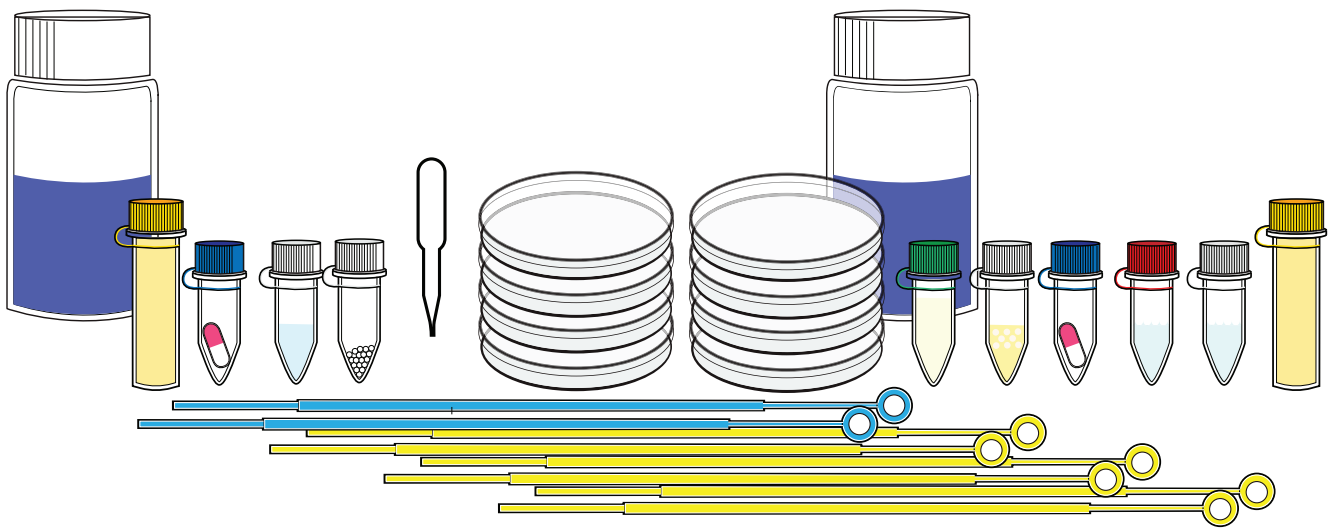
GENETIC ENGINEERING WITH

INDUCE-IT KIT™

User Manual



Make with biology

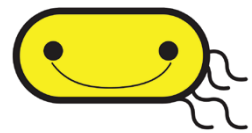
amino labs

Version 1.2 March 2019

INDUCE-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 Induce-it Kit™ and our entire ecosystem of easy-to-use, easy-to-succeed at products!

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

Practicing Safe Science

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

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

We recommend the system and kits for ages 12+, under adult supervision, and 14+ unsupervised.

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

- **Do not eat or drink near your experiments.** Keep your experiment at least 10 feet from food, drinks, etc. Under no circumstances should you consume any of the ingredients.

- **Immunocompromised persons:** While the ingredients in these kits are non-pathogenic, some persons, such as immunocompromised persons, can be affected by large numbers of bacteria and should wear extra protection, such as long sleeves and a face mask, to ensure no contact with the ingredients.

- **Wash your hands before and after** manipulating your experiment, the ingredients, or the hardware.

- **Wear gloves**, even when cleaning your station or handling the consumables (petri plates, loops, etc). This will protect you from your experiment, and your experiment from you. Any latex, nitrile, or general purpose gloves you can find at the pharmacy will do. Also, after you put your gloves on, be aware of what you touch. Try not to touch your face, scratch itches with your gloved fingers!

- **If using the DNA Playground™ or BioExplorer™ place it on a stable work surface.** Keep it level at all times.

- **Clean up your station, spills and work surface before and after use.** Use a 10% solution of chlorinated bleach generously sprayed onto a paper towel and rub onto any contaminated surfaces. (Careful! This can discolor your clothes). A chlorinated spray cleaner also works.

- **Find a container to hold the inactivation bag where you will discard used consumables**. An old 1L yogurt container, large plastic cup or the like will do. Used consumables will be loops, any tube or used petri dish.

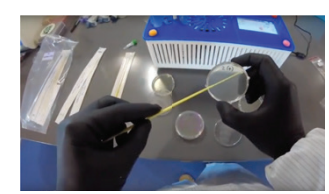
If you would like to do a short Online lab safety course for your edification, we recommend this Government of Canada course: https://training-formation.phac-aspc.gc.ca/course/index.php?categoryid=7

How will I learn?

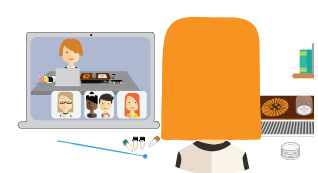
Learning and prototyping with genetic engineering and cells is becoming accessible to newcomers of all ages and backgrounds thanks to dedicated scientists and kits such as the one you are about to use. One of the easiest way to learn a new science, hobby or topic is by trying it, hands-on. Everything 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 Induce-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.

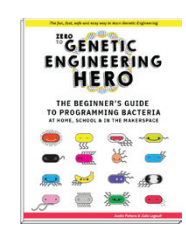
- View **real-time tutorials**, including video tutorials for each day of the genetic engineering experience on our Youtube channel. The genetic engineering days are under 'Engineer-it Kit,' the same procedures as the first 3 days of your Induce-it Kit experience . Subscribe! youtube.com/c/AminoLabs.



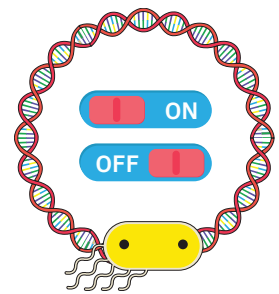
- Would you like for an Amino Labs team member to tutor you through your journey? Try the **Cyber Workshop & Tutoring**, a 3-day+ experience completed via video conferencing. www.amino.bio/products/cyberworkshop.



- Are you interested in the theory behind the experiment 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 Induce-it Kit™



The Induce-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 a chemical. 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 chemicals, or even environmental conditions like temperature and light. 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 Induce-it kit, a similar plasmid will be inserted into cells. Once grown, you will then turn the genetic switch 'on' using a chemical called Isopropyl β-D-1-thiogalactopyranoside or IPTG for short. IPTG is a common reagent used in laboratory experiments! You can learn more about it, and 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 Heat-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!

Kit Components

Transformation Buffer (Bag 1): A proprietary transformation buffer used in the colony transformation procedure to yield high transformation efficiencies. When you adhere strictly to the protocol, this buffer rivals other commercially available competent cells & procedures.[1]

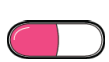
Recovery Media (Bag 1): Amino Labs' recovery media is used after the heat shock during the transformation protocol. This nutrient broth aids the cells in recovering and has a proprietary recipe that further boosts the cells ability to survive the transformation and begin dividing.[1]

Agar Powder (Bag 1 & 2): This LB agar powder is industry standard. Each tube can make 45 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 (Bag 1): 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 (Bag 1): 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.

DNA (Bag 1): A DNA plasmid to program your bacteria.



Antibiotics for Transformation (Bag 1 & 2): Amino Labs' proprietary antibiotic delivery system helps stabilize antibiotics for shipping and long-term storage. Each capsule has a measured amount of antibiotics for 45 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 (Bag 1 & 2): 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 (Bag 1): Small inoculating loops are used for transferring 1 uL of liquid and other tasks. These replace costly traditional pipettes.

Yellow Loops (Bag 1 & 2): 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 (Bag 1 & 2): 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 (Bag 1): 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.

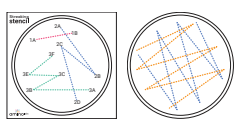


Plate Streaking Stencils (Bag 1 & 2): To help you grow the bacteria into a) separated, fast-growing colonies for engineering (Bag 1) or b) a maximum amount of bacteria to extract from or use for further experiments (Bag 2). Following these stencils will help you achieve this.

IPTG (Bag 2): A chemical that mimics the natural functions of lactose. Used to induce ('turn on') gene expression when a DNA program uses a particular genetic switch, the lac repressor**.**

Dissolving buffer (Bag 2): A chemical to dissolve the IPTG powder.

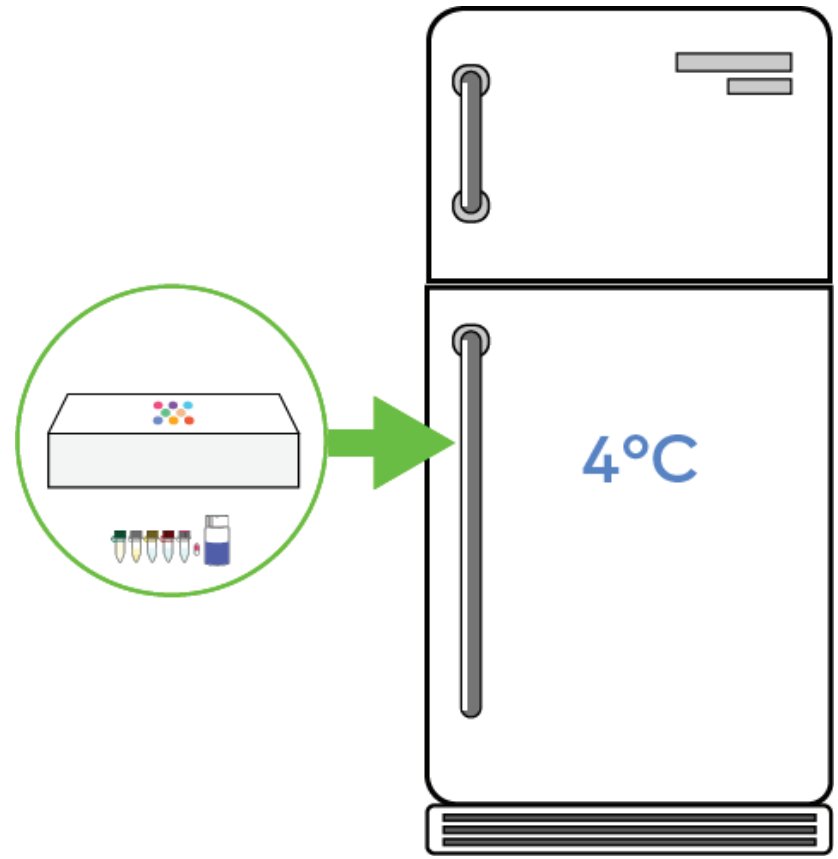
Pipet (Bag 2): Used to transfer the IPTG from the tube to the petri dishes.

[1] For education purposes only.

Unpacking and Storing your kit

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

If you can fit the whole bag, go ahead and store it all in the refrigerator. If you need to save space, please put all the tubes, selection marker (antibiotics) and waters in the refrigerator. The loops, plates, stencils, pipet can stay at room temperature.



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

Do not leave the tubes at room temperature!

Technical Specs

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

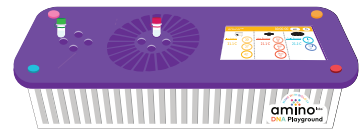
Transformation Buffer: 50 uL tubes
Recovery media: 350 uL tubes
IPTG: 1x100 uL (once dissolved)

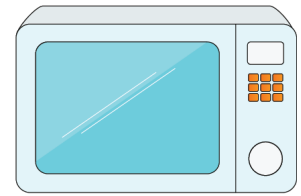
Growth plates: 6 cm petri dishes
Growth media:
LB agar powder (1.6 g)
50 mL sterile water

Necessary Equipment

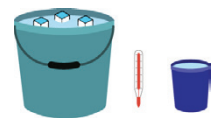
For Best results:

- DNA Playground™ or BioExplorer™
- Microwave





Alternative solution:



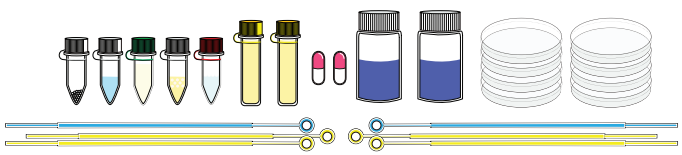
- **Microwave**
- **Ice bucket or bowl and ice:** This will become your "**Cold station**" "Ice" for the experiment. Make sure to keep the ice from melting too much during the experiment. You may need a fresh replacement during the experiment if it is warm where you are.
- **Hot water bath or bowl with 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.
- **Thermometer (**for 42°C**)**
- **Timer**
- **Incubator or warm environment!**: This will replace the **Incubator** set to **"37"**. If you do not have an incubator (biology or egg one, as long as they set to 37°C), you can create one using an online tutorial (ex: instructables.com/id/Low-cost-and-accurate-incubator-for-DIY-biology/.) If you have neither incubator or DIY version, you can try incubating the cells in a resealable bag in a warm environment. Your yield won't be as good as with an incubator but should work. Note that it will take a few more days to see results.

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 lightbulb and Tupperware as a DIY incubator. https://udemy.com/handsonbiology/

Necessary Safety Supplies

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

Timeline



Induce-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
Induce! Use the pipet to add the IPTG inducer to your grown bacteria.

Incubation
2-24 hours

Results!
Expression of the colored protein will happen 2 to 24 hours after induction.

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 in the Chrome App store is free to use, and will allow practicing the steps you are about to complete. Also available is a series of real-time video tutorials covering the experiment Day 1, 2 and 3. Find them on our youtube channel: youtube.com/c/AminoLabs

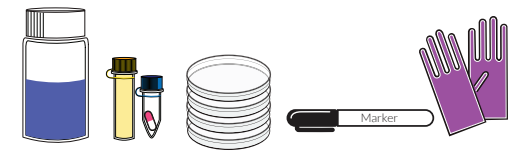
Experiment Protocol

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 Using a sharpie-type pen, label the bottom of the petri dishes like so : *(the bottom is the side with little tabs)*
1x N.S. [your initials] **3x** S. [your initials] of these three, label one **+,** one **-,** and one with an **"e"**

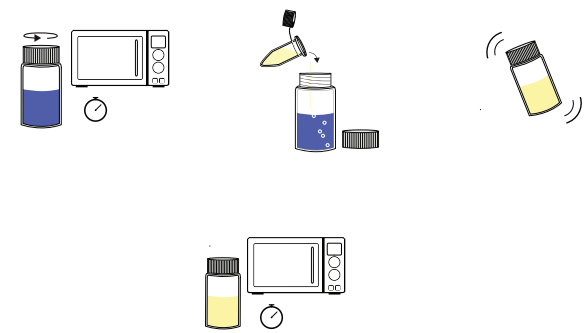
Mix the Agar

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 should see a rolling boil where many bubbles are rising constantly. Careful, the bottle will be hot!

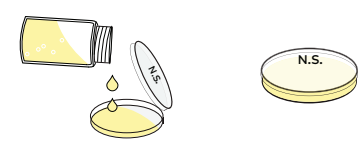
1.4 Add the tube of Agar powder to the boiling water. Careful, the water is hot! Some agar powder may "clump" around the lip of the agar tube. This is due to the water evaporation coming into contact with the agar powder as you pour it in. This is okay, we have accounted for this loss of powder.

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.



Make non-selective (N.S.) Plate

1.6 Pour molten LB agar in 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 back on.

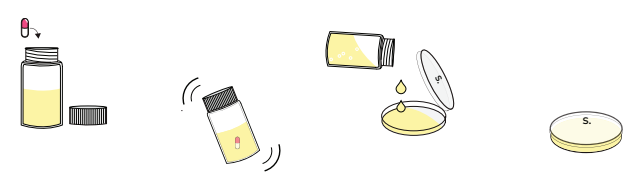


Make selective (S.) plates

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.

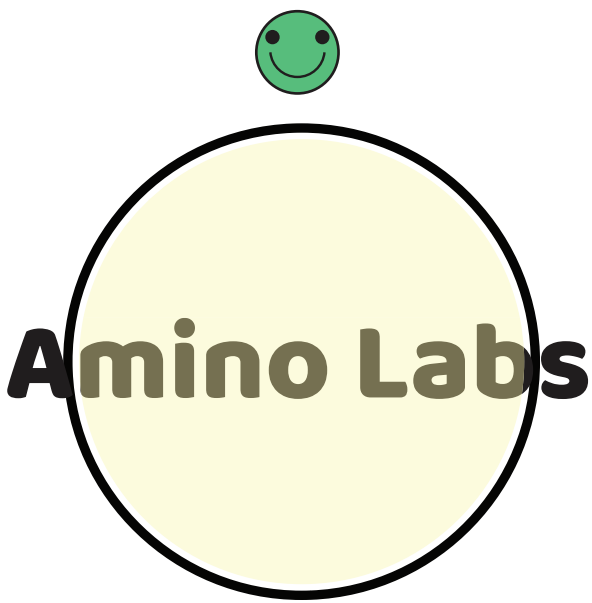
1.7 Once the pill is dissolved, pour the LB agar into the 3 petri dishes left. Place the lids partially back on to allow for some evaporation.

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



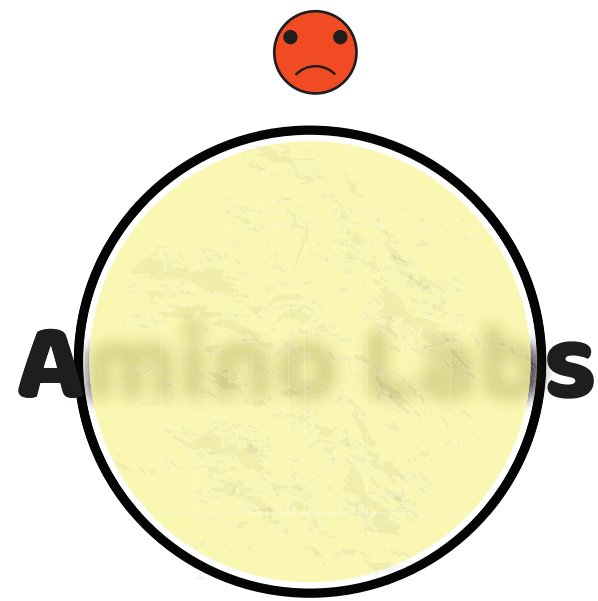
Checkpoint - Agar Plates

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.

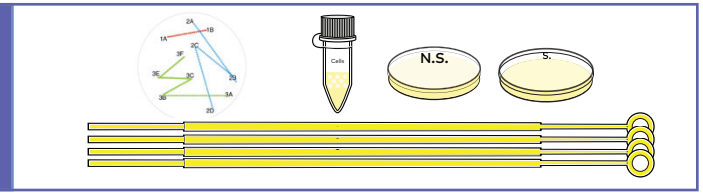
Unfortunately, this means you need to halt your experiment and complete the troubleshooting guide and follow the instructions at www.amino.bio/troubleshoot

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 1of 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.

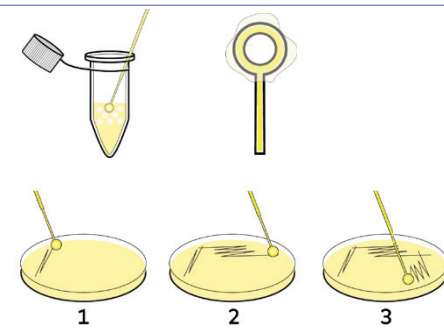
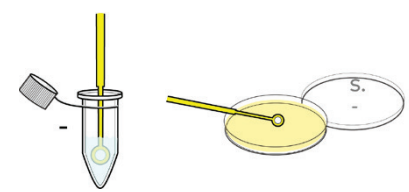


Plate your negative control ("-" plate)

2.6 Take your Selective LB Agar plate labeled "-". Using a single yellow loop, dip into the 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.

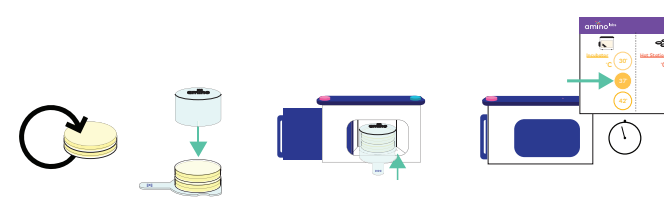


Incubate Overnight

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.

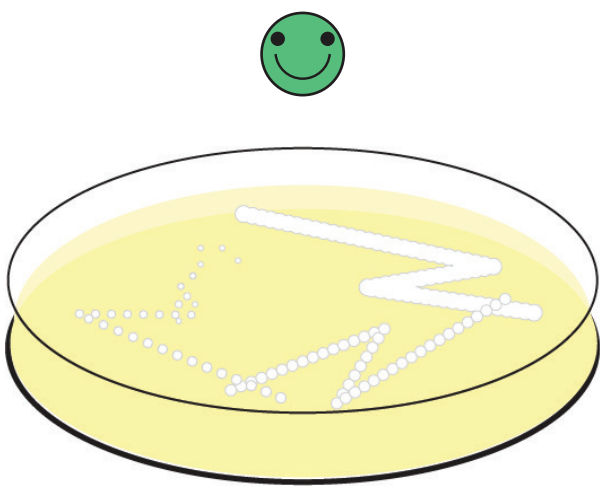
If you are using The DNA Playground or a commercially-made incubator at 37°C:
It is important to do the next steps in 16 to 24 hours so that your cells will be in their optimal growth phase.

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 48hrs until you see small colonies on your plate.

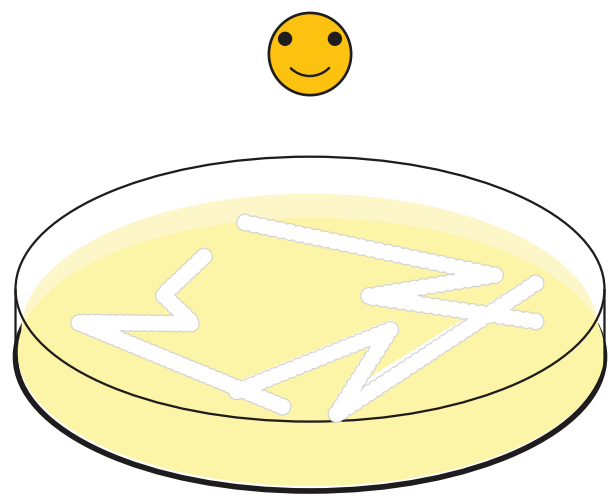


Checkpoint - Non-selective plate & blank cells

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

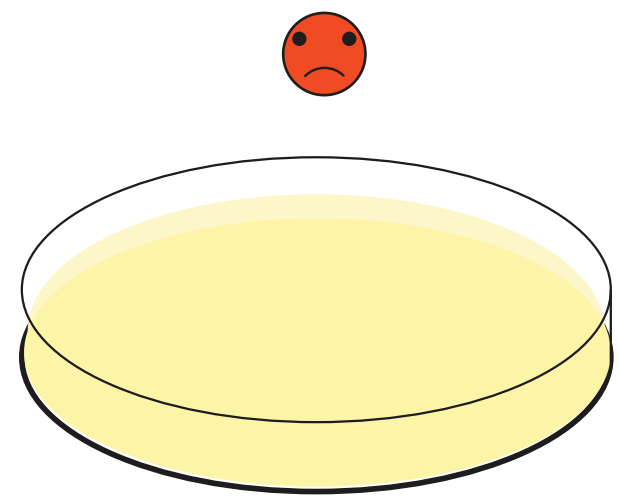


A perfect N.S. plate has lots of small individual white colonies (dots) of fast-growing bacteria after incubation. Proceed to the next page.



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:

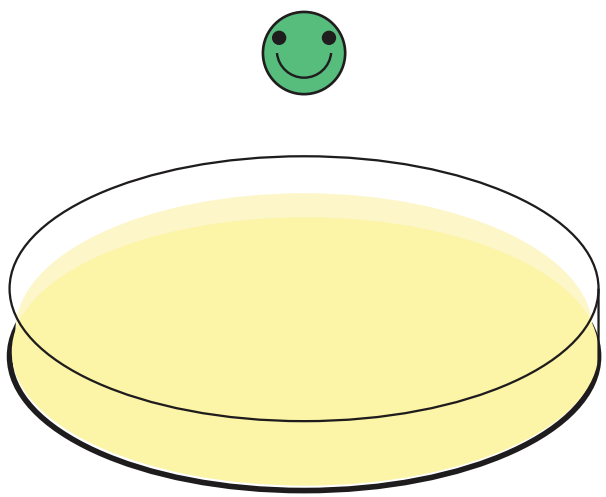
1. If your incubator was not at 37°C or is homemade, incubate for another 24hrs.

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

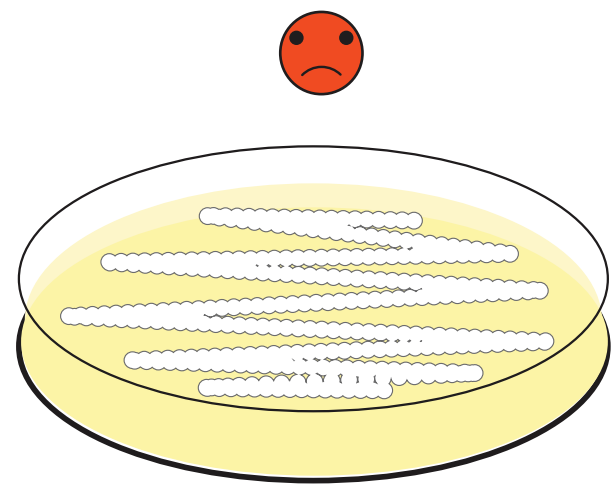
Checkpoint - Selective plate and “-” control

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.

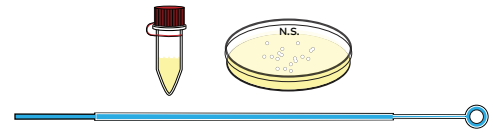
Continue the exercise with your other selective plates. Note that your results may not be as good as if you had the right amount of antibiotics. Don’t worry though, practice makes perfect, and our Success Guarantee means you will have the chance to be successful if this try doesn’t work.

3. Making Chemically Competent Cells Day2, 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

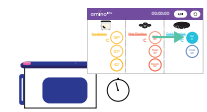
(1) Streaked N.S. Plate
(1) Blue Loop
(1) T. Buffer Tube



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 Get your Ice bucket or turn on the "Ice" (4°C) setting on your Amino Lab's Minilab™

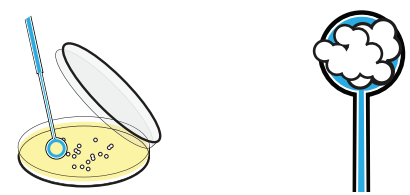


Mix the cells and T. Buffer

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 a surface. You should have 5mm of liquid in the bottom of the tube. Set it on "ice" for 2 minutes so that it cools down.

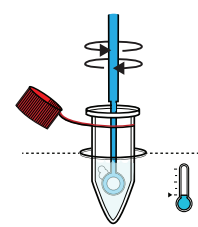
3.4 Take a blue inoculating loop and gently scrape it over small, well-separated colonies on your N.S. plate. Colonies that are ~1mm in diameter work the best. You want to collect ~10 or 20 of these colonies on your loop, enough so that you can see that the center of the loop is full.

Tip: A colony is one of the white "dots" or "mounds" you see on your N.S. 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.

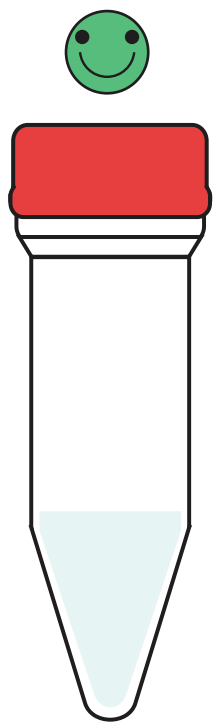
3.6 Twist the loop like a blender (or like you are trying to start a fire) to mix and suspend the cells in the liquid while keeping the tube in the cold station.



When successful, the solution should be 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 through, but replace it quickly into the cold station to keep it cool.

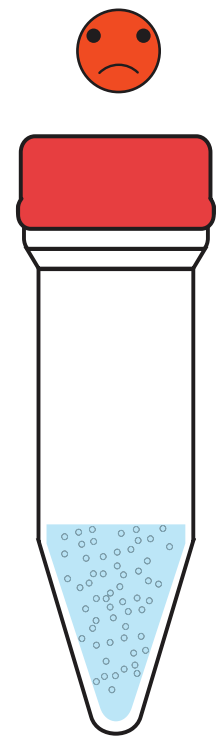
Checkpoint - Competent cells

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

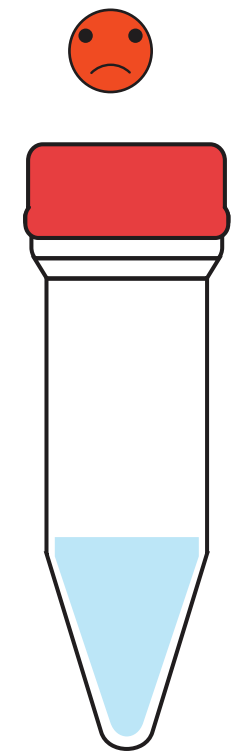


Cloudy liquid - Perfect!
The cells can now take up DNA.

Move on to the next step.



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



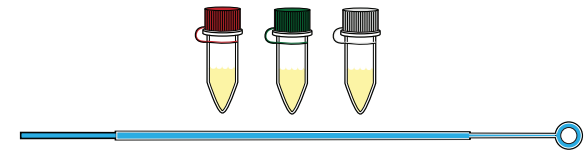
Clear liquid? Add more cells and mix until the liquid is cloudy like the image on the left.

4. Transformation Day 2, 45-60 minutes

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

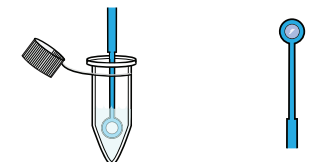
Materials from your kit - **BAG 1**

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



Take the DNA

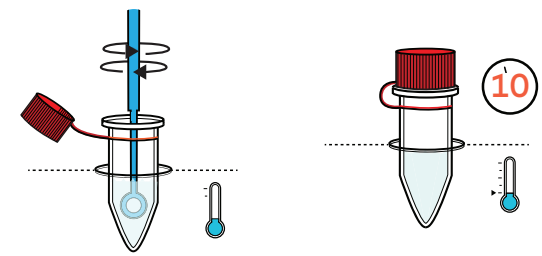
4.1 Dip a Blue inoculating loop into the DNA tube and twist a few times. When you pull the loop out of the DNA tube, the hole of the loop should have liquid in it. This is the DNA you will use to engineer your cells. Make sure you can see liquid in the loop!



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 5 seconds or more 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 10 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.

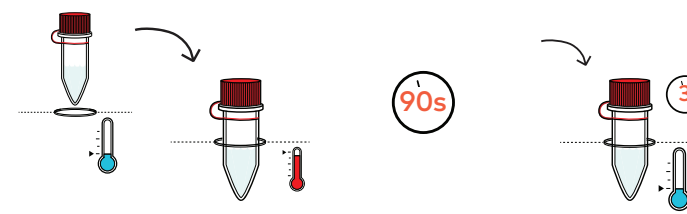


HeatShock

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 3 minutes.

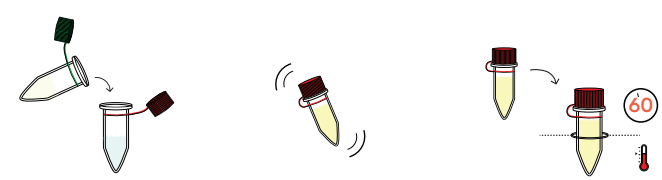
4.6 The next step is recovery which will happen at 37°C. Turn on your Minilab Hot station to Heat 37°C or adjust your water bath temperature to 37°C.



Recovery

4.7 Pour the tube of Recovery media (~350 uL) into your tube of cells, T. Buffer and 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.



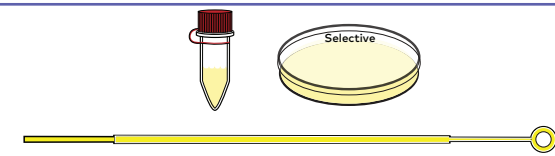
After the initial 30 minutes of recovery, you can wait up to an extra 24 hours before moving onto the next step.

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}
(3) Yellow Loop
(1) Transformed Cells {from prior step}
(1) "+ Cell" Positive Control tube



Prepare

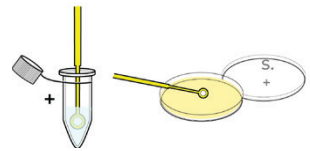
5.1 Turn on your Incubator at 37°C.

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 discard.

Plate your positive control ("+" plate)

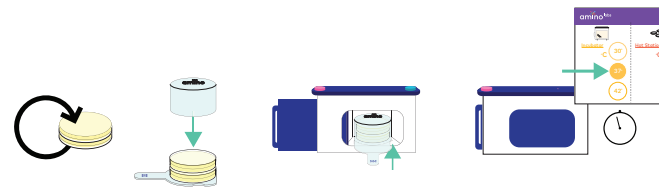
5.3 Get the stab of "+ cells" and your Selective LB Agar plate labeled "+". Using a single yellow loop, dip into the + cells stab and spread them across your agar plate in any pattern of your choosing.



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.

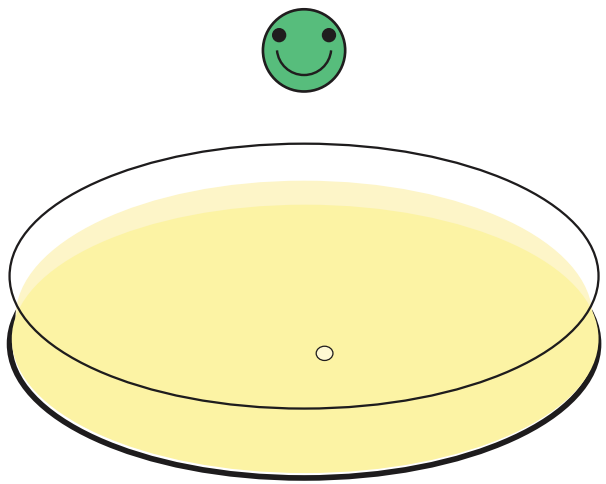
5.5 Incubate for 24-48 hours until you see one or more colonies.



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!

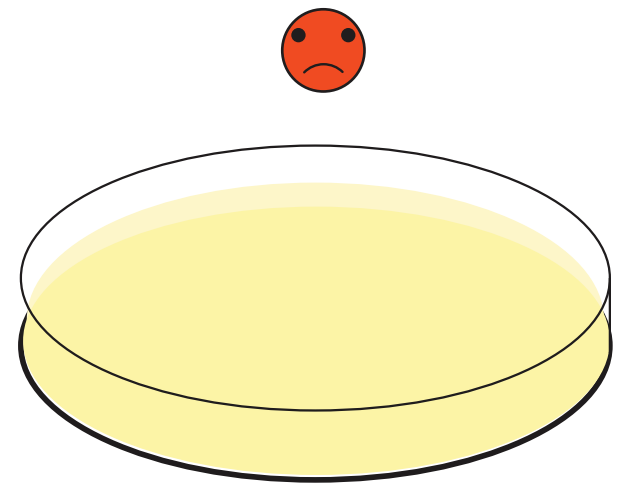
Checkpoint - Did your engineered cells grow?

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



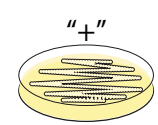
A perfect “e” plate has one or more colonies on it. Remember, even a single colony is a success. One colony is all you need to move onto the next step. Congratulations!

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, 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*.



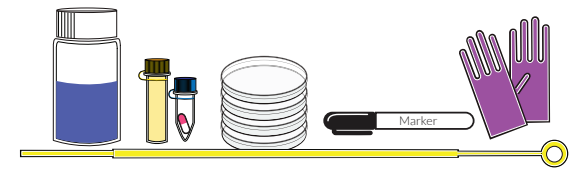
You can continue your experiment with a colony from your “+” plate if the + cells grew. Try to select a single, isolated colony if you can.

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] if doing this in groups with multiple kits. (*The bottom is the side with little tabs)*

Mix the Agar

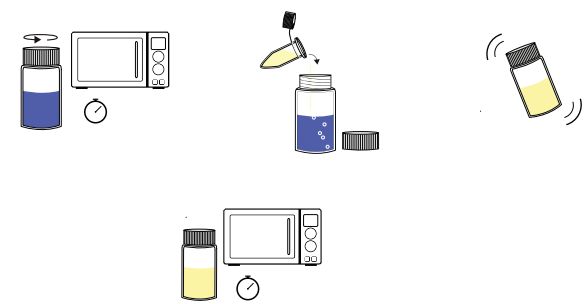
1.2 Unscrew the lid from the sterile water bottle and keep it loosely on top of the bottle to prevent any contaminants from entering the water, but allowing air to escape. This will prevent pressure build-ups.

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

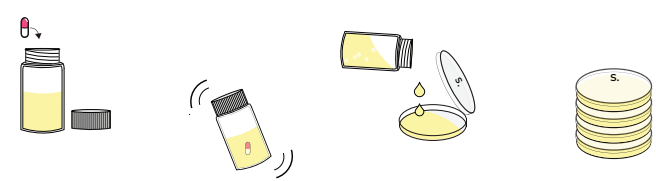


Make selective (S.) plates

1.6 Add the antibiotic pill to the bottle of agar and gently swirl for a few minutes until the contents of the pill have dissolved. Do not introduce bubbles into the LB agar, 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.

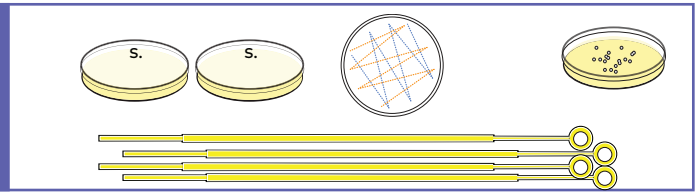


7. Amplify (Culture) engineered cells 15 minutes + 24-48 hours wait time

Goal **Grow a large quantity of cells on plates to induce after growth.**

Materials from your kit - **BAG 2**

(2) Agar plates from previous step
Your engineered cells
(1) Positive control cells
(2) Yellow Loops
(1) Double streak stencil



Prepare

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

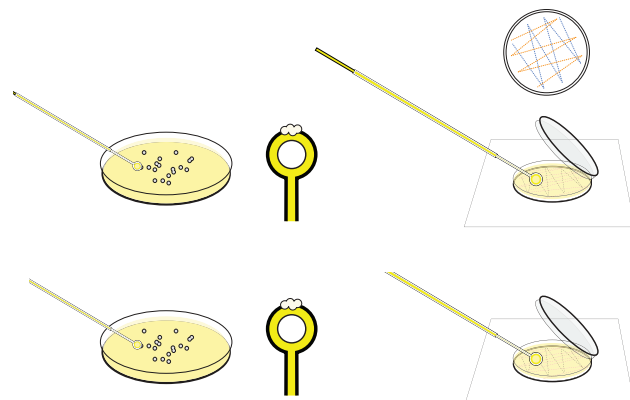
Streak

7.2 Take your engineered cells. Place one of your fresh petri dish on top of the double zigzag pattern stencil. Take one yellow loop, pick one or more colonies of engineered cells on your plate. You pick a colony by touching the end of the loop to it, gently rubbing it.

7.3 With your picked colony(ies) on your loop, trace one of the zig zag across the fresh selective agar plate.

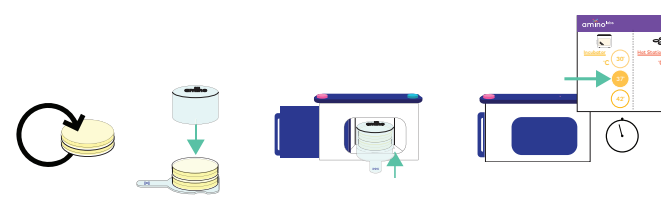
7.4 Using the same yellow loop, trace the second zigzag, which is at 90° of the first. This will ensure you will have lots of cells growing across your plate. Discard the loop.

7.5 Using the same yellow loop, repeat the same exercise on the second fresh selective agar plate.



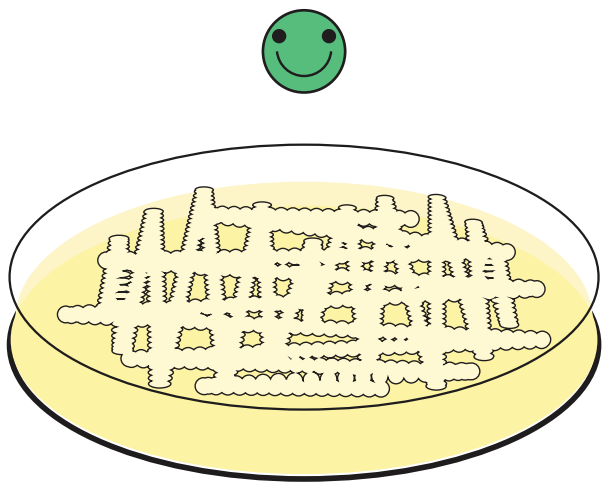
Incubate Overnight

7.6 Incubate your streaked plate **upside down** at ~37°C for up to 12 - 24 hours: Flip your plates upside down so that the agar is up and the lid down. If you have an Amino Labs' minilab, remember to close the incubator door and lock it!



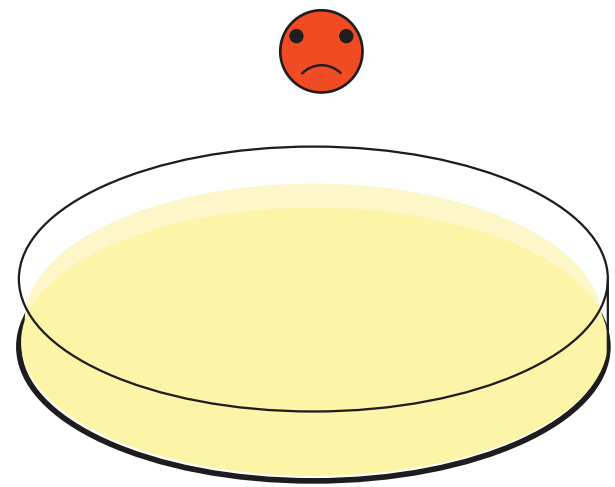
Checkpoint - Did your cultured cells grow?

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



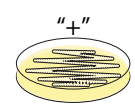
A perfect cultured plate will have many colonies or a lawn of bacteria on it. Congratulations!

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.



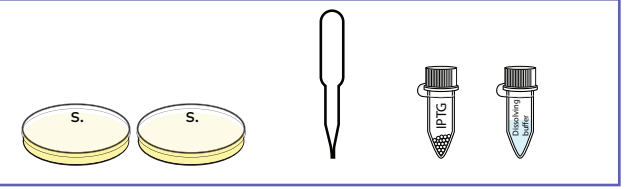
You can continue your experiment using your positive control "+" plate from step 5.

8. Induce your cells and see your results! 25 minutes + 2-24 hours wait.

Goal **Induce your incubated cells and see the colored protein expressed!**

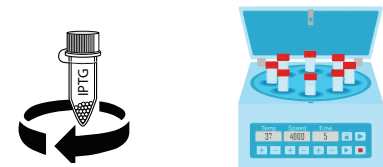
Materials from your kit

Your incubated experiment plates
(1) Pipet
(1) IPTG tube
(1) Dissolving buffer
(1) Sharpie marker



Prepare your IPTG

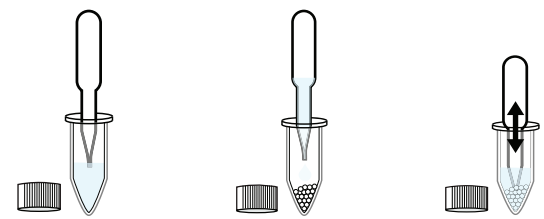
8.1 If you have a microcentrifuge, place your IPTG tube in your centrifuge and spin at max speed for 10-30 seconds. This will ensure that all the IPTG powder is at the bottom. If you do not have a microcentrifuge, 'whip' the tube so that all the powder is at the bottom of the tube. Use the whip-it technique: amino.bio/whip-it.



Dissolve your IPTG

8.2 Take the tube of Dissolving buffer and pipet its entire content into the IPTG tube. Pipet the contents up and down 10 times to mix.

If you have a DNA Playground, set your tube of dissolved IPTG with the pipet still inside in one of the tube holders on one of the stations set to 'OFF'. If not, close the tube and set your pipet on a clean surface like a paper towel. You will be re-using the pipet in the next steps.

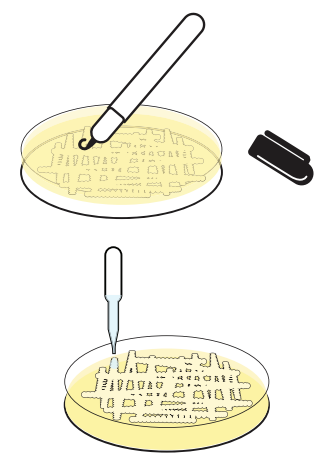


Prepare your plates for induction

8.3 Using a permanent marker, mark the part(s) of the plates where you want to place the inducer. Make a little circle or dot at the points where you will drop the inducer onto the plate.

8.4 Drop-by-drop, pipet all of the dissolved inducer onto your desired locations. The inducer will be at it's highest concentration at these points. However, it will dissolve outwards in a circular pattern through the LB agar. The higher the inducer concentration, the more the gene will be induced.

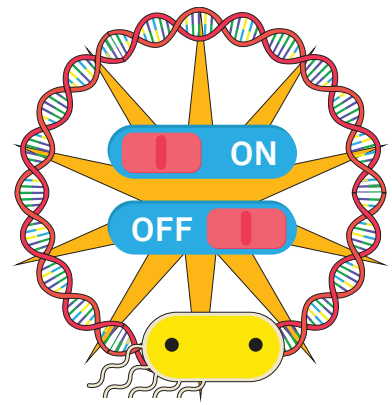
8.5 Incubate your plates upside-down at 37°C for the next 2 to 24 hours. The induction of the genetic switch relies both on time and concentration of IPTG. Check back on your plates often and take photos to see the color-change happen over time. You've manually turned on a genetic switch!



Note

If you cannot see any color change 24 hours after adding the inducer, you may not have fully dissolved the IPTG powder. Check the IPTG tube for remaining powder. You can try to re-dissolve it with distilled water and adding it to the plates. If nothing happens, 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 Induce-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 a chemical. Just like scientist and industries do every day inside their large laboratories! To learn more about genetic switches, IPTG, 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 see your results, you'll have bacteria petri dishes and tubes, loops, and ingredients in your inactivation bag, reusable or even unused components. Disposing of them responsibly is an integral part of your experiment:

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

1. **Reusable materials:** DNA and engineered cells (either on a petri dish or from stabs) can last up to 6 months when stored in a refrigerator. If you wish to keep them, close them tightly and store them in a zip-lock bag, inside a sealed plastic container in a refrigerator away from food items. If not, add them to the inactivation bag. Make sure the lids are off the tubes so that the inactivating liquid you will add can get inside. If you see any mold or strange bacteria growing inside then you should immediately inactivate the samples.

2. **Unused ingredients:** If you did not use all the agar petri dishes in your kit, store these for later use. Store them in their zip-lock bag, within a sealed container in the refrigerator for a few months. Keep away from food items. If you see any mold or strange bacteria growing inside then you should immediately inactivate the samples.

3. **Inactivation**: Dispose of any chemicals, ingedients, bacteria, agar, tubes, loops, paintbrushes, petri dishes, gloves and other non-paper material by adding all of it to the inactivation bag, including any petri dish with bacteria you are not keeping for a Keep it. Any paper packaging like loop packages and bags can go in the regular garbage.

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

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

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

Glossary

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

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

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

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

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

DNA: The DNA is the set of instructions that tell the cell how to function like a computer program tells your computer what to do.

DNA plasmid: A plasmid is a small circular piece of DNA (about 2,000 to 10,000 base pairs) that contains essential genetic information for the growth of bacteria. Bacteria share vital information by passing it among themselves in the form of genes in plasmids. By inserting a new plasmid in our bacteria, we

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

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

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

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

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

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

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

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

Transformation: See competent cells.

Troubleshooting

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

Here are some possible common issues:

Your agar is too wet/ doesn't solidify: The agar, if done correctly, will be the consistency of Jell-O. If not:
1. You might not have added all the powder from the tube, resulting in too much water vs. LB agar powder.
2. You may not have fully dissolved the powder, meaning it cannot turn into a gel and will look cloudy. You can practice by making Jell-O! Next time heat and swirl longer to ensure the powder is fully dissolved.

You don't have any colonies and its been 24+ hours: Don't worry, every scientist has experienced this, and it can take some practice before success.

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

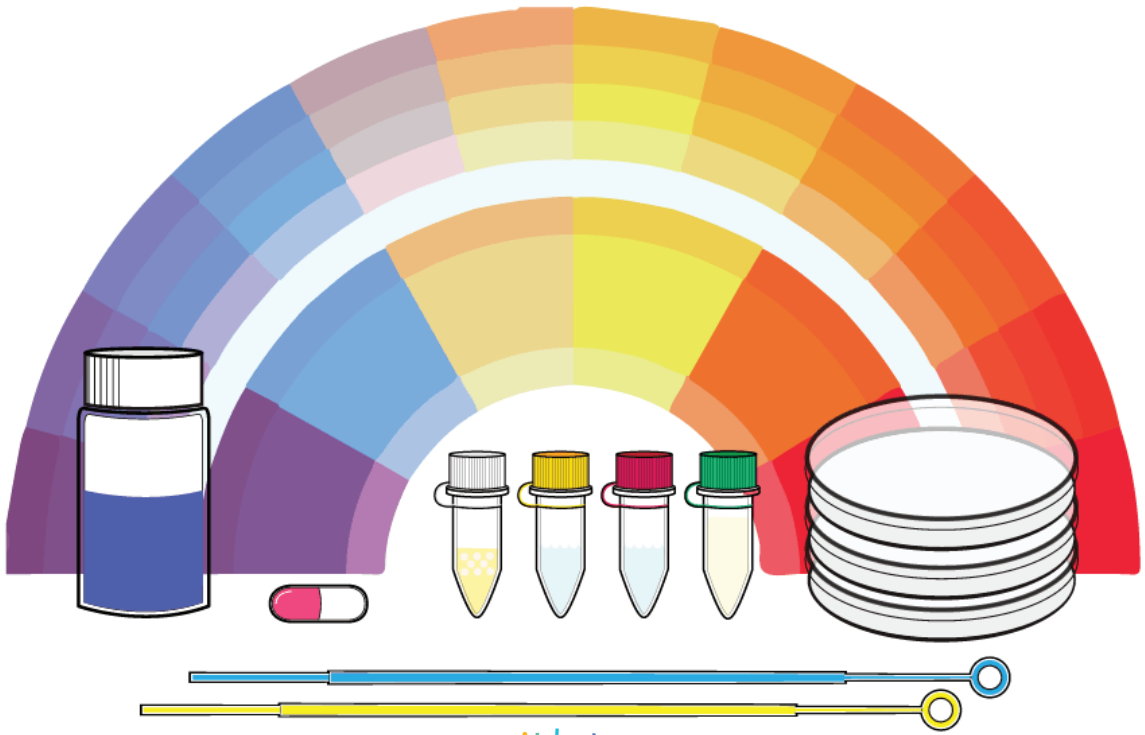
2. You may need to try again to hone your skills. See our Youtube videos for tips and tricks on how to improve your chances of success.

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

Pour 100% chlorinated bleach into the dish, put the lid on and let it sit for 24 hours before throwing it out: The strong oxidizing environment degrades any living organisms. After 24 hours, if there are still organisms present add more concentrated bleach until it is almost full, and let stand for a further 24 hours. *Always be aware that concentrated bleach is a strong oxidizing agent and if poured on the skin can cause irritation, and on clothes remove color. Follow the safety and handling protocol on the manufacturer's label.*

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

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



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