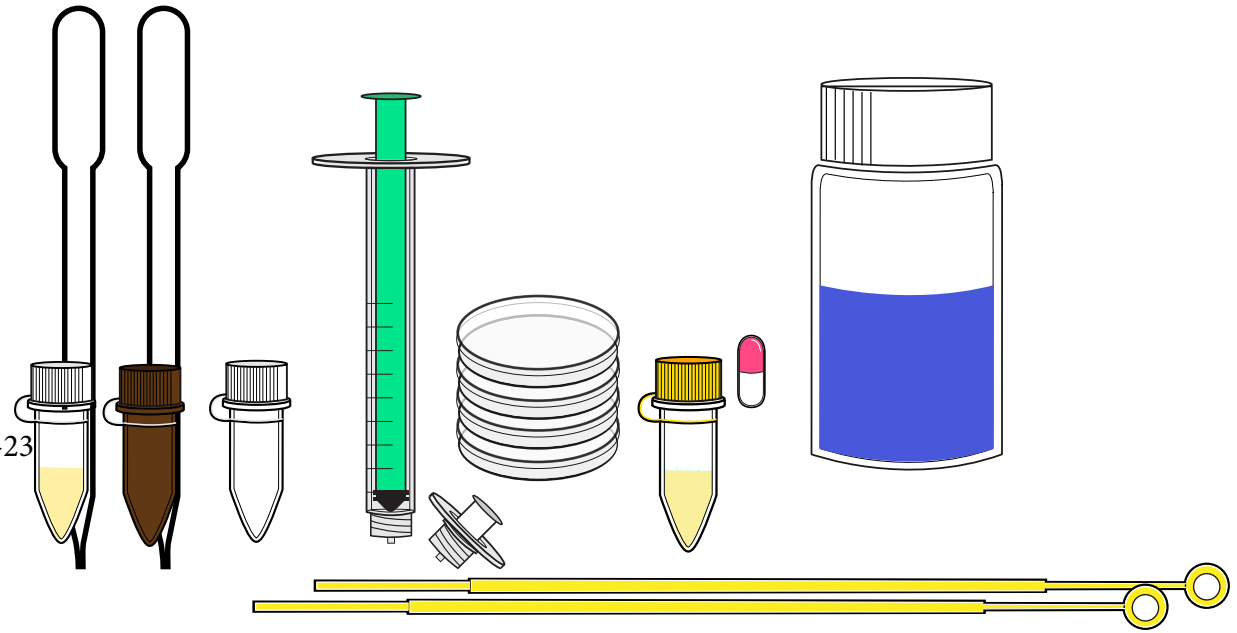
PLATE EXTRACT-IT KIT™

MANUAL



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www.amino.bio

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PLATE EXTRACT-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 importantly, know what to do in case of a spill or accident.

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

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

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

Practicing Safe Science

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

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

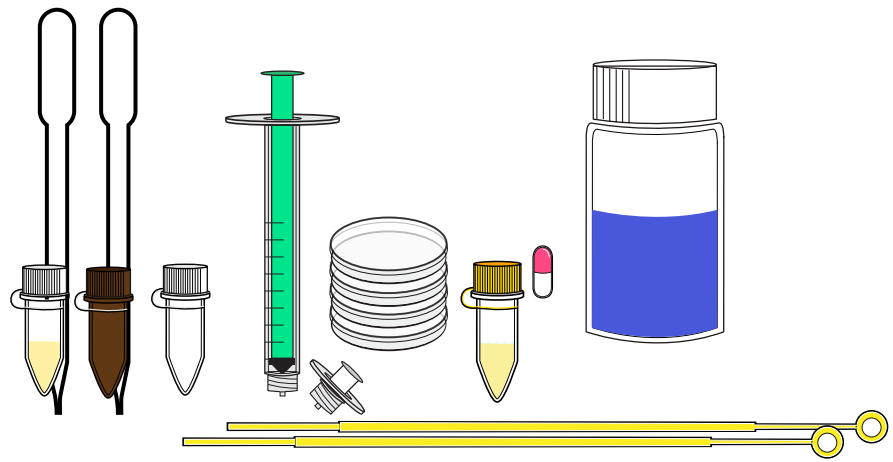
We recommend the system and kits for ages 12+, under adult supervision, and 14+ with or without supervision. We recommend that an adult empties the discard container. The cleaning instructions must be strictly followed for safety and experiment success. Make sure to store the kit per the instructions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

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

You can download a biosafety poster for your space from www.amino.bio/biosafetyinaction and complete a short safety quiz at www.amino.bio/biosafety-quiz

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

Discover your Plate Extract-it Kit™



For an end-to-end bioengineering experience, Amino Labs provides you with the means to extract and purify what your bacteria produced so you can use it outside of the system. In other words, The Extract-it kit allows you to take the product created by your DNA program plasmid (for example, a coloured protein) from within the bacteria so that you can use it.

First, the bacteria's cell wall will be broken open, and then filtered out so that you can obtain a solution of proteins. You will then filter this liquid for sterilization. What is cool about this is that the DNA program is still present within the product, so that if someone ever wanted to, they could copy it from there, and grow it once more in bacteria!

Specifically, your kit will allow you to complete the following hands-on exercises to successfully achieve protein extraction :

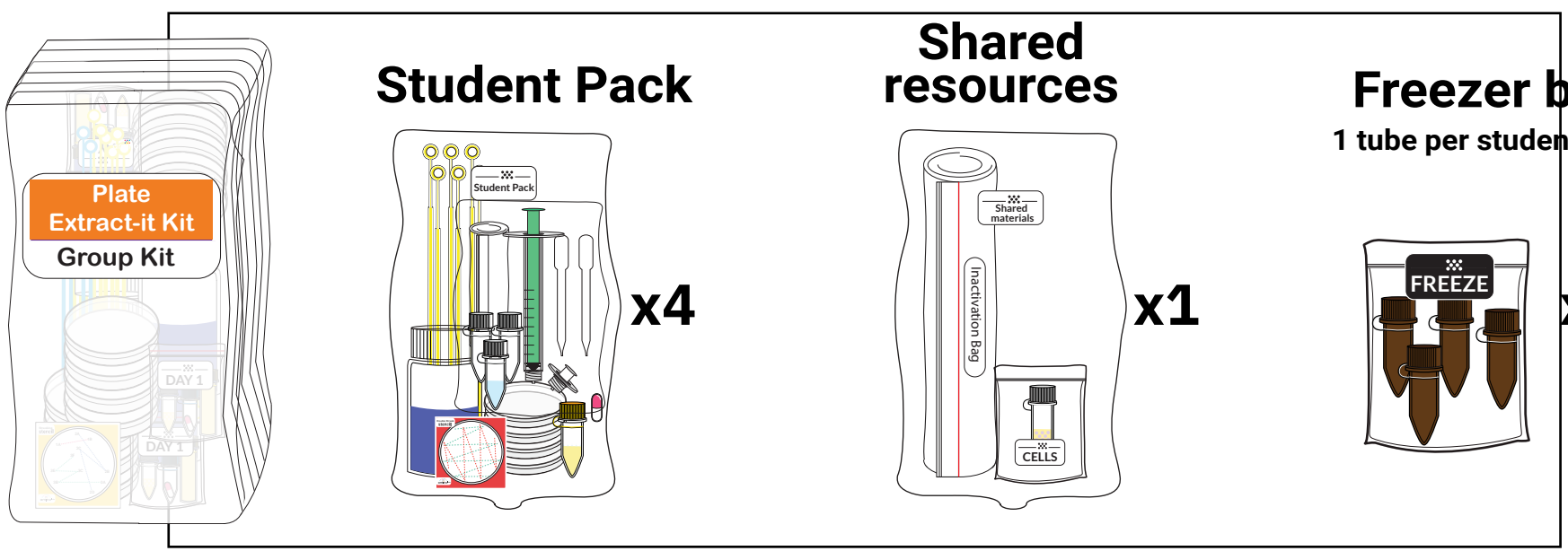
1. Grow more bacteria to "amplify" the amount of protein you can extract.
2. Collect bacteria and centrifuge it down into a "pellet".
3. Lyse (break open) the bacteria using surfactant and enzymes.
4. Collect and filter the proteins.

Individual kit size

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

Group kit size

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



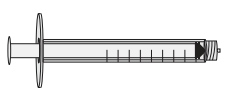
Kit Components

Inside the Extract-it Kit™, you will find these components:

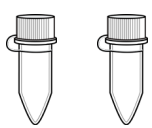
Lysis buffer: : softly breaks open (lyses) the cells to release the cell contents. This buffer should be used in concert with Lysis Accelerator.

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.

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.

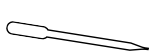


Syringe: Used to push unfiltered extract through a filter. Caution! Do not press to hard to avoid liquid mishaps. Goggles recommended when using the syringe.

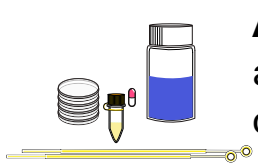


(2)1.5 mL Screw Cap Tube : Use one to store your final, extracted and filtered product, and one to use as a balancing tube when microcentrifuging.

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



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



Agar, Sterile Water, Plates, Antibiotics, Loops : Just like the Engineer-it Kit, these components are used to make selective LB agar. The loops are used to streaked your already engineered cells as well as to harvest your grown engineered cells into Lysis Buffer.

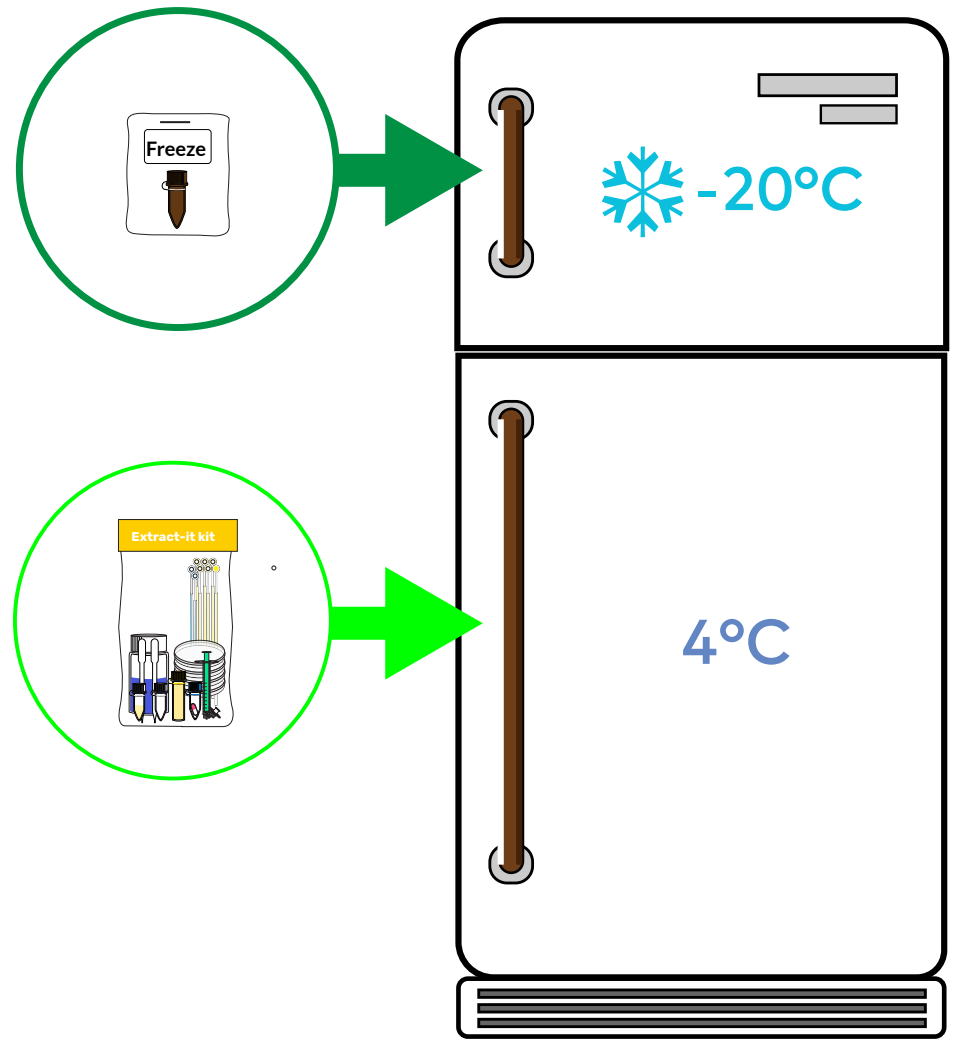
Unpacking and storing your kit

For a better shelf life and successful experiments:

- place your Extract-it Kit™ bag in a standard refrigerator at around 4°C.
- place the smaller Freezer bag with the brown tube(s) in a freezer.

Do Not Freeze all of your kit!

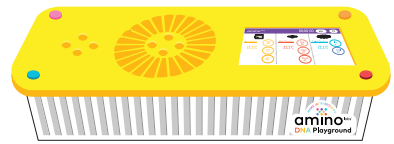
Do not leave tubes at room temperature!

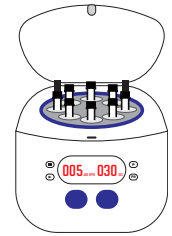


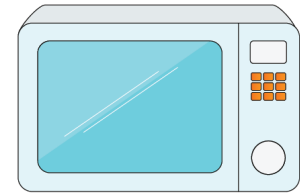
Necessary equipment

For Best results:

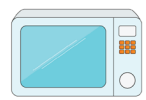
- DNA Playground™ or BioExplorer™
- Microwave
- Microcentrifuge

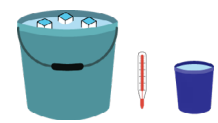






Alternative solution:






- **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"**. This will replace the **Incubator** set to **"37"**. If you do not have an incubator (biology or egg one, as long as they set to 37°C), you can create one using an online tutorial Search for DIY incubator on our youtube chanel - Youtube.com/aminolabs - or go to this direct link: https://www.youtube.com/watch?v=LEsv0Qvbczs

Necessary safety supplies

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

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

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

Bleach ~250 mL
to inactivate all the experiment materials at the end of the experiment.

Timeline

Plate Extract-it Kit

1st day

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

Incubation
24-48 hours

2nd day

Collect bateria, lyse bacteria.
(30-45 minutes)

Incubation
1-24 hours

3rd day

Centrifuge lyse bacteria, filter-sterilyze the extract.
Can be done on 2nd day.
(45 minutes)

4th day

See and use results!
Can be done on 2nd, 3rd day.
(45 minutes)

Experiment Protocol

An Experiment Protocol is the 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 and Tutorial videos.

In the next pages are detailed, step by step instructions to complete the experiment. **Please make sure to read all the steps in the section/sub-sections prior to starting the hands-on manipulation**; some steps will be done in rapid sequences.

Remember that a series of real-time video tutorials covering our different kits are available on our youtube channel : youtube.com/c/AminoLabs

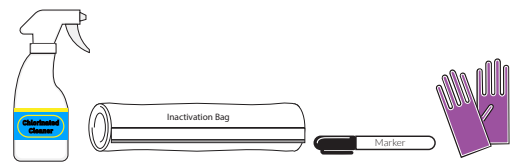
Experiment Protocol

0. Prepare your space

Goal Set yourself up for sucess.

Materials from the kit
(1) Inactivation bag

Materials not in your kit
(1) 1L discard container
Chlorinated bleach spray or wipes
Paper towels
(1/person) Pair of gloves



Make sure you have the necessary materials as explained on page 15, including gloves, microwave, and cleaner before you start.

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

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

- your tubes of cells if you are not saving them for a future experiment*,
- any used inactivation loops,
- bacteria paint palette once it is used (unless you are saving it to paint your other canvases later)*
- any empty tubes like the agar, buffer and selection tubes,
- any gloves that have touched bacteria.

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

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

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

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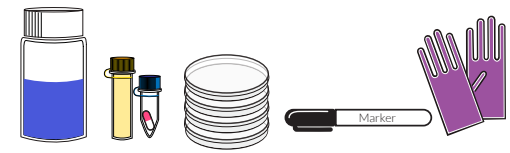
* If you are saving the tubes of cells or your painting palette for a future experiment, place them back in their ziploc bag after use and store them in a refrigerator. We recommend you use a sealed plastic container to store all your experiment materials inside a refrigerator if you also use this to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

1. Creating selective LB Agar Plates Day 1, 25 minutes

Goal Create selective LB agar plates.

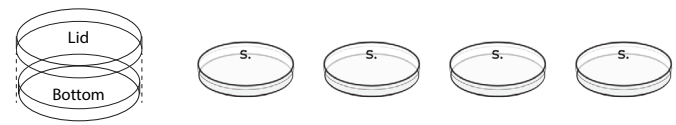
Materials from your kit
(1) 50 mL sterile water
(1) LB agar powder
(1) antibiotic pill
(4) 6 cm petri dishes

Materials not in your kit
(1) Sharpie marker



Prepare

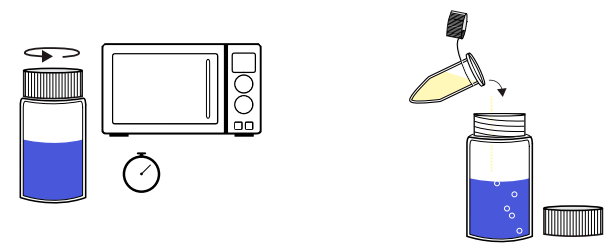
1.1 Label each petri dish with a sharpie-type pen. Make sure to label the bottom of the petri dishes (*the bottom is the part that has the smaller diameter of the two: the bottom fits inside the lid)*. Label **4x** S. (for selective) + Add [your initials] if doing this in groups with multiple kits.



Mix the Agar

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

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



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

1.5 Microwave the water and agar powder in 4 seconds intervals until you see it boil again. Instead of a rolling boil, you will see more of a foam forming above the molten LB agar liquid. ***Careful, the liquid will boil over if you microwave in more than 4 sec. increments.*** After you see the liquid foaming, swirl to mix for 10 seconds. Try not to shake vigorously as this will create bubbles in your agar and make the surface of your agar uneven.

Note: If you've 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: don't swirl too vigorously. The gelatin capsule may not fully dissolve. The important thing is that the contents of the capsule do dissolve.

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

Pro-tip: If there are water droplets on the surface of the LB agar, this can disrupt your art. Bacteria that you will be painting with can enter a droplet and spread throughout the droplet therefore 'smudging' your art. To avoid this

make sure the lid is partially over top to allow for evaporation and a dry LB agar surface.

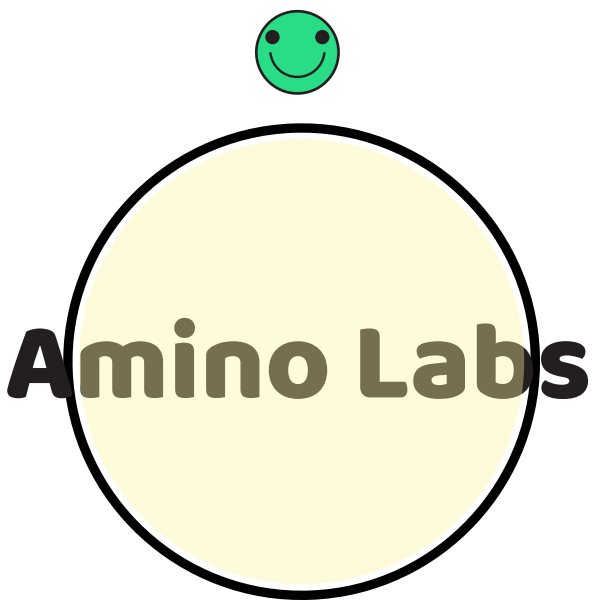
1.8 Let the LB agar harden. This can take up to 20 minutes depending on how warm and humid your environment is. You will use 1 plate in the next step. You can store the remaining 3 plates in the ziploc bag in the refrigerator for day 2.

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.

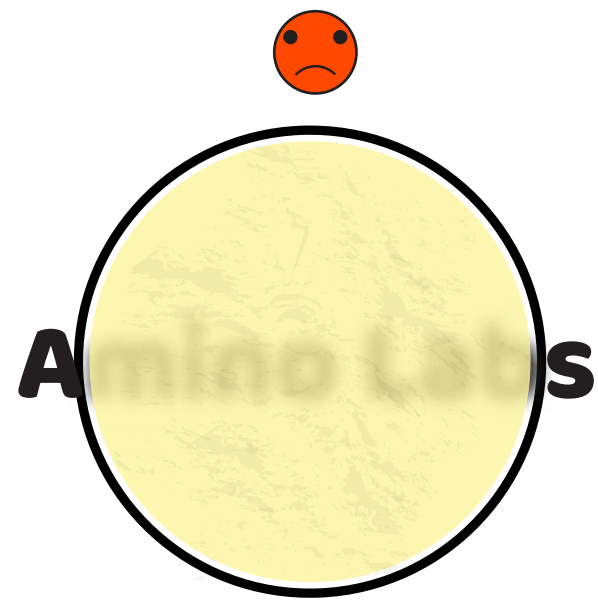
Checkpoint - Agar Plates

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



A perfect Agar plate is completely clear and solid - if you set it 4” above some image or text, you should be able to read it / see it clearly.

Move on to the next step!



An agar plate that is cloudy and/or bumpy and/or soft is not ideal - if you set your plate 4” above some text or image and cannot see clearly through it, it means you needed more boiling or mixing.

Troubleshooting tip

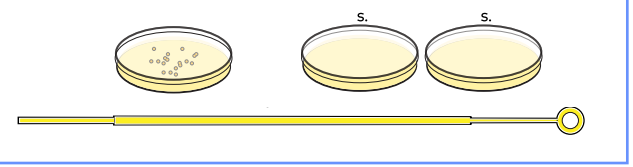
If your plates do not solidify after 30 minutes it is very likely that the water was not boiled enough to dissolve the agar powder. As a ‘hack’, you can pour all of the petri dish content back into the water bottle and microwave until you see it boil. Swirl to mix and re-pour your plates.

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

2. Amplify (Culture) engineered cells Day 1, 15 minutes + 24 hours wait time

Goal **Create living paintings**

Materials from your kit
(2) Selective Agar plates | Your engineered bacteria | (2)Yellow Loops



Prepare

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

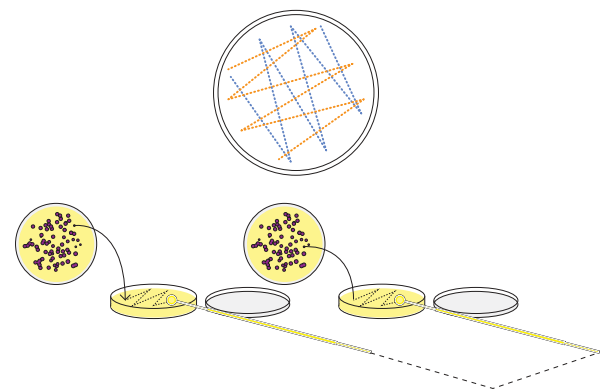
Streak

2.2 Take your engineered cells from your Engineer-it Kit experiment. Place your 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.

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

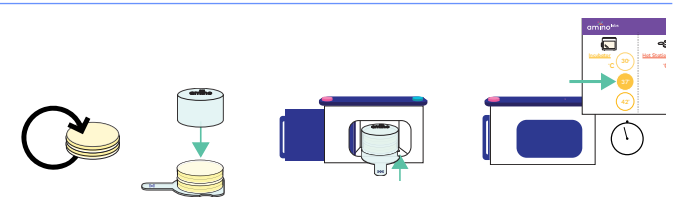
2.4 Using the same yellow loop, trace the second zigzag, which is at 90° of the first. This will ensure you will have lots of cells growing across your plate. Discard the loop.

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



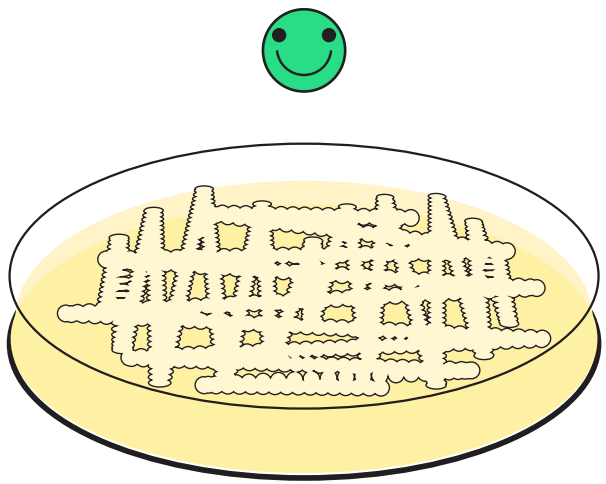
Incubate Overnight

2.6 Incubate your streaked plate **upside down** at ~37°C for up to 24 hours: Flip your plates upside down so that the agar is up and the lid down. Set your plate in the incubator or in a plastic bag in a warm location if you do not have an incubator. Incubate for 16-24 hours (it may take longer if you do not have an incubator. Bacteria prefer 37°C to grow optimally). If you have Amino Labs' minilab, remember to close the incubator door and lock it!



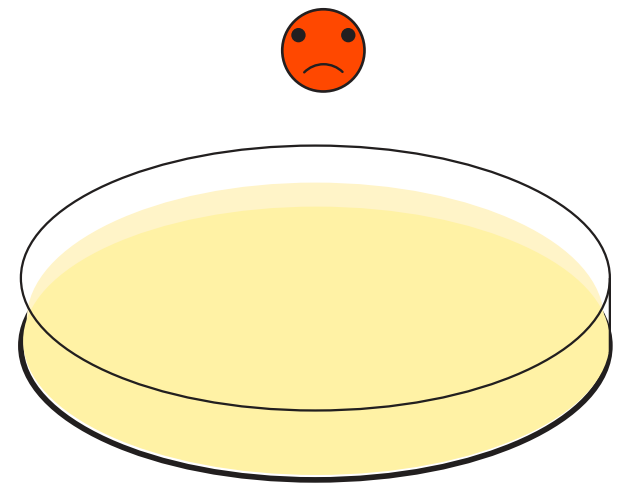
Checkpoint - Did your cultured cells grow?

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.

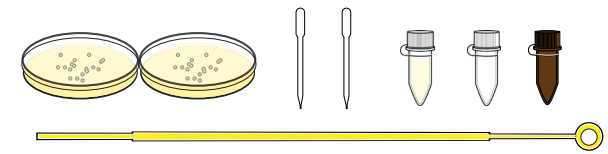
If you cannot see any growth after 48 hours, repeat step 2 on the 2 unused plates from step 6. If you still cannot see growth on these after incubating 48 hours, your experiment may have failed. Don't be discouraged. In science, failure is a chance to learn more.
Complete the troubleshooting guide at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

3. Harvest & lyse the bacteria Day 2, 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 engineered bacteria plates
(1)Yellow Loop
(1) Lysis Buffer tube
(1) Lysis Accelerator
(1) 1 mL Pipet



Harvest

3.1 Verify if your cells have grown and are fully expressing their traits. If they have not yet, keep incubating. If they have, take them out of the incubator.

3.2 Turn on the Cold Station to Ice, and set the tube of Lysis buffer in the cold station.

3.3 Get your tube of Lysis Accelerator from the freezer and also place it in the Cold station.

3.4 Take a yellow inoculating loop and gently drag the loop across the surface of the LB agar to collect the cells from the petri dish. The cells will collect inside the loop.

3.5 Once the loop is full, dip it into the Lysis buffer tube and twist it to dislodge and mix in the cells in the buffer.

3.6 Repeat collecting and blending cells in the buffer until you've collected all of the cells on the two petri dishes.

3.7 Using the yellow loop, blend the cells and buffer for a further 60 seconds to make sure they are fully suspended. This will help the surfactant in the Lysis Buffer begin lysing the cells.

3.8 Open the tube of Lysis Accelerator that you have placed on the Cold Station. Using one of the plastic pipette included in the your kit, suck up all of the Lysis Buffer and cells mixture and gently add it into the Lysis Accelerator tube. You may have to repeat this a few times to get all the mixture into the Lysis Accelerator tube.

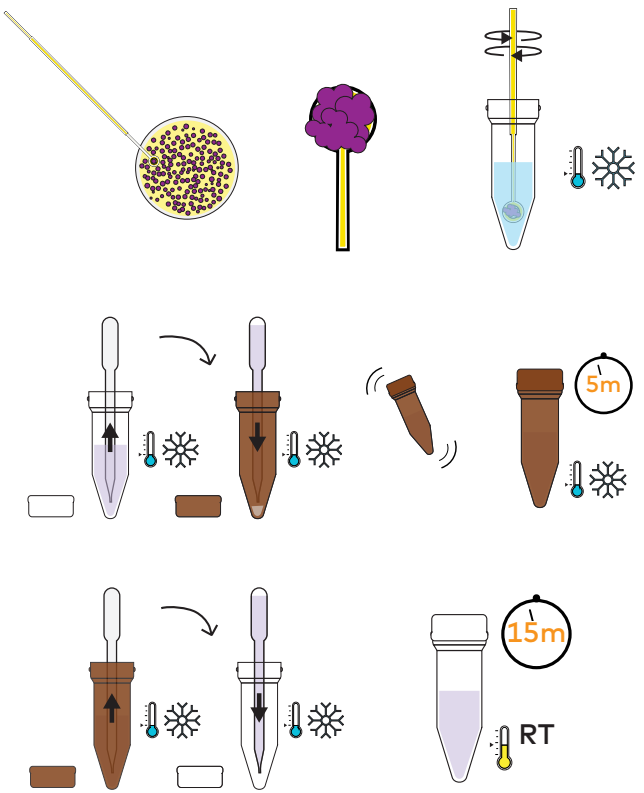
3.9 Leave your empty Lysis Buffer tube in the cold station and place your pipet inside to hold it until you use it again in the next few steps.

3.10 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.

3.11 Place it on the cold station to cool for 5 minutes.

3.12 After 5 minutes, use your pipette to move all the liquid in the Lysis Accelerator tube back into the clear Lysis Buffer tube. Be careful as the liquid will be bubbly. You can wait a few seconds for the bubbles to go down before putting on the lid.

3.13 Turn off the cold station and let the mixture incubate at room temperature for 15 minutes, or up to 24 hours. You can now dispose of the Lysis Accelerator tube and pipette in the inactivation bag.

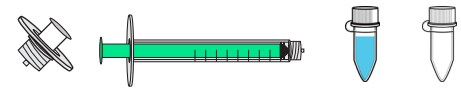


4. Collect & Filter-Sterilized bacteria Day 3, 30 minutes + 24 hours wait time

Goal **Passing the extracted pigment through a 0.22 um filter to get rid of cells and other debris (sterilize the products)**

Materials from your kit

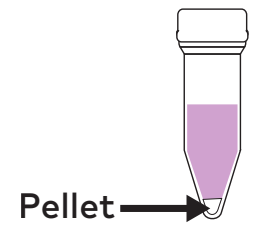
Your tube from the previous step
(1) Syringe
(1) Syringe Filter
(1) 1.5 mL tube for final product



Pellet

4.1 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 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.

4.2 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:

4.3 Using 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).

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

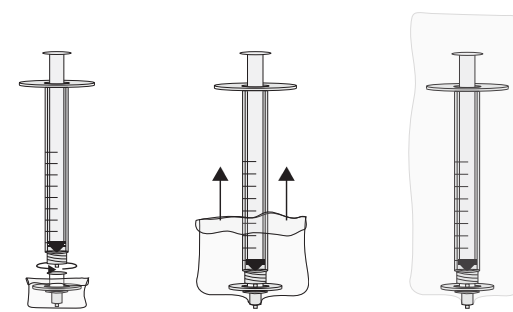
4.5 Open up the syringe filter by taking the paper cover off. DO NOT fully remove the filter! You want to make sure that you do not contaminate the other end, the one that will not screw into the syringe but will dispense your sterilized sample.

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

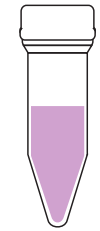
4.7 Place the syringe and filter inside the Burst Bag, with the tip of the filter poking out of the triangle cut at the bottom of the Burst Bag. You can lay this on the table and be sure not to touch the sterile end of the filter.

4.8 Once centrifuged, gently pour your centrifuged sample into the open syringe. Be careful to only pour the liquid. You can also use one of the plastic pipette to add the liquid to the syringe. If the the pellet of cell debris falls into the syringe, it will clog the filter! If this happens, and cell debris gets into the syringe, pour the entire mixture back into your tube and repeat the centrifugation.

4.9 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.



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



4.11 Close and tighten the lid on your Final Product tube. Congratulations! The solution in this tube is an extracted, filter-sterilize product you can now use!

You now have sterilized proteins you microfactured yourself with your genetic engineering skills! If you extracted color proteins, you can store your final pigment in the refrigerator, or at room temperature. Many colour pigments will keep their colour for more than a year if kept out of the sun!

Share your creations with us ! @aminobiolab

Storage, Disposal, Clean Up

After you sees your results, all experiment Petri dishes, tubes of cells and loops should be in the inactivation bag in your discard container. Disposing of experiment materials is an integral part of the experiment. **Always wear gloves for cleanup!**

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

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

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

D. Inactivation: Make sure all bacteria, agar, tubes, loops, paintbrushes, Petri dishes, contaminated gloves, and other non-paper material you are not keeping are in the inactivation bag. Remember that any paper packaging like loop wrappers, plastic bags, and gloves that have not touched bacteria go in the regular garbage or recycling.

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

Spray some chlorinated bleach cleaner in the discard container once emptied if it has become contaminated by experiment materials. Let it sit for an hour before wiping down. You can wait to wipe it down until you empty out your inactivation bags the next day.

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

Glossary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Transformation: See competent cells.

Troubleshooting

Here are some possible common issues:

Your agar is too wet/ doesn't solidify:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

More Information

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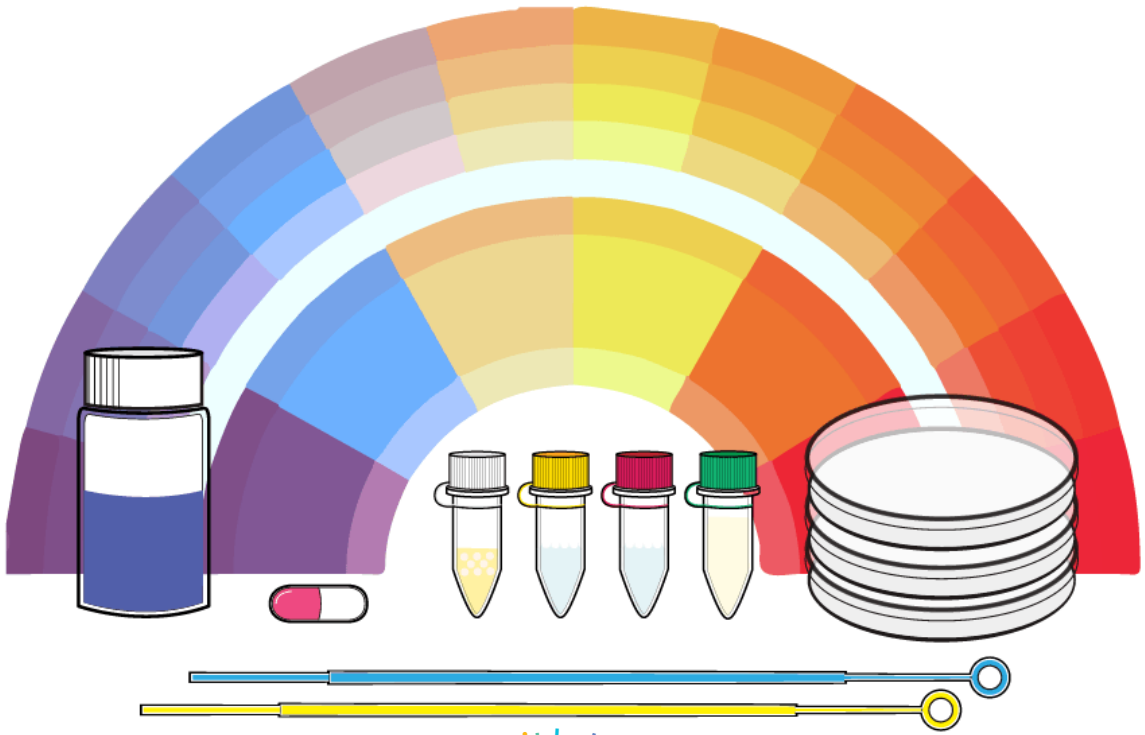

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