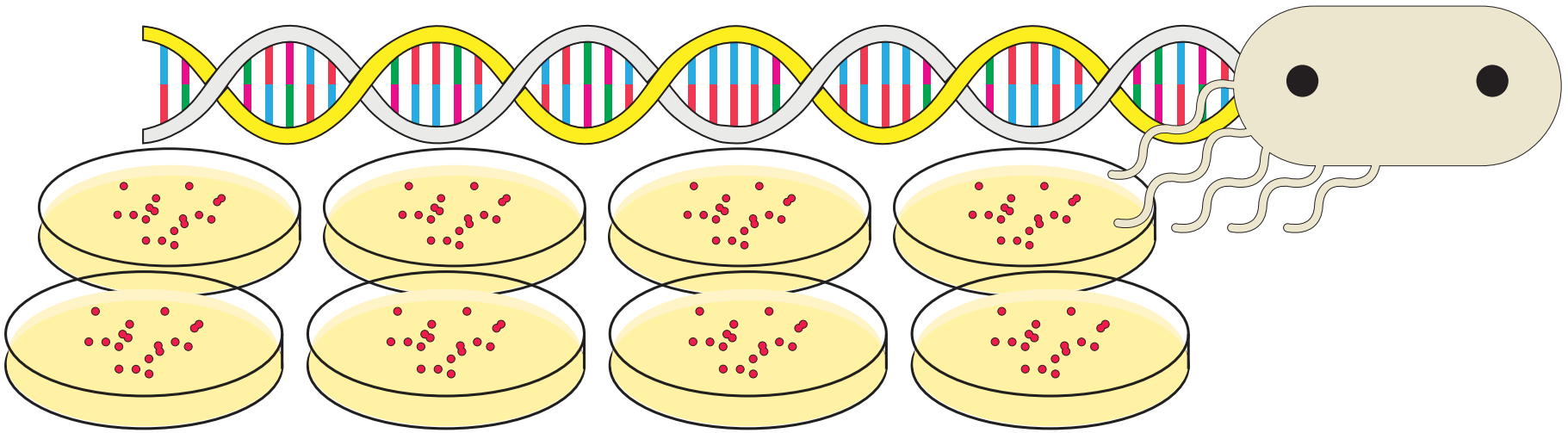
ENGINEER-IT KIT™

CLASSROOM & GROUP MANUAL

For use with the **group / large** kit size or the **Full Rainbow** pack

a (4x) 60-minute class periods experiment procedure



www.amino.bio

Version 2.5, Oct 2023

ENGINEER-IT KIT™

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Getting Ready - for educators

The Experiment - for educators & students

For educators

The student experiment protocol:

For educators and students

More information - for educators & students

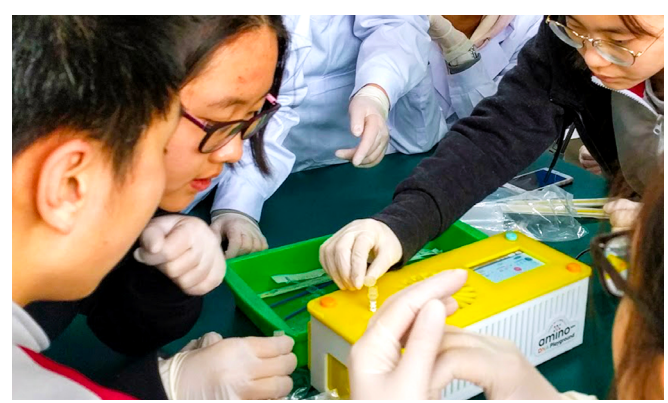
Welcome! Let's get started

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

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

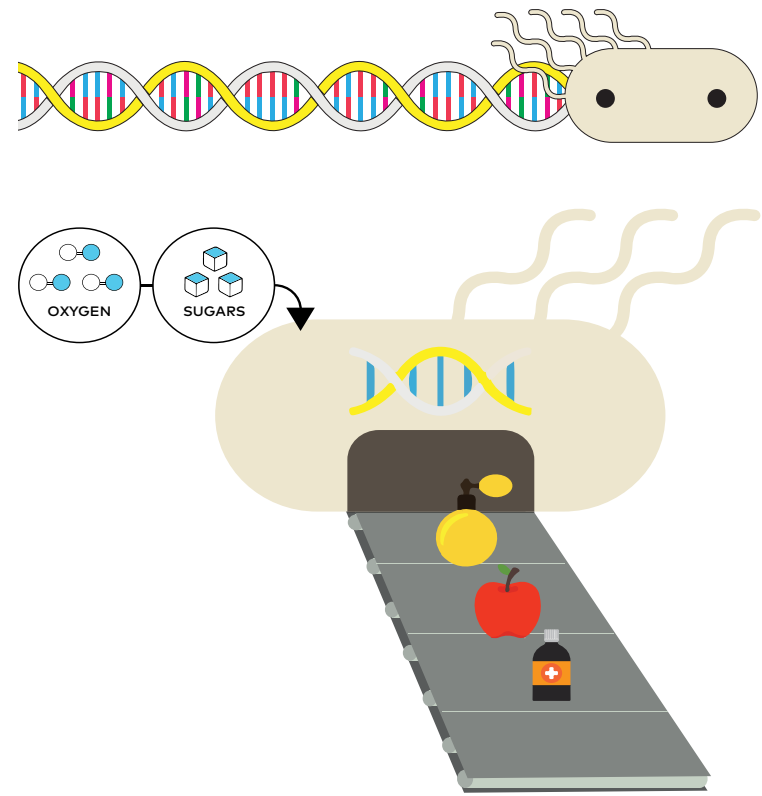
Amino Labs is excited to welcome you to the world of the genetic engineering with the Engineer-it Kit™, Canvas Kit™ and our entire ecosystem of easy-to-use, easy-to-succeed at products!

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

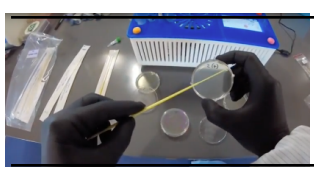
How will students learn?

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

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

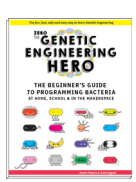
We also have additional resources to help students and teachers go further:

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



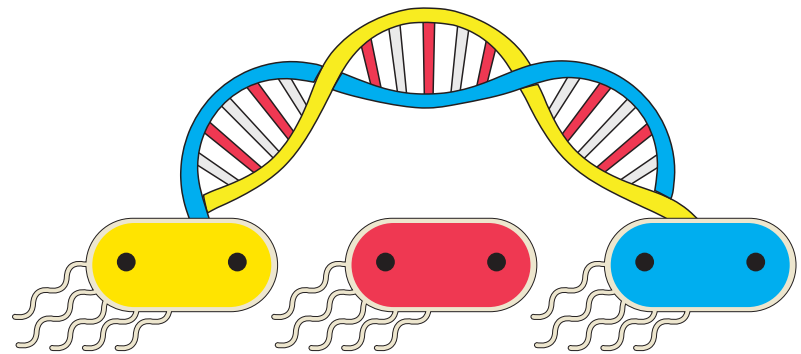
View **Real-time tutorials** videos at youtube.com/c/AminoLabs. These videos can even be viewed in class to help the students prepare for the hands-on experiments! See the pre-labs page (p.16) for more info.

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

Discover 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 classroom-specific manual is aimed at teachers using the group size of the Engineer-it kit. The group kit is perfect for a class of 32 divided into groups of 4. Diagrams on page 10-11 show how the kit breaks down into student packs and how to divide your students into groups.

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

What does the kit allow students 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." Learn more about DNA and genomes in the *What is DNA?* simulator on amino.bio

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***

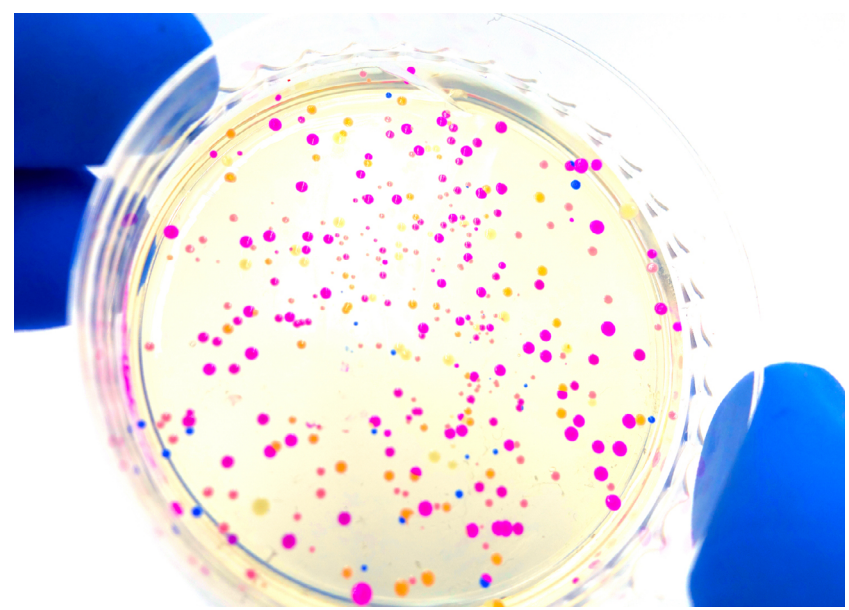
3. **Make *E. coli* cells chemically competent (able to take up DNA plasmids)**

4. **Transform the competent cells with DNA program**

5. **Recover the engineered cells**

6. **Grow the engineered cells on plates**

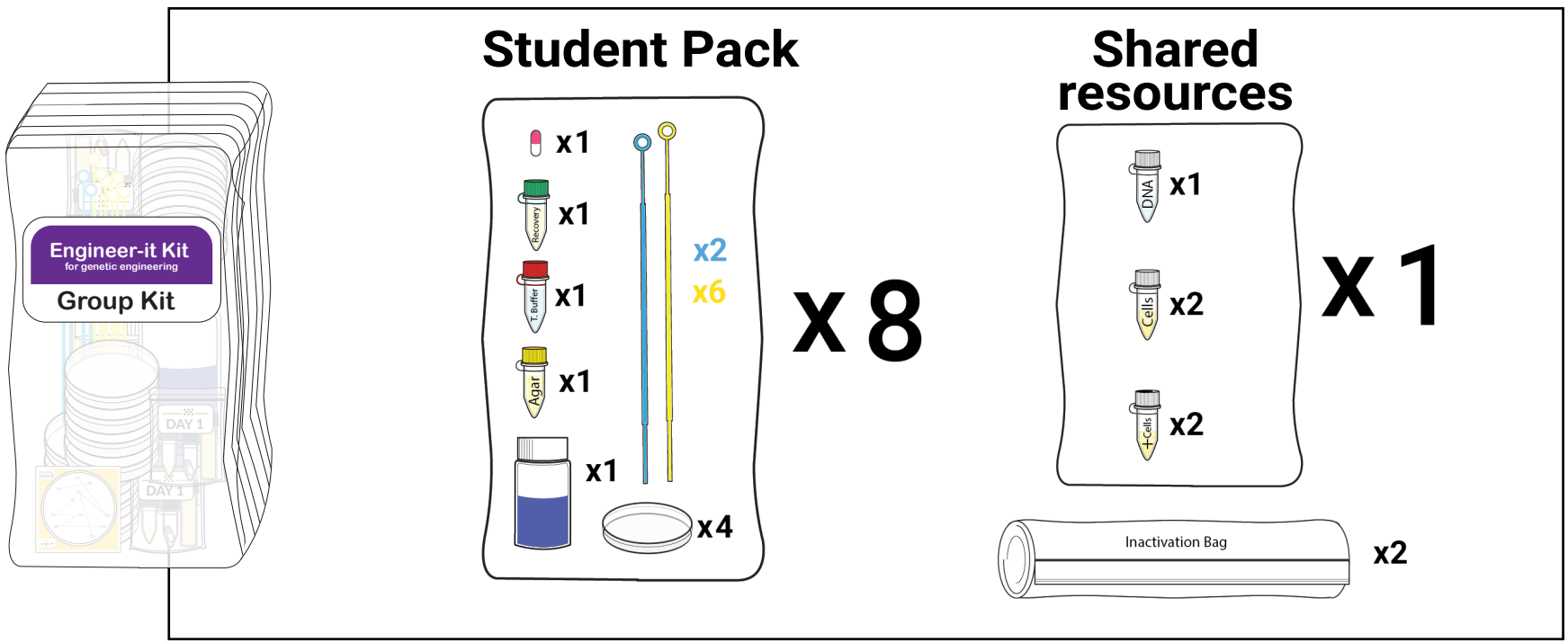
7. **View the results**



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

Group kit size: who gets what?

The Engineer-it Kit™ for groups contains 8 individually-wrapped student packs and one shared resources bag containting the blank cells, the positive control cells, the DNA program, and the inactivation bags which are shared by everyone. With 8 individual packs, you can group students in pairs, threes, or four to fit your total group size.



Using the Full-Rainbow Kit

The Engineer-it Kit™ Full-Rainbow kit is made up of 10 individually-packed kits, each containing everything needed to grow and engineer bacteria. Each kit has a different DNA program to produce different pigment in the cells. You can hand out one kit to each student group, reminding them they have their own cells and DNA in the kit. In the next pages, you will find descriptions of the each kit's content.

Kit components

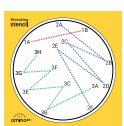
In each student pack:

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

Small Blue Loops: Small inoculating loops are used for transferring 1 uL of liquid and other tasks. uL stands for µL which means microliter, so one-millionth of a liter. These replace costly pipettes.

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

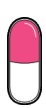
Petri Dish / Plate: 6cm Petri dishes are large enough for this lab experiment and help save on the cost of reagents and reduce waste.



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.

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% w/v). Agar is both the surface the bacteria grow on and the food they eat to grow.[1]



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

<u>In the shared resources bag:</u>

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]

+ 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 coloful pigments.

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.

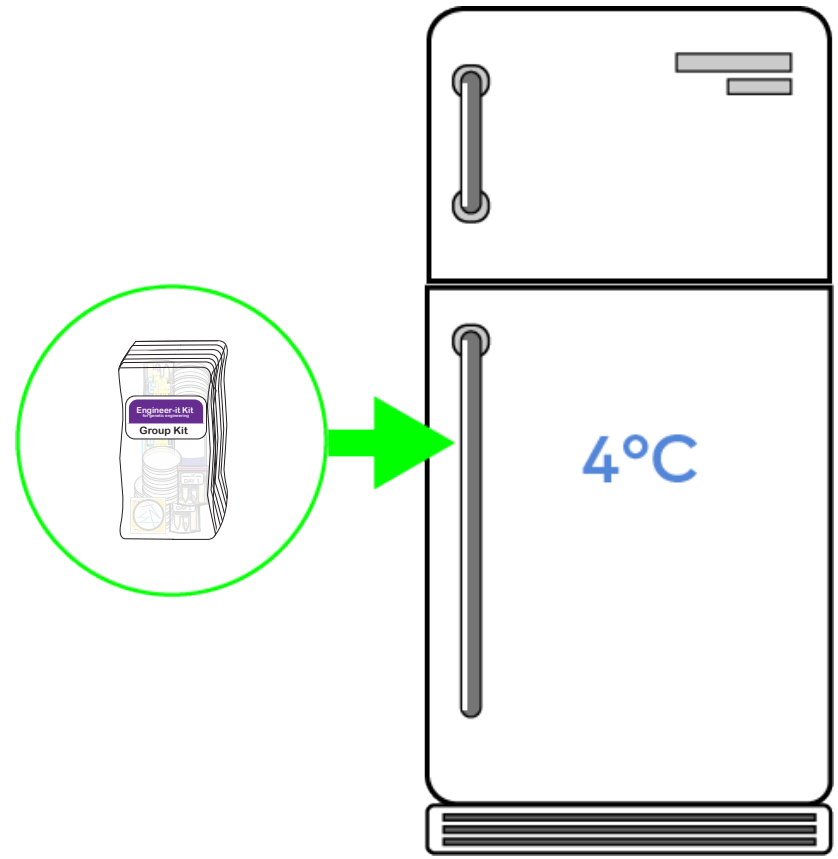
[1] For education purposes only.

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, the most important bag to refrigerate is the Shared Resources bag. Note that you need to keep your students packs and shared resources bags from different types of kits separate. For example, if you opened up your Engineer-it kit(s) and also have Canvas kit(s), or other group kits, you will need to identify them as the contents will be different.

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

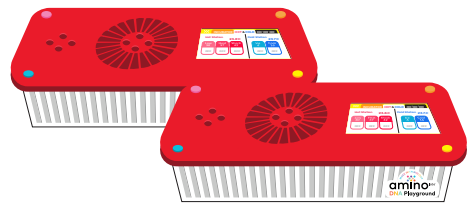
Transformation Buffer: 50 uL tubes
Recovery media: 350 uL tubes
Cells /+Cells: K12 *E. coli* Stab

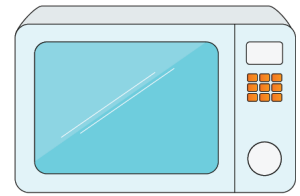
Solid growth media:
LB agar powder (1.6 g)
50 mL sterile water

Necessary equipment

For Best results:

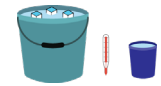
- **DNA Playground:** One DNA Playground Classroom size per up to 4 student groups or one DNA Playground Home size per student group.
- **Microwave:** 1 for the group
- **Sharpie-type marker:** 1 per group





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 - or go to this direct link: https://www.youtube.com/watch?v=LEsv0Qvbczs



Necessary safety supplies

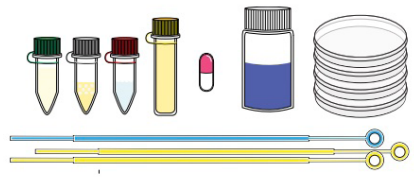
Disposable container 500ml-1L
to hold tubes, loops and other contaminated waste (e.g., yogurt container, plastic cup). 1 per station

Latex or nitrile gloves
like the ones found at a pharmacy. 1 pairs/student if students keep & reused each day, or 4 pairs/student if not saved & reused.

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

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

4-class period timeline

Pre-labs →  Engineer-it Kit

Pre-lab(s)

Students can prepare with:
1. Virtual Bioengineer Simulator (~25 mins)
2. Watch experiment 'real-time' videos & take quizz (~65 mins)
3. Read experiment procedure & do the quick test (~30 mins)

Day 1

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

Incubation
12-24 hours

Day 2

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

Recovery
12-24 hours

Day 3

Students plate transformed cells and incubate
(45 minutes)

Incubation
24-48 hours

Day 4

Students view colonies and discuss 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) which can be done in or out of class time.*

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

*Look at page 17, Step 2 for tips on making this work if your classes are more than 24 hours apart...

Recommended pre-labs

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

1. Virtual Bioengineer Simulator - Engineer-it Kit Edition

www.amino.bio/vbioengineer

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

2. Youtube follow along Engineer-it experiment video

https://youtu.be/ZADzCQVall0

The Engineer-it Kit experiment "follow along" video show your students the entire Engineer-it Kit's materials and procedure from the experimenter's point of view. The students can view the video as a group in class while answering the worksheets 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

Have students read through Practicing safe science, the Student experiment protocol and the Glossary pages of this manual. Following this, have the student complete this short activity to test whether your students read the experiment procedures and are ready to start the experiment. The students can complete the test online on a computer or mobile device or you can chose to print it out for the class.

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

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

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

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

Note: **If your classes are 1 day apart, but there is more than 24 hours in between them** (ex: you have a morning period on Day 1 and an afternoon period on Day 2)**:** 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 the class starts. 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.

If your classes are more than 1 day apart (ex: one class every week) see the next page for instructions.

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 students 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 the students see liquid in the loop before adding it to their tube. Make sure to twist the loop in the liquid for at least 10 seconds to mix the DNA.

Specialized timeline:

How to wait ~1 week between each 'Day' of experiment.

Scenario: Your class may not be able to meet for 4 days consecutively but rather, your class meets once a week. Here are a few additional steps you can do if this is the case for your class/group. This will require that you complete some preparatory materials before the second experiment class. You will also combine "Day 2" and "Day 3" into a one class period.

Materials needed: To use this modified timeline, you will need to either: keep one of the Engineer-it Kit student pack to use yourself or have a Petri Dish Refill Kit, or some LB agar and petri dishes you may already have on-hand.

DAY 1 session

a) Students start their Engineer-it kit(s) as per the manual instructions for DAY 1.

b) Students begin the incubation of streaked blank cells & negative controls petri dishes as per the manual. Keep one of the blank cell tube to use later on.

c) After 12 to 24 hours of incubation, remove the petri dishes from the incubator, place in a ziploc-type bag, and refrigerate until the next session with the student. This will allow students to see their results as if no extra time had passed. You will not use these streaked cells for the DAY 2 activities: they will only be used for students to see their results.

12 to 24 hours before the DAY 2 session

e)12 to 24 hours before the 'DAY 2' session takes place, make your own non-selective petri dishes using either a Student pack that you've kept, the Petri Dish Refill, or materials you have. Follow the *Step 1* instructions of the manual to make Non Selective agar petri dishes. Do not add any antibiotics pill.

f) Wait for the agar to solidify and streak the blank cells as per the manual *Step 2* instructions. If you have more than 4 student groups, the students will be able to share your 4 petri dishes of streaked cells as there are plenty of colonies. If you have enough material, streak as many petri dishes as there are student groups. Because you have a limited number of yellow loops to streak with, follow this instructional video: www.amino.bio/superstreak

g) Incubate as directed, 12 to 24 hrs MAXIMUM before the start of the DAY 2 session.

DAY 2 session

h) Students will look at their own streaked blank cells and negative control results on the refrigerated petri dishes.

i) Students will complete the DAY 2 steps, using the freshly incubated blank cells that you made.

j) Students will only recover their bacteria for 45 minutes or more, and continue with the Day 3 manipulations on the same day;

k) Students will incubate their experiment as directed for 48 to 72 hours.

l) After such time, or once you see bacteria grow and color on student Petri dishes, remove the Petri dishes from the incubator, place in a ziploc-type bag and refrigerate until the next session.

Last session

k) Students view their results on the refrigerated petri dishes and inactivate all the materials as per instructions.

With these modified instructions, it is not possible to use the 4-day timeline where the engineered cells are left in the recovery media for 24 hours before plating. Engineered cells cannot be left in the recovery media for more than 24 hours before being plated and cannot be refrigerated for more than 24 hours to "pause" the experiment. You can choose to plate the experiment for the students if you only have one hour classes.

For a more detailed instruction, go to page 35-36

Teacher Experiment Setup

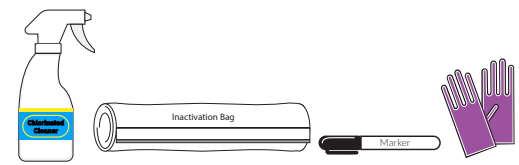
0. Prepare your classroom space

Goal Set yourself up for success.

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

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

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



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

0.0 Have students download/print the manual and read *Practicing safe science*, the 4 pitfalls to avoid, the *Student experiment protocol* (including the *Checkpoints)*, and the *Glossary* pages.

0.1 Set down the DNA Playground(s), or other lab equipment (37°C incubator, hot water bath, ice buckets. Ice is only needed on Day 2) on or near the students work stations. Make sure the equipment is level and on a stable surface. Refer to the instruction manual to make sure you know how to use your equipment safely.

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

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

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

- any used inactivation loops,
- all empty tubes like the agar, buffers and selection tubes,
- any gloves that have touched bacteria.

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

0.5 Ask the students to put on their gloves.

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

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

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

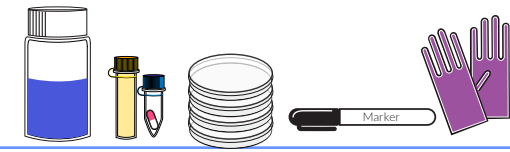
Student's Experiment Protocol

1. Creating LB Agar Plates Day 1, 25 minutes

Goal Create non-selective and selective LB agar plates.

Materials from your kit - **BAG 1**

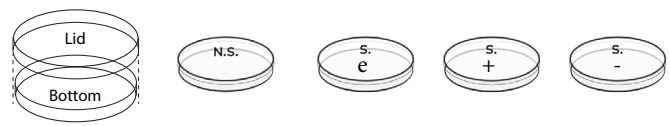
(1) 50 mL sterile water
(1) LB agar powder
(1) antibiotic pill
(4) 6 cm petri dishes
(1) Sharpie marker



Prepare

1.1 Label the bottom of each petri dish with a sharpie-type pen. Label **1x** N.S. [your initials] **3x** S. [your initials]. Of these three, label one "**+" for your positive control,** one"**-" for your negative control,** and one with an **"e" for the cells you will engineer.** (The e stands for experimental sample.)

Note: *the bottom is the part that has the smaller diameter of the two: the bottom fits inside the lid.*



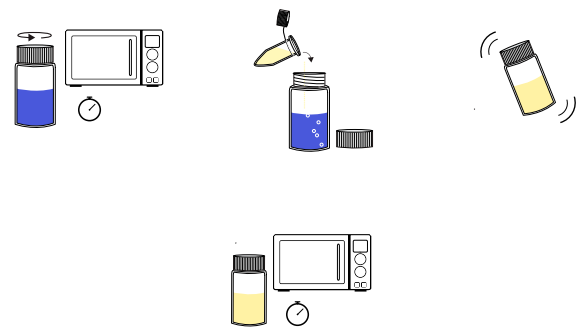
Mix the Agar

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 dissolved and your plates will not solidfy !!** Careful, the bottle will be hot.

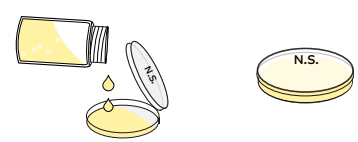
1.4 Add the tube of agar powder to the boiling water. Careful, the water is hot! Some agar powder may "clump" around the lip of the tube due to the water evaporation. This is okay, we have accounted for this possible loss.

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).

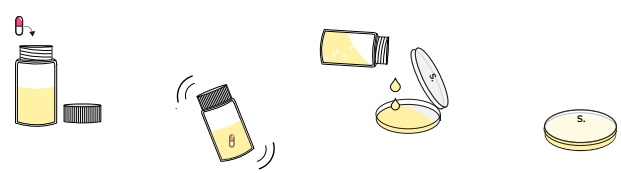


Make selective (S.) plates

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. Don't swirl too vigorously as it would create bubbles in the agr. The gelatin capsule may not fully dissolve. The important thing is that the contents of the capsule do dissolve.

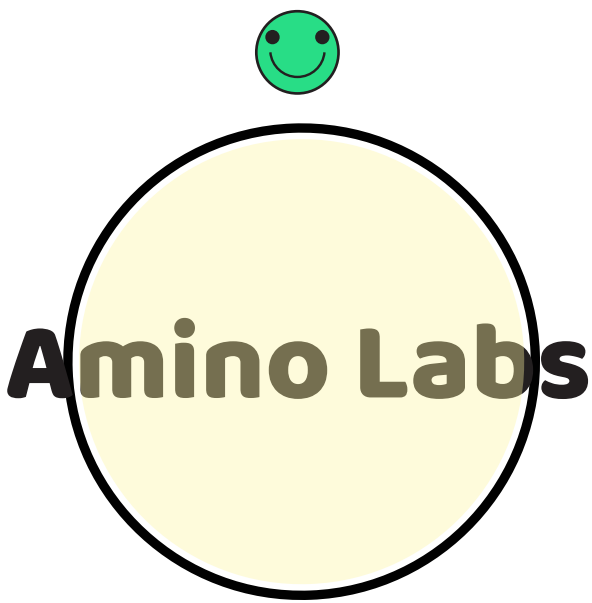
1.7 Once the antibiotic pill is dissolved, pour the molten LB agar into the **bottom half** of the 3 remaining petri dishes. Place the lids 3/4 of the way back on so that the agar can cool and dry (solidify).

1.8 Let the agar harden. The N.S. 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.



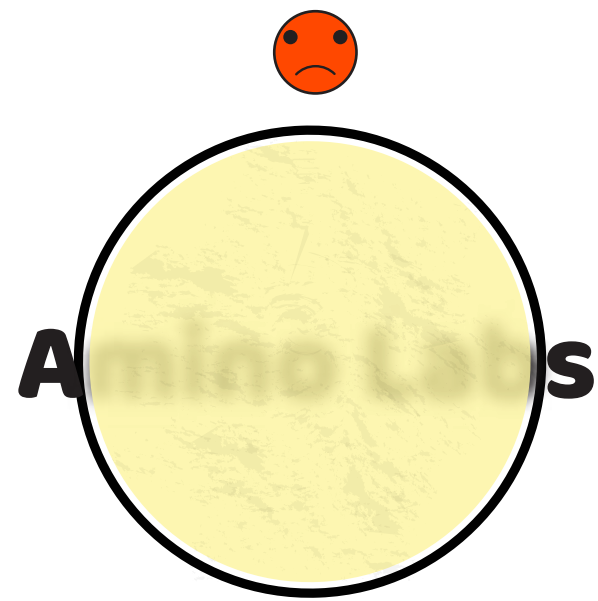
Checkpoint - Agar Plates

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.

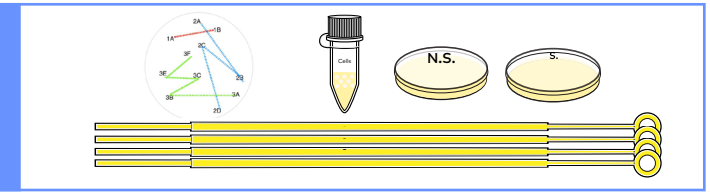
Unfortunately, if the agar does not solidify, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

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

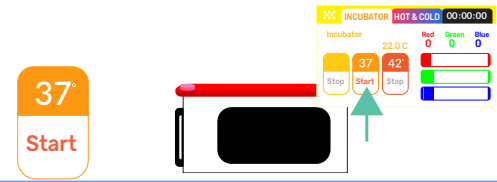
Non-selective & "-" selective plate
(1) Plate streaking stencil
(4) Yellow Inoculation Loops
(1) Stab of blank cells ***(Not Cells +)***



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 1of 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 time 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.

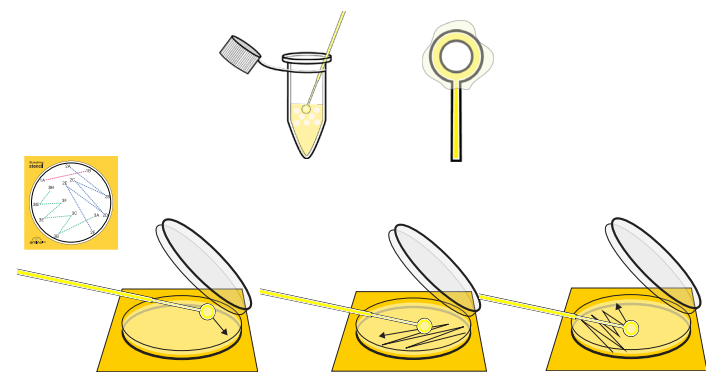
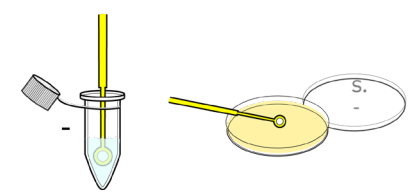


Plate your negative control ("-" plate)

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.

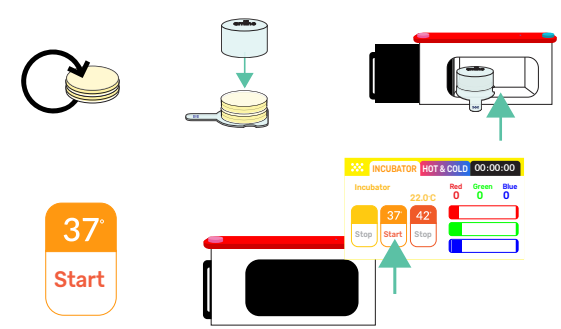


Incubate Overnight

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.

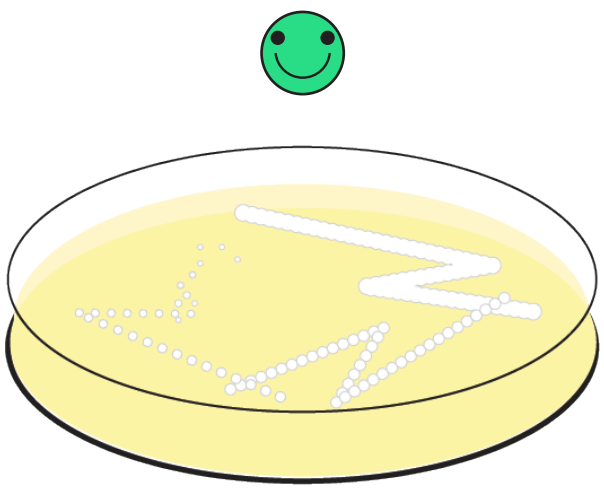
It is important to do the next steps in 12 to 24 hours so that your cells will be in their optimal growth phase. If you wait longer, your experiment will not work. Note that closer to 12 hours is better than closer to 24 hours!

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.

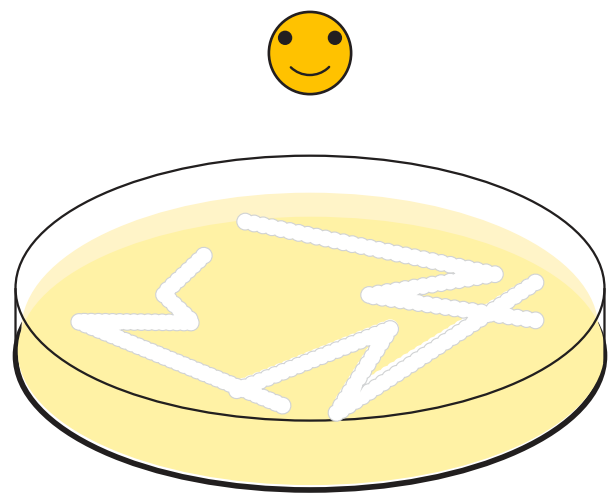


Checkpoint - Non-selective plate & blank cells

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

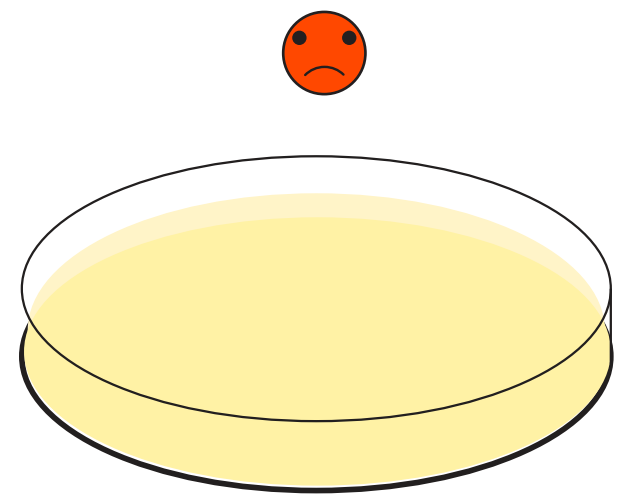


A perfect N.S. plate has lots of small individual white colonies (dots) of fast-growing bacteria after incubation. Proceed to the next page.



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:

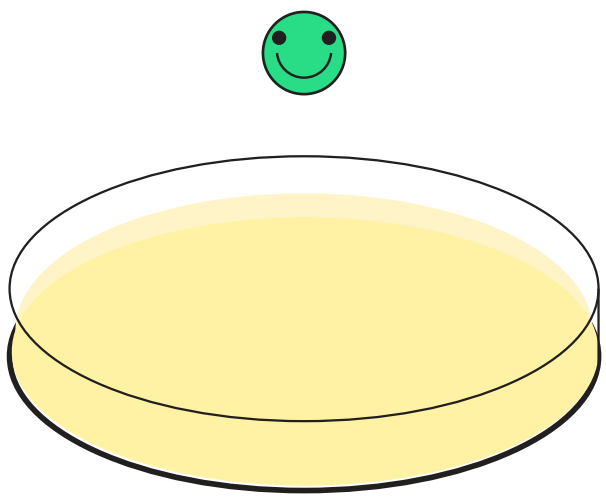
1. If your incubator was not at 37°C or is homemade, incubate for another 24hrs.

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

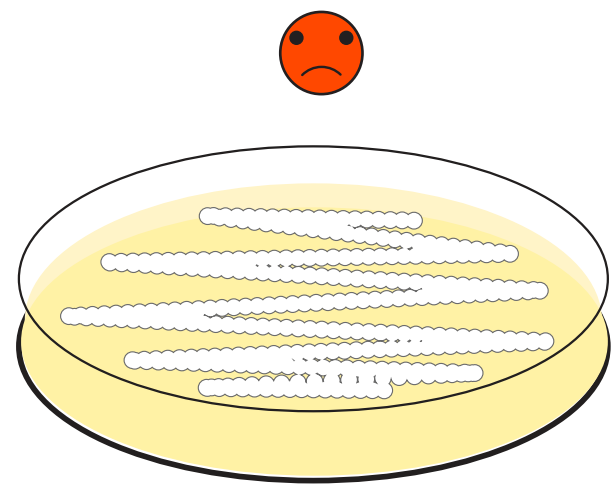
Checkpoint - Selective plate and “-” control

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.

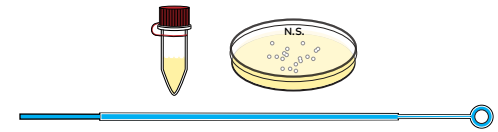
Continue the exercise with your other selective plates. Note that your results may not be as good as if you had the right amount of antibiotics. Don’t worry though, practice makes perfect, and our Success Guarantee means you will have the chance to be successful if this try doesn’t work.

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

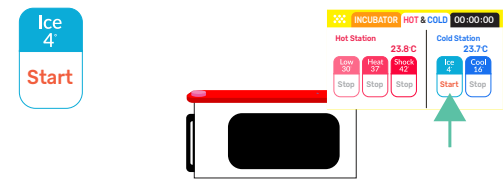
(1) Streaked N.S. Plate
(1) Blue Loop
(1) T. Buffer Tube



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.*



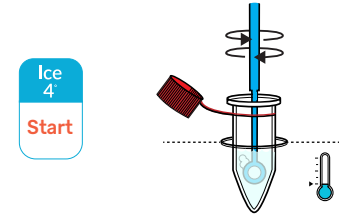
Mix the cells and T. Buffer

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.



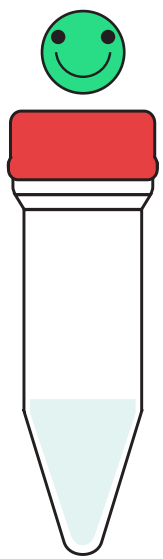
3.6 Twist the loop like a blender (or like you are trying to start a fire) to mix and suspend the cells in the liquid **while keeping the tube in the cold station.**

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.

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

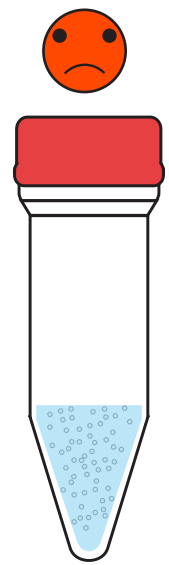
Checkpoint - Competent cells

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

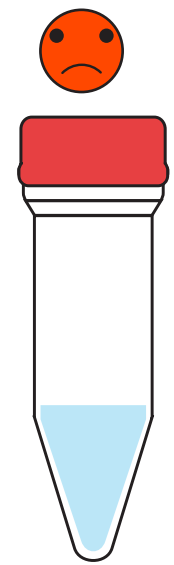


Cloudy liquid - Perfect!
The cells can now take up DNA.

Move on to the next step.



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



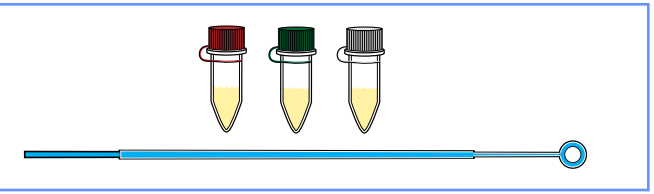
Clear liquid? Add more cells and mix until the liquid is cloudy like the image on the left.

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

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

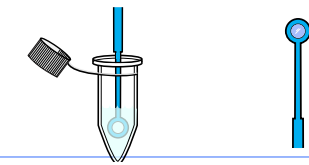
Materials from your kit

(1) DNA plasmid tube
(1) Blue Loop
(1) Competent Cells {from prior step}
(1) Recovery Media tube



Take the DNA

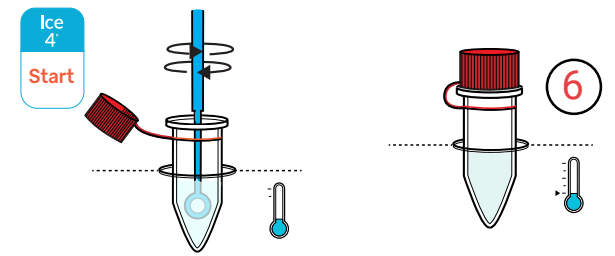
4.1 Dip a blue inoculating loop into the DNA tube and twist a few times. When you pull the loop out of the DNA tube, the hole of the loop should have liquid in it. This is the DNA you will use to engineer your cells. Make sure you can see liquid in the loop!



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.

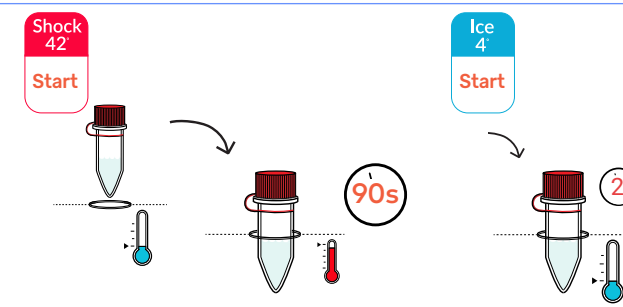


HeatShock

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).

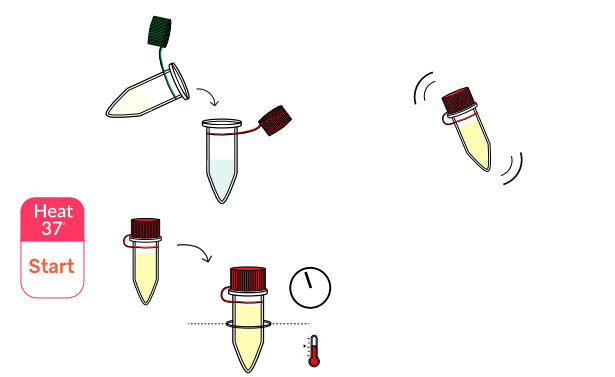


Recovery

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.



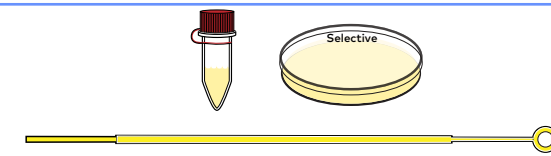
Teacher Timing Tip: If due to unforseen circumstances tomorrow's class is cancelled, allow the tubes to recover overnight as per the instructions and then place them in a fridge until they can be plated. This will temporarily put the cells in suspended animation. The final results will not be as good as plating freshly recovered cells, but you will still get results!

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 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.

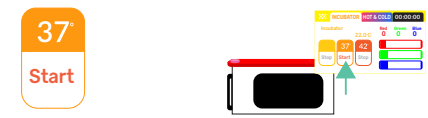
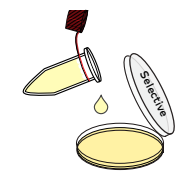


Plate your transformed cells ("e" plate)

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.

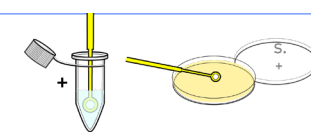


5.3 Leave the lid of the plate partially off to allow for evaporation. You will be ready to incubate when there is no pooling of liquid on the plate. It can take anywhere between 5 to 30+ minutes for the liquid to evaporate... it depends on the humidity and temperature of the room you are in.

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..

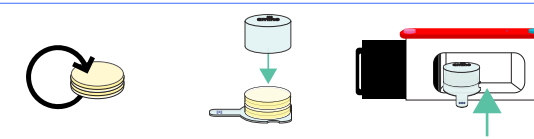
Plate your positive control (“+” plate)

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.

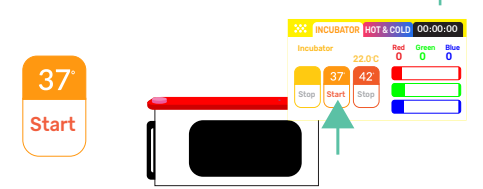


Incubate

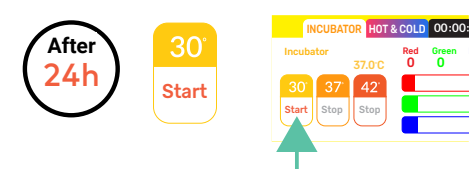
5.4 Once there is no liquid pooling on any of the plates, place the plates upside down in the Incubator. If you are using a Minilab incubator, make sure to use the humidity chamber as well. Inverting the plates ensures that water vapor in the plates keeps the LB agar surface moist.



5.5 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.



Teacher 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



6. Did your cells grow? Understanding your results. Day 4+

Goal **Verify if you have any cells that have grown**

The cells you engineered will grow in colonies and start producing their new DNA program in the next 24-48 hours. Keep an eye out, and your camera ready to document!

As you saw in Virtual Bioengineer simulator, you will see colonies (dots) of engineered bacteria appear on your petri dish over time. 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 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.

Understanding your results: the "+" petri dish and "e" petri dish

The "e" petri dish: This is the petri dish on which you will see your newly engineered cells grow. This is the petri dish on which you will have poured and spread out your transformed cells in the recovery media after the recovery period. Remember that in this case, "e" stands for "experimental sample." Your engineered cells will start out as white dots on your plates (the colonies) and as they grow bigger, they will also start producing their color. The color of the cells depends on the DNA program that was included in your kit. You can see what each color is expected to look like below.

The "+" petri dish: You'll remember that on your petri dish identified with +, you streaked your positive control (+) cells. These cells should be visible if your selective plates were properly made. The + cells will develop color much faster than your newly engineered cells on your "e" petri dish. The positive control cells may be a different color than your experiment DNA. The (+) plate bacteria are purple or pink. That's ok! The "+" plate is there to help you troubleshoot in case your experiment did not go according to plan. 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 cells at all after 48 hours on your petri dishes, your experiment may have failed. Don't be discouraged! 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!

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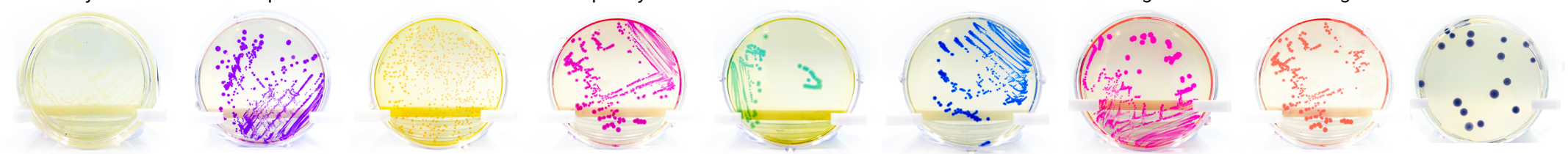
7. Expected colors on the "e" petri dish, depending on your kit's DNA.

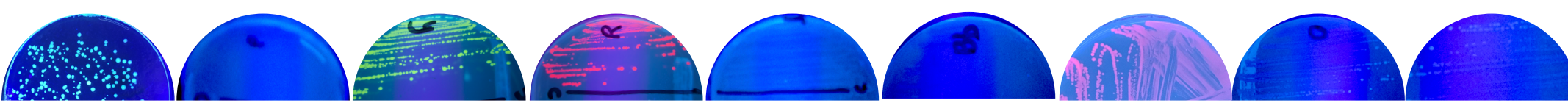
Each DNA program produces its own color pigment. Most can be seen under regular lighthing, and some fluoresce ("glow") under UV/black light. Note that cyan can only be seen under a UV light - it will appear white under regular light, the same color as your non-engineered cells. Have a look below to see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

Under regular light

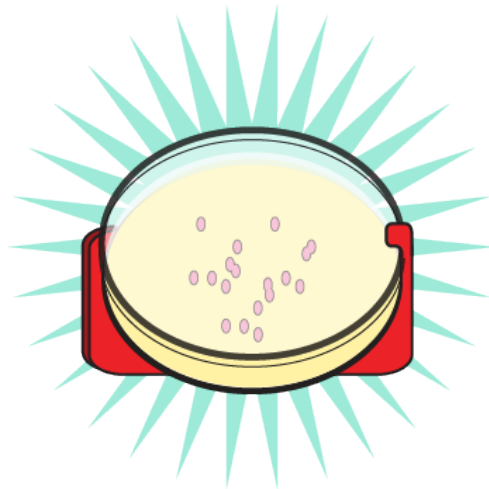
Cyan | Purple | Yellow | Raspberry Red | Teal | Blue | Magenta | Orange | Pearl



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

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

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

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

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

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

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

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

Glossary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Transformation: See competent cells.

Troubleshooting

Here are some possible common issues:

Your agar is too wet/ doesn't solidify:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

HOW TO USE THE ENGINEER-IT KIT & PETRI DISH REFILLS TO COMPLETE THE GENETIC ENGINEERING EXPERIMENT ON NON-CONSECUTIVE DAYS.

FOR EXAMPLE, IF YOU ONLY HAVE YOUR STUDENTS ONCE A WEEK.

Extended instructions

First class: Experiment "DAY 1": making LB agar Petri dishes, streaking blank cells, and negative control.

a) Students start the Engineer-it kit following the instructions for DAY 1: making their Non-Selective and Selective LB agar Petri dishes, streaking and incubating blank cells, and their negative control.

b) Students start the incubation of their blank cells (N.S. petri dish) & negative control (S (-) petri dish) as per the manual. Then, place the students' leftover kit material and unused Petri dishes in the refrigerator until the next class. Keep one tube of blank cells.

c) After 16 to 24 hours, remove the Petri dishes from the incubator, place them in a ziplock-type bag, and refrigerate them until the next class. This way, your students can see their grown cells and negative control as if no extra time had passed. Do not use these streaked cells for the experiment's DAY 2 activities. The cells will no longer be suitable for genetic engineering. Instead:

16 to 24 hours before the students return to class for Experiment "DAY 2.""

d)16 to 24 hours before the 'DAY 2' session takes place, use the Petri Dish Refill to make non-selective Petri dishes. (Follow the Engineer-it kit manual instructions to make and pour Non-Selective agar Petri dishes (do not add the antibiotics pill).

e) Once the agar has solidified, streak these new non-selective Petri dishes with the blank cells and three inoculation loop method as per the instructions. Streak as many Petri dishes as there are student groups*.

f) Incubate the petri dish at 37 °C, as per the manual, for 16 to a maximum of 24 hours before your students start "Day 2" of the experiment.

** If you have more student groups than you have LB agar Petri dishes, student groups can share the blank cells Petri dishes*

Second class: Experiment DAY 2 & DAY 3: competent cells, adding DNA, recovering cells, and plating.

g) Hand out your students' refrigerated kit materials and their incubated Petri dishes from Day 1, so they can look at their results. Once students have observed their blank cells and negative controls, have them discard the Petri dishes (to prevent any confusion at the next step).

h) Hand out the freshly incubated blank cells you made for them. They will use these for today's experimental steps (instruction manual "Day 2" and "Day 3").

i) Students will follow the "Day 2" instructions to make their cells competent, add the DNA, and Recover their cells. Complete this part at the start of class to leave as much time as possible for the cells to recover.

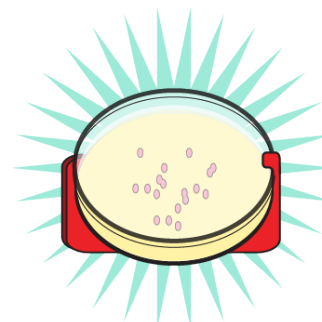
j) Leave the cells to recover at 37 °C for 45 minutes or more.** Then, 10 to 15 minutes before the end of class, have your students plate their recovered cells on the S(e) petri dish and streak their positive control on S(+) by following the "Day 3" instructions.

k) Students start the incubation at 37 °C per the manual.

l) After 48 to 72 hours of incubation, remove the students' Petri dishes from the incubator and place them in a zip-lock-type bag. Refrigerate until the next class.

Third class: Experiment DAY 4: viewing the results

m) Students view their results on the refrigerated Petri dishes. Congratulations!



** While a recovery time of 45 minutes to a few hours works, we've found that letting the cells recover for 12-24 hours provides the best results. You can plate the cells for your students if you wish. Do not let the cells recover for more than 24 hours, as they will no longer be viable.

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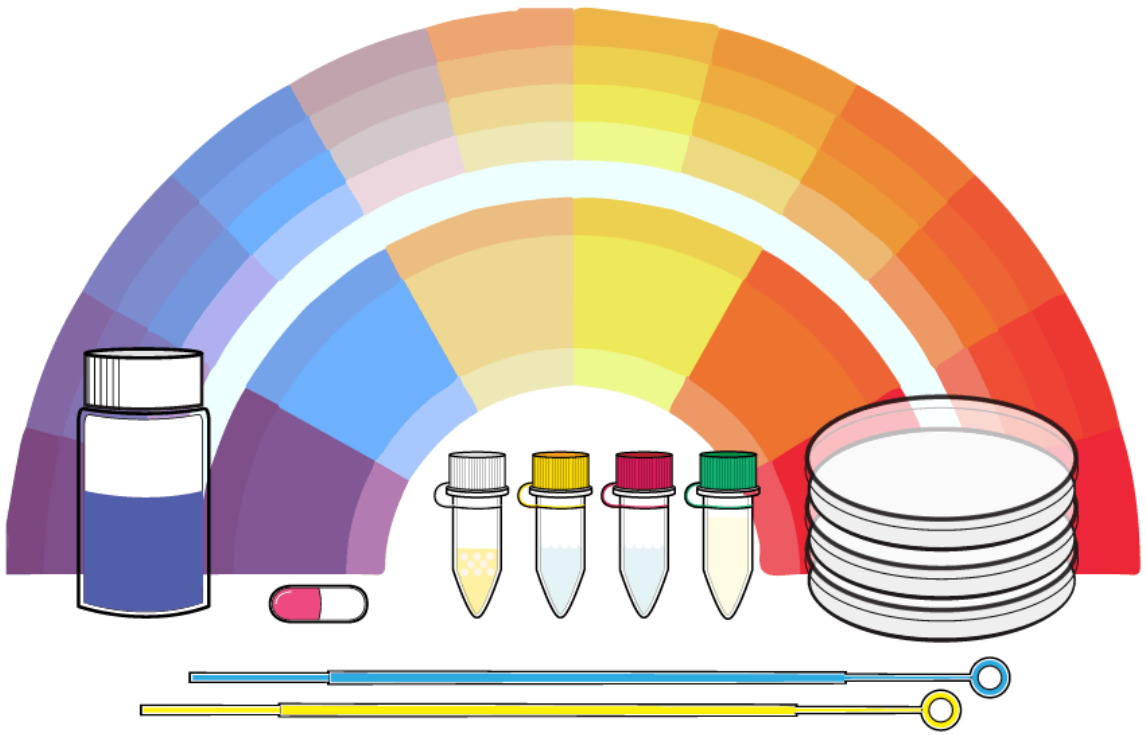
All Amino Labs products, from the hardware to DNA, are invented, designed, manufactured in and shipped from our laboratory-workshop in Canada. We'd love to hear your feedback and suggestions so that we can continue to make our products better and more fitting to your needs. Answers to your questions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

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



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