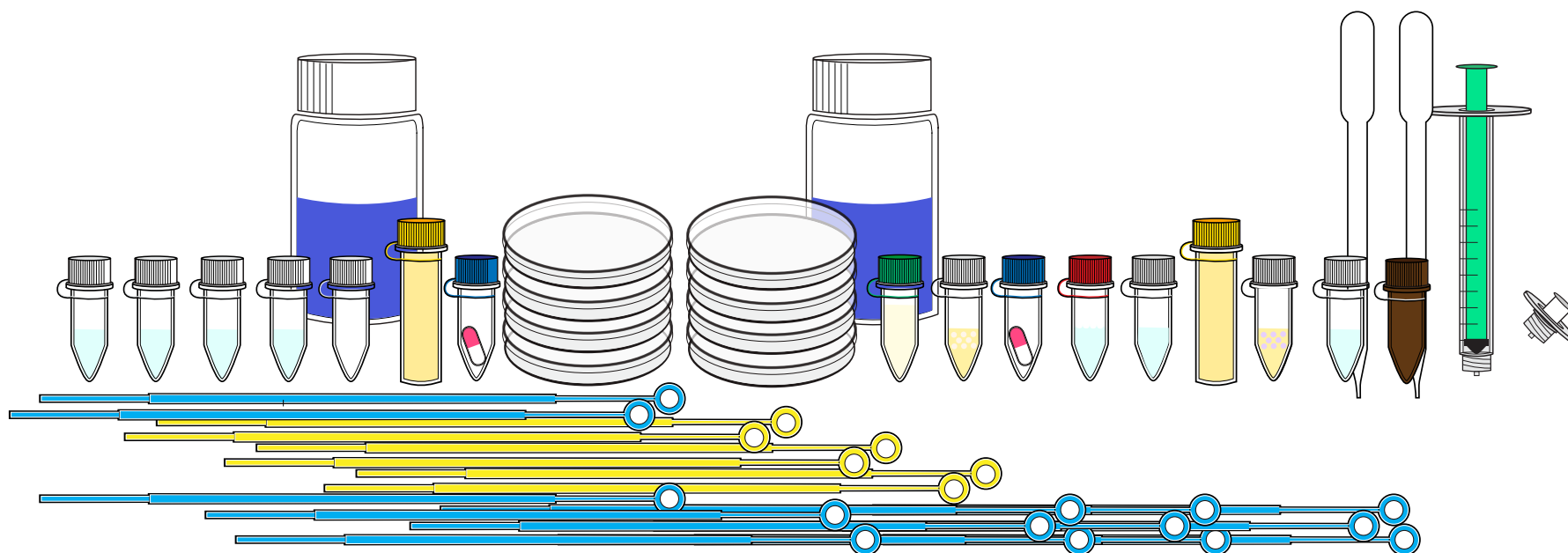


GENETIC ENGINEERING WITH

# BLUE-IT KIT™

— User Manual —



# BLUE-IT KIT™

## User Manual

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# Welcome! Let's get started

This User Guide was created to help you get the most out of your Amino Labs Experience. Even if you are familiar with genetic engineering, science or other Amino Labs™ products, please take the necessary time to read through this guide. This will ensure you practice safe science, store, use and get the most out of your Kit and 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. You can also learn more about enzymatic reactions, including the specific reaction for the Blue-gal reaction, by reading Chapter 6 of the Zero to Genetic Engineering Hero book, [www.amino.bio/book](http://www.amino.bio/book).

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



# 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, also known as 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 seek medical opinions prior to completing the exercises.
- **Wash your hands before and after** manipulating your experiment, ingredients or 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 phar-

macy 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 chlorinated spray cleaner or make a 10% solution of chlorinated bleach generously and sprayed onto a paper towel and rub onto any contam-

inated surfaces. To make a 10% solution, add 9 parts water to 1 part chlorinated bleach. Careful! Chlorinated sprays and bleach can discolor your clothes.

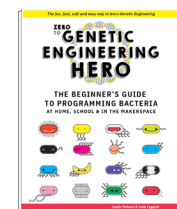
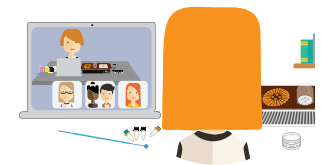
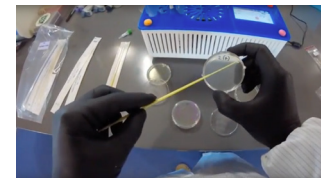
- **Find a container to hold the inactivation bag where you will discard used consumables.** An old 1L yogurt container, large plastic cup or the like will do. Used consumables will be loops, any tube or used petri dish. Used gloves can go in regular trash if you do not get any samples (micro organisms, DNA) on your hands.

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

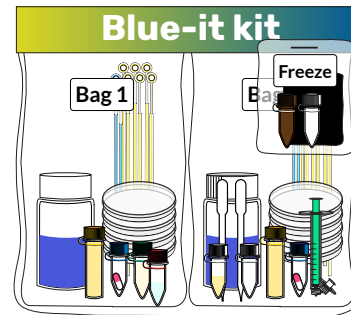
# How will I learn?

Learning and prototyping with genetic engineering and cells is becoming accessible to newcomers of all ages and backgrounds thanks to dedicated scientists and kits such as the one you are about to use! One of the easiest ways to learn a new science, hobby or topic is by trying it, hands-on. Our Amino Labs Kits make it easy to add a DNA program into living cells by following the instructions in this booklet. Everything you need to complete the science is included; each ingredient in the kit is pre-measured and labeled for a stress-free experience. Our all-in-one stations decrease setup time, mess, guesswork and the need to collect and calibrate multiple machines. The included instructions should be easy-to-follow for everyone but may contain some new terms. For your reference, we have added a glossary at the end. We also have additional resources to help you go further in your learning:

- An essential addition to our ecosystem is the free **Virtual Bioengineer™ simulation** developed with the educators at the Biobuilder Educational Foundation. A 20 minutes guided experience that makes it easy to practice using a DNA Playground™ and kits beforehand. While the main simulator focuses on the Engineer-it kit™ experience, many manipulations you will be doing are very similar. It also includes additional information on the manipulations and a more in-depth look into DNA and genetic engineering. We recommend it strongly! Complete it online at [www.amino.bio/vbioengineer](http://www.amino.bio/vbioengineer).
- View **Real-time tutorials** on our Youtube channel. Subscribe! [youtube.com/c/AminoLabs](https://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](http://www.amino.bio/products/cyberworkshop).
- Are you interested in the theory behind the experiment? In going deeper on the science, learning pro-tips and eventually completing more advanced genetic engineering? The **Zero to Genetic Engineering Hero book** is for you. Find out more at [www.amino.bio/book](http://www.amino.bio/book)



# Discover your Blue-it Kit™



The Blue-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 protein-enzyme that is able to catalyze a chemical reaction. The enzyme, beta-galactosidase which comes from the gene LacZ, is able to convert substrate molecule called X-gal which is a colorless white powder into a colorful product. Within this kit, there are two different X-gal substrates, Yellow-gal and Blue-gal.

First, you will engineer the bacteria to produce the beta-galactosidase enzyme. Second, you will culture the engineered bacteria so that lots of enzymes are produced within the bacteria. Third, you will collect the bacteria, lyse them open to release the enzymes and sterilize the extract. Fourth, you will dissolve some X-gal substrates in reaction buffer tubes, add your extracted enzymes, and watch the chemical reactions happen!

You can learn more about enzymatic reactions, including the specific reaction for the Blue-gal reaction, by reading Chapter 6 of the Zero to Genetic Engineering Hero book, [www.amino.bio/book](http://www.amino.bio/book). You can also have a look at the Smell-it Kit™ in our store to explore another enzymatic reaction experiments. [www.amino.bio](http://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. **Caution: one of the kit components has a strong odor. If you are sensitive to odors, this kit may not be ideal for you.**

# Kit Components

## Bag 1



**Transformation Buffer:** 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.<sup>1</sup>



**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.<sup>1</sup>



**Agar Powder:** 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.<sup>1</sup>



**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.<sup>1</sup>



**+ 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.



**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 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!<sup>1</sup>





**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 is used with LB agar powder is enough to make 4 LB agar plates.<sup>1</sup>



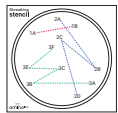
**Blue Loops:** Small inoculating loops are used for transferring 1 uL of liquid and other tasks. These replace costly traditional pipettes.



**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.

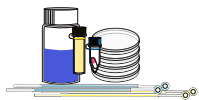


**Plate Streaking Stencil:** To help you grow the bacteria into separated, fast-growing colonies for engineering . Following this stencil will help you achieve this.

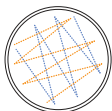


**Inactivation Bag:** A heavy duty bag to put all of the kit waste in. After your experiment, add bleach and water to the bag to inactivate all the samples and practice safe science.

## Bag 2



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



**Plate Double-Streak Stencil:** To help you grow bacteria into large quantities to use for further experiments or for extracting products. Following this stencil will help you achieve this.



**Lysis buffer:** : softly breaks open (lyses) the cells to release the cell contents. This buffer should be used in concert with Lysis Accelerator.



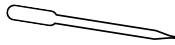
**0.22 um filter** : this filter has pore sizes that are 0.22 um which are smaller than bacteria. This means bacteria cannot pass through, but your pigment (smaller than 0.22 um) can.



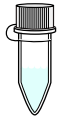
**Syringe**: used to push unfiltered extract through a filter. Caution! Do not press too hard to avoid liquid mishaps. Goggles recommended when using the syringe.



**1.5 mL Screw Cap Tube** : Use one to store your final, extracted and filtered product.



**Pipets** : Pipets are used to transfer the cells between tubes.



**Balancing tube\***: use this tube as a balancing tube when microcentrifuging. Always be sure to verify that this tube has the same weight as the tube you are microcentrifuging.



**Burst bag**: a plastic bag to use over the syringe-filter sterilization to minimize possible mess.



**Enzyme stabilizer**: this reducing agent called DTT helps to maintain protein-enzyme structure and function. DTT is similar to Beta-mercaptoethanol but is a safer component for you to handle.

## Freezer Bag



**Lysis Accelerator**: includes enzymes that break down the cell wall of bacteria and works with Lysis Buffer to release the contents of cells into their environment.



**Reaction Buffer**: Enzyme reactions require a specific chemical environment and pH to occur. This reaction buffer is designed for the beta-galactosidase enzyme to function.



**Substrate 1 & 2**: Blue-gal and Yellow-gal are substrate molecules that change colors when in contact with beta-galactosidase.

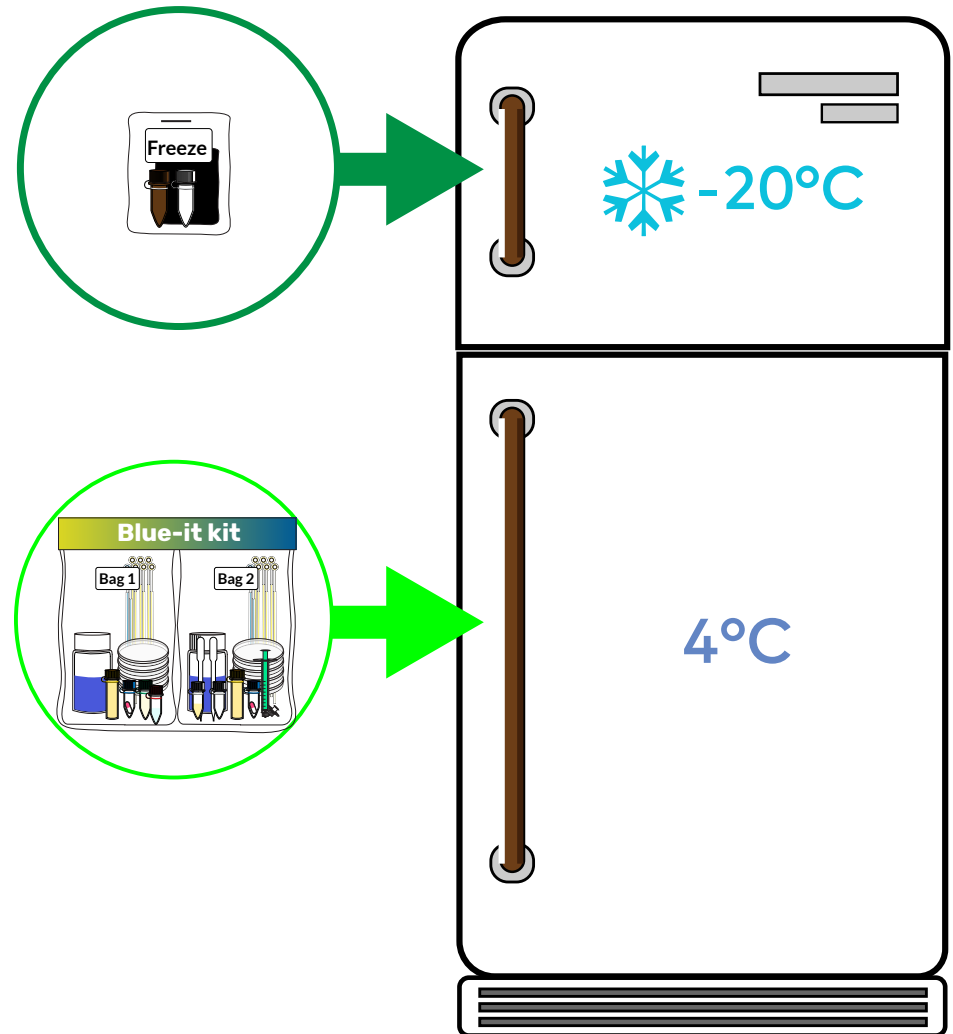
# Unpacking and Storing your kit

For a better shelf life and successful experiments:

- place your Blue-it Kit™ Bag 1 & Bag 2 in a standard refrigerator at around 4°C.
- place the smaller Freezer bag in a freezer.

**Do Not Freeze all of your kit!**

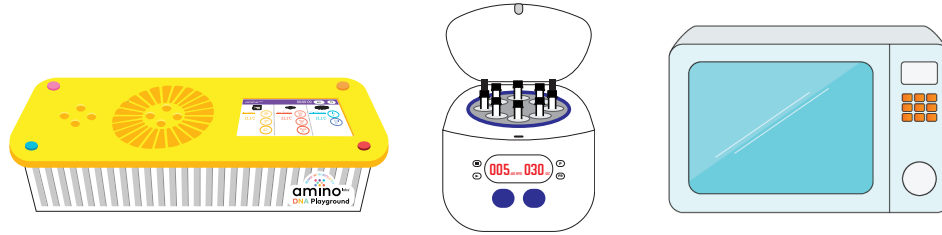
**Do not leave tubes at room temperature!**



# Necessary Equipment

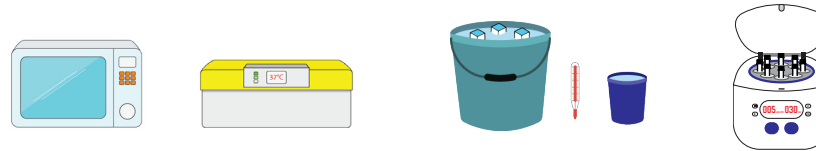
## For Best results:

- DNA Playground™ or BioExplorer™
- Microwave
- Microcentrifuge



## Alternative solution:

- **Microwave**
- **Microcentrifuge**
- **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 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/](https://www.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 light bulb 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 spray or 10% bleach solution** (mix a 10% solution: 1 part bleach to 9 parts water)

# Timeline

## 1<sup>st</sup> day

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

Incubation  
12-24 hours

## 2<sup>nd</sup> day

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

Recovery  
1-24 hours

## 3<sup>rd</sup> day

Plate transformed cells and incubate.  
*Can be done on 2<sup>nd</sup> day.* (45 minutes)

Incubation  
24 hours

Bag 1

## 4<sup>th</sup> day

Create culture plates, double-streak engineered colonies, incubate. (45 minutes)

Incubation  
24-48 hours

## 5<sup>th</sup> day

Collect bacteria, lyse bacteria and put in the fridge (30-45 minutes)

Incubation  
24-72 hours

## 6<sup>th</sup> day

Centrifuge lyse bacteria, filter-sterilize the extract. (45 minutes)

## 7<sup>th</sup> day

Prepare reaction buffer tubes. Start reactions.  
*Can be done on 6<sup>th</sup> day.* (45 minutes)

**Yellow-gal reaction:**  
happens in minutes  
**Blue-gal reaction:**  
happens over 24 hrs.

Bag 2 & Freezer bag

Can be completed on the same day

# 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 Day 1, 2 and 3. Find them on our youtube channel: [youtube.com/c/AminoLabs](https://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) antibiotic pill (1) Sharpie marker  
(1) LB agar powder (4) 6 cm petri dishes



### 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"**



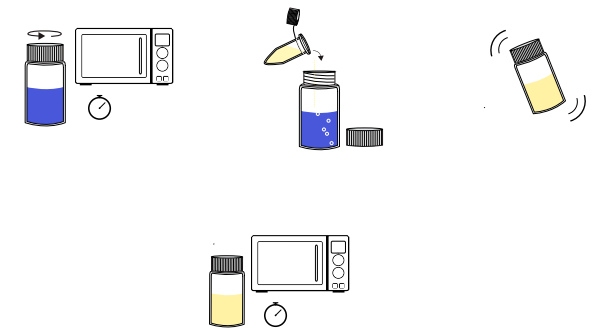
### Mix the Agar

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. Place the lid 3/4 of the way 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.

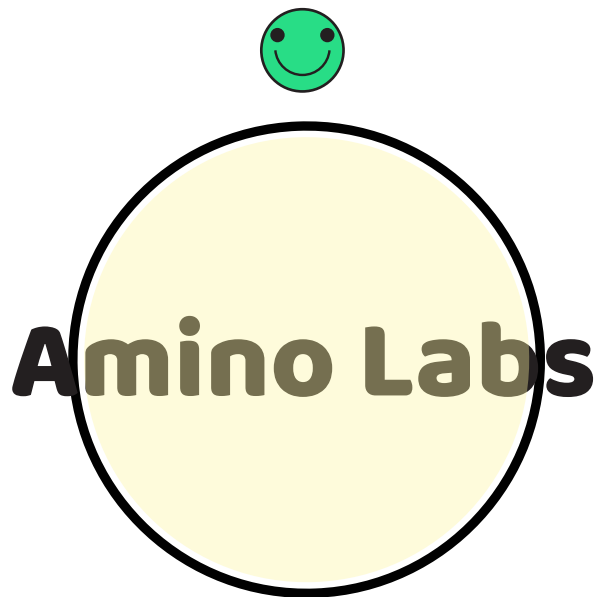
1.7 Once the pill is dissolved, pour the LB agar into the 3 petri dishes left. Place the lids 3/4 of the way back on to allow for some evaporation.



1.8 Let the LB agar cool and harden. The non-selective plate and the "-" selective 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.

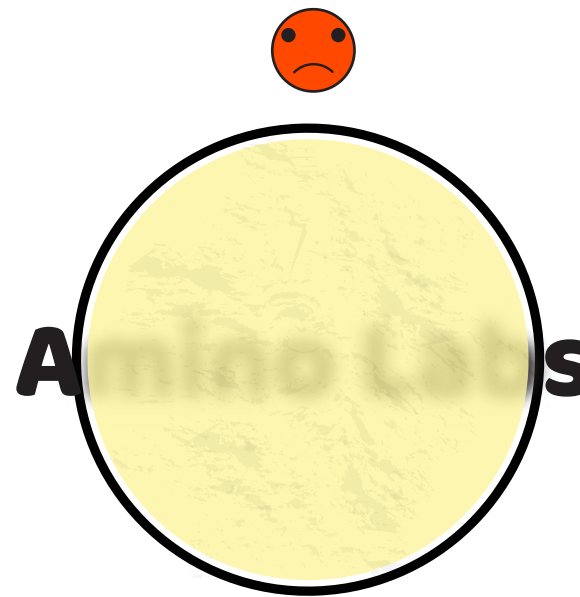
# Checkpoint - Agar Plates

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, 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](http://www.amino.bio/troubleshoot)

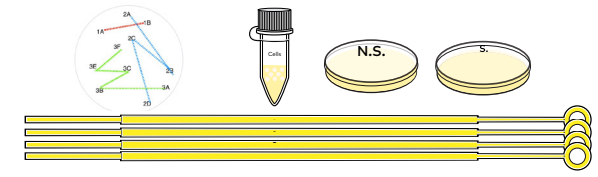


## 2. Growing Blank Cells Day 1, 20-45 minutes + 16-24 hours wait time

**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

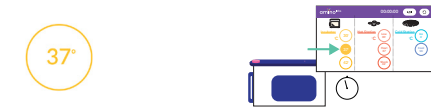
Non-selective & "-" selective plate (4) Yellow Inoculation Loops  
(1) Plate streaking stencil (1) Stab of cells (**Not Cells +**)



### 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



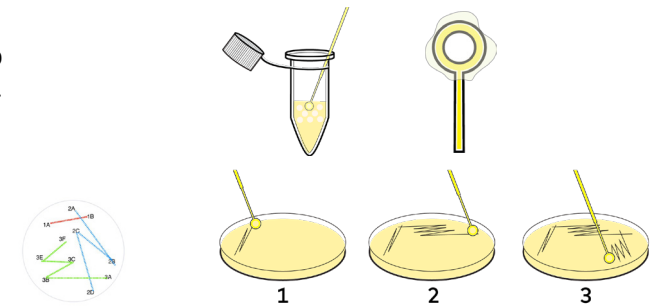
### Streak your Plate

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.

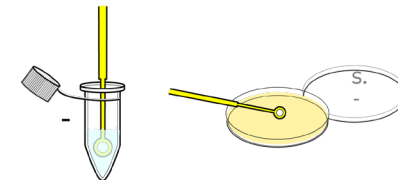
2.5 Using a new yellow loop, trace line 3. Discard the loop. Close your petri dish and set aside.



### 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

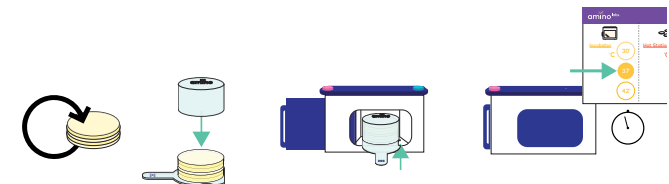
2.8 Flip your streaked N.S. & S. "-" plates upside down and stack them in a pile in an incubator set to 37°C. If you are using the DNA Playground, put your stack of plates on top of the incubator paddle, set the incubator humidity chamber on top and slide in the incubator. Close the incubator door and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

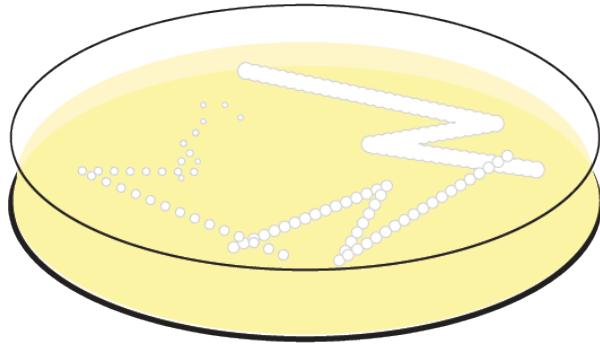
It must incubate between 35°C and 37°C. If the cells are below 37°C, it will take longer for them to incubate - you may have to wait up to 48 hrs until you see small colonies on your plate.



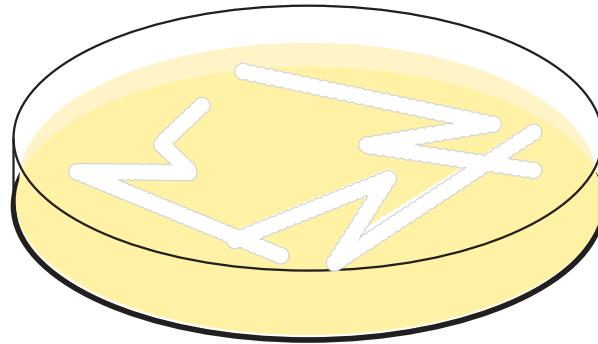
**Teacher Tip!** If you are in a classroom setting and your class is 24 hours-30 hours apart or more, you can initially grow your cells at 30 C overnight and then increase the temperature to 37 the day of making competent cells.

# Checkpoint - Non-selective plate & blank cells

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

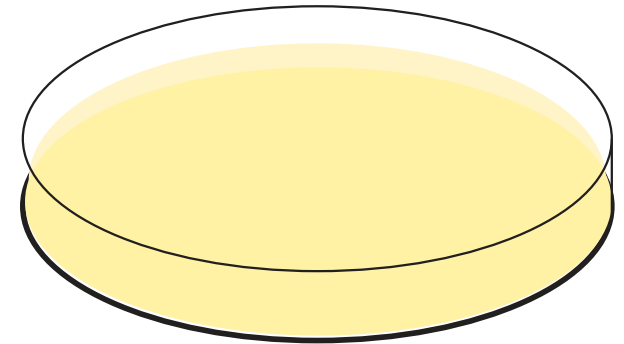


A perfect N.S. plate has lots of small individual white colonies (dots) of fast-growing bacteria after incubation. Proceed to the next page.



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.

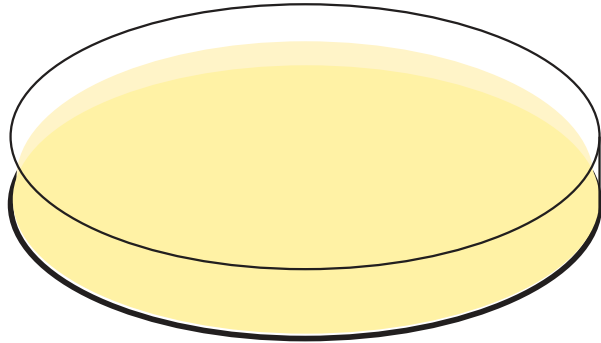


If you see no growth on your N.S. plate:

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](http://www.amino.bio/troubleshoot)

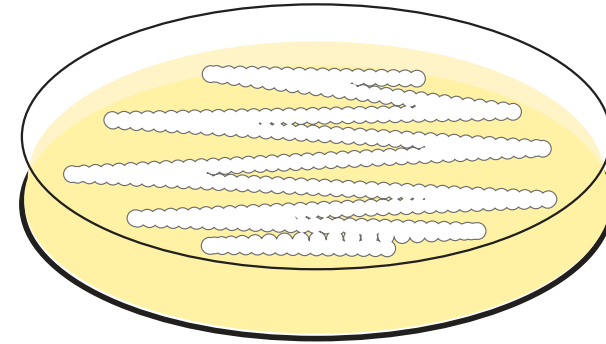
# Checkpoint - Selective plate and “-” control

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

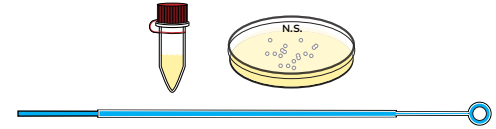
**Goal** Pick small colonies, and suspend the bacteria in cold transformation buffer, enabling bacteria to better take up DNA.

#### Materials from your kit

(1) Streaked N.S. Plate

(1) Blue Loop

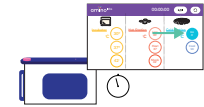
(1) T. Buffer Tube



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

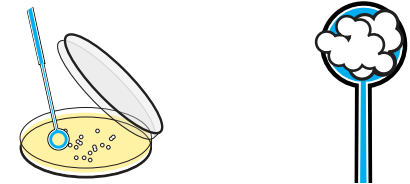


#### Mix the cells and T. Buffer

3.3 Take your T. Buffer tube and make sure all the liquid is in the bottom of the tube by briefly putting 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.

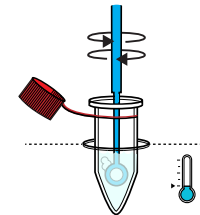
Tip: A colony is one of the white "dots" or "mounds" you see on your N.S. LB agar. The separated colonies are those that look like individual dots, not streaks or solid lines of white.



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.

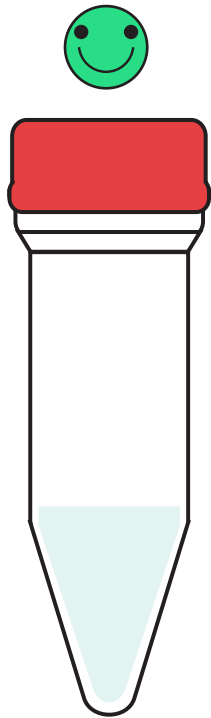
When successful, the solution should be slightly cloudy, and there should be no clumps floating in the solution. You may have to mix vigorously for up to 45 seconds. If you see clumps, keep blending. You can lift the tube out of the cold station to see whether the T. Buffer is cloudy and the clumps are gone, but replace it quickly into the cold station to keep it cool.



3.7 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.

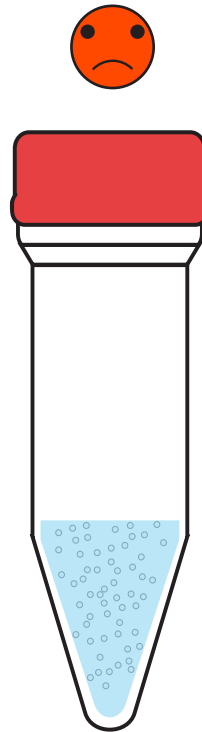
# Checkpoint - Competent cells

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

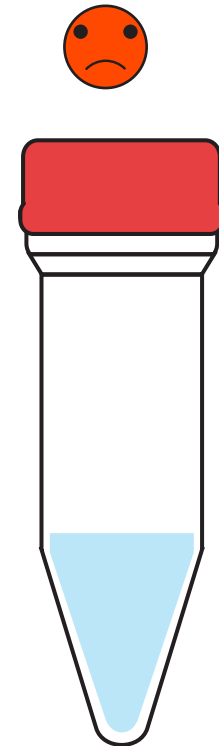


Cloudy liquid - Perfect!  
The cells can now take up DNA.

Move on to the next step.



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



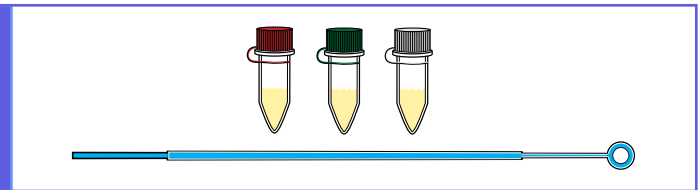
Clear liquid? Add more cells and  
mix until the liquid is cloudy like  
the image on the left.

# 4. Transformation Day 2, 45-60 minutes

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

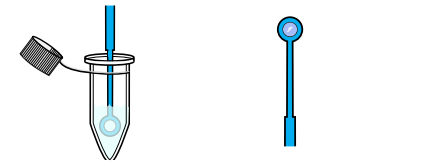
## Materials from your kit - BAG 1

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



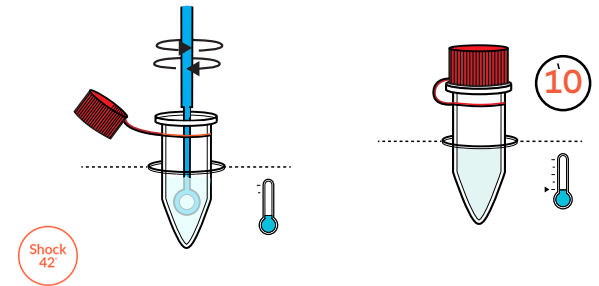
## Take the DNA

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

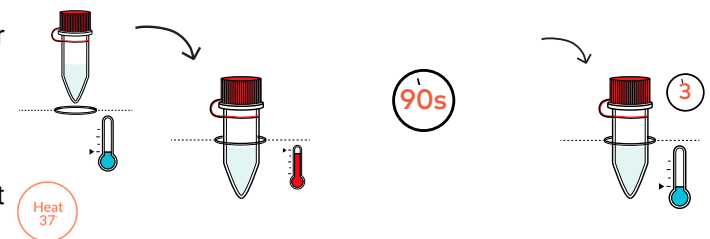
4.2 Slowly dip and spin the inoculating loop containing DNA into the competent cells you made in the previous step. Stir/swirl for 5 seconds to fully mix. Do this while the tube is on Ice / Cold Station. Discard the loop. \* Do not reuse the inoculating loop! \*



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

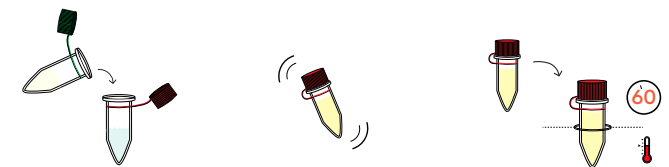


4.5 After 90 seconds, immediately place your tube back on Ice / Cold Station for 2 minutes.

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](http://www.amino.bio/whip-it) to learn the Whip-it method for moving liquid inside a tube.

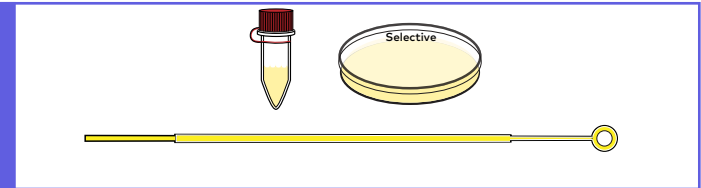
**After the initial 30 minutes of recovery, you can wait up to 24 hours before moving onto the next step.**

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



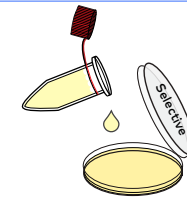
**Prepare**

5.1 Turn on your Incubator at 37°C.



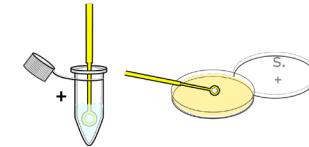
**Plate your transformed cells ("e" plate)**

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 them or keep them just incase you do not get colonies. You can always pour the remaining recovered cells onto the selective plate and incubate them.



**Plate your positive control ("+" plate)**

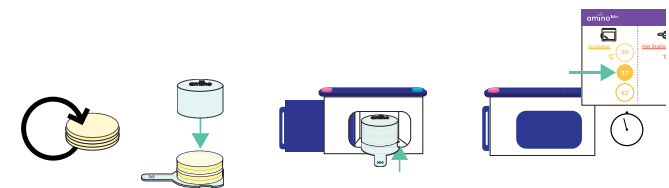
5.3 Get the stab of "+" cells" and your Selective LB Agar plate labeled "+". Using a single yellow loop, dip into the + cells stab and spread them across your agar plate in any pattern of your choosing.



**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.

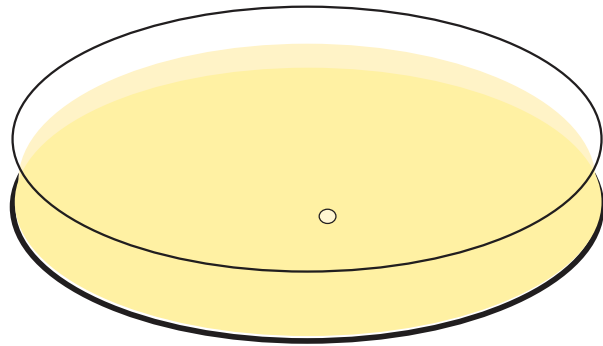


The cells will grow in colonies and start producing their new DNA program in the next hours. The DNA program can take up to 48 hours to develop. Keep an eye out, and your camera ready to document!

# Checkpoint - Did your engineered cells grow?

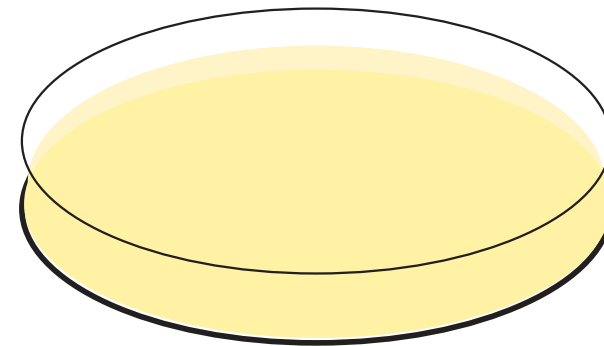
+

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



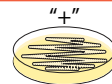
A perfect “e” plate has one or more colonies on it. Remember, even a single colony is a success. One colony is all you need to move onto the next step. Congratulations!

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](http://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 more selective LB agar plates 25 min.

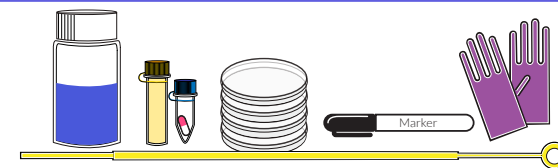
**Goal** Create a new batch of selective LB agar plates to amplify (culture) your engineered cells.

### 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*)



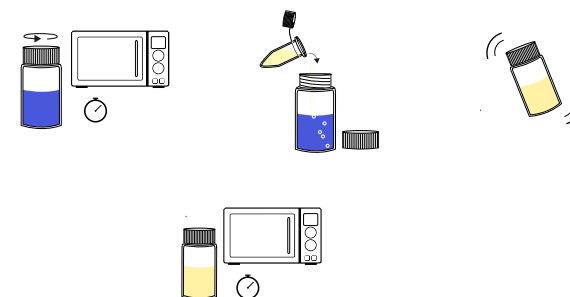
### Mix the Agar

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.



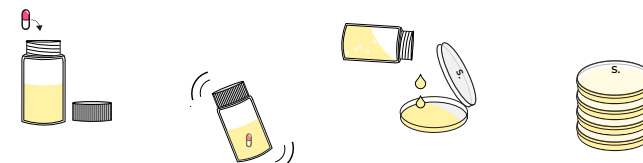
*Note: you will not be making a non-selective plate. All 4 plates will be selective agar.*

### 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**

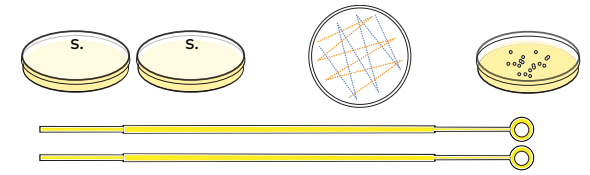
(2) Agar plates from previous step

Your engineered cells

(1) Positive control cells

(2) Yellow Loops

(1) Double streak stencil



## Prepare

7.1 Turn on your incubator to 37°C.

37°

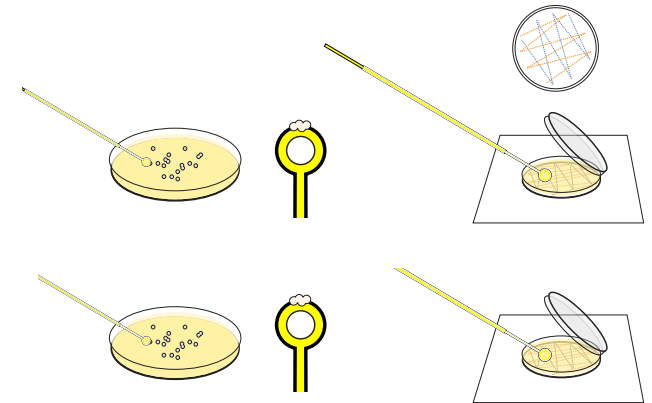
## Streak

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 colony 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 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.

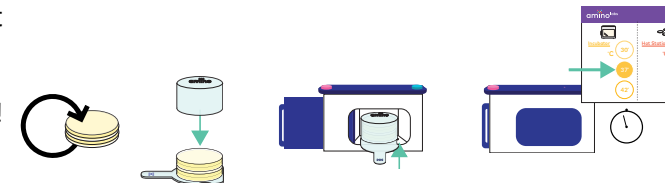
7.5 Using the **same yellow loop**, repeat the same exercise on the second fresh selective agar plate.



## Incubate

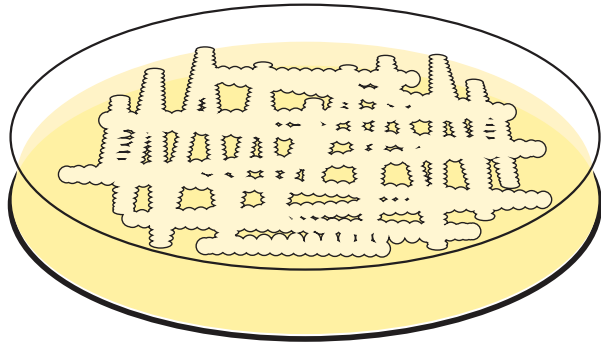
7.6 Incubate your streaked plate **upside down** at ~37°C for 24 to 48 hours: Flip your plates upside down so that the agar is up and the lid down.

If you have an Amino Labs' minilab, remember to use the humidity chamber, close the incubator door and lock it!



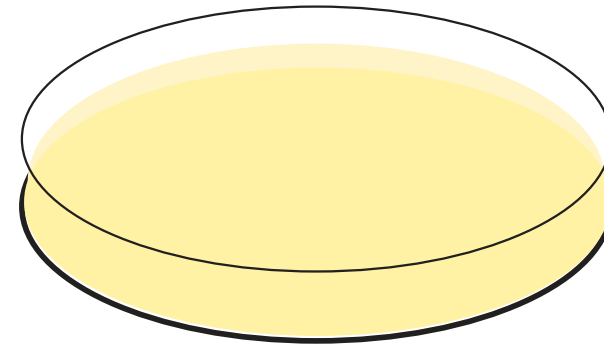
# Checkpoint - Did your cultured cells grow?

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



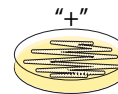
A perfect cultured plate will have many colonies or a lawn of bacteria on it. Congratulations!

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](https://amino.bio/troubleshoot) to claim your free *Success Guarantee* kit.



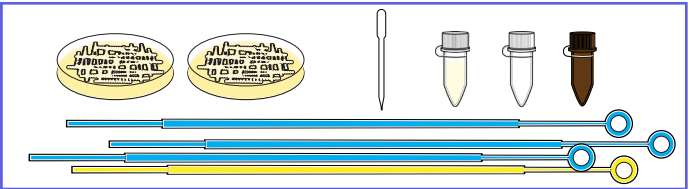
You can continue your experiment using your positive control "+" plate from step 5.

## 8. Harvest & lyse the bacteria 15-30 minutes

**Goal** Suspend the cells in lysis buffer and enzyme in order to break down the cell wall and release the product

### Materials from your kit

(2) Your amplified bacteria plates	(1) Lysis Buffer tube	(1) 1 mL Pipet
(1) Yellow Loop	(1) Lysis Accelerator	(1) Enzyme stabilizer
(3) Blue Loops		



### Harvest

8.1 Verify if your cells have grown. If they have not, keep incubating. If they have, move onto the next step

8.2 Take the Freezer bag out of the freezer and let the tubes thaw at room temperature.

8.3 Centrifuge the enzyme stabilizer tube for 5 seconds to make sure all the powder is at the bottom of the tube.

8.4 On your small pipet, notice the small line partway up its length. This line indicates 100  $\mu$ L of volume. Using the pipet, add 100  $\mu$ L of the reaction buffer from one of your reaction tubes to the enzyme stabilizer powder tube and pipet up and down until the powder is dissolved.

8.5 Add 3  $\mu$ L of Enzyme Stabilizer to the lysis buffer: Dip a blue loop into the stabilizer, spin it to fill the center of the loop with liquid. Transfer this liquid to the lysis buffer tube and spin the loop to mix. Discard the loop. Repeat twice more with 2 new blue loops. **Close and store your Enzyme Stabilizer tube in the FREEZER immediately after use.** You will need it again for step 10.

8.6 Gently drag your inoculating loop across the surface of the LB agar to collect the cells inside the loop. Once the loop is full, dip it into the Lysis buffer tube and twist the inoculating loop like a blender to dislodge and mix in the cells, just like you did when creating competent cells during the transformation process.

8.7 Repeat the scraping and blending process until you've collected all of the cells. Blend the cells and buffer for a further 60 seconds to make sure they are fully suspended. This helps the Lysis Buffer surfactants lyse the cells.

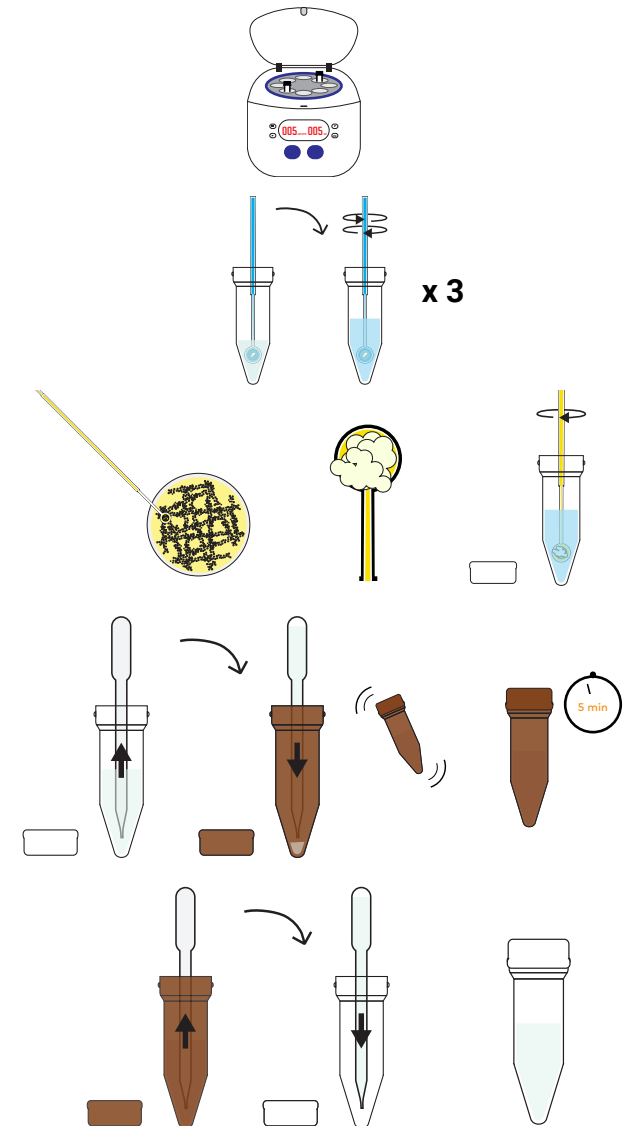
8.8 Open the Lysis Accelerator. Using one of the pipette included, suck up all of the Lysis Buffer and cells and gently pipet it into the Lysis Accelerator tube.

Note: Keep the pipette as you will use it again in the next steps. Set it on a clean surface.

8.9 Once you have moved all the buffer over to the Lysis accelerator tube, firmly close the lid of the Lysis accelerator tube and vigorously shake it for 30 seconds to ensure that it is fully mixed.

8.10 Let sit for 5 minutes.

8.10 Using the pipette, move all the liquid in the Lysis Accelerator tube back into the clear Lysis Buffer tube. Let the mixture incubate in the refrigerator for 24 to 72 hours.



## 9. Collect & filter-sterilized bacteria 30 minutes + 24 hours wait time

**Goal** Passing the extracted protein-enzymes through a filter to get rid of cells and other debris (sterilize the products)

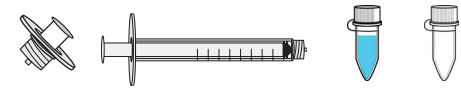
### Materials from your kit

Your tube from step 8

(1) Syringe

(1) Syringe Filter

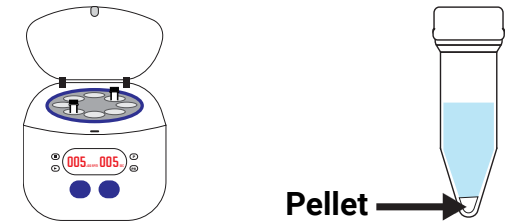
(1) 1.5 mL tube for final product



### Pellet

**9.1** Balance your microcentrifuge according to the manufacturer's instruction by adding a similarly weighted tube directly opposite your tube. The kit provides a balancing tube for your help. This tube includes a volume of liquid that should be close to what you have inside your Lysis Buffer tube (Lysis buffer, cells, Lysis Accelerator). If you have spilled any of your Lysis buffer and cells, you will need to use the pipet to remove some liquid from the balance tube.

**9.2** Add your tube of Lysis Buffer and cells into the centrifuge. Spin at maximum speed (13,000 x g to 15,000 x g) for 20 minutes. Refer to the centrifuge manufacturer's instruction for additional centrifuging help.



### Filter-Sterilize

While your solution is centrifuging, prepare your next step:

**9.3** Using a tube rack or your DNA Playground as a tube holder, get the Final Product Tube, remove the lid and place in on the Hot tube station (in the off setting).

**9.4** Remove the syringe plunger from the syringe and lay it on a clean surface.

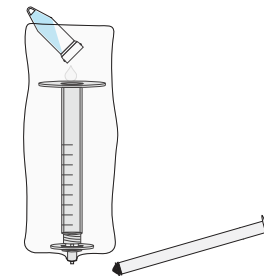
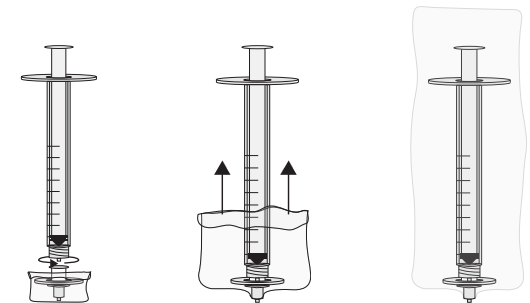
**9.5** Open up the syringe filter by taking the paper cover off. DO NOT fully remove the filter! You want to make sure that you do not contaminate the other end, the one that will not screw into the syringe but will dispense your sterilized sample.

**9.6** Holding the filter via the plastic container, screw on the syringe to the filter so it is firmly connected.

**9.7** Place the syringe and filter inside the Burst Bag, with the tip of the filter poking out of the triangle cut at the bottom of the Burst Bag. You can lay this on the table and be sure not to touch the sterile end of the filter.

**9.8** Once centrifuged, gently pour your centrifuged sample into the open syringe. Be careful to only pour the liquid - If the pellet of cell debris falls into the syringe, it will clog the filter! If any cell debris gets into the syringe, pour the entire amount back into the tube and repeat the centrifugation.

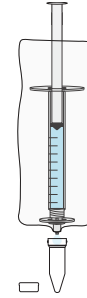
**9.9** With the sample in the syringe, hold it so that the sterile end that will release your filtered solution is pointing into the Final Product Tube.



9.10 Replace the syringe plunger into the syringe and GENTLY press down. If you have effectively centrifuged your sample, the plunger should slowly push until all the solution passes through. Any cell debris or bacteria that were in the sample will be trapped in the filter, while small molecules such as your protein-enzymes will pass through.

9.11 Close and tighten the lid on your Final Product tube. Congratulations! The solution in your Final Product tube is an extracted, filter-sterilize protein-enzyme you can use to create chemical reactions.

You now have sterilized protein-enzymes you microfactured yourself with your genetic engineering skills. Store the final product tube into the refrigerator until you need it to start your chemical reactions.



# 10. Catalyze a chemical reaction using enzymes 25-45 min. + 24 hours

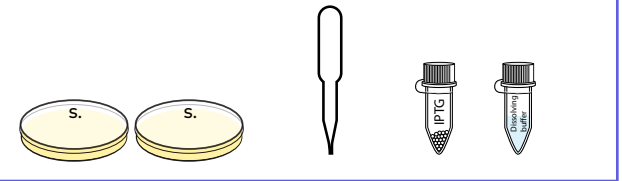
**Goal** Create a suitable chemical environment for your enzymes to function and catalyze a chemical reaction and turn colorless substrates into colorful products.

## Materials from your kit

Your final product tube  
(1) Pipet

(2) Substrate tubes  
(2) Reaction buffer tubes

(1) Enzyme stabilizer

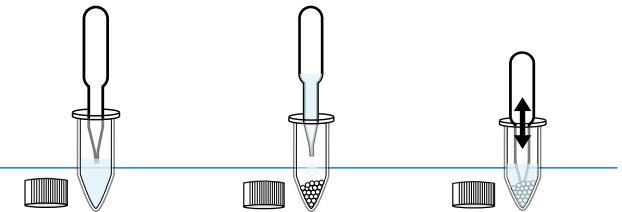


## Prepare your substrates

**10.1** Pour the liquid from one of the reaction buffer tube into one of the X-gal substrate tube. Repeat with the other reaction buffer and X-gal substrate. Let them stand for 5 minutes at room temperature. Mix by inverting a few times per minute. This will dissolve the X-gal powders.

**Note:** The Blue-gal will not fully dissolve and you may see some white powder floating around. This is ok.

**10.2** Add 3  $\mu$ L of enzyme stabilizer to each of the X-gal substrate tubes: Dip a blue loop into the stabilizer, spin it to fill the center of the loop with liquid. Transfer this liquid to the one of the X-gal tube and spin the loop to mix. Discard the loop. Repeat twice more with 2 new blue loops for the first tube, and then repeat the entire step for the second tube of X-gal.



## Start your reactions

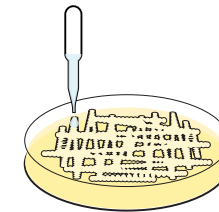
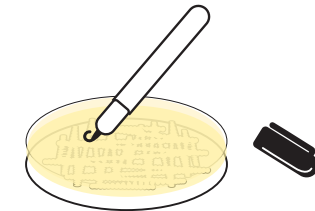
**10.3** Using the smaller pipet included in the freezer bag, pipet up 100  $\mu$ L of your enzyme from your final product tube. **Note:** The smaller pipet has a line halfway to the bulb which indicates 100  $\mu$ L volume.

**10.4** Pipet this enzyme into the substrate tube marked Blue-gal. Return the pipet to the final product tube, you will use it in the next steps.

**10.5** Close the lid of the Blue-gal tube. Mix by inverting a few times and set it on your DNA Playground's hot station turned on to 37°C. If you do not have a DNA Playground, leave it upright at room temperature.

The Blue-gal reaction happens over 24 hours. Check back on your tube often and take photos to see the color-change happen over time. You've started up a chemical reaction!

**10.6** Pipet up another 100  $\mu$ L of your enzyme from your final product tube and add it to the Yellow-gal tube. The Yellow-gal reaction happens over a few minutes so keep your eyes on your reaction. Mix your tube by inverting a few times and set it on your DNA Playground's hot station turned on to 37°C. You will see the clear liquid change color... that means your reaction is happening!



You can learn more about chemical reactions in Chapter 6 of the Zero to Genetic Engineering Hero guide book.

## Note

If you cannot see any color change, complete the troubleshooting guide at [www.amino.bio/troubleshoot](http://www.amino.bio/troubleshoot)

# Congratulations!



Using your Blue-it Kit™, you inserted a DNA Program inside single-celled organisms, bacteria, and had the bacteria execute that DNA Program to produce beta-galactosidase enzyme, which you then extracted from the bacteria and used to catalyze a reaction. Just like scientist and industries do every day inside their large laboratories! To learn more about substrates, enzymes, and enzymatic reactions, look at Chapter 6 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, ingredients, 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](https://www.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](mailto:help@amino.bio)  
Feedback, Suggestions, Comments: [info@amino.bio](mailto: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](https://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!

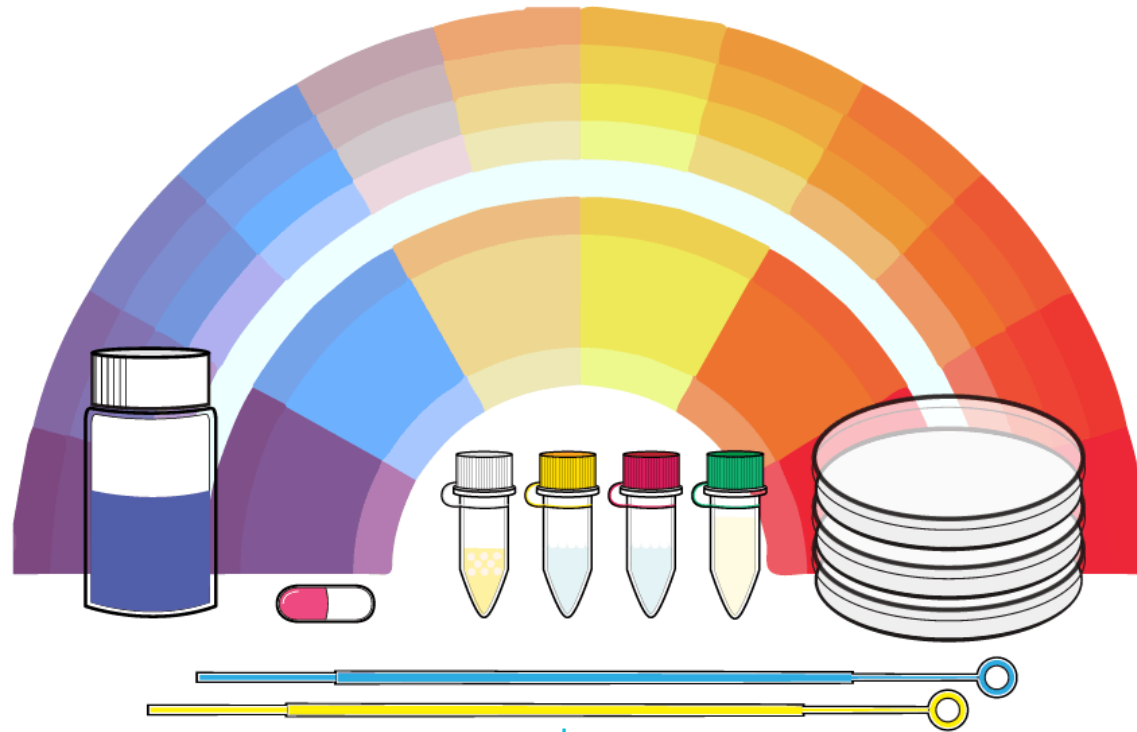
2. You may need to try again to hone your skills. See our Youtube videos for tips and tricks on how to improve your chances of success.

**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](mailto:help@amino.bio)



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