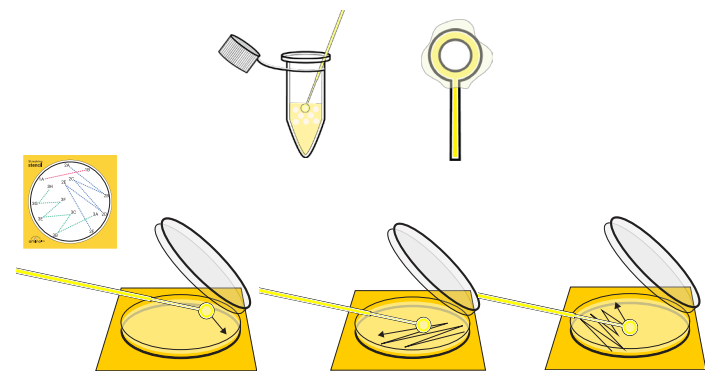
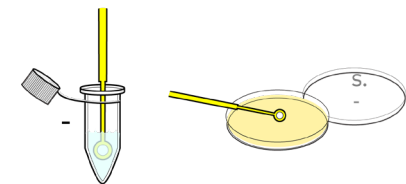


Plate your negative control ("-") plate)

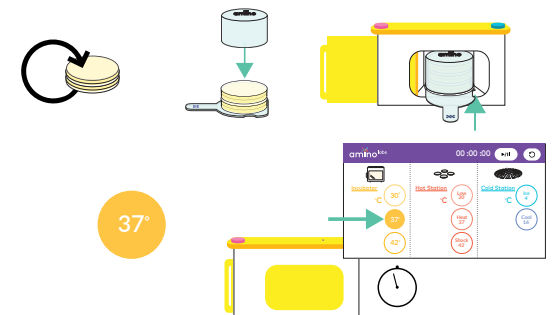
2.6 Take your Selective LB Agar plate labeled "-". Using a single yellow loop, dip into the blank cells and spread them across your agar plate in any pattern of your choosing. This is your negative control. On this selective "-" plate, the blank cells should not grow.

2.7 Close your tube of bacteria and place it back in the fridge in a ziploc bag if you want to keep them, discard them in the Inactivation bag if you do not. You no longer need them for this experiment.



Incubate Overnight

2.8 Flip your streaked N.S. & S. "-" plates upside down and stack them in a pile in an incubator set to 37°C. If you are using the DNA Playground, put your stack of plates on top of the incubator paddle, set the incubator humidity chamber on top and slide in the incubator. Close the incubator door and lock it using the incubator key.

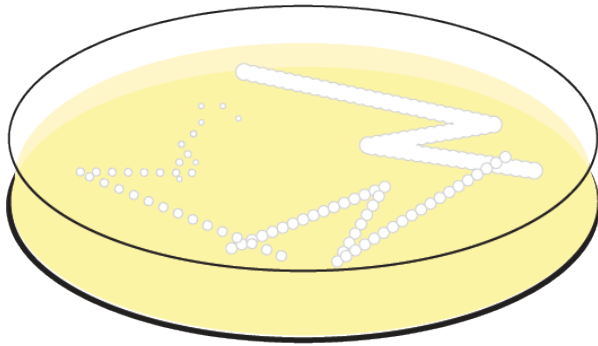


It is important to do the next steps in 12 to 24 hours so that your cells will be in their optimal growth phase. If you wait longer, your experiment will not work. Note that closer to 12 hours is better than closer to 24 hours!

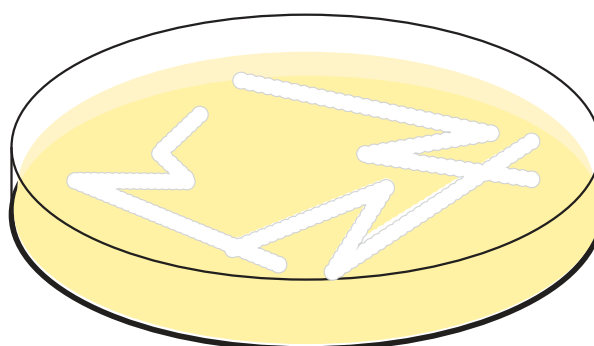
Timing Tip: If you are continuing the next day, but more than 24 hours later, start incubating the petri dishes at 30°C and either right before the end of day, or first thing in the morning, turn the temperature up to 37°C. Your cells will be optimal for the next steps. By growing cells initially at 30°C, they grow slowly. By then changing to 37°C, they grow optimally. Make sure you grow your cells at 37°C for at least 3 hours prior to starting the experiment again.

Checkpoint - Non-selective plate & blank cells

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

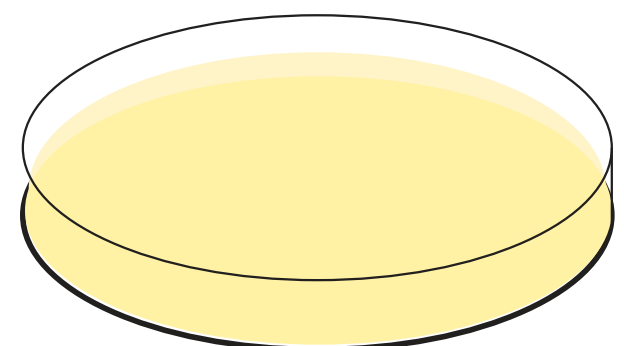


A perfect N.S. plate has lots of small individual white colonies (dots) of fast-growing bacteria after incubation. Proceed to the next page.



An okay N.S. plate has bacteria growth but few or no individual colonies (dots) after incubation. You will continue with the experiment by selecting the colonies that are on the edges of the dense lines of bacteria.

Note that your results may not be as good as when you start with a perfect plate. Don't worry though, practice makes perfect, and our Success Guarantee means you will have the chance to be successful if this try doesn't work.

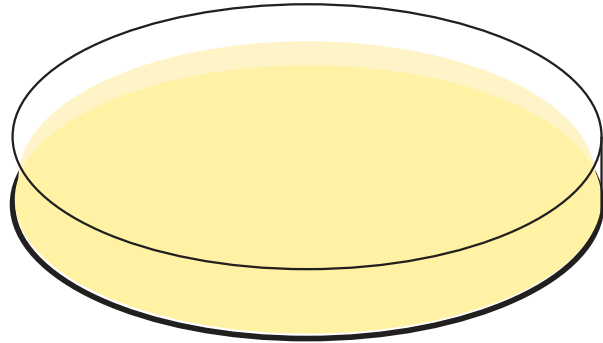


If you see no growth on your N.S. plate:

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: Growing Blank cells on this plate.
3. If you still have no colonies after repeating Step 2, complete the guide at www.amino.bio/troubleshoot

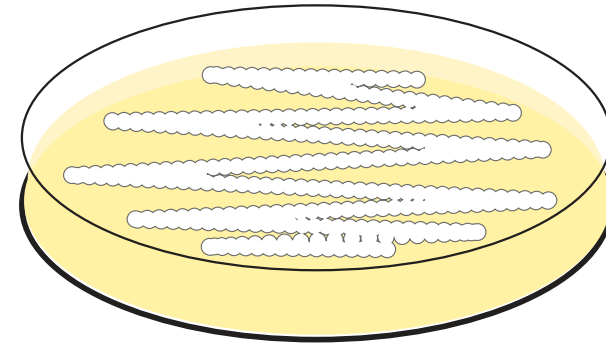
Checkpoint - Selective plate and “-” control

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



A perfect “-” plate has no growth on it. This is because the antibiotics do not allow the blank cells to grow. This means that you made your selective plates properly.

Move on to the next step.



If you see growth on your “-” plate, this suggests that there is not enough antibiotics.

Continue the exercise with your other selective plates. Note that your results may not be as good as if you had the right amount of antibiotics. Don't worry though, practice makes perfect, and our Success Guarantee means you will have the chance to be successful if this try doesn't work.

3. Making Chemically Competent Cells Day 2, 10-15 minutes

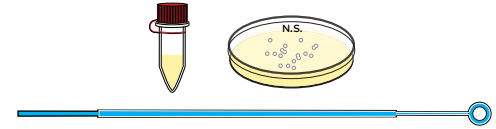
Goal Pick small colonies, and suspend the bacteria in cold transformation buffer, enabling bacteria to better take up DNA.

Materials from your kit

(1) Streaked N.S. Plate

(1) Blue Loop

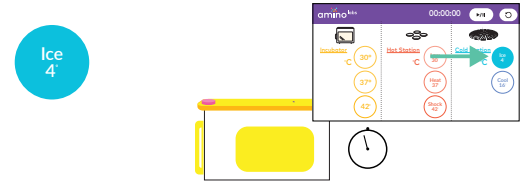
(1) T. Buffer Tube



Prepare

3.1 Make sure you have completed the Non-Selective Plate and “-” control Checkpoints. You can discard the “-” plate in your discard container.

3.2 Turn on the “Ice 4°C” setting on your DNA Playground™ or get your Ice bucket. *You can also prepare for next steps by turning on “Shock 42°C” on the Hot Station of your DNA Playground or setting your water bath to 42°C.*



Mix the cells and T. Buffer

3.3 Take your T. Buffer tube and make sure all the liquid is in the bottom of the tube by tapping it gently on the table surface. You should have approximately 5 mm of liquid in the bottom of the tube. Place it on the Cold station that has reached between 2-5°C or in your ice bucket for 2 minutes so that it cools down.

3.4 Take a blue inoculating loop and gently scrape it over small, well-separated colonies on your N.S. plate. Colonies that are ~1mm in diameter work the best. You want to collect ~10 or 20 of these colonies on your loop, enough so that you can see that the center of the loop is full.

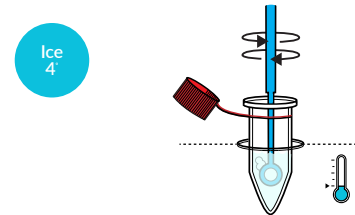
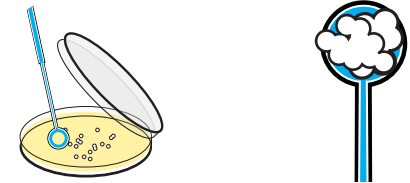
Tip: A colony is one of the white “dots” or “mounds” you see on your N.S. agar. The separated colonies are those that look like individual dots, not streaks or solid lines of white.

3.5 Immerse the loop with the bacteria in the cold Transformation Buffer without touching the sides of the tubes.

3.6 Twist the loop like a blender (or like you are trying to start a fire) to mix and suspend the cells in the liquid **while keeping the tube in the cold station.**

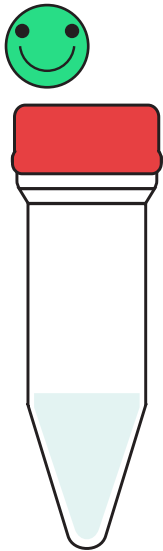
When successful, the solution should be cloudy, and there should be no clumps floating in the solution. You may have to mix vigorously for 30 seconds. If you see clumps, keep blending. You can lift the tube out of the cold station to see through, but replace it quickly into the cold station to keep it cool. Have a look at the next page to see what it should look like.

This step should take no more than 2 minutes! You need to move on to the next step of adding DNA quickly!



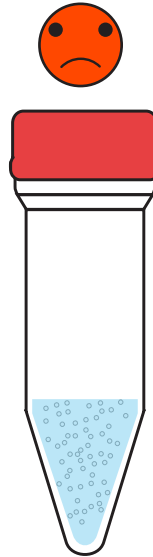
Checkpoint - Competent cells

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

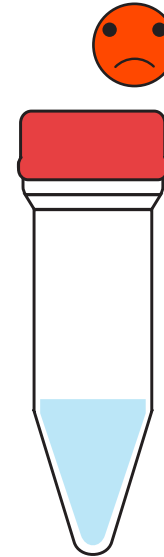


Cloudy liquid - Perfect!
The cells can now take up DNA.

Move on to the next step.



You can still see clumps... Keep
mixing until liquid is cloudy like
the image on the left.



Clear liquid? Add more cells and
mix until the liquid is cloudy like
the image on the left.

4. Transformation: HeatShock & starting the recovery Day 2, 15 min.

Goal Introduce a DNA plasmid into competent bacteria and recover the cells.

Materials from your kit

(1) DNA plasmid tube
(1) Blue Loop

(1) Competent Cells {from prior step}
(1) Recovery Media tube

Take the DNA

4.1 Dip a blue inoculating loop into the DNA tube and twist a few times. When you pull the loop out of the DNA tube, the hole of the loop should have liquid in it. This is the DNA you will use to engineer your cells. Make sure you can see liquid in the loop!

Mix the competent cells & the DNA

4.2 Slowly dip and spin the inoculating loop containing DNA into the competent cells you made in the previous step. Stir/swirl for 10 seconds to fully mix. Do this while the tube is on Ice / Cold Station set to 'Ice 4°C'. Discard the loop. * Do not reuse the inoculating loop! *

4.3 You will incubate your tube for 6 minutes on Ice / Cold Station, or until your Hot Station or water bath reaches 42°C. So, turn on "Shock 42°C" on your DNA Playground or set your water bath to 42°C if you have not done so. You can close and put the DNA tube back in the fridge.

HeatShock

4.4 Once the temperature is at 42°C - it is now time to get the DNA into the cells. Heat shock your cells, T. Buffer, and DNA solution by moving your tube to the Hot Station (42°C) / Water Bath (42°C) for 90 seconds.

4.5 After 90 seconds, immediately place your tube back on ice or on your Cold Station set to Ice 4°C for 2 minutes, or until your Hot Station/Water bath is at 37°C. Your cells are now engineered with the DNA! These are now called 'Transformed cells.'

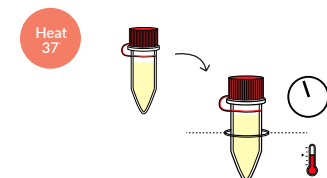
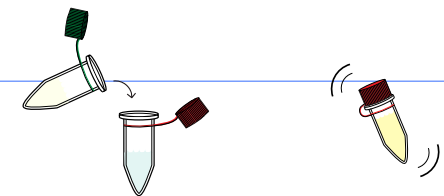
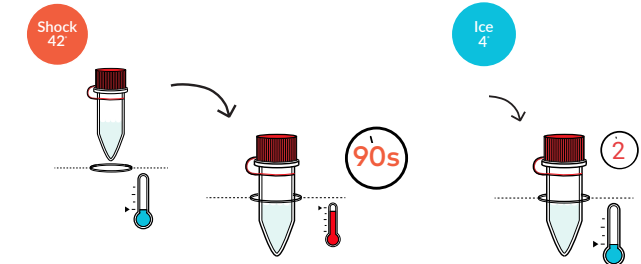
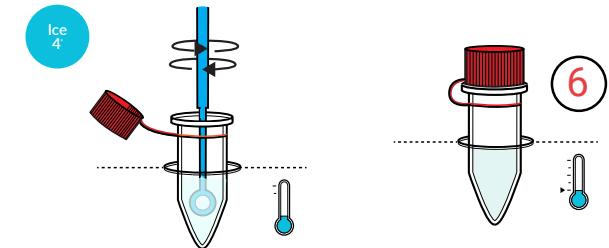
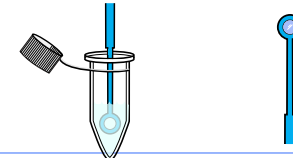
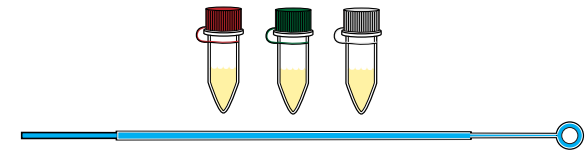
4.6 For the next step, turn your DNA Playground's Hot station to 'Heat 37°C' or adjust your water bath temperature to 37°C. (You can open the door of the incubator to help your DNA Playground's Hot Station reach 37°C faster).

Recovery

4.7 Pour the tube of Recovery media (~350 uL) into your tube of Transformed cells. Mix gently by inverting 10 times. Some Recovery media liquid will stay in the recovery tube. That is acceptable.

4.8 Place your tube with your Transformed cells in your DNA Playground's Hot station or water bath after making sure it is now at 37°C. You must also make sure the liquid is in the bottom of your tube, not on the sides. See www.amino.bio/whip-it to learn a fun technique, the Whip-it method, for moving liquid inside a tube.

4.9 Leave your tube to recover at 37°C for up to 24 hours (minimum of 30 minutes, with best results after at least 12 hours). If possible, briefly shake/mix the cells a couple times over the period. This step allows the Transformed cells to recover and start expressing their new DNA programs.



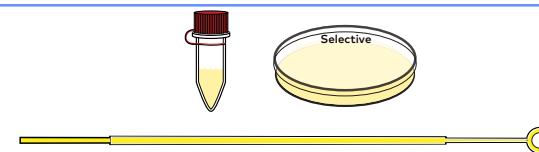
5. Plating Cells Day 3, 20 minutes + 24-72 hours wait

Goal Spread your transformed bacteria ("e"), positive control cells ("+") on selective LB agar plates

Materials from your kit

(2) Selective Plate (from step 1)
(2) Yellow Loop

(1) Transformed Cells (from prior step)
(1) "+ Cell" Positive Control tube



Prepare

5.1 After your 30 minutes to 24 hours of recovery period, turn on your Incubator at 37°C. Note that it can take about 1 hour to reach 37°C, but you can place your petri dishes in the incubator before it reaches temperature.

37°

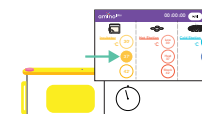


Plate your transformed cells ("e" plate)

5.2 On the Selective LB Agar plate labeled "e", pour 1/2 of your recovered Transformed cells from the previous step. Spread with a yellow inoculation loop so that the liquid covers the entire plate. Be gentle as not to puncture the agar! Discard the Loop.

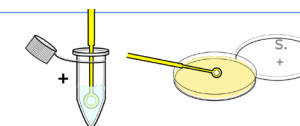


5.3 Leave the lid of the plate partially off to allow for evaporation. You will be ready to incubate when there is no pooling of liquid on the plate. It can take anywhere between 5 to 30+ minutes for the liquid to evaporate... it depends on the humidity and temperature of the room you are in.

5.4 Close the tube with your Transformed cells and discard or, if you like, you can keep the rest of your Transformed cells in a fridge and if you do not see any colonies of engineered cells after incubation, you can pour the rest on the plate and incubate again.

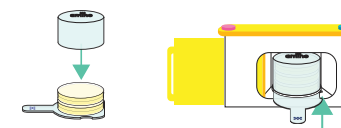
Plate your positive control ("+" plate)

5.5 Get the stab of "+" cells" and your Selective LB Agar plate labeled "+". Using a single yellow loop, dip into the + cells stab and spread them across your agar plate in a zig zag or any pattern of your choosing.



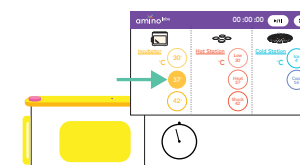
Incubate

5.6 Once there is no liquid pooling on any of the plates, place the plates upside down in the Incubator. If you are using a Minilab incubator, make sure to use the humidity chamber as well. Inverting the plates ensures that water vapor in the plates keeps the LB agar surface moist.



5.7 Incubate for 24-72 hours until the trait is expressed (for example, until you see a bright color if you selected a DNA program for color pigments). Swap the order of the plates, (i.e. the top one goes to the bottom and the bottom one to the top) every 24 hrs. **After the first 24 hours, switch the incubator temperature to 30°C for optimal color production** for the remainder of the incubation.

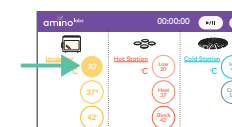
37°



Timing Tip: It is possible that petri dishes can dry out after 48 hours incubation, especially if you are located in a dry climate. If you are in a dry climate and you are incubating your cells over a weekend (72 hours or more), consider incubating your petri dishes at 30°C after an initial 24 hours incubation at 37°C. By incubating at a slightly cooler temperature later on, there will be less water evaporation from the LB agar. If you are unable to come back to lower the temperature to 30°C after 24 hours, you can simply incubate at 30°C the entire time.

After
24h

30°



6. Did your cells grow? Day 4+

Goal Verify if you have any "e" cells that have grown and are producing your specific DNA Program.

The cells will grow in colonies and start producing their new DNA program in the next 24-48 hours. Certain programs can take up to 72 hours to fully develop. Keep an eye out, and your camera ready to document!

Like you saw in Virtual Bioengineer, you will see colonies (dots) of engineered bacteria. Getting a single colony is a success! Many scientists doing real research often hope for a single colony! If you get more than one colony, this means you followed the procedure very well. If you repeat this experiment, you will very likely get more colonies than you did this time, because you will have practiced the procedure and like most things in life, practice makes perfect!

Your positive control: "+" cells" should be visible if your selective plates were properly made. The + cells will develop color much faster than your newly engineered cells and may be a different color than your experiment DNA. That's ok! The S. "+" plate is there to help you troubleshoot in case your experiment did not go according to plan. Note that the (+) plate bacteria are often purple or pink. The important part of the positive (+) control is that cells grow in selective LB agar plates and change color.

Problems? If you cannot see any growing cells at all after 48 hours, your experiment may have failed. Don't be discouraged! In science especially, failure is a chance to learn more. Ask your teacher to help you complete the troubleshooting guide at www.amino.bio/troubleshoot to get a new chance to try the experiment, thanks to the success guarantee!

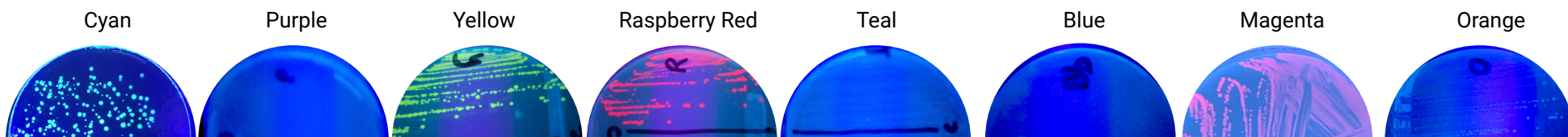
7. "e" Plate Expected Results

Each DNA program produces its own color pigment. Most of these pigments can be seen under regular lighting, and some fluoresce "glow" under UV light (also known as a black light). The cyan pigment can only be seen under a UV light - it will appear white under regular light, the same color as your non engineered cells. Have a look below to see what to expect.

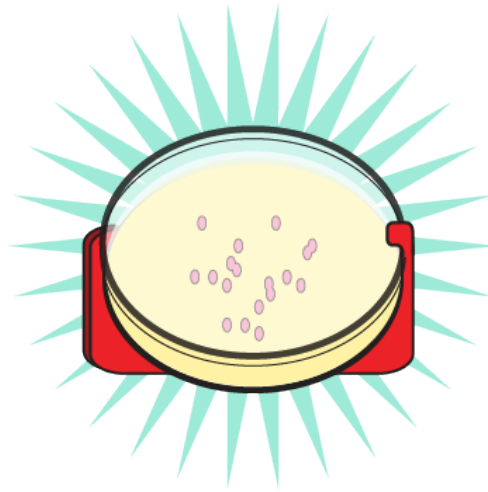
Under regular light



Under blacklight



CONGRATULATIONS



You did it! Using your Engineer-it Kit™, you inserted a DNA Program inside single-celled organisms called bacteria and had the bacteria execute that DNA Program to produce something for you. Just like scientist and industries do every day inside their large laboratories. Great! We hope you enjoyed the experience and will continue to experiment in the field of genetic engineering. You can preserve your results with our Keep-it Kit™ or create living art from your colored bacteria with the Canvas Kit™. If you plan to complete a Plate Extract-it Kit, then you'll want to keep your cells in the fridge, or move directly onto the Plate Extract-it Kit instructions

Show off your results with your friends, our community and us too! We'd love to see your work. Our Twitter, Instagram, Facebook, and Youtube can be found under 'aminobiolab'

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

Storage, Disposal, Clean Up

After you see your results, all experiment Petri dishes, tubes of cells and loops should be in the inactivation bag in your discard container. 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: Make sure all bacteria, agar, tubes, loops, paintbrushes, Petri dishes, contaminated gloves, and other non-paper material you are not keeping are in the inactivation bag. Remember 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](https://www.youtube.com/c/AminoLabs).

Spray some chlorinated bleach cleaner in the discard container once emptied if it has become contaminated by experiment materials. 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 **Deoxyribonucleic acid**.

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, like 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.bio

Heatshock: is when the cells are moved from ice-cold to warm temperature, typically 42°C, 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 tradi-

tional 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

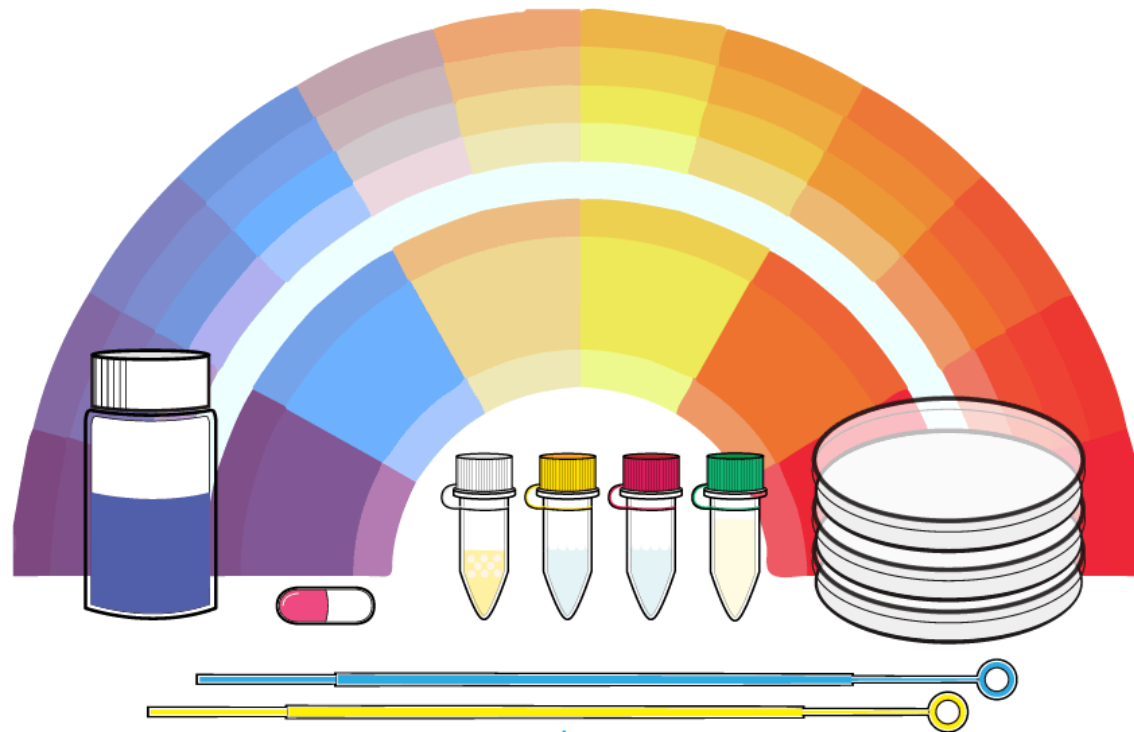
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