

Will-it Grow KitTM INSTRUCTION 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 as well as store, use, and get the most out of your kit. It will also let you know what to do in case of a spill or accident.

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

Amino Labs is excited to welcome you to the world of the genetic engineering with the Streak-it Kit[™], Canvas Kit[™], Engineer-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. 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 or scratch itches with your gloved hands!
- If using the DNA Playground[™] or BioExplorer[™] place it on a stable work surface. Keep it level at all times.
- Clean up your station, spills and work surface before and after use. Use a 10% solution of chlorinated bleach generously sprayed onto a paper towel and rub onto any contaminated surfaces. (Careful! This can discolor your clothes). A chlorinated spray cleaner also works.
- Find a container to hold the inactivation bag where you will discard used items. An old 1L yogurt container, large plastic cup or the like will do. Used items (in science, these are often called consumables) will be loops, tubes or used petri dish.
- Eye-wear is not provided but can be worn.

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

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

How will I learn?

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

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

We also have additional resources to help you go further:



Our Lessons and Resource Center at <u>wiki.amino</u>.bio includes everything from discussion prompts to quizzes, activites, lessons and links to other cool biotechnology content.



View Real-time tutorials videos at youtube.com/AminoLabs.



Would you like for an Amino Labs team member to guide you through your journey? Try the **Virtual Tutoring or Teacher Professional Development**, a multi-day experience completed via video conferencing. <u>https://amino.bio/collections/virtual-sessions</u>



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

Discover your Kit[™]



With the Will-it-Grow? Kit, you'll explore the hidden world of microorganisms—tiny living things too small to see without help. By growing them on petri dishes, you'll reveal their presence through colorful, uniquely shaped colonies. Each colony begins from a single cell that multiplies into a visible group you can see with the naked eye!

In this experiment, you'll swab surfaces from around your home or school, transfer them onto the agar in your petri dishes, and use the included identification chart and online tools to investigate what's growing. Just remember—always keep your petri dishes sealed to stay safe!

This method of collecting and growing microbes is a real scientific technique used in microbiology labs around the world. Scientists use it to study bacteria from hospitals, farms, oceans—even outer space! It helps them understand where microbes live, how they behave, and whether they are helpful, harmful, or just part of everyday life. By practicing this skill, you're stepping into the shoes of a microbiologist and learning how to observe and investigate the living world we usually can't see.

Kit Components



Sterile Water: Sterility is critical when doing biology/biotechnology experiments. This water is sterilized in an autoclave to ensure there are no contaminating organisms present. This 100 mL volume, when used with our agar powder tubes is enough to make 10 petri dishes.¹



Petri Dish / Plate: A Petri dish is a transparent lidded dish use to grow cells in. Petri dish will be filled with a solid media that the cells can eat and grow on.



Inactivation Bag: A heavy duty bag to hold the experiment waste. Once finished, add bleach and water to the bag to inactivate the samples as per *Storage, disposal & clean up* Instructions.



Streaking stencil: These stencils help you streak your cells in different patterns to obtain individual colonies or lawns. Place the stencil under the petri dish to trace.



Sterile cotton swabs: Used to transfer cells onto the agar surface in a sterile way. Each swab is individually wrapped and sterile to prevent contamination during streaking.

Plastic bags: These resealable bags are used to safely store the petri dishes after streaking. Keeping plates sealed ensures safety during incubation.



Starter liquid: This tube contains sterile water used to help collect microorganisms from surfaces. Dip a sterile swab into the starter liquid before swabbing the surface you're sampling to loosen and suspend the cells.

Agar Powder: This LB agar powder is industry standard. Each tube of agar powder can make 50 mL of molten agar. Use both tubes to make 100 mL of agar you need in this experiment. Agar is both the surface the bacteria grow on and the food they eat to grow.

Unpacking and storing kits

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

Once refrigerated upon arrival, your kit will be best by the date found on the 'Best by' sticker on the outer packaging of the kit. The date on the sticker is in Month/Year format.

If your refrigerator is not a science-only refrigerator, we recommend placing your science experiments inside a sealed plastic container before placing them in the refrigerator, especially once your kit is open.

Do Not Freeze your kit!



Necessary Equipment

For Best results:

- 30-37°C incubator or DNA Playground (recommended but optional)
- Microwave
- Permanent marker (like a Sharpie)





Alternative solution:

- DIY Incubator/ thermometer or warm environment: This will replace the Incubator set to "30/37". If you do not have an incubator (biology or egg one, as long as they set to 37°C), you can create one using our online tutorial: <u>https://www.youtube.com/watch?v=LEsv0Qvbczs</u>
- If you have neither incubator or DIY version, you can try incubating your petri dishes in a resealable bag in a warm environment. Note that it will take a few more days to see results at room temperature.

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. 1 pairs/person if you will keep & reused each day, or 4 pairs/person if not saved & reused.

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

Will-it Grow Kit

Day 1

Create LB agar petri dishes, swab surfaces, transfer bacteria and incubate (45-60 minutes)

Day 2-4+

Document and identify the organisms that grew with the identification chart. Inactivate the petri dishes.

(45-120 minutes)

Incubation 24-72 hours

Key pitfalls to avoid!

In the next pages are detailed, step-by-step instructions to complete the experiment. **Please make sure you/your stu**dents read all the steps before starting the hands-on manipulation; some steps will be done in rapid sequence.

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

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

Experiment Protocol

0. Prepare your space



Make sure you have the necessary materials as explained on the *Necessary Equipment and Necessary Safety Supplies* page, 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:

- any used cotton swabs
- · incubated petri dishes you are finishes with,
- 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.

How to sterilize a lab bench or surface *Pro-tip*

If you are using an alcohol solution (70% isopropyl alcohol), spray your work surface. Wait 30 seconds and then wipe down the surface.

If you are using a bleach solution, spray your work surface. Wait 10 minutes and then wipe down the surface.

0.4 If you have equipment like an incubator or DNA Playground, Set them down 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.

Creating LB Agar Petri dishes Day 1, 20 minutes Goal Create selective agar petri dishes. Materials from your kit: Bacteria Bag Materials from your kit: Bacteria Bag Materials not in your kit

(1) Sharpie/permanent marker

Prepare

(1) 100 mL sterile water

(2) LB agar powder

3.1 Label each petri dish with a permanent marker. 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*). Number them from 1 to 10 and add your initials if doing this in groups with multiple kits or in a shared space/incubator.

(10) 6 cm petri dishes

Make the agar

3.2 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.

3.3 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 petri dishes will not solidfy !!**

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

3.5 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.

3.8 Pour the molten agar into the bottom of all 10 petri dishes. Place the lids 3/4 of the way back on so that the agar can cool and dry (solidify).

3.9 Let the agar harden. Once the petri dishes have fully cooled and the agar is no longer liquid or "wobbly", close the lids. This can take up to 20 minutes depending on how warm and humid your environment is. You will use the petri dishes in the next step.

Troubleshooting tip

If your petri dishes 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 petri dishes.









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



A perfect Agar petri dish 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 petri dish that is cloudy and/or bumpy and/or soft is not ideal - if you set your petri dish 4" above some text or image and cannot see clearly through it, it means you needed more boiling or mixing.

Troubleshooting tip

If your petri dishes 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 petri dishes.

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

2. Swab surfaces and incubate Day 1, 30 minutes + 24-72 hours wait time

<u>Goal</u> Grow the microorganisms from different locations around you.

Materials from your kit (10) LB petri dishes (10) Sterile cotton swabs

(1) Starting Liquid

Prepare

2.0 If you have an incubator, set it to 37°C

2.1 Find 10 surfaces you are curious to swab. Using your marker, write each surface name on the bottom of your petri dishes, one per petri dish. Suggested locations: Phones, door knobs, keyboards, desks, light switches. You can also do 'before' and 'after': swab the surface for the 'before' petri dish, wipe with a clearning cloth or other cleaning spray/gel, and swab again using a new swab for the 'after' petri dish to see if your results change.



Swab and transfer

2.2 Place a petri dish on top of the zigzag pattern on the streaking stencil. Open the lid.

2.3 Take one of the sterile cotton swabs and dip it into the starter liquid. Then, gently rub the cotton swab on the surface that matches your petri dish (for example, if you wrote 'phone' on your petri dish, swab your phone). This will transfer any microorganisms on the surface to the swab.

2.4 Place the corresponding petri dish on top of the zigzag pattern on the streaking stencil. For example, if you just swabbed your phone, place the 'phone' petri dish on top of the stencil. Open the lid

2.5 Gently run the end of the cotton swab that touched the surface onto the agar of the petri dish, following the double zig zag pattern on the stencil. The agar is a soft surface, you do not need to press into it.

2.6 Place the lid back on your petri dish then place it into one of plastic zipper bag. Seal the bag carefully, flip it so that the petri dish is upside down and place it in the incubator. The last petri dish you will swab will be stored in the bag in which your sterile swabs were packaged.

2.7 Repeat steps 2.2-2.6 for all your petri dish/surfaces. You do not need to do them all now. To keep some for later, place the unused the petri dishes back in their bag (use a piece of tape to seal the bag) and put them in the refrigerator until you are ready to swab more locations. You can keep them in the refrigerator for a week or two. Placing them in a tupperware-style container will help keep them from drying out or getting contaminated.

Incubate Overnight

4.19 Incubate for 24-72 hours, until you see colonies grow. If you are not using an incubator, it will take longer.

Important Safety Instruction: If you see any black mold (black dots, black fuzzies) growing, stop your incubation and proceed to the analysis for that petri dish. Take some photos and place the petri dish in the inactivation bag without touching it. Do not open your petri dishes after starting your incubation. Always keep them sealed until you inactivate. After you finish your observations, make sure to dispose of your petri dishes properly by following the inactivation steps detailed at the end of this manual.

3. Results and analysis Day 2, 45-120 minutes

Goal Observe and Describe Your Bacterial Colonies (Morphology)

Materials Incubated petri dishes Pen/paper/printed page

Prepare

For this next part, you will need to access the identification table online. You can also print or use your computer to fill out the next pages for your analysis.

The identification tables: https://wiki.amino.bio/en/Resources/By-Kit/Will-itGrow

The steps you will complete for each petri dish* are:

- 1. Record Your Plate Information
- 2. Observe and Sketch Colonies
- 3. Choose a Colony to Identify
- 4. Identify Your Microorganisms using the tables provided
- 5. Safely Dispose of Your Petri Dishes

Important: Do not touch or open your petri dish to observe your colonies—this can be unsafe! Remember, the organisms growing are unknown! Always keep your dish sealed in its plastic bag.

*If you did not get any colonies on some of your petri dishes, you can use multiple analysis charts for the petri dishes where you did have colonies. Pick different colonies for each chart.



Name:	Date:
Experiment Details:	
Swab Location:	
Incubation Period:	
Growth media used:	

Colony Observations: Sketch colonies observed on agar:

Safety Reminder:

Do not open your petri dishes after the microorganisms have grown! Always keep them sealed to stay safe. After you finish your observations, make sure to dispose of your petri dishes properly by following the inactivation (disposal) steps detailed in your instruction guide.

Colony Shape (circle all that apply):

Circular | Irregular | Filamentous | Rhizoid

Colony Surface (circle all that apply):

Concentric | Radial Stripes | Granulated

Colony Edges (circle all that apply):

Plain | Filiform | Undulate | Folded | Serrated | Lobate | Filamentous

Colony Side View (circle all that apply):

Convex | Umbonate | Crateriform | Convex and Folded | Concave | Pseudomycellium | Folded and flattened | Flat | Raised

Colony Color (circle all that apply):

White/Cream | Yellow | Tan/Beige | Grey | Red/Pink | Orange | Blue/Violet/Purple | Black | Green | Other: _____

Colony Texture (circle all that apply):

Smooth | Mucoid | Dry | Buttery | Granular | Waxy

Use the data above and the identification tables to find its potential identity <u>https://wiki.amino.bio/en/Resources/By-Kit/Will-itGrow</u>

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Circular | Irregular | Filamentous | Rhizoid

Colony Surface (circle all that apply):

Concentric | Radial Stripes | Granulated

Colony Edges (circle all that apply):

Plain | Filiform | Undulate | Folded | Serrated | Lobate | Filamentous

Colony Side View (circle all that apply):

Convex | Umbonate | Crateriform | Convex and Folded | Concave | Pseudomycellium | Folded and flattened | Flat | Raised

Colony Color (circle all that apply):

White/Cream | Yellow | Tan/Beige | Grey | Red/Pink | Orange | Blue/Violet/Purple | Black | Green | Other:

Colony Texture (circle all that apply):

Smooth | Mucoid | Dry | Buttery | Granular | Waxy

Use the data above and the identification tables to find its potential identity <u>https://wiki.amino.bio/en/Resources/By-Kit/Will-itGrow</u>

Potential identity: _____

Name:	Date:
Experiment Details:	
Swab Location:	
Incubation Period:	
Growth media used:	

Colony Observations: Sketch colonies observed on agar:

Safety Reminder:

Do not open your petri dishes after the microorganisms have grown! Always keep them sealed to stay safe. After you finish your observations, make sure to dispose of your petri dishes properly by following the inactivation (disposal) steps detailed in your instruction guide.

Colony Shape (circle all that apply):

Circular | Irregular | Filamentous | Rhizoid

Colony Surface (circle all that apply):

Concentric | Radial Stripes | Granulated

Colony Edges (circle all that apply):

Plain | Filiform | Undulate | Folded | Serrated | Lobate | Filamentous

Colony Side View (circle all that apply):

Convex | Umbonate | Crateriform | Convex and Folded | Concave | Pseudomycellium | Folded and flattened | Flat | Raised

Colony Color (circle all that apply):

White/Cream | Yellow | Tan/Beige | Grey | Red/Pink | Orange | Blue/Violet/Purple | Black | Green | Other: _____

Colony Texture (circle all that apply):

Smooth | Mucoid | Dry | Buttery | Granular | Waxy

Use the data above and the identification tables to find its potential identity <u>https://wiki.amino.bio/en/Resources/By-Kit/Will-itGrow</u>

Potential identity: _____

Microorganisms Investigation

Use your observations, <u>https://microbewiki.kenyon.edu/</u> and internet search to identify some of the microbes growing on your petri dish. (MicrobeWiki should have most of the answers!). Note: You might only be able to identify the general type of microbe, not the exact strain. Use the tips provided on the identification tables to narrow down your choices.

1. Based on your observations and the information you found online, list the microorganism(s) you think are on your petri dish and explain your reasoning::

2. Pick one microorganism from your petri dish and write its name below. You'll use this microorganism to answer questions 3 to 6.

Class:_____

Species:_____

Size of a cell of this microorganism:_____

3. What health risks might be associated with exposure to this microorganism?

4. Where can you find this microorganism in nature?

5. What other interesting characteristics or behaviors might this microorganism have, and under what conditions would these features appear?

6. What does this microorganism do naturally or in our daily lives? How does it help or harm us? (For example, Micrococcus helps reduce nitrates, which is beneficial in food processing and meat curing.)

7. Use the CDC Public Health Image Library (<u>https://phil.cdc.gov/</u>) to look up your microorganism. Describe what it looks like under a microscope. Include shape, size, color, arrangement, and gram-staining information. For example, Micrococcus cells are small, round (cocci-shaped), pink (gram-positive), and form clusters.

Cell Shape:	Cell Arrangement:	Other structures:	Gram staining:
Cocci (Spherical, round)	Diplo: Paired	Flagella (Whip-like appendage)	Gram-positive: Purple
Bacilli (Rod-shaped)	Strepto: Chains	Pili (Has hair-like appendages)	Gram-negative: Pink
Spirilla (Spiral-shaped)	Staphylo: Clusters	Capsules (Layer surrounding the cell)	Nucleus (Internal structure)
Spirochetes (Corkscrew-shaped)	Tetrads: Groups of four		
Vibrios (Comma-shaped)	Sarcinae: Cubical pack of eight cells		

8. Discuss how the microscopic description you gave relates to the name of your microbe, if applicable. For example, Micrococcus gets its name from its cocci or round shape and micro which means small.

Reflection Questions:

1. What surprised you about your microbial growth results?

2. Why do you think microbes from different locations gave different results?

3. How could you use what you've learned from this experiment in real-life situations?

4. Why is it important never to open the petri dishes after microbes have grown?



You've successfully completed your experiment-great work!

Excited about your results? Share your discoveries with friends and our growing community on Instagram, Twitter, and Facebook @aminobiolab. We'd love to see how your streaking experiment turned out!

Remember to properly dispose of and store any remaining materials by following the instructions ont the next page. And don't forget—this is just the beginning of what you can learn and achieve with microbiology. Keep exploring and experimenting!

Storage, Disposal, Clean Up

After you sees your results, it is time to dispose of everything safely. Disposing of experiment materials is an integral part of the experiment. **Always wear gloves for cleanup!**

A. Unused ingredients: If you did not use all the agar Petri dishes you poured, store these for later use. Store them in a ziploc bag within a sealed container in the refrigerator for up to a month. 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.

B. Inactivation: Place all petri dishes, agar, tubes, swabs, contaminated gloves, and other non-paper material in the inactivation bag. Paper packaging, plastic bags, and gloves go in the regular garbage or recycling.

Make sure you have gloves on and open the zipper on your petri dish bags. **Do not open the petri dishes or touch them.** Place them immediately in the inactivation bag once their protective bag is open. Once everything is in the inactivation bag, add a solution of 1 part bleach to 4-6 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.

Watch this inactivation video on youtube to see the process; <u>https://www.youtube.com/watch?v=FLVqFSwL4Kk.</u>

C. 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.

D. 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 your equipment 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.

Biotechnology 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 Deoxyribonucleic acid.

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 icecold to warm temperature, typically 42°C, 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 tradi-

tional 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 72+ hours:

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

1. Your surface did not have any microorganisms on it: its unlikely, but possible. You can try to swab it again, or swab something else

2. The organisms on the surface do not grow at 37°C, or on this type of agar: swab a different surface!.

3. If you are growing at room temperature, then it can take much longer to see the bacteria colonies. Keep waiting!

If you see black mold on your petri dish You should 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.

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

More Information





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



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



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