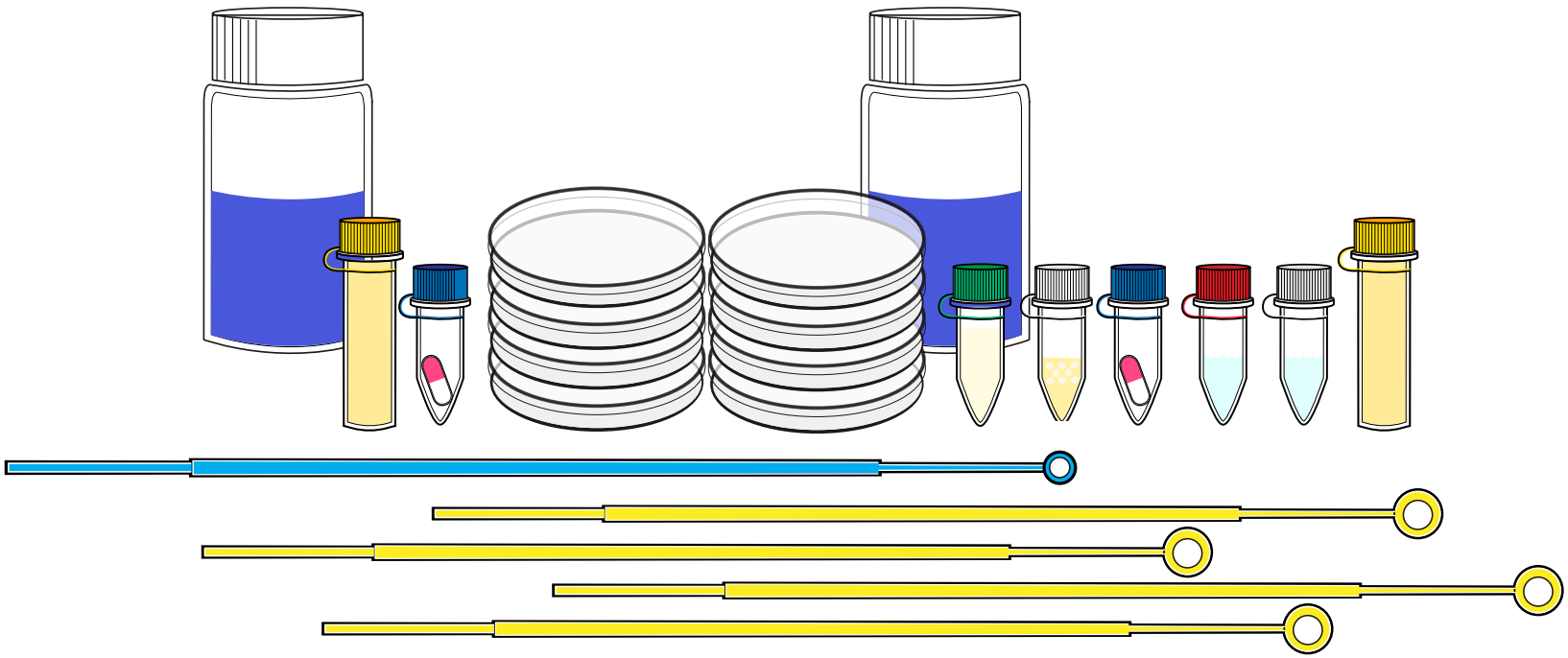
HEAT-IT KIT™

MANUAL



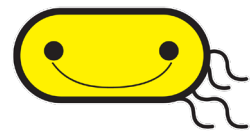
www.amino.bio

Version 3.3, Oct 2023

HEAT-IT KIT™

User Manual

Welcome! Let's get started



This User Guide was created to help you get the most out of your Amino Labs Experience. Even if you are familiar with genetic engineering, science or other Amino Labs™ products, please take the necessary time to read through this guide. This will ensure you practice safe science, store, use and get the most out of your Kit and 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. And, 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 advanced genetic engineering with the Heat-it Kit™ and our entire ecosystem of easy-to-use, easy-to-succeed at products!

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

Practicing Safe Science

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

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

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

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

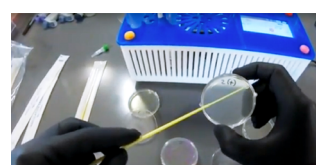
If you would like to do a short Online lab safety course for your edification, we recommend a Government of Canada course: www.amino.bio/biosafety

How will I learn?

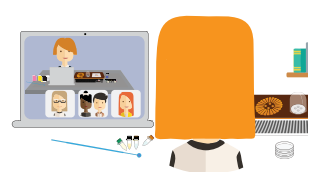
Learning and prototyping with genetic engineering and cells is becoming accessible to newcomers of all ages and backgrounds thanks to dedicated scientists and kits such as the one you are about to use. One of the easiest way to learn a new science, hobby or topic is by trying it, hands-on. Everything you need to complete the science is included; each ingredient in the kit is pre-measured and labeled for a stress-free experience and our minilabs decrease setup time, mess, guesswork and the need to collect and calibrate multiple machines. These instructions aimed to be easy-to-follow for everyone but may contain some new terms. Therefore, we have added a glossary at the end. We also have additional resources to help you go further in your learning:

- An essential addition to our ecosystem is the free **Virtual Bioengineer™ simulation** developed with the educators at the Biobuilder Educational Foundation. A 20 minutes guided experience that makes it easy to practice using your DNA Playground™ and Heat-it Kit™ beforehand. It also includes additional information on the manipulations and a more in-depth look into DNA and genetic engineering. We recommend it strongly! Complete it online at www.amino.bio/vbioengineer.

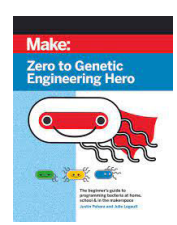
- View **real-time tutorials**, including video tutorials for each day of the genetic engineering experience on our Youtube channel. The genetic engineering days are under 'Engineer-it Kit,' the same procedures as the first 3 days of your Heat-it Kit experience . Subscribe! youtube.com/aminobiolab



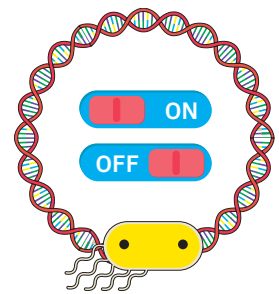
- Would you like for an Amino Labs team member to tutor you through your journey? Try the **Virtual Tutoring or Teacher Professional Development sessions**, a multi-day experience via video www.amino.bio/collections/virtual-sessions



- Are you interested in the theory behind the experiment and in going deeper on the science, learning protips and completing more advance genetic engineering?The ***Zero to Genetic Engineering Hero*** **book** is for you. Find out more at www.amino.bio/book

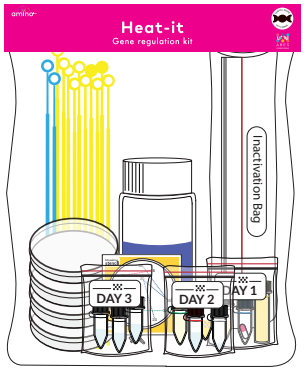


Discover your Heat-it Kit™



The Heat-it Kit™ has everything needed to insert a DNA Program (plasmid) into bacteria, and with your help, have them produce what the DNA "tells" them. In this case, a colored protein that won't appear until you 'turn it on' using temperature. That is because, in the plasmid, there is a 'genetic switch' that is 'turned off'. That's right! Just like in computer programming, behaviors in the cells can be turned 'on' and 'off' using temperature, chemicals, light and other environmental conditions. If you've used one of our Engineer-it kits before, you'll have engineered cells to produce colored proteins which you can see as colored colonies on the petri dish. In that case, the colored proteins were created automatically because the genetic switch was in the 'on' position. With the Heat-it kit, a similar plasmid will be inserted into cells. Once grown, you will then turn the genetic switch 'on' using a higher temperature than what the cells were incubated under. You can learn more about genes, genetic switches, and how to use chemicals, light, and temperature to activate DNA programs by reading Chapter 7 of the *Zero to Genetic Engineering Hero* book, www.amino.bio/book. You can also have a look at the Induce-it Kit™ and the RGB kit™ in our store to explore our other genetic switch experiments. www.amino.bio

Note! Amino Labs kits contain the same or similar ingredients in most of its kits but it is important to remember that the bacteria, the antibiotics, DNA plasmid, and the different buffers are made to fit perfectly together. Make sure you keep kit components separated!

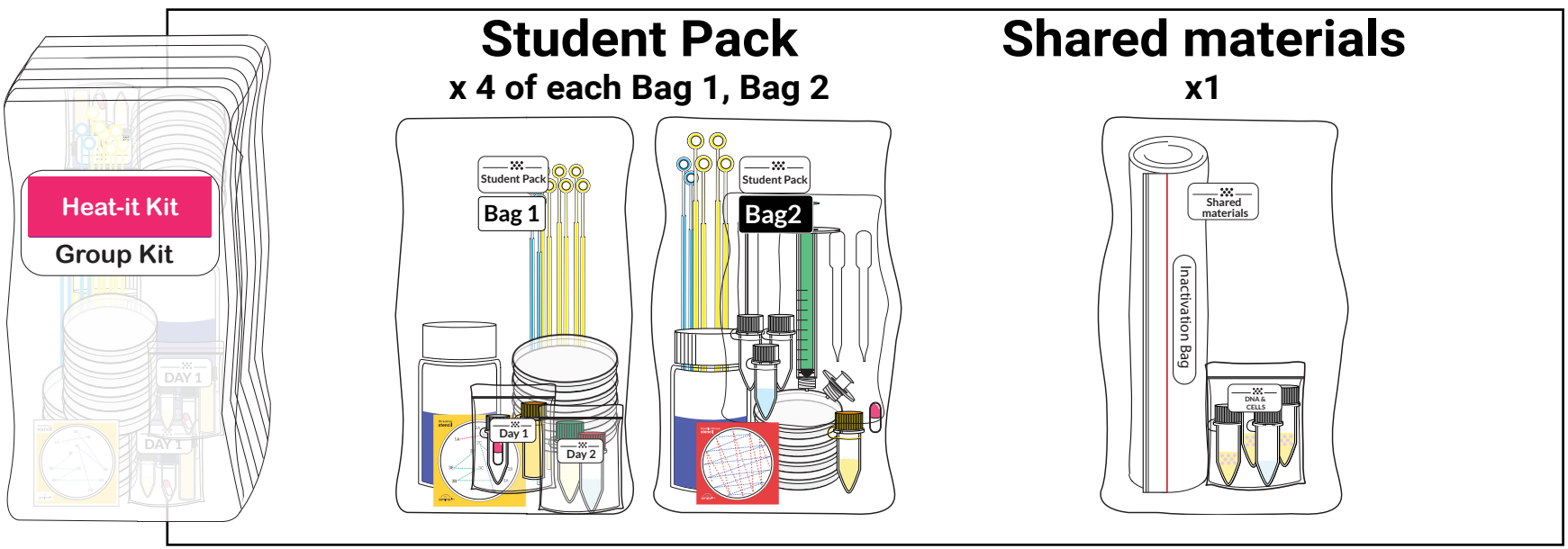


Individual kit size

The Individual kit size will lets you complete the experiment in full, one time! This kit can be used alone, (with parent supervision if necessary) or in a small group.

Group kit size

The Group kit size contains **4** individually-wrapped student packs and one shared materials bag that contains blank cells, positive control cells, DNA program, and the inactivation bags. These items will be shared by the group. In the students packs, you will have everything else you need for the experiment to be done 4 times by student groups or individuals.



Kit Components

Bag 1

Transformation Buffer: A proprietary transformation buffer used in the bacteria transformation procedure to yield high efficiencies. When you adhere strictly to the protocol, this buffer rivals other available competent cells & procedures.[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]

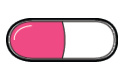
Agar Powder: This LB agar powder is industry standard. Each tube can make 50 mL of molten LB agar (3.5% w/v). Agar is the surface the bacteria grow on and the food they eat to grow.[1]

Cells *: A standard K12 strain of E. Coli, non-pathogenic and the typical strain used by thousands of labs around the world. This strain comes as a "stab," in a small tube of agar.[1]

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

- Cells *: This stab of E. coli K12 is non-pathogenic and allows you to create a negative control sample by growing it on a plate to to compare to your experimental sample.

DNA *: A DNA plasmid to program your bacteria.



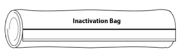
Antibiotics for Transformation: Amino Labs' proprietary antibiotic delivery system helps stabilize antibiotics for shipping and long-term storage. Each capsule has a measured amount of antibiotics for 50 mL of molten LB agar. In such small quantities, these antibiotics are very safe, even if ingested by accident. Do not ingest them, however![1]

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 5 LB agar plates.[1]

Blue Loops: Small inoculating loops are used for transferring 1 uL of liquid and other tasks. These replace costly traditional pipettes.

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 typical lab experiments and help save on the cost of reagents as well as reduce waste.



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

Plate Streaking Stencil: To help you grow the bacteria into separated, fast-growing colonies for engineering

Bag 2

Agar Powder, Antibiotics for Transformation, Sterile Water, Yellow Loops, Petri Dishes:
Just like Bag 1, Bag 2 has these necessary items to help you grow cells.

---

[1] For education purposes only.

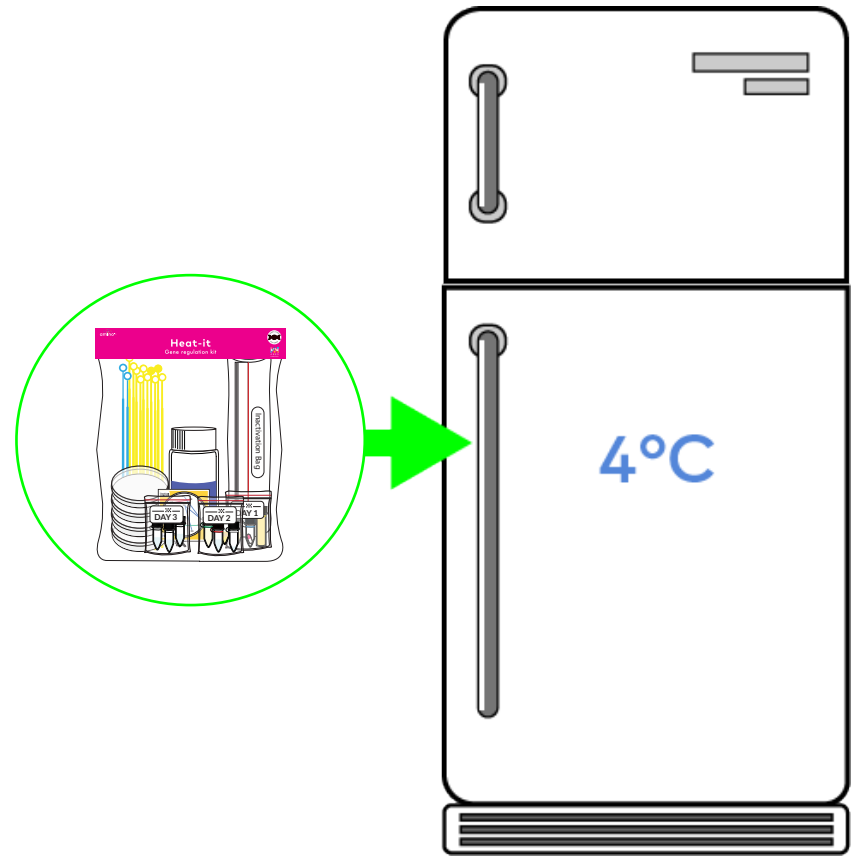
* If you have the group kit version, all cells, DNA and inactivation bags will be in the 'Shared materials' bag to be shared by students

Unpacking and Storing your kit

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

Once refrigerated, your kit will be good until the "Best by" date on the sticker on the outer packaging.

<u>Do Not Freeze</u> your kit!



Technical Specs

DNA plasmid 250 ng
Selection/Antibiotic: variable
Cells /+Cells: K12 E. coli stab

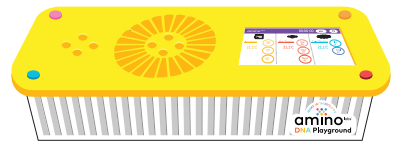
Transformation Buffer: 50 uL tubes
Recovery media: 350 uL tubes
IPTG: 1x100 uL (once dissolved)

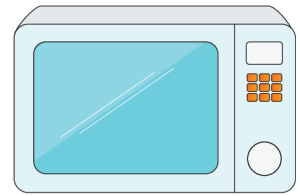
Growth plates: 6 cm petri dishes
Growth media:
LB agar powder (1.6 g)
50 mL sterile water

Necessary Equipment

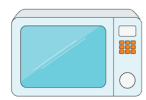
For Best results:

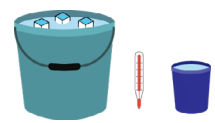
- DNA Playground™ or BioExplorer™
- Microwave





Alternative solution:





- **Microwave**
- **Thermometer** (for 42°C)
- **Timer**
- **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 it is warm where you are.
- **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.
- **Incubator:** 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

If you are using this solution, our online Udemy course will be an excellent resource for you - in this video series, Dr. Pahara completes an Engineer-it Kit using this alternative set up and shows how to use a light bulb and Tupperware as a DIY incubator. The Engineer-it kit experiment is exactly the same as what you will do with Bag 1 of your Blue-it Kit. https://udemy.com/handsonbiology/

Necessary Safety Supplies

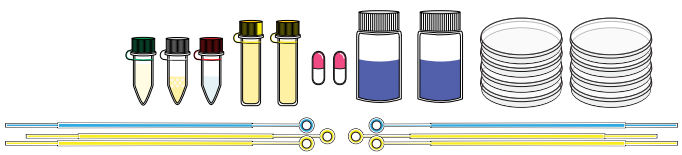
Disposable container 500ml-1L
to hold tubes, loops and other contaminated waste (e.g., yogurt container, plastic cup).

Latex or nitrile gloves
like the ones found at a pharmacy.
at least 10 pairs

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.

Timeline



Heat-it Kit

Day 1
Create petri dishes, LB agar, streak blank bacteria and incubate
(45 minutes)

Incubation
12-16 hours

Day 2
Make cells chemically competent & transform with DNA plasmid. Start recovery
(45 minutes)

Recovery
0-24 hours

Day 2 or 3
Plate transformed cells and incubate
(45 minutes)

Incubation
24-48 hours

Bag 1

Day 3 or 4
Create culture plates, double streak engineered colonies, incubate
(45 minutes)

Incubation
12-24 hours

Day 4 or 5
Increase the temperature!
(5 minutes)

Incubation
24+ hours

Results!
Expression of the colored protein will happen during the incubation at increased temperature.

Bag 2

Experiment Protocol

An Experiment Protocol is a scientific way to talk about your instructions for completing the exercises. These will not include any theory or background information on the why of each step. You can find that in the Virtual Bioengineer Simulator, the *Zero to Genetic Engineering Hero* book and the tutorial videos.

In the next pages are detailed, step by step instructions to complete the experiment and genetically engineer your bacteria with DNA. **Please make sure to read all the steps in the section before starting the hands-on manipulation;** some steps will be done in rapid sequences.

Remember that the Virtual Bioengineer Simulator available on amino.bio is free to use, and will allow practicing the engineering steps you are about to complete. Also available is a series of real-time video tutorials covering the engineering experiment part of your kit. Look for the Engineer-it follow along experiment video!
Find it and more on our youtube channel: youtube.com/c/AminoLabs

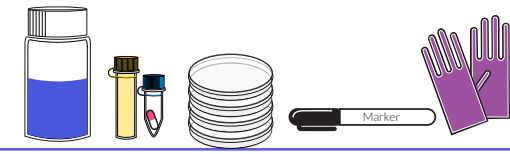
Experiment Protocol

1. Creating LB Agar Plates Day 1, 25 minutes

Goal Create non-selective and selective LB agar plates.

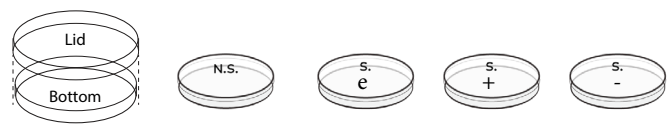
Materials from your kit - **BAG 1**

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



Prepare

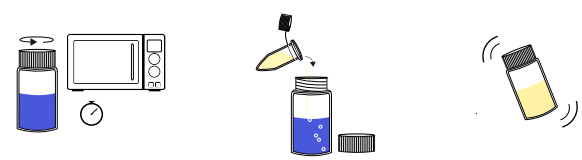
1.1 Label the bottom of the petri dishes with a marker (*the bottom is the part that has the smaller diameter of the two: the bottom fits inside the lid)*:
1x N.S. [your initials] **3x** S. [your initials].Of these three, label one **+,** one **-,** and one with an **"e"**



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

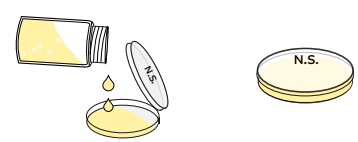


1.4 Add the tube of agar powder to the boiling water. Careful, the water is hot! Some agar powder may "clump" around the lip of the tube due to the water evaporation. This is okay, we have accounted for this possible loss.

1.5 Microwave the water and agar powder in 4 seconds intervals until you see it boil again. Instead of a rolling boil, you will see more of a foam forming above the molten LB agar liquid. ***Careful, the liquid will boil over if you microwave in more than 4 sec. increments.*** After you see the liquid foaming, swirl to mix for 10 seconds.

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

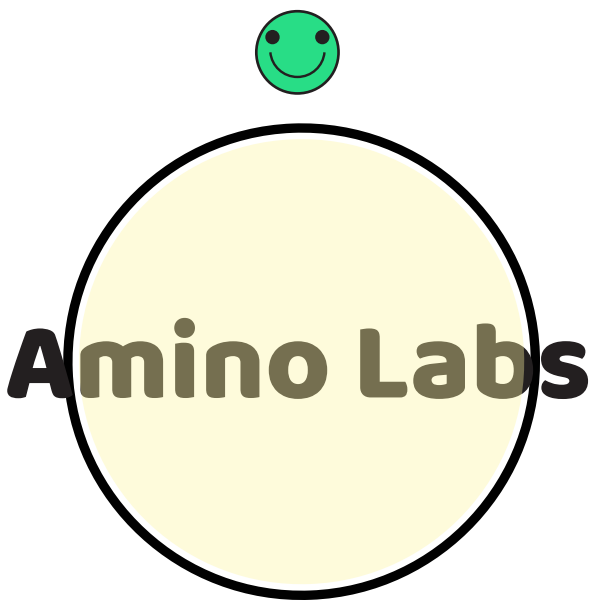
1.6 Add the antibiotic pill to the bottle of agar and gently swirl for a few minutes until the contents of the pill have dissolved. Do not introduce bubbles into the LB agar: don't swirl too vigorously. The gelatin capsule may not fully dissolve. The important thing is that the contents of the capsule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

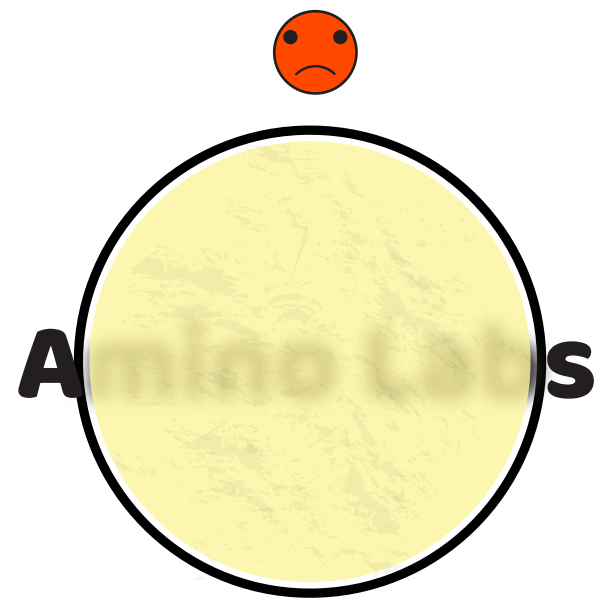
Checkpoint - Agar Plates

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.

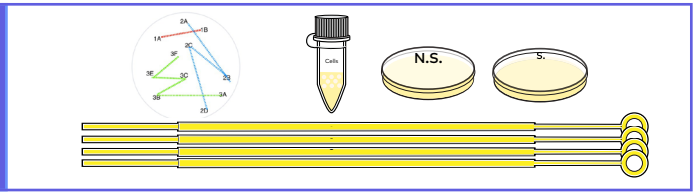
Unfortunately, if the agar does not solidify, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

2. Growing Blank Cells Day 1, 20-45 minutes + 16-24 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 - **BAG 1**

Non-selective & "-" selective plate
(1) Plate streaking stencil
(4) Yellow Inoculation Loops
(1) Stab of 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

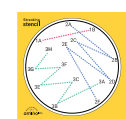
Streak your Plate

2.2 Place your N.S. Petri dish on top of the zigzag pattern on the Plate streaking stencil. Take one yellow loop and dip it into the stab of cells. Inspect your loop to make sure it appears wet to confirm you have collected cells.

2.3 Trace the line 1 of the stencil with this loop. Discard the Loop in your Inactivation Bag.

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

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



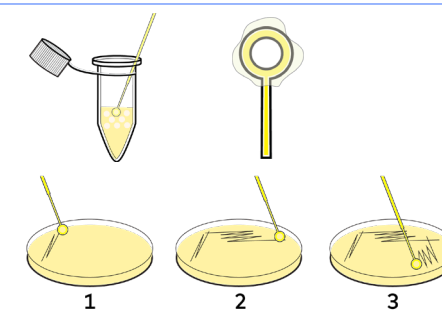
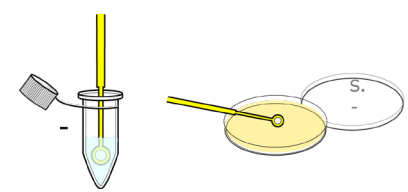


Plate your negative control ("-" plate)

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

2.7 Close your tube of bacteria and place it back in the fridge in a zip-lock 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.

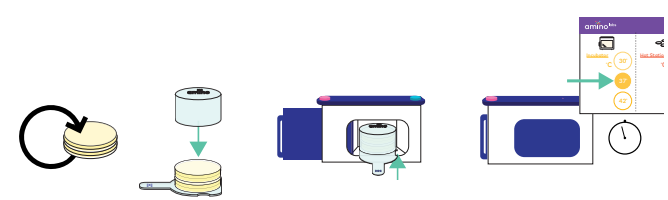


Incubate Overnight

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.

If you are using The DNA Playground or a commercially-made incubator at 37°C:
It is important to do the next steps in 12 to 24 hours so that your cells will be in their optimal growth phase.

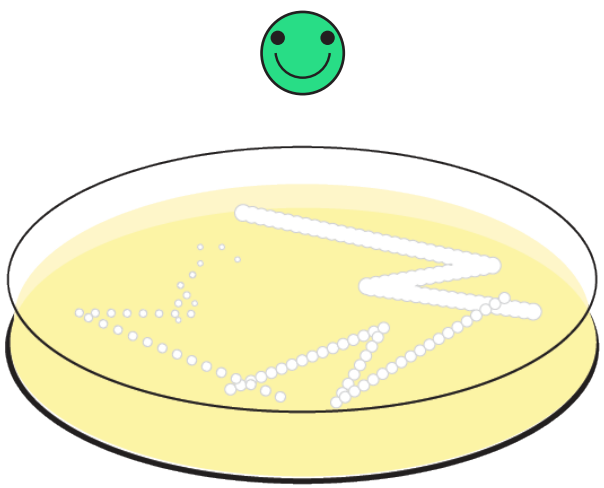
If you are using a homemade incubator:
It must incubate between 35°C and 37°C. If the cells are below 37°C, it will take longer for them to incubate - you may have to wait up to 48 hrs until you see small colonies on your plate.



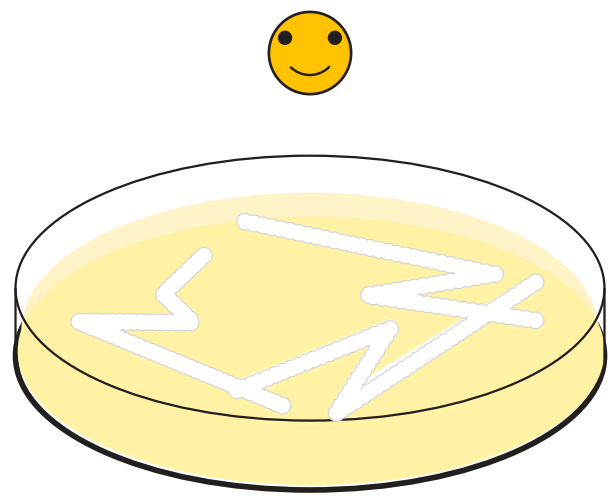
Teacher Tip! If you are in a classroom setting and your class is 24 hours-30 hours apart or more, you can initially grow your cells at 30 C overnight and then increase the temperature to 37 the day of making competent cells.

Checkpoint - Non-selective plate & blank cells

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

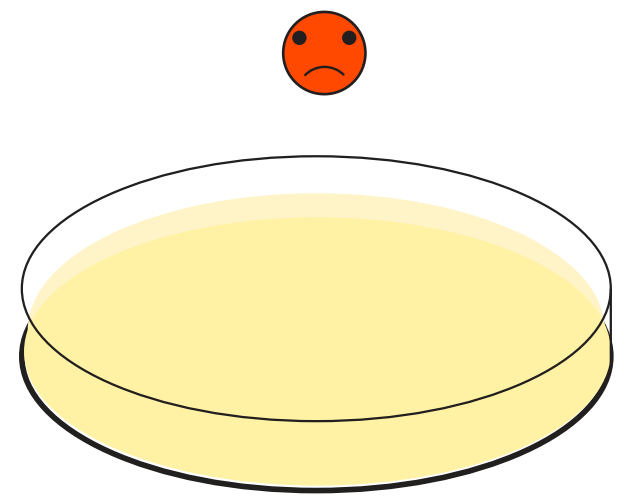


A perfect N.S. plate has lots of small individual white colonies (dots) of fast-growing bacteria after incubation. Proceed to the next page.



An okay N.S. plate has bacteria growth but few or no individual colonies (dots) after incubation. 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:

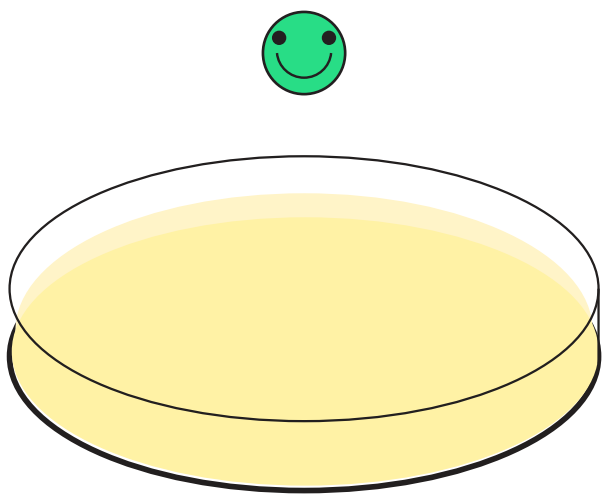
1. If your incubator was not at 37°C or is homemade, incubate for another 24 hrs.

2. If you are certain you incubated at 37°C, or incubated for 48 hrs 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

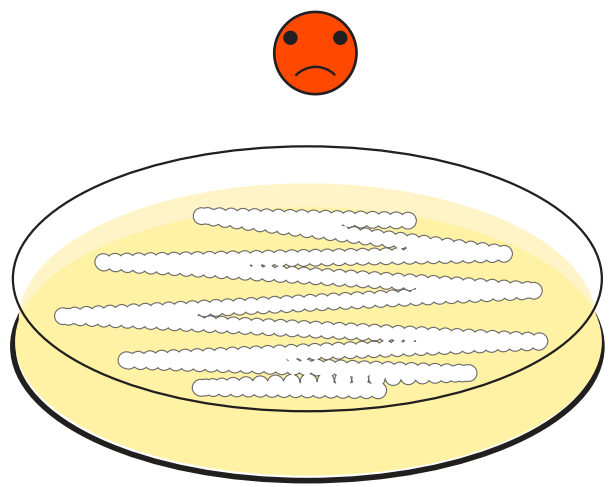
Checkpoint - Selective plate and “-” control

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.

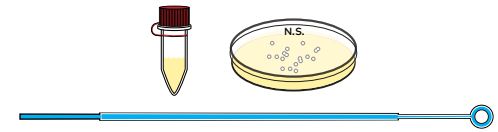
Continue the exercise with your other selective plates. Note that your results may not be as good as if you had the right amount of antibiotics. Don’t worry though, practice makes perfect, and our Success Guarantee means you will have the chance to be successful if this try doesn’t work.

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 & Blank cell + Selective Plate and "-" control Checkpoints. You can discard the S. "-" plate in your inactivation bag.

3.2 Turn on the cold station to the "Ice" (4°C) setting on your Amino Lab's Minilab™ or get your Ice bucket.

Mix the cells and T. Buffer

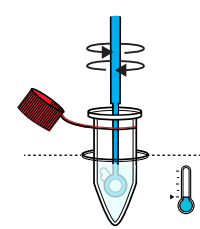
3.3 Take your T. Buffer tube and make sure all the liquid is in the bottom of the tube by briefly puting it in your microcentrifuge (don't forget to put a balance tube!), or by tapping it gently on a surface. You should have 5mm of liquid in the bottom of the tube. Set it on your cold station set to "ice" or in real crushed ice 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. LB 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 While keeping the T. Buffer tube on the cold station, 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. You don't have to twist too vigorously as this could break off the loop.

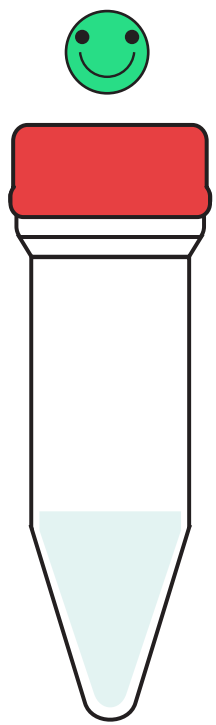


When successful, the solution should be slightly cloudy, and there should be no clumps floating in the solution. You may have to mix vigorously for up to 45 seconds. If you see clumps, keep blending. You can lift the tube out of the cold station to see whether the T. Buffer is cloudy and the clumps are gone, but replace it quickly into the cold station to keep it cool. Have a look on the next page to see what your tube should look like. When you have successfully mixed in your cells to the T. Buffer, discard the loop in the inactivation bag.

Without delay, move on to the next step to add your DNA! **Ideally you should add your DNA less than 1 minute after making your competent cells.**

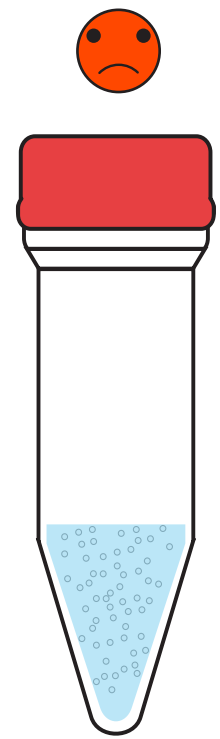
Checkpoint - Competent cells

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

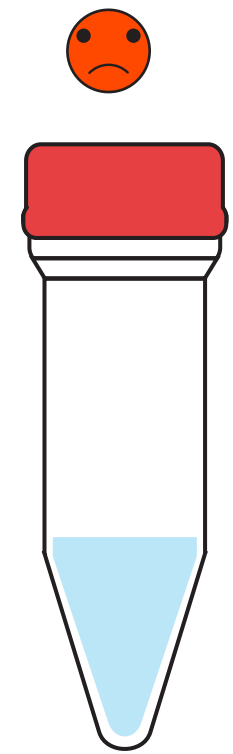


Cloudy liquid - Perfect!
The cells can now take up DNA.

Move on to the next step.



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



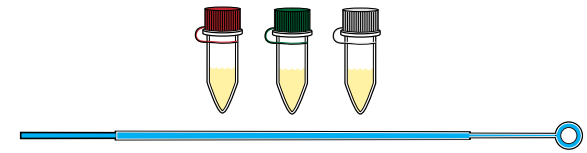
Clear liquid? Add more cells and mix until the liquid is cloudy like the image on the left.

4. Transformation Day 2, 45-60 minutes

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

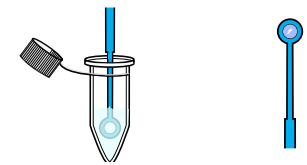
Materials from your kit - **BAG 1**

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



Take the DNA

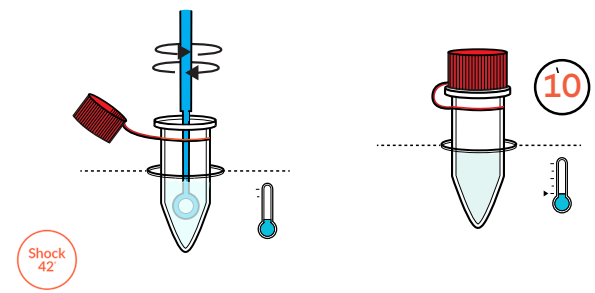
4.1 Tap your DNA tube on the table or briefly microcentrifuge to make sure most of the DNA is at the bottom. Dip a Blue inoculating loop into the DNA tube and twist a few times. When you pull the loop out of the DNA tube, the hole of the loop should have liquid in it. Each tiny droplet of the liquid contains thousands of DNA plasmids, small circles of DNA programs that 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 5 seconds to fully mix. Do this while the tube is on Ice / Cold Station. Discard the loop. * Do not reuse the inoculating loop! *

4.3 You will incubate your tube for 5 minutes on Ice / Cold Station. While this is happening, turn on "Shock 42°C" on your Minilab or set your water bath to 42°C. You can close and put the DNA tube back in the fridge. If stored in a fridge, and you have not accidentally contaminated the DNA, it should last a month or more and can be used again in a future engineering experiment.

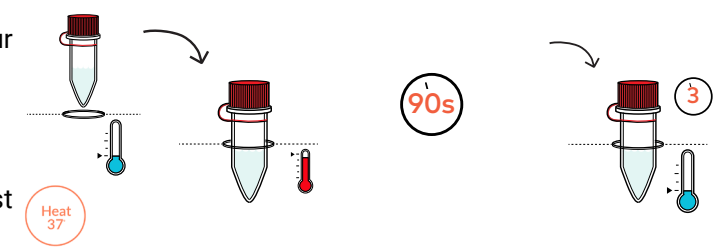


HeatShock

4.4 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 / Cold Station for 2 minutes.

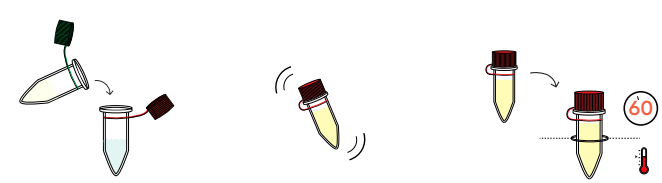
4.6 The next step is recovery which will happen at 37°C. Turn on your Minilab Hot station to Heat 37°C or adjust your water bath temperature to 37°C.



Recovery

4.7 Tap the tube of Recovery Media on the table to ensure that most of the liquid is at the bottom of the tube and pour into your tube of cells + T. Buffer + DNA solution. Mix gently by inverting 10 times. Some liquid will stay in the recovery tube. That is acceptable.

4.8 Place your tube of solution in the Hot station set to "Heat 37°C" or your water bath for 30-45 minutes to allow the cells to recover and start expressing their antibiotic resistance proteins. Mix the cells by inverting them every 15 minutes. * Make sure the liquid is in the bottom of the tube when you place it in the tube heaters! * See www.amino.bio/whip-it to learn the Whip-it method for moving liquid inside a tube.



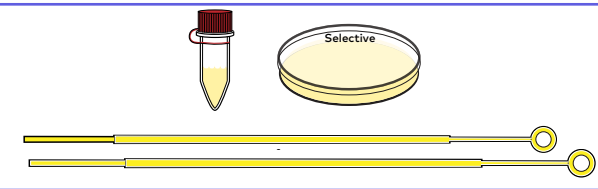
After the initial 30 minutes of recovery, you can wait up to 24 hours before moving onto the next step.

5. Plating Cells Day 2 or 3, 10-15 minutes + 24-72 hours wait

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

Materials from your kit - **BAG 1**

(1) 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.

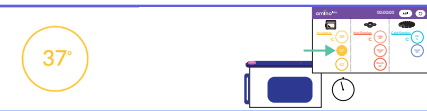


Plate your transformed cells ("e" plate)

5.2 On the Selective LB Agar plate labeled "e", pour 1/2 of your Transformed cells solution 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. 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. Close the tube with your transformed cells and keep them them just in case you do not get colonies since you can always pour the remaining recovered cells onto the selective plate and incubate them after your step 6. incubation.

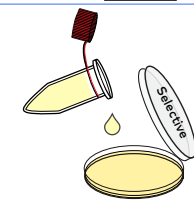
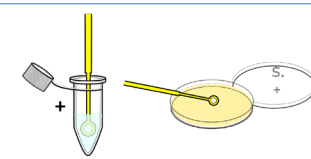


Plate your positive control ("+" plate)

5.3 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 any pattern of your choosing.

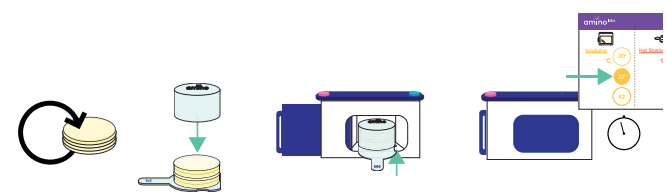


Incubate

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.

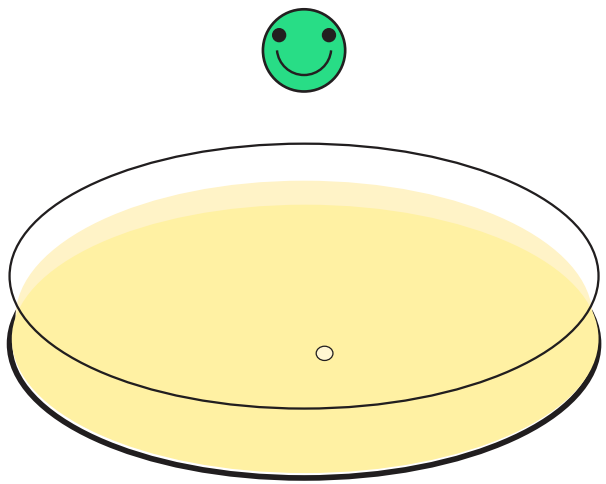
5.5 Incubate for 24-48 hours until you see one or more colonies.

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



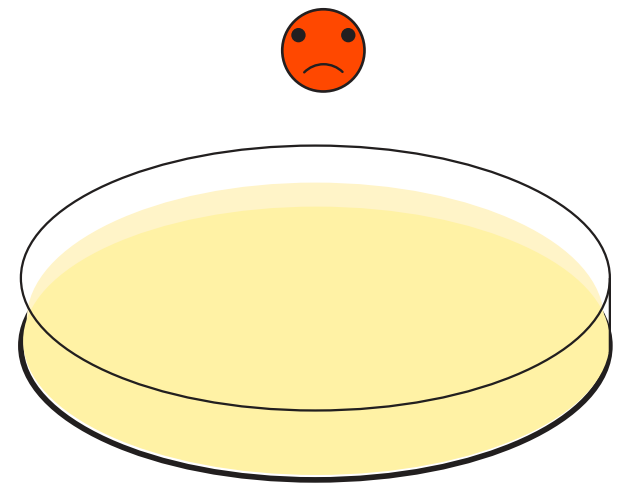
Checkpoint - Did your engineered cells grow?

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



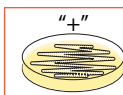
A perfect “e” plate has one or more colonies on it. Remember, even a single colony is a success. One colony is all you need to move onto the next step. Congratulations!

Move on to the next step.



If you see no growth on your “e” plate, this means that your engineering may not have worked, or that you haven’t incubated long enough.

If you cannot see any growing cells after 48 hours, you can pour the rest of your engineered, recovered cells onto the “e” plate and continue incubator for another 24 hours. If you still don’t see any growth after this, your experiment may have failed. Don’t be discouraged. In science, failure is a chance to learn more. Complete the troubleshooting guide at amino.bio/troubleshoot to claim your free kit to try again, as part of our *Success Guarantee*.



You can continue your experiment with a colony from your “+” plate if the + cells grew. Try to select a single, isolated colony if you can.

6. Create Selective LB Agar Plates 25 min.

Goal Create a new batch of selective LB agar plates to amplify (culture) your engineered cells.

Materials from your kit - **BAG 2**

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

Prepare

1.1 Using a sharpie-type pen, label the bottom of the petri dishes as follows: **4x** S. (for selective) + Add [your initials]. On one of the petri dish write (-) as this will be your negative control, and (e) as this will be your experimental test. (*Remember, the bottom of the petri dish is the side with little tabs)*

Mix the Agar

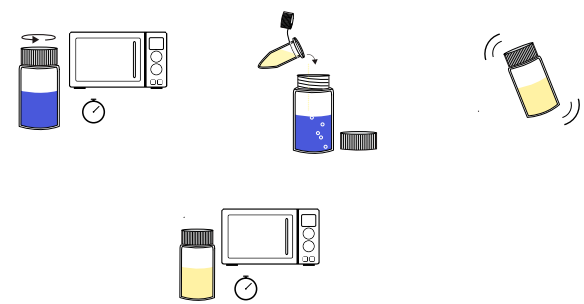
1.2 Unscrew the lid from the sterile water bottle and keep it loosely on top of the bottle to prevent any contaminants from entering the water, but allowing air to escape. This will prevent pressure build-ups.

1.3 Place the bottle in the microwave and heat the water **until you see it boil**. You should see a rolling boil where many bubbles are rising constantly. Careful, the bottle will be hot!

1.4 Add the tube of Agar powder to the boiling water. Careful, the water is hot! Some agar powder may "clump" around the lip of the agar tube. This is due to the water evaporation coming into contact with the agar powder as you pour it in. This is okay, we have accounted for this loss of powder.

1.5 Microwave the water and agar powder in 4 seconds intervals until you see it boil again. Instead of a rolling boil, you will see 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.

Note: you will not be making a non-selective plate. All 4 plates will be selective agar.

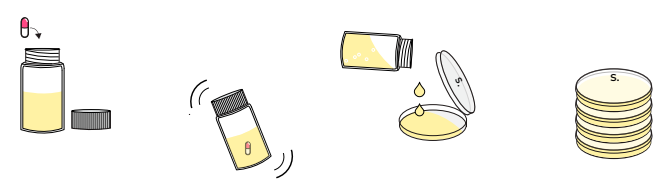


Make selective (S.) plates

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

1.7 Once the antibiotic pill is dissolved, pour the molten LB agar into the 4 petri dishes. Place the lids back on.

1.8 Let the LB agar harden. You will use 2 plates in the next step. You can store the remaining plates in the zip-lock bag in the refrigerator.

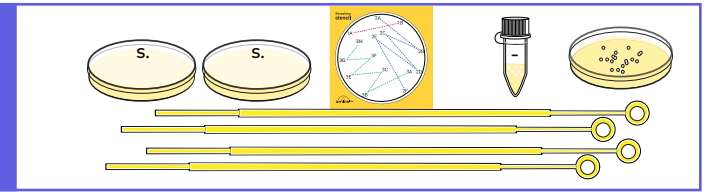


7. Amplify (Culture) engineered cells 15 minutes + 24 hours wait time

Goal **Grow a large quantity of cells on plates to turn on the genetic switch with temperature.**

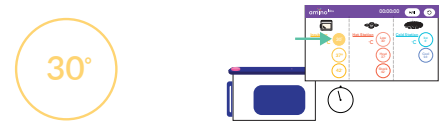
Materials from your kit - **BAG 2**

(2) Agar plates from previous step, marked (-) and (e).
Your engineered cells
(1) Tube of negative control cells
(4) Yellow Loops
(1) Streaking stencil



Prepare

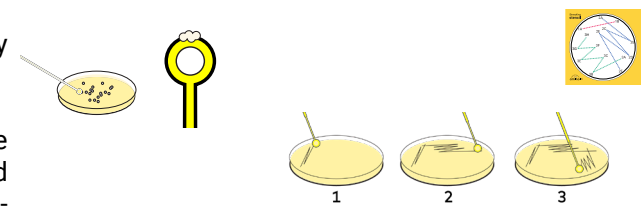
7.1 If you have an incubator, turn it on to 30°C.



7.2 Watch the "superstreak" video at www.amino.bio/superstreak - you are ready to learn pro streaking methods!

Streak

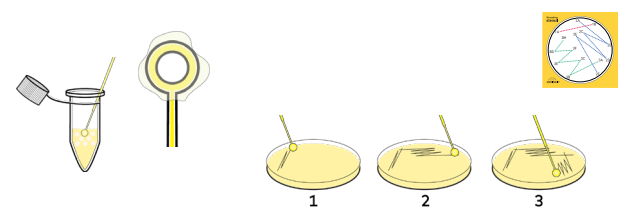
7.2 Place your new (e) petri dish on top of the streaking pattern yellow stencil, like you did in Bag 1. (You may have a red "double streak" stencil with two zig zags in your bag - ignore this stencil - you don't need it here)



7.3 Take one yellow loop, and following the instructions in the superstreak video you watched, "pick" one or more colonies of the engineered cells you made in Bag 1. You pick a colony by touching the end of the loop to it and gently rubbing it to make sure it transfers to your loop. Note: If you did not have any colonies grow from the engineering experiment you did with Bag 1, you can use colonies from your S (+) petri dish you already incubated.

7.4 With your colony on the end of your loop, trace one the first line of the stencil across the selective agar plate.

7.5 Turn your loop to a unused side of the loop and trace the second line of the stencil. With a third side of the loop, streak the the third line to obtain seperated colonies on your plate. Discard your loop after use.



7.6 Take your new (-) petri dish and tube of negative control cells. Place the petri dish on the yellow stencil.

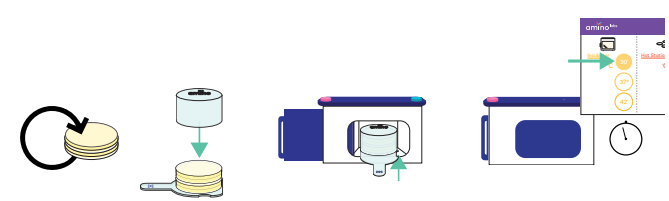
7.7 Dip a new yellow loop in the stab of cells, and streak the first stencil line with this loop. Since the loop was dipped in the tube of cells, all sides are coated with bacteria. Discard this loop.

7.8 Take a new yellow loop and, with one side of the loop, streak the second line from the stencil. Flip your loop to a new side of the loop and streak the third line of the stencil. Discard the loop after use.

Congratulations! You just learned pro tricks to streaking cells and conserving loops!

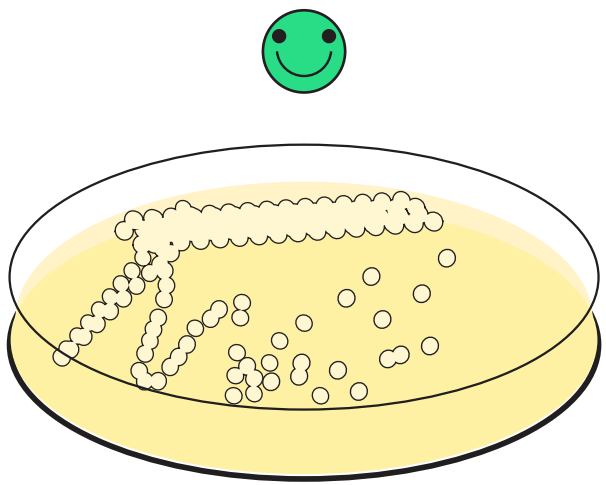
Incubate Overnight

7.8 Incubate your streaked plates **upside down** at ~30°C for up to 24 hours: If you have the DNA Playgroun remember to close the incubator door and lock it. After 24 hours you should see lots of growth. The colonies on your (e) plate should be mostly white in color, whereas the colonies on your (-) plate may be beginning to express their colors as they do not have the temperature genetic switch in their DNA. The (-) cells express the color protein as they grow, no matter the temperature. The Heat-it engineered cells on your (e) plate mostly express their color proteins at 37 to 42°C, once the genetic switch turns on. In the next step, you will turn this switch on.



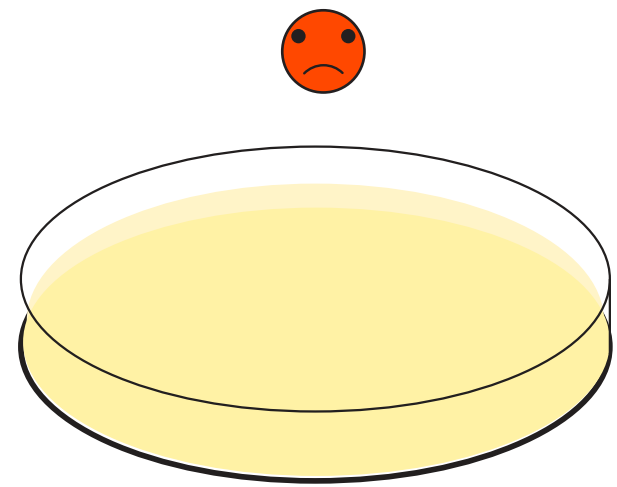
Checkpoint - Did your cultured cells grow?

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



A perfect cultured plate will have many colonies on it. Congratulations!

Move on to the next step.



If you see no growth on your plates, your amplification of cells may not have worked or you haven't incubated long enough. Try to incubate longer.

If you cannot see any growth after 48 hours, repeat step 7 on the 2 unused plates from step 6. If you still cannot see growth on these after incubating 48 hours, your experiment may have failed. Don't be discouraged. In science, failure is a chance to learn more. Complete the troubleshooting guide at amino.bio/troubleshoot to claim your free *Success Guarantee* kit.

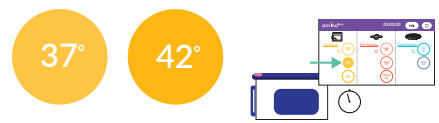
8. Increase the temperature & see the results! 5 minutes + 24 hours wait.

Goal **Increase the temperature on your incubated cells and see the colored protein expressed!**

Materials from your kit
Your incubated experiment plates

Increase the temperature

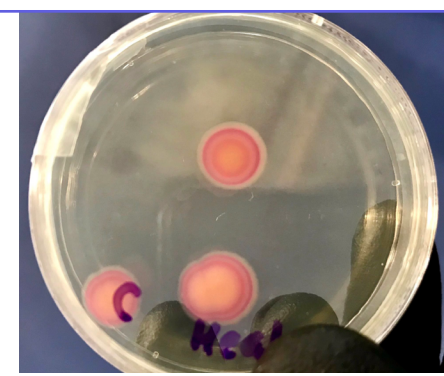
8.1 For temperature-based genetic switches, there are no external factors that need to be prepared other increasing the temperature of the environment. After your cells have grown and colonies are visible increase your DNA Playground incubator to 37 °C or 42 °C.



Keep incubating!

8.2 Incubate your cells at 37°C or 42 °C for another 24 hours to see the color proteins being expressed.

8.3 For the best result, continue incubating your petri dish and alternating the temperature between room temperature or 30 °C and 37 °C/ 42 °C every 24 hours. This will extend your experiment timeline, and you will need to watch out for any contaminants growing on your plate, and for your agar drying out, but alternating temperature over a week will create interesting results on the S (e) petri dish, like on the photo on the right.



Alternating between room temperature and 37 °C during a week-long incubation will create colorful patterns as your (e) plate cells grow!

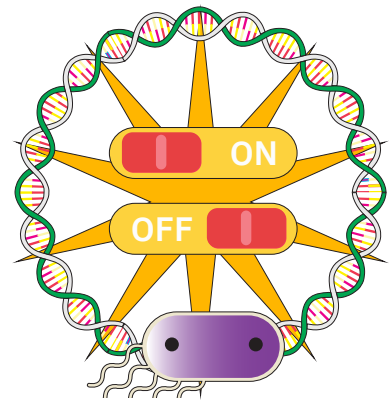
At room temperature or 30 °C, your engineered cells will continue to grow without expressing color whereas your negative control will always express colors, while at 37 °C/ 42 °C, both will express their coloring. Alternating between low temperature and 37 °C/ 42 °C will create colorful patterns as your cells grow!

8.4 You can repeat steps 7 and 8 on the 2 remaining selective plates and incubate the cells at different alternating temperatures to experiment with creating different patterns. Have fun with genetic switches!

Note

If you cannot see any color change 24 hours after changing the temperature, your experiment might have failed. Don't be discouraged. In science especially, failure is a chance to learn more. Complete the troubleshooting guide at www.amino.bio/troubleshoot and claim your free kit to try again, as part of our *Success Guarantee*.

Congratulations!



Using your Heat-it Kit™, you inserted a DNA Program inside single-celled organisms, bacteria, and had the bacteria execute that DNA Program to produce something for you, which you then controlled the expression of using temperature. Just like scientist and industries do every day inside their large laboratories! To learn more about genetic switches and inducible plasmids, look at Chapter 7 of the *Zero to Genetic Engineering Hero* book. We hope you enjoyed the experience and will continue to experiment in the field of genetic engineering.

Show off your results with your friends, our community and us too on Twitter, Instagram, Facebook, and youtube (@aminobiolab)

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

Storage, Disposal, Clean Up

After you sees 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.

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 **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.bio

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.

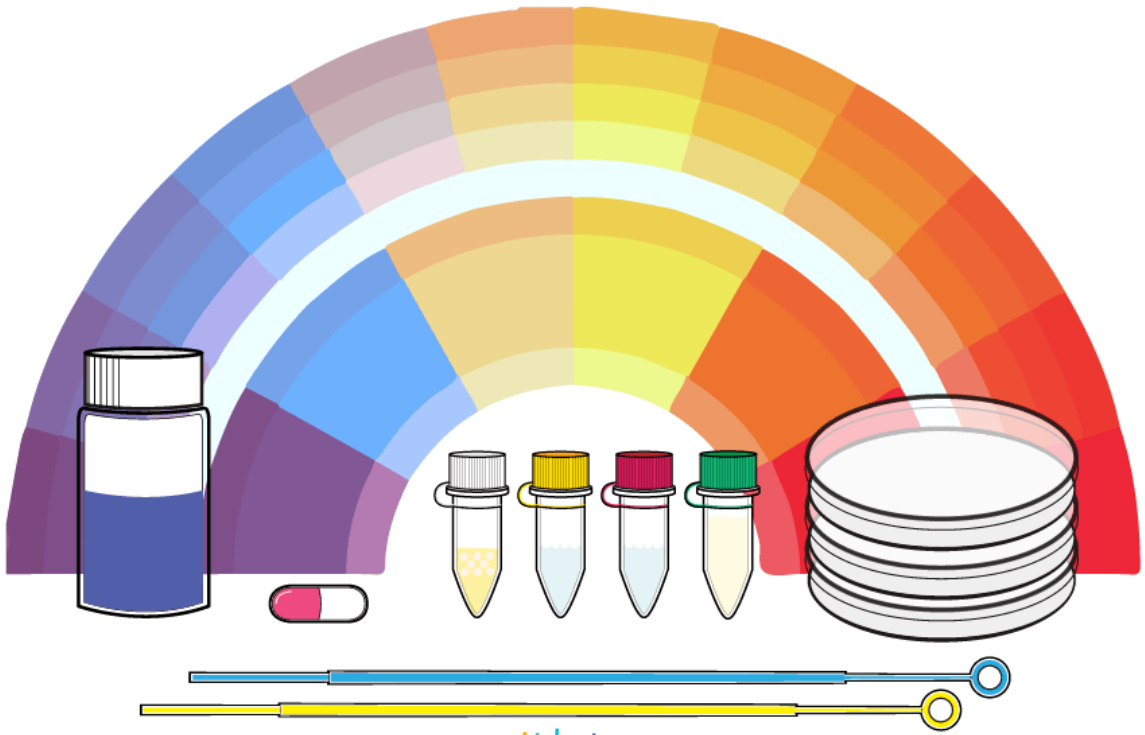
If anything else causes you issues, please contact us : help@amino.bio

More Information

Made in Canada

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

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



Make with biology

amino labs

www.amino.bio